The occurrence of 19-hydroxylation in mammalian tissue was recognized for the first time in incubation experiments with androstenedione. The formation (4) and subsequent identification (5) by Meyer of 19-hydroxy-Δ^4-androstene-3,17-dione, a hitherto unknown steroid, has been announced.

One gram of DOC was incubated as previously described [see "Corticosterone" p. 178 (1)]. The silica gel chromatography fractions containing material more polar than corticosterone were combined and subjected to a second chromatography. The ethyl acetate eluates were processed by paper chromatography in the toluene–propylene glycol system of Zaffaroni *et al.* (6). Material migrating at a rate approximately one-third that of corticosterone was separated and crystallized twice to give 5 mg., m.p. 155–159°C. (corrected). The functional groups absorbed near 3333 cm.$^{-1}$ (—OH); 1703 cm.$^{-1}$ (C_{20}=O); 1642 and 1610 cm.$^{-1}$ (—C=C—C=O); whereas some fingerprint bands appeared near 1127, 1091, 1066, 1046, 1037, and 1011 cm.$^{-1}$ (solid film).

Identification of this compound as 19-hydroxy-11-deoxycorticosterone was established as stated above.

Further investigation of the enzymology of 19-hydroxylation is under way.

ACKNOWLEDGMENTS

The authors acknowledge the able assistance of Miss M. C. Lindberg in the laboratory and wish to thank Dr. H. Rosenkrantz for the infrared analysis.

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Received January 10, 1955

The Isolation of 19-Hydroxy-11-deoxycorticosterone and an Unknown, Active Mineralocorticoid from Bovine Adrenal Perfusions of Progesterone[1]

We have previously described the isolation of 17 α-hydroxyprogesterone, 11 β-hydroxyprogesterone, corticosterone and 17 α-hydroxycorticosterone from perfusions of progesterone through isolated bovine adrenal glands. We wish to report

[1] The investigations described in this paper were supported by grants from G. D. Searle and Co., the U. S. Public Health Service (A-247), and the American Cancer Society (Grant-in-Aid EDC-8-A and Institutional Grant 63-C).

the isolation of 2 other substances from such perfusion: the first, 19-hydroxy-11-deoxycorticosterone and the other, a compound which is highly active in the salt retention assay but whose complete structure is as yet unknown.

Ninety grams of progesterone in 600 l. of blood were perfused through 600 bovine adrenal glands. A crude extract of steroids was obtained by adsorption from the perfusate on active charcoal and then elution from the charcoal by acetone (1, 2). The crude extract was dissolved in ethyl acetate and washed free of acids with 0.5 *N* sodium bicarbonate. The residue of the ethyl acetate solution was then subjected to a series of adsorption chromatograms on silica gel (1, 2). Certain of the syrups from the eluates in which 17-hydroxycorticosterone appeared, and from which most of this substance was removed by crystallization, were then chromatographed on a Celite 545 partition column with methanol-water, 1:1, as the stationary phase and benzene as the mobile phase. This column is based on that described by Morris and Williams (3).

One of the substances crystallized from the syrupy residues of the eluates by means of ethyl acetate consisted of a series of solids totaling 26 mg. with a melting range of 143–154°C. Recrystallizations from methanol raised the melting point to 154–157°, uncorrected (intact crystal, heated stage). This substance had an α,β-unsaturated carbonyl group [λ maximum (methanol) 242 mμ, ν maximum (film) 1662, 1625 cm.$^{-1}$], an additional carbonyl group [ν maximum (film) 1715 cm.$^{-1}$] and a hydroxyl group [ν maximum (film) 3425 cm.$^{-1}$]. A positive blue tetrazolium test suggested the presence of an α-ketol group. A negative Porter-Silber test indicated the absence of a 17 α-hydroxyl group. Its mobility in the propylene glycol–toluene paper partition chromatography system was about four times that of 17 α-hydroxycorticosterone. Its solution in concentrated sulfuric acid was light blue-green against a white background and light blue-green with a burgundy-red fluorescence against a black background. This solution, after 2 hr. at room temperature, had a strong absorption peak at 285 mμ and 2 weak peaks at 372 and 445 mμ.

When the substance was reacted with acetic anhydride and pyridine at room temperature, it formed another compound, m.p. 127–130°C., uncorrected, whose infrared absorption spectrum showed the presence of an acetate group [ν maximum (film) 1738, 1232 cm.$^{-1}$] and the absence of an hydroxyl group (no absorption maximum at about 3425 cm.$^{-1}$).

These data suggested that the isolated substance was a C_{21}-steroid with an α,β-unsaturated carbonyl group in ring A and with an α-ketol side chain, that it possessed one or more additional hydroxyl or carbonyl groups, and that any other hydroxyl groups must be readily esterified. In particular, its melting point and that of its acetate suggested that it might be 19-hydroxy-11-deoxycorticosterone (reported m.p. 163–165°C. with sintering at 153–158°; 19,21-diacetate, m.p. 127°) which Barber and Ehrenstein had synthesized (4). A sample of the authentic 19-hydroxy-11-deoxycorticosterone (m.p. 152–156°C. on our apparatus), generously supplied by Dr. Ehrenstein, was found to have an infrared absorption spectrum identical with that of the isolated substance. Further, the infrared spectrum of the 19,21-diacetate prepared from the known substance was identical with that of the acetate of the isolated compound.

19-Hydroxy-11-deoxycorticosterone has also been isolated by Hayano and

Dorfman from the products of the incubation of deoxycorticosterone with adrenal homogenates (5).

Another substance of interest has been isolated from one of the silica gel chromatograms of the extract of the progesterone perfusion. It was obtained from certain of the benzene–ethyl acetate, 1:1, eluates as a group of crude solids, totaling 29 mg., with a melting range of 208–218°C. Recrystallizations from methanol and a partition chromatogram on Celite 545, using methanol–water, 1:1, as the stationary phase and benzene–ethyl acetate, 15:1, as the mobile phase, achieved the pure solid, m.p. 240–244°C., uncorrected. About 10 mg. of solids melting from 230–236° to 240–244° were obtained. The substance has an α,β-unsaturated carbonyl group [λ maximum (methanol) 242 mμ, ν maximum (film) 1660, 1625 cm.$^{-1}$], an additional carbonyl group [ν maximum (film) 1705 cm.$^{-1}$], a hydroxyl group [ν maximum (film) 3525 cm.$^{-1}$], an α-ketol group (blue tetrazolium test) and a 17 α-hydroxyl group (Porter-Silber test; sodium bismuthate oxidation and Zimmermann test of the product for the 17-carbonyl group). Its solution in concentrated sulfuric acid is bright red and, after 2 hr. at room temperature, has absorption peaks at 285, 395, and 540 mμ. Its mobility in the propylene glycol–toluene paper partition chromatography system is about one-fourth that of 17 α-hydroxycorticosterone and about 5 times that of 6 β,17 α-dihydroxycorticosterone. These data suggest that the substance is a $C_{21}O_5$ steroid containing an α,β-unsaturated carbonyl group in ring A, an α-ketol side chain, and a 17 α-hydroxyl group.

When reacted with acetic anhydride and pyridine at room temperature, it forms an acetate [ν maximum (film) 1730, 1230 cm.$^{-1}$] whose mobility in the benzene–formamide paper partition chromatography system is about 2½ times that of 17 α-hydroxycorticosterone 21-monoacetate. This suggests that the fifth oxygen function is a readily esterified hydroxyl group. Further work toward the elucidation of the structure of the unknown metabolite is in progress. This substance is of interest in that it is 1 and 10 times as active as deoxycorticosterone in doses of 6 and 0.5 μg., respectively, in the sodium retention assay. It has less than one-tenth the activity of cortisone acetate in effecting a decrease of eosinophils.

Note Added in Proof

The unknown mineralocorticoid has been identified as 17α,19-dihydroxy-11-deoxycorticosterone by oxidizing it with sodium bismuthate and comparing the product, m.p. 170–171°, with Δ^4-androsten-19-ol-3,17-dione, reported m.p. 168–170°, a substance which has been isolated from adrenal incubation of Δ^4-androsten-3,17-dione (6). The infrared spectra were identical.

Acknowledgments

We are grateful to Dr. A. L. Raymond for arranging for the perfusions at G. D. Searle and Co., to Dr. E. B. Romanoff for some of the paper chromatographic data; to Dr. M. Ehrenstein for his reference sample; to Dr. H. Rosenkrantz and Mr. P. Skogstrom for determining and interpreting some of the infrared absorption spectra; to Dr. G. Pincus and Mr. M. Cook for the electrolyte assay; and to Dr. R. S. Speirs for the eosinophil assay.

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Received January 10, 1955

Iron as a Stabilizer of Vitamin B_{12} Activity in Liver Extracts and the Nature of So-called Alkali-stable Factor

Although it is generally assumed that the alkali-stable activity represents deoxyribosides, some workers (1, 2) postulated the existence, in crude liver extracts, of other growth-promoting factors for the lactobacilli and *E. coli* mutant used in vitamin B_{12} assay. Questioning this hypothesis, Smith (3) suggested the possibility of part of the vitamin B_{12} being "protected from such relatively mild alkali-treatment by unknown components in some crude sources such as liver extract," a suggestion supported by more recent data (4, 5).

It was observed by us (6) that the microbiological (*L. leichmannii* 313) vitamin B_{12} activity of unfractionated aqueous liver extract (Liver Concentrate, N.F. IX) was far more thermostable (120°C.; 30 min.) than that of dilute solutions of the 70% alcohol-soluble fraction (Liver Fraction 1, N.F. IX) derived from it and that the vitamin stability in the latter was restored on mixing it with the 70% alcohol-insoluble material (Liver Fraction 2, N.F. IX), or raising the concentration of Liver Fraction 1 solution to more than 30% solids. Further investigation in the light of the work of Frost *et al.* (7) and Skeggs (8) showed that this stabilizing property of Liver Fraction 2, N.F. resided in the ash which contained an appreciable amount of iron and could be duplicated by the addition of an equivalent amount of iron in the form of ferrous sulfate or ferric chloride.

When the study was extended to a wider range of pH, the surprising observation was made that added iron gave excellent protection to the vitamin B_{12} activity of liver extracts when heated at pH 10.0 and 120°C. for 30 min. On the other hand, aqueous solutions of crystalline vitamins B_{12} and B_{12b} were not protected by iron at pH 10.0. In order to confirm this interesting finding, the experiment was repeated using a dilute solution of Liver Fraction 1, N.F., which was rendered free from vitamin B_{12} activity by heating for 2 hr. at pH 10.0 and 120°C. The results (see Table I) showed that crystalline vitamins B_{12} and B_{12b} added to this solution were unstable on heating at pH 10 but were remarkably stabilized by the further addition of ferric chloride.

It is clear that heating for 30 min. at pH 10.0 and 120°C. is not an effective procedure for destruction of the vitamin since it depends upon the iron content of the extract. This possibly explains the variable results reported in the litera-