Distinction Between 17-Epimeric Hydroxy Steroids of the 3,17-Dioxygenated Androstane Series by Chemical Ionization

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The distinction between 17-epimeric 3,17-dioxygenated hydroxyandrostanes has been made by comparison of both their methane or ammonia positive and OH^- negative chemical ionization (CI) mass spectra. In the methane or ammonia positive CI, the 17α-configuration in the eight stereoisomeric 5ξ-androstane-3ξ,17ξ-diols can be determined by the relative abundances of the ion $[MH-2H_2O]^+$. In the ammonia CI spectra, the ion $[M+NH_4-H_2O]^+$ possesses only a low abundance, but a comparison of the relative rates of the loss of water v. the loss of ammonia from $[M.NH_4]^+$ in the second field-free region allows a clear distinction to be made between the 17α- and 17β-series. In the OH^- negative CI mass spectra, the 5ξ-androstane-3-one-17ξ-ols produce an intense ion $[M-H-H_2O]^-$ in the 17α-series only.

INTRODUCTION

The stereochemical differentiation of isomeric oxygenated steroids by chemical ionization mass spectrometry has been the subject of several studies, both in the positive ionization mode[1–4] and in the negative mode.[5–7] Nevertheless, except in recent publications[6,7] where a bifunctional interaction was shown in 14β,17ξ-androstane diols, nothing has been reported concerning the distinction between 17-epimeric androstanes hydroxylated in position 17. We report in this paper the use of methane and ammonia positive and OH^- negative chemical ionization for the determination of the stereochemistry of position 17 in 5ξ-androstane-3-one-17ξ-ols and 5ξ-androstane-3ξ,17ξ-diols.

EXPERIMENTAL

Instrumentation

A VG-ZAB 2F mass spectrometer equipped with a high-pressure ion source, working at 8 kV, was used. In the positive ion chemical ionization mode, the reagent gas pressure was adjusted to minimize the abundance of the odd-electron ions. In particular when ammonia was used, the ratio between the abundance of the ions m/z 17 and 18 was less than 0.1. In the negative mode, the generation of the OH^- ions was made by a careful control of the relative flow rate of the reagent gases CH_4 and N_2O, and the ratio between $O^{-\cdot}$ (m/z 16) and OH^- (m/z 17) was less than 0.1. In these conditions the ratio between the flow rates of methane and N_2O was approximately 10.

The source temperature was left to equilibrate without external heating (*c.* 200 °C) and the samples were introduced via a direct inlet probe.

Chemicals

The androstane-3,17-diols and androstane-3-one-17-ols belonging to the 17β-hydroxy series were purchased from Sigma, together with the sample of epitestosterone which was the starting material for the chemical synthesis of all the steroids of the 17α-hydroxy series.

Chemical synthesis

The androstane-3-one-17α-ols were synthesized by a catalytic hydrogenation of epitestosterone over a 10% Pd/C catalyst, in ethanol. The separation of the 5α- and 5β-epimers was performed by HPLC, using a Porasil column (Waters-7.8 × 300 mm) and a mixture of hexane and ethyl acetate 6:4 as eluent. The 5α-epimer ($k' = 1.83$, yield = 13%) was eluted before the 5β-epimer ($k' = 3.58$, yield = 67%); spectral data: $[M]^{+\cdot}$ at m/z 290, $\nu_{CO} = 1720\ cm^{-1}$. The respective methylene values on a capillary column were 24.95 and 24.64.

The synthesis of the 5α-androstane-3ξ,17α-diols was made by catalytic hydrogenation of the 5α-androstane-3-one-17α-ol over platinum in acetic acid. The two epimers were separated by reverse phase HPLC (μC_{18} Bondapak from Waters—3.9 × 300 mm) using methanol–water 3:1 as eluent. The 3β-epimer

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CCC-0030–493X/85/0020–0525$02.50

($k' = 1.55$, yield = 20%) was eluted before the 3α-epimer ($k' = 3.10$, yield = 70%). On capillary GLC (OV 1) the two isomers were only slightly differentiated (methylene values 24.60 and 24.58, respectively).

The synthesis of the 5β-androstane-3ξ,17α-diols was made by sodium borohydride reduction of the corresponding 3-keto derivative. The two epimers were separated by reverse phase HPLC, using the same conditions as above. The 3β-epimer ($k' = 2.06$, yield = 11%) was eluted before the 3α-epimer ($k' = 3.50$, yield = 80%). The respective methylene values on GLC (OV 1) were 25.10 and 24.60.

RESULTS AND DISCUSSION

Methane and ammonia positive chemical ionization of androstane-3,17-diols: double dehydration process

It has been shown[2] that under methane chemical ionization conditions the quasi-molecular ion $[MH]^+$ of 3ξ,17β-androstane-diols is of very low intensity and that the two most abundant ions are $[MH-H_2O]^+$ and $[MH-2H_2O]^+$. We have obtained similar spectra with the androstane-3,17-diols in the 17α-series, but have also observed that the intensity ratio of the two daughter ions depends on the stereochemistry of the asymmetric centres 3, 5 and 17 (see Table 1). Indeed the intensity of ions from double dehydration is much higher for 17α-epimers. This is also the case under NH_4^+ chemical ionization conditions where the $[MH-2H_2O]^+$ ion abundance is clearly larger in the 17α-series than in the 17β-series (Table 1). The mechanism of this stereodependent elimination of two water molecules is unknown. The loss of the first water molecule is directly related to the protonation process in which a carbocation at positions 3 or 17 is formed. The second water loss occurs from the $[M-H_2O]^+$ ion as shown by the observation of the corresponding metastable ion.[2] In these rigid molecules a proton transfer from a position close to the carbocation formed to the remaining hydroxyl group cannot be taken into account for the loss of a second water molecule. Two other possibilities for this mechanism are either a charge transfer via a 'backbone rearrangement' which would invert all asymmetric centres of the skeleton,[8,9] or the reprotonation of a neutral molecule formed after the loss of H_3O^+ from $[MH]^+$. In the former mechanism, the positive charge would be transferred from ring *A* to ring *D*, since the driving force is the strain release of the *trans*-hydrindane junction of the rings *C* and *D*. In such a process, the final elimination of water from the 17-OH group might be stereoselective.

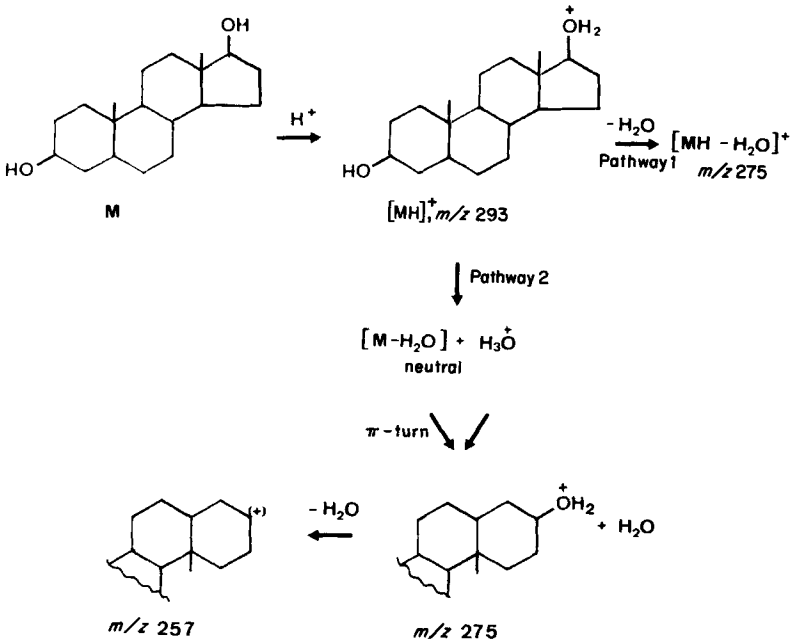
Alternatively, reprotonation of a neutral fragment by the reagent gas is highly unlikely. Nevertheless it is possible for a positive ion formed in the first step to protonate its neutral counterpart after a 'π turn' of the latter, before they move apart completely. Such an interaction has been demonstrated by Longevialle and Botter[10,11] and accounts for proton transfer between spatially remote positions in the fragmentation reactions of steroids after electron impact. Thus the observed stereodependence of the double loss of H_2O from protonated androstane-3,17-diols could well originate from the formation of the neutral fragment in the first reaction step (Scheme 1, reaction pathway 2). It has been shown[2] that none of the hydrogen atoms α to the hydroxyl groups are involved in the consecutive losses of water molecules. The higher abundance of $[MH-2H_2O]^+$ ions in the 17α-series can be explained by the closer distance of the axial hydroxyl group to the other hydrogen atoms of the ring than the 17β-OH group. It has to be noted that the symmetrical process in which the first reaction step would occur at the hydroxyl group in position 3 should reflect the stereochemistry in this position. Indeed the double elimination of water is a favoured reaction when the cycle's junction is *trans* and the hydroxyl group in position 3 is axial (3α) (see Table 1); but this differentiation is not possible in the 5β-series or when ammonia is used as the reagent gas.

Table 1. Chemical ionization mass spectra of the androstane-3,17-diols with methane and ammonia as reagent gas: relative abundance of the ion from double dehydration of $[MH]^+$

	$[MH-2H_2O]^+/[MH-H_2O]^+$		$[MH-2H_2O]^+/[M+NH_4]^+$
	CH_4	NH_3	
5α-Androstane-3α,17α-diol	9.6	8.1	1.39
5α-Androstane-3α,17β-diol	3.6	3.8	0.19
5α-Androstane-3β,17α-diol	5.7	10	1.20
5α-Androstane-3β,17β-diol	1.8	2.8	0.17
5β-Androstane-3α,17α-diol	8.8	7.6	0.58
5β-Androstane-3α,17β-diol	2.3	3.7	0.19
5β-Androstane-3β,17α-diol	7.3	7.6	1.15
5β-Androstane-3β,17β-diol	2.7	4	0.17

Ammonia positive chemical ionization of 3,17-diols: nucleophilic substitution reaction

The source spectra of androstane-3,17-diols, in NH_4^+ chemical ionization, show an intense ion at *m/z* 292 (Fig. 1). This ion may correspond either to an $[M]^{+\cdot}$ ion formed by a charge transfer reaction, or to a $[M+NH_4-H_2O]^+$ ion resulting from a nucleophilic substitution reaction: the mechanism of the latter reaction has already been demonstrated to be of the S_N2 type,[12] from which one could expect a stereodependence from position 17. In order to determine the nature of the ion at *m/z* 292, we used ND_3 in place of NH_3.[4] As can be seen in Table 2 more than 75% of the corresponding ions have their mass number displaced by 2 mass units and 22% of them by 4 mass units. A mass displacement of 4 mass units is expected for the $[M+NH_4-H_2O]^+$ ions resulting from a nucleophilic substitution reaction $[M+ND_4-D_2O]^+$. Thus the majority of the ions present at *m/z* 292 are $[M]^{+\cdot}$ ions. The relatively low abundance of ions from nucleophilic substitution in the spectra of saturated alcohols has already been noted[1] and seems to be observable only if the molecule possesses another



Scheme 1

electropositive site contributing to the stabilization of the ion.[1,7,12]

The occurrence of a nucleophilic substitution reaction is clearly reflected in the mass-analysed ion kinetic energy (MIKE) spectra of the $[M.NH_4]^+$ ions from the androstane-3,17-diols as can be seen in Table 3, which reports the intensity ratio of the $[M.NH_4-H_2O]^+$ and $[M.NH_4-NH_3]^+$ ions. The loss of a water molecule yields an ion isobaric to the molecular ion, while loss of NH_3 leads to $[MH]^+$ ions. The nucleophilic reaction (loss of water) is seen to be the major process in the 17α-series, so that the simple measurement of the intensity ratios of the ions from these two competitive reactions allows the determination of the stereochemistry in position 17, whatever the configurations of centres 3 and 5 are.

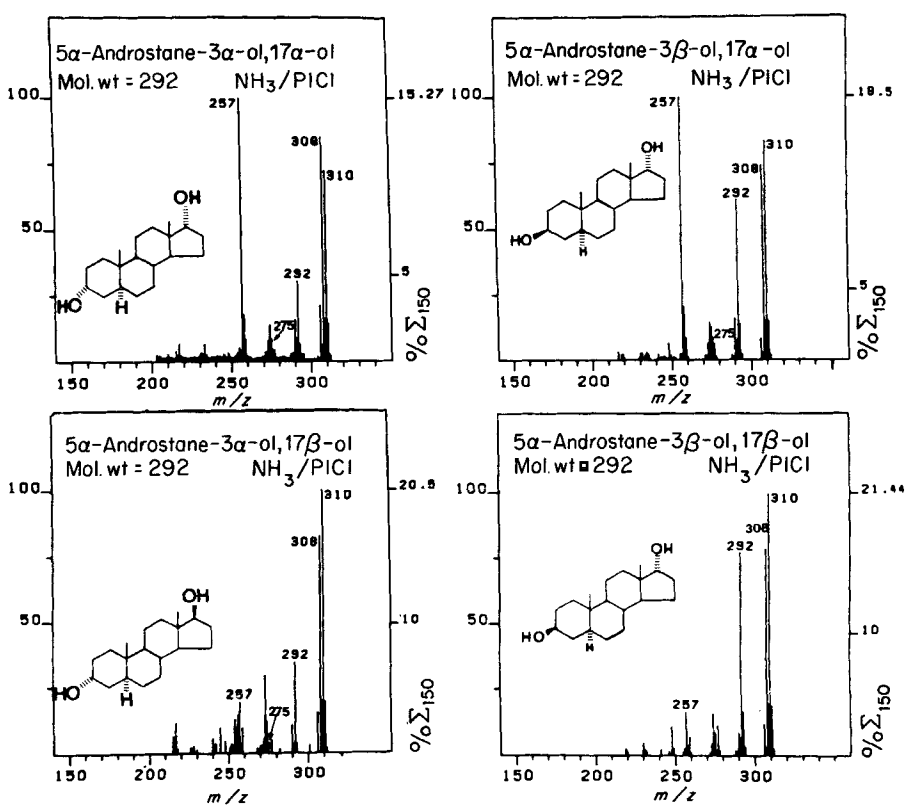


Figure 1. Mass spectra of the four isomers of 5α-androstane-3ξ,17ξ-diol under NH_4^+ chemical ionization.

Table 2. Partial chemical ionization mass spectra of the 5α-androstane-3β,17α-diol using NH_3 and ND_3 as reagent gas. M_d and M_h represent the androstane-diol molecule in which the hydroxylic hydrogen atoms are exchanged or remain unexchanged with deuterium, respectively. The values for the abundance of the ions have been corrected from ^{13}C contributions, if any, and calculated relative to the intensity of the adduct $[M_h.NH_4]^+$ or $[M_d.ND_4]^+$, respectively.

Reagent gas	m/z = 316	313	310	308	296	294	293	292
NH_3	—	—	100	90	—	—	<5	73
(Assignment)	—	—	$[M_h.NH_4]^+$	$[M_h.NH_4-H_2]^+$	—	—	$[MH]^+$	$[M_h]^{+\cdot}$ or $[M_h.NH_4-H_2O]^+$
ND_3	100	36	—	—	12	42	—	—
(Assignment)	$[M_d.ND_4]^+$	$[M_d.ND_4-HD]^+$	—	—	$[M_d.MD_4-D_2O]^+$ or $[M_dD]^+$	$[M_d]^{+\cdot}$	—	—

Table 3. MIKE spectra of the $[M.NH_4]^+$ ion from androstane-diols: ratio between the loss of water and ammonia

	$[M.NH_4-H_2O]^+/[M.NH_4-NH_3]^+$
5α-Androstane-3α,17α-diol	9.7
5α-Androstane-3α,17β-diol	0.44
5α-Androstane-3β,17α-diol	20.0
5α-Androstane-3β,17β-diol	0.25
5β-Androstane-3α,17α-diol	16.5
5β-Androstane-3α,17β-diol	3.0
5β-Androstane-3β,17α-diol	10.0
5β-Androstane-3β,17β-diol	2.0

This metastable reaction is unimolecular, thus only a S_Ni type mechanism can be taken into account. The stereochemical effect could be due to a minor steric hindrance of the α-face which would facilitate the reaction for the hydroxyl group at 17α.

Negative chemical ionization of androstane-3-one-17ξ-ols by OH^-

In contrast to androstane-3,17-diols, the 3-keto,17-hydroxy compounds do not show significant differences related to the configuration of the 17-site in their methane or ammonia chemical ionization spectra. In the MIKE spectra the signal corresponding to the reaction $[M.NH_4]^+ \rightarrow [M-NH_4-H_2O]^+$ is masked by that of an intense loss of NH_3 originating from the desolvation of the carbonyl function.

Under negative chemical ionization conditions using OH^- (generated by electron impact of a 10:1 mixture of CH_4 and N_2O,[13] the source spectra of 3-keto,17-hydroxyandrostane derivatives are very sensitive to the stereochemistry of position 17 (Fig. 2 and Table 4). The compounds of the 17β-series virtually only show a $[M-H]^-$ anion, while their homologues from the 17α-series are seen to give an intense $[M-H-H_2O]^-$ ion.

This water loss from the $[M-H]^-$ ion is also observed in the metastable spectra. It is more important by a factor of five in the 17α-series than in the 17β. This reaction involves a successive transfer of two protons to the hydroxylic oxygen, the first one being a 1,3-process. The stereochemical effect can be explained by the closer proximity of the transferable hydrogen atoms in the 17α-isomer (axial) relative to the 17β-isomer. However, the mechanism is probably more complex, as no difference in the water loss from the $[M-H]^-$ has been observed for androstane-17ξ-ols which do not contain another oxygenated function.[6] The oxygenated site in position 3 indeed plays an important role in this reaction which can be compared to the long-distance effects observed in the condensed phase[14] in which the functional groups of ring A influence the rate of the elimination reactions of the groups at position 17, probably by transfer of conformational modifications.

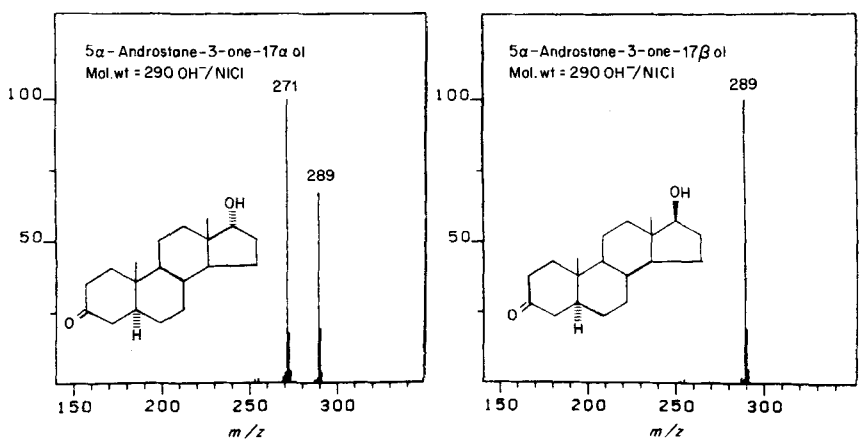


Figure 2. Mass spectra of the two isomers of 5α-androstane-3-one-17ξ-ol under OH^- chemical ionization.

Table 4. Negative chemical ionization mass spectra of some 17-hydroxyandrostanes using OH^- as reagent ion

	$[M-H-H_2O]^-/[M-H]^-$ (×100)
5α-Androstane-17α-ol	2.3[a]
5α-Androstane-17β-ol	4.5[a]
5α-Androstane-3-one-17α-ol	150
5α-Androstane-3-one-17β-ol	7
5β-Androstane-3-one-17α-ol	115
5β-Androstane-3-one-17β-ol	6

[a] Ref. 6.

CONCLUSION

Various reactions occurring under chemical ionization conditions, i.e. elimination and substitution reactions, allow a clear-cut distinction between 17-epimeric androstanes oxygenated at positions 3 and 17. In these molecules, where bifunctional interaction is not possible, the stereochemical effects are due either to differences in the steric hindrance of the two faces, or to regio- or stereo-selectivity in the hydrogen transfers. Some of the differences are large enough to allow the assignment of the stereochemistry of position 17 while ignoring that of centres 3 and 5.

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Received 4 December 1984; accepted 17 January 1985