MICROBIOLOGICAL EPOXIDATION OF STEROIDS

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Bloom and Shull (J. Am. Chem. Soc. **77**:5767, 1955) observed that microorganisms convert isolated double bonds into oxides rather than alcohols. Through a limited number of experiments, their data strongly suggest that this type of transformation is a predictable one. When 9(11)-dehydrocortexolone was incubated with an 11-β-hydroxylating organism, 9-β, 11-β-oxidocortexolone was formed (Shull and Kita, J. Am. Chem. Soc. **77**:763, 1955). Similarly, 14-dehydrocortexolone was converted by a 14-α-hydroxylating organism into 14-α, 15-α-oxidocortexolone (Bloom et al., Experientia **12**:27, 1956). From these results they postulated that "a microorganism capable of introducing an axial hydroxyl function at C_n of a saturated steroid also effected the introduction of an epoxide grouping axial at C_n in the corresponding unsaturated substrate." Thus, incubation of 9(11)-dehydrocortexolone with an equatorial 11-α-hydroxylating culture failed to produce the 9-α,11-α-epoxide. The purpose of this study was to test the validity of this hypothesis by the incubation of $\Delta^{9(11)}$ steroids with a 9-α-hydroxylating organism.

Nocardia sp. ATTCC 13934 is an organism capable of introducing a 9-α-hydroxyl (axial) group into C_{19} and C_{21} steroids (Dodson and Muir, J. Am. Chem. Soc. **83**:4631, 1961). This organism was grown in 250-ml Erlenmeyer flasks with 50 ml of Difco nutrient broth. After 24 hr of incubation at 25 C on a rotary shaker, 0.5 g of 9(11)-dehydroandrostenedione in 4 ml of dimethylformamide was distributed equally among 20 flasks to give a final concentration of 250 μg per ml. The fermentation was continued for 72 hr, and the culture broth was filtered and extracted three times with 100-ml portions of chloroform. The chloroform extract was dried with sodium sulfate and concentrated to dryness; the residue weighed 0.68 g. Three recrystallizations from acetone afforded 280 mg of a compound: mp, 273 to 275 C; λ_{max}^{alc} 236 mμ (ϵ 16,000); $[\alpha]_D^{25}$ = + 180° (c = 0.9 in chloroform); $\lambda_{max}^{CHCl_3}$ 5.76, 6.02, 6.18 μ. Analysis calculated for $C_{19}H_{24}O_3$ (300.38): C, 75.97; H, 8.05. Found: C, 76.03; H, 7.98; identical in all respects to an authentic sample of 9-α,11-α-oxidoandrostenedione obtained by chemical synthesis (Sih, J. Org. Chem. **26**:4716, 1961). Similar conversion of 9(11)-dehydrocortexolone gave 9-α,11-α-oxidocortexolone in 20% yield, mp, 213 to 215 C; $[\alpha]_D^{25}$ = + 85° (c = 0.9 dioxane); λ_{max}^{alc} 238 mμ (ϵ 16,600); λ_{max}^{Nujol} 2.98, 5.85, 6.06 μ. Analysis calculated for $C_{21}H_{28}O_5$ (360.44): C, 70.02; H, 7.77. Found: C, 70.27; H, 8.02.

The results presented herein provide additional evidence in favor of the hypothesis advanced by Bloom and Shull (J. Am. Chem. Soc. **77**:5767, 1955). It is quite likely that the same enzyme system is involved in hydroxylation and epoxidation.

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ACCUMULATION OF CYSTATHIONINE IN A HOMOCYSTEINE-REQUIRING MUTANT OF *AEROBACTER AEROGENES*

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The position of cystathionine as an intermediate in bacterial methionine biosynthesis has not been conclusively established. Roberts et al. (*Studies of biosynthesis in Escherichia coli*, Carnegie Institution of Washington, Publication no. 607, 1957), using the isotopic competition method, concluded that cystathionine did not appear to be on the pathway of methionine