METABOLITES OF 11-DEHYDROCORTICOSTERONE: PREGNANE-3(α), 20-DIOL-11-ONE

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A study which involved the administration of relatively large amounts of synthetic 11-dehydrocorticosterone (Ia) and its acetate (Ib) to a woman (Case 1) and to a man (Case 2) with Addison’s disease afforded an opportunity to determine what urinary products might be related to this hormone. Thus far it has not been possible to isolate from the urine, in cases of hyperfunctioning lesions of the adrenal cortex, any 11-oxygenated pregnane derivatives. In the present study pregnane-3(α), 20-diol-11-one and another unidentified compound were isolated from the urine collected while 11-dehydrocorticosterone and its acetate were being given. These compounds could not be found in the urine during another period when 17-hydroxy-11-dehydrocorticosterone was being given.

DISCUSSION

The urine was processed, the neutral extracts were separated into “ketonic” and “non-ketonic” fractions, and these fractions were subjected to chromatographic analysis essentially as previously described (1). The only crystalline fractions of any consequence were isolated from the non-ketonic fractions. Only a trace of crystalline material was obtained from the ketonic fraction. It was necessary to rechromatograph the crystalline material first obtained from the non-ketonic fraction in order to separate it into two compounds. The compound that was not identified had a melting point a little lower than that of pregnanediol, and its melting point was not depressed by admixture of pregnanediol. However, the acetate of the newly isolated compound melted at 147–149°, whereas a specimen of pregnanediol diacetate melted at 160–162°.

Analysis of the other compound indicated the formula C$_{21}$H$_{34}$O$_3$. When the compound was heated 1 hour at 90° with pyridine and acetic anhydride, a diacetate was formed. The formula, C$_{21}$H$_{34}$O$_3$, indicated the presence of a ketone group or of a carbon-carbon double bond. A double bond would require the presence of a third alcohol group in a hindered position, since it


2 These substances were provided through the courtesy of Merck and Company, Inc., Rahway, New Jersey.
was not acetylated. If a ketone group were present, it also must be in a hindered position such as C-11, since it did not combine with Girard's reagent. (The ketonic fraction was chromatographed, but there was no evidence of this compound in that fraction.) Since it is known that 11-desoxycorticosterone can be converted physiologically to pregnanediol (2-4), it seemed likely that this new compound might be 11-ketopregnanediol (II) (pregnane-3(α),20-diol-11-one). Consequently, it was oxidized so that the ketone formed might be compared with pregnane-3,11,20-trione (III). This latter compound had been prepared by Dr. R. B. Turner in Dr. E. C. Kendall's laboratory; I am indebted to them for this preparation. The melting points of the two preparations were identical and there was no depression in the melting point of a mixture. Likewise there was no depression in the melting point of a mixture of the oximes.

Lieberman reported at the Laurentian Hormone Conference, September 10, 1947, the isolation of pregnane-3(α),20-diol-11-one from the urine of normal individuals. The excretion of pregnane-3(α),20-diol-11-one as the result of administration of 11-dehydrocorticosterone definitely identifies this latter hormone as the precursor of the urinary compound.

During the administration of 11-dehydrocorticosterone and its acetate there was no increase in the excretion of 17-ketosteroids as determined by the Zimmermann reaction. Furthermore, it was not possible to isolate any crystalline substance from the ketonic fraction. It may be concluded that 11-dehydrocorticosterone is not a precursor of 17-ketosteroids, but that it is converted in small part to pregnane-3(α),20-diol-11-one and in large part to unrecognized substances. Possibly this latter part is destroyed completely.

**EXPERIMENTAL**

In Case 1, 50 mg. of 11-dehydrocorticosterone acetate were given daily for 8 days and then 200 mg. for 11 days. The urine collected during administration of the acetate (Period 1) and during the 2 days following withdrawal of the hormone was pooled. Later, 100 mg. of the free hormone were given daily for 11 days (Period 2). The urine collected during this period was processed apart from that collected during Period 1. In Case 2, 100 mg. of 11-dehydrocorticosterone were given daily for 5 days.
After the addition of 50 ml. of chloroform, the 24 hour specimens of urine were stored in the refrigerator at 5° for 2 days. They were then extracted with chloroform for determination of “cortin-like” substances. The urine residues were acidified with 5 ml. of concentrated HCl and stored in the refrigerator until the collection was complete. The pool of urine was then concentrated and worked up in the manner that has been described previously (1).

In Case 1, 20 mg. of 17-hydroxy-11-dehydrocorticosterone also were given daily for 11 days. Nothing crystalline could be isolated from the urine collected during this period, perhaps because of the relatively small daily dose of hormone. However, since the results were completely negative, this period served as a control for the periods during which 11-dehydrocorticosterone was administered. Any steroids isolated during the latter periods must have been derived from 11-dehydrocorticosterone. The amount of steroids normally excreted by these patients, as measured by the Zimmermann reaction, was negligible.

The chloroform extracts obtained before acid hydrolysis also were examined. The ketonic portion of the fraction that was removed from benzene by repeated extraction with water was used for the estimation of cortin-like substances. Not enough remained for any attempt to isolate compounds. The non-ketonic fraction, however, yielded a crystalline substance which melted at 272° and which appeared to be too soluble in water to be of steroid nature. It was also present when 17-hydroxy-11-dehydrocorticosterone was given. It will not be considered further in this report, since it evidently bore no relation to 11-dehydrocorticosterone.

**Ketonic Fraction**—The ketonic fractions of the neutral extract of the urine that had been boiled with HCl gave only traces of a crystalline compound. This result was expected, since the excretion of 17-ketosteroids as determined by the Zimmermann reaction was 0.3 to 1.5 mg. per day and was not increased by administration of the hormone.

**Non-Ketonic Fraction**—The non-ketonic fractions obtained in Case 1 during administration of 11-dehydrocorticosterone acetate and during administration of the free hormone were 370 and 200 mg., respectively, and that obtained in Case 2, 145 mg. These three non-ketonic fractions were chromatographed separately on alumina. Benzene containing increasing amounts of alcohol was used for elution of the various fractions.

In Period 1, Case 1, a few crystals were obtained from the fraction eluted with 0.2 per cent of alcohol by volume. After recrystallization from acetone they melted at 200–210°. Not enough was available for further work. The fraction eluted with 1 per cent alcohol weighed 90 mg. Analysis of the recrystallized fraction (50 mg.) and its acetate indicated that it was not a single compound. Consequently the remainder was combined
with the corresponding fraction obtained in Period 2 (total weight 80 mg.) and rechromatographed.

**Compound with Melting Point of 232-233°**—In the second chromatogram 11 mg. of a crystalline fraction were eluted with the fourth to ninth 20 ml. portions of 0.3 per cent alcohol by volume in benzene. The substance melted at 232-233°. A mixture with pregnane-3(α), 20(α)-diol (m.p. 235-237°) melted at 231-233°. However, the acetate of this substance melted at 147-149° and therefore could not be the acetate of pregnanediol. A mixture with etiocholane-3(α), 17(α)-diol (m.p. 233°) melted at 216-222°. There was not sufficient material for further characterization.

**Pregnane-3(α), 20-diol-11-one (I)**—Beginning with the sixteenth 20 ml. portion of 0.3 per cent alcohol in benzene, 100 ml. of solvent removed 7 mg. of crystalline material; 100 ml. of 0.5 per cent and 100 ml. of 1 per cent alcohol in benzene together eluted 36 mg. of the same material. It was best recrystallized by addition of acetone to its solution in a little hot methanol. It melted at 217-219°; \( [\alpha]_D^2 = +59° \pm 2.2° \) (c = 0.407 in alcohol). It failed to give a precipitate with digitonin in 90 per cent alcohol. For analysis a sample was dried 1 hour at 100° and 0.1 mm.

**Analysis**—C\(_{21}\)H\(_{30}\)O\(_3\). Calculated, C 75.33, H 10.14; found, C 75.40, H 10.25

**Pregnane-3(α), 20-diol-11-one Diacetate**—The acetate was prepared by heating 10 mg. with a few drops of pyridine and 3 drops of acetic anhydride for 1 hour at 90°. Water was added while cooling; then sufficient concentrated HCl was added to make the mixture acid to Congo red. The precipitate was filtered out, washed with dilute HCl and water, dried, and recrystallized from methanol. The first crop was recrystallized, and it then weighed 7 mg. and melted at 233°.

**Analysis**—C\(_{33}\)H\(_{42}\)O\(_5\). Calculated, C 72.08, H 8.98; found, C 71.74, H 9.15

**Oxidation of Pregnane-3(α), 20-diol-11-one**—To a solution of 12 mg. of this compound (0.144 milliequivalent) in 1 ml. of glacial acetic acid was added 0.26 ml. of CrO\(_3\) in acetic acid containing 0.158 milliequivalent (10 per cent excess). The mixture was allowed to stand overnight. Benzene was added and the solution was washed three times with water, then with sodium carbonate solution, and finally with water. Removal of the benzene and crystallization of the residue from anhydrous ether gave crystals which melted at 156-157°. A mixture with pregnane-3, 11, 20-trione (III) (m.p. 157-158°) melted at 156-157°.

The oximes also were prepared for comparison. The ketone (2 mg.), 0.5 ml. of a solution of hydroxylamine hydrochloride (4 mg. per ml.) in 50 per cent alcohol, and 2 drops of a saturated solution of sodium acetate were heated in an open tube in a water bath until the alcohol had evapo-
rated. Heating was continued while alcohol was added dropwise until the precipitate had redissolved. The crystals that separated on cooling were washed with water and dried. The oxime prepared from the authentic specimen of pregnane-3,11,20-trione melted at 240–243° when placed on the block at 230°. The other oxime melted at 243–245°. A mixture of the two melted at 240–244°.

SUMMARY

Pregnane-3(α),20-diol-11-one was isolated from the urine of two patients with Addison’s disease after administration of 11-dehydrocorticosterone and its acetate. Another substance melting at 232–233° was isolated but not identified.

Microanalyses were performed by J. Alicino. Melting points were determined with the Fisher-Johns electrically heated block and are recorded as read.

BIBLIOGRAPHY

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