Hyalodendrin: a New Fungitoxic Epidithiodioxopiperazine produced by a *Hyalodendron* Species

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A new fungitoxic metabolite, designated hyalodendrin, isolated from a culture medium which had supported growth of *Hyalodendron* sp., an imperfect fungus, was identified as 3-benzyl-6-hydroxymethyl-1,4-dimethyl-3,6-epidithiopiperazine-2,5-dione on the basis of spectrometric and chemical investigations.

ANTIFUNGAL properties have recently been observed to be associated with a species of *Hyalodendron*, an imperfect fungus.[1] A search for the principles responsible for this activity has led to the isolation of a new fungitoxic metabolite, designated hyalodendrin, for which the structure (1) has been deduced as follows.

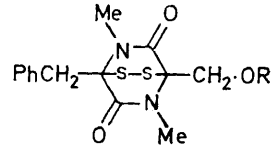
Hyalodendrin, $C_{14}H_{16}N_2O_3S_2$, is optically active. A prominent feature in its mass spectrum is the loss of sulphur,[2] which results in ions at *m/e* 260 $(M - S_2)^{+\cdot}$ and 64, and is also manifest in other major fragment ions. Intense peaks at *m/e* 91 and 169 $(M - S_2 - C_7H_7)^+$, as well as i.r. absorption characteristic of a monosubstituted benzene ring[3] (see Experimental section) suggested the presence of a benzyl group, which was confirmed by the n.m.r. spectrum [δ 7·33 (5H, s) and 3·86 (2H, ABq, *J* 16 Hz, $PhCH_2$)]. The nature of the latter signal indicated that the benzyl group is attached to a tetrasubstituted carbon atom.

The presence of alcohol functionality was indicated by i.r. absorption at 3550 cm^{-1}, and confirmed by the mass spectrum, in which the base peak at *m/e* 242 corresponds to the ion $(M - S_2 - H_2O)^{+\cdot}$. The single (exchangeable) hydroxy-proton gives rise to an n.m.r. multiplet at δ 3·68, and two hydrogen nuclei on the hydroxy-bearing carbon atom are revealed as a broad singlet at δ 4·35. In the spectrum of hyalodendrin monoacetate (2), $C_{16}H_{18}N_2O_4S_2$, the latter signal was shifted downfield, and appeared as an AB quartet at δ 4·89 (*J* 13 Hz), demonstrating the tetrasubstituted nature of the neighbouring carbon atom. The absence in hyalodendrin monoacetate of OH and NH groups was evident from the i.r. spectrum.

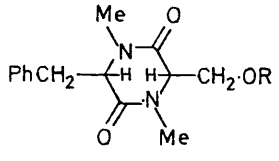
Amide functionality was evidenced by intense absorption at 1675 cm^{-1} in the i.r. spectrum of hyalodendrin, and n.m.r. singlets (3H each) at δ 2·98 and 3·22 were attributed to methyl groups on the amide nitrogen atoms.

Treatment of hyalodendrin with aluminium amalgam effected reductive removal of the disulphide bridge,[4] and an optically inactive product (3), $C_{14}H_{18}N_2O_3$, was isolated.† This transformation was accompanied by a lowering of the i.r. amide carbonyl band frequency to 1655 cm^{-1}. The signals in the n.m.r. spectrum corresponding to the newly introduced methine protons [δ 3·79 (dd) and 4·20 (t)] are characteristic of the X proton in an ABX or AMX system, and an A_2X system respectively.[5] The two pairs of methylene protons (see before) with which these protons are coupled give rise to a multiplet at δ *ca.* 2·9—3·6, and singlets at δ 2·88 and 2·97 correspond to the two sets of *N*-methyl protons.

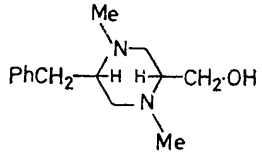
Detailed first-order analysis was more readily accomplished in the case of the spectrum of didethiohyalodendrin t-butyl ether (4), $C_{18}H_{26}N_2O_3$. Besides the



(1) R=H
(2) R=Ac



(3) R=H
(4) R=Bu^t



(5)

appropriate absorptions corresponding to phenyl, *N*-methyl, and t-butyl protons, this spectrum displayed an eight-line multiplet at δ 2·92—3·68, and a doublet of doublets at 3·86—3·97, together constituting a typical AMX pattern (*J* 10, 4, and 3 Hz).[5] The remaining protons give rise to a doublet at δ 3·32 (2H, *J* 6 Hz) and

† Partially racemized didethio-products were isolated in some runs. The *cis* t-butyl ethers derived from them displayed low positive optival rotations.

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a triplet at 4·14 (1H, J 6 Hz), a characteristic A_2X pattern.[5]

Further reduction of didethiohyalodendrin (3) with lithium aluminium hydride afforded the corresponding piperazine (5), $C_{14}H_{22}N_2O$, in which the n.m.r. signals for the N-methyl groups are appropriately shifted upfield to δ 2·30 and 2·43.[5]

These data are best rationalized in terms of the structures indicated for hyalodendrin and its derivatives. The structure assignments were rigorously confirmed by comparison of didethiohyalodendrin t-butyl ether (4) with a synthetic sample prepared from L-phenylalanine and L-serine.[6] Naturally derived and synthetic material exhibited identical chromatographic behaviour and solution spectra.*†

Hyalodendrin is a new member of the class of epidithiodioxopiperazines comprising gliotoxin,[7-9] the sporidesmins,[8,10-14] the aranotins,[2,15-18] chaetocin,[19] and verticillin A.[20]‡

EXPERIMENTAL

M.p.s were determined on a hot-stage apparatus. I.r. and u.v. spectra were recorded on Beckman IR-10 and DK-2A spectrophotometers, respectively. N.m.r. spectra were measured on a Varian T-60 spectrometer. Mass spectra, other than the high resolution spectrum of hyalodendrin, were obtained with a Hitachi–Perkin-Elmer RMU-6D spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan, U.S.A.

Hyalodendrin (*3-Benzyl-6-hydroxymethyl-1,4-dimethyl-3,6-epidithiopiperazine-2,5-dione*).—The production and isolation of hyalodendrin are described in detail elsewhere.[1] Material extracted with chloroform from filtered culture medium which had supported growth of *Hyalodendron* sp. was chromatographed on a column of Kieselgel (100—200 mesh, Gebr. Herrmann, Köln). Hyalodendrin (1) was eluted with benzene–chloroform (7 : 3), and crystallized from ether to give *prisms*, m.p. 100·5—101·5°, $[\alpha]_D^{22}$ +26° (*c* 1·06 in $CHCl_3$), $\nu_{max.}$ (KBr) 3490, 3060, 3030, 1675, 1603, 1500, 1453, 740, 705, and 690 cm^{-1},[3] $\nu_{max.}$ (CCl_4) 3550 and 1685 cm^{-1}, $\lambda_{max.}$ (EtOH) <220 and 260 nm (slight inflection, ε *ca.* 1000); for n.m.r. and mass spectral data see Discussion section; *m/e* 260·1166 ($M - S_2$, $C_{14}H_{16}N_2O_3$ requires 260·1161) and 91·0548 (C_7H_7 requires 91·0548) (Found: C, 51·7; H, 4·95; N, 8·65; O, 14·6; S, 19·75. $C_{14}H_{16}N_2O_3S_2$ requires C, 51·85; H, 4·95; N, 8·65; O, 14·8; S, 19·75%).

Hyalodendrin Acetate (2).—A solution of hyalodendrin (74 mg, 0·228 mmol) in acetic anhydride (0·5 ml) and pyridine (1 ml) was stirred at 25° under anhydrous conditions for 12 h. The resulting pale yellow solution was poured into ice–water and the mixture was extracted thoroughly with ether. The extracts were washed successively with *ca.* 1N-hydrochloric acid, water, and saturated aqueous solutions of sodium hydrogen carbonate and sodium chloride. The organic phase, after drying ($MgSO_4$) was evaporated *in vacuo* to give 67 mg of yellow oily product. Chromatography on preparative layer silica gel plates afforded pure *hyalodendrin acetate* (2) as a gum which resisted crystallization attempts, $\nu_{max.}$ (CCl_4) 1760 and 1700 cm^{-1}, δ ($CDCl_3$; 60 MHz) 2·17 (3H, s), 2·98 (3H, s), 3·17 (3H, s), 3·86 (2H, q, J_{AB} 16 Hz), 4·89 (2H, q, J_{AB} 13 Hz), and 7·33 (5H, s), *m/e* 302 $(M - S_2)^{+\cdot}$, 242 $(M - S_2 - CH_3COOH)^{+\cdot}$ (Found: C, 52·45; H, 5·05; N, 7·55; O, 17·45. $C_{16}H_{18}N_2O_4S_2$ requires C, 52·45; H, 4·95; N, 7·65; O, 17·45%).

Didethiohyalodendrin (3).—To a solution of hyalodendrin (403 mg, 1·24 mmol) in absolute ethanol (300 ml) was added aluminium amalgam [4] prepared from aluminium turnings (4 g).[21] Water (1 ml) was added and the hydrogen sulphide evolved during the reduction was swept out by slow passage of nitrogen through the mixture. The mixture was stirred vigorously for 24 h after which hydrogen sulphide could no longer be detected in the emerging gas stream. The resulting thick grey suspension was filtered, and the residual solids were washed thoroughly with ethanol. The yellow oily mixture obtained on evaporation of the filtrates *in vacuo* was chromatographed on preparative layer silica gel plates [solvent benzene–hexane–acetone (25 : 16 : 9)]. Elution of material close to the origin with chloroform–acetone (7 : 3) afforded crude didethiohyalodendrin as an oil (208 mg) which crystallized from benzene. Recrystallization from benzene gave pure crystalline *material* (3) (65 mg), m.p. 132—135°, $[\alpha]_D^{22}$ 0° (*c* 0·64 in $CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 3400 and 1655 cm^{-1}, $\nu_{max.}$ (KBr) 1660, 1630, 1492, 747, and 708 cm^{-1}, δ ($CDCl_3$; 60 MHz) 2·88 (3H, s), 2·97 (3H, s), 2·9—3·6 (4H, m), 3·79 (1H, dd, J 4 and 5 Hz), 4·20 (1H, t, J 5 Hz), and 7·25 (5H, m), *m/e* 262 (M^+), 244 $(M - H_2O)^{+\cdot}$, 232 $(M - CH_2O)^{+\cdot}$, 171 (base peak) $(M - C_7H_7)^+$, 141 $(M - C_7H_7 - CH_2O)^+$, and 91 $(C_7H_7)^+$. The product from another run, recrystallized from benzene, had m.p. 156—159° (Found: C, 64·15; H,

* Partially racemized didethio-products were isolated in some runs. The *cis* t-butyl ethers derived from them displayed low positive optical rotations.

† *trans*-Didethiohyalodendrin t-butyl ether, a minor product in some runs, was readily separated by chromatography.

‡ *Added in proof.*—The circular dichroism curve of hyalodendrin was kindly determined by Dr. J. A. Verpoorte and Dr. A. Taylor: c.d. (*c* 0·376 in MeOH, 1 mm cell) 234 nm (Δ ε 22·5), 252 (0), 264 (−8·36), and 310 (0). The absolute configuration of the bridged dioxopiperazine system thus corresponds to that of chaetocin and verticillin A, and is antipodal to that of gliotoxin, the sporidesmins, and the aranotins.

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6·9; N, 10·65. $C_{14}H_{18}N_2O_3$ requires C, 64·1; H, 6·9; N, 10·7%).

2-Benzyl-5-hydroxymethyl-1,4-dimethylpiperazine (5).—Hyalodendrin (390 mg, 1·20 mmol) was reduced to the didethio-derivative (3) with aluminium amalgam [from aluminium foil (6 g)] in ethanol (150 ml), as just described. The crude product (246 mg) was refluxed with lithium aluminium hydride (500 mg, 13·2 mmol) in dry tetrahydrofuran (100 ml) for 42 h. Aqueous 10% sodium hydroxide was then added dropwise until vigorous reaction no longer accompanied the addition. The organic layer was decanted and the residual suspension of salts was washed several times with tetrahydrofuran. The combined organic solutions were evaporated under reduced pressure and the residue was taken up in chloroform. The desired basic material was separated by extraction of the chloroform solution with *ca.* 1N-hydrochloric acid. Work-up in the usual manner afforded a brown oil (151 mg), which was chromatographed on preparative layer alumina plates to give a pale yellow oil (5) (62 mg) which crystallized from ether. The *product* had m.p. 81—84°, $\nu_{max.}$ (CCl_4) 3300br, 3085, 3060, 3030, and 2960—2800 cm^{-1} (no carbonyl band), $\nu_{max.}$ (KBr) 1602, 1496, 1455, 756, and 711 cm^{-1}, δ ($CDCl_3$; 60 MHz) 2·10—3·17 (14H, m, including *N*-methyl singlets at δ 2·30 and 2·43), 3·47—4·23 (3H, m, including OH), and 7·22 (5H, s), *m/e* 234, 233, 232 (low intensity) [M^+, $(M - H)^+$, and $(M - 2H)^{+\bullet}$], 203 $(M - CH_2OH)^+$, 143 (base peak) $(M - C_7H_7)^+$, and 70 [possibly $(C_4H_8N)^+$] (Found: C, 71·5; H, 9·55; N, 11·7. $C_{14}H_{22}N_2O$ requires C, 71·75; H, 9·45; N, 11·95%).

Didethiohyalodendrin t-Butyl Ether (4).—A mixture of didethiohyalodendrin (59 mg, 0·225 mmol) and concentrated sulphuric acid–dioxan (1 : 10; 5 ml) in a pressure bottle was cooled in a solid carbon dioxide–acetone bath. Isobutene (*ca.* 3 ml) was distilled into the bottle while precautions were taken to exclude moisture. The bottle was closed and the contents were allowed to attain room temperature. The mixture was then stirred vigorously at 25° for 12 h. It was cooled again before the bottle was (cautiously) opened. The mixture was set aside at room temperature until the excess of isobutene had evaporated; it was then neutralized by addition of saturated sodium hydrogen carbonate solution and extracted thoroughly with methylene chloride. The extracts were washed with water, dried ($MgSO_4$), and evaporated to give a pale yellow oil (72 mg). Chromatography on preparative layer silica gel plates gave the ether (4) (65 mg), m.p. 97—98° (from cyclohexane), $\nu_{max.}$ (CCl_4) 1663, 1403, and 1365 cm^{-1} (no OH), $\nu_{max.}$ (KBr) 1652, 1403, 1365, 1347, 1110, and 1086 cm^{-1}, δ ($CDCl_3$; 60 MHz) 1·17 (9H, s), 2·72 (3H, s), 2·98 (3H, s), 2·92—3·68 (2H, 8-line multiplet, *J* 10, 4, and 3 Hz), 3·86—3·97 (1H, dd, *J* 4 and 3 Hz), 3·32 (2H, d, *J* 6 Hz), 4·14 (1H, t, *J* 6 Hz), and 7·30 (5H, s), *m/e* 318 (M^+), 232 $(M - C_3H_{10}O)^{+\cdot}$, 171 $(M - C_4H_8 - C_7H_7)^+$, and 141 (base peak) $(M - C_4H_8 - C_7H_7 - CH_2O)^+$, *m** 169·5 (318 ⟶ 232) and 116·5 (171 ⟶ 141) (Found: C, 68·0; H, 8·15; N, 8·85. Calc. for $C_{18}H_{26}N_2O_3$: C, 67·9; H, 8·25; N, 8·8%), identical [i.r. (CCl_4), n.m.r., and mass spectra, and t.l.c. behaviour] with a synthetic sample,[6] m.p. 117—121°, $[\alpha]_D^{23}$ −87° (*c* 1·0 in $CHCl_3$).

The chromatographic separation yielded a second product (1 mg), identified tentatively as *trans*-didethiohyalodendrin t-butyl ether on the basis of its spectral properties and greater mobility on t.l.c.

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