Isolation of 17-Hydroxy 11-Dehydro Corticosterone (Kendall's Compound E) from Urine of Normal Males

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In the course of attempting to recover adrenocortical compounds from urine it has been possible to isolate 17-hydroxy 11-dehydro corticosterone from a chloroform extract of pooled normal male urine obtained prior to hydrolysis.

Urine was collected daily and extracted with chloroform without prior adjustment of pH. The extracts were concentrated to a small volume *in vacuo* and stored in the cold. At weekly intervals the pooled extracts were washed with cold 0.1 *N* NaOH and water, taken to dryness *in vacuo*, and stored under nitrogen in the cold. Ultimately the dried residues from 1000 l of urine were combined and part of the caffeine was removed by crystallization from benzene. The noncrystalline portion was divided into 70% alcohol-soluble and petroleum ether-soluble components, and after reduction to dryness *in vacuo* the alcohol-soluble fraction was dissolved in benzene and repeatedly extracted with equal volumes of water. The ketonic parts of both the benzene-soluble and water-soluble fractions were recovered and in turn repeatedly partitioned between benzene and water, using the method outlined by Mason, Myers, and Kendall (*2*). The fraction which passed readily from benzene to water and from water to benzene (designated fraction III by Mason *et al.*) weighed 161 mg and upon being reduced to dryness from chloroform solution crystallized spontaneously.

The first and subsequent crops were recrystallized from absolute ethanol to give 32 mg of colorless rhombohedra, mp 215–218° C. On admixture with an authentic sample of 17-hydroxy 11-dehydro corticosterone (mp 216–218° C) the mp was 215–218° C. Analysis: Calculated for $C_{21}H_{28}O_5$—C = 69.96%, H = 7.83%. Found—C = 69.50, 69.80%, H = 7.61, 7.70%. The addition of concentrated sulfuric acid to a small amount of the crystalline compound gave a yellow solution with a faint green fluorescence. Methanolic solutions of the substance rapidly reduced ammoniacal silver in the cold and formed a bright red precipitate upon the addition of a few drops of a saturated solution of 2,4 dinitrophenylhydrazine in 2 *N* HCl. $[\alpha]_D^{26} = +214° \pm 2°$ (concentration, 0.604 in 95% ethanol). Mason, Myers, and Kendall (*3*) have recorded from 17-hydroxy 11-dehydro corticosterone $[\alpha]_{5461}^{25} = +248° \pm 4°$ (concentration, 0.1 to 0.2), Kuizenga and Cartland (*1*) $[\alpha]_D^{25} = +195°$ (concentration, 1.89), and Wintersteiner and Pfiffner (*4*) $[\alpha]_D^{25} = +209° \pm 1°$ (concentration = 1.2) (all in 95% ethanol).

The compound readily formed an acetate on treatment with a mixture of pyridine and acetic anhydride at room temperature. Recrystallization from absolute ethanol yielded fine needles, mp 236–239° C. On admixture with an authentic preparation of 17-hydroxy 11-dehydro corticosterone acetate (mp 237°–239° C), the mp was 236°–239° C. Analysis of the acetate: Calculated for $C_{23}H_{30}O_6$—C = 68.65%, H = 7.46%. Found—C = 68.41%, H = 7.14%. The compound showed an absorption maximum in the ultraviolet at 237–238 mμ. ε = 13,870 (absolute ethanol). Mason, Myers, and Kendall (*2*) observed an absorption maximum at 237 mμ and have recorded a molecular extinction coefficient of 16,150. The biological activity of the substance is now being determined. A more detailed account of this work will appear at a later date.

References

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2. Mason, H. L., Myers, C. S., and Kendall, E. C. *J. biol. Chem.*, 1936, **114**, 613.
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Large Scale Paper Chromatography

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In view of the excellent separation of microquantities that can be achieved by paper partition chromatography (*1*), an attempt has been made to apply these same principles on a larger scale.

Two obstacles were encountered initially in large scale chromatography, the first being the difficulty of applying several milliliters of solution to a sheet of paper in a narrow straight band, and the second, obtaining a thick paper which could handle large quantities and still give good separation.

Attempts to apply several milliliters of solution from a capillary by hand were unsatisfactory, since it was virtually impossible to maintain a narrow band. It was found, however, that the application of solutions to paper could be greatly facilitated by the use of an automatically revolving drum to which the paper could be attached. The liquid could then be fed onto the paper, using a narrow capillary tube.

Such an apparatus can be readily set up in the laboratory using a kymograph, modified as shown in Fig. 1. The lower drum (A) of the kymograph is fixed at any convenient height by means of the latch (G). A second drum (B) is then put onto the shaft above (A), but is separated from (A) by means of a one-hole rubber stopper (C) which serves to provide a 1½-in. clearance between the two drums. This is desirable in order to prevent blotting between the paper and the drum when liquid is applied to the paper. A sheet of Whatman No. 1 paper, 17 in. high and 20 in. wide, is wrapped around the drums and fastened by means of two elastic bands. These can be closed most readily and held tightly around the paper if hooks and eyes are sewn on the ends. The paper is fastened in such a way that it overlaps in a direction opposite to that of the rotation of the kymo-