Metabolites of *Hyalodendron* sp.: Bisdethiodi(methylthio)hyalodendrin

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Spectroscopic studies and chemical correlation with hyalodendrin show that a new metabolite of *Hyalodendron* sp. is bisdethiodi(methylthio)hyalodendrin (3-benzyl-6 hydroxymethyl-1,4-dimethyl-2,5-dioxo-3,6-di(methylthio)piperazine).

Les études spectroscopiques et une correlation chimique avec l'hyalodendrine démontrent qu'un métabolite nouveau d'une espèce d'*Hyalodendron* est la bisdethiodi(méthylthio)hyalodendrine (3-benzyl-6-hydroxyméthyl-1,4-diméthyl-2,5-dioxo-3,6-di(méthylthio) piperazine).

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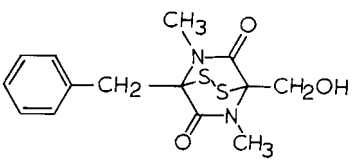
A recent addition to the group of biologically active epidithiodiketopiperazine fungal metabolites (1) is hyalodendrin (**1**),[1] produced by a *Hyalodendron* sp. (2, 3). We report here the isolation and identification of a new, related metabolite, bisdethiodi(methylthio)hyalodendrin (**2**), produced in culture by this organism.



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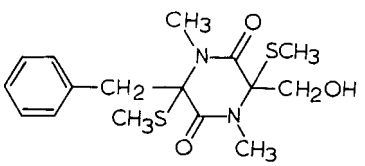


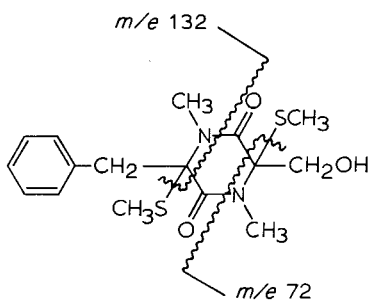
2

Comparison of the spectroscopic characteristics of the new metabolite, $C_{16}H_{22}N_2O_3S_2$, with those of hyalodendrin (**1**), indicated that the former compound is also an *N*-methylserine-*N*-methylphenylalanine diketopiperazine, bearing substitution at the 3 and 6 positions. Thus, **2** displayed i.r. absorption indicative of amide and hydroxyl functionality, as well as peaks attributable to a monosubstituted phenyl group (see Experimental section) (2, 4). In the n.m.r. spectrum (220 MHz, $CDCl_3$), the phenyl hydrogens give rise to multiplets at δ 7.12 (2H) and 7.26 p.p.m. (3H). An AB quartet centered at δ 3.44 (2H, J = 14 Hz) represents the benzylic protons, and demonstrates the tetrasubstituted character of the neighboring carbon atom. Coupling with the hydroxyl proton (J = 6 and 7 Hz), as well as geminal coupling (J = 12 Hz) is observed for the two hydrogens attached to the primary carbinol center, and they appear as doublets of doublets at δ 3.14 and 3.85 p.p.m. The hydroxyl proton is manifested as a broadened unsymmetrical "triplet" at δ 1.43; as expected, irradiation at 314 Hz collapsed the signal for the carbinol protons to an AB quartet (J = 12 Hz). Singlets (3H each) at δ 3.03 and 3.29 p.p.m. represent the methyl groups on the amide nitrogens, and singlets (3H each) at δ 2.13 and 2.30 p.p.m. are assigned to two methylthio groups (5–7), which can only be located at the 3 and 6 positions of the diketopiperazine system, as in structure **2**. The presence of benzyl and —CH_2OH functionality, as well as the two methylthio groups was confirmed by the mass spectral fragmentation pattern (see Experimental section). Ions at *m/e* 132 and 72 can be rationalized in terms of the cleavages indicated in Scheme 1 (*c.f.* ref. 8).

Correlation with hyalodendrin corroborated the formulation **2**. Thus, treatment of the former with sodium borohydride in the presence of methyl iodide and pyridine (5) at 0° gave a di(methylthio) ether, identical in all respects with the metabolite **2**. Sodium borohydride reduction of epidithiodiketopiperazines proceeds with retention of configuration (1, 5).

Isolation of bisdethiodi(methylthio) modifications of epidithiodiketopiperazine metabolites

[1]The absolute configuration of the bridged diketopiperazine system of hyalodendrin is antipodal to that of gliotoxin (2).



SCHEME 1

has precedents in similar derivatives of sporidesmin (5), acetylaranotin (6), and acetylapoaranotin (7). Since these metabolites lack the striking biological activity of their bridged counterparts (1, 9), it is not surprising that **2** was devoid of antifungal activity in a series of bioassays (3).

Experimental

See ref. 1 for description of instrumentation, etc. The 220 MHz n.m.r. spectrum of **2** was recorded for a solution in deuteriochloroform on a Varian HR-220 instrument at the Canadian 220 MHz N.M.R. Center, Ontario Research Foundation.

Isolation of Metabolites

The production and isolation of hyalodendrin have been detailed elsewhere (2, 3). Material extracted with chloroform from filtered culture medium which had supported growth of *Hyalodendron* sp. for 10 to 12 days was chromatographed on a column of silica gel (Kieselgel: 100–200 mesh, Gebr. Herrmann, Köln). Elution with benzene–chloroform (7:3) afforded hyalodendrin (after crystallization, *ca.* 100 mg/l of culture medium) as described previously (2, 3). Bisdethiodi(methylthio)hyalodendrin (**2**) was subsequently eluted with benzene–chloroform (1:1) and further purified by recrystallization from methylene chloride – cyclohexane to give colorless needles, m.p. 140–140.5°; $[\alpha]_D^{23}$ +64° (*c*, 1.071 in $CHCl_3$); ν_{max} (KBr) *inter alia*, 3375, 1660, 1644, 1636, 1499, 1375, 735, and 700 cm^{-1}; ν_{max} (CCl_4) 3570, 1657, and 1380 cm^{-1}; λ_{max} (EtOH) <220, *ca.* 260 nm (inflection, $\varepsilon \sim 900$). The n.m.r. data are given in the text. Mass spectrum *inter alia*, *m/e* 354 (low intensity, M^+), 323 $(M - CH_2OH)^+$, 307 (base peak) $(M - SCH_3)^+$, 277 $(M - SCH_3 - CH_2O)^+$, 276 $(M - SCH_3 - CH_2OH)^+$, 263 $(M - C_7H_7)^+$, 260 $(M - SCH_3 - SCH_3)^+$, 132 $(C_9H_{10}N)^+$, 91 $(C_7H_7)^+$, and 72 $(C_3H_6NO)^+$. Accurate mass measurements: 307.1107 ($C_{15}H_{19}N_2O_3S$ requires 307.1116), 132.0809 ($C_9H_{10}N$ requires 132.0813).

Anal. Calcd. for $C_{16}H_{22}N_2O_3S_2$: C, 54.21; H, 6.26; O, 13.54; N, 7.90; S, 18.10. Found: C, 54.18; H, 6.30; O, 13.41; N, 7.95; S, 18.02.

While growth of *Hyalodendron* sp. in liquid culture as described above (3) leads to isolation of **1** as the major metabolite, longer fermentation periods appear to favor the formation of **2**.

Conversion of Hyalodendrin into 2

A solution of hyalodendrin (200 mg) in pyridine (1 ml) was treated with methyl iodide (5 ml) at 0° in a nitrogen atmosphere. An ice-cold solution of sodium borohydride (100 mg) in methanol (7 ml) was added dropwise to the stirred suspension and the resulting pale yellow solution was stirred for 30 min at 0°, during which the color faded. Additional methyl iodide (1 ml) was added and the solution was allowed to attain room temperature during 3 h. The resulting pale yellow solution was evaporated *in vacuo* at 30 to 40° and the residue was taken up into methylene chloride and water. The aqueous phase was adjusted to p*H* 6 by addition of 1 *N* HCl and the organic layer was then washed with water, dried ($MgSO_4$), and evaporated. Preparative layer chromatography of the residue on a silica gel plate using the solvent system benzene–hexane–acetone (5:4:1) afforded 215 mg of crude crystalline bisdethiodi(methylthio)hyalodendrin, which was recrystallized from methylene chloride – cyclohexane to give 188 mg (86%) colorless needles, m.p. 138–140°. Crystals melting at 139.5–140° were obtained on further recrystallization from methylene chloride – cyclohexane. Comparison of chromatographic behavior and spectra, and mixture melting point determination established the identity of the product with the natural metabolite **2**.

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