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THREE NEW *p*-BENZOQUINONES OBTAINED DURING ISOLATION OF SHANORELLIN FROM CULTURES OF *SHANORELLA SPIROTRICHA*

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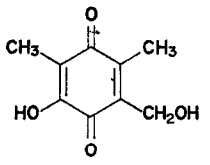
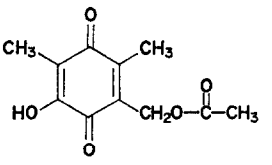
Abstract—During the isolation of shanorellin (2,6-dimethyl-3-hydroxymethyl-5-hydroxy-1,4-benzoquinone) from the culture media of *Shanorell spirotricha* Benjamin, three other *p*-benzoquinones have been obtained and identified. These are a monoacetate and an ethyl ether of shanorellin as well as a dimer with an ether linkage at the primary alcohol function These three new benzoquinones are proved to be artifacts derived from shanorellin during the isolation process

INTRODUCTION

SHANORELLIN (I) is the major pigment excreted into culture media by the Ascomycete *Shanorella spirotricha* Benjamin.[1] In the course of isolating shanorellin, three other chemically related colored compounds were observed and these have been isolated. Their structures have been elucidated (II, III and IV) and shown to be closely related to shanorellin.



(I)



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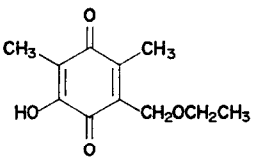

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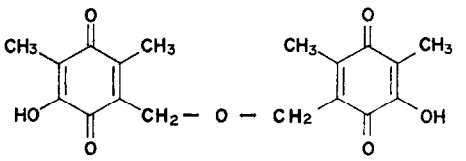

(IV)

Since ethyl acetate was used to extract the acidified culture filtrate, the possibility that II, III, and IV were formed from shanorellin during the isolation process was examined. TLC of an ether extract of the broth at pH 2 yielded shanorellin alone, while the residue obtained by evaporating an acidic solution of shanorellin in ethyl acetate showed all four compounds. Thus, the new benzoquinones are artefacts derived from shanorellin and are not produced by the fungus.

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[1] C-K WAT, A. TSE, R J BANDONI and G. H N. TOWERS, *Phytochem* 7, 2177 (1968).

RESULTS

Identification of Shanorellin Acetate (II)

The compound crystallized from benzene–petroleum as bright orange-yellow needles, m.p. 128°, and sublimed at 100° under high vacuum. As with shanorellin its solutions were decolorized by $NaBH_4$ and sodium dithionite and, on basifying and aerating, turned purple. The u.v. spectrum showed a strong band at 269 nm with a shoulder at 274 nm and a weak band at 395 nm, similar to that of many hydroxybenzoquinones.[2] The i.r. spectrum showed bands characteristic of shanorellin, with addition of an ester function (C=O stretching at 1740 cm^{-1}). The NMR spectrum revealed five types of protons (all singlets), in the proportion of 1·2:3:3:3 at τ 3·04, 5·00, 7·86, 7·97 and 8·07. The peak at τ 3·04 disappeared on addition of D_2O. The mass spectrum gave a molecular ion at *m/e* 224·0681, as required for shanorellin monoacetate ($C_{11}H_{12}O_5$, 224·06848), and showed fragment ions at *m/e* 53, 55, 67, 80, 83, 108, 136, 154 and 182 which are also observed in the mass spectrum of shanorellin. The acetate ion (*m/e* 43) was the base peak. Hydrolysis with dilute NaOH for one minute gave a compound with the same u.v. spectrum and R_f values on TLC in solvent systems A and D (Table 1) as shanorellin.

TABLE 1. R_f VALUES ON SILICA GEL G PLATES

Compounds	A	B	C	D
II	0 55	0 79	0 45	0 79
III	0 60	0 83	0 51	0 85
IV	0 49	0·77	0 44	0·76

Solvent Systems (all mixtures by volume)· A, C_6H_6–HOAc(9·1), B, $CHCl_3$–HOAc(500:37:5), C, Cyclohexane–EtOAc–HOAc (20 10·1), D, $CHCl_3$–HOAc (9·1).

Identification of Shanorellin Ethyl Ether (III)

This compound was obtained as orange-yellow needles, m.p. 43–44°, and sublimed readily at 78° under high vacuum. As with shanorellin it changed color reversibly from orange to purple by altering the pH of the solvent. The u.v. spectrum showed λ_{max} 269·5 (log ϵ 4·14) and 404 nm (log ϵ 2·76). The i.r. spectrum showed stretching vibrations at 3400 (O—H), 2895, 2940 and 2985 (alkane C—H), 1655 and 1645 (quinone C=O), 1630 (C=C), and 1105 cm^{-1} (ether C—O). The NMR spectrum resembled that of shanorellin acetate, except that the acetyl (CH_3CO) signal was replaced by signals for an ethyl function, with methylene protons at τ 6·43 (quartet, J = 7 Hz) and methyl protons at τ 8·80 (triplet, J = 7 Hz). The signal at τ 2·86, disappearing on addition of D_2O, could be assigned to nuclear hydroxyl, and those at τ 5·58, 7·83 and 8·07 to the nuclear methylene and two methyl protons respectively. The mass spectrum gave a molecular ion at *m/e* 210·0891, corresponding to a molecular formula $C_{11}H_{14}O_4$ (210·08921). The fragmentation pattern was similar to that of shanorellin with ions at *m/e* 39, 53, 55, 67, 80, 83, 108 and 136. The base peak at *m/e* 31 could be derived by α,β-cleavage of an ethyl ether with a single hydrogen transfer.[3] All of this information can be accommodated by structure III.

[2] R. A. MORTON, in *Biochemistry of Quinones* (edited by R A MORTON), p. 23, Academic Press, London (1965).

[3] J. H. BEYNON, R. A SAUNDERS and A E WILLIAMS, in *The Mass Spectra of Organic Molecules*, p. 231, Elsevier, New York (1968).

Identification of the bis-*Benzoquinone (IV)*

This compound crystallized from benzene-petroleum as fine needles of a dull orange-yellow color, m.p. 170°, and sublimed at 145° under high vacuum. Its behavior to treatment with $NaBH_4$ and dithionite was similar to that of shanorellin. The u.v. spectrum had two peaks with λ_{max} 268 (log ϵ 4·41) and 405 nm (log ϵ 3·07). The i.r. spectrum showed stretching vibrations at 3330 (O—H), 1620 (C=C), 1660 and 1640 (quinone C=O), and 1085 cm^{-1} (ether C—O). The NMR spectrum showed four types of protons (all singlets) at τ 2·95, 5·50, 7·825 and 8·02 in the proportions 1 2.3 3. The τ values are quite similar to those of shanorellin[1] and the peak at τ 2·95 disappeared on addition of D_2O. The mass spectrum showed a molecular ion at *m/e* 346·1045, corresponding to a molecular formula of $C_{18}H_{18}O_7$ (346·10526). The base peak at *m/e* 83 and those at *m/e* 53, 55, 67, 108 and 136 also occur in the mass spectrum of shanorellin. These properties are accommodated by structure IV.

EXPERIMENTAL

Culture

Shanorella was cultured in 1 l. flasks with 400 ml of modified Czapek–Dox medium under the conditions described previously [1]

Isolation of Metabolites

The isolation procedure described for shanorellin was followed [1] The three metabolites described in this communication ran together as the second orange-yellow band, preceding shanorellin on the silicic acid· Hyflo Super Cel column. They were separated from one another by preparative TLC on silica gel G plates. Their R_f values in four solvent systems are listed in Table 1 The material eluted from TLC was purified further by sublimation and crystallization from benzene–petroleum (b p. 60–120°).

Formation of Artifacts

Shanorellin (10 mg) in ethyl acetate (5 ml) was treated with a drop of conc. HCl at ambient temperature, and evaporated to dryness under an air jet

Instrumentation

M.ps were determined with a Thomas–Hoover Capillary Melting Point Apparatus, and are uncorrected. Electronic absorption spectra were obtained in $CHCl_3$. I.r. spectra were recorded in KBr discs A Varian model HA-100 spectrometer was used to obtain NMR spectra with $CDCl_3$ as solvent and tetramethylsilane as internal standard. Accurate mass measurements were made with a Consolidated Electrodynamics Corporation model 110-B mass spectrometer using perfluorokerosene as reference.

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