10.00 h, a portion of the liver was freeze-clamped and stored under liquid N_2 until processing. Total carnitine, the sum of free and esterified carnitine, was measured after alkaline hydrolysis, using a radiochemical method[7] with the following modification; thiol groups were trapped with 0.2 mM N-aethyl-maleinimide. Total AA, the sum of AA and dehydro-AA, was measured as described[8]. Authentic L-carnitine was a kind gift from Sigma-Tau (Rome).

Results and discussion. The table shows a marked decrease of liver carnitine in AA deficient guinea-pigs (groups 5 and 6), which is consistent with our early findings. On the other hand, a depletion of liver carnitine is also seen in the underfed ('pair-fed') animals receiving a normal AA dose (group 2). This finding confirms our previous report[6], although in that experiment[6] the food restriction was more severe (10 g/day) and as expected the decrease in liver carnitine was more pronounced. However, these results are in contrast with those[9] which did not find a decrease of liver carnitine either in 'pair-fed' or even in scorbutic guinea-pigs. That paper[9] reported a carnitine decrease only in the muscles of AA deficient guinea-pigs. The reason for this discrepancy is not yet clear.

The action of AA deficiency is easily conceivable on the basis of in vitro studies[2,5]. It is of importance that limited feeding can evoke a decrease of AA level in the liver (table, groups 2 and 4). This finding raises the possibility that underfeeding acts via depression of tissue AA level in lowering the carnitine level. Whether the AA depression seen in group 2 ($p < 0.01$) was sufficient in itself to lower the carnitine level is difficult to investigate. So this finding cannot exclude some other kind of mechanism operating in the underfed state. Yet the result that an overdose of AA prevented the effect of underfeeding in depressing the carnitine level (compare group 3 with group 4) gives support to the view that in the combined effect of AA deficiency and underfeeding the ascorbic acid deficiency is the primary factor.

1 Brosnan, J.T., Kopec, B., and Fritz, I.B., J. biol. Chem. *248* (1973) 4075.
2 Hulse, J.D., Ellis, S.R., and Henderson, L.M., J. biol. Chem. *253* (1978) 1954.
3 Hoppel, C.L., Cox, R.A., and Novák, F., Biochem. J. *188* (1980) 509.
4 Rebouche, C.J., and Engel, A.G., Biochim. biophys. Acta *630* (1980) 22.
5 Lindstedt, G., and Lindstedt, S., J. biol. Chem. *245* (1970) 4178.
6 Sandor, A., Kerner, J., and Alkonyi, I., Experientia *37* (1981) 933.
7 McGarry, J.D., and Foster, D.W., J. Lipid Res. *17* (1976) 277.
8 Roe, J.H., and Kuether, C.A., J. biol. Chem. *147* (1943) 399.
9 Nelson, P.J., Pruitt, R.E., Henderson, L.L., Jenness, R., and Henderson, L.M., Biochim. biophys. Acta *672* (1981) 123.

0014-4754/83/050512-02$1.50 + 0.20/0

A further naphthoquinone derivative from the fungus *Hendersonula toruloidea*

G.W. van Eijk and H.J. Roeymans[1,2]

Centraalbureau voor Schimmelcultures, P.O. Box 273, NL-3740 AG Baarn (The Netherlands), August 18, 1982

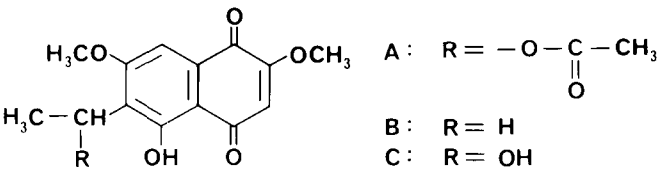
Summary. A further yellow naphthoquinone derivative was isolated as a metabolite from several human pathogenic strains of *Hendersonula toruloidea* Nattrass. It was identified as 2,7-dimethoxy-5-hydroxy-6-(1-hydroxyethyl)-1,4-naphthoquinone by means of physico-chemical methods.

In a previous paper[3] the isolation and identification of 2,7-dimethoxy-5-hydroxy-6-(1-acetoxyethyl)-1,4-naphthoquinone (compound A) and 2,7-dimethoxy-6-ethyl-5-hydroxy-1,4-naphthoquinone (compound B) from several human pathogenic strains of *Hendersonula toruloidea* Nattrass have been described. The compounds showed R_f-values of 0.31 and 0.47 on silica gel thin-layer plates using the solvent system toluene/acetone = 95:5 (v/v). During further investigations a yellow spot (R_f 0.19) of a minor metabolite (C) from some strains was detected.

The identification was undertaken with *H. toruloidea* CBS 137.77, since this strain produced compound C in sufficient quantities to warrant successful isolation. The strain was grown on malt agar in 225 petri dishes for 20 days at 24 °C. To avoid possible hydrolysis, the cultures were extracted with chloroform instead of ethyl acetate, which was used in previous experiments. The evaporated extract (496 mg) was chromatographed on a 30 × 3 cm silica gel/kieselguhr (2:1 w/w) column using toluene/acetone = 4:1 (v/v). The eluted yellow fraction was applied to several 2 mm silica gel thick-layer plates (Merck) and developed with toluene/acetone = 95:5 (v/v).

The yellow bands comprising compound C were scraped off and eluted with chloroform/methanol = 2:1 (v/v). For a final purification the fraction was chromatographed after concentration on 0.5 mm silica gel plates with the solvent system toluene/acetone = 4:1 (v/v). C was obtained as optically inactive orange needles by twice recrystallizing from toluene/light petroleum 60–80 °C (1:1, v/v). C showed an UV-visible spectrum similar to those of A and B, indicating the same chromophoric system.

High resolution MS of C gave the formula $C_{14}H_{14}O_6$. The MS- and UV-visible data indicated that C compared with B must have a hydroxyl group in the ethyl side-chain. Since the mass-spectrum showed the elimination of an aliphatic methyl it was concluded that the side-chain was the 1-hydroxyethyl group. Confirmation of this conclusion was obtained by examination of the ^{1}H-NMR-spectrum. Typical resonances were observed: a doublet ($-CH_3$) at 1.55 (δ in ppm, J = 6.75 Hz), a doublet (−OH) at 3.72 (J = 11.95 Hz), a double quartet (1H) at 5.30 (J = 11.95, 6.75 Hz) and a singlet (−OH) at 12.88. The signals at 3.72 and 5.30 pointed to a slow rate of exchange of the proton of



the OH at the ethyl group, due to hydrogen bonding with the oxygen of the *peri*-OH group. Addition of a drop of D_2O caused the disappearance of the signals at 3.72 and 12.88 and the signal at 5.30 showed a normal quartet. The difference between the 2 OH groups was clearly demonstrated by measuring the NMR-spectrum of the original $CDCl_3$ solution after standing for 45 days. The signal at 3.72 was not observed, whereas the signal at 12.88 was still present (no exchange, due to strong hydrogen bonding of the *peri*-OH with the quinone carbonyl group). Thus the structure of C had been established as 2,7-dimethoxy-5-hydroxy-6-(1-hydroxyethyl)-1,4-naphthoquinone. Using the same gas chromatographic conditions as described earlier[3] compound C could be easily detected (retention time A = 4.40; B = 3.80; C = 4.75 min). In this way the presence of C was also demonstrated in the strains CBS 136.77 and M 48[3].

Efforts to synthesize C by hydrolysis of A in alkaline methanol failed. Only very small amounts of C were formed. The main product was a naphthoquinone derivative with a molecular weight of 292. IR, UV-vis. and PMR[4] studies indicated that the compound was 2,7-dimethoxy-5-hydroxy-6-(1-methoxyethyl)-1,4-naphthoquinone.

Since C showed no optical activity, the question was considered, whether the compound had been obtained as an artefact of the purification process. In our opinion this was not the case. Extraction procedures were carried out rapidly throughout this study. The presence of C in extracts of CBS 137.77 could already be demonstrated by TLC after an extraction period of only 2 min, whereas C was not found in extracts of cultures of CBS 131.78 and CBS 145.78, both good producers of A and B[3], even after a storage period of 1 week in the refrigerator. Therefore, we concluded that C is a natural compound.

Otomo et al.[5] recently reported the isolation of B and C (also optically inactive) from *Guignardia laricina* (Sawada) Yamamoto & Ito, a fungus belonging to the Botryosphaeriaceae[6] and considered to be the cause of shoot blight of larches. Its anamorph is classified as *Macrophoma* spec. by the authors[7] (*Phyllosticta* ss van der Aa[8]). The Botryosphaeriaceae include never conidial fungi belonging to *Hendersonula* and related genera, and *Scytalidium*. Campbell and Mulder[9] have described several strains belonging to the *Hendersonula toruloidea* complex as *Scytalidium hyalinum*. Although *H. toruloidea* is not strictly congeneric with the type species of the genus, *H. australis* (Sutton[10]), it can be concluded that neither *H. toruloidea* nor *Scytalidium* is related to *G. laricina* although they produce the same naphthoquinone pigments.

Physico-chemical data. Compound C: m.p. 202–204 °C; optically inactive; mol.wt 278.0795 (calculated for $C_{14}H_{14}O_6$ 278.0790). MS: 278 (M^+), 263 (100%), M* 209.98: transition $263^+ \rightarrow 235^+ + 28$ (CO); λ_{max} (MeOH): 222.5 (log ε 4.38), 232sh (4.19), 258 (4.18), 262 (4.18), 310 (3.98), 426 nm (3.62); ν_{max} (KBr): 1688, 1640, 1605 cm^{-1}; PMR (90 MHz, $CDCl_3$): 1.55 ($-CH_3$, d, J = 6.75 Hz), 3.72 (−OH, d, J = 11.95 Hz), 3.92 ($-OCH_3$, s), 4.00 ($-OCH_3$, s), 5.30 (>CH−, dq, J = 11.95, 6.75 Hz), 6.02 (>C=CH−, s), 7.27 (aromatic H, s), 12.88 δ_{TMS} (−OH, s).

1 The authors are indebted to Mr C. Versluys, Analytical Laboratory, State University of Utrecht, for measuring the mass-spectra.
2 Thanks are due to Mr A. George, Laboratory for Organic Chemistry, State University of Utrecht, who measured and interpreted the PMR-spectrum.
3 van Eijk, G.W., and Roeymans, H.J., Experientia *34* (1978) 1257.
4 We thank Dr H. Sato, Hokkaido University, Sapporo for PMR-data of this major product.
5 Otomo, N., Sato, H., and Sakamura, S., Agric. Biol. Chem. *43* (1982) 861.
6 von Arx, J.A., and Müller, E., Stud. Mycol. *9* (1975) 1.
7 Yamamoto, W., Sci. Rep. Hyogo Univ. Agric. Ser. Agric. Biol. *5* (1961) 1.
8 van der Aa, H.A., Stud. Mycol. *5* (1973) 1.
9 Campbell, C.K., and Mulder, J.L., Sabouraudia *15* (1971) 161.
10 Sutton, B.C., The Coelomycetes, p. 347. Commonwealth Mycological Institute, Kew 1980.

0014-4754/83/050513-02$1.50 + 0.20/0

Disruption of the goblet cell intercellular junction following histamine infusion of the rabbit ileum[1,2]

M. Porvaznik[3,4], W. Baker and R.I. Walker[5]

Experimental Hematology Department, Armed Forces Radiobiology Research Institute, Bethesda (Maryland 20814, USA), September 28, 1982

Summary. Histamine infused into the mesenteric artery of the rabbit ileum ruptured tight junctional complexes linking goblet cells with neighboring absorptive epithelial or other goblet cells. Loss of tight junctional integrity induced by histamine could contribute to extra-intestinal endotoxemia reported after a variety of injuries.

Histamine and other vasoactive substances, increased in mammalian tissues following trauma, could enhance intestinal permeability[6]. For example, infusion of vasoactive substances into rabbits permitted endotoxin from gram negative enteric microorganisms to move transmurally into the peritoneal cavity[7]. A mechanism for increased permeability of the intestine to endotoxin has been suggested. Following challenge with endotoxin[8], radiation trauma[9,10], or full dermal thickness wounds (Porvaznik, unpublished), the tight junctional barriers (zonulae occludens) between goblet-epithelial cells in the ileum became disrupted. In irradiated animals this disruption was associated with the presence of endotoxin in extra-intestinal tissues[11].

If disruption of junctional barrier provides a pathway for the escape of endogenous endotoxin present in the intestine, a substance such as histamine, which has been associated with escape of endotoxin from the intestine, may also mediate the disruption of these junctional barriers. We tested the hypothesis that histamine could be responsible for disruption of tight junctions by infusing normal rabbits with histamine and subsequently determining the integrity of their junctional barriers.

New Zealand White male rabbits weighing 1.5–2.0 kg were anesthetized by delivery of methoxyflurane (Metofane, Pittman-Moore, Inc., Treton, NJ 08619) and oxygen via a Frazier-Sweatman gas anesthesia apparatus. The descend-