An Epitetrathiodioxopiperazine with 3*S*,6*S* Configuration from *Hyalodendron* sp.

GEORGE M. STRUNZ, MASATOSHI KAKUSHIMA, AND MERLYN A. STILLWELL

Canadian Forestry Service, P.O. Box 4000, Fredericton, New Brunswick E3B 5G4

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(3*S*,6*S*)3-Benzyl-6-hydroxymethyl-1,4-dimethyl-3,6-epitetrathiopiperazine-2,5-dione has been isolated from cultures of *Hyalodendron* sp. The same compound is produced when hyalodendrin is heated with methanol – hydrochloric acid.

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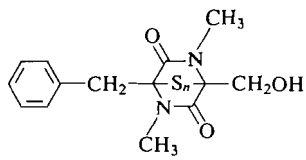
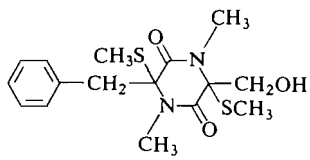
La (3*S*,6*S*) 3-Benzyl-6-hydroxyméthyl-1,4-diméthyl-3,6-epitetrathiopiperazine-2,5-dione a été isolé de cultures d'une espèce *d'Hyalodendron*. Le même composé est produit en chauffant l'hyalodendrine avec une solution de méthanol et d'acide chlorhydrique.

The fungitoxic epidithiodioxopiperazine hyalodendrin, **1**, and the corresponding di(methylthio) ether, **2**, both with 3*S*,6*S* configuration have recently been isolated from culture filtrates of *Hyalodendron* sp. (1–3). Metabolites **1** and **2**, and the trisulfide **3**, of undetermined absolute configuration, were obtained from cultures of an unidentified fungus (NRRL 3888) (4). The metabolite **1**, with *R* configuration at positions 3 and 6, and compounds **2** and **4**, evidently belonging to the same stereochemical series, are produced by *Penicillium turbatum* (5). Gliovictin, a metabolite of *Helminthosporium victoriae* also has structure **2** (6) and is likewise enantiomeric (3*R*,6*R*) with the corresponding *Hyalodendron* product.



1 $n = 2$
3 $n = 3$
4 $n = 4$



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We record here some observations made during a study of the 3*S*,6*S* tetrasulfide **4**, isolated from fermentations of *Hyalodendron* sp. This compound, $C_{14}H_{16}N_2O_3S_4$, was present in chloroform extracts of the filtered culture medium in amounts which ranged up to 3% of the yield of pure hyalodendrin. It was slightly more polar than the latter, and could be separated and purified by preparative-layer chromatography, followed by crystallization. Comparison of the spectral characteristics of **4** (see Experimental section) with those of its co-metabolites **1** and **2** leave no doubt as to its constitution. Comparison of the circular dichroism curve of the *Hyalodendron* tetrasulfide (see Experimental section) with that published for the *Penicillium* metabolite (5) establishes that these compounds are enantiomeric at positions 3 and 6. A detailed discussion of the geometry of the tetrasulfide system of **4** is deferred at this stage, although it is noted that the synthetic compound *N*,*N*′-dimethyl-3,6-epitetrathiopiperazine-2,5-dione and sporidesmin G have been shown by X-ray analysis to be structurally similar with respect to the epitetrathiodioxopiperazine moiety (7, 8).

A tetrasulfide, identical in all respects (including c.d. spectrum) with the *Hyalodendron* tetrasulfide, was obtained in modest yield when hyalodendrin was refluxed in methanolic hydrochloric acid until no starting material remained (t.l.c.). By-products from this transformation, depleted in sulfur, resisted preliminary purifica-

tion attempts and have not been investigated further at this stage. Tetrasulfide formation was also effected in low yield by shaking a solution of hyalodendrin in the culture medium (3) (initial p*H* 3.5, methanol co-solvent) for a period comparable with the fermentation time. This result raises the possibility that the tetrasulfide isolated could be an artifact, formed from hyalodendrin by a similar acid-catalyzed mechanism. Although such a possibility cannot be excluded at present, the fact that the *P. turbatum* tetrasulfide is produced in a substantially different medium (5), and that sporidesmin G appears to be a true metabolite[1] seems to attest the 'natural' origin of at least part of the *Hyalodendron* tetrasulfide.

Refluxing hyalodendrin in aqueous methanol solution without addition of hydrochloric acid also led to the formation of a tetrasulfide. This transformation occurred more slowly than that in acid solution and the yield of tetrasulfide obtained was substantially higher. The product formed under these conditions was *racemic*.

Conversion of epidithiodioxopiperazines to the corresponding tetrasulfides (with retention of configuration) has previously been effected by the action of dihydrogen disulfide (9, 10). In bioassays, the 3*S*,6*S* tetrasulfide **4** was in general comparable with hyalodendrin (in some cases slightly less effective) in its ability to inhibit the growth of selected test fungi.[2]

Experimental

See refs. 1 and 2 for description of instrumentation, etc.

Isolation of 'Natural' Tetrasulfide 4

The production and isolation of hyalodendrin and bisdethiodi(methylthio) hyalodendrin have been detailed elsewhere (1–3). In a typical isolation sequence, material extracted with chloroform from 37 l of filtered culture medium which had supported growth of *Hyalodendron* sp. for 12 days was chromatographed on a column of silica gel (500 g Kieselgel, 100–200 mesh, Gebr. Herrmann, Köln). The eluates were monitored by t.l.c. Elution with benzene–chloroform (65:35) afforded initially 2 g of hyalodendrin. In the later fractions, the emergence of a second compound, with slightly lower R_f became evident (t.l.c.). Fractions containing the new compound were combined and rechromatographed on a preparative-layer plate of silica gel (benzene–acetone, 9:1, $R_f \sim 0.5$). This separation provided 78 mg of crude tetrasulfide, which gave 60 mg of crystalline material from benzene–cyclohexane. Recrystallization from benzene–cyclohexane afforded colorless crystals, m.p. 133–139° (pulverized crystals, 125–132°); c.d. (*c* 2.81 × 10^{-3} *M* in MeOH, 1 mm cell) λ nm (Δε): 370(0), 328(+0.9), 316(0), 297(−4.4), 287(0), 263(+13.6), 225(inflection +12.1), 230(0), and <220 (−*ve* max); ν_{max}(KBr) *inter alia* 3400, 1666, 1648, 1375, 1060, 742, 735, 701, and 657 cm^{-1}; n.m.r. ($CDCl_3$, 220 MHz) δ 3.03 (3H, s), 3.07 (3H, s) 3.25 (2H, d, $J \sim 15$ Hz), broadened base), 3.83 (1H, d, $J \sim 12$ Hz), 4.02 (1H, d, $J \sim 15$ Hz), 4.20 (1H, d, $J \sim 12$ Hz), 7.09–7.27 (5H, m). The signal for the hydroxyl proton (broad absorption around δ 3.25) disappeared on deuterium exchange. Mass spectrum *inter alia*, *m/e* 260 (base peak) $(M - S_4)^+$, 242 $(M - S_4 - H_2O)^+$, 169 $(M - S_4 - C_7H_7)^+$, and 64.

Anal. Calcd. for $C_{14}H_{16}N_2S_4O_3$: C, 43.27; H, 4.15; N, 7.21; S, 33.01. Found: C, 43.42; H, 4.11; N, 7.20; S, 33.02.

Acid Treatment of Hyalodendrin

Methanol – Hydrochloric Acid

A solution of hyalodendrin (582 mg, 1.79 mmol) in methanol (30 ml) containing 1 *N* aqueous hydrochloric acid (3.0 ml) was heated under reflux for 24 h, when no starting material remained. (Longer reaction times, up to 16 days, did not appear to have much effect on either the yield or c.d. properties of the tetrasulfide product.) After addition of saturated sodium chloride solution, the mixture was extracted thoroughly with chloroform. The extracts were washed with saturated sodium bicarbonate solution, dried ($MgSO_4$), and evaporated *in vacuo*. The tetrasulfide **4** was separated and purified by repeated chromatography on preparative-layer plates of silica gel (benzene–acetone, 94:6). Crystallization from benzene–cyclohexane gave 100 mg (0.257 mmol) of colorless crystals of **4**, m.p. 132–136° (pulverized crystals, 125–132°); mixture melting point with 'natural' **4**: 125–132°; c.d., i.r. (KBr), n.m.r. ($CDCl_3$, 220 MHz), and mass spectra identical with 'natural' **4** (*vide supra*). An analytical sample was prepared by recrystallizations from benzene: colorless prisms, m.p. 135–138°.

Anal. Calcd. for $C_{14}H_{16}N_2S_4O_3$: C, 43.27; H, 4.15; N, 7.21; S, 33.01. Found: C, 43.30; H, 4.16; N, 7.28; S, 32.99.

Culture Medium, pH 3.5 (3)

Because of the low solubility of hyalodendrin in water, methanol was used as co-solvent. An aliquot (1 ml) of a solution of hyalodendrin (2 g) in methanol (40 ml) was added to each of 40 1-l Erlenmeyer flasks, each containing 500 ml of synthetic culture medium at p*H* 3.5 (3). The flasks were stoppered with foam-plastic plugs (as used in fermentation) and shaken on a gyratory shaker at 22° for 14 days (final p*H* ~ 4). Unchanged hyalodendrin and tetrasulfide **4** were recovered from the chloroform extracts by chromatography as described above (isolation of 'natural' tetrasulfide **4**). Recrystallization from benzene–cyclohexane afforded 37 mg of **4** as almost colorless crystals, m.p. 133–142°; c.d. (*c* 2.75 × 10^{-3} *M* in MeOH, 1 mm cell) λ nm (Δε): 370 (0), 330 (+0.9), 318 (0), 298 (−4.2), 288 (0), 264 (+12.8), 254 (inflection + 10.4), 231 (0), and <220 (−*ve* max); i.r. (KBr), and mass spectra identical with those of 'natural' **4**.

Formation of Racemic Tetrasulfide 4

A solution of hyalodendrin (1.0 g, 3.08 mmol) in methanol (40 ml) and water (4 ml) was heated under reflux for 18 days, when no unchanged starting material remained (t.l.c.). A saturated aqueous solution of sodium

[1]Dr. A. Taylor. Personal communication.
[2]M. A. Stillwell. Unpublished data.

chloride was added and the mixture was extracted with chloroform. Material from the chloroform extracts was chromatographed on preparative layer plates of silica gel (benzene–acetone, 9:1), and the product corresponding in R_f with the tetrasulfide **4** was collected. Recrystallization from benzene afforded 410 mg (1.05 mmol, 68% theoretical) of tetrasulfide as almost colorless crystals, m.p. 155–166°; c.d. (c 2.92 × 10^{-3} M in MeOH, 1 mm cell) 600–250 nm, $\Delta\varepsilon = 0$; n.m.r. ($CDCl_3$, 220 MHz) and mass spectra identical with those of 'natural' tetrasulfide **4**. The main peaks in the i.r. (KBr) spectrum were essentially as quoted for 'natural' **4**: minor differences were detectable in the fingerprint region. An analytical sample was prepared by recrystallizations from benzene–chloroform, m.p. 159–166°.

Anal. Calcd. for $C_{14}H_{16}N_2S_4O_3$: C, 43.27; H, 4.15; N, 7.21; S, 33.01; 0, 12.35. Found: C, 43.26; H, 4.20; N, 7.22; S, 32.97; 0, 12.28.

Mol. Wt. Calcd. for $C_{14}H_{16}N_2S_4O_3$: 388.6. Found (osmometer, $CHCl_3$ solvent): 399.

NOTE ADDED IN PROOF: An additional correlation of the *Hyalodendron* tetra- and disulfide has been effected by sodium borohydride reduction of the former, followed by oxidation with iodine–iodide solution (*cf.* ref. 11). This treatment yielded a product, identical in all respects, including c.d. spectrum, with hyalodendrin.

The enantiometric relationship of bisdethiodi(methylthio)hyalodendrin and gliovictin was confirmed by direct comparison of the former with a sample of the latter, kindly provided by Professor Arigoni.

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