141. The Synthesis of Stero-bile Acids. XXVII. The Metabolism of Dehydrocholic Acid by *Penicillium islandicum*. 1

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In the previous reports, metabolism of bile acid derivatives by *Aspergillus oryzae*^{1,2)} and *Neurospora crasser*³⁾ has been verified. The present paper deals with the metabolism of dehydrocholic acid by *Penicillium islandicum*.

Experimental

Cultivation of *P. islandicum*: In the Czapeck's culture medium modified by Fujiwara¹⁾ *P. islandicum* spores were planted. Changes of developmental behavior and pH of culture medium were shown in Fig. 1. Culture medium turned to turbid at about 2 days after plantation and then produced slight yellow-white color barm and pH lowered to 7.4 and then 6.0 at 4th day. At 9th day, produced color barm was more increased, but pH rose to 7.6 and 8.2 at 13th day. After plantation of *P. islandicum*, a week was developmental stadium, next 4-5 days were abortive stadium and then self-digestive stadium continued. Even in developmental stadium, pH of this medium decreased. It was known that decrease of pH was not suitable for experiment of metabolism of bile acid, because this medium was lowered to pH 5.4 in developmental stadium of *P. islandicum*. As *P. islandicum* produced H₂S and nitrous acid by reduction of nitric acid, component of the above culture medium was modified to 3.0 g of Na₂HPO₄, 2.0 g of NaNO₃, 0.5 g of KCl, 0.05 g of MgSO₄·7H₂O, 0.01 g of FeSO₄, 3.0 g of glucose, and 1000 ml of water at pH 7.8. When in this culture medium *P. islandicum* was cultured, favourable growth, slight lowering of pH in developmental stadium (from 7.4 to 8.0), and alkaline definite level of pH of the medium in abortive stadium were proved (Fig. 2). Owing to the determination of concentration of bile acid in medium, *P. islandicum* was cultured in this medium containing dehydrocholic acid in the ratio of 0.1, 0.5, 1.0, and 2.0 per cent, and so it was made up clear that 1 per cent bile acid culture medium was able to use to metabolic experiment.

Metabolism of dehydrocholic acid: Four liters of the above modified Czapeck's medium were poured into an aseptic bottle with a draught tube, and one unit of *P. islandicum* spore and 40 g of Na-dehydrocholate were added and cultured with draughting for 2 months

Fig. 1

Fig. 2

at 30°. *P. islandicum* spores and 12 g of glucose were added with every 5–8 days in the medium. After culture, culture medium adding sodium carbonate was boiled on a water bath and filtered. The filtrate was concentrated to about 700 ml on a water bath and under acidification with HCl, produced precipitate was filtered. Precipitate was dissolved in ethyl acetate, washed with water, dried with sodium sulfate, and concentrated to dryness. Resulting residue, from which fat removed with petroleum ether, was recrystallized from ethyl acetate and divided into crystalline substance (a) and filtrate (b).

Recrystallization of the crystalline (a) from acetone gave crystals with m.p. 260°, which was recrystallized five times from methanol water to yield 200 mg of prisms with m.p. 268°. Melting point was lowered when this crystal was mixed with β -reductodehydrocholic acid, m.p. 268°.

The filtrate (b) was concentrated to dryness on a water bath and obtained residue was dissolved in 150 ml of 8 per cent methanolic H_2SO_4 , heated on a water bath for 3 hours, poured into 1500 ml of water and produced precipitate was extracted with ether. From ether extract inreactionable acid was removed by washing with 5 per cent sodium carbonate and ether soluble fraction was dried with sodium sulfate and concentrated to dryness. The residue was dissolved in ethyl acetate and chromatographed on an alumina eluted with ethyl acetate and then methanol.

Ethyl acetate elute was divided into 19 fractions: Crystals with m.p. 168° were obtained from the first elute and from the second elute crystals with m.p. 180° were obtained. Recrystallization of both crystals from methanol gave prisms with m.p. 182°. As crystal was not yielded from 3, 4, 5, 6th fractions, these fractions were dissolved in ether again and chromatographed on an alumina eluted with ether (9 fractions) and ethyl acetate (13 fractions). From the 7–19 fractions crystal was not yielded by any procedures and only glutinous substance was yielded. Glutinous substances of the 3, 4, 5, 6th fractions were again dissolved in ether and divided into ether fractions and ethyl acetate fractions by chromatography. From this ether elute yielded crystal indicated yellow-green color fluorescence in methanol solution. Repeated recrystallization from methanol gave 1.2 g of non-color

prisms with m.p. 182° . This methyl ester was identical with crystal which was obtained from ethyl acetate elute previously. The characterization of the crystal as methyl 3·12-diketo- $\Delta^{4,6}$ -bisorcholadienate was confirmed by mixed melting point test with Hayakawa's sample⁵⁾ and infrared absorption data (yield 1.2 g).

Analysis Calcd. for $C_{23}H_{30}O_4$	C 74.59	H 8.11
Found	C 74.24	H 8.15

Infrared absorption of methyl 3·12-diketo- $\Delta^{4,6}$ -bisorcholadienate

When 450 mg of above crystals with m.p. 182° was dissolved in 50 ml of 2 per cent methanolic sodium hydroxide, boiled on a water bath for 2 hours, color of this hydrolyzate turned to black brown. After evaporation of methanol, the hydrolyzate was acidified with dilute HCl. Recrystallization of produced black brown precipitate from acetone water gave crystals of 3·12-diketo- $\Delta^{4,6}$ -bisorcholadienic acid with m.p. 250° . From methanol elute of the first chromatography in the filtrate (b), 110 mg of crystals with m.p. 209° was obtained.

Summary and Conclusion

As degradation products of dehydrocholic acid by *P. islandicum*, 3·12-diketo- $\Delta^{4,6}$ -bisorcholadienic acid, the acid with m.p. 268° and methyl ester with m.p. 209° have been isolated. It is well known that ketone groups at C_3 , C_7 , and C_{12} of bile acid nucleus are reduced to the secondary alcohol by microbiological reduction,⁴⁾ and 7- α -hydroxy-3-keto- Δ^4 -ene groups with steroid nucleus are easily dehydrated to derive $\Delta^{4,6}$ -3-ketone structure by mineral acid.^{5,6)} So in this study, the pathway of β -oxidation and reduction of dehydrocholic acid to 3·12-diketo- $\Delta^{4,6}$ -bisorcholadienic acid by *P. islandicum* will be deduced as follows.

References

- 1) K. Fujiwara: J. Jap. Biochem. Soc., **26**, 532 (1954).
- 2) K. Fujiwara: J. Jap. Biochem. Soc., **28**, 111 (1956).
- 3) H. Matsubara: Proc. Japan Acad., **32**, 516 (1956).
- 4) M. Machida: J. Biochem., **40**, 435 (1953).
- 5) S. Hayakawa: Proc. Japan Acad., **30**, 128, 133, 139 (1954).
- 6) C. W. Greenhalgh, H. B. Henbest, and E. R. H. Jones: J. Chem. Soc., **1952**, 2375.