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The selective detection of some steroid acetates using the electron capture detector

Electron capture detectors are used in gas chromatography for the detection of sub microgram quantities of halogen containing molecules and in particular for chlorinated pesticide residues. To measure similar quantities of naturally occurring steroids, which have a low affinity for electrons, halogenated derivatives have been formed, *e.g.* chloroacetates[1,2], heptafluorobutyrates[3,4], chloromethyldimethylsilyl ethers[5], and bromomethyldimethylsilyl ethers[6].

A draw-back to the use of such derivatives for steroids in biological extracts is their lack of selectivity, considerable purification often being necessary prior to gas chromatography.

The electron capture detector is known to give a response with some corticosteroid acetates[7]. In a study of the response of the detector to the acetates of a number of steroids that occur in biological fluids it was found that those with the α-ketol grouping gave a high response, *i.e.* that some selection occurred.

TABLE I

RESPONSE OF THE ELECTRON CAPTURE DETECTOR TO SOME STEROID ACETATES

Steroid	*Response per ng*	*Relative retention time (OV-1)*
1 Cholestane	—	1.00 (4.4 min)
2 Dehydroepiandrosterone chloroacetate	1.00[a]	1.16
3 Dehydroepiandrosterone acetate	—[b]	0.58
4 Androst-5-ene-3β,17β-diacetate	—	0.84
5 3β,16α-Diacetoxyandrost-5-en-17-one	1.50	1.24
6 3α,16α-Diacetoxy-5α-androstan-17-one	1.33	1.13
7 Testosterone chloroacetate	3.98	1.62
8 Androst-4-ene-3,17-dione	0.12	2.22
9 16α-Acetoxyandrost-4-ene-3,17-dione	6.30	1.19
10 3β,21-Diacetoxypregn-5-en-20-one	0.79	2.38
11 21-Acetoxypregn-5-en-3β-ol-20-one	0.61	1.76
12 3β,16α-Diacetoxypregn-5-en-20-one	—	1.89
13 11-Deoxycorticosterone-21-acetate	3.11	2.57
14 Corticosterone-21-acetate	6.26	3.84
15 17α-Acetoxyprogesterone	0.21	1.65
16 3,16α-diacetoxyoestrone	2.58	1.46
17 Oestrone acetate	—	0.67

[a] Absolute response $2.5 \cdot 10^{-10}$ coulombs per ng.
[b] No response with quantities up to 100 ng.

Table I gives the response of the acetates relative to dehydroepiandrosterone chloroacetate and their retention times on a 1% OV-1 column (column 210 × 0.6 cm; support Supasorb 80–100; temp. 230°; nitrogen flow 50 ml per min). The gas chromatograph was a Pye series 104 model 74 with a Nickel-63 source electron capture detector. The detector was operated at 240° without purge or quench gas in the pulse mode.

Pulse amplitude was > 47 V, pulse width 0.75 μS, and pulse space 150 μS. The amplifier was set to give a full scale deflection of 5×10^{-10} A.

The response of the detector to each steroid was obtained from the linear plot of the recorder responses to three or four different quantities injected directly onto the column.

From Table I the selective response is very apparent between steroids 3 and 5, 8 and 9, 16 and 17. The ease with which the acetates are made and their stability to further purification and to gas chromatography makes them preferable in some circumstances to halogenated derivatives—especially when the selective response applies to steroids that are of particular interest. Fig. 1 illustrates this point. It compares the chromatograms of an acetylated extract of pregnancy urine obtained simultaneously with a flame ionisation and electron capture detector.

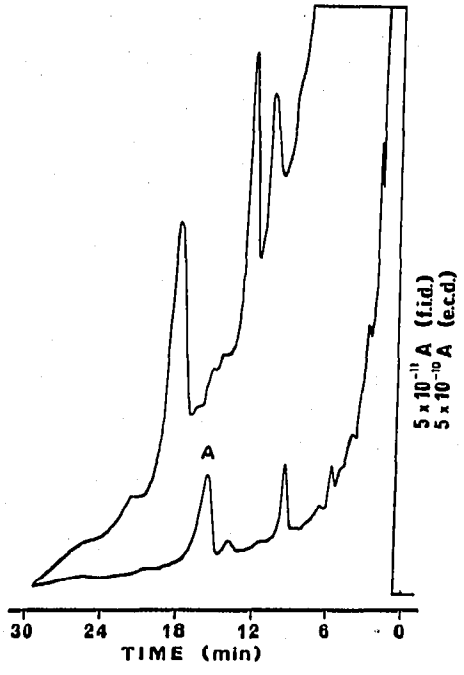

Fig. 1. Chromatograms of an acetylated extract of pregnancy urine. Upper trace, flame ionisation detector (f.i.d.). Lower trace, electron capture detector (e.c.d.). Pye Series 104, Model 84 gas chromatograph. Column 1%OV-17, 150 × 0.6 cm; support Supasorb 100–120; temperature 230°; nitrogen flow 60 ml per min. Effluent split to f.i.d. and e.c.d. in ratio 10:1, respectively. Peak A corresponds to the 3,16-diacetate of 16α-hydroxydehydroepiandrosterone.

Peak A, clearly visible on the electron capture tracing only, is that given by the 3,16-diacetate of 16α-hydroxydehydroepiandrosterone, the estimation of which was the aim in mind.

The selectively high response of the electron capture detector to certain steroid acetates may be of value not only in the quantitative analysis of steroids in biological fluids, but also in qualitative analysis using the technique previously described by LOVELOCK[8].

Acknowledgements

The author wishes to thank Dr. G. W. PENNINGTON for his advice and encourage-

ment in the preparation of this communication. The work was supported by the Medical Research Council of Great Britain.

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Received August 14th, 1968

J. Chromatog., 38 (1968) 139–141

CHROM. 3750

Thin-layer and gas–liquid chromatography of phenolic diterpene acetates

In a current study of extractives of the outer bark of western red cedar (*Thuja plicata* Donn), we have gathered several phenolic diterpenes as reference compounds. Chromatographic data on individual compounds have been published, but there has not been a collection of these data. The phenolic diterpenes were examined as their acetate derivatives because these compounds were more stable than the parent and, also, reference samples most often were donated as the acetates. Although nimbiol is not a diterpene, it is included because of its close structural relationship to the others.

Table I shows the chromatographic behaviour of these compounds. The first four columns of Table I give the thin-layer chromatography data and the last two columns give the gas–liquid chromatography data. The three developing solvents for thin-layer chromatography were petroleum ether (PE) (b.p. 65° to 110°), methylene dichloride (MD) and ether (E). The two columns used for gas–liquid chromatography were one 5 ft. $\times$ $^1/_8$ in. containing silicone gum SE-30 (25%) on Gas Chrom Q (100–200 mesh) and another, same size, containing diethylene glycol succinate (DEGS) (20%) on the same absorbent. A flame ionization detector was used in the gas chromatograph.

Table I shows that these compounds can be divided into groups according to the variety of functional groups present. Thus compounds with only the acetoxy group developed with the least polar PE solvent. Compounds with a carbonyl and acetoxy group were intermediate with the polar E solvent. Similar considerations applied to the gas chromatographic behaviour of the compounds. These data have been used to predict the amount of substitution on unknown compounds isolated from western red cedar bark.