the hexagonal structure of phase F, when, with the pure caprylate middle soap as a starting point, the decanol content of the system is increased at the expense of its caprylate and water concentration. It is more likely that the reason is a change in the layer of hydrophilic groups at the interfaces between the hydrocarbon and water regions. Whereas the negative carboxyl groups of the caprylate ions repel each other, hydrogen bonds are formed between the hydroxyl groups of the decanol molecules and the oxygen atoms of the carboxyl groups. The incorporation of hydroxyl groups of decanol molecules between the ionized carboxyl groups of caprylate may thus lead to a denser packing in the layer of hydrophilic groups. Calculations reveal that this is actually the case but that in the region of a homogeneous mesomorphous phase the density of the packing varies between rather narrow limits. The differences in the packing between the different phases are, however, quite large. The area per polar group is much smaller in phase D than in phase E and again much smaller in phase F than in phase D. The existence of the various mesomorphous phases hence seems to be determined by the density of the packing of the end groups which in each case cannot deviate greatly from a certain characteristic value.

Acknowledgements. One of the authors, K.F., is indebted to Prof. Rolf Hosemann and Dr Richard Bonart, Fritz Haber-Institut der Max Planck Gesellschaft, Berlin, for valuable discussions.

1. Marsden, S. S., Jr. and McBain, J. W. *J. Am. Chem. Soc.* **70** (1948) 1973.
2. Luzzati, V., Mustacchi, H. and Skoulios, A. *Nature* **180** (1957) 600; Discussions Faraday Soc. **25** (1958) 43; Luzzati, V., Mustacchi, H., Skoulios, A. and Husson, F. *Acta Cryst.* **13** (1960) 660; Husson, F., Mustacchi, H. and Luzzati, V. *Acta Cryst.* **13** (1960) 668.
3. Ekwall, P., Danielsson, I. and Mandell, L. *Kolloid-Z.* **169** (1960) 113.
4. Ekwall, P., Danielsson, I. and Mandell, L. *3rd Intern. Conf. Surface Activity*, Vol. I, Section A, pp. 189 and 193, Cologne 1960; Ekwall, P., Danielsson, I., Fontell, K. and Mandell, L. *Finska Kemistsamfundets Medd.* **70** (1961) 51.
5. Rosevear, F. B. *J. Am. Oil Chemist's Soc.* **12** (1954) 628.

Received September 29, 1962.

The Biosynthesis of Gentisic Acid

S. GATENBECK and I. LÖNNROTH

Institute of Biochemistry, University of Lund, Lund, Sweden

In the series of reactions leading to the formation of the antibiotic patulin in *Penicillium urticae*, 6-methylsalicylic acid occupies a key position. Some other products synthesized by the same organism are gentisalcohol, gentisaldehyde and gentisic acid [1]. The acetate origins of 6-methylsalicylic acid [2] and patulin [3] are well established, and furthermore, good evidence has been obtained for the biological transformation of 6-methylsalicylic acid to patulin [4]. These results are consistent with Birkinshaws' suggestion [5] that patulin is formed by a ring-opening process of gentisaldehyde. However, the acetate origin of gentisaldehyde or gentisic acid has never been confirmed. On the contrary, indications have been obtained that gentisic acid is formed along other pathways, presumably *via* shikimic acid.

In this communication results will be presented which clearly show that gentisic acid is derived from acetate in *P. urticae*. In a preliminary experiment acetate-2-^{14}C was given to a growing culture and 6-methylsalicylic acid and gentisic acid were isolated from the culture medium. The specific radioactivity of 6-methylsalicylic acid was found to be six times that of gentisic acid which could be explained by a larger nonlabelled pool of gentisic acid at the moment the labelled substrate was added. Decarboxylation of gentisic acid showed that the carboxyl group contained 25 % of the total radioactivity, indicating the acetate origin of gentisic acid and supporting the hypothesis that it is formed by oxidation and decarboxylation of 6-methylsalicylic acid. This hypothesis if further emphasized by time aspect studies of the production of the two substances as the amount of 6-methylsalicylic acid is reaching a maximum before that of gentisic acid.

A more extensive degradation of gentisic acid labelled from acetate-1-^{14}C was performed to confirm the alternate labelling of the ring. After tosylating the gentisic acid in 5-position, the toluenesulphonic acid group was removed by refluxing with Raney nickel in alcohol according to the method of Kenner and Murray [6]. Nitration of the formed salicylic acid yielded picric acid which was further degraded with ba-

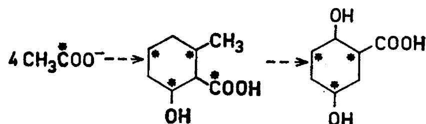

Fig. 1.

rium hypobromite to bromopicrin and carbon dioxide. Of the total radioactivity of gentisic acid 93.5 % were found in the bromopicrin and 2.8 % in the carbon dioxide (Table 1). These values show that the labelled positions in gentisic acid are those expected when formed from 6-methylsalicylic acid (Fig. 1).

The production of gentisic acid in nature is not restricted to *P. urticae* but has been found in many other organisms as well, e.g. *Polyporus tumulosus* [7]. This organism produces along with gentisic acid *p*-hydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid, 2,5-dihydroxyphenylglyoxylic acid and some other acids. As these types of aromatic acids are known to be derived from shikimic acid *via* phenylpyruvic acid, it is possible that gentisic acid in *P. tumulosus* is not formed from acetate but, e.g., by an oxidative decarboxylation of 2,5-dihydroxyphenylglyoxylic acid.

Table 1.

Substance	Cpm/mmole
Gentisic acid	13.85×10^3
Bromopicrin (C1, C3, C4 in gentisic acid)	12.92×10^3
Carbon dioxide (C2, C4, C6 in gentisic acid)	0.39×10^3

Experimental

Culture conditions. Penicillium urticae Bainier was incubated as shake culture at 27°C in Czapek Dox medium. When the production of the phenolic substances had started labelled tracer substrates (0.5 mC acetate-2-^{14}C and 0.5 mC acetate-1-^{14}C) were added to separate cultures in 500 ml Erlenmeyer flasks containing 150 ml of medium, and the cultures were harvested after another two days incubation.

Isolation of gentisic acid and 6-methylsalicylic acid. The culture filtrates were acidified and extracted with ether. The residues of the ether extracts were separated on paper chromatograms (Whatman 3 MM) with ethylmethylketone-water-diethylamine (921:77:2) as the solvent and gentisic acid (R_F 0.36) and 6-methylsalicylic acid (R_F 0.62) eluted from the paper with acetone. After sublimation of the two substances their specific radioactivities were determined in a liquid scintillation counter using internal standard.

Degradation of gentisic acid. The radioactive gentisic acid was recrystallized with nonlabelled carrier from acetone-ligroin to constant specific radioactivity. A part of the sample derived from acetate-2-^{14}C was oxidized to carbon dioxide by wet combustion and the remaining portion decarboxylated by refluxing with aqueous ferri chloride. 105 mg of the labelled gentisic acid obtained from acetate-1-^{14}C was treated with an equivalent amount of *p*-toluenesulphonyl chloride in 3 ml of 1 N sodium hydroxide at room temperature until a clear solution was obtained. Acidification with concentrated hydrochloric acid gave a precipitate that was filtered off, washed with water and recrystallized from benzene. Yield 88.5 mg, m.p. 169°C. The formed tosylderivative was refluxed for 3 h in an alcoholic solution with a large excess of Raney nickel. The reaction mixture was made acidic and the catalyst sucked off and thoroughly washed with ethanol. Evaporation of the filtrate to dryness and subsequent sublimation of the residue yielded a substance that proved to be identic with salicylic acid (18.5 mg, m.p. 159°C). The radioactive salicylic acid was recrystallized from water with 150 mg of nonlabelled carrier and 130 mg of the dry substance heated with 0.5 ml of nitric acid (*d* 1.52) to dryness on the steam bath. The procedure was repeated with the same amount of nitric acid. Extraction of the dry residue with benzene and evaporation of the solvent gave picric acid which was recrystallized from water (130 mg, m.p. 122°C). The degradation of picric acid with barium hypobromite to bromopicrin and carbon dioxide and the subsequent combustion of the bromopicrin has been described elsewhere [8].

Measurements of radioactivity. All measurements of the radioactivities in the degradation series were performed in a liquid scintillation counter with the samples as barium carbonate suspended in a gel of Aerosil in a toluene solution of 2,5-diphenyloxazol.

1. Ehrensvärd, G. *Exptl. Cell Research Suppl.* **3** (1955) 102.
2. Birch, A. J., Massey-Westropp, R. A. and Moye, C. J. *Australian J. Chem.* **8** (1955) 539.
3. Bu'Lock, J. D. and Ryan, A. J. *Proc. Chem. Soc.* **1958** 222.
4. Bassett, E. W. and Tanenbaum, S. W. *Biochim. Biophys. Acta* **40** (1960) 535.
5. Birkinshaw, J. H. *Ann. Rev. Biochem.* **22** (1953) 371.
6. Kenner, G. W. and Murray, M. A. *J. Chem. Soc. Suppl.* **1949** 178.
7. Crowden, R. K. and Ralph, B. J. *Australian J. Chem.* **14** (1961) 475.
8. Gatenbeck, S. *Acta Chem. Scand.* **12** (1958) 1985.

Received October 29, 1962.