instead less potent and less active than daunorubicin, **VIa** and **VIb.**

The biological properties of the new analogues indicate that considerable modification on ring D of the antitumour anthracyclines is compatible with the exhibition of antitumour efficacy. In particular, the 2,3-dimethyl-4-demethoxy analogue showed promising results in the animal tests.

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Naphthoquinone derivatives from the fungus *Hendersonula toruloidea*

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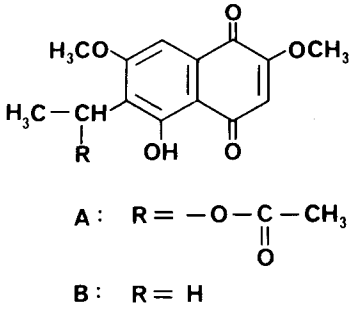
Summary. 2 yellow naphthoquinone derivatives were isolated from several pathogenic strains of *Hendersonula toruloidea* Nattrass. They were identified as 2,7-dimethoxy-6-ethyl-5-hydroxy-1,4-naphthoquinone and 2,7-dimethoxy-5-hydroxy-6-(1-acetoxyethyl)-1,4-naphthoquinone by means of physico-chemical methods.

A fungus isolated from a patient (foot) was found to produce yellow crystalline material on malt agar. The fungus (CBS 131.78) was provided by M.K. Moore of St. John's Hospital for Diseases of the Skin in London and identified as *Hendersonula toruloidea* Nattrass by G.A. de Vries of the Department of Medical Mycology of our institute. The strain M 56 (CBS 145.78 = IMI 198935), one of several other isolates from patients supplied by C.K. Campbell of the M.R.C. Unit on the Experimental Pathology of the Skin in Birmingham, showed the same phenomenon as described above. TLC of an ethyl acetate extract of a culture of both strains revealed the presence of 2 yellow main pigments. Since only 1 pigment formed by *H. toruloidea* has been described[4], it was decided to investigate the nature of the 2 metabolites.

Each strain was grown on malt agar in 100 petri dishes for 20 days at 24 °C. Cultures were extracted with ethyl acetate. Purification was accomplished by means of column chromatography and preparative TLC using toluene/acetone (85:15, v/v) as the developing system. Merck silica gel 60 26×3 cm columns and Merck 2 mm silica gel thick-layer plates were used. Separation of the 2 pigments was successful on the silica gel plates.

Yellow bands comprising compound A, R_f 0.43–0.52 and compound B, R_f 0.53–0.63 were scraped off and eluted with chloroform/methanol (2:1, v/v). A and B were obtained as orange needles by twice recrystallizing from toluene/light petroleum 60–80 °C (1:1, v/v). A and B showed identical UV-visible spectra indicating the same chromophoric system for both pigments. The absorptions measured were similar to those described[5] for the substance originally isolated from *H. toruloidea.* Further study (MS, IR and PMR) revealed that B was identical with the known *Hendersonula* pigment, which was identified as 2,7-dimethoxy-6-ethyl-5-hydroxy-1,4-naphthoquinone (2,7-dimethoxy-6-ethyljuglone, $C_{14}H_{14}O_5$). High resolution MS of A gave the formula $C_{16}H_{16}O_7$. Important peaks were observed at m/e 278 ($M-CH_2CO$) and m/e 260 ($M-CH_3COOH$). The IR-spectrum of A in CCl_4 compared with that of compound B



A: R = —O—C(=O)—CH3

B: R = H

Formation of compounds A and B by other strains of *H. toruloidea*

Strain No.	Origin	Yield mg/20 petri dishes A	B
CBS 204.33	Plant	–	–
CBS 251.49	Plant	–	–
CBS 136.77	Man	1.4	0.4
CBS 137.77	Cow	10.4	1.7
CBS 661.77*	Man	–	–
CBS 662.77*	Man	0.2	–
M 38**	Man	–	–
M 48**	Man	0.2	0.9
M 52**	Man	–	–

* Isolates from M.K. Moore; ** isolates from C.K. Campbell.

showed an extra CO absorption peak at 1742 cm^{-1}. The MS and IR data suggested the presence of an acetate group in A. The final structure of A was determined on the basis of ^{1}H-NMR study. The PMR-spectra of A and B showed some resemblance, although several significant differences were observed. In the spectrum of A an A_3X quartet (J = 7 Hz) centred at 6.36 (δ in ppm), a singlet (3H) at 2.05 and a doublet at 1.60 (J = 7 Hz) were detected, whereas in the spectrum of B an A_3X_2 quartet at 2.75 (J = 7.2 Hz) and a triplet at 1.13 (J = 7.2 Hz) were measured. The CH_3 signal at 2.05 was absent in this spectrum. It was concluded from the PMR and spectral data that A can only have the structure shown in the figure (2,7-dimethoxy-5-hydroxy-6-(1-acetoxyethyl)-1,4-naphthoquinone). Since the molecule has an asymmetric C atom, it was interesting to investigate the optical activity of the pigment. A specific rotation of −46.0° was determined. The stereochemistry at the asymmetric C atom has remained unsolved. A good distinction between the 2 compounds can also be obtained by gas-liquid chromatography without derivatization. Retention times of 3.80 and 4.75 min were measured for B and A respectively using a 1.7 m glass column packed with 3% SE-30 on Gas-Chrom Q (225 °C isothermally; gas flow rate 30 ml/min).

Compound A was the major pigment of the strain M 56, whereas B was the main product of CBS 131.78. The yield was 38 mg A and 103 mg B from 1.47 g EtOAc extract of CBS 131.78 and 660 mg A and 305 mg B from 3.4 g EtOAc extract of M. 56. Other strains of *H. toruloidea* were also examined for their capability of forming A and B. The results are summarized in the table. Recently Campbell and Mulder[6] described the strains M 38, M 48, M 52 and M 56 (type culture) and some other isolates as *Scytalidium hyalinum*. The genus *Scytalidium* was chosen since the isolates exhibited the same mode of conidial production as *S. lignicola viz.* arthroconidia. They also suggested that the 'torula' state of *H. toruloidea* would be better placed in *Scytalidium*. A pycnidial form which is shown by the more typical strains of *H. toruloidea* has not been seen in *S. hyalinum*.

Physico-chemical data. Compound A: m.p. 160–163 °C (dec.), $[\alpha]_{589}^{25} = -46.0°$ (c 0.3 in $CHCl_3$); mol. wt 320.0912, calc. for $C_{16}H_{16}O_7$ 320.0896; transition $320^+ \rightarrow 260^+ + 60$ (AcOH) found 260.0702, calc. 260.0685; λ_{max} (MeOH): 220 (logε 4.51), 257 sh (4.14), 263 (4.15), 306 (3.97), 425 nm (3.55); ν_{max} (CCl_4): 1742, 1690, 1632 cm^{-1}; PMR (90 MHz, $CDCl_3$): 1.60 ($-CH_3$, d, J = 7 Hz), 2.05 ($-CO-CH_3$, s), 3.90 ($-OCH_3$, s), 4.00 ($-OCH_3$, s), 6.01 (<C=CH-, s), 6.36 (<CH-, q, J = 7 Hz), 7.23 (aromatic H, s), 12.80 δ_{TMS} (-OH, s).

Compound B: m.p. 187–190 °C; mol. wt 262.0864, calc. for $C_{14}H_{14}O_5$ 262.0841; λ_{max} (MeOH): 220.5 (logε 4.47), 258 sh (4.22), 263.5 (4.24), 306 (4.00), 424 nm (3.64); ν_{max} (CCl_4): 1688, 1632 cm^{-1}; PMR (90 MHz, $CDCl_3$): 1.13 ($-CH_3$, t, J = 7.2 Hz), 2.75 ($-CH_2-$, q, J = 7.2 Hz), 3.90 ($-OCH_3$, s), 3.97 ($-OCH_3$, s), 5.99 (<C=CH-, s), 7.20 (aromatic H, s), 12.50 δ_{TMS} (-OH, s).

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Effect of tryptophan on hepatic nuclear free and engaged RNA-polymerases in young and adult rats

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Summary. Whereas in young rats (2 weeks old), administration of typtophan produced marked enhancement in the activity of both engaged and free polymerases of nuclei, in adult rats (10 weeks old) only the engaged polymerases showed higher activities following tryptophan force-feeding.

In the light of recent observations that a single tube-feeding of L-tryptophan enhances hepatic protein synthesis[2–5], several investigators, besides ourselves, have studied the activity of hepatic nucelar RNA polymerases[6–8]. The results show that tryptophan stimulates both Mg^{2+} (polymerase I) and $Mn^{2+}/(NH_4)_2SO_4$-dependent (polymerase II) activities. However, in these experiments endogenous DNA-template-directed RNA synthesis in nuclei was measured, which essentially reflects the activity of different polymerases, bound to chromatin. In recent years it has been demonstrated that, in intact liver, nuclei RNA polymerases exist both as 'engaged' (chromatin-bound) and 'free' states[9–11], and free polymerase population can account for as much as 50% of the total polymerase activity[11]. In view of large populations of 'engaged' and 'free' RNA polymerases in nuclei, we decided to investigate the activity of different polymerases in these 2 population groups in young and adult rat livers after a single tube-feeding of tryptophan.

Materials and methods. Young and adult (2 and 10 weeks old) male Wistar rats were fasted for 24 h, and were then fed by stomach-tube either L-tryptophan (30 mg/100 g) or an equivalent volume of water, and killed 1 h later. Livers were excised and homogenized immediately in 10 vols. of cold 2.3 M sucrose 3.3 mM $CaCl_2$. Nuclei were isolated according to the procedure of Yu and Feigelson[12]. DNA concentration was measured by the method of Burton[13].

RNA-polymerase activity was measured in a 0.25 ml incubation medium containing (μmole): Tris-HCl (pH 8.0), 25, 2-mercaptoethanol 5; $MnCl_2$ 0.4; $(NH_4)_2S/_4$ (pH 8.0 with NH_3) 16.25; each of ATP, GTP and CTP 0.0625; UTP 0.00625; and 0.5 μCi of [5,6-^{3}H]UTP (41 Ci/mmole, Radiochemical Centre, Amersham, England.) When required, as in the case for exogenous template, 4 μg actinomycin-D and 10 μg poly (dA-dT) were also added to the incubation medium; α-amanitin was added at a concentration of 2 μg/ml. The reaction was initiated by addition of 0.05 ml of nuclei and incubated for 10 min at 37 °C. The reaction was terminated by addition of $Na_4P_2O_7$ and trichloroacetic acid (TCA) as described earlier[14]. The acid-insoluble material was collected on glass fibre filter (GF/C), and processed for radioactivity measurements as described previously[15].

Results and discussion. In the present investigation, all incubations were performed in the presence and absence of α-amanitin, a fungal toxin which specifically inhibits polymerase II activity[16,17]. Although polymerase III activity is