REVIEW ARTICLE

THE STRUCTURE AND PROPERTIES OF AQUEOUS SOLUTIONS OF SOAP

By

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The sodium and potassium salts of the higher members of the homologous series of saturated fatty acids have different properties from those of the lower members. For example, there is no reason to suspect the existence of colloidal particles in solutions of sodium acetate, but it is impossible to deny their existence in solutions of sodium stearate or palmitate[1]. McBain and Taylor[2] found that the equivalent conductivity curve of potassium palmitate shows a pronounced minimum at 0·2N and a maximum at 0·5N. Such a phenomenon had not previously been met in aqueous solutions. Similar maxima and minima were found by McBain, Cornish and Bowden[3] in the equivalent conductivity curves of sodium myristate and sodium laurate. An examination by McBain, Laing and Titley[4] of the whole range of potassium soaps from the acetate to the stearate showed that the equivalent conductivity-concentration curves are smooth in form for the soaps below the laurate, whereas the laurate, myristate, palmitate and stearate all exhibit pronounced maxima and minima.

Experiments[5,6] showed that the equivalent conductivity of solutions of soap cannot be attributed to the presence of free hydroxyl ions produced by hydrolysis. The latter could only account for a few per cent. of the observed conductivity, which is nearly as great for solutions of ammonium soaps as it is for the corresponding potassium soaps[7]. The belief that hydrolysis could account for the conductivity was finally disposed of by Reychler[8] who found that solutions of cetyltriethylammonium iodide behaved similarly.

McBain's Micelle Theory

McBain believed that the discrepancy between the conductivity and osmotic properties of the sodium and potassium salts of the higher members of the homologous series of fatty acids, must be due to the colloidal matter that such solutions undoubtedly contain. He postulated that groups of fatty ions or " ionic micelles " developed in the solutions[9], and that they are responsible for the colloidal character of the solutions. Their formation would, he thought, also explain the minima which are observed in the equivalent conductivity-concentration curves of aqueous solutions of soap because, according to Stokes' Law, the micelles would be more mobile, and therefore, more highly conducting than the simple

ions from which they are formed. That is, the micelles would produce a rise of equivalent conductivity.

Hartley[10] maintained that the comparison of osmotic activity and equivalent conductivity led McBain to the correct conclusion that the discrepancy could be accounted for by the formation of micelles, but did not agree with McBain that micelles begin to be formed at the soap concentration at which the equivalent conductivity is a minimum. He explained that the aggregation of fatty ions produces a considerable local concentration of negative charge in the solutions, as a result of which there must be a corresponding local increase of cations. The cations cease to play their normal part in conduction and, instead, carry positive charges towards the anode, resulting in a lower mobility and a *fall* in the equivalent conductivity of the solution.

Hartley sought to prove his point by determining the "Wien Effect"[11,12] of a long-chain salt in aqueous solution. He subjected solutions of cetylpyridinium bromide to electric fields of such high intensity that the effect of the bromide ions on the micelles, composed of cetylpyridinium ions, was completely annulled[13]. The result was a rise in equivalent conductivity, indicating that the formation of micelles can only be accompanied by a rise in equivalent conductivity when the atmosphere of " gegenions " is sufficiently removed from the micelles. Hartley obtained further evidence that the micelles begin to form at the soap concentration at which the total conductivity falls, by calculating the equivalent conductivity of the paraffin radical alone[14]. He showed that when the total equivalent conductivity falls, that of the paraffin radical rises steeply. The fall of total equivalent conductivity is due to the atmosphere of oppositely charged " gegenions " around the micelles, and the rise of equivalent conductivity of the paraffin radicals due to their aggregation as micelles, with a resulting Stokes' Law-like effect. The concentration of soap at which the transition from simple ions or molecules to micelles takes place is known as the " critical concentration for micelles "[15,16].

Physical Properties Affected by Micelle Formation

Equivalent conductivity is only one of several factors affected by the formation of micelles. Others include surface tension, interfacial tension, density, transport number, depression of freezing point, osmotic coefficient, diffusion coefficient and capacity to solubilise water-insoluble material. The decrease in surface tension that accompanies an increase in the concentration of aqueous solutions of soap is arrested at the critical concentration, in excess of which there is no further fall. A pronounced dip in surface tension-concentration curves has been noted by several workers[17,18,19] who show that the soap concentration at which it occurs is influenced by the length of the hydrocarbon chain of the soap, and by the presence of electrolytes, which shift it to a lower concentration. Solutions containing only pure soaps do not exhibit the dip in their surface tension-concentration curves, but the presence of trace quantities of electrolytes[20] or carbon dioxide[21] are sufficient to produce it. Powney and Addison[22] and Lottermoser and

Püschel[23] showed that critical concentrations deduced from surface tension measurements were in good agreement with those deduced from conductivity and interfacial tension measurements. They obtained values of 0·008 molar for C_{12} compounds and 0·00017 molar for C_{18} compounds. The relation between the interfacial tension of aqueous solutions of soap and their concentration has been studied by Powney and Addison[22] and Alexander and Trim[24] who noted a change in the slope of the curves at the critical concentration.

Bury and Parry[25] examined the densities of aqueous solutions of potassium laurate and found an abrupt change of slope in the density-concentration curves at about 0·025 molar. This concentration of potassium laurate agrees well with that reported by others as the critical concentration. Transport numbers of the long-chain ions in solutions of soap have been shown by Hartley, Collie and Samis[14] to be affected by the formation of micelles and therefore to change in magnitude at the critical concentration. The variation of osmotic and diffusion coefficients with concentration have also been shown[26,27] to change sharply at the critical concentration.

Factors Affecting the Critical Concentration

The critical concentration for a particular soap is dependent upon several factors, and the value obtained experimentally appears to vary somewhat with the physical property used to detect micelle formation[10,28]. Nevertheless, it is apparent that the length of the hydrocarbon chain of a soap molecule influences the concentration of soap at which the transition from ions to micelles takes place. Measurements of conductivity[23], refraction[29] and transport number[30] have shown that the longer the paraffin-chain the lower the concentration of soap at which the change occurs. Table I shows the critical concentrations for C_{10}, C_{12}, C_{14}, C_{16} and C_{18} compounds as determined by different methods.

TABLE I

Critical concentration, molar, of C_{10}, C_{12}, C_{14}, C_{16} and C_{18} compounds

	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}
Equivalent conductivity[23] ...	—	0·0076	0·0028	0·0011	0·00039
Refraction[29]	0·098	0·0255	0·0066	—	—
Transport number[30]	0·032	0·013	0·0045	—	—
Interfacial tension[22]	—	0·008	—	—	0·00017

The addition of two $-CH_2$ groups to a hydrocarbon chain seems to reduce the critical concentration by about 65 per cent. The discrepancies for the critical concentrations for a particular chain length may be, in part, due to the use by different workers of compounds with different hydrophilic groups although the effect of the latter is quite small.

Lottermoser and Püschel were unable to detect any difference between the critical concentrations of potassium and sodium alkyl sulphates.

Klevens[29] and Ekwall and Otterstrom[31] have shown that an increase in temperature decreases the amount of colloid present in the solutions at any concentration. That is, an increase in temperature increases the critical concentration.

Hartley [32,33] demonstrated by the indicator displacement phenomenon that the addition of electrolytes to an aqueous solution of a soap depresses the critical concentration. His method consisted of buffering methyl red at about *p*H 3·5, at which it has a red colour, and slowly adding a solution of cetylpyridinium chloride, when the colour changed abruptly to yellow. Dilution with water until the concentration of the cetylpyridinium chloride was below its critical concentration restored the red colour of the indicator. The yellow colour could then be regained by the addition of either more cetylpyridinium chloride or a neutral salt such as potassium or sodium chloride, indicating that the chloride lowers the critical concentration. Hartley found that the addition of sodium chloride to a solution of cetylpyridinium chloride so that the concentration of the former is 0·032M lowers the critical concentration of the cetylpyridinium chloride from about 0·0009M to 0·0001M, or approximately ten-fold.

Determination of the Critical Concentration

Clearly, any physical property which is affected by the formation of micelles can be used as a means of detecting their formation. The magnitude of the physical property under consideration is plotted on a graph as ordinate and the concentration of the soap in solution as abscissa and the curve examined for the characteristic "break" or change of slope that is associated with the critical concentration. As explained earlier in this Review, the value obtained for a particular soap will depend on the physical property being examined[10,28].

The Structure of the Micelles

McBain's early belief[9] was that soap in dilute aqueous solution behaves as a normal electrolyte, molecules of undissociated soap being in equilibrium with paraffin-chain ions and metallic ions, and that at the critical concentration the paraffin-chain ions re-associate to form comparatively simple ionic micelles. Later McBain[34] and Lottermoser and Püschel[23] believed that neutral colloid, which makes little or no contribution to the conductivity of soap solutions, is formed simultaneously with the ionic micelles. Ralston[35] upheld this view as he thought it was not logical to suppose that ionic micelles can first produce a drop in equivalent conductivity, and later account for an appreciable rise.

Hartley[10] attacked this contention and maintained that the conclusions had been drawn without consideration of the effect of Coulomb forces between the ions and the micelles. He believed that the rise in conductivity in the more concentrated solutions could be explained by the liberation of some of the attached gegenions from the more closely

packed micelles, and that it in no way suggested the formation of a second type of micelle. He regarded the micelle as being symmetrically spherical and consisting of an aggregate of hydrophobic hydrocarbon chains jumbled together and away from the water, and with their end-groups projecting into the surrounding water, and the whole aggregate surrounded by an atmosphere of the hydrophilic ions of the soap, which he called " gegenions." Figure 1 shows a diagram of Hartley's representation of an ionic micelle.

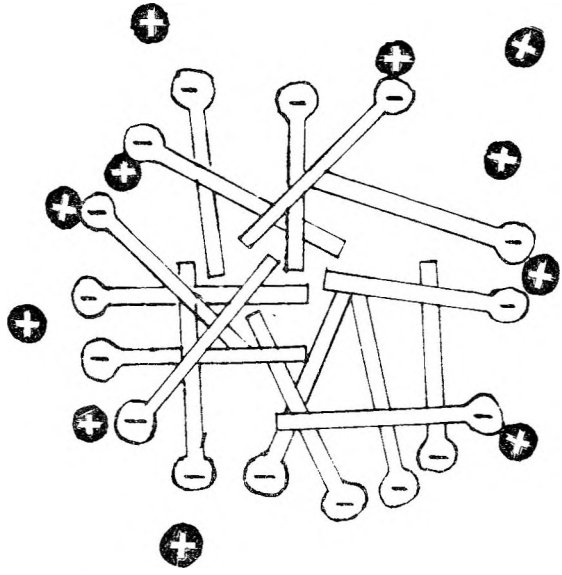


FIG. 1. Diagrammatic Representation of a Spherical Ionic Micelle. (From Hartley.)

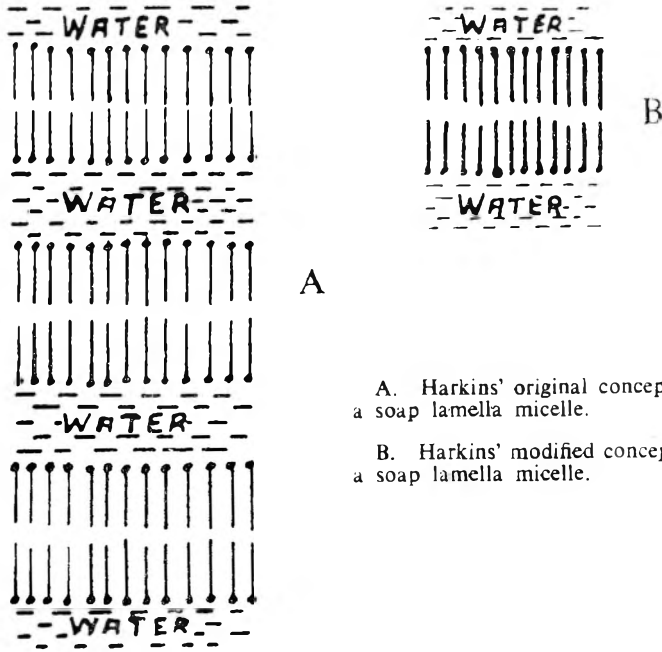
Lawrence[36] considered that aqueous solutions of soap contain both " primary " and " secondary " micelles, both of which have a liquid structure. His " secondary " micelles are to be regarded as filamentous groups of " primary " micelles rather than as micelles of a different type.

The development of X-ray absorption analysis enabled Stauff[37] and Kiessig and Phillipoff[38] to show that 0·2M solutions of sodium laurate and 0·1M solutions of sodium tetradecyl sulphate contain much larger particles than had hitherto been believed. Stauff called these large particles " large micelles " to distinguish them from the smaller colloidal aggregates present in the solutions, and regarded them as consisting of double layers of soap *molecules* closely packed side by side and at right angles to the lamellæ. McBain[39] coupled Stauff's work with a suggestion from Meyer and van der Wyk[40] and regarded it as proof that aqueous solutions of soap contain two kinds of colloidal particles. He now believed that ionic micelles begin to form in very dilute solution and steadily increase with concentration, and that the " lamella micelles arise from ion-pairs and higher aggregates formed at the critical con-

centration for micelles, increasing in size and amount until their development is sufficient to produce an X-ray pattern in the solution."

X-ray studies of soap solutions have been continued by Harkins and his co-workers. In general, they have confirmed the findings of the German workers as regards the structure of the micelles. Unfortunately, X-ray measurements have as yet only been made with fairly concentrated soap solutions (i.e. several times the critical concentration) and hence we are left to infer that the soap lamellæ, the presence and structure of which has been determined in the concentrated solutions, are similar, except perhaps in dimension, to those produced at the critical concentration from ion-pairs and smaller aggregates.

Harkins, Mattoon and Stearns[41] and Harkins, Mattoon and Corrin[42] examined 5 per cent. to 30 per cent. aqueous solutions of potassium laurate by means of X-ray diffraction, and concluded that the lamella micelles consist of four or more double layers of soap molecules oriented like the ions of the spherical micelles of McBain and Hartley, with their hydrophilic portions projecting into the surrounding water, and the paraffin chains towards one another, forming a liquid structure in the plane of the layer. The double layers of soap molecules are separated by layers of water and are kept apart by repulsion of the positive ions produced by ionisation of the molecules. Harkins representation of such a soap micelle is shown in Figure 2A.



A. Harkins' original conception of a soap lamella micelle.

B. Harkins' modified conception of a soap lamella micelle.

FIG. 2. Diagrammatic Representation of a Lamella Micelle. (From Harkins *et. al.*)

More recent work by Mattoon, Stearns and Harkins[43] has led to the recognition of a new X-ray diffraction band, from which the authors conclude that the micelles probably have a somewhat simpler structure

than they originally proposed. They now believe them to consist, as shown in Figure 2B, of only one double layer of soap molecules and to increase in size and number as the concentration increases.

The work of Harkins *et al.*, on the one hand, has done little to settle the dispute over the structure of the micelles in the neighbourhood of the critical concentration. Hartley's postulation of spherical micelles on the other, fails to explain the X-ray diffraction observed in the more concentrated solutions. Vetter[44] considers that neither theory accounts satisfactorily for all the known properties of solutions of soaps. He believes that a slight modification of the Hartley picture is more acceptable than the present McBain viewpoint, at any rate, for solutions below the concentration at which X-ray diffraction has been observed. He offers no suggestions as to the origin of the lamella micelles in the concentrated solutions.

The Size of the Micelles

It is generally accepted that micelles begin to form at the critical concentration from ions and ion-pairs, and that the transition is not so abrupt that micelles of maximal size are produced over an infinitely small concentration range. Hartley[10] suggests that in aqueous solutions of a 16 carbon atom soap, the micelles which commence to form at about 0·001M (the critical concentration) are probably composed of about 10 ions, and that they simultaneously increase in size and number until at about 0·005M the hydrocarbon ions exist almost entirely of micelles containing about 50 ions each.

The diameter of spherical micelles cannot be less than the length of the paraffin chains unless part of the chain protrudes into the surrounding water, nor can it be greater than twice the length of the chain unless the centre is vacuous. These possibilities are unlikely[10]. The diameter, then, will be approximately twice the length of the paraffin chain, and as the homologous series is ascended from C_{12} to the C_{18} soap, it will increase. Hartley gives the diameter of micelles formed in solutions of the C_{16} soap as approximately 36Å.

Measurements by X-ray diffraction of the size of lamella micelles made by Harkins *et al.*[42], indicate that the thickness of the lamellæ in 15 per cent. aqueous solutions of potassium laurate is about 32·4Å which is similar to the diameter of the Hartley micelles.

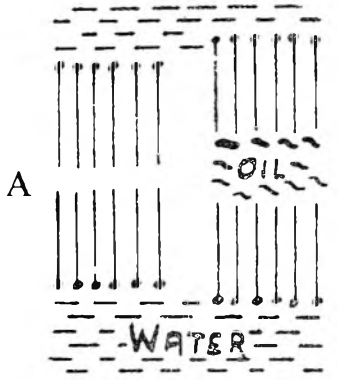
Soap Solutions as Solvents for Water-insoluble Substances

The earliest record of the use of soap as a solvent appears to be by Engler and Dieckhoff[45] in 1892 who prepared clear aqueous solutions of cresol and water by the addition of soap. Their work was quickly followed by British Patent No. 13,201 of 1895 which described the use of soft soap to solubilise water-insoluble materials such as naphthalene.

The explanation usually offered for clear lysol solutions is that the soap lowers the critical solution temperature of cresol and water to such an extent that they become completely miscible at normal temperatures. Some doubt was cast upon this explanation by Angelescu and Popescu[46] who measured the solubility of *o*-cresol in several soaps of the fatty acid

series and found it greater than in solutions of sodium hydroxide. A number of other organic liquids have been shown to be more soluble in solutions of soap than they are in water[47,48] and Hartley[10] suggested that they dissolve in the soap micelles. This view has been elaborated somewhat by Lawrence[49] who proposed that solubilisation of organic substances by aqueous solutions of soap takes place by two different methods. Water-soluble substances have their solubility enhanced by becoming attached to the exterior of the micelles by dipole attraction, and water-insoluble substances form an internal solution in the hydrocarbon interior of the micelles. Most organic substances, though oil-soluble, contain polar groups and thus both mechanisms will operate simultaneously.

X-ray diffraction measurements in aqueous solutions of soap offer practical evidence of the correctness of the suggestions of Hartley and Lawrence. Mattoon, Stearns and Harkins[43] demonstrated that the thickness of lamella micelles of potassium myristate is about 40Å, but when oil has been dissolved in the soap solution the micelles are found to have expanded to about 53Å in thickness to incorporate sheets of oil between the hydrocarbon layers of the lamellæ (Fig. 3).



A. Micelle of Potassium Myristate.

B. Micelle of Potassium Myristate in which oil has been dissolved.

FIG. 3. Cross Section of Highly Idealised Micelle of Potassium Myristate in which Oil has been Dissolved. (From Mattoon, Stearns and Harkins.)

These conclusions are confirmed with an extremely different system by the X-ray work of Palmer[50] on nerve lipide emulsions containing cephalin. Figure 4 shows the expansion of a lamella of typical lipide material as water is progressively taken up as a layer within the leaflets.

THE RELATION BETWEEN THE SOAP CONCENTRATION AND THE AMOUNT OF WATER-INSOLUBLE MATERIAL SOLUBILISED

If solubilisation of water-insoluble substances by aqueous solutions of soap is due to internal solution within the micelles, it can only occur when the concentration of the soap solution is in excess of the critical concentration. Although, in general, this is borne out by practical experience, McBain, Merrill and Vinograd[51] report that they found that solutions of potassium laurate and laurylsulphonic acid dissolved sufficient of the water-insoluble dye Yellow AB to colour them yellow,

even though they were below the critical concentration. Similarly Bean and Berry[52] record that when aqueous solutions of potassium laurate and benzylchlorophenol are diluted with water to below the critical concentration, the amount of benzylchlorophenol remaining in solution

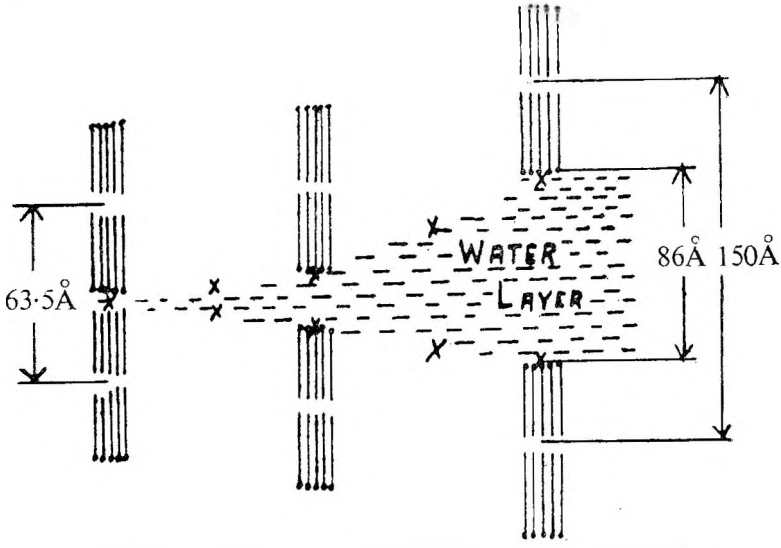


FIG. 4. Expansion of Lamellæ of Nerve Lipides as Water is Taken Up. (From Palmer.)

X[s] represent values when water content is 0 (dry), 25, 50, 67 and 75 per cent.

is greater than can be accounted for by its water-solubility. The solvent action shown by aqueous solutions of soap below their critical concentration is attributed by Corrin, Klevens and Harkins[53] to the presence in the solution of some elementary organised structures such as the ion-pairs referred to by McBain.

One of the first studies of the relation between the concentration of a soap solution and its solubilising ability was carried out with cetylpyridinium chloride and *trans*-azobenzene[33], the latter being chosen because it is not sufficiently water-soluble to modify materially the properties of the micelles. Reference to the solubility curve (Fig. 5) will show that there is no appreciable solvent action when the concentration of the cetylpyridinium chloride is below 0·001M. Above that concentration the solubility of the *trans*-azobenzene rises rapidly, and reaches a value more than half that at five hundred times the concentration within the first part of this range.

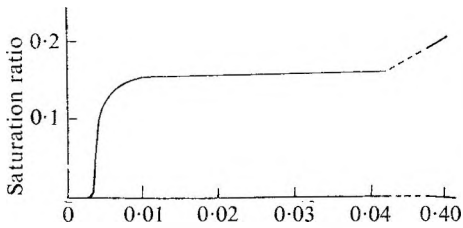


FIG. 5. The Solubility of *trans*-azobenzene in Cetylpyridinium Chloride. (From Hartley.)

A curve of very similar shape was obtained for the solubility of Yellow AB in laurylsulphonic acid[51], for chlorophyll and Yellow AB in sodium deoxycholate[26], and for benzylchlorophenol in potassium laurate[54] and it may, therefore, be regarded as typical for water-insoluble materials solubilised by aqueous solutions of soap and other long-chain surface-active compounds.

McBain, Merrill and Vinograd[51] record the interesting fact that solubilisation of water-insoluble materials by aqueous solutions of soap is greater at high temperatures than at room temperature, in spite of there being far less colloid at the higher temperature[29,31]. The increase in the solubility of the so-called water-insoluble materials in the aqueous phase of soap solutions which is brought about by elevation of the temperature, must therefore be relatively greater than the reduction caused by the smaller number of micelles at the higher temperature.

The very abrupt rise at the critical concentration, of the solubility of dyes and other water-insoluble compounds, provides an extremely simple and sensitive method for the determination of the critical concentration[55].

The Relation between the Length of the Soap Molecule and the Amount of Material Solubilised

The solubilisation of the water-insoluble dye Orange OT by the homologous series of pure potassium fatty acid soaps was examined by McBain and Johnson[56], who found that the amount of dye solubilised increases as the number of CH_2 groups in the hydrocarbon chain increases. As the homologous series is ascended not only does the dye commence to go into solution at a lower soap concentration, due to the critical concentration decreasing as the chain length increases, but when the micelles are fully formed the amount of dye necessary to saturate the solution increases considerably (Fig. 6).

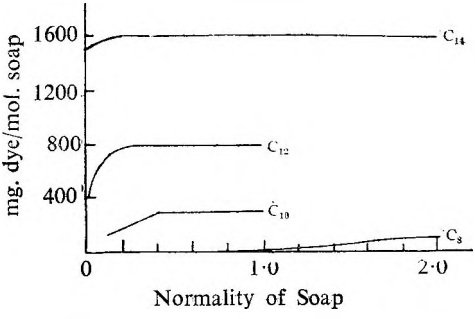


Fig. 6. The Solubility of Orange OT in the Homologous Series of Pure Potassium Fatty-acid Soaps. (From McBain and Johnson.)

McBain and Johnson draw special attention to the fact that for an increase of 50 per cent. in the number of carbon atoms from caprylate to the laurate, solubilisation increases 6·7-fold. The authors maintain that this increase cannot be accounted for if it is assumed that the dye merely dissolves in the hydrocarbon interior of the micelles. The discrepancy is used to lend weight to the argument that soap solutions, in excess of the critical concentration, contain lamella micelles and the authors suggest that solubilisation is probably brought about by packing the spaces between the parallel sheets of hydrocarbon molecules together with adsorption on the exteriors of the micelles.

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RESEARCH PAPERS

THE BACTERICIDAL ACTIVITY OF PHENOLS IN AQUEOUS SOLUTIONS OF SOAP

PART I.—THE SOLUBILITY OF A WATER-INSOLUBLE PHENOL IN AQUEOUS SOLUTIONS OF SOAP

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INTRODUCTION

THE investigation described in the present and subsequent papers is concerned with the bactericidal activity of phenols when dissolved in aqueous solutions of soap. It shows that the solubility of sparingly water-soluble phenols in aqueous solutions of potassium laurate is related to the formation of micelles in the solutions, and that the mechanism of solubilisation is the same as that quoted by Lawrence[1], McBain and Johnson[2] and Hartley[3] for the solubilisation of water-insoluble dyes by potassium laurate and other soaps. It seeks to prove that the bactericidal activity of water-insoluble phenols solubilised by aqueous solutions of soap is a function of the concentration of the phenol within the micelles of the soap solution, rather than the concentration of the phenol in the solution as a whole. A number of attempts have been made previously to ascertain the effect of soap and other paraffin-chain surface-active compounds on the activity of phenolic bactericides. The results of the earlier attempts appear to be contradictory, but they can be reconciled with the present knowledge of the properties of soap in aqueous solution.

Hamilton[4] showed that a solution containing 1·3 per cent. of phenol and 1·3 per cent. of soap had only the same activity as a 1·2 per cent. solution of phenol alone, while Lange[5] found that the activity of aqueous solutions of cresol is improved by the addition of soap. Hampil[6] found that the addition of 0·2 to 0·5 per cent. of sodium oleate to solutions of phenol, *m*-cresol or hexylresorcinol had a marked depressant effect on the bactericidal activity. The author suggested that the observed depression might be due (1) to the occurrence of a true chemical reaction between the soap and the phenol, resulting in a non-active substance, (2) a protective colloidal action by the soap on the bacteria, (3) the ability of the soap to remove the phenolic material from the solution in a definite partition coefficient ratio or (4) a combination of physico-chemical factors which may or may not have been expressed in the previous statements. Frobisher[7] reported that the effect of sodium oleate on the bactericidal activity of aqueous solutions of phenol depended upon its concentration therein. The addition of up to 0·05 per cent. of sodium oleate to a 1 per cent. solution of phenol increased the activity of the latter, but the addition of 0·1 per cent. decreased it. A similar

effect was observed by Cade[8] who noted that a 0·05 per cent. solution of phenol exhibited maximal activity when it contained 0·25 per cent. of sodium ricinoleate. Further experiments by Cade also established that the concentration of soap that must be added to a solution of a phenol to produce the maximal bactericidal activity varies according to the constitution of the phenol.

Studies by Alexander and Trim[9] on the essentially similar problem of the anthelmintic activity of aqueous solutions of hexylresorcinol and soap, showed that the biological activity of such solutions can be related to the interfacial tension between the solution and liquid paraffin. The interfacial tension is lowest and the anthelmintic activity highest when the soap concentration corresponds with the " critical concentration for the formation of micelles." At the critical concentration, which is a characteristic of each soap, the relation between a number of physical properties and the soap concentration changes abruptly. These changes have been described elsewhere[10]. Immediate interest lies in the fact that at the " critical concentration " of an aqueous solution of a soap, the paraffin-chain ions of the soap agglomerate to form groups of ions or " micelles." The micelles are liquid structures and hydrocarbon in nature. They increase in size with increase in the concentration of the soap solution until the upper critical concentration or " critical concentration for completion of micelle formation " is reached. The micelles have been shown to be centres of solubilisation of water-insoluble materials[1,2,3,11,12,13,14,15,16,17]. The latter are insoluble in aqueous solutions of soap which are below their critical concentration. At the critical concentration these substances become soluble and their solubility per molecule of soap increases rapidly as the soap concentration is increased up to the critical concentration for completion of micelle formation, after which there is no further increase. The present paper describes the solubility of a sparingly water-soluble phenol in aqueous solutions of potassium laurate, and shows that the mechanism of solubilisation is similar to that described by other workers for the solubilisation of fixed and volatile oils and water-insoluble dyes.

Experimental

1. Choice of Materials

(a) *The Phenol.* The phenol selected for study was 5-chloro-2-hydroxydiphenylmethane (benzylchlorophenol), which has a solubility in water of about 1 in 15,000. Because of its low water-solubility it provides the simplest type of system, and, when dissolved in soap solutions, does not affect materially the proportions of the micelles[3]. Another important factor contributing to the choice of this compound is that even a saturated aqueous solution of it is shown to possess no appreciable bactericidal action. In a later paper, the properties of 2-chloro-5-hydroxy-1 : 3-dimethylbenzene (chloroxylenol) in similar circumstances will be described. This phenol is slightly more soluble in water (1 in 3,000).

(b) *The Soap.* The soap selected for the investigation was potassium laurate. It is the lowest member of the homologous series of potassium fatty acid soaps exhibiting advanced micellar structure[18], and therefore

the lowest member possessing marked solubilising action. Soaps of the higher members of the series have a greater solubilising action per molecule[2], but their water-solubility at 20°C. is too low for them to be of any practical value as solvents. The physical properties of aqueous solutions of potassium laurate have been the subject of several investigations, and those properties which are affected by micelle formation are well plotted. In particular, the critical concentration of potassium laurate has been determined by various methods and is, therefore, known with some certainty. The experimentally obtained value depends on the physical property considered[11,19], and is given as being between 0·008M[20] and 0·076M[21] but most frequently as about 0·025M[22,23].

2. Preparation of the Potassium Laurate

The potassium laurate was prepared from lauric acid with the following characteristics: acid value, 278·0, iodine value, 0·04, m.pt. 39·2°C., collected at 160° to 163°C. A molar solution of potassium laurate was prepared by adding the calculated volume of 2N potassium hydroxide solution to 400·4 g. of grated lauric acid and warming the mixture to about 50°C. When the reaction was complete the solution was cooled to 20°C. and sufficient freshly boiled and cooled distilled water added to produce 2·0 litres. The soap solution thus prepared was stored under nitrogen in glass-stoppered bottles of 250 ml. capacity. For use the molar solution was diluted to 0·1M with distilled water that had been freshly boiled and cooled under soda lime. Each ml. of the 0·1M solution contained 0·02383 g. of potassium laurate.

3. The Determination of the Solubility of Benzylchlorophenol in Aqueous Solutions of Potassium Laurate

The experiments of McBain, Merrill and Vinograd[16] show that the solubility of water-insoluble compounds in aqueous solutions of soap is independent of whether saturation is reached from under-saturation or over-saturation. The latter method was used in our determinations of the solubility of benzylchlorophenol in aqueous solutions of potassium laurate, because it is easier to dilute a concentrated solution until precipitation of the benzylchlorophenol occurs, than to saturate a solution of potassium laurate by the gradual addition of weighed amounts of the phenol. The method consisted essentially of preparing a series of solutions of benzylchlorophenol in potassium laurate such that each solution contained a smaller proportion of benzylchlorophenol potassium laurate than the preceding one, and diluting each solution with freshly boiled and cooled distilled water until precipitation of the benzylchlorophenol occurred. The concentrations of potassium laurate necessary to keep in solution the various proportions of benzylchlorophenol/potassium laurate were calculated, and a solubility curve was constructed to show the number of molecules of benzylchlorophenol that can be solubilised per molecule of potassium laurate at different concentrations of the latter.

(a) *Preparation of a "Stock" Solution.* For convenience, a concentrated solution of potassium laurate almost saturated with benzylchlorophenol was prepared. The concentrated reference or "stock" solution

contained : benzylchlorophenol, 25·5 g., M potassium laurate solution. 250·0 ml., distilled water (freshly boiled and cooled), to 1,000·0 ml.

The benzylchlorophenol was dissolved in the potassium laurate solution with the aid of gentle heat. When solution was complete, it was cooled to 20°C. and the volume adjusted to 1,000·0 ml. with freshly boiled and cooled distilled water. The solution was stored under nitrogen in glass-stoppered bottles of 250 ml. capacity, each bottle being sealed with a " viscose " cap.

(b) *Preparation of the " Working " Solution.* Preliminary trials showed that a dilution of 2 parts by volume of the " stock " solution with 3 parts by volume of freshly boiled and cooled distilled water, produced a more convenient " working " solution, each millilitre of which contained : benzylchlorophenol, 0·0102 g., potassium laurate, 0·0238 g.

(c) *Experimental Details.* A known volume of the " working " solution was run from a micro-burette into each of a series of screw-capped, wide-mouthed bottles of 1 fl. oz. capacity. From a second micro-burette, a measured volume of 0·1M potassium laurate solution was run into each of the bottles, and from a third burette a gradually increasing volume of distilled water was added to each successive bottle of the series. A sufficient number of experiments were set up so that towards the dilute end of the series were systems to which sufficient distilled water had been added to produce turbidity due to benzylchlorophenol coming out of solution. The bottles were closed and incubated for 24 hours at 20°C. After incubation they were examined for the presence of benzylchlorophenol crystals. Further series of experiments were set up so that in each successive series, the proportion by weight of benzylchlorophenol to potassium laurate was less, i.e. a greater volume of potassium laurate solution was added per unit volume of " working " solution. A sufficient number of series of experiments were set up so that a series was eventually reached in which no crystals of benzylchlorophenol developed on incubation for 24 hours. The last experiment in each series in which the benzylchlorophenol remained in solution was taken as the end-point. The weight of benzylchlorophenol which can be dissolved in a solution of potassium laurate of known concentration was calculated from a knowledge of the volumes of " working " solution, 0·1M potassium laurate and distilled water added to the bottle corresponding with the end-point. The amount of benzylchlorophenol which can be dissolved in potassium laurate solutions of different concentrations is calculated in Table I. Figure 1 shows the number of molecules of benzylchlorophenol which can be solubilised per molecule of potassium laurate at different concentrations of the latter. Reference to Figure 1 will show that the solubility of benzylchlorophenol remains at a constant low level for all concentrations of potassium laurate below about 0·015M. Its solubility over this range is about 1 in 2,650 which is in excess of the solubility in water. At about 0·015M potassium laurate the solubility of the benzylchlorophenol per molecule of potassium laurate increases sharply with increases in the concentration of the potassium laurate solution. This increase continues up to about 0·04M potassium laurate, in excess of which the

TABLE I

THE SOLUBILITY OF BENZYLCHLOROPHENOL IN SOLUTIONS OF POTASSIUM LAURATE

Series	Working Solution	Weight of Benzyl-chlorophenol in Working Solution	Weight of Potassium Laurate in Working Solution	0·1M Potassium Laurate Added	Weight of Potassium Laurate Added	Total Weight of Potassium Laurate	Mols. Benzyl-chlorophenol /Mol. Soap	Maximum Volume of Water that may be added	Total Volume	Molar Concentration of Potassium Laurate at end-point
F	1·0 ml.	0·0102 g	0·02383 g	0·05 ml.	0·001191 g	0·02502 g	0·44459	1·0 ml.	2·05 ml.	0·0512
G	1·0 ml.	0·0102 g	0·02383 g	0·10 ml.	0·002383 g	0·02621 g	0·42440	1·5 ml.	2·60 ml.	0·0423
H	1·0 ml.	0·0102 g	0·02383 g	0·20 ml.	0·004766 g	0·02860 g	0·38893	2·0 ml.	3·20 ml.	0·0375
I	1·0 ml.	0·0102 g	0·02383 g	0·30 ml.	0·007149 g	0·03098 g	0·35904	3·0 ml.	4·30 ml.	0·0326
J	1·0 ml.	0·0102 g	0·02383 g	0·40 ml.	0·009532 g	0·03346 g	0·33244	3·5 ml.	4·90 ml.	0·0287
K	1·0 ml.	0·0102 g	0·02383 g	0·50 ml.	0·011955 g	0·03574 g	0·31123	4·0 ml.	5·50 ml.	0·0273
L	1·0 ml.	0·0102 g	0·02383 g	0·60 ml.	0·014298 g	0·03813 g	0·29173	4·5 ml.	6·10 ml.	0·0262
M	1·0 ml.	0·0102 g	0·02383 g	0·70 ml.	0·016681 g	0·04051 g	0·27495	5·0 ml.	6·70 ml.	0·0254
N	1·0 ml.	0·0102 g	0·02383 g	0·80 ml.	0·019064 g	0·04289 g	0·25935	6·0 ml.	7·80 ml.	0·0231
O	1·0 ml.	0·0102 g	0·02383 g	1·00 ml.	0·02383 g	0·04766 g	0·23340	7·0 ml.	9·00 ml.	0·0222
P	1·0 ml.	0·0102 g	0·02383 g	1·20 ml.	0·028596 g	0·05243 g	0·21216	8·0 ml.	10·20 ml.	0·0216
Q	1·0 ml.	0·0102 g	0·02383 g	1·40 ml.	0·033362 g	0·05719 g	0·19450	9·0 ml.	11·40 ml.	0·0210
R	1·0 ml.	0·0102 g	0·02383 g	1·60 ml.	0·038128 g	0·06196 g	0·17952	11·0 ml.	13·60 ml.	0·0191
S	1·0 ml.	0·0102 g	0·02383 g	1·80 ml.	0·04289 g	0·06672 g	0·16672	12·0 ml.	14·80 ml.	0·0189
T	1·0 ml.	0·0102 g	0·02383 g	2·00 ml.	0·04766 g	0·07149 g	0·15560	14·0 ml.	17·00 ml.	0·0176
U	1·0 ml.	0·0102 g	0·02383 g	2·20 ml.	0·052426 g	0·07626 g	0·14587	15·0 ml.	18·20 ml.	0·0175
V	1·0 ml.	0·0102 g	0·02383 g	2·50 ml.	0·05959 g	0·08342 g	0·13335	18·0 ml.	21·50 ml.	0·0163
W	1·0 ml.	0·0102 g	0·02383 g	2·75 ml.	0·065527 g	0·08936 g	0·12448	21·0 ml.	24·75 ml.	0·0152
X	1·0 ml.	0·0102 g	0·02383 g	3·00 ml.	0·07149 g	0·09532 g	0·11670	23·0 ml.	27·00 ml.	0·0148
Y	2·0 ml.	0·0204 g	0·04766 g	0·025 ml.	0·00059 g	0·04825 g	0·46095	1·5 ml.	3·52 ml.	0·0574

rate of increase begins to fall. In excess of approximately 0·06M potassium laurate, there is negligible increase in the solubility of the benzylchlorophenol per molecule of potassium laurate.

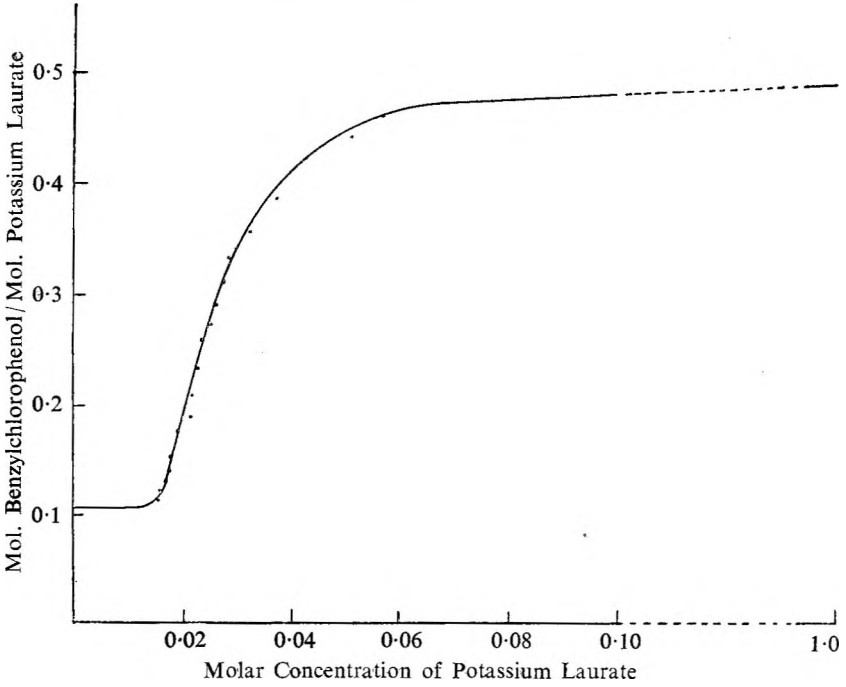

FIG. 1. The Solubility of Benzylchlorophenol in Potassium Laurate Solution.

DISCUSSION

The general form of the solubility curve shown in Figure 1 corresponds very closely to those published by Hartley[3], McBain, Merrill and Vinograd[16], McBain and Johnson[2] and others for the solubility of water-insoluble dyes in aqueous solutions of soap. Correlation of the solubility curves for the dyes and the equivalent conductivity curves of the soaps used as solvents, provided ample evidence that the soap micelles are responsible for the solubilisation. The similarity in shape of the curves for the solubility of benzylchlorophenol in potassium laurate, and for the dyes in potassium laurate and other soaps in solution, together with the fact that the marked increase in the solubility of benzylchlorophenol per molecule of potassium laurate occurs within the limits quoted as the critical concentration for potassium laurate[22,25], can again be accepted as sufficient evidence that the soap micelles are responsible for the solubilisation of the benzylchlorophenol.

The constant low level of solubility of the benzylchlorophenol in all concentrations of potassium laurate up to about 0·015M is accounted for by the non-micellar nature of these solutions. The solubility of the phenol in these solutions is of the order of 1 in 2,650. This is somewhat greater than can be accounted for by its water-solubility. A similar

phenomenon was reported by McBain[14] and Corrin, Klevens and Harkins[24] who proposed the existence of some pre-micellar particles on which water-insoluble material might be adsorbed.

The sudden increase in solubility at about 0·015M potassium laurate is obviously due to the formation of micelles at this concentration. The increase in the solubility of the benzylchlorophenol per molecule of potassium laurate continues until the concentration of the latter is about 0·06M, at which concentration the micelles presumably reach maximal size. When they have reached their maximal size they are unable to solubilise any further benzylchlorophenol. That is, the solubility of benzylchlorophenol per molecule of potassium laurate reaches a maximum when the micelles are completely formed.

Thus, it is possible to have a micellar solution of potassium laurate in which the micelles (1) contain no benzylchlorophenol, (2) are saturated with benzylchlorophenol or (3) contain some intermediate proportion. The authors have previously suggested that the bactericidal activity of benzylchlorophenol in aqueous solutions of potassium laurate is a function of the concentration of the benzylchlorophenol in the micelles of the potassium laurate[25]. Subsequent papers will present the evidence on which this statement was based.

Summary

The solubility of benzylchlorophenol in aqueous solutions of potassium laurate has been plotted, and is shown to be dependent on the presence of micelles in the soap solution.

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THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART IV.—BENZIMINAZOLE GLYCOSIDES

(1) THE PREPARATION AND PROPERTIES OF SOME *o*-NITROANILINE GLYCOSIDES

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IN experiments on the degradation of Vitamin B_{12}, Ellis, Petrow and Snook[1] extracted the coloured cobalt-containing complex formed on acidolysis and examined the colourless cobalt-free phase spectroscopically. Selective absorption in the ultraviolet was observed in the region of 2850 Å, the material responsible being designated " the 285-component." Subsequent work, by Beavan *et al.*, outlined in Part III[2] revealed the complex character of " the 285-component " which was resolved by paper chromatography into three related compounds designated *components* α, β and γ. Spectroscopic and chemical studies indicated the identity of *component* γ with 5 : 6-dimethylbenziminazole and of *components* α and β with 1-substituted 5 : 6-dimethylbenziminazoles. It was therefore inferred that *components* α, β and γ represented different stages of degradation of a common precursor. The isolation of 5 : 6-dimethylbenziminazole from acid hydrolysates of vitamin B_{12} was announced simultaneously[3] by Brink and Folkers[4].

The formation by acid hydrolysis of vitamin B_{12} of two 1-substituted 5 : 6-dimethylbenziminazoles as well as 5 : 6-dimethylbenziminazole itself, led to the conclusion that the 1-substituent was capable of stepwise degradation by acid. This fact, together with the structural similarity between vitamin B_{12} and riboflavine implicit in the existence of an N-substituted 4 : 5-dimethyl-*o*-phenylenediamine residue in both compounds, led to the further conclusion that the grouping may be glycosidic in character. The synthesis of benziminazole glycosides was accordingly undertaken with the object of examining their behaviour on acidolysis. In addition, such compounds were required for phosphorylation studies following the discovery that, on hydrolysis, the α-component passed smoothly into the β-component and phosphate.

The synthesis of sugar derivatives of benziminazole has not hitherto been reported in the literature. The synthesis of nucleosides, a group which bears a formal analogy to the benziminazole glycosides, however, has formed the subject of detailed study by a number of workers. Thus Fischer and Helferich[5] prepared the D-glucosides of adenine, guanine and hypoxanthine from the reaction product of acetobromglucose and trichloropurine silver, a method subsequently exploited by Levene and Sobotka[6] and by Davoll, Lythgoe and Todd[7]. A major contribution to the synthesis of nucleosides, however, is contained in a series of publications by Todd, Lythgoe and their collaborators[8]. In addition to extending the silver salt route, Todd *et al.* elaborated a new synthesis of nucleosides in which a 5-amino-4-glycosidamino pyrimidine is treated with dithio-

formic acid[9] when a 4-glycosidamino-5-thioformamidopyrimidine is obtained, which is converted into the purine by treatment with basic reagents[9,10,11].

Our own synthetic experiments followed, in outline, the pattern established for the nucleosides, methods analogous to the silver salt route and Todd's glycosidaminopyrimidine route being successfully developed.

o-Phenylenediamine glycosides (e.g.E) were required for the latter study and their preparation forms the subject of the present communication.

The glycosidation of *o*-nitroanilines by boiling with hexose and pentose sugars in ethyl alcoholic solution in the presence of ammonium chloride was described by Kuhn and Strobële[12] in 1937. As the reaction involves the anomeric centres of the sugar components it is not surprising that two D-arabinosides and two L-arabinosides of 5-nitro-*o*-4-xylidine were obtained, whilst the other glycosides described melted over a range of temperatures. Kuhn and Strobële (*loc. cit.*) claimed, however, that the isomerism disappeared on acetylation, the same 5-nitro-*o*-4-xylidine-triacetyl-L-arabinoside being obtained, for example, from the two modifications of the unacetylated glycoside.

We have now extended these observations to include the preparation of glycosides from D-glucose, D-mannose, D-galactose, L-arabinose, D-xylose, D-ribose and L-rhamnose as the sugar component and *o*-nitroaniline, *m*-nitro-*p*-toluidine, 3- and 5-nitro-*o*-4-xylidines as the aglycone. As nearly all these compounds proved heterogeneous in the crude state, it appears that a mixture of isomers is invariably formed in this reaction. The relative proportions obtained, however, vary over a wide range with the nature of the reactants and the experimental conditions employed. Furthermore one isomer is occasionally formed to the almost complete exclusion of the other.

When L-arabinose was employed as the sugar component the isomeric L-arabinosides were formed in approximately equal amounts and were conveniently separated by crystallisation from alcohol. Following the nomenclature devised by Kenner, Lythgoe and Todd[13] for the analogous aminopyrimidine glycosides, we differentiate these by the suffixes I and II. The distinction is an arbitrary one and in our usage is based solely upon rotational data, the L-arabinoside with the highest positive rotation being designated the series I isomer. Structural similarity within the series is assumed. The rotations obtained are recorded in Table I, column (i). Serious attempts to fractionate the isomeric glycosides were limited to those derived from L-arabinose. In all other instances the reaction products were directly acetylated with acetic anhydride in pyridine solution. In our hands, however, and in contrast to the observations recorded by Kuhn and Strobële (*loc. cit.*), these acetylated products were usually found to consist of two isomeric acetates. Thus, for example, acetylation of 5-nitro-*o*-4-xylidine-L-arabinosides (mixed isomers) gave the series I triacetate, m.pt. 213° to 214°C., $[\alpha]_D$ +142° (in chloroform), already described by Kuhn and Strobële, together with smaller quantities of 5-nitro-*o*-4-xylidine-triacetyl-L-arabinoside II, m.pt. 143° to 144°C., $[\alpha]_D$ +24° (in chloroform). Some of these results are collected in Table I, column (ii).

Following these unexpected observations we turned our attention to the direct acetylation of the pure *o*-nitroaniline glycosides listed in column (i) of Table I, and obtained, in each instance, only the pure

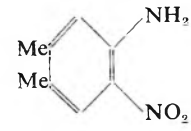
TABLE I

Aglycone		(i) L-arabinosides		(ii) triacetyl-L-arabinosides	
		M.pt.	$[\alpha]_D$ in pyridine c=1	M.pt.	$[\alpha]_D$ in chloroform c=1
o-Nitroaniline	Series I	159° to 161°C.	+55·5°	154°C.	+159·1°
	Series II	173° to 175°C.	+21·1°	90° to 91°C.	−23°
m-Nitro-p-toluidine ...	I	133° to 135°C.	+83·6°	194°C.	+146·5°
	II	141° to 142°C.	−10°	77° to 79°C.	−22·6°
5-Nitro-*o*-4-xylidine ...	I	187·5°C.	+71°	213° to 214°C.	+142°
	II	108° to 109°C.	−7·8°*	143° to 144°C.	+ 24°

* Kuhn and Strobele (*loc. cit.*) give $[\alpha]_D$ +26°.

triacetate of the same series. The view expressed by Kuhn and Strobele that isomerisation of *o*-nitroaniline glycosides takes place on acetylation is thus shown to be erroneous and to lack experimental foundation. Their sample of 5-nitro-*o*-4-xylidine-L-arabinoside II which yielded the series I triacetate on acetylation must have been admixed with the Series I isomer and it is significant that the rotation of our sample of 5-nitro-*o*-4-xylidine-L-arabinoside II is considerably lower than the value recorded by the German authors.

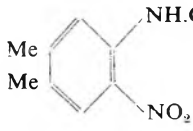
We have, *inter alia*, made the interesting observation that the triacetyl-L-arabinosides II are converted into the series I compounds by simply



(A)

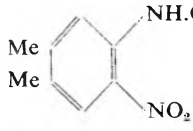
+ HO.CH.(CHOH)$_3$.CH$_2$
O

(B)



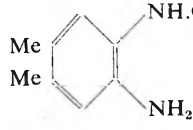
(C)

5-Nitro-*o*-4-xylidine-L-arabinosides I and II.



(D)

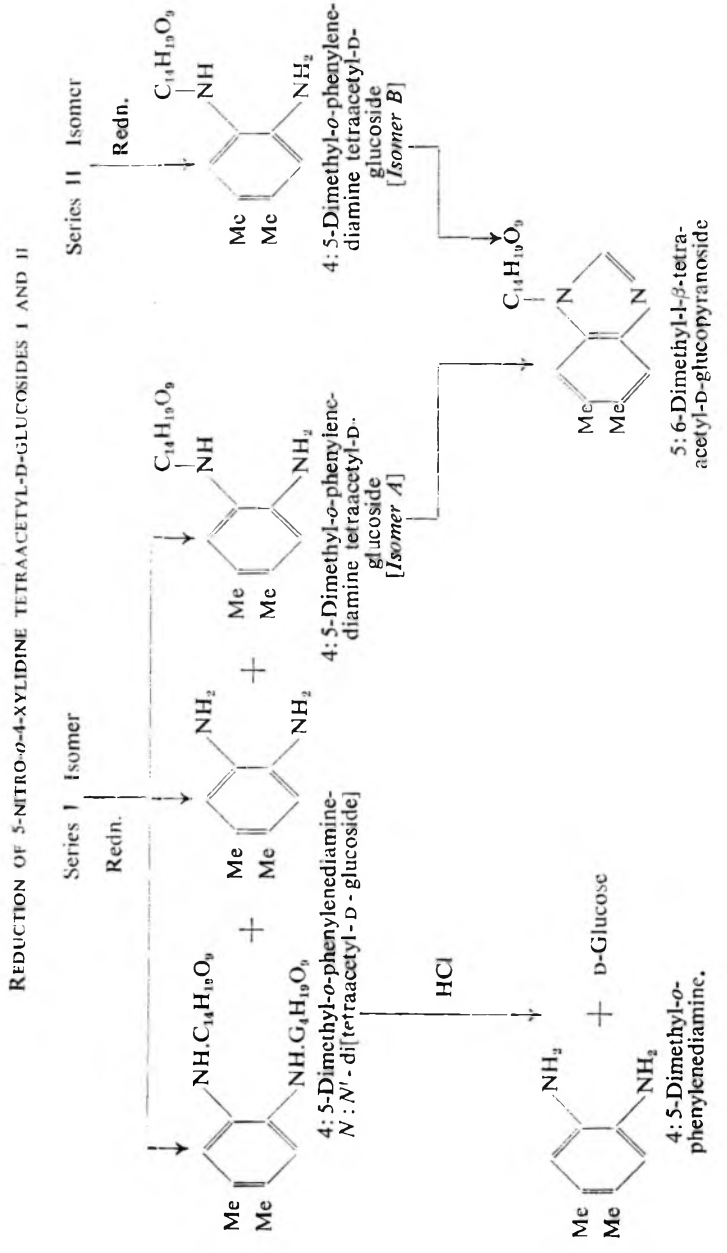
5-Nitro-*o*-4-xylidine triacetyl-L-arabinosides I and II.



(E)

4: 5-Dimethyl-*o*-phenylenediamine triacetyl-L-arabinoside

heating for a few seconds with 2N hydrochloric acid containing a little ethyl alcohol. The change is irreversible and prolonged heating results in fission of the molecule with regeneration of the components. This behaviour was found to be characteristic of all the Series II acetylated glycosides examined and indicates that the difference between the Series



I and Series II isomers involves only the anomeric centres of the sugars. It also excludes the possibility that the difference might be due to an Amadori rearrangement[14].

Catalytic reduction of *m*-nitro-*p*-toluidine-tetraacetyl-D-glucosides I and II gave the same 4-methyl-*o*-phenylenediamine-tetraacetyl-D-glucoside. It is thus evident that in this instance the isomerism existing between the two series of acetylated *o*-nitroaniline glycosides disappear upon reduction of the nitro-grouping. Similar results have been recorded in the pyrimidine series by Kenner, Lythgoe and Todd[13], who observed that reductive fission of the isomeric 6-amino-4-triacetyl-D-xylosidamino-5-(2′ : 5′-dichlorobenzeneazo)-pyrimidines I and II led to the same 5 : 6-diamino-4-triacetyl-D-xylosidaminopyrimidine.

Experiments on the reduction of 5-nitro-*o*-4-xylidine tetraacetyl-D-glycosides I and II gave somewhat different results. The Series II isomer, $[\alpha]_D+111°$ (in chloroform) passed into a crystalline 4 : 5-dimethyl-*o*-phenylenediamine tetraacetyl-D-glucoside [*isomer B**], m.pt. 128° to 129°C. $[\alpha]_D-31{\cdot}6°$ (in chloroform) in excellent yield. The Series I isomers $[\alpha]_D-62°$ (in chloroform), in contrast, gave rise to a product. $C_{26}H_{48}O_{18}N_2$, m.pt. 180° to 181°, $[\alpha]_D-19{\cdot}4°$ (in chloroform) together with smaller quantities of a 4 : 5-dimethyl-*o*-phenylenediamine tetraacetyl-D-glucoside (*isomer A*). The latter resembled *isomer B* in giving the same 5 : 6-dimethylbenziminazole-1-β-tetraacetyl-D-glucopyranoside (see Part IV (2)), but differed from it in the facility with which this transformation could be effected.

The constitution of a 4 : 5-dimethyl-*o*-phenylene-diamine-*N* : *N*′-di[tetraacetyl-D-glucoside], has been assigned to the main reduction product, m.pt. 180° to 181°C. of the Series I isomer. This formulation is based on its conversion by acid hydrolysis, into D-glucose, identified as the osazone, and 4 : 5-dimethyl-*o*-phenylenediamine identified as 5 : 6-dimethylbenziminazole. In addition, its ultraviolet absorption spectrum shows the typical characteristics of a substituted 4 : 5-dimethyl-*o*-phenylenediamine (see Fig. 1). Its formation presumably involves the dismutation of a tetraacetyl glucose residue between two *o*-phenylenediamine glycoside groupings, with parallel formation of the parent base, identified as present in the products of reduction by conversion into 5 : 6-dimethyl-benziminazole and isolation as the picrate (see page. 494).

The other *o*-phenylenediamine glycosides prepared during this phase of the work proved to be compounds of low crystallising power. In all the cases examined, however, the same benziminazole glycoside was obtained from both Series I and Series II nitroglycosides (see Parts IV (2) and IV (3)), so that merging of the two series must have occurred either during the reduction of the nitroaniline glycosides, or during the conversion of the resulting *o*-phenylenediamine glycosides into the corresponding benziminazole glycosides. As the latter proved to be glyco-

* The terminology Series I and II has not been employed in this instance as the $[\alpha]_D$ of *isomer B* would appear to indicate its adherence to Series I. Direct comparison with *isomer A* to settle this point was not possible, however, as attempts to isolate the latter compound in a state of purity proved unsuccessful. Its existence was inferred, however, by its facile conversion into the corresponding benziminazole glycoside (Part IV (2)).

pyranosides (see Parts IV (2) and IV (3)), the pyranose formulation must be adopted for both the *o*-nitroaniline- and *o*-phenylenediamine acetyl glycosides unless migration of an acetyl grouping be assumed, an eventuality which is, in our opinion, very slight. The nature of the isomerism in these two classes of compounds must consequently be of the α β type at the glycosidic centre, a view which accords well with the conversion of the Series II *o*-nitroaniline acetylglycosides into their Series I anomers

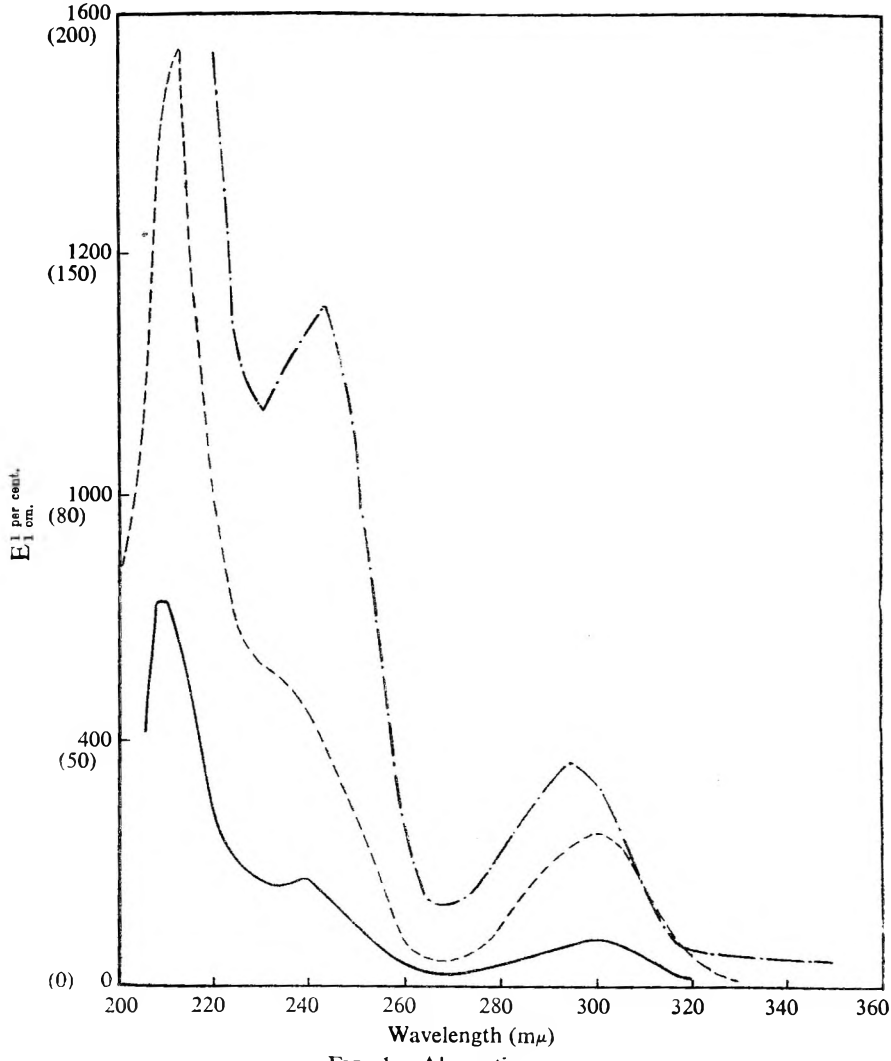


FIG. 1. Absorption curves.

———— 4 : 5-Dimethyl-*o*-phenylenediamine-tetraacetyl-D-glucoside.
—·—·— 4 : 5-Dimethyl-*o*-phenylenediamine-*N* : *N'*-di-(tetraacetyl-D-glucoside).
— — — 4 : 5-Dimethyl-*o*-phenylenediamine.

In order to represent the absorption curve for 4 : 5-dimethyl-*o*-phenylenediamine-*N* : *N'*-di (tetraacetyl-D-glucoside) on this figure it has been necessary to plot the $E^{1\ per\ cent.}_{1\ cm.}$ values for this compound on the scale 0-200.

on warming with dilute acid (*vide supra*). Unfortunately it cannot be argued that the parent *o*-nitroaniline glycosides likewise have the pyranose-structure, as furanose → pyranose changes are known to occur on acetylation[15]. Kuhn and Strobele (*loc. cit.*) originally assigned furano-structure to their *o*-nitroaniline glycosides, but the experimental foundation for this postulate, the formation of trityl-derivatives, is now known to be unreliable. Howard, Kenner, Lythgoe and Todd[15] have, in fact, demonstrated that *o*-nitroaniline-L-arabinoside has the pyranose structure. The complete elucidation of the structures of the *o*-nitroaniline glycosides described in this communication, with this one exception, must, therefore, await further investigation.

EXPERIMENTAL

Melting points are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford. The specific rotations of the glycosides and of the acetylated glucosides were measured (c=1) in pyridine and chloroform solutions, respectively.

Preparation of the o-*Nitroaniline glycosides.* The following general method was employed (cf. Kuhn and Strobele, *loc. cit.*). The sugar (0·1 mol.), the appropriate *o*-nitroaniline (0·2 to 0·3 mol.), ammonium chloride (2 g.) and carefully dried alcohol (200 to 300 ml.) were heated under reflux on the steam bath with exclusion of moisture until the sugar had dissolved. Heating was then continued for a further 30 minutes. A portion of the solvent was removed by distillation, and the residual liquor poured onto a column of alumina. Excess *o*-nitroaniline present was removed by washing with benzene, whereafter the glycoside was eluted with alcohol (50 per cent.) or with water. The eluate was concentrated under reduced pressure to small volume, or until the glycoside began to separate. After standing at 0° to 5°C. for 24 hours the product was collected, washed with ice-water, and dried *in vacuo*.

The following compounds were prepared in this way :—

m-*Nitro*-p-*toluidine*-D-*glucoside* (yield 50 per cent.), golden-yellow needles from water, m.pt. 120° to 128°C. Found : C, 49·7 ; H, 6·3 ; N, 9·0. $C_{13}H_{18}O_7N_2$ requires C, 49·7 ; H, 5·8 ; N, 8·9 per cent.

o-*Nitroaniline*-D-*mannoside* (yield 60 per cent.), yellow needles from alcohol, m.pt. 214° to 215° (decomp.). Found : C, 48·4 ; H, 5·3. $C_{12}H_{16}O_7N_2$ requires C, 48·0 ; H, 5·4 per cent.

m-*Nitro*-p-*toluidine*-D-*mannoside* (yield 55 per cent.), crystallising in fine yellow needles from alcohol, m.pt. 205° to 206°C. (decomp.). Found : C, 48·6 ; H, 6·2. $C_{13}H_{18}O_7N_2$, $\frac{1}{2}H_2O$ requires C, 48·3 ; H, 5·9 per cent.

3-*Nitro*-o-4-*xylidine*-D-*mannoside* (yield 85 per cent.) formed silky orange needles from alcohol, m.pt. 215° to 216°C. $[\alpha]_D^{20°C.}$ −28·5°. Found : C, 51·2 ; H, 6·0 ; N, 8·4. $C_{14}H_{20}O_7N_2$ requires C, 51·2 ; H, 6·1 ; N, 8·5 per cent..

5-*Nitro*-o-4-*xylidine*-D-*mannoside* crystallised in orange needles from alcohol which turned yellow at 110° to 210°C. and melted at 213°C. (decomp.), $[\alpha]_D^{25°C.}$ −35·0°. Found : C, 51·0 ; H, 6·4 ; N, 8·3. $C_{14}H_{20}O_7N_2$ requires C, 51·2 ; H, 6·1 ; N, 8·5 per cent.

m-*Nitro*-p-*toluidine*-D-*galactoside* (yield 33 per cent.), crystallised from alcohol in yellow needles, m.p. 204°C. (decomp.). Found : C, 49·2 ; H, 5·8 ; N, 8·5. $C_{13}H_{18}O_7N_2$ requires C, 49·7 ; H, 5·8 ; N, 8·9 per cent.

o-*Nitroaniline*-L-*arabinosides*. The aqueous eluate from the column (*vide supra*) was allowed to stand in a refrigerator and the crystalline deposit collected after 24 hours. The latter comprised almost pure o-*nitroaniline*-L-*arabinoside* II, which, when recrystallised from alcohol, formed hard, orange-yellow needles, m.p. 173° to 175°C. $[\alpha]_D^{22°C.}$ + 21·1°. Found : C, 48·8 ; H, 5·2 ; N, 10·6. $C_{11}H_{14}O_6N_2$ requires C, 48·9 ; H, 5·2 ; N, 10·3 per cent. The mother liquors were taken to dryness *in vacuo*, and the residue carefully fractionated from alcohol. After separation of a further crop of the Series II isomer, the Series I arabinoside was crystallised by addition of light petroleum. o-*Nitroaniline*-L-*arabinoside* I formed orange needles from a mixture of alcohol and light petroleum, m.p. 159° to 161°C., $[\alpha]_D^{23°C.}$ + 55·5°. Found : C, 49·3 ; H, 5·1 ; N, 10·0 per cent.

m-*Nitro*-p-*toluidine*-L-*arabinosides*. The crude mixture of arabinosides was fractionated in the manner described above. The series II L-arabinoside crystallised as the *hydrate* in orange needles from alcohol, m.pt. 141° to 142°C. $[\alpha]_D^{20°C.}$ − 10°. Found : C, 47·4 ; H, 6·0 ; N, 9·0. $C_{12}H_{16}O_6N_2$, H_2O requires C, 47·7 ; H, 6·0 ; N, 9·3 per cent. The Series II *isomer* formed rosettes of orange needles from alcohol, m.pt. 133° to 135°C. $[\alpha]_D^{25°C.}$ + 83·6°. Found : C, 50·9·; H, 5·7 : N, 9·7. $C_{12}H_{16}O_6N_2$ requires C, 50·7 ; H, 5·6 ; N, 9·9 per cent.

5-*Nitro*-o-4-*xylidine*-L-*arabinosides*. The mixture of isomers was fractionated from alcohol by the method described by Kuhn and Strobele (*loc. cit.*). The Series I compound had m.pt. 187·5°C. $[\alpha]_D^{24°C.}$ + 71·0° and the Series II, m.pt. 108° to 109°C. $[\alpha]_D^{22°C.}$ − 7·8°.

o-*Nitroaniline*-D-*xyloside*, yellow prisms from a mixture of alcohol and light petroleum, m.pt. 172° to 176°C. Found : C, 48·7 ; H, 5·2 ; N, 10·3. $C_{11}H_{14}O_6N_2$ requires C, 48·9 ; H, 5·2 ; N, 10·3 per cent.

m-*Nitro*-p-*toluidine*-D-*xyloside* crystallised from aqueous alcohol m.pt., *ca.* 170°C. Found : C, 50·3 ; H, 5·9 ; N, 9·7. $C_{12}H_{16}O_6N_2$ requires C, 50·7 ; H, 5·6 ; N, 9·9 per cent.

5-*Nitro*-o-4-*xylidine*-D-*xyloside*, orange prisms from a mixture of methyl alcohol and ethyl acetate, m.pt. 212° to 213°C. (decomp.). Found : N, 9·5. $C_{13}H_{18}O_6N_2$ requires N, 9·4 per cent.

o-*Nitroaniline*-L-*rhamnoside* (yield 50 per cent.) formed silky yellow needles from alcohol, m.pt. 225°C. (decomp.). Found : C, 50·9 ; H, 5·3 ; N, 9 7. $C_{12}H_{16}O_6N_2$ requires C, 50·7 ; H, 5·7 : N, 9·9 per cent.

m-*Nitro*-p-*toluidine*-L-*rhamnoside* crystallised from alcohol in yellow needles, m.pt. 215°C. (decomp.). Found : C, 52·7 ; H, 6·1 ; N, 9·4. $C_{13}H_{18}O_6N_2$ requires C, 52·4 ; H, 6·1 ; N, 9·4 per cent.

A number of the *o*-nitroaniline-glycosides were not purified as such, but were directly acetylated as described below :—

Acetylation of the o-*Nitroaniline-glycosides and Fractionation of the resulting mixtures of Acetates*. Acetic anhydride (1·0 to 1·2 mols.) was

added to a cooled solution of the glycoside (0·1 mol.) in pyridine (200 to 250 ml.). After standing at room temperature for 24 hours, the excess of acetic anhydride was destroyed by addition of alcohol, and the solvents removed under reduced pressure. The residue was re-evaporated once or twice with alcohol, and then recrystallised once from the minimum of alcohol (with charcoal if necessary) to give the mixture of acetates. The latter was fractionated by crystallisation from ethyl acetate. The first crop, which comprised the series I isomer, was collected and washed with a little ethyl acetate. The Series II acetate was recovered from the filtrate either by evaporation to dryness, or by addition of light petroleum. Both acetates were then crystallised to constant melting-point and rotation from the appropriate solvent. The total yield of the acetates generally exceeded 85 per cent. of the theoretical yield.

o-*Nitroaniline-tetraacetyl*-D-*glucoside* crystallised from a mixture of alcohol and ethyl acetate in yellow prisms, m.p. 184°C. (cf. Kuhn and Strobële *loc. cit.*). Evaporation of the filtrates gave only small amounts of β-pentacetyl-D-glucose, m.p. 129° to 130°C.

m-*Nitro*-p-*toluidine-tetraacetyl*-D-*glucoside* I crystallised from alcohol in yellow needles or prisms, m.p. 180°C. $[\alpha]_D^{23°C.}$ −51·7°. Found: C, 52·9; H, 5·3; N, 5·9. $C_{21}H_{26}O_{11}N_2$ requires C, 52·3; H, 5·4; N, 5·8 per cent. The Series II *isomer* separated from alcohol in yellow needles, m.p. 129°C. $[\alpha]_D^{23°C.}$ +107·7°. Found: C, 52·5; H, 5·3; N, 5·7 per cent.

3-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*glucoside* I formed prismatic yellow needles from alcohol, m.p. 157°C. $[\alpha]_D^{24°C.}$ −172·5°. Found: C, 53·4; H, 5·8; N, 5·7. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2; H, 5·7; N, 5·6 per cent.

5-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*glucoside* was described by Kuhn and Strobële (*loc. cit.*) as a compound of indefinite melting point. This mixture of isomers was readily separated by crystallisation from ethyl acetate. The Series I isomer formed yellow needles from ethyl acetate, m.p. 168° to 169°C. $[\alpha]_D^{24°C.}$ −62°. Found: C, 53·6; H, 5·5; N, 5·8. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2; H, 5·7; N, 5·6 per cent. The Series II compound separated from alcohol in prismatic yellow needles, m.p. 150° to 151°C. $[\alpha]_D^{22°C.}$ +111°. Found: C, 53·2; H, 5·6; N, 5·8 per cent.

No attempts were made to fractionate the following galactosides and mannosides :—

o-*Nitroaniline-tetraacetyl*-D-*galactoside* separated in fine yellow needles from alcohol, m.p. 178°C. $[\alpha]_D^{25°C.}$ −34·6°. Found: C, 51·3; H, 5·4; N, 5·9. $C_{20}H_{24}O_{11}N_2$ requires C, 51·3; H, 5·2; N, 6·0 per cent.

m-*Nitro*-p-*toluidine-tetraacetyl*-D-*galactoside* formed yellow platelets from alcohol, m.p. 190°C. $[\alpha]_D^{22°C.}$ −21·6°. Found: C, 52·6; H, 5·3; N, 5·8. $C_{21}H_{26}O_{11}N_2$ requires C, 52·3; H, 5·4; N, 5·8 per cent.

3-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*galactoside* crystallised in prismatic orange-yellow needles from ethyl acetate, m.p. 196° to 197°C. $[\alpha]_D^{23°C.}$ −149·2°. Found: C, 53·3; H, 5·5; N, 5·3. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2; H, 5·7; N, 5·6 per cent.

5-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*galactoside* formed yellow octahedra

from alcohol, m.p. 180°C. $[\alpha]_D^{23°C.}$ −23·4°. Found : C, 53·3 ; H, 5·6 ; N, 5·3. $C_{22}H_{28}O_{11}N_2$ requires C, 53·2 ; H, 5·7 ; N, 5·6 per cent.

o-*Nitroaniline-tetraacetyl*-D-*mannoside* formed lemon yellow prismatic needles, m.p. 127° to 128°C. $[\alpha]_D^{21°C.}$ −103·0°. Found : C, 51·7 ; H, 5·2 ; N, 6·1. $C_{20}H_{24}O_{11}N_2$ requires C, 51·3 ; H, 5·2 ; N, 6·0 per cent.

m-*Nitro*-p-*toluidine-tetraacetyl*-D-*mannoside* separated from a mixture of ethyl acetate and light petroleum in golden-yellow needles, m.p. 144° to 145°C. $[\alpha]_D^{21°C.}$ −97·8°. Found : C, 52·8 ; H, 5·2 ; N, 5·7. $C_{21}H_{26}O_{11}N_2$ requires C, 52·3 ; H, 5·4 ; N, 5·8 per cent.

3-*Nitro*-o-4-*xylidine-tetraacetyl*-D-*mannoside* crystallised in silky bright yellow needles from ethyl acetate, m.p. 154° to 155°C. $[\alpha]_D^{21°C.}$ −258°. Found : C, 53·0 ; H, 5·5 ; N, 5·9. $C_{22}H_{28}O_{11}N_2$ requires C, 52·2 ; H, 5·7 ; N, 5·6 per cent.

o-*Nitroaniline-triacetyl*-L-*arabinoside* I crystallised from a mixture of ethyl acetate and light petroleum in yellow needles, m.p. 154°C. $[\alpha]_D^{22°C.}$ +159° (Kuhn and Strobële *loc. cit.* give m.p. 151°C. $[\alpha]_D$ + 133·8°). The Series II isomer separated in pale yellow needles from light petroleum (b.p. 60° to 80°C.), containing a trace of ethyl acetate, m.p. 90° to 91°C. $[\alpha]_D^{21°C.}$ −23°. Found : C, 51·5 ; H, 5·3 ; N, 7·5. $C_{17}H_{20}O_9N_2$ requires C, 51·5 ; H, 5·1 ; N, 7·1 per cent.

m-*Nitro*-p-*toluidine-triacetyl*-L-*arabinoside* I, hard yellow needles from ethyl acetate, m.p. 194°C. $[\alpha]_D^{22°C.}$ +146·5°. Found : C, 53·0 ; H, 5·4 ; N, 6·5. $C_{18}H_{22}O_9N_2$ requires C, 52·7 ; H, 5·4 ; N, 6·8 per cent. and the Series II *isomer*, orange cubes from a mixture of ethyl acetate and light petroleum, m.p. 77° to 79°C. $[\alpha]_D^{21°C.}$ −22·6°. Found : C, 52·5 ; H, 5·5 ; N, 6·8 per cent.

3-*Nitro*-o-4-*xylidine-triacetyl*-L-*arabinoside* crystallised in yellow prisms from ethyl acetate, m.p. 167°C. $[\alpha]_D^{23°C.}$ −140·8°. Found : C, 53·6 ; H, 5·9 ; N, 6·3. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent.

5-Nitro-*o*-4-xylidine-triacetyl-L-arabinoside I, m.p. 213° to 214°C. $[\alpha]_D$ +142° has been described by Kuhn and Strobële *loc. cit.* The Series II glycoside separated from a mixture of ethyl acetate and light petroleum in prismatic needles, m.p. 143° to 144°C. $[\alpha]_D^{22°C.}$ +24°. Found : C, 53·9 ; H, 5·7 ; N, 7·2. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent.

o-*Nitroaniline-triacetyl*-D-*xyloside* I has been described by Kuhn and Strobële (*loc. cit.*). A second isomer appeared to be present in the mother liquors but could not be isolated in a crystalline state.

m-*Nitro*-p-*toluidine-triacetyl*-D-*xyloside* I formed fluffy yellow needles from alcohol, m.p. 183°C. $[\alpha]_D^{22°C.}$ −87·2°. Found : C. 52·8 ; H. 5·4 ; N, 6·8. $C_{18}H_{22}O_9N_2$ requires C, 52·7 ; H, 5·4 ; N, 6·8 per cent. The Series II *isomer*, yellow needles, m.p. 130°C. to 132°C. $[\alpha]_D^{26°C.}$ +7·9°. Found : C, 52·5 ; H, 5·4 ; N, 7·0 per cent.

5-*Nitro*-o-4-*xylidine-triacetyl*-D-*xyloside* separated from aqueous alcohol in yellow needles, m.p. 168° to 169°C. Found : C, 53·5 ; H, 5·7 ; N, 6·6. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent. The mother liquors were not investigated.

o-*Nitroaniline-triacetyl*-L-*rhamnoside* crystallised in yellow needles from alcohol, m.pt. 185°C. $[\alpha]_D^{23°C.}$ +117·6°. Found : C, 52·7 ; H, 5·4 ; N, 6·4. $C_{18}H_{22}O_9N_2$ requires C, 52·7 ; H, 5·4 ; N, 6·8 per cent.

m-*Nitro*-p-*toluidine-triacetyl*-L-*rhamnoside* formed yellow flakes from alcohol, m.pt. 161° to 162°C. $[\alpha]_D^{23°C.}$ +104·9°. Found : C, 54·5 ; H, 5·5 ; N, 6·7. $C_{19}H_{24}O_9N_2$ requires C, 53·8 ; H, 5·7 ; N, 6·6 per cent.

5-*Nitro*-o-4-*xylidine-triacetyl*-L-*rhamnoside* I, yellow prisms from ethyl acetate, m.pt. 169°C. $[\alpha]_D^{22°C.}$ +100·8°. Found : C, 55·0 ; H, 6·1 ; N, 6·4. $C_{20}H_{26}O_9N_2$ requires C, 54·7 ; H, 6·0 ; N, 6·4 per cent. A Series II compound was isolated in very low yield and crystallised in yellow needles from light petroleum, m.pt. 106° to 107°C.

Isomerisation of the o-*Nitroaniline-acetyl glycosides* II. The following examples illustrate the methods employed :—

(i) *m*-Nitro-*p*-toluidine-triacetyl-L-arabinoside II (50 mg.) and 2N hydrochloric acid (3 ml.) were boiled together. The molten solid rapidly resolidified and, after cooling, was collected and recrystallised from a mixture of ethyl acetate and light petroleum to give the Series I isomer, m.pt. and mixed m.pt. 194°C. Yield 40 mg.

(ii) 5-Nitro-*o*-4-xylidine-triacetyl-L-arabinoside II (60 mg.), and 2N hydrochloric acid (4 ml.) were heated to boiling and alcohol added dropwise until solution was complete. After rapid cooling, the product which separated was collected, recrystallised, and identified as the Series I isomer by m.pt. and mixed m.pt. determinations.

o-*Phenylenediamine-acetylglycosides* : The following general method was employed. The nitroglycoside, Series I or II (5g.) dissolved in ethyl acetate (100 ml.) was shaken with hydrogen in the presence of a 10 per cent. palladised charcoal catalyst at 40° to 50°C. When uptake ceased, the catalyst was separated and the solvent removed *in vacuo*. The crude amines were generally used directly for benziminazole synthesis (see Part IV (2)). The following compounds were examined in detail and obtained in crystalline form :—

4-*Methyl*-o-*phenylenediamine-tetraacetyl*-D-*glucoside* crystallised from a mixture of benzene and light petroleum in felted needles, m.pt. 130° to 131°C. $[\alpha]_D^{23°C.}$ −47·0°. Found : 55·4 ; H, 5·8 ; N, 6·4. $C_{21}H_{28}O_9N_2$ requires C, 55·7 ; H, 6·2 ; N, 6·2 per cent.

The same amine was obtained from both the Series I and Series II nitro-compounds.

3 : 4-*Dimethyl*-o-*phenylenediamine-tetraacetyl*-D-*glucoside* crystallised from alcohol in white prismatic needles, m.pt. 128° to 129°C. $[\alpha]_D^{22°C.}$ −36·4°. Found : C, 56·7 ; H, 6·5 ; N, 6·1. $C_{22}H_{30}O_9N_2$ requires C, 56·6 ; H, 6·5 ; N, 6·0 per cent.

4 : 5-*Dimethyl*-o-*phenylenediamine-tetraacetyl*-D-*glucoside* (*isomer* B) was prepared by reduction of the nitro-compound (Series II) and crystallised from a mixture of ethyl acetate and light petroleum in colourless needles, m.pt. 128° to 129°C. $[\alpha]_D^{20°C.}$ −31·6°. Found : C, 56·8 ; H, 6·8 ; N, 5·9. $C_{22}H_{30}O_9N_2$ requires C, 56·6 ; H, 6·5 ; N, 6·0 per cent.

Reduction of 5-*nitro*-O-4-*xylidine-tetraacetyl*-D-*glucoside* I. The nitroglycoside I (4 g.) was reduced in ethyl acetate and the product isolated in the usual way. 4 : 5-*Dimethyl*-O-*phenylenediamine*-*N* : *N'*-*ditetraacetyl*-D-*glucoside* separated from a mixture of ethyl acetate and light petroleum in silky white needles, m.pt. 181°C. $[\alpha]_D^{22°C.}$ −19·4°. Found : C, 54·4, 54·4 ; H, 6·1, 6·2 ; N, 3·7, 3·4. $C_{36}H_{48}O_{18}N_2$ requires C, 54·3 ; H, 6·1 ; N, 3·5 per cent. This compound (200 mg.) was heated with 4N hydrochloric (5 ml.) at 100°C. for 15 minutes, whereafter the solution was divided into two portions. One yielded glucosazone m.pt. 200°C. (decomp.), on treatment with phenylhydrazine and sodium acetate. The other portion yielded 5 : 6-dimethylbenziminazole, isolated as the picrate after heating with formic acid.

Summary and Conclusions

1. Glucosidation of *o*-nitroaniline gives, in general, a mixture of two isomers designated *o*-nitroaniline glycosides I and II.

2. In contrast to results reported by Kuhn and Strobele[12] acetylation of these isomers gives the corresponding *o*-nitroaniline acetylglycosides I and II.

3. Conversion of the Series II isomer into the Series I isomer has been effected in certain cases by very short contact with hot dilute hydrochloric acid.

4. Reduction of the *o*-nitroaniline acetylglycosides I and II is accompanied, in certain instances, by a merging of the two series and the formation of only one *o*-phenylenediamine acetylglycoside.

5. Similar results have been obtained and are here recorded, employing *m*-nitro-*p*-toluidine, 3-nitro-*o*-4-xylidine and 5-nitro-*o*-4-xylidine.

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THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART IV. BENZIMINAZOLE GLYCOSIDES

(2) SYNTHETIC ROUTES TO THE BENZIMINAZOLE-1-D-GLUCOPYRANOSIDES

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From the Research Laboratories, The British Drug Houses, Ltd., London, N.1

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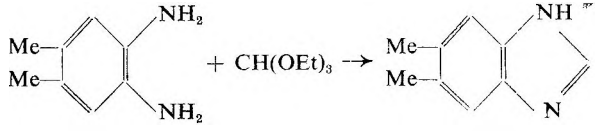
SUCCESSFUL completion of the investigation outlined in the preceding paper (Part IV (I))[1] furnished *o*-phenylenediamine glycosides (e.g., I) suitable for conversion into benziminazole glycosides (e.g., IV). Their instability to acids, however, precluded their conversion into the latter compounds by methods successfully used in Part III[2]. We therefore directed our attention, in the first instance, to the elaboration of milder cyclisation procedures employing *o*-phenylenediamine-tetraacetyl-D-glucopyranoside (I) for these model experiments.

Fundamentally the problem presented in the conversion of (I) into (IV) is the introduction of a methine group between two reactive centres. A similar problem was involved in the synthesis of the carbocyanine dyes from quinaldine alkiodides. Its solution by Hamer[3], who replaced formic acid or formaldehyde by ethyl orthoformate, furnished the essential clue to a new synthesis of benziminazoles[4] which forms the subject of the present communication.

Preliminary experiments showed that *o*-phenylenediamine and its *N*-alkylated derivatives underwent facile conversion into the corresponding benziminazoles on treatment with ethyl orthoformate:



The reaction could be conducted in an excess of the ester at an elevated temperature or in a solvent such as ethyl alcohol or ethyl acetate, the benziminazole being generally obtained in almost quantitative yield.

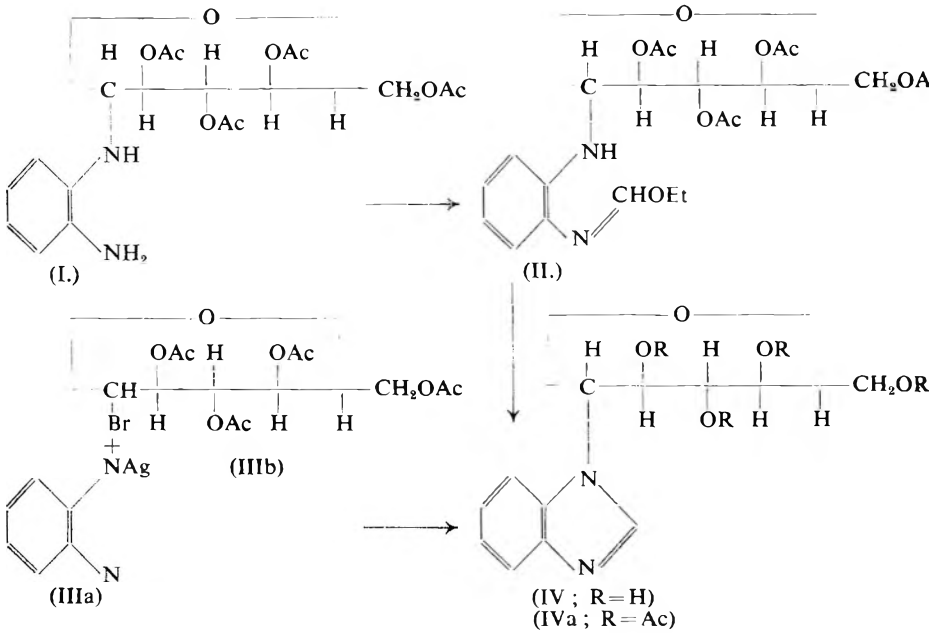
An unexpected difficulty was encountered, however, in the first application of this synthesis to an *o*-phenylenediamine glycoside. Reaction of *o*-phenylenediamine-tetraacetyl-D-glucoside (I) with ethyl orthoformate gave a crystalline product in high yield which, although giving reasonable analytical figures for (IVa), yet failed to exhibit the typical 1-substituted benziminazole absorption spectrum (Fig. I). Elemental analysis and molecular weight determinations finally led to its formulation as a 2-ethoxymethylene-*o*-phenylenediamine-tetraacetyl-D-glucoside (II). The formation of ethyl *iso*formanilides such as (II) from primary arylamines and orthoformates, it may be added, has previously been recorded by a number of workers[5].

The conversion of (II) into the benziminazole glycoside (IVa) likewise presented considerable initial difficulty[4]. Systematic study led ultimately to the discovery that carefully controlled treatment with very dilute

hydrochloric acid (0·05 to 0·1 N) at 100°C. resulted in smooth conversion into benziminazole-1-tetraacetyl-D-glucoside (IVa).

The constitution assigned to this product was confirmed by its ultraviolet absorption spectrum which bore the characteristics of a 1-substituted benziminazole (Fig. 1). The procedure employed for its synthesis, however, left undefined the stereochemical configuration of the glycosidic centre and the size of the lactol ring.

Two methods have hitherto been employed for the determination of the αβ-configuration of *N*-glycosides. The first, depending upon mutarotation studies[6], is not applicable in this instance owing to the quite remarkable stability of (IV) to acids (*vide infra*). The second depends



upon the interaction of a nitrogenous base, or of its metal derivatives, with an acetohalogeno-sugar and upon the reasonable assumption that a Walden inversion takes place in such a reaction[7]. This assumption appears to be warranted in many cases and is supported indirectly by the kinetic studies of Ingold, Hughes and their collaborators[8] to which Howard has recently drawn attention[9] in this connection. Nevertheless, as the latter author points out (*loc. cit.*), retention of configuration is occasionally encountered in the reaction of certain acetohalogeno-sugars with nucleophilic reagents. In so far as the present evidence allows, however, condensation of α-acetobromglucose with the silver salt of a base appears in general to lead to an *N*-glucoside possessing a β-configuration, and this view has been adopted in the present investigation.

Bearing the above theoretical consideration in mind, we examined the reaction between benziminazole silver[10] (IIIa) and α-acetobromglucose (IIIb) in xylene solution. Facile reaction occurred to give a benzimin-

azole-1 β-tetraacetyl-D-glucoside, identical in m.pt., mixed m.pt. specific rotation, and ultraviolet absorption spectrum (Fig. I) with the product obtained by the "orthoformate route." The latter must, therefore, be assigned the constitution of benziminazole-1-β-tetraacetyl-D-glucopyranoside (IVa).

A number of procedures were examined for the hydrolysis of (IVa), the products being investigated on unidimensional paper chromatograms by

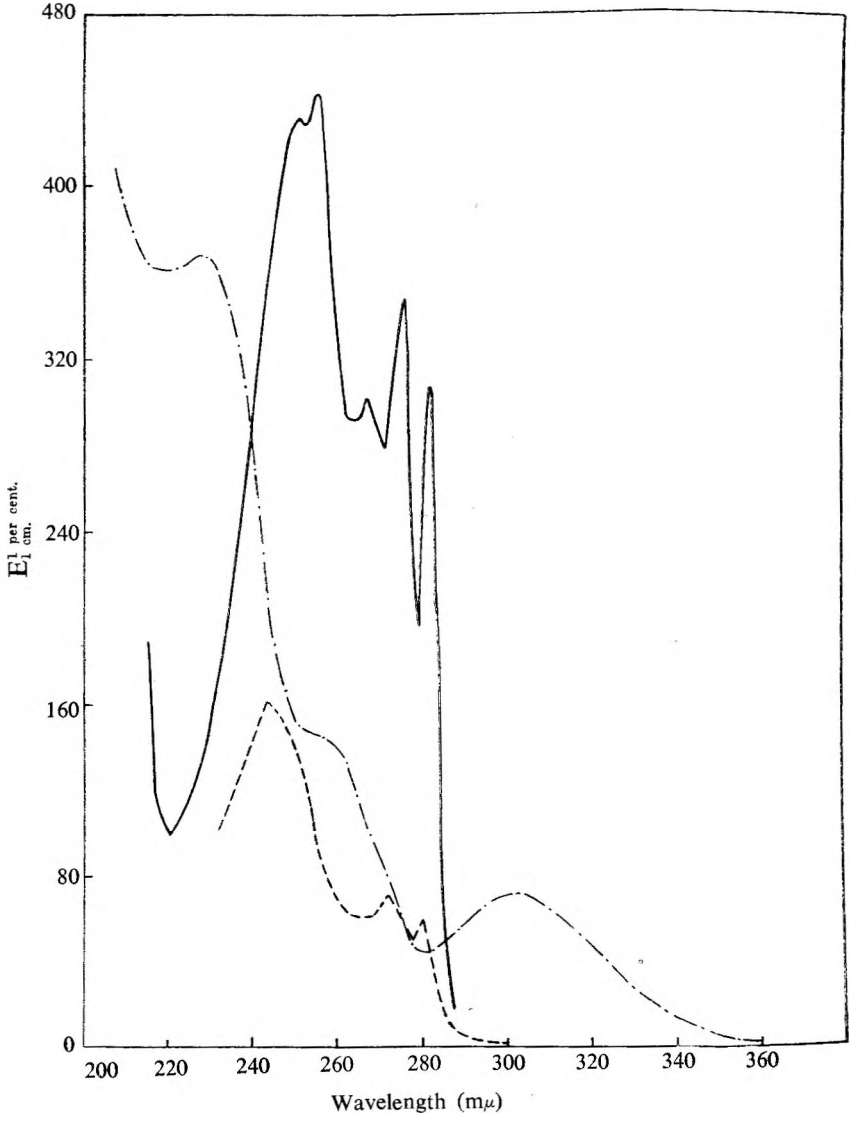


FIG. 1. Absorption curves

——— 1-Methylbenziminazole
—·—·— 2-Ethoxymethylene-*o*-phenylenediamine-tetraacetyl-D-glucoside
— — — Benziminazole-1-β-tetraacetyl-D-glucopyranoside

Mr. B. Ellis and Mr. G. Cooley of these laboratories. Hydrolysis with sodium ethoxide or ethyl alcoholic ammonia was found to be effective, but had limited practical value owing to the difficulty in separating the glycoside from concomitant inorganic matter. Heating with hydrochloric acid of strength from 2N to 6N at 100°C., however, proved satisfactory in every way, the benziminazole glucopyranoside ring system showing remarkable stability toward acidic reagents, a result in marked contrast to recorded observations in the nucleoside field. Rapid deacetylation occurred giving benziminazole-1-β-glucopyranoside hydrochloride, readily isolated as the monohydrate. Benziminazole-1-β-D-glucopyranoside (IV) itself was obtained by percolating an aqueous solution of the latter compound through the ion-exchange resin "Amberlite IR-4B" and was characterised by formation of the picrate. The pyranoside formulation was supported by periodate titrations when two molecules of the oxidant were consumed.

Having successfully developed two novel routes to the benziminazole glycosides, we next turned our attention to the preparation of some analogues of (IV) with the object of extending the range of synthetic compounds available for study. Both hexose and pentose sugars were employed for these experiments. For convenience, however, discussion of the pentosides is deferred at this stage and is reported upon separately in Part IV (3).

4-Methyl-*o*-phenylenediamine-tetraacetyl-D-glucoside reacted normally with ethyl orthoformate to give the *iso*formanilide derivative, converted by 0·1 N hydrochloric acid into 5-methylbenziminazole-1-tetraacetyl-D-glucoside. The latter compound could not be prepared, however, by the reaction of α-acetobromglucose with 5(6)-methylbenziminazole silver. A mixture of 5- and 6-methylbenziminazole-1-β-tetraacetyl-D-glucopyranosides was obtained from which attempts to isolate either isomer in a state of purity proved unsuccessful. It was thus impossible to determine the configuration of the anomeric centre in the 5-methyl acetylated glycoside by direct experiment. The results obtained with the parent base (IV), however, leave little doubt that this compound, too, must by analogy be formulated as the β-glycoside. Its hydrolysis with 6N hydrochloric acid furnished 5-methylbenziminazole-1-β-D-glucopyranoside.

3:4-Dimethyl-*o*-phenylenediamine-tetraacetyl-D-glucoside, in contrast to the foregoing glycosides, was surprisingly transformed directly into the benziminazole glycoside on heating with ethyl orthoformate. The same compound was also obtained by the action of α-acetobromglucose on 4:5-dimethylbenziminazole silver. It must, therefore, be formulated as 4:5-dimethylbenziminazole-1-β-tetraacetyl-D-glucopyranoside. On deacetylation with acid it gave 4:5-dimethylbenziminazole-1-β-D-glucopyranoside hydrochloride.

A further example of the direct conversion of an *o*-phenylenediamine glycoside into a benziminazole glycoside was encountered during experiments employing 4:5-dimethyl-*o*-phenylenediamine tetraacetyl-D-glucosides, *isomers A* and *B* (see Part IV (I)). Both compounds gave the same 5:6-dimethyl-benziminazole-1-β-tetraacetyl-D-glucopyranoside, the

constitution of which followed from its alternative preparation from α-acetobromglucose and 5:6-dimethylbenziminazole silver. Only *isomer B*, however, yielded the intermediate ethyl*iso*formanilide on treatment with ethyl orthoformate. Hydrolysis furnished 5:6-dimethylbenziminazole-1-β-D-glucopyranoside, characterised by formation of the picrate.

Attempts to convert *o*-phenylenediamine-tetraacetyl-D-galactoside into the corresponding benziminazole proved unsuccessful, resinification taking place. The reason for this failure is not evident, particularly as Andrews, Kenner and Todd[11] observed no difficulty in the preparation of 2-methylthioadenine-9-D-galactopyranoside. The results obtained in this and the succeeding communication, however, lead to the conclusion that the factors governing the synthesis of benziminazoles by the "orthoformate route" are exceedingly complex and cannot readily be defined at this stage.

EXPERIMENTAL

Melting points are corrected. Microanalyses and molecular weight determination are by Drs. Weiler and Strauss, Oxford.

Experiments on the Synthesis of Benziminazole Glycosides from o-Phenylenediamine Glycosides employing Sodium Dithioformate. (i) a methyl alcoholic solution of *o*-phenylenediamine-tetraacetyl-D-glucoside (prepared by catalytic reduction of 5 g. of the corresponding nitro-compound in the presence of palladium/charcoal; see Part IV (I) preceding communication) was treated with technical sodium dithioformate (for which the authors are indebted to Prof. A. R. Todd, F.R.S., and Dr. F. Bergel) (4·5 g.) in methyl alcohol (40 ml.), followed at once by acetic acid (1·3 g., 1 mol.). After standing at room temperature for 3 days, the mixture was boiled for 1 hour, filtered and evaporated to dryness under reduced pressure. The residue was extracted with ethyl acetate and the extract chromatographed on a column of alumina which was thoroughly washed with ethyl acetate. The crude thioformyl derivative (1 g.), isolated by evaporation to dryness, could not be crystallised and was, therefore, cyclised direct by heating under reflux for 5 hours with potassium acetate (5 g.) in acetonitrile (20 ml.). After filtration and evaporation *in vacuo* the product was isolated as the picrate (90 mg.) which crystallised from alcohol (95 per cent.) in yellow needles m.pt. 167°C. This was probably an impure sample of *benziminazole-1-β-tetraacetyl-D-glucopyranoside picrate* (Found: C, 48·1; H, 3·8; N, 10·7. $C_{21}H_{24}O_9N_2$, $C_6H_3O_7N_3$ requires C, 47·2; H, 4·0; N, 11·6 per cent.) as it showed no depression of the melting point in admixture with an authentic sample (see below).

(ii) 4:5-Dimethyl-*o*-phenylenediamine-tetraacetyl-D-glucopyranoside (1·6 g.) was converted into a resinous thioformyl-derivative (1·1 g.) by the procedure outlined above. This intermediate (500 mg.) was cyclised by heating with sodium methoxide (110 mg.) in absolute alcohol (20ml.) for 4 hours. The neutralised solution was taken to dryness and an alcoholic extract of the residue treated with picric acid. The picrate so obtained (120 mg.) was presumably 5:6-*dimethylbenziminazole*-1-β-D-*glucopyranoside picrate* as it did not depress the melting-point of an

authentic sample (see below), but repeated crystallisation failed to raise the m.pt. above 228°C. (decomp.). Found: C, 45·8; H, 4·4; N, 13·2. $C_{15}H_{20}O_5N_2$, $C_6H_3O_7N_3$ requires C, 46·9; H, 4·2; N, 13·0 per cent.

2-*Ethoxymethylene-o-phenylenediamine-tetraacetyl*-D-*glucoside*. A solution of *o*-nitroaniline-tetraacetyl-D-glucopyranoside (5 g.) in ethyl acetate (50 ml.) was shaken in hydrogen in the presence of palladium/charcoal until reduction was complete, whereafter the catalyst was filtered and the filtrate evaporated on the steam bath for 2 to 3 hours with ethyl orthoformate (8 ml.). The solvents were removed *in vacuo* and the residue recrystallised from a mixture of benzene and light petroleum. The product (4·5 g.) separated in colourless needles, m.pt. 141° to 142°C. $[\alpha]_D^{20°C.}$ − 84·6° (in chloroform c = 1). Found: C, 55·5; H, 6·1; N, 5·9; Mol.Wt. 497. $C_{23}H_{30}O_{10}N_2$ requires C, 55·6; H, 6·1; N, 5·7 per cent.; Mol.Wt. 494.

Benziminazole-1-β-*tetraacetyl*-D-*glucopyranoside*. (i) The foregoing *iso*-formanilide (5 g.), 0·1N hydrochloric acid (30 ml.), water (15 ml.) and a few drops of absolute alcohol were heated at 100°C. for 15 minutes with stirring. The solution was neutralised with potassium bicarbonate and cooled. *Benziminazole*-1-β-*tetraacetyl*-D-*glucopyranoside* 2·5 g.) separated and was recrystallised from a mixture of ethylacetate and light petroleum forming feathery needles, m.pt. 151° to 152°C. $(\alpha)_D^{24·6°C}$ − 22·8° (in chloroform c = 1). Found: C, 55·8; H, 5·7; N, 6·0. $C_{21}H_{24}O_9N_2$ requires C, 56·2; H, 5·4; N, 6·3 per cent.

(ii) A suspension of the silver salt of benziminazole (4·0 g., thoroughly dried and then finely ground) in dry xylene (120 ml.) was dried by azeotropic distillation of half of the solvent. α-Acetobromglucose (7·35 g.) was added, the mixture heated under reflux for 4 hours, and filtered from silver bromide. The product was isolated by evaporation of the filtrate *in vacuo*, purified by passage of a chloroform solution through a column of alumina, and finally converted into the picrate. Benziminazole-1-β-tetraacetyl-D-glucopyranoside picrate (2·5 g.) crystallised from ethyl alcohol in felted yellow needles, m.pt. 170° to 171°C. Found: N, 11·4; Calc. for $C_{21}H_{24}O_9N_2$, $C_6H_3O_7N_3$: N, 11·6 per cent. The picrate was conveniently decomposed by passing a chloroform solution through alumina. On treating the evaporated eluate with light petroleum, the base separated in felted white needles, m.pt. 151° to 152°C., identical in all respects with the material prepared by method (i). Found: C, 55·2; H, 5·5; N, 6·0 per cent.

Benziminazole-1-β-D-*glucopyranoside*. The foregoing tetraacetate (2·5 g.) and 6N hydrochloric acid (90 ml.) were heated at 100°C. for 3 hours. The solution was concentrated under reduced pressure until the product began to separate, when acetone was added. The hydrochloride (1·6 g.) separated as colourless needles of the mono-hydrate from water and acetone, m.pt. 196°C. (with foaming), $(\alpha)_D^{24·6°C.}$ + 17·3° (in water, c = 1). Found: C, 46·7; H, 6·0; N, 8·4; Cl, 10·6. $C_{13}H_{16}O_5N_2$, HCl. H_2O requires C, 46·7 H, 5·7; N, 8·4; Cl, 10·6 per cent.

A solution of the hydrochloride (1·0 g.) in distilled water (100 ml.) was percolated through a column of Amberlite IR-4B and the filtrate and

washings taken to dryness. The residue was dried at 100°C. (0·1 mm.) and crystallised once from a mixture of absolute alcohol and benzene, and subsequently from absolute alcohol. *Benziminazole*-1-β-D-*glucoside* (800 mg.) separated in fine colourless needles, m.pt. 212° to 213°C. $[\alpha]_D^{22°C.}$ – 3·4° (in water, c = 1·2). Found: C, 55·8; H, 5·9; N, 10·3. $C_{13}H_{16}O_5N_2$ requires C, 55·7; H, 5·7; N, 10·0 per cent. The *picrate* crystallised from water in flat yellow needles, m.pt. 146° to 148°C. (decomp). Found: C, 44·8; H, 4·0; N, 13·3. $C_{13}H_{16}O_5N_2$, $C_6H_3O_7N_3$ requires C, 44·8; H, 3·7; N, 13·8 per cent.

Periodate titrations. The glycoside (20 to 200 mg.) was dissolved in water, 0·242N periodic acid solution (50 to 100 per cent. excess) was added, and the solution diluted to a standard volume. At intervals, aliquots were withdrawn, and the remaining periodic acid determined by the method of Barneby[12]. Oxidation was generally complete within 72 to 96 hours.

Benziminazole-1-β-D-glucopyranoside consumed 2·01 moles. of periodic acid under these conditions, whilst the hydrochloride consumed 1·98 moles.

5-*Methylbenziminazole*-1-*tetraacetyl*-D-*glucopyranoside.* 4-Methyl-*o*-phenylenediamine-tetraacetyl-D-glucopyranoside (4·5 g.) was heated at 100°C. with ethyl orthoformate (10 ml.). Evaporation to dryness left a crystalline product which proved extremely soluble in the common organic solvents and could not be satisfactorily purified. It was, therefore, converted direct into the benziminazole by heating at 100°C. with 0·1N hydrochloric acid (30 ml.) After basification with potassium bicarbonate, the product was extracted with chloroform and converted into the *picrate* (3·0 g.), which crystallised from alcohol in yellow needles, m.pt. 185° to 186°C. Found: C, 49·0; H, 4·5; N, 10·0. $C_{22}H_{26}O_9N_2$,$C_6H_3O_7N_3$ requires C, 48·6; H, 4·2; N, 10·1 per cent. The regenerated 5-*methylbenziminazole*-1-*tetraacetyl*-D-*glucopyranoside* crystallised from a mixture of chloroform and light petroleum in colourless needles, m.pt. 175°C. $(\alpha)_D^{21°C.}$ – 37·8 (in chloroform c = 1. Found: C, 57·0; H, 5·6; N, 6·0. $C_{22}H_{26}O_9N_2$ requires C, 57·1; H, 5·6; N, 6·1 per cent.

5-*Methylbenziminazole*-1-D-*glucopyranoside.* The foregoing tetraacetate (500 mg.) was dissolved in 6N hydrochloric acid (20 ml.) and the solution heated at 100°C. for 90 minutes. The residue left on evaporation was freed from hydrogen chloride by evaporation with water and then dissolved in distilled water and passed through a column of ion exchange resin. Evaporation gave 5-*methylbenziminazole*-1-D-*glucopyranoside* (300 mg.), octahedra from alcohol, m.pt. 275° to 276°C. (decomp.), $(\alpha)_D^{25°C.}$ – 33·6° (in pyridine, c = 1). Found: C, 57·1; H, 6·1; N, 9·5. $C_{14}H_{18}O_5N_2$ requires C, 57·1; H, 6·1; N, 9·5 per cent. This compound consumed 1·97 moles of periodic acid.

5:6-*Dimethylbenziminazole*-1-β-*tetraacetyl*-D-*glucopyranoside.* (i) An ethyl acetate solution of 4:5-diamethyl-*o*-phenylenediamine tetraacetyl-D-glucopyranoside (isomer B), m.pt. 128° to 129°C. (2 g.) was heated under reflux for 3 hours with ethyl orthoformate (6 ml.) The

solvents were removed under reduced pressure and the residue heated on the steam bath with 0·05N hydrochloric acid (15 ml.) for 2 hours, by which time the resinous product had solidified. 5:6-*Dimethylbenziminazole*-1-β-*tetraacetyl*-D-*glucopyranoside* (800 mg.) crystallised from a mixture of benzene and light petroleum in cream-coloured needles, m.p. 189, 5° to 191°C., $(\alpha)_D^{26°C.}$ – 40·4° (in chloroform, c=1). Found: C, 57·5; H, 6·2; N, 6·0. $C_{23}H_{28}O_9N_2$ requires C, 58·0; H, 5·8; N, 5·9 per cent.

(ii) The silver salt of 5:6-dimethylbenziminazole (4·75 g.) was reacted with α-acetobromglucose (7·75 g.) in xylene solution in the manner described above. After chromatography of the crude product, the glucoside crystallised and was purified from a mixture of benzene and light petroleum, forming cream-coloured needles, m.p. 190° to 191°C., not depressed in admixture with the compound obtained by method (i) above. Found: C, 58·7; H, 5·8; N, 5·8 per cent.

(iii) The mother liquor from the crystallisation of 4:5-dimethyl-*o*-phenylenediamine-*N* : *N'*-di-(tetraacetyl-D-glucopyranoside) (preceding paper) was evaporated at 100°C. with ethyl orthoformate (6 ml.) for 3 hours. A crystalline solid separated on cooling and was identified as 5:6-dimethylbenziminazole-1-β-tetraacetyl-D-glucoside, m.p. 189° to 190°C., $(\alpha)_D^{20°C.}$ – 37·4°, after crystallisation from a mixture of chloroform and light petroleum. After the separation of this glucoside, 5:6-dimethylbenziminazole was isolated from the mother liquor as the picrate (cf. preceding paper). A trial experiment showed that 4:5-dimethyl-*o*-phenylenediamine-*N* : *N'*-di(tetraacetyl-D-glucopyranoside) failed to react with ethyl orthoformate.

5:6-*Dimethylbenziminazole*-1-β-D-*glucopyranoside*. The foregoing tetraacetate was deacetylated in the usual manner. The *glucoside* crystallised from alcohol in cream-coloured octahedra, m.p. 246° to 248°C. Found: C, 58·1; H, 6·7; N, 8·9. $C_{15}H_{20}O_5N_2$ requires C, 58·4; H, 6·5; N, 9·1 per cent. The *picrate* separated from water containing a little alcohol in yellow needles, m.p. 236°C. Found: C, 46·2; H, 4·7. $C_{15}H_{20}O_5N_2$, $C_6H_3O_7N_3$ requires C, 46·9; H, 4·3 per cent.

4:5-*Dimethylbenziminazole*-1-β-*tetraacetyl*-D-*glucopyranoside*. (i) 3:4-Dimethyl-*o*-phenylenediamine-tetraacetyl-D-glucopyranoside (2·0 g.) and ethyl orthoformate (6 ml.) were heated under reflux for 4 hours. The crystalline residue remaining after evaporation was recrystallised from a mixture of ethyl acetate and light petroleum giving 4:5-*dimethylbenziminazole*-1-β-*tetraacetyl*-D-*glucopyranoside* (1·5 g.) in colourless needles, m.p. 177·5° to 178·5°C. $(\alpha)_D^{23°C.}$ – 35·7° (in chloroform, c=1). Found: C, 57·8; H, 5·5; N, 6·1. $C_{23}H_{28}O_9N_2$ requires C, 58·0; H, 5·8; N, 5·9 per cent.

(ii) 4:5-Dimethylbenziminazole silver (3·5 g.) and α-acetobromglucose (6·1 g.) were condensed in boiling xylene solution and the product isolated in the usual way. The *picrate* (3·5 g.) which crystallised from alcohol in yellow needles, m.p. 148° to 150°C. (decomp.) (Found: N, 9·8. $C_{23}H_{28}O_9N_2$, $C_6H_3O_7N_3$ requires N, 9·9 per cent.), gave a tetraacethylglucoside in slender needles from a mixture of benzene and light

petroleum, m.pt. 178° to 178·5°C. Found: C, 58·3; H, 6·1 per cent., identical with the compound prepared by the "orthoformate route" (i) (above).

4:5-*Dimethylbenziminazole*-1-β-D-*glucopyranoside.* Hydrolysis of the foregoing tetraacetate afforded 4:5-*dimethylbenziminazole*-1-β-D-*glucopyranoside hydrochloride,* prismatic needles from aqueous alcohol, m.pt. 216° to 217°C. (decomp.), $(\alpha)_D^{27°C.}$ +11·5° (c=1, in water). Found: C, 52·2; H, 6·3; N, 8·1. $C_{15}H_{20}O_5N_2$, HCl requires C, 52·2; H, 6·; N, 8·1 per cent. This compound consumed 2·06 moles of periodic acid per mole. The *picrate* formed needles from water, m.pt. 202° to 204°C. Found: C, 45·2; H, 4·6; $C_{15}H_{20}O_5N_3$, $C_6H_3O_7N_3$, H_2O requires C, 45·4; H, 4·6 per cent.

SUMMARY AND CONCLUSIONS

1. By heating *o*-phenylenediamine-tetraacetyl-D-glucoside with ethyl orthoformate, 2-ethoxymethylene-*o*-phenylenediamine tetraacetyl-D-glucoside has been obtained.

2. The foregoing ethyl *iso*formanilide has been converted into benziminazole-1-β-tetraacetyl-D-glucopyranoside by the action of hot dilute hydrochloric acid.

3. The constitution assigned to this compound has been confirmed by an alternative synthesis from benziminazole silver and α-acetobromglucose.

4. Benziminazole-1-β-D-glucopyranoside has been obtained by hydrolysis of the acetylated glucoside with 6N hydrochloric acid and the pyranoside character of the lactol ring confirmed by periodate titrations.

5. The foregoing novel synthetic methods have been applied to the preparation of 5-methyl-, 4:5-dimethyl-, and 5:6-dimethylbenziminazole glucosides.

6. In certain cases reaction between an *o*-phenylenediamine acetylglucoside and ethyl orthoformate has led directly to the formation of the benziminazole acetylglucoside.

7. The benziminazole glucopyranosides prepared in the course of this work showed unexpected stability towards hot 6N hydrochloric acid.

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THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

PART IV. BENZIMINAZOLE GLYCOSIDES

(3) THE PREPARATION OF SOME BENZIMINAZOLE PENTOSIDES

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THE present paper describes the extension of the new synthetic methods elaborated in Part IV (2) to the preparation of some benziminazole pentosides[1]. The work herein contained completes the studies initiated in Part IV (1) of this series by providing potential routes to the ribose derivatives of benziminazole[2]. The synthesis of the latter compounds formed the *raison d'etre* for this series of investigations. Their preparation bears on the chemistry of vitamin B_{12}, however, and will form the subject of a subsequent communication.

The preparation of benziminazole pentosides by the "orthoformate route" presented certain points of difference from the observations recorded in Part IV (2). Thus the intermediate ethyl *iso*formanilides proved, in all cases, to be compounds of low crystallising power which could only be isolated as gums. Their subsequent conversion into benziminazole pentosides was effected by heating with 0·1N hydrochloric acid, or, more conveniently, by treatment with alcoholic picric acid. In the latter case the picrate separated only after the solution had been boiled for some minutes. This result leads to the conclusion that the benziminazole was not originally present in the mixture but was formed from the *iso*formanilide by the cyclising action of the picric acid. Picric acid, it may be added, failed to effect ring closure with such compounds as 2-ethoxymethylene-*o*-phenylenediamine-tetraacetyl-D-glucoside (see Part IV (2)) for which hot dilute hydrochloric acid is required.

o-Phenylenediamine-triacetyl-D-xyloside was converted by ethyl orthoformate into benziminazole-1β-triacetyl-D-xylopyranoside, the constitution of which followed from its alternative preparation employing benziminazole silver and α-acetobromxylose. Hydrolysis furnished benziminazole-1β-D-xylopyranoside, the pyranose character of the lactol ring being established by periodate titration. 4-Methyl-*o*-phenylenediamine triacetyl-D-xyloside behaved in a similar way, but the resulting 5-methylbenziminazole-1β-triacetyl-D-xylopyranoside could not, unfortunately, be prepared by the silver salt route. 5 : 6-Dimethyl-benziminazole-triacetyl-D-xyloside, in contrast, could not be prepared by either synthetic method, a failure no doubt due to an inability to form a picrate (cf. the corresponding glucoside), thereby rendering isolation extremely difficult.

Attempts to prepare the benziminazole-L-arabinosides by the orthoformate route proved only partly successful. Reaction of *o*-phenylenediamine-triacetyl-L-arabinoside and its 4-methyl derivative with ethyl orthoformate, with or without subsequent treatment with 0·1N hydrochloric acid, invariably gave intractable gums from which a crystalline product could not be isolated. When 4 : 5-dimethyl-*o*-phenylenediamine-

triacetyl-L-arabinoside was employed, however, reaction occurred to give 5 : 6-dimethyl-l-α-triacetyl-L-arabopyranoside, although in somewhat poor yield. The constitution assigned to this compound followed from its facile preparation from 5 : 6-dimethylbenziminazole silver and β-acetobrom-L-arabinose. The silver salt method likewise gave excellent yields of benziminazole-l-α-triacetyl-D-arabopyranoside (from β-acetobrom-D-arabinose), benziminazole-l-α-triacetyl-L-arabopyranoside (from β-acetobrom-L-arabinose) and 5-(or 6)-methylbenziminazole-l-α-L-triacetyl-arabopyranoside. The latter result is particularly interesting as previous attempts to employ 5-(6)-methylbenziminazole silver had invariably given mixtures of isomers which had proved incapable of separation. Hydrolysis with 6N hydrochloric acid furnished the corresponding benziminazole arabopyranosides.

5 : 6-Dimethylbenziminazole-l-triacetyl-L-rhamnoside was prepared in excellent yield by the orthoformate route and was smoothly de-acetylated to give 5 : 6-dimethylbenziminazole-l-L-rhamnopyranoside, the structure of which was confirmed by periodate titration.

The foregoing pentosides resembled the benziminazole glucosides in their remarkable stability to acids being recovered apparently unchanged after twelve hours' heating with 6N hydrochloric acid at 100°C. The bearing of these results on the structure of the "β-component" from vitamin B_{12} will be discussed in a subsequent communication.

The above observations, together with those recorded in Part IV (2) provide a basis for discussing the mechanism of the "orthoformate route." The first stage in the reaction between an *o*-phenylenediamine glycoside and ethyl orthoformate is assumed to be the formation of a 2-ethoxy-methylene-*o*-phenylenediamine glycoside (I). Reaction of the latter compound with hydrogen ions, supplied by picric acid or, where necessary, by hydrochloric acid, leads to formation of the benziminazole glycoside (II) with concomitant elimination of ethylalcohol. The *iso*-formanilide derived from 3 : 4-dimethyl-*o*-phenylenediamine-tetraacetyl-D-glucoside forms an exception, however, in passing spontaneously into the benziminazole glycoside on heating at 100° in excess orthoformate employed as a solvent.

Consideration of these facts leads to the reaction scheme which is presented in simplified form on page 514.

The facility with which the change (I) → (II) may be effected is thus directly related to the basicity of the glycosidic nitrogen atom[3] which, in turn, is influenced by the following factors :

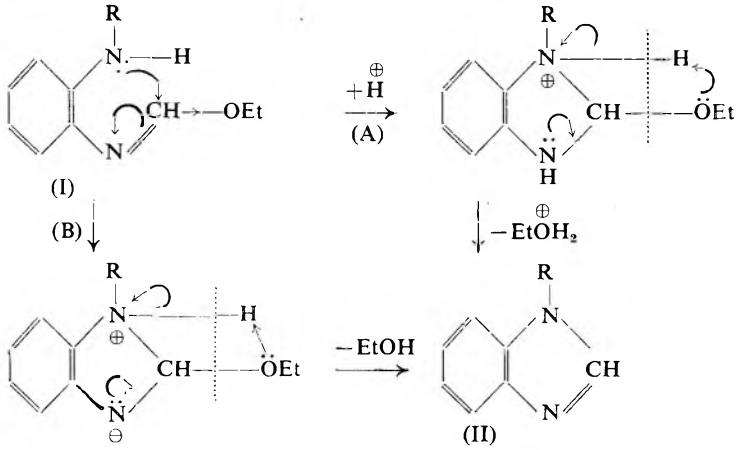
(i) The presence of substituents in the aryl nucleus of (I)

(ii) The presence of hydrogen ions which provide the driving force for reaction-scheme (A)

(iii) The possibility of chelation between an acetyl grouping of the sugar residue and glycosidic hydrogen which, as already pointed out by Brownlie, Sutherland and Todd[4], would have the effect of inducing a negative charge on the glycosidic nitrogen atom thereby increasing its basicity

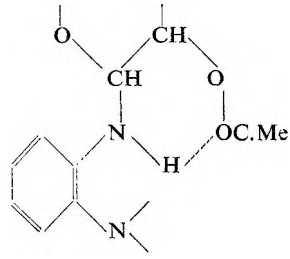
(iv) The nature of the sugar residue

(v) The α β-character of the glycosidic linkage.



(R = Hexose or Pentose Residue)

In so far as the present evidence allows, however, it seems clear that the inductive effect exerted by the sugar residue on the free electron pair of the glycosidic nitrogen represents the determining factor in the change



(I) → (II). The inductive effect, of course, will vary with the sugar residue, but its qualitative evaluation, unfortunately, is impossible at this juncture.

Experimental

Melting points are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford.

Benziminazole-1β-triacetyl-D-xylopyranoside. (i) *o*-Nitroaniline-triacetyl-D-xylopyranoside (1·4 g.) was reduced in warm ethyl acetate solution by shaking with hydrogen in the presence of 10 per cent. palladised charcoal. When hydrogen uptake was complete, the catalyst was filtered and ethyl orthoformate (5 ml.) added to the filtrate, which was then slowly evaporated on a steam bath for 3 hours. The remaining solvents were removed under reduced pressure and the residue heated with 0·1N hydrochloric acid (8 ml.) at 100°C. for 10 minutes. After basification with potassium bicarbonate, the product was extracted with chloroform and converted into the picrate (yield 800 mg.). *Benziminazole-1β-triacetyl-D-xylopyranoside picrate* separated from alcohol in flat yellow needles, m.pt. 192° to 193°C. Found : C, 47·8 ; H, 4·0 ; N, 11·4.

$C_{18}H_{20}O_7N_2$, $C_6H_3O_7N_3$ requires C, 47·6; H, 3·8; N, 11·6 per cent. Alternatively, the gum remaining after evaporation of the ethyl orthoformate was treated with alcoholic picric acid under reflux, when the picrate separated in slightly increased yield.

Percolation of a chloroform solution of the picrate through a column of alumina afforded the *triacetyl*-D-*xylopyranoside*, which separated from a mixture of chloroform and light petroleum in colourless cubes, m.p. 163°C. $[\alpha]_D^{25°C.}$ −44·8° (in chloroform, c=1). Found: C, 58·0; H, 5·2; N, 7·6. $C_{18}H_{20}O_7N_2$ requires C, 57·5; H, 5·3; N, 7·5 per cent.

(ii) A suspension of benziminazole silver (2·4 g.) in xylene (120 ml.) was dried azeotropically by distillation of one half of the solvent. α-Acetobromxylose (3·7 g.) was added and the mixture heated under reflux for 4 hours, whereafter the silver bromide was filtered, washed with xylene, and the filtrate and washings taken to dryness under reduced pressure. The crude xyloside was purified by passing an ethyl acetate solution through alumina, and was then isolated as the picrate, which separated from alcohol in yellow needles, m.p. 193°C. Found: C, 46·8; H, 3·8 per cent. This compound showed no depression in m.p. when mixed with a sample prepared by method (i).

Benziminazole-1β-D-*xylopyranoside*. The foregoing triacetate (700 mg.) and 6N hydrochloric acid (20 ml.) were heated at 100°C. for 2 hours. The solution was evaported to dryness, and the residue recrystallised from aqueous acetone. *Benziminazole*-1β-D-*xylopyranoside hydrochloride monohydrate* formed soft, colourless needles, m.p. 148° to 150°C., $[\alpha]_D^{25°C.}$ −25·4° (in water, c=1). Found: C, 47·4; H, 5·5; N, 9·4; Cl, 12·3. $C_{12}H_{14}O_4N_2$, HCl, H_2O requires C, 47·3; H, 5·6; N, 9·2; Cl, 11·7 per cent.

An aqueous solution of this compound was percolated through a column of Amberlite IR-4B, and the *xylopyranoside* recovered by evaporation to dryness. It crystallised from a mixture of benzene and alcohol in platelets, m.p. 237° to 238°, $[\alpha]_D^{21°C.}$ −89·1 (c=1, in pyridine). Found: C, 57·5; H, 5·6; N, 11·2. $C_{12}H_{14}O_4N_2$ requires C, 57·6; H, 5·6; N, 11·2 per cent. The pyranoside structure of this compound was confirmed by oxidation with periodic acid, when 2·05 moles were consumed.

5-*Methylbenziminazole*-1β-*triacetyl*-D-*xylopyranoside*. 4-Methyl-*o*-phenylenediamine-triacetyl-D-xylopyranoside (prepared by reduction of 2 g. of the corresponding nitro-compound I or II), ethyl acetate (50 ml.), and ethyl orthoformate (8 ml.), were heated on the steam bath for 3 hours. The solvents were removed *in vacuo* and the residue treated with alcoholic picric acid. The *picrate* (1·1 g.) crystallised from alcohol in fine yellow needles, m.p. 201°C. Found: C, 48·5; H, 3·5; N, 11·1. $C_{19}H_{22}O_7N_2$, $C_6H_3O_7N_3$ requires C, 48·5; H, 4·0; N, 11·3 per cent. Decomposition of this picrate on alumina afforded 5-*methylbenziminazole*-1α-*triacetyl*-D-*xylopyranoside*, colourless needles, m.p. 183°C. $[\alpha]_D^{23°C.}$ −67·0° (c=1, in chloroform). Found: C, 58·5; H, 5·6; N, 6·9. $C_{19}H_{22}O_7N_2$ requires C, 58·5; H, 5·6; N, 7·2 per cent.

5-*Methylbenziminazole*-1β-D-*xylopyranoside*. Deacetylation of the

foregoing compound (500 mg.) with 6N hydrochloric acid (12 ml.) at 100°C. followed by evaporation to dryness and passage of an aqueous solution of the resulting hydrochloride through an ion exchange resin, gave 5-*methylbenziminazole*-1β-D-*xylopyranoside*, fine felted needles from alcohol, m.p. 215° to 216°C. $[\alpha]_D^{20°C.}$ −50·7 (c=1, in water). Found: N, 10·4, $C_{13}H_{16}O_4N_2$ requires N, 10·4 per cent. Accurate analytical figures for carbon and hydrogen could not be obtained.

The lactol ring structure of this compound was proved to be of the pyranoside type by oxidation with periodic acid, when 2·09 moles were consumed.

Benziminazole-1α-*triacetyl*-L-*arabopyranoside*. Benziminazole silver (2·0 g.) was added to boiling xylene (70 ml.) and after distillation of 30 ml. of the solvent to remove traces of moisture, β-acetobrom-arabinose (3 g.) was added. The mixture was heated in an oil bath at reflux temperature for 4 hours, filtered from silver bromide, and the filtrate and washings taken to dryness *in vacuo*. The residue was dissolved in alcohol under reflux and picric acid (2 g.) added, when *benziminazole*-1-α-*triacetyl*-L-*arabopyranoside picrate* (2·2 g.) was obtained, yellow needles from alcohol, m.p. 182° to 185°C. Found: C, 47·2; H, 3·5; N, 11·1. $C_{18}H_{20}O_7N_2$, $C_6H_3O_7N_3$ requires C, 47·6; H, 3·8; N, 11·6 per cent. Filtration of a chloroform solution of this compound through a bed of alumina gave the *triacetyl*-L-*arabopyranoside*, which crystallised from a mixture of benzene and light petroleum in prismatic needles, m.p. 165°C. $[\alpha]_D^{25°C.}$ −3·7° (c=1, in chloroform). Found: C, 57·4; H, 5·2; N, 6·9. $C_{18}H_{20}O_7N_2$ requires C, 57·4; H, 5·4; N, 7·4 per cent.

Benziminazole-1α-L-*arabopyranoside* was prepared by deacetylation of the foregoing triacetate and removal of the hydrochloric acid in the usual way. It separated from alcohol in prismatic needles, m.p. 225°C. $[\alpha]_D^{20°C.}$ +22·6 (c=1 in water). Found: C, 57·9; H, 5·7; N, 11·1. $C_{12}H_{14}O_4N_2$ requires C, 57·6; H, 5·6; N, 11·2 per cent. This pyranoside consumed 1·93 moles of periodic acid.

Benziminazole-1α-D-*arabopyranoside* was prepared by an identical sequence of reactions starting from β-acetobrom-D-arabinose. It crystallised in colourless needles from a mixture of alcohol and light petroleum, m.p. 226°C. $[\alpha]_D^{15°C.}$ −24·9° (Found: C, 57·4; H, 5·8; N, 11·3 per cent.), and consumed 2·05 moles of periodic acid. The *triacetate* separated in colourless needles from a mixture of ethyl acetate and light petroleum, m.p. 165°C. $[\alpha]_D^{24°C.}$ +7·1° (Found: C, 57·7; H, 5·6; N, 7·5 per cent.), and was characterised as the *picrate*, yellow needles from a mixture of alcohol and β-ethoxyethanol, m.p. 181° to 183°C. Found: C, 47·2; H, 3·7; N, 12·3 per cent.

5 (or 6)-*Methylbenziminazole*-1α-L-*arabopyranoside*. 5 (or 6)-*Methylbenziminazole*-1α-*triacetyl*-L-*arabopyranoside*, prismatic needles from ethyl acetate/light petroleum, m.p. 181° to 183°, $[\alpha]_D^{24°C.}$ −15·7° (c=1, in chloroform) (Found: C, 58·2; H, 5·8 $C_{19}H_{22}O_7N_2$ requires C, 58·5; H, 5·7 per cent.) was prepared in 20 per cent. yield by reacting 5-methylbenziminazole silver (1·7 g.) with β-acetbromo-L-arabinose (2·7 g.) in xylene solution, and was characterised as the *picrate*, which formed yellow needles from β-ethoxyethanol, m.p. 217° to 218°C.

(decomp.). Found: C, 48·9; H, 4·1; N, 11·0. $C_{19}H_{22}O_7N_2$, $C_6H_3O_7N_3$ requires C, 48·5; H, 4·1; N, 11·3 per cent. Hydrolysis of the triacetate with 6N hydrochloric acid etc. gave 5 (or 6)-*methyl-benziminazole*-1α-L-*arabopyranoside*, white needles from a mixture of alcohol and light petroleum, m.p. 229°C $[\alpha]_D^{22°C.}$ +14·8°. Found: C, 59·7; H, 6·3. $C_{13}H_{16}O_4N_2$ requires C, 59·1; H, 6·1 per cent. This compound consumed 2·06 moles of periodic acid.

5:6-*Dimethylbenziminazole*-1α-*triacetyl*-L-*arabopyranoside*. (i) 5-Nitro-*o*-4-xylidine-triacetyl-L-arabinoside (series I or II, 3 g.) was reduced in the usual way and the solution, freed from catalyst, slowly evaporated with ethyl orthoformate (6 ml.) for 4 hours. The residue, after evaporation to dryness, was heated at 100°C. with 0·05N hydrochloric acid (15 ml.). After neutralisation, the product was isolated with chloroform and converted into the picrate (1·0 g.). 5:6-*Dimethyl-benziminazole*-1α-*triacetyl*-L-*arabopyranoside picrate* crystallised from a mixture of β-ethoxyethanol and alcohol in prismatic yellow needles, m.p. 234° to 236°C. (decomp.). Found: C, 49·0; H, 4·2; N, 10·9. $C_{20}H_{24}O_7N_2$, $C_6H_3O_7N_3$ requires C, 49·3; H, 4·3; N, 11·0 per cent.). The free *base* formed colourless needles from a mixture of benzene and light petroleum, m.p. 141° to 143°C. $[\alpha]_D^{25°C.}$ −31·9° (in chloroform). Found: C, 59·3; H, 6·0; N, 6·3. $C_{20}H_{24}O_7N_2$ requires C, 59·4; H, 6·0; N, 6·9 per cent.

(ii) 5:6-Dimethylbenziminazole silver (2·4 g.), β-acetobrom-L-arabinose (3·3 g.) and xylene (35 ml.) were heated under reflux in the usual way. The product was isolated as the picrate (2·2 g.), obtained in yellow needles from β-ethoxyethanol, m.p. 234° to 236°C. (decomp.). Found: C, 49·3; H, 4·2 per cent. $C_{20}H_{24}O_7N_2$, $C_6H_3O_7N_3$ requires C, 49·3; H, 4·3 per cent., not depressed in admixture with the compound prepared by the "ethyl orthoformate route." Regeneration of the base gave 5:6-dimethylbenziminazole-1α-triacetyl-L-arabopyranoside, m.p. 142° to 143°C.

5:6-*Dimethylbenziminazole*-1α-L-*arabopyranoside* crystallised from alcohol/light petroleum in rosettes of white needles, m.p. 280° to 281°C. (decomp.) $[\alpha]_D^{23°C.}$ −70·5° (c=1, in pyridine). Found: C, 60·0; H, 6·8; N, 10·3. $C_{14}H_{18}O_4N_2$ requires C, 60·4; H, 6·5; N, 10·1 per cent. It consumed 2·15 moles of periodic acid. The picrate formed rosettes of yellow needles from alcohol, m.p. 216° to 217°C. (decomp.).

5:6-*Dimethylbenziminazole*-1-*triacetyl*-L-*rhamnopyranoside*. 4:5-Dimethyl:*o*-phenylenediamine-triacetyl-L-rhamnoside (prepared by reduction of 5 g. of nitro-compound) in ethyl acetate (70 ml.) was heated on the steam bath with ethyl orthoformate (7 ml.) for 5 hours. Following treatment with 0·05N hydrochloric acid, the product was isolated as the *picrate* (3·3 g.), which crystallised from aqueous alcohol in slender yellow needles, m.p. 184°C. Found: C, 50·3; H, 4·8; N, 11·0. $C_{21}H_{26}O_7N_2$, $C_6H_3O_7N_3$ requires C, 50·1; H, 4·5; N, 10·8 per cent. 5:6-*Dimethyl-benziminazole*-1-*triacetyl*-L-*rhamnopyranoside* formed small white needles from light petroleum, m.p. 92° to 95°. Found: C, 59·8; H, 6·3. $C_{21}H_{26}O_7N_2$ requires C, 60·3; H, 6·3 per cent.

5:6-*Dimethylbenziminazole*-1-L-*rhamnopyranoside* crystallised in wisps

from alcohol/light petroleum, m.pt. 252°C. Found : C, 61·5 ; H, 6·9 ; N, 9·3. $C_{15}H_{20}O_4N_2$ requires C, 61·6 ; H, 6·9 ; N, 9·6 per cent. The pyranoside structure followed from periodate oxidation, when 2·2 moles of the oxidant were consumed.

Summary and Conclusions

1. New methods elaborated in Part IV (2) for the synthesis of benziminazole glucosides have been extended by the preparation of some benziminazole pentosides.

2. Results obtained employing the "orthoformate route" show that ethoxymethylene-*o*-phenylenediamine acetylpentosides undergo more facile ring closure than the corresponding glucosides, being readily converted into the benziminazole pentosides by the action of alcoholic picric acid.

3. The pyranoside structures assigned to the foregoing compounds have been confirmed by periodate titrations.

4. Benziminazole pentosides show unexpected stability to acids and are recovered unchanged after heating for 12 hours with 6N hydrochloric acid at 100°C.

The authors thank the Directors of The British Drug Houses Ltd. for permission to publish these results.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Adrenaline, Determination of, in Mixtures. C. O. Björling and H. Hellberg. (*Farm. Revy*, 1950, **49**, 69.) The method described below has been tested for adrenaline in admixture with ephedrine, nupercaine, procaine, tetracaine or xylocaine. 1 to 10 ml. of a solution, containing 10 to 50 μg. of adrenaline hydrochloride, is treated with 0·1 g. of trisodium citrate and passed through a column of alumina in a tube of 5 mm. diameter. The column is washed with 3 ml. of water, then with 10 ml. of alcohol, and finally with 5 ml. of water. This eluate contains the alkaloid or other base. The adrenaline is then recovered by washing the column with 10 ml. of 0·1N hydrochloric acid and 3 to 4 ml. of water. The adrenaline is determined fluorimetrically, with rivanol as standard, using the method of Ehrlens. Although the figures given show that the results are up to 50 per cent. too high, this appears to be due to the limitations of the fluorimetric method, the recovery of adrenaline being apparently quantitative. G. M.

Chlorine and Bromine, Microdetermination of. J. Grodsky. (*Anal. Chem.*, 1949, **21**, 1551.) A method is described for determining halogens in organic compounds by fusing with potassium and titrating the resulting potassium halide with silver nitrate using dichlorofluorescein as indicator; the method can be used for all types of compounds, including volatile liquids. A liquid sample (4 to 20 mg.) is weighed in a weighing capillary, the handle of which is a glass rod, 20 to 25 mm. long, and the capillary end is placed down in the reaction tube: a solid sample is weighed with a long handled weighing stick. About three shavings of potassium each about 1 cu. mm., are cut under ether, dried rapidly with clean tissue, and added to the microsample, using 2 to 5 times as much for semi-microsamples or in the presence of nitrogen or sulphur. The tube is heated at a point about 3 cm. below the open end then drawn to a thick walled capillary and when cool is evacuated and sealed off. The tube is shaken to break the inner capillary containing the liquid sample and is placed for 15 minutes in a furnace preheated to 400°C. When the tube is cool, it is washed, opened by cutting just below the shoulder and 1 ml. of ethyl alcohol is added to destroy excess of potassium and to wash the sides. To carry out the argentimetric titration the solution is transferred through a sintered-glass funnel into a flask containing 5 ml. of 0·01N potassium chloride. The reaction tube is washed four times with hot water containing a drop of nitric acid and the solution in the flask is brought to the boil at a low heat. About 5 mg. of barium carbonate is added to the hot solution, it is acidified if not already acid, and boiling is continued for at least 2 hours with replacement of evaporated water to assure quantitative removal of the hydrogen sulphide; excess of barium carbonate is then carefully added until an undissolved residue of about 50 mg. remains; 1 ml. of dichlorofluorescein indicator and 10 ml. of acetone are added to the cooled solution which is then titrated with 0·01N silver nitrate. R. E. S.

Morphine in Opium, Determination of. J. A. C. van Pinxteren and M. A. G. Smeets. (*Pharm. Weekbl.*, 1950, **85**, 1, 48.) The sources of error of the lime method were investigated. The amount of morphine remaining in the mother liquor was determined by extraction with benzene-butyl alcohol at *p*H 9, and determination as nitrosomorphine. The results showed that the quantity varied considerably with different methods, but was small under favourable circumstances. On the other hand, some abnormal types of opium gave considerably higher figures, so that it is not possible to apply a correction for this error. The amount of calcium carbonate precipitated with the morphine was determined after ashing of the precipitate. This also can lead to an appreciable error, although in those methods in which the morphine is purified before crystallisation it is small. Co-precipitation of other alkaloids may be followed by determining the methoxyl value of the recovered morphine. In the method of Mannich (determination as chlorodinitrophenyl ether) the purity of the product obtained, checked by a determination of methoxy and nitro groups in it, was found to be unsatisfactory. It appears, however, that better results are obtained by this method if the purification of the extract is better. Since Rusting's method gives the purest morphine, although it allows of a considerable loss in the mother liquors, a new combination of the last two methods was developed. 1 g. of morphine is rubbed down with 1 ml. of water, then mixed with 5 ml. of 5 per cent. solution of manganous chloride and 0·5 g. of calcium hydroxide. The mixture is filtered through a crucible 3G3 into a tared flask, being washed through with, in all, 15 ml. of water. During the filtration the residue must not be allowed to become quite dry. The filtrate is treated with 4 ml. of a solution of 20 g. of potassium oxalate and 10 ml. of N potassium hydroxide in 100 ml., and heated for 15 minutes on the water-bath. After cooling, the mixture is filtered on a porcelain filter crucible A_2, the residue on the filter being stirred to speed up filtration. After washing three times with 2 ml. portions of water, the filtrate is made up to 40 g. To this is added 250mg. of 4-chlor-1 : 3-dinitrobenzene dissolved in 30 ml. of acetone. After standing for 3 hours, with occasional shaking, the mixture is filtered through a weighed 3G3 crucible and the crystals are quantitatively transferred to the crucible, washed quickly with 3 × 2 ml. of acetone, then, with 2 × 2 ml. of water, dried at 70° to 80°C., and weighed. The factor 0·632 is used to convert the weight of chlorodinitrophenyl ether to morphine. A method of titrating the ether is also given. G. M.

Pyranisamine Maleate, Spectrophotometric Assay of. L. T. Anderson, W. C. Gakenheimer, C. Rosenblum and E. H. Smith. (*J. Amer. pharm. Ass. Sci. Ed.*, 1949, **38**, 373.) Pyranisamine maleate is obtained by neutralising 1 molecule of the free base *N*-(α-pyridyl)-*N*-(*p*-methoxybenzyl)-*N'*, *N'*-dimethylethylene-diamine with 1 molecule of maleic acid. As a substituted derivative of ethylenediamine, pyranisamine exhibits an ultraviolet absorption spectrum sufficiently intense and characteristic to distinguish it from substances commonly present in pharmaceutical preparations. The spectrum was found to change with acidity and with solvent; in aqueous solution at *p*H values below 5·5, it consisted of 2 bands at 2400 to 2430Å., and 3075 to 3100Å., whereas above *p*H 6 a third band was present at 2215 to 2240Å, probably due to a shift to longer wave lengths of a band existing in acid solutions below 2100Å. The spectra in distilled water and in alcohol (20 per cent.) were practically identical with the curve at *p*H 5·5, but in alcohol-light petroleum solution, three band maxima were

found at 2475, 2850 and 3075 Å. The absorption band most suitable for analytical determination was that *ca.* 2435Å. since the extinction coefficient remained constant at *p*H 4 to 6·5. The same curves were found in alcohol (20 per cent.) and in distilled water (*p*H 5·5), indicating an insensitivity of the spectrum to the presence of alcohol and salts from the buffer mixtures. For all work in water or alcohol (20 per cent.), therefore, an all-over average $E_{1\text{ cm.}}^{1\text{ per cent.}}$ of 422 was employed in calculating concentrations of pyranisamine maleate. For solutions in 1 : 1 absolute alcohol-light petroleum, the 2475Å. band with an $E_{1\text{ cm.}}^{1\text{ per cent.}}$ of 477 was employed. Methods are given for the determination of pyranisamine maleate in tablets, in elixirs, in anhydrous water-soluble ointments, in anhydrous petrolatum ointments, in aqueous emulsion type ointments and in parenteral solutions. In general the preparation was simply diluted with a suitable solvent before reading the extinction at the specified wave length in a suitable spectrophotometer. In the case of the elixir and the anhydrous petrolatum ointment a previous extraction was performed. R. E. S.

Silver, Colorimetric Determination of Small Quantities of. G. Saini. (*Ann. Chim. applic., Roma,* 1950, **40,** 55.) The method is based on the catalytic action of silver ions in the oxidation of manganous ions to permanganic acid by persulphates. The substance containing the silver should be dissolved in nitric acid, sulphuric acid added, and the solution heated until white fumes are evolved, thus removing reducing substances. Phosphates must be removed by precipitation with magnesium in ammoniacal solution. The reagents required are 25 per cent. sulphuric acid, 4 per cent. solution of borax ($Na_2B_4O_7$, $10H_2O$), solid potassium persulphate, 4 per cent. solution of manganous sulphate ($MnSO_4$, H_2O). The author used a Lange colorimeter with a green filter and 100 ml. containers. The solution of silver nitrate used for standardising contains 1·8 μg. of silver per ml. Run the silver solution into a 150 ml. beaker, add 12 ml. of manganous sulphate solution, 6 ml. of diluted sulphuric acid and 10 ml. of borax solution. Heat slowly to 90°C., adding gradually 2 g. of potassium persulphate and boil gently for 5 minutes (not longer). Filter off the precipitated manganese meta-hydroxide through a porous porcelain filter, wash with a little water and make up the filtrate to 150 ml. The water used for washing and making up should be redistilled or contain a trace of persulphate to remove reducing substances. The colour is then read in the colorimeter. The graph of the relation of colour to amount of silver is not a straight line. If 15 ml. of borax solution is used instead of 10 ml., the graph is straight but of lower values. Colourless cations do not interfere, but with coloured cations the standards should contain the same amount of them as is present in the solution to be tested. The method can be used for quantities between 12 μg. and 120 μg. per 100 ml. H. D.

Soft Paraffin, Peroxide in. M. J. Golden. (*J. Amer. pharm. Ass., Sci. Ed.,* 1949, **38,** 301.) A reagent is prepared by dissolving 2 g. of ferrous sulphate in 100 ml. of distilled water and 2 ml. of sulphuric acid, adding 200 ml. of acetone and 100 ml. of 2 per cent. aqueous ammonium thiocyanate, and heating under a reflux condenser with 0·2 g. of iron powder and 6 in. of No. 30 iron wire until the solution is colourless. Samples of soft paraffin are heated at 250°F. 2 ml. quantities are removed at hourly intervals, diluted with mineral oil at 250°F, cooled to 170°F, the reagent added under carbon dioxide, shaken and observed. The peroxide test time is taken as the time of heating of the first 2 ml. quantity which produces a darker pink to red

colour in the above test. A minimum peroxide test time of 2 hours is suggested for soft paraffin for pharmaceutical uses, or for cosmetics. The test can be made quantitative by measuring the transmission of the lower layer with a photoelectric colorimeter and a filter having a mean transmission of 560 mμ, and using heated mineral oil in a blank test. The colorimeter may be calibrated by using mixtures of reagent and hydrogen peroxide, the results being expressed in terms of peroxide, calculated as hydrogen peroxide.

G. B.

Strychnine and Brucine, Chromatographic Separation of. K. B. Jensen and A. B. Svendson. (*Pharm. Acta Helvet.*, 1950, **25**, 31.) Although direct partition of brucine and strychnine, from ether-water, should produce separation of the two alkaloids, the method is not suitable for practical application on account of the speed with which the compounds would pass through the column. The rate of passage can however be reduced by using a buffer solution. Conditions found suitable for separation of the alkaloids were as follows: the supporting phase consisted of 10 g. of kieselguhr, the immobile phase of 3 ml. of 0·2M. phosphate buffer of *p*H 7. The rate of elution was 10 ml. in 2 to 3 minutes. For the assay of nux vomica, the alkaloids were extracted from 30 g. of the drug with 200 g. of ether and 100 g. of chloroform, after the addition of 25 ml. of 10 per cent. solution of sodium carbonate. After shaking for 30 minutes, 50 ml. of water was added, and the ether-chloroform mixture was filtered. The solvent was distilled off, and the residue dissolved in chloroform and mixed with an equal volume of ether to a total volume of 50 ml. The solution was then passed through the column, and eluted with ether. The strychnine in the solution was determined by titration, while the brucine was recovered by elution with chloroform.

G. M.

Sulphates, Insoluble Inorganic, Microscopic Identification of. G. Denigès. (*Bull. Trav. Soc. Pharm., Bordeaux*, 1949, **87**, 101.) To distinguish the sulphates of calcium, barium, strontium and lead, a little of the finely powdered material is placed on a microscope slide with a drop of sulphuric acid, and warmed over a small flame, with stirring, until the acid commences to fume. After cooling, the preparation is covered with a cover glass and examined microscopically. The crystals observed are characteristic for the different sulphates.

G. M.

Sulphonamides, Identification of. C. J. de Wolff. (*Pharm. Weekbl.*, 1949, **84**, 717.) A table for the identification of the chief sulphonamides is based mainly on the following reactions: 1. Diazotisation sometimes gives a yellow colour, which may be so intense that it is easily seen in a 0·05 per cent. solution; 2. Ammonia, following diazotisation, gives a stronger yellow colour with many aromatic derivatives; 3. Insoluble compounds may be formed with formaldehyde; 4. Many sulphonamides react with Nessler's reagent. The complete scheme comprises the following compounds: albucid, cibazol, dagenan, elkosin, irgafen, irgamid, lucosil, marfanil, neo-uliron, percoccide, septazine, septosil soluble, soluseptazine, sulphadiazine, sulphaguanidine, sulphamethazine, sulphanilamide, sulphanilic acid, sulphaphthalidine, sulphasuxidine, uliron, ultraseptyl.

G. M.

Sulphonamides, Scheme for Identification of. G. Schallengerg-Heertjes. (*Pharm. Weekbl.*, 1949, **84**, 765.) A preliminary classification is effected by adding 1 drop of a saturated solution of potassium bromate to 10 mg. of the substance dissolved in 1 ml. of 4 N sulphuric acid. The

following appearances are observed:—sulphanilamide, sulphaguanidine, sulphamethazine, violet colour, changing to brownish; sulphathiazole, ultraseptyl, violet colour, changing to dark colour and brown precipitate liquid yellow; uliron, violet colour, then turbidity, later brown precipitate; neo-uliron,

10 mg./0·5N hydrochloric acid	Dragendorff's reagent	Silicotungstic acid	Bouchardat's reagent	Gold chloride	Eder's reagent	Picric acid	Picrolonic acid
Sulphanilamide	—	—	—	—	+	—	—
Sulphaguanidine	—	+	—	—	+	+	+
Sulphamethazine	+	+	+	weak	+	+	—
Sulphathiazole	+	+	+	+	+	+	+
Ultraseptyl	+	+	+	+	+	+	+
Uliron (in 4N sulphuric acid) ...	+	+	+	+	+		
Neo-Uliron	+	+	+	+	+	+	+
Septazine	—	—	—	—	weak	—	—
Sulphadiazine	+	+	+	+	+	—	+
Percoccide	+	+	+	+	+	+	+

rose colour, becoming brownish; septazine, turbidity and blue-violet colour, becoming violet; sulphadiazine, percoccide, yellow colour, becoming reddish-brown to red, turbidity, later brown precipitate; sulphapyridine, elkosin, lucosil, albucid, irgamid sodium, irgafen, sulphasuccidine, yellow to brown colours only. Further reactions are carried out with a series of alkaloidal reagents, as in the table. Identification is confirmed by the microscopic appearance of the crystals formed with various reagents. Photographs of the characteristic crystals are given. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Polypeptide with High Adrenocorticotrophic Activity. P. Morris and C. J. O. R. Morris. (*Lancet*, 1950, **258**, 117.) By ultrafiltration of pituitary-gland extracts under suitable conditions it is possible to prepare a polypeptide mixture of high adrenocorticotrophic activity. The authors describe the isolation of an apparently single, homogeneous peptide from this mixture by ultrafiltration and iontophoresis. Evidence of homogeneity comes from paper partition chromatography and iontophoresis at *p*H 2·3, 4·0, 6·0 and 8·0. Comparison of the adrenocorticotrophic activity of this substance with that of the Armour standard adrenocorticotrophin preparation No. La-l-a by the adrenal ascorbic acid depletion method shows it to be 8·5 times as active. E. N. I.

Theobromine in Theobromine and Sodium Salicylate, Determination of. C. W. Bell. (*J. Amer. pharm. Ass. Sci. Ed.*, 1949, **38**, 391.) The literature concerning the methods available for this determination is reviewed and an adaptation of Boie's acidimetric method based on the fact that silver nitrate reacts with theobromine to form insoluble silver theobromine with the simultaneous quantitative liberation of nitric acid, which can then be titrated with standard alkali, is recommended. The actual method is as follows. Transfer about 1 g., accurately weighed and previously dried at 110°C. to constant weight, to a 500-ml. flask, add 75 ml. of distilled water and 40 ml. of approximately 0·1N sulphuric acid. Boil for 2 to 3 minutes, washing down the sides of the flask with three 15 ml. quantities of distilled water. Cool quickly to about 40°C. and add 1 ml. of phenol red indicator. Add a slight excess of 0·1N sodium hydroxide to make alkaline and then carefully adjust the *p*H of the solution by adding the minimum amount of

0·1N sulphuric acid to produce a lemon-yellow colour. Add 35 ml. of 0·1N silver nitrate and titrate the liberated nitric acid slowly with 0·1N sodium hydroxide, to the first sign of a bluish red colour, the end-point being approached drop by drop. Each ml. of 0·1N sodium hydroxide is equivalent to 18·017 mg. of theobromine. Ten commercial samples were analysed by the proposed method, by the National Formulary VIII method, and by a modified total nitrogen (Kjeldahl) determination. The present N.F.VIII method gave high results while the acidimetric method gave results which were in close agreement with those calculated from the nitrogen content. It is contended that the acidimetric method is better than the present N.F.VIII method as regards ease of operation, saving of time, and accuracy and precision. R. E. S.

Terramycin, a new Antibiotic. A. C. Finlay. (*Science,* 1949, **111,** 85.) Terramycin is the name given to a crystalline antibiotic obtained from broth cultures of *Streptomyces rimosus*, isolated from soil. Terramycin is amphoteric and forms the crystalline hydrochloride and sodium salt. It has the following properties: m.pt. 185°C. (approx.) with decomposition, $[\alpha]_D^{25°C.}$ – 196° (1·0 per cent. in 0·1N hydrochloric acid), soluble in methyl alcohol, ethyl alcohol, acetone and propylene glycol, and in water to the extent of 0·25 mg./ml. at 25°C.; insoluble in ether and light petroleum. It is stable over long periods in aqueous solutions at about *p*H 2 to 5 at room temperature. It crystallises in several forms, one of which consists of thick hexagonal plates the refractive indices of which are $\alpha = 1{\cdot}636 \pm 0{\cdot}004$, $\beta = 1{\cdot}644 \pm 0{\cdot}004$, $\gamma = > 1{\cdot}700$. In 0·1M phosphate buffer (*p*H 4·5) it shows ultra-violet absorption maxima at approx. 247, 275 and 353 mμ; it also shows characteristic absorption in the infra-red region. It has a low degree of toxicity in animals and shows marked activity *in vitro* against a wide variety of organisms. It displays marked chemotherapeutic activity against experimental infections in mice due to *Streptococcus hæmolyticus, Diplococcus pneumoniæ, Klebsiella pneumoniæ, Salmonella typhosa,* and other organisms, and appears to have definite antirickettsial activity in the chick embryo, and, in high concentrations, to inhibit the infection of the chick embryo, with the PR8 strain of influenza A virus. S. L. W.

Vitamin B_{12}: Distribution in Natural Materials. U. J. Lewis, U. D. Register, H. T. Thompson and C. A. Elvehjem. (*Proc. Soc. exp. Biol., N.Y.,* 1949, **72,** 479.) The determinations were made by a rat assay process based on the fact that administration of thyroid-active substances increases the requirement of vitamin B_{12}. Figures are given for more than 30 materials. Two samples of yeast autolysate were found to be completely inactive. A sample of dried "slops" from streptomycin production contained at least 22 μg./100 g. Commercial condensed fish solubles contained more than 40 μg./100 g. Beef liver and beef kidney both contained more than 50 μg./100 g. of dry substance. Desiccated sheep rumen contents contained about as much as beef liver and kidney, suggesting the possibility of synthesis of the vitamin within the rumen. Plant materials showed no measurable activity. H. T. B.

BIOCHEMICAL ANALYSIS

Citric Acid, Micro-estimation of. H. W. Malherbe and A. D. Bone. (*Biochem. J.,* 1949, **45,** 377.) A method is given for the micro-estimation of citric acid by its conversion into pentabromoacetone. Vanadic acid is used

to replace the normal potassium permanganate oxidation making the method more adaptable and more specific. For quantities of 0·1 to 1·0 mg. of citric acid the solution (5 ml., containing not more than 1 mg. of citric acid) is mixed with 27N sulphuric acid (5 ml.) in a test tube and cooled to room temperature, saturated bromine water (2 ml.) and a 2 per cent. ammonium vanadate solution (3 ml.) being then added. The contents are well mixed, the stoppered tube is left in a water bath at 50°C. for 20 minutes, the tube is again cooled to room temperature and excess of bromine is removed by adding 10 to 12 drops of a 5 per cent. sodium thiosulphate solution. The solution is extracted with 6 ml. of light petroleum, the aqueous layer is blown out with the aid of a wash bottle head, a small amount of anhydrous sodium sulphate is added and the light petroleum extract is decanted off. A 5 ml. portion of the extract is shaken for 1 minute with 10 ml. of sodium sulphide solution (2 per cent.), the coloured aqueous layer is blown on to a funnel fitted with a dry filter-paper, and the absorption of the clear filtrate is measured in a suitable spectrophotometer using sodium sulphide solution as a solvent blank and determining a calibration curve in the usual way. For quantities of 0·02 to 0·2 mg. of citric acid the same procedure is followed except that the volume of the sodium sulphide solution extract is reduced. The calibration graph was a straight line and the standard deviation of an estimation was below 2 per cent.; a large number of substances of similar constitution did not interfere. For cerebro-spinal fluid (range 40 to 80μg./ml.) 1 volume is mixed with 30 per cent. trichloroacetic acid (0·1 vol.), and 1 to 3 ml. of the filtrate is analysed directly without further pre-treatment; for serum or plasma the protein is removed by treatment with 10 per cent. trichloroacetic acid solution; for normal urine no preliminary treatment is necessary and 1 to 5 ml. quantities diluted fivefold give satisfactory readings although protein, if present, must be removed with trichloroacetic acid. Recoveries of added citric acid were quantitative within experimental error. R. E. S.

Glucose, Microdetermination of. J. T. Park and M. J. Johnson. (*J. biol. Chem.*, 1949, **181**, 149.) A sensitive method for the estimation of glucose in quantities of 1 to 9 μg. is described, based on the ferricyanide reduction method. The sample, deproteinised and neutralised if necessary and containing 1 to 9 μg. of glucose, is placed in a test-tube and diluted to volume (1 to 3 ml. as desired); 1 ml. each of carbonate-cyanide solution (5·3 g. of sodium carbonate and 0·65 g. of potassium cyanide per l.) and of ferricyanide solution (0·5 g. of potassium ferricyanide per l.) is added and after mixing the tube is heated in a boiling water-bath for 15 minutes. 5 ml. of ferric iron solution (1·5 g. of ferric ammonium sulphate and 1 g. of Duponol in 1 l. of 0·05 N sulphuric acid) are mixed with the sample after cooling, and after 15 minutes the sample is then read against a reagent blank in a photoelectric colorimeter at 690 mμ., although any wave-length between 650 and 730 mμ. is suitable. Results are calculated after using a pure glucose sample as standard. In the final method adopted the quantities of reagents used were chosen to effect rapid reduction and colour development; without cyanide the reduction is only half completed in 15 minutes, but with 500 μg. or more of potassium cyanide it is complete in less than 15 minutes. The method is reproducible and the precision is such that over 90 per cent. of individual analyses of a known sample fall within 0·2 μg. of the expected result. R. E. S.

Hetrazan in Body Fluids, Estimation of. M. Lubran. (*Nature*, 1949, **164**, 1135.) In the estimation of hetrazan (1-dimethylcarbamyl-4-methyl

piperazine hydrochloride), blood serum or plasma, or other body fluid, is made strongly alkaline with one-fifth of its volume of 10N sodium hydroxide and extracted with twice to 3 times its volume of ethylene dichloride. The ethylene dichloride layer, after separation and filtration, is shaken for a few minutes with one-fifth of its volume (more may be required for urine) of 0·05 per cent. bromothymol blue solution in phosphate buffer at *p*H 7·0. The intensity of the yellow colour in the ethylene dichloride layer is compared with the colour produced by known amounts of hetrazan treated similarly. With low concentrations of hetrazan (1 μg./ml. or less) the ethylene dichloride is extracted with 2 ml. of N. sodium hydroxide and the blue colour compared with a standard, or alternatively a blue colour may be developed in the ethylene dichloride by addition of 0·1N alcoholic potash and sufficient ethyl alcohol to prevent turbidity. Blank values of the order of 1 μg./ml. are obtained with whole blood or with trichloracetic acid treated blood; urine shows a blank value of *ca.* 5 μg./ml. or occasionally, in concentrated urine up to 20 μg./ml. Although not specific for hetrazan the method is sufficiently sensitive to estimate 1 μg./ml. in blood. Results indicate that ingestion of 10 mg./kg. of body-weight of the hydrochloride causes a maximum plasma concentration of *ca.* 5 to 7 μg./ml. in 3 hours, the level falling slowly to zero in 24 hours, during which period about 20 per cent. of the drug is excreted in the urine. R. E. S

Paper Chromatography, Simplified Quantitative Procedures. R. J. Block. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **72,** 337.) Two procedures are described as applied to the determination of amino acids in protein hydrolysates. The first method is based on the finding that the concentration of a coloured substance can be determined with reasonable accuracy from a paper chromatogram by ascertaining the product of the coloured area and the greatest colour density. In the second method the determination is made simply from the maximum colour density obtained on a two dimensional chromatogram. In the application of the first method to the determination of histidine and tyrosine, the substances are separated on the strip by a mixture of *n*-butyl alcohol, 100 parts, and glacial acetic acid, 10 parts, saturated with water. After running the chromatogram in air-tight chambers for 3 hours, the paper is dried, sprayed with diazotised sulphanilamide in butyl alcohol solution, again dried for 5 minutes, and sprayed with saturated sodium carbonate solution. The maximum colour densities are determined by an electronic densitometer and the factors for converting the product, area × colour density, into mg. of the substances are ascertained by submitting weighed amounts of the amino acids to the same procedure. By the second method it is possible to determine 14 amino acids on approximately 0·3 mg. of hydrolysate of a protein with the usual amino acid pattern with an average error of less than 10 per cent. and only 5 hours working time. The hydrolysate is diluted so as to contain about 1·5 to 10·0 millimols of each amino acid per ml. The paper is "spotted" with 0·005 to 0·01 ml. and the constituents separated by two-dimensional chromatography. The first solvent used is water-saturated phenol in an atmosphere of 0·3 per cent. ammonia, with moistened sodium cyanide and coal gas. The sheets are dried overnight before an electric fan and then developed at right angles to the original direction with a mixture of 2:6-lutedine, 55 per cent. v/v, alcohol (95 per cent.), 20 per cent. v/v and water 25 per cent. v/v, in an atmosphere of diethylamine and moistened sodium cyanide. The completed chromatograms are dried and then sprayed with 0·1 to 0·2 per cent. ninhydrin solution and heated to develop the colour. To prepare the standard, mixtures containing

1·25, 2·5, 5·0 and 10·0 millimols/ml. of each amino acid except cystine, tryptophan, proline and hydroxyproline are chromatographed at least 25 times: from the chromatograms the average maximum colour density of *all* the spots is determined by means of the electronic densitometer (the " mean colour density "), and also the average maximum colour density of each amino acid. The latter divided by the former gives the " standard colour ratio " of the individual acids. " Experimental colour ratios " are then determined in the same way for each amino acid on the two dimensional chromatogram of the unknown solution, and these figures divided by the appropriate " standard colour ratios " give " corrected experimental colour ratios " which are proportional to the molar ratios of the amino acids on the chromatogram. If one amino acid can therefore be determined by some other method, or if any acid is known to be absent a weighed quantity can be added to serve as an internal standard, the quantities of each can be ascertained. Methionine, histidine and tyrosine can be determined by specific procedures and thus serve as standards. With 25 replicate chromatograms the average error for a mixture of 14 amino acids simulating β-lactoglobulin was −2 per cent. in one series and −4 per cent. in a second. The method is believed to be applicable to all substances giving colours in paper chromatography.

H. T. B.

Sodium in Biological Fluids, Determination of. G. C. H. Stone and J. W. Goldzieher. (*J. biol. Chem.*, 1949, **181,** (2), 511.) A modification of the previous method (*J. clin. Endocrinol.*, 1949, **9,** 95) based on the Rosenheim-Daehr reaction, in which the uranyl ion in strongly alkaline solution is treated with hydrogen peroxide to produce a complex of intensely reddish yellow colour, is given. The spectrophotometric characteristics of the uranium complex, the influence of temperature on the reaction, the selection of optimum wave-length for measurement, and the accuracy, reproducibility, and recovery obtained were investigated. In the final method 9·0 ml. of 10 per cent. trichloroacetic acid is added to 1·0 ml. of serum, drop by drop, the mixture being shaken and centrifuged. To 1·0 ml. of the protein-free filtrate in a 15 ml. graduated centrifuge tube 6·0 ml. of the uranyl zinc acetate reagent is added. After standing for 20 minutes the tube is centrifuged at high speed for 7 minutes, the supernatant liquid is decanted, and the tube allowed to drain for 1 minute. 5 ml. of the wash reagent is added, the contents of the tube are mixed, and again centrifuged for 7 minutes, decanted and drained. The precipitate is dissolved in a few drops of distilled water and 6 ml. of ammonium carbonate solution is added followed by 1 ml. of 30 per cent. hydrogen peroxide, the volume being adjusted to 15·0 ml. with distilled water. After mixing the solution is examined spectrophotometrically at 460 mμ. as, although the peak absorption occurred below 400 mμ., better precision was obtained at the higher wave-length. As regards stability of the complex ion no detectable change in the colour intensity was observed at varying concentrations after 2 hours; the effect of temperature variation between 20° and 30°C. on the final colour was also negligible. Good recovery was obtained in experiments with known amounts of sodium and the accuracy of the method was considered to be within 1 per cent.

R. E. S.

Streptomycin, Spectrophotometric Method for Determination of. W. Eisenman and C. E. Bricker. (*Anal. Chem.*, 1949, **21,** 1507.) A streptomycin sample (not over 4 ml. in volume and containing between 50

and 2500 units of streptomycin) is pipetted into a glass-stoppered test tube; if the volume of solution taken is less than 4 ml., the sample is diluted with water to this volume. To the solution is added 1 ml. of 5N sodium hydroxide solution and the tube and its contents are heated in a boiling water bath for 3 minutes. After cooling to room temperature and adding 2 ml. of 5N sulphuric acid and 1 ml. of ammonium sulphate, the resulting solution is distilled and a total of 10·0 ml. of distillate is collected. A description of the distillation apparatus, the process being essentially one of steam distillation, is given and a modification in which a slow stream of compressed air is passed through the solution in place of the steam is also quoted. If the sample contains over 500 units of streptomycin, 1·0 ml. of ferric chloride solution (2 g. of $FeCl_3.6H_2O$ in 100 ml. of 2N hydrochloric acid) is added to the distillate and the colour produced is measured at 550 mμ. against 1·0 ml. of the same reagent added to 10·0 ml. of distilled water; if the sample contains less than 500 units, 1·0 ml. of phenol reagent is added to the distillate and the solution is allowed to stand for 1 to 2 minutes before adding 3·0 ml. of 20 per cent. sodium carbonate solution. The optical density of this solution is measured at 775 mμ, against a reagent blank. From calibration curves prepared from known amounts of streptomycin and with both reagents, the number of units of streptomycin in the sample can readily be calculated. Results obtained with several impure solutions which were analysed by the maltol distillation method and by a biological assay method are given. The solutions differed widely in composition and were believed to contain relatively small amounts of mannosidostreptomycin; with one exception the values from the chemical procedure agreed to within less than 10 per cent. with those obtained by the biological method. The slightly higher values from the chemical method were considered to be due either to the presence of a small amount of mannosidostreptomycin or to inaccuracies of the biological method. Several broths of comparatively low activity were examined using both the ferric chloride and phenol reagents, the results with these two reagents agreeing to within 3 per cent. in all cases. If the phenol reagent is used samples with an activity as low as 10 units/ml. can be analysed. R. E. S.

Vitamin A, Determination of. D. Verhagen and R. W. Parent (*Anal. Chem.*, 1949, **21**, 1584.) A simplified method for the determination of vitamin A in the unsaponifiable fraction of fish liver oils is reported. The oil (0·1 to 0·25 g., depending on potency) is weighed into a test tube, 0·6 ml. of 50 per cent. potassium hydroxide solution and 6 ml. of alcohol are added and the mixture is refluxed on a steam bath under an air condenser for 15 minutes or until the oil is completely dissolved. The condenser is removed and the alcohol evaporated under vacuum with nitrogen. As soon as the solvent is removed about 20 ml. of 1·5 per cent. barium chloride solution saturated with chloroform is added, the mixture is allowed to cool and exactly 20 ml. of water-washed chloroform added. After shaking this mixture thoroughly and centrifuging until the chloroform layer is clear or nearly so (the barium soaps will form a layer at the interface) 10 ml. of the chloroform solution is pipetted into an amber or red volumetric flask. For an ultraviolet absorption determination 0·3 ml. of isopropyl alcohol is added and the mixture is evaporated to dryness under a stream of nitrogen. As soon as the last of the solvent has been removed, the flask is filled to its mark with isopropyl alcohol at room temperature and, if after standing for an additional period of 1 to 2 minutes the solution is cloudy, it is centrifuged.

For a Carr-Price determination a chloroform aliquot may be dried by the addition of a few grains of sodium sulphate and then used directly. When compared with the current method slightly greater recovery and better precision were obtained. It is not thought that the greater recovery was due to failure to remove extraneous absorbing materials as the absorption curves in the range 300 to 350 mμ were essentially identical. R. E. S.

PHARMACOLOGY AND THERAPEUTICS

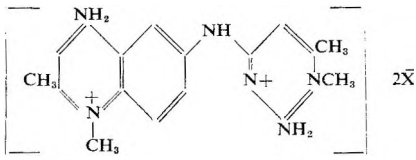
norAdrenaline, Action of. J. H. Burn and D. E. Hutcheon. (*Brit. J. Pharmacol.*, 1949, **4,** 373.) An important difference between the vascular action of noradrenaline and adrenaline is that the former causes constriction whereas the latter causes dilatation of the denervated hind limb of the cat, the difference being in the muscle vessels. Like adrenaline, noradrenaline dilates the coronary vessels of the cat and dog and, in small doses, the intestinal vessels. In the vessels of the rabbit ear the constrictor action of noradrenaline is as easily converted to a dilator action by 2-benzylimidazoline as is that of adrenaline. Denervation increases the action of noradrenaline on the nictitating membrane and on the pupil much more than it increases that of adrenaline. norAdrenaline causes contraction of the spleen *in situ*. It has a smaller constrictor action on renal blood flow than adrenaline; it inhibits intestinal movements recorded by a balloon in the duodenum; it has the same effect as adrenaline on skeletal muscle previously treated with neostigmine; and it has much less effect than adrenaline in dilating the bronchioles. S. L. W.

norAdrenaline; Assay of Substances from the Adrenal Medulla. J. H. Gaddum and F. Lembeck. (*Brit. J. Pharmacol.*, 1949, **4,** 401.) The concentrations of both adrenaline and noradrenaline in a mixture of these drugs can be roughly determined by parallel quantitative assays on the rat uterus and colon. The results may be misleading unless they are analysed statistically. The method used was based on that of Jalon, Bayo and Jalon. Rat's uterus or colon is suspended in a solution of the following composition (g./l): sodium chloride 9, potassium chloride 0·42, calcium chloride 0·06, sodium bicarbonate 0·2, glucose 0·5 at 30°C. Contractions are produced every 2 minutes by a choline ester, and the assay depends on the inhibition of these contractions by adrenaline or noradrenaline. Extracts of cats' adrenals, and plasma collected from the cat's adrenal veins during stimulation of the splanchnic nerves, were both shown to contain some other substance besides adrenaline. The results confirmed that this was noradrenaline and gave an estimate of the amount. S. L. W.

***p*-Aminobenzoic Acid and Thiouracil Compounds, a Comparison of the Anti-thyroid Activity of.** J. F. Goodwin, H. Miller and E. J. Wayne. (*Lancet*, 1949, **257,** 1211.) In a clinical trial of 10 thyrotoxic patients treated with *p*-aminobenzoic acid, 6 did not respond at all and in only one case was full control obtained. When 8 of these patients were treated with the thiouracil compounds, 2 did not respond and full control was obtained in 3 cases. The authors also compared the relative potencies of *p*-aminobenzoic acid and methylthiouracil in humans with normal thyroid function, by means of a tracer dose of radioactive iodine. Results showed that a single dose of 200 to 300 mg. of methylthiouracil was substantially

more effective in reducing the iodine uptake in the normal thyroid than a single dose of 4 g. of *p*-aminobenzoic acid. It is concluded that *p*-aminobenzoic acid has a slight but definite anti-thyroid action in safe dosage (4 g. a day). Other clinical evidence shows potentially serious side effects in larger doses; but to obtain an action comparable to that of the thiouracil group, toxic doses would be needed. E. N. I.

Antrycide, a new Trypanocidal Drug. F. H. S. Curd and D. G. Davey (*Brit. J. Pharmacol.*, 1950, **5**, 25.) Antrycide has the following constitution, where X is an anion :—



Four salts of antrycide have been prepared, the dibromide (m.pt. 316°C.), the dichloride (m.pt. 316°C.), the di-iodide (m.pt. 312°C.), and the dimethylsulphate (m.pt. 265 to 266°C.). They are white crystalline solids. The halides are sparingly soluble in water but the methylsulphate is readily soluble (up to about 33 per cent.). Toxicity, therapeutic and prophylactic experiments were conducted on mice, employing the chloride and the methylsulphate by subcutaneous injection. Absorption of the two salts, after subcutaneous injection, appears to be directly related to their solubility; a suspension of the chloride is absorbed slowly and a solution of the methylsulphate rapidly. A table is given showing the curative properties of antrycide in mice infected with various species of trypanosomes. It is most active against *T. congolense, T. evansi, T. equinum* and *T. equiperdum*, but also exhibits marked activity against *T. brucei, T. rhodesiense* and *T. gambiense*. No activity has been observed against *T. cruzi*. The substance may be used to protect mice for several weeks against *T. congolense*. The prophylactic properties of antrycide are shown to be due for the most part to the establishment of a reservoir of the drug beneath the skin from which absorption takes place slowly, and not to persistence of the drug in the body in the usually accepted sense of the term. S. L. W.

Aspirin, Enteric-coated, Value of. R. H. Talkov, M. W. Ropes and W. Bauer. (*New Engl. J. Med.*, 1950, **242**, 19.) On the administration of freshly prepared enteric-coated aspirin tablets to 32 patients, the analgesic effect was equal to that of ordinary aspirin, and the onset of action was only slightly delayed. Its use is specially indicated where ingestion of ordinary aspirin, in regular dosage, gives rise to gastric symptoms of local origin. Substitution by enteric-coated aspirin causes these symptoms to disappear. It is of value when maximal doses are required to control pain and in patients suffering with peptic ulcers and hæmorrhagic gastritis. E. N. I.

Chloramphenicol in Infantile Gastro-enteritis. K. B. Rogers, S. J. Koegler and J. Gerrard. (*Brit. med. J.*, 1949, **2**, 1501.) Previous authors have noted a close correlation between the same serologically specific type of *Bacterium coli* and epidemic infantile gastro-enteritis. The appearance of the organism has been studied in 86 infants under 1 year old, rectal swabs being taken on admission, and 5 days later, also if a case of gastro-enteritis appeared in the ward. The time between first isolation of

the organism and development of gastro-intestinal disturbance was calculated in 17 cases; it varied from 4 to 24 days with an average of 11. In 25 of the 86 infants, isolation of the organism was not followed by gastro-intestinal symptoms; of the remaining 61 infants, 13 died. Pencillin and sulphonamides and streptomycin were virtually without effect, although they helped to control parenteral infections such as otitis media; some infants developed the disease while actually receiving the medicaments. Chloramphenicol gave highly encouraging results. The average time to effect clearance of the organism was 4 days (range 2 to 8 days). In 2 cases, the organism reappeared in 1 and 7 days after cessation of treatment, but it was still sensitive to the drug; this may have been due to reinfection. Dosage was 75 mg./lb. of body weight per day in 6 or 8 divided doses, the material being removed from the capsules and given as a raspberry-flavoured suspension with tragacanth; the initial dose was twice the maintenance dose. The stools occasionally became coloured bright green. Four infants developed dermatitis, but it is not certain that the drug was responsible. No other toxic reactions were noted, blood and urine examinations giving normal results. This particular strain of *Bact. coli* was sensitive to 2 to 4 μg./ml. of antibiotic; no change in sensitivity occurred during treatment. Blood levels of up to 120 μg./ml. were obtained. H. T. B.

Chloromycetin; Antitreponemal Effect in Early Syphilis. M. J. Romansky, S. Olansky, S. R. Taggart and E. D. Robin. (*Science*, 1949, **110**, 639.) A series of 24 patients with early syphilis was treated with oral chloromycetin 30 mg./kg./day for 4, 6 or 8 days. The lesions in all cases showed evidence of initial healing within 24 hours and most of them showed complete healing by the end of the therapy. Quantitative serological tests were performed prior to therapy and at monthly intervals after treatment. There was a rapid decline in the serological titre in most cases at the end of one month following treatment. Chloromycetin seems to promote healing by a different process from penicillin; penicillin produces healing at the periphery of a lesion whereas healing with chloromycetin seems to be initiated from the base. The only toxic or outward reactions noticed with this series were an occasional mild diarrhœa and an occasional complaint of dryness of the mouth. Several patients complained of a generalised aching sensation 48 hours after the beginning of treatment but no fever or eruptions were noted. Since relatively small doses of chloromycetin will cure gonorrhœa, attention should be called to the possible danger of masking the diagnosis of syphilis. S. L. W.

Curarising Activity, Antagonism of Phenolic Substances. G. A. Mogey and P. A. Young (*Brit. J. Pharmacol.*, 1949, **4**, 359.) This investigation was undertaken in view of the finding that the use of *p*-chloro-*m*-cresol as a bacteriostatic agent in solutions of *d*-tubocurarine has an antagonistic action on the curarising activity. Observations on a series of hydroxy-derivatives of benzene showed them to antagonise the action of *d*-tubocurarine on the rat diaphragm. The relative potencies of the members of the series were expressed as the concentration of antagonist which reduced by half the effect of a concentration of *d*-tubocurarine which alone would cause two-thirds paralysis. The following antagonists were investigated: catechol, *p*-chlorophenol, *o*-cresol, *o*-chlorophenol, *m*-cresol, phenol, *p*-cresol, resorcinol, hydroquinone, guaiacol, and phloroglucinol, and it was found that there was a distinct fall of potency down the series in the order indicated, the last three substances being relatively inactive. The antagonism was

shown not to be due to cholinesterase inhibition or alteration of *p*H; neither was it thought to be due to a chemical combination of antagonist with *d*-tubocurarine. The authors suggest that there is attraction between phenols and proteins, causing steric hindrance. S. L. W.

Decamethonium Iodide and Related Compounds, Pharmacological Actions of. W. D. M. Paton and E. J. Zaimis. (*Brit. J. Pharmacol.*, 1949, **4,** 381.) The pharmacological actions of a series of polymethylene bistrimethylammonium salts, containing from 2 to 18 carbon atoms in the polymethylene chain, have been studied chiefly in regard to their activity in blocking neuromuscular transmission. C10 is the most active compound in causing neuromuscular block in the chloralosed cat, but the excitability of nerve and muscle is retained. Unlike *d*-tubocurarine, C10 can produce profound neuromuscular block without causing respiratory paralysis. The compound by mouth has 1/50 to 1/100 of the activity by intravenous injection. The order of decreasing sensitivity is cat, man, rabbit, monkey, mouse, rat, the variation being greater than with *d*-tubocurarine. C5 and C6 are effective antagonists, probably acting by competitive inhibition; previous administration of *d*-tubocurarine also reduces the effect of C10. Ganglionic transmission is blocked particularly by C5 and C6, higher and lower members of the series having a smaller activity. There is a less well-defined maximum for muscarine-like activity and anticholinesterase activity at C12. No member of the series shows any significant atropine-like activity or ability to stimulate autonomic ganglia. H. T. B.

Digitalis Glycosides, Influence of Saponins on Toxicity of. F. Neuwald and G. Zöllner. (*Arch. Pharm., Berl.*, 1950, **283,** 26.) Digitalis leaves, containing about 0·1 per cent. of total glycosides (determined chemically) should have an activity of 2 cat units/g Actually however the value found was about 10 units/g. It has also been observed that the clinical activity of digitalis is only one-fifth that of digitoxin, if doses of equivalent cat units are given. In order to determine whether this difference was due to the effect of digitalis saponins, determinations of M.L.D. were made with and without the addition of saponins in similar proportion to that of the leaf. The administration, both to cats and guinea-pigs, was intravenous. No significant differences were found, either with digitoxin-digitonin or with lanadigin-tigonin. Thus it appears that the toxicity of digitalis glycosides is not increased by the presence of the saponins of the leaf.
G. M.

Digitoxin. Renal Excretion Following Oral Administration. M. Friedman, S. O. Byers, R. Bine, Jr., and C. Bland. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **72,** 468.) The sensitivity of the embryonic duck heart preparation (Bine and Friedman, *Proc. Soc. exp. Biol., N.Y.*, 1948, **69,** 487) to minute amounts of digitalis glycosides in Tyrode's solution and human serum suggested the possibility of determining the urinary excretion of digitoxin. To extract the excreted glycoside, 200 ml. of urine was evaporated to 5 ml. which was absorbed by diatomite and dried. This powder was then extracted with chloroform and the dried chloroformic extract further extracted with ethyl alcohol, the final residue being taken up in Tyrode's solution. By adding known amounts of pure digitoxin to normal urine and extracting as above, it was found that the time of occurrence of the "digitalis effect" in the duck hearts was dependent upon the amount added. Five subjects received 1·2 mg. of digitoxin orally during 6 hours: the

urine was collected and a 200 ml. aliquot assayed. The rate of excretion varied considerably. During the first 24 hours, an average of 87 μg. was excreted (range, 44 to 144 μg.). During the second and third days, the average weights excreted were 55 μg. and 21 μg. respectively, the average total excretion being about 14 per cent. of the amount administered.

H. T. B.

Dimercaprol, Effect of, on Lead Poisoning in Mice. A. B. Anderson. (*Brit. J. Pharmacol.*, 1949, **4**, 348.) Lead acetate marked with a tracer of Pb^{210} (radium D) was given with a low calcium diet to mice, and after varying periods the lead content of the whole animal was determined by chemical analysis and count of β radiation. The lead content of mice which had received 50 mg./kg. of dimercaprol daily, and lead simultaneously for 10 to 14 days, averaged 0·48 mg. Pb/100 g. of mouse, and was significantly lower than that of controls receiving lead alone, which averaged 1·0 mg. Pb/100 g. When lead alone was administered for 8 to 10 days, subsequent treatment with dimercaprol during a recovery period of 1 to 2 weeks had no significant effect on the final lead content. The author concludes that these results provide no indications for the use of dimercaprol in the treatment of chronic lead poisoning.

S. L. W.

Morphine: Response of Duodenum. T. A. Loomis. (*Proc. Soc. exp. Biol., N.Y.*, 1948, **69**, 146.) Although the earliest investigations of the effect of morphine on intestinal muscle suggested that it produced relaxation, more recent investigations using balloon methods have indicated that the drug produces increased pressure and increased movements of various segments. In view of the possibility that erroneous conclusions may be drawn from the results of balloon experiments the action of morphine on a given segment of the duodenum in the intact anæsthetised dog has been investigated by a method giving simultaneous graphic recordings of both circular and longitudinal muscle activity. Circular contractions were recorded by means of a balloon not more than 2 cm. long which when fully expanded would come into contact with about 1 cm. length of intestinal mucosa. This gave no response to longitudinal movements. Longitudinal movements were recorded by the internal organ apparatus of Jackson (Experimental Pharmacology and Therapeutics, C. V. Mosby Co., 1939, page 89). The balloon was inserted in the duodenum through an incision in the stomach under pentobarbital anæsthesia and the Jackson apparatus was sutured to the mucosal surface of the same segment of the duodenum. All injections were given intravenously, the volume being always less than 5 ml. It was found that repeated equal doses of morphine did not produce equivalent responses and only one dose of the drug was therefore given. Doses of 0·01 to 1·0 mg./kg. body weight were used. The smallest dose consistently effective in altering muscular activity was 0·05 mg./kg. In 17 experiments on as many different animals doses of 0·1 mg./kg. elicited a response by the circular muscle in 16 animals but in 13 animals there was either no response by the longitudinal muscle or a decrease in activity. When the dose was increased to 0·5 mg./kg. all the animals showed increased activity of the circular muscle while in 15 there was either no response or decreased activity in the longitudinal muscle. With 1 mg./kg. a decreased activity in the longitudinal muscle was recorded in 5 out of 6 animals. The increased activity consisted of an increased level of tonus with either an increase or no change in the frequency and amplitude of normal spontaneous contractions. Decreased activity consisted of a

decreased level of tonus with either a decrease or no change in the frequency and amplitude. H. T. B.

Thiouracil and Propylthiouracil, Effect of Halides on Action of. R. H. Williams, H. Jaffe and B. Soloman. (*Amer. J. med. Sci.*, 1950, **219**, 1.) Both thiouracil and radioactive iodine have been observed to produce a slower clinical response if the patients have just been treated with potassium iodide for several weeks. It therefore seemed possible that the effectiveness of these treatments might be increased by first reducing the body store of iodine to subnormal levels. The effect of sodium chloride, bromide and fluoride on the goitrogenesis produced in rats by thiouracil and propylthiouracil was therefore investigated, on the hypotheses that non-iodide halides might reduce the body iodine level and that they might antagonise the synthesis of thyroid hormone. Male rats received the halide in their food in weighed amounts; some received thiouracil and propyl-thiouracil in their drinking water, dissolved with the aid of sodium hydroxide. The animals were killed after 12 days and the thyroid glands removed and weighed. All three halides increased goitrogenesis of the uracil compounds, the chloride being least active and the fluoride most active. Concentrations of the uracil compounds too small to produce goitre alone produced it when the halides were given simultaneously, although the latter alone gave no such effect. It is suggested that the action may be due, in part, to competition of the halide anions for reabsorption by the renal tubules, thus increasing the rate of excretion of iodide. On the basis of these experiments, clinical trial with sodium bromide is suggested.

H. T. B.

Vitamin B_{12} and Thyroid Function. E. J. Wayne, A. G. Macgregor and H. Miller. (*Lancet*, 1950, **258**, 327.) Patients receiving liver therapy for pernicious anæmia sometimes develop hyper-thyroidism. On the other hand, if patients receiving thyroid treatment for hypothyroidism develop pernicious anæmia and liver extract is administered, the dose of dried thyroid must be increased. For this reason the existence of a specific antithyroid principle in liver has been postulated and the effect of vitamin B_{12} on thyroid activity was therefore investigated using radioactive iodine. Doses of 60 μg. and 100 μg. respectively of vitamin B_{12} were given to two normal patients when iodine accumulation in their thyroid glands was proceeding at a steady rate. No effect whatever could be demonstrated and it was concluded that in the doses used vitamin B_{12} has no significant influence on thyroid function. H. T. B.

Vitamin C: Effect on Wound Healing. J. R. Penney and B. M. Balfour. (*J. Path. Bact.*, 1949, **61**, 171.) The effects of vitamin C on the healing of wounds was investigated in guinea-pigs. Muscle wounds were inflicted, and, at intervals thereafter, the animals were killed by stunning and bleeding and the wounds examined microscopically. The presence of acid mucopolysaccharide esters was shown by the production of meta-chromasia with toluidine blue. In animals receiving adequate vitamin C, the initial stage of the healing process, consisting of the invasion of the clot by fibroblasts, could be seen in 4 days. As the process continued, there was close correlation between the distribution of newly formed fibres and extracellular metachromasia with toluidine blue. In depleted animals, some slight fibroblastic invasion occurred, but the cells were very abnormal

(*Continued on page 536*)

LETTERS TO THE EDITOR

The "Ninhydrin-Reacting" Hydrolytic Fragment of Vitamin B_{12} and 1-Aminopropan-2-ol

SIR,—Ellis, Petrow and Snook[1] have previously reported that hydrolysis of vitamin B_{12} gives rise to a "ninhydrin-reacting fragment" not identical with any of the known amino-acids. Later work[2] led to the conclusion that the ninhydrin-reacting fragment was a volatile aliphatic base and, in all probability, an amino-alcohol. This view was strengthened by the observation that 2-aminopropan-1-ol and the ninhydrin-reacting fragment exhibited the same behaviour on paper chromatograms irrigated with four different solvent systems. Nevertheless, while drawing attention to this fact, Ellis, Petrow and Snook[2] pointed out that a final decision must rest on a rigid chemical comparison between the two compounds.

Subsequent work by Cooley, Ellis and Petrow[3] revealed a difference in the behaviour of 2-aminopropan-1-ol and the ninhydrin-reacting fragment on oxidation with acid permanganate. Microgram quantities were employed for these experiments, the products obtained being examined on chromatograms developed with the ninhydrin reagent. 2-Aminopropan-1-ol gave alanine under these conditions. The ninhydrin-reacting fragment, in contrast, gave an unidentified product which appeared as a yellow spot slowly turning purple at room temperature.

In continuation of this work, we have investigated the chromatographic behaviour of several amino-alcohols of low molecular weight, and have found that 1-aminopropan-2-ol, a structural isomer of 2-aminopropan-1-ol, and ninhydrin-reacting fragment are likewise indistinguishable on paper chromatograms irrigated with a number of different solvent systems. This observation is in complete agreement with that of Chargaff *et al.*[4] Furthermore, oxidation of 1-aminopropan-2-ol with acid potassium permanganate, followed by paper chromatography of the product, gives a yellow spot with the ninhydrin reagent, identical in every respect with that obtained from the ninhydrin-reacting fragment in a similar way. Thus, not only is the latter fragment chromatographically inseparable from 1-aminopropan-2-ol, but so also are their respective highly characteristic oxidation products.

We have previously stressed[3] the limitations attending the use of chromatographic methods for the identification of substances available only in microgram amounts. The evidence in this instance, however, appears to be more definite than is usually the case.

Dr. K. Folkers, during his recent visit to this country, was kind enough to inform us that independent studies by the Merck group had established the identity of the ninhydrin-reacting fragment with D-1-aminopropan-2-ol by the methods of classical organic chemistry.

The authors thank the Directors of The British Drug Houses, Ltd., for permission to publish these results.

G. COOLEY.
B. ELLIS.
V. PETROW.

Research Department,
The British Drug Houses, Ltd.,
London, N.1.
29th June, 1950.

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2. Ellis, Petrow and Snook, *ibid.*, 1949, **1**, 287.
3. Cooley, Ellis and Petrow, *ibid.*, 1950, **2**, 128.
4. Chargaff, Levine, Green and Kream, *Experientia*, 1950, **6**, 229.

BOOK REVIEWS

THE SULPHONAMIDES. F. Hawking and J. Stewart Lawrence. Pp. viii + 390 (including 46 illustrations, 12 plates and 17 tables). H. K. Lewis & Co., Ltd., London. 1950. 42s.

Fourteen years after its introduction, we are now at the beginning of the end of sulphonamide therapy. At first the introduction of new compounds proceeded apace and later the basic knowledge of the subject developed and became consolidated. Originally the pace was rather fast for the average clinician and the books which appeared on the subject tended soon to become out-of-date. It was not until the Medical Research Council War Memorandum No. 10 "The Medical Use of Sulphonamides" appeared in 1943, with a second edition in 1945, that a brief and accurate review of the principles of the subject and of the practical clinical considerations became available. These must have been best sellers among their kind. Further developments of importance are unlikely now, and with a detachment hitherto impossible, definitive review of the whole subject is both possible and called for. This, the work under consideration, magnificently provides.

There are 27 chapters, in which all aspects of the subject are described, followed by an extensive bibliography of the many original papers quoted and a less extensive, although apparently adequate, index.

The completeness and accuracy of this work leaves nothing to object to. Doubtless a second edition will be called for and, in that event, the authors might distinguish those names of sulphonamide drugs which are trade marks from those which are not. But few developments have occurred since this work was published; 4:4′-diamino-diphenylsulphone, stated on page 14 not to have come into clinical use, has recently been introduced for the treatment of leprosy, a reference to this being given on page 289. Pharmacists will note that the compound described on page 21 under a proprietary name is now included in the B.P.C. under the name sulphadimidine. Succinylsulphacetamide is not among the compounds described. For a work of this character the number of misprints is remarkably few, in fact the only one noted was 1·5 grammes on page 240 instead of 15 grammes.

The average pharmacist and practising clinician should find in this book the answer to almost any question on sulphonamide therapy. For all pharmaceutical and medical libraries it is obviously essential, and will doubtless become and remain a standard work. D. G. ARDLEY.

ABSTRACTS (*Continued from page 534*)

and little extracellular material was formed even up to 9 days after the wound. Striking changes occurred on giving vitamin C to the depleted group. Increase in the amount of metachromatic staining material was noted within 6 hours and the increase was large within 12 hours, isolated cells being surrounded by material showing metachromasia. Within 24 hours fibre formation was evident. The morphology of all the cells became normal in 48 hours. Treatment of the sections with hyaluronidase removed the substances responsible for the extracellular metachromasia, suggesting that they are mucopolysaccharides of the hyaluronic or chondroitin sulphate types. Treatment with ribonuclease removed the substances responsible for metachromasia in the cytoplasm, suggesting that they are ribo-nucleoproteins. The initial stage in fibre formation is probably the deposition of mucopolysaccharides, the production of which is dependent on the supply of vitamin C. H. T. B.