GAS CHROMATOGRAPHY OF ADRENAL CORTICAL STEROID HORMONES

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The possibility of separating adrenal cortical steroid hormones by gas chromatographic methods is of considerable interest to chemists, biochemists and endocrinologists dealing with steroid problems. The resolving power and sensitivity of this method is now well established, but individual problems are presented by each group of compounds. The chief problem associated with corticosteroid separations lies in the multiple functional groups of these substances. Although some poly-functional compounds may be carried through gas chromatographic conditions without difficulty, as for example estriol and methyl cholate (VandenHeuvel, Sweeley and Horning, 1960a) and allopregnane-3,11,20-trione (VandenHeuvel, Sweeley and Horning, 1960b), an increase in the number of oxygenated functional groups has been found to lead to an increased retention time and increased susceptibility to functional change or decomposition.

The corticosteroids were studied in two groups: the 20-one-21-ol series and the 20-one-17α,21-diol series. The behavior of three members of the 17α-hydroxy series was investigated. These were cortisone (17α-hydroxy-11-dehydrocorticosterone), cortisol (17α-hydroxycorticosterone) and Reichstein's compound S (17α-hydroxydeoxycorticosterone). With silicone polymer SE-30 as the liquid phase, 1.5% on Chromosorb W, 60-80 mesh, 20 psi., at 222°, all three compounds gave well defined single peaks with no evidence of decomposition on the column. However, the relative

retention times (cholestane reference) were not in agreement with what would be expected from our previous experience. The times were characteristic of androstane derivatives.

RELATIVE RETENTION TIMES[a]

	TIME, 222° 1.5%	TIME, 222° 0.75%
17 α-HYDROXY CORTICOSTEROIDS		
17-Hydroxydeoxycorticosterone	0.54	
17-Hydroxycorticosterone	0.91	
17-Hydroxy-11-dehydrocorticosterone	0.65	
17-KETO-4-ANDROSTENES		
4-Androstene-3,17-dione	0.54	
4-Androstene-11β-ol-3,17-dione	0.91	
4-Androstene-3,11,17-trione	0.65	
CORTICOSTERONES		
Deoxycorticosterone		1.40, 1.56
Corticosterone		1.27, 1.50 2.36, 2.76
REFERENCE COMPOUND		
Cholestane	1.00[b]	1.00[c]

[a] Argon ionization detector, 6 ft. x 4 mm. I.D. Columns.

[b] Time, 12.6 min. for 60-80 mesh Chromosorb W, 1.5% SE-30, 20 psi. (120 ml./min.).

[c] Time, 6.0 min. for 60-80 mesh Chromosorb W, 0.75% SE-30, 20 psi. (109 ml./min.).

The relative retention times were compared in each case with those for the corresponding 17-keto derivatives. There was an exact correspondence, as indicated in the Table. Further investigation suggested that the side chain was eliminated during the vaporization process. The "flash heating" step lasts only a few seconds, and thermally-induced reactions may occur under these circumstances without leading to peak distortion; decomposition in the heated column gives trailing effects which are readily recognized and which were not seen.

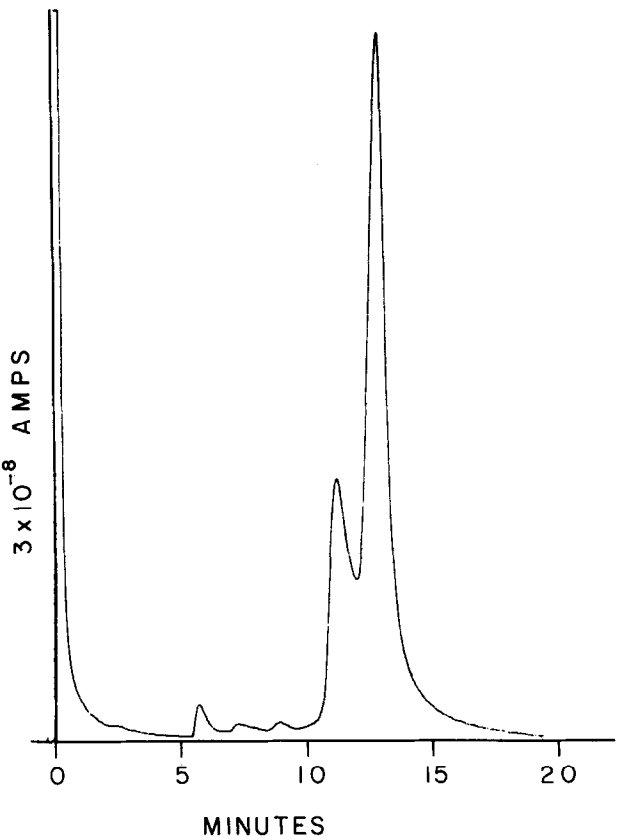

Figure 1. Analysis of deoxycorticosterone preparation. This separation was made with 0.75% SE-30 on 100-140 mesh Gas-Chrom support (Applied Science Laboratories).

The infrared spectra of the products from runs with cortisone and Reichstein's compound S were found to be identical with the spectra from the corresponding authentic 17-keto steroids. A second gas chromatographic determination gave no evidence of further change for the compounds derived from the cortical hormones or the authentic 17-keto compounds. In separate experiments it was found that 17α-hydroxyprogesterone did not undergo a similar change (relative retention time 1.22 with a 1.5% SE-30 phase under the same conditions), indicating that a 17α-hydroxy group is not the sole cause of the effect. There is no immediate evidence bearing on the mechanism of the reaction, but the absence of other steroidal products and the rapidity of the transformation suggests that an intramolecular process is involved.

Several samples of deoxycorticosterone and corticosterone were studied. Deoxycorticosterone gave two major peaks. The retention time relationships suggested that both components were in the pregnane series, but that functional or stereochemical differences were present. A typical result is shown. The infrared spectra of the initial and eluted materials were identical. Corticosterone samples gave four major peaks, with retention times suggesting that one pair had an 11β-hydroxy group and that the second pair did not. In no instance was there evidence of thermal decomposition, either during "flash heating" or on the column. The multiplicity of compounds observed for both deoxycorticosterone and corticosterone preparations make it difficult to assign relative retention times to individual structures. In order to clarify this behavior , it is necessary to determine the identity of each of these components.

Aldosterone and aldosterone-21-acetate did not survive the present separation conditions in intact form. About 60 steroids have been examined under a variety of conditions, and these are the first that have given evidence of multiple breakdown products. Further work is needed to determine if a suitable derivative of aldosterone can be prepared for gas chromatographic separation.

REFERENCES

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