Chemical Ionization Mass Spectrometry of Complex Molecules. XI.[1] Stereochemical and Conformational Effects in the Isobutane Chemical Ionization Mass Spectra of Some Steroidal Amino Alcohols

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Abstract: The chemical ionization (CI) mass spectra of several steroidal A ring 1,2- and 1,3-amino alcohols have been studied using isobutane as a reagent gas. The loss of water from the MH^+ ion protonated at the hydroxyl group occurs only when the distance between the oxygen and the nitrogen is too large to allow the formation of a hydrogen bond. Apart from a means of distinguishing between structural isomers, the CI mass spectra appear to permit conclusions regarding conformational equilibria. The results obtained in this way are consistent with those derived from ir data.

In CI mass spectrometry, introduced by Field and Munson in 1966,[2,3] the amount of energy available to produce ions is determined by the exothermicity of the reactions in which they are formed. Use of a reagent gas such as isobutane minimizes this exothermicity in proton transfer reactions, offering the best chance for stereochemical differences to manifest themselves. Several cases in which stereoisomers give different CI mass spectra have been reported, *e.g.*, esters of maleic and fumaric acids,[4] epimeric steroids,[5] and alkaloids.[6]

Amino alcohols are ideally suited to such studies in that their configurations and even their conformations can be readily ascertained by other means such as infrared spectrophotometry.[7] The first compounds that we studied by CI mass spectrometry were ephedrine[8] and pseudoephedrine. The isobutane CI mass spectra of these very flexible molecules are identical even at 25°, showing that differences between such isomers are too subtle to be detected by CI mass spectrometry. Within the more rigidly constrained steroids of the pregnane series shown below (Tables I, II, and IV), considerable differences were observed in the CI mass spectra of epimeric pairs.

Experimental Section

Compounds **1–4**,[9] **5–8**,[10] and **11–23**[11] were prepared by standard methods. The amino alcohols **9** and **10** were obtained by the action of dimethylamine on 5α-pregnane 2α,3α- and 3α,4α-oxides.[12] All CI mass spectra were measured on an MS-9 mass spectrometer modified[13] for use in the CI mode. Isobutane was used as the reagent gas, samples were admitted *via* a direct insertion probe, and the source temperature in all cases was 200 ± 10°. Under these conditions, the CI mass spectrum of isobutane is composed mainly of the *tert*-butyl ion (*m/e* 57)[14] which behaves as a weak Brønsted acid, protonating heteroatom-containing molecules with relatively little subsequent fragmentation.[15]

The ir frequency of the hydroxyl absorption band of most of these compounds was measured using a solution in carbon tetrachloride at an approximate concentration of 0.005 *M* in 2-cm cells on a Unicam SP-100. Under these conditions, intermolecular hydrogen bonding is minimized and the shifts eventually observed of the hydroxyl absorption bands are due to the intramolecular hydrogen bonds. Differences between the absorption frequencies of the free and the intramolecularly bonded species represent an evaluation of the proximity of the bonded groups.[16,17] The shifts ($\Delta\nu$) were calculated with respect to the frequency of the very weak free hydroxyl band when it was present. In the cases of **6** and **8**, which fail to show a free hydroxyl band, $\Delta\nu$ was calculated using the frequency of the free hydroxyl band in **5** (3640 cm^{-1}) and **7** (3639 cm^{-1}), respectively.

Results

The CI mass spectra of all these compounds are very simple. An ion at *m/e* (M + 1) is the base peak in all spectra except those of **19**, **20**, and **23**. In addition, most of the spectra have an intense peak at *m/e* (MH − 18) corresponding to the ion formed by loss of water following protonation of the hydroxyl group. Under the conditions used, no ion corresponding to the loss of the amine function is observed since a very stable ammonium ion is formed when the amino group is protonated. Such ions undoubtedly constitute a large proportion of those observed at *m/e* (M + 1). Many of the spectra also contain a small peak at *m/e* (M − 1) which is formed either from the protonated molecular

(1) Paper X in this series: G. W. A. Milne, H. M. Fales, and R. W. Colburn, *Anal. Chem.*, submitted for publication.
(2) F. H. Field and M. S. B. Munson, *J. Amer. Chem. Soc.*, **88**, 2621 (1966).
(3) F. H. Field, *Accounts Chem. Res.*, **1**, 42 (1968).
(4) H. M. Fales, G. W. A. Milne, and R. S. Nicholson, *Anal. Chem.*, **43**, 1785 (1971).
(5) J. Michnowicz and B. Munson, *Org. Mass Spectrom.*, **6**, 765 (1972).
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(7) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman, San Francisco, Calif., 1960, p 67.
(8) H. M. Fales, G. W. A. Milne, and M. L. Vestal, *J. Amer. Chem. Soc.*, **91**, 3682 (1969).
(9) M.-M. Janot, P. Longevialle, R. Goutarel, and C. Conreur, *Bull. Soc. Chim. Fr.*, 2158 (1964).
(10) P. Longevialle and R. Goutarel, *Bull. Soc. Chim. Fr.*, 3225 (1965).
(11) P. Longevialle, *Tetrahedron*, **25**, 3075 (1969).
(12) M. Svoboda, M. Tichy, J. Fajkos, and J. Sicher, *Tetrahedron Lett.*, 717 (1962).
(13) D. Beggs, M. L. Vestal, H. M. Fales, and G. W. A. Milne, *Rev. Sci. Instrum.*, **42**, 1578 (1971).
(14) F. H. Field, *J. Amer. Chem. Soc.*, **91**, 2827, 6334 (1969).
(15) G. W. A. Milne, H. M. Fales, and T. Axenrod, *Anal. Chem.*, **43**, 1815 (1971).
(16) M. Tichy, J. Sipos, and J. Sicher, *Collect. Czech. Chem. Commun.*, **27**, 2907 (1962).
(17) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Wiley, New York, N. Y., 1965, p 142.

Table I

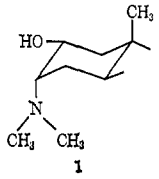
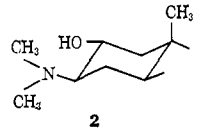
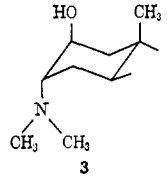
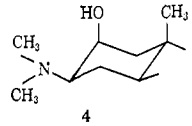
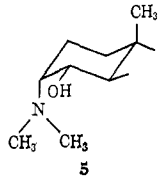
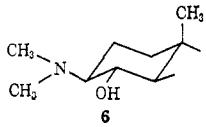
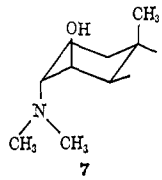
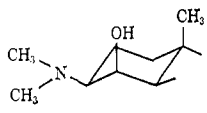
	MH^+	$MH^+ - H_2O$	Ir, cm^{-1}
 **1**	89.5		OH bonded 3360, $\Delta\nu$ = 270
 **2**	93		OH bonded 3493, $\Delta\nu$ = 137
 **3**	65	26.5	OH bonded 3465 OH free 3633 $\Delta\nu$ = 168
 **4**	93		OH bonded 3456, $\Delta\nu$ = 179
 **5**	92		OH bonded 3330, $\Delta\nu$ = 310
 **6**	98		OH bonded 3473, $\Delta\nu$ = 167
 **7**	51	41	OH free 3639
 **8**	92		OH bonded 3455, $\Delta\nu$ = 184

Table II

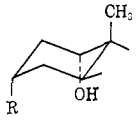
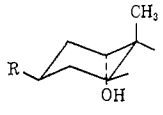
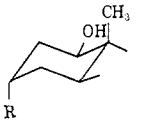
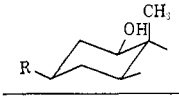
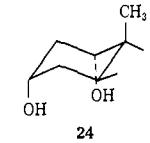
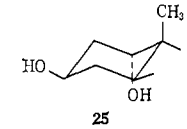
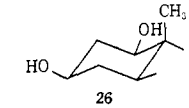
	R	MH^+	$\frac{MH^+}{H_2O}$	Ir, cm^{-1}
	**11** NH_2	96		
	12 $NHCH_3$	92.5		OH bonded 3306 $\Delta\nu$ = 324
	13 $N(CH_3)_2$	89		OH bonded 3280 $\Delta\nu$ = 350
	14 $NHCOCH_3$	96	2	
	**15** NH_2	66	32	
	16 $NHCH_3$	53.5	38.5	
	17 $N(CH_3)_2$	50	38	
	**18** NH_2	49	45	
	19 $NHCH_3$	42	51	OH free
	20 $N(CH_3)_2$	35	56	
	**21** NH_2	55	40	
	22 $NHCH_3$	44	39.5	
	23 $N(CH_3)_2$	36.5	52.5	

Table III

	MH^+	$MH^+ - H_2O$	$MH^+ - 2H_2O$
 **24**		2	94
 **25**		22.5	71
 **26**		14	80

ion by loss of a hydrogen molecule or from the neutral molecule M, by hydride abstraction.[2]

No fragment ions of mass less than *m/e* (M − 40) appear in any of these CI mass spectra and so the intensities of the MH^+ ions and of the ions at *m/e* (MH − 18) reported in Tables I–V are given as the percentage of the total ion current between *m/e* (M − 40) and *m/e* (M + 3), rather than as a percentage of the base peak, since in this way it can be shown what fraction of the total ion current is afforded by a given ion. The MH^+ ions of the primary, secondary, and tertiary amines are at *m/e* 320, 334, and 348, respectively, while the corresponding peak of the acylamino alcohol **14** is at *m/e* 362.

Examination of the results (CI mass spectra and ir spectra) given in Tables I, II, and IV clearly shows that the loss of water from the MH^+ ion is important when there is no hydrogen bonding between the hydroxyl and amino groups, as in, for example, **7**, **10**, and **15–23**. On the other hand, loss of water apparently does not occur when the two groups are close together (*e.g.*, in **1**, **2**, **4**, **5**, **6**, **8**, and **11–14**).

Thus protonation of an intramolecularly hydrogen-bonded species results in a complex that is more stable with respect to dehydration than one that is not so bonded. It seems eminently reasonable that this should

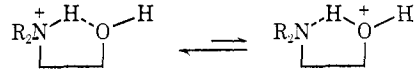


Table IV

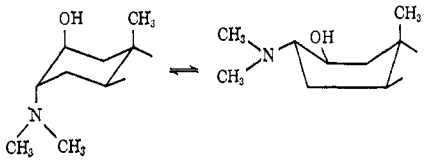
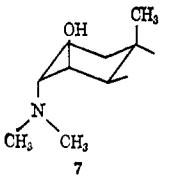
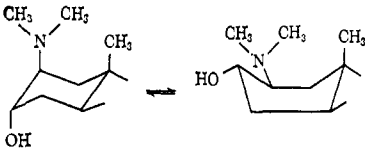
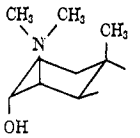
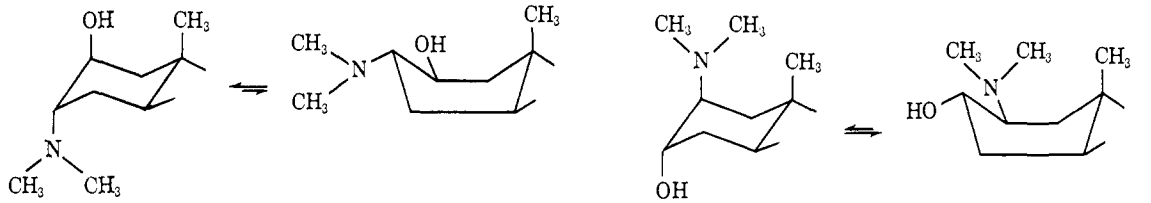
	MH^+	$MH^+ - H_2O$	Ir, cm^{-1}
 **3**	65	26.5	OH bonded 3465 OH free 3633 $\Delta\nu = 168$
 **7**	51	41	OH free 3639
 **9**	75	14	OH bonded 3455 OH free 3630 $\Delta\nu = 175$
 **10**	38.5	52.5	OH free 3628

Table V



3 **9**

Ir	60	40	30	70
CIMS	65	35	26.5	73.5

be so since the hydrogen bridge should remain intact regardless of which atom is protonated. Furthermore, considering the proton affinities of nitrogen and oxygen, the above equilibrium should lie far to the left, thus favoring a species that would appear to be less favorably disposed to lose water.

If this explanation is correct, replacement of the amino group by a hydroxyl group should lead to a diol whose protonated form loses water readily, whether or not the original diol is intramolecularly hydrogen bonded, since two protons must always be intimately associated with one of the oxygens resulting in a favorable intermediate for water loss. This difference between the diols and the amino alcohols will exist insofar as their stabilities depend upon the relative proton affinities of nitrogen and oxygen rather than on the bridged nature of the complex itself.

To check this hypothesis, we measured the CI mass spectra of the steroidal diols **24** and **25**[18] and **26**.[11] As expected (Table III), all fail to exhibit a protonated molecular ion. Peaks are seen at *m/e* values corresponding to $(MH - H_2O)^+$ (*m/e* values 275 and 303 in the androstane and pregnane series, respectively) and major ions are found at $(MH - 2H_2O)^+$ (*m/e* 257 and 285 in the same series).

These results are in apparent contrast to those reported by Dzidic and McCloskey[19] who found that long chain diols do show protonated molecular ions in spite of the fact that such compounds fail to show appreciable intramolecular hydrogen bonding in solution. On the other hand, as these authors note, the protonated diols in the gas phase may well be intramolecularly hydrogen bonded since they are flexible enough to permit the necessary conformations. The differences between the stabilities of the protonated diols of this sort and the above steroidal diols may lie in the strength of the hydrogen bond in question. The acyclic diols are able to form a much more linear bond than the steroid diols and as many workers have

(18) G. von Mutzenbecher, *Steroids*, **5**, 429 (1965).

(19) I. Dzidic and J. A. McCloskey, *J. Amer. Chem. Soc.*, **93**, 4955 (1971).

shown,[20] hydrogen bond strength is as much related to the bond angles involved as to the bond lengths.

Surprisingly, acetylation of the amino function of **11**, giving **14**, does not enhance significantly the dehydration process: the intensity of the ion at *m/e* $(MH - 18)^+$ in **14** is 2%. It is apparent that in the gaseous state, the acetamido group is still sufficiently more basic than the hydroxyl group to compete effectively for the proton of the reagent ion.

Also of interest is the fact that the peak corresponding to loss of water from the protonated molecular ions of the amino alcohols **18–23**, in which the hydroxyl group is β equatorial, is particularly important, it being the largest peak in the CI mass spectra of **19**, **20**, and **23**. This perhaps may be attributed to the steric bulk of the β-hydroxyl group on C-1, whose elimination as water brings about release of strain.

It seems possible to investigate steric effects even further than this. Our data on the trans-diaxial amino alcohols **3** and **9** (Table V) suggest that conformation may play a significant role in the behavior of these compounds under conditions of CI. Sicher, *et al.*,[12] have shown by infrared spectrophotometry that 2β-hydroxy-3α-dimethylaminocholestane is partially intramolecularly hydrogen bonded. The A ring of this compound will therefore be partially in a boat conformation since the alternative chair form will experience difficulty establishing a hydrogen bond between the 1,2-trans-diaxial substituents. Integration of the spectral bands led to the conclusion that approximately 40% of the molecules were in the boat (bonded) conformation, and the authors suggest that this deformation of ring A is probably due to the unfavorable 1,3 diaxial interaction between the hydroxyl group and the C-19 methyl group of the chair form.

These same authors also showed that the A ring of the isomeric 2β-dimethylamino-3α-hydroxycholestane is even more completely (70%) in the boat form due to the increased repulsion between the bulkier 2-dimethylamino and 19-methyl groups. In contrast to this, the A ring of 3α-hydroxy-4β-dimethylaminocholestane, in which hydrogen bonding is impossible, stays in the chair form in spite of the fact that it is subject to the same 1,3-diaxial repulsion between the 19-methyl group and the substituent at C-4. As was noted, the A ring deformation in this case is probably prevented by the strong interaction, which would exist in the boat form, between the C-4 amino function and the eclipsed C-5–C-6 bond.

We have obtained identical results on the analogous compounds in the pregnane series (**3**, **9**, and **10**) (Table IV). Also shown in Table IV are data derived for 3α-dimethylamino-4β-hydroxycholestane (**7**), which appears to exhibit no hydrogen bonding, presumably for reasons similar to those operating in the case of **10**.

In CI mass spectrometry, **3** and **9** show relatively little loss of water (26.5 and 14%, respectively) compared to the other isomers with free hydroxyl groups. This may be due to their existence partially in a hydrogen bonded, A ring boat conformation.

In evaluating the degree of dehydration observed in CI as a reflection of the boat (bonded) to chair (unbonded) conformations of **3**, it seems logical to suppose that MH^+ represents mainly species protonated on nitrogen in both boat and chair forms. On the other hand, the ion at *m/e* $(MH - H_2O)^+$ in the CI mass spectrum of **3** is assumed to arise only from its chair form which has been protonated on the hydroxyl oxygen. Since very similar stereochemistry is possessed by **7** which has no tendency to assume an A ring boat conformation, as evidenced by ir spectroscopy, it serves as an ideal model for evaluating the loss of water expected for **3** in the event that the A ring of **3** was exclusively in the chair conformation. In **7**, the ion at *m/e* $(MH - H_2O)^+$ accounts for 41% of the total ion current above *m/e* $(M - 40)^+$. Therefore, the ratio 26.5/41 × 100 (=65%) represents the proportion of **3** that has the A ring in the chair form. This compares well with the figure of 60% obtained by ir spectroscopy.

Compound **10** is an even better model for **9** since they differ only in the position of the amino group, the hydroxyl groups being 3-axial in both cases. In this case, the above calculation reveals that 25.6% of the compound has an A ring in the chair conformation, and the corresponding figure from ir spectroscopy is 30%.

This explanation further requires that after protonation in the ion source, the boat and chair forms of the ions do not interconvert before they are mass-analyzed (10–100 μsec) and that the rates of protonation of both isomers are either equivalent, or so rapid (compared to the interconversion rate) that all of the molecules of the steroids are protonated in the ion chamber.

Considering the differences in phase and temperature between the infrared and CI experiments, the agreement between these data seems (Table V) surprisingly good. Clearly, additional examples will be required to confirm the value of CI mass spectrometry in conformational analysis, but these results suggest that such investigations are at least worthwhile.

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(20) Reference 7, p 263.