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Use of New Silylating Agents for Identification of Hydroxylated Steroids by Gas Chromatography-Electron Impact-Mass Spectrometry

A difference of methylene unit value, Δ[MU], was used for the determination of hydroxyl group number of steroid by means of gas chromatography. This index is defined by the difference in the methylene unit value between trimethylsilyl and other dimethylalkylsilyl (DMAS) ether derivatives, namely dimethylethylsilyl (DMES) and dimethyl-*n*-propylsilyl (DMPS) ethers, of hydroxy-steroid. Mass spectra of these derivatives were characterized by the molecular ion cluster, M,M-15 and M-29 (or M-43), where the fragment ion of M-29 (or M-43) appeared in the most case as a base peak. The molecular ion cluster of these derivatives is of great use for estimating the molecular weight. Therefore, these DMAS ethers provide a valuable information for structural elucidation of hydroxy-steroids by gas chromatography-electron impact-mass spectrometry.

Gas chromatography-electron impact-mass spectrometry (GC-EI-MS) has been widely used in the study of drug metabolism and analysis of metabolic profile for endogenous substances. For the purpose of the use of GC-EI-MS, it is necessary that the sample to be analyzed can be readily converted into a volatile derivative without formation of the by-products. For this purpose trimethylsilylation has often been employed. However, the trimethylsilyl (TMS) derivative has an inevitable disadvantage in that little information on the molecular weight and the number of hydroxyl groups of steroids are obtainable from GC-EI-MS data owing to loss of the silanol group from their TMS ethers. In order to overcome this problem the retention indices such as steroid number,[1] methylene unit[2] and F-value[3] have been used as the supplemental informations. But, it may be unsuitable to apply these indices to an unknown compound. On the other hand, chloromethyldimethylsilylating and trialkylsilylating agents have been used for estimating the number of hydroxyl groups.[4,5] But, it seems that these agents remain the following problems; their derivatives result in longer retention time or the derivatization of sterically hindered compound is imcomplete.

The present communication deals with combination of a methylene unit value and characteristic fragment ions of the dimethylalkylsilyl (DMSA) ethers for estimating the number of hydroxyl groups and molecular weight of hydroxy-steroids.

Dimethylethylsilyl (DMES) and dimethyl-*n*-propylsilyl (DMPS) imidazole were synthesized as dimethylalkylsilylating agents used for the present study. The steroidal DMES and DMPS ethers were prepared in the same way as TMS ether. The reactivities of these silylating agents were nearly equal to those of TMS imidazole except for some sterically hindered steroids.

In the present study the following indices were used; $\Delta[MU]_E = MU_E - MU_M$, $\Delta[MU]_P = MU_P - MU_M$, where MU_M, MU_E and MU_P are the methylene unit values of TMS, DMES and DMPS ethers of hydroxy-steroid respectively. As these indices increase with increasing difference in the carbon number between two silylated derivatives of each steroid, the number of hydroxyl groups in steroid may possibly be estimated.

The Δ[MU] values of the typical steroids are listed in Table I. Each of $\Delta[MU]_E$ and $\Delta[MU]_P$ for mono-, di- and trihydroxylated steroids was roughly constant regardless of configuration and position of hydroxyl groups, and plotting these indices *vs* logarithm of the number

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Table I. Δ[MU] and Mass Spectral Data of TMS, DMES and DMPS Ether Derivatives

Compound	Δ[MU]				Mass spectral data					
	Δ[MU]$_E$		Δ[MU]$_P$		TMS derivative		DMES derivative		DMPS derivative	
	OV-101	OV-17	OV-101	OV-17	M^+	Base peak	M^+	Base peak	M^+	Base peak
5α-Androstane-3α-ol	1.00	1.03	1.80	1.75	348	258 (M^+–90)	362	257 (M^+–109)	376	257 (M^+–119)
5α-androstane-3β-ol	1.20	1.18	2.02	1.93	348	333 (M^+–15)	362	333 (M^+–29)	376	333 (M^+–43)
5β-androstane-3α-ol	1.06	1.17	1.85	1.83	348	258 (M^+–90)	362	333 (M^+–29)	376	333 (M^+–43)
5α-Cholestane-3α-ol	1.28	1.17	1.84	1.82	460	215	474	369 (M^+–105)	488	369 (M^+–119)
5α-Cholestane-3β-ol	1.22	1.43	2.05	2.10	460	215	474	445 (M^+–29)	488	445 (M^+–43)
5β-Cholestane-3α-ol	1.18	1.23	1.83	1.89	460	370 (M^+–90)	474	445 (M^+–29)	488	445 (M^+–43)
5β-Cholestane-3β-ol	1.13	1.23	1.83	1.89	460	370 (M^+–90)	474	445 (M^+–29)	488	445 (M^+–43)
5-Cholestene-3α-ol	1.19	1.19	1.93	1.82	458	129	472	443 (M^+–29)	486	443 (M^+–43)
5-Cholestene-3β-ol	1.22	1.34	2.12	2.16	458	129	472	143	486	443 (M^+–43)
Androsterone	1.21	1.17	1.76	1.76	362	272 (M^+–90)	376	271 (M^+–105)	390	271 (M^+–119)
Epiandrosterone	1.25	1.26	2.03	2.01	362	347 (M^+–15)	376	347 (M^+–29)	390	347 (M^+–43)
Dehydroepiandrosterone	1.28	1.27	1.99	2.02	360	129	374	143	388	157
Teststerone	1.25	1.29	2.04	2.04	360	129	374	143	388	345 (M^+–43)
Epiteststerone	1.16	1.21	2.03	2.01	360	129	374	143	388	345 (M^+–43)
Etiocholanolone	1.15	1.18	1.90	1.93	362	272 (M^+–90)	376	347 (M^+–29)	390	347 (M^+–43)
Estrone	1.26	1.26	2.05	2.01	342	342(M^+)	356	356(M^+)	370	370(M^+)
Estradiol	2.48	2.51	4.04	4.12	416	416(M^+)	444	444(M^+)	472	472(M^+)
Estriol	3.52	3.67	5.63	5.67	504	504(M^+)	546	546(M^+)	588	588(M^+)
Estetrol	4.08	4.14	6.38	6.17	592	191	648	219	704	247
5β-Pregnane-3α,20α-diol	2.19	2.40	3.81	3.87	464	117	492	131	520	103, 145 (90%)
5β-Pregnane-3α,20β-diol	2.04	2.32	3.69	3.78	464	117	492	131	520	103, 145 (60%)
5β-Pregnane-3α,17α, 20α-triol	3.32	3.52	—	—	552	255	594	255	—	—
Lithocholic acid methyl ester	1.07	1.18	1.90	1.95	462	372 (M^+–90)	476	447 (M^+–29)	490	447 (M^+–43)
Deoxycholic acid methyl ester	2.02	2.12	3.12	3.58	550	255	578	549 (M^+–29)	606	563 (M^+–43)
Chenodeoxycholic acid methyl ester	2.13	2.15	3.21	3.15	550	370 (M^+–90–90)	578	371	606	371
Ursodeoxycholic acid methyl dster	2.17	2.51	3.40	3.29	550	460 (M^+–90)	578	549 (M^+–29)	606	563 (M^+–43)
Cholic acid methyl ester	3.23	3.36	4.82	4.88	638	253	680	651 (M^+–29)	722	679 (M^+–43)
Average										
Monohydroxylated compound (n=17)	1.18	1.22	1.94	1.94						
Dihydroxylated compound (n=6)	2.17	2.34	3.54	3.63						
Trihydroxylated compound (n=3 or 2)	3.36	3.52	5.23	5.28						
Tetrahydroxylated compound (n=1)	4.08	4.14	6.38	6.17						

A Shimadzu GC-5A with FID and a Shimadzu LKB-9000 GC-MS system (Shimadzu GC-MS-PAC 300) were used. GC and GC-MS conditions: Column; 1.5% OV-101 lm and 1.5% OV-17 lm on Gas Chrom Q, column temp.; 210—250°, ionization current; 60 μA, ionization voltage; 70 eV, accerelating voltage; 3.5 kV, ion source temp.; 270°

of hydroxyl groups of steroids showed a good linearity. Conveniently, the round number of the $\Delta[MU]_E$ corresponded well to the number of hydroxyl groups. These results suggested that $\Delta[MU]_E$ was more favorable than $\Delta[MU]_P$ for estimating the number of hydroxyl groups.

On the other hand, the mass fragmentation pattern of DMES and DMPS ethers was closely related to that of TMS ether except for 14 and 28 mass unit shifts per one hydroxyl group. Figure 1 shows the mass spectra of TMS, DMES and DMPS ethers of cholesterol. The mass spectrum of DMES (or DMPS) ether was characterized by the molecular ion cluster, M, M-15 and M-29 (or M-43). Table I also listed the mass spectral data on TMS, DMES and DMPS ethers. The fragment ion of M-29 or M-43, due to loss of ethyl or *n*-propyl group from DMES or DMPS ether, was observed more prominently than that of M-51 in the corresponding TMS ether. This fact suggests that elimination of ethyl and *n*-propyl groups from these DMAS ethers takes place easier than that of the methyl group from TMS ethers. Therefore, it is recommended to use DMES or DMPS ether in place of TMS ether when TMS ether of hydroxy-steroid exhibits the weak intensity of molecular ion cluster.

It is hoped that the use of $\Delta[MU]$ value and molecular ion cluster may provide a valuable information of structural elucidation of hydroxy-steroids by means of GC-EI-MS.

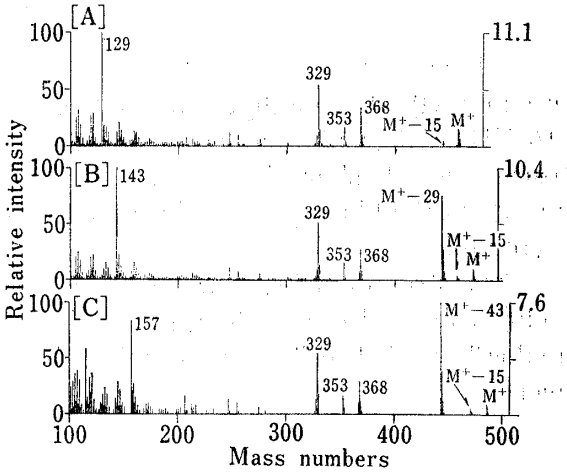

Fig. 1. Mass Spectra of Cholesterol TMS (A), DMES (B) and DMPS (C) Ether Derivatives

GC-MS was carried out under the same conditions as described in Table I.

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