phorus pentoxide *in vacuo* until it reached a constant weight. A loss in weight of 7% was observed. The results of the analysis agreed with the calculated values for the free base of 9-sarcosine oxytocin.

Anal. Calcd. for $C_{44}H_{68}N_{12}O_{12}S_2$: C, 51.8; H, 6.71; N, 16.5. Found: C, 52.0; H, 6.87; N, 16.2.

Biological Activity of 9-Sarcosine Oxytocin.—9-Sarcosine oxytocin was assayed for 5 biological effects and its activity was compared to that of oxytocin assayed under conditions which were as nearly the same as possible. The approximate activity of 9-sarcosine oxytocin in terms of units per mg. measured by the depression of blood pressure in the chicken[18] is 9, by the increase in mammary gland pressure in the lactating rabbit,[19,20] 55, by the contraction of the isolated rat uterus in the absence of magnesium,[17,18] 36, by the increase in blood pressure of the rat,[21] 0.01, and by the inhibition of diuresis in the hydrated rat,[22,23] 0.1. 9-Sarcosine oxytocin possesses about 2% of the avian depressor activity, 12% of the milk-ejecting activity, 5% of the oxytocic activity, 0.2% of the pressor activity and 2% of the antidiuretic activity of oxytocin.

Acknowledgments.—The authors are indebted to Mr. Joseph Albert for carrying out the microanalyses, to Mrs. Lorraine S. Abrash for performing the amino acid analyses, and to Miss Maureen O'Connell, Miss Shirley R. Pomeroy, Miss Lenore McAteer, Mr. Hans Holzhauser, Mr. Harald Aanning, and Mr. David N. Reifsnyder for carrying out the bioassays. Appreciation is also expressed to Dr. W. Y. Chan for helpful advice in regard to the bioassays.

Isomeric 2-Acetoxytropine Methiodides

S. ARCHER, A. M. LANDS, AND T. R. LEWIS

Sterling-Winthrop Research Institute, Rensselaer, New York

Received November 30, 1961

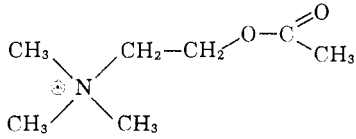
The α- and β-isomers of 2-tropanyl acetate methiodide have been prepared and the L-forms examined for muscarine- and nicotine-like action in biological preparations. It has been established that the α-configuration favors muscarinic action and that the β-configuration favors nicotinic action. Comparisons of various results obtained with 3-tropanyl derivatives characterized by blocking action also support the concept that these receptors are structured to favor the *transoid* form for action at the muscarine-sensitive sites and the *cisoid* form for the nicotine-sensitive sites.

Introduction

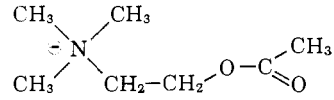
The fact that acetylcholine shows both muscarinic and nicotinic effects suggests that there are subtle but fundamental differences in

the nature of the responding receptor sites. The effects of structural modification on the biological activity of acetylcholine-like agents has been extensively explored. Most of these investigations have been concerned with the effect of varying the size of the cationic head and with the replacement of the ester portion of the molecule with chains of differing lengths and chemical composition.[1]

Schueler[2] has drawn attention to the importance of conformational lability and concluded that "flexibility of the acetylcholine molecule is an important attribute to be considered in collating structure with pharmacologic activity." The flexibility of acetylcholine has been further emphasized by the recent X-ray investigations of Sörum[3] who found that in the crystal acetylcholine bromide exists in two forms, the extended or *transoid* I, and the "ring" or *cisoid* II, and suggested that this ability accounted in some unspecified way for some of its pharmacological activities.

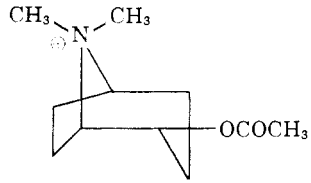


I (*transoid*)

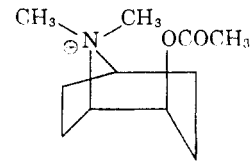


II (*cisoid*)

It occurred to us that the muscarinic properties of acetylcholine were associated with the *transoid* form I and the nicotinic effects were associated with the *cisoid* form II. To test this hypothesis we prepared 2α- and 2β-tropanyl acetate methiodides III and IV. The *transoid* III and *cisoid* IV forms corresponding to I and II are configurationally frozen and the relevant interatomic distances are essentially identical with those found in the two conformations of acetylcholine.



III



IV

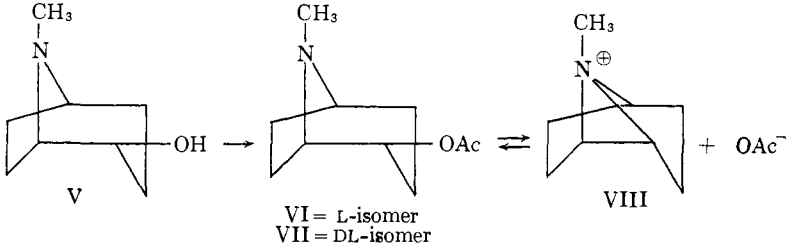
It was expected that III would be more muscarinic than IV and conversely IV would be more nicotinic than III.

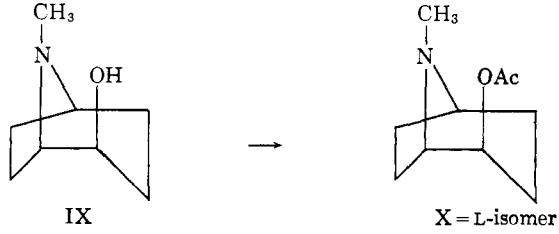
(1) For a recent review see C. J. Cavallito and A. P. Gray in "Progress in Drug Research, Vol. II, E. Jucker, Ed., Birkhäuser Verlag, Basel, 1960, p. 135.

(2) F. W. Schueler, *J. Am. Pharm. Assoc.*, **45**, 197 (1956).

(3) H. Sörum, *Acta Chem. Scand.*, **13**, 345 (1959).

Chemistry.—The preparation of L-2α-tropanol (V) and L-2β-tropanol (IX) from cocaine was described previously.[4] The required acetate VI was obtained when V was allowed to react with acetic anhydride at room temperature overnight. When V was refluxed for 3 hours in acetic anhydride two isomeric 2-tropanyl acetates were formed. One of these, VI, was optically active and the other, VII, was racemic. Prolonged reflux resulted in the formation of the racemic isomer only.[5]





It is believed that this racemization must proceed first to give VI which then afforded the symmetrical ion VIII. The reverse reaction of VIII with acetate ion yielded VII stereospecifically. This is supported by the observation that IX under similar conditions yielded X with no sign of racemization. The "wrong" stereochemistry of 2β-tropanol precludes the formation of VIII.

Quaternization of VI, VII and X with methyl iodide furnished the required salts III, DL-III, and IV, respectively.

Experimental

L-2α-Tropanyl Acetate.—A. Acetic Anhydride at Room Temperature.—One-half gram of L-2α-tropanol was dissolved in a solution of 5 ml. of acetic acid and 5 ml. of acetic anhydride containing one drop of 60% perchloric acid. The reaction mixture was allowed to stand overnight. The excess solvents were removed *in vacuo* and ice and 10% sodium hydroxide solution was added to the residue. The mixture was extracted with ether and the ether layer was dried

(4) M. R. Bell and S. Archer, *J. Am. Chem. Soc.*, **82**, 4642 (1960).

(5) Preliminary communication: S. Archer, T. R. Lewis, M. R. Bell and J. W. Schulenberg, *ibid*, **83**, 2386 (1961).

over magnesium sulfate and then evaporated to leave an oil. This was dissolved in dry ether and converted to the hydrochloride with alcoholic hydrogen chloride. The solid (300 mg.) was collected and recrystallized from acetone; m.p. 233–235.5°; $[\alpha]^{25}D + 39.7°$ (*c* 1.5% in H_2O).

Anal. Calcd. for $C_{10}H_{18}ClNO_3$: C, 54.66; N, 8.26; Cl, 16.14; N, 6.38. Found: C, 54.94; H, 8.58; Cl, 16.14; N, 6.94.

The methiodide melted at 261–262° after recrystallization from ethanol.

Anal. Calcd. for $C_{11}H_{20}INO_2$: C, 40.62; H, 6.20; N, 4.31. Found: C, 40.31; H, 6.22; N, 4.46.

B. Acetic Anhydride at Reflux for 3 Hr.—A solution of 1 g. of L-2α-tropanol in 5.0 ml. of acetic anhydride was refluxed for 3 hr. and taken to dryness *in vacuo* leaving a residue which was dissolved in dry ether and filtered (Supercel). This clear solution was treated with alcoholic hydrogen chloride and the salt that separated was crystallized from acetone–dry ether to furnish 1.1 g. of a mixture of hydrochlorides, m.p. 197–212°. Fractional crystallization from acetone and acetone–ether yielded three crops of crystals; the first of m.p. 210–217°, and the second of m.p. 217–220° were somewhat impure specimens of the L-hydrochloride; a third crop, m.p. 195–197°, proved to be the racemic hydrochloride identical with the sample prepared immediately below.

C. Acetic Anhydride at Reflux for 18 Hr.—One gram of the tropanol was refluxed in 10 ml. of acetic anhydride for 18 hr. and then the mixture was processed as described above to furnish 1.1 g. of crude hydrochloride, m.p. 168–178°. After one recrystallization from acetone the salt melted at 195–197°, unchanged after further crystallization from a large volume of tetrahydrofuran; $[\alpha]^{25}D$ 0.0° (*c*, 1.5%, H_2O).

Anal. Calcd. for $C_{10}H_{18}ClNO_3$: C, 54.66; H, 8.26; Cl, 16.14; N, 6.38. Found: C, 54.36; H, 8.00; Cl, 16.28; N, 6.27.

The methiodide melted at 263–265° (dec.) after recrystallization from ethanol.

Anal. Calcd. for $C_{11}H_{20}INO_2$: C, 40.62; H, 6.20: N, 4.31. Found: C, 40.19; H, 6.47, N, 4.33.

L-2β-Tropanyl Acetate.—A mixture of 1 g. of L-2β-tropanol was refluxed for 3 hr. in 5 ml. of acetic anhydride and then taken to dryness *in vacuo*. The residue was dissolved in ether and the solution was treated with alcoholic hydrogen chloride. The salt that separated melted at 219–222° after one crystallization from acetone, $[\alpha]^{25}D + 3.5°$ (*c*, 1.5, H_2O).

Anal. Calcd. for $C_{10}H_{18}ClNO_3$: C, 54.66; H, 8.26; N, 6.34; Cl, 16.14. Found: C, 54.94; H, 8.29; N, 6.17: Cl, 16.38.

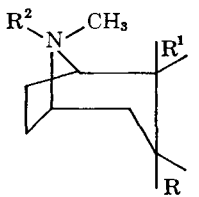
The methiodide melted at 223–228° after crystallization from ethanol.

Anal. Calcd. for $C_{11}H_{20}INO_2$: C, 40.62; H, 6.20; N, 4.31. Found: C, 40.94; H, 5.95; N, 4.43.

Pharmacology.—The pharmacological results obtained have been summarized in Table I. The muscarinic action of compound III is weak when compared with that of acetylcholine (ACh). A concentration of 1:50,000 to 1:100,000 induced contractions comparable to those produced by ACh at 1:5 million. Compound IV did not stimulate (1:50,000), and in three out of six preparations, relaxation of normal tones was observed. Similar results were obtained with the

TABLE I

A COMPARISON OF THE RELATIVE STIMULATING AND BLOCKING PROPERTIES OF VARIOUS TROPINE DERIVATIVES



Compound	R	R¹	R²	Config.	Action[a]	Pharmacological action Rel. pot.[b]	Test method used
III	H	CH_3CO_2	CH_3I	α	m+	1.0	Rat sigmoid colon
IV	H			β	m−	1.0	
				α	n+	0.25	Cat blood pressure
				β	n+	1.00[c]	and nictitating membrane
XIVa	C_6H_5	H	HCl	α	n+	0.25	Cat blood pressure
XIVb				β	n+	1.00	
XVa	$N(CH_3)(CH_2)_2N(C_2H_5)_2 \cdot CH_3I$	H	2-Chlorobenzyl	α	n−	0.24	Mouse mydriasis
XVb				β	n−	1.00	
XVIIa	$NH(CH_2)_2N(C_2H_5)_2 \cdot CH_3I$	H	CH_3I	α	n−	0.15	Mouse mydriasis
XVIIb		H		β	n−	1.0	
XVIIIa	$(C_6H_5)_2CHCO_2$	H	HCl	α	m−	1.00	Mouse mydriasis
XVIIIb				β	m−	0.29	

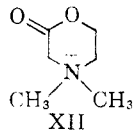
[a] Muscarinic (m) activity was assayed by use of the rat isolated sigmoid colon; nicotinic (n) activity by blood pressure and nictitating membrane responses after i.v. administration to the anesthetized cat; muscarine and nicotine blocking actions were determined by mouse mydriasis (P. Pulewka, *Arch. exptl. Path. Pharmakol.*, **168,** 307 (1932)). [b] Activity of the less potent isomer expressed as a fractional value. [c] Associated with skeletal muscle fasciculation.

racemic salt, DL-III as with L-III. Thus, the D-isomer could not be more active than its antipode.

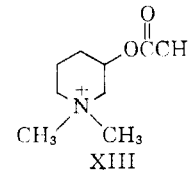
Nicotinic action was observed with both compounds following intravenous administration to the atropinized cat. However, in this test IV was clearly more potent than III, causing rises in blood pressure, contraction of the nictitating membrane and frequently some fasciculation at doses of 0.5–1.0 mg./kg. whereas III required doses of 1.0–2.0 mg/kg. for a pressor response and these doses had little effect on the nictitating membrane and did not cause fasciculation. Contraction of the nictitating membrane and rises in blood pressure were obtained consistently with ACh at 0.25 mg./kg.

Discussion.—The results obtained in this investigation support the hypothesis that the muscarine-like properties of ACh are associated with the *transoid*, and nicotine-like properties with the *cisoid* form, the receptor for each action being suitably structured for attachment with the corresponding form of ACh. The comparatively low pharmacological activity of these tropine acetate methiodides is not unexpected since it has been shown that in monoquaternary salts, cationic heads larger than tetramethylammonium have an adverse effect on stimulating action.[1] The muscarinic potency of III is of the same order as reported by Gyermek and Nador[6] for 3-tropanyl acetate methiodide.

Schueler[2] has examined the pharmacological action of the compounds shown below, with XII as the model for the *cisoid* form and XIII for the *transoid* form of ACh.



XII



XIII

He reported little difference between the two forms either in muscarinic activity or in susceptibility to hydrolysis by cholinesterase. The choice of these compounds as models is open to question inasmuch as XII and XIII are not structurally analogous. The ester portion of XII is a tetra-alkylammoniumacetyl group rather than acetyl which is present in XIII. In addition, the conformation of XII does not resemble the *cisoid* form of ACh, as revealed by X-ray studies.[3] It would have been more desirable to have the *cisoid* counterpart of XIII but this is not possible because of the conforma-

(6) L. Gyermek and K. Nador, *Acta Physiol. Acad. Sci. Hung.*, **4**, 341 (1953).

tional lability of this molecule. This difficulty has been obviated in the case of the 2-tropanyl acetate quaternaries III and IV.

Similar differences due to configurational change have been previously noted for the nicotine-like stimulating action of 3-phenyltropane in which it was pointed out that the β-isomer (XIVb) is clearly more potent than the corresponding α-isomer.[7] Table I includes results obtained by us for other 3-substituted tropines having blocking action at the nicotine- or muscarine-sensitive receptor sites. It will be noted that these results are in agreement with the generalization stated above. Nicotinic blockade is favored by the *cisoid* and muscarinic blockade by the *transoid* forms. Similar findings have been reported by Gyermek[8] for benzoyltropine in which the methiodides and ethiodides of the α-isomer are distinctly more effective muscarine blocking agents than the corresponding β-isomers. Results obtained by Gyermek[8] in the cat for ganglionic (nicotinic) blockade are not as distinct as those reported in this communication, possibly due to the fact that both of these quaternary salts show stimulating as well as blocking action. There is an indication from the above cited work, as well as that reviewed by Heusner,[9] that there is a favored configuration for local anesthetic action with blockage being obtained more readily with the *cisoid* form.

In a recent study of some analogs of muscarine, Waser[10] has pointed out that optimal muscarinic activity was obtained when the quaternary side chain extended away from the furan ring, *i.e.*, the molecule adopted a *transoid* conformation. However, to account for the relatively strong nicotinic action of muscarone he postulated that the ammonium ion side chain must be folded back toward the furan ring; in effect, the molecule assumed a *cisoid* conformation. Despite the obvious structural differences between the muscarine and 2-tropanol derivatives, a conformation-activity parallel appears to exist.

The results reported in this communication and those reviewed from other investigations, are consistent with the concept that the muscarine- and nicotine-sensitive receptors are structurally different and that *transoid* configurations are favorable for receptor interaction with the former and *cisoid* with the latter, particularly in compounds wherein the interatomic distances closely approximate those of acetylcholine.

(7) A. M. Lands and S. Archer, *J. Med. Pharm. Chem.*, **2**, 449 (1960).
(8) L. Gyermek, *Acta. Physiol. Acad. Hung.*, **4**, 333 (1953).
(9) A. Heusner, *Arzneimittel-Forschung*, **6**, 105 (1956).
(10) P. Waser, *Experientia*, **17**, 300 (1961).

Acknowledgments.—The authors wish to express their gratitude to Drs. M. R. Bell and D. Wood for samples of 2-tropanols. We wish also to acknowledge our gratitude for the technical assistance of Miss D. Fort and Mrs. L. Conklin in the pharmacological experimentation described in this communication.

Synthesis and Analgesic Activity of a New Bridged Heterocyclic System[1]

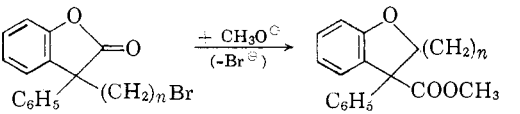
H. E. ZAUGG, R. W. DENET, AND E. T. KIMURA

Research Division, Abbott Laboratories, North Chicago, Illinois

Received December 23, 1961

Synthesis of a new bridged heterocyclic system, an aza-benzoxabicyclo-[3.3.1]-nonane derivative (VI) is described. Application of a new methoxide induced rearrangement of 3-(haloalkyl)-3-phenyl-2-benzofuranones which includes preferential and stereospecific displacement of one of two bromine atoms in the molecule, gives methyl *cis*-2-bromomethyl-4-chromancarboxylate (IIa). Heating this intermediate with primary amines produces the lactams V in good yields. The new heterocyclic system thus becomes readily accessible in five steps from phenol and mandelic acid or mandelonitrile. Structural analogy of this system to the analgesically active 6,7-benzomorphane ring system is pointed out, and preliminary pharmacological examination of several examples is reported.

A previous paper[2] described the methoxide ion induced rearrangement of the three homologous benzofuranones A to the corresponding methyl esters B.[3] The first two members of this series (n = 1, 2) rearranged with extreme rapidity (reaction could be accomplished under titration conditions); and, by contrast, the third member



A (n = 1,2,3) B (n = 1,2,3)

(1) Part IV of the series, "Neighboring Group Reactions." For Part III, *cf.* H. E. Zaugg, R. W. DeNet, R. J. Michaels, W. H. Washburn, and F. E. Chadde, *J. Org. Chem.*, **26**, 4753 (1961).

(2) H. E. Zaugg, R. W. DeNet, and R. J. Michaels, *ibid.*, **26**, 4821 (1961).

(3) This rearrangement, resulting from preferential nucleophilic attack of methoxide ion at the carbonyl carbon atom followed by intramolecular displacement of bromide ion, bears a formal resemblance to the suggested mode[4] of the rapid base-catalyzed solvolysis of the antibiotic, Antimycin-A.

(4) (a) A. J. Birch, D. W. Cameron, Y. Harada, and R. W. Richards, *J. Chem. Soc.*, 889 (1961); (b) E. E. van Tamelen, J. P. Dickie, M. E. Loomans, R. S. Dewey, and F. M. Strong, *J. Am. Chem. Soc.*, **83**, 1639 (1961).