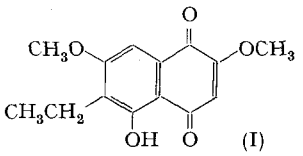
## 6-Ethyl-5-Hydroxy-2,7-Dimethoxynaphthoquinone, a Metabolite of *Hendersonula toruloidea* Nattrass

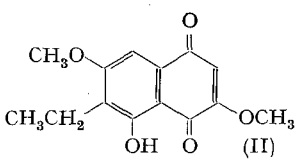
A yellow pigment, m.p. 187°, has been isolated in small yield (ca. 10 mg/l) from *Hendersonula toruloidea* Nattrass (IMI 135205). The organism was grown on a nutrient solution containing cerelose (dextrose monohydrate) (5%), ammonium tartrate (1%), $KH_2PO_4$ (0.25%), $MgSO_4.7H_2O$ (0.05%), minor elements concentrate[1] (0.1%), and yeast extract (0.1%) at natural pH. 500 ml flasks containing 200 ml sterilised nutrient solution were inoculated with *H. toruloidea* and then incubated on a rotary shaker at 25 °C for from 12–16 days. The contents of 20 flasks (3.7 l) were combined, adjusted to pH 2, and then extracted with ethyl acetate. A solution of the crude extract (2 g) in ether was washed successively with 5% aqueous sodium bicarbonate, 1 *N* sodium hydroxide, 1 *N* hydrochloric acid, and then water. The remaining ethereal solution afforded the neutral fraction (690 mg) of the initial extract. This neutral fraction was stirred with light petroleum (b.p. 40–60°) and sufficient ether to dissolve the gummy material but leave the crude pigment (53 mg) as a solid. The pigment was purified by chromatography on alumina (Grade III, neutral) using light petroleum (b.p. 60–80°) as eluting solvent, and then as crystallising solvent. Purification was monitored by TLC on silica gel GF plates. The pigment was visible as a yellow spot Rf 0.6 when the plates were developed with ethyl acetate, and Rf 0.8 with methanol-chloroform (1:9).

The mass spectrum revealed that the pigment had mol. wt. 262, and molecular formula $C_{14}H_{14}O_5$. NMR-signals ($CDCl_3$, TMS) were assigned as follows $\delta$ 12.50 (singlet, OH, 1), 7.30 (singlet, Ar-H, 1), 6.04 (singlet, $>C=C<^{H}$, 1), 4.00 (singlet, $OCH_3$, 3), 3.92 (singlet, $OCH_3$, 3), 2.75 (quartet, $J$ = 7.5 cps, $-CH_2CH_3$, 2), and 1.13 (triplet, $J$ = 7.5 cps, $-CH_2CH_3$, 3). The $C_{10}$ carbon skeleton, the colour of the compound, and the nature of the substituents suggested a naphthoquinone structure, and the evidence could be accommodated by either of structures (I) and (II)[2]. (I) has recently been obtained[3] by methylation of 6-ethyl-2,5,7-trihydroxynaphthoquinone, a product of the sodium borohydride reduction of spinachrome A. Comparison of NMR-spectra suggested that the pigment from *H. toruloidea* was in fact (I), and this was confirmed by comparison of mass spectra, by TLC, and by mixed m.p. with a sample of (I) kindly supplied by Professor P. J. Scheuer. Thus (I), although a known compound, is a new natural product. This appears to be the first isolation from a microorganism of an oxygenated naphthoquinone containing a C-ethyl substituent, a class of compound hitherto characteristic of echinoderms. 2,7-Dimethoxy-5-hydroxy-1,4-naphthoquinone (III) has recently been isolated from a strain of *Streptomyces*[4].

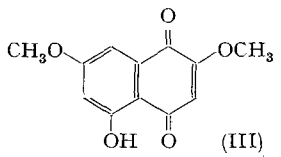
(I) is not active at 100 ppm in vitro against *Staphylococcus aureus* and *Salmonella dublin*, and it has no anticoagulant activity.



(I)



(II)



(III)

*Zusammenfassung.* Ein neuer Naturstoff, 6-Ethyl-5-hydroxy-2,7-dimethoxynaphtochinon ist aus *H. toruloidea* isoliert worden. Dieses Produkt wurde früher bei der Methylierung des 6-Ethyl-2,5,7-trihydroxynaphtochinons, welches bei der Reduktion des Spinachroms A mittels Natrium Borhydrid anfiel, erhalten.

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[3] R. E. Moore, H. Singh, C. W. J. Chang and P. J. Scheuer, J. org. Chem. *31*, 3638 (1966).
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## Induced Peroxidase Isoenzyme Patterns in Citrus Leaves

Decreased peroxidase activity of citrus leaves was suggested as an indicator for diagnosing iron deficiency in citrus leaves[1,2]. Infiltration of iron sulphate solution into excised but intact iron-deficient citrus leaves caused part or full restoration of the enzyme activity in these leaves. This iron-restored or induced enzyme activity was negatively associated with the iron level of the substrate and the growth response of the trees, and it was therefore proposed as a measure for the determination of the iron requirement of citrus trees[3]. The occurrence of peroxidase in multiple molecular form was recognized long ago[4], and more recently the molecular heterogeneity of this enzymes was studied in further details[5,6]. Hence, it wa thought that a study of the physico-chemical properties of the enzyme by means of the separation of its various isoenzymes, might improve the application of the enzyme assay as a diagnostic agent and bring about a better understanding of the process taking place during the induction procedure of the enzyme.

Excised, intact citrus leaves were placed in 0.5% ferrous sulphate solution, or in water as a blank treatment, in Buchner flasks under vacuum suction with slight bubbling, for 3 min. After this, they were removed from the solution and placed in petri dishes on Saran nets laid