C-1-DEHYDROGENATION OF STEROIDS BY SPORES OF *SEPTOMYXA AFFINIS*

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Vischer and Wettstein[1], in 1953 observed C-1-dehydrogenation of steroids by fusaria. Since then, the reaction has been reported with a large number of microorganisms[2-5]. With fusaria[1], *Streptomyces lavendulae*[2], *Cylindrocarpon radicicola*[2], *Septomyxa affinis*[6] and many other microorganisms investigated (using vegetative growth) C-1-dehydrogenation was often accompanied by the oxidative degradation of C-17 side chain, Transformation of steroids with microbial spores has been described previously[7,8]. In the present communication, we describe C-1-dehydrogenation of a few steroids of the pregnane series by spores of *Septomyxa affinis* ATCC 6737 and the influence of 17α-alkyl substituent in the steroid molecule on side chain degradation.

EXPERIMENTAL

Methods used for maintenance of the organism, spore production, steroid transformation and extraction were essentially the same as described previously[7]. Spores were suspended in a 1 % phosphate buffer (pH 7) to give 5 x 10^8 conidia per ml. To 50 ml of this suspension, in a 250 ml erlenmeyer flask, a 5 % steroid solution in dimethylformamide

was added to give 0.5 to 1.0 mg steroid per ml. Incubation was performed at 28 ° on a rotary shaker (240 rpm, 1" stroke) for 48 to 96 hours. The reaction mixture was extracted with ethylene dichloride and the solvent extract was evaporated to dryness. The products were tested by chromatography on thin layer silica gel plates (TLC), using isopropyl alcohol-benzene (1:6) or ethyl acetate-carbon tetrachloride (3:7) as solvents for development. The components of the steroidal mixture were isolated by elution from thick layer silica gel plates with methanol-chloroform (1:1) followed by crystallization from acetone-hexane or benzene-hexane mixtures. Known compounds were identified by comparison with the authentic standards. Other compounds were assigned the structure on the basis of the physical chemical data presented. The following tests were used for identification and characterization of the reaction products : R_f value and color with H_2SO_4 on TLC plates, Zimmermann test for 17-keto steroids, infrared spectra, ultraviolet spectra, melting point and in some cases rotation and elemental analysis. Most of the $\Delta^{1,4}$-3-keto steroids reported in this paper appeared as pink to red spots on TLC plates when sprayed with H_2SO_4 and heated.

Optical rotations were determined in 1 % $CHCl_3$ solution at room temperature and ultraviolet spectra were determined in ethanol. Infrared spectra were determined in 10 % $CHCl_3$ solution using a Model 21 Perkin-Elmer spectrophotometer and a 0.1 mm cell. Melting points were not corrected.

17α-Methyl-pregn-4-ene-21-ol-3,20-dione-21-acetate was prepared by Dr. D. Marshall of our laboratory according to Heusser et al[9]. The other 17-alkyl steroids were obtained from Dr. R. Deghenghi[10,11].

RESULTS

Spores of S. affinis dehydrogenated specifically in 1-position. However, with a number of steroids of the pregnane series this reaction was

accompanied by side chain degradation, with or without cleavage of ring D. The results presented below indicate that the 17α-alkyl substituted steroids, in contradistinction to 17α-hydroxy or 17-unsubstituted steroids, suffered no side chain degradation, but nevertheless were dehydrogenated in good yields. It seems, therefore, that an alkyl group in position 17 specifically blocks enzymatic reaction (s) responsible for side chain degradation by spore of S. affinis.

Transformation of pregn-4-ene-3,20-dione (I). Spores of S. affinis converted I to mainly androsta-1,4-diene-3,17-dione (II), and to small amounts of andro 1,4-diene-17β-ol-3-one (III) and 1-dehydrotestolactone (IV). Pregna-1,4-diene-3,20-dione (V) was detected in the reaction mixture during the earlier part of incubation (12 to 24 hours) but disappeared on prolonged incubation. The relative proportions of II, III and IV formed, varied with the period of incubation. In the earlier stages of incubation (24 to 48 hours) the predominant component was II with small amounts of III and traces of IV. On longer incubation (48 to 96 hours), larger amounts of IV accumulated. After a 72 hour incubation period employing 200 mg I, extraction with ethylene dichloride and chromatography on silica gel plates afforded 70 mg II, 32 mg III and 40 mg IV. Individual components were crystallized from acetone-hexane and were identified by comparison with the corresponding authentic standards.

Pregn-4-ene-17α-ol-3,20-dione (VI). Spores of S. affinis converted VI to II, III and IV. Employing the general procedure described above from 200 mg VI (incubation period 72 hours), 73 mg II, 24 mg III and 35 mg IV were isolated.

17α-Methyl-pregn-4-ene-3,20-dione (VII). Spores of S. affinis transformed VII to its 1-dehydro derivative (VIII). After a 72 hour incubation period employi 200 mg VII, extraction with ethylene dichloride, chromatography of the extrac on silica gel plates and crystallization from acetone-hexane yielded 110 mg

of crystalline product, m.p. 160°, $[\alpha]_D$ +37.8°, λ_{max} 245mμ (15,520), 17α-methyl-20-ketone absorption at 1691 cm^{-1}, and $\Delta^{1,4}$-3-ketone absorption at 1660, 1662 and 1605 cm^{-1}. Anal. calcd. for $C_{22}H_{30}O_2$: C, 80.93; H, 9.26. Found : C, 80.78; H, 9.32. On the basis of the above data VIII is assigned the structure : 17α-methyl-pregna-1,4-diene-3,20-dione.

21-Fluoro-pregn-4-ene-3,20-dione (IX). Spores of S. affinis converted IX to II, III and IV. Using the general procedure described above, transformation of 200 mg IX (incubation period 90 hours) afforded 80 mg II, 19 mg III and 64 mg IV.

17α-Methyl-21-fluoro-pregn-4-ene-3,20-dione (X). Spores of S. affinis dehydrogenated X to 17α-methyl-21-fluoro-pregna-1,4-diene-3,20-dione (XI). From 100 mg X (incubation period 96 hours) 60 mg XI isolated, was found to be identical with an authentic sample of the chemically prepared compound.

Pregn-4-ene-17α, 21-diol-3,20-dione (XII). Spores of S. affinis transformed XII to pregna-1,4-diene-17α,21-diol-3,20-dione (XIII), 70 to 80%; to II (10-15 %) and traces of III. C-1-Dehydrogenation of XII by spores of S. affinis will be described in greater detail in a subsequent communication.

Pregn-4-ene-21-ol-3,20-dione (XIV). Spores of S. affinis converted XIV to II, III and IV in approximate yields of 40, 20 and 20 % respectively (incubation period 72 hours).

Pregn-4-ene-17α-21-diol-3,11,20-trione (XV). The reaction of S. affinis spores with XV yielded three products. By the general procedure described above 200 mg XV was transformed. After an incubation period of 72 hours, the reaction mixture was extracted with ethylene dichloride. Separation of the individual steroidal components by chromatography on silica gel plates followed by crystallization from benzene-hexane yielded 66 mg XVI, 15 mg XVII and about 5 mg XVIII. The principal product (XVI) showed a positive

Zimmermann test, m.p. 186-190°, λ_{max} 239mμ (15,700). Infrared spectrum showed no hydroxyl absorption bands, but bands at 1710 cm^{-1} (6-membered ring ketone), 1740 cm^{-1} (5-membered ring ketone), 1665, 1625 and 1608 cm^{-1} ($\Delta^{1,4}$-3-ketone).Compound XVI was characterized as androsta-1,4-diene-3,11,17-trione[12] by comparison of its spectrum with that published by Jones et al.[13] (Spectrum No. 420). The second product (XVII), m.p. 208-210° with decomposition, has characteristic absorption bands at 3630 and 3420 cm^{-1} (OH group), 1708 cm^{-1} (6-membered ring ketone), and 1662, 1623, 1605 cm^{-1} ($\Delta^{1,4}$-3-keto). Acetylation of XVII with acetic anhydride and pyridine at room temperature afforded a product which does not show the hydroxyl absorption, but has the characteristic acetate bands (1725 and 1253 cm^{-1}), a band at 1710 cm^{-1} (6-membered ring ketone) and the $\Delta^{1,4}$-3-ketone absorption bands, indicating that the 17-side chain had been degraded. On the basis of the above information and by analogy to the reactions of S. affinis spores with steroids I, VI, IX, XII and XIV, compound XVII is characterized as androsta-1,4-diene-17β-ol-3,11-dione. The third product obtained by the action of S. affinis spores on XV was identified as pregna-1,4-diene-17α-21-diol-3,11,20-trione (XVIII) by comparison with an authentic standard.

Transformation of pregn-4-ene-11β,21-diol-3-one (XIX). Spores of S. affinis transformed XIX into two products. After an incubation period of 72 hours, employing 100 mg XIX, extraction with ethylene dichloride, chromatography on silica gel plates and crystallization from acetone-hexane afforded 48 mg XX and 11 mg XXI. The main product (XX), m.p. 185-186° (published : 181-182°)[12], λ_{max} 242mμ (19,500), showed a positive Zimmermann test. The infrared spectrum of XX shows a characteristic hydroxyl absorption bands at 3630 and 3470 cm^{-1}, a band at 1737 cm^{-1} (5-membered ring ketone) and a band system at 1660, 1621, 1605 cm^{-1} ($\Delta^{1,4}$-3-ketone) (see Jones et al.[13],

spectrum No. 528). Compound XX was identified as androsta-1,4-diene-11β-ol-3,17-dione.

The second product (XXI) showed a negative Zimmermann test and had a m.p. of 156°. The infrared spectrum (KBr) showed a strong absorption at 3400 cm^{-1} (OH) and 1658, 1615, 1601 cm^{-1} ($\Delta^{1,4}$-3-ketone). Acetylation with acetic anhydride and pyridine at room temperature yielded a product which shows characteristic acetate bands at 1725 and 1252 cm^{-1} and the $\Delta^{1,4}$-3-keto bands at 1659, 1620 and 1605 cm^{-1}. On the basis of the infrared spectra and by analogy to the reaction of S. affinis spores with some of the steroids described above, it is suggested that compound XXI is androsta-1,4-diene-11β,17β-diol-3-one.

17α-Ethyl-pregn-4-ene-3,20-dione (XXII). Spores of S. affinis transformed XXII into its 1-dehydro derivative (XXIII in about 50 % yield) which was more polar than XXII and showed as a red spot on TLC plates (when sprayed with H_2SO_4 and heated). Using the general procedure described above from 100 mg XXII (incubation period 72 hours), 25 mg of crystalline product (XXIII) was isolated, m.p. 150-151°, λ_{max} 245 mμ (18,500). Infrared spectrum shows absorption at 1692 cm^{-1} (17α-ethyl-20-ketone), 1660, 1622 and 1606 cm^{-1} ($\Delta^{1,4}$-3-ketone). On the basis of the above data, compound XXIII is indicated to be 17α-ethyl-pregna-1,4-diene-3,20-dione.

17α-Methyl-pregn-4-ene-21-ol-3,20-dione-21-acetate (XXIV). C-1-Dehydrogenation of XXIV by spores of S. affinis was accompanied by hydrolysis of the 21-acetate and the product formed (XXV) was assigned the structure : 17α-methyl-pregna-1,4-diene-21-ol-3,20-dione. The ethylene dichloride extracts from the transformation of 100 mg XXIV (incubation period 72 hours) were evaporated to dryness. Chromatography on silica gel plates followed by crystallization from acetone-hexane afforded 54 mg of the product which still

showed traces of impurities. A second chromatography on silicic acid plates and recrystallization from benzene-hexane yielded crystalline XXV, m.p. 154.5-156.5°, λ_{max} 245 mμ (17,000), no acetate absorption, but absorption at 1691 cm^{-1} (17α-methyl-20-ketone), 1660, 1620 and 1604 cm^{-1} ($\Delta^{1,4}$-3-ketone). Anal. calcd. for $C_{22}H_{30}O_3$: C, 77.15; H, 8.83. Found : C, 77.00; H, 9.09.

6α,17-Dimethyl-pregn-4-ene-3,20-dione (XXVI). Spores of S. affinis converted XXVI into its 1-dehydro derivative (XXVII) in yields of approximately 50 %. Using the general procedure described above, from 100 mg of XXVI (incubation period 72 hours) 27 mg of crystalline product XXVII was isolated, m.p. 118-119°, λ_{max} 245 mμ (16,650), absorption at 1690 cm^{-1} (17α-methyl-20-ketone), 1655, 1620 and 1605 cm^{-1} ($\Delta^{1,4}$-3-ketone), $[\alpha]_D$ +13.8°. Anal. calcd. for $C_{23}H_{32}O_2$: C, 81.13; H, 9.47. Found : C, 80.81; H, 9.26. Compound XXVII is assigned the structure : 6α,17-dimethyl-pregna-1,4-diene-3,20-dione.

6,17-Dimethyl-pregna-4,6-diene-3,20-dione (XXVIII). Spores of S. affinis transformed XXVIII into its 1-dehydro derivative (XXIX). After an incubation period of 72 hours, employing 100 mg XXVIII, extraction with ethylene dichloride and chromatography on silica gel plates yielded 79 mg of XIX. The product was crystallized from acetone-hexane and recrystallized from benzene-hexane, m.p. 121.0-121.5°, λ_{max} 228 mμ (14,150), 255 mμ (9,700) and 306 mμ (12,350). $[\alpha]_D$ +13.4; absorption at 1690 cm^{-1} (17α-methyl-20-ketone), 1650, 1608 and 1582 cm^{-1} ($\Delta^{1,4,6}$-triene-3-one). Anal. Calcd. for $C_{23}H_{30}O_2$:C, 81.06; H, 8.93. Found : C, 81.36; H, 8.88. The product XXIX is assigned the structure : 6,17-dimethyl-pregna-1,4,6-triene-3,20-dione.

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