Veratrum Alkaloids XLVI

Structure-Activity Relationships in a Series of Analogs of the Protoveratrines

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In view of the significant pharmacological differences between protoveratrine A and protoveratrine B when the substances are administered orally, and of our recent revelation that the structures of the two compounds differ only in the nature of the acid moiety of the ester at C_3, a series of analogs of the protoveratrines have been prepared and subjected to preliminary pharmacological evaluation. The results indicate that considerable alterations can be made in the structure of the ester affixed at C_3 without greatly altering hypotensive potency.

PROTOVERATRINE A and protoveratrine B, two ester alkaloids isolated from a number of veratrum species, have been shown to be useful agents in the treatment of hypertension (1, 2). The limiting factor in the clinical use of these drugs has been the narrow dosage range between hypotensive and emetic effects.

Recent clinical studies have established significant differences between protoveratrine A and protoveratrine B when the substances are administered orally (3–5). 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.

Structural studies in our laboratory recently culminated with the elucidation of the complete structures and configurations of protoveratrine A (I) and protoveratrine B (II) (6). It is evident that the structures of the two compounds 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 between protoveratrine A and protoveratrine B, it was deemed desirable that additional compounds which differ from the protoveratrines solely in the nature of the acid residue affixed at C_3 should be prepared and subjected to pharmacological evaluation. The present report details the synthesis and preliminary pharmacological evaluation of twenty-five analogs of the protoveratrines.

Received July 18, 1960, from the Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, and the Pharmacology Laboratories, Pitman-Moore Co., Indianapolis, Ind.

Accepted for publication August 4, 1960.

Part XLV in the series: Kupchan, S. M., Weaver, L. C., Ayres, C. I., and Hensler, R. H., THIS JOURNAL, 50, 52 (1961).

The investigation at the University of Wisconsin was supported in part by research grants from the Wisconsin Alumni Research Foundation and Pitman-Moore Co.

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Presented to the Scientific Section, A. PH. A., Chicago meeting, April 1961.

DISCUSSION

Protoveratrine B was the starting material for the preparation of all the new compounds reported herein. In one approach, the first step consisted of selective cleavage of the 2′,3′-dihydroxy-2′-methylbutyrate residue from C_3 by sodium periodate oxidation (6). The triester derivative which resulted, protoverine 6,7-diacetate 15-(*l*)-2′-methylbutyrate, proved to be an invaluable intermediate for elaboration of interesting compounds. For convenience, the latter compound has been assigned the name "desatrine," derived from *des*acylprotover*atrine*. Our earlier studies of selective acylation of related compounds had shown that the hydroxyl group at C_3 is more reactive than the C_{16}-hydroxyl (7). Acylation of desatrine under controlled conditions afforded a series of 3,6,7,15-tetraester analogs of the protoveratrines, Compounds IV–XV. Amination of desatrine 3-chloro-acetate (X) with diethylamine yielded desatrine N,N-diethylaminoacetate (XVI). Direct partial acylation of protoveratrine B yielded another series of protoveratrine analogs (XVII–XXIV). Our earlier work had revealed that controlled acylation of protoveratrine B led to selective reaction at the secondary hydroxyl group of the 2′,3′-dihydroxy-2′-methylbutyrate residue affixed at C_3 (6).

As indicated in Fig. 1, treatment of desatrine 3-(3′-tosyloxy-2′-hydroxy-2′-methylbutyrate (XXIV) (6) with methanol at reflux temperature or with potassium iodide in acetonitrile for five hours afforded a sulfur-free product. The latter product was assigned the desatrine 3-(2′,3′-epoxy-2′-methylbutyrate) structure (XXV) on the basis of its empirical formula and reactivity. Upon treatment with *p*-toluenesulfonic acid in acetonitrile, XXV reverted to XXIV. Reaction of XXV with anhydrous hydrogen chloride yielded a hydrogen chloride adduct. The latter compound consumed 1 oxygen equivalent of chromic acid, in accord with the desatrine 3-(3′-chloro-2′-hydroxy-2′-methyl-

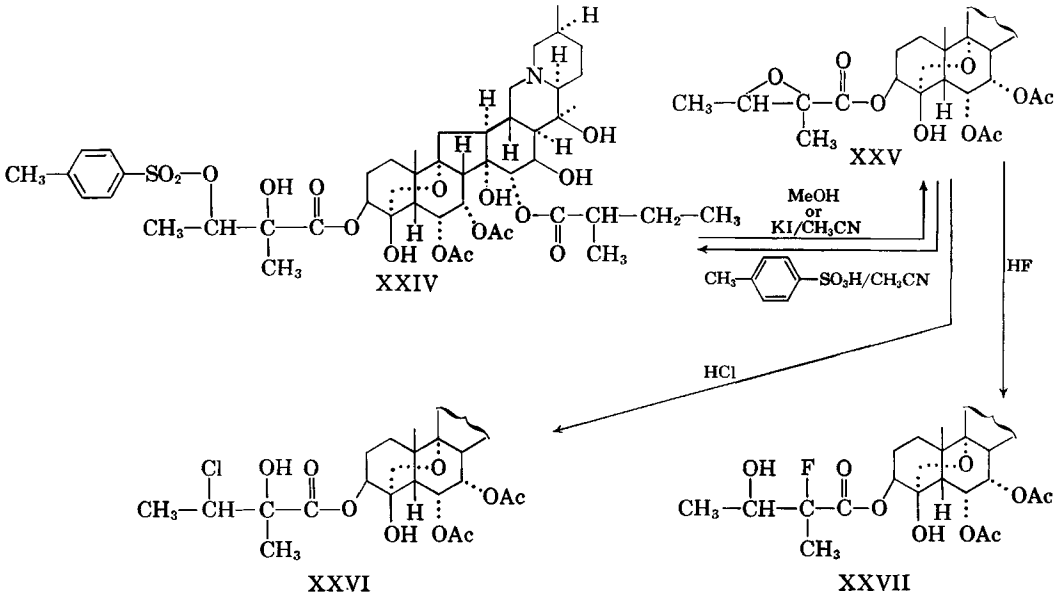


Figure 1

butyrate) structure (XXVI). Reaction of XXV with anhydrous hydrogen fluoride yielded a product assigned the desatrine 3-(3′-hydroxy-2′-fluoro-2′-methylbutyrate) structure (XXVII), in agreement with the observed chromic acid consumption of 2 oxygen equivalents. Amination of XXIII with diethylamine yielded desatrine 3-(3′-N,N-diethylaminoacetoxy-2′-hydroxy-2′-methylbutyrate) (XXVIII) and catalytic reduction of XXI gave desatrine 3 - (3′ - (4″ - aminobenzoxy) - 2′ - hydroxy - 2′ - methylbutyrate) (XXIX).

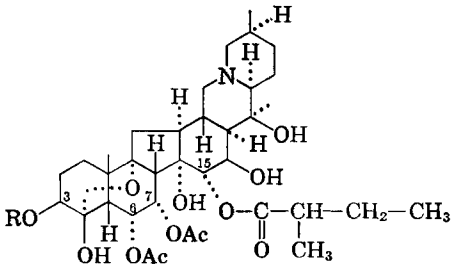
Twenty-six new compounds (see Table I and Fig. 2) have been investigated for hypotensive activity and the results are listed in Table II along with comparable earlier results for protoveratrine A (I), protoveratrine B (II), and escholerine (III) (8, 9).[1] The methods used have been described (10, 11). Adult mongrel dogs, unselected as to sex, were employed in all experiments described. Anesthesia was maintained at upper plane ii or lower plane i of Stage III by the judicious use of sodium pentobarbital. Test drug solutions were freshly prepared and injected intravenously. The ability 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 is an added indication of activity for this type of compound.

In our previous studies the compounds examined were protoverine derivatives with varying substitution at positions 3, 4, 6, 7, 14, 15, and 16 (7, 12). The results supported 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 for good activity, (*f*) esterification at position 7 with a branched-chain acid may be disadvantageous, (*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, (*i*) esterification at position 4 may be disadvantageous.

The protoveratrine analogs listed in Table II all have structures which generally fit the aforementioned "requirements" for high hypotensive activity, and, in accord with expectations, the compounds showed a high average order of activity. Among the series prepared by acylation of desatrine (i.e., compounds IV–XV), only the 3-diethylphosphate (XV) had suffered a profound loss of activity. The 3-acetate (V), 3-angelate (III), 3-trichloroacetate (IX), 3-tosylate (XIV), and 3,4′-nitrobenzoate (XII) show slightly diminished activity relative to the protoveratrines. There is not sufficient data to differentiate quantitatively among the remaining compounds produced by acylation of desatrine. Among the compounds derived directly from protoveratrine B (i. e., compounds XVII–XXIV), some decrease in activity was observed in the 3′-acetoxy-2′-hydroxy-2′-methylbutyrate (XVII) and the 3′-(4″-nitrobenzoxy) - 2′ - hydroxy - 2′ - methylbutyrate (XXI). The remaining compounds were not sufficiently different from protoveratrine B to indicate significant alterations in activity.

It is apparent from the data reported herein that considerable alterations can be made in the structure of the ester moiety affixed at position 3 in analogs of protoveratrine without greatly altering hypotensive potency. A future report will present data on the emetic properties of these compounds as they relate to the hypotensive activity in unanesthetized dogs.

[1] We are indebted to Shirley Study and Olive Johnston for technical assistance in pharmacology.



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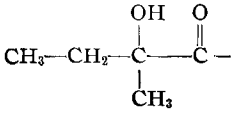
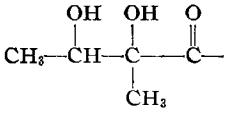
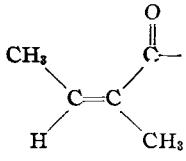
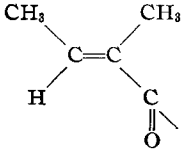
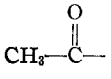
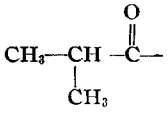
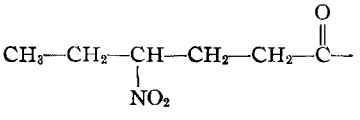
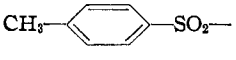
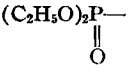
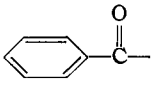
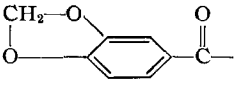
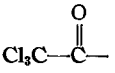
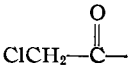
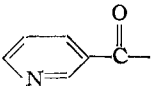
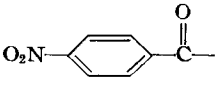
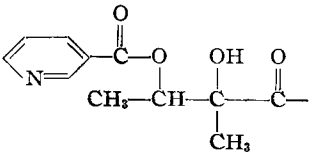
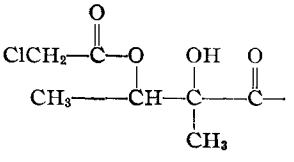
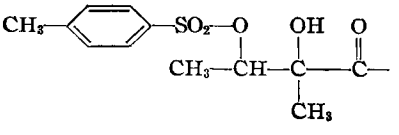
I	
II	
III	
IV	
V	
VI	
XIII	
XIV	
XV	

Compound	R
VII	
VIII	
IX	
X	
XI	
XII	
XXII	
XXIII	
XXIV	

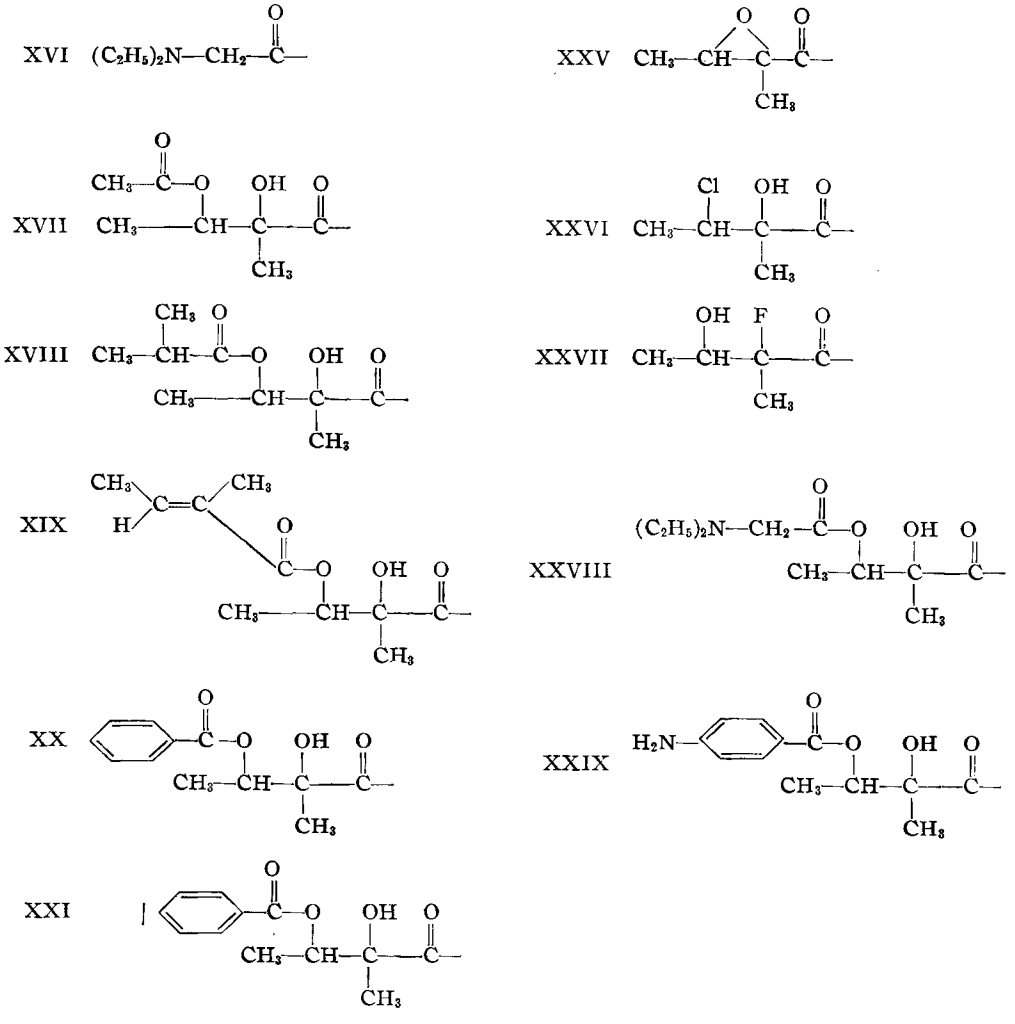


Figure 2.

EXPERIMENTAL

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% ethanol on a Cary recording spectrophotometer (model 11 MS). Infrared spectra were determined in chloroform on a Baird double beam recording spectrophotometer. Microanalyses[2] were carried out on samples dried under reduced pressure at 110°. "Petroleum ether" refers to the fraction of boiling point 60–80°.

Acylations.—The general procedure for the acylation reactions involved portionwise addition of acyl halide (2–3 equivalents) to a stirred solution of the dry alkaloid alcohol in 10 parts of dry reagent grade pyridine at ice bath temperature. The flask was protected from moisture with a calcium chloride tube and was allowed to warm gradually to room temperature. The course of the reaction was followed by paper chromatography[3] and interrupted when suitable, usually after twenty to forty-eight hours. The solution was treated with chloroform, ice water, and dilute ammonium hydroxide to pH 8–9. The solution was extracted with chloroform four times; the combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. To remove all traces of pyridine, the residue was repeatedly dissolved in benzene and evaporated to dryness. Occasionally, the paper chromatographic behavior of the crude residue was sufficiently promising so that direct crystallization at this point was attempted. More often, the mixtures obtained were separated by chromatography on Merck acid-washed alumina (20–25 Gm. per Gm. of alkaloid mixture). The solvent mixtures were selected on

[2] Microanalyses were made by Dr. S. M. Nagy and his associates at Massachusetts Institute of Technology.

[3] The solvent systems used were those of Levine, J., and Fischbach, H., This Journal, 44, 543(1955): (*a*) *n*-butyl acetate:*n*-butanol:formic acid (25:5:1 by volume); (*b*) the solution prepared by adding 1 cc. of formic acid to the separated solvent layer of the system *n*-butyl acetate:*n*-butanol:water (10:25:10 cc.).

TABLE I.—CHARACTERISTICS OF NEW COMPOUNDS

No.	Desatrine Derivative (3-Substituent)	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	M. p., ° C. (decompn.)	$[\alpha]_D^{25}$ pyr.	Yield, %	Crystal Form
IV	Tiglate[c]	$C_{41}H_{61}NO_{13}$	62.76[a]	62.87	7.96[a]	7.75	219–220	−23	24	ellipsoids
V	Acetate[c]	$C_{38}H_{57}NO_{13}$	60.53[b]	60.67	7.88[b]	7.71	253–254	−35	15	needles
VI	Isobutyrate[c]	$C_{40}H_{61}NO_{13}$	62.88	62.81	8.04	8.26	188–190	−42	16	prisms
VII	Benzoate	$C_{43}H_{59}NO_{13}$	64.00[a]	64.21	7.49[a]	7.14	240–241	−4	42	prisms
VIII	3′,4′-Methylene dioxybenzoate	$C_{44}H_{59}NO_{15}$	62.76	62.91	7.06	7.13	221–222	+23	50	micro needles
IX	Trichloroacetate[d]	$C_{38}H_{54}Cl_3NO_{13}$	54.57	54.67	6.50	6.31	185–190	−40	20	prisms
X	Chloroacetate[e]	$C_{38}H_{56}ClNO_{13}$	59.25	59.77	7.33	7.45	190–205	−39	22	prisms
XI	Nicotinate[f]	$C_{42}H_{58}N_2O_{13}$	62.42[a]	62.66	7.36[a]	7.26	237–239	−5	51	prisms
XII	4′-Nitrobenzoate[g]	$C_{43}H_{58}N_2O_{15}$	61.27	61.11	6.94	7.06	175–177	+11	45	needles
XIII	4′-Nitrohexanoate[h]	$C_{42}H_{64}N_2O_{15}$	60.27	60.11	7.71	7.58	187–188	−34	38	prisms
XIV	Tosylate[i]	$C_{43}H_{61}NO_{14}S$	60.90	60.86	7.25	7.36	213–215	−15	18	prisms
XV	Diethylphosphate[j]	$C_{40}H_{64}NO_{15}P$	57.88	58.23	7.77	7.77	203–204	−31	23	needles
XVI	N,N-Diethylaminoacetate[k]	$C_{42}H_{66}N_2O_{13}$	62.51	63.22	8.24	8.12	189–190	−40	43	rosettes
XVII	3′-Acetoxy-2′-hydroxy-2′-methylbutyrate[c]	$C_{43}H_{63}NO_{16}$	60.61	60.27	7.70	7.99	230–231	−18	29	needles
XVIII	3′-Isobutyroxy-2′-hydroxy-2′-methylbutyrate	$C_{45}H_{69}NO_{16}$	61.42	61.76	7.84	7.95	217–218	−21	61	needles
XIX	3′-Tigloxy-2′-hydroxy-2′-methylbutyrate[l]	$C_{47}H_{71}NO_{16}$	62.30	62.02	7.90	7.76	176–178	−19	32	rosettes
XX	3′-Benzoxy-2′-hydroxy-2′-methylbutyrate	$C_{48}H_{67}NO_{16}$	63.02	63.16	7.32	7.30	177–179	−22	71	plates
XXI	3′-(4″-Nitrobenzoxy)-2′-hydroxy-2′-methylbutyrate[m]	$C_{48}H_{66}N_2O_{18}$	59.02[b]	59.10	6.97[b]	6.65	169–171	−14	56	plates
XXII	3′-Nicotinoxy-2′-hydroxy-2′-methylbutyrate[n]	$C_{47}H_{66}N_2O_{16}$	60.52[b]	60.20	7.30[b]	7.01	162–163	−18	32	plates
XXIII	3′-Chloroacetoxy-2′-hydroxy-2′-methylbutyrate[o]	$C_{43}H_{64}ClNO_{16}$	58.56	59.43	7.42	7.21	201–203	−18	55	needles
XXV	2′,3′-Epoxy-2′-methylbutyrate[p]	$C_{41}H_{61}NO_{14}$	62.18	62.47	7.77	7.78	227–228	−26	40	prisms
XXVI	3′-Chloro-2′-hydroxy-2′-methylbutyrate[q]	$C_{41}H_{62}ClNO_{14}$	59.44	59.21	7.54	7.37	262–263	−37	53	prisms
XXVII	3′-Hydroxy-2′-fluoro-2′-methylbutyrate	$C_{41}H_{62}FNO_{14}$	60.65	60.87	7.70	7.78	257–259	−41	34	prisms
XXVIII	3′-N,N-Diethylaminoacetoxy-2′-hydroxy-2′-methylbutyrate[r]	$C_{47}H_{74}N_2O_{16}$	61.17	61.24	8.03	7.98	203–205	−19	60	plates
XXIX	3′-(4″-Aminobenzoxy)-2′-hydroxy-2′-methylbutyrate[s]	$C_{48}H_{68}N_2O_{16}$	61.48[a]	61.29	7.36[a]	7.43	193–195	−28	73	plates

[a] Calcd. with ½ H_2O. [b] Calcd. with 1 H_2O. [c] Equivalents of volatile acid found: 3.7. [d] Chlorine: calcd. 12.72; found, 12.79. [e] Chlorine: calcd., 4.60; found, 4.67. [f] Nitrogen: Calcd., 3.46; found, 3.60. [g] Nitrogen: calcd., 3.33; found, 3.08. [h] Nitrogen: calcd., 3.35; found, 3.08. [i] Sulfur: calcd., 3.78; found, 3.74. [j] Phosphorus: calcd., 3.73; found, 3.42. [k] Equivalents of volatile acid found: 2.9; nitrogen: calcd., 3.47; found, 3.60. [l] Prepared by Dr. A. D. J. Balon. [m] Nitrogen: calcd., 2.92; found, 2.98. [n] Nitrogen: calcd., 3.00; found, 3.10. [o] Calcd. with 1 CH_3COCH_3; chlorine: calcd., 3.76; found, 4.28. [p] Equivalents of volatile acid found: 2.7. [q] Chlorine: calcd., 4.28; found, 4.74. [r] Nitrogen: calcd., 3.04; found, 2.81. [s] Nitrogen: calcd., 3.02; found, 3.53.

TABLE II —COMPARATIVE HYPOTENSIVE ACTIVITY OF ANALOGS OF THE PROTOVERATRINES IN ANESTHETIZED DOGS

Compound No.	Desatrine Deriv. (3-Substituent)	No. Dogs	Dose γ/Kg.	Blood Pressure Chg., %	Blood Pressure Dur., min.	Carotid Occlusion Chg., %	Carotid Occlusion Dur., min.
I	2′-Hydroxy-2′-methylbutyrate	2	2	−49	>83	−68	>90
II	2′,3′-Dihydroxy-2′-methylbutyrate	2	2	−46	26	−55	38
III	Angelate	2	32	−47	>150	−90	>158
IV	Tiglate	2	2	−37	23	−50	30
V	Acetate	3	32	−44	>103	−80	>104
VI	Isobutyrate	3	8	−48	>130	−93	>94
VII	Benzoate	3	8	−40	>60	−100	>120
VIII	3′,4′-Methylenedioxybenzoate	2	8	−51	167	−100	230
IX	Trichloroacetate	4	32	−64	33	−38	80
X	Chloroacetate	1	2	−67	>120	−50	30
		2	4	−60	>76	0	...
XI	Nicotinate	2	2	−29	25	−36	38
XII	4′-Nitrobenzoate	4	8	−22	10	−15	60
XIII	4′-Nitrohexanoate	2	4	−64	>218	−82	142
XIV	Tosylate	1	8	−40	28	−6	32
		1	16	−55	210	−14	37
XV	Diethylphosphate	1	32	−7	4	0	...
		1	128	−69	16	0	...
XVI	N,N-Diethylaminoacetate	1	4	−19	33	−25	16
		1	8	−58	18	−100	21
XVII	3′-Acetoxy-2′-hydroxy-2′-methylbutyrate	2	16	−28	>66	−4	63
XVIII	3′-Isobutyroxy-2′-hydroxy-2′-methylbutyrate	3	4	−40	>70	−85	>105
XIX	3′-Tigloxy-2′-hydroxy-2′-methylbutyrate	2	8	−20	255	−57	138
XX	3′-Benzoxy-2′-hydroxy-2′-methylbutyrate	3	4	−25	>120	−90	187
XXI	3′-(4″-Nitrobenzoxy)-2′-hydroxy-2′-methylbutyrate	1	8	−20	84	−58	54
		2	16	−35	>108	−50	40
XXII	3′-Nicotinoxy-2′-hydroxy-2′-methylbutyrate	2	4	−20	130	−100	25
XXIII	3′-Chloroacetoxy-2′-hydroxy-2′-methylbutyrate	2	8	−45	53	−96	41
XXIV	3′-Tosyloxy-2′-hydroxy-2′-methylbutyrate	1	8	−23	>160	−57	>180
XXV	2′,3′-Epoxy-2′-methylbutyrate	1	16	−37	>120	−92	>96
		1	32	−79	>166	−100	>166
XXVI	3′-Chloro-2′-hydroxy-2′-methylbutyrate	2	2	−48	35	−69	65
XXVII	3′-Hydroxy-2′-fluoro-2′-methylbutyrate	2	4	−66	>134	−67	55
XXVIII	3′-N,N-Diethylaminoacetoxy-2′-hydroxy-2′-methylbutyrate	2	4	−24	80	−90	60
XXIX	3′-(4″-Aminobenzoxy)-2′-hydroxy-2′-methylbutyrate	2	8	−28	>113	−100	60

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 colored material was generally small in quantity and the major proportion was either retained by the alumina or eluted with the first few fractions. Initial fractions were usually kept very small in order to separate the yellow or brown impurities in the forerun from easily eluted colorless alkaloids. Most of the compounds were crystallized from acetone-petroleum ether. The only exceptions were VI (petroleum ether), VII (ether-petroleum ether), and XXIX (benzene-petroleum ether). Products were recrystallized for analysis at least once from the same solvents.

For the acylations involving chloroacetyl chloride (i. e., for the preparation of compounds X and XXIII), benzene was used as solvent. Extensive decomposition and color formation was observed in trial runs in pyridine.

Desatrine 3-(N,N-Diethylaminoacetate) (XVI).—A solution of desatrine 3-chloroacetate (260 mg.) (X) in dry benzene (10 cc.) was treated with diethylamine (1 cc.) and the mixture was allowed to stand in a stoppered flask at room temperature for forty-eight hours. Evaporation to dryness under reduced pressure yielded a residue which was treated with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The amorphous residue was crystallized from acetone-petroleum ether to yield colorless rosettes. The crystalline product was shown by paper chromatography to be homogeneous.

Desatrine 3-(2′,3′-Epoxy-2′-methylbutyrate) (XXV).—*By Methanolysis of Desatrine 3-(3′-Tosyloxy-2′-hydroxy-2′-methylbutyrate) (XXIV).*—A solution of desatrine 3-(3′-tosyloxy-2′-hydroxy-2′-methylbutyrate (XXIV) (6) (1.35 Gm.) in methanol (125 cc.) was heated under reflux for twenty-four hours. The methanol was evaporated under reduced pressure and the residue was chromatographed on Merck acid-washed alumina (30 Gm.). The column yielded to benzene-chloroform (50:50) and to chloroform a resin which was shown to be homogeneous by paper chromatography. Crystallization from ether afforded needles (550 mg.), m. p. 231–232° (decompn.). Recrystallization from acetone-petroleum ether gave prisms, m. p. 227–228° (decompn.).

By Sodium Iodide-Acetonitrile Treatment of Desatrine 3-(3′-Tosyloxy-2′-hydroxy-2′-methylbutyrate) (XXIV).—A solution of desatrine 3-(3′-

tosyloxy-2′-hydroxy-2′-methylbutyrate) (XXIV) (550 mg.) and sodium iodide (400 mg.) in acetonitrile (15 cc.) was heated under reflux for five hours. The precipitate (sodium tosylate) was filtered and the filtrate was reduced to a small volume. Water (10 cc.) was added and the solution was extracted with chloroform. The chloroform extract was dried and evaporated under reduced pressure to a resin. The resin was crystallized from acetone-petroleum ether to yield desatrine 3-(2′,3′-epoxy-2′-methylbutyrate) hydriodide in the form of needles (250 mg.), m. p. 232–233° (decompn.).

Anal.—Calcd. for $C_{41}H_{62}INO_{14}\cdot H_2O$: C,52.53; H,6.90; I, 13.55. Found: C, 52.42; H, 7.04; I, 13.23.

Treatment of the hydriodide with dilute ammonium hydroxide and chloroform and further workup in the usual manner yielded XXV in the form of free base. In a subsequent preparation, the hydriodide was not isolated as such; the concentrated reaction mixture was treated with chloroform-dilute ammonium hydroxide. Evaporation of the chloroform extract yielded a resin which was chromatographed on Merck acid-washed alumina. In this preparation, 710 mg. of crystalline XXV free base was obtained from 1.8 Gm. of XXIV.

Desatrine 3-(3′-Tosyloxy-2′-hydroxy-2′-methylbutyrate) (XXIV) from the Treatment of Desatrine 3-(2′,3′-Epoxy-2′-methylbutyrate) (XXV) with *p*-Toluenesulfonic Acid.—A solution of desatrine 3-(2′,3′-epoxy-2′-methylbutyrate) (XXV) (100 mg.) and *p*-toluenesulfonic acid hydrate (100 mg.) in dry acetonitrile (5 cc.) was allowed to stand at room temperature overnight. The mixture was cooled to 0°, basified to pH 8–9 with dilute ammonium hydroxide, and extracted with chloroform. The chloroform extract was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to yield an amorphous residue. Crystallization from acetone-petroleum ether yielded XXIV in the form of prisms (50 mg.). The identity of the material with that obtained by tosylation of protoveratrine B was confirmed by m. p., mixed m. p., infrared spectrum, optical rotation, and paper chromatographic behavior.

Desatrine 3-(3′-Chloro-2′-hydroxy-2′-methylbutyrate) (XXVI).—A solution of desatrine 3-(2′,3′-epoxy-2′-methylbutyrate) (XXV) (200 mg.) in dry ethereal hydrogen chloride (30 cc.) and benzene (15 cc.) was allowed to stand at room temperature for eighteen hours. The solvent was evaporated to dryness under reduced pressure and the residue was treated with dilute ammonium hydroxide and extracted with chloroform. The chloroform extract was dried over anhydrous magnesium sulfate and concentrated to a small volume. Addition of petroleum ether effected crystallization. Filtration of the material followed by recrystallization from acetone-petroleum ether yielded colorless prisms (110 mg.), shown to be homogeneous by paper chromatography.

Desatrine 3-(3′-Hydroxy-2′-fluoro-2′-methylbutyrate) (XXVII).—A solution of desatrine 3-(2′,3′-epoxy-2′-methylbutyrate) (XXV) (200 mg.) in dry ether (500 cc.) containing 50% hydrofluoric acid (0.2 cc.) was allowed to stand at room temperature for three hours. Chloroform was added and the solution was concentrated to a small volume. With cooling in an ice water bath, water (5 cc.) and dilute sodium carbonate solution were added (to pH 8–9), and the mixture was extracted with chloroform. The chloroform extract was dried over anhydrous magnesium sulfate and evaporated to yield an amorphous residue (200 mg.). The residue was crystallized from acetone to yield prisms (70 mg.) shown to be homogeneous by paper chromatography.

Desatrine 3-(3′-N,N-Diethylaminoacetoxy-2′-hydroxy-2′-methylbutyrate) (XXVIII).—To a stirred solution of desatrine 3-(3′-chloroacetoxy-2′-hydroxy-2′-methylbutyrate) (XXIII) (450 mg.) in dry benzene (10 cc.), diethylamine (1.05 cc., 20 mole-equivalents) was added, and the mixture was stirred at room temperature for forty-eight hours. Workup as described above for XVI yielded an amorphous crude product (410 mg.). Chromatography on Merck acid-washed alumina (10 Gm.) yielded paper chromatographically pure product. Crystallization from acetone-petroleum ether gave yellow-white plates, m. p. 203–205° (decompn.).

Desatrine 3-(3′-(4″-Aminobenzoxy)-2′-hydroxy-2′-methylbutyrate) (XXIX).—Desatrine 3-(3′-(4″-nitrobenzoxy)-2′-hydroxy-2′-methylbutyrate) (XXI) (500 mg.) in 95% ethanol (5 cc.) was hydrogenated over platinum oxide (100 mg.) at room temperature and atmospheric pressure. In two hours two mole-equivalents of hydrogen was absorbed and hydrogen uptake ceased. The reaction mixture was filtered and the residue washed with ethanol. Evaporation of the solution under reduced pressure afforded a residue which was crystallized from benzene-petroleum ether in the form of plates, m. p. 193–195° (decompn.).

Chromic Acid Titrations.—The method used in Part XLI (13) was used. The results obtained are summarized in Table III.

TABLE III.—CHROMIC ACID TITRATIONS

Alkaloid	Oxygen Equivalents Consumed		
	Theoretical	Found, 45 min.	Found, 90 min.
Protoveratrine A (I)	1	1.00	1.06
Protoveratrine B (II)	2	2.19	2.20
Desatrine 3-(3′-tosyloxy-2′-hydroxy-2′-methylbutyrate) (XXIV)	1	1.04	1.07
Desatrine 3-(3′-chloro-2′-hydroxy-2′-methylbutyrate) (XXVI)	1	1.02	1.05
Desatrine 3-(3′-hydroxy-2′-fluoro-2′-methylbutyrate) (XXVII)	2	2.03	2.24

Diosphenol Formation as Confirmation of Structure.—The analytical procedure of chromic acid titration followed by alkaline treatment as a criterion for protoveratrine-like structure, previously described for escholerine (14), was used to check the structures of several of the products. All of the materials tested (V, VI, X, XI, XIV, XVII, XXV) gave crude products with the characteristic diosphenol absorption spectrum, λmax. 328 mμ (ε 12,700) and λmax. 0.1 *N* NaOH 381 mμ (ε 8,800).

REFERENCES

(1) Goodman, L. S., and Gilman, A., "The Pharmacological Basis of Therapeutics," 2nd ed., The MacMillan Co., New York, N. Y., 1955, pp. 747–754.
(2) Krayer, O., see Drill, V. A., "Pharmacology in Medicine," 2nd ed., McGraw-Hill Book Co., New York, N. Y., 1958, pp. 515–524.
(3) Winer, B. M., *New Engl. J. Med.*, **255**, 1173(1956).
(4) Wyss, S., and Spühler, O., *Acta Med. Scand.*, **153**, 221(1956).
(5) Winer, B. M., *Circulation*, **14**, 1019(1956); **16**, 953(1957).
(6) Kupchan, S. M., and Ayres, C. I., *J. Am. Chem. Soc.*, **82**, 2252(1960).
(7) Kupchan, S. M., Hensler, R. H., and Weaver, L. C., *J. Med. Pharm. Chem.*, in press.
(8) Klohs, M. W., Draper, M., Keller, F., Koster, S., Malesh, W., and Petracek, F. J., *J. Am. Chem. Soc.*, **76**, 1152(1954).
(9) Kupchan, S. M., and Ayres, C. I., THIS JOURNAL, **48**, 735(1959).
(10) Abreu, B. E., Richards, A. B., Alexander, W. M., and Weaver, L. C., *J. Pharmacol. Exptl. Therap.*, **112**, 73(1954).
(11) Rubin, B., and Burke, J. C., *ibid.*, **105**, 409(1952).
(12) Kupchan, S. M., Weaver, L. C., Ayres, C. I., and Hensler, R. H., THIS JOURNAL, **50**, 52(1961).
(13) Kupchan, S. M., and Gruenfeld, N., *ibid.*, **48**, 737 (1959).
(14) Kupchan, S. M., and Ayres, C. I., *ibid.*, **48**, 735 (1959).

Investigation of Drug Release from Solids IV

Influence of Adsorption on the Dissolution Rate

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The influence of an adsorbent on the dissolution rate of a slightly soluble acidic solid was investigated. Experimental data indicated that the adsorbent was capable of increasing the dissolution rate observed in water under conditions of a decreased concentration gradient (Nernst-Brünner film theory) to the maximum rate obtained when a constant concentration gradient was maintained. The approximate amount of adsorbent required to increase the slower dissolution rate to the maximum was calculated with the aid of adsorption isotherms.

IN AN EXTREMELY large number of pharmaceutical preparations including tablets, capsules, and suspensions the drugs are found in the solid state. In addition to the active ingredients these preparations also contain substances which may or may not influence the release of the drugs from the physical system. Since many of the inactive ingredients are capable of adsorbing drugs and since dissolution of the drugs takes place following their introduction into the body, it is of interest to know the effect of adsorption on the dissolution rate. The contents of the gastrointestinal tract as well as stomach and intestinal walls may also be considered as potential adsorption sites for the drug molecules. The adsorption of many drugs of varying chemical structure by various adsorbents has been reported by several workers (1–8).

An attempt was made in this investigation to determine the influence of an adsorbent on the dissolution rate of a slightly soluble acidic solid.

Received August 15, 1960, from the University of Wisconsin, School of Pharmacy, Madison 6.
Accepted for publication September 16, 1960.
This paper is based on a dissertation submitted by Gerald P. Polli to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
Presented to the Scientific Section, A. PH. A., Washington, D. C., meeting, August 1960.
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PLAN OF STUDY

It was assumed in this study that dissolution of the solid solute involved a diffusion controlled process based on the Nernst-Brünner film theory (9–11). As the dissolution process takes place the concentration of the solute in solution increases, the concentration gradient, therefore, decreases, and subsequently the solution rate also decreases. If an adsorbent was present in the solution, solute molecules would be adsorbed from solution onto its surface. If, due to adsorption, the solute molecules were removed from the bulk solution, the concentration gradient would not decrease and thus, the dissolution rate, theoretically, would also not decrease. Therefore, if sufficient adsorbent is added to the bulk solution, the solution rate of a solid in its own solution should be equal to the solution rate when the concentration gradient is constant. Using adsorption isotherms, the amount of adsorbent required to produce this increase in the dissolution rate should be calculable.

The concentration gradient can be represented by $(C_s - C)/\delta$, where C_s is the concentration at saturation, C is the concentration at any time t, and δ is the Nernst-Brünner film thickness. Under existing conditions, δ could be considered constant, and C equal to zero or small compared to C_s. Therefore, if the presence of an adsorbent was to change the dissolution rate, it would have to alter C_s. An increase in solution rate would result if C_s increased, the solution rate would remain constant if C_s remained constant, and a decrease in solution rate would re-