Tetrahedron Vol. 39, No. 19, pp. 3171 to 3180, 1983
Printed in Great Britain

0040-4020/83 $3.00 + .00
Pergamon Press Ltd

CONFORMATIONAL ANALYSIS OF 5-MEMBERED RING COMPOUNDS HAVING CHOLINERGIC ACTIVITY

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(*Received in UK* 10 *December* 1982)

Abstract—A study of the conformational properties, both referred to the heterocyclic ring and to the ammonium substituent, has been carried out on dimethylaminomethylmethiodide derivatives of 2-methyl substituted 5-membered saturated heterocycles having cholinergic activity. The experimental method employed is NMR of the proton: the spectra were fully analyzed and all the multiplet parameters tentatively assigned. Vicinal coupling constants were employed for the conformational study by introducing them in a best fit procedure between experimental and calculated (by a semi-empirical method) values. The molecular conformations thus obtained were compared with results previously obtained for a few of the compounds here examined or for similar systems and employed for discussing the possible factors relating molecular geometry to their biological activity.

The study of ring conformations and substituent orientation in molecules of biological interest has been the object of several investigations, in order to acquire more knowledge on the structure-activity molecular correlations. This has also been performed for several cholinergic compounds related to acethylcholine and muscarine in order to study the molecular requirements of the active sites. Within molecules of this type several derivatives of saturated 5-membered rings are included,[1] such as substituted 1,3-dioxolanes, cyclopentanes and oxolanes.

For molecules in solution the experimental method which has proved most useful for studying the conformational properties is that based on vicinal H–H coupling constants, even though for 5-membered rings difficulties arise[2–7] in applying methods based on correlations between vicinal coupling constants and torsional angles. Furthermore the conformations assigned to the molecules in solution, in the case of 5-membered rings, may bear no relationship to the situation in the solid state, while most 6-membered rings assume the same conformation in the solid as in solution[2] (apart from axial-equatorial equilibrium which should not be present in the crystalline form). Even with this situation, conformational studies[8] performed on the radicals of a number of substituted 1,3-dioxolanes by means of electron spin resonance provide results very close to those obtained by ^{1}H NMR on strictly similar systems.

Recently we have reported[9] an attempt to determine the preferred conformations of a number of 4(5) substituted 1,3-dioxolanes by introducing an empirical scheme for employing the vicinal H–H coupling constants in the conformational analysis of 5-membered rings. The scheme was based on a comparison between coupling constants calculated by a semi-empirical method[10] and experimental values, since direct application of Karplus equation[11] or R-method[12] does not provide useful results in the conformational analysis of 5-membered rings.[2,13]

We report here a conformational study on homologous derivatives of 1,3-dioxolane, 1,3-oxathiolane and oxolane, by employing this procedure.[9] The compounds chosen are the *cis* and *trans* isomers of methiodides of 2-methyl 4-dimethylaminomethyl 1,3-dioxolane (**1**), 2-methyl 5-dimethylaminomethyl 1,3-oxathiolane (**2**), 2-methyl 4-dimethylaminomethyl oxolane (**3**), 2-methyl 5-dimethylaminomethyl oxolane (**4**), 4-methyl 2-dimethylaminomethyl 1,3-dioxolane (**5**) and, for comparison, DL muscarine (**6**).

Coupling constants are calculated[10] for several conformations of the whole ring expressed in terms of two puckering coordinates q and ϕ, namely the amplitude and phase coordinates.[14]

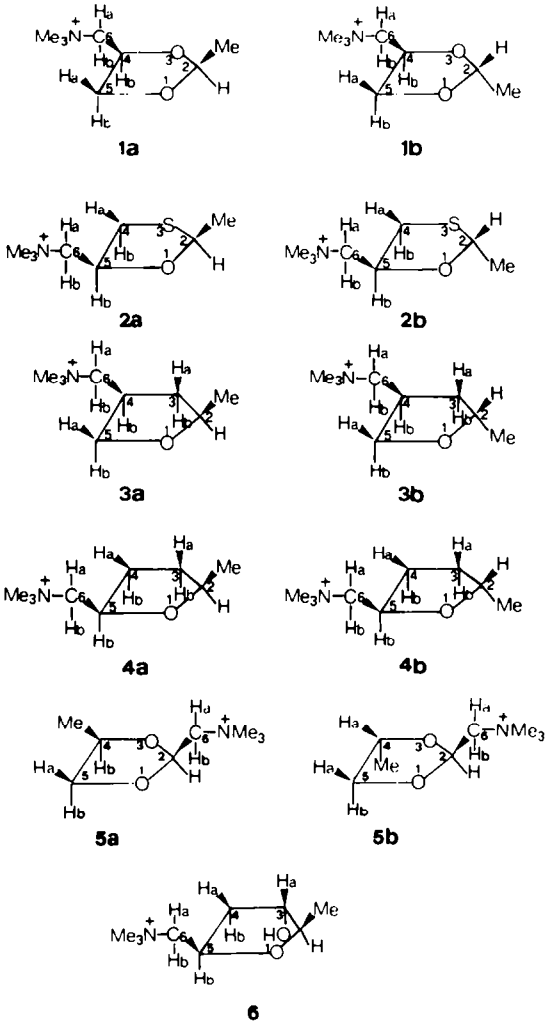
RESULTS AND DISCUSSION

The analysis of the ^{1}H NMR multiplets was carried out by the LAOCOON 3 program.[15] Chemical shifts and coupling constants are gathered in Tables 1 and 2.

As regards the proton on the C atom of the ring to which the $CH_2N^+Me_3$ substituent is bound, i.e. H(4b) or H(5b), the multiplet components were broad in all compounds and irradiation experiments on the protons did not reduce the line-width: the broadening was thus attributed to an unresolved coupling with the ^{14}N nucleus. A long-range coupling constant should therefore be present, of the type ^{14}N–C–C–H which should be of the order of magnitude of 1 Hz.

In several papers on substituted 1,3-dioxolanes assignments of ^{1}H chemical shifts and proton–proton coupling constants have been reported: the results are not always complete and the consistency between different literature sources is not, in general, satisfactory. We have therefore assigned the complete sets of NMR parameters by looking for the widest consistency among the compounds examined.

The assignment of protons H(5a) and H(5b) in compounds **1** and **3**, corresponding to H(4a) and H(4b) in compounds **2** and **4**, was made by assuming that the $CH_2N^+Me_3$ group may affect the shielding of the protons on adjacent C atoms acting both as an alkyl group and with the electrostatic effect of the positively charged N atom. As regards the anisotropic shielding due to the



C–C single bond, this should act in a different way on the *cis* and *trans* vicinal protons[16] but the entity of the effect should depend on the relative torsional angle of the ring. The orientation of the ammonium group should also be important[16a] in determining a different chemical shift of the geminal protons present in the heterocyclic rings, overcoming also the inductive effect of vicinal groups.

In order to define the situation with regards to the orientation of the $CH_2N^+Me_3$ group, it is interesting to note that the internal chemical shift differences of the two geminal protons on the vicinal C(4) or C(5) range within 0.4–0.7 ppm in all compounds. The electric field due to the dipole moment of the C–N^+ bond should have a determinant role in determining the shielding of these two protons. To acquire more quantitative details, even if only at an empirical level, we performed calculations of the changes in proton shielding due to this effect by employing the theory[17] elaborated by Buckingham and Musher. By adopting a dipole moment of the order of 1.63 D, it is found, as a result, that the proton holding a *cis* relationship with the ammonium substituent is in effect deshielded relative to its geminal partner (maximum value nearly 0.5 ppm) when the substituent is oriented in the region of the C atom bearing the two protons, i.e. C(4) or C(5) C atoms. From the choice operated on the chemical shifts on the basis of the deshielding effect of the ammonium group, all the other chemical shifts and coupling constants were consequently assigned, as reported in Tables 1 and 2.

The assignment of protons 3a and 5a in compounds **3** as those *cis* to the ammonium group is made on the basis of the deshielding effect of the C–N^+ group. The larger internal shift between the geminal partners on C(5) and those on C(3) in compound **3a**, which becomes nearly zero in compound **3b**, is probably to be ascribed to a combined effect of the orientation of the ammonium group and of the anisotropic shielding due to the C–C bond[16,18] (both C(4)–C(6) and C(2)–C(H_3)) probably in connection with different values of the torsional angle C(1)–C(2)–C(3)–C(4) in the two geometrical isomers.

In compound **4a** for the pair of geminal protons on C(4) and C(3) the resonance at lower field was assigned to the proton *cis* to the ammonium group. For compound **4b** the assignment of the protons on C(4) was reversed in order to assign the smallest and largest values (3.31 and 9.82 Hz) of the vicinal coupling constants between protons on C(3) and C(4) to *trans* couplings. This choice was made since the calculated values of coupling constants in function of torsional angles around the C–C bond show that the *trans* coupling spans a larger interval and can assume values of this order of magnitude.

In compounds **5** the assignment of the protons on C(5) was performed on the basis of the deshielding effect of the ammonium group on the *cis* proton. As a consequence of this assumption the Me group and the proton on C(4) are

Table 1. ^{1}H Chemical shifts (in ppm referred to the methyl peak of DDS) of the compounds examined. For the assignments see text

Compound	δ_{Me}	$\delta_{H(2)}$	$\delta_{H(4a)}$	$\delta_{H(4b)}$	$\delta_{H(5a)}$	$\delta_{H(5b)}$	$\delta_{H(6a)}$	$\delta_{H(6b)}$	$\delta_{H(3a)}$	$\delta_{H(3b)}$	$\delta_{N(Me)_3}$
1a	1.58	5.33	-	4.93	4.30	3.94	3.80	3.71	-	-	3.41
1b	1.56	5.45	-	5.01	4.59	3.82	3.68	3.90	-	-	3.45
2a	1.68	5.46	3.39	2.95	-	4.65	3.84	3.73	-	-	3.33
2b	1.67	5.58	3.50	3.01	-	5.05	3.67	3.78	-	-	3.33
3a	1.33	4.16	-	2.95	4.15	3.70	3.58	3.58	2.52	1.46	3.20
3b	1.46	4.42	-	3.12	4.48	3.70	3.82	3.75	2.19	2.19	3.39
4a	1.25	4.18	2.19	1.72	-	4.48	3.53	3.46	2.06	1.56	3.20
4b	1.24	4.21	1.62	2.26	-	4.61	3.40	3.51	2.10	1.47	3.19
5a	1.31	5.59	-	4.30	4.12	3.53	3.58	3.55	-	-	3.25
5b	1.25	5.60	4.36	-	4.19	3.50	3.49	3.49	-	-	3.21
6	1.20	4.05	2.00	2.10	-	4.66	3.59	3.47	4.12	-	3.20

Table 2. Proton–proton coupling constants (Hz) in the compounds examined. For the assignment see text

Compound	J_{Me-2}	J_{2-3a}	J_{2-3b}	J_{3a-3b}	J_{3a-4a}	J_{3a-4b}	J_{3b-4a}	J_{3b-4b}	J_{4a-5a}	J_{4a-5b}	J_{4b-5a}	J_{4b-5b}	J_{4a-4b} J_{5a-5b}	J_{6a-6b}	J_{4b-6a} J_{5b-6a}	J_{4b-6b} J_{5b-6b}
1a	4.90	-	-	-	-	-	-	-	-	7.46	-	5.24	-8.73	-14.03	3.31	7.49
1b	4.89	-	-	-	-	-	-	-	-	7.33	-	6.29	-9.18	-14.14	1.34	9.63
2a	5.68	-	-	-	-	-	-	-	-	5.54	-	9.10	-10.38	-14.23	1.85	9.17
2b	5.80	-	-	-	-	-	-	-	-	6.12	-	6.76	-11.42	-13.70	2.52	9.00
3a	6.20	5.30	9.90	-12.13	-	7.38	-	9.96	-	-	7.66	7.81	-8.80	-	6.20	6.20
3b	6.20	6.86	6.86	-	-	7.79	-	7.79	-	-	7.44	8.72	-8.68	-	6.04	5.77
4a	6.28	6.45	6.61	-11.98	8.19	6.03	7.73	8.68	-	7.55	-	6.49	-12.98	-13.96	1.43	9.81
4b	6.10	5.23	8.82	-11.77	7.78	3.31	9.82	8.18	-	7.46	-	7.26	-12.68	-14.03	1.84	9.68
5a	6.18*	-	-	-	-	-	-	-	-	-	7.41	6.20	-8.04	-14.01	4.53**	4.16**
5b	6.15*	-	-	-	-	-	-	-	5.99	7.14	-	-	-8.23	-	4.58**	4.58**
6	6.50	2.46	-	-	5.71	2.05	-	-	-	9.78	-	6.05	-13.73	-14.04	1.50	9.48

*Refers to the coupling of the methyl group with proton H(4). **Refers to the coupling of the methylene protons with H(2).

Table 3. Bond angles (θ) and bond distances (d) employed for constructing the molecular geometry for a number of conformations along the pseudorotational circuit of 1,3-dioxolane, 1,3-oxathiolane and oxolane

1,3-dioxolane	d_{C-O} = 1.42 Å; d_{C-C} = 1.54 Å; θ_2 = 109.05°; θ_3 = 104.29°.
1,3-oxathiolane	$d_{C(2)-O}$ = 1.39(9) Å; $d_{C(2)-S}$ = 1.85(2) Å; $d_{C(4)-S}$ = 1.81(9) Å; d_{C-C} = 1.49(9) Å; $d_{C(5)-O}$ = 1.42(5) Å; θ_4 = 105.20°; θ_5 = 107.81°.
oxolane[a]	d_{C-O} = 1.42(8) Å; d_{C-C} = 1.53(8) Å; θ_1 = 106.4° (ϕ = 0°); θ_1 = 110.5° (ϕ = 90°); θ_5 = 104.15° (ϕ = 0°); θ_5 = 106.15° (ϕ = 90°)

[a]While for 1,3 dioxolane and 1,3-oxathiolane the angles θ do not appreciably change along the pseudorotational circuit, for oxolane it was necessary to employ two sets of θ values in order to reproduce the molecular symmetries C_s (ϕ = 0°) and C_2 (ϕ = 90°) as reported in Ref. 14. Changes in the values of θ_1 and θ_5 were also necessary for oxolane for constructing the molecular geometries at different q values.

also deshielded when bearing a *cis* relationship with the ammonium group and the larger effect on the protons on C(5) should depend on the orientation of this group with respect to the rotation around the exocyclic C(2)–C(6) bond.

For purposes of comparison with a compound of known biological and conformational properties,[19] the NMR spectrum of DL-muscarine (**6**) was also recorded. The assignment of the several resonances is quite immediate apart those of the two protons on C(4). The internal shift among H(4a) and H(4b) amounts to only 0.1 ppm, while both protons are at higher field with respect to H(4a) and at lower field with respect to H(4b) of compounds **4**: with regard to the shift of H(4b) in compounds **4**, it may be assumed that the deshielding effect of the ammonium group still operates. The assignment of chemical shifts and coupling constants is close to that recently reported[20] for (+) muscarine.

CONFORMATIONAL ANALYSIS FROM PROTON-PROTON COUPLING CONSTANTS

In order to match experimental and calculated coupling constants, according to the procedure previously reported,[9] and to seek their best agreement as a function of conformational parameters, sets of proton–proton coupling constants were calculated for a large number of conformations of the heterocyclic ring as a function of the two puckering coordinates[14] q and ϕ.

As a first approach to the conformational problems, proton–proton coupling constants were calculated[10] for the unsubstituted heterocyclic rings with fixed amplitude q corresponding to the following values: for 1,3-oxathiolane q = 0.367,† obtained from the crystal structure of cholestan-4-one-3-spiro 2,5-oxathiolane,[21] for 1,3-dioxolane q = 0.265 and for oxolane q = 0.38 according to optimized values employed in *ab-initio* calculations.[14] The same amplitude values were retained in the calculations performed on substituted heterocyclic molecules, considering that experimental results[23,24] relative to solid substituted 1,3-dioxolanes show that the q value ranges within a narrow interval. By fixing all the bond distances and two values of bond angles, according to the figures reported in Table 3, and by assuming constant q values as specified above, molecular geometries were built up at selected values of ϕ chosen at regular intervals in the range from 0 to 360°.

The behaviour of the calculated vicinal coupling constants is well approximated by a Fourier series in the phase angle ϕ, and the expression (1), differing in the A and B constants

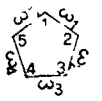
$$J = A_0 + \sum_i [A_i \cos i\phi + B_i \operatorname{sen} i\phi] \qquad (1)$$

for the different coupling constants, was employed for comparing calculated and experimental coupling constants. The comparisons were performed by employing calculated coupling constants referring to the unsubstituted compounds since it appears[9] that substituents on the ring do not appreciably affect coupling constants but rather operate in confining[2–6] the ring within preferred conformational situations.

The analysis supplies for each compound two sets of

†Recent X-ray analysis[22] of compound **2a** showed that the puckering of the 1,3-oxathiolane ring is higher (q = 0.46), but we have retained this value for our calculations.

Table 4. Torsional angles ω and θ (referred to the exocyclic C(6)–C(4) or C(6)–C(5) bond) for compounds **1–4** determined from vicinal proton–proton coupling constants. Δ refers to the mean deviation between calculated and experimental coupling constants, q and ϕ are the puckering coordinates referring to the five-membered ring:



Compound	Δ	q	Φ	ω_1	ω_2	ω_3	ω_4	ω_5	θ^* (X–C–C–N)
1a	±0.36	0.265	247.0	+20.51	-29.72	+26.55	-14.72	-3.23	-126.2 (X = O)
1b	±0.69	0.265	18.0	-31.39	+24.66	-9.34	-8.61	+23.28	-68.55 (X = O)
2a	±0.32	0.367	215.6	+40.58	-29.39	+16.17	+2.76	-31.65	-66.45 (X = O)
2b	±0.25	0.367	51.8	-34.52	+30.16	-22.95	+7.74	+19.84	-60.48 (X = O)
3a	±1.46	0.38	252.0	+26.39	-37.44	+35.03	-24.11	-0.93	-18.48 (X = C(5))
3b	±1.39	0.38	342.0	-34.94	+13.07	+11.44	-32.37	+42.91	-3.62 (X = C(5))
4a	±1.56	0.38	173.0	+39.0	-19.90	-4.50	+27.48	-42.26	-45.92 (X = O)
4b	±1.65	0.38	25.0	-42.82	+34.52	-15.74	-8.27	+31.90	+170.93 (X = O)
5a	±0.33	0.265	244.0	+21.7	-30.07	+25.94	-13.43	-4.69	+130.94 (X = O(3))
5b	±0.40	0.265	16.0	-31.40	+24.01	-8.38	-9.54	+23.82	+126.94 (X = O(3))
6	±1.36	0.38	150.5	+29.35	-5.39	-18.21	+36.26	-41.91	-53.73 (X = O)

* The angle θ is positive going in the clockwise direction from N to the X atom viewed down the exocyclic C–C bond.

values (q is maintained constant) which characterize two molecular geometries corresponding to the best agreement between calculated and experimental coupling constants. Of the two conformations, the one showing the substituents in the most crowded situation was discarded. The resulting molecular geometries, expressed in terms of torsional angles, are collected in Table 4.

By employing the vicinal coupling constants relative to the methylene protons on C(6) and the proton on the adjacent C, it was possible to calculate, with the same procedure, the dihedral angles X–C–C–N (X = O in compounds **1**, **2**, **4**, **5** and **6** while X = C(5) in compound **3**). These values are also reported in Table 4 and refer to the orientation of the ammonium substituent in respect to the atoms of the heterocyclic ring. In the comparison between calculated and experimental coupling constants in search of the best agreement, all the set of vicinal coupling constants relative to the protons of the ring and those relative to the methylene protons on C(6) with the vicinal proton attached to the ring carbon were employed: Δ refers to the mean deviation between calculated and experimental values.

The deviation between calculated and experimental coupling constants in compounds **3**, **4** and **6** is higher than for the remaining compounds, but in the former case the deviation Δ refers to eight coupling constants, those relative to three molecular fragments of the ring and to the *exo* C–C bond, while in the latter they are measurable only for one molecular fragment and for the *exo* C–C bond.

The conformations of the whole molecule thus determined for the compounds examined are schematically depicted[25] in Fig. 1. In the case of compound **2a** a recent X-ray determination[22] of crystal and molecular structure has shown that the angle θ amounts to 77.4° and is very close to that estimated in solution from NMR coupling constants (66.4°).

In a simplified picture of the general description of

S N

5-membered rings as a function of pseudorotation coordinates, a pentose ring may be represented in terms of N- and S-type conformers.[26–28] For compounds **4** and **6** it is possible to relate our results to this representation in order to have an immediate comparison with the results of conformational studies in the same or in strictly related compounds. Since we have assumed that our conformations refer to the predominant conformational species present in solution, we shall refer not to the S/N ratio but rather to a comparison deduced for our compounds with those of N- and S-type. By examining the torsional angle ω_3 reported in Table 4, and following the characterization[28] which assigns the N- and S-type conformation according to positive and negative values of this angle, compounds **4** and **6** (muscarine) turn out to have conformations which may be classified as of S-type, even if a more appropriate definition of the conformations of these molecules in terms of the pseudorotational pathway given by Altona and Sundaralingam[25] would place them in the region which spans from E (envelope) and S-type forms. By examining the problem of the muscarine ring as a function of an equilibrium between puckered N- and S-type conformers, it is found[20,29] that the S-type conformation is present in solution in amounts higher than 70%, a result which is coherent with our conclusions.

As regards the orientation of the ammonium group, from the values of dihedral angles X–C–C–N and the projection formulae[25] along the C(6)–C(4) or C(6)–C(5) bond, it appears that in compounds **1** and **2** the group points toward the C(5) or C(4) carbon atom supporting the hypothesis of a differential shielding due to elec-

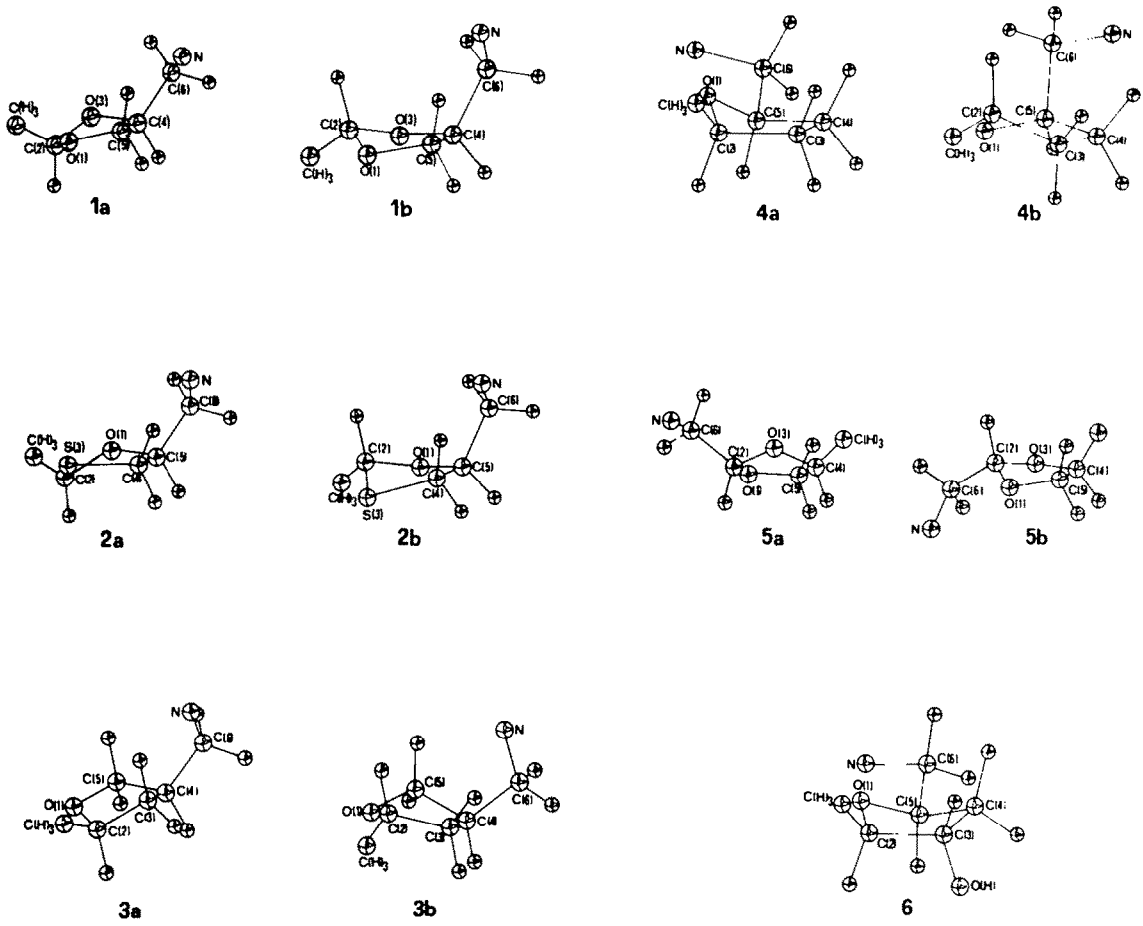


Fig. 1. Molecular conformation of derivatives **1–6** corresponding to the structural parameters reported in Table 4 and obtained in correspondence of a fixed q values for the ring.

trostatic effects on geminal protons on these C atoms. In compounds **1b**, **2a** and **2b** the group points slightly inside the ring, but this should not be identified with a Waser conformation, which seems not to be proper to muscarine analogues.[19] In compounds **3** the prevailing orientation is that with the group pointing toward the C(5) carbon.

In compound **4a** the ammonium group is *synclinal* with respect to the heterocyclic O atom but it points outside the ring, while in compound **4b** its orientation is of the *antiperiplanar* type. In compounds **5** the ammonium group is preferentially oriented in proximity to one of the O atoms of the ring: the deviation is smaller when the group points toward O(1). For muscarine (**6**) the orientation of the ammonium group is similar to that in compound **4a**: the angle N–C(6)–C(5)–O(1) of 54–56°, when compared with that found (73°) for muscarine in the solid state,[23,30] agrees in indicating the same *synclinal* relationship between the ammonium group and the O atom of the ring.

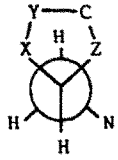
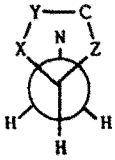
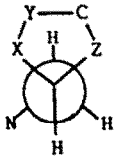
The orientation of the ammonium group corresponds to the X–C–C–N dihedral angle determined in the hypothesis of frozen conformations. Since no evidence is acquired in favour of this hypothesis, an analysis of the conformational situation relative to the exocyclic C–C bond in terms of conformer populations may equally well describe the orientation of the ammonium group. This has been done by employing for the conformations consisting of the classical staggered forms calculated coupling constants and simple equations containing their populations. The results are gathered in Table 5. The *gauche* N^+/O form prevails in all compounds where this situation is possible, except for compound **4b**, where the *anti* isomer is the predominant form. For DL muscarine the *gauche* conformation amounts to 98%, a value which compares well with the 88% of a recent estimate.[20]

Considerations relative to molecular conformations and biological activity

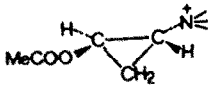
In terms of fixed conformations the situation of compound **1a** differs markedly from that of the other compounds: the dihedral angle O–C–C–N in this molecule is 126°, while in derivatives **1b**, **2a**, **2b** assumes values which range around 60°.

It is known[31,32] that for muscarinic activity in an agonist the presence of the quaternary N is essential, while the ether O seems not so important for high level activity.[33] When the O is present in β position with respect to the ammonium group, as here in the case of compounds **1**, **2**, **4**, **5** and **6**, the N atom seems to prefer a disposition *gauche* to this O atom, probably due to positive electrostatic interactions[34] between O and N^+ atoms. In open chain compounds related to acetylcholine[19] the torsional angle O–C–C–N in the solid state

Table 5. Rotamer populations calculated from vicinal coupling constants in compounds **1–6**

Compound	X	Y	Z	Rotamer populations 		
1a	C(5)	O(1)	O(3)	0.68	0.07	0.25
1b	C(5)	O(1)	O(3)	0.50	0.50	0.
2a	C(4)	S	O	0.58	0.42	0.
2b	C(4)	S	O	0.71	0.22	0.07
3a	C(3)	O	C(5)	0.61	0.	0.39
3b	C(3)	O	C(5)	0.62	0.	0.38
4a	C(4)	C(3)	O	0.92	0.	0.08
4b	C(4)	C(3)	O	0.	0.03	0.97
5a	O(1)	C(5)	O(3)	0.53	0.06	0.40
5b	O(1)	C(5)	O(3)	0.48	0.14	0.38
6	C(4)	C(3)	O	0.98	0.	0.02

amounts to 85–90° in agreement also with NMR measurements: for acetylcholine the *gauche* conformation prevails in aqueous solution.[35] The synclinal relationship between the N^+Me_3 group and the function containing O is not a critical requirement[19] for muscarinic activity, since in 2-acetoxycyclopropyltrimethyl-



7

ammonium (**7**), which shows high muscarinic properties, the torsional angle O–C–C–N is fixed and amounts to 137°. Further, in compound **5a**, where the N^+Me_3 group bears a *syn-periplanar* relationship with one O atom, O(1), and an *anti-clinal* relationship with the other, the biological activity is lower[36] than in compounds **1**. Even accounting for conformational changes of these molecules in the presence of a receptor, compound **1a** seems to possess a conformation very near to that of compound **7** which has fixed conformation and high activity. The change of conformation involving the ammonium group in order to reach binding conditions with the receptor molecules also requires an energy "price" to be paid[34] resulting in a decrease of the activity of the molecule: the conformational situation of compound **1a** seems to be in line with a high activity of this system. Following and accepting this conclusion, compound **1a** should possess an activity even higher than DL-muscarine itself and the cyclopentyl analogues,[33] assuming that the comparison may be made between the O–C–C–N torsional angles found for these compounds. Preliminary results indicate that these conclusions are correct.[36]

Even following a different reasoning in terms of rotamer populations, similar conclusions may be drawn. The conformation required for biological activity probably does not coincide with any one of the staggered forms[34] and it is rather difficult to predict whether more energy is required to open a *gauche* form or to close the *anti* form in order to reach the best bonding conditions, so that it is difficult to correlate the activity of the compounds to their rotamer populations. What is significant is that in compound **1a** the *gaughe*/*anti* situation, referred to the N^+/O atoms, differs from that of the other molecules where this comparison is possible. If binding to the receptor requires a peculiar arrangement of the O and N^+ atoms, i.e. that intermediate between the *gauche* and *anti* arrangements,[34] this seems better acquired from the *anti* arrangement since the *gauche* situation may involve electrostatic stabilizing interactions being the O and N^+ atoms. In compound **1a** the equilibrium between conformers is favourable to this condition. The same should also be true for compound **4b** over compound **4a**, but this is not supported from experimental results.[36] Factors other than the disposition of the O/N^+ atoms may influence the activity of these molecules.

One of these factors may be related to the presence of the Me group at position 2. A Me group is also present in acetylcholine, separated from the ammonium group by the same number of bonds as in the compounds here examined and in muscarine itself, while the highest muscarinic activity corresponds to a 5-bond separation between the Me group and the ammonium center.[32] The

Table 6. Distances between atoms in the compounds examined (Å).

Compound	$\overset{+}{N}\cdots X$		$\overset{+}{N}\cdots C(H_3)$	
1a	3.41	X = O(3)	4.82	
	3.66	X = O(1)		
1b	3.38	X = O(3)	4.73	
	3.54	X = O(1)		
2a	3.40	X = O(1)	5.03	
	3.84	X = S(3)		
2b	3.45	X = O(1)	5.06	
	3.98	X = S(3)		
3a	3.93	X = O	5.23	q = 0.38
	3.76	X = O	4.50	q = 0.08
3b	3.91	X = O	5.23	q = 0.38
	3.77	X = O	5.22	q = 0.13
4a	2.75	X = O	4.75	q = 0.38
	2.84	X = C	4.62	q = 0.13
4b	3.72	X = O	5.44	q = 0.38
	3.72	X = O	5.55	q = 0.15
5a	3.53	X = O(3)	4.98	
	2.53	X = O(1)		
5b	3.50	X = O(3)	5.18	
	2.52	X = O(1)		
6	2.82		4.83	q = 0.38
	2.84		4.57	q = 0.20

importance in biological activity of the relative disposition of the Me group with respect to other atoms of the ring and of the cationic head has also been proposed before.[1,37] By examining this factor through the interatomic distances $N^+ \ldots C(H_3)$ determined in the compounds here examined in correspondence of the conformations determined, from the results reported in Table 6 the following considerations may be advanced.

In compounds **1** this distance amounts approximately to 4.8 Å; a similar value is found only in compound **4a** and in muscarine, whereas the value in the remaining molecules is higher. If this value is an optimum for high biological activity, the distance $N^+ \ldots O$, where the O atom is the one in β position, may be assumed as a further critical parameter influencing the activity of the compounds, since it may condition the interaction of the ammonium group with the receptor. The $N^+ \ldots O$ distance which is found in compound **1** is present only in compounds **2**, where different values are found for the $N^+ \ldots C(H_3)$ distance.

In addition, the flexibility of the 5-membered rings, which in the presence of substituents assume different low-energy conformations, is also a factor influencing the activity of these molecules in solution. This factor is involved in determining the shape of the whole molecule, both as regards the topology of the atoms of the ring and the spatial arrangement of the substituents.

In conclusion, since strong agonists should interact simultaneously with different binding sites at the receptor level, the loss of one of the functional groups, the change of one heteroatom or of conformation in the heterocyclic ring and attenuation of the bonding properties of the groups as a consequence of their stereochemical orientation, may be at the origin of consistent changes in their activity.

EXPERIMENTAL

The ^1H NMR spectra were recorded both on a JEOL JNM-C60-HL spectrometer at 60 MHz and on a Varian XL200 at 200 MHz. Proton-proton decoupling was performed whenever necessary in order to determine the spectral parameters. The solns of the compounds were freshly prepared in D_2O (0.25 M) and DDS (sodium 2,2-dimethyl 2 silapentane-5-sulphonate) added as internal reference.

Compounds

DL-muscarine is a commercial product from SIGMA Chemical Company.

All m.ps were taken in sealed capillaries on a Büchi SMP-20

apparatus and are uncorrected. Elemental analyses were performed with Carlo Erba Elemental Analyzer mod. 1106. All compounds gave correct (C, H, N) analysis (±0.3%).

Compounds **1a** and **1b** were obtained according to the method previously described.[38]

Cis- and trans-2-methyl-5-trimethylammoniummethyl-1,3-oxathiolane iodide (**2a** and **2b**). The mixture of these *cis* and *trans* compounds was obtained according to Elferink and Salemink,[39] and separated using a Fractovap model P gas chromatograph with a 10-mm dia. and 4-m long column filled with 20% Carbowax 20 M on CPS 60–80 mesh, using the following operating conditions: the column oven temp was maintained isothermically at 170° for 30 min, then programmed from 170 to 190° at 3°/min, the final temp being maintained for 10 min, injection port temp 240°, temp of the detector 240°, N_2 flow-rate 60 ml/min. The chromatogram shows two peaks: A and B. An excess of MeI was added to an abs. EtOH soln of the tertiary amine corresponding to peak A. The precipitated ammonium salt was filtered, then crystallized from abs. EtOH: *cis*-2-methyl-5-trimethylammoniummethyl-1,3-oxathiolane iodide is thus obtained, m.p. 172–173° (lit.[40] 166–168°). Following the same procedure, from the tertiary amine corresponding to peak B, *trans*-2-methyl-5-trimethylammoniummethyl-1,3-oxathiolane iodide, m.p. 152–153°, was obtained. This ammonium salt is neither hygroscopic nor deliquescent at room temp.[41]

Cis- and trans-2-methyl-4-trimethylammoniummethyloxolane iodide (**3a** and **3b**). The mixture of these *cis* and *trans* compounds was obtained following the procedure reported[42] and separated using a Fractovap model P gas chromatograph with a 10-mm dia. and 4 m long column filled with 20% Carbowax 20 M on CGS 45–60 mesh, and the following operating conditions: column oven of the detector 240°, N_2 flow-rate 100 ml/min. The chromatogram shows two peaks: A and B. Peak A is identified as *cis*-2-methyl-4-hydroxymethyloxolane, peak B as its *trans* isomer, according to lit.[43] *p*-toluenesulphonates were prepared by reaction overnight with *p*-toluenesulphonyl chloride in pyridine at room temp. 0.01 moles of *cis* or *trans* 2-methyl-4-*para*-toluenesulphonyloxymethyloxolane are allowed to react, in a metal container, at 120° for 24 hr with 0.03 moles dimethylamine in 30 ml anhyd benzene. After heating, the solvent was removed under vacuum and the residue treated with dilute AcOH was extracted with ether. The water layer was made alkaline with a KOH aq and extracted with ether. After drying (K_2CO_3) the soln was evaporated and the residue distilled. *Cis*-2-methyl-4-dimethylaminomethyloxolane: b.p. 63–64° 18 mm (lit.[44] 108–111° 110 mm). *Trans*-2-methyl-4-dimethylaminomethyloxolane: b.p. 64–65° 18 mm. These tertiary amines were then quaternarized with MeI. *Cis*-2-methyl-4-trimethylammoniummethyloxolane iodide: from abs. EtOH, m.p. 193–194°. *Trans*-2-methyl-4-trimethylammoniummethyloxolane iodide: from abs. EtOH, m.p. 193–194°.

Cis- and trans-2-methyl-5-trimethylammoniummethyloxolane iodide (**4a** *and* **4b**). The mixture of these *cis* and *trans* compounds was obtained following the procedure reported in lit.[45] and separated using a Fractovap model P gas chromatograph with a 10-mm dia. and 4 m long column filled with 20% Carbowax 20 M on CGS 45–60 mesh, using the following operating conditions: the column oven temp was maintained isothermically at 65° for 20 min, then programmed from 65 to 160° at 3.5°/min, the final temp being maintained for 10 min; injection port temp 240°C, temp of the detector 240°, N_2 flow-rate 90 ml/min. The chromatogram shows two peaks: A and B in the ratio of about 4:1. An excess of MeI was added to an abs. EtOH soln of the tertiary amine corresponding to peak A. The precipitated ammonium salt was filtered off, then crystallized from abs. EtOH. *Cis*-2-methyl-5-trimethylammoniummethyloxolane iodide was obtained: m.p. 155–156° (lit.[45] 154–155°). Following the same procedure, from the tertiary amine corresponding to peak B, *trans*-2-methyl-5-trimethylammoniummethyloxolane iodide was obtained, m.p. 135–136°.

Cis- and trans-4-methyl-2-trimethylammoniummethyl-1,3-dioxolane iodide (**5a** *and* **5b**). The mixture of these *cis* and *trans* compounds was obtained following the procedure reported in lit,[46] and separated using a Fractovap model P gas chromatograph with a 10-mm dia. and 4 m long column filled with 12.5% Carbowax 20 M on CGS 60–80 mesh, using the following operating conditions: column oven temp 135°, injection port temp. 200°, temp of the detector 200°, N_2 flow-rate 180 ml/min. The chromatogram shows two peaks: A and B in the ratio of about 3:2. An excess MeI was added to an abs. EtOH soln of the tertiary amine corresponding to peak A. The precipitated ammonium salt was filtered off, then crystallized from abs EtOH. *Cis*-4-methyl-2-trimethylammoniummethyl-1,3-dioxolane iodide thus obtained has m.p. 136–137°. Following the same procedure, from the tertiary amine corresponding to peak B, *trans*-4-methyl-2-trimethylammoniummethyl-1,3-dioxolane iodide, m.p. 107–108°, was obtained.

Acknowledgements—The Authors wish to express their gratitude to the "Centro Strumenti" and "Centro di Calcolo" of Modena University for the use of the NMR spectrometer XL200 and for computing facilities. Cooperation of Dr. C. Bugatti in the preparation of the compounds is also warmly acknowledged.

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