New Piperazinedione Metabolites of *Gliocladium deliquescens*

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The phenol (3), its $\gamma\gamma$-dimethylallyl ether (7), and bisdethiobis(methylthio)dehydrogliotoxin (10), together with the hydroxy-acid (13), have been isolated from *Gliocladium deliquescens*.

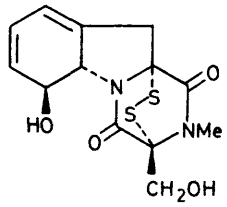
In the course of biosynthetic studies on the steroidal metabolites of *Gliocladium deliquescens*,[1] we have isolated some new metabolites related to the thiopiperazine-2,5-dione, gliotoxin (1).[2] The evidence for their structures forms the subject of this paper. Gliotoxin and bisdethiobis(methylthio)gliotoxin (2)[3] are known metabolites of *Gliocladium deliquescens*.

RESULTS AND DISCUSSION

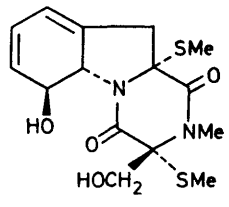
The gliotoxin and viridiol[4] were removed from the extract of the culture broth and the residue was then chromatographed on silica. A new phenolic metabolite (3) was obtained and crystallized as its methylene chloride solvate. The sporidesmins form similar solvates.[5] The phenol [$\nu_{max.}$ 3 680, 3 615, and 3 310 cm^{-1} (OH)] gave a typical alkali-induced bathochromic shift in the u.v. spectrum whilst the ^1H n.m.r. spectrum contained the AB quartet (δ 6.72 and 6.93, J 8 Hz) typical of a *para*-disubstituted aromatic ring. The ^1H and ^{13}C n.m.r. spectra contained resonances associated with two S–Me and two N–Me groups. In addition to the aromatic signals, the ^{13}C n.m.r. spectrum contained signals which could be assigned to the amide carbonyls of a piperazinedione.[6] The mass spectrum contained two significant ions at *m/e* 107 and 233 which were attributed to the fragments (4) and (5) respectively. Consequently structure (3) was assigned to the new metabolite.

Further evidence for the structure (3) was obtained by desulphurization of the methyl ether of the phenol with aluminium amalgam.[7] The product, 3-[(4-methoxyphenyl)methyl]-1,4-dimethyl-piperazine-2,5-dione (6) was prepared by cyclization and methylation of glycyl-L-tyrosine.[8] The n.m.r. and t.l.c. of the two samples were identical. However, the broad melting point and low optical activity of the degradation product indicated that partial racemization had occurred during desulphurization. Gliotoxin undergoes desulphurization with retention of configuration,[2] although in the case of hyalodendrin racemization occurs. The residual positive rotation in the degradation product suggests that enantiomer (6) is present in excess. Furthermore, like gliotoxin the phenol shows an increasingly negative rotation with wavelength. Hence it is assigned the configuration (3) comparable to the other metabolites of *Gliocladium deliquescens*.

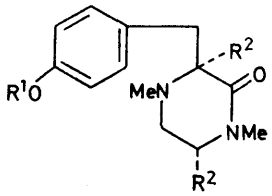
The $\gamma\gamma$-dimethylallyl ether (7) was also obtained from the culture filtrate. Its ^1H and ^{13}C n.m.r. spectra were similar to those of the phenol (3) with additional signals attributable to the $\gamma\gamma$-dimethylallyl group. The mass spectrum showed significant ions at *m/e* 175 (8), 233 (5), and 245 (9). As expected there was no shift in the u.v. spectrum on treatment with alkali. Hence the structure



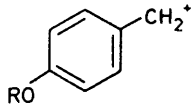
(1)



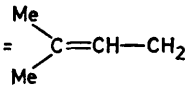
(2)

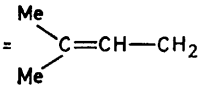


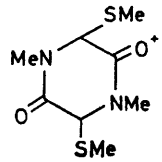
(3) R^1 = H, R^2 = SMe
(6) R^1 = Me, R^2 = H



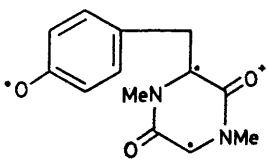
(4) R = H

(8) R = 

(7) R^1 =  R^2 = SMe



(5)



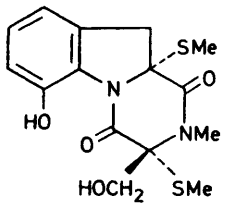
(9)

(7) was assigned to the metabolite. A similar dimethylallyl ether, phomamide, has recently been isolated[9] as a fungal metabolite.

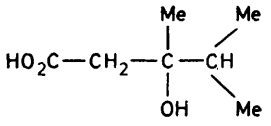
A third metabolite (10) showed ^1H n.m.r. signals assigned to two S–Me groups, one N–Me group, a primary alcohol, and three aromatic protons. The data were in accord with the structure, bisdethiobis(methylthio)-dehydrogliotoxin (10).[3] In confirmation of this, the

product was synthesized by treatment of dehydrogliotoxin with methyl iodide and sodium borohydride in pyridine–methanol. This compound has recently been described by Kirby *et al.*[3] in the degradation of bis-dethiobis(methylthio)gliotoxin (2).

The isolation of the metabolites with a *para*- rather than a *meta*-hydroxylated aromatic ring along with

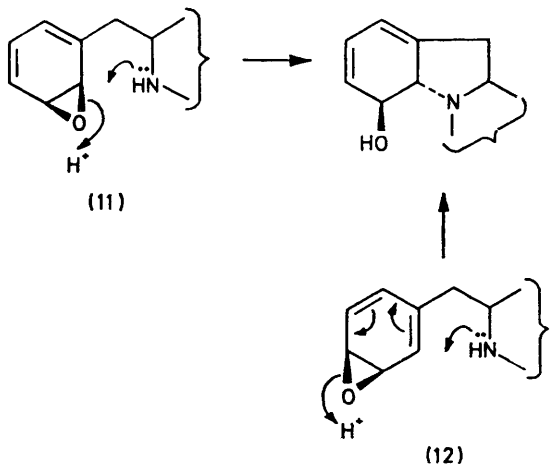


(10)



(13)

gliotoxin suggests a modification (see Scheme) of the biogenetic scheme involving an arene oxide (11) for the formation of gliotoxin.[10] The alternative arene oxide (12) which could arise from the known intermediate [11] cyclo-(L-phenylalanyl-L-seryl) or its glycyl analogue, could afford both *para*-substituted phenols and compounds of the gliotoxin type. On the other hand (11) would afford *meta*-substituted phenols. In this context



(11) (12)

SCHEME

biogenetically patterned cyclizations of model arene oxides related to (11) have failed to yield gliotoxin structures.[12]

The acid (13) was also isolated and characterized as its *p*-bromophenacyl ester. Its structure followed from the ^{1}H n.m.r. spectrum and from the preparation of its racemate by the Reformatski reaction between methyl bromoacetate and methyl isopropyl ketone.[13] Hydrolysis gave 3,4-dimethyl-3-hydroxypentanoic acid (13) identical (m.s., n.m.r. and i.r.) to the natural product. The structure of the acid is reminiscent of the side-chain of a steroid, possibly corresponding to part of a precursor of viridiol.

EXPERIMENTAL

I.r. spectra and optical rotations were determined in chloroform except where stated; ^{1}H and ^{13}C n.m.r. spectra were determined in deuteriochloroform at 90 and 25.15 MHz, respectively. Silica gel for chromatography was Merck 7734.

Gliocladium deliquescens (C.M.I. 101525) was maintained on potato dextrose agar slopes. The medium for growth was (per litre) sucrose (15 g), ammonium tartrate (1.5 g), potassium dihydrogenphosphate (0.5 g), ammonium sulphate (0.25 g), zinc sulphate (0.1 g), ferric chloride (0.01 g) peptone (0.2 g), and magnesium sulphate (0.4 g).

Isolation of the Metabolites.—Gliocladium deliquescens (70 l) was grown on shake culture in batches for 6 days. The combined broth was saturated with salt and extracted with ethyl acetate. The residue from the extract was triturated with hot ethyl acetate to give a precipitate of gliotoxin and viridiol (1.4 g). The mother liquors were concentrated and applied to a column of silica gel (174 g) which was then subjected to gradient elution with ether–ethyl acetate and then ethyl acetate–acetone mixtures to give the following compounds (in order of elution).

(i) 3-*Hydroxy*-3,4-*dimethylpentanoic acid* (13) (620 mg), which was further purified by extraction with sodium hydrogencarbonate. It was a colourless viscous oil, b.p. 100 °C (bath) at 0.05 mmHg (Found: C, 57.0; H, 9.6. $C_7H_{14}O_3$ requires C, 57.5; H, 9.65%), $[\alpha]_D$ −1.1° (*c* 2.7 in $CHCl_3$); δ(CCl_4) 0.95 (6 H, d, *J* 7 Hz), 1.26 (3 H, s), 1.9 (1 H, m), 2.53 (2 H, br s), and 6.9 (br s, exchanged by ^2H_2O); $\nu_{max.}$ (thin film), 3 700—2 400 (br) and 1 710 cm^{-1}; *m/e* 131 (5), 129 (5), 127 (5), 113 (5), 103 (40), 86 (25), 85 (70), 71 (35), 69 (15), and 43 (100). The *p-bromophenacyl ester* had m.p. 44—45 °C; $[\alpha]_D$ +4.7° (*c* 1.07 in $CHCl_3$) (Found: C, 52.3; H, 5.8. $C_{15}H_{19}O_4Br$ requires C, 52.5; H, 5.6%).

(ii) 1,4-*Dimethyl*-3[(4′-*γγ-dimethylallyloxyphenyl*)-*methyl*]-3,6-*bis*(*methylthio*)*piperazine*-2,5-*dione* (7) (200 mg), which was further purified by preparative t.l.c. as a colourless gum; $[\alpha]_D$ −26.8° (*c* 6.8 in $CHCl_3$) (Found: M^+ − SMe 361.160. $C_{19}H_{25}N_2O_3S$ requires 361.159); δ_H 1.7 and 1.77 (6 H, br s, =CMe_2), 2.13 and 2.26 (6 H, 2 × s, SMe), 2.93 and 3.20 (6 H, 2 × s, NMe), 3.04 and 3.53 (2 H, AB q, *J* 14 Hz, Ar–CH_2), 4.18 (1 H, s, H-6), 4.45 (2 H, d, *J* 7 Hz, CH_2O), 5.47 (1 H, br t, *J* 7 Hz, =CH–), and 6.79 and 6.98 (4 H, AB q, *J* 8.5 Hz, Ar-H); δ_C 13.5, 16.2, 18.2, 25.8, 30.1 and 33.5 (each q, Me), 41.9 ($ArCH_2$, t) 64.7 (t, OCH_2), 64.9 (d, C-6), 74.9 (s, C-3), 114.6 (d, 2 × Ar-C), 119.6 (d, =C) 125.9 (s, Ar-C), 130.5 (d, 2 × Ar-C), 138.0 (s, =C), 158.2 (Ar-C), and 164.3 and 164.8 (s, C=O); $\nu_{max.}$ 1 665 and 1 610 cm^{-1}; $\lambda_{max.}$ 283 (ε 13 240), 277 (16 340), and 228 (16 900); *m/e* 361 (25%), 314 (2), 293 (18), 246 (15), 245 (28), 233 (65), 218 (15), 186 (35), 175 (5), 158 (10), 107 (100), 83 (18), and 69 (30).

(iii) 3[(4′-*Hydroxyphenyl*)*methyl*]-1,4-*dimethyl*-3,6-*bis*(*methylthio*)*piperazine*-2,5-*dione* (3) (300 mg) which was purified by precipitation as its methylene chloride adduct, m.p. 68—69 °C; $[\alpha]_D$ −55.6° (*c* 1.06 in $CHCl_3$) (Found: C, 44.8; H, 5.3; N, 6.4. $C_{15}H_{20}N_2O_3S_2{\cdot}CH_2Cl_2$ requires C, 45.1; H, 5.2; N, 6.6%); δ_H 2.14 and 2.24 (6 H, 2 × s, SMe), 2.93 and 3.28 (6 H, 2 × s, NMe), 3.06 and 3.47 (2 H, AB q, *J* 14 Hz, $ArCH_2$), 4.16 (1 H, s, 6-H), 5.3 (2 H, s, CH_2Cl_2), 6.72 and 6.93 (4 H, AB q, *J* 8 Hz, Ar-H), and 7.2 (1 H, br, exchanged by ^2H_2O, OH); δ_C 13.5 and 15.8 (each q, SMe), 30.5 and 33.5 (each q, NMe), 42.0 (t, Ar–CH_2), 64.8 (d, C-6), 75.8 (s, C-3), 115.5 (d, 2 × Ar-C), 124.8 (s,

Ar-C), 130.6 (d, 2 × Ar-C), 156.3 (s, Ar-C), and 164.3 and 165.5 (each s, C=O); $\nu_{max.}$ 3 680, 3 615, 3 310, 1 665, and 1 615 cm^{-1}; $\lambda_{max.}$ 277 (ε 1 460) and 228 (12 100); $\lambda_{max.}$ (NaOH) 300 (2 000), and 252 (11 600); *m/e* no M^+, 293 (100, M^+ − SMe), 246 (20), 245 (20), 233 (43), 218 (23), 187 (14), 186 (14), 158 (15), 107 (57), and 42 (30); $[\alpha]_{546}$ −65.0°; $[\alpha]_{36}$ −104.5° $[\alpha]_{365}$ −146°.

(iv) 5a,6-*Dehydrobisdethio*-3,10a-*bis*(*methylthio*)*gliotoxin* (10) (20 mg) as a yellow gum which would not crystallize; δ_H 2.22 and 2.33 (6 H, SMe), 3.19 (3 H, s, NMe), 3.37 and 3.63 (2 H, AB q, *J* 17 Hz, Ar–CH_2), 3.97 and 4.54 (2 H, AB q, *J* 12 Hz, CH_2OH), and 6.75—7.5 (3 H, m, Ar-H); $\nu_{max.}$ 3 600, 3 400 (br), 1 670, 1 645, and 1 610 cm^{-1}; *m/e* 354 (M^+, 3%), 307 (83), 277 (12), 261 (40), 260 (100), 243 (20), 229 (48), 213 (15), 160 (70), 143 (30), and 43 (70).

Degradation of the Phenol (3).—The phenol (55 mg) in chloroform (1 ml) was treated with diazomethane until t.l.c. indicated that reaction was complete. The solvent was evaporated to afford a foam. This was dissolved in ethanol (10 ml) and treated with aluminium amalgam (from 100 mg aluminium [7]) and a few drops of water at room temperature for 14 h. The solvent was evaporated and the residue purified by preparative t.l.c. to afford 3[(4-*methoxyphenyl*)-*methyl*]-1,4-*dimethylpiperazine*-2,5-*dione* (6) (10 mg), m.p. 108—125 °C; $[\alpha]_D$ +3.8° (*c* 0.52 in $CHCl_3$) (Found: C, 64.1; H, 6.9; N, 10.5. $C_{14}H_{18}N_2O_3$ requires C, 64.1; H, 6.9; N, 10.7%); δ_H 2.72 and 3.03 (6 H, 2 × s, NMe), 3.32 and 2.39 (2 H, *J* 17 Hz, CH_2N), 3.1 (2 H, m, Ar–CH_2, collapses to a quartet, *J* 14 Hz, on irradiation at δ 4.14), 3.77 (OMe) 4.14 (1 H, br t, *J* 3 Hz), and 6.85 and 6.99 (4 H, AB q, *J* 8 Hz, Ar-H); $\nu_{max.}$ 1 660 cm^{-1}.

Synthesis of (6).—*cyclo*-Gly-L-Tyr was prepared as described [8] from glycyl-L-tyrosine, $[\alpha]_D$ +66.9° (*c* 1.5 in MeOH). Methyl iodide (150 μl) and excess of sodium hydride were added to a solution of cyclo(glycyl-L-tyrosyl) (66 mg) in dimethyl sulphoxide which was then left at room temperature for 18 h Recovery in ethyl acetate and purification by t.l.c. gave the piperazinedione (6), m.p. 138 °C, $[\alpha]_D$ +48 6 (*c* 1.21 in $CHCl_3$) identical (n.m.r. and t.l.c.) to the material described above.

Preparation of (10) *from Dehydrogliotoxin*.—Dehydrogliotoxin (80 mg) in pyridine (0.25 ml) was treated with methyl iodide (1.25 ml) and methanol (0.5 ml) at 0 °C. Sodium borohydride (25 mg) in methanol (0.5 ml) was then added and the mixture was allowed to attain room temperature during 4 h. The product was recovered in chloroform and purified by t.l.c. to afford bisdethiobis(methylthio)dehydrogliotoxin (10), identical (n.m.r., i.r. and t.l.c.) to the material obtained from the fungus (*cf.* ref. 3).

Preparation of the Hydroxy-acid (13).—A solution of methyl bromoacetate (1.4 g) and methyl isopropyl ketone (790 mg) in benzene (20 ml) was added to finely-cut pieces of zinc wool (780 mg) which had previously been activated by washing with 5% hydrochloric acid, water, and acetone, and flame-dried with iodine. The mixture was refluxed for 1.5 h, cooled, and poured into ice containing sulphuric acid (1 ml). The *methyl* 3-*hydroxy*-3,4-*dimethylpentanoate* was recovered in ether as an oil, b.p. 60 °C at 0.8 mmHg (Found: C, 59.9; H, 10.1. $C_8H_{16}O_3$ requires C, 60.0; H, 10.1%); δ 1.0 (6 H, d, *J* 6 Hz), 1.22 (3 H, s), 1.77 (1 H, m), 2.47 (2 H, br s), 3.4 (1 H, br, OH), and 3.72 (3 H, s); *m/e* 145 (M^+ − CH_3), 117, 87, 85, 71, and 69. The ester (1 g) and potassium hydroxide (1 g) in water (10 ml) were heated on a steam-bath for 1.5 h. The solution was cooled and washed with ether. It was then acidified and the 3-*hydroxy*-3,4-*dimethylpentanoic acid* was recovered in ether. It had b.p. 100 °C (bath) at 0.1 mmHg (Found: C, 57.5; H, 9.6. $C_7H_{14}O_3$ requires C, 57.5; H, 9.65%). The spectra (n.m.r., i.r., and m.s.) of this material were identical with those of the acid from the fungus.

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REFERENCES

[1] J. R. Hanson and H. J. Wadsworth, *J.C.S. Chem. Comm.*, 1979, 360.
[2] For reviews see: P. G. Sammes in ' Progress in the Chemistry of Organic Natural Products,' Springer, Vienna, 1975, vol. 32, p. 51; C. Leigh and A. Taylor, in 'Advances in Chemistry Series, No. 149,' ed. J. V. Rodricks, American Chemical Society, Washington, 1976, p. 228.
[3] G. W. Kirby, D. J. Robins, M. A. Sefton, and R. R. Talekar, *J.C.S. Perkin I*, 1980, 119.
[4] J. S. Moffatt, J. D. Bu'Lock, and T. H. Yuen, *Chem. Comm.*, 1969, 839.
[5] W. D. Jamieson, R. Rahman, and A. Taylor, *J. Chem. Soc.* (*C*), 1969, 1564.
[6] R. Deslauriers, Z. Grzonka, K. Schaumburg, T. Shiba, and R. Walter, *J. Amer. Chem. Soc.*, 1975, **97**, 5093.
[7] L. F. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' Wiley, New York, 1967, vol. 1, p. 22.
[8] K. D. Kopple and H. G. Ghazarian, *J. Org. Chem.*, 1968, **33**, 862.
[9] J.-P. Ferezou, A. Quesneau-Thierry, M. Barbier, A. Kollmann, and J.-F. Bousquet, *J.C.S. Perkin I*, 1980 113.
[10] N. Neuss, R. Nagarajan, B. B. Molloy, and L. L. Huckstep, *Tetrahedron Letters*, 1968, 4467.
[11] G. W. Kirby, G. L. Patrick, and D. J. Robins, *J.C.S. Perkin I*, 1978, 1336.
[12] W. H. Rastetter and L. J. Nummy, *Tetrahedron Letters*, 1979, 2217.
[13] L. E. Smith, W. L. Kohlaise, and R. J. Brotherton, *J. Amer. Chem. Soc.*, 1956, **78**, 2532.