Chemical and Biological Properties of Some Derivatives of *cis*- and *trans*-1-Hydroxyquinolizidine[1]

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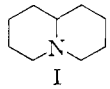
The syntheses of esters of *cis*- and *trans*-1-hydroxyquinolizidine are described. Data pertaining to the stereochemistry of the corresponding methyl halides are reported. Some preliminary pharmacological observations in experimental animals are recorded.

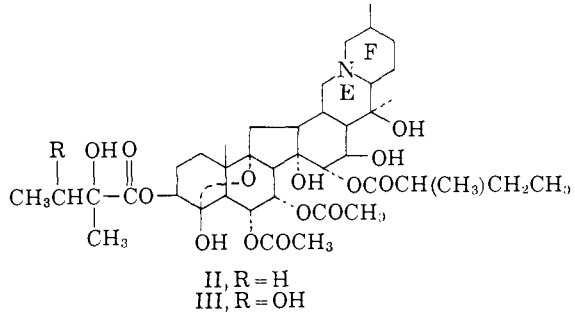
Chemical structures with fixed conformations have provided useful information regarding structure–activity relationships.[3] Quinolizidine[4] (I), which is present in many alkaloids, including veratrum[5] and rauwolfia,[6] served as a nucleus for our studies of the effect of structures on biological activity.



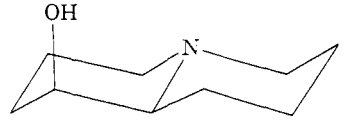
I

The *trans*-fused E–F ring system of protoveratrine[7] A (II) and B (III) is identical with that of *cis*-1-hydroxyquinolizidine (IV). Both the C_1 hydroxyl of the latter and the C_{21} hydroxyl of the protoveratrines are axial. Furthermore, *trans*-1-hydroxyquinolizidine contains a molecular fragment which has interatomic dimensions identical with the transoid conformation of the choline molecule, whereas the *cis* isomer is a 60° skew conformation of choline. The stereochemistry of the quaternary salts (V and VI) of *trans*-quinolizidin-1-yl acetate and the transoid conformation of acetylcholine, respectively, are likewise similar. This was of particular nterest in view of the observations of Belleau,[8a,b,c] Cavallito,[8d] Archer,[8e] Smissman[8f] and co-workers regarding the conformation of receptor-bound acetylcholine in biological systems. The relationship of V to VI and the fact that aromatic esters of 1-, 2-, and 3-hydroxyquinolizidines possess some antispasmodic and local anesthetic properties and also affect spontaneous motor activity[9] prompted further investigations in this area.

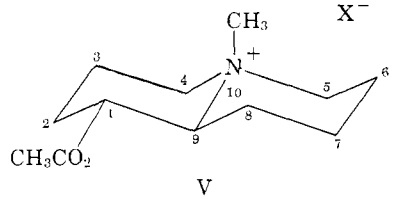
Our studies involved the preparation of esters of *cis*- and *trans*-1-hydroxyquinolizidine. The acids (as acid chlorides and anhydrides) employed in the esterification of the respective alcohols were mainly those that also



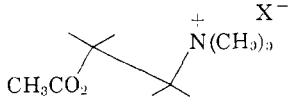
II, R = H
III, R = OH



IV



V



VI

occur in the veratrum ester alkaloids.[10] The alcohols were obtained from the cyclization of ethyl α-2-carbethoxypiperidinobutyrate[11] to 1-ketoquinolizidine[9] followed by reduction. The reduction procedures of Rader and co-workers[12] were employed to obtain a 93/7 *trans/cis* epimeric ratio and a 71/29 *cis/trans* ratio, respectively. The *cis*- and *trans*-1-hydroxyquinolizidines were obtained by preparative gas chromatography and elution chromatography.[13] The melting points of the geometrical isomers agreed with values reported by previous workers.[13,14]

Of particular interest in this investigation was the ring-fusion stereochemistry of the compounds reported in Table I. Aaron and associates[12,13,14c] have shown that the ring fusion in both *cis*- and *trans*-1- and -3-

(1) Taken from the dissertation presented by J. D. England, Jan 1966, to the Graduate School of the University of Mississippi, in partial fulfillment of the requirements for the Ph.D. degree.

(2) National Institutes of Health Predoctoral Fellow, 1963–1966.

(3) R. B. Barlow, "Introduction to Chemical Pharmacology," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1964.

(4) B. S. Thyagarajan, *Chem. Rev.*, **54**, 1019 (1954).

(5) R. H. Manske and H. L. Holmes, "The Alkaloids, Chemistry and Physiology," Vol. III, Academic Press Inc., New York, N. Y., 1953.

(6) R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey, and R. W. Kierstead, *J. Am. Chem. Soc.*, **78**, 2023 (1956).

(7) (a) S. M. Kupchan and C. I. Ayers, *ibid.*, **81**, 1009 (1959); (b) S. M. Kupchan and C. I. Ayers, *ibid.*, **82**, 2252 (1960).

(8) (a) D. J. Triggle and B. Belleau, *Can. J. Chem.*, **40**, 1201 (1962); (b) B. Belleau and J. Puranen, *J. Med. Chem.*, **6**, 325 (1963); (c) B. Belleau and G. Lacasse, *ibid.*, **7**, 768 (1964); (d) C. J. Cavallito and A. P. Gray, *Progr. Drug Res.*, **2**, 135 (1960); (e) S. Archer, A. M. Lands, and T. R. Lewis, *J. Med. Pharm. Chem.*, **5**, 423 (1962); (f) E. E. Smissman, W. L. Nelson, J. B. LaPidus, and J. L. Day, *ibid.*, **9**, 458 (1966).

(9) R. E. Counsell and T. O. Soine, *J. Am. Pharm. Assoc.*, **49**, 289 (1960).

(10) O. Wintersteiner, *Record Chem. Progr.* (Kresge-Hooker Sci. Lib.), **14**, 19 (1953).

(11) G. R. Clemo and G. R. Ramage, *J. Chem. Soc.*, 437 (1931).

(12) C. P. Rader, G. E. Wicks, Jr., R. L. Young, Jr., and H. S. Aaron, *J. Org. Chem.*, **29**, 2252 (1964).

(13) H. S. Aaron, G. E. Wicks, Jr., and G. P. Rader, *ibid.*, **29**, 2248 (1964).

(14) (a) N. J. Leonard, A. S. Hay, R. W. Fulmer, and V. W. Gash, *J. Am. Chem. Soc.*, **77**, 439 (1955); (b) G. A. Swan, *J. Chem. Soc.*, 2051 (1958); (c) H. S. Aaron and C. P. Rader, *J. Am. Chem. Soc.*, **85**, 3046 (1963).

TABLE I

ESTERS OF *cis*- AND *trans*-1-HYDROXYQUINOLIZIDINE

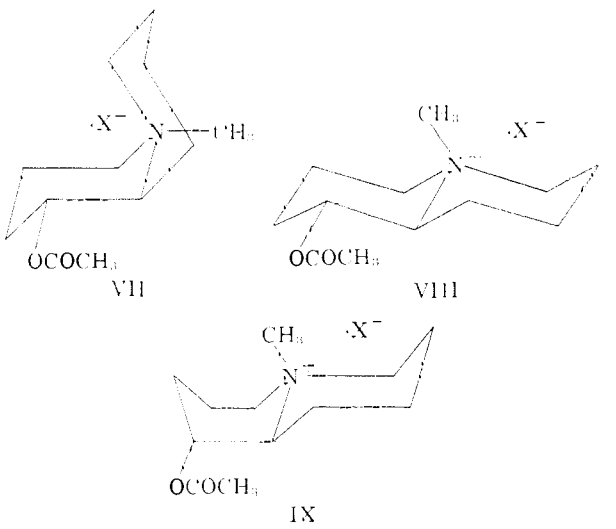
No.	Ester	Bp, °C (mm)	Method	Yield, %	n_D (t, °C)	Mp, °C (recrystn solvent[a]) Hydrochloride	Picrate[b]	Methobalide[c]	Formula[d]
1	Acetate[e]	65 (0.075)	A	84	1.4812 (28)	172–173 (EA)	180–182	...	$C_{21}H_{22}N_4O_8$[f]
2	Isobutyrate[e]	80–82 (0.075)	A	90	1.4669 (27)	218–220 (EA)	160.5–161	...	$C_{24}H_{26}N_4O_8$[f]
3	α-Methylbutyrate[e]	84 (0.3)	C	53	1.4704 (27)	Oil	Oil	...	$C_{14}H_{25}NO_2$
4	Tiglate[g]	75 (0.075)	C	44	...	...	134–135	...	$C_{26}H_{26}N_4O_8$[f]
5	Veratrate[e]	...	C	44	...	198.5 (EEt)	...	...	$C_{18}H_{20}ClNO_3$[f,g]
6	N,N-Dimethylcarbamate[e]	...	D	90	...	205.5–206.5 (EEt)	...	...	$C_{12}H_{22}ClN_3O_2$[f,h]
7	*trans*-4-Benzyloxy-3-methoxybenzoate[i]	...	C	20	...	180.2 (Et)	...	...	$C_{24}H_{30}ClNO_4$[f,i]
8	*trans*-Acetate[j,k]	75 (0.1)	A	79	1.4895 (28)	...	185–186	217–220[l,m] 190–199[n] (A)	$C_{17}H_{22}N_4O_8$[e]
9	*trans*-Propionate[j]	70 (0.05)	A	82	1.4751 (27)	...	164–166	189–191[l,m] 180–181[n] (A)	$C_{18}H_{24}N_4O_8$[e]
10	*trans*-n-Butyrate[j]	70 (0.25)	A	76	1.4782 (27)	...	162–163	178–180 (EaEt)	$C_{19}H_{26}N_4O_8$[e]
11	*trans*-Isobutyrate[j]	75 (0.05)	A	61	1.4775 (28)	...	160–161[o]	230–231[l,m]	$C_{19}H_{26}N_4O_8$[e,k]
12	*trans*-n-Valerate[j]	76 (0.25)	A	77	1.4782 (27)	...	140–141	161–163[l,m]	$C_{20}H_{28}N_4O_8$[e]
13	*trans*-n-Heptanoate[j]	70 (0.1)	A	78	1.4680 (27)	...	114–115	175–175 (Ea)	$C_{23}H_{32}N_4O_8$[e]
14	Acetate[p]	76 (0.06)	A	75	1.4789 (27)	155[m]	152–155	209–210[l,m]	$C_{21}H_{29}ClNO_2$[f,q]
15	Propionate[p]	70 (0.1)	A	77	1.4751 (30)	...	162–163	173–176[l,m]	$C_{18}H_{24}N_4O_8$[e]
16	Isobutyrate[p]	70 (0.05)	A	75	1.4705 (27)	223–224[m]	160	205–210[l,m]	$C_{19}H_{26}N_4O_8$[e]
17	N,N-Dimethylcarbamate[p]	...	D	76	...	244–245 (EEt)	...	...	$C_{12}H_{23}ClN_2O_2$[f,r]
18	*cis*-Acetate[r]	60 (0.1)	A	55	1.4798 (28)	...	176–178	209–210 (EEt) 174–176[n] (A)	$C_{17}H_{22}N_4O_8$[s]
19	*cis*-Propionate[r]	75 (0.25)	A	75	1.4667 (24)	...	...	168–170[n] (A)	

[a] A = acetone, E = ethanol, EA = ethanol–acetone, EEt = ethanol–anhydrous ether, EaEt = ethyl acetate–anhydrous ether, Ea = ethyl acetate. [b] All of the picrates were recrystallized from ethanol. [c] Prepared according to methods D and E; *trans* esters are mixtures of *cis* and *trans* ring-fused isomers, whereas *cis* esters are *cis* ring fused. [d] Prepared from 93% *trans*-1-hydroxyquinolizidine. [e] Picrate. [f] Hydrochloride. [g] Cl: calcd, 10.0; found, 9.3. [h] Cl: calcd, 13.4; found, 13.4. [i] Cl: calcd, 8.2; found, 8.2. [j] Prepared from 100% *trans*-1-hydroxyquinolizidine. [k] Lit.[14a] bp 78–79° (0.1 mm). [l] Methobromide. [m] Hygroscopic, triturated with anhydrous ether. [n] Methiodide. [o] Mixture melting point with **2** showed no depression. [p] Prepared from 71% *cis*-1-hydroxyquinolizidine. [q] Cl: calcd, 15.3; found, 15.4. [r] Prepared from 100% *cis*-1-hydroxyquinolizidine. [s] All compounds analyzed correctly for C, H, N. [t] Not analyzed.

hydroxyquinolizidine is primarily *trans*. This conclusion was supported by the presence of Bohlmann[15] absorption bands in the 2700–2800-cm^{-1} region and by Cookson's[16] analogy with the decalin system. Others[17] have demonstrated the existence of *cis* ring fusion in the quaternary salts of certain quinolizidines.

trans-1-Hydroxyquinolizidine was esterified with propionic and acetic anhydride and the resulting esters were converted to the methyl quaternary salts. The methyl quaternary salt of the *cis* acetate and propionate were likewise prepared. The nmr spectra of the acetate salts are recorded in Figures 1 and 2, respectively. The doublet centered at δ 2.21 of the acetate salt is due to the terminal methyl group of the ester moiety and was selected as the reference signal. The peaks at δ 3.10 and 3.35 were assigned to the N-methyl groups of the highly charged quaternary nitrogen of both the *cis* and *trans* isomers. The signal at δ 3.10 integrates to 1.5 protons and the signal at δ 3.35 integrates to 1.5 protons. The sum of these two integrated values (3 protons) is very close to the internal reference methyl group.

These observations provided evidence that the material represented in Figure 1 is approximately a 50:50 mixture of the methohalide of *cis*-fused (VII) and *trans*-fused (VIII) *trans*-quinolizidin-1-yl acetate. Structure IX, although possible, was not seriously considered because of the stereochemical interactions inherent in this conformation.[18]



VII VIII IX

The methobromide of *trans*-quinolizidin-1-yl acetate did not form readily; it was hygroscopic and difficult to handle. The methobromides of the other esters of the *trans* alcohol behaved in a similar manner. Moreover, the heptanoate derivative was easier to work with than the alkyl esters of less bulk. The methiodides of *trans*-quinolizidine-1-yl acetate and propionate on the other hand were crystalline, nonhygroscopic, and readily obtained.

The methohalide of *cis*-quinolizidin-1-yl acetate presented a somewhat different picture as seen in Figure 2. Only one quaternary methyl signal at δ 3.28 was present, indicating the presence of one isomer.[19] In contrast to the preparation of the *trans* methobromides, the methobromide of *cis*-quinolizidin-1-yl acetate was formed readily and crystalline and showed no hygro-

(15) F. Bohlmann, *Chem. Ber.*, **91**, 2157 (1958).

(16) R. C. Cookson, *Chem. Ind.* (London), 337 (1953).

(17) T. M. Moynehan, K. Schofield, R. A. Y. Jones, and A. R. Katritzky, *Proc. Chem. Soc.*, 218 (1961); T. M. Moynehan, K. Schofield, R. A. Y. Jones, and A. R. Katritzky, *J. Chem. Soc.*, 2637 (1962); C. D. Johnson, R. A. Y. Jones, A. R. Katritzky, C. R. Palmer, K. Schofield, and R. J. Wells, *ibid.*, 6797 (1965).

(18) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 8.

(19) K. L. Williamson, T. Howell, and T. A. Spencer, *J. Am. Chem. Soc.*, **88**, 325 (1966).

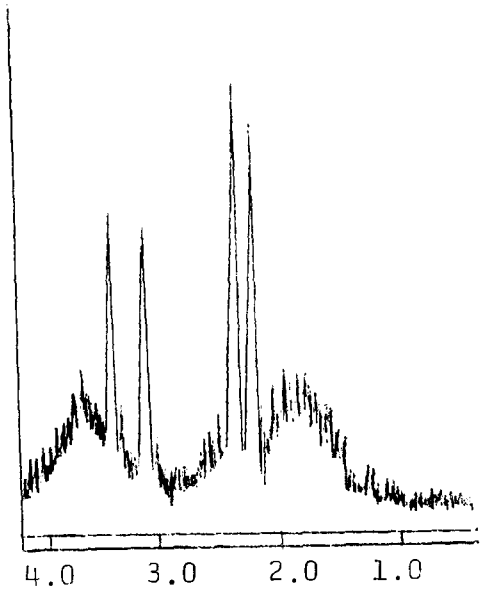


Figure 1.—Nmr spectrum (τ) of the methohalide of *trans*-quinolizidin-1-yl acetate in D_2O (10%) (mixture of *cis* and *trans* ring-fused salts).

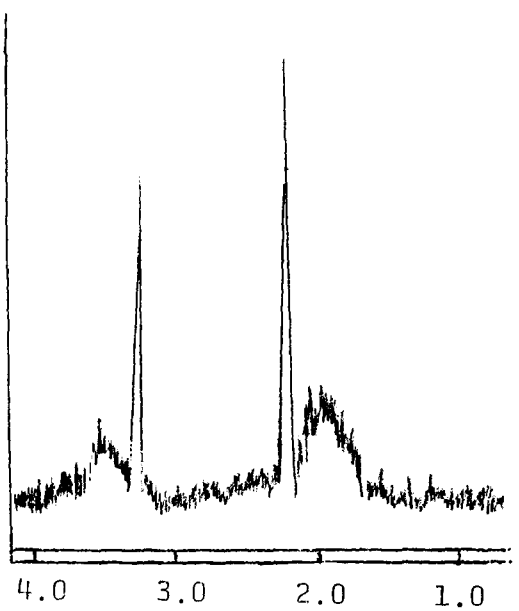


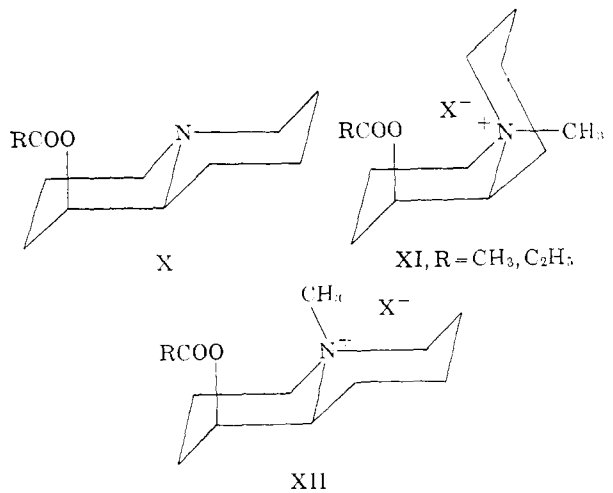
Figure 2.—Nmr spectrum (τ) of the methohalide of *cis*-quinolizidin-1-yl acetate in D_2O (10%).

scopic tendency. This was true also of the methiodide. The methohalides of *cis*- and *trans*-quinolizidin-1-yl propionate provided spectral data similar to the corresponding acetates. The observed data indicated that the original *trans*-fused ester (X) was converted entirely either to the *cis*-fused conformation (XI) or to the *trans*-fused salt (XII).

trans-Quinolizidine is analogous to *trans*-decalin with one tertiary hydrogen removed.[16] Turner[20] has shown that *trans*-decalin is more stable than *cis*-decalin by 2.7 kcal/mole. However, in 9-methyldecalin the introduction of additional skew interactions in the *trans* form lowers the energy difference between the *trans* and the *cis* isomers.[21] Because of the close similarity of the

(20) R. B. Turner, *J. Am. Chem. Soc.*, **74**, 2118 (1952).

(21) M. S. Newman, "Steric Effects in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1956, pp 30–31.



N-methylquinolizidines to 9-methyldecalin, structure XI tentatively has been assigned to the quaternary ammonium salts of the *cis* (axial) esters (X).

Pharmacological Results[22]

Toxicity and General Observations in Mice.—A number of similarities among the observable effects of this series of compounds were seen. Locomotor activity was reduced within several minutes after nontoxic doses of the hydrochlorides of **1–3**, **5**, **8**, **16**, **17** and the methobromides of **9–11**, **13**, **16**, **18**. Sedation with ptosis was noted with the hydrochlorides of **2**, **8**, **16**. Brief tremors or twitches appeared with the hydrochlorides of **1**, **3**, **6**, **14**, **17** and the methobromides of **9–13**, **15**, **16** with muscle fasciculations being prevalent in animals receiving the methobromides except for **18**. Clonic and/or tonic convulsions were associated with lethal dosage levels (Table II) of nearly all of the compounds. Deaths commonly occurred within 6 min and seldom were delayed later than 15 min after injection. The hydrochlorides of **1**, **2**, **6**, **16** caused cutaneous vasodilation which was, however, not of great intensity or

TABLE II

QUINOLIZIDINE DERIVATIVES. ACUTE TOXICITY IN MICE

No.[a]	LD_{50}, mg/kg ip (95% confidence limits) Hydrochlorides	Methobromides
1	709 (587–855)	...[b]
2	892 (765–1040)	...[c]
3	562	...
5	383	...
6	328 (272–396)	...
8	...	93 (64–135)
9	...	83
10	...	261
11	...	83
12	...	178 (143–221)
13	...	121 (97–150)
14	446 (370–539)	...[d]
15	...	77 (60–98)
16	242 (208–282)	72 (55–92)
17	383	...
18	...	154 (119–199)

[a] The numbers refer to the parent structure of the compounds of Table I. [b] See 8. [c] See 11. [d] See 18.

(22) The authors are grateful to Dr. Marvin Davis, Department of Pharmacology, School of Pharmacy, The University of Mississippi, for the pharmacological results. The numbers in the pharmacology sections refer to the compounds in the tables.

TABLE III

EFFECTS OF QUINOLIZIDINES ON THE CARDIOVASCULAR SYSTEM

Compd[a]	Dosage range, mg/kg	Hypotensive effect[b]	Interaction[c] effect with—		
			Histamine	Epinephrine	Methacholine
Protoveratines A and B	0.006–0.062	+3 to +4	0 to +1	0 to −2	0 to +1
6	19.9–109	+2 to +4	0 to −1	0 to −2	0 to −1
17	12.8–128	0 to +3	0 to −1	0 to −1	0
1	23.6–236	±2	0 to −1	−2 to +4	0 to −1
14	14.9–149	+2 to +3	−1 to −2	+1 to −2	−2
2	29.7–297	+2 to +4	0 to +2	+1 to +2	0
16	8.1–81.0	+1 to +3	0 to −2	+1 to +4	0 to −2
3	18.7–187	+1 to +4	0	0 to +1	0
5	12.8–128	+3 to +4	−1 to −2	0	0 to −1

[a] The numbers refer to compounds of Table I. All are hydrochlorides except **6** (base). [b] + = effect present, number = approximate degree on scale of 0–4 (+4 = *ca.* 80 mm). [c] + = synergism, − = antagonism, 0 = none or uncertain, number = degree on arbitrary scale of 0–4.

duration. The median lethal dosages of the methobromides were definitely lower than those of the hydrochlorides.

Cardiovascular Effects in Rats.—The observations of the effects of some of the quinolizidines on the cardiovascular system are recorded in Table III. The results show variability and some inconsistencies. All eight compounds caused some lowering of blood pressure. However, the duration of this action was generally brief, usually 5 min or less. Interactions with the three standard vasoactive substances seemed to occur in several cases. Inconsistencies between tests on different rat preparations with the same drug and dosage was seen in a number of instances.

Smooth Muscle Effects.—Of the nine methobromides available for testing, only two affected smooth muscle tissue even in the higher concentration under conditions described in the Experimental Section. Compound **8** was concluded to have a muscarinic action by the following observations: (1) a spasmogenic action on guts which was antagonized by atropine but not by hexamethonium, and (2) lack of spasmogenic action of frog rectus abdominus. On the contrary, the other active compound, **11**, was found to relax the gut at the higher test concentration.

Both the cholinergic compound **8** and the anticholinergic compound **11** are the *trans* isomers in pairs of which the corresponding *cis* isomers (**18** and **16**) were found to be inactive. In a third pair, both *cis* and *trans* isomers (**15** and **9**) were inactive. The three remaining compounds, **10**, **12**, **13**, were *trans* forms and were all inactive.

The difference in the activity of the methobromides of the *cis*-acetate (**14**, **18**) and the *trans*-acetate (**8**) probably is due not only to the different conformations of the acetate group but also to the differences in the fusion of the rings. Evidence was presented earlier for the formation of a mixture of *cis*-fused (VII) and *trans*-fused (VIII) rings from the *trans* (equatorial) acetate, and the formation of only a *cis*-fused ring acetate (XI) from the *cis* (axial) acetate. On the basis of Belleau's observation, one would expect the methobromide of **8** to be active and XI (methobromide of **14**, **18**) to be inactive.

Experimental Section[23]

1-Hydroxyquinolizidine.—1-Ketoquinolizidine was prepared from ethyl 2-(2-carbethoxypiperidino)butyrate[11] according to the method of Counsell and Soine[9] and reduced catalytically by the procedure of Rader, *et al.*[12] As reported by these workers, a Pd–C reduction gave 93/7 *trans/cis* ratio and a Ru/C reduction gave a 71/29 *cis/trans* ratio. The epimeric mixtures were separated by preparative gas chromatography, elution chromatography, and by fractional crystallization of their hydrochloride salts. The homogeneity of the samples was followed by thin layer chromatography; the observed melting points were consistent with those reported in the literature.[13,14]

Esters (Table I).—The procedures described below cover specific compounds; however, they are general and were utilized for the preparation of the compounds listed in Table I.

Method A. *trans*-Quinolizidin-1-yl Acetate.—The procedure described by Leonard, *et al.*,[14a] was followed. A solution containing 5 ml (0.053 mole) of freshly distilled Ac_2O, 2.0 g (0.020 mole) of *trans*-1-hydroxyquinolizidine, and 25 ml of anhydrous C_6H_6 was refluxed over a steam bath for 2 hr. The solution was poured over crushed ice; the mixture was saturated with K_2CO_3 and extracted with six 100-ml portions of ether. The ether extract was dried ($MgSO_4$) and distilled. An ir spectrum (liquid film) showed strong C=O absorption at 5.74 μ. The picrate was prepared in the usual manner.

Method B. Quinolizidin-1-yl α-Methylbutyrate.—A solution of 3.0 g (0.0247 mole) of *dl*-2-methylbutyryl chloride in 25 ml of dry C_6H_6 was added slowly (15 min), with stirring, to a 25-ml solution of dry C_6H_6 containing 3.0 g (0.0194 mole) of 93/7 *trans/cis* ratio of 1-hydroxyquinolizidine (from Pd–C reduction) and 3 ml of Et_3N. The resulting solution was kept at room temperature for 30 min and, thereafter, refluxed for 2 hr. The solution was cooled and then poured over crushed ice and saturated with K_2CO_3. An ether extract of the mixture was dried ($MgSO_4$). Evaporation of the ether left 3.9 g of crude product which was vacuum distilled. The infrared spectrum (liquid film) showed strong absorption at 5.74 μ and no OH absorption. The methiodide, hydrochloride, and picrate salts of this compound were prepared in the usual manner but each resisted crystallization. The ester was chromatographed using a 0.6-cm chromatographic column 3.048 m long and packed with 10% 20M Carbowax on Chromosorb W in an Aerograph Autoprep Model A-700 gas chromatograph. The column temperature was held at 200° and the carrier gas (He) flow rate was adjusted to 150 cc/min. The chromatogram indicated the presence of four components, one present in a quantity of about 90% of the total weight. An ir spectrum (liquid film) of this component showed strong CO absorption at 5.74 μ.

Method C. Quinolizidin-1-yl N,N-Dimethylcarbamate Hydrochloride.—A solution of 4.0 g (0.02 mole) of 1-hydroxyquinolizidine (from Pd–C reduction) in 10.0 g (0.1 mole) of freshly distilled N,N-dimethylcarbamoyl chloride was refluxed, with stirring, for 2 hr and thereafter cooled. The solid was filtered from the resulting dark red solution and washed with 50 ml of dry Et_2O. Tlc (4:1:1 BuOH–AcOH–H_2O) of the recrystallized product indicated a single component. The ir spectrum (Nujol) showed C=O absorption at 5.86 μ.

Methohalides (Table I). Method D.—The methobromides of the esters were prepared by adding excess ethereal MeBr to the

[23] All melting points were taken on a Thomas–Hoover Uni-Melt melting point apparatus and are corrected. Ir spectra were determined on a Perkin-Elmer Model 137 spectrophotometer using the technique indicated in the text. All absorption peaks have been corrected against the 5.138-μ absorption band of polystyrene film. The nmr spectra were taken using a Varian Model A-60A instrument.

ester in anhydrous Et_2O. Anhydrous MeOH (1 ml) was added and the solution was permitted to stand at room temperature. The precipitated salt was collected by filtration over a period of several weeks. This was the method utilized for the preparation of all of the methobromides reported in Table I. Quantitative yields were obtained but the salts were very hygroscopic (except the *cis* acetate) and generally resisted purification by recrystallization (see Table I).

Method E.—The methiodides were prepared in quantitative yield by refluxing for 12 hr a solution of the ester in Na-dried C_6H_6 with an excess of MeI.

Pharmacological Procedure. Toxicity in Mice.—All compounds were injected intraperitoneally in aqueous solution (made with aid of dilute HCl in the case of **6**) over a range of at least four dosages. Five albino mice were used at each dosage level and the proportion of animals dying was used to determine approximate LD_{50}'s, and confidence limits when the data permitted, by the method of Horn.[24]

Cardiovascular Effects in Rats.—Recordings of arterial blood pressure, respiration, and the electrocardiogram were made by means of an E & M Physiograph and appropriate transducers. Blood pressure was recorded *via* the cannulated carotid artery. Injections were made *via* a needle-cannula in the femoral vein. All materials administered were flushed in with a small volume of 0.9% saline solution.

(24) H. J. Horn, *Biometrics*, **12**, 311 (1956).

Male albino rats of the Holtzmann strain weighing 275–375 g were anesthetized with urethan (1.26 g/kg ip). The test compounds and a standard consisting of protoveratrines A and B were administered at dosages constituting 3, 10, and 30% of the approximate LD_{50} for mice. Observations for changes in the physiological parameters were made after each of these dosages. Then, upon equilibration from any changes evoked, standard doses of histamine, epinephrine, and methacholine were administered successively. Responses to these agents after the test compound were compared to responses to like quantities that had been observed prior to any administration of the test substance. In several cases the 30% dosage was lethal. In some others it was repeated or even a 60% dosage was administered if the preparation was still functioning.

Smooth Muscle Effects.—The compounds were tested on sections of rabbit ileum which were suspended in an oxygenated Ringer's solution in a 30-ml muscle bath maintained at 37°. The muscle strip was attached to a myograph transducer to record muscular activity on an E & M physiograph. Response to solutions of the test compound were observed in comparison to responses to 0.5-ml quantities of standard solutions of acetylcholine chloride (1:100,000) and epinephrine (1:10,000), or in conjunction with the cholinergic receptor blocking action of atropine sulfate (1:400). The two solutions (1:100 and 1:1000) of each compound which were tested yielded upon addition to the bath in 0.5-ml quantities final drug concentrations of about 15 and 150 μg/ml, respectively.

Synthesis and Pharmacological Evaluation of Some Tetrahydrooxadiazinones and Some Dihydroaminooxadiazines

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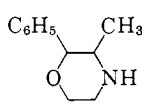
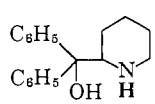
The synthesis of *cis*-(+)- and *cis*-(−)-tetrahydrooxadiazinone derivatives of (+)- and (−)-ephedrines and two related tetrahydrooxadiazinones is reported. The results of an attempted synthesis of a dihydroaminooxadiazine derivative of ephedrine and the successful synthesis of three related dihydroaminooxadiazines is also reported. The *cis*-(−)-tetrahydrooxadiazinone derived from (−)-ephedrine was found to be a monoamine oxidase inhibitor in pharmacological testing.

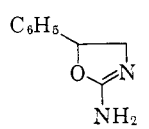
One widely used approach for the synthesis of new compounds that possess some type of central nervous system stimulant activity is the cyclization of substituted phenethanolamines into heterocycles, such as morpholine, piperidine, and 2-oxazoline in such a manner that more or less of the phenethanolamine skeleton becomes part of the heterocyclic ring. Well-known drugs of this type are phenmetrazine,[1] pipradol,[2] and aminorex.[3]



phenmetrazine



pipradol



aminorex

The ephedrines and norephedrines are useful starting materials for a study of this type because they possess some central activity and because all eight of the isomers are readily available. Morpholine,[1] 2-oxazoline,[4] oxazolidine,[5] di- and tetrahydro-1,3,4-oxadiazines,[6,7] 2-thiazoline,[8] thiazolidine,[9] dihydro-1,3,4-thiadiazine,[10] tetrahydro-*as*-triazine,[11] and imidazolidine[12] heterocyclic derivatives of the ephedrines and norephedrines have been reported. Certain of these heterocycles exhibit central-stimulating appetite-depressing,[1,4] monoamine oxidase inhibiting antidepressant,[6g,10b] central nervous system depressant,[6e,f,7,11] analgetic,[11] hypocholesterolemic,[7] antiinflammatory,[7] antimicro-

(1) L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," 3rd ed, The Macmillan Co., New York, N. Y., 1965, p 516.

(2) R. F. Gould, "Molecular Modification in Drug Design," Advances in Chemistry Series, No. 45, American Chemical Society, Washington, D. C., 1964, p 116.

(3) C. K. Cain, "Annual Reports in Medicinal Chemistry, 1965," Academic Press Inc., New York, N. Y., 1966, p 54.

(4) G. I. Poos, J. R. Carson, J. D. Rosenau, A. P. Roszkowski, N. M. Kelly, and J. McGowin, *J. Med. Chem.*, **6**, 266 (1963).

(5) H. Pfanz and G. Kirchner, *Ann.*, **614**, 149 (1958).

(6) (a) D. L. Trepanier, V. Spranemanis, and K. G. Wiggs, *J. Org. Chem.*, **29**, 668 (1964); (b) D. L. Trepanier and V. Spranemanis, *ibid.*, **29**, 673 (1964); (c) *ibid.*, **29**, 2151 (1964); (d) D. L. Trepanier, V. Spranemanis, D. S. Tharpe, and P. E. Krieger, *J. Heterocyclic Chem.*, **2**, 403 (1965); (e) D. L. Trepanier, P. E. Krieger, and J. N. Eble, *J. Med. Chem.*, **8**, 802 (1965); (f) D. L. Trepanier, V. Spranemanis, and J. N. Eble, *ibid.*, **9**, 753 (1966); (g) D. L. Trepanier, U. S. Patent 3,122,537 (1964).

(7) M. J. Kalm, U. S. Patent 3,251,838 (1966).

(8) M. Kojima, *Yakugaku Zasshi*, **79**, 1 (1959).

(9) A. P. Roszkowski and G. B. Koelle, *J. Pharmacol. Exp. Ther.*, **128**, 227 (1960).

(10) (a) D. L. Trepanier, W. Reifschneider, W. Shumaker, and D. S. Tharpe, *J. Org. Chem.*, **30**, 2228 (1965); (b) D. L. Trepanier, U. S. Paten 3,290,303 (1966).

(11) D. L. Trepanier, E. R. Wagner, G. Harris, and A. D. Rudzik, *J. Med. Chem.*, **9**, 881 (1966).

(12) M. Murakami and T. Fukumoto, *Nippon Kagaku Zasshi*, **76**, 270 (1955).