The data indicated 2.2 equiv of 1 and 4.1 equiv of aluminum per mole of tannic acid.

Zinc Tannate Complex of 2a. This was prepared in the same way as the complex of 1. Determination of **2a** content by measurement of absorbance of a 0.1 N HCl extract at 249 nm indicated the complex to contain 36.4% of **2a**. Zinc content was found to be 2.8%. This represents 2.6 equiv of **2a** and 2.4 equiv of zinc per mole of tannic acid.

Aluminum Tannate Complex of 2a. Prepared in the same way, this complex was found to contain 27.1% of **2a** and 4.2% of aluminum or 1.7 equiv of **2a** and 11.5 equiv of aluminum per mole of tannic acid.

Acknowledgment. We particularly wish to thank Dr. M. J. Karten (NICHD) for arranging for the appropriate testing of these compounds and for supplying us with the test results. This work was supported, in part, by Contract No. NO1-HD-4-2836 from the NICHD.

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Solution Conformations of Muscarine and Some Analogues

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Proton magnetic spectra have been recorded for muscarine and two biologically active cyclopentane analogues. In order to observe homonuclear intramolecular nuclear Overhauser effects, the $-N^+(CH_3)_3$ signal was irradiated and increases in integrated intensities for other key signals in the molecule were observed. The results indicate that the quaternary side chain in these compounds is in an extended conformation in aqueous solution.

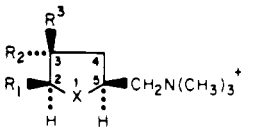
Muscarine (1) displays remarkable stereospecificity toward the cholinergic receptor system.[1] The solution conformation of muscarinic agents has long been of interest. Pullman[2] has calculated that the positive charge on the quaternary nitrogen atom is distributed over the *N*-methyl groups, and Waser[3] proposed that this cationic globe was held in position over the tetrahydrofuran ring system by electrostatic interaction with the ether oxygen. Jellinek[4] has determined the crystal structure of muscarine and Pauling and Petcher[5] that of muscarone. In both, the cationic center is directed away from the ring. Kier[6] has suggested on the basis of calculations that a similar conformation is likely in the gas phase. Furthermore, Melchiorre[7] and Givens[8] have demonstrated that the ether oxygen is not essential for high-level muscarinic activity, since cyclopentane analogues of muscarine are quite active. In cyclopentane analogues, cation–ether interaction is impossible.

The conformations of acetylcholine and some of its open-chain analogues have been previously investigated in aqueous solution by ^1H NMR[9,10] and by Raman–infrared spectroscopy.[11] Although ^{13}C NMR studies on similar systems have been published,[12] these do not afford conformational information; no previous ^1H NMR studies on muscarine analogues appear to have been carried out, although Belleau has recorded the ^1H NMR spectrum of muscarine itself.[13]

It is the purpose of this study to determine the approximate position of the cationic center in muscarine and several cyclopentane analogues in aqueous solution. This is accomplished by the homonuclear intramolecular nuclear Overhauser effect (NOE), in which the effect of irradiation of the *N*-methyl groups on the integrated intensities of other observable protons is measured.

Results and Discussion

^1H NMR signals for key protons in compounds **1–3** were



Compd	X	R_1	R_2	R_3
1	O	CH_3	OH	H
2	CH_2	CH_3	OH	H
3	CH_2	CH_3	=O	
4	CH_2	H	OH	H
5	CH_2	H	=O	

identified by comparison with ^1H NMR spectra for analogous molecules.[14] These are shown in the Experi-

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Table I. Intramolecular Nuclear Overhauser Enhancements

Compd	NOE enhancement (%) on $N(CH_3)_3$ irradiation				
	$-CH_3$	$-C(1)H_2$	$-C(4)H_2$	$-C(5)H$	$-CH_2N$
1	0		3	12	17
2	0	5	5	5	17
3	0	*a*	*a*	13	12

[a] Peaks insufficiently resolved in spectrum.

mental Section. Spectra were also recorded for compounds **4** and **5**; unfortunately, the only clearly defined peaks are the CH_2N^+ and $N^+(CH_3)_3$ groups, and without greater peak separation it is not possible to determine NOE enhancements for these compounds at 100 mHz. For **4**, irradiation of the proton at C-4 yielded very low NOE enhancement of the signal for $-N^+(CH_3)_3$, as expected.

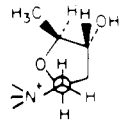
Homonuclear NOE enhancements for protons in compounds **1–3** are shown in Table I. Bell and Saunders[15] have listed the NOE's for the CH_3–H interaction as a function of internuclear distance and quote 17% enhancements for 2.88- and 2.90-Å separations. Dreiding models indicate that the distance between the CH_2 and $-N^+(CH_3)_3$ groups is of this order, so the observed NOE is as anticipated in **1** and **2**. A 12% enhancement was observed for the $-CH_2N^+$ group in **3**. As the distance should be similar to that in **1** and **2**, the reason for the smaller NOE is not known, but it may reflect the presence of intermolecular effects.

In all three compounds, NOE enhancements for the ring methyl group are very small. If the $-N^+(CH_3)_3$ were placed over the ring, the distance between it and the ring methyl group would be small, since these are oriented cis to each other. Even though the methyl group has an additional relaxation mechanism available, compared with protons (spin rotation),[16] a small residual NOE would still be expected if the $-N^+(CH_3)_3$ group were nearby.

Because of the stereochemistry, a NOE between the $-N^+(CH_3)_3$ group and the proton at the 1 position would not be expected. The very small enhancement observed for the methylene protons at C-4 indicates that the cationic group is far from these protons.

All samples have been prepared in D_2O at concentrations of about 10 mg/mL. Such dilute solutions are expected to minimize the influence of intermolecular interactions. For **1**, the NOE experiment in which the *C*-methyl group was irradiated and the *N*-methyl signal observed was also performed; zero enhancement was observed. This result is significant, since the trimethylammonio group would also possess an efficient spin-rotation relaxation mechanism but it offers no evidence in support of the Waser proposal for muscarine conformation.

These results indicate that in aqueous solution the $-N^+(CH_3)_3$ group in these compounds is located in an extended conformation away from the ring, similar to that shown in **6**, rather than the Waser conformation **7**. The



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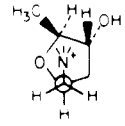
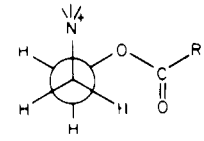


7

conformations of both open-chain and heterocyclic muscarinic agonists have long been of interest, and many studies using various techniques have evolved from this interest.[17] In general, conformations determined for open-chain compounds related to acetylcholine (**8**, R = Me) indicate that the $-N^+Me_3$ and –OCOR groups are synclinal, with torsional O–C–C–N angles of 85–90°.[18-21] Crystal-



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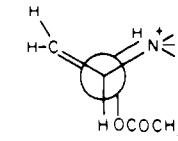

9

lographic and ^1H NMR determinations are in good agreement in almost all cases; the only exception is carbamylcholine (**8**, R = NH_2), which appears to have the $-N^+Me_3$ and –OCOR groups synclinal in solution but antiperiplanar in the crystalline state. However, it is not a requirement that the two critical functionalities exhibit a synclinal relationship, for the rigid cyclopropyl analogue **9**, in which the O–C–C–N torsional angle has been determined to be 137°,[22] is primarily a muscarinic compound possessing high activity.

In (2*S*,3*R*,5*S*)-muscarine, the O–C–C–N torsional angle has been determined to be 73° in the solid state. The present results indicate that muscarine, like other compounds, is found in solution in a conformation similar to that of the solid state, rather than the Waser conformation **7**; the very potent cyclopentyl analogues **2** and **3** adopt similar conformations. Although it can be argued that the receptor could constrain muscarinic agonists into conformations other than those exhibited in its absence, such a phenomenon appears unlikely in view of the marked tendency for muscarinic agonists of many types to adopt conformations similar to those found for the compounds in this study. Also, compound **9** would be unable to adopt a conformation similar to **7**. The stereochemistry involved in muscarinic agonist–receptor interactions cannot be precisely known until the receptors are better characterized. In view of the very high activity of **2** and **3** and the fact that their solution conformations are similar to that of muscarine, it is clear that the chemical factors involved in drug–receptor interactions of this type are also imperfectly understood at the present time.

Experimental Section

Solutions for ^1H NMR measurement were prepared by dissolving 6 mg of sample in 0.5 mL of D_2O. A trace of CH_3CN (δ 2.02 ppm) was added as internal standard; the samples were then degassed by several freeze–pump–thaw cycles and sealed under high vacuum.

A Jeolco PS-100 spectrometer was used at 100 MHz at 21 °C. An external reference capillary tube, containing benzene or another suitable compound, e.g., toluene, was used to ensure that the irradiation levels were suitable for the exclusive irradiation of the desired signals only in the NOE experiments. These were conducted by positioning the irradiating frequency in a "vacant" region of the spectrum and then, without altering any other settings, irradiating the desired signal. The external reference served as integration standard. Setting of the instrument was considered satisfactory only when the intensity of the integration standard signal did not change detectably between the "on" and "off" experiments. Major ^1H NMR signals were obtained as follows.

DL-**Muscarine** (**1**): δ 4.06 [3 H, m, C(2)H, C(3)H, and C(5)H], 3.46 (2 H, m, $=CH_2N$), 3.15 [9 H, s, $-N(CH_3)_3$], 1.16 (3 H, d, CCH_3).

2-Methyl-3-hydroxy-5-(dimethylaminomethyl)cyclopentane methiodide (**2**): δ 3.77 [1 H, q, –C(3)H], 3.33 (2 H, d, $-CH_2N$), 3.06 [9 H, s, $-N(CH_3)_3$], 0.96 (3 H, d, CCH_3).

2-Methyl-3-oxo-5-(dimethylaminomethyl)cyclopentane methiodide (**3**): δ 3.47 (2 H, d, $-CH_2N$), 3.12 [9 H, s, $-N(CH_3)_3$], 1.50 (1 H, m, $-CHCH_2N$), 1.02 (3 H, d, CCH_3).

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Book Reviews

Radiotracer Techniques and Applications. Volume 2. Edited by E. Anthony Evans and Mitsuo Muramatsu. Marcel Dekker, New York, N.Y., and Basel. 1977. xiii + 524 pp. 16 × 23.5 cm. $49.75.

Volume 2 of this two-volume work is concerned with applications of the radiotracer technique to the life sciences. A diverse field is covered, including biosynthesis, drug distribution and metabolism, pure and applied cytology, enzyme and enzymatic assays, agricultural chemicals, marine biology, competitive binding assays, medical diagnosis, and cancer treatment.

The book is compelling evidence of the power of the radiotracer method in pure and applied biology and of its utility in the solution of biomedical problems. The chapters are generally broad enough in their scope to provide an overview of applications in each of the fields addressed and to make the volume serve also as a reference text.

As in the applications section of the first volume, each chapter is written by a specialist with considerable first-hand laboratory experience, often a major contributor to his field. Thus, the limitations and pitfalls of the methods are as well elaborated as are the uses and advantages.

Except for occasional parochialisms the book is general enough to be used as a text reference for courses in the biological applications of radioactive isotopes. Some chapters taken separately or together will appeal to specialized audiences (e.g., those on biosynthetic studies and drug metabolism); others will give outsiders insight into fields with which they may not be familiar (e.g., the applications to agriculture and marine biology; medical uses as radioimmunoassays, diagnostic procedures, and potential cancer treatments). It should certainly be available to those who use radionuclides regularly in the laboratory.

Harvard Medical School **S. James Adelstein**

Clofibrate and Related Analogs. A Comprehensive Review. By Donald T. Witiak, Howard A. I. Newman, and Dennis R. Feller. Marcel Dekker, New York, N.Y. 1977. ix + 287 pp. 15.5 × 23.5 cm. $27.50.

This monograph reviews clinical efficacy of the hypolipidemic drug clofibrate, its activity in various animal models, structure–activity relationships of close analogues, and studies to elucidate its mechanism of action. Although comprehensive, one hesitates to call this review authoritative. The efficacy of clofibrate for treatment of heart disease is a matter of controversy and its mechanism of action remains unestablished. The authors do little beyond listing the conflicting pieces of evidence and, for better or worse, make no attempt to present a unifying view as to how clofibrate works.

This book is Volume 7 of the Medicinal Research Series, a new edition in modified format of A. Burger's textbook, "Medicinal Chemistry". While earlier volumes of this series continued more or less in the format of the textbook, the present volume does not. The subject matter is too narrow and too comprehensively treated to lend the book any of the qualities of a textbook. Its usefulness for teaching and learning purposes is therefore limited. Its primary use is as a factual literature survey for biomedical researchers and reviewers. References (774), a good index, and a glossary of selected terms are included. The book is printed by a photocopying process of a double-spaced typewritten manuscript. The 171 pages of text and the 92 pages of references could have been reduced to half or less. At $27.50 this is hardly a bargain.

Richardson-Merrell, Inc. **J. Martin Grisar**

Topics in Antibiotic Chemistry. Volume 1. Edited by Peter Sammes. Ellis Harwood Limited, Chichester, Sussex, England. 1977. 217 pp. 14.5 × 22.7 cm. $28.50.

This book is the foundation volume for a projected series designed to keep interested workers abreast of antibiotic progress. While seeking a broad disciplinary coverage, the emphasis is to be chemical in describing mechanisms of biosynthesis, the modes of action, and mechanisms of resistance development for the ever-increasing number of antibiotics being characterized today. The achievement of this volume is the review of two families of antibiotics, the aminoglycosides and the ansamycins. It would seem that more classes of compounds could be considered in each volume to assure a reasonable coverage of this large field.

Part A, comprising 99 pages and 214 references, reviews the aminocyclitol antibiotics quite fully from a theoretical viewpoint through the year 1975. It is subdivided on topics of structure, biotransformation, resistance, structure activity, chemistry, and synthesis. Little attention is given to the methodology of recognition, so important for dereplication in the search for new