protoverine 3-isobutyrate 6,7,16-triacetate 15-nicotinate (XVI) in 57 ml. of methanol was allowed to stand at room temperature for 16 hours. Evaporation to dryness left a resin which resisted attempts at purification by column chromatography, but which yielded to preparative paper chromatography. The resin was dissolved in chloroform and applied to Whatman No. 4 paper (19 sheets, 6 × 18 inches) pretreated with phosphate buffer of pH 3.5. After development for 2.5 hours with the isooctane solvent system[4] and drying, a chloroform solution of Bromphenol Blue was sprayed over the papers as usual. The desired band was cut from the sheets and extracted with 2% methanol-chloroform in a Soxhlet extractor for 2 hours. The extract was shaken with dilute ammonium hydroxide to liberate the free base and remove the dye, and the chloroform solution was washed with water. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to yield a colorless resin weighing 205 mg., which was shown to be homogeneous by paper chromatography.[4] Crystallization from acetone-ether gave colorless prisms weighing 139 mg., m.p. 227–229° decompn. Recrystallization gave prisms, m.p. 229–230° decompn., $[\alpha]_D^{27}$ − 1° (c 1.08, pyr.).

Anal.—Calcd. for $C_{41}H_{56}N_2O_{13} \cdot 1/2\ H_2O$: C, 62.04; H, 7.18; N, 3.53. Found: C, 61.89; H, 7.11; N, 3.84.

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Veratrum Alkaloids LI

Hypotensive-Emetic Relationships in the Unanesthetized Dog Among Analogs of the Protoveratrines

By LAWRENCE C. WEAVER†, W. RALPH JONES†, and S. MORRIS KUPCHAN

A study aimed at elucidation of the relationship between hypotensive and emetic activities in the unanesthetized dog among synthetic analogs of the protoveratrines is reported. Preliminary data indicate that a partial dissociation of hypotensive and emetic activities is demonstrable.

VERATRUM and related plants have been used for medicinal purposes for hundreds of years. Galenical preparations were used in the Middle Ages for purposes of sorcery and mystical rites. Subsequently, the crude extracts have been used in the treatment of fevers, as local counterirritants in neuralgia, as cardiac tonics, as emetics, as crow poisons, and as insecticides (1, 2). The use of veratrum in the control of hypertension, at least in the United States, dates from the report of Baker in 1859 (3). Several attempts were made to introduce the use of veratrum extracts into medical practice during the second half of the nineteenth century, but these attempts were unsuccessful. The treatments during this period continued to employ crude extracts containing many alkaloids. The results achieved with these crude extracts were erratic and the treatments fell into disrepute. Some 50 years later, during the late 1930's, purified alkaloidal preparations responsible for the hypotensive activity of veratrum became available for the first time. Poethke in Germany (4) and Jacobs and Craig in the United States (5) improved the extraction and purification procedures and made available the first crystalline powerfully hypotensive alkaloid preparation, protoveratrine. Careful pharmacological investigation of the crystalline preparations, spearheaded by Krayer and his associates, demonstrated that the drugs were suitable for clinical trials (6). These clinical

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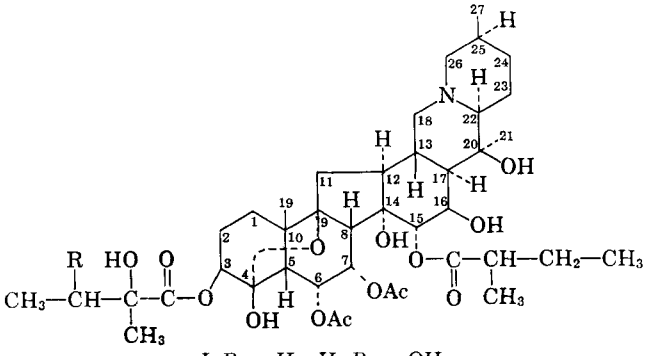


I, R = H II, R = OH

trials were followed by introduction of protoveratrine and related preparations into clinical use in the treatment of certain types of hypertension (7–9). The limiting factor in the clinical use of these drugs has been the narrow dosage range between hypotensive and emetic effects.

In 1952–1953, four groups reported almost simultaneously that protoveratrine was not a homogeneous entity, but a mixture of two closely related ester alkaloids, protoveratrine A and protoveratrine B (10–13). Subsequent clinical studies have established significant differences between protoveratrine A and protoveratrine B when the substances are administered orally (14–16). Protoveratrine A is a potent hypotensive agent with a narrow therapeutic dosage range. Protoveratrine B, on the other hand, is inactive orally in doses several times the hypotensive doses of protoveratrine A. However, studies of divided doses of more than 10 mg. a day have indicated that protoveratrine B has strong hypotensive activity, which may be prolonged and not accompanied by emetic effects (14–16).

Structural studies culminated in 1960 with elucidation of the complete structures and configurations of the alkamine protoverine and of the tetraesters protoveratrine A (I) and protoveratrine B (II) (17, 18). It is evident that the structures of the two protoveratrines are exceedingly similar, and, indeed, differ only in the nature of the acid moiety of the ester at C_3. In view of the aforementioned pronounced difference in activity which accompanies this minor difference in structure, it was deemed desirable that new analogs of the protoveratrines should be prepared and subjected to pharmacological evaluation. It was hoped that such a study might point the way to the preparation of new protoverine derivatives with more favorable hypotensive-emetic ratios.

To study the relationship between hypotensive activity and structural isomerism among polyesters of protoverine, the first phase of the program involved 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 (19). Pharmacological evaluation of the synthetic esters in *anesthetized* dogs led to formulation of the following generalizations: (*a*) esterification at position 16 with acetate or isobutyrate is accompanied by a profound loss in activity, (*b*) esterification at positions 3 and 15 is required for high activity, (*c*) esterification at position 15 with a branched-chain acid is advantageous, (*d*) the ester grouping at position 3 need not be branched, (*e*) positions 6 and 7 need not be esterified, and (*f*) esterification at position 7 with a branched-chain acid may be disadvantageous.

Our second study involved a broader group of structural variants, and the results supplemented and extended earlier generalizations (20). New relationships which evolved from the second study were: (*g*) oxidation of the alcohol group at position 16 to a ketone group is accompanied by a loss in activity, (*h*) acetonide formation at positions 14 and 15 is accompanied by a profound loss in activity, and (*i*) esterification at position 4 may be disadvantageous.

In our third study, 25 new protoverine tetraesters which differ from the protoveratrines solely in the nature of the acid residue affixed at position 3 were described (21). Pharmacological evaluation in anesthetized dogs indicated that considerable alterations can be made in the structure of the ester affixed at position 3 without greatly altering hypotensive potency.

The most recent study involved the synthesis and evaluation of a series of protoverine tetraesters which differ from each other only in the nature of the acid residue at position 15. Pharmacological evaluation of the new protoverine tetraesters showed substantial diminution in hypotensive activity when compared to the protoveratrines (22).

METHODS

The present report is concerned with an evaluation of selected active compounds in the *unanesthetized* dog. Adult mongrel dogs, unselected as to sex, were used for these studies. Mean blood pressure was recorded on a smoked kymogram from the catheterized femoral artery of the dog maintained in a standing position. All drugs were administered rapidly as aqueous solutions, usually acidic, by the intravenous route. The dogs were observed for approximately 2 hours to determine duration of changes in systemic blood pressure and signs of emesis. It should be emphasized that only one dose of one drug was given to any dog. Thus, tolerance or cumulation were not variables. When evaluating compound effects, one should consider hypotensive degree and duration, as well as the presence or absence of emesis.

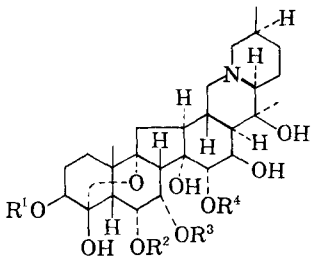
RESULTS AND DISCUSSION

Ten of the synthetic protoverine tetraesters of the "first study" showed activity in anesthetized dogs in dosages less than 128 mcg./Kg. i.v. (19). These compounds have been studied in the unanesthetized dog and the results are presented in Table I. The compounds are numbered as in ref. 19 in order to make comparisons with data in anesthetized dogs convenient. Generally, the compounds at equivalent dosages had less hypotensive effect than was observed in the anesthetized dog. This is not unexpected, since a barbiturate was the anesthetic agent used in the early studies. Only compound VI had a margin between hypotensive and emetic effects greater than protoveratrine A or B; it is questionable whether this margin is significant. It should be pointed out that when emesis occurred, the duration of the hypotensive response as recorded was frequently decreased because of the changes in blood pressure secondary to this emesis. The hypotensive-emetic ratio of the other compounds appeared similar to those of the protoveratrines.

None of the compounds from the "second study" exhibited sufficient activity to qualify them for investigation in unanesthetized dogs.

All of the 26 new protoveratrine analogs of the

TABLE I.—HYPOTENSIVE-EMETIC RELATIONSHIPS OF PROTOVERINE DERIVATIVES IN UNANESTHETIZED DOGS

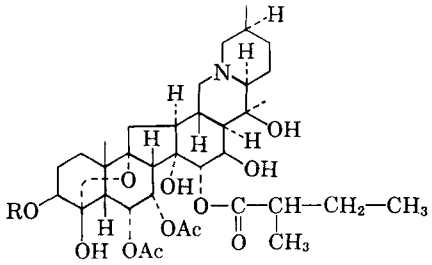


Compound No.	R^{1a}	R^2	R^3	R^4	Dose, mcg./Kg. i.v.	No. Dogs	Blood Pressure—Change, %	Blood Pressure—Duration, min.	Emesis—No. Vomiting/No. Tested	Emesis—Onset, min.
III	B	B	H	B	32	3	−53	8	0/4	
					64	3	−62	28	1/5	11
					128	2	−59	1	1/2	30
VI	A	B	A	B	16	2	−41	75	0/6	
					32	2	−60	225	3/6	5, 5, 10
VII	B	B	A	B	32	3	−41	19	3/5	1, 5, 7
					64	2	...	...	2/2	6, 11
XII	A	A	B	B	32	3	−46	3	1/3	97
XIII	A	A	A	B	4	3	−85	2	1/3	28
					8	1	−58	2	1/1	98
XVIII	A	B	A	A	128	3	−15	2	1/3	16
XXXIX	B	A	B	B	64	3	−44	22	0/3	
					128	4	−77	32	1/3	94
					256	1	−59	26	1/1	1
XL	B	A	A	B	4	3	−67	13	0/3	
					8	4	−62	8	0/4	
					16	2	−77	>30	2/2	17, 112
XLVIII	B	A	H	B	2	3	−50	8	0/3	
					4	4	−75	75	3/5	7, 55, 110
XLIX	B	H	H	B	4	3	−47	27	0/3	
					8	3	−71	9	2/3	3, 114
Protoveratrine	A				1	5	−22	39	1/5	87
					2	3	−37	75	2/3	24, 61
Protoveratrine	B				1	6	−33	25	4/6	2, 21, 95, 95

[a] A, acetate; B, isobutyrate; H, hydrogen.

"third study," *i.e.*, the compounds differing from the protoveratrines solely in the nature of the ester at position 3, showed significant hypotensive activity in anesthetized dogs at dosages of 128 mcg./Kg. i.v. or less (21). The results obtained with the latter compounds when administered to unanesthetized dogs is presented in Table II. The compounds are numbered as in ref. 21 to make comparisons with data in anesthetized dogs convenient. Further, an "a" is added (*e.g.*, Ia) to prevent confusion with compounds mentioned earlier in this report. The data for escholerine (IIIa) are included, as well. As was noted above in comparing data obtained from anesthetized and unanesthetized dogs, differences were usually in the direction of lesser activity in the unanesthetized dog. Test compounds were less active than protoveratrine A and B in most cases. Exceptions were compounds IVa, VIa, XIa, XVIIIa, XXIIa, and XXVIa. Increased hypotensive-emetic ratios or margins of safety appeared to be present with compounds IXa and XIVa. Thus, preliminary data indicate that a partial dissociation of hypotensive and emetic activities among synthetic analogs of the protoveratrines is demonstrable in the unanesthetized dog. Compounds XXa, XXIVa, and XXIXa failed to show hypotensive activity at the doses tested, although emesis was evoked. The latter results may be considered to constitute support for the thesis that hypotensive-emetic ratios can be altered. Several of the new analogs will be investigated in human hypertensives to determine whether the improved margin is demonstrable in man as well.

TABLE II.—HYPOTENSIVE-EMETIC RELATIONSHIPS OF DESATRINE DERIVATIVES IN UNANESTHETIZED DOGS



Compound No.	R	Dose, mcg./Kg. i.v.	No. Dose	Blood Pressure: Change, %	Blood Pressure: Duration, min.	Emesis: No. Vomiting/ No. Tested	Emesis: Onset, min.
IIIa	Angelate	4	7	−49	>61	4/7	2, 3, 7, 15
		16	2	...	...	2/2	...
IVa	Tiglate	2	2	−33	31	0/2	
		8	4	−49	>64	1/4	4
Va	Acetate	16	2	−62	16	1/2	16
		32	2	...	...	2/2	...
VIa	Isobutyrate	1	2	−65	6	1/2	50
		2	2	−68	68	1/2	15
		4	2	−51	171	2/2	2, 4
		8	2	...	...	1/2	...
VIIa	Benzoate	8	2	−57	4	2/2	2, 16
VIIIa	3′,4′-Methylene dioxybenzoate	8	2	−57	25	1/2	16
IXa	Trichloroacetate	4	1	14	30	0/1	
		8	2	−12	60	0/2	
		16	4	−45	12	0/4	
		32	3	−68	12	2/3	5, 11
Xa	Chloroacetate	2	1	−39	5	1/1	105
		4	2	−74	5	0/2	
		8	2	−52	21	1/2	2
XIa	Nicotinate	1	3	−19	7	1/3	19
		2	4	−57	37	1/4	11
		4	4	−59	47	4/4	4, 9, 13, 24
		8	2	−75	>60	2/2	3, 5
XIIa	4′-Nitrobenzoate	4	2	−39	56	0/2	
		8	3	−39	6	0/3	
		16	6	−64	>53	3/6	28, 48, 70
XIIIa	4′-Nitrohexanoate	4	3	−63	11	0/3	
		8	2	−66	23	1/2	8
XIVa	Tosylate	8	2	−21	34	0/2	
		16	2	−20	88	0/2	
		32	1	−45	120	0/1	
XVa	Diethylphosphate	32	3	−41	3	0/3	
		64	2	−73	17	0/2	
		128	2	−45	40	2/2	1, 1

TABLE II (*continued*)

Compound No.	R	Dose, mcg./Kg. i.v.	No. Dogs	Blood Pressure		Emesis	
				Change, %	Duration, min.	No. Vomiting/ No. Tested	Onset, min.
XVIa	N,N-Diethylamino-acetate	4	3	0	0	0/2	
		8	2	−61	19	1/3	44
		16	1	−34	4	0/1	
		32	3	−62	38	2/3	1, 3
XVIIa	3′-Acetoxy-2′-hydroxy-2′-methylbutyrate	4	3	−34	95	2/3	60, 100
		16	2	...	...	2/2	...
XVIIIa	3′-Isobutyroxy-2′-hydroxy-2′-methylbutyrate	4	4	−26	3	2/4	4, 24
		16	1	−72	2	lethal	
XIXa	3′-Tigloxy-2′-hydroxy-2′-methylbutyrate	8	3	−22/22	29	1/3	11
		32	1	−54	31	1/1	10
XXa	3′-Benzoxy-2′-hydroxy-2′-methylbutyrate	4	2	0	0	0/2	
		8	1	0	0	0/1	
		16	1	0	0	1/1	26
XXIa	3′-(4″-Nitrobenzoxy)-2′-hydroxy-2′-methylbutyrate	16	2	−19	40	1/2	7
XXIIa	3′-Nicotinoxy-2′-hydroxy-2′-methylbutyrate	4	7	−12	30	3/7	18, 19, 30
		8	8	−31	30	6/8	10, 14, 17 20, 20, 27
		16	2	−62	>45	1/1	4 (1 died)
XXIIIa	3′-Chloroacetoxy-2′-hydroxy-2′-methylbutyrate	8	3	−50	48	1/3	20
		16	4 2	−50	20	1/4	30
XXIVa	3′-Tosyloxy-2′-hydroxy-2′-methylbutyrate	4	2	10	9	1/2	18
		8	2	...	...	2/2	...
		16	2	...	...	2/2	...
XXVa	2′,3′-Epoxy-2′-methylbutyrate	8	2	−34	22	0/2	
		16	2	...	...	2/2	...
XXVIa	3′-Chloro-2′-hydroxy-2′-methylbutyrate	2	3	−25	30	1/3	120
		4	4	−55	50	2/4	3, 11
XXVIIa	3′-Hydroxy-2′-fluoro-2′-methylbutyrate	4	3	−34	>16	3/3	6, 10, 31
XXVIIIa	3′-N,N-Diethylamino-acetoxy-2′-hydroxy-2′-methylbutyrate	4	1	−21	12	0/1	
		8	4	−27	32	2/4	7, 67
		16	1	−100	...	lethal	
XXIXa	3′-(4″-Aminobenzoxy)-2′-hydroxy-2′-methylbutyrate	4	1	0	0	0/1	
		8	2	8	18	0/2	
		16	4	21	5	2/4	8, 15

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