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Intermediates of the oxidative breakdown of ricinoleic acid by Candida genus

In our previous paper[1] it was reported that a certain soil bacterium and some strains of ***Escherichia coli*** converted ricinoleic acid to three specific metabolites, which were chemically characterized as 10-hydroxyhexadec-*cis*-7-enoic (I), 8-hydroxytetradec-*cis*-5-enoic (II), and 6-hydroxydodec-*cis*-3-enoic (III) acids. The latter two acids were excreted into the culture medium and accumulated as final products. These hydroxy acids were also isolated from adipose tissue of rats after feeding of ricinoleic acid[2].

In the present communication evidence was found that Candida metabolizes ricinoleic acid in a special manner. It is converted to intermediate hydroxy acids, which finally disappear from the culture fluid. Under appropriate culture conditions these intermediates could be isolated from the medium.

All strains of Candida examined, such as *Candida albicans*, *C. parakrusei*, *C. guilliermondi*, *C. stellatoidea*, *C. tropicalis*, *C. pseudotropicalis*, and *C. krusei* which were grown in the medium containing 2 % meat extract and 0.3 % ricinoleic acid, were able to oxidize ricinoleic acid. The metabolites did not accumulated as the final products, but the amount of each intermediate varied with the duration of cultivation as illustrated in Fig. 1.

Fig. 2 shows a gas–liquid chromatogram of hydroxy acids which were extracted

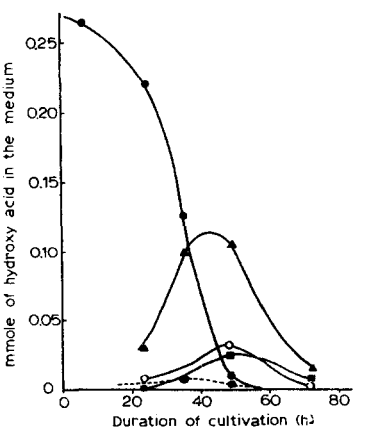

Fig. 1. Amounts of hydroxy acids isolated from the culture medium of *Candida guilliermondi*. ●—●, ricinoleic acid; ▲—▲, decanoic acid-γ-lactone; O—O, 6-hydroxydodecenoic acid; ■—■, 2-hydroxyoctanoic acid; ●---●, 8-hydroxytetradecenoic acid.

with ether repeatedly from the medium of *C. guilliermondi* after 48 h culture. The acids were separated by silicic acid column chromatography followed by methylation as described previously[1]. Components IV and V, which were not produced by *E. coli*, were separated by thin-layer chromatography of silica gel using the solvent system of benzene–methanol–acetic acid (45:8:4)[3] and 2′,7′-dichlorofluorescein as indicator. Each component was scraped off the plate and extracted with acetone. From five plates 25 mg of Component IV and 17 mg of Component V were recovered. Each of them showed a single peak when examined by gas–liquid chromatography. From their infrared spectra and elementary analyses, it appears that Components IV and V are decanoic acid γ-lactone and 2-hydroxyoctanoic acid, respectively. The former can be easily obtained from 4-hydroxydecanoic acid under acidic condition.

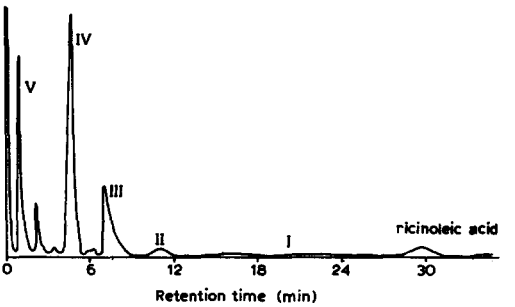

Fig. 2. Gas–liquid chromatogram of metabolites of ricinoleic acid by *Candida guilliermondi*. Fatty acids were extracted with ether from the culture fluid after 48 h cultivation at 27°. Hydroxy acids were separated by column chromatography on silicic acid, employing 30% ether in hexane for elution after removal of contaminants with 10% ether in hexane. Gas–liquid chromatography: 4 mm × 3 m column of 20% diethyleneglycol succinate polyester on 100 mesh Celite-545; operated at 198°; flow rate, 60 ml of He per min. Peak I, 10-hydroxyhexadec-7-enoic acid; Peak II, 8-hydroxytetradec-5-enoic acid; Peak III, 6-hydroxydodec-3-enoic acid; Peak IV, decanoic acid-γ-lactone; Peak V, 2-hydroxyoctanoic acid.

The intermediates thus obtained are shown in Fig. 2. It is generally considered that fatty acids are metabolized by β-oxidation. However, this does not explain the fact[1] that Component II cannot be oxidized to Component III by *E. coli*, although an appreciable amount of III was isolated. Furthermore, on the way of oxidative breakdown of naturally occurring fatty acids, it has been believed that the metabolic intermediates could never be present in detectable concentration since the active forms of fatty acids are thiol esters of coenzyme A. So it is of interest that these metabolites were isolated in the non-esterified form in a fairly good yield.

These microbiological conversions will also be useful for the production of these hydroxy acids because chemical syntheses or degradations of hydroxy acids are often troublesome.

No other microorganisms capable of converting ricinoleic acid to the metabolites mentioned above under the experimental conditions described have been found. This suggests that the fatty acid oxidation enzyme system of Candida strains may somewhat differ from those of *E. coli* and other microorganisms. Concerning this point the substrate specificity is now under investigation.

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A time study of the hydrolysis of lecithin by snake-venom phospholipase A

It has been established[1–3] that snake-venom phospholipase A (phosphatide acylhydrolase, EC 3.1.1.4) specifically hydrolyses the ester bond at the β-position of the lecithin molecule irrespective of the type of fatty acid occupying the β-position[3]. Although lecithin obtained from most natural sources is generally believed to consist of molecules with saturated acids occupying the α-position and unsaturated acids occupying the β-position, there have been suggestions[2,4] that a small proportion of native lecithin molecules does not possess this configuration. The results of the study now reported lend support to these suggestions and in addition indicate that phospholipase A hydrolyses certain types of lecithin molecules more readily than others.

The lecithin used in these experiments was prepared from the yolks of fresh eggs by the method of SAUNDERS[5] and was found to give rise to one spot only when chromatographed either on paper impregnated with silicic acid[6] or on thin-layer chromatoplates of Silica Gel G containing 10% (w/w) ammonium sulphate[7] with a solvent system of chloroform–methanol–water (65:25:5, v/v). Enzyme hydrolysis of the lecithin was carried out essentially as described by LONG AND PENNY[8]. To each reaction vessel containing 17.5 μmoles of lecithin and 0.25 μmole of ammonia dissolved in 5 ml of peroxide-free diethyl ether was added 0.05 ml of a solution (0.1%, w/v) of dried snake venom (*Crotalus adamenteus*, L. Light Ltd., Colnbrook, Great Britain) in aqueous 0.005 M $CaCl_2$. After shaking, the reaction mixtures were incubated for periods varying from 2 to 80 min at 18°. The reactions were stopped by the addition of 10 ml of methanol and the mixtures taken to dryness by means of a rotary film evaporator attached to a supply of nitrogen. As rapidly as possible the dry residues were dissolved in chloroform and the resulting solutions filtered. To determine the extent of hydrolysis during the reaction, portions of the chloroform solutions were analysed by thin-layer chromatography with the system described above. The bands of Silica Gel G containing the lysolecithin and unchanged lecithin were then eluted separately with methanol. After removal of the methanol, the phospholipids were digested with $HClO_4$ and the phosphorus content of the digests