Stereochemistry of Gaseous Anions: OH^- Negative Chemical Ionization of 17ξ-R-5α,14β-androstane-14,17ξ-diols

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Abstract: The decomposition of $(M - H)^-$ alkoxide anions from 17ξ-R-5α,14β-androstane-14,17ξ-diols (R = CH_3 (**5**), C_2H_5 (**6**), C_2H_3 (**7**), and C_2H (**8**)) formed in OH^- negative chemical ionization (OH^-/NCI) has been studied by means of ion source, metastable (MIKE), and collisional activation (CID/MIKE) mass spectra. The stereochemistry is unambiguously assigned, as only trans-diol isomers lose a RH molecule. The resulting $(M - H - RH)^-$ ions are shown to have a ketolate structure. By using a ND_3/N_2O mixture, which allows for gas-phase H/D isotope exchange of labile hydrogen atoms in the neutral species prior to ionization, it is possible to identify the H atom involved in the RH loss and to propose a mechanism for this highly stereospecific reaction.

Introduction

Mass spectrometric differentiation of stereoisomeric compounds from their fragmentation behavior in chemical ionization (CI) spectra is well known.[1] In contrast to the importance of stereochemistry in positive ion mass spectrometry, there are few examples of investigation on the stereochemistry of gas-phase anions. Negative chemical ionization (NCI) is of continuously increasing use since its first presentation by Dougherty.[2] A number of papers by Bowie,[3] Field,[4] Hunt,[5] Jennings,[6] and Dillard[7] show the potential of this technique for analytical and structural studies.

With $O^-\cdot$ and OH^- as reactant anions,[8] information can be obtained on the environment of the alcohol functional group[9] which is reflected in the spectra by the presence or lack of molecular hydrogen, alkanes, and water losses from the $(M - H)^-$ alkoxide anion, which is usually the most abundant ion. Moreover, it is possible to study the environment of charged functional groups and to distinguish between isomers by using appropriate deuteration reactants, such as ND_3, D_2O, and ROD.[10] The decomposition of $(M - H)^-$ anions has been shown to be sensitive to the stereochemistry of the alcoholate group. Bruins[11] reports large effects on water loss in stereoisomeric monoterpenoids such as *cis*- and *trans*-carveol. Stereochemical effects on anion mass spectra of cyclic diols, which are model compounds for natural products, have been reported by Winkler and Stahl.[12] The stability of $(M - H)^-$ anions in cyclohexane- and cyclopentanediols is attributed to the possibility of intramolecular hydrogen bond ($OH\cdots{}^-O$) formation.[13,14] The study of OH^-/NCI spectra of 35 steroids by Field[15] demonstrates that stereochemical effects are not always so clearcut. Spectra of 5α- and 5β-cholestane-3β,5-diols show only minor differences on which stereochemistry assignment would not be reliable. On the other hand, spectra of positional isomers are characteristic (Table I, values from ref 15). Spectra of androstanol stereoisomers obtained by Djerassi et al.[16] in negative desorption chemical ionization are not substantially different. However, there are only a few studies of steroids with an alcohol function on ring D.[17]

In the present paper we report results obtained in OH^- negative chemical ionization of steroids with an OH on ring D, in particular, 14β,17ξ-diol cis and trans isomers.

Experimental Section

The compounds listed in Figure 1 have been investigated. Their recent synthesis will be published elsewhere.[18]

Spectra were obtained with a ZAB-2F double-focusing instrument of reverse geometry (VG Analtytical Ltd.). A CI source was used at a pressure of about 1.5 torr. The instrument settings were: 8 kV accelerating voltage, 500 μA total emission current, source temperature 180 °C. OH^- reactant anion was generated in a mixture of CH_4/N_2O (10:1). The hydrogen/deuterium exchange reactions were carried out using a mixture of ND_3/N_2O. Deuterated ammonia gas was obtained from C.E.A. Saclay (France). Decompositions occurring in the first field-free

Scheme I

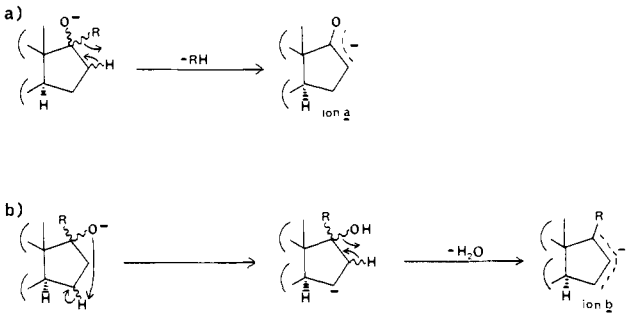

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Table I. NCI Spectra of Stereoisomers in the Cholestane Series[15]

	$(M-H)^-$	$(M-H-H_2)^-$	$(M-H-H_2O)^-$	$(M-H_3-H_2O)^-$
5α-cholestane-3β,5-diol	65		18	
5β-cholestane-3β,5-diol	64		18	
cholest-5-ene-3β,7α-diol	18	4	26	8
cholest-5-ene-3β,7β-diol	22		31	8
cholest-5-ene-3β,4β-diol[a]	5	5	73	12

[a] The loss of two hydrogen molecules from $(M-H)^-$ is also observed.

Table II. OH^-/NCI Source Spectra of Steroidal Alcohols[a]

compd	$(M-H)^-$	$(M-H_2)^-$	$(M-H_3)^-$	$(M-H-CH_4)^-$	$(M-H-H_2O)^-$	$(M-H_3-H_2O)^-$	$(M-H-RH)^-$	$(M-H-RH-H_2O)^-$	$(M-H-2H_2O)^-$
1a	71.0	7.7	11.6	2.9	3.2	3.6			
1b	68.8	10.1	18.2	<1	1.6	1.0			
2a	87.7	3.0	2.6	<1	1.7	<1	3.0	1.3	
2b	75.6	6.3	4.2	2.1	2.9	1.3	6.3	1.3	
3b	10.8	<1	<1	<1	2.0		83.5	<1	
4b	47.7		43.7	1.0	5.5	2.1			
5a	97.0	1	<1	<1	1.0				
5b	52.0			(47.0)[b]	1.0		47.0		
6a	80.2	2.5	7.4	<1	6.2	1.0	<1		1.4
6b	48.3	<1	4.1	<1	7.2	1.0	38.0		<1
7a	97.6			<1	<1		<1		<1
7b	53.4	<1	<1	1.4	2.5	1.5	37.6		2.2
8a	95.2	1.2	1.1	<1	<1		1.2		
8b	11.0	1.6	1.9	1.4	<1	<1	82.2		

[a] The intensities are given in % of the total ionization, neglecting fragments of very low abundance which correspond to fragmentation of the steroid skeleton. [b] For compound **5** $(M-H-CH_4)^- = (M-H-RH)^-$.

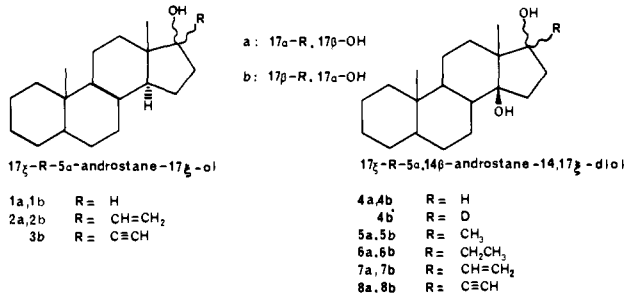

Figure 1.

region of the instrument were investigated using the high-voltage scan technique (HV scan).[19] Metastable transitions in the second field-free region and collision-induced decomposition (He as collision gas) of $(M-H)^-$ anions were studied by the mass analyzed ion kinetic energy technique (MIKE).[20]

Results and Discussion

1. Fragmentation Reactions Occurring in the Ion Source. The OH^-/NCI spectra of all compounds studied are given in Table II. The following observations can be made: (a) The spectra of monoalcohol stereoisomers (17ξ-R-**5**α-androstan-17ξ-ol) are very similar. In these compounds no interaction between different functional groups is possible. (b) Alkoxide anions from secondary alcohols (R = H) lose molecular hydrogen or a water molecule to form the enolate ion a and the allylic ion b, respectively (Scheme I). (c) Similarly, $(M-H)^-$ ions from tertiary alcohols eliminate ethylene and a water molecule when R = C_2H_3 (**2**), while the acetylenic substituted analogue (**3**) loses only an acetylene molecule (Table II).

The formation of ions of types a and b has already been used for characterization of alcohols.[14] Moreover, when R is an unsaturated moiety (**2**, **3**), it is suprising that no cleavage of ring D is observed, but rather reactions such as loss of C_2H_4 or C_2H_2 requiring cleavage at a vinylic bond are favored. Scheme Ib is a possible illustration of the formation of $(M-19)^-$ ions (loss of H_2O) by a mechanism similar to that proposed by Field.[15] The $(M-H-H_2O)^-$ anions of structure b are highly resonance stabilized by the presence of an unsaturated substituent in position 17 (α or β).

Unlike 17-androstanol compounds, 17ξ-R-5α,14β-androstane-14,17ξ-diols exhibit very strong differences in the spectra of their stereoisomers: only anions from 14β,17α-diols (**5b**, **6b**, **7b**, and **8b**) eliminate a hydrocarbon molecule to a significant extent (see Table II). The stereochemistry is clearly reflected by the presence in the spectra of the 14β,17α-diols of a peak at m/z 289 corresponding to RH loss from the alkoxide anion. Therefore, it is possible to assign unambiguously the stereochemistry of 17ξ-substituted 14β,17ξ-diols from their OH^-/NCI spectra.

2. Decomposition of Long-Lived Ions (>10 μs). Ions leaving the ion source with some excess internal energy can decompose during their flight through the mass spectrometer. Fragmentation reactions occurring in the second field-free region of the instrument can be observed by the MIKE technique. Distinction between steroid epimers has been achieved by measuring the translational energy released in decompositions of metastable positive ions[21] or by analyzing the reaction sequence in this time range.[22]

The mechanism of formation of $(M-H-RH)^-$ ions has been investigated via the MIKE spectra. These are reported in Table III along with those obtained after collisional activation of the $(M-H)^-$ parent ions (CID/MIKES). MIKE and CID/MIKE spectra of diastereoisomers in the androstanol series are similar. The allylic alcohol **2** eliminates spontaneously only a water molecule, while for the two other analogues **1** and **3** loss of RH (R = H and R = C_2H) is by far the major process observed in metastable decompositions. After collisional activation of the $(M-H)^-$ ion, both reactions (loss of RH and loss of H_2O) are observed for each compound, indicating that the activation energy barrier for either reaction is overcome.

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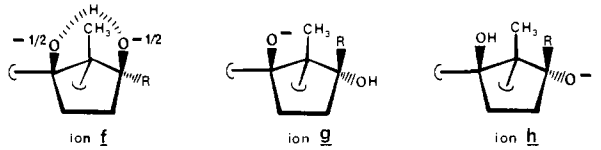
Table III. MIKE and CID/MIKE Spectra of $(M - H)^-$ [a]

	MIKES							CID/MIKES						
compound (m/z)	$-H_2$	$-HD$	$-H_2O$	$-RH$	$-2H_2O$	$-(RH + H_2O)$	$-(C_3H_5OR)$	$-H_2$	$-HD$	$-H_2O$	$-RH$	$-2H_2O$	$-(RH + H_2O)$	$-(C_3H_5OR)$
1a, 1b[b] (275)	0.95		0.05					0.80		0.20				
2a, 2b[b] (301)			1.00							0.10	0.90			
3b (299)			0.02	0.98						0.44	0.56			
4b (291)	0.80		0.20					0.39		0.43	0.04			0.14
4b' (17-D) (292)		0.80	0.20						0.51	0.33	0.04			0.12
5 (305) **5a**			0.85	0.15						0.78	0.10			0.12
5b			0.85	0.15						0.89	0.07			0.04
6 (319) **6a**			0.97	0.03						0.81	0.02			0.17
6b			0.55	0.45						0.65	0.33			0.02
7 (317) **7a**			0.56	0.04	0.40					0.59	0.03	0.18	0.06	0.13
7b			0.10	0.78	0.09	0.03				0.07	0.57	0.20	0.10	0.06
8 (315) **8a**			0.13	0.83			0.04			0.27	0.13		0.12	0.48
8b										0.15	0.74		0.09	0.02

[a] Intensities given in % of the sum of fragments. [b] No significant difference between cis and trans isomers.

Scheme II



In sharp contrast, stereospecific reactions are observed in the metastable decomposition of 14β,17ξ-androstanediol derivatives. Only 17ξ-R-substituted trans diols (R = CH_3, C_2H_5, C_2H_3, C_2H) give rise to $(M - H)^-$ ions which lose an RH molecule. If water loss is always observed, consecutive reactions such as loss of (RH + H_2O) or (H_2O + H_2O) are minimized. The MIKE spectra obtained after collisional excitation (CID/MIKES) show the same stereochemical effect (loss of RH only for trans diols), but also consecutive reactions, as well as loss of ring D ($-C_3H_5OR$). All daughter ions from reactions induced by collision are resonance stabilized, and no stereochemical effects are observed for reactions other than loss of RH molecules.

The behavior of the stereoisomers can be explained by the different stability of the $(M - H)^-$ anions of the 14β,17ξ-diols, as proposed by Winkler and Stahl.[13] Alkoxide anions from the cis compounds are stabilized by the possibility of intramolecular hydrogen bond formation (Scheme II, ion f), which is not the case for trans derivatives, independently of the negative charge location (Scheme II, ions g and h).

Such an interpretation, which explains the differences for the reaction involving loss of water, cannot account for the stereochemical effect in the case of RH loss. By analogy with results from androstanols **2** and **3** it is difficult to understand why the RH elimination is a "forbidden" process for alkoxide of structure f if the resulting daughter ion is an enolate i (Scheme III). The best rationalization for the differences between cis and trans diols is that the remaining hydroxyl hydrogen in a structure of type h (Scheme II) is involved in the RH loss. In fact, this reaction is equivalent to an intramolecular oxidation with degradation of the site in position 17. Such a mechanism calls in question the formation of an enolate daughter ion (structure i) and suggest the formation of a ketolate anion j (Scheme III) which is not, a priori, the most stable species. One possible way to test the validity of this assumption is to compare CID/MIKE spectra of the resulting daughter ions $(M - H - H_2)^-$ and $(M - H - RH)^-$ (m/z 289) from secondary and tertiary trans diols with the corresponding spectrum of the $(M - H)^-$ anion obtained by proton abstraction from 17-ketoandrostanol-14β (**9**). The CID/MIKE spectra of the ions at m/z 289 from these different precursors are given in Table IV. All spectra are identical within experimental error so that it can be concluded that all daughter ions at m/z 289 have a common structure, probably that of the ketolate anion j.

3. Isotope Exchange between Neutrals into the Ion Source. H/D exchange reactions have been studied in both the negative and positive chemical ionization modes. In the case of negative ions, different gases, including mixtures, have been used. One can classify them according to their gas-phase acidity ($\Delta H°_{acid}$ (kcal/mol):[23] ND_3, 404; D_2O, 391; CH_3OD, 379; C_2H_5OD, 376; and CF_3CD_2OD, 364. Several techniques have been employed to establish the most important rules governing isotope exchange between pseudomolecular anions $(M - H)^-$ or fragment ions and the reactant gas XD: ion cyclotron resonance by Nibbering,[24] flowing afterglow by de Puy,[25] and NCI conventional mass

Table IV. CID/MIKE Spectra[a] of $(M - H - RH)^-$ from Trans Isomers of 17β-R-5α-Androstane-14β,17α-diol and of $(M - H)^-$ from 17-Ketoandrostanol-14β (**9**), m/z 289

fragments m/z (neutral lost)	4b	4b'	5b	6b	7b	8b	9
231 ($-C_3H_4O, -H_2$)	5.5	4.6	2.2	6.0	5.2	4.6	4.9
233 ($-C_3H_4O$)	18.2	17.5	18.8	17.1	17.7	17.5	17.8
255 ($-CH_4, -H_2O$)	2.2	2.6	2.7	2.4	2.5	2.5	2.5
269 ($-H_2, -H_2O$)	3.8	3.8	3.5	4.0	5.0	5.0	4.0
271 ($-H_2O$)	56.0	56.5	57.9	57.2	56.9	57.8	58.0
274 ($-CH_3$)	1.0	0.8	0.8	0.9	0.5	0.8	0.6
287 ($-H_2$)	6.3	7.2	6.9	7.3	7.1	6.0	6.3
288 ($-H$)	7.0	7.0	7.2	5.1	5.1	5.8	5.9

[a] Intensities in % of the sum of fragments (relative error ±0.6%).

Scheme III

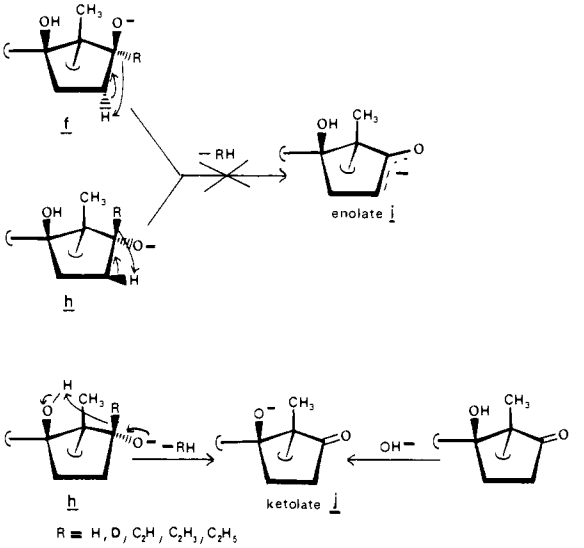


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Table V. OD^- Source Spectra (ND_3/N_2O) of Trans Diastereoisomers from 17β-R-5α,14β-Androstane-14,17α-diols

	compound					
	4b	4b′	5b	6b	7b	8b
molecular zone[a]						
$(M_{d_3} - D)^-$		0.90				0.70
$(M_{d_2} - H)^-$		0.05				
$(M_{d_2} - D)^-$	0.95	0.05	0.92	0.95	0.95	0.30
$(M_d - H)^-$	0.02_5		0.04	0.02_5	0.02_5	
$(M_d - D)^-$	0.02_5		0.04	0.02_5	0.02_5	
	fragments					
	R = H, D		R = CH_3	R = C_2H_5	R = C_2H_3	R = C_2H
m/z 290						
$(M_{d_3} - D - R_dH)^-$		0.25				0.12
$(M_{d_2} - D - R_hH)^-$	0.15		0.30	0.20	0.20	
m/z 289						
$(M_{d_3} - D - R_dD)^-$		0.75				0.88
$(M_{d_2} - D - R_hD)^-$	0.85		0.70	0.80	0.80	

[a] Intensities normalized to 100% in each group (absolute error ±2%). Values corrected for natural isotope abundances. $(M_{d_2} - H)^-$ and $(M_d - H)^-$ supposed to have the same intensities as $(M_{d_2} - D)^-$ and $(M_d - D)^-$.

spectrometry by Hunt.[26] As reported by de Puy,[27] the process involves the formation of an addition complex $((M - H), XD)^-$ according to the following sequence:

$$(M - H)^- + XD \rightleftarrows [(M - H), XD]^- \rightleftarrows (M_d, X)^- \rightleftarrows [(M_d - H), XH]^- \rightleftarrows (M_d - H)^- + XH$$

where M_d represents the molecule after insertion of deuterium. To be exchanged, the proton of the $(M - H)^-$ anion must be less acidic than those of the XD molecule and the difference between the basicities of $(M - H)^-$ and X^- must be in order of 10 to 15 kcal/mol.[24] The replacement of *x* hydrogen atoms by deuterium in $(M - H)^-$ will be denoted as $(M_{dx} - H)^-$.

The present work deals with H/D exchange between neutrals, using a mixture of ND_3/N_2O. Hydroxylic protons of different 17-substituted 14β,17ξ-androstanediols and 17-keto-androstanol-14β are replaced by deuterons. In ND_3/N_2O mixtures OD^- is produced by the reactions:

$$N_2O + e^- \rightarrow N_2 + O^-\cdot \quad (1)$$

$$O^-\cdot + ND_3 \rightarrow ND_2\cdot + OD^- \quad (2)$$

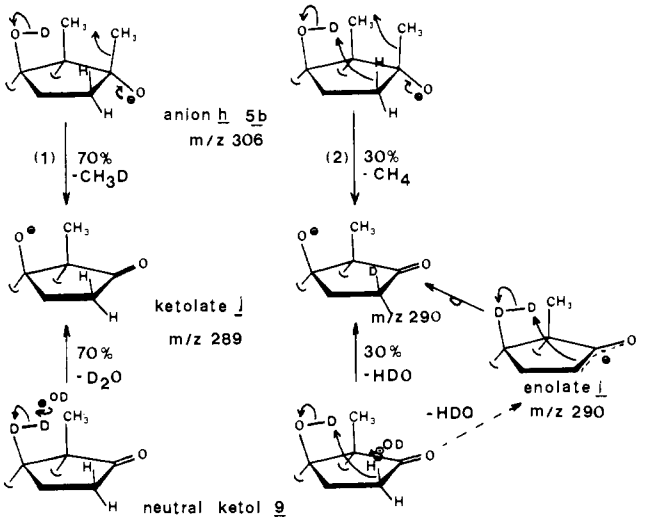
while reaction 3 gives a small amount of ND_2^- (in our experimental conditions $OD^-/ND_2^- = 100$).

$$ND_3 + e^- \rightarrow ND_2^- + D\cdot \quad (3)$$

Under these conditions no H/D isotopic exchange by ion–molecule reaction $[(M - H)^-/ND_3$ or $(M - H)^-/D_2O]$ can occur, as the acidity of D_2O (or HDO) is lower, by some 15 kcal/mol, than that of ketones.

For diols, proton abstraction by OD^- after H/D isotopic exchange produces $(M_{d2} - D)^- = (M_{d1})^-$. The spectra obtained when using ND_3/D_2O as reactant gas with 17β-R-5α,14β-androstane-14,17α-diols are reported in Table V. In all cases, except for compound **8b** (acetylenic substituent), two deuterium atoms have been incorporated in the molecule with a yield of 95%. When R = –C≡CH the pseudomolecular anion contains two deuterium atoms. This means that in the neutral molecule three acidic protons have been exchanged: the two hydroxylic protons and the hydrogen atom of the acetylenic substituent. An ion–molecule reaction for this H/D exchange between the alkoxide $(M_{d2} - D)^-$ and D_2O or HDO formed in these reactions cannot, a priori, be excluded as the acidity of acetylene (~375 kcal/mol) is close to that of water. Nevertheless, this is highly unlikely since the concentrations of D_2O and HDO (formed by ionic reactions) will be much too low.

Scheme IV



For the loss of RX molecule in the ion source (R = H, D, CH_3, C_2H_5, C_2H_3, and C_2D for compounds **4b**, **4b′**, **5b**, **6b**, **7b**, and **8b**, respectively, and X = H or D), formation of a daughter ion at *m/z* 289 shows that loss of RD occurs with a probability of at least 70%. This result is a valuable proof for the transfer of the second hydroxylic proton in the elimination of molecular hydrogen or hydrocarbon molecule, even if a proton of another origin is involved to some extent to give RH elimination (*m/z* 290). In particular, this is the case for 17β-methyl-5α,14β-androstane-14,17α-diol (**5b**), as shown in Scheme IV. As already mentioned, $(M_{d2} - D)^-$ and $(M_{d2} - D - RD)^-$ are not able to give the ion–molecule isotopic exchange so that the formation of *m/z* 290 by incorporation of one deuterium in the ion at *m/z* 289 by this process can be ruled out. On the other hand, all daughter ions at *m/z* 289 have the same structure according to their CID/MIKE spectra (Table IV); thus, the only explanation for this label retention must involve a migration of the deuterium atom from the OD group in position 14 in the ring D in a quasi-concerted mechanism in which stereochemistry plays a role (Scheme IV, reaction path 2).

MIKE and CID/MIKE spectra of $(M_{dx} - D)^-$ anions of trans stereochemistry (Table VI) show that only an RD molecule is eliminated, except for compound **5b** (R = CH_3). For this product, gas-phase elimination of CH_4 (40%) and CH_3D (60%) is a confirmation that the ion at *m/z* 290 in the ion source is the result of a concerted elimination of hydrocarbon, with migration of the 14-D atom on carbon 16 α to the carbonyl group and not the product of an ion–molecule isotope exchange reaction. Moreover, this is reinforced by the similarity of MIKE and CID/MIKE spectra of *m/z* 289 and 290 daughter ions from **5b** and **4b′** (17-d_1) (Table VII) (the very small differences are due to experimental errors and to possible isotope effect).

Furthermore, CID/MIKE spectra of the ions at *m/z* 290 are characteristic of the location of the deuterium atom. (a) Only loss of H_2O is observed (yielding *m/z* 272), which reflects that no enolate structure i is present (if this were the case, loss of HDO would be observed, as for deuterated compounds of trans diols). (b) Fragments at *m/z* 233 (loss of C_4H_7OD) and at *m/z* 231 (loss of H_2 from the ion at *m/z* 233) correspond to the loss of carbons 15, 16, 17, and 20 from the D ring, to give an enolate ion (*m/z* 233) and locate the deuterium on ring D. (c) Loss of H· (giving *m/z* 289) and H_2 (*m/z* 288) exclusively indicates that the deu-

(25) (a) Stewart, J. H.; Shapiro, R. H.; de Puy, C. H.; Bierbaum, V. M. *J. Am. Chem. Soc.* **1977**, *99*, 7650. (b) De Puy, C. H.; Bierbaum, V. M.; Kuy, G. K.; Shapiro, R. H. *Ibid.* **1978**, *100*, 2921.
(26) Hunt, D. F.; Sethi, S. K. *J. Am. Chem. Soc.* **1980**, *102*, 6953.
(27) Squires, R. R.; de Puy, C. H.; Bierbaum, V. H. *J. Am. Chem. Soc.* **1981**, *103*, 4256.

Table VI. MIKE and CID/MIKE Spectra of $(M_{d*} - D)^-$ from *trans*-Androstanediols OD^-/NCI[a]

	MIKES[b]						CID/MIKES[b]					
neutral lost	4b (292)	4b′ (293)	5b (306)	6b (320)	7b (318)	8b (317)	4b (292)	4b′ (293)	5b (306)	6b (320)	7b (318)	8b (317)
$-H_2$	0.20						0.15					
$-HD$	0.80	0.18					0.85	0.22		0.65	0.60	
$-D_2$		0.82						0.78		0.35	0.40	
$-H_2O$	0.20	0.20	0.15	0.70	0.60	0.90	0.18	0.20				0.10[e]
$-HDO$	0.80	0.80	0.85	0.30	0.40	0.10	0.82	0.80	1.00			0.60[e]
$-D_2O$												0.30[e]
$-RH$			0.40[c]	0.00	0.00	0.00			*d*			
$-RD$			0.60[c]	1.00	1.00	0.00			*d*	1.00		
$-R_DD$						1.00						1.00

[a] Intensities are normalized within each group of fragments. [b] *m/z* in parentheses. [c] Measurements perturbed by the presence of an artifact peak. [d] Too weak for significant measurement; see footnote *c*. [e] Broad peak of weak intensity.

Table VII. CID/MIKE Spectra[a] of Anions at *m/z* 289 and 290 from 17β-Methyl-5α,14β-androstane-14,17α-diol and 17-Ketoandrostan-14β-ol in $ND_3/N_2O/NCI$

	5b		4b′	9b	
m/z of fragments	*m/z* 289	*m/z* 290	*m/z* 290	*m/z* 289	*m/z* 290
231	2.6	4.0	3.5	3.5	2.1
233	17.0	17.5	17.9	18.0	16.6
255	2.2			2.1	
256		1.5	2.1		2.3
269	4.7			4.0	
270		5.0	3.9		3.2
271	59.0			56.0	
272		58.0	55.4		58.0
274	0.8			1.3	
275		0.6	1.2		1.8
287	6.5			7.3	
288	7.2	6.2	7.5	7.8	7.5
289		7.2	8.2		8.5

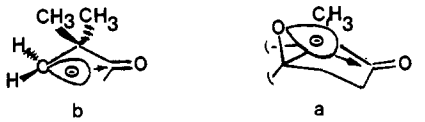
[a] Intensities in % of the sum of the fragments.

terium atom is not in the accessible positions (α position to the hydroxyl); otherwise it would be eliminated (D· or HD loss).

Thus the labeling is unambiguously localized in a position α to the carbonyl group which does not participate in water or hydrogen loss, corroborating the ketolate structure j of the ion at *m/z* 289.

For 17-ketoandrostanol-14β (**9**), 30% of the deuterium exchanged is not eliminated to form the pseudomolecular anion $(M_d - D)^-$. The only mechanism which can account for this observation is the elimination of a proton in position α to the carbonyl group, accompanied by a simultaneous transfer of the hydroxylic deuterium to form the anion of structure j (16-D, *m/z* 290) (Scheme III). This is supported by the analogy between CID/MIKE spectra of ions at *m/z* 289 and 290 from **9** and those of the same masses from **5b** and **4b′**, (Table VII); furthermore, the localization of the deuterium in the ion at *m/z* 290 is also α to the carbonyl group, although the reaction is an intramolecular D transfer accompanying the enolic proton abstraction by OD^-. In fact, in this bifunctional cyclic compound (1-keto, 3-ol), the hydrogen of the OH group is seen to be more acidic than those α to the carbonyl group, contrary to what is expected from monofunctional compounds ($\Delta H°_{acid}(EtOH) - \Delta H°_{acid}(Me_2CO) = 7.3$ kcal mol^{-1}). A tentative explanation for this astonishing behavior is that the dipole of the carbonyl group is able to stabilize the alkoxide as shown in Scheme V, a. This hypothesis is similar to that proposed by Nibbering[24] to explain the high acidity of 2,2-dimethylpropionaldehyde (*t*-BuCHO); he suggested an interaction between the sp^3 orbital of the carbanion homoallylic to the carbonyl group and the dipole of the C=O group (Scheme V, b).

Scheme V



b a

Conclusion

In general, conventional spectra obtained in OH^-/NCI for compounds of the 17ξ-R-5α,14β-androstane-14,17ξ-diol series are characterized by abundant pseudomolecular anions $(M - H)^-$. These compounds behave like alicyclic alcohols[14] and lose, competitively, a water molecule and an exocyclic hydrocarbon molecule for tertiary alcohols or hydrogen for secondary alcohols.

No stereochemical effect for OH groups in position 17 (α or β) is observed in conventional or metastable spectra of 17-androstanols. H_2 or RH elimination leads to the formation of an enolate daughter ion.

Quite different is the behavior of $(M - H)^-$ from stereoisomers in the series of 17ξ-R-5α,14β-androstane-14,17ξ-diol. Only trans-diol stereoisomers lose a hydrocarbon or hydrogen molecule to form an ion at *m/z* 289, which in every case has the ketolate structure.

The use of isotopic labeling of the hydroxylic groups by exchange between neutral molecules in the ion source allows one to prove that, beside the lack of stabilization of trans diols by intramolecular hydrogen bond, a real driving force exists for the elimination of RH; this is due to the assistance of the alkoxy group and the abstraction of the proton of the 14-OH group in a sterically favorable position.

Results from 17-ketoandrostanol-14β show that the ketolate structure is more stable than the enolate, despite the respective values of gas-phase acidity of ketones and alcohols. These bifunctional molecules yield a pseudomolecular anion $(M - H)^-$ with the alcoholate group stabilized by the carbonyl.

The potential of $OH^-(OD^-)/NCI$ is again demonstrated for analytical as well as for mechanistic studies; it allows one to establish the stereochemistry of diols in ring D of steroids. Such stereochemical effects are very seldom seen in positive chemical ionization.[28]

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Registry No. 1a, 1225-43-0; **1b**, 19037-37-7; **2a**, 73483-78-0; **2b**, 73522-39-1; **3b**, 84415-09-8; **4b**, 84369-79-9; **4b′**, 84369-80-2; **5a**, 84369-81-3; **5b**, 84369-82-4; **6a**, 84369-83-5; **6b**, 84415-10-1; **7a**, 84369-84-6; **7b**, 84415-11-2; **8a**, 79405-75-7; **8b**, 79435-43-1.

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