Phytochemistry, 1968, Vol. 7, pp. 1741 to 1742. Pergamon Press. Printed in England

SHORT COMMUNICATION

THE METABOLISM OF PHENYLACETIC ACID BY *ASPERGILLUS NIGER*

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(*Received* 15 *May* 1968)

Abstract—2-Hydroxyphenylacetic acid was the only major metabolite obtained from the metabolism of phenylacetic acid by *Aspergillus niger* Van Tiegh. (Mulder strain); no homogentisic acid could be detected.

THE METABOLISM of phenylacetic acid has been investigated in a variety of biological systems[1–3] and two alternative pathways have been suggested for the bacterial oxidation.[1,4] The adaptive pattern for strain K-1 of *Pseudomonas fluorescens* supported the route via 4-hydroxy- and 3,4-dihydroxyphenylacetic acids, and this was confirmed by the isolation of these acids from the metabolic liquor. Results with strain K-2 of the same organism suggested a scheme involving 2-hydroxyphenylacetic and homogentisic (2,5-dihydroxyphenylacetic) acids, both of which had been reported[5,6] in the culture filtrate of *Penicillium chrysogenum* Q176, to which phenylacetic acid had been added as a penicillin precursor.

In the present work using *Aspergillus niger*, 2-hydroxyphenylacetic acid was the only major metabolite which could be detected in the metabolism of phenylacetic acid though the production of oxalic acid was noted. The absence of homogentisic acid, easily detectable in the presence of monohydroxyphenylacetic acids by thin-layer chromatography followed by a diazo-spray and also by GLC, contrasts sharply with the work of Kluyver and van Zijp,[7] who only mentioned the formation of homogentisic acid from an unnamed strain of *A. niger*. More recently Bocks,[8] using the Mulder strain of *A. niger*, reported 2-hydroxyphenylacetic acid as the main monohydroxylated product, though the 3- and 4-isomers were identified as minor products, together with homogentisic acid. After the publication of these results by Bocks, our experiments were repeated but we were unable to find any evidence for the formation of homogentisic acid. Whilst strain differences have been previously shown to be important in the metabolism of phenoxyacetic acid[9] by *A. niger*, this can hardly be the explanation of the present difference. It is also perhaps significant that homogentisic acid has not been found amongst the products from the metabolism of tyrosine by *A. niger*,[10] although it has been reported by Utkin.[11]

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EXPERIMENTAL

Phenylacetic acid (10^{-3} M in aqu. NaOH, pH adjusted to 7, 8 l.) was incubated at 26° for 24 hr under mycelial mats of *Aspergillus niger* (Mulder strain, obtained from C.B.S., Delft), grown in penicillin flasks as previously described.[12] After concentration *in vacuo*, the fungal liquor was acidified and extracted with ether. Examination of the ether-soluble material (0·1 g) using paper and thin-layer chromatography showed the presence of one major metabolite, R_f 0·59, together with unchanged phenylacetic acid R_f 0·80. Both were isolated by means of TLC on Kieselgel HR (Merck) with ether/petrol. ether (b.p. 60–80°)/formic acid (50:50:2) as solvent. The appropriate band was outlined in u.v. light and a diazo-coupled marker strip (obtained using *p*-nitrobenzene diazonium fluoroborate), scraped from the plate and the silica extracted with ether in a soxhlet for 2 hr. After removal of the solvent, the major metabolite (0·018 g), m.p. 143–146°, was identified as 2-hydroxyphenylacetic acid by mixed m.p. with an authentic specimen and comparison of i.r. spectra. No homogentisic acid (R_f 0·16 in the solvent system used) could be detected in the fungal substrate obtained from 6, 24 or 96 hr incubation experiments, though the formation of oxalic acid was substantiated; no homogentisic acid could be detected using the conditions employed by Kluyver and van Zijp.[7]

The fungal metabolic product was also analysed by gas–liquid chromatography, using a 6 ft × ¼ in. stainless-steel column packed with 2·5 per cent LAC 2R 446 on chromosorb G (acid-washed and silanized), column temperature 192°, and a helium flow rate of 100 ml/min. By this means it was shown that any 3- and 4-hydroxyphenylacetic acids present represented less than 3 per cent of the amount of the 2-hydroxyphenylacetic acid formed; no homogentisic acid could be detected.

Acknowledgements—We thank Dr. D. A. M. Watkins for the GLC data and Mrs. C. S. James for valuable technical assistance.

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