(+)-(5*R*)-Methyl-4-oxo-octane-1,8-dioic Acid, Microbiological Degradation Product from Rings c and d of Cholic Acid

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Recently, several reviews and papers on the microbiological degradation of the steroid skeleton into the perhydroindane nucleus have been published (Talalay, 1965; Schubert, 1967; Hayakawa, Kanematsu & Fujiwara, 1967; Schubert, Böhme, Ritter & Hörhold, 1968; Sih & Whitlock, 1968). On the further metabolism of the resulting perhydroindane nucleus, Lee & Sih (1967) and Schubert *et al.* (1968) have proposed some possible metabolic pathways. However, no key degradative intermediates that give an insight into the mechanism of ring-opening have been isolated thus far.

The present communication deals with the isolation and identification of (+)-(5*R*)-methyl-4-oxo-octane-1,8-dioic acid (IV) as a microbiological degradation product of 3 α -hexahydro-7 α β -methyl-1,5-dioxind-4 α -ylpropionic acid (II).

Results and discussion. Although 2,3,4,6,6 α β ,7,8-,9,9 α ,9 β β -decahydro-6 α β -methyl-1*H*-cyclopenta-[*f*]quinoline-3,7-dione (III), which was obtained from cholic acid (I) with *Streptomyces rubescens* (Hayakawa, Hashimoto, Onaka & Fujiwara, 1967), was the most degraded product among the cholic acid metabolites that have been isolated so far, product (III) was not further metabolized at a significant rate by this organism. However, compound (II), which might be a precursor of product (III) in the metabolic sequence from cholic acid, was utilized as the sole source of carbon and converted into several metabolites by this organism. One of these was shown to have the formula C₉H₁₄O₅ by elementary analysis and molecular-weight determination by mass spectrometry (*m/e* 202). The i.r. spectrum showed the presence of a carboxyl group (approx. 2500–3000 and 1706 cm.⁻¹). The n.m.r. spectrum exhibited bands at 7.02 (2H, two carboxyl groups) and 1.13 (3H, doublet, one secondary methyl group) p.p.m. The function of a remaining oxygen atom in the formula was assigned as a carbonyl group from the positive 2,4-dinitrophenylhydrazine test. These data are consistent with the structure 5-methyl-4-oxo-octane-1,8-dioic acid. This structure was confirmed by synthesis. Since cyclic β -diketones, which are completely substituted at the common α -position, are extremely prone to ring-cleavage by

alkali (Wendler, Slates & Tishler, 1951), 1-methyl-2,5-dioxocyclopentane-1-propionic acid was treated with alkali and ($\pm$)-5-methyl-4-oxo-octane-1,8-dioic acid was obtained as expected. Both i.r. spectra in chloroform solution and mass spectra of this racemic acid and the optically active C₉ degradation product were identical. Further, the stereochemistry of the product was assigned as (+)-(5*R*)-methyl-4-oxo-octane-1,8-dioic acid (IV) from its optical rotatory dispersion (o.r.d.) curve, which exhibits a negative Cotton effect (Djerassi & Geller, 1959).

Experimental. Bacteriological procedures were carried out essentially as reported by Hayakawa, Saburi & Tamaki (1958) with the use of the acid (II) instead of cholic acid as the sole carbon source. The incubation was continued for 90 hr. and the culture broth was filtered to remove mycelium. The filtrate was concentrated to one-tenth of its original volume, acidified and extracted with ethyl acetate. In a typical run 30 g. of the acid (II) was used and 5.62 g. of the ethyl acetate extracts was obtained. The extracts were chromatographed on 224 g. of silicic acid (Mallinckrodt 2847) that had been deactivated with water (15%, w/w). The eluate (339 mg.) with 20% (v/v) acetone in dichloromethane was rechromatographed on silicic acid (see above). The eluate (96 mg.) with 14% (v/v) acetone in dichloromethane was subjected to preparative thin-layer chromatography with four plates (20 cm. $\times$ 20 cm., 0.5 mm. thick) of silica gel HF₂₅₄ (E. Merck A.-G., Darmstadt, Germany) and ethyl acetate–iso-octane–acetic acid (15:5:1, by vol.). An upper area (*R_F* 0.38, 1 cm. wide) of the narrow band, which consists of an unknown metabolite as detected by u.v. light (253.7 nm.), was scraped from the plates and extracted with chloroform–methanol–0.01*N*-HCl (10:2:0.1, by vol.). The extracts were recrystallized from ether–light petroleum to yield 12 mg. of (+)-(5*R*)-methyl-4-oxo-octane-1,8-dioic acid (IV), m.p. 66–69.5° (Found: C, 53.4; H, 7.1. C₉H₁₄O₅ requires C, 53.5; H, 6.98%), [α]_D²⁰ +13.5 $\pm$ 1.3° (c 0.430 in chloroform), mass spectrum (Hitachi RMU-6 single-focus spectrometer at 70 ev with the source at 254°) *m/e* 55, 101 (base peak), 184 and 202 (molecular ion), i.r. maxima (in chloroform) approx.

2500–3000 (CO_2H) and 1706 ($\text{C}=\text{O}$ and CO_2H) cm^{-1} , n.m.r. absorptions (Varian A-60 spectrometer with tetramethylsilane as internal standard in CDCl_3) at 1.13 (doublet; J 6.8 cyc./sec.; CH_3 at C-5) and 7.02 (broad singlet; CO_2H at C-2 and C-7) p.p.m., o.r.d. (Nihonbunko ORD/UV-5 spectrometer, c 0.355 in chloroform) $[\phi]_{400} + 57$, $[\phi]_{303} - 365$ and $[\phi]_{260} + 1106$.

A synthesis of 1-methyl-2,5-dioxocyclopentane-1-propionic acid from 2-methylcyclopentane-1,3-dione was carried out as reported by Brown, Lustgarten, Stanaback & Meltzer (1966). This acid (5 g.) was dissolved in aq. 2% (w/w) NaOH solution (200 ml.) and the solution was kept at room temperature for 2 hr. The reaction mixture was acidified, salted with NaCl and extracted with ether. The ether extracts (4.424 g.), on recrystallization from ether, gave 1.409 g. of crystals, m.p. 45–50°. A further 991 mg. of crystals, m.p. 53–56°, was recovered from the mother liquor through silicic acid chromatography. The crystalline fractions were further recrystallized from ether-light petroleum to afford ($\pm$)-5-methyl-4-oxo-octane-1,8-dioic acid, m.p. 55.5–57° (Found: C, 53.5; H, 6.97. $\text{C}_9\text{H}_{14}\text{O}_5$ requires C, 53.5; H, 6.98%).

Identity with the C_9 acid obtained from growing cultures was determined by i.r. mass and n.m.r. comparisons.

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