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Notes on Methodology

Gas-liquid chromatography of adrenal cortical steroid hormones

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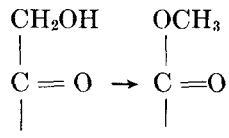
» The possibility of separating adrenal cortical steroid hormones by gas chromatographic methods has been investigated by VandenHeuvel and Horning (1). They established that an increase in the number of oxygenated functional groups in the steroid molecule leads to an increased retention time and increased susceptibility to decomposition. The corticosteroids were divided by these authors into two groups. When steroids belonging to the 20-one-17α, 21-diol series (cortisone, cortisol, cortexolone) were chromatographed at 222° on an SE-30 column, the two-carbon side chain was eliminated and corresponding 17-keto derivatives resulted. In the absence of a 17α-hydroxyl group, however, the 20-one-21-ol corticosteroids (deoxycorticosterone, corticosterone, aldosterone) gave several major peaks. Obviously it is difficult to assign the relative retention times of these multiple peaks to individual structures.

In view of the physiological and pharmacological importance of these corticosteroids, an effort was made to prepare suitable derivatives of them for gas-liquid chromatographic separation.

Periodic acid oxidation is a specific reaction for the 20, 21-ketol steroids (2, 3). In this instance, equimolar quantities of periodic acid and steroid in aqueous alcohol or dioxane solution were kept at room temperature for 15 hours. The products were formaldehyde and the corresponding 17β-carboxylic acids (4-etiocholenic acids). After most of the solvent had been evaporated *in vacuo* the acids precipitated. The residue was extracted with ether and the ether solution was then extracted with aqueous sodium bicarbonate (4). After addition of dilute hydrochloric acid to the bicarbonate solution, the acids were finally extracted into chloroform. Each derivative was prepared using a purified steroid in micromole quantities.

Since the free acids were not suitable for gas-liquid chromatography, their methyl esters were prepared using diazomethane. Thus, the final products were structural isomers of the starting materials, the ω-oxymethyl groups having been replaced by methoxy groups:



Since aldosterone exists as an 18,11-hemiacetal, oxidation of the side chain to a 17β-carboxylic acid results in γ-lactone formation (5, 6). The lactone is not soluble in sodium bicarbonate solution and is therefore separated from other etiocholenic acids at this step. The absence of a free carboxyl group enables one to chromatograph the lactone directly.

Three stationary phases were used in this work: silicone SE-30 (General Electric), polyether Pluronic F-68 (Wyandotte), and polyester neopentyl glycol succinate

TABLE 1. RELATIVE RETENTION TIMES OF SOME 4-ETIOCHOLENIC ACID DERIVATIVES*

Starting Material	Final Product	Relative Retention Times (Progesterone = 1.00) SE-30	Pluronic F-68	Neopentyl Glycol Succinate
Deoxycorticosterone	17β-Carbomethoxy-3-keto-4-androstene	1.00	0.88	0.78
Corticosterone	—, 11β-ol	1.63	3.56	3.13
Cortexolone	—, 17α-ol	1.28	1.81	1.68
Cortisone	—, 11-one, 17α-ol	1.57	3.58	3.64
Cortisol	—, 11β,17α-diol	2.15	7.90	6.67
Aldosterone†	17β-Carboxy-11β-hydroxy-3-keto-4-androstene-18-al-18,11-hemiacetal-γ-lactone	2.2	8.5	10.6

Column Characteristics	SE-30	Pluronic F-68	Neopentyl Glycol Succinate
Stationary phase, %	0.8	0.5	0.3
Inlet pressure, psi	20	25	25
Flow rate, ml/min	80	100	100
Time for progesterone, min	8.5	12.2	10.6

* Column size 180 cm × 6 mm, column temperature 220°, flash heater 250°. Solid support Chromosorb W 60 to 80 mesh pretreated with dichlorodimethylsilane vapor (7). Argon ionization detector.

† The author is indebted to Dr. E. B. Hershberg, Schering Corporation, for supplying a sample of aldosterone.

(Eastman). In all cases, well defined single peaks were obtained. Of the three stationary phases, the neopentyl glycol succinate is to be preferred because it separated all the methyl esters in a mixture. The results are shown in Table 1.

Infrared absorption spectra and melting point determinations of the compounds, before and after separation on the column, showed no differences. It is evident, therefore, that no structural changes occurred.

This qualitative separation of adrenal cortical hormone derivatives might serve as a basis for the development of a quantitative method.

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