119. *The Synthesis of Stero-bile Acids.*¹⁾ XXV. *The Metabolism of Dehydrocholic Acid by Neurospora Crasser*

By Hiroomi MATSUBARA

Department of Biological Chemistry, Hiroshima University
School of Medicine, Hiroshima, Japan

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Okada²⁾ reported that two-third of oral administered bile acid was degraded in the enterohepatic circulation. But the metabolic substance of bile acid has never been found in urine and blood, and metabolic pathway of bile acid in the liver or the intestine has not been clear. Because very little is known concerning the mechanism which controls the production and destruction of bile acid in the body, and there is a fact which is due largely to the lack of a satisfactory method for their quantitative estimation in the blood serum and in the urine.

In the previous reports, metabolism of cholic acid and dehydrocholic acid by *Aspergillus oryzae*,³⁾ and of brom-dehydrocholic acid¹⁾ in animal body has been verified. The present paper deals with the metabolism of dehydrocholic acid by *Neurospora crasser*.

Experiment

In ten liters of Czapek's culture medium regulated at pH 7.2 with sodium phosphate, 30 g of sodium dehydrocholate was dissolved in the flask with the air ventilator and sterilized three times by heating at 100° for half an hour in a sterilizer. *Neurospora crasser* was cultured in the medium for 2 months in an incubator at 28°. Aseptic air was let always in the culture medium from the bottom of the flask. When the pH value decreased, the medium was regulated with normal sodium hydroxide solution to pH 7.2, and spores and 10 g of glucose were added every five days. When culture was over, culture medium was alkalized with sodium carbonate solution, and the filtrate, after removal of microorganism by filtration, was concentrated until about 1000 ml of total amount on a water bath, and extracted with ether repeatedly under acidification with diluted HCl, and divided into ether soluble fraction and ether insoluble liquid.

A) Ether soluble fraction: The ether solution was washed with water, dried, and distilled off. The residue, from which fat was removed with petroleum ether, was extracted with ether again and divided into ether soluble fraction and ether insoluble fraction.

a) Ether soluble fraction: The ether was distilled off and obtained

residue was esterized with methanol in usual way. This ester was chromatographed on alumina, eluted with ether, ethyl acetate, and methanol. Recrystallization of ether elute from methanol-water gave 150 mg of needles with m.p. 241°. Melting point was not lowered when mixed with pure methyl dehydrocholate. The filtrate of the above crystal was hydrolyzed with 2 per cent methanolic sodium hydroxide solution and the obtained acid was dissolved in ethyl acetate to yield crystal. Recrystallization of produced crystal from methanol-water gave 100 mg of needles with m.p. 265°. Melting point was not lowered when mixed with β -reductodehydrocholic acid. Recrystallization of ethyl acetate elute from various solvents did not give any crystal. Concentration of methanol from methanol elute gave a few prisms which were recrystallized from methanol to yield crystals with m.p. 154°. Melting point was not lowered when mixed with pure methyl cholate.

Analysis Calcd. for $C_{25}H_{42}O_5$, C 71.09 H 9.95

Found C 69.97 H 10.12

b) Ether insoluble fraction: This residue was recrystallized from methanol to yield crystals with m.p. 250–260°, which were esterized with diazomethane. Methyl ester was chromatographed on alumina eluted with ethyl acetate to yield needles with m.p. 157°. This crystal was hydrolyzed with 2 per cent methanolic sodium hydroxide solution on a water bath for 50 minutes. Recrystallization of obtained acid from methanol-water gave one gram of needles with m.p. 266°. When mixed with β -reductodehydrocholic acid, melting point was not lowered.

Analysis Calcd. for $C_{24}H_{36}O_5$, C 71.29 H 8.91

Found C 71.10 H 8.73

B) Ether insoluble mother liquid: Ether insoluble liquid was salted out with sodium chloride and produced black brown glutinous precipitate was extracted with ethyl acetate. The extract was washed with water, dried, and ethyl acetate was distilled off. The residue was esterized with diazomethane and chromatographed on alumina, eluted with ether, ethyl acetate, and methanol. The acid obtained by hydrolyzation of ether elute was recrystallized from methanol-water to yield a few needles with m.p. 266°. When mixed with β -reductodehydrocholic acid, melting point was not lowered. Ethyl acetate elute was hydrolyzed by the same way, and the obtained acid was recrystallized from methanol-water to yield 50 mg of prisms with m.p. 219°. Melting point was not lowered, when mixed with pure $3\alpha\cdot7\alpha$ -dihydroxy-12-ketocholanic acid.

Analysis Calcd. for $C_{24}H_{38}O_5$, C 70.94 H 9.36

Found C 70.70 H 9.22

This acid was esterized with diazomethane and was recrystallized from methanol-water to yield plates with m.p. 159°.

Analysis Calcd. for $C_{25}H_{40}O_5$, C 71.43 H 9.52

Found C 71.29 H 9.42

Methanol elute was hydrolyzed to yield crystalline acid. Repeatedly recrystallization from alcohol gave 30 mg of crystals with m.p. 197°. Melting point was not lowered when mixed with pure cholic acid.

Summary and Conclusion

Dehydrocholic acid is reduced by *Neurospora crasser* to β -reductodehydrocholic acid and $3\alpha\cdot7\alpha$ -dihydroxy-12-ketocholanic acid, and still more to cholic acid by reduction of 12-ketone group. As reductodehydrocholic acid was not isolated from culture medium, when 3-ketone group is reduced to β -form, ketone groups at 7th and 12th positions may not be reduced by *Neurospora crasser*. If ketone at 3rd position is reduced to α -form, ketones at 7th and 12th positions are reduced to cholic acid easily. By Fujiwara's report,^{4,5)} ketone groups at 7th and 12th positions were not reduced by *Aspergillus oryzae*, so it is thought that *Neurospora crasser* may have stronger reductase than *Aspergillus oryzae*. It is interest that ketones at 7th and 12th of cholanic acid nucleus are reduced by *Neurospora crasser*, when this result is compared with the Okunobo's experiment¹⁾ that if ketone at 3rd position of dehydrocholic acid is reduced to α -configuration, ketones at the 7th and 12th positions are also reduced in the liver of a dog. In a body, metabolism of β -reductodehydrocholic acid takes a different metabolic pathway from that of α -reductodehydrocholic acid in the liver.

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