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Mass spectrometry of fluorinated corticosteroidal 1,4-dien-3-ones of the British Pharmacopoeia

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The mass spectrum of each of the B.P. 1968 fluorinated corticosteroidal 1,4-dien-3-ones is shown to be sufficiently characteristic to be of value as a means of identification. Mechanisms for the principal fragmentations are discussed.

Mass spectrometry has received significant recognition in the pharmaceutical literature as a powerful analytical tool (Coutts, 1968; Coutts and Locock, 1968, 1969; Dreyfuss, Cohen & Hess, 1968; Shipchandler & Soine, 1968; Lee & Soine, 1969). It is our experience that the mass spectrum of a corticosteroid is characteristic for any particular compound, and can serve as an unequivocal means of identification. One particular advantage of this technique is that the molecular weight of the compound is indicated.

Although in a recent communication (Lodge & Toft, 1970) we described a fundamental difference between the mass spectra of betamethasone and dexamethasone, a comparative study of all the fluorinated corticosteroidal 1,4-dien-3-ones of the B.P. has not been reported. Reference standards are provided in the B.P. for betamethasone (9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-dien-3,20-dione), betamethasone 17-valerate, betamethasone 21-valerate, dexamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-dien-3,20-dione), fluocinolone acetonide (6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-dien-3,20-dione) and triamcinolone acetonide (9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-dien-3,20-dione). In addition, we have included triamcinolone acetonide 21-t-butylacetate for comparison purposes with the other acetonides.

Significant differences in the mass spectra of isomeric pairs of compounds can often be seen, as, for example, with betamethasone (Fig. 1a) and dexamethasone (Fig. 1b), which are configurational isomers at C-16 (Lodge & Toft, 1970). The isomeric pair of esters betamethasone 17-valerate (Fig. 1c) (the ester of a tertiary alcohol) and betamethasone 21-valerate (Fig. 1d) (the ester of a primary alcohol) show several differences in a comparison of their respective mass spectra. The 17-valerate produces a relatively large amount of a fragment of m/e 315, apparently due to loss of one molecule of valeric acid plus carbons 20 and 21 with their attendant groups. The molecular ion (m/e 476) is hardly seen at all. The 21-valerate has a much more significant molecular ion, and relatively much less of the m/e 315 fragment. The base peak in the spectrum of the 21-valerate is at m/e 122, arising from cleavage of the 6–7 and 9–10 bonds and transfer of two hydrogens to the charged fragment (the A-ring plus C-6 and C-10); there is rather less of the fragment of m/e

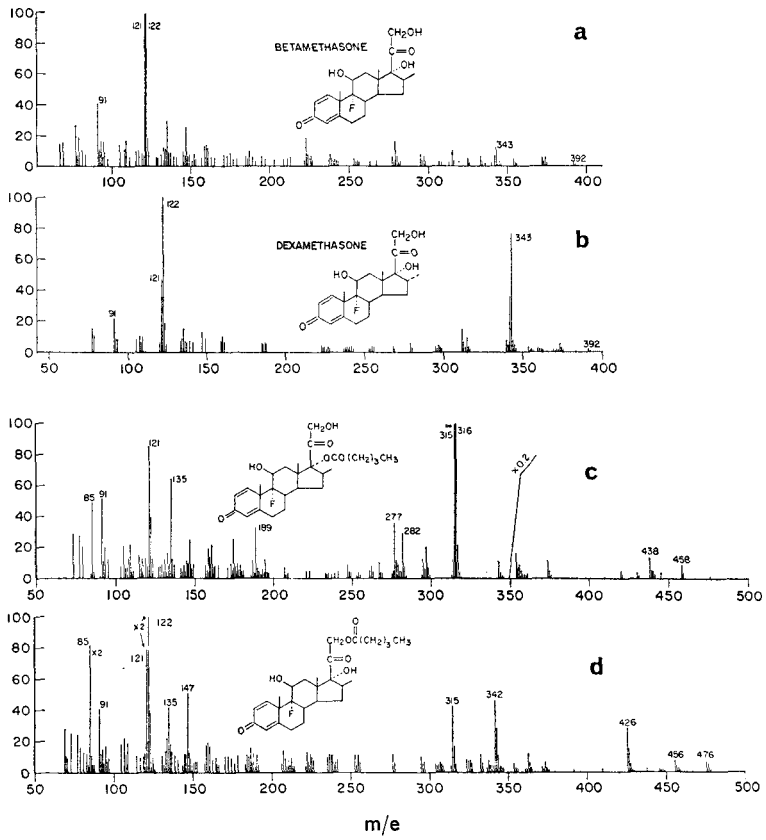
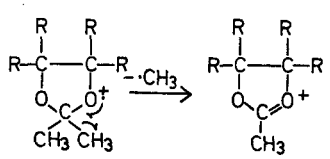


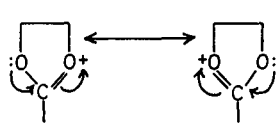
FIG. 1a. Betamethasone—9α-fluoro-11β,17α,21-trihydroxy-16β-methylpregna-1,4-dien-3,20-dione. b. Dexamethasone—9α-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1, 4-dien-3,20-dione. c. Betamethasone 17-valerate. d. Betamethasone 21-valerate.

121, formed from the same cleavage but with the transfer of only one hydrogen. With the corresponding peaks in the spectrum of the 17-valerate, the situation is reversed, the m/e 121 fragment predominating.

It has been reported (Budzikiewicz, Djerassi & Williams, 1967) that molecular ion peaks are not detectable in the mass spectra of acetonides, and that the peak of highest mass is the M–15 species, which arises as shown in scheme *a*. The ion formed is stabilized by resonance of the positive charge between the two oxygen atoms (scheme *b*). In the case of the three corticosteroid acetonides a molecular ion is seen. This difference is probably due to the fact that the earlier spectra were obtained on instruments with indirect inlet systems which involved heating the sample before entering the ion chamber. In our work the samples were vaporized directly in the



Scheme *a*



Scheme *b*

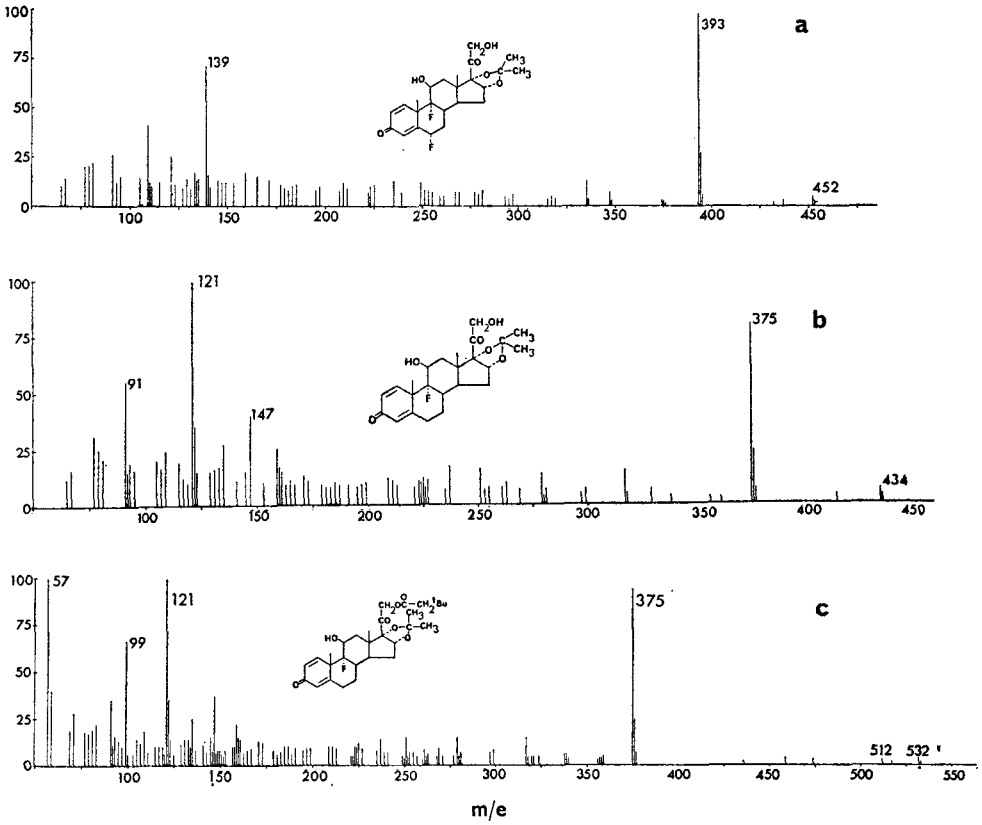
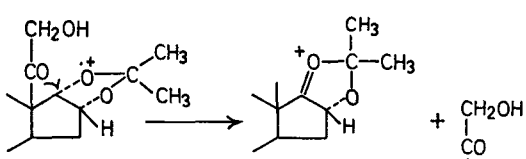


FIG. 2. a. Fluocinolone acetonide—6α,9α-difluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-dien-3,20-dione. b. Triamcinolone acetonide—9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregna-1,4-dien-3,20-dione. c. Triamcinolone acetonide 21-t-butylacetate.

ion chamber thus minimizing thermal decomposition before ionization. (For identification purposes it is preferable to compare the spectrum of an unknown with the spectrum of the standard obtained under identical conditions and on the same instrument.) In two of the spectra a small peak (Fig. 2a and c) is seen at M–15 (m/e 517 for triamcinolone acetonide 21-t-butylacetate and m/e 437 for fluocinolone acetonide). This is presumably due to loss of methyl from the acetonide by scheme *a*. Such a fragment from triamcinolone acetonide was not significant enough to be measured on this scale.

There is apparently an alternative competing fragmentation (scheme *c*) which allows the formation of a different stable ion. Loss of the two carbon side chain



Scheme *c*

from C–17 is probably preferred because the fragment $O{=}C{-}CH_2OH$ is relatively much more stable than CH_3.

Triamcinolone acetonide (Fig. 2b) and its 21-t-butylacetate (Fig. 2c) both show fragments at m/e 375, corresponding to cleavage of the 17–20 bond. Fluocinolone acetonide (Fig. 2a), being in effect 6-fluoro-triamcinolone acetonide, shows a corresponding fragment at m/e 393, the additional fluorine having, and indeed expected to have, no effect on this fragmentation.

The other principal fragmentation is again cleavage of the 6–7 and 9–10 bonds, with the transfer of one hydrogen to the charged fragment. In triamcinolone acetonide and its ester this causes a fragment at m/e 121; in the fragmentation of fluocinolone acetonide the 6α-fluorine atom remains with the charged fragment, which thus appears at m/e 139. The fluorine does not appear to affect the hydrogen-transfer process.

All three acetonides show a small fragment at M–20 (loss of HF) and this is common to all the compounds under consideration (except betamethasone 17-valerate which shows a peak at (M–18)–20 but not at M–20.

The spectrum of triamcinolone acetonide 21-t-butylacetate has two large peaks which do not appear in the spectra of the other two acetonides; both may be attributed to the ester moiety. The fragment of m/e 99 represents cleavage of the O–CO bond, and that of m/e 57 cleavage of the $CH{-}C(CH_3)_3$ bond, forming a relatively stable t-butyl ion. Betamethasone 21-valerate undergoes a similar cleavage of the O–CO ester bond to form a comparable fragment of m/e 85.

Conclusion

Mass spectrometry affords a means of distinguishing between corticosteroids which are closely related chemically. In our experience, the mass spectrum of each of these compounds is unique, and thus the technique is a powerful analytical tool.

METHOD

Mass spectra were recorded on a Hitachi-Perkin-Elmer Model RMU-6D or an AEI model MS-12 mass spectrometer with an ionization voltage of 70 eV. The compounds were introduced directly into the ion chamber, and the temperature of the probe was raised until a sufficient vapour pressure of compound was obtained that the spectrum could be measured. Probe temperatures were in the range 180–250°.

The compounds are part of the Food and Drug Directorate collection of reference steroids.

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