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Veratrum Alkaloids—XLIV.* Structure–Activity Relationships in a Series of Synthetic Hypotensive Esters of Protoverine†

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Ester derivatives of protoverine have been shown to constitute some of the most potent hypotensive alkaloids isolated from *Veratrum* species.[1-8] The structures of the naturally-occurring hypotensive protoverine esters have recently been elucidated in our Laboratory.[9-11] Two of the alkaloids, protoveratrine A and protoveratrine B, have been used in recent years for the treatment of hypertension. The deterrent to the wider use of these agents in therapy is the narrow range between their therapeutic and emetic doses.[12, 13]

Germine esters occur with the esters of protoverine in all *Veratrum* species examined to date,[1-8] and the germine polyesters have been shown to possess pharmacological activity similar to that of the active protoverine derivatives.[12, 13] Weisenborn and coworkers[14] have reported the preparation of a number of synthetic esters of high hypotensive activity by stepwise esterification of germine. Largely on the basis of optical rotational values and of pharmacological activity, it was proposed that direct esterification of germine introduced acid residues on the same

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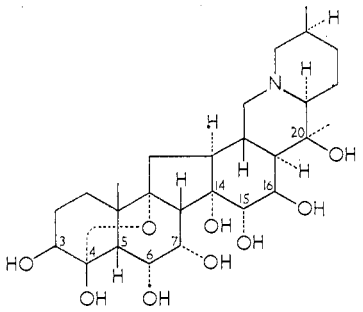
hydroxyl groups found esterified in the natural potently-hypotensive germine ester alkaloids such as germitrine and neogermitrine. Since the elucidation of the structure of germine and its ester derivatives was not completed until several years later,[15-19] no systematic structure–activity relationship could be defined among the synthetic esters of germine.

The present investigation was undertaken to study the relationship between hypotensive activity and structural isomerism in a series of synthetic hypotensive esters of protoverine (I).[20]



(I)

It was hoped that such a study might point the way to the preparation of new protoverine esters which would possess a desirable degree of hypotensive activity accompanied by lower emetic effects. We report herein the synthesis of all the possible protoverine 3,6,7,15-tetraesters which contain one, two or three isobutyryl residues and in which the remaining acyl groups are acetyl (see Table I).

Acylation of protoverine with two to three mole-equivalents of isobutyryl chloride afforded a mixture of diisobutyrate and triisobutyrate, separated by chromatography. The diisobutyrate consumed 1·0 mole-equivalent of periodic acid. Furthermore, the amorphous oxidation product showed absorption at 3·65, 5·62 and 5·80 μ characteristic of the aldehydo-γ-lactone resulting from periodic acid cleavage of the ring A glycol.[20] This result showed that the product was either a 6,15- or a 7,15-diisobutyrate. Acetylation of the diisobutyrate yielded a triacetate which was *not* identical with protoverine 3,6,16-triacetate 7,15-diisobutyrate (X). Consequently, the diisobutyrate prepared from protoverine

Table I.

Compound no.	Protoverine derivative	Formula	Calcd. %		Found %		m.p., °C	$[\alpha]_D^{25}$ (py.)	Equiv. vol. acid found
			C	H	C	H			
II	6,15-diisobutyrate	$C_{35}H_{55}NO_{11}$	63·14	8·33	62·88	8·41	190–191	−34	1·8
III	3,6,15-triisobutyrate	$C_{39}H_{61}NO_{12}$					amorph.	−11	—
IV	3,7,16-triacetate 6,15-diisobutyrate	$C_{41}H_{61}NO_{14}$	62·18	7·76	61·74	7·79	254–255	−49	4·7
V	3,6,15-triisobutyrate 7,16-diacetate	$C_{43}H_{65}NO_{14}$	62·98	7·99	62·95	8·00	234–236	−44	4·7
VI	3,7-diacetate 6,15-diisobutyrate	$C_{39}H_{59}NO_{13}$	62·46	7·93	62·28	7·66	237–238	−46	3·9
VII	3,6,15-triisobutyrate 7-acetate	$C_{41}H_{63}NO_{13}$	63·30	8·16	62·97	8·13	234–235	−46	3·8
IX	3,6,16-triacetate 15-isobutyrate	$C_{37}H_{55}NO_{13}$	61·56	7·68	61·44	7·84	228–229	−3	3·8
X	3,6,16-triacetate 7,15-diisobutyrate	$C_{41}H_{61}NO_{14}$	62·18	7·76	62·50	7·97	224–225	−45	4·7
XI	3,6,7,16-tetraacetate 15-isobutyrate	$C_{39}H_{57}NO_{14}$	60·60[a]	7·56	60·19	7·57	258–259	−46	4·6
XII	3,6-diacetate-7,15-diisobutyrate	$C_{39}H_{59}NO_{13}$	62·46	7·93	62·50	7·99	252–253	−46	3·7
XIII	3,6,7-triacetate 15-isobutyrate	$C_{37}H_{55}NO_{13}$	60·80[a]	7·72	60·67	8·89	248–249	−40	3·7
XVIII	3,7,15-triacetate 6-isobutyrate	$C_{37}H_{55}NO_{13}$	61·56	7·68	61·90	7·77	265–266	−41	3·7
XIX	3,7,15,16-tetraacetate 6-isobutyrate	$C_{39}H_{57}NO_{14}$	60·60[a]	7·56	60·59	7·29	219–220	−51	4·3

Table I—*continued*

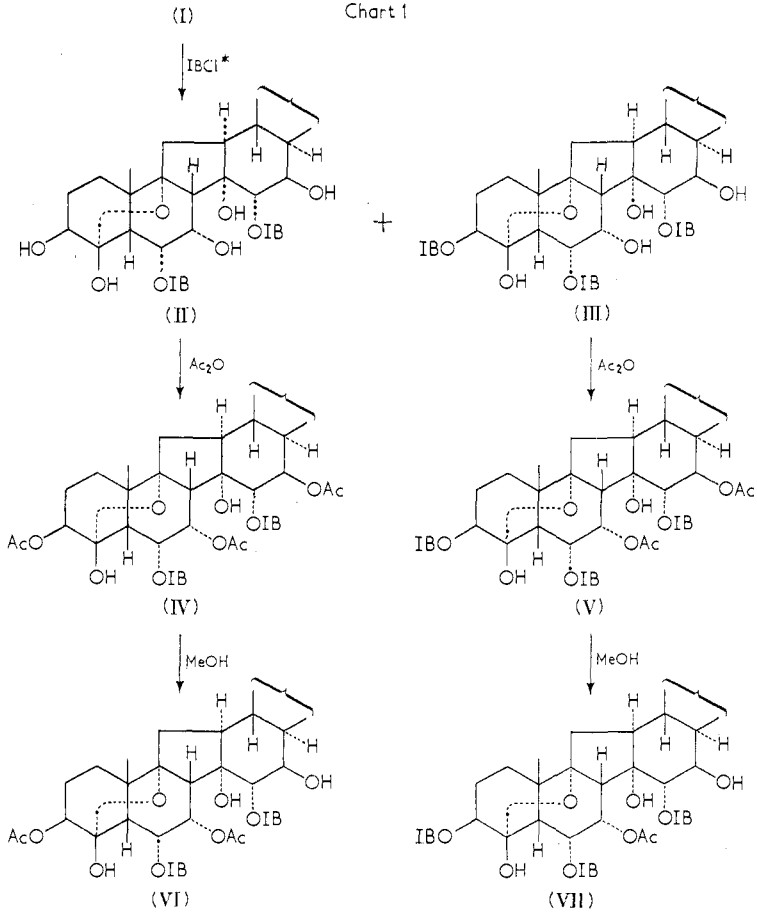
Compound no.	Protoverine derivative	Formula	Calcd. %		Found %		m.p., °C	$[\alpha]_D^{25}$ (py.)	Equiv. vol. acid found
			C	H	C	H			
XX	3,6-diisobutyrate	$C_{35}H_{55}NO_{11}$	60·67[b]	8·44	60·84	8·36	160–170	−10	1·9
XXI	3,6-diisobutyrate 7,15,16-triacetate	$C_{41}H_{61}NO_{14}$	60·80[c]	7·84	60·30	7·71	210–212	−44	4·3
XXII	3,6-diisobutyrate 7,15-diacetate	$C_{39}H_{59}NO_{13}$	62·46	7·93	62·79	8·00	262–263	−40	3·7
XXIII	14,15-acetonide 6-isobutyrate 3,16-diacetate	$C_{38}H_{57}NO_{12}$	62·61	8·02	62·67	8·03	252–253	+21	3·0
XXIV	3,15,16-triacetate 6-isobutyrate	$C_{37}H_{55}NO_{13}$	60·06[c]	7·76	59·87	8·08	177–179	−4	3·8
XXV	3,16-diacetate 6-isobutyrate	$C_{35}H_{53}NO_{12}$	61·03	7·90	60·93	7·87	232–233	−7	2·8
XXVI	3,16-diacetate 6,7,15-triisobutyrate	$C_{43}H_{65}NO_{14}$	62·98	7·99	63·91	8·34	233–234	−39	4·1
XXVII	3,15,16-triacetate 6,7-diisobutyrate	$C_{41}H_{61}NO_{14}$					amorph.	−50	—
XXVIII	3,15-diacetate 6,7-diisobutyrate	$C_{39}H_{59}NO_{13}$	62·46	7·93	62·38	7·97	227–228	−42	—
XXIX	3-acetate 6,7,15-triisobutyrate	$C_{41}H_{63}NO_{13}$					amorph.	−38	—
XXX	3,6-diisobutyrate 15-acetate	$C_{37}H_{57}NO_{12}$	62·78	8·12	62·72	8·18	165–168	−7	2·8
XXXI	3,6,7-triisobutyrate 15-acetate	$C_{41}H_{63}NO_{13}$	62·57[a]	8·20	62·61	7·88	239–241	−40	4·1

XXXIII	3,6,15,16-tetraacetate 7-isobutyrate	$C_{39}H_{57}NO_{14}$	61·32	7·52	61·39	7·39	257–259	−46	4·5
XXXIV	3,6,15-triacetate 7-isobutyrate	$C_{37}H_{55}NO_{13}$	61·56	7·68	61·65	7·47	243–244	−48	3·9
XXXVI	3,15-diisobutyrate 6,16-diacetate	$C_{39}H_{59}NO_{13}$					amorph.	−16	—
XXXVII	3,7,15-triisobutyrate 6,16-diacetate	$C_{43}H_{65}NO_{14}$	62·98	7·99	63·26	7·90	232–233	−37	4·5
XXXVIII	3,15-diisobutyrate 6,7,16-triacetate	$C_{41}H_{61}NO_{14}$	62·18	7·76	62·27	8·14	259–260	−48	4·7
XXXIX	3,7,15-triisobutyrate 6-acetate	$C_{41}H_{63}NO_{13}$					amorph.	−30	—
XL	3,15-diisobutyrate 6,7-diacetate	$C_{39}H_{59}NO_{13}$	62·46	7·93	62·04	7·82	222–223	−42	3·8
XLI	6,15,16-triacetate	$C_{33}H_{49}NO_{12}$	59·18[c]	7·68	59·51	7·63	242–243	−18	3·0
XLII	3,7-diisobutyrate 6,15,16-triacetate	$C_{41}H_{61}NO_{14}$					amorph.	−40	—
XLIII	3,7-diisobutyrate 6,15-diacetate	$C_{39}H_{59}NO_{13}$	61·00[c]	8·01	61·18	7·97	214–216	−37	3·6
XLIV	6,15-diacetate	$C_{31}H_{47}NO_{11}$	60·17[a]	7·82	60·02	7·89	235–236	−27	1·9
XLV	3-isobutyrate 6,15-diacetate	$C_{35}H_{53}NO_{12}$					amorph.	−10	—
XLVI	3-isobutyrate 6,7,15-triacetate	$C_{37}H_{55}NO_{13}$	61·56	7·68	61·28	7·55	249–250	−39	3·9
XLVII	6-acetate	$C_{29}H_{45}NO_{10}$	60·40	8·04	60·23	7·96	180–190	−15	0·8
XLVIII	3,15-diisobutyrate 6-acetate	$C_{37}H_{57}NO_{12}$					amorph.	−12	—
XLIX	3,15-diisobutyrate	$C_{35}H_{55}NO_{11}$	63·14	8·33	63·78	8·56	185–190	−10	1·6

[a] Calculated with $\frac{1}{2}$ H_2O. [b] Calculated with $1\frac{1}{2}$ H_2O. [c] Calculated with 1 H_2O.

was the 6,15-diester (II) and the acetylation product of (II) was protoverine 3,7,16-triacetate 6,15-diisobutyrate (IV). The triisobutyrate prepared from protoverine was stable to periodate, and this fact, coupled with the known greater ease of acylation at C_3

Chart 1

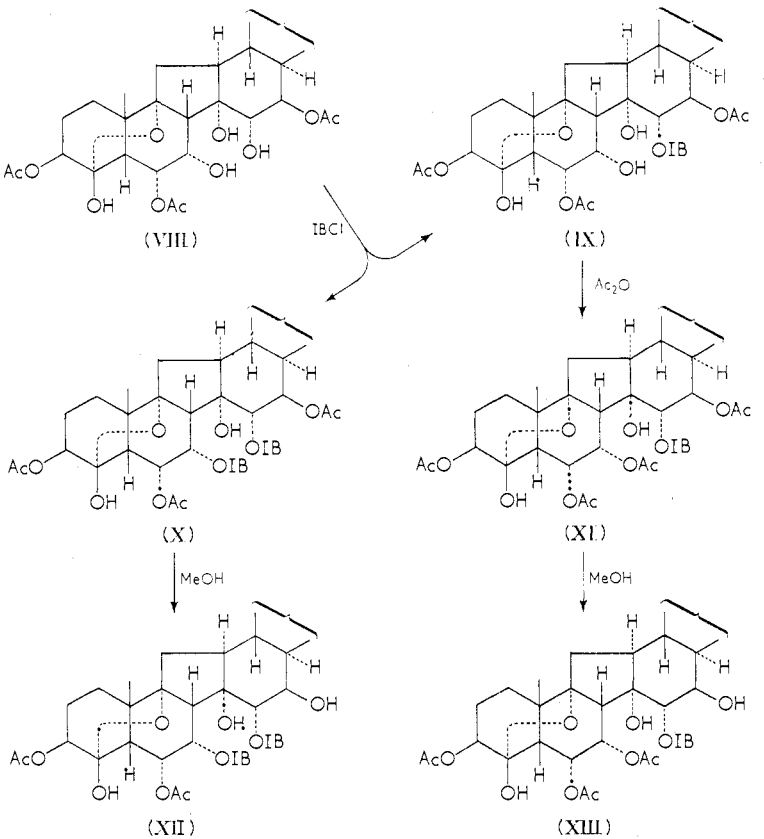


over C_7 and C_{16} (*cf.* for example, references 10 and 18) led to the assignment of the 3,6,15-triisobutyrate structure (III) to the triester. Acetylation of (III) gave protoverine 3,6,15-triisobutyrate 7,16-diacetate (V). Methanolysis of (V) yielded a 3,6,15-triisobutyrate monoacetate, assigned structure (VII) on the basis

of the known exceptionally facile methanolysis of C_{16} acetate esters of protoverine[20] and closely related alkaloids[15, 21] (Chart 1).

Protoverine 3,6,16-triacetate (VIII)[20] afforded a mixture of monoisobutyrate triacetate and diisobutyrate triacetate upon

Chart 2



isobutyrylation. The pentaester was assigned the 3,6,16-triacetate 7,15-diisobutyrate structure (X) by analogy with the structure of the known protoverine pentaacetate.[20] Methanolysis of (X) gave the 3,6-diacetate 7,15-diisobutyrate (XII). The monoisobutyrate derivative was assigned the 3,6,16-triacetate 15-isobutyrate structure (IX) in accordance with the known preferential

acylation at C_{15} over C_7.[11] Acetylation of (IX) gave the 3,6,7,16-tetraacetate 15-isobutyrate (XI), and methanolysis of (XI) yielded the 3,6,7-triacetate 15-isobutyrate (XIII) (Chart 2).

Acylation of protoverine 14,15-acetonide (XIV) with isobutyryl chloride has been shown to yield 14,15-acetonide 6-isobutyrate (XV) and 14,15-acetonide 3,6-diisobutyrate (XVII).[20] Hydrolysis of (XV) with dilute mineral acid gave protoverine 6-isobutyrate (XVI) and acetylation of (XVI) yielded the 3,7,15,16-tetraacetate 6-isobutyrate (XIX). Methanolysis of (XIX) afforded protoverine 3,7,15-triacetate 6-isobutyrate (XVIII). Mineral acid hydrolysis of (XVII) gave protoverine 3,6-diisobutyrate (XX) (periodate consumption: 1·8 mole-equivalents). Acetylation of (XX) yielded the 3,6-diisobutyrate 7,15,16-triacetate (XXI) which underwent methanolysis to yield the 3,6-diisobutyrate 7,15-diacetate (XXII) (Chart 3).

Acetylation of (XV) yielded a diacetate, assigned the 14,15-acetonide 3,16-diacetate 6-isobutyrate structure (XXIII) on the basis of the known resistance to acylation at C_7 in acetonide derivatives.[20] Mineral acid hydrolysis gave the 3,16-diacetate 6-isobutyrate (XXV). Controlled acetylation of (XXV) yielded a monoisobutyrate triacetate, assigned structure (XXIV) on the basis of the ease of acylation at C_{15}. Acylation of the 3,15,16-triacetate 6-isobutyrate (XXIV) with isobutyryl chloride yielded the 3,15,16-triacetate 6,7-diisobutyrate (XXVII). Methanolysis of (XXVII) gave protoverine 3,15-diacetate 6,7-diisobutyrate (XXVIII). Acylation of protoverine 3,16-diacetate 6-isobutyrate (XXV) with isobutyryl chloride afforded the 3,16-diacetate 6,7,15-triisobutyrate (XXVI), which underwent methanolysis to yield protoverine 3-acetate 6,7,15-triisobutyrate (XXIX) (Chart 4).

Controlled acetylation of protoverine 3,6-diisobutyrate (XX) yielded a monoacetate derivative, assigned the 3,6-diisobutyrate 15-acetate structure (XXX) in accordance with the previously mentioned analogy for preferential acylation at C_{15}. Acylation of (XXX) with isobutyryl chloride yielded a triisobutyrate monoacetate, tentatively assigned the protoverine 3,6,7-triisobutyrate 15-acetate structure (XXXI) on the basis of the apparent selectivity of acylation at C_7 over C_{16} in germine derivatives.[18]

Isobutyrylation of protoverine 3,6,15,16-tetraacetate (XXXII)[11]

Chart 3

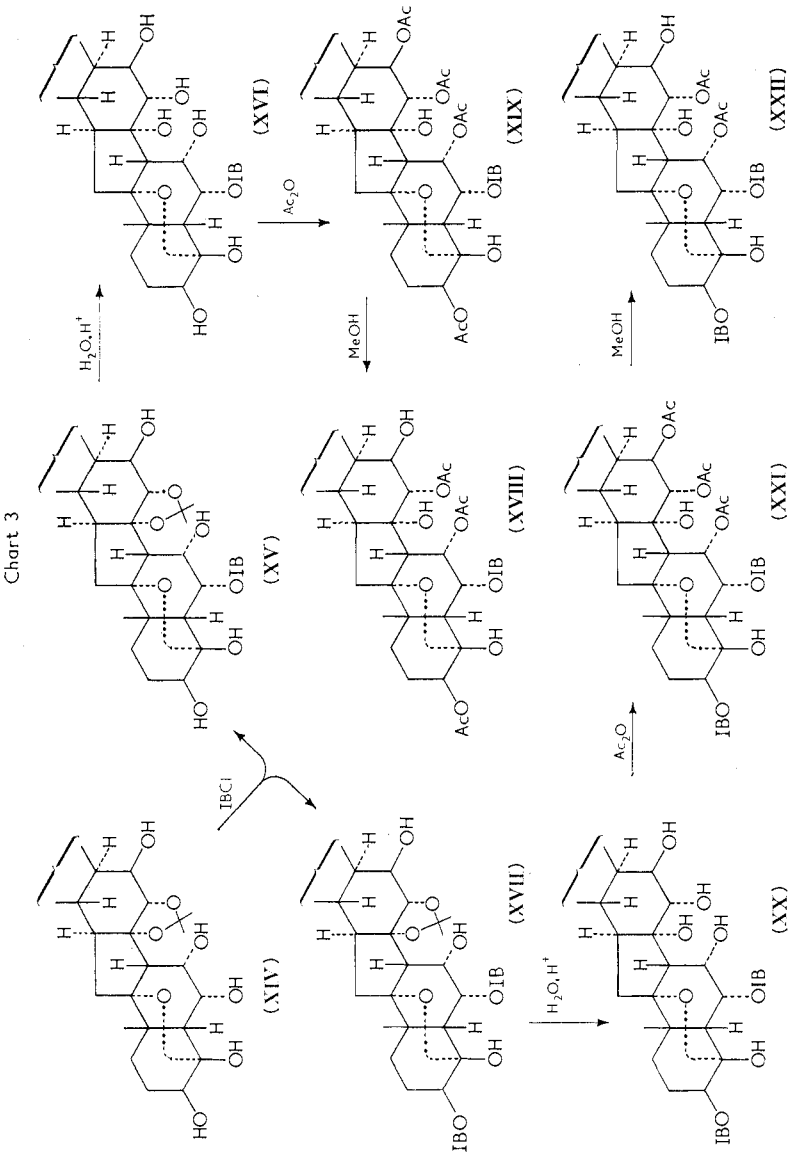


Chart 4

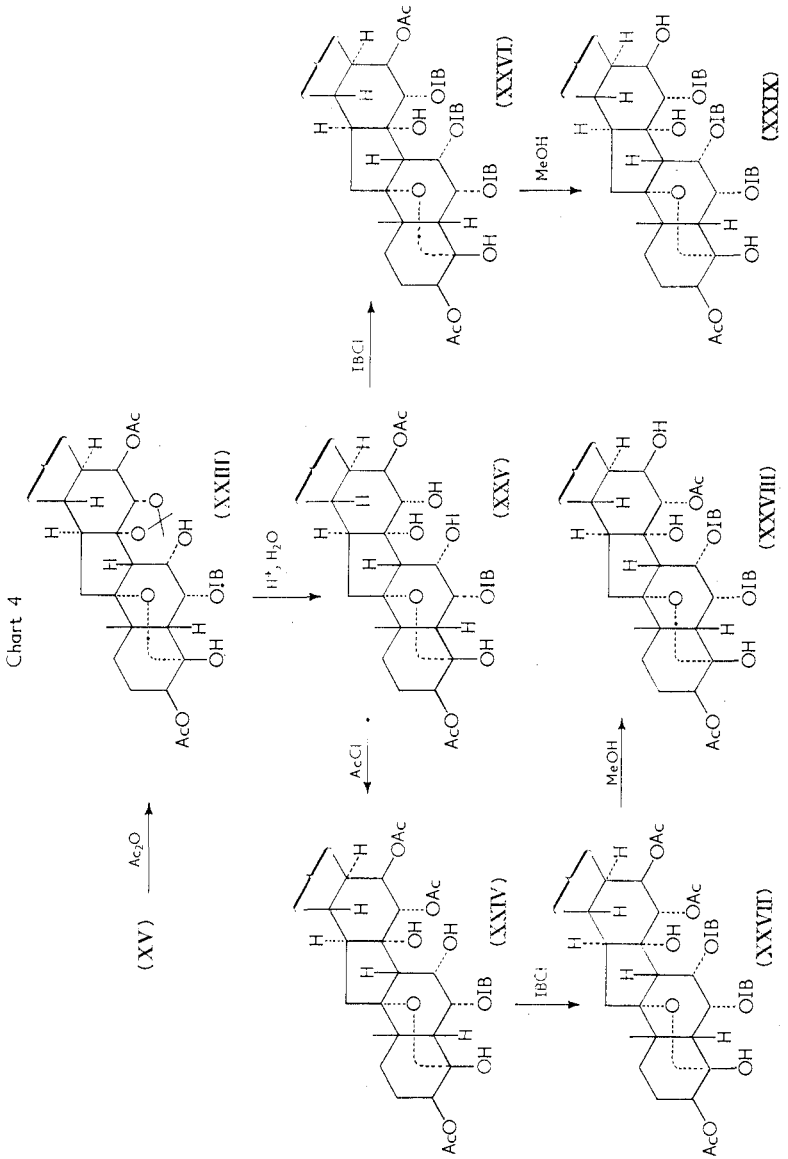
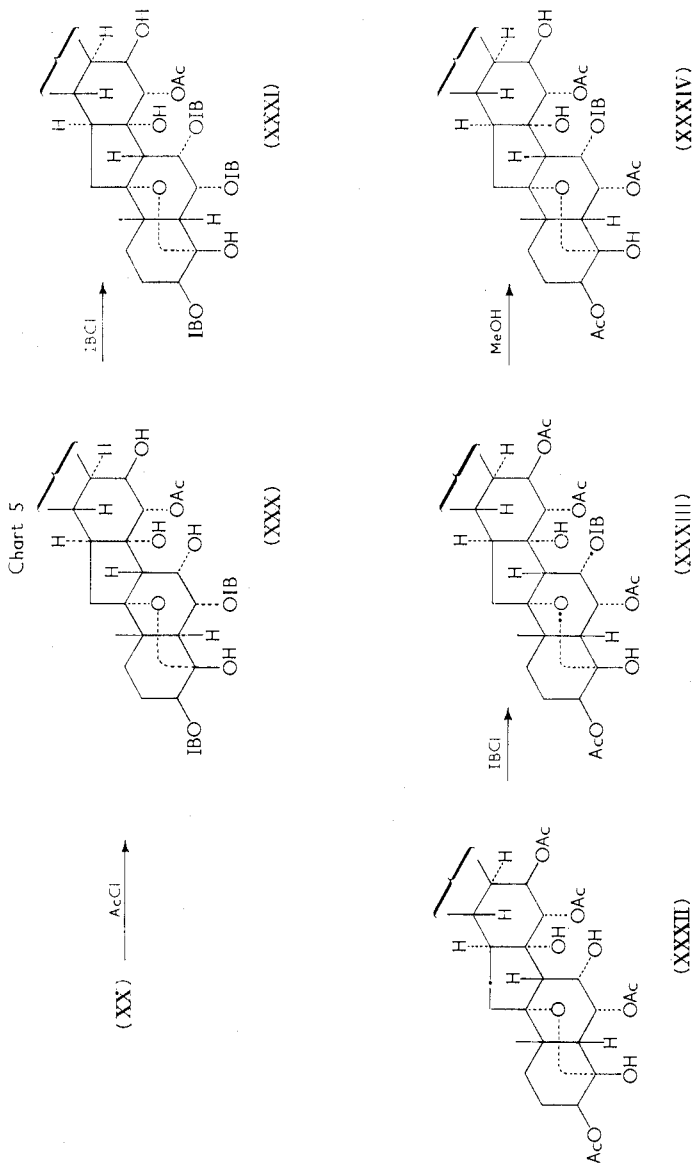

(XV)
Ac2O
(XXIII)
H+, H2O
(XXV)
IBCl
(XXVI)
MeOH
(XXIX)
AcCl
(XXIV)
IBCl
(XXVII)
MeOH
(XXVIII)

Chart 5



gave a monoisobutyrate derivative [assigned the 3,6,15,16-tetra-acetate 7-isobutyrate structure (XXXIII)]. Methanolysis of (XXXIII) yielded protoverine 3,6,15-triacetate 7-isobutyrate (XXXIV) (Chart 5).

Acylation of protoverine 6,16-diacetate (XXXV)[11] with isobutyryl chloride gave a diisobutyrate and a triisobutyrate, separated by chromatography. The pentaester, assigned the protoverine 3,7,15-triisobutyrate 6,16-diacetate structure (XXXVII) underwent methanolysis to yield the 3,7,15-triisobutyrate 6-acetate (XXXIX). The diisobutyrate was assigned the protoverine 3,15-diisobutyrate 6,16-diacetate structure (XXXVI) in accordance with the known facility of acylation at C_3 and C_{15} relative to C_7.[11,18] Acetylation of (XXXVI) yielded protoverine 3,15-diisobutyrate 6,7,16-triacetate (XXXVIII) and methanolysis of (XXXVIII) yielded the 3,15-diisobutyrate 6,7-diacetate (XL) (Chart 6).

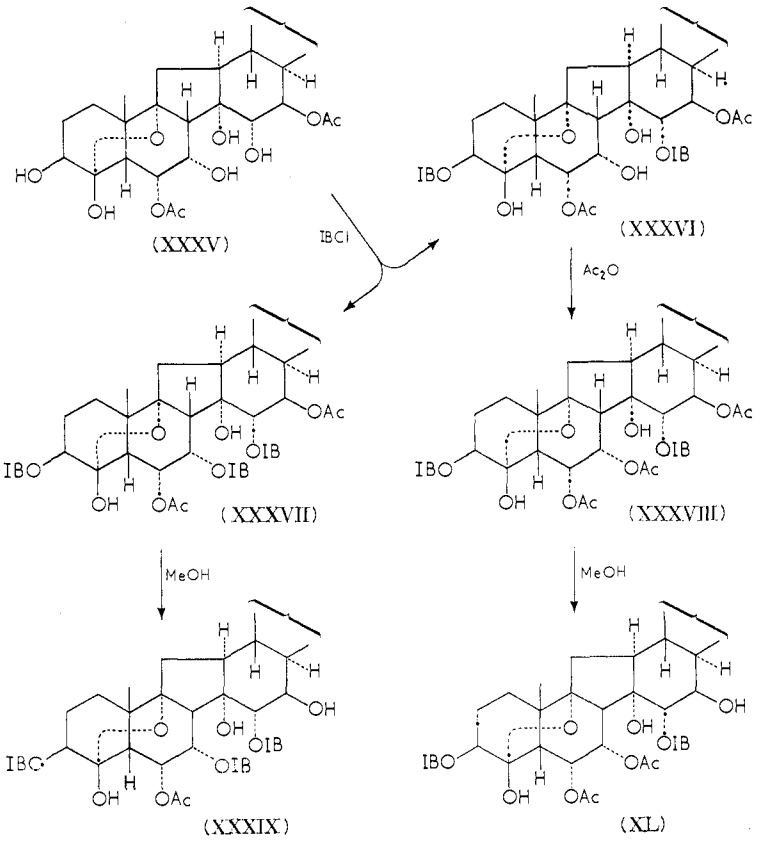
Controlled acetylation of protoverine 6,16-diacetate (XXXV) yielded a triacetate which consumed 1·3 mole-equivalents of periodate to yield a product with aldehydo-γ-lactone absorption in the infrared. These facts and the aforementioned reactivity of C_{15} toward acylation led to assignment of the protoverine 6,15,16-triacetate structure (XLI). Isobutyrylation of (XLI) afforded a pentaester, assigned the 3,7-diisobutyrate 6,15,16-triacetate structure (XLII). Methanolysis of (XLII) gave protoverine 3,7-diisobutyrate 6,15-diacetate (XLIII).

Controlled acetylation of protoverine (I) gave a diacetate and a monoacetate. The diester was assigned the 6,15-diacetate structure (XLIV) on the basis of its conversion by periodate (consumption 1·0 mole-equivalent) to a product which showed aldehydo-γ-lactone absorption in the infrared, and by analogy to the 6,15-diisobutyrate (II). Acylation of (XLIV) with isobutyryl chloride yielded a monoisobutyrate derivative, assigned the protoverine 3-isobutyrate 6,15-diacetate structure (XLV) on the basis of the preferential acylation at C_3 relative to C_7 or C_{16}. Controlled acetylation of (XLV) gave an isobutyrate triacetate, assigned the protoverine 3-isobutyrate 6,7,15-triacetate structure (XLVI) in agreement with the apparent selectivity of acylation at C_7 over C_{16}.

The monoacetate obtained by partial acetylation of protoverine

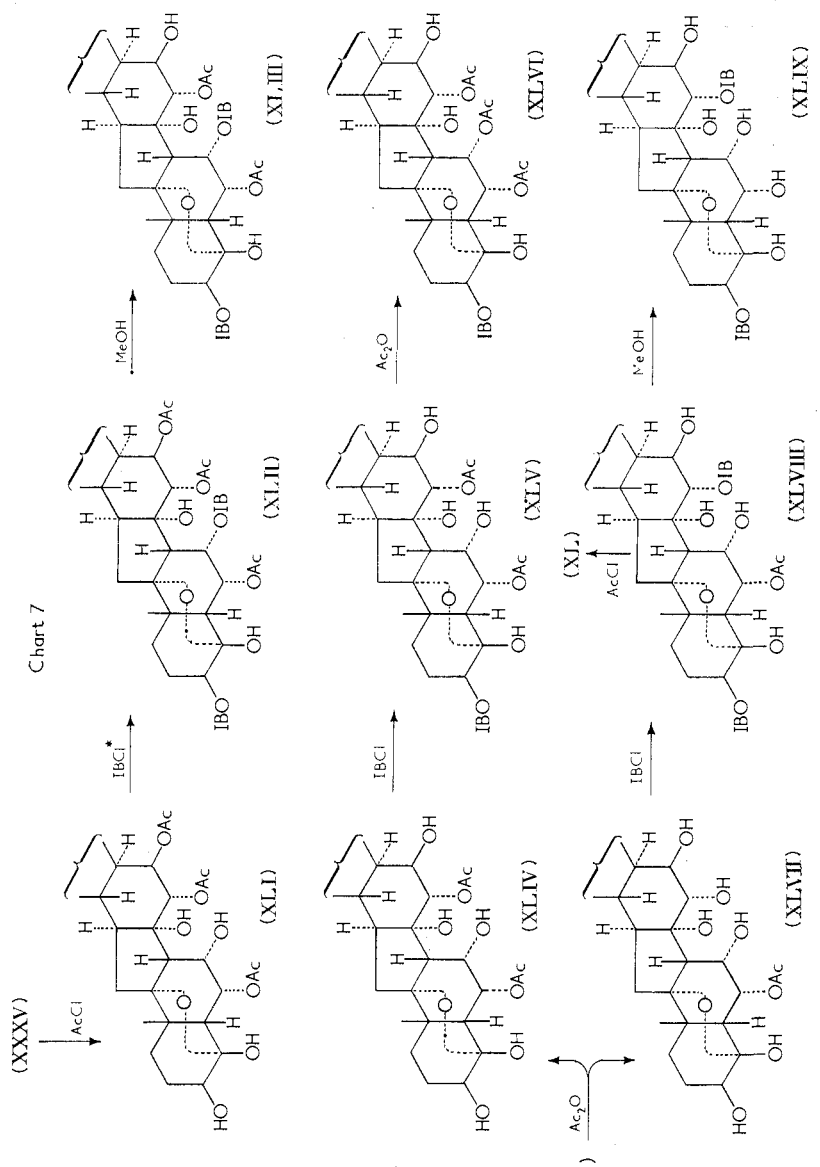
was assigned the 6-acetate structure (XLVII) on the basis of periodate consumption (2·6 mole-equivalents), and by analogy to the previously observed facile acylations at C_6.[20] Isobutyrylation of (XLVII) afforded a triester, assigned the protoverine

Chart 6



3,15-diisobutyrate 6-acetate structure (XLVIII) in accordance with the facility of acylation at C_3 and C_{15}. Support for the assignment of structure (XLVIII) to the triester was obtained by partial acetylation to protoverine 3,15-diisobutyrate 6,7-diacetate (XL). Methanolysis of (XLVIII) gave protoverine 3,15-diisobutyrate (XLIX) (Chart 7).

Chart 7


(XXXV)
AcCl
(XLI)
IBCl
(XLII)
MeOH
(XLIII)
Ac2O
(XLIV)
IBCl
(XLV)
Ac2O
(XLVI)
(I)
AcCl
(XLVII)
IBCl
(XLVIII)
MeOH
(XLIX)
(XL)

Thirty-seven compounds have been investigated for hypotensive activity and the results are presented in Table II.* The methods used have been described.[22, 23] Adult mongrel dogs, unselected as to sex, were employed in all experiments described. Anaesthesia was maintained at upper plane (ii) or lower plane (i) of Stage III by the judicious use of sodium pentobarbital. Test solutions of drugs were prepared by dissolving sufficient amounts of each compound in distilled water made acid by the addition of glacial acetic acid. Drug solutions were freshly prepared and injected intravenously. The abilities of these drugs to lower systemic blood pressure, and, in most cases, to decrease the carotid occlusion response have been investigated; the carotid occlusion response gives further indication of activity.

When considering the results presented, it should be realized that duration of effect is an important property of the drug under investigation. Protoveratrine A [protoverine 3-(+)-2′-methylbutyrate 6,7-diacetate 15-(−)-2′-methylbutyrate][9] at 1 μg/kg in the anaesthetized dog would produce a decrease of 30 per cent in blood pressure with a duration of about 45 min.[22]

Certain structure–activity relationships are apparent in the data recorded in Table II.

(1) *Esterification at position 16 is accompanied by a profound loss in activity.* Compare compound (VI) *vs.* compound (IV), (VII) *vs.* (V), (XII) *vs.* (X), (XIII) *vs.* (XI), (XVIII) *vs.* (XIX), (XXII) *vs.* (XXI), (XXIX) *vs.* (XXVI), (XXXIX) *vs.* (XXXVII), and (XLVIII) *vs.* (XXXVI).

(2) *Esterification at positions 3 and 15 is required for high activity.* Diesters (II) (the 6,15-diisobutyrate) and (XX) (the 3,6-diisobutyrate) are essentially inactive, whereas (XLIX) (the 3,15-diisobutyrate) is one of the most active compounds studied.

(3) *Esterification at position 15 with a branched-chain acid is advantageous.* Protoveratrine A has a 2-(−)-methylbutyrate residue at 15; in the present series an isobutyrate grouping produced greatest activity. Compare (III) *vs.* (XXX), (VI) *vs.* (XVIII), (XII) *vs.* (XXXIV), (XXXIX) *vs.* (XLIII), (XL) *vs.* (XLVI), and, possibly, (VII) *vs.* (XXII).

* We are indebted to Betty Alvey and Luise Schoene for technical assistance in the pharmacological investigations.

Table II. Comparative hypotensive activities of protoverine derivatives in anaesthetized dogs

Compound no.	Substitution at position[a]					No. dogs	Dose, μg/kg	Blood pressure		Carotid occlusion	
	3	6	7	15	16			% change	duration, min	% change	duration, min
II		B		B		8	128	−41	4	—	—
III	B	B		B		7	16	−54	15	−6	38
IV	A	B	A	B	A	11	128	−6	9	−44	15
V	B	B	A	B	A	10	128	−12	3	—	—
VI	A	B	A	B		3	8	−60	>15	−85	>28
VII	B	B	A	B		4	64	−35	33	—	—
IX	A	A		B	A	3	128	0	0	—	—
X	A	A	B	B	A	5	128	−22	7	−40	27
XI	A	A	A	B	A	8	128	−4	4	−14	14
XII	A	A	B	B		2	16	−60	4	−40	20
XIII	A	A	A	B		4	4	−60	>12	−46	>22
XVI		B				3	128	−2	0	—	—
XVIII	A	B	A	A		6	64	−55	25	−64	>55
XIX	A	B	A	A	A	5	128	−9	2	−40	>30
XX	B	B				2	128	0	0	0	0
XXI	B	B	A	A	A	5	128	−2	0	—	—
XXII	B	B	A	A		10	128	−59	19	−80	36
XXIV	A	B		A	A	4	128	−22	12	−76	90

10 XXV	A	B			A	3	128	−21	10	−40	43
XXVI	A	B	B	B	A	2	128	−6	5	−23	15
XXIX	A	B	B	B		2	128	−69	10	−36	55
XXX	B	B		A		2	128	−13	20	−2	>12
XXXI	B	B	B	A		3	128	−14	8	−16	16
XXXIII	A	A	B	A	A	2	128	−5	5	10	15
XXXIV	A	A	B	A		3	128	−12	9	−40	60
XXXV		A			A	3	128	−14	7	−12	15
XXXVI	B	A		B	A	2	128	−6	6	−34	30
XXXVII	B	A	B	B	A	2	128	4	4	34	15
XXXVIII	B	A	A	B	A	2	128	5	3	−8	22
XXXIX	B	A	B	B		3	128	−41	90	−100	>165
XL	B	A	A	B		5	4	−64	>22	−85	>30
XLI		A		A	A	3	128	−8	5	10	>50
XLIII	B	A	B	A		2	128	−25	10	−20	27
XLV	B	A		A		5	128	−35	4	−57	>31
XLVI	B	A	A	A		6	128	−64	>13	−58	>21
XLVIII	B	A		B		6	8	−58	>13	−53	>27
XLIX	B			B		6	4	−64	>23	−63	>36

[a] A = acetate; B = isobutyrate.

(4) *The ester grouping at position 3 need not be branched.* Good activity was realized with compounds (VI), (XII) and (XIII) in which C_3 bears an acetate residue.

(5) *Positions 6 and 7 need not be esterified.* Compound (XLIX) was highly active.

(6) *Esterification at position 7 with a branched-chain acid may be disadvantageous.* Compare (XII) *vs.* (XIII), (XXIX) *vs.* (VI), and (XXXIX) *vs.* (XL). Esterification at position 6 with a branched-chain acid does not appear to be particularly disadvantageous. Compare (XIII) *vs.* (VI), and (XLVIII) *vs.* (III).

Our earlier studies[17,18] of selective stepwise acylation of germine have indicated the following order of reactivity toward acylation: $C_{15} > C_3 > C_7 > C_{16}$. These relationships and analogies to the known structures of the naturally-occurring polyesters of germine[16-19] now make possible the assignment of probable structures to the synthetic germine esters prepared by Weisenborn and coworkers. Thus, the synthetic germine monoisobutyrate is most probably germine 15-isobutyrate; the diisobutyrate is most probably the 3,15-diisobutyrate, and the very active diisobutyrate monoacetate is, in all likelihood, germine 3,15-diisobutyrate 7-acetate. Examination of structure–activity relationships in the germine ester series reveals that most of the generalizations summarized above for the protoverine esters apply as well to the germine series.

(1) The 3,7,15,16-tetraesters derived either from germine or from a naturally-occurring germine polyester[24,16] were essentially inactive.

(2) The 3,15-diesters, whether naturally-occurring or derived from germine, showed relatively high activity.[14]

(3) The 3,15-diesters bearing branched-chain acid residues at position 15 were more active than those with straight chain acid residues.[14]

(4) The ester grouping at position 3 need not be branched. Neogermitrine, one of the most active of the naturally-occurring esters, is germine 3,7-diacetate 15-(−)-2′-methylbutyrate.[16]

(5) Position 7 need not be esterified; the activity of the 3,15-diesters has been mentioned in generalization 2 above.

(6) Esterification at position 7 with a branched-chain acid may

be disadvantageous, as exemplified by the decrease in activity noted in a comparison of the 3,15-diisobutyrate 7-acetate with the 3,7,15-triisobutyrate.[14]

On the basis of the data reported here, it would appear that certain limited alterations can be made in the nature and number of esterified groups in protoverine derivatives without greatly changing the hypotensive potency. A future report will present data obtained in an attempt to determine whether any of the more potent compounds possessed a greater margin between the hypotensive and emetic activities than that observed for protoveratrine A.

Experimental*

Acylations with limited amounts of isobutyryl chloride or acetyl chloride. To the dry starting material in reagent grade pyridine (1 g in *ca.* 10 ml of pyridine) cooled in an ice bath, was added slowly the specified quantity of acyl chloride. The flask was protected from moisture with a calcium chloride tube and placed in an ice-water bath which was allowed to warm gradually to room temperature. After 15–30 h the solution was transferred to a separatory funnel and treated with chloroform, ice-water, and dilute ammonium hydroxide to pH 8–9. The aqueous solution was extracted with chloroform four times; the combined extracts were dried (Na_2SO_4 anhyd.) and evaporated to dryness under reduced pressure. To remove all traces of pyridine, the residue was repeatedly dissolved in benzene and evaporated to dryness.

Methanolyses of 16-acetate esters. The ester (5–10 mg) was dissolved in methanol (0·1–0·3 ml) and left at room temperature in a corked flask. The course of the reaction was followed by

* Melting points are corrected for stem exposure. Values of $[\alpha]_D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined in 95 per cent ethanol on a Cary recording spectrophotometer (model 11 MS). Infrared spectra were determined in chloroform on a Baird double beam recording spectrophotometer. Microanalyses were carried out by Dr. S. M. Nagy and his associates at the Massachusetts Institute of Technology on samples dried under reduced pressure at 110°. The periodate oxidations were run as described in reference 20.

paper chromatography* and the methanolysis was interrupted when the mixture appeared to be most suitable for a column separation. The preparative-scale reaction was then run in a similar manner.

Column separations on alumina. The partial acylations and methanolyses afforded mixtures of products. In all cases, separations were effected by chromatography on Merck acid-washed alumina (20–25 g per gram of alkaloid mixture). The solvent mixtures were selected on the basis of the R_f-values of the alkaloids, and generally ranged from benzene, benzene–chloroform, and chloroform, to mixtures of chloroform–methanol containing gradually increasing proportions of methanol. The acylations were usually accompanied by some discoloration. The coloured material was generally small in quantity and the major proportion was either completely retained by the alumina or eluted with the first few fractions. Initial fractions collected were usually kept very small in order to separate the yellow or brown impurities in the forerun from easily eluted colourless alkaloids.

Recrystallizations. Most of the compounds obtained in crystalline form separated from acetone–petroleum ether or ether–petroleum ether, and the products were recrystallized for analysis at least once from the same solvents. Frequently, the resinous fractions obtained by evaporation of the solutions eluted from the columns contained slight residual amounts of yellow to brown contaminants. The coloured material was separated from each resin by dissolution in a little ether or acetone and dropwise addition of petroleum ether to turbidity; filtration at this point removed the coloured impurity and gave a colourless filtrate from which crystalline product separated on standing.

Protoverine 6,15-diisobutyrate (II) and protoverine 3,6,15-triisobutyrate (III). These esters were obtained as the principal products of acylation of protoverine with 2·3 mole-equivalents of isobutyryl chloride for 17 h. No indication of more highly acylated products was noted.

* The solvent systems used were those of Levine, J. and Fischbach, B. *J. Amer. pharm. Ass., Sci. Ed.*, **44**, 543 (1955); (*a*) *n*-butyl acetate: *n*-butanol: formic acid (25 : 5 : 1 by volume); (*b*) the solution prepared by adding 1 ml of formic acid to the separated organic layer of the system *n*-butyl acetate: *n*-butanol: water (10 : 25 : 10).

Protoverine 3,7,16-triacetate 6,15-diisobutyrate (*IV*). Protoverine 6,15-diisobutyrate (II) (500 mg) was treated with pyridine (3 ml) and acetic anhydride (10 ml) and the mixture was heated for 2½ h in a water bath at 80°. Excess of acetic anhydride was cautiously decomposed by dropwise addition of methanol (7 ml) and the reaction mixture was worked up in the usual manner with ice-water, chloroform and ammonia. The residue obtained by evaporation of the chloroform solution to dryness was crystallized from acetone–petroleum ether in the form of colourless needles; yield of (IV), 90 per cent.

Protoverine 3,7-diacetate 6,15-diisobutyrate (*VI*). Protoverine 3,7,16-triacetate 6,15-diisobutyrate (IV) (1·8 g) in methanol (75 ml) was allowed to stand at room temperature for 60 h. Evaporation of the product to dryness and column separation gave fractions which were crystallized from acetone–petroleum ether and yielded (IV) as large prisms (0·83 g).

Protoverine 3,6,15-triisobutyrate 7,16-diacetate (*V*). Acetylation of (III) by the procedure described for the preparation of (IV) afforded the crystalline diacetate from acetone–petroleum ether; yield 85–90 per cent.

Protoverine 3,6,15-triisobutyrate 7-acetate (*VII*). Methanolysis of (V) (1·8 g) by the procedure described for the preparation of (VI) yielded (VII) (760 mg) in the form of colourless needles.

Protoverine 3,6,16-triacetate 7,15-diisobutyrate (*X*) *and protoverine 3,6,16-triacetate 15-isobutyrate* (*IX*). Protoverine 3,6,16-triacetate (VIII[20]) (3·1 g) in pyridine (100 ml) was acylated with isobutyryl chloride (2·0 ml, *ca.* 4 moles). Work-up and column separation in the usual manner yielded (X) (1·07 g, needles from acetone–petroleum ether) and (IX) (730 mg, prisms from acetone–petroleum ether).

Protoverine 3,6-diacetate 7,15-diisobutyrate (*XII*). Methanolysis of (X) (700 mg) followed by column separation yielded (XII) (170 mg, prisms from acetone–petroleum ether).

Protoverine 3,6,7,16-tetraacetate 15-isobutyrate (*XI*). Acetylation of (IX) (500 mg) by the procedure described for the preparation of (IV) yielded crystalline (XI) (495 mg from chloroform–petroleum ether).

Protoverine 3,6,7-triacetate 15-isobutyrate (*XIII*). Methanolysis

of (XI) (300 mg) followed by column separation yielded (XIII) (120 mg, crystallized from acetone–petroleum ether).

Protoverine 3,7,15,16-tetraacetate 6-isobutyrate (XIX). Acetylation of protoverine 6-isobutyrate (XVI[20]) (1·0 g) by the procedure described for the preparation of (IV) yielded (XIX) (1·2 g, clusters of prisms from acetone–petroleum ether).

Protoverine 3,7,15-triacetate 6-isobutyrate (XVIII). Protoverine 3,7,15,16-tetraacetate 6-isobutyrate (XIX) (1·0 g) in acetone (20 ml) and methanol (40 ml) was allowed to stand at room temperature for 60 h. Column separation yielded (XVIII) (380 mg, prisms from acetone–petroleum ether).

Protoverine 3,6-diisobutyrate (XX). A solution of protoverine 14,15-acetonide 3,6-diisobutyrate (XVII[20]) (1·0 g) in 2 per cent hydrochloric acid (50 ml) was allowed to stand at room temperature for 17 h. The solution was made alkaline with ammonium hydroxide and extracted exhaustively with chloroform. The chloroform extract was dried (Na_2SO_4 anhyd.) and evaporated to dryness under reduced pressure. The residue was crystallized from ether (yield 618 mg). Recrystallization from acetone–petroleum ether gave 475 mg of (XX).

Protoverine 3,6-diisobutyrate 7,15,16-triacetate (XXI). Acetylation of (XX) (1·0 g) by the procedure described for the preparation of (IV) yielded (XXI) (1·12 g, crystallized from ether).

Protoverine 3,6-diisobutyrate 7,15-diacetate (XXII). Methanolysis of (XXI) (1·1 g) by the procedure described for the preparation of (XVIII) yielded (XXII) (460 mg, prisms from acetone–petroleum ether).

Protoverine 14,15-acetonide 3,16-diacetate 6-isobutyrate (XXIII). Acetylation of protoverine 14,15-acetonide 6-isobutyrate (XV) (6 g) in pyridine (40 ml) with acetic anhydride (50 ml) by the procedure described for the preparation of (IV), followed by column purification, yielded (XXIII) (6·1 g, from ether). Recrystallization from acetone–petroleum ether afforded hexagonal prisms.

Protoverine 3,16-diacetate 6-isobutyrate (XXV). Mineral acid hydrolysis of (XXIII) (6 g) by the procedure described for the preparation of (XX), followed by column separation, yielded (XXV) (4·4 g). Recrystallization from acetone–petroleum ether afforded long colourless prisms.

Protoverine 3,15,16-triacetate 6-isobutyrate (*XXIV*). Protoverine 3,16-diacetate 6-isobutyrate (XXV) (1·8 g) in pyridine (40 ml) was acetylated with acetyl chloride (0·3 ml, *ca.* 1·7 moles). Work-up and column separation in the usual manner gave (XXIV) (720 mg crystallized from ether).

Protoverine 3,15,16-triacetate 6,7-diisobutyrate (*XXVII*). Protoverine 3,15,16-triacetate 6-isobutyrate (XXIV) (600 mg) in pyridine (30 ml) was treated with isobutyryl chloride (0·8 ml, *ca.* 9 moles). Work-up and column fractionation in the usual manner gave 400 mg of (XXVII) which resisted all attempts at crystallization.

Protoverine 3,15-diacetate 6,7-diisobutyrate (*XXVIII*). Methanolysis of (XXVII) (210 mg) by the procedure described for the preparation of (XIII) yielded 120 mg of chromatographically pure (XXVIII). Crystallization from ether–petroleum ether gave 40 mg of analytical sample.

Protoverine 3,16-diacetate 6,7,15-triisobutyrate (*XXVI*). Protoverine 3,16-diacetate 6-isobutyrate (XXV) (1·55 g) in pyridine (50 ml) was treated with isobutyryl chloride (1·6 ml, *ca.* 7 moles). Work-up and column separation in the usual manner yielded 900 mg of chromatographically homogeneous (XXVI). Crystallization from ether yielded colourless prisms (220 mg).

Protoverine 3-acetate 6,7,15-triisobutyrate (*XXIX*). Methanolysis of (XXVI) (650 mg) followed by column separation yielded chromatographically homogeneous amorphous (XXIX) (330 mg).

Protoverine 3,6-diisobutyrate 15-acetate (*XXX*). Protoverine 3,6-diisobutyrate (XX) (1·1 g) in pyridine (40 ml) was treated with acetyl chloride (0·3 ml, *ca.* 2·5 moles). Work-up and column separation in the usual manner yielded (XXX) (450 mg, needles from ether).

Protoverine 3,6,7-triisobutyrate 15-acetate (*XXXI*). Protoverine 3,6-diisobutyrate 15-acetate (XXX) (300 mg) in pyridine (10 ml) was treated with isobutyryl chloride (0·3 ml, *ca.* 7 moles). Work-up and column separation in the usual manner yielded (XXXI) (80 mg, prisms from ether).

Protoverine 3,6,15,16-tetraacetate 7-isobutyrate (*XXXIII*). Protoverine 3,6,15,16-tetraacetate (XXXII[11]) (850 mg) in pyridine (10 ml) was treated with isobutyryl chloride (0·9 ml, *ca.* 7·5 moles) and the solution was kept at room temperature for 12 h and then

at 60° for 6 h. Work-up in the usual manner, followed by two column separations, yielded 180 mg of (XXXIII).

Protoverine 3,6,15-triacetate 7-isobutyrate (XXXIV). Methanolysis of (XXXIII) (130 mg) by the procedure used for the preparation of (XIII) yielded (XXXIV) (75 mg, prisms from ether).

Protoverine 3,7,15-triisobutyrate 6,16-diacetate (XXXVII) and protoverine 3,15-diisobutyrate 6,16-diacetate (XXXVI). Protoverine 6,16-diacetate (XXXV[11]) (5·3 g) in pyridine (60 ml) was treated with isobutyryl chloride (2·7 ml, 3 moles). Work-up and column separation in the usual manner yielded (XXXVII) (2·4 g) and (XXXVI) (1·1 g). Crystallization of (XXXVII) from acetone–petroleum ether yielded needles.

Protoverine 3,7,15-triisobutyrate 6-acetate (XXXIX). Methanolysis of (XXXVII) (1·4 g) followed by column separation yielded chromatographically homogeneous amorphous (XXXIX) (250 mg).

Protoverine 3,15-diisobutyrate 6,7,16-triacetate (XXXVIII). Acetylation of (XXXVI) (900 mg) by the procedure used for preparation of (IV) yielded (XXXVIII) (760 mg, prisms from acetone–petroleum ether).

Protoverine 3,15-diisobutyrate 6,7-diacetate (XL). Methanolysis of (XXXVIII) (300 mg) for 40 h, followed by column separation yielded (XL) (140 mg, prisms from ether).

*Protoverine 6,15,16-triacetate (XLI).** Protoverine 6,16-diacetate (XXXV[11]) (5·1 g) in pyridine (60 ml) was treated with acetyl chloride (0·7 ml, *ca.* 1·2 moles). Work-up in the usual manner and crystallization from acetone–petroleum ether afforded *ca.* 2 g of starting material. The latter material was reacylated with 1·2 moles of acetyl chloride, and the products of both acylations were combined. Chromatography on alumina yielded chromatographically homogeneous (XLI) (2·2 g). Crystallization from chloroform–ether yielded small prisms.

Protoverine 3,7-diisobutyrate 6,15,16-triacetate (XLII). Protoverine 6,15,16-triacetate (XLI) (500 mg) in pyridine (10 ml) was treated with isobutyryl chloride (0·4 ml, *ca.* 5 moles). Work-up and column separation in the usual manner yielded 430 mg of (XLII) which resisted all attempts at crystallization.

* Experiment by Dr. C. Ian Ayres.

Protoverine 3,7-diisobutyrate 6,15-diacetate (XLIII). Methanolysis of (XLII) (300 mg) followed by column separation yielded (XLIII) (160 mg prisms from ether).

Protoverine 6,15-diacetate (XLIV) and protoverine 6-acetate (XLVII). Protoverine (I) (6·3 g) in pyridine (75 ml) was treated with acetyl chloride (1·6 ml, *ca.* 1·8 moles). Work-up and column separation in the usual manner gave (XLIV) (800 mg, crystallized from chloroform–petroleum ether) and (XLVII) (790 mg, crystallized from acetone–petroleum ether).

Protoverine 3-isobutyrate 6,15-diacetate (XLV). Protoverine 6,15-diacetate (XLIV) (1·2 g) in pyridine (50 ml) was treated with isobutyryl chloride (1·2 ml, 4 moles). Work-up and column separation in the usual manner yielded chromatographically homogeneous amorphous (XLV) (850 mg).

Protoverine 3-isobutyrate 6,7,15-triacetate (XLVI). Protoverine 3-isobutyrate 6,15-diacetate (XLV) (600 mg) in pyridine (4 ml) was treated with acetic anhydride (0·17 ml, *ca.* 2 moles). After 15 h at room temperature, work-up and column separation in the usual manner gave (XLVI) (260 mg, prisms from acetone–petroleum ether).

Protoverine 3,15-diisobutyrate 6-acetate (XLVIII). Protoverine 6-acetate (XLVII) (1·7 g) in pyridine (30 ml) was treated with isobutyryl chloride (1·6 ml, *ca.* 5 moles). Work-up and column separation in the usual manner yielded chromatographically homogeneous amorphous (XLVIII) (1·25 g).

Acetylation of protoverine 3,15-diisobutyrate 6-acetate (XLVIII) to protoverine 3,15-diisobutyrate 6,7-diacetate (XL). Protoverine 3,15-diisobutyrate 6-acetate (XLVIII) (750 mg) in pyridine (20 ml) was treated with acetyl chloride (0·5 ml, *ca.* 7 moles). After 14 h, work-up and column separation yielded (XL), 240 mg, m.p. 221–222°(d.), $[\alpha]_D^{24}$ −41° (*c*, 1·00 in pyridine); the infrared spectrum in chloroform was identical with that of the sample described above.

Protoverine 3,15-diisobutyrate (XLIX). Methanolysis of (XLVIII) (250 mg) for 40 h yielded (XLIX) (120 mg, crystallized from ether).

Summary. A study aimed at the elucidation of the relationship between hypotensive activity and structure in a series of synthetic hypotensive

esters of protoverine is reported. The syntheses of all the possible protoverine 3,6,7,15-tetraesters which contain one, two or three isobutyrate residues and in which the remaining acyl residues are acetates are described. Preliminary pharmacological evaluation of the compounds is reported, and certain structure–activity relationships evolving from the study are discussed.

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