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A Novel Approach for Determining the Orientation of 3-Hydroxyl Group of Some Steroids by CIMS

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Abstract: Trimethylchlorosilane was used as a stereoselective reagent to determine the e or a orientation of 3-hydroxyl group of some steroids by chemical ionization mass spectrometry.

Keywords: Orientation of 3-hydroxyl group; steroids; mass spectrometric determination.

Stereochemical analysis is an important subject in organic structure elucidation, and mass spectrometry proves to be a promising tool for this propose due to its high sensitivity (the limit of detection is about 10^{-14} mole), rapidity, *etc*[1]. In our previous work, a series of studies on the stereochemical effects in mass spectrometry have been discussed[2-6]. In this communication we report a novel approach for determination the e or a orientation of 3-hydroxyl group of some steroids directly by chemical ionization mass spectrometry using trimethylchlorosilane as a stereoselective reagent.

The mass spectra were measured with a VG-7070E mass spectrometer using chemical ionization (CI) source (methane as reagent gas). The temperature of ion source and probe was 200°C. Prior to the introduction to the mass spectrometer, a few μg of sample was mixed thoroughly with Me_3SiCl at room temperature. The following six pairs have been tested and the results are summarized in **Table 1.**

A1: 5β-cholestan-3α-ol (e)	A2: 5β-cholestan-3β-ol (a)
B1: 5α-cholestan-3β-ol (e)	B2: 5α-cholestan-3α-ol (a)
C1: 5β-cholestan-3α, 20-diol (e)	C2: 5β-cholestan-3β, 20-diol (a)
D1: 5α-cholestan-3β, 20-diol (e)	D2: 5α-cholestan-3α, 20-diol (a)
E1: 5β-cholestan-3α-ol-20-one (e)	E2: 5β-cholestan-3β-ol-20-one (a)
F1: 5α-cholestan-3β-ol-20-one (e)	F2: 5α-cholestan-3α-ol-20-one (a)

In general, the usual CIMS can not provide the information to distinguish the orientation of 3-hydroxyl group of the steroids, but when the stereoseletive reagent was introduced into the CI ion resource, the steroids containing an equatorial hydroxyl group (e-OH) reacted with the reagent -- $(CH_3)_3SiCl$ (the reaction is shown in **Scheme 1**) to give the characteristic ions $[M+72]^+$ and $[M+72-CH_3]^+$, and in the mass spectra of those compounds containing an axial hydroxyl group (a-OH), none of these characteristic ions

appeared, this indicated that these compounds containing an axial hydroxyl groups did not react with the reagent. This phenomenon may be explained as being due to the stereo-effects in the steroids.

Table 1. The Differences of the CIMS of Compounds A1--F2

Compounds	3-OH	Mw.	Relative abundances (%)			
			M^+	$[M-H_2O]^+$	$[M+72]^+$	$[M+72-CH_3]^+$
A1	e	388	14	62	12	28
A2	a	388	20	100	--	--
B1	e	388	18	43	25	100
B2	a	388	12	100	--	--
C1	e	320	--	30	18	22
C2	a	320	6	25	--	--
D1	e	320	--	28	23	12
D2	a	320	--	50	--	--
E1	e	318	18	100	8	13
E2	a	318	28	90	--	--
F1	e	318	80	100	10	17
F2	a	318	82	70	--	--

By this method, the a or e orientation of 3-OH group of these steroids can be definitely differentiated at ultra-low level with CI MS.

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