RESEARCH PAPERS

ANALGESIC PROPERTIES OF 4-ETHOXYCARBONYL-1-(2-HYDROXY-3-PHENOXYPROPYL) 4-PHENYLPIPERIDINE (B.D.H.200) AND SOME RELATED COMPOUNDS

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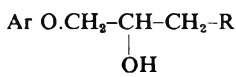
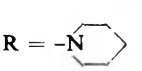
Twenty-one 3-aryloxy or 3-alkoxy-2-hydroxy-n-propyl derivatives of norpethidine were tested subcutaneously for analgesic activity in mice. Many of them are more potent than pethidine. The 2-hydroxy-3-phenoxypropyl derivative (B.D.H. 200) is at least three times more active than morphine and ten times more active than pethidine in this species with a therapeutic index slightly better than morphine and very much better than pethidine. The duration of analgesia is similar to morphine and pethidine and it is less constipating than pethidine. Its effects on respiration and the cardiovascular systems are counteracted by nalorphine. The decrease in activity after oral administration is probably due to a more rapid metabolic breakdown than poor absorption, as this decrease in activity can be modified by pretreatment with iproniazid and BAL while subcutaneous administration is unaffected.

Some 3-aryloxy-2-hydroxypropylamines with weak analgesic properties were described by Beasley, Petrow and Stephenson[1] and having the general formula (I) where Ar = phenyl or substitued phenyl, and R an alkyl or dialkyl amino group.



(I)



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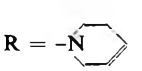
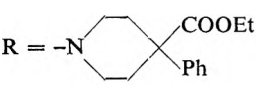


(III)

The analgesic activity was increased by replacing the amino group by cyclic structures such as piperidine (II), pyrrolidine and morpholine. The replacement of the piperidine group by Δ^3-piperideine (III), further enhanced the analgesic activity[2], and led to the synthesis of *N*-(2-hydroxy-3-*o*-toloxypropyl)-Δ^3 piperideine (Tolpronine) whose pharmacological properties were described by David, Leith-Ross and Vallance[3].

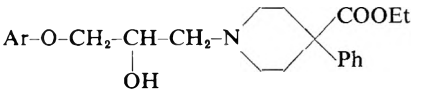
The study of the analgesic properties of structures related to (II) was further extended by preparing the 4-ethoxycarbonyl-4-phenyl derivatives (IV) of *N*-(3-aryloxy-2-hydroxypropyl)piperidine[4].



(IV)

These compounds are analogues of pethidine being substituted derivatifes of norpethidine where the *N*-methyl group has been substituted by the *N*-aryloxyhydroxypropyl group.

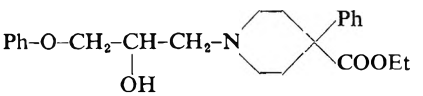
Pethidine



Series (IV)

Since the discovery, by Schaumann[5], of the analgesic properties of the 4-ethoxycarbonyl-4-phenyl-*N*-alkyl piperidines, *N*-methyl substitution has been regarded as essential for the analgesic activity in the pethidine molecule[6–8].

Some doubts on the validity of this conclusion have recently been raised by the discovery that some *N*-aralkyl analogues of pethidine show an analgesic activity superior to that of pethidine itself[9–15], as do the *N*-morpholinoethyl analogue[16,17], and the *N*-2-(2-hydroxyethoxy)ethyl analogue[18]. The results obtained with the *N*-aryloxyhydroxypropyl analogues of pethidine and particularly with 4-ethoxycarbonyl-1-(2-hydroxy-3-phenoxypropyl) 4-phenylpiperidine (B.D.H. 200) provide



further support for the view that *N*-methyl substitution in the pethidine molecule is not the best for analgesic activity. In the following experiments B.D.H. 200 was used as the hydrochloride or hypophosphite.

Physical Properties

The hydrochloride of B.D.H. 200 is a white crystalline compound with a molecular weight of 419·7. It has a melting point of 174·2–175°. It is sparingly soluble in water 0·5 per cent at 25°. A 0·1 per cent solution at 21·5° has a pH of 5·49.

B.D.H. 200 hypophosphite is a white crystalline compound with a molecular weight of 449·3. It has a melting point of 115·5–116·5°. It is soluble 4·9 per cent in water at 22°. A 1 per cent solution at 20° has a pH of 3·93.

Methods

Analgesic Activity in Mice

The subcutaneous and oral analgesic activities were estimated in male albino mice, weighing between 15 and 20 g., using Haffner's method as described by Bianchi and Franceschini[19]. The animals were fasted overnight before the oral tests but in the subcutaneous experiments were allowed free access to food and water. The sensitivity of each mouse was determined immediately before administration by placing a bulldog artery clip covered with catheter tubing to the base of the tail; only those mice making continuous attempts to remove the clip within 15 seconds were included in the experiments. The hydrochloride and hypophosphite were given in aqueous solutions, the volumes being adjusted to 0·5 ml./20g. weight. At 30, 60 and 90 minutes after administration the clip was applied to each mouse in turn. A positive analgesic response was recorded if no attempt was made to remove the clip at any one of the three observation

times. The ED50 and the activity ratios with fiducial limits (P = 0·05) were calculated using Litchfield and Wilcoxon's method[20].

Duration of Analgesic Action in Mice

This was estimated in sensitive male albino mice after subcutaneous or oral administration of a single submaximal analgesic dose. Tests for analgesia were made as before at 15 or 30 minute intervals for as long as analgesia was present. The duration of analgesic effect, the ET50, and the activity ratio, with fiducial limits (P = 0·05), were calculated according to Litchfield[21]. The compounds were given as aqueous solutions, the volumes being adjusted to 0·5 ml./20 g. weight.

Analgesic Activity in Rats

This was investigated to confirm that the analgesic activity of B.D.H. 200 hydrochloride was not confined to mice. Analgesia was tested using a thermal stimulus[22], 600 W, 165 V, 3·6 A applied from 1 to 1½ inches from the base of the tail, previously blackened with indian ink, for not more than 6 seconds.

The individual reaction time, that is the time taken for each animal to remove its tail from the region of the stimulus, was recorded by means of a stop watch. A period of training was carried out twice a day for 2 days before and once on the day of the experiment. Insensitive or hypersensitive rats were not used. Doses were administered subcutaneously and all volumes adjusted to 0·5 ml./200 g. weight. The thermal stimulus was applied at 30, 60 and 90 minutes following administration and those rats showing an increase of 2 seconds or more over their normal reaction time were regarded as showing analgesia.

Acute Toxicity

The subcutaneous and oral toxicities were estimated in male albino mice weighing between 15 and 20 g. each. The LD50, calculated from the seven days mortalities, the toxicity ratios, and their fiducial limits (P = 0·05), were estimated according to Litchfield and Wilcoxon's method[20].

The compounds were administered in distilled water and all volumes were adjusted to 0·5 ml./20 g. weight.

Effect on Blood Pressure and Respiration

The effects on blood pressure and respiration were determined in male rabbits weighing between 2·4 and 3 kg. and in male cats weighing between 2·4 and 4·3 kg.

The rabbits were anaesthetised with 2 g./kg. of urethane subcutaneously and the cats with 500 mg./kg. of urethane plus 50 mg./kg. of chloralose given intraperitoneally. The carotid blood pressure was recorded by means of a mercury manometer, and respiratory movements by means of a lever attached to a rubber tambour connected by a side arm directly to the trachea.

B.D.H. 200 was given subcutaneously to rabbits in 0·5 ml./kg. of normal saline and in cats through the cannulated femoral vein. The rabbits were

observed for 3 hours after administration and the percentage variation in respiration estimated at intervals. The cats were used chiefly in the experiments designed to estimate the antagonistic effect of nalorphine and B.D.H. 200.

TABLE I

THE SUBCUTANEOUS ED50, LD50, WITH FIDUCIAL LIMITS (P = 0·05), AND THERAPEUTIC INDICES IN MICE OF MORPHINE, PETHIDINE AND B.D.H. 200

Compound	No. of mice	ED 50 mg./kg.	No. of mice	LD 50 mg./kg.	Therapeutic index
Morphine hydrochloride	295	5·80 (4·83–6·96)	160	505·0 (459·0–555·5)	87·0
Pethidine hydrochloride	80	17·00 (11·48–25·16)	50	130·0 (97·7–172·9)	7·6
B.D.H. 200 hydrochloride	300	1·38 (1·16–1·62)	70	145·0 (117·9–178·3)	105·0
B.D.H. 200 hypophosphite	200	1·70 (1·33–2·16)	80	150·0 (127·1–177·0)	88·2

Constipating Effect

This was investigated by Lou's method[23] in unfasted mice weighing approximately 20 g. each. Groups of nine or ten mice were given three doses of B.D.H. 200 or pethidine hydrochloride subcutaneously and

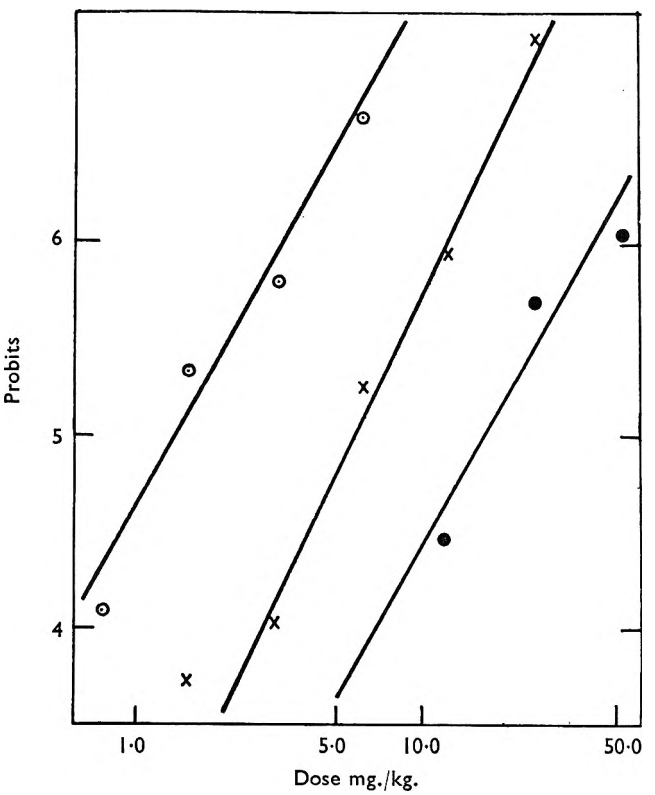


FIG. 1. The subcutaneous analgesic activity of morphine X, pethidine ● and B.D.H. 200 ⊙ in mice.

15 minutes later placed in separate compartments over a wire grid and faeces collected on blotting paper. The number of faecal pellets was counted at 8 and 24 hours and the mean number recorded. The mice were allowed free access to a paste of Rat Diet 41 and water.

RESULTS

Toxicity and Analgesic Properties

The subcutaneous toxicities, analgesic activities and therapeutic indices in male albino mice of morphine hydrochloride, pethidine hydrochloride, and the hydrochloride and hypophosphite of B.D.H. 200 are recorded in Table I.

The percentages of mice insensitive to the pressure stimulus after the administration of morphine, pethidine and B.D.H. 200 were converted

TABLE II

THE SUBCUTANEOUS ANALGESIC AND TOXICITY RATIOS OF B.D.H. 200, PETHIDINE AND MORPHINE IN MICE

Compound	Analgesic ratio (fiducial limits P = 0·05)	Acute toxicity ratio (fiducial limits P = 0·05)
Pethidine : Morphine	0·34 (0·22–0·53)	3·88 (2·87–5·24)
B.D.H. 200 HCl : Morphine	4·20 (3·28–5·37)	3·48 (2·78–4·35)
B.D.H. 200 hypophos : Morphine.	3·41 (2·52–4·60)	3·36 (2·77–4·06)
B.D.H. 200 HCl : Pethidine	12·31 (7·94–19·08)	0·89 (0·62–1·26)
B.D.H. 200 hypophos : Pethidine	10·00 (6·25–16·00)	0·86 (0·61–1·20)
B.D.H. 200 hypophos : B.D.H. 200 HCl	0·81 (0·62–1·04)	0·96 (0·74–1·25)

TABLE III

THE ORAL ED50, LD50 AND RELATIVE ACTIVITIES WITH FIDUCIAL LIMITS (P = 0·05) OF PETHIDINE AND B.D.H. 200 HYDROCHLORIDE IN MICE

Compound	No. of mice	ED 50 mg./kg.	No. of mice	LD 50 mg./kg.	Relative activity
Pethidine hydrochloride	80	27·00 (20·00–36·45)	30	230·0 (178·1–296·7)	1
B.D.H. 200 hydrochloride	100	48·00 (30·96–74·40)	40	419·0 (335·2–523·7)	0·56 (0·32–0·95)
Pethidine hydrochloride	50	28·00 (19·31–40·60)	—	—	1
B.D.H. 200 hydrochloride	50	53·00 (36·55–76·85)	—	—	0·52 (0·30–0·87)

into probits and plotted against log dose in Figure 1. The responses to the hydrochloride and hypophosphite were similar and therefore the latter was not plotted. The regression lines do not deviate significantly from parallelism and the activity ratios were estimated. The subcutaneous activity and toxicity ratios are recorded in Table II.

In this species, B.D.H. 200 is at least three times more active than morphine hydrochloride and ten times more active than pethidine hydrochloride. It is approximately three times more acutely toxic than

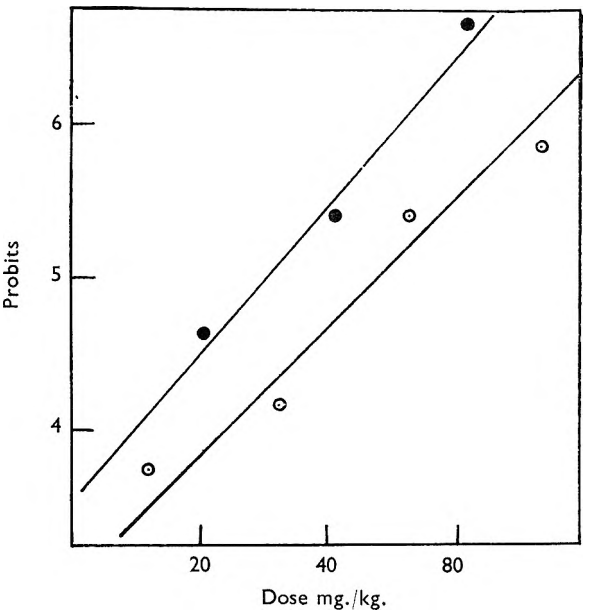


FIG. 2. The oral analgesic activity of pethidine ● and B.D.H. 200 ○ in mice.

morphine and has a similar toxicity to pethidine. Its therapeutic index is slightly better than morphine and much better than pethidine.

B.D.H. 200 also possesses oral analgesic properties in mice but there is a reduction in activity compared to subcutaneous administration. Table III and Figure 2 record the oral toxicity, the analgesic activity and ratio with pethidine hydrochloride.

TABLE IV

THE DURATION OF ANALGESIA (ET50) IN MICE AFTER SUBCUTANEOUS OR ORAL ADMINISTRATION OF MORPHINE, PETHIDINE AND B.D.H. 200

Compound	Route	Dose mg./kg.	No. showing analgesia at intervals (min.)							ET 50* min. (limits P = 0·05)	Significance of difference P = 0·05
			15	30	60	90	120	150	180		
Morphine hydrochloride	Subcut.	12·5		22/25	21/25	15/25	6/25	4/25	0/25	100·0 (86·9–115·0)	None
B.D.H. 200 hydrochloride	Subcut.	2·75		20/25	17/25	10/25	7/25	5/25	0/25	95·0 (81·2–111·1)	
Pethidine hydrochloride	Subcut.	35·0		21/25	10/25	3/25	0/25	0/25	0/25	59·0 (50·0–69·6)	None
B.D.H. 200 hydrochloride	Subcut.	3·5		23/25	16/25	7/25	3/25	1/25	0/25	73 (61·8–86·1)	
			15	30	45	60	75	90	120		
Pethidine hydrochloride	Orally	40·0	10/10	7/10	5/10	2/10	1/10	1/10	0/10	37·0 (26·4–51·8)	None
B.D.H. 200 hydrochloride	Orally	80·0	10/10	8/10	5/10	4/10	2/10	1/10	0/10	47·0 (34·0–64·8)	

* Calculated only from those showing analgesia.

The analgesic ratio of oral to subcutaneous administration is 1·6 for pethidine and 36·6 for B.D.H. 200. The toxicity ratio of oral and subcutaneous administration is 1·8 for pethidine and 2·9 for B.D.H. 200. The mean durations of analgesia of subcutaneous equi-effective doses of B.D.H. 200, morphine and pethidine are similar, but the dose of B.D.H.

TABLE V

THE NUMBER OF RATS DEVELOPING ANALGESIA AT INTERVALS FOLLOWING SUBCUTANEOUS ADMINISTRATION OF B.D.H. 200 HYDROCHLORIDE AND PETHIDINE HYDROCHLORIDE

Compound	Dose mg./kg.	No. developing analgesia at intervals (minutes)		
		30	60	90
B.D.H. 200 hydrochloride	0·5	2/4	1/4	0/4
	1·0	3/4	2/4	0/4
	2·0	4/4	4/4	0/4
Pethidine hydrochloride	5·0	0/4	0/4	1/4
	10·0	0/4	0/4	0/4
	20·0	0/4	0/4	1/4
Controls	—	0/4	0/4	0/4

200 is only one-quarter that of morphine and one-tenth that of pethidine. After oral administration of equi-effective doses of pethidine and B.D.H. 200 the durations of analgesia are similar but the dose of B.D.H. 200 was twice that of pethidine. Table IV records the results.

Analgesic Effect in Rats

The analgesic response in rats after subcutaneous injection of B.D.H. 200 and pethidine hydrochloride are recorded in Table V. These results demonstrate that B.D.H. 200 possesses analgesic properties in rats and is much more active than pethidine hydrochloride by the method used.

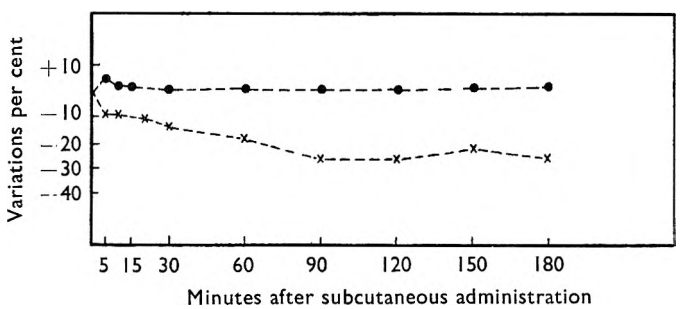


FIG. 3. The variations per cent in respiration of rabbits given 0·5 mg./kg. of B.D.H. 200 or 0·5 ml./kg. of normal saline.

Effect on Blood Pressure and Respiration

B.D.H. 200 causes respiratory depression in rabbits after subcutaneous administration with no apparent effect on blood pressure compared to controls. Figure 3 records the mean per cent variation in respiration in two groups of three rabbits after subcutaneous injection of 0·5 mg./kg. of B.D.H. 200 and control rabbits given a similar volume of normal saline.

In cats the intravenous administration of 0·5 mg./kg. causes a rapid respiratory depression with a concomitant fall in blood pressure. These effects were, however, reversed by intravenous injection of 3 mg./kg. of nalorphine. Figure 4 records the results.

Constipating Effect

At analgesic doses B.D.H. 200 has no constipating effect in mice, for example at 4 mg./kg., which is three times its subcutaneous ED50, there is

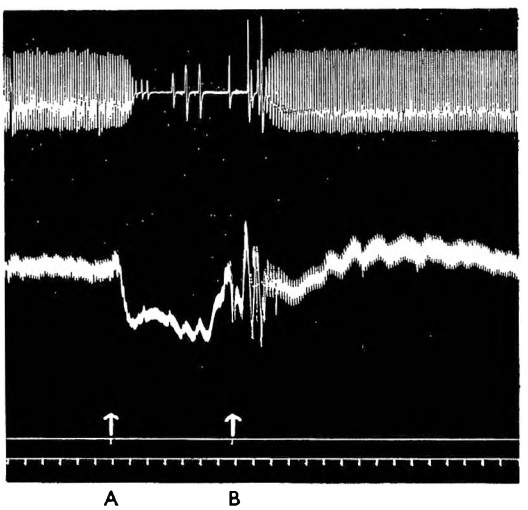


FIG. 4. Cat. Urethane and chloralose anaesthesia. Respiration and carotid blood pressure record. Time = 30 sec. Line above, zero of mercury manometer. At A, B.D.H. 200, 0·5 mg./kg. i.v.; at B, nalorphine, 3·0 mg./kg. i.v.

TABLE VI

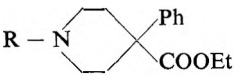
THE EFFECT OF B.D.H. 200 AND PETHIDINE GIVEN SUBCUTANEOUSLY TO MICE ON THE NUMBER OF FAECAL PELLETS PASSED IN 24 HOURS

Compound	Dose mg./kg.	Mean no. of faecal pellets	
		0–8 Hours	8–24 Hours
B.D.H. 200 hydrochloride	4	14	66
	13	8	58
	40	3	39
	120	1	19
Pethidine hydrochloride	4	13	64
	13	17	59
	40	10	54
	120	4	51
Controls	—	15	66

no difference between the treated and control animals. Table VI records the results after various doses of B.D.H. 200 and pethidine. On a weight for weight basis B.D.H. 200 is more constipating than pethidine but in terms of equi-effective analgesic doses it is probably less constipating.

TABLE VII

THE RELATIVE SUBCUTANEOUS ANALGESIC ACTIVITIES IN MICE OF TWENTY-ONE ANALOGUES OF PETHIDINE



Compound	R	Analgesic activity
Pethidine	$-CH_3$	1
B.D.H. 200	$-CH_2-CH(OH)-CH_2-O-Ph$	12·31
1638	$-CH_2-CH(OH)-CH_2-O-C_6H_4-o-Me$	5·1
1947	$-CH_2-CH(OH)-CH_2-O-C_6H_4-m-Me$	2·0
1944	$-CH_2-CH(OH)-CH_2-O-C_6H_4-p-Me$	0·25
1959	$-CH_2-CH(OH)-CH_2-O-C_6H_4-o-O.Me$	5·8
1899	$-CH_2-CH(OH)-CH_2-O-C_6H_4-o-Cl$	5·0
1969	$-CH_2-CH(OH)-CH_2-O-C_6H_4-m-Cl$	0·93
1982	$-CH_2-CH(OH)-CH_2-O-C_6H_4-p-Cl$	0·32
1954	$-CH_2-CH(OH)-CH_2-O-C_6H_4-o-F$	4·9
1957	$-CH_2-CH(OH)-CH_2-O-C_6H_4-o-O.CH_2.CH=CH_2$	1·3
2006	$-CH_2-CH(OH)-CH_2-O-Ph$	6·1
1932	$-CH_2-CH(OAc)-CH_2-O-C_6H_4-o-Me$	0·5
1951	$-CH_2-CH(O.COEt)-CH_2-O-C_6H_4-p-COOEt$	0·1
1985	$-CH_2-CH(OH)-CH_2-N$ (piperidine)	0·60
2042	$-CH_2-CH(OH)-CH_2-N$ (morpholine)	0·43
1945	$-CH_2-CH(OH)-CH_2-OH$	0·1
1993	$-CH_2-CH(OH)-CH_2-OEt$	0·71
3132	$-CH_2-CH(OH)-CH_2-O-CMe_3$	3·3
2040	$-CH_2-CH(OH)-CH_2-O-Bu$	2·0
1965	$-CH_2-CH(OH)-CH_2-O-CHPh_2$	0·1
1949	$-CH_2-C(OH)(Me)-CH_2-O-C_6H_4-o-Me$	0·1

Analogues of B.D.H. 200

In addition, a number of analogues of B.D.H. 200 were examined and are recorded in Table VII.

Discussion

The analgesic activities of several *N*-(2-hydroxy-3-phenoxypropyl) derivatives of norpethidine confirm recent studies that a methyl substitution on the *N*-group of norpethidine does not necessarily give the best analgesic activity in animals. Table VII shows that many derivatives are more potent than pethidine in mice; for example, B.D.H. 200 is twelve times more active than pethidine.

We have no direct evidence of the importance of the carbon chain length or of the ether link in the *N*-arylalkyl chain for the activity of B.D.H. 200. It is also difficult to draw valid comparisons from the results of other

TABLE VIII

The relative analgesic activity of B.D.H. 200 and of the *N*-phenoxypropyl analogue of pethidine (B.D.H. 3022) in mice after subcutaneous administration

Compound	Number of mice	Relative analgesic activity (fiducial limits P = 0·05)
Pethidine	90	1·00
B.D.H. 200	90	15·71 (9·88–24·97)
B.D.H. 3022	90	6·87 (4·40–10·71)

TABLE IX

Oral and subcutaneous analgesic activity of B.D.H. 200 and pethidine in mice pretreated with iproniazid 100 mg./kg. i.p. or BAL 40 mg./kg. i.p. injected 60 minutes before administration

Compound	Pretreatment	Oral ED50 mg./kg.	Subcutaneous ED50 mg./kg.
B.D.H. 200 ..	—	49·51	1·46
B.D.H. 200 ..	Iproniazid	14·21	1·43
B.D.H. 200 ..	BAL	75·00	1·96
Pethidine ..	—	29·75	18·38
Pethidine ..	Iproniazid	27·10	16·51
Pethidine ..	BAL	74·36	9·19

workers with other series using different techniques. However, Elpern, Gardner and Grumbach[12] and Grumbach[13] found in rats that the *N*-phenylpropyl derivative of norpethidine was 10 times more active than the *N*-phenylethyl derivative, and that the *N*-phenoxypropyl derivative was slightly less active than the corresponding *N*-phenylpropyl derivative. Moreover the *N*-phenoxyethyl derivative of norpethidine is only three times more active than the *N*-phenylethyl derivative. Winter, Orahovats and Lehman[24], who examined in rats many *N*-analogues of morphine synthetised by Clark, Pessolano, Weyland and Pfister[25], found that the *N*-phenylethyl derivative of normorphine is six times more active than morphine and that the *N*-phenoxyethyl derivative is inactive.

These studies indicate that the length of the carbon chain may be more important for analgesic activity than the presence of an oxygen ether linkage whether in a ring system or not.

We know that the presence of the 2-hydroxy group in the B.D.H. 200 molecule is important for its analgesic properties. The relative subcutaneous activities of B.D.H. 200 and B.D.H. 3022, the phenoxypropyl analogue, to pethidine are recorded in Table VIII where B.D.H. 200 is more than twice as active as B.D.H. 3022.

The thirty-six fold fall in analgesic activity of B.D.H. 200 from subcutaneous to oral administration in mice cannot be explained entirely by poor alimentary absorption as the corresponding fall in toxicity is less than three. In contrast the decrease in toxicity and analgesic activity of pethidine from subcutaneous to oral administration is similar, the oral LD50 and ED50 values being approximately two-thirds of the subcutaneous values.

A marked decrease in analgesic activity after oral compared to subcutaneous administration[10,15] has been reported by others. With the *N*-phenylethyl and the *N*-2-hydroxy-2-phenylethyl analogues of norpethidine, the decrease in oral activity is about the same as B.D.H. 200[10].

The marked decrease in activity of B.D.H. 200 on oral administration compared to pethidine can possibly be explained by differences in metabolism. Table IX demonstrates an increase in oral analgesic activity of B.D.H. 200 in mice after pre-treatment with iproniazid and a decrease after pre-treatment with BAL. Neither of these compounds modified the responses to subcutaneous administration.

After this work had been completed the following papers dealing with the analgesic activity of norpethidine derivatives were published. Janssen and Eddy, *J. med. pharm. Chem.*, 1960, 2, 31. Blair and Stephenson, *Brit. J. Pharmacol.*, 1960, 15, 247. Lister, *ibid.*, 1960, **15**, 254.

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THE USE OF *S*-ALKYL-*N*-PHENYLTHIURONIUM PICRATES, STYPHNATES AND PICROLONATES FOR THE CHARACTERISATION OF ALKYL HALIDES

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A series of *S*-alkyl-*N*-phenylthiuronium picrates, styphnates and picrolonates has been prepared and the melting points determined. By use of these derivatives it is possible to identify any of the lower primary and secondary alkyl halides examined. This has not previously been possible with *S*-alkylthiuronium salts. The reaction between tertiary alkyl halides and thiourea has been re-examined.

Primary and Secondary Alkyl Halides

S-Alkylthiuronium picrates are well known as derivatives for characterising primary and secondary alkyl halides[1,2]. However, it was pointed out by Schotte[3] that the usefulness of these derivatives is limited by the fact that derivatives of different homologues have the same or similar melting points. In an attempt to improve the analytical usefulness of the reaction, Schotte[3] prepared the *S*-alkylthiuronium styphnates of many of the lower primary and secondary alkyl halides. While the combination of melting points of the picrate and the styphnate increased the usefulness of the method, it did not permit unambiguous indentification of alkyl halides (Table I). Schotte[3] also examined 2,4-dinitrophenol and 2,4-dinitroresorcinol, but reported that they did not form crystalline salts with *S*-alkylthiuronium halides. Other acidic precipitating reagents which have been examined[4] are picrolonic acid, 3,5-dinitrobenzoic acid, *p*-toluenesulphonic acid, perchloric acid, oxalic acid and nitric acid. Jurecek[6] selected 3,5-dinitrobenzoic acid as the most satisfactory and he reported the melting points of a series of *S*-alkylthiuronium 3,5-dinitrobenzoates. But the situation is not completely satisfactory (Table I).

We have now examined 3-nitrobenzoic acid, hexanitrodiphenylamine, flavionic acid, two sulphonic acids of fairly high molecular weight, R acid and H acid, and ammonium reineckate, as well as the acids listed above. From this work picric acid, styphnic acid and picrolonic acid were selected as the best precipitating agents. (For a detailed discussion of each precipitating agent see Baker[5]). A series of *S*-alkylthiuronium picrolonates was prepared and the melting points determined (Table I), in the vain hope that a combination of the melting points of *S*-alkylthiuronium picrate, styphnate and picrolonate would allow unambiguous identification.

A second method by which the general reaction may be modified is to replace the thiourea by a thiourea substituted on the nitrogen and determine the melting points of their picrates, styphnates and picrolonates. *N*-Phenylthiourea, *N*-ethylthiourea, 1,3-diphenylthiourea and 1,3-di-n-butylthiourea have been examined for their suitability for characterising alkyl halides. Both the disubstituted thioureas and *N*-ethylthiourea yielded thiuronium derivatives which had low melting points; several of

the picrates and styphnates were oils which could not be induced to crystallise. *N*-Phenylthiourea condensed smoothly with alkyl halides and produced *S*-alkyl-*N*-phenylthiouronium salts whose picrates, styphnates and picrolonates were readily crystallisable and had sharp melting points. The melting points were scattered over a wide range of temperature (Table II) and in every example derivatives were obtained by which it is possible to identify an alkyl halide without ambiguity. It has been reported[2] that *N*-phenylthiourea does not react with branched chain alkyl halides; this has now been shown to be incorrect.

TABLE I

MELTING POINTS OF *S*-ALKYLTHIURONIUM PICRATES, STYPHNATES, PICROLONATES AND 3,5-DINITROBENZOATES. THE *S*-ALKYLTHIURONIUM PICROLONATES WERE PREPARED BY THE AUTHORS

Radical	Melting point °C			
	Picrate	Styphnate	Picrolonate	3,5-Dinitrobenzoate
Methyl	224[3]	226[3] (decomp.)	243	205–206[6]
Ethyl	188	179	224	118
n-Propyl	177	161	221	176
Isopropyl	196	187	212	184
n-Butyl	177	164	197 (decomp.)	169
Isobutyl	167	150	127	158
s-Butyl	166	163	184	176
n-Pentyl	155	147	200	156
Isopentyl	173	159	225	—
2-Pentyl	155	112	174	—
3-Pentyl	159	114	177	—
n-Hexyl	157	153	190	—
Isohexyl	147	144	—	—
Heptyl	143	144	191	157–158
Octyl	134	122	191–195	—
Allyl	155	154	209	163
Benzyl	187	190	217	174–175
p-Nitrobenzyl	205	166	218–219 (decomp.)	—
Cetyl	137[1]	—	—	—
Ethylene	260 (267[14] decomp.)	—	—	—
Trimethylene	229	—	—	—
1-Phenylethyl	167	—	—	—
Phenylethyl	139	—	—	—
o-Bromobenzyl	222	—	—	—
m-Bromobenzyl	205	—	—	—
p-Bromobenzyl	219	—	—	—
o-Chlorobenzyl	213	—	—	—
m-Chlorobenzyl	200	—	—	—
p-Chlorobenzyl	194	—	—	—
2-Octyl	131[14]	—	—	—
Nonyl	131	—	—	144
Decyl	137	—	188	145–146
Dodecyl	139	—	—	143
2-Hydroxyethyl	155–156	—	—	—
Pentamethylene	247	—	—	—
Hexamethylene	208	—	—	—
Octamethylene	214	—	—	—
Nonamethylene	193	—	—	—

Both the nature of the halide and of the alkyl radical influence the time required for any particular alkyl halide to react with thiourea and different times of reaction have been recommended for chlorides, bromides and iodides[1,3]. To rationalise the method, a number of solvents and conditions have been investigated. Ethanol (absolute, 95 and 50 per cent) and acetone (80 and 50 per cent) were used for refluxing times varying from 5 minutes to 3 hours. Thiourea and *N*-phenylthiourea were found to react with all primary and secondary alkyl bromides and iodides examined when they were refluxed with any alkyl bromide or iodide in

ethanol (50 per cent) for 1 hour. The reaction with alkyl chlorides is slower, but may be accelerated by adding sodium iodide to the solution.

Tertiary Alkyl Halides

Levy and Campbell[1] reported that the *S*-ethylthiuronium salt was obtained when t-butyl iodide was refluxed with thiourea in ethanol as solvent. It was suggested that the t-butyl iodide reacted with ethanol to form ethyl iodide which then condensed with thiourea. This anomalous reaction of tertiary alkyl halides has since been quoted in books on organic analysis[8,9], even though the evidence is based on only two observations. It has been generally regarded that thiourea will not react with

TABLE II

MELTING POINTS OF *S*-ALKYL-*N*-PHENYLTHIURONIUM PICRATES, STYPHNATES AND PICROLONATES PREPARED AS DESCRIBED

Radical	Melting point °C		
	Picrate	Styphnate	Picrolonate
Methyl	179	205 (decomp.)	212 (decomp.)
Ethyl	198	176	206
n-Propyl	169	144	159
n-Butyl	144	125	154
n-Pentyl	142	131	173
n-Hexyl	128	112	143
n-Heptyl	127	112	163
n-Octyl	130	100	153
n-Nonyl	165	153	150
n-Decyl	128	97	94
s-Propyl	181	155	196–197
Isobutyl	134	105	160
s-Butyl	149	131	178
Isopentyl	153	149	165
2-Pentyl	130	86–87	151
3-Pentyl	137	84–86	145
Isohexyl	126	113	130–131 (decomp.)
Cetyl	113	101	102
Allyl	157	146	183
1-But-3-enyl	134	129	170
1-Pent-4-enyl	138	124	154
Benzyl	147	143	194 (decomp.)
p-Nitrobenzyl	195	162	192 (decomp.)

t-alkyl halides. However, Schotte[3] was able to prepare *S*-t-alkylthiuronium salts by condensing a t-alkyl halide with thiourea using the corresponding tertiary alcohol as solvent. Later Schotte and Veibel[10] obtained *S*-t-alkylthiuronium salts using aqueous ethanol (35 per cent) as solvent. The reactions between t-alkyl halides and thiourea and *N*-phenylthiourea have now been examined. T-alkyl halides will react with thiourea to produce the *t*-alkylthiuronium salts in aqueous ethanol, dioxan or acetone. The latter two solvents are better because there is no possibility of an abnormal product being formed and no solubility problems. Schotte and Veibel[10] found that higher molecular weight t-alkyl halides were only slightly soluble in aqueous ethanol (35 per cent) with the consequence that the reaction was slow. No product was obtained when t-alkyl halides were refluxed with *N*-phenylthiourea in a number of solvents for 12 hours.

With the failure of *N*-phenylthiourea to react with t-alkyl halides, the possibility was considered of preparing *S*-alkyl-*N*-phenylthiuronium salts

directly from alcohols by refluxing together an alcohol, a t-alkyl halide and *N*-phenylthiourea with either dioxan or acetone as solvent if required. With methanol, ethanol and propanol *S*-methyl-, *S*-ethyl- and *S*- propyl-*N*-phenylthiuronium picrates, respectively, have been prepared by this method.

Other applications of thiuronium and N-*phenylthiuronium salts.* Alkoxyl groups have been identified by conversion with hydrogen iodide to the alkyl iodides which were then characterised as the thiuronium picrates[6,12].

Analysis of S-*alkyl*-N-*phenylthiuronium picrates, styphnates and picrolonates.* A spectrophotometric method has been developed for the determination of the picrate, styphnate and picrolonate ions present in the respective *S*-alkyl-*N*-phenylthiuronium salts[13].

EXPERIMENTAL

Melting points. All melting points are corrected and were taken on a Kofler block.

Materials

All alkyl halides, except pent-3-yl bromide and isohexyl bromide, were obtained commercially and redistilled before use.

TABLE III

ANALYSIS OF *S*-ALKYL-*N*-PHENYLTHIURONIUM PICRATES, STYPHNATES AND PICROLONATES

	Picrate				Styphnate				Picrolonate			
	Found		Required		Found		Required		Found		Required	
	C	H	C	H	C	H	C	H	C	H	C	H
Methyl	42·6	3·41	42·5	3·29	40·6	3·25	40·9	3·16	50·2	4·52	50·0	4·63
Ethyl	44·2	3·8	44·0	3·67	42·2	3·38	42·3	3·53	51·1	4·9	51·1	4·93
n-Propyl	45·3	3·91	45·4	4·02	43·6	3·95	43·7	3·87	52·1	5·1	52·1	5·21
n-Butyl	46·5	4·36	46·7	4·35	45·2	4·0	45·0	4·19	53·0	5·4	53·1	5·49
n-Pentyl	48·2	4·63	47·9	4·66	46·4	4·6	46·3	4·5	54·1	5·69	54·1	5·74
n-Hexyl	49·0	4·8	49·0	4·95	47·2	4·66	47·4	4·78	55·0	5·82	55·0	5·98
n-Heptyl	50·1	5·4	50·1	5·22	48·7	5·01	48·5	5·05	55·8	6·0	55·8	6·2
n-Octyl	51·3	5·4	51·1	5·48	49·5	5·2	49·5	5·3	56·7	6·3	56·6	6·41
n-Nonyl	51·9	5·8	52·0	5·71	50·5	5·4	50·5	5·54	57·3	6·54	57·4	6·62
n-Decyl	53·1	5·82	53·0	5·95	51·6	5·76	51·4	5·77	58·3	6·64	58·1	6·81
s-Propyl	45·6	3·98	45·4	4·02	43·8	5·8	43·7	5·87	51·9	4·97	52·1	5·21
Isobutyl	46·5	4·26	46·7	4·35	45·1	4·38	45·0	4·19	52·9	5·4	53·1	5·49
s-Butyl	46·7	4·49	46·7	4·35	45·0	4·21	45·0	4·19	53·3	5·35	53·1	4·49
Isopentyl	48·0	4·46	47·9	4·66	46·1	4·54	46·3	4·5	54·4	5·61	54·1	5·74
2-Pentyl	47·7	4·84	47·9	4·66	46·5	4·32	46·3	4·5	54·2	5·63	54·1	5·74
3-Pentyl	47·6	4·71	47·9	4·66	46·4	4·5	46·3	4·5	54·1	5·65	54·1	5·74
Isohexyl	48·9	4·87	49·0	4·95	47·6	4·6	47·4	4·78	55·0	5·91	55·0	5·98
Cetyl	57·5	7·23	57·5	7·11	56·3	6·93	56·0	6·93	61·8	7·76	61·7	7·79
Allyl	45·5	3·58	45·6	3·56	44·0	3·46	43·9	3·44	52·5	4·61	52·4	4·8
1-But-3-enyl	47·0	4·1	46·9	3·91	45·0	3·68	45·2	3·77	53·7	4·99	53·4	5·09
1-Pent-4-enyl	47·9	4·13	48·1	4·23	46·3	3·97	46·4	4·09	54·4	5·15	54·3	5·35
Benzyl	50·9	3·5	51·0	3·61	49·1	3·7	49·3	3·49	56·8	4·66	56·7	4·72
p-Nitrobenzyl	46·7	3·2	46·5	3·1	45·1	2·87	45·1	3·01	52·2	3·9	52·1	4·16

Pent-3-yl bromide. Diethyl ketone was reduced by the Meerwein-Ponndorf-Verly reduction to give pentan-3-ol b.p. 114–117°. Pentan-3-ol was treated with hydrobromic acid and sulphuric acid. Pent-3-yl bromide b.p. 116–119° was collected.

Isohexyl bromide. Isohexanol was treated with potassium bromide and sulphuric acid. Isohexyl bromide b.p. 138–141° was collected.

Thiourea and all *N*-substituted thioureas were obtained commercially. Thiourea was recrystallised from aqueous ethanol m.p. 176°. *N*-Phenylthiourea was recrystallised from aqueous ethanol m.p. 153°. All the precipitating reagents except styphnic acid were obtained commercially. Picric acid m.p. 122° and picrolonic acid m.p. 118° (decomp.) were recrystallised from ethanol before use. A few samples of picrolonic acid were found to contain a dark yellow crystalline impurity which decomposed between 220–250°. This can be removed by crystallisation. Styphnic acid was prepared by the method of Merz and Zetter[11] by nitration of resorcinol. Recrystallisation from ethanol gave yellow crystals m.p. 175° (Lit.[11] 175°).

Standard procedure for preparing S-*alkyl*-N-*phenylthiuronium picrates, styphnates and picrolonates.* *N*-Phenylthiourea (1 g.) and the alkyl halide

TABLE IV

ANALYSIS OF *S*-ALKYLTHIURONIUM PICROLONATES

Radicals	Found		Required	
	C	H	C	H
Methyl	40·3	4·6	40·4	4·5
Ethyl	42·1	4·81	42·15	4·86
n-Propyl	43·9	5·27	43·8	5·21
n-Butyl	45·2	5·6	45·2	5·53
n-Pentyl	46·6	5·8	46·6	5·82
n-Hexyl	47·7	6·07	47·9	6·1
n-Heptyl	49·2	6·32	49·1	6·36
n-Octyl	50·0	6·7	50·2	6·6
n-Decyl	52·2	7·12	52·3	7·06
s-Propyl	43·6	5·2	43·8	5·21
Isobutyl	45·3	5·51	45·2	5·53
s-Butyl	45·3	5·46	45·2	5·53
Isopentyl	46·6	5·8	46·6	5·82
2-Pentyl	46·4	5·71	46·6	5·82
3-Pentyl	46·5	5·78	46·6	5·82
Allyl	43·7	4·75	44·0	4·71
Benzyl	50·1	4·58	50·0	4·63
p-Nitrobenzyl	45·4	4·01	45·3	3·99

(1 g.) were dissolved in turn in ethanol (10 ml. 50 per cent). The solution was refluxed for 1 hour and then divided into three aliquots.

Picrate. One aliquot was poured into a saturated aqueous solution of picric acid (25 ml.). The picrate was allowed to crystallise for half an hour then collected, washed and recrystallised from aqueous ethanol (50 per cent).

Styphnate. Styphnic acid (0·3 g.) was added to a second aliquot and the solution brought to the boil. Sufficient ethanol (50 per cent) was added dropwise to bring the styphnic acid into solution. The solution was allowed to cool when the *S*-alkyl-*N*-phenylthiuronium styphnate crystallised out. It was collected, washed and recrystallised from ethanol (50 per cent).

Picrolonate. The third aliquot was treated with picrolonic acid (0·3 g.) as described for styphnate.

Thiuronium picrates, styphnates and picrolonates were prepared by a similar procedure substituting thiourea for *N*-phenylthiourea.

S-*T-butylthiouronium picrate, styphnate and picrolonate.* T-butyl chloride (1·5 ml.) and thiourea (1 g.) were dissolved in dioxan (15 ml.) and water

(1 ml.) and refluxed for 2 hours. The picrate, styphnate and picrolonate were prepared as described above. *S*-T-butylthiuronium picrate m.p. 150·5° (Lit.[10] 151°). Found: C, 37·0; H, 4·3; N, 19·35. Calc. for $C_{11}H_{15}O_7N_5S$; C, 36·56; H, 4·15; N, 19·4.

S-T-butylthiuronium styphnate m.p. 149°—150°. $C_{11}H_{15}O_8N_5S$ requires N, 18·56. Found: N, 18·3.

S-T-butylthiuronium picrolonate m.p. 178°—179° (decomp.). $C_{15}H_{20}O_5N_6S$ requires N, 21·2. Found: N, 21·0.

Identical products were obtained when the reaction was repeated using (*a*) ethanol (95 per cent), (*b*) ethanol (50 per cent) and (*c*) acetone (very low yield).

Attempted preparation of S-*t-butyl*-N-*phenylthiuronium picrate, styphnate and picrolonate.* *N*-Phenylthiourea (1 g.) and t-butyl bromide (1 ml.) were dissolved in ethanol (10 ml.) (95 per cent) and the solution refluxed for 2 hours. The three salts were prepared as described above.

Picrate m.p. 198°, styphnate m.p. 176°, picrolonate m.p. 205–206°. These all correspond to the *S*-ethyl-*N*-phenylthiuronium salts (see Table II). *S*-Ethyl-*N*-phenylthiuronium picrate $C_{15}H_{15}O_7N_5S$ requires N, 17·11. Found N, 16·95.

The above experiment was repeated using (*a*) dioxan, (*b*) acetone, (*c*) formamide, (*d*) dimethylformamide as solvent and reaction times of up to 12 hours, but in no case was any product obtained. *S*-Methyl-*N*-phenylthiuronium picrate m.p. 179° and *S*-*n*-propyl-*N*-phenylthiuronium picrate m.p. 169° were obtained when the ethanol was replaced by methanol and n-propanol respectively in the above reaction. No product was obtained with butanol or isopropanol.

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AN ULTRA-VIOLET SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF PICRATE, STYPHNATE AND PICROLONATE IN *S*-ALKYL-*N*-PHENYLTHIURONIUM SALTS

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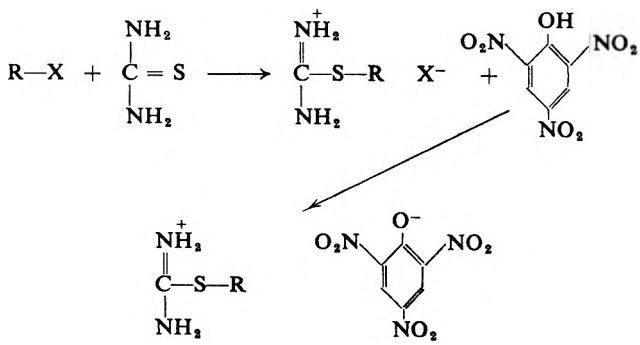
By selecting suitable wavelengths to measure the extinction, it has been found possible to determine the percentage of picrate, styphnate and picrolonate in *S*-alkyl-*N*-phenylthiuronium salts in ethanol solution by an ultra-violet spectrophotometric method. The method is rapid and accurate to ± 1 per cent.

In a previous communication[1] we reported that *S*-alkyl-*N*-phenylthiuronium picrates, styphnates and picrolonates were useful as derivatives for the characterisation of alkyl halides. It has been shown previously[2] that alkyl halides react with thiourea to form *S*-alkylthiuronium halides which precipitate as *S*-alkylthiuronium picrates with picric acid, Scheme 1.



Scheme 1

Elemental analysis of the *S*-alkyl-*N*-phenylthiuronium picrates, styphnates and picrolonates[1] suggested that these salts were of the same type as the *S*-alkylthiuronium picrates. To check the composition of all the salts which were prepared and to widen the analytical scope of the method, we have developed a spectrophotometric method of determining the picrate, styphnate and picrolonate content of the respective *S*-alkyl-*N*-phenylthiuronium salts.

N-Phenylthiourea does not absorb in the wavelength region 350 to 400 mμ whereas picric acid absorbs strongly in this region. The total absorption of *S*-alkyl-*N*-phenylthiuronium picrate in the region 350 to 400 mμ will therefore be due to the picrate ion only. An application of this principle to the determination of the molecular weight of amine picrates has been reported[3]. Since both styphnic acid and picrolonic acid absorb in the region 350 to 400 mμ, then the principle should be applicable to *S*-alkyl-*N*-phenylthiuronium styphnates and picrolonates.

Experimental

Apparatus

A Hilgar and Watts Uvispek H.700.304 equipped with a quartz prism was used. Matched pairs of silica cuvettes of 1 cm. optical path length

were used for the determination of absorption spectra (for readings below 360 mμ). Matched pairs of glass cuvettes of 1 cm. optical path length were used for the assay readings above 360 mμ.

Melting points were taken on a Kofler block and are corrected.

Material

Solvent. Ethanol (95 per cent) which complied with Appendix IV H of the British Pharmacopoeia 1958 was used. Picric acid, styphnic acid and picrolonic acid were of the purity described previously[1]. *N*-Phenylthiourea was of M.A.S. grade (Hopkin and Williams). *S*-Alkyl-*N*-phenylthiuronium picrates, styphnates and picrolonates were prepared as described previously[1].

Piperidine picrate. Piperidine (0·85 g.) and picric acid (2·3 g.) were dissolved in hot ethanol (95 per cent). On cooling, the product crystallised out. The precipitate was collected and recrystallised twice from

TABLE I

ABSORPTION OF *N*-PHENYLTHIOUREA IN ETHANOL (95 PER CENT) (6·44 MG. IN 100 ML. OF SOLUTION). 1 CM. MATCHED SILICA CUVETTES

Wavelength (mμ)	Extinction
400	0·000
385	0·000
380	0·000
370	0·000
360	0·000
350	0·000
340	0·003
330	0·028
320	0·05
310	0·153
300	0·535
290	1·671

ethanol (95 per cent). Yellow crystals m.p. 151° (Lit.[4] 151°). Found: N, 17·8. Calc. for $C_{11}H_{14}O_7N_4$: N, 17·84. The following compounds were all prepared in a similar manner.

cis-2,6-*Dimethylpiperidine picrate* m.p. 165° (Lit.[5] 162—164°). Found: N, 16·3. Calc. for $C_{13}H_{18}O_7N_4$: N, 16·4.

Trimethylamine picrate m.p. 226° (Lit.[4] 225°). Found: N, 19·5. Calc. for $C_9H_{12}O_7N_4$: N, 19·45.

Tropane picrate m.p. 281° (Lit.[6] 281°). Found: C, 47·31; H, 5·101. Calc. for $C_{14}H_{17}O_7N_4$: C, 47·45; H, 5·08.

Piperidine styphnate. Recrystallised twice from ethanol (50 per cent) m.p. 204–206° (decomp.). Found: N, 17·1. Calc. for $C_{11}H_{14}O_8N_4$: N, 17·0.

cis-2,6-*Dimethylpiperidine styphnate.* Recrystallised twice from ethanol (50 per cent) m.p. 208—210° (decomp.). $C_{13}H_{18}O_8N_4$ requires N, 15·6. Found: N, 15·45.

Tropane styphnate. Recrystallised from ethanol (50 per cent) m.p. 266°. Found: N, 15·1. Calc. for $C_{14}H_{18}O_8N_4$: N, 15·14.

Quinine styphnate. Recrystallised from ethanol (95 per cent) m.p. 155—156° (Lit.[6] 154°). Found: N, 12·3. Calc. for $C_{26}H_{27}O_{10}N_5$: N, 12·3.

Tropane picrolonate. Tropane (2 ml.) was poured into a saturated solution of picrolonic acid in ethanol (95 per cent). After thirty minutes the crystals were filtered off and recrystallised from ethanol (95 per cent) m.p. 207°. Found: N, 18·1: Calc. for $C_{18}H_{22}O_5N_5$: N, 18·0.

The following picrolonate salts were similarly prepared.

Diethylamine picrolonate m.p. 256—260° (decomp.). Found: N, 20·5. Calc. for $C_{14}H_{19}O_5N_5$: N, 20·77.

cis-2,6-*Dimethylpiperidine picrolonate* m.p. 262–265° (decomp.). $C_{17}H_{23}O_5N_5$ requires N, 18·57. Found: N, 18·6.

Piperidine picrolonate m.p. 270—273° (decomp.). Found: N, 19·7. Calc. for $C_{15}H_{19}O_5N_5$: N, 19·6.

Measurements

N-*Phenylthiourea.* The extinction of a solution of *N*-phenylthiourea (6·44 mg. in 100 ml. of solution) was determined between the wavelengths 290 to 400 mμ. The results are given in Table I.

Picrate ion. The absorption of a solution of piperidine picrate (1·36 mg. in 100 ml. of solution) was determined between the wavelengths 340 to

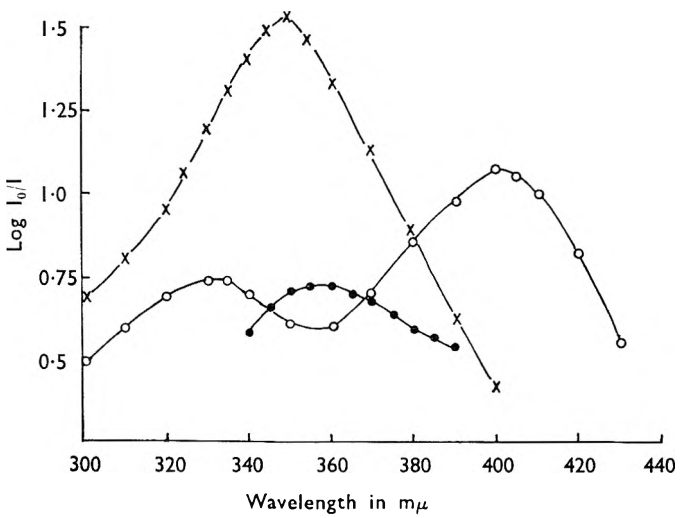


FIG. 1. Absorption spectra of picrate, styphnate and picrolonate in ethanol, 95 per cent. 1 cm. silica cuvettes.
Piperidine picrate, 1·36 mg. in 100 ml. ●—●
Piperidine styphnate, 2·05 mg. in 100 ml. ○—○
Diethylamine picrolonate, 2·124 mg. in 100 ml. ×—×

390 mμ (Fig. 1). The wavelength 385 mμ was selected as suitable for the determination of picrate.

Validity of Beer-Lambert Law for picrate in ethanol. Solutions of different concentrations of an authentic sample of *S-n*-butyl-*N*-phenylthiuronium picrate in ethanol were prepared and the extinction determined at 385 mμ in 1 cm. glass cuvettes (Fig. 2).

Molecular extinction coefficient of picrate at 385 *mμ in ethanol.* Solutions in ethanol were prepared of a number of salts of picric acid. The extinctions of these solutions were determined at 385 mμ using 1 cm. matched glass cuvettes. The molecular extinction coefficient was calculated for each compound and the mean taken for assay purposes (Table II).

Determination of picric content of S-*alkyl*-N-*phenylthiuronium picrates.* A weighed amount of each picrate was dissolved in ethanol (1 to 4 mg.

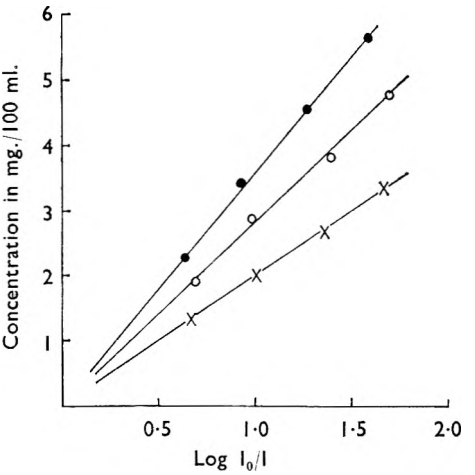


FIG. 2. Verification of Beer-Lambert law for picrate, styphnate and picrolonate in 95 per cent ethanol.

S-n-Butyl-*N*-phenylthiuronium picrate, 385mμ ●—●.
S-n-Pentyl-*N*-phenylthiuronium styphnate, 400 mμ ○—○.
S-n-Pentyl-*N*-phenylthiuronium picrolonate, 350 mμ ×—×.

in 100 ml. of solution). The extinction was determined using 1 cm. glass matched cuvettes at 385 mμ. The precentage picrate was calculated using the following formula.

$$\text{Per cent picrate} = \frac{E \times 228{\cdot}1 \times 100}{\epsilon \times C}$$

where 228·1 = ionic weight of picrate ion
E = Extinction of solution
ϵ = Molecular extinction coefficient of picrate
C = Concentration of picrate in g./l.

A list of values obtained is given in Table III.

Styphnate ion. The absorption of a solution of piperidine styphnate (2·05 mg. in 100 ml. of solution) was determined between 300 to 440 mμ (Fig. 1). The wavelength 400 mμ was selected as the most suitable for the determination of styphnate.

Validity of Beer-Lambert Law for styphnate ion in ethanol. Solutions of different concentrations of an authentic sample of *S-n*-pentyl-*N*-phenylthiuronium styphnate in ethanol (95 per cent) were prepared and the extinction determined at 400 mμ in a 1 cm. glass cuvette (Fig. 2).

Molecular extinction coefficient of styphnate at 400 *mμ in ethanol.* Solutions in ethanol were prepared of a number of known styphnate salts. The extinctions of these solutions were determined at 400 mμ using 1 cm. matched glass cuvettes. The molecular extinction coefficient was calculated for each compound and the mean taken for assay purposes (Table II).

Determination of styphnate content of S-*alkyl*-N-*phenylthiuronium styphnates.* A weighed amount of each styphnate was dissolved in ethanol (1 to 4 mg. in 100 ml. of solution). The extinction was deter-

TABLE II

MOLECULAR EXTINCTION COEFFICIENTS OF PICRATE, STYPHNATE AND PICROLONATE IN ETHANOL (95 PER CENT)

Compound	Molecular extinction coefficient
Picrate—385 *mμ*	
Picric acid	12,310
Piperidine picrate	12,280
2,6-Dimethylpiperidine picrate	12,340
Tropane picrate	12,230
Trimethylamine picrate	12,310
Mean	12,300
Styphnate—400 *mμ*	
Styphnic acid	16,990
Piperidine styphnate	17,000
Tropane styphnate	16,900
2,6-Dimethylpiperidine styphnate	16,950
Quinine styphnate	16,930
Mean	16,950
Picrolonate—350 *mμ*	
Picrolonic acid	24,190
Tropane picrolonate	24,140
Diethylamine picrolonate	24,160
Piperidine picrolonate	24,200
2,6-Dimethylpiperidine picrolonate	24,190
Mean	24,170

mined using 1 cm. glass matched cuvettes at 400 mμ. The percentage styphnate was calculated using the formula given for picrate, but the molecular weight of the styphnate ion (244) was substituted for that of the picrate ion. A list of results is given in Table III.

Picrolonate ion. The absorption of a solution of diethylamine picrolonate (2·124 mg. in 100 ml. of solution) was determined between 300 to 420 mμ (Fig. 1). The wavelength 350 mμ was selected as the most suitable for the determination of picrolonate.

Validity of Beer-Lambert Law for the picrolonate ion in ethanol. Solutions of different concentrations of an authentic sample of *S-n*-pentyl-*N*-phenylthiuronium picrolonate in ethanol were prepared and the extinctions determined at 350 mμ in a 1 cm. silica cuvette (Fig. 1).

Molecular extinction coefficient of picrolonate at 350 *mμ in ethanol.* Solutions in ethanol were prepared of a number of salts of picrolonic acid. The extinctions of these solutions were determined at 350 mμ using 1 cm. matched silica cuvettes. The molecular extinction coefficient was calculated for each compound and the mean taken for assay purposes (Table II).

Determination of picrolonate content of S-*alkyl*-N-*phenylthiuronium picrolonates.* A weighed amount of each picrolonate was dissolved in ethanol (1 to 2·5 mg. in 100 ml. of solution). The extinction was determined using 1 cm. silica matched cuvettes at 350 mμ. The percentage picrolonate was calculated and is given in Table III, using the formula given above but substituting the molecular weight of the picrolonate ion (263) for that of the picrate ion.

TABLE III

DETERMINATION OF PICRATE, STYPHNATE AND PICROLONATE IN *S*-ALKYL-*N*-PHENYLTHIURONIUM PICRATES, STYPHNATES AND PICROLONATES*

Compound	Picrate		Styphnate		Picrolonate	
	Calc.	Found	Calc.	Found	Calc.	Found
Methyl	57·71	57·23	59·34	59·55	61·15	60·84
Ethyl	55·73	55·50	57·42	57·46	59·21	59·33
n-Propyl	53·89	54·36	55·55	55·12	57·41	57·45
n-Butyl	52·14	51·88	53·84	54·06	55·75	55·44
n-Pentyl	50·53	50·27	52·22	52·20	54·12	53·96
n-Hexyl	49·00	50·07	50·70	50·74	52·59	53·01
n-Heptyl	47·57	48·03	49·26	48·71	51·16	51·05
n-Octyl	46·22	46·78	47·92	48·76	49·80	49·72
n-Nonyl	44·95	45·68	46·63	46·49	48·52	48·67
n-Decyl	43·75	43·22	45·51	45·32	47·30	47·11
Allyl	54·14	54·01	55·81	55·98	57·66	57·51
1-But-3-enyl	52·40	52·38	54·08	53·51	55·95	56·03
1-Pent-4-enyl	50·76	50·71	52·43	52·47	54·39	54·40
Benzyl	48·28	48·83	49·97	50·02	51·87	51·40
p-Nitrobenzyl	44·08	44·84	45·76	45·93	47·64	47·66
Cetyl	37·66	37·31	39·27	39·01	41·09	41·26
s-Propyl	53·89	53·18	55·55	55·81	57·41	57·69
s-Butyl	52·14	52·19	53·84	54·26	55·75	55·50
Isobutyl	52·14	52·89	53·84	53·46	55·75	55·68
2-Pentyl	50·53	49·99	52·22	51·78	54·12	53·96
3-Pentyl	50·53	50·51	52·22	51·50	54·12	54·46
Isopentyl	50·53	50·89	52·22	51·99	54·12	54·46
Isohexyl	49·00	48·63	50·70	51·25	52·59	52·67

* Picrate λ = 385 mμ, 95 per cent ethanol, 1 cm. glass cuvettes.
Styphnate λ = 400 mμ, 95 per cent ethanol, 1 cm. glass cuvettes.
Picrolonate λ = 350 mμ, 95 per cent ethanol, 1 cm. silica cuvettes.

DISCUSSION

The ultra-violet spectrophotometric determination of picrates, styphnates and picrolonates is a quick and accurate method. The results (Table III) indicate that the *S*-alkyl-*N*-phenylthiuronium salts consisted of one mole of *S*-alkyl-*N*-phenylthiuronium ion and one mole of picrate, styphnate or picrolonate. The method is not limited to the determinations of these ions only, but could be used for the determination of the molecular weight of a cation of a picrate, styphnate or picrolonate provided that the molar relation between the cation and the acid was known and that the cation did not absorb at the wavelengths at which the determinations were made. Since milligram quantities of the salts are used for the spectrophotometric determination, then it is possible to use this method for the determination of molecular weights on a micro scale.

Acknowledgements. One of us, W. A. Baker, wishes to thank the Pharmaceutical Society for a research grant.

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ANATOMICAL STUDIES IN THE GENUS *RUBUS**

PART III. THE ANATOMY OF THE LEAF OF *Rubus loganobaccus* L. H. BAILEY

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The anatomy of the leaf of *Rubus loganobaccus* L. H. Bailey, in the whole and powdered condition, is described and illustrated. A summary of the anatomical characters is given, and the anatomical features which distinguish leaves of *R. loganobaccus* from those of *R. idaeus* L. and *R. fruticosus* L.[1,2] are briefly discussed.

No reference has appeared to date concerning the use of loganberry leaves in medicine, but the plant is closely allied to the raspberry and blackberry, and its leaves resemble those of these plants. Moreover, loganberries have become a well-known article of commerce, and the plants are frequently cultivated near to raspberry and blackberry crops. The present investigation was therefore undertaken to show the anatomical characters by which loganberry leaves may be distinguished.

The loganberry plant originated in 1881 by hybridisation between *R. idaeus* ssp. *strigosus* (Michx.) Focke, an American species of red raspberry, and *R. vitifolius* Cham. & Schlecht, the Californian dewberry[3,4]. It is a hexaploid, now behaving as a species, and was introduced into this country about 1900. Warburg[4] includes it, with *R. idaeus* L., in the subgenus IDEOBATUS.

MATERIAL

The material investigated consisted of the leaves of *R. loganobaccus* cultivated at the Gardens of the Royal Horticultural Society, Wisley, Surrey.

METHODS OF INVESTIGATION

A brief treatment with warm chloral hydrate solution sufficed to show the epidermises in surface view, but it was profitable to shave off the hairs before examining the lower epidermis, and also to soak the pieces of lamina in ether for several hours before testing for lignin with phloroglucin and hydrochloric acid or aniline sulphate solution. The hairs of the upper epidermis gave a positive reaction for lignin, but some of the hairs forming the tomentum on the lower epidermis of the lamina gave a positive reaction only after the above treatment, or alternatively, after preliminary heating in chloral hydrate solution. Other histological methods used were similar to those described earlier[2].

* The subject matter of this communication forms part of a thesis by one of us (K.R.F.) accepted by the University of London for the degree of Doctor of Philosophy.

Anatomical Structure

The leaf of *R. loganobaccus* is imparipinnately compound; there are usually five leaflets, lateral leaflets being sessile on the rachis. Paired linear stipules are adnate at the lower end of the rachis (Fig. 1, *A*; Fig. 5, *A* and Fig. 6, *A*).

(*a*) Leaflets

No anatomical differences were detected between lateral and terminal leaflets; the following description, therefore, applies to either of these.

(i) *Lamina, interneural region* (Fig. 1, *C* and *D*; Fig. 2, *A*, *B*, C and *D*; Fig. 3, *C*; Fig. 4, *A*).

The Upper Epidermis is covered with a fairly thick, smooth cuticle and consists of a layer of polygonal cells having wavy, well-beaded, anticlinal walls; they measure* about H 20 to 24μ and Lev L and B 16 to 68 μ. Stomata are absent, but numerous, prominent, oval *hydathodes*, about 22 to 30 μ long and 14 to 20 μ wide, are present on each of the marginal teeth (Fig. 1, *C*). *Covering trichomes* occur frequently; they arise over or in close proximity to the veins and around the edges of the marginal teeth (Fig. 1, *B*; Fig. 2, *C*). They are unicellular, with thick, lignified walls, tapering and acutely pointed, with heavily thickened bases exhibiting well-marked linear pits; they measure about 180 to 1,400 μ long and 15 to 42 μ wide at the base. The lumen is wide in the basal part of the trichome, but narrows gradually, often becoming obliterated about one-third of the way along the trichome. The bases are surrounded by about 8 to 10 radiating epidermal cells; these radiating cells frequently show a striated cuticle.

The Mesophyll is well differentiated. The *palisade* is double, with the upper layer very slightly more well-formed than the lower. Both layers are continuous when seen in thin transverse sections, and consist of cylindrical cells measuring about H 20 to 35 μ, Lev 4 to 10 μ in the upper layer, and H 16 to 30 μ, Lev 4 to 10 μ in the lower. The cells of both layers contain chloroplasts, about 4 to 6 μ in diameter. Scattered irregularly in both the palisade and the spongy mesophyll (more commonly in the latter) are occasional rounded idioblasts containing well-defined cluster crystals of *calcium oxalate*, about 14 to 36 μ in diameter; there is no well-defined crystal layer between the palisade and the spongy mesophyll. The *spongy mesophyll* consists of about 2 to 4 layers of cells, which in surface view are rounded, elongated, triangular or trabeculate and measure about H 8 to 16 μ and Lev 10 to 30 μ; they contain chloroplasts, about 4 to 6 μ in diameter; numerous air-spaces occur (Fig. 3, *C*; Fig. 4, *A*).

The Lower Epidermis has a smooth, thin cuticle. Its cells have wavy, slightly beaded walls about H 8 to 14 μ, Lev L and B 20 to 60 μ. *Stomata* are very numerous, and, when the hairs are shaved off, are seen to be of

* The symbols H, Lev, Lev L and Lev B are suggested for the purpose of describing organs showing bilateral symmetry by Moll and Janssonius. The symbol H = height, in a direction perpendicular to the surface of the organ; Lev = in the direction of the surface of the organ; Lev L and Lev B = parallel to the surface and at the same time in a longitudinal or transverse direction respectively.

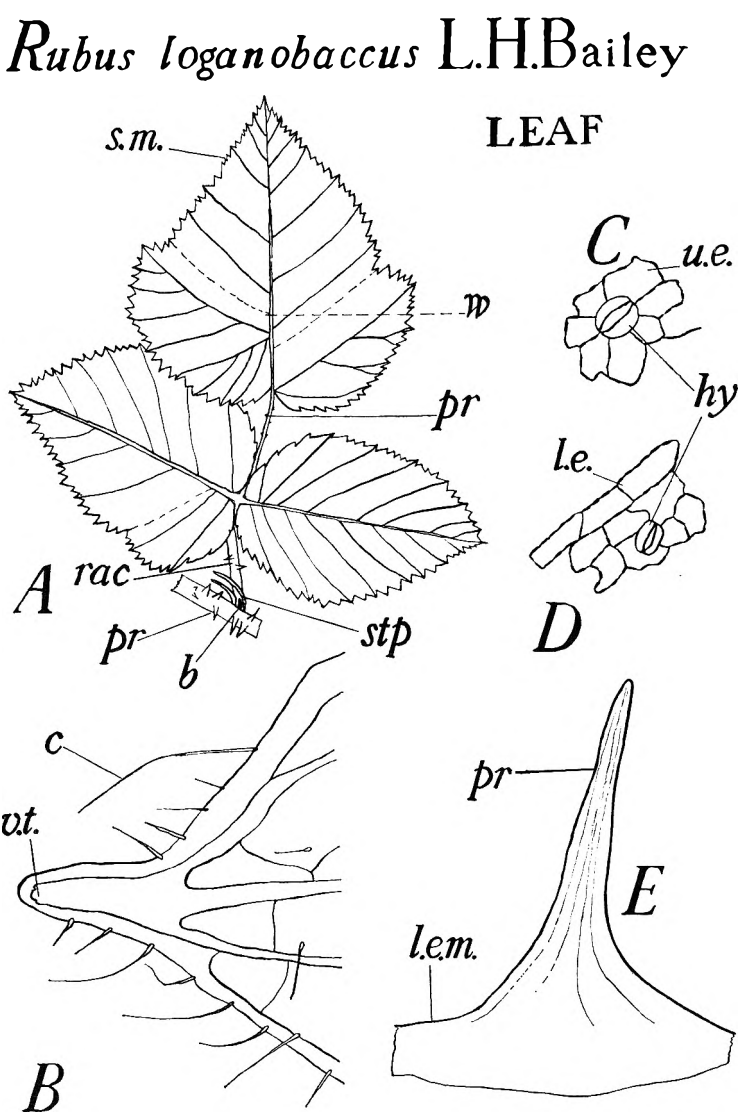

FIG. 1. Leaf of *Rubus loganobaccus* L. H. Bailey. *A*, complete, compound leaf with terminal and two lateral leaflets and paired stipules adnate to the rachis. *B*, marginal tooth of leaflet. *C*, hydathode on upper epidermis of marginal tooth of leaflet. *D*, hydathode on lower epidermis of marginal tooth of leaflet. *E*, prickle. *A*, x $\frac{1}{3}$; *B*, x 33; *C* and *D*, x 200; *E*, x 18. *b*, bud; *c*, covering trichome; *hy*, hydrathode; *l.e.*, lower epidermis of marginal tooth; *l.e.m.*, lower epidermis of midrib; *pr*, prickle; *rac*, rachis; *s.m.*, serrate margin; *stp*, stipule; *u.e.*, upper epidermis of marginal tooth; *v.t.*, vein termination; *w*, position at which transverse section in Fig. 3, *A* was made.

the anomocytic type and raised very slightly above the level of the epidermis; they exhibit prominent ostioles, are oval in outline and measure about 23 to 30 μ long and 18 to 22 μ wide (Fig. 2, *B*). Occasional oval *hydrathodes*, about 24 to 30 μ long and 18 to 22 μ wide, occur on the lower epidermis of the marginal teeth (Fig. 1, *D*). Long *covering trichomes* are present in great numbers; they form a virtual tomentum which is not so dense, however, as that observed on the leaf of *R. idaeus*. They are unicellular, lignified (but only stain satisfactorily after preliminary treatment of the lamina with ether or chloral hydrate solution), are commonly 300 to 700 μ long and 12 to 30 μ wide at the base, the lumen is sometimes obliterated in the upper two-thirds of the hair and they are curled, and to some extent, intertwined with each other; the bases are thickened and slightly pitted and the apices are bluntly pointed; spiral markings were not observed (Fig. 2, *B* and *D*).

The lamina has a coarsely dentate-serrate margin, individual teeth being acutely pointed. The minute ends of the secondary and tertiary veins extend to within about 60 μ of the teeth apices and terminate in a few, very small, spiral elements. Two fine veinlets, one on either side, converge towards the central veinlet and unite with it about 0·6 mm. from the tip of the tooth (Fig. 1, *A* and *B*).

(ii) *Midrib* (Fig. 2, *E* and *F*; Fig. 3, *A*, *B* and *D*; Fig. 4, *B* and *C*).

The midrib has a typically dicotyledonous structure; one significant variation was noted in transverse sections cut serially from apex to base in most (but not all) leaflets examined, namely that groups of pericyclic fibres appear in sections cut between the base of the leaflet and a point which varied from about one-quarter to about one-third of the way up the midrib (Fig. 3, *A* and *B*).

The UPPER EPIDERMIS consists of a single layer of elongated, slightly beaded, well-cuticularised, straight-walled cells measuring about H 14 to 24 μ, Lev B 8 to 30 μ, and Lev L 16 to 60 μ; stomata are absent (Fig. 2, *E*; Fig. 3, *D*). *Covering trichomes* are present in moderate numbers, and are similar to those of the interneural epidermis. Occasional *glandular trichomes* about 100 to 180 μ long occur on the upper epidermis of both midrib and secondary veins; they comprise a multicellular, biseriate stalk about 6 cells long, and a multicellular, subspherical, glandular head about 60 to 70 μ in diameter (Fig. 2, *E*).

The LOWER EPIDERMIS consists of strongly cuticularised, longitudinally elongated, beaded, straight-walled cells, measuring about H 14 to 28 μ, Lev B 8 to 28 μ and Lev L 25 to 90 μ; occasional anomocytic stomata are present (Fig. 2, *F*; Fig. 3, *D*). *Covering trichomes* arise frequently and are in general of similar structure to those of the upper interneural epidermis.

Laterally compressed, slightly curved, elongated-conical *prickles* occur frequently on the lower surface of the midrib. They are commonly 2 to 5 mm. high and 1 to 2 mm. long at the base, and consist of lignified, fibre-like sclerotic cells, about 140 to 900 μ long and 14 to 30 μ wide, their walls, in general, are thin, with oblique pits; the lumen is wide (Fig. 1, *A* and *E*; Fig. 3, *A*; Fig. 4, *B*).

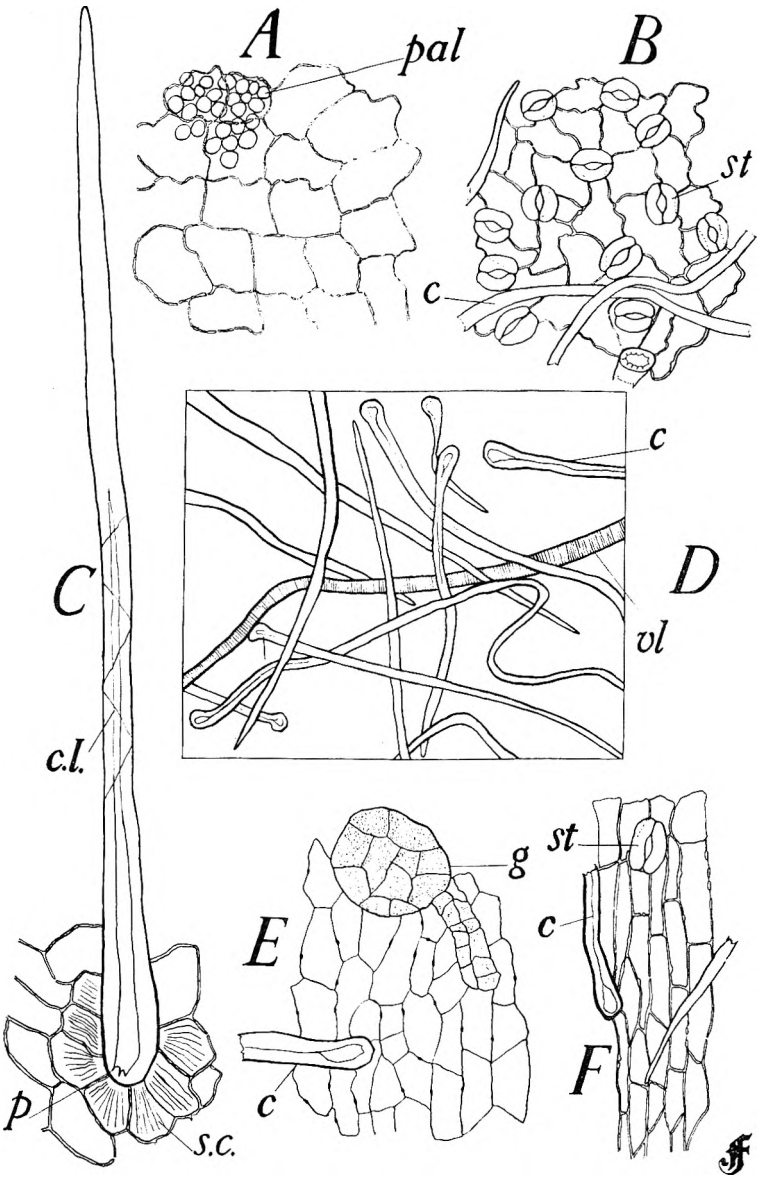

FIG. 2. Leaflet of *Rubus loganobaccus* L. H. Bailey. *A*, upper epidermis of leaflet; *B*, lower epidermis of leaflet; *C*, covering trichome on upper epidermis of lamina; *D*, covering trichomes forming partial tomentum on lower epidermis of lamina; *E*, upper epidermis of midrib; *F*, lower epidermis of midrib. All x 200. *c*, covering trichome (or portion of); *c.l.*, crossed-line effect; *g*, glandular trichome; *p*, pit; *pal*, palisade; *s.c.*, striated cuticle; *st*, stoma; *vl*, veinlet.

The CORTEX contains a few rows of supporting hypodermal *collenchyma* beneath both surfaces of the midrib, that towards the lower surface being the greater in extent. The cells are normally very heavily thickened, particularly in the angles, and measure about L 26 to 120 μ, R and T 14 to 40 μ; chloroplasts are present, measuring about 6 μ in diameter. The interior of the cortex is of *parenchyma* which is often slightly collenchymatous, the cells being sometimes thickened at the corners and occasionally exhibiting small pits in their walls; individual cells measure about L 30 to 150 μ, R and T 14 to 64 μ. Idioblasts occur fairly frequently in this tissue, each containing a cluster or rosette crystal of *calcium oxalate* about 10 to 40 μ in diameter.

Endodermal tissue could be detected only with the aid of chloral-iodine solution—minute starch grains like those described in the analogous tissue of the leaves of *R. idaeus* and *R. fruticosus*[1],[2] were observed in about half of the leaflets examined—in the innermost layers of the cortex. In none of the sections examined, however, did this layer continue over the upper side of the meristele. No lignified endodermal tissue could be demonstrated (Fig. 3, *D*).

PERICYCLIC FIBRES, in those leaflets where they occur, are arranged in small groups (Fig. 3, *B*) and commonly measure about 800 μ long and 20 μ in diameter; they exhibit thin, lignified walls with occasional small, oblique pits, a wide lumen and pointed apices (Fig. 4, *C*).

The MERISTELE is crescent-shaped and well-defined.

The PHLOEM consists of strands of sieve-tissue and small-celled parenchyma, alternating with medullary rays. The sieve-tubes are small, individual segments being about 70 μ long and about 2 to 6 μ in diameter, with transverse or oblique sieve-plates. The medullary rays are clearly defined and are usually one or two cells wide (Fig. 3, D).

The XYLEM is well-developed and the conducting elements are radially arranged. The component elements resemble those found in the midrib of *R. fruticosus*[2] to a marked extent. *Tracheids*, *fibre-like tracheids* and *vessels* are present, which exhibit annular, spiral, reticulate and pitted thickenings; they are all lignified, and measure about 8 to 50 μ in diameter (Fig. 3, *A*, *B* and *D*; Fig. 4, *C*).

In longitudinal sections, files of microclusters of *calcium oxalate* crystals about 3 to 10 μ in diameter are frequently seen in the parenchyma of the meristele.

The *lateral veins* exhibit similar anatomy to that of the midrib, all features progressively diminishing towards the margin.

Transverse sections mounted in ferric chloride solution exhibit a dark greenish-black coloration in the phloem and medullary rays of the meristele; the parenchyma below the meristele and the cortical collenchyma show a weak reaction. The mesophyll of the lamina reacts strongly, but there is no reaction in the epidermis.

(*b*) RACHIS

The rachis is about 6 to 14 cm. long and 2 to 5 mm. wide; prickles are prominent throughout the length of the lateral and abaxial surfaces

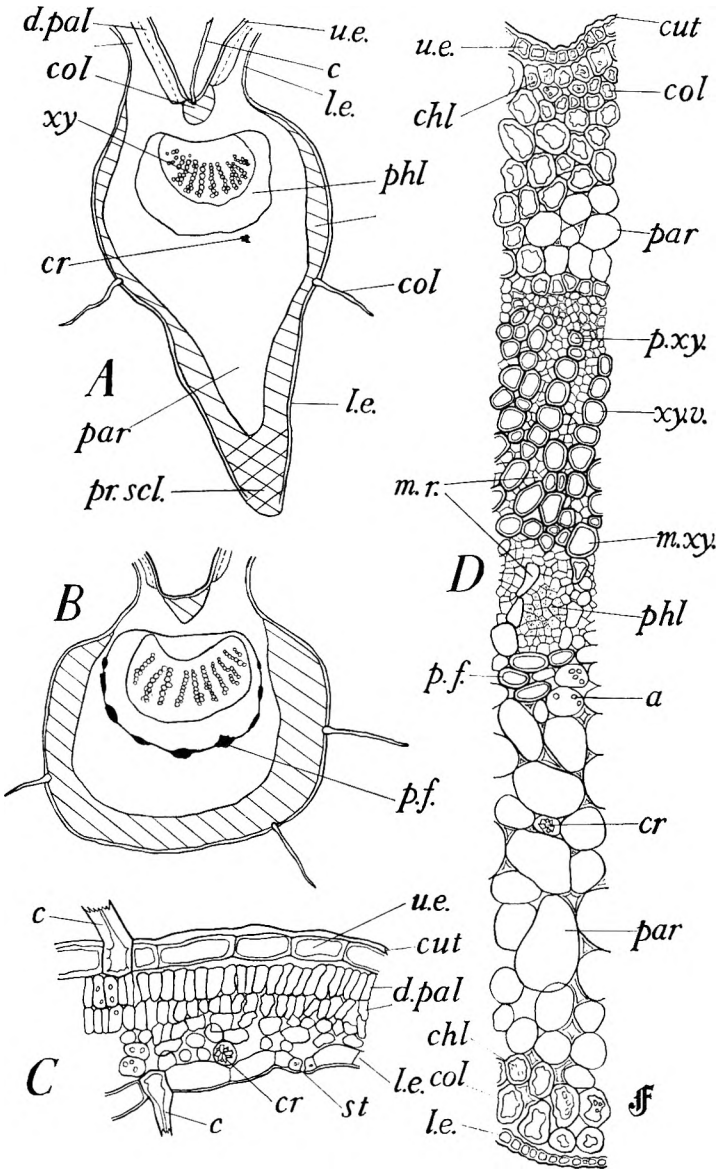

FIG. 3. Leaflet of *Rubus loganobaccus* L. H. Bailey. *A*, transverse section of midrib of terminal leaflet, cut at the position *w* (see Fig. 1, *A*) showing absence of pericyclic fibres; this section is cut through a prickle. *B*, a similar section cut from another leaflet, showing pericyclic fibres. *C*, transverse section of lamina, interneural region. *D*; central region of Fig. *B*. *A* and *B* x 20; *C* and *D*, x 100. *a*, starch; *c*, covering trichome; *chl*, chloroplast; *col*, collenchyma; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *d. pal.*, double palisade; *l.e.*, lower epidermis; *m.r.*, medullary ray; *m.xy.*, metaxylem; *par*, parenchyma; *p.f.*, pericyclic fibre; *phl*, phloem; *pr. scl.*, area of prickle sclereids; *p.xy.*, protoxylem; *st*, stoma; *u.e.*, upper epidermis; *xy*, xylem; *xy.v.*, xylem vessel.

(Fig. 1, *A*; Fig. 5, *A*). It is grooved along the adaxial surface, particularly in the part between the lateral and terminal leaflets. Transverse sections cut in the upper part present an almost oval outline apart from the groove (Fig. 5, *B*) whereas those cut in the lower part are nearly circular (Fig. 5, *C*). The arrangement of the vascular system in the upper part resembles that found in *R. idaeus*[1], but simple variations from this were observed in transverse sections cut in the lower part; usually, 4 to

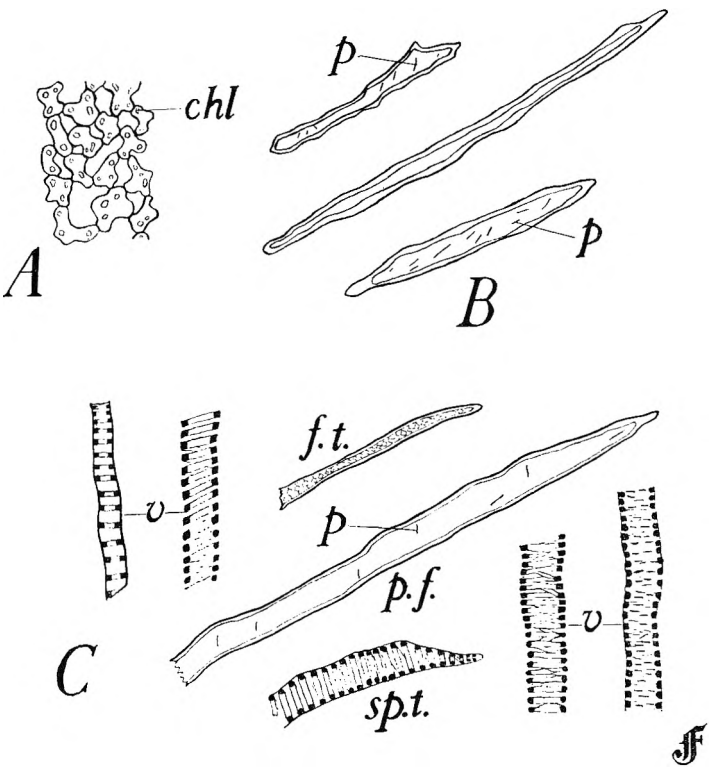

FIG. 4. Leaflet of *Rubus loganobaccus* L. H. Bailey. *A*, spongy mesophyll in surface view. *B*, elongated sclereids isolated by maceration of prickles. *C*, isolated elements obtained by maceration of the midrib. All x 200. *chl.*, chloroplast; *f.t.*, fibre-like tracheid; *p.*, pit; *p.f.*, pericyclic fibre; *sp.t.*, end-portion of a spiral tracheid; *v*, fragments of annular, spiral, reticulate and pitted vessels.

6 small meristeles, 2 or 3 on each side, occur above the central arc (Fig. 5, *C*). Pericyclic fibres are generally abundant below the phloem of all meristeles (Fig. 5, *B*, *C* and *H*).

The EPIDERMIS consists of cells having similar structure to those of the epidermis of the midrib. They are heavily cuticularised, elongated longitudinally, and measure about H 12 to 20 μ, Lev B 16 to 40 μ and Lev L 20 to 140 μ (Fig. 5, *D*, *E* and *H*); *stomata* of the anomocytic type are present; they are elliptical in shape and measure about 40 μ in length and 24 μ in width (Fig. 5, *D* and *E*). *Covering trichomes*, generally similar in detailed structure to those on the lower surface of the midrib, occur

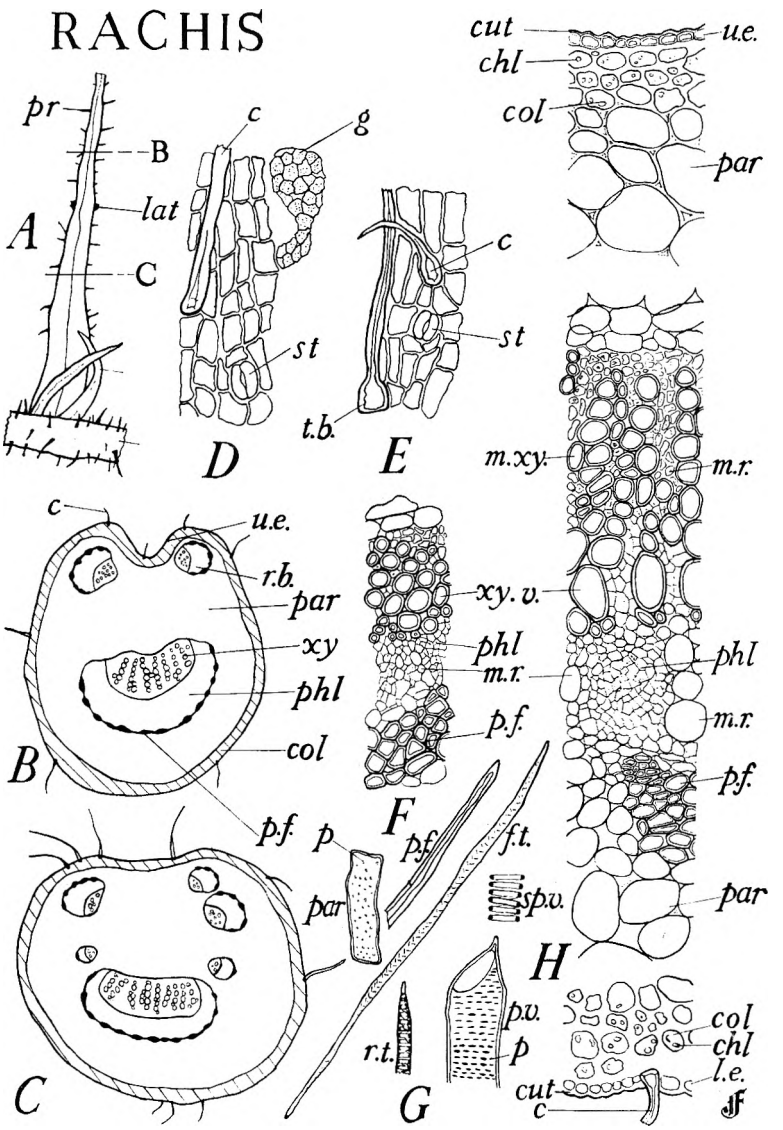

FIG. 5. Rachis of *Rubus loganobaccus* L. H. Bailey. *A*, rachis, denuded of leaflets. *B* and *C*, transverse sections cut at the positions indicated in Fig. *A*. *D*, upper epidermis of rachis with glandular trichome and stoma. *E*, lower epidermis of rachis with covering trichome and stoma. *F*, transverse section of a ridge bundle. *G*, isolated elements obtained by maceration. H, transverse section through central region of rachis at the point B, Fig. *A*. *A*, x $\frac{1}{2}$; *B*, x 20; *C*, x 12; *D*–*H*, x 150. *c*, covering trichome; *chl*, chloroplast; *col*, collenchyma; *cut*, cuticle; *f.t.*, fibre-like tracheid; *g*, glandular trichome; *lat*, point of attachment of lateral leaflet; *l.e.*, lower epidermis; *m.r.*, medullary ray; *m.xy.*, metaxylem; *p*, pit; *par*, parenchyma; *p.f.*, pericyclic fibre; *phl*, phloem; *pr*, prickle; *p.v.*, portion of a segment of a pitted vessel; *r.b.*, ridge bundle; *sp.v.*, fragment of spiral vessel; *st*, stoma; *r.t.*, end-fragment of reticulated tracheid; *t.b.*, trichome base; *u.e.*, upper epidermis; *xy*, xylem; *xy.* v., xylem vessel.

frequently, and those of the bi-cellular type occur occasionally; they vary enormously in size—from about 120 μ to well over 1,500 μ in length, and 10 to 26 μ wide at their bases. Some trichomes are curled (Fig. 5, *E*). *Glandular trichomes* occur frequently on the upper epidermis and resemble very closely those seen on the upper epidermis of the midrib (Fig. 5, *D*). The *prickles* are generally larger than, but have similar structure to those on the lower surface of the midrib (Fig. 5, *A*).

The CORTEX, like that of the midrib, consists of two layers of tissue. There is an outer hypodermal layer of *collencyhyma*, several cells wide, similar in character to the corresponding layer of the midrib; the cells measure about L 40 to 140 μ, R and T 12 to 46 μ, and contain chloroplasts, about 5 μ in diameter (Fig. 5, *B*, *C* and *H*). The inner cortex is *parenchymatous*, consisting of cells measuring about L 30 to 150 μ, R and T 40 to 120 μ; they frequently possess finely pitted walls; occasional idioblasts occur containing small cluster crystals of *calcium oxalate*, about 10 to 30 μ in diameter.

PERICYCLIC FIBRES occur below the arc of the meristele; they measure up to several mm. in length and 10 to 25 μ in diameter; they exhibit thick, smooth walls, a variable lumen, and pointed apices (Fig. 5, *G*).

The vascular tissue of the central MERISTELE is arranged in a crescent and in general the structure of the vascular elements approximates closely to those of the midrib, except that those of the rachis are all somewhat larger. The PHLOEM consists of groups of sieve-tubes about 80 μ long and 4 to 10 μ wide, frequently accompanied by small rectangular or cubical parenchymatous cells arranged in longitudinal files, many of which contain microclusters of *calcium oxalate* about 8 to 10 μ in diameter. Medullary rays traverse the phloem; they are usually one or two cells wide and their cells often contain minute starch grains about 4 μ in diameter. The XYLEM is well-defined, the elements being arranged in radial rows. Its general structure resembles that of the xylem of the midrib; both tracheids and vessels are present, exhibiting annular, spiral, reticulate and pitted thickenings (Fig. 5, *B*, *C*, *G* and *H*).

The fibrovascular bundles on the adaxial side each exhibit similar structure to that shown by the main meristele.

Transverse sections mounted in ferric chloride solution show a marked greenish-black coloration in the cortical collenchyma and the parenchyma of the meristeles, due to tannin.

(*c*) STIPULES

The paired stipules are adnate to either side of the base of the rachis. They are about 10 to 20 mm. long and 1 to 2 mm. wide, subulate or linear, and hairy (Fig. 1, *A*, Fig. 5, *A* and Fig. 6, *A*).

EPIDERMAL CELLS of both upper and lower surfaces are small, elongated or polygonal, and sometimes with slightly wavy and slightly beaded anticlinal walls, measuring about H 12 to 30 μ, Lev B 12 to 26 μ and L 20 to 80 μ. *Stomata* are present on both surfaces; they are elliptical in outline and measure about 30 μ in length and 20 μ in width (Fig. 6, *D*, *E* and *F*). Very numerous covering trichomes occur, especially on the midrib

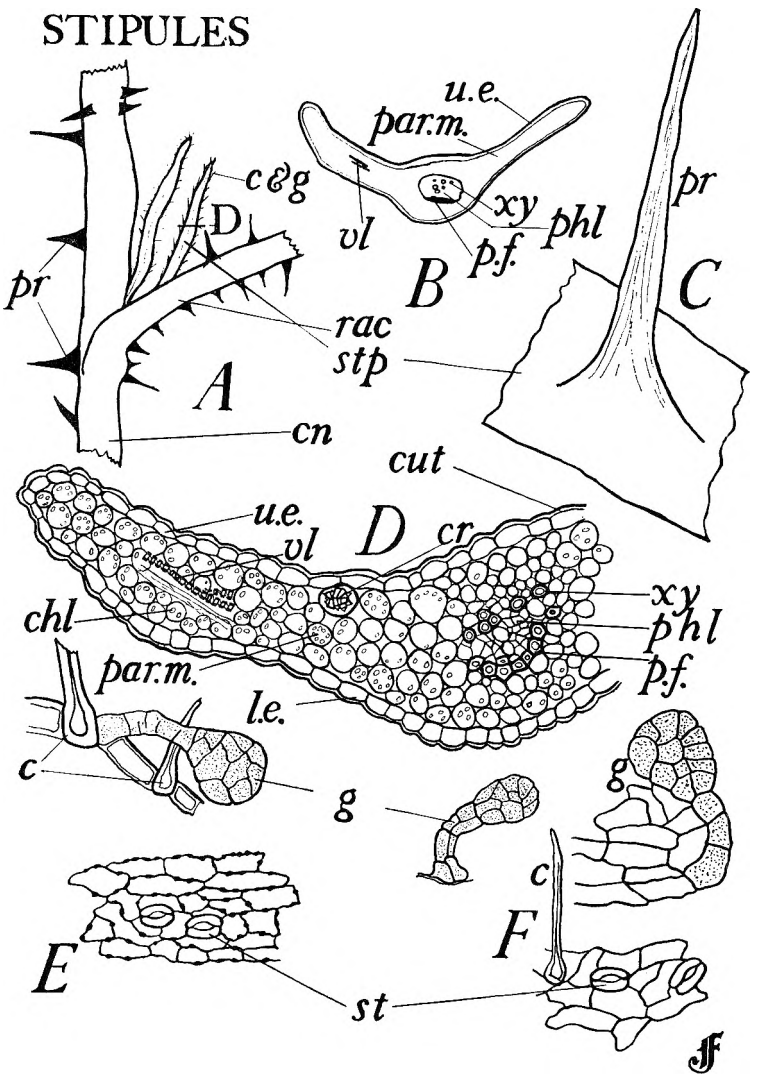

FIG. 6. Stipules of *Rubus loganobaccus* L. H. Bailey. *A*, paired stipules at base of rachis. *B* and *D*, transverse sections cut at the position D, Fig. *A*. *C*, prickle arising from lower surface of stipule. *E*, upper epidermis, showing stomata, also covering and glandular trichomes at the edge of the epidermis. *F*, lower epidermis, showing stomata, also covering and glandular trichomes, one of the latter from the edge of the stipule. *A*, x 1; *B* and *C*, x 40; *D*, *E* and *F*, x 200. *c*, covering trichome; *chl*, chloroplast; *cn*, cane; *cr*, cluster crystal of calcium oxalate; *cut*, cuticle; *g*, glandular trichome; *l.e.*, lower epidermis; *par.m.*, parenchymatous mesophyll; *p.f.*, pericyclic fibres; *phl*, phloem; *pr*, prickle; *rac*, rachis; *st*, stoma; *stp*, stipule; *u.e.*, upper epidermis; *vl*, veinlet seen in longitudinal section; *xy*, xylem.

and around the edges of the stipules; they possess the general characters of those of the upper epidermis of the leaflets, but vary greatly in size, measuring from about 90 μ to about 1,100 μ in length, and about 10 to 28 μ wide at the bases (Fig. 6, *E* and *F*). Occasional *glandular trichomes* are present, often occurring around the edges; they have the general character of those found on the rachis and leaflets, but their stalks are sometimes uniseriate (Fig. 6, *E* and *F*).

Very small, slender, elongated pointed *prickles* were observed on the upper and lower surfaces of the midrib of some of the stipules examined; they were from 0·5 to 1·25 mm. in length (Fig. 6, *C*).

The MESOPHYLL has very simple structure, is undifferentiated and consists of rounded cells about 8 to 26 μ in diameter; almost all of these cells contain chloroplasts, about 4 μ in diameter. Many widely distributed idioblasts occur, containing cluster or rosette crystals of *calcium oxalate* about 10 to 30 μ in diameter (Fig. 6, *D*).

The VENATION is simple, consisting of a central midrib and very minute, pinnate, secondary veinlets. The midrib consists of a very few lignified, mainly spirally thickened xylem elements about 8 to 10 μ in diameter; there is but little phloem. The pericycle is evident, and here consists of a few, lignified, smooth-walled fibres, usually about 280 μ in length and 8 μ in diameter; these fibres are evident throughout the length of the stipule (Fig. 6, *D*).

POWDER

A No. 60 powder is green in colour; it has but little odour and a slightly astringent taste. When some of the powder is mixed with ferric chloride solution, a greenish-black colour is observed.

To examine the powder for structural features, it should be mounted in either 50 per cent glycerol solution, solution of chloral hydrate, or phloroglucin and hydrochloric acid. The diagnostic characters of the powder (Fig. 7) are as follows.

Numerous straight, or slightly curved, lignified fragments of *covering trichomes from the upper surface of the lamina*, up to about 45 μ wide, apical fragments being solid, whilst basal fragments are hollow and pitted with simple pits; spiral markings are visible on some of these hairs but are not particularly prominent; curled fragments of *covering trichomes from the lower surface of the lamina*, up to about 30 μ wide, which stain only feebly, or not at all, in mounts made with phloroglucin and hydrochloric acid (unless the powder is previously treated with ether); numerous *fragments of the lamina*, showing a transverse sectional view, about 100 μ thick, with a *double palisade layer* in which there are rounded idioblasts, each containing a cluster crystal of calcium oxalate about 14 to 36 μ in diameter; particles showing in surface view the slightly wavy-walled, beaded cells of the *upper interneural epidermis* of the lamina and usually, immediately beneath it, the palisade with its idioblasts; fragments showing the *lower interneural epidermis* consisting of wavy-walled, often slightly beaded cells with scattered anomocytic stomata and cicatrices of trichomes; fragments of the *veins* showing small, lignified vascular elements showing

annular, spiral, reticulate and pitted thickenings, and occasional files of small parenchymatous cells containing microclusters of *calcium oxalate*; fragments of the *prickles* consisting of elongated, *lignified sclereids* with simple, linear pits; infrequent fragments of *glandular trichomes* consisting of a multicellular, biseriate stalk, and a multicellular, subspherical, glandular head about 60 to 70 μ in diameter.

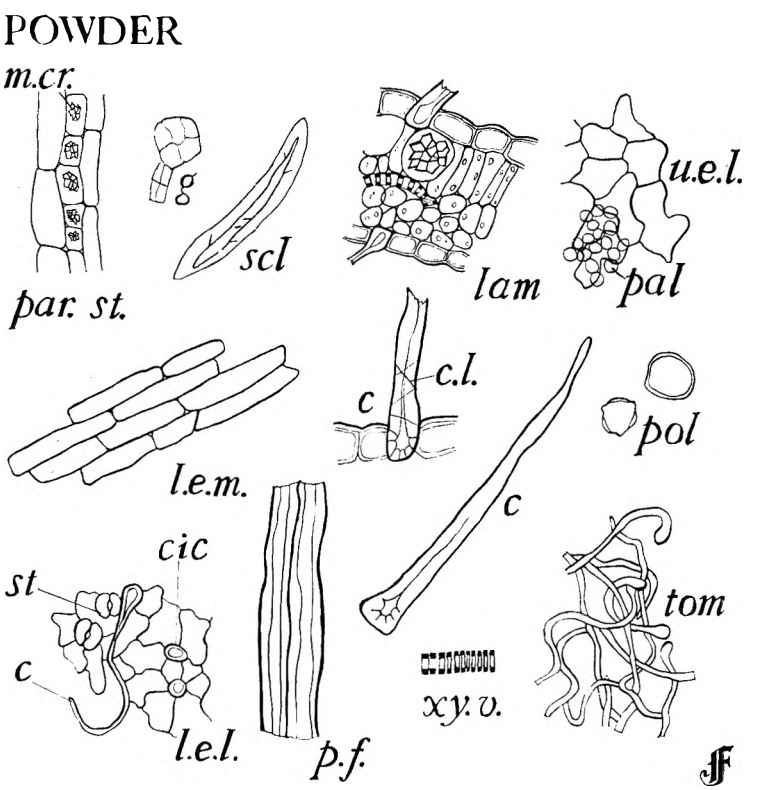

FIG. 7. Powder of the leaf of *Rubus loganobaccus* L. H. Bailey. All x 200. *c*, covering trichome; *g*, isolated head of a glandular trichome; *lam*, fragment of lamina in transverse section, showing upper and lower epidermises, idioblasts with cluster crystal of cacium oxalate, double palisade and spongy mesophyll; *l.e.l.*, lower epidermis of lamina; *l.e.m.*, lower epidermis of midrib; *m.cr.*, files of microcluster crystals of calcium oxalate, adjacent to phloem tissue; *pal*, palisade; *p.f.*, fragments of pericyclic fibres; *phl*, phloem; *scl*, sclereid of prickle; *sp.t.*, fragment of spiral tracheid; *st*, stoma; *t.b.*, trichome base; *tom*, fragment of curled, covering trichome from tomentum of lower epidermis of lamina; *u.e.l.*, upper epidermis of lamina.

SUMMARY OF THE ANATOMICAL CHARACTERS OF THE LEAF

1. The epidermises of the leaflets are characteristic. The *upper epidermis* consists of cells with slightly wavy, beaded anticlinal walls, and bears scattered, unicellular, lignified *covering trichomes* with acute, solid apices and thickened, pitted bases. The *lower epidermis* consists of cells with slightly beaded, wavy anticlinal walls, and bears curled, *covering trichomes* in sufficiently large numbers that they form a virtual tomentum;

these trichomes can be shown to be lignified only by first soaking in ether for several hours, before testing with phloroglucinol and hydrochloric acid. *Glandular trichomes* are present on the upper epidermises of midribs and secondary veins; they consist of a multicellular, biseriate stalk of about 5 to 8 cells in length, and a multicellular, subspherical, glandular head about 60 to 70 μ in diameter. *Stomata*, of the anomocytic type, occur in the lower epidermis only, and are oval in outline.

2. The *lamina* of the leaflet is thin and dorsiventral, with a well-developed, *double* palisade in which are rounded idioblasts, each containing a cluster crystal of *calcium oxalate*.

3. The midrib of the leaflet contains a meristele consisting of a *xylem* composed of lignified *tracheids* and *vessels* which exhibit annular, spiral, reticulate and pitted thickenings, a *phloem* consisting of simple sieve tubes with transverse or oblique sieve-plates, and rows of parenchymatous cells arranged in longitudinal files, each cell containing a microcluster of *calcium oxalate*.

4. *Prickles* of the midrib and rachis are composed of lignified, elongated sclereids with thick walls exhibiting linear pits.

5. Lignified pericyclic fibres are abundant, always occur in the rachis, but occasionally occur also in the midribs of leaflets.

6. The arrangement of the vascular bundles in the rachis is somewhat intermediate between that observed in the case of *R. idaeus* and that in *R. fruticosus*[2]. There are usually two small ridge meristeles in the upper part of the rachis, and usually from 4 to 6 in the lower part.

Discussion

It is seen that the morphological and anatomical characteristics of the leaves of *R. idaeus* L., *R. fruticosus* L. and *R. loganobaccus* L. H. Bailey are similar in most general respects, but there are differences in detailed structures, some of which are diagnostic pharmacognostically. A fuller discussion of the common and differential characters of these leaves, also leaves of (*a*) other species of bramble and (*b*) cultivated varieties of *R. idaeus* and *R. loganobaccus* will be presented in a later communication; the following characters serve to distinguish leaves of *R. loganobaccus* from those of the two species described earlier[1,2]:—

1. Most diagnostic are the *covering trichomes of the lower epidermis of the lamina*, which are somewhat intermediate in character between those on the lower epidermises of the leaves of *R. idaeus* and *R. fruiticosus*. They are 300 to 400 μ long and form a virtual tomentum which is, however, by no means as dense as that found in the case of *R. idaeus*. Lignification can be demonstrated only with phloroglucin and hydrochloric acid after preliminary treatment with ether or chloral hydrate solution; this fact affords further differentiation from leaves of *R. idaeus*, where the corresponding trichomes are unlignified, also from those of *R. fruticosus*, where they give a positive reaction for lignin without difficulty.

2. The *covering trichomes of the upper epidermis of the lamina* resemble, in general, those in the analogous position in the other two species, but

frequently show a greater maximal length—up to 1,400 μ in specimens examined, compared with a maximum of about 500 μ in the case of *R. idaeus*, and 1,000 μ in the case of *R. fruticosus*.

3. The *epidermal cells of the interneural lamina* (both upper and lower) show a very well-marked beaded effect; this is not seen on leaves of *R. idaeus*, and only slightly on those of *R. fruticosus*.

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A STUDY OF THE TOXICITY OF SULPHITE. I.

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The effect of sodium metabisulphite, 750 p.p.m. as SO_2, in the drinking water, has been studied through three generations of rats in experiments lasting for nearly 3 years. The metabisulphite was without effect on growth, the intake of food and fluid and the output of faeces. It did not influence fertility, the weight of the newborn or lactation: neither did it increase the frequency with which tumours developed.

It is well known that sulphite reacts with aneurine[1] and that the sulphiting of foodstuffs reduces their aneurine activity[2,3]. This alone, however, cannot fully explain the effects observed by Fitzhugh, Knudsen and Nelson[5] in the course of the only published long-term investigation of the toxicity of a sulphited diet. These workers fed a partially synthetic diet to young rats for periods of 12 weeks or 1 year and observed the effects of mixing sodium bisulphite with the food on the growth and health of the animals. The addition of 0·1 per cent sodium bisulphite, 615 p.p.m. as SO_2, produced multiple histopathological changes, only some of which were reduced in incidence or severity by the bi-weekly injection of 100 μg. of aneurine per rat. Deficiency of aneurine was therefore not the full explanation of the findings. The present work is a first step in the search for additional causes of the long-term toxicity of sulphite. We have examined the toxicity of sodium metabisulphite itself, and have administered it in the drinking water to prevent prior interaction between the metabisulphite and the constituents of the diet. The experiments have extended over three generations of rats.

Methods

Eleven male and 39 female newly weaned rats, not differing in age by more than 5 days, and of a uniform strain bred for cancer research by the Chester Beatty Research Institute, London, received individual earmarks for identification and were divided at random into three groups. Each group contained 13 females: there were in addition 5 males in group I and 6 males in group II. The animals of different groups and the sexes were segregated. All were kept in standard cages of Boot's type, not more than 7 in a cage, in the same room under similar conditions of lighting and humidity, at a temperature maintained within the range of 21·5° to 24·5°.

Solid diet. All rats received cube diet 41 b of Stein in wire baskets. A typical sample of this diet was assayed for us by Vitamins Ltd. and was found to contain 3·1 μg. of aneurine per g.

Fluids. All rats drank freely from graduated bottles. Those of group I were provided with tap water, those of group II received tap water containing sodium metabisulphite equivalent to 750 p.p.m. SO_2, and those of group III tap water containing metabisulphite equivalent to 375 p.p.m. SO_2. Calculated weights of sodium metabisulphite assayed

for SO_2 equivalent were added to tap water daily to yield solutions equivalent to 750 p.p.m. SO_2. The solutions containing 375 p.p.m. SO_2 were initially prepared every other day. Only later was it found that the SO_2 content of these had dropped by nearly 20 per cent in 48 hours, so that the female rats of group III had received only 90 per cent of the SO_2 intended in the period before their first mating. Thereafter all solutions of metabisulphite were prepared daily, and were found to deteriorate in SO_2 content by approximately 10 per cent in the 24 hours before a fresh solution was provided.

Estimation of growth rate. Each rat was weighed twice weekly. Curves relating body weight in g. to age in days were found to be linear from the time of weaning to the 70th to 80th day of life for both males and females. Rates of growth were therefore expressed as the slopes of these lines. Standard statistical methods, based on the normal curve of distribution were used to compare the rates of growth of rats of the same sex which had received different drinking fluids since the males and females were found to constitute two different populations in respect of growth, each homogeneous within itself.

Estimation of food intake, fluid intake and faecal output. The daily food consumption of the rats in each cage was measured as the loss in weight of the food basket in 24 hours and was expressed in g./100 g. weight of rat per day. The bottles containing the drinking fluid were graduated at 5 ml. intervals. Fluid consumption was therefore measured daily to the nearest 5 ml. and was expressed as ml. fluid drunk per 100 g. weight of rat per day.

The faeces produced in a cage were sifted from the sawdust, twice weekly, through a No. 6 sieve. The weight of faeces produced was expressed as a percentage of the weight of food eaten in the corresponding period.

Mean values for the consumption of food and fluids and for the output of faeces, and the standard errors of these means were obtained for the rats of each sex in each group, and the corresponding mean values for control and sulphite treated rats were compared by means of *t* tests.

Tests of ability to reproduce. The 50 rats with which the experiment began were designated Generation I. Male and female rats from group II, Generation I, which had been treated with sulphite (750 p.p.m. SO_2) for 11 weeks were paired and each pair was left to run in a separate cage for a week. Males and females from control group I, Generation I, were similarly paired and mated. If no litter resulted from the first mating period, a second period of 2 weeks was allowed. The litters resulting from these matings of Generation I constituted Generation II (i). Newborn rats were weighed individually and the six heaviest were retained; this was to reduce the burden of suckling for the mother. Weaning was effected 20 to 25 days after birth, when the newly weaned were segregated according to sex. Six to 8 weeks after the birth of Generation II (i), the same pairs of rats from Generation I were again mated, allowing not more than two periods each of 2 weeks duration: the resulting litters constituted Generation II (ii).

The members of Generation II (i) were paired and mated within their dose groups when they had attained their plateau weights. Not more than two periods of 2 weeks were allowed for this purpose. The litters so produced constituted Generation III. They were weaned 21 days after birth. All the young were retained for suckling tests in Generation II (ii) and Generation III.

Postmortem and histological examinations. Weighed rats were killed with ether. The heart, lungs, liver, spleen, adrenals, testes or ovaries, seminal vesicles or uterus, and the kidneys, were removed and weighed. Parts of these, the stomach, ileum, colon, a gastrocnemius muscle and a sciatic nerve were fixed in formol saline for not less than three days before they were blocked in paraffin. By routine, all sections were stained in Ehrlich's haematoxylin and eosin except for those of sciatic nerve for which a modified Weigert-Pal technique was used with 1 per cent neutral red as the counter stain.

RESULTS

Sodium metabisulphite has been added to the drinking water of rats throughout an experiment lasting for 2½ years and extending over three generations of animals. The concentrations of metabisulphite used corresponded to 350 and 750 p.p.m. SO_2. Observation has been made of the effect of these concentrations of metabisulphite on growth, food consumption, fluid intake, faecal output, reproduction, lactation and the incidence of tumours. Water drinking rats of Generation II were derived from experimental matings between water drinking rats of Generation I. Sulphite treated rats of Generation II were the product of experimental matings between rats of Generation I which had received sulphite (750 p.p.m. as SO_2) in their drinking water from the start of the experiment, throughout mating, pregnancy and lactation, and until they were subjected to histological examination. Rats of generation III were similarly derived from Generation II.

The Effect of Metabisulphite in the Drinking Water on Growth

Curves relating body weight in g. to postnatal age in days were linear for this strain of rats from shortly after weaning until the 8th to 12th week of life; then, rather abruptly, as the animals matured sexually, the curves flattened to a plateau on which growth advanced only slowly. It was therefore possible to measure the growth rate of each animal as the slope of the steep part of this curve, and to compare the growth rates of water drinking and sulphite-treated animals in each generation by mean slopes (Table I, column 4). Males and females have been entered separately since the males grew the faster. There was no significant difference between the growth rates of the water drinking rats and those which received sulphite in their drinking water in any generation, but the growth rate of the stock as a whole accelerated from one generation to the next. This was more marked amongst the females, and was accompanied by shortening of the steep linear part of the curve relating body weight to time. Thus, whereas female rats of Generation I grew rapidly for the

TABLE I

THE EFFECT OF SULPHITE IN THE DRINKING WATER ON THE GROWTH, THE INTAKE OF FOOD AND FLUIDS AND THE FAECES OF RATS

Generation	Sex	Treatment	Slope of growth curve Mean ± SE	Values per 100 g. rat per day Means ± SE		Faeces weight as per cent of food intake Mean ± SE
				Food intake g.	Fluid intake ml.	
I	Male	Water	5·89 ± 0·38 (5)*	7·2 ± 0·5 (22)†	9·3 ± 0·5 (22)†	22·7 ± 0·46 (22)†
		SO_2 750 p.p.m.	5·01 ± 0·39 (6)	6·7 ± 0·4 (22)	9·8 ± 0·6 (22)	22·2 ± 0·42 (22)
	Female	Water	2·45 ± 0·13 (12)	6·9 ± 0·3 (22)	10·4 ± 0·4 (22)	21·4 ± 0·42 (22)
		SO_2 375 p.p.m.	2·55 ± 0·10 (13)	7·1 ± 0·3 (22)	11·3 ± 0·5 (22)	21·3 ± 0·33 (22)
		SO_2 750 p.p.m.	2·21 ± 0·12 (13)	7·1 ± 0·3 (22)	12·0 ± 0·5 (22)	22·0 ± 0·36 (22)
II (i)	Male	Water	6·19 ± 0·42 (12)	10·1 ± 0·8 (22)	13·1 ± 0·9 (22)	19·9 ± 0·63 (20)
		SO_2 750 p.p.m.	5·18 ± 0·24 (11)	9·7 ± 0·6 (22)	15·4 ± 1·1 (22)	20·9 ± 0·67 (21)
	Female	Water	3·55 ± 0·27 (8)	11·6 ± 1·4 (17)	16·3 ± 2·3 (17)	20·3 ± 0·46 (11)
		SO_2 750 p.p.m.	3·23 ± 0·38 (6)	8·9 ± 0·6 (21)	15·0 ± 0·9 (21)	19·8 ± 0·73 (17)
II (ii)	Male	SO_2 750 p.p.m.	6·41 ± 0·57 (7)	8·6 ± 0·7 (18)	15·4 ± 1·0 (18)	24·2 ± 0·70 (18)
	Female	SO_2 750 p.p.m.	4·12 ± 0·14 (13)	9·4 ± 0·5 (15)	15·8 ± 0·9 (15)	21·2 ± 0·66 (15)
III	Male	Water	6·94 ± 0·51 (10)	13·6 ± 1·3 (9)	17·7 ± 1·2 (9)	19·9 ± 0·58 (9)
		SO_2 750 p.p.m.	6·81 ± 0·36 (5)	13·5 ± 1·3 (9)	19·8 ± 1·6 (9)	20·5 ± 0·89 (9)
	Female	Water	5·34 ± 0·15 (9)	13·9 ± 1·3 (6)	19·3 ± 1·6 (6)	21·1 ± 0·20 (6)
		SO_2 750 p.p.m.	5·16 ± 0·12 (8)	14·7 ± 1·3 (7)	23·3 ± 2·0 (7)	23·6 ± 0·92 (6)

* Number of animals.
† Number of observations.

first 80 days of life, those of the third generation reached the end of this phase in 60 days. The plateau weights of the stock (Table II) did not alter significantly. There was no significant difference between the adult weights of water drinking and sulphite drinking male rats in any generation. By contrast, the female sulphite drinking rats of Generations II (ii) and III grew at a normal rate (Table I) but matured more rapidly than the water drinking controls, so that the plateau weights of the sulphite drinking females were significantly lower than those of the water drinking females in the third generation, (t calc. = 2·5, where n = 15 : P = $<$0·05).

TABLE II

COMPARISON OF THE WEIGHTS OF FULL GROWN RATS OF THE FIRST, SECOND AND THIRD GENERATION TREATED AND UNTREATED WITH METABISULPHITE IN THE DRINKING WATER

Generation	Treatment	Mean body weight in g. ± S.E.	
		Male	Female
I	Water	530·0 ± 20·2 (5)*	301·7 ± 9·4 (12)*
	SO_2 375 p.p.m.		313·9 ± 8·7 (13)
	SO_2 750 p.p.m.	471·7 ± 27·1 (6)	295·4 ± 9·2 (13)
II (i)	Water	498·2 ± 28·4 (11)	320·0 ± 7·6 (8)
	SO_2 750 p.p.m.	430·5 ± 24·0 (11)	310·0 ± 10·5 (7)
(ii)	Water		353·3 ± 9·3 (3)
	SO_2 750 p.p.m.	494·3 ± 21·3 (7)	295·8 ± 7·5 (13)
III	Water	442·5 ± 10·7 (10)	287·2 ± 5·7 (9)
	SO_2 750 p.p.m.	428·0 ± 17·3 (5)	269·4 ± 5·5 (8)

* Number of animals.

The Effect of Metabisulphite in the Drinking Water on the Consumption of Food and Fluid

The spontaneous intake of food was unaffected by the addition of metabisulphite (to 750 p.p.m. as SO_2) to the drinking water (Table I, column 5) throughout three generations.

The female rats of the first generation which received metabisulphite (750 p.p.m. as SO_2) in their drinking water had a significantly higher fluid intake than did those rats of the same generation which drank only water (t calc. = 2·3, n = 42, P = $<$ 0·05), but this difference was not maintained amongst the females of later generations and was absent throughout amongst the males (Table I, column 6).

There was a progressive increase in the consumption of food and of fluid from generation to generation in the stock as a whole, so that rats of the third generation ate and drank twice as much during rapid growth as did those of the first generation. This difference can be correlated with the steady increase in the growth rate of the stock (see above).

The Effect of Metabisulphite in the Drinking Water on the Output of Faeces

The net weights of faeces excreted by each group of rats expressed as a percentage of the weight of food eaten during the period of faecal collection, followed by the standard errors of the means, are shown in Table I, column 7. There was no difference for the values for normal male and female rats, or between the generations. The only difference appearing between comparable groups of water drinking and sulphite-treated animals

was amongst the females of Generation III, where mean percentage found for the sulphite-drinking female significantly exceeded that for the group of water drinking controls (t calc. = 2·2, n = 11, P = < 0·05). This difference may have reflected a greater water content in the stools or a lesser degree of digestion or absorption of foodstuffs by the groups receiving sulphite (750 p.p.m. as SO_2) in the drinking water: in either case greater than normal speed of passage through the intestines is indicated.

The Effects of Metabisulphite in the Drinking Water on Fertility

The fertility of sulphite-treated animals was compared with that of control animals by comparing the number of periods paired animals had to be run together to produce pregnancies and the number of young per

TABLE III

THE EFFECT OF METABISULPHITE (750 P.P.M. AS SO_2) IN THE DRINKING WATER ON THE REPRODUCTION OF RATS

	Sulphite-treated rats	Control rats
Generation I		
First reproduction test		
Number of matings required/litter	1·2 ± 0·20 (5)	1·5 ± 0·07 (6)
Number of young/litter	12·6 ± 2·04 (5)	10·7 ± 1·66 (6)
Number surviving lactation/litter (six only returned to each mother)	3·6 ± 1·17 (5)	4·5 ± 1·12 (6)
Second reproduction test		
Number of matings required/litter	1·0 ± 0·00 (3)	1·0 ± 0·00 (2)
Number of young/litter	9·0 ± 0·58 (3)	10·5 ± 1·50 (2)
Number surviving lactation/litter (all the young were retained)	6·7 ± 1·50 (3)	2·5 ± 0·50 (2)
Generation II		
Single reproduction test		
Number of matings required/litter	1·3 ± 0·33 (3)	1·3 ± 0·33 (3)
Number of young/litter	8·3 ± 2·85 (3)	14·6 ± 2·19 (3)
Number surviving lactation/litter	4·3 ± 3·38 (3)	6·3 ± 3·48 (3)

All results are expressed as means ± S.E. (number of pregnancies).

litter. Table III shows that there was no difference in the numbers for the sulphite-treated and control pairs of Generation I. The young of litters from the first mating of Generation I were weighed within 24 hours of birth, and only six young were returned to each mother. Six to 8 weeks after the birth of the first litter (Generation II, (i)) some of the same pairs of rats from Generation I were remated. All the young born remained with the mothers and were not handled for the first week of life (Generation II (ii)). Reduction in the handling of the young did not increase the proportion of those surviving lactation. The chief cause of loss was cannibalism by the mother. This was most marked amongst the water drinking animals.

A similar fertility test was made using rats of Generation II (i). Again there was no significant difference for rats of these two groups. All the young were weighed within 24 hours of birth, and were returned to their mothers. The proportion of the young surviving to the end of lactation did not differ significantly between control and sulphite-treated animals. The weights of the young are compared in Table IV. A significant difference appears (P = < 0·05) between the two groups of young at birth

after the first mating of Generation I, but this does not recur in the larger samples of young born to Generation II (i).

The growth of the young during the first ten days did not differ for sulphite-treated and control animals either in the first reproduction test on Generation I or in the reproduction test on Generation II (i). An apparent difference shown in the second test on Generation I should be attributed to a failure of lactation in the control water drinking animals (Table IV).

The Effect of Metabisulphite in the Drinking Water on the General Health of Rats

The general health of the control and sulphite-treated animals remained excellent throughout the first 9 months of the experiment. Occasional

TABLE IV

THE EFFECT OF METABISULPHITE IN THE DRINKING WATER SUPPLIED TO RATS ON THE YOUNG BORN TO THEM

	Treated rats 750 p.p.m. as SO_2	Control rats
Generation I		
First test		
Weight of newborn in g.	7·2 ± 0·23 (23)	5·8 ± 0·14 (14)
Weight of young at 10 days in g.	21·2 ± 1·97 (11)	19·6 ± 1·64 (6)
Second test		
Weight of young at 10 days in g.	20·2 ± 0·50 (24)	14·1 ± 1·46 (6)
Generation II (i)		
Weight of newborn in g.	6·7 ± 0·12 (25)	6·4 ± 0·11 (44)
Weight of young at 10 days in g.	19·8 ± 0·92 (15)	20·2 ± 1·27 (23)

All results are means ± S.E. (number of animals).

snuffles and sticky eyes developed, but these were as frequent amongst the controls as the experimental animals.

An epidemic respiratory infection broke out in the tenth month of the trial. This killed some of the rats of the first generation, but nearly all of those of the second and third generations recovered. Incidence of infection was spread evenly between the control and sulphite-treated animals. The latter could not, therefore, be claimed to show either increased or decreased susceptibility to the infection. There were as many deaths among the control as among the experimental animals.

Postmortem and histological findings. Four or five rats were selected at random from each group in Generation I for postmortem and histological examination of their organs a few days after the onset of the respiratory infection in the tenth month of the experiment. Metabisulphite treatment was maintained throughout the infection and thereafter. All survivors of the epidemic were kept until they had either reached 2 years of age or developed a well established tumour, then they were killed and examined.

There were no significant differences between the body weights of rats killed at 10 months and at 2 years of age. Therefore the weights of all animals subjected to postmortem examination are shown together in Table V combined according to the treatment they had been given. The

drinking of sodium metabisulphite solution (750 p.p.m. SO_2) throughout life affected neither the weights of the rats (Table V) nor the percentage of the weight contributed by the various organs (Table VI).

There was no evidence of clinical polyneuritis before death, nor were spectacle eyes, blanching of the incisor teeth, or browning of the uteri evident in animals which had received 750 p.p.m. SO_2 in their drinking water. These changes were described by Fitzhugh, Knudsen and Nelson[5] in rats fed solid diets containing sulphite.

TABLE V

POSTMORTEM WEIGHTS OF ADULT RATS IN G.

Sex	Drinking fluid supplied throughout life		
	Water	Solution of sodium metabisulphite	
		375 p.p.m. SO_2	750 p.p.m. SO_2
Females	409 ± 13·1 (20)*	360 ± 25·5 (10)	408 ± 10·0 (22)
Males	636 ± 26·1 (13)	—	599 ± 23·3 (18)

* Number of animals

TABLE VI

POSTMORTEM WEIGHTS OF THE ORGANS OF ADULT RATS EXPRESSED AS MEAN PERCENTAGE BODY WEIGHT ± S.E.

Organ	Drinking fluid supplied throughout life		
	Water	Solution of sodium metabisulphate	
		375 p.p.m. SO_2	750 p.p.m. SO_2
FEMALES			
Liver	4·1 ± 0·15 (20)*	3·8 ± 0·23 (10)*	3·8 ± 0·13 (22)*
Heart	0·45 ± 0·06 (20)	0·52 ± 0·05 (10)	0·48 ± 0·02 (22)
Spleen	0·45 ± 0·08 (20)	0·38 ± 0·05 (10)	0·31 ± 0·02 (22)
Kidneys ..	0·91 ± 0·08 (20)	0·99 ± 0·64 (10)	0·86 ± 0·03 (22)
Lungs	1·48 ± 0·28 (20)	2·08 ± 0·84 (10)	1·12 ± 0·19 (22)
Adrenals ..	0·03 ± 0·002 (20)	0·04 ± 0·004 (10)	0·03 ± 0·03 (22)
Uterus	0·34 ± 0·03 (20)	0·21 ± 0·15 (10)	0·38 ± 0·08 (22)
MALES			
Liver	3·40 ± 0·02 (13)	—	3·30 ± 0·09 (18)
Heart	0·40 ± 0·03 (13)	—	0·49 ± 0·04 (18)
Spleen	0·27 ± 0·01 (13)	—	0·23 ± 0·01 (18)
Kidneys	0·92 ± 0·08 (13)	—	0·84 ± 0·02 (18)
Lungs	0·84 ± 0·09 (13)	—	1·02 ± 0·01 (18)
Adrenals ..	0·02 ± 0·002 (13)	—	0·014 ± 0·001 (18)
Testes	0·73 ± 0·06 (13)	—	0·69 ± 0·06 (18)

* Number of animals.

Histological examination was made of the tissues of thirteen females and four males from each group, water drinking and sulphite drinking (750 p.p.m. as SO_2), 10 months after the beginning of treatment. No abnormalities were found in the spleens, adrenal glands, stomachs, ileums, colons, gastrocnemius muscles, sciatic nerves, uteri or testes or in the seminal vesicles of any of these animals. The nine cases of focal nephtitis and two of cloudy swelling of the liver were almost evenly distributed between sulphite treated and control animals. These changes should most probably be ascribed to the infection prevalent at that time because

there was evidence of acute infection in the lungs of every one of these animals irrespective of their treatment group. None had developed tumours.

Thirty-seven per cent of the 54 animals kept for 2 years developed tumours. The incidence was higher among the females (44 per cent) than among the males (27 per cent), and was unaffected by the addition of sodium metabisulphite (750 p.p.m. as SO_2) to the drinking water. Of 16 females drinking water, one developed a lipoma, two had tumours of lymphoid type, and three tumours which had arisen from the duct of a sebaceous gland. Among the 16 females drinking sulphite in their water, three developed lymphoid tumours, four had tumours arising from sebaceous ducts, and one a fibroid. There were two lymphoid tumours, one duct tumour and one lipoma among nine untreated males; two lymphoid tumours arose in a group of 13 sulphite drinking males.

The full histological examination of the tissues of the 54 animals kept for 2 years under test revealed no abnormalities of the spleens, adrenal glands, ileums, colons, gastrocnemius muscles, sciatic nerves, ovaries or testes, or of the uteri or seminal vesicles, except when these were involved in a growth. Twenty-seven cases of excess fat, three of cloudy swelling and seven of infiltration of the portal tracts in liver were evenly distributed among the males and females of both control and treatment groups. So were the 28 cases of chronic and seven of acute infection of lung. Renal scarring with old nephron damage was found in nineteen animals of either sex, spread evenly over control and treatment groups. Hyaline degeneration of heart muscle was confined to two treated and two untreated males. Two small acute gastric ulcers were found in water drinking males and one in a sulphite drinking male. Evidence of hyperplasia of the gastric mucosa, which occurred when rats were fed a solid diet containing 650 p.p.m. SO_2[5], was absent.

Discussion

The toxicity of sodium bisulphite (615 p.p.m. as SO_2) mixed in a solid diet was only in small part prevented by biweekly injection of 100 μg. aneurine to each rat[5]. The residual toxicity could have been due to the sulphite itself or to the products of its interaction with constituents of the solid food. The first of these two possibilities has now been excluded because sodium metabisulphite (750 p.p.m. as SO_2) in drinking water has proved non-toxic and the weight of fluid drunk has exceeded that of diet eaten by each generation of rats.

This work was undertaken during the tenure, by I. L. Natoff, of a studentship for training in research, presented by Carter and Co. Ltd., who also defrayed the expenses of the work.

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STUDIES IN THE FIELD OF DIURETICS

PART III. SOME SYMMETRICAL BENZENE-1,3-DISULPHONALKYLAMIDES

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The symmetrical benzene-1,3-disulphonalkylamides described herein are found to be virtually devoid of carbonic anhydrase inhibiting activity. Certain dialkylamides nevertheless retain significant diuretic activity, which is consequently regarded as an intrinsic property of the 1,3-disulphamylbenzene ring system and not as solely an expression of carbonic anhydrase inhibiting activity.

IN Part II of this series[1] the preparation of a number of 1,3-disulphamyl derivatives of benzene was reported. Most of these compounds were found to show some diuretic activity in the saline-loaded rat when administered by mouth. For reasons outlined in the earlier publication, 5-chloro-2,4-disulphamyltoluene (disulphamide) was selected for further study.

In the course of this work it was found that diuretic activity in this series was not related in simple manner to the carbonic anhydrase inhibiting activity *in vitro*. This observation seemed to indicate that their diuretic properties stemmed from structural features active in their own right as well as from ability to inhibit the enzyme carbonic anhydrase. To test this hypothesis attention was directed to the related benzene-1,3-disulphonalkylamides; which group of compounds was expected to possess negligible carbonic anhydrase inhibiting activity by virtue of the substituents on their sulphamyl groups[2].

The benzene-1,3-disulphonalkylamides recorded in Table I were prepared by standard methods involving reaction of the appropriate disulphonchloride (cf.[1]) with the amine in aqueous or aqueous:ethanolic solution, or under anhydrous conditions in a variety of solvents. In most instances the use of a two-phase system, e.g., carbon tetrachloride or chloroform and water, led to readier control of the reaction and improved yields. Precautions were necessary in those reactions in which a halogen atom, particularly bromine or fluorine, was situated *o*- to a sulphonchloride group, when the reaction had to be performed at low temperatures using only theoretical amounts of amine, otherwise replacement of the halogen atom by substituted amine readily occurred.

The alkylamides listed in Table I proved to be, without exception, essentially inactive ($<$ than 1/5000 $\times$ acetazolamide) as inhibitors of carbonic anhydrase. Their diuretic study kindly made by Dr. A. David and his colleagues, in saline-loaded rats, showed clearly the retention of significant activity in the methylamides and dimethylamides. Increase in the size of the sulphonamide alkyl substituent beyond methyl led to rapid diminution of diuretic activity, which had essentially disappeared in the 2,4-disulphonbutylamides. Replacement of the $^{-}NH_2$ moiety of the

TABLE I

SYMMETRICAL BENZENE-1,3-DISULPHONALKYLAMIDES

No.	R	Substituent at position				m.p. °C	Formula	Found					Required				
		2	4	5	6			C	H	N	S	Cl	C	H	N	S	Cl
1	MeNH	—	Me	—	—	127–129	$C_9H_{14}O_4N_2S_2$	38·7	4·6	10·0	—	—	38·6	5·0	10·1	—	—
2	,,	—	Et	—	—	120–121	$C_{10}H_{16}O_4N_2S_2$	41·4	5·2	9·6	21·7	—	41·1	5·5	9·6	21·9	—
3	,,	—	Me_2CH	—	—	136–137	$C_{11}H_{18}O_4N_2S_2$	43·3	5·7	8·8	20·6	—	43·1	5·9	9·1	20·9	—
4	,,	—	F	—	—	144–145	$C_8H_{11}O_4N_2S_2F$	33·7	3·8	9·8	22·7	—	34·0	3·9	9·9	22·7	—
5	,,	—	Cl	—	—	141–143	$C_8H_{11}O_4N_2S_2Cl$	32·4	3·8	9·1	21·2	11·7	32·2	3·7	9·4	21·5	11·9
6	,,	—	Br	—	—	139–141	$C_8H_{11}O_4N_2S_2Br$	27·8	3·4	8·7	—	—	28·0	3·2	8·2	—	—
7	,,	—	Me	Me	—	178–180	$C_{10}H_{16}O_4N_2S_2$	41·0	5·2	9·5	—	—	41·1	5·5	9·6	21·9	—
8	,,	—	Me	—	Me	176–178	,,	41·2	5·5	9·4	21·2	—	41·1	5·5	9·6	21·9	—
9	,,	—	Me	—	F	144–146	$C_9H_{13}O_4N_2S_2F$	37·0	4·4	9·6	21·5	—	36·5	4·4	9·5	21·6	—
10	,,	—	Me	—	Cl	194–195	$C_9H_{13}O_4N_2S_2Cl$	34·9	4·5	9·0	20·1	11·0	34·6	4·2	9·0	20·5	11·4
11	EtNH	—	Me	—	Cl	125	$C_{11}H_{17}O_4N_2S_2Cl$	38·7	4·9	8·1	—	10·1	38·8	5·0	8·2	—	10·4
12	n-PrNH	—	Me	—	Cl	107–108	$C_{12}H_{21}O_4N_2S_2Cl$	42·1	5·6	8·0	—	10·1	42·3	5·7	7·6	17·4	9·6
13	iso-PrNH	—	Me	—	Cl	220	,,	42·3	5·5	7·6	17·3	—	42·3	5·7	7·6	17·4	9·6
14	n-BuNH	—	Me	—	Cl	97–98	$C_{13}H_{25}O_4N_2S_2Cl$	45·2	6·0	7·4	16·1	—	45·4	6·3	7·1	16·1	—
15	C_6H_5NH	—	Me	—	Cl	129–130	$C_{15}H_{17}O_4N_2S_2Cl$	42·6	4·6	—	17·6	—	42·8	4·7	—	17·6	—
16	$C_6H_4(Me)NH$	—	Me	—	Cl	124–125	$C_{16}H_{19}O_4N_2S_2Cl$	45·4	5·1	6·9	15·9	—	45·9	5·4	7·1	16·3	—
17	Me_2N	—	Me	—	Cl	150–152	$C_{11}H_{17}O_4N_2S_2Cl$	39·3	5·2	8·3	19·1	10·6	38·7	5·0	8·2	18·8	10·4
18	MeNH	—	Me	Cl	—	153–155	$C_9H_{13}O_4N_2S_2Cl$	34·7	4·0	9·1	20·7	11·3	34·6	4·2	9·0	20·5	11·4
19	Me_2N	—	Me	Cl	—	128–130	$C_{11}H_{17}O_4N_2S_2Cl$	38·8	5·0	8·0	19·1	—	38·8	5·0	8·2	18·8	—
20	MeNH	—	Cl	Me	—	157–160	$C_9H_{13}O_4N_2S_2Cl$	34·9	4·2	—	—	—	34·6	4·2	—	—	—
21	Me_2N	—	Cl	Me	—	127–129	$C_{11}H_{17}O_4N_2S_2Cl$	38·7	5·0	8·4	18·5	10·4	38·8	5·0	8·2	18·8	10·4
22	$N<(CH_2)_5$	—	Cl	Me	—	135–136	$C_{17}H_{25}O_4N_2S_2Cl$	48·7	5·6	6·5	—	8·3	48·6	6·0	6·7	—	8·4
23	MeNH	—	Me	—	Br	189–190	$C_9H_{13}O_4N_2S_2Br$	30·5	3·7	7·7	18·1	—	30·3	3·6	7·8	17·9	—
24	EtNH	—	Me	—	Br	117–118	$C_{11}H_{17}O_4N_2S_2Br$	33·8	4·3	7·0	16·7	—	34·3	4·4	7·3	16·6	—
25	n-PrNH	—	Me	—	Br	128–129	$C_{12}H_{21}O_4N_2S_2Br$	37·5	5·0	6·7	—	19·2*	37·8	5·1	6·8	—	19·4*
26	iso-PrNH	—	Me	—	Br	222–223	,,	38·0	5·0	7·2	—	—	37·8	5·1	6·8	—	19·4*
27	n-BuNH	—	Me	—	Br	96–97	$C_{13}H_{25}O_4N_2S_2Br$	—	—	6·4	14·8	—	40·8	5·7	6·4	14·5	—
28	C_6H_5NH	—	Me	—	Br	125–126	$C_{15}H_{17}O_4N_2S_2Br$	38·4	4·2	6·6	15·8	—	38·1	4·2	6·9	15·7	—
29	$C_6H_4(Me)NH$	—	Me	—	Br	128	$C_{16}H_{19}O_4N_2S_2Br$	41·6	4·6	6·5	—	18·5*	40·2	4·8	6·4	—	18·3*
30	MeNH	—	Cl	Cl	—	147–149	$C_9H_{12}O_4N_2S_2Cl_2$	28·8	3·4	7·9	—	20·7	28·9	3·0	8·4	—	21·3
31	,,	—	Cl	—	Cl	192–194	,,	28·7	3·3	8·6	—	—	28·9	3·0	8·4	—	21·3
32	,,	—	Cl	—	NH_2	180–183	$C_9H_{12}O_4N_3S_2Cl$	31·1	4·1	13·5	—	11·3	30·6	3·9	13·4	—	11·3
33	,,	—	MeNH	—	—	166–168	$C_9H_{15}O_4N_3S_2$	30·6	5·1	14·5	—	—	36·8	5·2	14·3	—	—
34	,,	—	MeNH	—	Me	206–207	$C_{10}H_{17}O_4N_3S_2$	39·0	5·7	13·9	21·0	—	39·1	5·6	13·7	20·8	—
35	,,	—	MeNH	—	Cl	213–124	$C_9H_{14}O_4N_3S_2Cl$	32·7	4·3	12·4	—	—	32·9	4·3	12·8	—	—
36	,,	—	$N<(CH_2)_5$	—	Me	208–209	$C_{14}H_{23}O_4N_3S_2$	46·4	6·3	12·0	—	—	46·5	6·4	11·6	—	—
37	n-BuNH	—	n-BuNH	—	Me	107	$C_{15}H_{31}O_4N_3S_2$	52·8	8·4	9·7	—	—	52·6	8·2	9·7	—	—
38	$N<(CH_2)_5$	—	$N<(CH_2)_5$	—	Me	139–140	$C_{22}H_{35}O_4N_3S_2$	56·6	7·3	9·2	—	—	56·3	7·5	9·0	—	—
39	$NH.NH_2$	—	Me	—	—	147 (d)	$C_7H_{19}O_4N_3S_2$	30·1	4·5	20·3	22·8	—	30·0	4·3	20·0	22·9	—
40	MeNH	—	OH	Cl	—	152–154	$C_9H_{11}O_5N_2S_2Cl$	30·7	3·5	9·4	—	—	30·6	3·5	8·9	—	—
41	,,	OH	—	SO_2NHMe	—	217–218	$C_9H_{16}O_7N_2S_3$	29·5	4·3	10·8	—	—	28·9	4·1	11·3	—	—
42	,,	Me	OH	SO_2NHMe	—	222–224	$C_{10}H_{17}O_7N_2S_3$	31·5	4·6	10·8	—	—	31·0	4·4	10·9	—	—
43	,,	OH	Me	Cl	Me	198–200	$C_{10}H_{15}O_5N_2S_2Cl$	35·4	4·5	8·2	18·3	10·3	35·0	4·4	8·2	18·7	10·4
44	Et.O.CO.NH	—	Me	—	—	103–105	$C_{10}H_{22}O_6N_2S_2$	42·5	5·5	6·6	—	—	42·6	5·2	6·6	—	—
45	MeNH	MeNH	—	SO_2NHMe	—	159–160	$C_{10}H_{18}O_6N_4S_3$	31·3	4·7	14·3	—	—	31·1	4·7	14·5	—	—
46	,,	—	Br	—	MeNH	228–229	$C_9H_{14}O_4N_3S_2Br$	29·1	3·6	10·8	—	22·1*	29·1	3·8	11·3	—	21·5*

* Bromine

sulphamyl groups by a heterocyclic radical likewise led to almost complete loss of diuretic potency.

Comparison of the diuretic activities of the 1,3-disulphamyl benzene derivatives described in Part II with their alkylated analogues recorded herein showed that alkylation was accompanied always by loss of potency and by virtual disappearance of carbonic anhydrase inhibiting activity. Significant diuretic activity was retained, however, by certain methylamides and dimethylamides (Nos. 5, 6, 9, 17, 18 and 46). The hypothesis that 1,3-disulphamylbenzene derivatives may be diuretically active *per se* and independently of their carbonic anhydrase inhibiting properties may consequently be regarded as proven.

The authors thank Mr. B. G. Overell and Miss S. Condon for the carbonic anhydrase inhibiting activities quoted herein.

Experimental

The following illustrate the preparative methods involved.

5-*Chlorotoluene*-2,4-*disulphonmethylamide*. 5-Chlorotoluene-2,4-disulphonchloride (20 g.) was added in portions with stirring and cooling below 20° to a mixture of 25 per cent w/v aqueous methylamine (100 ml.) and carbon tetrachloride (100 ml). After the addition was complete, stirring was continued for 15 minutes at room temperature. The product (19 g.) was collected and crystallised from ethanol; m.p. 194–195°.

5-*Methylaminotoluene*-2,4-*disulphonmethylamide*. (*a*) The foregoing compound (15·6 g.) was dissolved in 33 per cent ethanolic methylamine (50 ml.) and the solution heated at *ca.* 60° for 10 hours when excess of amine and solvent was boiled off. The residual *product* had m.p. 205–206° after crystallisation from aqueous ethanol.

(*b*) 5-Fluorotoluene-2,4-disulphonchloride (20 g.) was added in portions with stirring and cooling to liquid methylamine (100 ml.). The excess of amine was allowed to evaporate overnight at room temperature. The viscous residue was dissolved in hot water and the solution acidified with hydrochloric acid. The *product* which separated had m.p. 206–207° after crystallisation from aqueous ethanol and was identical with that described in (*a*).

Toluene-2,4-*disulphonylaminoacetate diethyl ester*. A solution of toluene-2,4-disulphonchloride (28·8 g.) in chloroform (150 ml.) was added with stirring and cooling below 10° to a mixture of ethyl aminoacetate hydrochloride (35 g.) and triethylamine (50·5 g.) in chloroform (500 ml.) Stirring was continued for 30 minutes after the addition was complete then excess of triethylamine was extracted with dilute hydrochloric acid. Concentration of the chloroform furnished the solid *product* which had m.p. 103°—105° after crystallisation from aqueous ethanol.

Fluorobenzene-2,4-*disulphonmethylamide*. To a stirred solution of fluorobenzene-2,4-disulphonyl chloride (29·3 g.) in carbon tetrachloride (120 ml.) was added slowly, with cooling to 0°, a 10 per cent aqueous solution of methylamine (125 ml.). After the addition was complete stirring was continued at 0° for 4 hours when the aqueous layer was

separated and acidified with concentrated hydrochloric acid. The *product* which separated on cooling had m.p. 144–145° after crystallisation from water.

5-*Methylaminochlorobenzene*-2,4-*disulphonmethylamide*. 1,5-Dichlorobenzene-2,4-disulphonchloride (20 g.) was added in portions with stirring and cooling below 30° to a 30 per cent aqueous solution of methylamine (150 ml.). Stirring was continued for 30 minutes and excess of methylamine was boiled off. The residual liquors were cooled and acidified with hydrochloric acid. The *product* (70 per cent yield), had m.p. 213° after crystallisation from aqueous ethanol.

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THE ANTICONVULSANT PROPERTIES OF 2-METHYL-3-*p*-BROMOPHENYL-3*H*-4-QUINAZOLONE HYDROCHLORIDE (B.D.H. 1880) AND SOME RELATED COMPOUNDS

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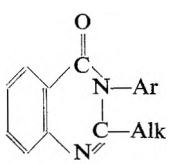
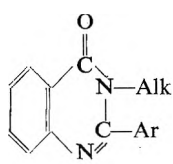
Twenty-five 2-alkyl-3-aryl-3*H*-4-quinazolone derivatives, eight 2-aryl-3-alkyl-3*H*-4-quinazolone derivatives and six 2-alkyl-3-aryl-3*H*-4-thioquinazolone derivatives were examined for orala nticonvulsant properties against leptazol induced convulsions in mice. 2-Methyl-3-*p*-bromophenyl-3*H*-4-quinazolone hydrochloride (B.D.H. 1880) was the most active compound and was therefore examined more fully. It is fourteen times more active than troxidone against leptazol and eight times more active against electroshock induced convulsions. B.D.H. 1880 also has a better therapeutic index than troxidone. It has approximately one-quarter the activity of phenytoin against leptazol and one-twelth the activity against electroshock-induced convulsions. B.D.H. 1880 and primidone have similar anticonvulsant properties against leptazol induced convulsions but B.D.H. 1880 is only one-third as active as primidone against electroshock induced convulsions.

A number of 2-alkyl-3-aryl-3*H*-4-quinazolones (I), 2-aryl-3-alkyl-3*H*-4-quinazolones (II), and some 2-alkyl-3-aryl-3*H*-4-thioquinazolones (III) were examined for oral anticonvulsant activity following Gujral, Saxena and Tiwari's[1] findings that some related compounds possessed central nervous system depressant properties.



(I)



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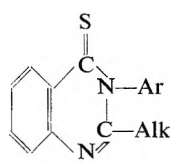
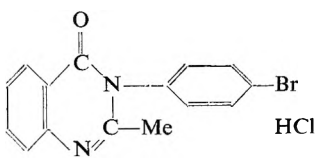


(III)

Forty compounds, prepared by Jackman, Petrow and Stephenson[2], were examined for anticonvulsant properties against leptazol induced convulsions in mice. The most active compound in this test, 2-methyl-3-*p*-bromophenyl-3*H*-4-quinazolone hydrochloride B.D.H. 1880 (IV) was also examined for its anticonvulsant activity in electroshock induced convulsions in mice. Troxidone, phenytoin and primidone were used as reference compounds. The new compound is a stable white powder, slightly soluble in water with a molecular weight of 351·6.



(IV)

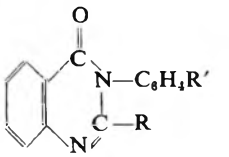
METHODS

Acute Oral Toxicity

The acute oral toxicity of troxidone and B.D.H. 1880 was estimated in unfasted albino mice weighing approximately 20 g. Ten animals were used at each of the seven doses and the mortalities after 7 days recorded. The LD50 was estimated using Kärber's formula[3]. B.D.H. 1880 and troxidone were given as suspensions in 10 per cent gum acacia and all volumes were adjusted to 0·5 ml./20 g. weight.

TABLE I

ANTICONVULSANT ACTIVITY OF SOME 2-ALKYL-3-ARYL-3*H*-4-QUINAZOLONE COMPOUNDS



Compound	R	R'	Relative activity to troxidone
2-Methyl-3-*o*-tolyl	-Me	-*o*-Me	3·3
2-Methyl-3-*o*-anisyl	-Me	-*o*-OMe	2·2
2-Methyl-3-*p*-anisyl	-Me	-*p*-OMe	2·1
2-Methyl-3-*o*-phenetyl	-Me	-*o*-OEt	2·0
2-Methyl-3-*m*-phenetyl	-Me	-*m*-OEt	<0·5
2-Methyl-3-*p*-phenetyl	-Me	-*p*-OEt	<0·3
2-Methyl-3-*o*-chlorophenyl	-Me	-*o*-Cl	10·0
2-Methyl-3-*m*-chlorophenyl	-Me	-*m*-Cl	7·0
2-Methyl-3-*p*-chlorophenyl	-Me	-*p*-Cl	13·0
2-Methyl-3-*o*-bromophenyl	-Me	-*o*-Br	14·0
2-Methyl-3-*m*-bromophenyl	-Me	-*m*-Br	1·5
2-Methyl-3-*p*-bromophenyl	-Me	-*p*-Br	14·0
2-Methyl-3-*p*-iodophenyl	-Me	-*p*-I	6·7
2-Methyl-3-*p*-fluorophenyl	-Me	-*p*-F	2·6
2-Methyl-3(2,4-dichlorophenyl)	-Me	2,4-di-Cl	2·1
2-Methyl-3(2,5-dichlorophenyl)	-Me	2,5-di-Cl	14·0
2-Methyl-3(2,3-xylyl)	-Me	2,3-di-OMe	inactive at 600 mg./kg.
2-Methyl-3(2,4-xylyl)	-Me	2,4-di-Me	—
2-Methyl-3(2,5-xylyl)	-Me	2,5-di-Me	—
2-Methyl-3(2,6-xylyl)	-Me	2,6-di-Me	4·5
2-Methyl-3(3,4-xylyl)	-Me	3,4-di-Me	—
2-Methyl-3(3,5-xylyl)	-Me	3,5-di-Me	1·3
2-Methyl-3(3,4,5-trimethoxyphenyl)	-Me	3,4,5-tri-OMe	inactive at 600 mg./kg.
2-Methyl-3(4-bromo-2,3-dimethylphenyl)	-Me	4Br-2,3-di-Me	2·5
2-Ethyl-3-phenyl	-Et	H	2·0
2-Ethyl-3-*o*-chlorophenyl	-Et	-*o*-Cl	2·0
-2-Ethyl-3-*p*-bromophenyl	-Et	-*p*-Br	<2·5
-2-Propyl-3-*p*-bromophenyl	-Pr	-*p*-Br	2·4

Anticonvulsant Activity

This was estimated in unfasted male albino mice weighing between 15 and 20 g. The compounds, with the exception of phenytoin sodium which is water soluble, were administered by stomach tube as suspensions in 10 per cent gum acacia or in the following aqueous suspending medium.

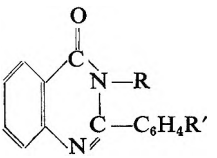
Sodium carboxymethylcellulose 1·2 g. Tween 80 1·5 g. Methyl *p*-hydroxybenzoate 0·06 g. Propyl *p*-hydroxybenzoate 0·03 g. Distilled water to 100 ml.

Doses were administered to groups of five to ten animals 2 hours before the convulsive stimuli. The volumes were adjusted to 0·5 ml./20 g. weight.

Leptazol convulsions were induced by 50 mg./kg. given intravenously. Electroshock seizures were induced through ear electrodes by a current of 15 mA for a 0·2 seconds duration using an apparatus described by Woodbury and Davenport[4].

TABLE II

ANTICONVULSANT ACTIVITY OF EIGHT 2-ARYL-3-ALKYL-3*H*-4-QUINAZOLONE COMPOUNDS



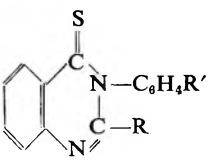
Compounds	R	R′	Relative activity to troxidone
2-Phenyl-3-methyl	-Me	-H	3·0
2-*o*-Tolyl-3-methyl	-Me	-*o*-Me	7·4
2-*p*-Chlorophenyl-3-methyl	-Me	-*p*-Cl	<0·7
2-*o*-Bromophenyl-3-methyl	-Me	-*o*-Br	6·3
2-*p*-Bromophenyl-3-methyl	-Me	-*p*-Br	<1·0
2-Phenyl-3-ethyl	-Et	-H	1·3
2-Phenyl-3(βγ-dihydroxypropyl)	$-CH_2.CHOH.CH_2OH$	-H	2·3
2-*p*-Bromophenyl-3-ethyl	-Et	-*p*-Br	<0·7

Positive protection was recorded if the tonic hindleg extensor component of the seizure was abolished. From the number of mice protected the ED50's values were estimated according to Kärber's formula[3] or Litchfield and Wilcoxon's method[5].

The duration of effect of B.D.H. 1880 against electroshock induced convulsions was estimated in four groups of twenty mice given varying amounts orally. The ED50 value was estimated from the number of mice

TABLE III

ANTICONVULSANT ACTIVITY OF SIX 2-ALKYL-3-ARYL-3*H*-4-THIOQUINAZOLONE COMPOUNDS



Compound	R	R′	Relative activity to troxidone
2-Methyl-3-*o*-tolyl	-Me	-*o*-Me	2·3
2-Methyl-3-*p*-chlorophenyl	-Me	-*p*-Cl	<0·7
2-Methyl-2-*o*-bromophenyl	-Me	-*o*-Br	<0·7
2-Methyl-3-*p*-bromophenyl	-Me	-*p*-Br	<0·7
2-Methyl-3-*p*-fluorophenyl	-Me	-*p*-F	Inactive at 600 mg./kg.
2-Ethyl-3-*p*-bromophenyl	-Et	-*p*-Br	Inactive at 600 mg./kg.

protected at 1, 2, 3, 5, 7 and 23 hours after administration. The convulsions were induced by a current of 30 mA for 0·2 seconds applied through ear electrodes.

RESULTS

Table I records the oral anticonvulsant activity of twenty-five 2-alkyl-3-aryl-3*H*-4-quinazolone derivatives taking troxidone as unity. Tables II and III record the anticonvulsant activity of eight 2-aryl-3-alkyl-3*H*-4-quinazolone derivatives and of six 2-alkyl-3-aryl-3*H*-4-thioquinazolone derivatives respectively.

In the 2-alkyl-3-aryl-3*H*-4-quinazolone group a number of compounds, particularly the halo phenyl derivatives, are more active than troxidone.

TABLE IV

THE ORAL ANTICONVULSANT ACTIVITY OF B.D.H. 1880, TROXIDONE, PHENYTOIN SODIUM AND PRIMIDONE IN MICE, TEN PER GROUP

Compound	*ED50 mg./kg. (Fiducial limits P = 0·05)		Therapeutic index	
	Leptazol	Electroshock	Leptazol	Electroshock
B.D.H. 1880	30 (23–38)	140 (104–189)	11·7	2·5
Troxidone	425 (371–480)	1200 (876–1644)	5·3	1·9
Phenytoin sodium	12 (9–17)	12 (8–18)	—	—
Primidone	41 (29–59)	40 (21–75)	—	—

* Litchfield and Wilcoxon's method.

Table III shows that the related thioquinazolone derivatives are invariably less active. The 2-aryl-3-alkyl-3*H*-4-quinazolone compounds are also less active than the related 2-alkyl-3-aryl compounds with the exception of the *o*-tolyl derivative which is more active.

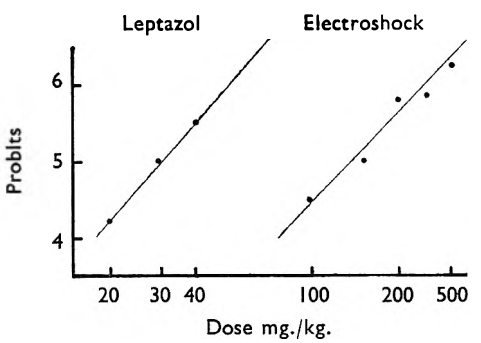


FIG. 1. The oral anticonvulsant activity of B.D.H. 1880 against leptazol and electro-shock induced convulsions in mice.

Acute Toxicity and Anticonvulsant Activity of B.D.H. 1880

This compound, one of the most active against leptazol induced convulsions, was examined more fully. It has an oral LD50 in mice of 353·5 mg./kg. compared to 2291·0 mg./kg. for troxidone. The ED50 values against leptazol and electroshock induced convulsions for B.D.H. 1880, troxidone, phenytoin and primidone are recorded in Table IV.

The anticonvulsant activity of B.D.H. 1880 has been repeatedly confirmed and Table IV records the results of a typical experiment. There is a linear relation between log dose and the probits of the animals protected against both leptazol and electroshock induced convulsions (Fig. 1). The anticonvulsant activity of B.D.H. 1880 is still present 7 hours after administration. Table V records the ED50 values at intervals.

TABLE V

THE ORAL ED50 OF B.D.H. 1880 IN MICE AGAINST ELECTROSHOCK INDUCED CONVULSIONS AT INTERVALS FOLLOWING ADMINISTRATION

Time in hours	ED50 mg./kg.
1	>175
2	108
3	115
5	120
7	125
23	>175

DISCUSSION

The results reported in this paper show that the 2-alkyl-3-phenyl-3*H*-4-quinazolone structure can yield compounds with marked anticonvulsant properties: for example, the 2-methyl-3-*p*-bromophenyl derivative, B.D.H. 1880, has a similar or a greater activity than most commonly used anti-epileptic drugs.

The thioquinazolone analogue of B.D.H. 1880 has less anticonvulsant properties than troxidone which suggests that the oxygen in the fourth position in the quinazolone ring is essential for good anticonvulsant activity.

There is some reduction in activity when the 2-methyl group in B.D.H. 1880 is substituted by an ethyl or butyl group. There is, however, a marked reduction in activity when the *p*-bromophenyl group is moved from the third to the second position in the quinazolone nucleus as in 2-*p*-bromophenyl-3 methyl-3*H*-4-quinazolone.

The introduction of a bromo radical in the benzene ring of the heterocyclic nucleus as in 6-bromo-2-methyl-3-*o*-tolyl-3*H*-4-quinazolone does not influence anticonvulsant activity.

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AN INVESTIGATION OF THE ALKALOIDS OF SOME BRITISH SPECIES OF *EQUISETUM*

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From the Department of Pharmacy, University of Manchester

Received April 5, 1960

The alkaloids nicotine and palustrine have been separated from authentic mixtures by paper partition chromatography, paper electrophoresis and the use of buffered cellulose columns. These techniques were applied to extracts of *E. arvense*, *E. telmateia* and *E. sylvaticum* from the last two of which no alkaloids have previously been isolated. Nicotine was found in all three species but palustrine was not detected.

Equisetum species, popularly referred to as "Horsetails", have been known to medicine for centuries and *E. arvense* (common horsetail) has been included in several European pharmacopoeias. The principal use of the herb is as a diuretic, but it has also been recommended for haemoptysis, haemorrhoids, varicose ulcers and tuberculosis. In the field and as contaminants of hay, *Equisetums* have caused fatalities among farm stock, the toxicity being attributed to alkaloids[1], although more recent work suggests that a thiaminase may be responsible[2–4]. The compounds which have been identified in *Equisetums* include alkaloids, flavonoids, amino-acids, thymine, 3-methoxypyridine, dimethylsulphone, tannins, saponins, silica, silicic acids, vitamin C and several organic acids.

The presence of alkaloids in *Equisetum* species was first reported by Lohmann[1], in 1903, but it was not until 1936, that Glet and others[5], obtained from *E. palustre* 0·95 per cent of an alkaloid which they named palustrine. This was isolated from the same species in pure form by Karrer and Eugster[6], in 1948, and given the molecular formula $C_{17}H_{29}O_2N_3$. In the following year they obtained it from *E. arvense* and *E. hiemale* together with traces of nicotine[7]. In 1950, Wohlbier and Beckman[8] obtained from *E. palustre* 0·01 per cent of an alkaloid equisetine which proved to be identical with palustrine. They also found traces (0·0002 per cent) of a second alkaloid equisetonine. Further work by Eugster and others[9], 1953, recorded the isolation from *E. palustre* of another alkaloid palustridine, molecular formula $C_{18}H_{31}O_3N_3$, which was thought to be identical with equisetonine. Both palustrine (equisetine) and palustridine (equisetonine) yielded degradation products related to the lupinane alkaloids.

The only record of alkaloids in *E. telmateia* and *E. sylvaticum* appears to be the early report of Lohmann, 1903; hence the present work was undertaken to investigate and compare these species with *E. arvense*. All three are abundant in the Manchester area.

Experimental Methods

Separation of Nicotine and Palustrine

Owing to the low yields of alkaloids previously reported[6–11], methods for the microseparation of nicotine from palustrine were investigated.

Paper partition chromatography by the descending method using butanol: concentrated hydrochloric acid: glacial acetic acid: water (100: 5: 5: 22) (solvent system A) gave results similar to those of Eugster[9], R_F nicotine 0·08, R_F palustrine 0·15–0·20. A better separation was obtained with butanol: glacial acetic acid: water (4: 1: 5) (solvent system B), R_F nicotine 0·5–0·56, R_F palustrine 0·37–0·44.

Electrophoresis on acid impregnated paper produced well separated round compact spots. The best separations were obtained on Whatman No. 1 paper impregnated with 0·1M tartaric acid using initial currents of 3 to 5 mA at 500 to 850 V. An initial current of 3 mA at 540 V moved nicotine 14·2 cm. and palustrine 10·4 cm. after 60 minutes.

Chromatograms were sprayed with freshly prepared Dragendorff's reagent (potassium bismuth iodide) which revealed the alkaloids as orange-red spots on a yellow background.

TABLE I

R_F VALUES AND CHARACTERS OF SPOTS AFTER CHROMATOGRAPHY OF EXTRACTS FROM THREE SPECIES OF *Equisetum*. REFERENCE COMPOUNDS NICOTINE AND PALUSTRINE

Solvent system	Butanol: hydrochloric acid: acetic acid: water	Butanol: acetic acid: water
Main compact spots	0·08	0·53
Small diffuse spots	0·56	0·59
Small diffuse spots	—	0·69
Large diffuse spots	0·89	0·90–0·93
Nicotine compact spots ..	0·08	0·53
Palustrine compact spots ..	0·19–0·21	0·38

Extraction of the Alkaloids

The material used consisted of the sterile aerial stems of *E. arvense*, *E. telmateia* and *E. sylvaticum* collected locally during June to September, dried at room temperature and coarsely powdered.

Soft extracts were prepared from 5 kg. quantities by percolation with 20 l. of 95 per cent ethanol, evaporation to low volume at 55°, precipitation of non-alkaloidal matter in the presence of 1 per cent hydrochloric acid and extraction with ether after making alkaline.

Examination of the Extracts

Chromatography of the extracts using the equivalent of 100 g. of dry plant in ethanolic solution yielded spots corresponding to nicotine but not to palustrine. Typical results are given in Table I.

Preliminary trials with nicotine on buffered cellulose columns[12] using ether saturated with water as eluant showed that the alkaloid remained on the column at pH 5·1 but was readily eluted after addition of a small amount of diethylamine. Recovery estimated by ultra-violet absorption was about 80 per cent. Application of this technique to the extracts using quantities equivalent to 5 kg. of dry plant gave ultra-violet absorption spectra similar to that of nicotine eluted from a comparable column.

Electrophoresis of the eluate produced spots with the same mobility as nicotine.

Further portions of the eluate were spotted on filter paper bearing reference spots of nicotine and (*a*) sprayed with 1 per cent *p*-aminobenzoic acid in ethanol, (*b*) moistened with 30 per cent aqueous acetic acid and sprayed with 1 per cent α-naphthylamine in ethanol. The papers were dried and after exposure to cyanogen bromide, the extracts developed colours similar to the nicotine. Another portion of the eluate after heating with epichlorhydrin gave the same colour as nicotine similarly treated.

Results

Palustrine, the main alkaloid of *Equisetum*, was not detected in the three British species examined, but all contained small amounts of nicotine.

Discussion

Alkaloids have been isolated from *Equisetum* species of continental origin, but no reference to their presence in British species appears to exist. The absence of alkaloids (other than traces of nicotine) from the British species reported in this work may be due to climatic effects or may be genotypical.

Paper electrophoresis proved to be a useful technique for the separation of mixtures of alkaloids, its main advantage compared with paper partition chromatography being a saving of time. It was less suitable for plant extracts which tended to form streaks the full length of the paper, unless previously purified on buffered cellulose columns. Paper partition chromatography of the same extracts without special purification produced well defined spots with reproducible R_F values.

The amount of nicotine obtained from 5 kg. of dried plant material was estimated by ultra-violet absorption spectrophotometry to be not more than 2 mg. Such small quantities were readily separated from much larger amounts of impurities by the use of buffered cellulose columns following the technique of Carless[12].

Acknowledgements. The authors are grateful to Professor P. Karrer of Zürich University for the generous donation of palustrine alkaloid.

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NEW APPARATUS

A TWO-STAGE MICRO-EVAPORATOR

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Two devices for concentrating solutions are described. The first reduces a volume of 1 ml. to one of 0·05 ml. and the second a volume of 0·05 ml. to a few microlitres.

Recent developments in microchemical techniques make possible the identification of sub-microgram quantities of various compounds including alkaloids[1], sugars[2,3], steroids[4] and inorganic radicals[5]. These techniques need a moderately concentrated solution. Therefore to obtain high sensitivity, a very small volume (in general 0·1 μl.[6]) has to be employed. This may give rise to difficulties. For example, if a test will detect 0·01 μg. of a compound dissolved in one micro-drop (0·1 μl.), a solution containing 1·0 μg. in one ml. will have to be concentrated 100 times (i.e., to a volume of 10 μl.) to give a positive result. To effect such concentration is quite impracticable by ordinary means, and is even beyond the limit of the device described by Tryhorn and Curry[7]. To resolve this problem the two pieces of apparatus described below have been developed.

It is assumed that there is no difficulty in reducing a volume to 1 ml. by ordinary methods.

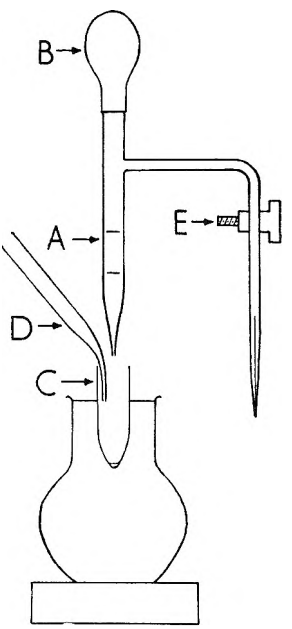

Fig. 1. First-stage evaporator.

Description of Apparatus

The first-stage evaporator will reduce the volume from 1 to 0·05 ml. The solution to be evaporated is drawn up into the tube A by means of the rubber bulb B (Fig. 1). It is allowed to drip slowly into the small conical tube C, which is maintained at a temperature of about 65° by means of a miniature water bath made from a small, flat-bottomed, bolt-head flask standing on a Simmerstat-controlled hotplate. Evaporation is assisted by means of a jet of air or nitrogen from the tube D. The optimum rate of dripping, which is controlled by the adjustable air-leak E, is such that one drop is evaporated to dryness before the arrival of the next. This has the effect of concentrating the residue on a small area at the bottom of the tube. It is usually more convenient to take the solution to complete dryness, and to redissolve in a volume of 0·05 ml. The device works best

with a solvent such as chloroform or methanol which boils at about 65°, but may also be used with less volatile liquids such as ethanol or water.

In many cases this 20-fold concentration is sufficient, but if tests carried out with micro-drops of the final solution are still negative, further concentration may be effected with the second-stage evaporator (Fig. 2).

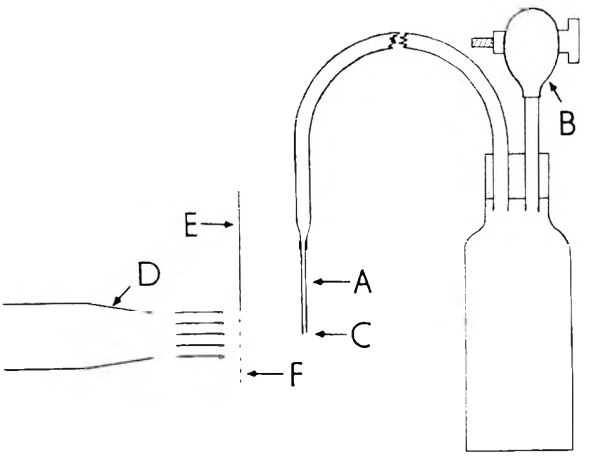

FIG. 2. Second-stage evaporator.

The solution (0·05 ml.) is drawn up into the melting point tube A by manipulation of the screw-controlled rubber bulb B, which is then adjusted so that there is a small positive meniscus at C. This is subjected to a stream of hot air from the blower D. A metal shield E prevents overheating of the liquid in A, while a gauze F moderates the air-flow to prevent the drop at C being blown away. The position of E and F respectively may be varied in relation to A. The bulb B is adjusted continuously to maintain the pendant drop at C. This drop should be kept small to avoid loss of material on the outside of the tube. When the column of liquid in A is reduced to less than 0·25 cm. in length the air blower may be switched off. Micro-drops of the concentrated solution in A may be taken for use with colour or crystal tests, or the whole of the remaining liquid may be transferred to a suitable surface and used to carry out a single reaction.

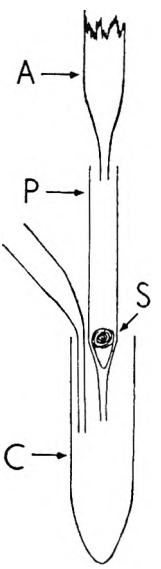

Fig. 3. Elution from paper chromatograms.

APPLICATIONS

By this method it has been found possible to demonstrate the presence of 1 part of strychnine in 10^6 parts of ethanol[1], 1 part of progesterone in 10^6 parts of methanol[4] and one part of glucose in 10^4 parts of water[2,3]; one millilitre of the solution having been used in each instance.

The first stage evaporator is especially useful in the elution of material from a paper chromatogram. For this purpose a Pasteur pipette P is inserted between the dropper A and the conical tube C (Fig. 3). The chromatogram spot is cut out and, after appropriate treatment, is folded up and forced into the neck of the pipette (S). A suitable solvent is drawn into A and allowed to drop slowly via P into C, eluting the substance from S as it passes. As before, the eluate is deposited at the bottom of C and may be redissolved in a minimal volume of liquid for subsequent examination.

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BOOK REVIEW

SYNTHETIC ANALGESICS. PART I. DIPHENYLPROPYLAMINES. By Paul A. J. Janssen. Pp. vii + 183 (including Index). Pergamon Press, Oxford, 1960. 45s.

It is no fault of Dr. Janssen that this book, the third in a series of chemical monographs and Part I on synthetic analgesics, makes rather dull reading. Much of chemical pharmacology is routine synthesis undertaken only with a view to compensation by the chance of producing a notable drug. It is inevitable that a complete review of any aspect of chemical pharmacology must approximate to a catalogue. The synthetic chemistry of drugs related to methadone, substantially an essay in the problems posed by steric hindrance, is relatively uninteresting with the possible exception of the "methadone rearrangement". Methadone was described in 1948 and the author has summarised the subsequent work to July, 1958, including his own considerable unpublished material. Synthetic methods used to prepare 576 3,3-diphenylpropylamines without substituents on the aromatic nuclei, and their published physical properties (52 have no recorded m.p. or b.p.), are briefly described. The book is worth the outlay for the time it can save in literature searching alone.

As befits a chemical monograph, biological topics have been severely pruned and this may account for the 190 references of the total of 564 that are not mentioned in the text. In view of this surfeit it is perhaps unfortunate that reference 344 is missing. Analgesia is dismissed in two pages and analgesic assay methods severely criticised in two more pages, but to assist speculation on structure-activity relationships there is a table of 31 pages giving analgesic ED50 doses and potency ratios relative to morphine, codeine, methadone and pethidine (the latter expressed in error as mg./kg.). Three tables in sequence totalling 92 pages and covering physical properties, analgesic potencies in animals, and structure, seems a bit unwieldy in the middle of a chapter, especially when compared to an average chapter length of two and a half pages and one of a mere half page. Thus the casual reader scanning Table V might find it difficult to locate the definitions of its symbols in the body of a chapter some 56 pages away, only then to find that some symbols lack definition. There is an occasional looseness of expression such as "other nitriles—are solid bases" (p. 125), or "heavier alkyl halides" (p. 135). Paragraph 4 on p. 125 is unintelligible as it stands and listing quaternary ammonium salts as alkyl halide salts of trivalent bases is perhaps convenient for tabulation but Figure 5.5 (p. 117) would be better drawn as a quaternary salt. These and a number of misprints however are minor points and do not detract from a well presented concise and factual account. There is only a modicum of hypothetical matter, this mainly limited to configurational studies.

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