Characterisation of Steroid Ketones by Mass Spectrometry in Conjunction with Deuteriation by Gas–Liquid Chromatography

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Summary Gas-chromatographic columns suitable for deuteriation of steroids have been developed and applied to the characterisation of saturated and unsaturated monooxo-steroids by combined gas chromatography–mass spectrometry.

TECHNIQUES for deuteriation of organic compounds by gas–liquid chromatography (g.l.c.) have been reported[1–4] as convenient and efficient methods for introducing deuterium into small amounts of material. Hydroxylic and other readily exchangeable hydrogen atoms can be selectively replaced by deuterium by g.l.c. with a suitable stationary phase [*e.g.* poly(ethylene glycol)] which has been previously saturated with deuterium oxide.[3] Enolic hydrogen atoms have been effectively replaced by deuterium on such columns only in the presence of strongly acidic or

basic catalysts, *e.g.* phosphoric acid,[1] potassium hydroxide.[2] The catalyst may be incorporated either into the column support material before this is coated with stationary phase, or into a separate column preceding the main column.[1] We now report an adaptation of the former technique, suitable for application to steroids and related compounds.

somewhat less satisfactory results, but ions representing exchange of enolic states were invariably observed. The $\beta\gamma$-unsaturated ketone, cholest-5-en-3-one, was isomerised (by the barium hydroxide column) and the resulting mass spectrum was that of the deuteriated $\alpha\beta$-isomer.

The mass spectral fragmentations of many saturated and

70ev *Mass spectra of deuteriated steroid ketones*

Steroid ketones	M.w.	Deuterium incorporation[a] d_1	d_2	d_3	d_4	d_5	Diagnostic fragments[b,c,d] d_o	d_n	d_o	d_n	Refs.
A: *Saturated*											
5α-Cholestan-1-one	386	13	87	—	—	—	124	126	343	345[e]	7
5α-Cholestan-2-one	386	—	9	24	67	—					7
5α-Cholestan-3-one	386	—	—	19	81	—					7
5α-Cholestan-6-one	386	3	23	74	—	—	123	123	331	334	8
5α-Cholestan-7-one	386	—	19	81	—	—	178	181	191	194	7
5α-Pregnan-11-one	302	—	24	76	—	—	192	195	205	208	7
5α-Cholan-12-one ..	344	14	86	—	—	—	233	235			9
5α-Androstan-17-one	274	9	91	—	—	—	230	230	218	218	10, 11
5α-Pregnan-20-one	302	—	4	23	73	—	217	217	43	46	12
							84	88			
B: *Conjugated*											
Cholest-4-en-3-one	384	—	—	14	37	49	124	129	342	345	7
Cholesta-1,4-dien-3-one	382	—	17	83	—	—	122	125			13
Cholesta-4,6-dien-3-one	382	12	70	5	6	7	136	138	247	247	13
Cholesta-3,5-dien-7-one	382	21	12	26	29	3	174	178	187	191	14

[a] Expressed as percentages of total deuteriated species, assessed in terms of peak heights (corrected for natural abundance of ^{13}C).
[b] Only ions considered to be characteristic of the ketone position are cited.
[c] d_o = m/e of fragment from undeuteriated species, d_n = m/e of fragment incorporating deuterium.
[d] Deuteriated fragment quoted as m/e of most prominent ion.
[e] Loss of CO and CH_3.

We considered that a convenient deuteriation method would be a valuable analytical tool for the determination of carbonyl environment in the steroid nucleus, using combined gas chromatography–mass spectrometry. The increase in molecular weight upon deuteriation serves in itself to distinguish between certain steroid ketones, whilst knowledge of the expected mode of fragmentation under electron impact may yield supplementary structural evidence. We have investigated base-catalysed hydrogen–deuterium exchange. As poly(ethylene glycols) are too polar for g.l.c. of steroids, Apiezon L has been selected initially as stationary phase: it has low polarity and good thermal stability, and has been shown to be compatible with potassium hydroxide in the g.l.c. of free amines.[5,6]

The Figure illustrates g.l.c. of steroids containing various oxygen functions examined on 1% Apiezon L on Gas Chrom Q and on a similar column containing 0·5% potassium hydroxide. Satisfactory results have also been obtained with a weaker base, barium hydroxide, as catalyst: this causes less column "bleed" and is therefore more suitable for columns coupled directly to the mass spectrometer. Columns containing barium hydroxide have been found suitable for qualitative use in the presence of functional derivatives such as trimethylsilyl ethers and *O*-methyloximes.

Mass spectra of saturated and unsaturated steroid ketones were recorded with the LKB 9000 combined gas chromatograph–mass spectrometer, using as stationary phases OV-1 (1%), and Apiezon L (1%) incorporating barium hydroxide (1%). Helium was used as carrier gas. Results are summarised in the Table. For unconjugated steroid ketones, the data indicate good deuterium incorporation at all enolic sites. The $\alpha\beta$-unsaturated ketones gave

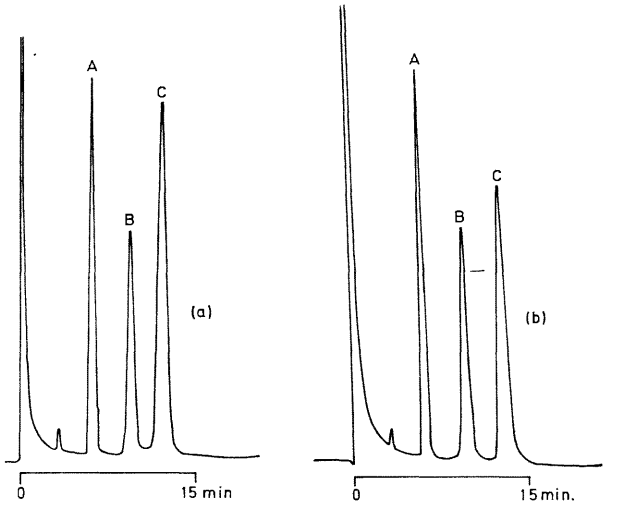

FIGURE. *G.l.c. of steroid mixture* (a) *on* 1% *Apiezon L and* (b) 1% *Apiezon L*/0·5% KOH, *coated on Gas Chrom Q* (100—120 *mesh*). *Carlo Erba Fractovap GB Chromatograph, "silanised" glass columns* 6 *ft* × 4 *mm.I.D*; *nitrogen carrier gas* 60 *ml./min.*; *column temperature* 210°. A = *Oestr-4-en-17-one*; B = 5α-*Androstan-17β-ol*; C = *Androst-4-en-3-one*.

some conjugated steroid ketones are well established,[7–14] and the deuterium incorporation in the principal fragments (Table) agrees with that expected. Fragments characteristic of the nuclear location of particular ketonic groups, although prominent in some monofunctional steroids, may of course be less easily recognisable in the spectra of polyfunctional steroids. Mass increments accompanying

deuteriation strengthen the identification of such diagnostic fragments, and thus aid interpretation of the mass spectrum of an unknown steroid.

We are engaged in further development of this technique with the aim of applying it to the characterisation of urinary steroids.

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