Short Communication

Isoprenoid Quinone Precursors of Ubiquinone-10(X-H₂) in *Aspergillus flavus*

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Whistance, Brown & Threlfall (1969, 1970) isolated a number of 6-methoxy-2-polyprenyl-1,4-benzoquinones and 5-demethoxyubiquinones (6-methoxy-3-methyl-2-polyprenyl-1,4-benzoquinones) from the bacteria *Pseudomonas ovalis* Chester and *Proteus mirabilis*, and showed that in these organisms they are precursors of ubiquinones. More recently, Ah Law, D. R. Threlfall & G. R. Whistance (unpublished work) have isolated 5-demethoxyubiquinones from the yeasts *Saccharomyces cerevisiae* and *Rhodotorula glutinis* and the mould *Phycomyces blakesleeanus*, and have shown they are precursors of ubiquinones in these organisms also.

In the present communication we describe the isolation from the mould *Aspergillus flavus* [a mould that contains ubiquinone-10(X-H₂) (where the roman numeral indicates that the tenth isoprene unit from the nucleus is saturated) (III) as its principal isoprenoid quinone (Lavate & Bentley, 1964)] of 6-methoxy-2-decaprenyl(X-H₂)-1,4-benzoquinone (I) and 5-demethoxyubiquinone-10(X-H₂) (II), compounds hitherto not reported to occur in Nature. In addition, we present evidence that in *A. flavus* these quinones are precursors of ubiquinone-10(X-H₂).

Experimental and results. (a) Isolation and characterization of compounds (I) and (II). *A. flavus* I.M.I. 16145 (1.4 kg wet wt.) was grown for 3 days at 28°C in shake culture on the medium described by Lavate & Bentley (1964). At the end of the growth period it was harvested by filtration and then saponified (Lavate, Dyer, Springer & Bentley, 1965). The non-saponifiable lipid was extracted from the saponification mixture by the procedure of Spiller, Threlfall & Whistance (1968).

After precipitation of most of the sterol with ice-cold light petroleum (b.p. 40–60°C) (Spiller *et al.* 1968), the non-saponifiable lipid (1.2 g) was chromatographed on a 60 g column of acid-washed alumina (Brockmann grade III) developed by stepwise elution with 0.25%, 1%, 3%, 5%, 8% and 12% (v/v) diethyl ether in light petroleum (b.p. 40–60°C) (Threlfall & Goodwin, 1967). In this chromatographic system compound (III) and small amounts of compound (II) are eluted by 5% diethyl ether in

light petroleum, and compound (I) and most of compound (II) are eluted by 8% diethyl ether in light petroleum.

Fractionation of the fraction eluted by 8% diethyl ether in light petroleum by the procedures employed in the isolation of 6-methoxy-2-polyprenyl-1,4-benzoquinones from *Ps. ovalis* Chester and *Prot. mirabilis* (Whistance *et al.* 1969, 1970) allowed the isolation of 140 µg of compound (I) and 480 µg of compound (II). The identities of compounds (I) and (II) were established on the basis of the following criteria. (1) Their u.v. spectra in cyclohexane [compound (I), $\lambda_{\max}$ 264.5 nm; compound (II), $\lambda_{\max}$ 266 nm] and ethanol [compound (I), $\lambda_{\max}$ 267 nm; compound (II), $\lambda_{\max}$ 270 nm] are qualitatively identical with those of 6-methoxy-2-polyprenyl-1,4-benzoquinones and 5-demethoxyubiquinones respectively (Imamoto & Senoh, 1968; Daves, Wilczynski, Friis & Folkers, 1968; Whistance *et al.* 1969, 1970). Further, the u.v. spectrum ($\lambda_{\max}$ 273, 278, and 340 nm) of the chromenol of compound (II) [prepared by base (pyridine)-catalysed cyclization (Whistance *et al.* 1970)] is identical with that of 7-demethoxyubichromenol (Daves *et al.* 1968; Whistance *et al.* 1970). [This establishes that the quinone is a 5-demethoxyubiquinone(H₂) and not a 6-demethoxyubiquinone(H₂).] (2) On paraffin-impregnated thin layers of silica gel developed with aq. 90% (v/v) acetone they migrated (as does compound III) with lower R_F values than the corresponding decaprenyl analogues (compound I, R_F 0.29, cf. 6-methoxy-2-decaprenyl-1,4-benzoquinone, R_F 0.36; compound II, R_F 0.22, cf. 5-demethoxyubiquinone-10, R_F 0.30; compound III, R_F 0.14, cf. ubiquinone-10, R_F 0.20). (3) Their mass spectra show peaks at m/e 820 (M) and 191 (base peak) and at 834 (M) and 205 (base peak) respectively. (4) They are precursors of compound (III) in *A. flavus* (see below). [This shows that they possess a decaprenyl(X-H₂) side chain.]

Fractionation of the fraction eluted by 5% diethyl ether in light petroleum by t.l.c. showed that it contained 26 mg of compound (III), 0.5 mg of ubiquinone-9(H₂) and trace amounts of ubiquinone-8(H₂) and compound (II).

(b) Conversion of compounds (I) and (II) into

Scheme 1. Partial pathway for the biosynthesis of ubiquinone-10(X-H₂) in *A. flavus*.

compound (III). Evidence that compounds (I) and (II) are precursors of compound (III) in *A. flavus* was obtained from a pulse-labelling experiment with *p*-hydroxy[U-¹⁴C]benzoic acid. Mycelia (250 g wet wt.) from a 3-day-old culture of *A. flavus* were resuspended in 2 litres of 0.1 M-potassium phosphate buffer, pH 6.0, containing 8 μ Ci of *p*-hydroxy[U-¹⁴C]benzoic acid (10 mCi/mmol) and incubated for 4 h at 28°C in a Gallenkamp orbital shaker (300 rev./min). At the end of this time the mycelia were harvested, washed thoroughly with water and then divided into two equal portions. One portion was extracted immediately, and compounds (I), (II) and (III) were isolated and assayed for radioactivity. The other portion was resuspended in 4 litres of 0.1 M-potassium phosphate buffer, pH 6.0, containing 3 mmol of unlabelled *p*-hydroxybenzoic acid and incubated for a further 20 h at 28°C with vigorous aeration. Compounds (I), (II) and (III) were then isolated and assayed for radioactivity.

At the end of the incubation with *p*-hydroxy-[U-¹⁴C]benzoic acid compounds (I) (sp. radioactivity 450 000 c.p.m./mg), II (sp. radioactivity 228 000 c.p.m./mg) and III (sp. radioactivity 120 000 c.p.m./mg) contained 5400, 9120 and 285 830 c.p.m. respectively. At the end of the incubation with unlabelled *p*-hydroxybenzoic acid the amount of radioactivity in compounds (I) (sp. radioactivity 71 400 c.p.m./mg) and (II) (sp. radioactivity 90 125 c.p.m./mg) had fallen to 856 and 3600 c.p.m. respectively, whereas that in compound (III) (sp. radioactivity 125 000 c.p.m./mg) had risen to 297 900 c.p.m.

Discussion. Two new isoprenoid quinones have been isolated from the mould *A. flavus*. U.v. spectroscopy, t.l.c., mass spectrometry and biogenetic studies have established that they have structures (I) and (II).

In a pulse-labelling experiment it was shown that the radioactivity content of compounds (I) and (II) in mycelia that had been incubated for 4 h with *p*-hydroxy[U-¹⁴C]benzoic acid fell when the mycelia were incubated for a further 20 h with unlabelled *p*-hydroxybenzoic acid. Further, it was shown that this fall in radioactivity content was accompanied by a corresponding rise in the radioactivity content of compound (III). There would therefore seem to be little doubt that compounds (I) and (II) are precursors of compound (III) (Scheme 1). Indirect support for this comes from the facts that analogues of compounds (I) and (II), 6-methoxy-2-polyprenyl-1,4-benzoquinones and 5-demethoxyubiquinones, have been shown to be precursors of ubiquinones in some bacteria, yeasts and moulds (see the introduction).

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