Differentiation between 19-Methyl- and 19-Nor-steroids by Mass Spectrometry

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THE differentiation between 19-methyl- and 19-nor-steroids of incompletely defined structure is a problem of considerable chemical and biological significance. Methods available at present frequently involve extensive chemical investigation or the application of n.m.r. spectroscopy, which may be equivocal.

We report the application of high-resolution mass spectrometry to this problem. The 3-keto-derivative of the steroid is converted into the 2-spiro-2′-(1,3-dithian),[1] the mass spectral fragmentation pattern of which is determined. The peaks at *m/e* 145 and *m/e* 159, which are particularly significant in this context, are often multiplets occasioned by the presence of the ions $C_{10}HO_9^+$, and $C_{11}H_{13}^+$ (*m/e* 145) and $C_{11}H_{11}O^+$ and $C_{12}H_{15}^+$ (*m/e* 159). The Table records the intensities relative to the base peak of those components of the peaks at *m/e* 145 and *m/e* 159, which accurate mass measurement shows are due to the ions $C_6H_9S_2^+$ and $C_7H_{11}S_2^+$ respectively.

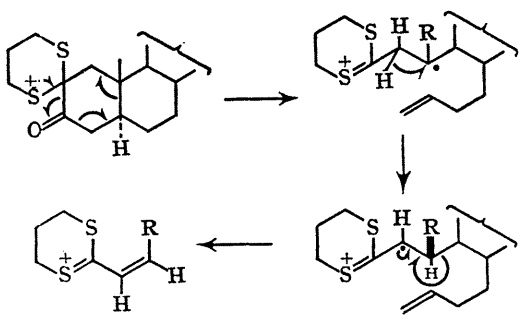
The data clearly indicate that for a ring A saturated steroid having a 19-methyl group the ratio of the intensities of the peaks due to the $C_7H_{11}S_2^+$ and $C_6H_9S_2^+$ ions is greater than 20 : 1; in the 19-nor-compounds this ratio is reversed. Hence this ratio is apparently diagnostic of the substitution pattern at C-10.

The results (Table) with steroids having a double bond in ring A or B are not so sharply defined, since the unsaturation has a pronounced effect on both the overall intensities of the diagnostic peaks relative to the base peaks and the

TABLE

2-Spiro-2′-(1,3-dithian) of:—	Intensity of $C_6H_9S_2^+$ (*m/e* 145)	Intensity of $C_7H_{11}S_2^+$ (*m/e* 159)	(*m/e* 145) : (*m/e* 159)
17β-Hydroxy-19-nor-5α-androstan-3-one*	18·0	0·71	25/1
17β-Hydroxy-5α-androstan-3-one	2·0	46·0	1/23
17β-Acetoxy-4α-methyl-19-nor-5α-androstan-3-one*	32·0	1·4	23/1
17β-Acetoxy-4α-methyl-5α-androstan-3-one	1·4	30·0	1/21
17β-Acetoxy-4,4-dimethyl-19-nor-5α-androstan-3-one*	35·2	0·9	39/1
17β-Acetoxy-4,4-dimethyl-5α-androstan-3-one*	1·7	88·1	1/51
19-Nortestosterone	1·5	0·0	High
Testosterone	0·4	0·8	1/2
17β-Hydroxy-4,4-dimethyl-19-norandrost-5(6)-en-3-one	1·5	0·1	15/1
17β-Hydroxy-4,4-dimethylandrost-5(6)-en-3-one	0·3	2·0	1/7

* J. M. Midgley, W. B. Whalley, and (in part) G. F. Katekar, and B. A. Lodge, *Chem. Comm.*, 1965, 169.



m/e 145 : *m/e* 159 ratio. Any potential ambiguity may, however, be readily eliminated by reduction of the unsaturated steroid to the 5α-derivative prior to preparation of the dithian. The following scheme might well represent a major pathway in the saturated series:

Since the reactions involved in the preparation of the dithian derivatives proceed readily, in high yield, this technique is particularly applicable to steroids obtained (usually in small amounts only) from biological sources.

All new compounds had the requisite spectral and analytical characteristics.

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[1] R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Chem. Soc.*, 1957, 1131.