REVIEW ARTICLE

BIOLOGICAL ACTIVITY IN STEROIDS POSSESSING NITROGEN ATOMS

PART II. STEROIDAL ALKALOIDS‡

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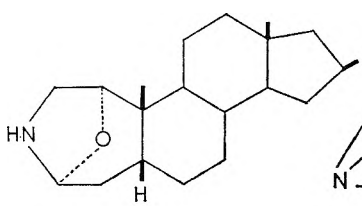
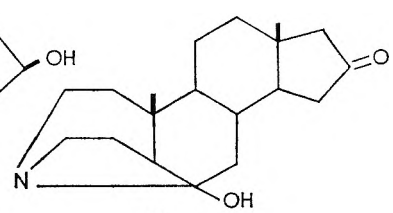
CHEMICALLY the steroidal alkaloids form a complex group, the individual members displaying much diversity in molecular structure (Fieser and Fieser, 1959; Goutarel, 1961; Jeger and Prelog, 1960; Morgan and Barltrop, 1958; Schöpf, 1961). Some occur unconjugated as the free alkamines in nature, but others occur as glycosides or esters. It has been customary to base classification of the group on botanical origin, but with the number of representatives now known, it is more convenient to consider them according to the nature of the skeleton of the alkamine. Such a classification in point of fact does not diverge too greatly from the botanical classification, but it should serve to give greater emphasis to possible structure-action relationships. Chemically, four main groups can be recognised.

3-Aza-A-homoandrostane derivatives.
Bases formally derived from the pregnane skeleton.
Bases formally derived from the unrearranged cholestane skeleton.
Bases possessing the "jervi" skeleton.

The first group is small and the only known representatives are the four salamander alkaloids samandarine (I), samandarone, samandaridine and cycloneosamandione (II) (Schöpf, 1961; Habermehl, 1962). Their pharmacological properties have been studied in detail by Gessner and his co-workers (1948 and earlier papers) who showed that these compounds exhibit analeptic activity, producing convulsions in mice and antagonising the narcotic effects of barbiturates, ethylurethane and tribromoethanol in salamander larvae and small fish.



I



II

Their action on smooth muscle appears to be variable. Thus they produce vasoconstriction of the Löwen-Trendelenburg preparation of

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isolated frog vessels, relax the carotid artery of calves, relax the guinea-pig uterus and antagonise the action of adrenaline on certain preparations. Intracutaneous injection in man produces pain and hyperaemia.

Bases Formally Derived from Pregnane

The alkaloids of this group occur in various plants belonging to the family Apocynaceae and are characterised by the possession of amino functions at C(3) or C(20), or at both positions. Usually the members

TABLE I

MONOACID BASES DERIVED FROM PREGNANE

3-AMINOPREGNANES

Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Funtumine $C_{21}H_{35}NO$	3α-Amino-20-oxo-5α-pregnane	126	+95	*Funtumia latifolia*	1
Funtumidine $C_{21}H_{37}NO$	3α-Amino-20α-hydroxy-5α-pregnane	182	+10	*F. latifolia*	1
Holamine $C_{21}H_{33}NO$	3α-Amino-20-oxo-pregn-5-ene	135	+23	*Holarrhena floribunda*	2
Holaphyllamine $C_{21}H_{33}NO$	3β-Amino-20-oxo-pregn-5-ene	260 as HCl salt	+33 as HCl salt	*H. floribunda*	2, 3
Holaphylline $C_{22}H_{35}NO$	3β-Methylamino-20-oxo-pregn-5-ene	128	+23	*H. floribunda*	3
Paravallarine $C_{22}H_{33}NO_2$	3β-Methylamino-20α-hydroxypregn-5-en-18-carboxylic acid lactone	181	−52	*Paravallaris microphylla*	4

20-AMINOPREGNANES

Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Funtuphyllamine A $C_{21}H_{37}NO$	20α-Amino-3β-hydroxy-5α-pregnane	173	+13	*Funtumia africana*	5
Funtuphyllamine B $C_{22}H_{39}NO$	20α-Methylamino-3β-hydroxy-5α-pregnane	214	+24	*F. africana* and *Malouetia bequaertiana*	5, 6
Funtuphyllamine C $C_{23}H_{41}NO$	20α-Dimethylamino-3β-hydroxy-5α-pregnane	172	+24	*F. africana*	5
Funtumafrine B $C_{22}H_{37}NO$	20α-Methylamino-3-oxo-5α-pregnane	160	+43	*F. africana*	5
Funtumafrine C $C_{23}H_{39}NO$	20α-Dimethylamino-3-oxo-5α-pregnane	174	+45	*F. africana* and *M. bequaertiana*	5
Gluco-alkaloid $C_{27}H_{45}NO_6$	20α-Amino-3β-hydroxy-pregn-5-ene-β-D-glucoside	—	—	*Conopharyngia pachysiphon*	7
Holafebrine $C_{21}H_{35}NO$	20α-Amino-3β-hydroxy-pregn-5-ene	177	−61	*Holarrhena febrifuga* and *Kibatalia arborea*	8a

ALKALOIDS NOT FULLY CHARACTERISED

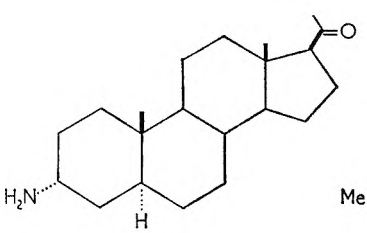
Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Holadysamine $C_{23}H_{37}NO$	—	173	−78	*Holarrhena antidysenterica*	8
Holadysine $C_{23}H_{37}NO$	—	120	−199	*H. antidysenterica*	8
Irehine $C_{23}H_{39}NO$	—	163	−30	*Funtumia elastica*	8
Latifoline $C_{23}H_{37}NO$	—	135	−4	*F. latifolia*	8*

1. Janot, Qui Khuong Huu and Goutarel (1959). 2. Janot, Cavé and Goutarel (1960). 3. Janot, Cavé and Goutarel (1959). 4. Le Men (1960). 5. Janot, Qui Khuong Huu and Goutarel 1960). 6. Janot, Lainé and Goutarel (1960). 7. Dickel, Lucas and MacPhillamy (1959). 8. Goutarel (1961). 8a. Janot and others (1962b)

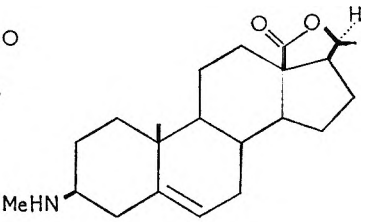
*Latifoline is now known to be the 3β-hydroxy compound corresponding to conamine (Table II) (Janot, Qui Khuong Huu and Goutarel, 1962).

of this group occur in nature as the free alkamine but at least one representative is found in glycosidic combination (Dickel, Lucas and MacPhillamy, 1959) and at least two others are found in the form of pyroterebate ester conjugates (Rostock and Seebeck, 1958).

The chemistry of the monoacid bases has recently been reviewed (Goutarel, 1961) and the known alkaloids belonging to this group are listed in Table I. Examples of the monoacid bases with the nitrogen atom in the 3-position are funtumine (III) (Janot, Qui Khuong Huu and Goutarel, 1959), and paravallarine (IV) (Le Men, 1960) which possesses a saturated lactone ring, and thus bears some structural resemblance to the dihydrocardenolides. A typical example of the monoacid bases possessing the nitrogen atom in the 20-position is afforded by funtuphyllamine A (V) (Janot, Qui Khuong Huu and Goutarel, 1960).

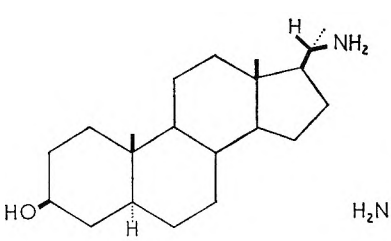


III

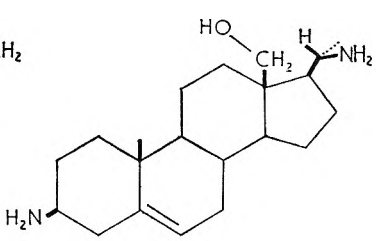


IV

The alkaloids containing two nitrogen atoms in the molecule can be subdivided into three main groups. Where the nitrogen atom on C(20) is not incorporated in a ring the alkaloids belong to the holarrhimine class, which is exemplified by holarrhimine itself (VI) (Černý, Lábler and Šorm, 1957). Where the nitrogen atom on C(20) forms a bridge to C(18) the conarrhimine and conkurchine groups result. In the conarrhimine group the nitrogen ring is fully saturated whilst in the conkurchine group the nitrogen ring possesses a double bond in the 17-20-position (Tschesche and Roy, 1956). The most extensively investigated alkaloids of the conarrhimine and conkurchine groups are conessine and conessidine respectively. The known diacid alkaloids of the pregnane group are listed in Table II.



V



VI

Two main pharmacological actions appear to be characteristic of the pregnane group of alkaloids. These are hypotensive activity and local

anaesthetic activity and both properties do not appear to be dependent upon either the number or the position of the nitrogen atoms. Thus hypotensive activity has been reported in kurchicine (Chopra, Gupta and Chopra, 1933) (later shown to be impure holarrhimine (Bertho, 1939)) and conessine (Bakhsh, 1936; Burn, 1915; Paris, 1938) as well as in funtumine, funtumidine and related alkaloids (Quévauviller and Blanpin, 1960, and earlier refs.) and in 20α-amino-3β-hydroxy-5-pregnene-βD-glucoside (Dickel and others, 1959). The activity of this last compound inspired the synthesis of several related glycosides (Lucas and others, 1960) and some of these synthetic compounds also exhibited hypotensive activity when administered intravenously to dogs, although like the parent alkaloid, they were inactive by the oral route. For similar reasons the 20-glucoside of funtumidine (glucofuntumidine) was prepared synthetically for pharmacological studies (Quévauviller and Blanpin, 1960).

More detailed studies have indicated that the hypotensive properties stem from direct actions on the heart and blood vessels. Conessine and holarrhimine, in the anaesthetised cat (Bakhsh, 1936; Burn, 1915) show a preliminary rise in blood pressure before a prolonged depression and this has been attributed to an initial stimulation of the smooth muscle of the blood vessels, followed by slowing and incoordination of the heart. Section of the vagi has no influence on the drop in blood pressure (Bakhsh, 1936; Chopra and others, 1933) although the magnitude of the fall is smaller in decerebrate cats (Chopra and others, 1933), indicating that the medullary centres are playing some rôle.

Conessine and holarrhimine have been shown to produce a dilatation of the splanchnic vessels but to contract the renal vessels (Bakhsh, 1936; Chopra and others, 1933) whilst funtumine and funtumidine have been shown to dilate both peripheral and coronary vessels (Quévauviller and Blanpin, 1958), and to exhibit a positive inotropic and negative chronotropic action on the isolated rabbit heart. Conessine and holarrhimine have been reported to stimulate intestinal and uterine contractions (Bakhsh, 1936; Chopra and others, 1933) but later work (Stephenson, 1948) has shown that conessine has a quinidine-like action and antagonises the action of acetylcholine on skeletal, cardiac and smooth muscle. In this connection it is interesting that funtumidine has been reported to slightly inhibit peristalsis of the dog intestine *in situ* (Quévauviller and Blanpin, 1958).

The local anaesthetic activity exhibited by the pregnane group of alkaloids (Burn, 1915; Chopra and others, 1933; Quévauviller and Blanpin, 1958) is in most compounds more pronounced than that of cocaine (Quévauviller and Blanpin, 1960; Stephenson, 1948; Stephenson and Dutta, 1948; Trevan and Boock, 1927) but as the compounds produce necrosis on injection (Stephenson, 1948; Stephenson and Dutta, 1948) they are without clinical value.

Other actions which have been shown to be present in the group include antipyretic activity (Quévauviller and Blanpin, 1960; Paris, 1938) and ability to potentiate barbiturate hypnosis (Quévauviller and Blanpin, 1960). Holarrhimine and conessine exert a direct narcotic effect on frogs, but

this action is absent in mammals (Bakhsh, 1936; Burn, 1915). Although funtumidine has been classed as a tranquilliser on the basis of its ability to depress motility in rats (La Barre and Desmarez, 1959) it is possible that the effect could be produced by a direct paralysis of the peripheral motor nerves rather than by a reserpine-like action. Conessine has been shown to inhibit certain enzymes (Chopra and others, 1927; Kaushiva and Ghatak, 1956) and holamine on intraperitoneal administration gives rise to Parkinsonian-like tremors (Quévauviller and Blanpin, 1960).

The structural similarity of the funtumia alkaloids to the steroid hormones inspired an investigation of these agents for hormonal activity (Blanpin and Quévauviller, 1960). The results showed that all the alkaloids studied were devoid of positive hormonal properties, but there were some indications that the bases exhibited a degree of antagonism towards a limited number of specific effects of the natural hormones.

Conessine has been termed a general protoplasmic poison since it exhibits marked toxicity towards various micro-organisms, especially protozoans (Bertho, 1944b; Chopra and others, 1927; Goyal, 1935; Henry and Brown, 1923; Paris, 1938). It appears to have little or no activity against the malaria parasite (Stephenson, 1948) or helminths (Janot and Cavier, 1949; Mackie and others, 1955) although it has been reported to show weak antituberculous properties (Lambin and Bernard, 1953; Meissner and Hesse, 1930). Its toxicity towards *Entamoeba dysenteriae* has led to a limited clinical use (see for example Acton and Chopra, 1933; Lavier, Crosnier and Merle, 1948; Tanguy, Robin and Raoult, 1948) particularly on the Indian subcontinent and there have been several studies (see for example Durieux, Trenous and Tanguy, 1948; Kaushiva, 1957; Muhlpfordt and Martinez-Silva, 1956; Piette, 1950) in which its efficacy has been compared to that of emetine. The results indicate that it is inferior to emetine as an amoebicide, but not such a potent emetic. Studies have also been made on the distribution and fixing of conessine in the monkey (Auffret and Tanguy, 1950) and on its rate of elimination in man, which is very slow (Pluchon and Pille, 1950). Several reviews concerning the clinical potentialities of conessine in the treatment of amoebiasis have been published (Duviau, 1953; Kerny, 1948; Leake, 1932) and the authors all agree that conessine is not a suitable drug.

Alkaloids Formally Derived from Cholestane

Members of this group have been isolated as the free alkamines or as glycosides of mono-, di-, tri- and tetrasaccharides but it is possible that at least some of the alkamines and lower glycosides are produced by the hydrolysis of higher glycosides during the isolation procedure. The group embraces steroidal alkaloids occurring in various *Solanum spp.* and at least three alkaloids occurring in *Veratrum spp.*, namely rubijervine (12β-hydroxysolanidine), isorubijervine (18-hydroxysolanidine) and isorubijervosine, which is the 3-glucoside of isorubijervine. These alkaloids are all characterised by a hexacyclic skeleton incorporating a piperidine

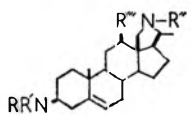
TABLE II

DIACID BASES DERIVED FROM PREGNANE

A. HOLARRHIMINE GROUP

Alkaloid	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Chonemorphine $C_{23}H_{40}N_2$	3β-Amino-20α-dimethylamino-5α-pregnane	145	+25	*Chonemorpha fragrans* *C. penangensis*	9
Holarrhidine $C_{21}H_{38}N_2O$	3α,20α-Diamino-18-hydroxy-pregn-5-ene	181–2	−23	*Holarrhena antidysenterica*	10
Holarrhimine (*syn* kurchicine) $C_{21}H_{36}N_2O$	3β,20α-Diamino-18-hydroxy-pregn-5-ene	183–6	−14·2	*H. antidysenterica*	11–13
Malouetine $C_{27}H_{52}N_2^{++}$	3β,20α-Bistrimethylammonium-5α-pregnane	264 (picrate)	+3 (chloride in water)	*Malouetia bequaertiana*	14
(3)-*N*-Methylholarrhimine $C_{22}H_{38}N_2O$	20α-Amino-18-hydroxy-3β-methylamino-pregn-5-ene	above 360 (dihydrochloride)	−28 (dihydrochloride in MeOH)	*H. antidysenterica*	15
(20)-*N*-Methylholarrhimine $C_{22}H_{38}N_2O$	3β-Amino-18-hydroxy-20α-methylamino-pregn-5-ene	163–164	−19	*H. antidysenterica*	15
NNN'N'-Tetramethylholarrhimine $C_{26}H_{44}N_2O$	3β,20α-Bis(dimethylamino)-18-hydroxy-pregn-5-ene	227–229	−35	*H. antidysenterica*	15, 16

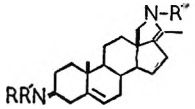
B. CONARRHIMINE GROUP



Alkaloid	R	R'	R''	R'''	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Conarrhimine $C_{21}H_{34}N_2$	H	H	H	H	Impure preparation	—	*H. antidysenterica*	13
Conimine $C_{22}H_{36}N_2$	Me	H	H	H	134	−30 (EtOH)	*H. antidysenterica*	17, 18
Conamine $C_{22}H_{38}N_2$	H	H	Me	H	130	−21	*H. antidysenterica*	13, 18
Conessimine $C_{23}H_{38}N_2$	Me	Me	H	H	100	−22·3	*H. antidysenterica*	19, 20
Isoconessimine $C_{23}H_{38}N_2$	Me	H	Me	H	92	+30 (EtOH)	*H. antidysenterica*	21
Conessine (*syn* wrightine) $C_{24}H_{40}N_2$	Me	Me	Me	H	125	−2	*Holarrhena spp.*	16, 22–24
Holarrhenine $C_{24}H_{40}N_2O$	Me	Me	Me	OH	198	−7	*H. congolensis*	25, 26
Holafrine $C_{29}H_{46}N_2O_2$	Me	Me	H	$OCOC_6H_9$	116–117	−19·1	*H. africana DC*	25
Holarrhetine $C_{30}H_{48}N_2O_2$	Me	Me	Me	$OCOC_6H_9$	74–75	−14·9	*H. africana DC*	25

TABLE II *continued*

C. CONKURCHINE GROUP



Alkaloid	R	R′	R″	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Conkurchine $C_{21}H_{36}N_2$	H	H	H	152	−67·4	*H. antidysenterica*	27
Conessidine $C_{22}H_{38}N_2$	Me	H	H	123	−52·2	*H. antidysenterica*	18, 28
Trimethylconkurchine $C_{24}H_{42}N_2$	Me	Me	Me	125–127	+12·0	*H. antidysenterica*	18

D. ALKALOIDS INCOMPLETELY CHARACTERISED

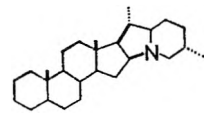
Alkaloid	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Base $C_{22}H_{34}N_2$	129·5	—	*H. antidysenterica*	29
Base $C_{22}H_{38}N_2$	87–88	—	*H. antidysenterica*	30
Conkurchinine $C_{23}H_{38}N_2$	161	−47 (EtOH)	*H. antidysenterica*	28
Holarrhessimine $C_{22}H_{36}N_2O$	160–164	−30	*H. antidysenterica*	20
Holarrhine $C_{20}H_{38}N_2O_2$	240	−17 (MeOH)	*H. antidysenterica*	19
α-Hydroxyconessine $C_{24}H_{40}N_2O$	—	−9 (EtOH)	*H. antidysenterica*	22
Kurchamine $C_{23}H_{38}N_2$	115–117	−16	*H. antidysenterica*	15
Kurchenine $C_{21}H_{32}N_2O_2$	335–336	−78 (H_2SO_4 in water)	*H. antidysenterica*	31
Kurchessine $C_{24}H_{40}N_2$	132–133	−36	*H. antidysenterica*	15
Kurchine (*syn* Curchine, nor-Conessine) $C_{20}H_{36}N_2$	75	−7	*H. antidysenterica*	12, 28, 32
Letiocine $C_{17}H_{25}NO_2$	350–352	—	*H. antidysenterica*	33
Malouphylline $C_{24}H_{40}N_2O_2$	259	−10	*M. bequaertiana*	34*

9. Janot and others (1962a). 10. Černý, Lábler and Šorm (1959). 11. Černý, Lábler and Šorm (1957). 12. Ghosh and Bose (1932). 13. Siddiqui (1936). 14. Janot, Lainé and Goutarel (1960). 15. Tschesche and Wiensz (1958). 16. Lábler and Černý (1959). 17. Siddiqui and Siddiqui (1934). 18. Tschesche and Roy (1956). 19. Siddiqui and Pillay (1932). 20. Tschesche and Petersen (1954). 21. Siddiqui (1935). 22. Bertho and Gotz (1958). 23. Favre and others (1953). 24. Siddiqui (1934). 25. Rostock and Seebeck (1958). 26. Uffer (1956). 27. Bertho (1951). 28. Bertho (1939). 29. Bertho (1947). 30. Bertho (1944a). 31. Bertho, von Schuckmann and Schönberger (1933). 32. Haworth (1932). 33. Peacock and Chowdhury (1935). 34. Goutarel (1961).

* The structure of malouphylline is now known to be 3β — acetamido-20α-dimethylamino-5α-pregnan-18-al (Janot, Lainé and Goutarel, 1962).

TABLE III

BASES DERIVED FROM SOLANIDANE



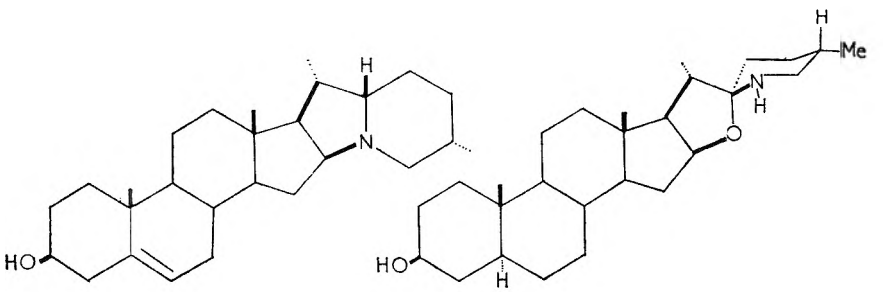
Alkamine	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids	m.p. °C.	$[\alpha]_D$ in pyridine	Sugar	Source	Refs.
Acetylleptinidine $C_{29}H_{45}NO_2$	3β-Hydroxy-x-acetoxy-solanid-5-ene	192–196	—	Leptine I $C_{47}H_{75}NO_{16}$	230	−85	Trisaccharide of 2 moles L-rhamnose and 1 mole D-glucose	*Solanum chacoense*	35
				Leptine II				,,	
				Leptine III				,,	
				Leptine IV				,,	
Demissidine $C_{27}H_{45}NO$	3β-Hydroxy-5α-solanidane	220–222	+21	Demissine (*syn* Solanine d) $C_{50}H_{83}NO_{20}$	305–308	−20	Branched tetrasaccharide of 1 mole D-xylose 1 mole D-galactose and 2 moles D-glucose (lycotetraose)	*S. demissum*	36, 37
Leptinidine $C_{27}H_{45}NO_2$	3β,-x-Dihydroxy-solanid-5-ene	247–248	−24	Leptinine I $C_{45}H_{73}NO_{15}$	230	−90	Trisaccharide of 1 mole D-glucose and 2 moles L-rhamnose	*S. chacoense*	35
				Leptinine II $C_{45}H_{73}NO_{16}$	225	−62	Trisaccharide of 1 mole D-galactose 1 mole L-rhamnose and 1 mole D-glucose	,,	35
				Leptinine III	—	—	—	,,	35
				Leptinine IV				,,	35
Rubijervine $C_{27}H_{43}NO_2$	3β,12α-Dihydroxysolanid-5-ene	242–243	+8					*Veratrum album*	38, 39
Isorubijervine $C_{27}H_{43}NO_2$	3β,18-Dihydroxysolanid-5-ene	235–237	+6·5 (EtOH)	Isorubijervosine $C_{32}H_{53}NO_7$	279–280	−20	D-Glucose	*V. viride* *V. album*	38, 40, 41

TABLE III—*continued*

Alkamine	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids	m.p. °C.	$[\alpha]_D$ in pyridine	Sugar	Source	Refs.
Solanidine (*syn* Solatubine Solanidine t) $C_{27}H_{43}NO$	3β-Hydroxysolanid-5-ene	219	−27	α-Chaconine $C_{45}H_{73}NO_{14}$	242	−85	α-L-Rhamnopyranosyl (1 → 2 glucose)-α-L-rhamnopyranosyl (1 → 4 glucose)-D-glucose	*Solanum tuberosum* *S. nigrum* *S. dulcamara* *S. chacoense*	42
				β-Chaconine $C_{39}H_{63}NO_{10}$	255	−61·5	α-L-Rhamnopyranosyl (1 → 4) D-glucose	"	42
				γ-Chaconine $C_{33}H_{53}NO_6$	243–244	−40	D-Glucose	"	42
				α-Solanine (*syn* Solanine Solanine t Solatunine) $C_{45}H_{73}NO_{15}$	285	−59	α-L-Rhamnopyranosyl (1 → 2 galatose)-β-D-glucopyranosyl (1 → 3)galactose D-galactose	"	37, 43, 44
				β-Solanine $C_{39}H_{63}NO_{11}$	295	−31 (MeOH)	β-D-Glucopyranosyl (1 → 3) D-galactose	"	42
				γ-Solanine $C_{33}H_{53}NO_6$	240–250	−26 (MeOH)	D-Galactose	"	42
				Tetroside	—	—	Tetrasaccharide of 1 mole D-xylose 2 moles D-glucose 1 mole D-galactose	*S. acaulia*	45
				Solacauline $C_{45}H_{69}NO_{14}$	260–265	−30	Trisaccharide of 1 mole D-glucose and 2 moles D-xylose	*S. acaulia*	37
Soladulcamaridine $C_{27}H_{43}NO$	—	220–222	−78 (MeOH)	Soladulcamarine $C_{45}H_{73}NO_{17}$	193–197	—	Tetrasaccharide of 1 mole D-glucose 1 mole L-rhamnose 2 moles L-arabinose	*S. dulcamara*	46

35. Kuhn and Löw (1961 a,b). 36. Kuhn, Löw and Trischmann (1957). 37. Schreiber (1954). 38. Jacobs and Craig (1945). 39. Pelletier and Locke (1957). 40. Klohs and others (1953b). 41. Weisenborn and Burn (1953). 42. Kuhn, Löw and Trishmann (1955,b,c). 43. Kuhn, Löw and Trishmann (1955c). 44. Uhle and Jacobs (1945). 45. Schreiber (1957b). 46. Rasmussen and Boll (1958).

ring and they may be divided into two subclasses according to the immediate environment of the piperidino nitrogen atom. These subclasses are the solanidane and spirosolane groups and are exemplified by the alkamines solanidine (VII) and tomatidine (VIII) respectively. The known alkaloids belonging to the solanidane group are listed in Table III and those belonging to the spirosolane group are shown in Table IV.



VII VIII

The literature contains a number of references to the poisonous nature of various *Solanum spp.* and this can be attributed to the presence of cholestane-type alkaloids (see for example Griebel, 1923; Lowe, 1929; Rühl, 1951; Schowalter and Hartmann, 1924; Sirotina and Spirina, 1948). Potato sprouts and potatoes which have turned green through exposure above the ground develop a detectable amount of solanine and its aglycone solanidine and human consumption of such potatoes or potato shoots has led to a number of outbreaks of potato poisoning, several of which have been discussed by Willimott (1933). The most extensively investigated alkaloid of the cholestane group from a biological point of view is α-solanine (solanine), but it is to be noted that commercial samples of solanine have been shown to consist of 6 components (Kuhn, Löw and Trischmann, 1955b).

The cholestane group of alkaloids show certain similarities in their pharmacological properties to the pregnane group. Thus solanocapsine has been shown to slow the heart and induce incoordination by a direct action on cardiac muscle (Watt, Heimann and Epstein, 1932) and solanine, like conessine, has been shown to possess local anaesthetic properties (Weill, 1913). Rubijervine and several of its synthetic esters possess hypotensive properties (Poethke and Kuntze, 1958). Both solanine and solasonine induce haemolysis (de Lavergne and Kissel, 1935; Fischer, 1929; Macht, 1933) whilst solanine diminishes blood catalase (Levi, 1936), and inhibits non-specific cholinesterase (Pokrovskii, 1956). Solanine is also active as a mitotic poison (Danneberg and Schmähl, 1953) and has been shown to inhibit the oxygen uptake of mouse ascites tumour cells (Schmitz, 1951).

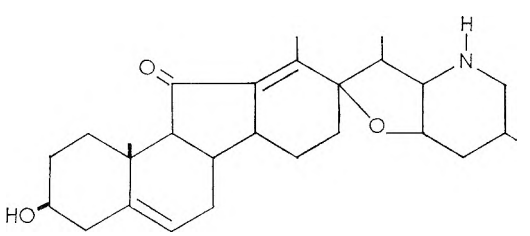
The discovery that extracts of tomato leaves exhibited antifungal and antibacterial activity led to the isolation of tomatine (Fontaine and

others, 1948) and similarly the observation that the leaves of *Solanum demissum* were resistant to the attacks of the larvae of the potato beetle *Leptinotarsa decemlineata* had as a result the isolation of demissine (Kuhn and Gauhe, 1947). Later work showed that other cholestane-type glycosidic alkaloids possessed the ability to prevent the ravages of the potato beetle and as tested on the leaves of solanum *tuberosum*, the order of potency was leptine I, then tomatine, then demissine, then α-solanine, and finally α-chaconine (Kuhn and Löw, 1961a). Independent work showed demissine to be more active than solacauline, which was more active than solanine (Shreiber, 1954). The insecticidal activity of various preparations of solanum steroidal alkaloids has, however, been shown to be but slight (Bergmann, Levinson and Mechoulam, 1958; Pollacci and Gallotti, 1940; Sievers and others, 1949).

Several studies have been devoted to the investigation of the antimicrobial properties of the group. Tomatine and several other alkaloids are antifungal (Chanussot, 1957; McKee, 1959; Sackman, Kern and Wiesman, 1959) and solanocapsine is claimed to possess *in vitro* activity against *Mycobacterium tuberculosis* (Boll and others, 1955–56). A number of synthetic solanine-type glycosides have been prepared, but they do not appear to have been investigated biologically (Schreiber, 1955).

Alkaloids possessing the "Jervi" Skeleton

The completely characterised alkaloids possessing the modified or "jervi" steroid skeleton in which ring C is 5-membered and ring D is 6-membered, are conveniently divided into two subclasses with the fritillaria alkaloids whose chemical constitution is as yet incompletely established, forming a third subclass. The first group consists of alkaloids whose alkamines possess a secondary nitrogen atom and contain only two or three atoms of oxygen. They occur in nature as the free alkamine or as D-glucosides and may be termed the "jerveratrum" alkaloids, as suggested by Fieser and Fieser (1959). Representative alkamines of this class are jervine (IX) and veratramine (X). The second subclass, which may be termed the "ceveratrum" group, consists of alkaloids whose alkamines are polyhydroxy tertiary bases possessing seven to nine atoms of oxygen and incorporating a quinolizidine ring system.

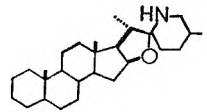


IX

The alkamine germine (XI) and the closely related alkamines, protoverine, veracevine and zygadenine possess a masked α-ketal system and

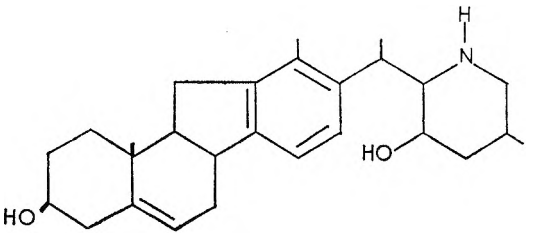
TABLE IV

DERIVATIVES OF SPIROSOLANE



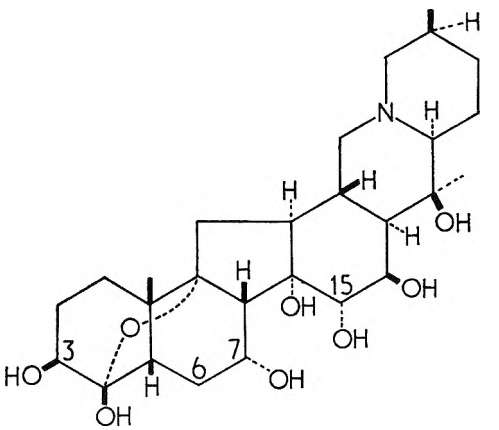
Alkamine	Structure	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids	m.p. °C.	$[\alpha]_D$ in pyridine	Sugar	Source	Refs.
Solasodine (*syn* Solanidine s Solancarpidine Purapuridine) $C_{27}H_{43}NO_2$	3β-Hydroxy-22a, 25-L-spirosol-5-ene	202	−80 (MeOH)	Solasodamine $C_{51}H_{83}NO_{20}$	298–302	−72 (MeOH)	L-Rhamnosido-L-rhamnosido-D-galactosido-D-glucose	*Solanum sodomeum* *S. auriculatum* *S. marginatum* *S. aviculare*	47
				Solasonine (*syn* Solanine s Solancarpine Purapurine γ-solanigrine) $C_{45}H_{73}NO_{16}$	301–303	−88	L-Rhamnosido-D-galactosido-D-glucose	*S. aviculare* *S. sodomeum* *S. xanthocarpum* *S. nodiflorum* *S. torvum* *S. laciniatum*	48–52
				Solamargine (*syn* δ-solanigrine) $C_{45}H_{73}NO_{15}$	301–310	−114	L-Rhamnosido-L-rhamnosido-D-glucose	*S. marginatum* *S. nigrum*	47, 48, 50, 52a
Tomatidine $C_{27}H_{45}NO_2$	5α,22b,25-L-Spirosolan-3β-ol	210–211	−8 (MeOH)	Tomatine $C_{50}H_{83}NO_{21}$	263–267	−19	Branched tetrasaccharide of 1 mole D-xylose 1 mole D-galactose and 2 moles D-glucose	*Lycopersicum pimpinellifolium* *L. esculentum* *L. peruvianum* *L. hirsutum*	52b, 53
				Trioside	—	—	Trisaccharide of 2 moles D-xylose and 1 mole D-glucose	*S. polyadenum*	52a
Solanocapsine $C_{27}H_{44}N_2O_2$	3α-Amino-x-hydroxy spirosolane	222	+25·5	—	—	—	—	*S. pseudocapsicum*	54
Solauricidine $C_{27}H_{43}NO_2$	—	219	−90 (MeOH)	Solauricine $C_{45}H_{73}NO_{16}$	270	—	L-Rhamnosido-D-galactosido-D-glucose	*S. auriculatum*	55
Solangustidine $C_{27}H_{43}NO_2$	—	amorphous (above 315 (HCl))	—	Solangustine $C_{33}H_{53}NO_7.H_2O$	235	—	D-Glucose	*S. angustifolium*	56
Soladulcidine $C_{27}H_{45}NO_2$	5α,22a,25-D-Spirosolan-3β-ol	206·5	52·6	α-Soladulcine	—	—	—	*S. dulcamara*	57–59
				β-Soladulcine	—	—	—		
				γ-Soladulcine	—	—	—		
				Tetroside $C_{50}H_{83}NO_{21}$	268–270	53	2 moles D-glucose, 1 mole D-galactose and 1 mole D-xylose	*S. dulcamara*	60
5,6-Dehydrotomatidine $C_{27}H_{43}NO_2$	3β-Hydroxy-22b, 25-L-spirosol-5-ene	206	−45	—	—	—	—	*S. tuberosum*	61

47. Briggs and Brooker (1958). 48. Boll (1958). 49. Briggs and Cambie (1958). 50. Kuhn, Löw and Trischmann (1955a). 51. Taylor (1958). 52. Uhle (1954). 52a. Schreiber (1957b). 52b. Fontaine and others (1948). 53. Kuhn, Löw and Trischmann (1957). 54. Boll and Lillevik (1959). 55. Briggs and Carroll (1942). 56. Tutin and Clewer (1914). 57. Briggs and O'Shea (1952). 58. Shreiber (1958). 59. Tuzson and Kiss (1957). 60. Alkemeyer and Sander (1959). 61. Shreiber (1957a).



X

undergo rearrangement with base. In veracevine, the rearrangement is particularly facile to yield first cevagenine and then cevine and for a time it was believed that cevine was the actual alkamine of the veracevine ester alkaloids. The ceveratrum alkaloids occur in various *Veratrum*, *Zygadenus* and *Schoenocaulon* species and are the agents responsible for the poisonous nature of these plants (Bealth and others, 1933; Reinhardt, 1909).



XI

The ceveratrum group have been isolated as the free alkamines or as mono-, di-, tri- or tetra- esters of various organic acids. Partial deacylation of the ester alkaloids occurs readily, however, and it is possible that some of the lower esters which have been isolated are in fact artefacts. It is the ceveratrum ester alkaloids, more particularly the tri- and tetra-esters which are the agents responsible for the hypotensive properties present in crude extracts of veratrum alkaloids. The known alkaloids of the ceveratrum group, together with the known jerveratrum alkaloids, are shown in Table V.

The jerveratrum alkaloids (both the alkamines and the glucosides) are characterised by an ability to antagonise the cardioaccelerator action of sympathetic nerve stimulation or of sympathomimetic amines (Krayer, 1952). This effect is thought to arise from a highly selective action upon the pacemaker of the heart and is not shown by adrenergic blocking agents. Accordingly the term "anti-accelerator agent" has been coined to describe

TABLE V

ALKALOIDS POSSESSING THE JERVI SKELETON

A. JERVERATRUM ALKALOIDS

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived alkaloids (3-glucosides)	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Source	Refs.
Jervine $C_{27}H_{39}NO_3$	240–245	−167·5	Pseudojervine $C_{33}H_{49}NO_8$	300–301	−131	*Veratrum album* *V. viride* *Schoenocaulon officinale*	62–66
Veratramine $C_{27}H_{39}NO_2$	204–207	−71	Veratrosine $C_{33}H_{49}NO_7$	242–243 (decomp.)	−55 (EtOH/$CHCl_3$)	*V. album* *V. viride*	67–69

B. CEVERATRUM ALKALOIDS

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived ester alkaloids	Esterifying acids—position of substitution in brackets	m.p. °C.	$[\alpha]_D$ in pyridine	Source	Refs.
Germine $C_{27}H_{43}NO_8$	218–221	+4 (EtOH)	Germanitrine $C_{39}H_{59}NO_{11}$	Acetic (7): (−)-2-Methylbutyric (15): Angelic (3)	228–229	−61	*Veratrum fimbriatum*	70–72
			Germbudine $C_{37}H_{59}NO_{12}$	(−)-2-Methylbutyric (15): (+)-*threo*-2,3-Dihydroxy-2-methylbutyric (3)	160–164	−8	*V. viride*	73, 74
			Neogermbudine $C_{37}H_{59}NO_{12}$	(−)-2-Methylbutyric (15): (−)-*erythro*-2,3-Dihydroxy-2-methylbutyric (3)	149–152	−12	*V. album* *V. viride*	75, 76
			Germerine $C_{37}H_{59}NO_{11}$	(−)-2-Methylbutyric (3) (+)-2-Hydroxy-2-methylbutyric (15)	200–203	−14	*V. album* *V. viride* *V. nigrum*	77–81
			Germidine $C_{34}H_{53}NO_{10}$	Acetic (3) (−)-2-Methylbutyric (15)	230–231	−11	*V. viride* *Zygadenus venenosus*	78, 81, 82
			Isogermidine (*syn* neogermidine) $C_{34}H_{53}NO_{10}$	Acetic (7) (−)-2-Methylbutyric (15)	221–223	−63	*V. viride* *Z. paniculatus* *Z. venenosus*	78, 79, 81, 83
			Germinitrine $C_{39}H_{57}NO_{11}$	Acetic Angelic Tiglic	175–176	−36	*V. fimbriatum*	63, 70
			Germitetrine (*syn* germitetrine B) $C_{41}H_{63}NO_{14}$	Acetic (7): (−)-2-Methylbutyric (15): 2-Hydroxy-2-methyl-3-acetoxybutyric (3)	229–230	−74	*V. album*	75, 84, 85, 92
			Deacetylgermitetrine $C_{39}H_{61}NO_{13}$	(−)-2-Methylbutyric (15): 2-Hydroxy-2-methyl-3-acetoxybutyric (3)	143–149	−8	*V. album*	75, 76
			Germitrine $C_{39}H_{61}NO_{12}$	Acetic (7): (−)-2-Methylbutyric (3): (+)-2-Hydroxy-2-methylbutyric (15)	216–219	−69	*V. viride*	77, 78
			Neogermitrine $C_{36}H_{55}NO_{11}$	2 moles Acetic (3,7): (−)-2-Methylbutyric (15)	234–235	−78	*V. viride* *V. fimbriatum* *V. escholtzii* *Z. paniculatus* *Z. venenosus*	78, 82, 86, 92
			Protoveratridine $C_{32}H_{51}NO_9$	(−)-2-Methylbutyric (3)	272–273	−9	*V. album* *V. viride* *Z. venenosus*	78, 83

TABLE V—*continued*

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived ester alkaloids	Esterifying acids—position of substitution in brackets	m.p. °C.	$[\alpha]_D$ in pyridine	Source	Refs.
Protoverine $C_{37}H_{43}NO_9$	195–200	−11 (EtOH)	Escholerine $C_{32}H_{41}NO_8$	2 moles Acetic (6,7): (−)-2-Methylbutyric (15): Angelic (3)	235–236	−30	*V. escholtzii*	87, 88
			Deacetylprotoveratrine $C_{39}H_{61}NO_{13}$	Acetic (6): (−)-2-Methylbutyric (15): (+)-2-Hydroxy-2-methylbutyric (3)	191–192	−15	*V. album*	76, 89
			Deacetylneoprotoveratrine (*syn* germbutrine deacetylprotoveratrine B protoveratetrine) $C_{39}H_{61}NO_{14}$	Acetic (6): (−)-2-Methylbutyric (15): (+)-*threo*-2,3-Dihydroxy-2-methylbutyric (3)	182–183	−9	*V. album* *V. viride*	73, 89, 90
			Protoveratrine A $C_{41}H_{65}NO_{14}$	2 moles Acetic (6,7): (−)-2-Methylbutyric (15): (+)-2-Hydroxy-2-methylbutyric (3)	267–269	−40	*V. album* *V. viride* *Z. venenosus*	85, 91–93
			Protoveratrine B (*syn* neoprotoveratrine veratetrine) $C_{41}H_{63}NO_{15}$	2 moles Acetic (6,7): (−)-2-Methylbutyric (15): (+)-*threo*-2,3-Dihydroxy-2-methylbutyric	268–270	−37	*V. album* *V. viride* *Z. venenosus*	79, 85, 91, 93, 94
Neo-Sabadine $C_{27}H_{43}NO_8$	140–150	−33 (EtOH)	Sabadine $C_{29}H_{45}NO_9$	Acetic	120–140	−9·5 (EtOH)	*V. sabadilla*	95
Sabine $C_{27}H_{45-47}NO_7$	173–176	—	Sabatine $C_{29}H_{47-49}NO_8$	Acetic	256–258	—	*Schoenocaulon officinalie*	96

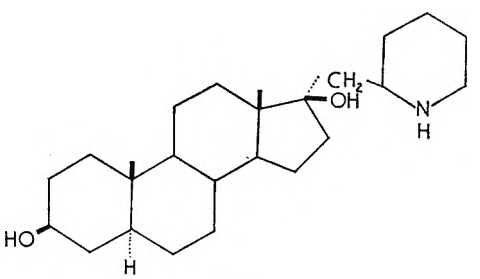
[*continued overleaf*

TABLE V—*continued*

Alkamine	m.p. °C.	$[\alpha]_D$ in $CHCl_3$	Derived ester alkaloids	Esterifying acids—position of substitution in brackets	m.p. °C.	$[\alpha]_D$ in pyridine	Source	Refs.
Veracevine (*syn* protocevine) $C_{27}H_{43}NO_8$	181–183	−33	Cevacine $C_{29}H_{45}NO_9$	Acetic (3)	205–207	−27 ($CHCl_3$)	*V. sabadilla*	97–100
			Cevadine (*syn* α-veratrine crystalline veratrine pure veratrine) $C_{32}H_{49}NO_9$	Angelic (3)	208–215	+6	*V. sabadilla* *V. viride*	101, 102
			Vanilloylveracevine $C_{35}H_{49}NO_{11}$	Vanillic (3)	256–257	+43	*V. sabadilla*	103
			Veratridine (*syn* amorphous veratrine) $C_{36}H_{51}NO_{11}$	Veratric (3)	160–180	−19 ($CHCl_3$)	*V. album* *V. sabadilla* *V. viride*	100, 101, 104
Zygadenine $C_{27}H_{43}NO_7$	201–204	−45	Angeloylzygadenine $C_{44}H_{69}NO_{11}$	Angelic (3)	222–224	−35 ($CHCl_3$)	*V. album*	105, 106
			Vanilloylzygadenine $C_{36}H_{49}NO_{10}$	Vanillic (3)	258–259	−27 ($CHCl_3$)	*Z. paniculatus* *Z. venenosus*	107
			Veratroylzygadenine $C_{36}H_{51}NO_{10}$	Veratric (3)	270–271	−27 ($CHCl_3$)	*V. album* *V. fimbriatum* *V. escholtzii* *V. nigrum* *Z. paniculatus* *Z. venenosus*	105, 108, 109
			Zygacine $C_{29}H_{45}NO_8$	Acetic (3)	Amorphous	−22 ($CHCl_3$)	*Z. paniculatus* *Z. venenosus*	110, 111

62. Jacobs and Craig (1944). 63. Klohs and others (1953a). 64. Okuda and Tsuda (1961). 65. Poethke (1938). 66. Tsukamoto and Kishimoto (1954). 67. Jacobs and Sato (1949). 68. Klohs and others (1953b). 69. Tamm and Wintersteiner (1952). 70. Klohs and others (1952b). 71. Kupchan and Afonso (1959). 72. Kupchan and Narayanan (1959). 73. Myers and others (1955). 74. Kupchan and Gruenfeld (1959b). 75. Kupchan and Ayres (1959). 76. Myers and others (1956). 77. Fried, White and Wintersteiner (1950). 78. Kupchan (1959a). 79. Myers and others (1952). 80. Poethke (1937). 81. Weisenborn and Bolger (1954). 82. Fried, Numerof and Coy (1952). 83. Kupchan and Deliwala (1952b). 84. Glen and others (1952). 85. Nash and Brooker (1953). 86. Klohs and others (1954). 87. Klohs and others (1952c). 88. Kupchan and others (1960). 89. Kupchan, Ayres and Hensler (1960). 90. Klohs and others (1953c). 91. Kupchan and Ayres (1960). 92. Kupchan and Deliwala (1953). 93. Stoll and Seebeck (1953a). 94. Klohs and others (1952a). 95. Möhrle and Auterhoff (1959). 96. Mitchner and Parks (1959). 97. Barton and others (1954). 98. Kupchan, Johnson and Rajagopalan (1959). 99. Kupchan and others (1953). 100. Vejdelek, Macek and Kahac (1956). 101. Ikawa and others (1945). 102. Kupchan and Afonso (1960). 103. Morgan and Barltrop (1958). 104. Blount (1935). 105. Kupchan (1959b). 106. Suzuki and others (1957). 107. Kupchan and Deliwala (1952a). 108. Klohs and others (1953a). 109. Stoll and Seebeck (1953b). 110. Kupchan, Lavie and Zonis (1955). 111. Shimizu (1958).

compounds exhibiting this particular pharmacological action (Krayer, 1950). Since antiaccelerator activity is absent in the tertiary veratrum alkaloids, it was concluded that such activity was associated with a secondary nitrogen atom incorporated in a piperidine ring (Krayer, Uhle and Ourisson, 1951) and attention was devoted to the preparation of synthetic steroids possessing a piperidine ring in the side chain (Krayer and Briggs, 1950; Uhle, 1951), among which were included several compounds prepared by cleavage of the ether link in derivatives of spirosolane. These compounds indeed proved active, but later work showed antiaccelerator activity to be present both in tertiary amino-steroids (Gould, Shapiro and Herschberg, 1954) and in secondary amino-steroids in which the nitrogen atom was not part of a piperidine ring (Gould and others, 1954; Margolin and others, 1954). These observations serve to emphasise the dangers of postulating structure-action relationships from a consideration of an inadequate number of compounds of insufficient chemical diversity. Quinidine was also shown to possess dual anti-accelerator and antifibrillatory activity and in this connection it is interesting that certain nitrogenous steroids (Gould and others, 1954; Robson and Trounce, 1955; Schallek and others, 1957) such as 17α-(2-piperidylmethyl)-3β,17β-dihydroxyandrostane (XII) and 16α-cyclohexylamino-3β-hydroxy-20-oxopregn-5-ene show quinidine-like properties.



XII

Detailed investigations have shown that veratramine decreases the oxygen consumption of atrial tissue without any initial augmentation of uptake (Reiter, 1950). In high doses veratramine produces excitation of the central nervous system (Krayer, 1949) whilst it is claimed that jervine in high doses produces hypotension in dogs (Wood, 1906).

Preparations of the ceveratrum ester alkaloids of varying purity have been employed medicinally from the time of the ancient Greek herbalists until the present day, the modern interest lying in their hypotensive properties, but as their pharmacology is covered in standard texts and has been extensively reviewed elsewhere (Krayer and Acheson, 1946; Stoll, 1954), a relatively brief summary will suffice in the present article. No attempt will be made to cover the literature pertaining to each individual ceveratrum ester.

Much of the earlier work was done with a preparation known as veratrine, which was first obtained by Pelletier and Caventou (1820),

but as this proved to be a complex mixture (Auterhoff, 1955; Blount, 1935), care must be taken in assessing this work owing to the great variation in potency exhibited by the alkamines and their esters (Krayer and Acheson, 1946; Krayer, Moe and Mendez, 1944). Other mixtures of alkaloids which have been employed in biological studies or medicinally, are cevadilline (also called sabadilline), cryptenamine (Kupchan and Gruenfield, 1959), protoveratrine, sabadine and sabatrine. As a broad generalisation it would appear that the alkamines are almost devoid of hypotensive activity, the naturally-occurring monoesters are feebly active, the diesters more active and the tri- and tetra-esters very active (Wintersteiner, 1953). In this connection it is to be noted that the highly active ester alkaloid germitetrine, although giving rise to four molecules of organic acids per molecule on hydrolysis, is really only a triester, as one of the esterifying acids is 2-hydroxy-2-methyl-3-acetoxybutyric acid (Kupchan and Ayres, 1959). Similar potency relationships have been found amongst synthetic esters of germine, where it was also discovered that several synthetic tetraesters were virtually inactive (Weisenborn and others, 1954).

More recent structure-action studies of a large number of synthetic esters of protoverine (Kupchan, Hensler and Weaver, 1961) have shown that esterification at positions 3 and 15 is necessary for high activity and that esterification at position 16 is accompanied by a profound loss of activity. Positions 6 and 7 need not be esterified for high activity. Esterification by a branched chain acid is advantageous at position 15, of no great import at position 3 and disadvantageous at position 7. Moreover, these relations were found to be broadly true for both the naturally-occurring and the previously prepared synthetic esters of germine (Weisenborn and others, 1954) whose structures were unknown at the time of the original experiments.

The pharmacological actions exhibited by the ceveratrum ester alkaloids are complex, making it difficult to analyse the exact contribution each makes to the total response, but there would now seem to be general agreement that in low doses they act by triggering reflex mechanisms.

The most pronounced pharmacological effect of the ceveratrum esters at therapeutic doses is the production of a rapid fall in arterial pressure, which is mediated by a reflex general vasodilatation, and a fall in heart rate. Thus they act in a unique fashion, differing from all other hypotensive agents. They are without any direct action on the blood vessels. The drop in blood pressure is also accompanied by respiratory depression. These effects have been demonstrated in various species of animals as well as in man and the experiments have indicated the existence of species differences (Rothlin and Cerletti, 1954), rodents being much more resistant to the hypotensive effect than man, the dog, or the cat.

The experimental evidence indicates that the afferent sensory receptors, upon which the ceveratrum esters act to produce the reflex fall in blood pressure, lie in the lungs and the heart (Heymans and Neil, 1958), with their afferent fibres lying in the vagi. Elicitation of the Benzold-Jarisch reflex (Aviado and Schmidt, 1955; Jarisch and Richter, 1939; von

Benzold and Hirt, 1867) as it is now known, however, is not the only reflex action produced by the ceveratrum esters, as vagotomised animals may still show a fall in blood pressure, indicating that other receptors are also involved (Heymans and Vleeschhouwer, 1950; Wang, Ngai and Grossman, 1955). The most important of these other receptors appear to be the baroreceptors situated in the region of the carotid bifurcation (see for example Aviado and others, 1955; Martini and Calliauw, 1955). Experiments with dogs would seem to indicate that stimulation of receptors within the nodose ganglion are responsible for the production of emesis (Borison and Fairbanks, 1952).

In higher concentrations the ceveratrum esters induce vasoconstriction and so exert a pressor effect. This action is mediated in part at least by the liberation of adrenaline from the adrenal medulla (Krayer, Moe and Mendez, 1944; Mendez and Montes, 1943).

At high doses the ceveratrum esters also produce changes in the electrophysiological state of nerve and muscle (see for example Shanes, 1958) which manifest themselves in the elicitation of a series of repetitive responses to a single stimulus—the so-called "veratrinic" response (see for example Acheson and Rosenbleuth, 1941; Gregor, 1904). It is thought that these electrophysiological changes result from alterations in the concentration of calcium ions on the cell membrane (Gordon and Welsh, 1948; Straub, 1954) with disruption of the normal ion transport mechanisms across the membrane (Shanes, 1952 and earlier papers; Straub, 1956) although muscle and nerve do not show the same changes in ionic migration when exposed to ceveratrum esters. Thus there is an increase of potassium ion efflux from nervous tissue (Shanes, 1952) and cardiac muscle (Lister and Lewis, 1959; Vick and Kahn, 1957) but no increase from skeletal muscle (Lister and Lewis, 1959). In view of the general assumption that ionic exchange occurs by similar mechanisms in all tissues (Davson and Danielli, 1952; Heilbrunn, 1956; Hodgkin, 1951) these facts are disturbing. It has also been shown that the ceveratrum esters do not promote potassium ion influx into potassium-poor cold-stored human erythrocytes (Kahn, Acheson and Cohen, 1955).

Whatever the detailed mechanism, it would appear safe to conclude that the ceveratrum esters interfere with the functioning of the cell membrane and it is probable that similar changes occur at the sensory afferent receptors (Jarisch and Zotterman, 1948) which would appear to have a far greater sensitivity than nerve or muscle cells.

Experimentally veratrine has been used to induce auricular arrhythmias in order to screen drugs for antifibrillatory activity (Scherf and Chick, 1951).

Other Actions of Ceveratrum Esters

Pronounced insecticidal activity is present in the ceveratrum group and dusts and extracts prepared from the seeds of *Schoenocaulon spp.* (sabadilla seed) have been tried as insecticides (see for example Anderson, 1945; Frazier, 1945; Filmer and Smith, 1946; Ikawa and others, 1945; Walton, 1946), over eighty publications dealing with their efficacy against

various insect species appearing in the years 1944–1956. These studies have also been extended to include ceveratrum alkaloid preparations from *Veratrum spp.* (see for example Fisher, 1940; Jaretzky and Janecke, 1940; Krupp, Lendle and Stapenhorst, 1952; Seiferle, Johns and Richardson, 1942). A particularly active preparation is produced by treating sabadilla seed with lime (Allen and Brunn, 1945; Walton, 1945). As is true for hypotensive activity, the alkamines appear virtually inactive as insecticides (Allen and others, 1945). In attempts to elucidate the exact mechanism by which the ceveratrum esters act upon the insect, several studies have been concerned with their effect on various enzyme systems (Collias, McShan and Lilly, 1952; Hartley and Brown, 1955).

Other studies have been concerned with their ability to induce mutations in *Drosophila funebris* (Tinyakov, 1947) and their ability to produce C-mitotic effects (Burroni, 1955).

TABLE VI

FRITILLARIA ALKALOIDS

Alkaloid	m.p. °C	$[\alpha]_D$ in $CHCl_3$	Refs.
Alginine $C_{33}H_{39}NO_3$	271–272	+108 (EtOH)	112
Amianthine $C_{27}H_{41}NO_2$	251–253	−87	113
Base $C_{27}H_{45}NO_3$	256	—	114
Beilupeimine $C_{27}H_{43}NO_3$	155–157	−53 (EtOH)	114
Chinpeimine $C_{27}H_{43}NO_2$	247–248	−21	114
Fritiminine	258–260	—	114
Imperoline $C_{27}H_{45}NO_3$	237–238	−8	115
Imperonine $C_{27}H_{43}NO_3$	239	−65	115
Peimidine $C_{27}H_{45}NO_2$	222	−74 (EtOH)	116
Peimine (*syn* Peimunine Verticine apo Verticine) $C_{27}H_{45}NO_3$	223–224	−26 (EtOH)	117–120
Peiminine (*syn* Peimiphine Peimitidine Verticilline Fritillarine) $C_{27}H_{43}NO_3$	212–213	−78	116, 120, 121
Peimissine $C_{27}H_{43}NO_4$	270	−51 (EtOH)	116
Sipeimine (*syn* Imperialine) $C_{27}H_{43}NO_3$	267	−36	122–124
Sonpeimine $C_{27}H_{43}NO_4$	256–258	—	114

112. Yunusov, Konovalova and Orekhov (1939). 113. Neuss (1953). 114. Chu and Loh (1956b). 115. Paul and Boit (1958). 116. Chou (1947). 117. Chou and Chu (1941). 118. Chu and Loh (1956a, b, and earlier papers). 119. Ito and others (1961). 120. Wu (1944). 121. Chi, Kao and Chang (1940). 122. Bauer and others (1958). 123. Boit (1954). 124. Chu and Loh (1955).

The Fritillaria Alkaloids

The fritillaria group on present indications would appear to possess the same skeleton as the ceveratrum group, but to possess only two or three hydroxyl groups (Chu and Loh, 1956b and earlier papers). The group deserves more research, especially on the inter-relations of the

alkaloids which are claimed to be individual entities. These alkaloids are listed in Table VI.

The pharmacology of the group is also in need of re-investigation with modern techniques. The Chinese drug pei-mu (Chou, 1954), from which many of the alkaloids have been isolated, was used quite irrationally for widely diverse conditions (Chi, Kao and Chang, 1936). Claims (Liu, Chang and Chang, 1936) that one of the alkaloids, peimine (also called peimunine) resembles atropine in its pharmacological properties seem surprising, as do claims of veratrine-like activity (Narumi, 1935), since these alkaloids do not possess the ester groups now known to be essential for this activity. On the other hand, reports (Chen, Chen and Chou, 1933; Narumi, 1936) that peimine and peiminine possess hypotensive properties and induce incoordination in the heart are more in line with the known activity of the pregnane group of alkaloids and may further illustrate the lack of dependence of these properties upon the position and configuration of the nitrogen atom in nitrogenous steroids. Additional evidence on this point would be provided by the reports (Zolotukhina, 1945) that alginine, the alkaloid from *Fritillaria sewerzowii*, possesses pronounced local anaesthetic activity, comparable to that of cocaine and exhibits vasodilatatory properties—if one assumes that the originally proposed molecular formula (Yunusov, Konovalova and Orekhov, 1939) is in error and that alginine does indeed have a cevane skeleton.

Amianthine, whose admission to the group is by no means certain, is obtained from the plant known as staggergrass or fly poison (*Amianthium muscaetoxicum*) (Neuss, 1953). This alkaloid has been shown to depress respiration and lower blood pressure and to be definitely without a typical veratrinic action on muscle (Alsberg, 1914).

There are a number of other alkaloids which, in all probability, belong to the steroid group, but their purity, identity or chemical constitution, are still unknown. Three such alkaloids which would appear to be pure entities are geralbine (Stoll and Seebeck, 1952), which is a C_{22} compound and raddeanine (Aslanov and Sadykov, 1956) and veratrobasine (Stoll, Stauffacher and Seebeck, 1955), which are C_{24} compounds. Raddeanine has been shown to stimulate the central nervous system of cats, rabbits and dogs in small doses, but in larger doses to be a depressant (Zolotukhina, 1944).

Conclusions

Despite the shortcomings of current theories of drug action and the lack of a simple correlation of chemical structure and biological activity, it is nevertheless clear from the foregoing account that much of the recent interest in the biological properties of nitrogenous steroids has a rational basis. Two examples may be quoted. The first is the application of the conclusions drawn from the receptor theory to the synthesis of the anabolic steroidal [3,2-c] pyrazoles and [2,3-d] isoxazoles, and the second is the synthesis of new drugs suggested by the supporting moiety theory. Both represent significant steps forward. Only within the last ten years

has interest developed in the synthetic nitrogenous steroids and, in view of the encouraging progress in the field, it would seem that even greater attention will be devoted to these compounds in the future. This is especially true now that aza-steroid hormone analogues have been synthesised (Zderic, Carpio and Limon, 1962) and with the interesting demonstration that the anabolic steroid 17β-hydroxy-17α-methylandrostano-[3,2-c]-pyrazole loses its ability to promote nitrogen retention on introduction of a double bond into the 4-position whilst it is converted into an oestrogenic compound showing no anabolic or androgenic properties when the 4,-6-diene system is introduced (Beyler, Potts and Arnold, 1961). With the recent discoveries that anabolic properties are present in a number of steroidal Schiff's bases (Irmscher, 1962) and that hypotensive properties are present in certain steroidal enamines (Clinton and others, 1962), it can be confidently predicted that nitrogenous steroids will play a further rôle in studies of drug action.

Moreover, the nitrogenous steroids as a group faithfully reflect the biological properties of the steroids in general, affording a broad spectrum of biological action and emphasizing changes in pharmacological properties with species, and routes of administration. Certain individual members, notably the ceveratrum ester alkaloids, display a variety of pharmacological actions which in themselves have led the pharmacologist to disentangle basic mechanisms of action, thus contributing to a better understanding of biological phenomena. Nitrogenous steroids are not unique in this respect, but they do present a happy choice with which to illustrate the slow but certain development of the theoretical aspects of pharmacology.

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RESEARCH PAPERS

CARDIOTONIC SUBSTANCES FROM *BERSAMA ABYSSINICA* FRES. SUB. SPECIES *ABYSSINICA*

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Three crystalline materials have been isolated from the leaves of *Bersama abyssinica* sub. sp. *abyssinica*, N.O. Melianthaceae. Some chemical and pharmacological evidence is presented that these materials are bufodienolide aglycones. The material isolated in greatest quantity has a toxicity similar to that of scillaridin.

Bersama abyssinica, N. O. Melianthaceae, is a tree with a wide distribution in Africa occurring from S. Rhodesia to Ethiopia. Leaves from this tree have been shown to be toxic to cattle (Harker and Gourlay, 1959) and the clinical and pathological findings in cattle, rabbits and mice after poisoning by leaves and crude leaf extracts have been described (Gourlay and Harker, 1960). Investigation of the leaves of this tree have resulted in the isolation of several toxic materials: the most potent of these have produced in cattle the characteristic symptoms of poisoning by the leaves (Gourlay, Harker and Lock, 1962). The method of isolation of some of these materials and some of their chemical and pharmacological properties are described in this paper.

Materials and Methods

Plant materials were air dried and reduced to moderately fine powder in a disintegrator.

Chromatographic purification and separation; materials. Alumina, chromatographic grade, was heated for 1 hr. at 110° and 14 mm. Hg, and then equilibrated with water vapour for a week over aqueous saturated sodium bromide solution. Chloroform was rendered low in ethanol by washing four times with water, drying over calcium chloride and distilling. Isopropanol was dried over anhydrous sodium sulphate and distilled.

Identification of active fractions. From 0·1 ml. to 1·0 ml. of eluate was evaporated to dryness on a staining tile and the residue covered with concentrated sulphuric acid; characteristic colours developed.

Chromatography on paper. Whatman No. 1 or No. 4 paper was soaked in 20 per cent formamide in acetone for 5 min. and the acetone blown off the paper. After applying the samples, the paper was run in 50 per cent xylene and methylethyl ketone by the ascending method at the ambient temperature of this laboratory (ca. 22°) (Kaiser, 1955). After drying at 110°, the paper was layered on to a thick film of concentrated sulphuric acid on a glass plate; characteristic colours developed.

Pharmacological solutions. Crystalline materials were dissolved at a concentration of 1 per cent in ethanol and the ethanol diluted with 9 parts of water. This solution was subsequently diluted with physiological solutions as required.

Toxicity determinations. Mice. White mice, 16–22 g., were dosed intraperitoneally, intravenously or orally. Observations for deaths were made for 24 hr. Approximate LD50 determinations were obtained graphically.

Cats and Vervet monkeys. Animals were anaesthetised with ether and infused intravenously with dilutions of the drugs in normal saline at the rate of 1 ml./min. until death (de Lind Van Wijgaarden, 1926). Electrocardiographic records were taken from some animals. Respiration was recorded by Gaddum's (1941) method.

Isolated hearts from rabbits and guinea-pigs were perfused with Ringer's solution by Langendorff's method.

RESULTS

Extraction. 5 kg. of powdered leaves were percolated with 100 litres of 75 per cent ethanol. After concentration to 10 litres extraction with chloroform yielded 35 g. of oily semi-solid material. Preliminary purification of this was achieved by chromatography on a column 8 cm. diameter by 18 cm. high containing 800 g. alumina. Some inert oily material was removed by the passage of 3 litres of chloroform. Active material was then eluted with 3 litres of 10 per cent isopropanol in chloroform. Removal of the solvent left 4·2 g. of slightly oily solid residue. Subsequent elution of the column with methanol yielded 2·8 g. of material containing only small amounts of toxic activity. In Table I is summarised the toxic activity of the various stages so far described.

TABLE I

THE TOTAL TOXIC ACTIVITIES OBTAINED FROM LEAVES OF *B. abyssinica* DURING PRELIMINARY EXTRACTIONS AND PARTIAL PURIFICATION BY CHROMATOGRAPHY

Stage	Total amount of sample	kg.-mouse units*
Concentrate of percolate from 5 kg. leaves	10 litres	2490
Total residue from chloroform extract of percolate ..	35 g.	2200
Partially purified material from 1st chromatography	4·2 g.	1240
Methanol eluate from 1st chromatography	2·3 g.	5

* A kg.-mouse unit is defined as ONE median lethal dose per kg. administered intraperitoneally to white mice.

Fractionation of active components. The partially purified material was dissolved in chloroform and applied to a column of alumina, 330 g., of dimensions 2·8 cm. diameter and 54 cm. length. Linear gradient elution with 0 to 10 per cent isopropanol in chloroform was then started. After the passage of 1·25 litres of eluent, 25 ml. samples were fractionally collected. Samples of individual fractions were tested with sulphuric acid and combined in groups according to the colour reaction. Solvent was removed and the residues weighed. Aliquotes of the individual samples were taken for paper chromatography and toxicity tests. In Table II is summarised the results obtained by the fractionation.

Treatment of individual fractions. Only those fractions showing major amounts of toxic activity have so far been investigated, namely, Nos. 4, 7 and 8 in Table II.

TABLE II

A SUMMARY OF THE RESULTS OF GRADIENT ELUTION, WITH ISOPROPANOL IN CHLOROFORM, FROM A COLUMN OF ALUMINA, OF 4·2 G. OF PARTIALLY PURIFIED TOXIC MATERIAL FROM BERSAMA LEAVES

Combined fraction No.	Gradient range per cent isopropanol	Tube numbers 25 ml. fractions	Colour* with sulphuric acid**	Total wt. residue of fraction g.	*RF* and colour* of spot on paper with sulphuric acid†	kg.-mouse units	Designation of material crystallised from fraction
1	0·625–1·25	1–17	B V,	0·792	0·64 V, 0·73 Pc, 0·81 YB, 0·72 B, 0·91 BOr,	5	
2	1·25–2·5	18–28	P Or,				
3	1·25–2·5	29–38	B Or,				
4	1·25–2·5	39–53	Rd,	1·020	0·71 R	913	R
5	2·5–5	54–60	B V,	0·210	0·4 V, 0·51 R, 0·73 Pc,	3	
6	2·5–5	61–70	Rd,		0·29 V, 0·37 Y, 0·49 ROr, 0·61,		
7	2·5–5	71–84	Or Rd,	1·190	0·47 POr,	16	O
8	2·5–5	85–104	Or Rd,	0·986	0·43 Or,	49	Q
9	5·0–10	105–120	Or,	0·202	0·27 B, 0·36 Or, 0·75 Y, 0·69 V, 0·22 BOr,	5	
10	5·0–10	121–200	Y Or,				

* B, brown; Or, orange; Pc, peach; Rd, red; V, violet; Y, yellow.
** The colours attempt to express the maximal colour developing with H_2SO_4 from an aliquote of the fraction evaporated to dryness. Thus B V, indicates a brownish violet coloration.
† *RF* values of entities separated on paper by Kaiser's method, the colour being that developed by immersion of the paper in sulphuric acid.

Fraction 4. The residue from this fraction tended to crystallise. Repeated washing with ether removed oil and green colouring matter. Almost white crystals were left. Yield: 350 mg. The crystals were dissolved in ethanol, and an equal quantity of water added and the solution treated with charcoal (BDH decolourising acid washed) and the recovered material recrystallised from methanol. The purified material is designated in this paper as substance "R".

Fraction 7. This residue which was oily was also treated with ether, in which the active material was found to be moderately soluble. Repeated crystallisation from this solvent removed much oily and green matter and the greenish white crystals (74 mg.) were treated with charcoal and recrystallised from methanol. The resulting crystals are designated as "O".

Fraction 8. This material was treated as for fraction 7 and designated as "Q".

A summary of some chemical and physical properties of R, O and Q is shown in Table III.

Pharmacological: Toxicity Determinations

Mice. Lethal and sublethal doses produced violent and prolonged clonic convulsions in this species. With lethal doses the mice frequently died in a tonic convulsion with the head drawn down onto the chest and the hind legs extended. With sublethal doses convulsions were prolonged

for as long as 24 hr. followed by recovery. Convulsions began within 5 min. after intraperitoneal injection, and within 10, orally.

TABLE III

SOME CHEMICAL AND PHYSICAL PROPERTIES OF MATERIALS R, O AND Q

Test	Material R	Material O	Material Q
Melting point ° (uncorrected) ..	237–241	224–226	224–226
Molecular wt.*	421	420	—
Optical rotation* $[\alpha]_D^{23°}$	+43	+43	—
Maximum ultra-violet absorption Å	300	300	300
Liebermann Immediate	pale yellow	red	bright yellow
Burchard After 30 sec.	pale green	olive green	blue green
Legal	negative	negative	negative
Raymond	negative	negative	negative

* This information is derived from results obtained by the Microanalytical Laboratories, Oxford

In Table I is shown the total toxic activity obtained during extraction of the leaves and preliminary purification of the extract. Table IV shows the approximate median lethal doses of the crystalline materials R, O and Q; the results for digitoxin are included for comparison.

Knee jerk. During the administration of toxic fractions in increasing amounts up to lethal doses, no increase in knee jerk reflex was observed.

TABLE IV

THE APPROXIMATE MEDIAN LETHAL DOSES FOR MICE OF MATERIALS R, O, Q AND DIGITOXIN BY THE INTRAPERITONEAL AND ORAL ROUTES

Material	Median lethal dose mg./kg. Intraperitoneal	Oral
R	0·51	0·84
O	30·75	83·00
Q	9·30	16·20
Digitoxin	12·60	250·00

Blood pressure and respiration. Non-lethal doses of toxic materials in cats and monkeys produced a pressor response of up to 50 mm. Hg, recovery taking about 20 min. With lethal doses, heart irregularities supervened and the blood pressure fell abruptly to zero. Respiration continued after cessation of the heart beat (Fig. 1).

Isolated hearts. Non toxic doses produced on isolated hearts an increased contractility and tone with irregularities of rhythm due to extrasystoles. Large doses produced heart block, independent beating of auricles and ventricles and finally ventricular fibrillation (Fig. 2).

Lethal doses, Vervet monkeys and cats. Table V shows the lethal doses obtained by intravenous infusion of material R in cats and monkeys; material Q was not included owing to the small amount isolated. ECG recordings obtained simultaneously (Fig. 3) showed cardiac changes

sometimes beginning with slight bradycardia with an increased P—Q interval, and then, invariably, the development of ectopic beats, bundle branch block, gross irregularities and cardiac arrest following ventricular fibrillation. Results obtained with substance O and Q were similar.

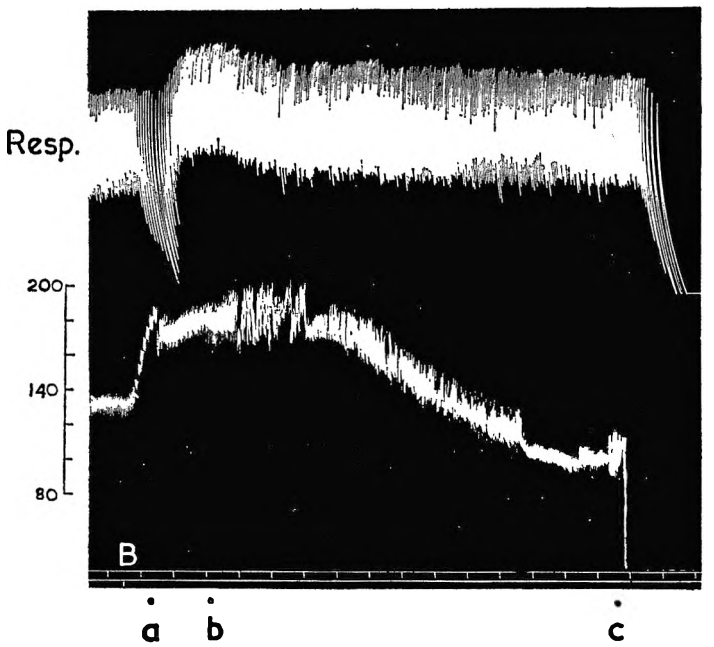

FIG. 1. Vervet monkey, 4·5 kg., anaesthetised with ether. At B, 0·5 mg. material R was given intravenously. At a, a pressor response of 50 mm. of mercury is seen; at b, irregularities of the heart's action occurred; at c, the blood pressure fell to zero, preceding the failure of respiration.

DISCUSSION

The preliminary investigations into the toxic activity of leaves of *B. abyssinica* lead to the suspicion that a strychnine-like substance was involved, mice, rabbits and cattle showing a convulsive response varying in intensity with the species. The absence, however, of any enhancement of the knee jerk reflex in cats indicated stimulation of the central nervous system at a level higher than the spinal cord. The results obtained on blood pressure and respiration in monkeys showed clearly a cardiotoxic effect, the heart invariably failing before the respiration. The cardiac effect was confirmed on isolated hearts; an initial increase of amplitude was seen first, followed by partial and then complete heart block, culminating, if the dose was large enough, in ventricular fibrillation. These responses, together with the initial rise of blood pressure shown in Fig. 1 and the convulsant effects, particularly prominent in mice, are all characteristic of a digitalis-like substance. Confirmation of this hypothesis is afforded by the ECG recordings (Fig. 3). The successive changes consisting of bradycardia with an increased P-Q interval, the development of

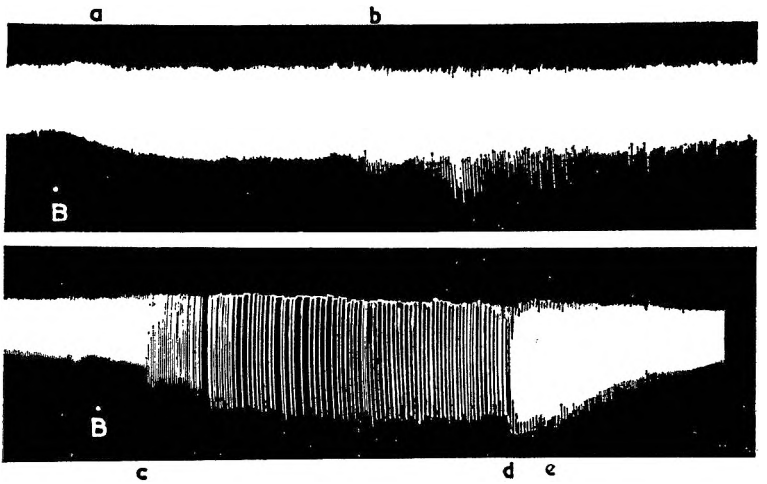

FIG. 2. The effect of material R on the isolated rabbit heart; Langendorff's preparation. Upper tracing: At B, 60 μg. of material R were injected into the perfusion fluid immediately above the heart. At a, an increase in amplitude occurred together with an increase in tone; there was no significant change in rate. At b, irregularities of rhythm occurred, due to extrasystoles.

Lower tracing: At B, 400 μg. of material R were injected. At c, heart block and loss of tone developed; at d, auricles and ventricles began to beat independently; at e, ventricular fibrillation occured.

ectopic beats, increasing degrees of block and finally ventricular fibrillation are typical of digitaloid substances.

The chemical evidence indicates that the isolated materials are bufodienolides. The negative responses to the Legal and Raymond tests point to the absence of the five membered lactone ring, whereas ultraviolet absorption at 300 Å is characteristic of the six membered bufodienolide ring. The small molecular weights found for R and O indicate

TABLE V

THE LETHAL DOSES OF MATERIAL R BY CONTINUOUS INTRAVENOUS INFUSION INTO ETHERISED VERVET MONKEYS AND CATS

Species. Doses in μg./kg.	
Vervet monkey	Cat
93·0	110
78·0	127
93·5	130
90·5	110
98·8	
91·5	
94·2	
81·7	
Mean 90·1	119
S.E. ± 2·43	± 5·38

that the substances are genins and evidence has not been found of the occurrence of glycosides during the isolation of these materials. The similarity of the intraperitoneal and oral median lethal doses for mice,

particularly for material R, and the rapid onset of convulsions by the latter route, indicate a ready absorption by the gastrointestinal tract.

The vervet monkey was found to be more susceptible to the toxic action of material R than the cat and in this laboratory, on a small number of animals, the mean lethal dose for the cat (Table V) is similar to that reported by Chen (1950) for scillarenin viz. 110 μg./kg.; the elucidation of the structure of R would be of interest. It is of note that unlike most cardenolides and bufodienolides, the materials isolated from *B. abyssinica* produce a prolonged burning sensation on mucous membranes, but no bitter taste.

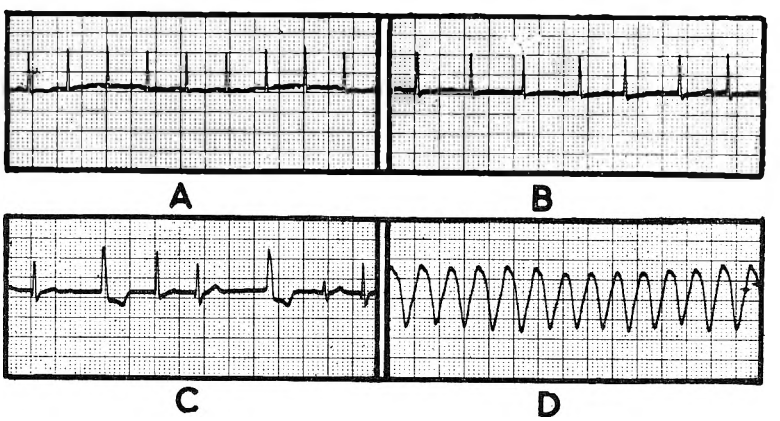

FIG. 3. Vervet monkey, 3·5 kg. anaesthetised with ether. Electrocardiographic records taken during continuous intravenous infusion with material R. At A, after 12·5; at B, 25; at C, 75; and at D, 87·5 per cent of the lethal dose.

Toxic activity has not been found in the fruit, seeds or bark of *B. abyssinica* sub. sp. *abyssinica*; the leaves of *B. abyssinica* sub. sp. *paullinioides* have been found to contain a toxic factor producing typical convulsions in mice. This and allied varieties of *Bersama* are being investigated.

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THE RELATIONSHIP BETWEEN ELECTRICAL RESISTANCE AND DISPERSED PHASE CONCENTRATION IN OIL IN WATER EMULSIONS

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The electrical resistance of a series of oil-in-water emulsions has been measured, and used to test the validity of four equations relating resistance with the concentration of dispersed phase. A modification of one of the equations was found to give the best relationship.

Although the electrical conductivity (or its reciprocal, the resistance) of emulsions has been used qualitatively as a means of determining emulsion type since about 1920 (Clayton, 1918, Bhatnagar, 1920), little work has been published on the factors which influence the quantitative results.

Maxwell (1904) calculated that when a medium of specific resistance (R′) contains a volume fraction (ϕ) of spheres of specific resistance (R″), then the specific resistance of the complete system (R) can be calculated from the equation:

$$R = \frac{2R'' + R' + \phi (R'' - R') R'}{2R'' + R' - 2\phi (R'' - R')} \quad \ldots\ldots\ldots\ldots \quad \text{(i)}$$

Oils have a much greater specific resistance than most aqueous solutions so that for oil-in-water emulsions R″ approaches infinity, and equation (i) can be simplified to:

$$\frac{R'}{R} = \frac{2 (1 - \phi)}{2 + \phi} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad \text{(ii)}$$

Maxwell placed two limitations on his equation, (*a*) the spheres must not be too close together, and (*b*) R″ must not be considerably greater than R′. These limitations imply that equation (ii) is not valid for oil-in-water emulsions, particularly those containing large amounts of oil. However, Fricke and Morse (1925), who separated cream from milk, and determined the specific resistance of the cream, the skimmed milk, and mixtures of the two, obtained agreement within $\pm 0{\cdot}5$ per cent for emulsions containing a volume fraction of oil of up to 0·62, when using this equation.

More recently Mashovets (1951) studied the conductance of electrolyte solutions containing glass spheres, and found that for any arrangement, and all volume concentrations up to closest packing (0·7405), the results agreed with the empirical equation:

$$\frac{R'}{R} = 1 - 1{\cdot}78\phi + \phi^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad \text{(iii)}$$

Equations (ii) and (iii) apply to systems containing spheres of uniform size. Bruggeman (1935) derived the equation:

$$1 - \phi = \frac{R'/R - R/R''}{(R'/R)^{\frac{1}{2}} (1 - R/R'')} \quad \ldots\ldots\ldots\ldots\ldots \quad \text{(iv)}$$

for systems containing spheres of an infinite variety of diameters. When the disperse phase is non-conducting, this reduces to:

$$\frac{R'}{R} = \sqrt{(1-\phi)^3} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (v)$$

De la Rue and Tobias (1959) applied this equation to dispersions of glass spheres in an aqueous solution of zinc bromide, and found that better agreement was obtained with dispersions consisting of a variety of sizes of spheres, than with uniform dispersions. Meredith and Tobias (1961) modified Bruggeman's equation to allow for equal volumes of spheres of two different diameters. For a non-conducting disperse phase this reduced to:

$$\frac{R'}{R} = \frac{8\,(2-\phi)\,(1-\phi)}{(4+\phi)\,(4-\phi)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (vi)$$

Meredith and Tobias recommended this equation for systems containing a volume fraction of over 0·2 of disperse phase, and equation (ii) for weaker emulsions.

The literature thus contains four equations which appear to be applicable to the electrical resistances of oil-in-water emulsions, and three of these have been derived from theoretical considerations. Three of the four equations have been utilised in studies of ideal systems. The purpose of the present work was to determine which of these equations best fitted the data obtained from a number of emulsions of possible pharmaceutical interest.

Experimental

Materials. Analar potassium chloride was used to prepare the 0·1 Demal solution used in the determination of cell constants. The polyvinyl alcohol was a commercial sample, Gelvatol 20–30 (Shawinigan Ltd.). All the other materials used in this preliminary investigation were of B.P. quality.

Apparatus. The resistances were measured using a Cambridge Conductivity Bridge, in conjunction with Mullard dip cells. Platinum black was removed from the electrodes because it would be difficult to clean them adequately after immersion in the emulsions. The cells were cleaned by rinsing with tap, and then purified water, before immersion in acetone. Cell constants were determined periodically and showed that the cleaning process was effective. The cell was always air dried before immersion in the emulsion to avoid dilution effects and to ensure good contact between the emulsion and the electrodes. All weighings and measurements were made at 25 $\pm$ 0·1°.

Methods

(*a*) *Preparation of emulsions.* In order that R′ and ϕ could be most accurately ascertained the following procedure was adopted to prepare the emulsions. A solution of the emulgent was prepared in purified water and, if necessary, strained to remove foreign matter. The weight per ml.

(at 25°) and the specific resistance of this solution were determined. The weight per ml. of the oil was similarly found. Known weights of the oil and continuous phase were triturated together in a mortar and pestle. With emulsions stabilised either by acacia or polyvinyl alcohol this treatment produced emulsions of suitable stability, but emulsions containing either cetrimide or sodium lauryl sulphate were coarse, and were passed through a hand-operated homogeniser to obtain stable preparations. In each instance, the orifice was set at the same distance. The completed emulsion was usually divided into two portions, each of which was transferred to a dry tube, capped, and placed in the water bath. The volumes of oil and aqueous phase were calculated, and the volume of oil divided by the total volume of the emulsion to obtain ϕ.

(*b*) *Measurement of resistance.* The dry dip cell was placed in the emulsion, care being taken to avoid the introduction of air bubbles, and the resistance measured at about 5 min. intervals. The resistance usually decreased gradually, becoming constant after about 20 min. This decrease was attributed to temperature changes brought about by the introduction of the cell. Duplicate measurements were made on each emulsion. Emulsions which showed a tendency to cream were stirred gently with the cell between measurements. The ratio R′/R found experimentally for each emulsion was compared with the values obtained by solving the various equations using the known values of ϕ.

Systems Studied

Unless otherwise stated olive oil was used as dispersed phase.

(i) *Acacia emulsions.* A total of 57 emulsions were made using 40 per cent w/v acacia solution as continuous phase. Oil contents varied between 0·09 and 0·60. Stronger emulsions could not be prepared. The more dilute systems (ϕ <0·2) tended to cream slowly.

Similar series of emulsions containing either castor oil or liquid paraffin dispersed in 40 per cent w/v acacia solution were prepared and measured, to determine whether the results were affected by the oil used. A series of emulsions containing olive oil dispersed in 30 per cent acacia solution, and another in 50 per cent acacia solution were also prepared.

(ii) *Polyvinyl alcohol emulsions.* Polyvinyl alcohol was chosen as an emulgent because it is non-ionic and forms an aqueous solution of fairly high resistance and also because of its reported protective colloid properties (Capitani and Pirrone, 1956) it was expected to behave in a similar way to acacia. A 10 per cent solution of polyvinyl alcohol in purified water was prepared and used as continuous phase in the preparation of olive oil emulsions. Although the emulsions were finely dispersed, they creamed visibly over several hours and were stirred gently at intervals between measurements of the resistances.

(iii) *Ionic emulgents.* Cetrimide and sodium lauryl sulphate were examined. In both cases a 5 per cent solution was used as continuous phase. All these emulsions tended to cream, and were stirred gently between measurements.

The results obtained with sodium lauryl sulphate are given in Table I and are typical of the whole series.

TABLE I

CALCULATED VALUES OF R′/R FOR SODIUM LAURYL SULPHATE STABILISED EMULSIONS OF OLIVE OIL

Equation		(ii)		(iii)		(v)		(vii)	
ϕ	R′/R Experimental	R′/R Calculated	Percentage error	R′/R Calculated	Percentage error	R′/R Calculated	Percentage error	R′/R Calculated	Percentage error
0·0959	0·8425	0·8627	+2·4	0·8385	−0·5	0·8597	+2·0	0·8521	+1·1
0·1395	0·7761	0·8044	+3·5	0·7712	−0·6	0·7982	+2·8	0·7885	+1·6
0·1439	0·7663	0·7986	+4·1	0·7646	−0·2	0·7921	+3·3	0·7822	+2·8
0·2053	0·6894	0·7207	+4·4	0·6767	−1·9	0·7084	+2·7	0·6968	+1·1
0·2213	0·6552	0·7011	+6·6	0·6551	−0·0	0·6872	+4·7	0·6753	+3·0
0·2274	0·6699	0·6937	+3·4	0·6469	−3·6	0·6791	+1·4	0·6672	−0·4
0·2987	0·5513	0·6102	+9·7	0·5575	+1·1	0·5873	+6·1	0·5756	+4·2
0·3078	0·5403	0·5999	+9·9	0·5469	+1·2	0·5759	+6·2	0·5643	+4·3
0·3344	0·5097	0·5703	+10·6	0·5166	+1·3	0·5430	+6·1	0·5321	+4·2
0·3813	0·4411	0·5196	+15·1	0·4667	+5·5	0·4867	+9·4	0·4771	+7·5
0·3900	0·4521	0·5105	+11·4	0·4579	+1·3	0·4764	+5·1	0·4673	+3·3
0·3994	0·4411	0·5006	+11·9	0·4486	+1·7	0·4655	+5·2	0·4567	+3·4
0·4448	0·3671	0·4542	+19·2	0·4061	+9·6	0·4137	+11·3	0·4071	+9·8
0·4816	0·3482	0·4178	+16·7	0·3747	+7·1	0·3732	+6·7	0·3688	+5·6
0·5091	0·3188	0·3913	+18·5	0·3530	+9·7	0·3439	+7·3	0·3410	+6·5
0·5410	0·3014	0·3613	+16·6	0·3297	+8·6	0·3110	+3·1	0·3100	+2·8
0·5972	0·2488	0·3102	+19·8	0·2936	+15·3	0·2556	+2·0	0·2588	+3·9
0·6696	0·1986	0·2475	+19·8	0·2565	+22·6	0·1899	−4·6	0·1985	0·0
0·6716	0·1907	0·2458	+22·4	0·2556	+25·4	0·1882	−1·3	0·1967	+3·1
0·7476	0·1411	0·1837	+23·2	0·2282	+38·2	0·1268	−11·3	0·1395	−1·2
0·7765	0·1173	0·1610	+27·1	0·2208	+46·9	0·1057	−11·0	0·1189	+1·3

DISCUSSION

Since the pattern of the results varied only slightly on changing the emulgent or the disperse phase, all the systems are considered together.

The results obtained with the various equations are analysed in Table II, which shows that only 30 per cent of the 143 values of R′/R are within

TABLE II

ANALYSIS OF RESULTS

ϕ	Number of results	Number of results within 95 to 105 per cent of calculated value			
		Equation (ii)	Equation (iii)	Equation (v)	Equation (vii)
0·0000 to 0·1999	22	22	21	22	22
0·2000 to 0·2999	26	14	21	20	25
0·3000 to 0·3999	28	6	24	22	27
0·4000 to 0·4999	32	1	26	27	26
0·5000 to 0·5999	23	0	16	13	12
0·6000 to 0·8100	12	0	0	3	9
	143	43	108	107	121

±5·0 per cent of the results calculated from equation (ii). Best agreement occurred when ϕ was less than 0·3, and at all concentrations the results tended to be low. As the concentration of oil phase increased, the differences between true and calculated results increased also.

Since the experimental work reported in this paper was completed, Hanai (1960) published results on the conductivities of emulsions stabilised

with non-ionic emulgents. Results were compared with the theoretical values obtained using equations (ii) and (v), and showed that with equation (ii) poor agreement was obtained when ϕ exceeded 0·3.

Equation (v) fits the results for emulsions in which ϕ is less than 0·6, but for more concentrated emulsions the agreement is less impressive. Thus, Table II shows that although 79 per cent of those emulsions below $\phi = 0{\cdot}6$ fell within $\pm 5{\cdot}0$ per cent of the calculated results, only 25 per cent of those above $\phi = 0{\cdot}6$ fell within these limits. This agrees with Hanai's results in which 100 per cent fell within $\pm 5{\cdot}0$ per cent when ϕ was less than 0·6, and 17 per cent when ϕ exceeded 0·6. Equation (vi) would not be expected to be applicable since it applies to systems containing two globule sizes only. This was actually the case, since although it gave better agreement than equation (ii), it was inferior to equations (iii) and (v).

Although it has been stated that the value of equation (iii) is questionable because it was obtained empirically from a few results from an ideal system (De la Rue, 1959), it was used in the present work because it had not previously been applied to emulsions. The equation has been found to fit the experimental results reasonably well, 76 per cent of the results being within $\pm 5{\cdot}0$ per cent. Poor agreement was obtained, however, when ϕ was greater than 0·6. The emulgent used seemed to affect the results, for example in acacia emulsions the equation fitted the results when ϕ was less than 0·6 (88 per cent of the values fell within the limits), but for cetrimide and sodium lauryl sulphate emulsions the results only fitted the equation up to values of $\phi = 0{\cdot}5$ and 0·4 respectively.

Equation (iii) could be the first three terms of a power series. The best coefficients for ϕ up to the fourth power to fit the results in each series were calculated using a Stantec Zebra electronic computer, but a better fit could not be obtained. (The whole 143 results could not be examined together since the programme could not cater for more than 100 terms.) Constants calculated by the computer for a quadratic equation were as follows:

Acacia	$R'/R = 1{\cdot}00 - 1{\cdot}66\phi + 0{\cdot}75\phi^2$
Polyvinyl alcohol	$= 0{\cdot}97 - 1{\cdot}32\phi + 0{\cdot}24\phi^2$
Cetrimide	$= 0{\cdot}98 - 1{\cdot}53\phi + 0{\cdot}55\phi^2$
Sodium lauryl sulphate ..	$= 1{\cdot}00 - 1{\cdot}74\phi + 0{\cdot}78\phi^2$

These gave good results within their own series but, as is evident from the considerable variation in the coefficients from series to series, none was any better than equation (iii) when applied to all 143 emulsions. An equation using the weighted means of each coefficient was no more successful; this was due to the fact that the system providing the greatest contribution to the mean coefficients (90 acacia emulsions) was not represented by any emulsions in which ϕ exceeded 0·62, the concentration at which equation (iii) breaks down.

The equation:

$$R'/R = 1{\cdot}0 - 1{\cdot}60\phi + 0{\cdot}6\phi^2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad \text{(vii)}$$

was derived by trial and error, and fitted our results better than any equations considered so far. Thus, Table II shows that 121 of the 143 results were within $\pm 5{\cdot}0$ per cent: these included 9 of the 12 results for emulsions in which ϕ was greater than 0·6. The results quoted by Hanai also fitted equation (vii) more favourably than the other equations. The fact that this equation fits the results better than equation (iii) does not suggest that the coefficients derived by Mashovets (1951) are incorrect, since his equation was derived from the study of an ideal system in which the interface was much simpler than that between an oil and an aqueous solution of a surface-active agent.

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EFFECT OF IMIPRAMINE, AMITRIPTYLINE AND THEIR MONOMETHYL DERIVATIVES ON RESERPINE ACTIVITY

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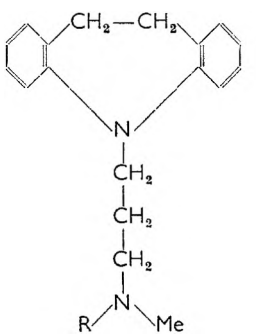
Impramine, amitriptyline and their respective *N*-monomethyl derivatives (desmethylimipramine and desmethylamitriptyline) prevent reserpine activity to varying degrees. Desmethylimipramine and desmethylamitriptyline are more effective than are imipramine and amitriptyline in reducing the hypothermia induced by reserpine in rats. Imipramine and desmethylimipramine are more effective than are amitriptyline and desmethylamitriptyline in decreasing the severity of gastric ulcers induced by reserpine in restrained rats. Desmethylimipramine does not prevent the lowering of brain amines by reserpine. The antagonistic effect of imipramine toward leptazol convulsions in mice is not shared by desmethylimipramine. Imipramine and desmethylimpramine do not potentiate the central effects of 5-hydroxytryptophan and tryptamine and do not prevent the hypothermia induced by 5-hydroxytryptamine, α-methyl-dopa or chlorpromazine. The activity of desmethylimipramine may be differentiated therefore on a pharmacological basis from that of the monoamine oxidase inhibitors and amphetamine.

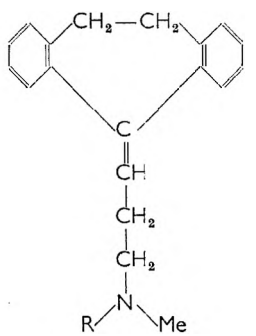
IN several animal species, imipramine causes a weak phenothiazine-like tranquillisation (Domenjoz and Theobald, 1959) in contrast to its action on the symptoms of endogenous depression reported first by Kuhn (1957) and confirmed by others. In our previous work we reported the anti-reserpine action of imipramine suggesting that this could be a clue to its antidepressant action (Garattini, 1959; Costa, Garattini and Valzelli, 1960). The anti-reserpine action of imipramine was more effective when the antagonist was given chronically; unlike chlorpromazine, imipramine greatly potentiated the anti-reserpine action of a monoamine oxidase inhibitor (Costa, 1960). We observed also that imipramine increases the brain 5-hydroxytryptamine (5-HT) concentration but we could not interpret the significance of this finding because the effect was transient, it was present in rats but not in rabbits; and imipramine was not amonoamine oxidase inhibitor (Costa, Garattini and Valzelli, 1960; Costa, 1960); Domenjoz and Theobald (1959), Sulser and Watts (1960) and Sigg, Gyermek and Soffer (1961) confirmed our pharmacological findings. More recently Brodie and his group (Sulser, Watts and Brodie, 1961; Gillette, Dingell, Sulser, Kuntzman and Brodie, 1961) have elucidated the problem of the anti-reserpine action of imipramine by showing that the antagonism was due to the accumulation in brain of the desmethylimipramine previously described and isolated among many other urinary metabolites by Hermann, Schindler and Pulver (1959). The great merit of Brodie's group (Brodie, Bickel and Sulser, 1961) was to show that this imipramine metabolite not only antagonises but reverses the reserpine syndrome, eliciting an endogenous excitation when given before reserpine; the drug is not active *per se* in normal animals. Moreover, chronic administration of imipramine is more effective against reserpine than

single injections because of the accumulation in brain of the imipramine metabolite. Finally, the fact that in clinical use desmethylimipramine acts more rapidly against endogenous depression than imipramine (Brodie, Bickel, Sulser, 1961) stresses the importance of the anti-reserpine effect as a test for antidepressant action.

This discovery by Brodie's group prompted us to investigate the effect of desmethylimipramine on a number of pharmacological and biochemical effects of reserpine in order to prove the specificity of the antagonism existing between reserpine and desmethylimipramine. For comparison amitriptyline (Vernier, 1961) and its *N*-monomethyl analogue (desmethylamitriptyline) (Hucker and Porter, 1961) were also evaluated as reserpine antagonists.



R = Me = Imipramine
R = H = Desmethylimipramine



R = Me = Amitriptyline
R = H = Desmethylamitriptyline

Experimental

Animals. Female Sprague-Dawley rats weighing 170 g., female Swiss mice, weighing 20 g. and the Zebra fish, *Brachydanius rerius* were used in these experiments.

Methods

Body temperature was measured by inserting an electric thermometer into the rectum. Heart rate was measured from an electrocardiogram record (speed 50 cm./min.). Experimental ulcers were produced by giving reserpine, 5 mg./kg., i.p., to rats kept under restraint according to the technique of Rossi, Bonfils, Lieffogh, and Lambling (1956). Leptazol (0·5 per cent acqueous solution) was infused into the tail vein at a constant speed of 0·14 ml./min. according to Orloff, Williams and Pfeiffer (1949) and Fingl and McQuarrie (1960).

Adrenal hypertrophy was studied 6 days after unilateral adrenalectomy under light ether anaesthesia.

Brain 5-HT and noradrenaline levels were measured by a spectrofluorimetric method according to Shore (1959).

The expansion of melanophores was observed in *Brachydanius rerius* kept in beakers containing 50 ml. of distilled water at 18–20°.

Drugs. Reserpine was kindly supplied by Ciba; imipramine and desmethylimipramine by Geigy; amitriptyline, desmethylamitriptyline,

and α-methyl-dopa by Merck, Sharp and Dohme; tetrabenazine by Hoffman La Roche; chlorpromazine by Farmitalia and 5-HT creatinine sulphate by Vister.

The doses and the route of administration used are given in the Tables.

Results

Hypothermia by reserpine. All four compounds tested, when given i.p. 1 hr. before the administration of reserpine, 2·5 mg./kg. i.v., prevent the hypothermia induced by reserpine. The results obtained are in Table I from which it is evident that imipramine and amitriptyline are less

TABLE I

EFFECT OF IMIPRAMINE AND ITS DERIVATIVES ON HYPOTHERMIA INDUCED BY RESERPINE

No. of rats	Treatment in mg./kg. i.p. (1 hr. before reserpine 2·5 mg./kg. i.v.)	Body temperature (° ± S.E.) hours after reserpine			
		0	4	6	24
13	Saline	36·8 ± 0·16	34·4 ± 0·33	33·0 ± 0·35	26·8 ± 1·49
5	Imipramine 7·5	36·0 ± 0·20	36·1 ± 0·18	36·3 ± 0·46	31·2 ± 0·94
11	Imipramine 15	35·7 ± 0·12	37·7 ± 0·30	37·0 ± 0·24	37·5 ± 0·5
6	Imipramine 30	35·0 ± 0·43	37·4 ± 0·27	37·1 ± 0·31	36·1 ± 0·32
11	*DMI 7·5	36·5 ± 0·11	37·8 ± 0·13	37·3 ± 0·16	36·3 ± 0·2
11	DMI 15	36·1 ± 0·23	38·2 ± 0·07	37·4 ± 0·14	37·3 ± 0·3
5	Amitriptyline 7·5	35·8 ± 0·36	35·1 ± 0·32	34·4 ± 0·65	30·2 ± 2·6
11	Amitriptyline 15	35·9 ± 0·30	36·3 ± 0·27	34·7 ± 0·20	33·5 ± 0·5
6	Amitriptyline 30	35·2 ± 0·67	36·3 ± 0·35	35·3 ± 0·32	31·7 ± 1·35
11	†DMA 7·5	36·3 ± 0·07	36·2 ± 0·24	35·1 ± 0·21	34·8 ± 0·2
10	DMA 15	36·7 ± 0·20	37·7 ± 0·16	35·5 ± 0·18	32·9 ± 1·0

* Desmethylimipramine.
† Desmethylamitriptyline.

effective than the respective desmethyl derivatives in preventing the hypothermia induced by reserpine.

Ulcer by restraint. Reserpine, 5 mg./kg. i.p., increases the incidence of formation of gastric ulcers in restrained rats. Pretreatment with any of the four compounds prevent this effect as it is shown in Table II.

TABLE II

EFFECT OF IMIPRAMINE AND ITS DERIVATIVES ON GASTRIC ULCER INDUCED BY RESERPINE IN RESTRAINED RATS

No. of rats	Treatment in mg./kg. i.p. (30 min. before 5 mg./kg. i.p. reserpine)	Severity of ulcer
25	Controls	100 ± 5
10	Imipramine 15	25 ± 7
10	Imipramine 7·5	50 ± 14
10	DMI 15	39 ± 15
10	DMI 7·5	64 ± 14
5	Amitriptyline 30	57 ± 18
5	Amitriptyline 15	72 ± 9
5	DMA 30	45 ± 12
5	DMA 15	93 ± 12

Other pharmacological effects. Imipramine and its desmethyl derivative, 15 mg./kg., but not amitriptyline, prevent the bradycardia induced by reserpine, 2·5 mg./kg. i.p. The compounds were injected 30 min. before, and the heart rate was measured 8 hr. after, giving reserpine.

Reserpine decreases the threshold in mice for tonic convulsions during infusion with leptazol. Imipramine and amitriptyline, 50–75 mg./kg. i.p., 3 hr. before reserpine, 5 mg./kg. i.p., and 1 hr. before leptazol, but not desmethylimipramine, counteract the effect of reserpine on leptazol-induced convulsions. However imipramine and amitriptyline at the doses tested exert a direct anticonvulsant activity, while desmethyl-imipramine is inactive.

Desmethylimipramine does not inhibit the stimulation of adrenal hypertrophy found when reserpine was given after monoadrenalectomy at a dose of 200 μg./kg. daily for 6 days.

Imipramine and desmethylimipramine differ from monoamine oxidase inhibitors, in that given in doses of 15–30 mg./kg. i.p., they do not increase the hyperthermia and tremors produced by 5-hydroxytryptophan, 50 mg./kg. i.p., in mice or the convulsions produced by tryptamine, 40 mg./kg. i.v., in rats.

Effect on melanophore expansion. Reserpine produces expansion of the melanophores of *Brachydanius rerius* (zebra fish) at doses of 0·1–0·5 μg./ml. Both desmethyl compounds, but not imipramine and amitriptyline at a dose of 2 μg./ml., prevent the effect induced by reserpine. Amitriptyline alone acts on melanophores in a manner similar to that of reserpine.

Effect on brain amines. As is shown in Table III, desmethylimipramine does not prevent the lowering of brain 5-HT and noradrenaline induced by reserpine.

TABLE III

EFFECT OF DESMETHYLIMIPRAMINE ON DEPLETION OF BRAIN 5-HT AND CATECHOLAMINE INDUCED BY RESERPINE IN RATS

No. of rats	Treatment	Brain		Body temperature	Blepharospasm
		5-HT	NOR.		
8	Controls	100 ± 3	100 ± 5·2	37·3 ± 0·22	—
8	DMI, 15 mg./kg. i.p.	104 ± 1·8	103 ± 3·4	36·8 ± 0·18	—
8	Reserpine, 2·5 mg./kg. i.v.	35 ± 2·4	22 ± 1·5	34·3 ± 0·4	+
8	DMI, 15 mg./kg. i.p. + reserpine 2·5 mg./kg.. i.v.	35 ± 2·6	25 ± 1·6	37·6 ± 0·3	—

DMI was given 1 hr. before reserpine.

Effect on other types of hypothermia. To establish the specificity of the anti-reserpine effect exerted by imipramine and desmethylimipramine, other drugs capable of inducing hypothermia were tested. The results obtained are in Table IV.

The only hypothermia prevented by imipramine and desmethylimipramine is that induced by tetrabenazine, a short acting reserpine analogue (Pletscher, Besendorf and Gey, 1959). The lowering of body temperature produced by chlorpromazine, α-methyl-dopa and 5-HT are not antagonised.

DISCUSSION

Imipramine, amitriptyline and the desmethyl derivatives prevent, with differing potency, some pharmacological effects induced by reserpine. The hypothermia induced by reserpine is, on the whole, inhibited more by the desmethyl derivatives than by imipramine and amitriptyline. This is

of interest in relation to the findings described above, that the desmethyl derivatives are the metabolites occurring in the tissues after the administration of imipramine and amitriptyline. It may therefore be suggested that the anti-reserpine effects observed after the administration of imipramine and amitriptyline are mediated through the formation of the respective desmethyl derivatives.

TABLE IV

EFFECT OF IMIPRAMINE AND DESMETHYLIMIPRAMINE ON VARIOUS AGENTS INDUCING HYPOTHERMIA

No. of rats	Treatment mg./kg. i.p.	Body temperature after hr.			
		0	2	4	6
6	Tetrabenazine 40	37·2 ± 0·18	35·8 ± 0·56	35·0 ± 0·51	36·6 ± 0·26
6	Imipramine 15 + tetrabenazine 40	35·7 ± 0·18	36·9 ± 0·13	37·5 ± 0·15	37·0 ± 0·05
6	DMI 15 + tetrabenazine 40	35·5 ± 0·47	36·6 ± 0·11	36·9 ± 0·20	36·6 ± 0·26
		0	1	4	6
15	α-Methyl-dopa 500	36·9 ± 0·18	34·7 ± 0·14	33·3 ± 0·24	34·4 ± 0·37
7	Imipramine 15 + α-methyl-dopa 500	34·9 ± 0·34	33·7 ± 0·23	33·7 ± 0·53	34·6 ± 0·75
12	DMI 30 + α-methyl-dopa 500	34·7 ± 0·33	34·3 ± 0·22	34·2 ± 0·33	34·4 ± 0·26
		0	1	2	3
6	5-HT 20	36·8 ± 0·05	32·5 ± 0·23	34·5 ± 0·60	36·0 ± 0·50
6	Imipramine 15 + 5-HT 20	35·1 ± 0·18	31·0 ± 0·40	32·2 ± 0·57	33·5 ± 0·36
6	DMI 15 + 5-HT 20	35·0 ± 0·22	30·2 ± 0·98	31·6 ± 0·73	32·7 ± 0·55
		0	1	2	5
5	Chlorpromazine 10	37·7 ± 0·19	34·1 ± 0·62	33·9 ± 0·94	35·1 ± 0·83
5	DMI 15 + chlorpromazine 10	36·2 ± 0·25	33·2 ± 0·31	32·2 ± 0·30	34·9 ± 0·48

The two derivatives counteract the expansion of melanophores induced by reserpine in zebra fish while imipramine and amitriptyline are almost inactive at the same concentration.

Imipramine and desmethylimipramine are more effective than are amitriptyline and desmethylamitriptyline in preventing the gastric ulcers induced by reserpine in restrained rats.

However not all the pharmacological effects exerted by imipramine and amitriptyline may necessarily be mediated by their *N*-monomethyl derivatives. For example, the anticonvulsant activity shown by imipramine is not shared by its derivative.

Again not all the effects of reserpine are counteracted by imipramine or its congeners. The stimulation exerted on adrenal hypertrophy is not inhibited by desmethylimipramine. This is consistent with the observation that this derivative does not prevent the release of ACTH by reserpine (B. B. Brodie, personal communication).

The antireserpine activity exerted by the derivative is different from that of the monoamine oxidase inhibitors.

Desmethylimipramine does not prevent the lowering of brain amines induced by reserpine. Furthermore, as opposed to monoamine oxidase inhibitors, it does not increase the tremors and convulsions induced respectively by 5-hydroxytryptophan (Horita and Gogerty, 1958) in mice, and tryptamine (Tedeschi, Tedeschi and Fellows, 1959) in rats.

The effects of imipramine and its derivatives on the hypothermia induced by reserpine seem to be specific since the hypothermia induced by chlorpromazine, 5-HT, and α-methyl-dopa is not prevented by imipramine or desmethylimipramine. Tetrabenazine, being a short acting reserpine-like drug, is antagonised by imipramine and its metabolite.

These results differentiate desmethylimipramine from the stimulants. Amphetamine counteracts not only reserpine and tetrabenazine, but also chlorpromazine, 5-HT and α-methyl-dopa (experiments to be reported).

In conclusion, imipramine and its congeners exhibit a pharmacological pattern which is different both from monamine oxidase inhibitors and amphetamine. This may be of interest in assessing the mechanism of action of the antidepressant effect shown by these drugs.

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THE ANTIVERATRINIC ACTION OF SOME LOCAL ANAESTHETICS

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The activity of fifteen local anaesthetics against the "veratrine response" induced in the frog's sartorius muscle by 0·1 μg./ml. of veratridine was investigated. The suppression of potassium efflux by these agents, and the role played by this ion in the action of veratridine has also been examined.

Cocaine and procaine and its close analogues block nerve conduction without influencing membrane potentials (Bennett and Chinburg, 1946), whereas the blocking action of veratrine is accompanied by a depolarisation of the nerve membrane. Bishop (1932) and Shanes (1958) have, therefore, classified local anaesthetics and compounds with similar properties as membrane "stabilisers" in contrast to membrane "labilisers" like veratrine. It has also been reported that local anaesthetics stabilise the normal membrane potential of the nerve to depolarisation by veratrine. The action of veratrine and membrane stabilisers on nerves has been further studied by Herr and Akcasu (1960).

Since the *ortho*-substituted benzoic acid esters of dialkylaminoalkanols which resemble procainamide exhibited antiveratrinic activity (Arora and Das, 1956), other local anaesthetic agents have been investigated for their activity against the veratrine-induced skeletal muscle response.

Experimental

The following drugs were investigated.

I 2-(Pyrrolidin-l-yl)propyl 2,6-dimethyl-4-propoxybenzoate hydrochloride.
II 2-Methyl-2-(pyrrolidin-1-yl)propyl 2,6-dimethyl-4-propoxybenzoate hydrochloride.
III 2-(Pyrrolidin-l-yl)ethyl *p*-propoxybenzoate hydrochloride.
IV 3-(2-Methylpiperidino)propyl *p*-butoxybenzoate.
V 3-(2-Methylpyrrolidin-1-yl)propyl *p*-cyclohex-2-enyloxybenzoate hydrochloride.
VI α-(Diethylaminomethyl)-*p*-methoxyphenethyl *p*-butoxybenzoate.
VII 2-Diethylaminoethyl 3,4,5-trimethoxybenzoate hydrochloride.
VIII αα-Di(*o*-methoxybenzyl)methylamine lactate.
IX Di-(*o*-methoxy-α-methylphenethyl)amine lactate.
X 2-Di(*o*-methoxy-α-methylphenethyl)aminoethanol hydrochloride.
XI *p*-Butoxy-β-piperidinopropiophenone hydrochloride.
XII 3-Dimethylamino-2-phenoxypropiophenone hydrochloride.
XIII 3-Dimethylamino-2-phenoxy-1-phenylpropanol hydrochloride
XIV 2-Diethylaminoethyl *p*-hexyloxybenzilate hydrochloride.
Lignocaine.

These drugs are white, crystalline, odourless powders, soluble in distilled water. Aqueous solutions were stored in a refrigerator, and not used after 7 days; 1 per cent solutions of these compounds had a pH value ranging from 6 to 7·5, except those of XIII and VII, which had a pH value of 4. Quinidine was used as a standard drug for comparison.

Methods

Male frogs (*Rana tigrina*) weighing between 40 and 60 g. were used. Both the sartorius muscles were removed, suspended in twin chambers and attached to tension levers.

The fluid in which the muscles were placed contained: sodium chloride, 0·5; potassium chloride, 0·014; calcium chloride anhydrous, 0·011 and sodium bicarbonate, 0·23 per cent. The bathing fluid was gassed with 95 per cent oxygen and 5 per cent carbon dioxide throughout the experiment. Electrical stimuli were applied to the muscle in the bath by a Grass Stimulator (Model 34B). One electrode was attached to the clip holding the upper end of the muscle and the other to the silver outlet of the chamber. A small length of the muscle (about 5 mm.) was kept above the surface of the bathing fluid to ensure the completion of the circuit through the muscle at each stimulus. The strength of the stimulus was 40 V, its duration 0·5 msec. and its frequency every 2 min.

Prevention of the veratrine response. After keeping the sartorius muscles in the bicarbonate buffer for about 15 min., the stimulus was applied seven times at about 2 min. intervals. The bicarbonate solution containing a suitable concentration of the test drug was now substituted in one of the chambers. Seven stimuli, at 2 min. intervals, were then applied to both muscles. After this, both muscles were subjected to a concentration of veratridine (0·1 μg./ml.). Stimuli were applied again to see whether the drug under study afforded protection against the development of a veratrine response.

Abolition of the veratrine response. After obtaining a few 'normal' twitches of the sartorius muscles, veratridine was added to the bath fluid to give a concentration of 0·1 μg./ml. The veratrine response was allowed to develop fully in the two sartorius muscles. The test substance in a concentrated solution was then added to one muscle to give the desired concentration in the bicarbonate buffer. The muscle treated with veratridine alone served as control. After the test substance had abolished the fully developed veratrine response, the treated muscle was washed thoroughly with bicarbonate buffer solution, followed by the addition of veratridine, 0·1 μg./ml. The development of a typical veratrine response indicated that the effect was reversible.

Determination of K^+ efflux in the veratrinised sartorius muscle. The muscles were suspended in the usual manner in the twin chamber and left in the bath for 5 min., after which the fluid was withdrawn and preserved, to act as a "control" for the K^+ content every withdrawal was followed by the addition of fresh Ringer's solution. Fresh Ringer's fluid was added,

a single stimulus was applied, and, during the contraction, the fluid was withdrawn. After 5 min. 14 stimuli were applied in succession and the fluid was again withdrawn. The veratrine response was then developed with 0·1 μg./ml. veratridine. Sufficient time was allowed for the veratrine response to develop to its maximum, then a single stimulus and multiple stimuli were applied. Fluid was withdrawn during the contraction after a single stimulus and again after repeated stimuli. Every withdrawal was followed by the addition of fresh Ringer's solution. The antiveratrinic drug was then added, and when the veratrine response was abolished, the process of fluid withdrawal after a single stimulus, and after successive stimuli was repeated. The potassium content was determined spectrophotometrically.

RESULTS

The concentrations of each drug were 10 μg./ml.; 3 μg./ml.; 1 μg./ml. and 0·3 μg./ml. Three experiments were made with each drug to observe the preventive action, and another 3 to test the abolition of the veratrine response. The evidence is given in Table I.

TABLE I

THE RELATION BETWEEN THE CONCENTRATION WHICH PREVENTED THE VERATRINE RESPONSE AND THAT WHICH ABOLISHED THE VERATRINE RESPONSE IN THE FROG SARTORIUS MUSCLE

Compound No.	Prevention of veratrine response		Abolition of veratrine response		
	No. positive experiments	Conc. (μg./ml.) which prevents	No. positive experiments	Conc. (μg./ml.) which abolishes	Average time min.
I	1 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	26 8
II	3 out of 3	3·3	3 out of 3	3·3	20
III	3 out of 3	0·33	3 out of 3	0·33	6
IV	2 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	26 6
V	1 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	30 4
VI	3 out of 3	3·3	3 out of 3	3·3	12
VII	3 out of 3	10·0	3 out of 3	10·0	12
VIII	2 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	18 8
IX	3 out of 3	1·0	3 out of 3	1·0	30
X	3 out of 3	3·3	3 out of 3	3·3	10
XI	3 out of 3	1·0	3 out of 3	1·0	10
XII	3 out of 3	1·0	3 out of 3	3·3	12
XIII	3 out of 3	3·3	3 out of 3	3·3	18
XIV	3 out of 3	3·3	3 out of 3	3·3	20
Lignocaine	0 out of 3 3 out of 3	3·3 10·0	0 out of 3 3 out of 3	3·3 10·0	— 2
Quinidine	2 out of 3 3 out of 3	3·3 10·0	1 out of 3 3 out of 3	3·3 10·0	26 12

Prevention of veratrine response. Compounds I, III, IV, V, VIII, IX, XI and XII exhibited strong antiveratrinic activity antagonising the veratrine response in concentrations of 0·3 and 1 μg./ml. Compounds II, VI, X, XII and XIV were effective in 10 μg./ml. concentrations. Compound VII and lignocaine were effective in 10 μg./ml. concentrations. Quinidine exhibited a greater antiveratrinic activity than VII and lignocaine, but was less potent than the rest of the drugs tested. Fig. 1 shows the prevention of the veratrine response with I in a concentration of 1 μg./ml.

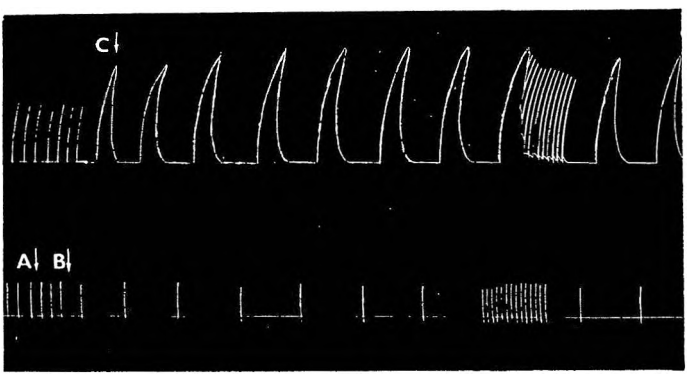

FIG. 1. Prevention of veratrine response by compound I. At Ag lower muscle was exposed to compound I (10^{-6}). After 7 stimuli at 2 min. intervals the upper muscle was treated with veratridine (10^{-7}) at C.
At B compound I was replaced by another solution containing compound I+ veratridine (10^{-7}).

Abolition of veratrine response. The drugs which were found to be more active in preventing the veratrinic response were also more potent in abolishing the response itself. However, in general it may be said that a higher concentration is necessary to bring about an abolition of the fully developed response. The effects were reversible as indicated by the re-establishment of the veratrine response.

TABLE II

POTASSIUM EFFLUX IN THE VERATRINISED SKELETAL MUSCLE OF FROGS

	K^+ efflux in m-equiv./litre in sartorious muscle			Significance of K^+ increase after veratridine	Significance of K^+ decrease after drugs
	Mean ± Standard deviation Range				
	Control	After veratridine	After drugs		
Single stimulus	0·028 ± 0·006 0·012 — 0·048	0·031 ± 0·010 0·014 — 0·055	0·021 ± 0·007 0·011 — 0·036	$P < 0.05$	$P < 0.001$
After 14 stimuli	0·065 ± 0·008 0·05 — 0·09	0·12 ± 0·01 0·1 — 0·17	0·060 ± 0·007 0·046 — 0·094	$P < 0.001$	$P < 0.001$

Probability was calculated by applying Student's '*t*' test.

The abolition of the fully developed veratrine response using veratridine 0·1 μg./ml. by compound V is shown in Fig. 2.

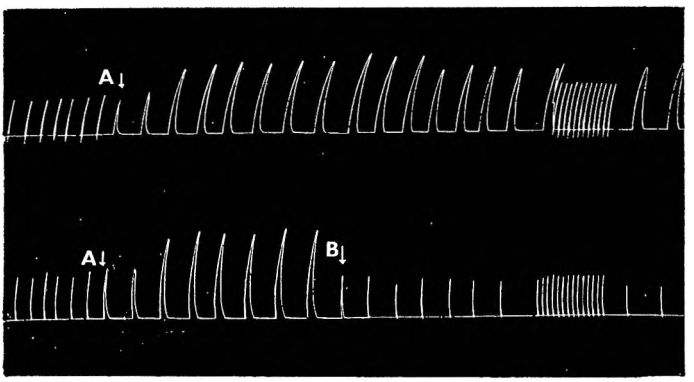

FIG. 2. (i) Abolition of the fully developed veratrine response.

(ii) The Veratrine response was allowed to develop fully in both muscles with veratridine (10^{-7}) added at A.

(iii) At B in the lower muscle a concentrated solution of compound V was added so as to make a desired concentration of the drug in the bicarbonate buffer solution. Soon after, the veratrine response was completely abolished.

Potassium efflux. In 35 experiments, the average potassium efflux during contraction of the sartorius muscle following a single stimulus and after applying 14 repeated stimuli was 0·028 and 0·065 m-equiv. per litre respectively. After the veratrine response was fully developed, following a single stimulus an average potassium efflux was 0·031 instead of 0·028 m-equiv., thereby showing that in the veratrinised muscle the efflux of potassium is increased. After bringing about abolition of the veratrine response with drugs, the diminution of potassium efflux was significant,

TABLE III

THE RELATION BETWEEN ANTIVERATRINE ACTIVITY AND LOCAL ANAESTHETIC POTENCY TESTED ON THE RABBIT CORNEA, AND RABBIT INTRADERMAL TEST

	Antiveratrinic activity			Local anaesthetic activity		
				MEC cornea conc. per cent	Intradermal	
Drug	Prevention	Abolition	Time in min.		Conc. per cent	Score
I	1×10^{-6}	1×10^{-6}	8	0·06	0·2	16
II	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	20	0·157	0·1	26
III	$3·3 \times 10^{-7}$	$3·3 \times 10^{-7}$	6	0·6	0·1	21
IV	1×10^{-6}	1×10^{-6}	6	0·06	0·05	9
V	1×10^{-6}	1×10^{-6}	4	0·03	0·25	22
VI	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	12	0·03	0·05	10
VII	1×10^{-5}	1×10^{-5}	12		10·0	20
VIII	1×10^{-6}	1×10^{-6}	8	0·01	0·01	19
IX	1×10^{-6}	1×10^{-6}	30	0·03	0·5	25
X	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	10	0·15	5·0	20
XI	1×10^{-6}	1×10^{-6}	10	0·05	0·4	12
XII	1×10^{-6}	$3·3 \times 10^{-6}$	12	0·3	0·5	20
XIII	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	18		0·5	12
XIV	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	20	1·5	0·4	22
Lignocaine	1×10^{-5}	1×10^{-5}	8	0·06	0·2	8

both after a single stimulus as well as after repeated stimuli. The average figures on addition of antiveratrinic drugs after single and repeated stimuli were 0·021 (before: 0·028) and 0·060 (before: 0·065) m-equiv./litre respectively (Table II).

Discussion

All the drugs investigated possessed both local anaesthetic and antiveratrinic activity. The rank correlation coefficient (R) was calculated between these two biological properties by applying the equation $R = 1 - \frac{6\Sigma d^2}{n^3 - n}$, where n is the number of drugs, d is difference between the ranks for the two different properties. For antiveratrinic activity, the concentrations for prevention and abolition of response were taken as a measure of potency; on the other hand the median effective concentration (MEC) and the intradermal anaesthetic score, determined by the methods elaborated by Chance and Lobstein (1944) and Bülbring and Wajda (1945) respectively, were taken as a suitable gauge for local anaesthetic activity. The actual values for the four said observations are given in Table III, which summarises the relative potencies of the various drugs. The rank correlation coefficient value on calculation was found to be 0·64.

Most of the strong antiveratrinic drugs were found to possess strong local anaesthetic activity. To cite a few examples, compounds I, III, IV, V, IX and XI were found to be potent in both respects.

The present investigation shows that the average potassium efflux during contraction of the frog's sartorius muscle following single and repeated stimuli was 0·028 and 0·065 m-equiv./litre respectively. After the development of the veratrine response the potassium efflux increased to 0·031 and 0·12 m-equiv./litre after single and repeated stimuli respectively. All the drugs exhibited an antiveratrinic activity and brought about a significant reduction in the potassium efflux both after a single stimulus and repeated stimuli, as is evident from Table II. The local anaesthetic action of drugs has also been partially explained by their ability to depress the potassium efflux from the nerve and thus bring about an interruption in nerve conduction.

Thus the potassium ion may be the common point for attack by the drugs for the biological activities, and the degree of impedence of potassium efflux through the plasmatic membrane may be responsible partly for the individual potency. In this study a parallelism between the two properties has been shown to exist, probably due to a common point of attack. This parallelism, however, is in no way fully explained, and demands a further solution.

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HYPOGLYCAEMIC AGENTS. PART III

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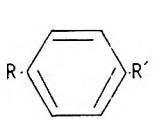
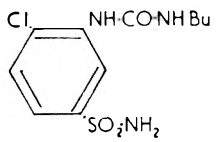
New variants of tolbutamide are described.

Our work on hypoglycaemic agents is extended herein to some new variants of 1-butyl-3-*p*-toluenesulphonylurea (I; R = Me, R′ = SO_2·NH·CO·NHBu):

(*a*) 1-Cyclohexyl-3-*p*-fluorobenzenesulphonyl- and 3-*p*-fluorobenzenesulphonyl-1-phenyl-urea (I; R = F, R′ = SO_2·NH·CO·NH– cyclohexyl or Ph) have been prepared by reaction of the appropriate isocyanate with the sodium salt of *p*-fluorobenzenesulphonamide in aqueous acetone. The corresponding propyl and butyl analogues were previously described by Marshall and Sigal (1958).



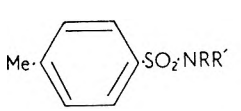
I



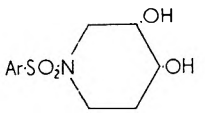
II

(*b*) 1-Butyl-3-*p*-methylsulphamoylphenylurea (I, R = SO_2·NHMe; R′ = NH·CO·NHBu) was prepared by reaction of *p*-aminobenzenesulphonmethylamide with butyl isocyanate in dioxan solution. 1-Butyl-3-(2-chloro-5-sulphamoylphenyl)urea (II) was similarly obtained from 3-amino-4-chlorobenzenesulphonamide (Petrow, Stephenson and Wild, 1960).

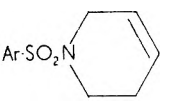
(*c*) Compounds containing a 3-arylsulphonylacetyl-1-butylurea group (I; R = F, Cl and Me; R′ = SO_2·CH_2·CO·NH·CO·NHBu) were prepared by reaction of 1-butyl-3-chloroacetylurea with the sodium salts of fluorobenzene-, chlorobenzene- and toluene-*p*-sulphinic acids.



III



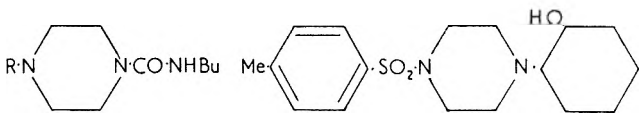
IV



V

(*d*) Budesinsky, Emr, Musil, Svab and Zikmund (1959) prepared a series of *p*-toluenesulphonamido-carboxylic acids. They reported that certain analogues of glycine (III, R = n-, iso- and t-butyl or t-pentyl; R′ = CH_2CO_2H) and of alanine (III, R = Pr; R′ = $CH(Me)CO_2H$) possessed up to 60 per cent of the hypoglycaemic activity of tolbutamide. We prepared the related *p*-fluoro- and *p*-chloro-benzenesulphonyl derivatives of α-alanine and α-amino-α-methylpropionic acid by reaction of the sodium salts of the acids with the appropriate sulphonyl chlorides in a two-phase 1,2-dichloroethane/water medium.

Work on amino-acid types was also extended to include the preparation of arylsulphonyl derivatives of di- and tri-hydroxy amines. Such compounds are relatively water-soluble and might be expected to metabolise *in vivo* to active amino-acids. Sulphonyl derivatives of the readily available 2-amino-2-methylpropane-1,3-diol and 2-amino-2-hydroxymethylpropane-1,3-diol were obtained by reaction with the appropriate arylsulphonyl chlorides in isopropanol or t-butanol in the presence of anhydrous sodium carbonate. 1-Arylsulphonyl derivatives of *cis*- and *trans*-piperidine-3,4-diol (IV) were prepared by hydroxylation of the 1-arylsulphonyl-1,2,3,6-tetrahydropyridine (V) under varied conditions. The *trans* diols (IV) were readily prepared by hydroxylation of the tetrahydropyridines with peracetic acid. The preparation of *cis*-cyclohexane-1,2-diol by hydroxylation of cyclohexene with sodium chlorate in aqueous solution using osmium tetroxide as catalyst was described by Böeseken and van Giffen (1920) and later by Clarke and Owen (1949). We found that the arylsulphonyltetrahydropyridines (V) were too insoluble in the aqueous medium to undergo hydroxylation by this method. The use of aqueous dioxan offered no advantages. However, high yields of *cis* diols were obtained using *ca* 65 per cent ethanol as hydroxylating medium at 50–60° progressive dilution of the mixture being carried out as the reaction proceeded.



VI VII

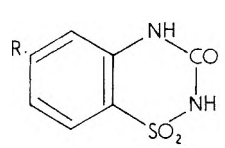
(*e*) Derivatives of butylcarbamoylpiperazine were prepared in which the 4-position was substituted by aroyl, furoyl, arylsulphonyl and diphenylcarbamoyl groups (cf. VI). Most of these were obtained by reaction of the appropriate acid chloride with 1-butylcarbamoylpiperazine (VI; R = H) but the *p*-methylbenzoyl derivative (VI, R = *p*-methylbenzoyl) was also obtained by reaction of 1-*p*-methylbenzoylpiperazine with butyl isocyanate.

Additionally, 1-formyl-4-*p*-toluenesulphonylpiperazine and 1-(*trans*-2-hydroxycyclohexyl)-4-*p*-toluenesulphonylpiperazine (VII) were synthesised.

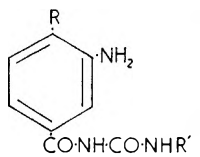
(*f*) The preparation of some 6-substituted 3,4-dihydro-3-oxobenzo-1,2,4-thiadiazine 1,1-dioxides (VIII) was undertaken as these compounds may be regarded as cyclised sulphonyl ureas.

They were readily obtained using the method first described by Parke and Williams (1950) whereby an amino-sulphonamide is heated with urea at 200–210°. Compounds were prepared in which the substituent R (VIII) was a methyl, methoxyl, trifluoromethyl group, or a fluorine, chlorine or bromine atom and the first of these was oxidised to the 6-carboxy derivative (VIII; R = CO_2H) using aqueous alkaline potassium permanganate.

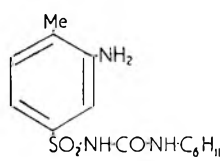
(*g*) Some 1-alkyl-(3-amino-4-methylbenzoyl)- and 1-alkyl-(3-amino-4-chlorobenzoyl)-ureas (IX, R = Me or Cl; R′ = Bu or C_6H_{11}) which bear a formal resemblance to the active hypoglycaemic agent, metahexanamide (X) (Haack, 1958), were obtained by reaction of the appropriate 4-methyl(or chloro)-3-nitrobenzoyl chloride with the alkylurea in benzene-pyridine followed by reduction of the nitro-compounds to the required amines.



VIII



IX



X

Biological study of the above compounds by Dr. A. David and his colleagues did not reveal significant hypoglycaemic activity.

Experimental

1-*Cyclohexyl*-3-p-*fluorobenzenesulphonylurea.* A solution of *p*-fluorobenzenesulphonamide (15·5 g.) in acetone (200 ml.) was cooled with stirring to 0° and treated successively with a solution of sodium hydroxide (3·6 g.) in water (10 ml.) and cyclohexyl isocyanate (12·1 g.). Stirring was continued at 0° for 30 min. then at 50–60° for 1 hr. when the mixture was cooled and poured into ice-water (600 ml.). After filtration to remove a small amount of insoluble material the filtrate was acidified to pH 6. The *product* (24·7 g.) had m.p. 144–145° after crystallisation from aqueous ethanol. Found: C, 52·1; H, 5·5; N, 9·7; S, 11·0. $C_{13}H_{17}FN_2O_3S$ requires C, 52·0; H, 5·7; N, 9·3; S, 10·7 per cent.

3-p-*Fluorobenzenesulphonyl*-1-*phenylurea,* had m.p. 154–156° after crystallisation from aqueous ethanol. Found: C, 53·1; H, 4·0; N, 9·5; S, 10·8. $C_{13}H_{11}FN_2O_3S$ requires C, 53·1; H, 3·8; N, 9·5; S, 10·9 per cent.

1-*Butyl*-3-p-*methylsulphamoylphenylurea.* To a solution of *p*-aminobenzenesulphonmethylamide (9·3 g.) in dioxan (50 ml.) was added butyl isocyanate (5·5 g.) and the mixture heated on the steam-bath for 4 hr. when excess of solvent was distilled off at reduced pressure. Crystallisation of the residue from aqueous ethanol yielded the *product* (7·3 g.), m.p. 165–167°. Found: C, 50·6; H, 6·7; N, 15·2; S, 11·2. $C_{12}H_{19}N_3O_3S$ requires C, 50·5; H, 6·7; N, 14·7; S, 11·2 per cent.

1-*Butyl*-3-(2-*chloro*-5-*sulphamoylphenyl*)*urea,* was prepared by reaction of 3-amino-4-chlorobenzenesulphonamide (11·36 g.) with butyl isocyanate (6 g.) in dioxan at 100° for 8 hr. The *product* (6·6 g.) had m.p. 188–190° (from aqueous ethanol). Found: C, 43·1; H, 5·3; Cl, 11·7; N, 13·8; S, 10·6. $C_{11}H_{16}ClN_3O_3S$ requires C, 43·2; H, 5·3; Cl, 11·6; N, 13·7; S, 10·5 per cent.

1-*Butyl*-3-(2-*methyl*-5-*sulphamoylphenyl*)*urea,* had m.p. 163–165° (from aqueous ethanol). Found: C, 50·1; H, 6·8; N, 14·3; S, 11·2. $C_{12}H_{19}N_3O_3S$ requires C, 50·4; H, 6·7; N, 14·7; S, 11·2 per cent.

1-*Butyl*-3-p-*toluenesulphonylacetylurea.* A solution of sodium toluene-*p*-sulphinate dihydrate (18·5 g.) and 1-butyl-3-chloroacetylurea (16·6 g.) in ethanol (200 ml.) was heated under reflux for 8 hr. The *product*, obtained on dilution, had m.p. 179–181° (from ethanol). Found: C, 53·6; H, 6·2; N, 8·9; S, 10·3. $C_{14}H_{20}N_2O_4S$ requires C, 53·8; H, 6·5; N, 9·0; S, 10·3 per cent.

1-*Butyl*-3-p-*fluorobenzenesulphonylacetylurea*, had m.p. 192–194° (from ethanol). Found: C, 49·5; H, 5·6; N, 8·4; S, 9·6. $C_{13}H_{17}FN_2O_4S$ requires C, 49·4; H, 5·4; N, 8·9; S, 10·1 per cent.

1-*Butyl*-3-p-*chlorobenzenesulphonylacetylurea*, had m.p. 212–214° (from ethanol). Found: C, 47·2; H, 5·2; Cl, 8·4; N, 11·0; S, 9·2. $C_{13}H_{17}ClN_2O_4S$ requires C, 46·9; H, 5·2; Cl, 8·4; N, 10·7; S, 9·6 per cent.

N-p-*Fluorobenzenesulphonyl*-DL-α-*alanine.* A solution of fluorobenzene-*p*-sulphonyl chloride (21·4 g.) in 1,2-dichloroethane (100 ml.) was added dropwise with stirring to a solution of DL-α-alanine (8·9 g.) in 2N sodium hydroxide (55 ml.) and stirring was continued for 4 hr. after the addition was complete. The aqueous layer was separated, cooled in ice and acidified with hydrochloric acid. The *product* (10·0 g.) had m.p. 116–117° (from benzene). Found: C, 43·6; H, 4·2; N, 5·9; S, 12·7. $C_9H_{10}FNO_4S$ requires C, 43·7; H, 4·1; N, 5·7; S, 13·0 per cent.

N-p-*Chlorobenzenesulphonyl*-DL-α-*alanine* was similarly prepared. It had m.p. 155° (from water). Found: C, 40·9; H, 3·5; Cl, 13·5; N, 5·5; S, 11·9. $C_9H_{10}ClNO_4S$ requires C, 41·0; H, 3·8; Cl, 13·5; N, 5·3; S, 12·2 per cent.

α-p-*Chlorobenzenesulphonamido*-α-*methylpropionic acid*, was obtained in 20 per cent yield by reaction of a solution of chlorobenzene-*p*-sulphonyl chloride in 1,2-dichloroethane with α-amino-α-methylpropionic acid in 2N sodium hydroxide at 30–35°. It had m.p. 168–170° (from water). Found: C, 43·2; H, 4·5; Cl, 12·7; N, 4·8; S, 11·2. $C_{10}H_{12}ClNO_4S$ requires C, 43·2; H, 4·4; Cl, 12·8; N, 5·0; S, 11·5 per cent.

2-*Methyl*-2-p-*toluenesulphonamidopropane*-1,3-*diol.* To a hot solution of 2-amino-2-methylpropane-1,3-diol (21 g.) in isopropanol (300 ml.) containing anhydrous sodium carbonate (16 g.), toluene-*p*-sulphonyl chloride (38·1 g.) was added in portions with stirring. The mixture was then heated under reflux for 30 min. and filtered hot. The *product* (34·5 g.) separated on cooling, it had m.p. 124–126° after crystallisation from water or ethyl acetate-light petroleum (b.p. 60–80°). Found: C, 51·0; H, 6·6; N, 5·2; S, 12·0. $C_{11}H_{17}NO_4S$ requires C, 51·0; H, 6·6; N, 5·4; S, 12·4 per cent. A better yield of product (38 g.) was later obtained using t-butanol in place of isopropanol.

5-*Methyl*-5-*p-toluenesulphonamido*-1,3-*dioxol*-2-*one.* A solution of the foregoing diol (25·9 g.) in ethyl carbonate (100 ml.) containing anhydrous sodium carbonate (1 g.) as catalyst was heated under reflux for 10 hr. when the solvent was removed at reduced pressure. The residual solid was extracted with boiling ethyl acetate to yield the *product* (17·9 g.), m.p. 149–150° after crystallisation from ethyl acetate-light petroleum (b.p. 60–80°). Found: C, 50·4; H, 5·1; N, 5·0; S, 11·0. $C_{12}H_{15}NO_5S$ requires C, 50·5; H, 5·3; N, 4·9; S, 11·2 per cent.

2-p-*Chlorobenzenesulphonamido*-2-*methylpropane*-1,3-*diol* had m.p. 134–135° (from ethyl acetate). Found: C, 42·8; H, 4·7; Cl, 13·0; N, 5·1; S, 11·8. $C_{10}H_{14}ClNO_4S$ requires C, 42·9; H, 5·0; Cl, 12·7; N, 5·0; S, 11·5 per cent.

2-*Hydroxymethyl*-2-p-*toluenesulphonamidopropane*-1,3-*diol.* To a hot stirred solution of 2-amino-2-hydroxymethylpropane-1,3-diol (36·3 g.) in isopropanol (400 ml.) containing anhydrous potassium carbonate (31·1 g.), a solution of toluene-*p*-sulphonyl chloride (57 g.) in toluene (100 ml.) was added during 20 min. The mixture was then refluxed for 2 hr., filtered hot and the residue washed with hot toluene (50 ml.). The filtrate was evaporated to dryness at reduced pressure and the residual solid dissolved in boiling ethyl acetate (300 ml.). The solid (14 g.) which separated on cooling was collected and had m.p. 139–140° (from ethyl acetate-ethanol). It proved to be the toluene-*p*-sulphonic acid salt of 2-amino-2-hydroxymethylpropane-1,3-diol (m.p. not depressed on admixture with authentic material). Further concentration of the filtrate yielded the *product* (30 g.), m.p. 107–108° (from ethyl acetate). Found: C, 48·2; H, 6·2; N, 5·0; S, 11·7. $C_{11}H_{17}NO_5S$ requires C, 47·9; H, 6·3; N, 5·1; S, 11·6 per cent.

2-*Acetoxymethyl*-1,3-*diacetoxy*-2-p-*toluenesulphonamidopropane.* A solution of the foregoing triol (15 g.) in acetic anhydride (75 ml.) was heated under reflux for 4 hr. After removal of the solvent at reduced pressure, the *product* (16·4 g.) had m.p. 100–102° [from ethyl acetate-light petroleum (b.p. 60–80°)]. Found: C, 51·1; H, 6·0; N, 3·4. $C_{17}H_{23}NO_8S$ requires C, 50·9; H, 5·8; N, 3·5 per cent.

1,2,3,6-*Tetrahydro*-1-p-*toluenesulphonyl-pyridine.* A solution of toluene-*p*-sulphonyl chloride (1 mole.) in chloroform (500 ml.) was added with stirring during 20 min. to a solution of 1,2,3,6-tetrahydropyridine (2 mole.) in water (600 ml.). After 4 hr. the mixture was acidified with hydrochloric acid, the chloroform layer was separated, washed with water, concentrated to half-bulk and diluted with light-petroleum (b.p. 60–80°). The *product* (90 per cent yield) had m.p. 102–104° [from light petroleum (b.p. 80–100°)]. Found: C, 60·7; H, 6·3; N, 5·7. $C_{12}H_{15}NO_2S$ requires C, 60·7; H, 6·4; N, 5·9 per cent.

3,4-*Dibromo*-1-p-*toluenesulphonylpiperidine* was prepared by reaction of the foregoing compound with bromine in acetic acid, had m.p. 116–117° (from aqueous ethanol). Found: C, 36·3; H, 3·4; N, 3·6; S, 8·3. $C_{12}H_{15}Br_2NO_2S$ requires C, 36·3; H, 3·8; N, 3·5; S, 8·1 per cent.

Trans-1-p-*Toluenesulphonylpiperidine*-3,4-*diol.* A solution of 1,2,3,6-tetrahydro-1-*p*-toluenesulphonylpyridine (47·4 g.) in acetic acid (50 ml.) at 80° was added to a solution of peracetic acid [prepared from acetic acid (150 ml.) and 30 per cent hydrogen peroxide (46 ml.)] at the same temperature and heating was continued for 4 hr. The solution was concentrated to one third bulk at reduced pressure, diluted with saturated salt solution and the oil isolated with chloroform. The chloroform extract was washed free from acid and peroxide and the solvent removed at reduced pressure. The residual product was boiled with 80 per cent ethanol (60 ml.) containing sodium carbonate (20 g.) for 30 min. when it

was diluted with water and extracted with chloroform. Concentration of the extract yielded the *product* (28·5 g.) m.p. 126–127° [from ethyl acetate-light petroleum (b.p. 60–80°)]. Found: C, 53·2; H, 6·1; N, 5·2; S, 11·5. $C_{12}H_{17}NO_4S$ requires C, 53·1; H, 6·3; N, 5·2; S, 11·8 per cent.

trans-3,4-*Diacetoxy*-1-p-*toluenesulphonylpiperidine*. A solution of the foregoing diol (8·8 g.) in acetic anhydride (50 ml.) was heated under reflux for 3 hr. when excess of anhydride was distilled at reduced pressure. The *product* (11 g.) had m.p. 106–107° from ethyl acetate-light petroleum (b.p. 60–80°). Found: C, 54·0; H, 6·1; N, 4·0; S, 8·7. $C_{16}H_{21}NO_6S$ requires C, 54·1; H, 6·0; N, 3·9; S, 9·0 per cent. It was reconverted into the original *trans*-diol by short warming with 0·5N ethanolic hydrochloric acid.

cis-1-p-*Toluenesulphonylpiperidine*-3,4-*diol*. A solution of 1,2,3,6-tetrahydro-1-*p*-toluenesulphonylpyridine (59·3 g.) in warm ethanol (600 ml.) was stirred, treated with a solution of sodium chlorate (35·5 g.) in water (200 ml.), heated to 60° and a solution of osmium tetroxide (0·25 g.) in water (125 ml.) added. Water (450 ml.) was added in portions to the mixture at intervals as the hydroxylation proceeded care being taken not to precipitate the starting material. Heating was continued for 4 hr. when the solution was filtered from osmic oxide and concentrated at reduced pressure until separation of solids occurred when the *product* (53·2 g.) was collected. Saturation of the filtrate with sodium chloride followed by extraction with chloroform yielded a further quantity (10·8 g.) of material. It had m.p. 138–139° (from ethyl acetate). Found: C, 52·9; H, 6·3; N, 5·3; S, 11·7. $C_{12}H_{17}NO_4S$ requires C, 53·1; H, 6·3; N, 5·2; S, 11·8 per cent. The *diacetyl* derivative had m.p. 131–133° (from ethanol). Found: C, 54·4; H, 5·8; N, 4·0; S, 8·6. $C_{16}H_{21}NO_6S$ requires C, 54·1; H, 6·0; N, 3·9; S, 9·0 per cent. It was reconverted into the *cis*-diol by heating at reflux temperature with N ethanolic hydrochloric acid.

1-p-*Chlorobenzenesulphonyl*-1,2,3,6-*tetrahydropyridine*, had m.p. 67–68° [from light petroleum (b.p. 60–80°)]. Found: C, 51·0; H, 4·8; Cl, 13·7; N, 5·2; S, 12·3. $C_{11}H_{12}ClNO_2S$ requires C, 51·3; H, 4·7; Cl, 13·8; N, 5·4; S, 12·5 per cent.

trans-1-p-*Chlorobenzenesulphonylpiperidine*-3,4-*diol* was prepared by hydroxylation of the foregoing compound with peracetic acid. It had m.p. 157–158° (from ethyl acetate). Found: C, 45·0; H, 5·0; N, 4·8; S, 11·1. $C_{11}H_{14}ClNO_4S$ requires C, 45·3; H, 4·8; N, 4·8; S, 11·0 per cent. In one reaction 4-*acetoxy*-3-*hydroxy*-1-p-*toluenesulphonylpiperidine* was isolated along with the required diol after hydrolysis of the crude reaction product with dilute ethanolic hydrochloric acid. It had m.p. 166–168° (from ethanol). Found: C, 47·0; H, 4·7; N, 4·5; S, 9·5. $C_{13}H_{16}ClNO_5S$ requires C, 46·8; H, 4·8; N, 4·2; S, 9·6 per cent. Infra-red spectra confirmed the presence of hydroxyl and acetoxy groups.

trans-3,4-*Diacetoxy*-1-p-*chlorobenzenesulphonylpiperidine* was obtained by heating the foregoing diol or its monoacetate with acetic anhydride. It had m.p. 152–154° [from ethyl acetate-light petroleum (b.p. 60–80°)]. Found: C, 47·7; H, 4·9; Cl, 9·8; N, 4·0; S, 8·4. $C_{15}H_{18}ClNO_6S$ requires C, 47·9; H, 4·8; Cl, 9·4; N, 3·7; S, 8·5 per cent.

cis-1-p-*Chlorobenzenesulphonylpiperidine*-3,4-*diol* was prepared by hydroxylation of 1-*p*-chlorobenzenesulphonyl-1,2,3,6-tetrahydropyridine with sodium chlorate-osmium tetroxide in aqueous ethanol. It (90 per cent yield) had m.p. 167–168° (from ethyl acetate). Found: C, 45·2; H, 4·6; N, 5·0. $C_{11}H_{14}ClNO_4S$ requires C, 45·3; H, 4·8; N, 4·8 per cent. The *diacetyl* derivative had m.p. 131–133° [from ethyl acetate-light petroleum (b.p. 60–80°)]. Found: C, 48·2; H, 4·9; N, 3·8; S, 8·2. $C_{15}H_{18}ClNO_6S$ requires C, 47·9; H, 4·8; N, 3·7; S, 8·5 per cent.

1-*Formyl*-4-p-*toluenesulphonylpiperazine*. A solution of 1-formylpiperazine (25 g.) (cf. Horrom, Freifelder and Stone, 1955), in benzene (100 ml.) was treated with a solution of toluene-*p*-sulphonyl chloride (20·75 g.) in benzene (50 ml.), when the mixture was heated under reflux for 1 hr. and filtered hot to remove formylpiperazine hydrochloride (15·5 g.). The product (22·9 g.) separated on cooling and had m.p. 144–145° (from benzene). Found: C, 54·0; H, 5·7; N, 10·4; S, 12·3. $C_{12}H_{16}N_2O_3S$ requires C, 53·7; H, 6·0; N, 10·4; S, 12·0 per cent.

1-*Butylcarbamoylpiperazine hydrochloride*. To a stirred solution of anhydrous piperazine (86 g.) in ethanol (900 ml.) was added concentrated hydrochloric acid (87·5 ml.). The solution was heated to 60° and treated with butyl isocyanate (100 g.) added during 20 min. The mixture was allowed to stand overnight and filtered to remove piperazine dihydrochloride [26 g., m.p. 345° (decomp.)]. The filtrate was concentrated to about 200 ml. and the *product* (136 g.) separated on cooling. It had m.p. 242–244° (from ethanol). Found: C, 48·7; H, 9·2; Cl, 15·8; N, 18·7. $C_9H_{20}ClN_3O$ requires C, 48·8; H, 9·1; Cl, 16·0; N, 19·0 per cent.

1-*Butylcarbamoyl*-4-p-*chlorobenzenesulphonylpiperazine*. A solution of the foregoing hydrochloride (22·15 g.) in pyridine (60 ml.) at about 60° was treated with chlorobenzene-*p*-sulphonyl chloride (21·1 g.) added during 15 min. When the addition was complete the mixture was allowed to cool and was diluted with water (500 ml.). The *product* (27 g.) which separated had m.p. 175–176° (from aqueous methanol). Found: C, 50·3; H, 6·3; Cl, 10·2; N, 12·1; S, 9·2. $C_{15}H_{22}ClN_3O_3S$ requires C, 50·0; H, 6·2; Cl, 9·9; N, 11·7; S, 8·9 per cent.

1-*Butylcarbamoyl*-4-p-*toluenesulphonylpiperazine* was obtained in 80 per cent yield using the foregoing method. It had m.p. 133–135° (from aqueous methanol. Found: C, 56·2; H, 7·2; N, 12·5; S, 9·8. $C_{16}H_{25}N_3O_3S$ requires C, 56·6; H, 7·4; N, 12·4; S, 9·4 per cent.

1-p-*Methylbenzoylpiperazine hydrochloride*. A solution of piperazine monohydrochloride, prepared from piperazine hexahydrate (77·6 g.) and concentrated hydrochloric acid (35 ml.), in ethanol (150 ml.) was stirred at 20° and treated with *p*-toluoyl chloride (30·9 g.) added during 20 min. The mixture was stirred for a further hour at room temperature and then for 30 min. at 70° when solvent was removed at reduced pressure. Water (2 vol.) was added to the residue to dissolve piperazine dihydrochloride and the insoluble *product* purified by crystallisation from methanol-acetone. It had m.p. 283–285° (decomp.). Found: C, 59·7; H, 7·2; Cl, 14·9; N, 11·6. $C_{12}H_{17}ClN_2O$ requires C, 59·9; H, 7·1; Cl, 14·7; N, 11·6 per cent.

1-*Butylcarbamoyl*-4-p-*methylbenzoylpiperazine*. (*a*) A solution of the foregoing hydrochloride (12·0 g.) in acetone (100 ml.) and water (40 ml.) was treated with sodium carbonate (2·7 g.) and butyl isocyanate (5 g.) and the mixture heated under reflux for 30 min. The acetone was boiled off and the residual solid crystallised from aqueous ethanol to yield the *product* (14 g.), m.p. 137–138°. Found: C, 67·2; H, 8·3; N, 13·0. $C_{17}H_{25}N_3O_2$ requires C, 67·3; H, 8·3; N, 13·8 per cent. (*b*) A suspension of 1-butylcarbamoylpiperazine (22·15 g.) in pyridine (50 ml.) was stirred and treated with *p*-toluoyl chloride (15·45 g.) added during 15 min. Reaction was completed by heating on the steam bath for 10 min. The mixture was cooled, diluted with ice-water (150 ml.) and acidified with 2N hydrochloric acid (about 180 ml.). The *product* (20 g.) had m.p. 137–138° when crystallised from aqueous methanol or ethyl acetate.

1-*Butylcarbamoyl*-4-*furoylpiperazine* was obtained in 63 per cent yield using the foregoing method. It has m.p. 135–137° [from ethyl acetate-light petroleum (b.p. 60–80°)]. Found: C, 60·4; H, 7·6; N, 15·0. $C_{14}H_{21}N_3O_3$ requires C, 60·2; H, 7·6; N, 15·0 per cent.

1-*Butylcarbamoyl*-4-*diphenylcarbamoylpiperazine*, had m.p. 168–169° (from ethyl acetate). Found: C, 69·6; H, 7·5; N, 14·7. $C_{22}H_{28}N_4O_2$ requires C, 69·4; H, 7·4; N, 14·7 per cent.

1-(2-*Hydroxycyclohexyl*)*piperazine dihydrochloride* (cf. Mousseron, 1932). A solution of 2-chlorocyclohexanol (67·3 g.) and piperazine hexahydrate (194 g.) in ethanol (400 ml.) was treated with a solution of potassium hydroxide (28 g.) in methanol (80 ml.) and the mixture heated under reflux for 4 hr. It was then concentrated to half bulk when 1,4-di(2-hydroxycyclohexyl)piperazine (15 g., m.p. 203–205°) separated on cooling and was removed. The filtrate was treated with carbon disulphide (25 ml.) and the resultant *dithiocarbamate* collected and washed with ethanol. This was suspended in ethanol (400 ml.), concentrated hydrochloric acid (60 ml.) added and the mixture heated under reflux for 1 hr. It was then concentrated at reduced pressure to yield the *product* (56·7 g.) m.p. 264–266° (from methanol). Found: C, 46·9; H, 8·5; Cl, 27·6; N, 11·2. $C_{10}H_{22}Cl_2N_2O$ requires C, 46·7; H, 8·6; Cl, 27·6; N, 10·9 per cent.

1-(2-*Hydroxycyclohexyl*)-4-p-*toluenesulphonylpiperazine*. A suspension of the foregoing dihydrochloride (21·5 g.) in pyridine (100 ml.) was stirred and treated with toluene-*p*-sulphonyl chloride (15·9 g.) and heated on the steam-bath for 15 min. The solution was cooled and poured into ice-water (500 ml.). The *product* (23 g.) was collected and washed with water. It had m.p. 169–171° (from aqueous ethanol). Found: C, 60·5; H, 7·7; N, 7·9; S, 9·3. $C_{17}H_{26}N_2O_3S$ requires C, 60·3; H, 7·7; N, 8·3; S, 9·5 per cent.

4-*Chlorosulphonyl*-3-*nitrotoluene* (compare Zincke and Rose, 1914). A solution of 4-amino-3-nitrotoluene (60·8 g.) in 24 per cent hydrochloric acid (480 ml.) was diazotised at 0° with a solution of sodium nitrite (30·8 g.) in water (72 ml.). The filtered solution was added with stirring to a saturated solution of sulphur dioxide in acetic acid (640 ml.) containing cupric chloride dihydrate (28 g.) at 15°. After 20 min. the mixture was diluted with ice-water and the *product* (90 g.) collected and washed with

ice water. It had m.p. 99–101° [from 1,2-dichloroethane-light petroleum (b.p. 60–80·)]. Found: C, 36·0; H, 2·5; Cl, 15·2; N, 6·0; S, 13·4. Calc. for $C_7H_6ClNO_4S$: C, 35·7; H, 2·6; Cl, 15·1; N, 5·9; S, 13·6 per cent.

3-*Nitrotoluene*-4-*sulphonamide*. The foregoing sulphonyl chloride was added with stirring during 20 min. to a mixture of ammonia solution (700 ml., d = 0·880) and chloroform (300 ml.) at 15–20°. After 1 hr. excess of ammonia and chloroform were boiled off and the residual liquid acidified. The *product* (68 g.) had m.p. 170–172° (from 40 per cent ethanol). Found: 39·5; H, 3·8; N, 13·0; S, 14·5. Calc. for $C_7H_8N_2O_4S$: C, 38·9; H, 3·7; N, 13·0; S, 14·9 per cent.

3-*Aminotoluene*-4-*sulphonamide*. A mixture of the foregoing nitro-compound (32·4 g.), iron powder (30 g.), water (200 ml.) 20 per cent acetic acid (9 ml.) and n-octanol (1 ml.) was heated under reflux for 3 hr. and filtered hot. The filtrate was boiled with charcoal, filtered and the solution cooled to 0°. The product (22 g.), had m.p. 126–127° (from water). Found: C, 45·6; H, 5·3; N, 15·1; S, 17·4. $C_7H_{10}N_2O_2S$ requires C, 45·2; H, 5·4; N, 15·1; S, 17·2 per cent.

3,4-*Dihydro*-6-*methyl*-3-*oxobenzo*-1,2,4-*thiadiazine* 1,1-*dioxide*. A mixture of the foregoing amine (18·6 g.) and urea (6·6 g.) was heated at 200° for 30 min. The residual solid was dissolved in hot water (150 ml.), cooled, and acidified to Congo red with concentrated hydrochloric acid. The *product* (18·5 g.), had m.p. 291–293° (from ethanol). Found: C, 45·4; H, 3·4; N, 13·4; S, 15·0. $C_8H_8N_2O_3S$ requires C, 45·3; H, 3·8; N, 13·2; S, 15·1 per cent.

6-*Carboxy*-3,4-*dihydro*-3-*oxobenzo*-1,2,4-*thiadiazine* 1,1-*dioxide*. A stirred solution of the foregoing compound (9·9 g.) in N/2 sodium hydroxide (150 ml.) was warmed to 60–70° and treated during 2 hr. with powdered potassium permanganate (14·7 g.). The mixture was filtered hot and the insoluble sludge washed with hot water. The combined filtrate and washings were cooled and acidified to pH 2 with concentrated hydrochloric acid. The *product* (9·75 g.) which separated, had m.p. 304–305° (decomp.) after crystallisation from water. Found: C, 39·9; H, 2·7; N, 11·6; S, 13·5. $C_8H_6N_2O_5S$ requires C, 39·7; H, 2·5; N, 11·6; S, 13·2 per cent.

4-*Methoxy*-2-*nitrobenzenesulphonamide*. A solution of 4-amino-3-nitroanisole (50·4 g.) in 24 per cent hydrochloric acid (360 ml.) was diazotised at 0–5° with a solution of sodium nitrite (23·2 g.) in water (54 ml.). The filtered solution was added to a stirred solution of acetic acid saturated with sulphur dioxide containing cupric chloride dihydrate (21·0 g., dissolved in a minimum of water) at 15°. Reaction was completed by warming to 25° for 20 min. when the mixture was diluted with ice water (1 litre). The *sulphonyl chloride* (82 g. moist) was used for the next stage of the reaction. A sample crystallised from 1,2-dichloroethane-light petroleum (b.p. 60–80°) had m.p. 80–82°. Found: C, 33·6; H, 2·5; Cl, 14·1; N, 5·4; S, 12·5. $C_7H_6ClNO_5S$ requires C, 33·4; H, 2·4; Cl, 14·1; N, 5·6; S, 12·8 per cent. Reaction with ammonia solution (d = 0·880)-chloroform as described earlier, furnished the *sulphonamide* (32 g.) m.p. 143–145° (from aqueous ethanol). Found: C, 36·4; H, 3·6;

N, 12·1; S, 13·7. $C_7H_8N_2O_5S$ requires C, 36·2; H, 3·5; N, 12·1; S, 13·8 per cent.

2-*Amino*-4-*methoxybenzenesulphonamide*. Reduction of the foregoing nitro-sulphonamide with iron powder in acidulated water as described earlier furnished the *product*, m.p. 141–142° after crystallisation from water. Found: C, 41·2; H, 5·1; N, 14·1; S, 16·0. $C_7H_{10}N_2O_3S$ requires C, 41·6; H, 5·0; N, 13·9; S, 15·9 per cent.

3,4-*Dihydro*-6-*methoxy*-3-*oxobenzo*-1,2,4-*thiadiazine* 1,1-*dioxide* was obtained in 82 per cent yield when the foregoing amino-sulphonamide (12·0 g.) was heated with urea (3·9 g.) at 210° for 30 min. It had m.p. 290–291° (decomp.) after crystallisation from ethanol. Found: C, 42·3; H, 3·3; N, 12·4; S, 14·2. $C_8H_8N_2O_4S$ requires C, 42·1; H, 3·5; N, 12·3; S, 14·1 per cent.

4-*Fluoro*-2-*nitrobenzenesulphonamide*. A solution of 4-fluoro-2-nitroaniline [cf. Swarts (1915)] in 24 per cent hydrochloric acid was diazotised and converted into the *sulphonyl chloride* as described earlier. This did not crystallise readily and was therefore dissolved in 1,2-dichloroethane, washed acid-free and added with stirring to aqueous ammonia (d = 0·880) to yield the *product*, m.p. 155–156° (from water). Found: C, 32·6; H, 2·2; N, 12·7. $C_6H_5FN_2O_4S$ requires C, 32·4; H, 2·3; N, 12·7 per cent.

2-*Amino*-4-*fluorobenzenesulphonamide*. A solution of the foregoing nitro-sulphonamide (4·47 g.) in ethanol (50 ml.) was hydrogenated in the presence of 5 per cent palladised charcoal (0·5 g.). The *product* (3·4 g.) had m.p. 126–128° (from water). Found: C, 37·8; H, 3·6; N, 14·8; S, 16·9. $C_6H_7FN_2O_2S$ requires C, 37·9; H, 3·7; N, 14·7; S, 16·9 per cent.

6-*Fluoro*-3,4-*dihydro*-3-*oxobenzo*-1,2,4-*thiadiazine*-1,1-*dioxide* was obtained by reaction of the foregoing amino-sulphonamide with urea at 200° for 30 min. It had m.p. 272–274° after crystallisation from water. Found: C, 38·8; H, 2·4; N, 12·8; S, 14·7. $C_7H_5FN_2O_3S$ requires C, 38·9; H, 2·3; N, 13·0; S, 14·8 per cent.

2-*Amino*-4-*chlorobenzenesulphonamide* was obtained in 80 per cent yield by reduction of 2-nitro-4-chlorobenzenesulphonamide [Meerwein *et al.* (1957)] with iron powder in 70 per cent ethanol containing 1 per cent of acetic acid. It had m.p. 139–141° after crystallisation from dilute ethanol. Found: C, 35·2; H, 3·2; Cl, 17·0; N, 13·5; S, 15·4. $C_6H_7ClN_2O_2S$ requires C, 34·9: H, 3·4; Cl, 17·2; N, 13·6; S, 15·5 per cent.

6-*Chloro*-3,4-*dihydro*-3-*oxobenzo*-1,2,4-*thiadiazine* 1,1-*dioxide* was prepared by heating the foregoing amino-sulphonamide with urea at 200° for 45 min. It had m.p. 304° (decomp.) (from ethanol). Found: C, 36·5; H, 2·1; Cl, 15·0; N, 12·3; S, 14·0. $C_7H_5ClN_2O_3S$ requires C, 36·1; H, 2·2; Cl, 15·2; N, 12·0; S, 13·8 per cent.

4-*Bromo*-2-*nitrobenzenesulphonamide* was obtained by the diazo-route already described from 4-bromo-2-nitroaniline [Hartley (1928)]. The intermediate 4-*bromo*-2-*nitrobenzenesulphonyl chloride* had m.p. 100–102° [from 1,2-dichloroethane-light petroleum (b.p. 60–80°)]. Found: C, 23·9; H, 1·2; Total halogen, 38·5; N, 4·9; S, 10·7. $C_6H_3BrClNO_4S$ requires C, 24·0; H, 1·0; Total halogen, 38·4; N, 4·7; S, 10·7 per cent. The *sulphonamide*, obtained by reaction of the sulphonyl chloride in

aqueous ammonia (d = 0·880)-chloroform medium, had m.p. 176–178° (from aqueous ethanol). Found: C, 25·6; H, 1·9; Br, 28·4; N, 10·1. $C_6H_5BrN_2O_4S$ requires C, 25·6; H, 1·8; Br, 28·4; N, 10·0 per cent.

2-*Amino*-4-*bromobenzenesulphonamide*, obtained by reduction of the foregoing nitro-sulphonamide in 70 per cent ethanol containing 0·5 per cent acetic acid, had m.p. 146–148° (from water). Found: C, 28·7; H, 2·6; N, 11·2; S, 12·6. $C_6H_7BrN_2O_2S$ requires C, 28·7; H, 2·8; N, 11·2; S, 12·8 per cent.

6-*Bromo*-3,4-*dihydro*-3-*oxobenzo*-1,2,4-*thiadiazine* 1,1-*dioxide*, obtained by heating the foregoing amino-sulphonamide with urea at 200° for 45 min., had m.p. 313–315° (decomp.) (from aqueous ethanol). Found: C, 30·4; H, 1·9; N, 10·3; S, 11·8. $C_7H_5BrN_2O_3S$ requires C, 30·3; H, 1·8; N, 10·1; S, 11·6 per cent.

3,4-*Dihydro*-3-*oxo*-6-*trifluoromethylbenzo*-1,2,4-*thiadiazine* 1,1-*dioxide* was obtained by heating 2-amino-4-trifluoromethylbenzenesulphonamide (Holdrege, Babel and Cheney, 1959), with urea at 200° for 30 min. It had m.p. 233–235° (from aqueous ethanol). Found: C, 36·2; H, 2·3; N, 10·5. $C_8H_5F_3N_2O_3S$ requires C, 36·1; H, 1·9; N, 10·5 per cent.

1-*Butyl*-3-(4-*methyl*-3-*nitrobenzoyl*)*urea*. A solution of 4-methyl-3-nitrobenzoyl chloride (61·4 g.) (King and Murch, 1925) in benzene (120 ml.) was added dropwise to a stirred solution of butylurea (38·3 g.) in pyridine (23·7 g.) and benzene (300 ml.) during 30 min. The mixture was warmed to 60° for 1 hr., cooled to below 5° and the solids collected and washed with water. The *product* (32·2 g.) had m.p. 119–120° (from ethanol). Found: C, 56·0; H, 6·3; N, 14·8. $C_{13}H_{17}N_3O_4$ requires C, 55·9; H, 6·1; N, 15·0 per cent.

3-(3-*Amino*-4-*methylbenzoyl*)-1-*butylurea* was prepared by hydrogenation of the foregoing nitro-compound (20·6 g.) with 5 per cent palladium-charcoal catalyst (4 g.) in ethanol (500 ml.). It (13·6 g.) had m.p. 134–136° (from ethanol). Found: C, 62·4; H, 7·7; N, 16·7. $C_{13}H_{19}N_3O_2$ requires C, 62·6; H, 7·7; N, 16·9 per cent.

1-*Cyclohexyl*-3-(4-*methyl*-3-*nitrobenzoyl*)*urea* was obtained in 52 per cent yield by reaction of 4-methyl-3-nitrobenzoyl chloride with cyclohexylurea in pyridine-benzene. It had m.p. 210–212° (from ethanol or benzene). Found: C, 58·8; H, 6·3; N, 14·0. $C_{15}H_{19}N_3O_4$ requires C, 59·0; H, 6·3; N, 13·8 per cent.

3-(3-*Amino*-4-*methylbenzoyl*)-1-*cyclohexylurea* was obtained in 47 per cent yield by reduction of the foregoing nitro-compound with iron powder-ferrous sulphate in 60 per cent ethanol. It had m.p. 203–205° (from ethanol). Found: C, 65·2; H, 7·4; N, 14·9. $C_{15}H_{21}N_3O_2$ requires C, 65·4; H, 7·7; N, 15·3 per cent.

1-*Butyl*-3-(4-*chloro*-3-*nitrobenzoyl*)*urea* was obtained in 75 per cent yield by reaction of 4-chloro-3-nitrobenzoyl chloride (Montagne, 1900) with butylurea in benzene-pyridine. It had m.p. 143–145° (from ethanol). Found: C, 48·1; H, 4·8; Cl, 11·8; N, 14·1. $C_{12}H_{14}ClN_3O_4$ requires C, 48·1; H, 4·7; Cl, 11·8; N, 14·0 per cent.

3-(3-*Amino*-4-*chlorobenzoyl*)-1-*butylurea*, obtained in 80 per cent yield by reduction of the foregoing nitrocompound with iron powder-ferrous

sulphate in 80 per cent ethanol, had m.p. 157–158° (from ethanol). Found: C, 53·5; H, 5·9; Cl, 13·0; N, 15·6. $C_{12}H_{16}ClN_3O_2$ requires C, 53·4; H, 6·0; Cl, 13·2; N, 15·6 per cent.

3-(4-*Chloro*-3-*nitrobenzoyl*)-1-*cyclohexylurea*, obtained in 73 per cent yield, had m.p. 205–206° (from ethanol). Found: C, 51·8; H, 4·9; Cl, 10·9; N, 12·8. $C_{14}H_{16}ClN_3O_4$ requires C, 51·6; H, 5·0; Cl, 10·9; N, 12·9 per cent.

3-(3-*Amino*-4-*chlorobenzoyl*-1-*cyclohexylurea*, obtained in 71 per cent yield by iron powder-ferrous sulphate reduction of the foregoing nitro-compound in 80 per cent ethanol, had m.p. 204–206° (from aqueous ethanol). Found: C, 57·3; H, 6·1; Cl, 12·3; N, 14·5. $C_{14}H_{18}ClN_3O_2$ requires C, 56·9; H, 6·1; Cl, 12·0; N, 14·2 per cent.

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LETTERS TO THE EDITOR

Calophyllolide, a complex coumarin anticoagulant from *Calophyllum inophyllum* Linn

SIR,—*Calophyllum inophyllum* Linn (Guttiferae) is an Indian medicinal plant commonly growing in the coastal regions of South India, Andaman islands, Burma and Ceylon. Ormancy, Potier, Buzas, and Lederer (1951) isolated a lactone, Calophyllolide from the defatted kernels of the nuts. Polonsky (1956) established its chemical structure as 3,4-dihydro-5-methoxy-2,2-dimethyl-6-(2-methylcrotonoyl)-10-phenyl-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*']dipyran-8-one.

The anticoagulant activity of calophyllolide, a coumarin derivative, was compared in 78 rabbits with dicoumarol, ethyl biscoumacetate and nicoumalone at the single dose levels of 50 mg./kg. by mouth for the hypoprothrombinaemic property, by determining the prothrombin time by Quick's (1935) one-stage method. The coagulation valency (the percentage prothrombin in plasma) in plasma from 35 normal Indian rabbits was established by the procedure of Montigel and Pulver (1952) in these laboratories for the evaluation of the results thus obtained. The technique used for determining the effect of calophyllolide on platelet clumping time was that of Mills, Nechles and Chu (1928) as modified by Sharp (1958). Whole blood coagulation time by Lee and White's method and bleeding time by Duke's (1910) technique were also studied and compared with dicoumarol. The results are summarised in Table I.

TABLE I

ANTICOAGULANT ACTIVITY OF CALOPHYLLOLIDE AND OTHER ANTICOAGULANTS 50 MG./KG. IN RABBITS

Property	No. of expts. with each drug	Calophyllolide	Dicoumarol	Ethyl biscoumacetate	Nicoumalone
Hypoprothrombinemic activity ..	12				
Coagulation valency, per cent		20*	10	50	35
Onset of action		24 hr.	24 hr.	24 hr.	24 hr.
Peak of action		48 hr.*	144 hr.	24 hr.	48 hr.
Duration of action		168 hr.	288 hr.	72 hr.	96 hr.
Platelet clumping time	5	35†	46†		
Whole blood coagulation time ..	5	11·2†	15·7†		
Bleeding time	5	18·35†	22·42†		

* Peak of hypoprothrombinemic action.
† Increase per cent, in time from normal.

It is evident from the results that the onset of hypoprothrombinaemic activity was achieved earlier with calophyllolide than with dicoumarol and nicoumalone, but more slowly than with ethyl biscoumacetate. Its duration of action is longer than ethyl biscoumacetate and nicoumalone but shorter than dicoumarol. It lowered the coagulation valency more than nicoumalone and ethyl biscoumacetate but slightly less than dicoumarol. The properties of calophyllolide, therefore, permit a relatively rapid onset of hypoprothrombinaemia like nicoumalone with less likelihood of the very rapid fluctuations of prothrombin time. In this sense, calophyllolide strikes a balance between the slow, long acting dicoumarol and the very fast, short acting ethyl biscoumacetate.

The whole blood coagulation, bleeding and platelet clumping times were also increased moderately like dicoumarol.

Calophyllolide was given to five male rabbits for a period of eight weeks. A first dose of 50 mg./kg. brought down the coagulation valency to between 18 to 22 per cent after 48 hr. The subsequent doses of 30 mg./kg. on alternate days thereafter maintained the coagulation valency between 20 and 30 per cent. These rabbits were then killed after eight weeks and on macroscopic or microscopic examination of liver, kidney, spleen, skin and mucous membrane, revealed no evidence of haemorrhage. Further studies are in progress.

No fatalities were observed in rats when calophyllolide was administered orally in doses up to 1,750 mg./kg. Its intraperitoneal LD50 in rats was found to be 560 mg./kg.

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Effect of Hydrocortisone on Capillary Permeability Changes Induced by *Echis carinatus* (Saw-scaled Viper) Venom in the Rat

SIR,—During recent years the corticosteroids have been reported to be very useful, alone and in combination with the specific antivenene, in the treatment of snake poisoning (Wig and Vaish, 1960; Gupta, Bhargava and Sharma, 1960; Benyajati, Keoplung and Sribhibhadh, 1961; Arora, Wig and Somani, 1962). Since a widespread capillary endothelial damage, with the resultant haemorrhages in various viscera, in the skin, in the mucous membranes and the accompanying shock, determine to a large extent the ultimate outcome of viperine snake poisoning in man (Ahuja and Singh, 1956), we have examined the effect of hydrocortisone on the capillary permeability changes induced by the *Echis carinatus* (the saw-scaled viper, a common poisonous snake in India) venom in an attempt to elucidate the exact mechanism of protective action.

Azovan blue dye, 20 mg./kg., was administered intravenously in male albino rats, Haffkine strain, weighing 150–200 g., and the extent and intensity of blue discoloration produced by 20 μg. each of 5-hydroxytryptamine (5-HT), histamine and the *E. carinatus* venom (freeze-dried form, dissolved fresh before use) administered in 0·1 ml. distilled water intradermally in the previously depilated abdominal skin of the rat was observed. An interval of 15 min. was allowed for the dye to accumulate at the site of the drug administration when the rats were killed and the skin removed (see Parratt and West, 1957).

The extent of capillary permeability, as indicated by the area of blue discoloration which developed due to the leakage of the vital dye-plasma protein complex (Spector, 1958) induced by 5-HT, histamine and the venom in the control and the hydrocortisone pretreated group of rats is summarised in Table I.

TABLE I

Drug (20 μg. in 0·1 ml. distilled water)	Diameter in mm. of blue discoloration 15 min. after the drug administration in groups of 10 rats	
	Control	Hydrocortisone acetate 10 mg./kg. i.p. 30 min. before the test
5-HT	20 (18–22)	14 (12–16)
Histamine	6 (5–7)	nil
E. carinatus venom	18 (16–20)	4 (3–5)

The results thus obtained show that hydrocortisone pretreatment inhibited the increased capillary permeability induced by the *E. carinatus* venom in the rat abdominal skin. From the evidence available at present, it appears that the corticosteroids protect against the viperine snake poisoning mainly by suppressing the increased capillary permeability induced by the venom, though other factors, particularly the favourable effect of corticosteroids against haemorrhages and shock, may also be contributing, because once the capillary endothelial damage has been produced by the viperine venoms, it cannot be reversed even by the administration of a large amount of the specific antivenene (Ahuja and Singh, 1956) though it is checked if the venom is neutralised early by the antivenene, suggesting that probably it is not the venom itself but some substance liberated by the venom in the body which leads to the increased capillary permeability. That the *E. carinatus* venom itself does not affect the capillary permeability has been shown by Somani and Arora (1962) in the rat skin, confirming experimentally that the increased capillary permeability induced by the venom is mediated mainly through a release of histamine and to a lesser extent through 5-HT. Our results suggest that the corticosteroids in some way inhibit the effect of the antigen-antibody complex on the capillary permeability. Nevertheless, the general response of the capillary wall also appears to be depressed, as the response to 5-HT is also decreased, both in extent and intensity of blue discoloration.

The present findings, moreover, confirm our earlier observations that the *E. carinatus* venom acts mainly through a release of histamine, since hydrocortisone almost completely blocked the response to the venom and that to histamine, though there was only a slight reduction in the response to 5-HT. If, on the other hand, the action of the venom was mediated through 5-HT release, the inhibiting effect of hydrocortisone against the venom would have been less marked.

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The Effects of Ether on Potassium Flux in Skeletal Muscle Preparations

SIR,—In anaesthetic concentrations, ether increases and in higher concentrations, decreases the response of striated muscle to potassium (Torda, 1944). Lorkovic (1959) has noted that in potassium-stimulated frog muscle, ether depresses the twitch fibres and potentiates the response of the slow fibres. Ether also alters the blood potassium levels. It decreases serum potassium in the dog (Gerschman and Marenzi, 1933), an effect preceded by an initial rise (Kiersz, 1948) and confirmed in man (Goodman and Gilman, 1955). Using cat erythrocytes, Davson and Reiner (1942) showed that ether increased the rate of efflux of potassium and decreased the inward movement of sodium. The effects of ether upon serum sodium levels are, however, irregular, and small (Kiersz, 1948; Gerschman and Marenzi, 1933). The ether-induced depolarisation of nervous elements (Lorente de Nó, 1947) and the suggestion that ether had a depolarising action at the neuromuscular synapse (Secher, 1951) led us to re-investigate the effects of ether and some volatile anaesthetics on potassium flux in skeletal muscle preparations.

Saturated solutions of the volatile liquids were freshly prepared by shaking with the appropriate saline for 15 min. at room temperature. The clear, anaesthetic-saturated layer was decanted and used.

Uptake and release of potassium-42 ($^{42}K^+$) from paired frog sartorius muscles and from rectangular strips of rat diaphragm were measured by a method similar to that of Lister and Lewis (1959) and Ahmad and Lewis (1962).

The effects of ether upon the twitch height, in response to both direct and indirect stimulation, in the isolated rat phrenic-nerve diaphragm preparation (Bülbring, 1946) and, using this preparation the actions of ether and tubocurarine (1 to 3 μg./ml.) on the $^{42}K^+$ efflux from $^{42}K^+$-loaded rats were observed.

In pentobarbitone-anaesthetised cats, the effects of adrenaline (50–100 μg./kg.), neostigmine (0·25 mg./kg.) and tubocurarine (100–200 μg./kg.) on ether-induced neuromuscular block and at the same time on blood serum levels of $^{42}K^+$ were studied.

The effects of ether, methyl n-propyl ether, chloroform and halothane upon acetylcholine-induced contractions of the isolated frog rectus abdominis muscle were also investigated.

In the isolated frog sartorius muscle and isolated strips of rat diaphragm, ether decreased the uptake ($P < 0{\cdot}001$) and increased the release of $^{42}K^+$. Qualitatively similar results were obtained using saturated solutions of chloroform, halothane and methyl n-propyl ether. Methyl n-propyl ether and ether also increased the release of $^{42}K^+$ from the frog rectus abdominis muscle. Ether

increased the blood levels of $^{42}K^+$ in pentobarbitone-anaesthetised cats. Neither on this preparation nor on the isolated rat phrenic nerve-diaphragm preparation did tubocurarine (0·2–0·5 mg./kg. and 1–2 μg./ml. respectively) alter the release of $^{42}K^+$. On the rat diaphragm-phrenic nerve preparation, ether reduced or abolished the response to indirect stimulation and increased the block produced by tubocurarine (1–2 μg./ml.) or decamethonium (10–20 μg./ml.). The latter effect confirms the findings of Secher (1951). The effects of ether were antagonised by 2–10 μg./ml. of neostigmine. Qualitatively similar results were obtained on the cat gastrocnemius muscle-sciatic nerve preparation. Inhalation of the vapour of from 5–10 ml. of ether reduced the magnitude of contractions of the muscle elicited by sciatic nerve stimulation. Adrenaline (20–50 μg./kg.) and neostigmine (0·25 mg./kg.) both antagonised the action of ether and at the same time, increased serum $^{42}K^+$ levels.

Ether solution (2–10 ml.) caused a contraction of the isolated frog rectus abdominis muscle, confirming the findings of Torda (1943). This was followed by a decline in sensitivity to acetylcholine (0·1–1·0 μg./ml.). Repeated exposure to ether further reduced the response to acetylcholine. Soaking in Ringer's fluid containing three times the normal quantity of potassium did not reverse this effect.

Solutions of methyl n-propyl ether (2–10 ml.), halothane (2–5 ml.) and chloroform (2–5 ml.) also depressed the response of the frog rectus abdominis muscle to acetylcholine. Methyl n-propyl ether had in addition a direct stimulant effect accompanied by an increase in the release of potassium-42.

Although ether and the other anaesthetics increase potassium release from skeletal muscle preparations in a similar manner to depolarising muscle relaxants (Ahmad and Lewis, 1961), ether potentiates non-depolarising (Foldes, 1957) more consistently than depolarising drugs (Paton, 1953). Synergism between ether and decamethonium is not difficult to interpret on this basis, but it seems very unlikely that the ether-potentiation of tubocurarine is due to a high local concentration of potassium causing intense depolarisation sufficient to override the competitive effects of tubocurarine and then exert a depolarising block of its own. On the other hand, loss of large amounts of intracellular potassium may weaken the contractile mechanism of the cell. A further possibility is that ether-induced potassium loss may render more easy the access of tubocurarine to receptor sites.

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BOOK REVIEWS

BENTLEY'S TEXTBOOK OF PHARMACEUTICS. 7th Edition. By Harold Davis. Pp. xiii + 1091 (including 309 illustrations and Index). Baillière, Tindall and Cox, London, 1961. 52s. 6d.

Bentley's Textbook of Pharmaceutics has been a standard reference book on pharmacists' bookshelves for over 35 years and during that time has been the vade mecum of countless pharmacy students.

In his preface to the first edition. Bentley remarked that no book had then been published on this aspect of the training of a pharmacist. It now seems remarkable that the whole field of Pharmaceutics should be included within the covers of one volume. The fact that the book is now in its seventh edition shows that it is in demand and that there is a need for frequent revision; this revision would surely be simplified if the work were in three or four volumes. In this manner justice would be done to each section of the subject.

The present edition has been extensively revised by Dr. Davis in co-operation with academic and industrial specialists who have been able to effect the revision with only a slight increase in the overall size. A new section on Radio-activity is included and there is a very welcome chapter on Containers and Packaging. Certain rearrangements have also been effected although they do not always assist in the use of the book. Containers, preservatives and incompatibilities which are dealt with in widely separated sections are all facets of the one problem —*formulation*—which warrants a complete section of its own. Similarly eye drops and eye lotions (neither of which appear in the index) should not be separated by 80 pages.

The introduction of a specific section on Unit Dosage Forms for Oral use is welcome but it seems a pity that it is restricted to tablets and capsules. Pills and cachets are dealt with elsewhere, the former occupying almost as much space as tablets; some of this space could well have been devoted to delayed action formulations and the associated processes for producing them.

The present edition contains an increased number of line diagrams which are most helpful, but there is still a need for further diagrams—the two illustrations of a steamer which appeared in the first edition might well be replaced by diagrams of an ethylene oxide steriliser, pre-vacuum autoclave system and spray cooling autoclave for bulk injections. Although there are five pictures of tablet machines there is none which illustrates how a simple tablet machine works.

The authors are to be congratulated in having placed between the covers of one book the present state of a rapidly developing subject and the publishers have done well to produce it so well at a very reasonable price. The above comments are made in the hope that they will help to keep this important work abreast with modern trends.

The inclusion of an increased number of references to other works and to original publications is very commendable, but the usefulness of "Bentley" as a book of reference could be enhanced by the amplification of its own index.

J. C. PARKINSON.

THE CHROMATOGRAPHY OF STEROIDS. By I. E. Bush. Pp. xxi + 437 (including Index). Pergamon Press, Oxford, 1961. 80s.

Chromatography has revolutionised biochemistry probably more than any other technique. The isolation, identification and structural analysis of complex substances are particularly impressive in the steroid field and whether the interest be in biosyntheses, metabolism or analysis of steroids the use of chromatographic methods is inevitably now involved. This comprehensive monograph on the chromatography of steroids will therefore be highly valued by all workers in the field. And yet, not by them only, for Professor Bush, who has contributed so much technically to methods for solution of particular problems has also delved deeper into the methodology. He has demonstrated the relevance in the steroid field of the general theory and background of partition chromatography built up with other families of organic compounds and has thereby demonstrated the ideal nature of steroids as a group for the study of the general theory of chromatographic behaviour. This monograph therefore contains a great deal of interest to biochemists and chemists generally. By drawing on work on fatty acids, flavonoids, sugars and amino-acids to demonstrate general principles underlying technical details of chromatographic work with steroids, he has also emphasised in consequence the relevance of progress in the chromatography of steroids to applications of the technique in other fields. His demonstrations of the quantitative treatment of steroid behaviour on chromatograms in typical and atypical solvent systems and his tables of, and calculations with, R_F and R_M values are illustrative of their potentiality in other fields. While basic theory and general principles provide the framework and the structure of this monograph, practical aspects are also fully discussed. Techniques and apparatus are described in most interesting detail, and include the preparation of extracts of animal tissues, blood and plasma, sweat, faeces and urine for chromatography with details of some typical analytical problems of steroid biochemistry. Appendices include notes on the purification of reagents and materials, on methods of detection and personally checked details of microchemical reactions for steroids. The systematic investigation of unknown steroids is described and future developments are envisaged. If indeed precise analytical details are not provided for the detection and limitation of any foreign steroid or steroid intermediate in any particularly important steroid, the basis for approaching the problem and determining whether it is capable of solution by chromatographic means is largely provided, though special problems arising from the development and use of variously substituted fluoro-derivatives may require additional experimental study. The importance of colour reactions, fluorescence and spectroscopy are carefully considered and their importance recognised but properly their limitations are also noted and underline the need to consider them in conjunction with, and not in substitution for, chromatographic properties. Perhaps in some respects the special value of infra-red spectroscopy of the steroids may seem, in consequence, to be unintentionally under-represented. Chromatography, however, remains the technique of major importance in the isolation and separation of steroids and has contributed greatly to our understanding of metabolism and steroid transformations. This monograph will help and encourage many to contribute still further knowledge.

FRANK HARTLEY.