Esters of Bicyclic Aminoalcohols V: Duration of Corneal Anesthesia *versus* Enzymatic Hydrolytic Rate of Benzoates of 1-, 2-, and 3-Methyl-2-hydroxyquinolizidines

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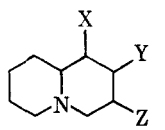
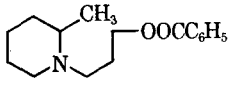
Abstract □ Benzoates of the 1-, 2-, and 3-methyl-2-hydroxyquinolizidines were prepared, and the relative *in vitro* rates of enzymatic hydrolysis of these esters together with quinolizidin-2-yl benzoate and piperocaine by pseudocholinesterase were determined. The duration of corneal anesthesia on rabbits was measured for these compounds. A comparison of the relative rate of enzymatic hydrolysis with duration of corneal anesthesia within the group of compounds and enzyme system studied was made and indicates no correlation between duration of corneal local anesthesia and rate of hydrolytic cleavage.

Keyphrases □ Aminoalcohol esters, bicyclic—synthesis □ Enzymatic hydrolysis—bicyclic aminoalcohol esters □ Corneal anesthesia—bicyclic aminoalcohol esters □ UV spectrophotometry—analysis, enzymatic hydrolysis □ IR spectrophotometry—identification □ TLC—separation □ NMR spectroscopy—structure □ GLC—identification

Metabolic inactivation of aminoester-type local anesthetics usually involves enzymatic cleavage at the ester linkage (1), presumably by nonspecific esterase activity. Prolongation of activity was achieved in a number of cases (2–4) by sterically inhibiting hydrolysis of the ester linkage. Kalow (5) correlated the duration of activity with the hydrolytic rate using human serum cholinesterase. In contrast to these observations, piperocaine hydrochloride was shown to be a longer acting local anesthetic than procaine (6), in spite of being hydrolyzed six times more rapidly by human serum cholinesterase (5). Most studies to date concern the effects of serum cholinesterases on local anesthetics; few have been conducted to determine whether duration of local anesthetic action in the cornea can be correlated with enzymatic hydrolytic rates. The literature is not very revealing as to the esterase activity of corneal tissue, but it can be presumed that any esterase activity would very likely be nonspecific. Thus, termination of corneal anesthesia could occur by enzymatic inactivation of an aminoester local anesthetic, by simple diffusion from the corneal tissue, or by protein binding, which then, in effect, becomes the rate-limiting factor in the diffusion process.



I*a* X = $OOCC_6H_5$, Y = Z = H
I*b* X = Z = H, Y = $OOCC_6H_5$
I*c* X = Y = H, Z = $OOCC_6H_5$



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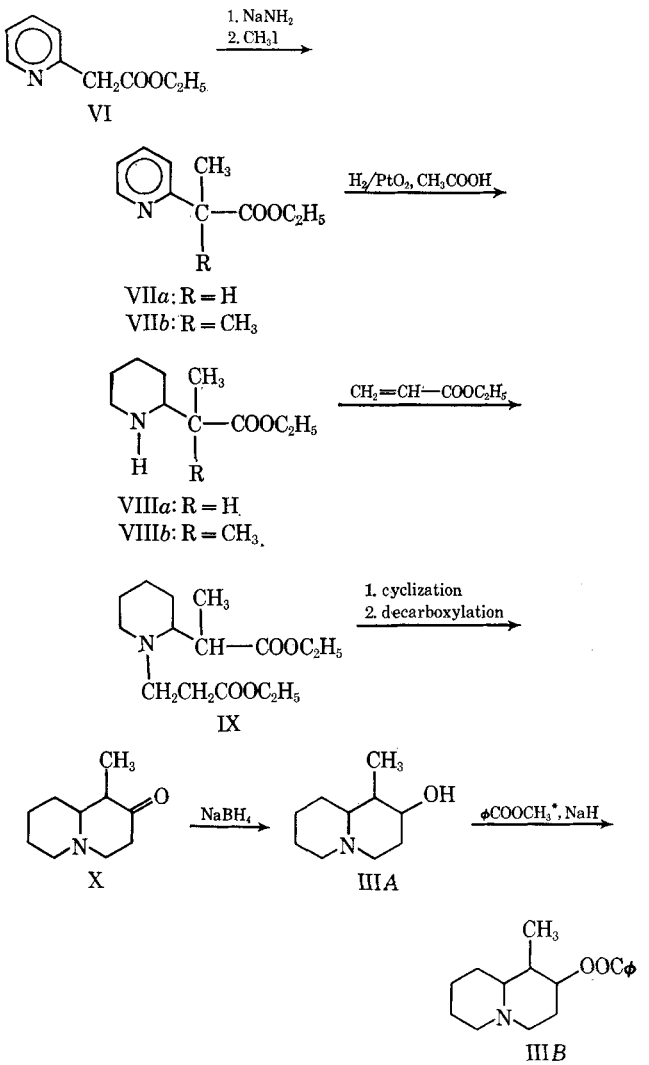
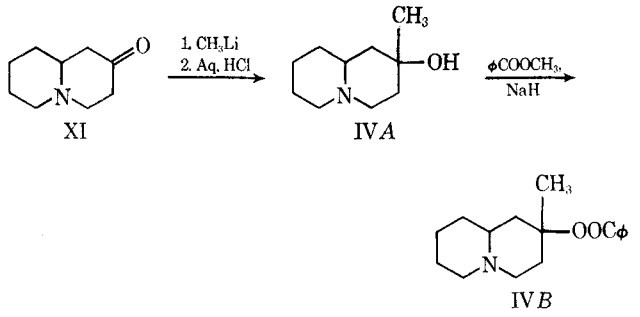


Scheme I—Synthesis of 1-Methyl-2-hydroxyquinolizidine Benzoate

The objective of the present work was to examine the relationship between *in vitro* enzymatic hydrolytic rate and corneal duration of action, using selected aminoesters with varying degrees of steric complexity around the hydrolytic site. The esters selected arose initially from a study (7) of the isomeric hydroxyquinolizidine benzoates (I*a*,*b*,*c*) in which the esters were compared with piperocaine (II), an open-chain analog. I*b* was found to be 1.72 times as active as II in the intradermal wheal test, whereas I*a* and I*c* were markedly less active. This suggested that I*b* and modifications of it might be useful for the indicated studies where the modification caused steric hindrance to es-

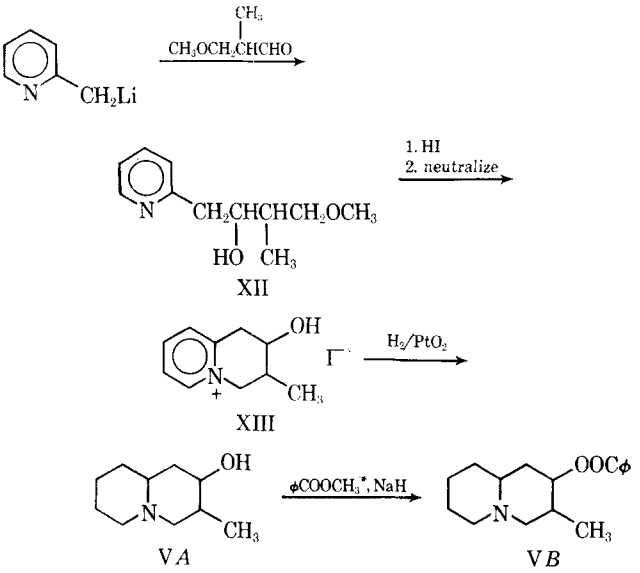


Scheme II—Synthesis of 2-Methyl-2-hydroxyquinolizidine Benzoate

terase hydrolysis through introduction of suitably placed methyl groups. Thus, the synthesis of the 1-, 2-, and 3-methyl-2-hydroxyquinolizidines (III*A*, IV*A*, V*A*) and the corresponding benzoates (III*B*, IV*B*, V*B*) was undertaken. Two racemates of IV*B* and V*B* were obtained, whereas III*B* provided only one. These racemates, together with I*b* and II, were the compounds used for the present study. No stereochemical designations were attempted, since it was obvious from the pharmacological results that stereochemistry was a minimal factor affecting activity and hydrolytic rate.

DISCUSSION

Synthesis—The synthesis of 1-methyl-2-hydroxyquinolizidine (III*A*) (Scheme I) was initiated by alkylating ethyl 2-(α-pyridyl) acetate (VI) to obtain ethyl 2-(α-pyridyl)-2-methylacetate (VII*a*) which, however, was contaminated with starting material and the corresponding dialkylated product [ethyl 2-(α-pyridyl)-2-dimethylacetate (VII*b*)] as determined by chromatographic procedures. Separation of the mixture by fractional distillation or chromatography could not be achieved. Therefore, the mixture was reduced catalytically to provide a mixture of the desired ethyl 2-(α-piperidyl)-2-methylacetate (VIII*a*) and the corresponding reduced forms of VI and the dialkylated contaminant (VIII*b*). A single product was not achieved, although fractional distillation provided a product without the reduced form of VI and with a much lower concentration of VIII*b*, as demonstrated by GLC. Further purification of VIII*a* could not be effected by the usual techniques and, therefore, it was subjected to Michael addition with ethyl acrylate in the impure form. Ethyl piperidyl-2α-propionate-1β-propionate (IX) was obtained in good yield, since VIII*b* apparently failed to react and was



Scheme III—Synthesis of 3-Methyl-2-hydroxyquinolizidine Benzoate

separated by fractional distillation. Dieckmann cyclization of IX followed by decarboxylation yielded the desired aminoketone (X), which was then reduced with sodium borohydride to yield III*A* after chromatographic purification. The NMR showed a doublet (J = 6 Hz.) in the C-methyl region and showed homogeneity under GLC and TLC examination.

The synthesis of 2-methyl-2-hydroxyquinolizidine (IV*A*) (Scheme II) was initiated by the preparation of 2-ketoquinolizidine (XI) by the method of Rhodes and Soine (8), which had been shown to be superior to that of Clemo *et al.* (9). Conversion to IV*A* was effected by the addition of methyl lithium according to McElvain and Berger (10) and Leonard *et al.* (11). The product had a broad melting point (60–74°) and was not separable into its isomeric forms by distillation or chromatographic procedures. However, when used as such for the preparation of the benzoate ester (IV*B*), it provided two isomeric racemic benzoates. Coincidentally, the melting points of the ester hydrochlorides were identical, but a mixed melting point showed marked depression; furthermore, the IR spectra differed significantly.

The synthesis of 3-methyl-2-hydroxyquinolizidine (V*A*) (Scheme III) could not be effected by the procedure used for III*A* and IV*A*. It was initiated by a modification of the Boekelheide and Gall (12) synthesis of 2-hydroxyquinolizidine in which β-methoxyisobutyraldehyde, prepared by the method of Elderfield *et al.* (13), was treated with α-picolyl lithium to yield 4-methoxy-1-(α-pyridyl)-3-methyl-2-butanol (XII). Hydriodic acid cleavage of XII yielded the cyclization product, 2-hydroxy-3-methyl-1,2-dihydro-3*H*,4*H*-quinolizinium iodide (XIII), which, upon catalytic reduction, yielded V*A* as a mixture of its isomers. Fractional distillation and chromatographic methods failed to provide a separation of the isomers. However, conversion to the benzoate form (V*B*) provided an easy separation of two isomeric racemic esters as the hydrochloride salts.

With respect to the conversion of the aminoalcohols to the benzoate esters, it was found, in keeping with the experiences of Feldkamp (14) and of Counsell and Soine (7), that the ester-interchange method was far superior to the usual Schotten–Baumann procedure.

Hydrolytic Rate Studies—An appropriate enzyme system was used in an attempt to approximate, by *in vitro* studies, the metabolic inactivation of a select group of topically active local anesthetics. Pseudocholinesterase was chosen for this study because of its possible role in synaptic transmission (15), its abundance in a variety of tissues in man and other animals (16), and its nonspecific nature. It is thought that the properties of this enzyme would allow a meaningful measure of the effects on enzymatic hydrolysis produced by the steric restrictions imposed when the alkyl ester chain of piperocaine (II) is fixed into the relatively rigid nucleus of quinolizidin-2-yl benzoate (I) and when additional restriction is produced by placing a methyl group in the 1-, 2-, and 3-positions of the quinolizidine nucleus. The type of kinetic data found in this study is predictable from classical theoretical considerations of enzyme catalysis (17) according to the Michaelis–Menten scheme. Both the Michaelis constant, K_m, and the maximum or zero-order rate were determined.

Although reaction velocities can be considered a function of K_m at low substrate concentrations, local anesthetics are injected in such high concentrations that the maximum or zero-order rate would seem to provide the best set of rates for comparison with duration of local anesthetic activity. The K_m values were determined as a measure of the effects produced by synthetic molecular manipulation on the ability of the enzyme and drug molecule to form a complex. K_m represents an upper limit to the value of the enzyme-substrate dissociation constant. The method used to measure hydrolysis was based on the UV absorption spectra of the local anesthetics and their hydrolytic products.

The results of the rate studies shown in Table I indicate that introduction of the quinolizidine nucleus reduces the rate of enzymatic hydrolysis considerably. A 300-fold reduction in rate is produced when the alkyl ester chain of piperocaine hydrochloride is fixed into the rigid nucleus of quinolizidin-2-yl benzoate hydrochloride. The Michaelis constants for the two compounds are of the same order of magnitude, suggesting that the reduction in rate is possibly due to a misalignment of the ester group in the complex with the enzymatic moieties functioning in hydrolysis rather than to a significantly reduced ability of the latter compound to form an enzyme–substrate complex.

Table I—Enzymatic Hydrolytic Data

Compound	Relative Hydrolytic Rates (Maximum or Zero-Order Velocity) with Piperocaine Arbitrarily Set at 1000.0	Michaelis Constants[a] (K_m) as Determined by Lineweaver–Burk Treatment of Data from Hydrolytic Curves
Piperocaine hydrochloride (II)	1000.0	5.0×10^{-6}
Quinolizidin-2-yl benzoate hydrochloride (I*b*)	2.83	7.6×10^{-6}
1-Methylquinolizidin-2-yl benzoate hydrochloride (III*B*)	0.56	9.5×10^{-5}
2-Methylquinolizidin-2-yl benzoate hydrochloride (IV*B*, isomer *a*)	0.11	—
2-Methylquinolizidin-2-yl benzoate hydrochloride (IV*B*, isomer *b*)	0.05	—
3-Methylquinolizidin-2-yl benzoate hydrochloride (V*B*, isomer *a*)	1.66	3.5×10^{-5}
3-Methylquinolizidin-2-yl benzoate hydrochloride (V*B*, isomer *b*)	1.54	3.8×10^{-5}

[a] Molar concentration units.

Introducing a methyl group into the 1- and 3-positions of the quinolizidine nucleus does, however, affect the attraction between enzyme and substrate, as is suggested by the higher K_m values for these compounds. The reduction in rate for these compounds as compared with piperocaine hydrochloride is most likely due to a combination of reduced complex formation and relative inaccessibility of the catalytic groups of the enzyme to the ester portion of the drug molecule.

The approximately 10,000-fold decrease in rate noted when comparing IV*B* (isomers *a* and *b*) with II is probably due to both of the previously mentioned factors. The extent of this produced by reduced attraction of the molecule to the enzyme surface cannot be estimated, since the K_m values for these compounds were not determined.

Differences in stereochemistry about the ester grouping (IV*B* isomers and V*B* isomers) did not affect the hydrolytic rate to a significant extent. The very slow reactions caused some difficulty in that the enzyme–substrate solutions became turbid before completion of the reaction. The rate data from the last portion of the reaction curves, which are important in K_m calculations, were erratic so that only the zero-order rates could be calculated for the IV*B* isomers in Table I.

Pharmacological Testing—The testing procedure employed parallels that of Patel and Soine (18) in most respects, with the only probable exception occurring in interpretation of the end-point of anesthesia. This may account for the fact that the duration ratio, as found for I*b* in this study, is approximately 20% below that found by the previous authors. It is assumed, however, that interpretation of the end-point in this study was consistent and that relative values for duration of activity are as accurate as this type of testing will permit. The testing results are tabulated in Table II.

The first and most striking observation is that, if the rates found using pseudocholinesterase are taken as representative of nonspecific esterase activity, no differences in duration of activity can be attributed to differences in rates of hydrolytic cleavage of the ester grouping.

The possibility remains, however, that an enzyme or group of enzymes with properties distinct from pseudocholinesterase, capable of cleaving all of the esters at nearly the same rate, could be present in the rabbit cornea and surrounding tissues. The literature contains little information pertaining to the isolation of esterases from this area.

At any rate, it would seem that the steric restrictions imposed in the quinolizidinyl esters of Table II should cause an enzyme some difficulty if proximity of one or more side chains of the enzyme to the carboxy portion of the ester is necessary for esterase activity. If metabolic inactivation of this type is not a dominant factor in termination of local anesthesia, other factors remain to be considered.

Table II—Local Anesthetic Activity

Compounds	Duration of Local Anesthesia, min.[a]	Duration Ratio[b]
Piperocaine hydrochloride (II)	15	—
Quinolizidin-2-yl benzoate hydrochloride (I*b*)	19	1.22 (±0.18)[c]
1-Methylquinolizidin-2-yl benzoate hydrochloride (III*B*)	21	1.38 (±0.27)
2-Methylquinolizidin-2-yl benzoate hydrochloride (IV*B*, isomer *a*)	24	1.60 (±0.43)
2-Methylquinolizidin-2-yl benzoate hydrochloride (IV*B*, isomer *b*)	19	1.24 (±0.43)
3-Methylquinolizidin-2-yl benzoate hydrochloride (V*B*, isomer *a*)	17	1.12 (±0.14)
3-Methylquinolizidin-2-yl benzoate hydrochloride (V*B*, isomer *b*)	25	1.64 (±0.51)

[a] Expressed as the mean duration of activity. [b] This ratio represents the mean ratio of activity. Each ratio used in calculating the mean is equal to the duration of activity of the compound divided by the duration of activity of piperocaine hydrochloride on the same rabbit. [c] 95% confidence limits.

Of these, the physicochemical factors involved in the removal or diffusion of drug from its site of action would appear to be potentially most influential. These factors, however, were not investigated in the current study.

EXPERIMENTAL

UV spectra were determined on a Bausch and Lomb 505 recording spectrophotometer, IR spectra on a Perkin-Elmer 237B grating IR spectrophotometer, and NMR spectra on a Varian Associates A-60 instrument in CCl_4 or $CDCl_3$ using tetramethylsilane as the internal standard. Melting points were determined in capillary tubes in a Thomas-Hoover melting-point apparatus and are uncorrected[1]. Fractional distillation was carried out on a Nester-Faust semimicro spinning band unit rated at 23 theoretical plates. GLC studies were done on a F&M model 500 instrument, equipped with a flame-ionization detector, using 0.63 × 60.9-cm. (0.25 × 24-in.) copper tubes. Preparatory scale gas chromatography (PGC) was attempted with 1.91 × 243.8-cm. (0.75 × 96-in.) copper columns using a thermal conductivity detector. Unless otherwise specified, columns were packed with 10% Carbowax on Chromosorb W. Silica gel for column chromatography (CC) refers to Baker Analyst 3405 activated at 120°, and silica gel for TLC refers to silica gel G[2] activated at the same temperature. The neutral form of alumina[2] for columns was used in all cases.

Ethyl 2-(α-Pyridyl)-2-methylacetate (VII*a*)—Sodium amide (14.4 g., 0.37 mole) in ether (200 ml.) was stirred and treated dropwise with ethyl 2-(α-pyridyl)acetate (VI) (60 g., 0.36 mole) without heating over a 1-hr. period. The mixture was then heated over a steam bath for 15 hr. followed by portionwise addition of methyl iodide (52.5 g., 0.37 mole) and an additional 5-hr. heating period. On cooling, the mixture was filtered, stripped of solvent, and fractionally distilled to yield 45 g. of a yellow liquid, b.p. 87° (1.25 mm.). GLC examination of the liquid indicated that it was a three-component mixture in the ratio of 10:4:1, with the lowest concentration being unreacted VI as shown by potentiation of its peak when added to the liquid. The peak of the intermediate concentration was shown to be ethyl 2-(α-pyridyl)-2,2-dimethylacetate (VII*b*) by again subjecting a small portion of the liquid product to the original alkylating conditions, since this peak in the GLC of the treated mixture was augmented. The peak in greatest abundance was, therefore, assumed to be VII*a* and this was confirmed by the NMR spectrum of the liquid which clearly showed the expected doublet for the methyl group. Further fractional distillation and attempts at PGC failed, although TLC confirmed the GLC results by showing three spots. These spots, developed with petroleum ether (30–60°)–acetone–ether (8:2:1), showed a similar pattern of behavior, although

[1] Microanalyses were determined by the Microanalytical Laboratory, School of Chemistry, University of Minnesota, Minneapolis, Minn., or by the Schwartzkopf Microanalytical Laboratories, Woodside, N. Y.
[2] Supplied by Brinkmann Instruments, Inc., Great Neck, N. Y.

Table III—Analytical Data

Compound	Molecular Formula[a]	M.p. or B.p. (mm. Hg)	Analysis, % Calcd.	Found
III*A*	$C_{10}H_{19}NO \cdot HCl$	259.5–260°	C, 58.38	57.98
			H, 9.80	9.69
III*B*	$C_{17}H_{23}NO_2 \cdot HCl$	254–254.5°	C, 65.90	66.05
			H, 7.90	7.86
IV*A*	$C_{10}H_{19}NO \cdot 1/2H_2O$	74–79°	C, 67.41	67.79
			H, 11.23	11.23
IV*B* (isomer *a*)	$C_{17}H_{23}NO_2 \cdot HCl \cdot 1/2H_2O$	190–191°	C, 64.04	64.31
			H, 7.90	7.89
IV*B* (isomer *b*)	$C_{17}H_{23}NO_2 \cdot HCl \cdot 1/2H_2O$	190–191.5°	C, 64.04	64.48
			H, 7.90	7.86
V*A*	$C_{10}H_{19}NO \cdot HCl \cdot$	190–215°	C, 58.38	58.26
			H, 9.80	9.98
V*B* (isomer *a*)	$C_{17}H_{23}NO_2 \cdot HCl \cdot 1/4H_2O$	282–282.5°	C, 64.97	64.70
			H, 7.72	7.95
V*B* (isomer *b*)	$C_{17}H_{23}NO_2 \cdot HCl \cdot 1/4H_2O$	262–262.5°	C, 64.97	64.67
			H, 7.72	8.18
IX Picrate	$C_{11}H_{21}NO_2 \cdot C_6H_3N_3O_7$	129°	C, 47.65	47.55
			H, 5.65	5.66
XII	$C_{11}H_{17}NO_2$	96° (0.25)	C, 67.66	67.41
			H, 8.78	9.00
XIII	$C_{10}H_{14}NO \cdot I$	124–128°	C, 41.25	41.19
			H, 4.84	4.95

[a] Samples containing water of crystallization were dried in a desiccator following crystallization without the application of heat and then analyzed. Heating the samples in an abderhalden pistol at 110° resulted in loss of weight but, on exposure to the atmosphere, the samples rapidly regained the weight lost.

this system applied to a silica gel column failed to achieve separation. Since attempts at separation proved ineffective, the impure product was used as such for the following reaction.

Ethyl 2-(α-Piperidyl)-2-methylacetate (VIII*a*)—The above product (41 g.), dissolved in glacial acetic acid (100 ml.), was reduced with platinum oxide (1 g.) and hydrogen at 60 p.s.i. in a conventional Parr hydrogenation apparatus for 3 hr. The catalyst was removed by filtration, an additional 0.25 g. of platinum oxide was added, and the hydrogenation was resumed until hydrogen uptake ceased. Following removal of the catalyst by filtration and distillation of most of the acetic acid under reduced pressure, the residue was made alkaline with 15% (w/v) potassium hydroxide solution and extracted with ether (3 × 50 ml.). The dried (anhydrous Na_2SO_4) ethereal extracts were stripped of solvent and fractionally distilled to yield 32 g. of a colorless liquid, b.p. 74° (0.8 mm.). GLC examination of this liquid on a column containing 10% zinc stearate on Chromosorb W showed two peaks of differing magnitude (5:1), whose retention times did not correspond to that of ethyl 2-(α-piperidyl) acetate[3] since the latter showed a third peak when mixed with the product. Further fractional distillation and CC failed to show substantial separation; therefore, this product was used as such in the next step.

Ethyl Piperidyl-2α-propionate-1β-propionate (IX)—The above product (25 g.) was dissolved in absolute ethanol (200 ml.) to which was added freshly distilled ethyl acrylate (15 g., 0.15 mole), and the mixture was allowed to stand for 20 days at room temperature. Stripping off of the solvent and fractional distillation yielded two fractions, the first weighing 4 g., b.p. 75° (0.8 mm.), and the second weighing 33 g., b.p. 130° (0.8 mm.). The lower boiling fraction showed N—H stretching in the IR and corresponded in GLC retention time to the material in lowest concentration in the impure VIII*a* from the previous step. The higher boiling fraction showed no N—H stretching in the IR and was assumed to be the addition product (IX). Its homogeneity was demonstrated by a single GLC peak when a 10% silicone gum rubber on Chromosorb W packing was used. A picrate, m.p. 129°, was prepared in the usual manner.

1-Methyl-2-ketoquinolizidine (X)—Sodium hydride (6 g., 0.25 mole) in a 50.2% mineral oil dispersion was added to dry xylene (120 ml.) under a nitrogen atmosphere followed by 1 ml. of absolute ethanol, and the whole was raised to reflux temperature. To this, IX (27.1 g.) was slowly added over a 1-hr. period followed by 3 hr. of reflux. The reaction mixture was cooled in ice and treated with glacial acetic acid (16 g., 0.27 mole) followed by 50 ml. of water to dissolve the formed sodium acetate. The xylene layer was separated from the aqueous phase, and the latter was extracted with xylene (2 × 25 ml.), the extractions being added to the original xylene phase. The combined xylene extracts were then extracted with 6 *N* hydrochloric acid (3 × 80 ml.), and the combined acid extracts were heated under reflux for 8 hr. On cooling, the acid solution was neutralized with 20% (w/v) potassium hydroxide solution, saturated with potassium carbonate, and extracted with ether (4 × 25 ml.). The dried (anhydrous Na_2SO_4) ether extracts were stripped of solvent and fractionally distilled to yield 10.5 g. of product, b.p. 68.5° (0.6 mm.).

1-Methyl-2-hydroxyquinolizidine (III*A*)—Compound X (10 g.) was dissolved in water (25 ml.) and added to a cool solution of sodium borohydride (0.6 g.) in water (30 ml.); the whole was allowed to stand at room temperature for 5 hr. Ammonium hydroxide solution (10 ml.) was added, and the solution was extracted with hot benzene (4 × 25 ml.). The dried (anhydrous Na_2SO_4) benzene solution was stripped of solvent and distilled to yield 8.5 g. of product, b.p. 94° (0.6 mm.), which was free of C=O absorption in the IR. GLC and TLC (on alumina) examination showed a major component in approximately 85% concentration and two minor components. The solvent system for TLC was petroleum ether (30–60°)–acetone–ether (5:4:1) and was also the solvent system used to effect separation of 500 mg. of the product on an alumina column [100 g. alumina in a 1.91 × 45.7-cm. (0.75 × 18-in.) column]. GLC showed the column-chromatographed material to be devoid of the minor peaks, and its NMR clearly showed a doublet (J = 6 Hz.) in the *C*-methyl region (1.1 p.p.m.) which integrated for three protons. The hydrochloride salt, m.p. 259.5–260°, was prepared by adding an ethereal solution of HCl to a solution of the compound in ether and recrystallization from ethanol–ethyl acetate.

2-Methyl-2-hydroxyquinolizidine (IV*A*)—2-Ketoquinolizidine (9.4 g., 0.06 mole) was dissolved in dry ether (150 ml.) and treated by dropwise addition of methyl lithium (2.7 g., 0.12 mole) in a nitrogen atmosphere accompanied by stirring and reflux. Following the addition, the lithium complex was decomposed with a mixture of hydrochloric acid and water (11.5 ml. : 7 ml.), after which the ether layer was washed successively with water (10 ml.) and diluted hydrochloric acid (5 ml. acid:10 ml. water). The aqueous extracts were alkalinized with potassium carbonate and extracted with ether to obtain an ethereal solution which was dried (anhydrous Na_2SO_4) and stripped of solvent. Fractionation of the residue yielded a fraction, b.p. 123° (7 mm.), with a C=O free spectrum which solidified on standing and, following recrystallization from isopropyl ether and sublimation, gave 1.74 g. (18.5% theory) of IV*A*, m.p. 74–79°. Further attempts at fractionation and crystallization did not improve the product.

4-Methoxy-1-(α-pyridyl)-3-methyl-2-butanol (XII)—This compound was prepared smoothly according to the general method of

[3] Obtained from previous studies in these laboratories.

Walter (19), employing β-methoxyisobutyraldehyde prepared by the Elderfield *et al.* (13) method. The yield was 36.5% of theory, b.p. 96° (0.25 mm.).

2-Hydroxy-2-methyl-1,2-dihydro-3*H*,4*H*-quinolizinium Iodide (XIII)—Compound XII (10 g., 0.05 mole) was refluxed for 15 hr. with concentrated hydriodic acid (225 ml.) and then concentrated to 25 ml. under vacuum. The residue was placed in a separator with 25 ml. of chloroform; to this was added dropwise a saturated solution of potassium carbonate. The liberated oil dropped into the chloroform layer, and this layer was then withdrawn, cooled in a refrigerator overnight, and filtered. The yellow granular material (6 g., 42% of theory) was recrystallized twice from ethanol–ether, m.p. 124–128°.

3-Methyl-2-hydroxyquinolizidine Hydrochloride (V*A*)—Compound XIII (2 g., 0.007 mole), dissolved in ethanol (25 ml.) and combined with platinum oxide (0.15 g.), was subjected to atmospheric pressure hydrogenation until 3 equivalents of hydrogen was absorbed (3 hr.). The catalyst was removed by filtration, the residue was dissolved in water, and the solution was saturated with potassium carbonate. The alkaline solution was extracted thoroughly with ether; the ether extract was dried (CaH_2), filtered, and treated with ethereal hydrogen chloride. Recrystallization of the precipitated salt from ethyl acetate–ethanol yielded 0.6 g. (42% of theory) of white crystals, m.p. 190–215°.

1-Methylquinolizidin-2-yl Benzoate Hydrochloride (III*B*)—This ester was prepared from III*A* by the ester-interchange method of Counsell and Soine (7) and recrystallized from ethyl acetate–petroleum ether (30–60°) to give an 81% yield of white crystals, m.p. 254–254.5°. TLC examination of the ester prior to hydrochloride formation was carried out on alumina using petroleum ether (30–60°)–acetone–ether (5:4:1) and acetone–petroleum ether (30–60°) (1:9). No evidence for a second isomer was noted.

2-Methylquinolizidin-2-yl Benzoate Hydrochloride (IV*B*)—This ester was prepared from IV*A* by the ester-interchange method of Counsell and Soine (7). However, instead of converting the ester directly to the hydrochloride form, it was examined by TLC on alumina using the solvent systems employed for examination of III*B*. In the case of IV*B*, the TLC procedure indicated a mixture of two closely related materials. The acetone–petroleum ether (30–60°) (1:9) system seemed to be the most suitable and was applied to a 100-g. alumina column, 1.91 × 45.7-cm. (0.75 × 18-in.), for separation of the isomeric forms. The fastest moving material (isomer *a*), monitored by the use of Mayer's reagent, was collected, stripped of solvent, dissolved in ether, and treated with ethereal hydrogen chloride. The white crystals so obtained (10% of theory) were recrystallized from ethyl acetate–petroleum ether (65–70°), m.p. 190–191°. The slower moving fraction (isomer *b*), monitored in the same way and similarly converted to the hydrochloride form, was recrystallized from ethanol–ethyl acetate–petroleum ether (65–70°) and yielded 30% of theory of white crystals, m.p. 190–191.5°. A mixed melting point with the hydrochloride of the first fraction showed a 30° depression, and IR spectra of the two hydrochlorides showed significant differences.

3-Methylquinolizidin-2-yl Benzoate Hydrochloride (V*B*)—This ester was prepared from V*A* by the ester-interchange method of Counsell and Soine (7). Instead of converting the free base to the hydrochloride form directly, it was examined using a TLC system of 5% acetone in petroleum ether (30–60°) on alumina. This system was useful for examining both the aminoalcohol (V*A*) and its benzoate (V*B*). Separation of the isomeric esters was achieved on an alumina column as described for 2-methylquinolizidin-2-yl benzoate, and conversion to the hydrochloride form was similarly effected. The faster moving benzoate (isomer *a*), after conversion to the hydrochloride, was obtained in 14% yield, m.p. 282–282.5°, after recrystallization from isopropanol–isopropyl ether. The slower moving benzoate (isomer *b*) was obtained in 10% yield as the hydrochloride and, after recrystallization from isopropanol–petroleum ether (65–70°), gave m.p. 262–262.5°.

Kinetic Studies—One milliliter of a solution (at 37°) of pseudocholinesterase[4] in 0.05 *M* phosphate buffer (pH = 7.23 ± 0.02) was pipeted into a cell (1-cm. pathlength) containing 2 ml. of a solution (at 37°) of the ester to be studied. The cell was then stoppered, shaken to mix the solutions thoroughly, and placed into a jacketed cell holder (at 37 ± 0.02°) contained in a Bausch & Lomb model 505 recording spectrophotometer. The enzyme solution, ester solution, and pipets used to withdraw samples were kept in a constant-temperature bath at 37° before each run. The ester solution was pipeted into a cell, stoppered, and placed in the cell holder 10 min. before mixing. A blank was prepared in the same manner, substituting 2 ml. of the buffer solution for the ester solution. The ester concentrations were calculated to give an absorbance of about 0.9 when mixed with the enzyme solution.

With the instrument set at 240 nm., the decrease in absorption was followed as a function of time. The hydrolysis of II was rapid enough to allow a continuous recording to be made on the Bausch & Lomb 505. The hydrolysis of I*b* was followed on an external Honeywell recorder with a chart drive of 2.54 cm. (1 in.) per minute. The remaining esters were studied by taking single absorbance measurements at definite intervals of time. Each compound was studied at a minimum of two enzyme concentrations, with a minimum of two runs per specific enzyme concentration. All data from these runs were used in preparing absorbance *versus* time plots. The curves obtained were then analyzed to give the relative rates and Michaelis constants listed in Table I.

The observed zero-order rates are directly proportional to the enzyme concentration. To obtain a set of rates that would provide comparison on an equal basis, all rates were corrected to the enzyme concentration used in a standard run.

The standard run employed a 7.925 mg. % enzyme solution which hydrolyzed piperocaine hydrochloride at the rate of 4.80×10^{-6} moles/min. Besides providing a basis for comparison in this study, the standardization, which represents an average of three very close runs, allows other investigators to compare the potency of the particular lot of crystalline pseudocholinesterase they are using to that used in this study. This certainly would be essential if any comparison of rates to those shown in Table I were to be made. Hydrolysis of all the esters by the buffer solution alone at 37° was checked and found negligible for a period of 96 hr.

Pharmacological Testing—One-half milliliter of a 1% solution of the ester salt was introduced into the conjunctival sac from a blunt-tipped syringe and allowed to act upon the cornea for 1 min. before the measurements of duration of anesthesia were started. The compounds tested all induced anesthesia within the 1-min. period, as evidenced by the absence of the wink reflex. This was determined by attempting stimulation of the cornea with a blunt glass rod. The length of time required for the animal to regain the wink reflex was recorded. Six rabbits were used, as a minimum, to determine the duration of activity for each compound. To arrive at a duration ratio, each measurement was divided by the duration of anesthesia for piperocaine hydrochloride on the same rabbit. The mean of these ratios was determined, and the 95% confidence limits of the mean were calculated assuming a normal distribution for the mean and a χ-square distribution for the squares of the deviations (20). During the testing, no corneal irritation was observed and at no time were both eyes subjected to anesthesia simultaneously. One-percent solutions of the ester salts tested were prepared in distilled water immediately prior to use. Young adult white rabbits, male and female, were used for testing. The eyelids were carefully trimmed to remove the eyelashes. Each animal was allowed a 24-hr. rest period between tests.

[4] Carefully weighed crystalline human pseudocholinesterase from Cohn fraction IV-458 (Cutter Laboratories, Berkeley, Calif.) was dissolved in the buffer to yield solutions ranging in concentration from 7.925 mg. % for the hydrolysis of piperocaine hydrochloride to 80.0 mg. % for hydrolysis of the stereoisomers of 2-methylquinolizidin-2-yl benzoate hydrochloride. One milligram of this preparation contains the pseudocholinesterase activity of 5 ml. of normal human plasma.

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Anti-Inflammatory and Liver Sulfhydryl Content-Altering Effects of Certain Nonsteroids in the Rat

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Abstract □ Selected agents were evaluated orally in the male rat for their capacity to prevent carrageenin-induced pedal edema and to alter liver sulfhydryl levels. Indomethacin was the most potent anti-inflammatory agent, followed by tetrabenazine and chlorpromazine. Cryogenine and phenylbutazone were equieffective, with cyproheptadine, aspirin, and sparteine being least potent. Carrageenin-induced pedal edema alone did not lower liver sulfhydryl content. Aspirin elevated the sulfhydryl concentration both in the presence and absence of carrageenin-induced edema, while chlorpromazine, indomethacin, phenylbutazone, cryogenine, tetrabenazine, and sparteine were without significant effect at anti-inflammatory dosages. Cyproheptadine lowered liver sulfhydryl levels, but this was considered to be a nonspecific or toxic effect resulting from the high dosages employed.

Keyphrases □ Anti-inflammatory action—nonsteroids □ Sulfhydryl content alteration, liver—nonsteroids □ Carrageenin-induced pedal edema, rats—anti-inflammatory testing

The work of Marozzi and Malone (1) indicated that a correlation might exist between a compound's ability to protect against stress-induced hepatic sulfhydryl depletion and its potential in inhibiting carrageenin-induced acute pedal edema in the rat. The present study is concerned with a comparison of such activities, using both clinically useful antirheumatic agents (aspirin, phenylbutazone, and indomethacin) and compounds that are potent inhibitors of several models of inflammation in animals but which are not used clinically as anti-inflammatory agents (chlorpromazine and cyproheptadine). Cryogenine (vertine), an alkaloid shown to be equipotent to phenylbutazone in both carrageenin-induced acute inflammation and in adjuvant-induced (*Mycobacterium butyricum*) chronic inflammation (2), as well as sparteine and tetrabenazine, was also investigated. The latter two agents bear some structural resemblances to possible degradation products of cryogenine.

EXPERIMENTAL

Carrageenin-Induced Pedal Edema—Adult male rats of the Wistar strain[1] were allowed to equilibrate in air-conditioned quarters for at least 1 week after receipt of shipment. Animals were allowed free access to Purina laboratory chow and tap water at all times. Experimental procedures used were similar to those described by Van Arman *et al.* (3). On the day of the experiment, rats were removed from food at −1 hr. and dosed orally to avoid the parenteral "counterirritant" effect. The test drugs were administered either dissolved or suspended in 0.25% agar solution at a constant dosage volume of 10 ml./kg. Control animals received the agar vehicle alone. Plantar injections of 0.1 ml. of aged, 1% carrageenin were made into the rat hind paw at 0 hr. The paw volume of all animals was recorded plethysmographically immediately afterward and again at +3 hr. (peak edematous response) just prior to sacrifice and removal of liver samples. On the basis of preliminary testing, a predictable high and a predictable low effective oral anti-inflammatory dose were selected for each experimental compound[2].

Each experimental run consisted of 60 animals divided into six interrelated groups: Group I, agar control (10 ml./kg. of 0.25% aqueous agar orally at −1 hr.); Group II, carrageenin control (agar solution at −1 hr. followed by plantar injection of carrageenin at 0 hr.); Group III, low drug control (at −1 hr. the lower dosage of the test drug shown by preliminary experimentation to be effective orally in reducing carrageenin-induced acute inflammation); Group IV, high drug control (the higher dosage of the test drug); Group V, low drug experimental (the lower oral dose of the drug being evaluated at −1 hr., followed by plantar injection of carrageenin at 0 hr.); and Group VI, high drug experimental (the higher dosage of the test drug followed by carrageenin injection).

[1] Obtained from E. G. Steinhilber Co., Oshkosh, Wis.

[2] Sources of the test agents were: aspirin, Merck and Co., Inc., Rahway, N. J.; indomethacin and cyproheptadine HCl, Merck Sharp & Dohme Research Institute, West Point, Pa.; phenylbutazone, Geigy Pharmaceuticals, Ardsley, N. Y.; chlorpromazine HCl, Smith Kline & French Labs., Philadelphia, Pa.; tetrabenazine, Hoffmann–La Roche, Inc., Rahway, N. J.; sparteine SO_4, K and K Labs., Inc., Plainview, N. Y.; and cryogenine, Dr. A. E. Schwarting, Division of Pharmacognosy, University of Connecticut, Storrs, Conn. The cryogenine (mol. wt. = 435.53) used in this study is an alkaloid isolated from *Heimia salicifolia* Link and Otto, and not the trade name product Cryogénine (phenylsemicarbazide, mol. wt. = 151.2), a specialty of Lumière of Lyons, France, and distributed by Laboratoires Sarbach of Châtillon, France.