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SHORT COMMUNICATION

SHANORELLIN: A NEW SUBSTITUTED BENZOQUINONE FROM *SHANORELLA SPIROTRICHA* (ASCOMYCETES)

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Abstract—The isolation and identification of shanorellin (I) (2,6-dimethyl-3-hydroxymethyl-5-hydroxy-1,4-benzoquinone) from the culture medium of an ascomycete, *Shanorella spirotricha* (Benjamin) is described.

INTRODUCTION

BENZOQUINONES are distributed widely in nature. By virtue of their metabolic function they are often considered in two categories: those that are essential, such as the ubiquinones and plastoquinones, and those to which no essential metabolic function has as yet been ascribed, such as the substituted benzoquinones of fungi, higher plants, insects and arachnids.[1–3]

Benzoquinones which have been isolated from fungi are either simple benzoquinones, bibenzoquinones or terphenylquinones.[2, 4] Shanorellin, which is described in this paper, is a monobenzoquinone obviously related to the compounds which have been isolated from species of *Aspergillus* and *Penicillium*.[2, 4] It was discovered as an extracellular purple pigment, diffused through the agar, in cultures of the organism.

RESULTS AND DISCUSSION

Shanorellin ($C_9H_{10}O_4$) crystallizes as bright orange–yellow needles (m.p. 121°) from benzene-light petroleum; sublimes at 100° under vacuum; and has two broad maxima in the u.v., at 272 nm (log ϵ 4·05) and 406 nm (log ϵ 2·07) respectively. Its solutions are decolorized by sodium borohydride or sodium dithionite and the color is restored on aeration after adjusting the pH to 8.[5] The colorless form of the compound shows λ_{max} at 353 nm. The mass spectrum (Fig. 1) showed a molecular weight of 182. These properties are consistent with those of a substituted hydroxybenzoquinone.[6]

The i.r. spectrum shows two bands at 1660 and 1640 cm^{-1} (carbonyl stretching), one at 1620 cm^{-1} (C=C stretching),[7] another two at 3180 and 3450 cm^{-1} (hydroxyl stretching) and two more in the region of 2900–3000 cm^{-1} (alkane C—H stretching).

[1] R. A. MORTON, in *Biochemistry of Quinones*, p. 3, Academic Press, New York (1965).
[2] R. H. THOMSON, in *Naturally Occurring Quinones*, p. 6, Butterworths, London (1957).
[3] C. MATHIS, in *Comparative Phytochemistry* (edited by T. SWAIN), p. 245, Academic Press, New York (1966).
[4] S. SHIBATA, S. NATORI and S. UDAGAWA, in *List of Fungal Products*, p. 65, C. C. Thomas, Illinois (1964).
[5] J. CASON, *Org. Reaction* **4**, 305 (1948).
[6] R. A. MORTON, in *Biochemistry of Quinones*, p. 38, Academic Press, New York (1965).
[7] P. YATES, M. I. ARDAO and L. F. FIESER, *J. Am. Chem. Soc.* **78**, 650 (1956).

The NMR spectrum reveals the presence of five types of protons in the proportion of 1:2:1:3:3 (τ, 3·06, 5·475, 7·75, 7·885 and 8·075). On addition of D_2O the peak at τ 3·06 and the broad band around τ 7·75 disappear indicating the presence of two exchangeable protons. Of the remaining peaks, that at τ 5·475 can be assigned to methylene protons and the other two, at τ 7·885 and 8·075, to protons of two methyl groups. On expansion of the sweep width of the spectrum (10×) the methylene protons appear as a quartet, and the methyl protons at τ 8·075 appear as a triplet. These two groups of protons are thus coupled with each other (coupling constant $J = 0{\cdot}5$ Hz). This coupling constant is too small for a CH_3CH_2 group but could account for a methyl and a methylene group on adjacent carbons, on the same side of the quinone nucleus.

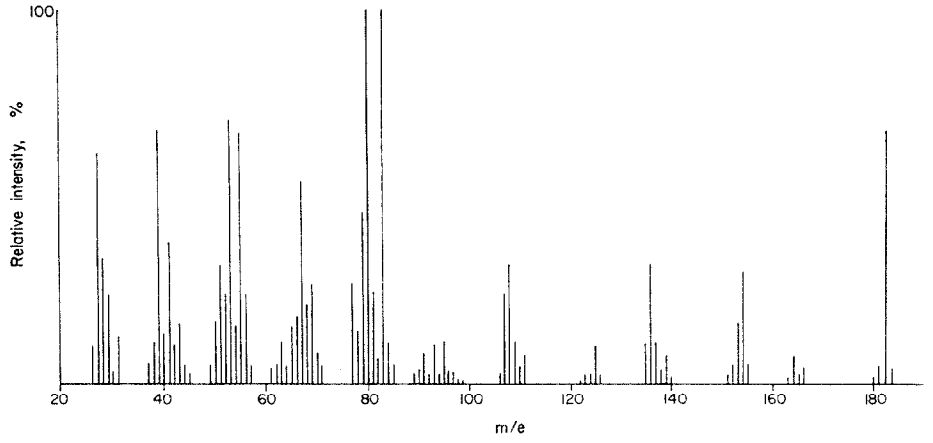
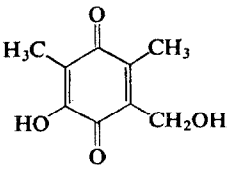


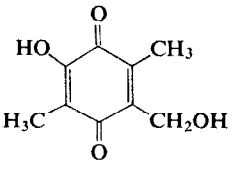
FIG. 1

A diacetate and a tetraacetate of shanorellin were prepared and the NMR spectra obtained. The preparation of these derivatives confirms the presence in the molecule of two hydroxyl groups in addition to the two carbonyl functions of the benzoquinone nucleus.

With the above information it was possible to assign either of the two isomeric structures shown below to shanorellin.



(I)



(II)

The correct structure (I) was obtained by X-ray crystallographic analysis of a chloro derivative (2,6-dimethyl-5-hydroxy-3-chloromethyl-1,4-benzoquinone). This was determined by Dr. E. Subramanian of the Chemistry Department and is to be the subject of a separate communication.

EXPERIMENTAL

Organism and Culture Conditions

The organism *Shanorella spirotricha* (Benjamin) UBC240, was cultured in Roux bottles with 100 ml of the following modified Czapek-Dox medium per bottle: glucose 50 g, $NaNO_3$ 2 g, K_2HPO_4 1 g, KCl 0·5 g, $MgSO_4.7H_2O$ 0·5 g, $FeSO_4.7H_2O$ 0·01 g, yeast extract (Difco) 2 g, distilled water 1 l. Each bottle was innoculated with 2·5 ml of a spore preparation. All cultures were maintained at 25° in a Sargent Incubator.

Isolation of Shanorellin

After 18 days' growth, the mycelial mat was filtered off and washed with distilled water until the filtrate became colorless. The filtrate was aerated for 30 min, acidified with dil HCl to pH 2 and extracted with ethyl acetate. After the removal of ethyl acetate with a flash evaporator, the residue was dissolved in $CHCl_3$ and the solution passed through a column of silicic acid with $CHCl_3$ as the eluting solvent. The shanorellin band, which appeared as the major one on the column, was collected. The residue obtained on removal of the solvent from this fraction was sublimed at 100° under high vacuum. The sublimate was crystallized three times from benzene and light petroleum (b. range 65–120°)[m.p. 121°, MW 182 (mass spectrum Fig. 1). Found: C, 58·81; H, 5·56. $C_9H_{10}O_4$ required: C, 59·3; H, 5·5 per cent.]

Preparation of Derivatives

Shanorellin diacetate. To shanorellin (100 mg) in acetic anhydride (2 ml) was added conc. H_2SO_4 (2 drops) and the solution warmed for a few sec. The diacetate separated out as a brownish yellow oil on addition of ice water and was extracted with ethyl ether. On evaporation of the ether, a viscous brownish yellow oil (93·8 mg) was obtained which could not be crystallized from either light petroleum or methanol. TLC on silica gel G with benzene:acetic acid (9:1 v/v) showed the presence of one compound at R_f 0·55. The R_f of shanorellin under the same conditions was 0·14. (NMR spectrum: $2CH_3$ at τ 7·87, 8·03; CH_2 at τ 5·4 and $2CH_3CO$ at τ 7·68 and 7·98.)

Shanorellin tetra-acetate. Shanorellin (100 mg) in ethanol (5 ml) was treated with $NaBH_4$ until the yellow color disappeared. Water (20 ml) was added, and the solution extracted four times with ethyl ether (20 ml). The ether extract was dried (Na_2SO_4) and the solvent removed. Acetylation with acetic anhydride (2 ml) and pyridine (1 ml) overnight at room temperature followed by addition of ethanol (10 ml) and evaporation *in vacuo* below 40° until the pyridine was removed gave a slightly yellowish gummy residue. Crystallization from methanol afforded the colorless tetra-acetate. (M.p. 112°, yield 47 mg. NMR spectrum: 2 CH_3 at τ 7·87 and 8·05; CH_2 at τ 4·95; 4 CH_3CO at τ 7·7, 7·74, 7·18 and 8·03).

Shanorellin chloride. Thionyl chloride (65·45 mg) was added dropwise to shanorellin (91 mg) in $CHCl_3$ (20 ml) and pyridine (39·5 mg) in an ice bath. After the addition the solution was cooled to room temperature and then refluxed for 2·5 hr. The solvent was removed *in vacuo* and the residue chromatographed on silicic acid column with $CHCl_3$ as eluting solvent. The major band was collected and further purified on silica gel G plates with benzene:acetic acid (9:1 v/v) as the developing system. The chloride band had a R_f of 0·6. It was crystallized from hexane and $CHCl_3$ giving orange-yellow needles. (Yield: 46 mg, m.p. 69°, MW 200·5. Found: C, 53·67; H, 4·4; Cl, 17·66. $C_9H_9O_3Cl$ required. C, 53·86; H, 4·48; Cl, 17·71 per cent. NMR spectrum: 2-CH_3 at τ 7·8 and 8·03; —CH_2— at τ 5·53; —OH at τ 2·985.)

Spectroscopic Determinations

U.v. and i.r. spectra were taken with a Unicam SP. 800 and a Unicam SP. 200G respectively. The NMR spectrum was obtained with a Varian model HA-100 and the mass spectrum with an Associated Electrical Industries Model MS9 instrument.

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