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PRELIMINARY COMMUNICATION

Transformation of steroids by Pseudomonas

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DESPITE extensive studies on the transformations of steroids by a wide variety of micro-organisms, little has been described on the steroid transformations by *Pseudomonas* species. There are several interesting transformations of steroids by *Pseudomonas*, and in this communication the conversions of 4-pregnene-17α,21-diol-3,20-dione (I, Reichstein's Substance S) to 1,4-pregnadiene-17α,20β,21-triol-3-one (II) and 4-pregnene-11β,17α,21-triol-3,20-dione (III, hydrocortisone) by *Pseudomonas* sp. 109[*], to II and 1,4-pregnadiene-17α,21-diol-3,20-dione (IV) by *Pseudomonas* sp. 125[*], and to 4-pregnene-17α,20β,21-triol-3-one (V) by *Pseudomonas* sp. M8[*] are reported.

Pseudomonas sp. 109: This organism was isolated from soil and identified as closely akin to *Pseudomonas boreopolis*.[1] Incubation of I was carried out with a 24 hr culture of this organism at 30° for 17 hr in the synthetic medium which was employed in the study of pyoluteorin.[2] Extraction of the culture filtrate with ethyl acetate, followed by acetylation with acetic anhydride and pyridine afforded 20β,21-diacetoxy-1,4-pregnadiene-17α-ol-3-one (the 20,21-diacetate of II), m.p. 178–179°; $[\alpha]_D^{20}$ +100° (chloroform); M_D +430°; $\lambda_{max}^{ethanol}$ 243·5 mμ (15,900); ν_{max}^{Nujol} 3509 (OH), 1739 (acetate), 1664, 1626, 1608, 886 cm^{-1} ($\Delta^{1,4}$-diene-3-one) (Found: C, 69·52; H, 7·92. $C_{25}H_{34}O_6$ requires C, 69·74; H, 7·96%), which on treatment with potassium hydrogen carbonate was reverted to II, m.p. 194–195°; $[\alpha]_D^{20}$ +33° (chloroform); M_D +114°; $\lambda_{max}^{ethanol}$ 244·5 mμ (14,200); ν_{max}^{Nujol} 3333 (OH), 1667, 1613, 1600, 885 cm^{-1} ($\Delta^{1,4}$-diene-3-one) (Found: C, 72·53; H, 8·43. $C_{21}H_{30}O_4$ requires C, 72·80; H, 8·73%). Chromatography on Florisil of the mother liquors from the diacetate of II gave, besides an additional amount of the diacetate of II, 21-acetoxy-4-pregnene-11β,17α-diol-3,20-dione [the 21-acetate of hydrocortisone (III)], m.p. 214–216°; ν_{max}^{Nujol} 3390, 3279 (OH), 1739 (acetate), 1709 (carbonyl), 1626 cm^{-1} (Δ^4-ene-3-one) (Found: C, 67·91; H, 7·86. $C_{23}H_{32}O_6$ requires C, 68·29; H, 7·97%), which was identical in every respect with an authentic sample. The production of III by *Ps*. sp. 109 constitutes one of the rare examples of 11β-hydroxylation accomplished by micro-organisms other than fungi. The approximate ratio of the formation of II and III was 10 to 1.[†]

Structural assignment for II[‡][3] is based upon the following facts. The colour reaction and the infra-red spectrum clearly indicated the presence of a $\Delta^{1,4}$-diene-3-one system and the absence of any isolated carbonyl group. Oxidation of II by periodic acid yielded 1,4-androstadiene-3,17-dione,[4] m.p. 137°; $[\alpha]_D^{20}$ +112° (chloroform); ν_{max}^{Nujol} 1736 (carbonyl), 1656, 1618, 1600, 890 cm^{-1} ($\Delta^{1,4}$-diene-3-one) (Found: C, 79·94; H, 8·33. $C_{19}H_{24}O_2$ requires C, 80·24; H, 8·51%), which was identical with the compound derived from 1,4-pregnadiene-17α,21-diol-3,20-dione by chromic acid oxidation. Further, the preferential reduction[5] of the 20-carbonyl group in 21-acetoxy-1,4-pregnadiene-17α-ol-3,20-dione with sodium borohydride was effected, and the product obtained after

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[*] The numbers are of R. Takeda's collection.

[†] In each case a small amount of I was recovered.

[‡] Sutter *et al.*[3] reported the microbiological formation of this compound, but gave only the melting points and the infra-red spectral data of the free steroid and its diacetate.

[1] R. S. Breed, E. G. D. Murray and N. R. Smith, *Bergey's Manual of Determinative Bacteriology* (7th Ed.) pp. 101, 105, 113. Williams and Wilkins Co., Baltimore (1957).

[2] R. Takeda and I. Nakanishi, *J. Fermentation Technol.* In press.

[3] D. Sutter, W. Charney, P. L. O'Neill, F. Carvajal, H. L. Herzog and E. B. Hershberg, *J. Org. Chem.* **22**, 578 (1957).

[4] H. H. Inhoffen, G. Zühlsdorff and Huang-Minlon, *Chem. Ber.* **73**, 451 (1940); H. H. Inhoffen, *Angew. Chem.* **59**, 207 (1947); J. Fried, R. W. Thomas and A. Klingsberg, *J. Amer. Chem. Soc.* **75**, 6764 (1953).

[5] J. K. Norymberski and G. F. Woods, *J. Chem. Soc.* 3426 (1955).

acetylation was found identical in all respects with the diacetate of II. The 20β-hydroxy configuration in II was assigned on the basis of the well-known mode of the sodium borohydride reduction[5,6] and of the molecular rotation data.[7]

Pseudomonas sp. 125: This organism was isolated from soil and identified as closely akin to *Pseudomonas oleovorans.*[1] Incubation of I with this organism was performed under conditions similar to those used in the case of *Ps.* sp. 109. Acetylation and subsequent chromatography over Florisil gave 21-acetoxy-1,4-pregnadiene-17α-ol-3,20-dione (the 21-acetate of IV), m.p. 215–218°; $\nu_{\max}^{\text{Nujol}}$ 3333 (OH), 1739 (acetate), 1718 (carbonyl), 1658, 1613, 1600, 893 cm^{-1} ($\Delta^{1,4}$-diene-3-one) (Found: C, 71·26; H, 7·78. $C_{23}H_{30}O_5$ requires C, 71·48; H, 7·82%), and the diacetate of II, both of which were identified by rigorous comparison with respective authentic samples. The formation ratio of IV and II was roughly 2 to 1.†

Pseudomonas sp. M8: This organism was isolated from water and identified as closely akin to *Pseudomonas fluorescens.*[1] Compound I was subjected to the action of this organism under conditions similar to those used in the above cases.† Acetylation followed by chromatography on Florisil furnished 20β,21-diacetoxy-4-pregnene-17α-ol-3-one (the 20,21-diacetate of V),[8] m.p. 191–192°; $[\alpha]_D^{20}$ + 133° (dioxane); M_D + 575°; $\nu_{\max}^{\text{Nujol}}$ 3390 (OH), 1733 (acetate), 1650, 1610 cm^{-1} (Δ^4-ene-3-one) (Found: C, 69·60; H, 8·34. $C_{25}H_{36}O_6$ requires C, 69·42; H, 8·39%). The structure of the product was confirmed by direct comparison with the compound prepared by the sodium borohydride reduction of I. Hydrolysis of the diacetate of V with potassium hydrogen carbonate afforded V, m.p. 188–189°; $[\alpha]_D^{20}$ + 67° (dioxane); M_D + 233°; $\nu_{\max}^{\text{Nujol}}$ 3390 (OH), 1645, 1610 cm^{-1} (Δ^4-ene-3-one) (Found: C, 72·21; H, 9·18. $C_{21}H_{32}O_4$ requires C, 72·38; H, 9·26%).

Other species of *Pseudomonas* genus have also effected similar patterns of transformations. A detailed account of these findings will be published later.

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[6] E. P. Oliveto and E. B. Hershberg, *J. Amer. Chem. Soc.* **75**, 488 (1953).

[7] L. H. Sarett, *J. Amer. Chem. Soc.* **71**, 1175 (1949); W. Klyne and D. H. R. Barton, *Ibid.* **71**, 1500 (1949).

[8] W. Logemann, *Naturwissenschaften* **27**, 196 (1939); C. W. Shoppee, *Helv. Chim. Acta* **23**, 925 (1940).