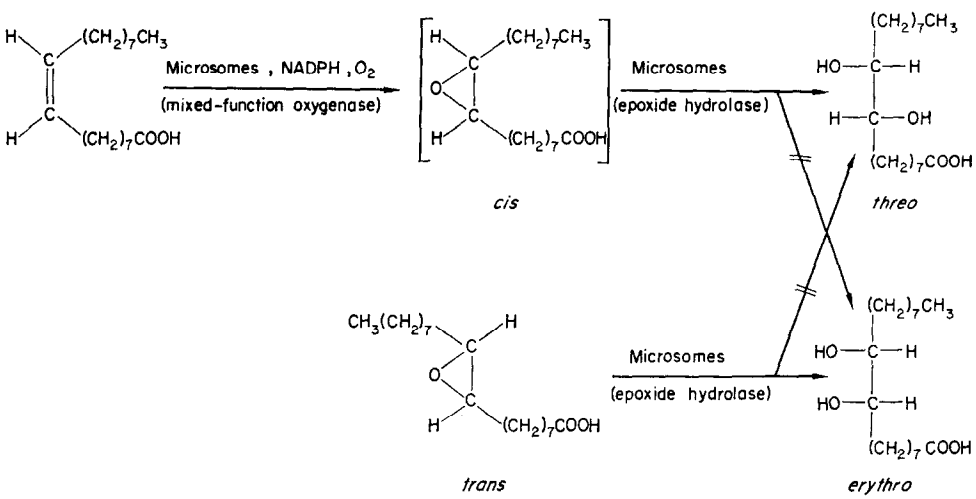
Biochemical Pharmacology Vol. 20 pp. 912–913. Pergamon Press 1971. Printed in Great Britain

Conversion of oleic acid into *threo*-dihydroxystearic acid by rat liver microsomes

(*Received* 29 *June* 1970; *accepted* 19 *August* 1970)

THE CONVERSION of foreign olefins[1] and aromatic compounds[2,3] into vicinal glycols by liver microsomes in the presence of NADPH and molecular oxygen has recently been demonstrated to proceed through a two-step enzymatic process which is the epoxidation of the double bond by an NADPH-dependent oxygenase, followed by the hydrolysis of the resulting epoxides by a hydrolase to the corresponding glycols. The hydrolase, named epoxide hydrolase,[4] has very recently been obtained in soluble and partially purified form from rabbit liver microsomes and demonstrated to be separable from the epoxidizing enzyme.[5] However, apart from the case of the oxidation of estratetraenol by rat liver slices,[6] little is known of the biological role of this microsomal epoxidation–hydrolysis pathway. It might be reasonable to predict that the pathway would also involve the metabolism of biologically important olefins which occur in animal body. The present communication is concerned with the conversion of oleic acid, the major component fatty acid in mammals, into *threo*-9,10-dihydroxystearic acid and with the stereoselective hydrolysis of *cis*-9,10-epoxystearic acid, which is reasonably regarded as a possible intermediate in the enzymatic conversion, and its *trans*-isomer to *threo*- and *erythro*-9,10-dihydroxystearic acids, respectively, by rat liver microsomes.



A mixture of washed microsomes, obtained from 2 g of rat liver by the previous method,[7] 20 μmoles of oleic acid dissolved in 0·2 ml of ethanol, 10 μmoles of NADP, 50 μmoles of glucose-6-phosphate, 10 I.U. of glucose-6-phosphate dehydrogenase, 50 μmoles each of $MgCl_2$ and nicotinamide, and 0·1 M phosphate buffer, pH 7·4, to make a final volume of 10 ml, was incubated aerobically at 37° for 30 min and extracted with peroxide-free ether.

The extract was treated with an ethereal solution of diazomethane and analyzed by TLC using boric acid-impregnated silica gel plates and hexane–ether (3:2). Cochromatography of the diazomethane-treated and untreated extracts with authentic methyl oleate, *threo*- and *erythro*-9,10-dihydroxystearates[8] (R_fs 0·40 and 0·27, respectively) and *cis*- and *trans*-9,10-epoxystearates[9,10] (R_fs 0·78 and 0·71, respectively) showed that the extract contained the *threo*-dihydroxy acid but neither the *erythro*

isomer nor the epoxy acids. Further identification of the *threo* acid formed was carried out by dissolution of the diazomethane-treated extract in acetone containing a catalytic amount of perchloric acid followed by cochromatography with an acetonide of the *threo* acid methyl ester: as a result of this treatment the spot assigned as the *threo* acid methyl ester disappeared, and a new spot appeared at the area corresponding to the authentic acetonide (R_f 0·85). Identity of the methyl ester of the *threo* acid, isolated from the diazomethane-treated extract by preparative TLC, with that of the authentic one was also confirmed by mass spectroscopy: *m/e* 330 (M^+), 187 ($M^+ - CH(OH)\text{-}(CH_2)_7\text{-}CH_3$), 155 (187 − CH_3OH). VPC analysis of the diazomethane-treated extract on an SE-30 column at the column temperature of 190° indicated the rate of conversion of oleic acid into the *threo* acid (at the retention time of 5·4 min as methyl ester) to be 4–5%.

Oleic acid did not yield the *threo* acid when the mixture was incubated anaerobically, when boiled microsomes were used, or when they were omitted or replaced in the NADPH-generating system by either NADH or NADP alone.

In order to know whether the *cis*-epoxy acid, a possible intermediate to the *threo* acid, is hydrolyzed by the microsomes or not, 30 μmoles of the *cis*-epoxy acid dissolved in 0·2 ml of ethanol were incubated for 30 min with microsomes, from 2 g of liver, suspended in 0·1 M phosphate buffer, pH 7·4, to make a final volume of 10 ml, and the mixture was extracted with ether. Treatment of the extract with diazomethane and subsequent analysis by TLC showed that the *cis*-epoxy acid was hydrolyzed stereoselectively to the *threo* acid without formation of any detectable amount of the *erythro* isomer. The extent of conversion of the *cis*-epoxy acid to the *threo* acid was 35 per cent by VPC.

The *trans*-epoxy acid, examined qualitatively in the same manner, yielded the *erythro* acid but not the *threo* isomer.

Both epoxy acids were stable in water at pH 3·0–9·0 and were not hydrolyzed to any extent to the corresponding dihydroxy acids when incubated with boiled microsomes for 3 hr.

The above results appear strongly to suggest that the mode of the enzymatic hydrolysis of the epoxy acids is similar to that of the acid hydrolysis,[11] which involves protonation to the oxygen atom of the oxirane ring and a concerted back-side attack of hydroxyl anion to its carbon atom, and also, in view of stereochemistry of the reaction products, that the *cis*-epoxy acid might be an obligatory intermediate in the conversion of oleic acid into the *threo* acid. This indirect evidence for the epoxide intermediate is in stereochemically good accordance with the previous demonstrations concerning the metabolism of chlorinated cyclodiene type insecticides,[12] cyclohexene,[13] aromatic compounds,[2,3,14] and estratetraenol[6] to the corresponding *trans*-glycols.

Biochemical Laboratory,
Division of Organic Chemistry,
Tokyo College of Pharmacy,
Ueno-sakuragi, Taito-ku, Tokyo 110, *Japan*

TADASHI WATABE
YUKO UENO
JUNKO IMAZUMI

REFERENCES

1. T. WATABE and E. W. MAYNERT, *Pharmacologist* **10**, 203 (1968).
2. J. HOLTZMAN, J. R. GILLETTE and G. W. MILNE, *J. Am. chem. Soc.* **89**, 6341 (1967).
3. D. M. JERINA, J. W. DALY, B. WITKOP, P. ZALTZMAN-NIRENBERG and S. UDENFRIEND, *J. Am. chem. Soc.* **90**, 6525 (1968).
4. T. WATABE and E. W. MAYNERT, *Fedn Proc.* **27**, 302 (1968).
5. T. WATABE and S. KANEHIRA, *Chem. Pharm. Bull.* (*Tokyo*) **18**, 1295 (1970).
6. H. BREUER and R. KNUPPEN, *Biochim. biophys. Acta* **49**, 620 (1961).
7. T. WATABE, H. YOSHIMURA and H. TSUKAMOTO, *Chem. Pharm. Bull.* (*Tokyo*) **12**, 1151 (1964).
8. T. P. HILDITCH, *J. chem. Soc.* **1926**, 1828.
9. D. SWERN, T. W. FINDLEY and J. T. SCANLAN, *J. Am. chem. Soc.* **66**, 1925 (1944).
10. K. H. BAUER and O. BÄHR, *J. prakt. Chem.* **122**, 201 (1929).
11. D. SWERN, *J. Am. chem. Soc.* **70**, 1235 (1948).
12. K. C. LEIBMAN and E. ORTIZ, *Pharmacologist* **10**, 203 (1968).
13. G. T. BROOKS, A. HARRISON and S. E. LEWIS, *Biochem. Pharmac.* **19**, 255 (1970).
14. D. M. JERINA, H. ZIFFER and J. W. DALY, *J. Am. chem. Soc.* **92**, 1095 (1970).