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THE ISOLATION AND THE CHARACTERIZATION OF 1β-HYDROXY-11-DEOXYCORTISOL AS A BY-PRODUCT OF A MICROBIAL 11-HYDROXYLATION

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ABSTRACT

From the microbial 11-hydroxylation of 17α,21-dihydroxypregn-4-ene-3,20-dione with Absidia orchidis, 1β,17α,21-trihydroxypregn-4-ene-3,20-dione (I) was isolated as a by-product and identified. The possible pathway of formation is discussed. Also, some structural features of 21-acetoxy-17α-hydroxypregn-4-ene-1,3,20-trione (V), an oxidation product of I-acetate, are outlined on the basis of spectral properties.

Following the course of microbial 11-hydroxylation of 11-deoxycortisol (17α,21-dihydroxypregn-4-ene-3,20-dione) with Absidia orchidis[3], we observed repeatedly a spot on thin-layer chromatograms, indicating the presence of a substance, which was different from any of the usual products of this transformation, i.e. cortisol (11β,17α,21-trihydroxypregn-4-ene-3,20-dione), 11α-epicortisol (11α,17α,21-trihydroxypregn-4-ene-3,20-dione), and cortisone (17α,21-dihydroxypregn-4-ene-3,11,20-trione). In a preparative experiment, the crude transformation product was extracted with ethyl acetate, and the substance was isolated from the ex-

tract by fractional crystallization successively from a methanol-chloroform mixture, chloroform, and acetone.

This chromatographically individual substance (I), m.p. 203-8°, $/\alpha/_D^{19}$ + 85±3°, corresponded to the elemental composition of $C_{21}H_{30}O_5$ and showed the characteristic absorption in ultraviolet region of Δ^4-3-ketone at 241 mμ (log ε 4.18). The substance I was strongly positive toward blue tetrazolium and ammoniacal silver oxide solution. By oxidation with sodium bismuthate in 50% aqueous acetic acid at room temperature, it afforded a mixture of products, from which only androsta-1,4-diene-3,17-dione (VI) was isolated in pure state. Acetylated under very mild conditions, i.e. with acetic anhydride in acetone in the presence of triethylamine, it afforded monoacetate II, whereas by the customary acetylation with acetic anhydride in pyridine, it gave rise to diacetate III isolated in amorphous form as its solvate with methanol. By heating at 80-85° for several hours with glacial acetic acid, the monoacetate II was dehydrated to the known 21-acetoxy-17α-hydroxy-pregna-1,4-diene-3,20-dione (IV). The easy dehydration under very mild conditions accounts for a β-ketol grouping, and the structure of the dehydration product IV located the hydroxyl in question in the 1-position. The constants of the substance I fit well with the constants of 1β-hydroxy-11-deoxycortisol (1β,17α,21-trihydroxypregn--4-ene-3,20-dione), already obtained from 11-deoxycortisol by 1-hydroxylation with Rhizoctonia ferrugena[4] or from rusogenine by a partial synthesis[5]. The molecular rotation

differences for 1β-hydroxy- or 1β-acetoxy-group in the substances I-III are similar as in other known compounds of this structure (cf. Table I).

By oxidation of 21-acetate II with chromium trioxide in acetic acid, 21-acetoxy-17α-hydroxypregn-4-ene--1,3,20-trione (V) was formed in an excellent yield, and this behaviour is in accordance with the ready oxidizability of 1β-hydroxyl with this reagent[6]. The β-diketone grouping with the neighbouring conjugated double bond offers various possibilities of enolization demonstrated by the partial formulae Va-d. As we are not aware of a closer related model structure, the wave-length of ultraviolet maxima of substance V in methanolic solution at 245 and 282 mμ (log ε 3.56 and 3.71) may be well compared to those of a saturated β-diketone, viz. a 16,20-diketopregnane derivative, showing maxima at 241 and 285 mμ (log ε 4.23 and 3.73)[8,9]. Those maxima are best interpreted by the tautomeric structure Vb, in which the Δ^4-double bond should not contribute to a bathochromic shift because it forms a cross--conjugated system. A plausible possibility cannot also be ruled out that the substance is present, at least in part, in its β-diketo form Va. - In a basic medium, i.e. in a 1:1 mixture of methamol and 1N-aqueous sodium hydroxide, a very strong bathochromic shift of the longer-wave maximum is observed of magnitude of 65 mμ (from 282 to 347 mμ; log ε 3.85), the shorter-wave maximum being unchanged at 245 mμ (log ε 3.85) as a shoulder (see Figure I). This very

Table I

Molecular rotations differences

Compound	[M]	Δ[M]
Pregn-4-ene-3,20-dione	+ 635° 7	+ 169°
1β-Hydroxypregn-4-ene-3,20-dione	+ 466° 5	
17α,21-Dihydroxypregn-4-ene-3,20-dione	+ 458° 7	+ 149°
1β,17α,21-Trihydroxypregn-4-ene-3,20-dione	+ 309°	
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	+ 447° 7,a	+ 140°
21-Acetoxy-1β,17α-dihydroxypregn-4-ene-3,20-dione	+ 307°	
Pregn-4-ene-3,20-dione	+ 635° 7	+ 371°
1β-Acetoxypregn-4-ene-3,20-dione	+ 264° 5	
21-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	+ 571° 7,b	+ 377°
1β,21-Diacetoxy-17α-hydroxypregn-4-ene-3,20-dione	+ 194° x	

x Corrected for methanol of solvation

a Acetone, b chloroform

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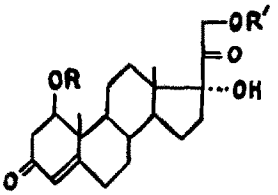
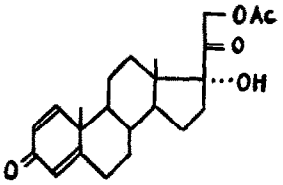
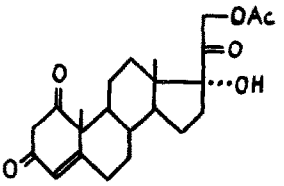
significant shift permits us to conclude that conjugation with a further double bond occured together with a change from an heteroannular system to an homoannular one. Moreover, a relatively very small increase of extinction accounts



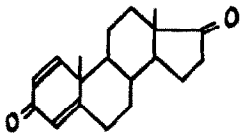
I, R=R´=H
II, R=H, R´=Ac
III, R=R´=Ac



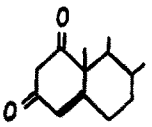
IV



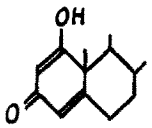
V



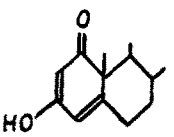
VI



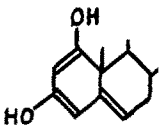
Va



Vb



Vc



Vd

for the latter system, too. All these spectral properties are in best accord with the fully enolized structure Vd. Under similar conditions of full enolization, the saturated reference 16,20-diketone shows maxima at 241 and 308 mμ (log ε 4.22 and 4.30, resp.), i.e. a bathochromic shift of 23 mμ only.

To the best of our knowledge, no example of 1-hydroxylation was observed as a side reaction of microbial 11--hydroxylation as yet. A probable clue to our results may be seen in a rather small stereospecificity of the enzymic system of Absidia orchidis apparent also from the mixed 11α- and 11β-hydroxylation. Bearing in mind the closed steric proximity of $C_{(1)}$ and $C_{(11)}$, it is possible that the less stereospecific enzyme brings the oxygen function (whatever it is) in the neighbourhood of both mentioned carbons and allows it to be distributed between them. A possibility of

substitution into the axial 1α-position must be ruled out in this case. A steric competition of the 1β-hydroxyl with 11α- or 11β-hydroxyl is demonstrated by the absence of even a trace of 1,11-dihydroxy-compound among the reaction products. Moreover, all attempts to achieve 11-hydroxylation of 1β-hydroxy-11-deoxycortisol (I) were unsuccessful [10]. From this point of view, it seems probable that the products of 1-hydroxylation of 9α-fluorocortisol (9α-fluoro--11β,17α,21-trihydroxypregn-4-ene-3,20-dione) and related substances with Rhizoctonia ferrugena[4] or microorganisms of the genera Streptomyces[11] and Mortierella[12], for which the configuration of the 1-hydroxy group was not estimated, have the 1-hydroxyl in the sterically more accessible α-configuration.

EXPERIMENTAL

Melting points were determined on a Kofler microstage. Thin-layer chromatography was carried out over silica gel with 5% of plaster of Paris as a binder using chloroform-ethanol mixtures in various proportions as developing system. The samples for analysis were dried at 76° and 0.01 mm. for 5 hours. Ultraviolet spectra were run on Spectralphotometer Zeiss, Model VSU (quartz cell of 1 cm. thickness) in ethanol. Infrared spectra were recorded on a double-beam apparatus Zeiss, Model UR-10 (NaCl-prism), in about 6% chloroform solution.

The Isolation of 1β,17α,21-Trihydroxypregn-4-ene-3,20-dione (I)

Crude fermentation product (200 g) containing cortisol (11β,17α,21-trihydroxypregn-4-ene-3,20-dione) and 11α-epicortisol (11α,17α,21-trihydroxypregn-4-ene-3,20-dione) as the main components, was dissolved in 8 l of hot ethyl acetate,

and allowed to stand at room temperature overnight. The crystalline mixture was filtered off, and the mother liquor was concentrated to a small volume. A crystalline mixture resulted upon cooling, consisting (thin-layer chromatography) predominantly of the expected compound I. The residual quantities of the main products, which separated in crystalline form upon crystallization from chloroform-methanol, were filtered off, and the concentrated mother liquor was purified by repeated crystallization successively from chloroform and acetone. Chromatographically pure 1β-hydroxy-11-deoxycortisol (2.3 g, 1.2%) was obtained, m.p. 203-8°; $[\alpha]_D^{19}$ + 85° ±3° (dioxan, c 1.9); λ_{max}^{EtOH} 241 mμ (log ε 4.18); ν 3530,3390,3320,1710,1650,1609,888 cm^{-1}. Literature[4,5] records m.p. 203-7°, $[\alpha]_D$ + 89° (dioxan). Anal. Calc'd for $C_{21}H_{30}O_5$ (362.5): C, 69.58; H, 8.34. Found: C, 69.36; H, 8.40.

21-Acetoxy-1β,17α-dihydroxypregn-4-ene-3,20-dione (II)

A solution of 300 mg. of 1β-hydroxy-11-deoxycortisol (I) in 15 ml. of acetone and 0.5 ml. of triethylamine was treated with 0.6 ml. of acetic anhydride under reflux for 8 hours. A part of the solvent mixture was evaporated, and the product was precipitated with water and worked up in the usual manner. Crystallization from methanol afforded 310 mg. of the 21-acetate II, m.p. 257-260°; $[\alpha]_D^{19}$ + 76° ±3° (acetone, c 0.35); λ_{max}^{EtOH} 241 mμ (log ε 4.18); ν 3470,3320, 1754,1722,1645,1607,1236,888 cm^{-1}. Anal. Calc'd for $C_{23}H_{32}O_6$ (404.5): C, 68.29; H, 7.97. Found: C, 68.34; H, 8.13.

1β,21-Diacetoxy-17α-hydroxypregn-4-ene-3,20-dione (III)

1β-Hydroxy-11-deoxycortisol (I) (206 mg.) was dried by distillation with a few ml. of benzene and acetylated with 0.5 ml. of acetic anhydride in 1 ml. of pyridine at room temperature overnight. Worked up as usual, the reaction mixture afforded 208 mg. of an oily residue which solidified under methanol upon standing in a refrigerator to give a single amorphous product (by thin-layer chromatography) m.p. 115-7°; $/\alpha/_D^{19}$ + 43.5° ±2° (chloroform, c 2.2); λ_{max}^{EtOH} 241 mμ (log ε 4.17); ν 3480,1740,1730,1725,1665, 1615 cm^{-1}. By repeated analyses, the substance was shown to be solvated with 0.5 mol of methanol. Anal. Calc'd for $C_{25}H_{34}O_7$. 0.5 MeOH (446.5 +16): C, 66.24; H, 7.87. Found: C, 66.20; H, 8.06.

21-Acetoxy-17α-hydroxypregna-1,4-diene-3,20-dione (IV)

A solution of 50 mg. of monoacetate II in 2 ml. of glacial acetic acid was heated at 80-85° for 6 hours. The reaction mixture was evaporated under diminished pressure to dryness, and the crystalline residue was crystallized from aqueous methanol to give 26 mg. of IV, m.p. 215-221°; $/\alpha/_D^{19}$ + 94° ±2° (chloroform, c 0.5); λ_{max}^{EtOH} 244 mμ (log ε 4.14); literature records[13] m.p. 224-6°; $/\alpha/_D$ + 91°; λ_{max}^{EtOH} 244 mμ (log ε 4.16). A second crop of 13 mg. of IV, m.p. 213-8°, was obtained from the mother liquor. Anal. Calc'd for $C_{23}H_{30}O_5$ (386.5): C, 71.48; H, 7.82. Found: C, 71.36; H, 7.94.

Oxidation of I with Sodium Bismuthate

A mixture of 200 mg. of I, 3.6 g. of sodium bismuthate, 30 ml. of acetic acid, and 30 ml. of water was shaken at room temperature for 3 days. The solids were filtered off and washed with acetic acid, and the filtrate was evaporated under diminished pressure. The oily residue (170 mg.) consisting (thin-layer chromatography) of several components, was chromatographed on a column of 17 g. of silica gel. By elution with a 2:1-mixture of benzene-ether, a fraction was obtained which afforded, upon preparative thin-layer chromatography over alumina, 26 mg. of an individual substance. This was identified (mixed melting point) as androsta-1,4-diene-3,17-dione, m.p. 136-9°; literature[14] records m.p. 140-1°. By elution with ether, 98 mg. of an oily product was obtained which could not be brought into a crystalline state, in spite of the fact that it showed a single spot on thin-layer chromatogram. Judging from the chromatographic mobility as well as from the ultraviolet spectrum (λ_{max}^{EtOH} 240 mμ, log ε 4.06), this fraction should be the corresponding 1β-hydroxyandrostene derivative.

21-Acetoxy-17α-hydroxypregn-4-ene-1,3,20-trione (V)

A solution of 1.0 g. of monoacetate II in 40 ml. of glacial acetic acid was treated dropwise with a solution of 0.4 g. of chromium trioxide in 0.2 ml. of water and 1 ml. of acetic acid. The reaction mixture was allowed to stand at room temperature for 48 hours, and methanol was added

to destroy an excess of oxidant. The reaction mixture was diluted with water, and the crystalline precipitate was taken up into ether and worked up in the usual manner. Upon crystallization from methanol, 770 mg. of 1-ketoderivative V was obtained as light-yellow needle-like crystals, m.p. 232.4°; $[\alpha]_D^{23}$ + 46° ±2° (chloroform, c 0.9), λ_{max}^{EtOH} 245 and 282 mμ (log ε 3.56 and 3.71, resp.), $\lambda_{max}^{MeOH-1N-NaOH(1:1)}$ 245 (ahoulder) and 347 mμ (log ε 3.85 and 3.85); ν 3510,1745, 1710,1593,1241 cm^{-1}. Anal. Calc'd for $C_{23}H_{30}O_6$ (402.5): C, 68.63; H, 7.51. Found: C, 68.43; H, 7.41.

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REFERENCES

1. Part XXXIV.: This Journal, in the press (September, 1964).
2. Present address: Chemopharma n.p., Ústí nad Labem.
3. Hanč,O., Čapek,A. and Tadra,M.: ČESKOSLOV.FARM. 11, 181 (1962).
4. Greenspan,G., Schaffner,C.P., Charney,W., Herzog,H.L. and Hershberg,E.B.: J.AM.CHEM.SOC. 79, 3922 (1957).
5. Nussbaum,A.L., Carlon,F.E., Gould,D., Oliveto,E.P., Hershberg,E.B., Gilmore,M.L. and Charney,W.: J.AM.CHEM. SOC. 81, 5230 (1959).
6. Schreiber,J. and Eschenmoser,A.: HELV.CHIM.ACTA 38, 1529 (1955).

7. Mathieu,J.P. and Petit,A.: CONSTANTES SÉLECTIONNÉES. POUVOIR ROTATOIRE NATUREL. I. STÉROÏDES. Masson and C^{ie}, Paris, 1956.

8. Bernstein,S., Heller,M. and Stolar,S.M.: J.AM.CHEM.SOC. 77, 5327 (1955).

9. Inhoffen,H.H., Blomeyer,F. and Brückner,K.: CHEM.BER. 87, 593 (1954).

10. Protiva,J.: private communication.

11. McAleer,W.J., Kozlowski,M.A., Stoudt,T.H. and Chemerda, J.M.: J.ORG.CHEM. 23, 508 (1958).

12. Feldman,L.I., Rigler,N.E. and Shay,A.J.: U.S.pat. 2,962,423, CHEM.ABSTR. 55, 8756 (1961).

13. Ringold,H.J., Rosenkranz,G. and Sondheimer,F.: J.AM. CHEM.SOC. 78, 820 (1956).

14. Djerassi,C. and Scholz,C.R.: J.ORG.CHEM. 13, 697 (1948).