Table II. Urinary Metabolites of [^{14}C]I in the Dog

R_3, R_2, $S(CH_2)_3-N-CH_3$, R_1, $NHCONHCH_3$

Metabolite	R_1	R_2	R_3	% of dose
1	CH_3			6.8
2	H			8.1
3	CH_3	0		35.6
4	CH_3	0	0	3.3
5	OH			2.7
6	CH_3		0	2.3
				58.8

of methylation of the molecule of 283 amu that arises by the thermal elimination of oxygen from the molecule of 299 amu.

An ion is found in the CIMS of metabolites **4** and **6** at *m/e* 227 (Figure 4) that is not found for these metabolites in the EIMS. On the other hand, there is an ion at *m/e* 211 in the CIMS of I that corresponds to the protonated ion at *m/e* 210 in the EIMS. It is reasonable to assume that the ion at *m/e* 227 of metabolites **4** and **6** is the oxygenated analog of the ion at *m/e* 211 for I, a consequence of the formation of a sulfoxide. A small ion is present for **4** at *m/e* 239 that corresponds to the MH^+ of the Cope elimination product, which was absent in the EIMS of **4**; the ion at *m/e* 62 corresponds, again, to Me_2NOH. Interestingly, thermolysis occurs by both the loss of O and the elimination of Me_2NOH, but more readily by the former, since ions are found at *m/e* 86 (for the loss of O) and at *m/e* 62 and 239 (for the Cope elimination). Metabolites **4** and **6**, but none of the others nor I, exhibited a band in the ir spectra that was indicative of the presence of the SO function. A summary of the metabolites of I isolated and identified from dog urine is shown in Table II.

Discussion

Metabolic products resulting from the N-oxidation or N-hydroxylation of methylamino alkyl side chains can be difficult to identify. Thus, the *N*-oxide function is not readily detected by uv or ir spectroscopy. Nmr spectrometry of these compounds does produce characteristic downfield shifts of neighboring protons, but the significance of these shifts is difficult to interpret in the absence of spectra of authentic *N*-oxides. Mass spectrometry, however, can provide diagnostic information on the presence of *N*-oxy- or *N*-OH-containing metabolites if certain fragments characteristic of the Cope elimination are found, even if the M^+ or MH^+ are not observed. The presence of such olefinic fragment ions can be further studied in the EIMS or CIMS by varying the temperature of the source.

The thermolysis of *N*-oxides by gas chromatography has been employed as a technique to detect the presence of *N*-oxides of chlorpromazine.[5] Unlike chlorpromazine, I bears a dimethylaminopropyl*thio* side chain. Apparently, the presence of the S atom does not influence the formation of olefinic products from a simple *N*-oxy or *N*-OH compound, but when an SO function is present together with NO, the tendency for the Cope elimination to occur is markedly reduced. Thus, the absence of olefinic products does not rule out the presence of a metabolite due to N-oxidation, particularly if a neighboring SO function is also present. In conclusion, CIMS appears to be a very useful supplementary technique for determining the structure of those compounds that produce weak or nonexistent fragment ions in the high-mass region of the mass spectrum.

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References

(1) D. B. Evans, R.J. Laffan, and R. J. Lee, *Pharmacologist,* **12,** 573 (1970).
(2) A. C. Cope and E. R. Trumbull, *Org. React.,* 11, 317 (1960).
(3) B. Loev and M. M. Goodman, *Chem. Ind. (London),* 2026 (1967).
(4) G. W. A. Milne, T. Axenrod, and H. M. Fales, *J. Amer. Chem. Soc.,* **92,** 5170 (1970).
(5) J. C. Craig, N. Y. Mary, and S. K. Roy, *Anal. Chem.,* **36,** 1142 (1964).

Conformational Aspects of Systems Related to Acetylcholine. 4. The Syntheses of the *dl*-2-Dimethylamino-*trans*-decalin Methiodides

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In order to ascertain if the muscarinic activity of ACh analogs in the *trans*-decalin series was due only to the quaternary function and if the inability of certain of these analogs to act as substrates for AChE was due to selective inhibition by the quaternary group, the axial and equatorial 2-dimethylamino-*trans*-decalin methiodides were prepared and tested. Neither of the compounds prepared inhibited AChE to the degree necessary to account for the inability of certain of the decalin ACh analogs to act as substrates. The pharmacologic results indicate that 4° N alone was not responsible for the action observed with the decalin ACh analogs but that the activity was due to conformational relationship between both ACh pharmacophoric functions.

Earlier reports[1,2] from this laboratory indicate that a conformational preference exists at the various receptor sites for acetylcholine (ACh). Marked differences were found in the rigid analogs of ACh in the *trans*-decalin[1] and *trans*-decahydroquinoline[2] series with respect to acetyl cholinesterase (AChE) substrate activity and muscarinic activity. The possibility existed that the muscarinic activity was due only to the quaternary function and that the inability of certain isomers to act as substrates for AChE was due to selective inhibition by the quaternary group with the

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acetyl function having little or no effect.

In an effort to study further the conformational requirements of the ACh receptor sites, the syntheses and preliminary testing of 2(a)-dimethylamino-*trans*-decalin methiodide (1) and 2(e)-dimethylamino-*trans*-decalin methiodide (2) was undertaken.

$^{+}NMe_3 \cdot I^-$ H

1 2

trans-Decalin 2,3-oxide[3] (3) was reduced with LAH to yield *trans*-2(a)-decalol (4), which was converted to the corresponding tosylate 5. Displacement of the tosylate group using Me_2NH afforded 2(e)-dimethylamino-*trans*-decalin which was isolated as the HCl salt 6. Quaternization of 6 with MeI yielded the desired 2(e)-dimethylamino-*trans*-decalin methiodide (2).

3

4, R = H
5, R = $-SO_2C_6H_4CH_3$

6

Reduction of *trans*-2-decalone with LAH afforded pure *trans*-2(e)-decalol (7), which was converted to the corresponding tosylate 8. Treatment of 8 with Me_2NH at elevated temperature and pressure afforded the desired 2(a)-dimethylamino-*trans*-decalin which was isolated as the HCl salt 9. Quaternization of 9 with MeI afforded the desired 2(a)-dimethylamino-*trans*-decalin methiodide (1).

7, R = H
8, R = $SO_2C_6H_4CH_3$

9

Results

Enzymatic Results. The kinetics of the hydrolysis of ACh in the presence of electric eel AChE and horse serum cholinesterase (ChE) were studied at pH 7.2 and 25° with and without added inhibitor 1 and 2 by the method described previously.[4] The Michaelis-Menten parameters $V_{max}(app)$ and $K_m(app)$ are presented in Table I together with the inhibitor constants, K_i, calcd for reverstible, competitive inhibition.† It will be noted that within experimental error the maximum veloxity remains unchanged in the presence of inhibitor but that $K_m(app)$ increases with increasing inhibitor concn, behavior characteristic of competitive reversible inhibition.

For a given enzyme, the K_i values for each of the 2 inhibitors, 1 and 2 are clearly of the same order of magnitude; within experimental error they are, in fact, equal. The results thus indicate that the $N^+(CH_3)_3$ group does not selectively (depending on its axial or equatorial conformation) bind to the enzyme. K_i values for each of the isomeric *dl*-3-dimethylamino-*trans*-2-decalol methiodides obtained in our laboratories are also of the same order of magnitude and within experimental error of each other for a given enzyme ($4\text{-}12 \times 10^{-5}$ *M* and $1\text{-}8 \times 10^{-6}$ *M* for eel AChE and horse serum ChE, respectively)‡ and confirm the above results.

Data reported previously[4] show that the relative normalized maximum velocities for the hydrolysis of compd **10-13** with eel AChE are 1.00: $\leqslant$ 0.003:§ 0.014: 0.013, respectively.

10 11

12 13

For selective inhibition of the enzyme by the equatorial quaternary function to be the only cause of the $\geqslant$300-fold reduction in reactivity of **11** over **10**, and assuming no inhibitory binding by the axial quaternary function in **10** (*i.e.*, a K_i of ∞), the K_i for **11** would have to be $\leqslant 1 \times 10^{-6}$ *M*. The difference in K_i values observed for **1** and **2** is clearly much smaller and therefore cannot account for the difference in reactivity of **10** and **11**. Furthermore, if selective binding were to explain this reactivity difference, a similar difference would be expected for **12** and **13** (assuming no effect due to the change in OAc orientation). In fact, however, the latter 2 compds exhibit the same substrate reactivity. The relative reactivities of **10-13** are best accounted for in terms of dihedral angle between the 2 functional groups.[4]

Pharmacolgic Results. Compds **1** and **2** when assayed on the guinea pig ileum caused weak contraction in a dose of 100 μg to 1 mg. This was more than 10 times less than the effect observed with ACh. When low concns of ACh were used in conjunction with 100 μg/ml of **1** or **2**, the contraction observed was potentiated over that observed with ACh alone. At higher concn of ACh an antagonist effect was observed with both **1** and **2** at 100 μg/ml. Compd **1** was slightly more effective than **2** and its effects were longer lasting than the equatorial isomer **2**.

In the chicken *musculus biventer cervicis* both **1** and **2** caused contraction at a dose of 1 mg/ml which was comparable to 0.03 mg/ml of nicotine. The maximal contraction observed with **1** and **2** was only 50% of the maximal contraction observed with nicotine in the same preparation. At a dose of 100 μg/ml both compds blocked the effects of nicotine noncompetitively. The axial isomer **1** was more effective than **2** and its effect was still observed after the preparation had been washed twice. The effect of **2** was

†For reversible, competitive inhibition, the reaction velocity is given by $v = (V_{max} \cdot s)/\{s + K_m[1 + (i/K_i)]\} = (V_{max} \cdot s)/\{s + [K_m(app)]\}$, where *s* is the substrate concentration, *i* is the inhibitor concentration, and K_i is the dissociation constant for the enzyme-inhibitor complex.[5]

‡Details of these and other studies will be reported in a later paper.

§Further reductions in reaction rate cannot be determined accurately by our methods.

Table I. Michaelis-Menten Parameters and Inhibitor Constants for the Hydrolysis of ACh with Eel AChE and Horse Serum ChE in the Presence and Absence of 2(a)- and 2(e)-dimethylamino-*trans*-decalin Methiodide Inhibitors at pH 7.2 ± 0.1 and 24.90 ± 0.05[a]

Enzyme	Inhibitor	Conc, mM	K_m(app), mM	V_{max}(app)[b]	K_i, mM[c]
AChE	H; $^{+}NMe_3 \cdot I^-$	0.0000	0.127 ± 0.015	1.81 ± 0.04	
		0.0005	0.14 ± 0.01	1.91 ± 0.06	0.01 ± 0.02
		0.0052	0.153 ± 0.009	1.89 ± 0.04	0.02 ± 0.02
	$\overset{+}{N}Me_3 \cdot I^-$; H	0.0000	0.127 ± 0.015	1.81 ± 0.04	
		0.0005	0.12 ± 0.01	1.83 ± 0.05	
		0.0052	0.175 ± 0.006	1.87 ± 0.02	0.014 ± 0.006
ChE	H; $^{+}NMe_3 \cdot I^-$	0.0000	0.63 ± 0.04	0.67 ± 0.02	
		0.0005	0.66 ± 0.05	0.66 ± 0.24	0.01 ± 0.03
		0.0052	0.99 ± 0.16	0.63 ± 0.07	0.009 ± 0.006
	$\overset{+}{N}Me_3 \cdot I^-$; H	0.0000	0.63 ± 0.04	0.67 ± 0.02	
		0.0005	0.81 ± 0.07	0.73 ± 0.05	0.002 ± 0.001
		0.0052	1.4 ± 0.3	0.61 ± 0.10	0.004 ± 0.002

[a]Error limits are standard deviations. [b]Experimental V_{max} (app) values have been normalized by dividing by the enzyme concentration in μM units/ml, and thus have units of mM min^{-1} μM unit^{-1} ml. [c]For dissociation of the enzyme inhibitor complex for competitive, reversible inhibition.

present after one wash but not after a second bath.

Both **1** and **2** depressed blood pressure in the rat at doses of 1 to 4 mg/kg. The axial isomer **1** was more effective than the equatorial isomer **2**. This effect was abolished by the ganglionic blocking agent hexamethonium administered at 30 mg/kg. When atropine was administered at 1 mg/kg it did not change the effect on the blood pressure observed with **1** and **2**. In some experiments an increased effect was observed when atropine was administered simultaneously.

From the above experiments it can be concluded that **1** and **2** have no specific muscarinic effect and they affect the nicotinic receptors noncompetitively. The axial 4° compd **1** is somewhat more effective and has a more prolonged action *in vitro* than the equatorial isomers **2**.

These results indicate the muscarinic action observed with **10** in the earlier work[1] was not a function of the 4° N alone but was a function of the conformation of both of the ACh pharmacophoric functions.

Experimental Section#

trans-**Decalin 2,3-Oxide (3).** The procedure used is modified from that of Hibbert and Burt.[3] To a cold (0°) soln of *m*-chloroperbenzoic acid (30.2 g, 0.15 mmole) in $CHCl_3$ (400 ml) was added cautiously *trans*-Δ^2-octalin[6] (18.7 g, 0.137 mole) in $CHCl_3$ (50 ml). The reaction mixt was stirred at 0° for 2 hr after which the excess peracid was destroyed by addn of a soln of 10% Na_2SO_3. The organic layer was washed with 5% NaOH soln, H_2O, and satd NaCl soln and dried ($MgSO_4$). The solvent was removed and the residual oil distd affording 18.5 g (80%) of **3**: bp 50-52° (0.5 mm); 1.4832 [lit.[1] bp 136° (730 mm), n^{22}D 1.4828].

trans-**2(a)-Decalol (4).** To a soln of LAH (2.25 g, 0.06 mole) in 100 ml of anhyd Et_2O previously refluxed for 1 hr was added dropwise a soln of epoxide **3** (18.5 g, 0.12 mole) in 100 ml of Et_2O. The reaction mixt was heated at reflux for 2 hr after which "wet" Et_2O followed by a soln of 2% HCl was added dropwise to decomp the excess LAH. The aqueous layer was exted several times with Et_2O and the combined Et_2O fractions were washed with 2% HCl soln, H_2O, and satd NaCl soln and dried ($MgSO_4$). The Et_2O was removed and crystn (hexane) afforded 15.5 g (84%) of **4**: mp 52-54° (lit.[7] mp 53°).

trans-**2(a)-Decalyl Tosylate (5).** To alcohol **4** (6.0 g, 0.04 mole) in 45 ml of anhyd C_5H_5N was added TsCl (7.50 g, 0.040 mole). The reaction mixt was cooled at 10-15° for 18 hr after which excess H_2O was added. The resulting ppt was collected by filtration and dried, and recrystn (hexane) afforded 6.2 g (53%) of **5**: mp 110-112°; nmr ($CDCl_3$) δ 7.81-7.23 (m, 4 H, aromatic), 4.81 (m, 1 H, $w_{1/2}$ = 7.0 Hz, C-2 CH), 2.43 (s, 3 H, aryl CH_3). *Anal.* ($C_{17}H_{24}O_3S$) C, H.

2(e)-Dimethylamino-*trans*-decalin Hydrochloride (6). To tosylate **5** (4.0 g, 0.013 mole) in a steel reaction vessel cooled in a Dry Ice-Me_2CO bath was added approx 50 ml of Me_2NH. The vessel was sealed and heated at 135-140° for 20 hr. The vessel was cooled to Dry Ice-Me_2CO temp and opened and the excess Me_2NH removed. The residue was dissolved in Et_2O and the Et_2O washed with 5% NaOH soln, H_2O, and satd NaCl soln and dried ($MgSO_4$). The solvent was removed to yield a colorless oil. Formation of the HCl salt and recrystn (EtOH-Et_2O) afforded 1.21 g (43%) of **6**: mp 228-229°; nmr (CD_3OD) δ 3.26 (m, 1 H, C-2 CH), 2.83 (s, 6 H, CH_3). *Anal.* ($C_{12}H_{24}NCl$) C, H, N.

2(e)-Dimethylamino-*trans*-decalin Methiodide (2). To 2(e)-dimethylamino-*trans*-decalin (0.670 g, 3.69 mmoles) at 0° was added MeI (5 ml) and the reaction mixt stirred in the cold for 30 min, then at 25° for 2 hr. Et_2O was added and the resulting ppt collected by filtration. Recrystn (EtOH-EtAc) afforded 1.01 g (84%) of **2**: mp 277-278°; nmr (CD_3OD) δ 3.55 (m, 1 H, C-2 CH), 3.16 (s, 9 H, CH_3). *Anal.* ($C_{13}H_{26}IN$) C, H, N.

trans-**2(e)-Decalol (7).** *trans*-2-Decalone[8] (45.0 g, 0.30 mole) was reduced with LAH (5.7 g, 0.15 mole) according to the procedure for **4**. Recrystn (hexane) afforded 40.1 g (87%) of **7**: mp 72-73° (lit.[7] 75°).

trans-**2(e)-Decalyl Tosylate (8).** A soln of **7** (6.54 g, 0.0425 mole in 40 ml of anhyd C_5H_5N was treated with TsCl (15.8 g, 0.083 mole) as described in the synthesis of **5** to yield after recrystn (hexane) 11.1 g (84%) of **8**: mp 62-63.5°; nmr ($CDCl_3$) δ 7.92-7.22 (m, 4 H, aromatic), 4.45 (m, 1 H, $w_{1/2}$ = 17 Hz, C-2 CH), 2.43 (s, 3H, aryl CH_3). *Anal.* ($C_{17}H_{24}O_3S$), C, H.

2(a)-Dimethylamino-*trans*-decalin Hydrochloride (9). Tosylate **8** (6.0 g, 0.02 mole) was treated with Me_2NH according to the procedure for **6** to yield after formation of the HCl salt and recrystn (EtOH-Et_2O) 2.52 g (58%) of **9**: mp 232-233°; nmr (CD_3OD) δ 3.43 (m, 1 H, $w_{1/2}$ = 7.5 Hz, C-2 CH), 2.92 (s, 6 H, CH_3).

2(a)-Dimethylamino-*trans*-decalin Methiodide (1). To 2(a)-

#Melting points were obtained on a calibrated Thomas-Hoover Uni-Melt and are corrected. Ir data were recorded on Beckman IR-10 and Perkin-Elmer 421 spectrophotometers, and nmr data on a Varian Associates Model A-60 A spectrophotometer (Me_4Si). Microanalyses were conducted by Widwest Microlab, Inc., Indianapolis, Ind., on an F and M Model 185 C, H, N Analyzer, University of Kansas, and Microanalytical Laboratory, NIH, Bethesda, Md. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

dimethylamino-*trans*-decalin (0.816 g, 4.45 mmoles) at 0° was added MeI (5 ml) and the reaction mixt stirred in the cold for 30 min, then at 25° for 4 hr. Et_2O was added and the resulting ppt collected by filtration. Recrystn (EtOH-EtAc) afforded 1.20 g (86%) of 1: mp 258-259°; nmr (CD_3OD) δ 3.84 (m, 1 H, C-2 CH), 3.18 (s, 9 H, CH_3). *Anal.* ($C_{13}H_{26}IN$) C, H, I, N.

Enzyme Kinetics. The kinetic measurements were performed as described previously.[4] The concn of AChE (Sigma Chemical Co. electrical eel Type III) was 0.094 μM units/ml of salt soln. The concn of ChE (Sigma Chemical Co., from horse serum, Type IV) was 0.41 μM units/ml. One μM unit hydrolyzes 1 μmole of ACh/min at pH 8.0 and 37°. The salt soln was 0.16 M and 0.002 M in NaCl and $MgCl_2$, respectively, and contained 50 mg of bovine serum albumin per l. Sigma acetylcholine chloride was used in 6 different concns ranging from 3×10^{-4} to 10×10^{-4} M for each detn of K_m and V_{max}. Inhibitor concns were 5×10^{-7} and 5.2×10^{-6} M. Measurements were performed by titrating for liberated AcOH under a stream of N_2 using an automatic pH Stat assembly at pH 7.2 ± 0.1 and 24.90 ± 0.05°. Titrant was 0.0100 M NaOH. Deionized H_2O was used throughout. Raw data, consisting of a chart trace of per cent of full buret *vs.* time was fitted by an iterative least-squares technique directly to the Michaelis-Menten equation.

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References

(1) E. E. Smissman, W. L. Nelson, J. L. Day, and J. B. LaPidus, *J. Med. Chem.*, 9, 458 (1966).
(2) E. E. Smissman and G. S. Chappell, *ibid.*, 12, 432 (1969).
(3) H. Hibbert and P. Burt, "Organic Syntheses," Collect. Vol. I., Wiley, New York, N. Y., 1941, p. 494.
(4) W. F. Stephen, Jr., E. E. Smissman, K. B. Schowen, and G. W. Self, *J. Med. Chem.*, 15, 241 (1972).
(5) M. S. Dixon and E. C. Webb, "Enzymes," 2nd ed, Academic Press, New York, N. Y., 1964, pp 318-319.
(6) W. S. Johnson, V. J. Bauer, J. L. Margrave, M. A. Grisch, L. H. Dreger, and W. N. Hubbard, *J. Amer. Chem. Soc.*, 83, 606 (1961).
(7) W. G. Dauben, R. C. Tweit, and C. Mannerskantz, *ibid.*, 76, 4420 (1954).
(8) E. E. Smissman and W. H. Gastrock, *J. Med. Chem.*, 11, 860 (1968).

Notes

Spirans. 19. Spirans with Functional Groups[1,†]

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In previous studies,[2,3] it was found that alicyclic spirans A (R = NH_2) substituted in the 2 or 3 position displayed interesting pharmacological properties such as analgetic, analeptic, and local anesthetic actions. In another investigation, a group of N-substituted azaspirans B (R = alkyl, dialkylaminoalkyl, etc.) was synthesized and tested for cytotoxic effects. A number of these compds were found to be highly active.[3,4]

R NR R' R" $(CH_2)_X$ $(CH_2)_Y$

A B C

We would now like to report the preparation and testing of some new substituted spirans of type C where R" is not an amino group but contains a variety of functional groups such as carboxy and higher homologous acids and their amine derivatives. The general synthetic steps are shown in Schemes I and II in which for simplicity the synthesis of only unsubstituted spirans are given. Many of the spirans prepared have substituents R' in the first ring of C such as Me, *gem*-Me_2, and CF_3 groups. Besides the preparation of this new class of spiro acids and their intermediates (Table I, **1-18**), we were interested in preparing various amides and amines of these spiro acids in which the spiran nucleus was separated from the N moiety by 1, 2, or 3 C atoms.

Scheme I

CH_2CH_2Br, $(CH_2)_X$, $(CH_2)_YBr$ + $H_2C(COOC_2H_5)_2$ —NaOEt, EtOH→

X = 1 or 2
Y = 1 or 2

I ($COOC_2H_5$, $COOC_2H_5$) —1. alc KOH, 2. H^+→ II (COOH, COOH) —Δ, $-CO_2$→ III (COOH) —NaN_3, H_2SO_4→ IV (NH_2)

III —1. $(COCl)_2$, 2. R_2NH→ VII ($CON R_2$), R = alkyl —LAH→ VIII (CH_2NR_2)

III —1. $(COCl)_2$, 2. ROH→ V (COOR), R = alkyl —LAH→ VI (CH_2OH)

The key compds from which the desired compds could be prepared were spiran carboxylic acid III and spiran acetic acid XII. The acids of type III were synthesized by

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