New Co-metabolites of Gliotoxin in *Gliocladium virens*

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Chromatographic separation of extracts of *Gliocladium virens*, grown on a medium containing [^{35}S]sulphate, has led to the identification of 5 co-metabolites of gliotoxin (**1a**), new to this fungus, *viz.* the epitrisulphide, gliotoxin E (**1b**), the 3-hydroxymethylbut-2-enyl ether (**3c**), didehydrogliotoxin (**5**), bis-*N*-norgliovictin (**6**), and the 3-methylbut-2-enyl ether of *cyclo*-(glycyl-L-tyrosyl) (**7**); the metabolites (**3c**), (**6**), and (**7**) are new natural products. The synthesis of the epitri- and epitetra-sulphides, gliotoxin E (**1b**) and gliotoxin G (**1c**), from gliotoxin (**1a**) is described, as is that of the 3-methylbut-2-enyl ether (**7**) from *cyclo*-(glycyl-L-tyrosyl). The biosynthetic significance of the dioxopiperazines (**6**) and (**7**) is briefly discussed.

While studying the biosynthesis[1] of gliotoxin (**1a**) in the fungus *Gliocladium virens* (*deliquescens*)† we isolated a minor metabolite,[2] bisdethiobis(methylthio)gliotoxin (**2**). Successive reduction and methylation of gliotoxin gave a substance identical with the new natural product (**2**), which has since been reported[3] to act as a specific inhibitor of the platelet activating factor (PAF). The transformation (**1a**)⟶(**2**) was shown[2] to occur also in *G. virens*. More recently, Hanson and O'Leary[4] have isolated 3 other sulphur-containing dioxopiperazines from cultures of the same fungus; the phenol (**3a**) and its dimethylallyl ether (**3b**) were new compounds whereas the dihydroindole (**4**) had been prepared earlier[2] by dehydrogenation of the cyclohexadienol (**2**). To facilitate biosynthetic studies, the metabolites of *G. virens* have now been investigated in more detail. We report here the identification of 5 compounds, *viz.* (**1b**), (**3c**), (**5**), (**6**), and (**7**), previously undetected in cultures of this fungus, and give details[5] of the partial synthesis of gliotoxin E (**1b**) and gliotoxin G (**1c**).

The fungus was grown in the usual medium to which had been added [^{35}S]sulphate to aid detection of sulphur-containing metabolites. After 5 d growth the culture medium was extracted with dichloromethane and the extracts were concentrated. Gliotoxin (**1a**), the major metabolite, was crystallised to a constant specific activity (29.6 μCi mmol^{-1}) which was approximately twice that of the [^{35}S]sulphate (14.5 μCi mmol^{-1}) in the culture medium. It appeared therefore that essentially all the sulphur in gliotoxin was derived from sulphate in the medium and the radioactivity of chromatographic fractions would be a quantitative guide to the presence of other metabolites of interest. Chromatography was carried out with silica plates and by preparative h.p.l.c. ^{35}S-Labelled compounds were detected on t.l.c. plates by radioscanning and autoradiography. The quantitative results are summarised in the Table. Individual metabolites will be discussed sequentially, as follows.

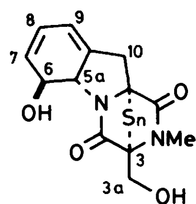
Bis-N-*norgliovictin* (**6**).—Until recently, sulphur-containing dioxopiperazines lacking substituents on nitrogen were not known.[6] The first example[7] of this 'primitive' structural type was obtained by feeding the unnatural analogue (**8a**) of *cyclo*-(L-Phe-L-Ser) (**8b**), the natural precursor[8] of gliotoxin (**1a**), to *G. virens*. Three 'unnatural' metabolites were formed in approximately equal amounts, one being the ethyl derivative (**6**; CH_2OH = Et). This experiment showed that *G. virens* was able to introduce sulphur into a dioxopiperazine ring, albeit of an unnatural precursor, without prior *N*-methylation or oxidative cyclisation onto the phenyl group. Gratifyingly, the derivative (**6**) of *cyclo*-(L-Phe-L-Ser) (**8b**) itself has now been isolated and characterised. The corresponding bis-*N*-methyl derivative, gliovictin, is a known metabolite of *Penicillium turbatum*[9] and *Helminthosporium victoriae*.[10] Bis-*N*-norgliovictin (**6**), m.p. 231 °C (decomp.), was obtained in amounts too small (*ca.* 0.15% the yield of gliotoxin) to permit combustion analysis. However, h.r.m.s. established the elemental composition and showed the expected, ready fragmentation with loss of MeS and CH_2OH. Further, the specific ^{35}S activity (26 μCi mmol^{-1}) was close to that of gliotoxin (29.6 μCi mmol^{-1}) thus confirming the presence of 2 sulphur atoms. The ^{1}H n.m.r. spectra (200 MHz) in $CDCl_3$ and $(CD_3)_2CO$ together gave identifiable signals for all the distinct protons, including sharp singlets at δ 2.21 and 2.35 ($CDCl_3$) for the *S*-methyl groups and broad singlets at δ 5.88 and 6.08 ($CDCl_3$) for the NH groups. Signals for the hydroxymethyl group were clearly resolved in $(CD_3)_2CO$ and for the benzylic methylene group in both solvents. The phenyl group gave a complex multiplet. Bis-*N*-norgliovictin, $[\alpha]_D$ −32° (*c* 0.1 in MeOH), is assigned the stereochemistry (**6**) by analogy with that of its co-metabolites. Possibly the corresponding epidisulphide or bisthiol is an intermediate on the biosynthetic pathway from *cyclo*-(L-Phe-L-Ser) (**8b**) to gliotoxin (**1a**).

Table. Metabolites of *Gliocladium virens*, grown on [^{35}S]sulphate

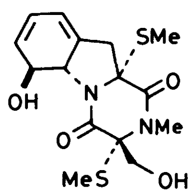
	Yield (mg) per litre	R_F In system[a] A	B
Gliotoxin (**1a**)	97.0	0.47	0.35
Bisdethiobis(methylthio)gliotoxin (**2**)	6.5	0.34	0.16
Phenol (**3a**)	6.0	0.41	0.31
3-Methylbut-2-enyl ether (**3b**)	5.6	0.67	0.65
Gliotoxin E (**1b**)	2.1	0.47	0.32
Didehydrogliotoxin (**5**)	0.51	0.58	0.61
Bis-*N*-norgliovictin (**6**)	0.15	0.27	0.15
Bisdethiobis(methylthio)-didehydrogliotoxin (**4**)	0.13[b]	0.47	0.37
3-Hydroxymethylbut-2-enyl ether (**3c**)	<0.1	0.34	0.17
cyclo-(Glycyl-*O*-3-methylbut-2-enyl-L-tyrosyl) (**7**)	<0.1	0.03	0.00

[a] T.l.c. (SiO_2) in solvent systems: A, toluene–acetone (2:1); B, toluene–ethyl acetate (2:1). [b] Calculated from ^{35}S activity in relevant chromatographic fractions.

† Cultures of the fungus obtained from the Commonwealth Mycological Institute, now the C.A.B. International Mycological Institute (Kew), were described as *Gliocladium deliquescens* Sopp (CMI 101525, NRRL 1828), formerly *Trichoderma viride*. Dr. R. W. Jones (Agricultural Experiment Station, University of California) kindly informed us that the fungus NRRL 1828 is properly described as *Gliocladium virens*. Dr. M. A. J. Williams (Kew) has now confirmed that their isolate (IMI 101525) is *G. virens* J. Miller Giddens and Foster.



(1) a; $n = 2$
b; $n = 3$
c; $n = 4$



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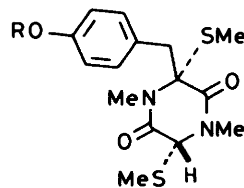
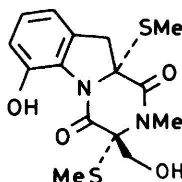


(3) a; R = H
b; R = $Me_2C{=}CH.CH_2$
c; R = $HOCH_2$(Me)C=C(H)CH_2



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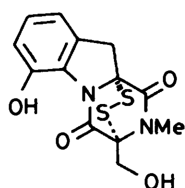

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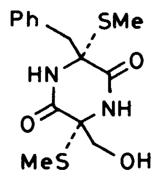

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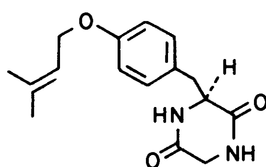

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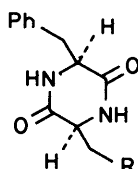

(8) a; R = Me
b; R = OH

The 3-Hydroxymethylbut-2-enyl Ether (**3c**).—The metabolites (**3a**) and (**3b**) described by Hanson and O'Leary[4] were readily identified by their spectroscopic properties. However, a new, closely related derivative (**3c**) was also isolated, although in much smaller quantities. The ^{1}H n.m.r. spectrum of (**3c**) resembled that of (**3b**) but one vinyl methyl signal was absent and a new 2-proton doublet at δ 4.07 (J 5 Hz) was observed. This collapsed to a singlet upon treatment of the compound with D_2O, suggesting the presence of a hydroxymethyl group. No molecular ion was observed in the mass spectrum of compound (**3c**) but a fragment ion had an accurate mass corresponding to M^+ − MeS. Other fragment ions amply confirmed the composition and structure (**3c**). The (E)-configuration for (**3c**) was established by nuclear Overhauser effect experiments.

cyclo-(*Glycyl*-L-*tyrosyl*) *Dimethylallyl Ether* (**7**).—The most polar chromatographic fractions yielded a small amount of a highly crystalline, sulphur-free, compound. The structure (**7**) was assigned on the basis of its ^{1}H n.m.r. and mass spectra and confirmed by comparison with material synthesized from *cyclo*-(glycyl-L-tyrosyl)[11] by treatment with sodium hydride and dimethylallyl bromide in dimethyl sulphoxide. The presence of the dioxopiperazine (**7**) in extracts of *G. virens* suggests that *cyclo*-(glycyl-L-tyrosyl) may be a precursor for the 4 metabolites (**3a**—**c**) and (**7**).

Didehydrogliotoxin (**5**).—Didehydrogliotoxin (**5**) was described[12] in 1966 as a co-metabolite of gliotoxin in *Penicillium terlikowskii*. Its detection now in extracts of *G. virens* is therefore unremarkable. The quantity didehydrogliotoxin appears to increase with lengthening incubation times, but this effect has not been studied systematically.

Gliotoxin E (**1b**).— While the present study was in progress, Waring *et al.* reported[13] the isolation of small amounts of the epitetrasulphide, gliotoxin G (**1c**), from *Aspergillus fumigatus*. We devised the following preparation[5] of gliotoxin G to provide quantities of the new derivative for further biological testing[14] and to facilitate its possible detection in extracts of *G. virens*. Safe and Taylor[15] converted didehydrogliotoxin (**5**) into a mixture containing the corresponding epitri- and epitetra-sulphides by heating with dihydrogen disulphide. Similarly, Curtis *et al.*[16] converted the epidisulphide, sirodesmin A, into the corresponding epitri- and epitetra-sulphides with sulphur in pyridine. We elected to introduce sulphur into gliotoxin under mild conditions using a catalytic amount of a thiolate to effect cleavage of S–S bonds. Thus, a suspension of gliotoxin in carbon disulphide containing an excess of rhombic sulphur was treated with lithium phenylmethanethiolate at room temperature. The gliotoxin dissolved as the reaction proceeded. After 17 h the mixture was chromatographed on silica to give gliotoxin (**1a**) (13%), gliotoxin E (**1b**) (26%), and gliotoxin G (**1c**) (60%). The physical properties of gliotoxin G agreed well with those reported[13] for the metabolite of *A. fumigatus*. A sample was sent to Dr. P. Waring, who kindly confirmed the identify of the natural and partially synthetic materials. At the same time, he informed us of his recent isolation[17] of the epitrisulphide, gliotoxin E (**1b**), from extracts of, *inter alia*, *Penicillium terlikowskii*, and provided a reference sample. We demonstrated the identity of the synthetic and natural samples of gliotoxin E by comparison of their ^{1}H n.m.r. spectra ($CDCl_3$) at similar concentrations. With a reference sample to hand, we were then able to isolate gliotoxin E from extracts of *G. virens*. To date, gliotoxin G has not been detected in extracts of this fungus.

The ^{1}H n.m.r. spectrum of gliotoxin E (**1b**) indicated the presence of two conformations. This was most clearly seen in dilute solution ($CDCl_3$; 200 MHz), the spectrum then showing, *inter alia*, two sets of ABX multiplets, of similar intensity, for the hydroxymethyl groups, as well as two N-methyl singlets. The existence of two conformations has been noted before[15,16,18] for epitrithiodioxopiperazines, whereas the corresponding epidi- and epitetra-thio derivatives give simple ^{1}H n.m.r. spectra. X-Ray structure determinations of epidi- and epitetra-sulphides show conformations[19] illustrated schematically in the Figure. The disulphide bridges are skewed, thereby relieving strain that otherwise would arise from eclipsed C–S bonds. Also, the bridges are always skewed such that the sulphur atoms are displaced towards the carbonyl groups (**9**). Possibly,[19] this allows an energetically favourable interaction between the sulphur lone-pairs and the electrophilic, carbonyl carbon atoms.[9] Conversely, the epitetrasulphides all adopt conformations (**10**) having S(2) and S(3), calculated[19] to carry small

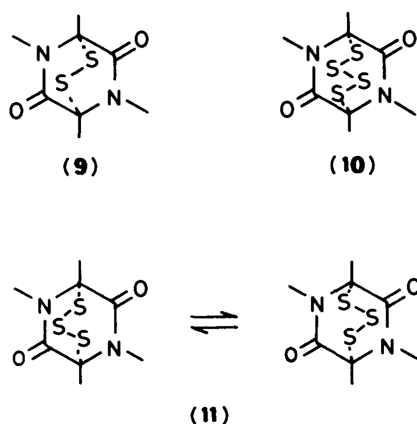

Figure.

positive charges, close to the negative nitrogen atoms. No *X*-ray structures have been published for epitrithiodioxopiperazines, but one would expect little difference in energy between the conformations (**11**) having S(3) approximately equidistant from the carbonyl groups and nitrogen atoms. Indeed, the conformations (**11**) are identical for symmetrically substituted dioxopiperazines, but are diastereoisomeric, and therefore distinguishable by n.m.r. spectroscopy, but for the naturally-occurring epitrisulphides.

Experimental

Radiochemical Methods.—[^{35}S]Sulphate was obtained as an aqueous solution from Amersham International plc. Radiolabelled samples were counted with a Philips PW 4700 liquid scintillation counter in either toluene or, for aqueous solutions, Ecoscint (National Diagnostics). Specific activities were corrected for radiodecay from the date of inoculation. Dioctyl [^{35}S]sulphide (Amersham) was used for standardisation. Radioactive compounds were detected on t.l.c. plates with a Panax thin layer scanner or by autoradiography on Kodak direct exposure *X*-ray film.

Chromatographic Methods.—Analytical and preparative t.l.c. was carried out with precoated Merck Kieselgel GF_{254} plates of 0.25 and 2 mm thickness, respectively. Epipolysulphides were revealed as brown spots by spraying the plates with 2% silver nitrate in aqueous acetone. The solvent systems toluene–acetone (2:1) and toluene–ethyl acetate (1:1) were used routinely (Table), and dichloromethane–ethyl acetate (2:1), diethyl ether–ethyl acetate (2:1), and dichloromethane–methanol (95:5), occasionally. Final separation and purification was effected by h.p.l.c. with a Perkin-Elmer 400 Series liquid chromatograph using a LiChrosorb silica 60 (Merck) column (250 × 16 mm; particle size, 7 μm) eluted with hexane–propan-2-ol (5 ml min^{-1}), and a u.v. detector (254 nm).

Fermentation Conditions and Isolation of Metabolites.—*Gliocladium virens* (NRRL 1828), obtained from the C.A.B. International Mycological Institute (Kew) (IMI 101525, listed as *G. deliquescens*), was maintained on potato dextrose agar and grown for 5 d at 27 °C in shake-culture (250 ml flasks containing 100 ml medium shaken at 160 r.p.m.) in a defined medium [20] at pH 3.0—3.5 (falling to pH 2 after 5 d). [^{35}S]Sulphate (2 mCi) was added to the medium, which contained inactive sulphate salts (total volume, 6.3 l), immediately before inoculation to give a specific activity of 14.5 μCi mmol^{-1} (calcd.). The culture filtrate was extracted with dichloromethane (5 × 600 ml) and the extracts were washed with water, dried (Na_2SO_4), and evaporated to give a gummy residue (1.50 g) (105 μCi). Crystallisation from methanol gave the bulk of the gliotoxin (**1c**) (*ca.* 500 mg) directly; a further quantity was obtained by chromatography of the mother liquors (total, 611 mg). The gliotoxin was crystallised to constant specific activity (29.6 μCi mmol^{-1}). The mother liquors were subjected to repeated preparative t.l.c., monitored by radioscanning and autoradiography. Final purification of minor metabolites was achieved by h.p.l.c. The results are summarised in the Table. The combined yield (by weight) of separated metabolites accounts for 64% of the ^{35}S activity (105 μCi) in the crude extract (calculated with the assumption that each metabolite had the same specific activity as gliotoxin).

*Bis-*N*-norgliovictin* (**6**).—This *metabolite* formed needles, m.p. 231 °C (decomp), $[\alpha]_D$ −32° and $[\alpha]_{546}$ −96° (*c* 0.1 in MeOH); ^{35}S activity, 26 μCi mmol^{-1}; mass spectrum (EI; 70 eV) (values required for $C_{14}H_{18}N_2O_3S_2$ and its fragment ions are given in parentheses); *m/z* 326.0783 (*M*) (326.0759), 279.0807 (*M* − MeS) (279.0803), 248.0618 (*M* − MeS − CH_2OH) (248.0620), 203.0819 (*M* − 2MeS − CHO) (203.0821), 202.0741 (*M* − 2MeS − CH_2O) (202.0742), 201.0661 (*M* − 2MeS − CH_2-OH) (201.0664), and 91.0546 ($C_7H_7^+$) (91.0548); $\nu_{max.}$(KBr) 3 450, 3 190, 3 060, and 1 675 cm^{-1}; δ_H(200 MHz; $CDCl_3$), 2.21 and 2.35 (2 × s, 2 × MeS), 3.01 and 3.60 (ABq, *J* 13.6 Hz, $PhCH_2$), 3.45 (m, CH_2OH), 5.89 and 6.08 (2 × br m, 2 × NH), and 7.18—7.35 (m, Ph); δ_H[200 MHz; $(CD_3)_2CO$] 2.19 and 2.34 (2 × s, 2 × MeS), 3.09 and 3.64 (ABq, *J* 13.4 Hz, $PhCH_2$), 3.22 (dd, *J* 11.4 and 7.6 Hz, CH_AH_BOH), 3.45 (dd, *J* 11.4 and 5.4 Hz, CH_AH_BOH), 3.84 (dd, *J* 7.6 and 5.3 Hz, CH_2OH), 7.23 (m, Ph), and 7.78 (br m, NH).

The 3-*Hydroxymethylbut*-2-*enyl Ether* (**3c**).—This *metabolite* did not crystallise. The mass spectrum (EI; 70 eV) did not show a molecular ion peak [usually weak in bis(methylthio) derivatives], but the following fragment ions supported the composition $C_{20}H_{28}N_2O_4S_2$ (required values in parentheses); *m/z* 377.1533 (*M* − MeS) (377.1535), 327.1351 (*M* − MeS − MeSH − H_2) (327.1345), 245.0918 [*M* − 2MeS − C_5H_9O (3-hydroxymethylbut-2-enyl)] (245.0927), 233.0417 [*M* − $C_{12}H_{15}O_2$ ($C_5H_9O_2C_6H_4CH_2$)] (233.0419), 217.0960 (*M* − 2MeS − C_5H_9O − CO) (217.0977), 186.0459 (*M* − MeS − $C_{12}H_{15}O_2$) (186.0463), 158.0513 (*M* − MeS − C_{12}-$H_{15}O_2$ − CO) (158.0513), 107.0491 ($C_7H_7O^+$) (107.0496), and 91.0552 ($C_7H_7^+$) (91.0548); δ_H(200 MHz; $CDCl_3$) 1.75 (d, *J* 0.5 Hz, vinyl-Me), 2.15 and 2.27 (2 × s, 2 × MeS), 2.96 and 3.23 (2 × s, 2 × MeN), 3.07 and 3.54 (ABq, *J* 14.0 Hz, $PhCH_2$), 4.07 (br d, *J* 5 Hz, collapsed to br s upon D_2O exch., CH_2OH), 4.15 (s, NCH), 4.55 (d, *J* 6.0 Hz, CH_2OAr), 5.72 (t, *J* 6.0 Hz, with fine splitting, vinyl-H), and 6.76 and 6.96 (ABq, *J* 8.7 Hz, ArH); in n.O.e. experiments, irradiation at δ 5.72 caused signal enhancement at δ 4.07 but not 1.75, irradiation at δ 4.55 caused enhancement at δ 1.75 but not 4.07, and irradiation at δ 1.75 caused enhancement at δ 4.55 but not 5.72.

cyclo-(*Glycyl*-L-*tyrosyl*) 4,4-*Dimethylallyl Ether* (**7**).—This highly polar *metabolite* formed plates, m.p. 224—228 °C (from acetone), $[\alpha]_D$ +50° and $[\alpha]_{546}$ +73° (*c* 0.14 in MeOH) (more precise values are reported below for synthetic material) [Found: *m/z* (EI; 70 eV) 288.1459, 220.0849, and 107.0497. $C_{16}H_{20}N_2O_3$ requires *M*, 288.1473; *M* − C_5H_8, 220.0847; and *M* − $C_9H_{13}N_2O_2$, 107.0497]; δ_H[200 MHz; $(CD_3)_2CO$] 1.72 and 1.74 (2 × s, 2 × Me), 2.81 and 3.48 (2 × dd, *J* 17.5 and 3.0 Hz, CH_2NH), 2.96 and 3.12 (2 × dd, *J* 13.5 and 4.7 Hz, CH_2Ar), 4.14 (m, collapsing to t, *J* 4.5 Hz, after D_2O exch., *CH*NH), 4.52 (d, *J* 6.5 Hz, CH_2O), 5.43 (br t, *J* 6.5 Hz, vinyl-H), 6.84 and 7.13 (ABq, *J* 8.5 Hz, aryl-H), and 6.94 and 7.11 (2 × br s, 2 × NH,

exch. with D_2O); δ_H[200 MHz; $(CD_3)_2SO$] 1.68 and 1.72 (2 × s, 2 × Me), 2.55 and 3.33 (ABq, 17.5 Hz, after D_2O exch., CH_2ND), 2.78 and 3.00 (2 × dd, *J* 13.5 and 4.7 Hz, CH_2Ar), 4.00 (m, *CH*NH), 4.47 (d, *J* 6.7 Hz, CH_2O), 5.39 (br t, *J* 6.7 Hz, vinyl-H), 6.83 and 7.03 (ABq, *J* 8.5 Hz, ArH), and 7.87 and 8.11 (2 × br s, 2 × NH).

cyclo-(Glycyl-L-tyrosyl)[11] (55 mg, 0.25 mmol), and 3,3-dimethylallyl bromide (112 mg, 0.75 mmol) in dimethyl sulphoxide (0.5 ml) were treated with sodium hydride (60% suspension in mineral oil) (10 mg, 0.25 mmol) at 0 °C. The mixture was kept for 20 h at room temperature and then diluted with water. The resulting precipitate was collected and washed with water and then dried and washed with hexane. Crystallisation twice from acetone removed an unidentified by-product. The resulting *3-methylbut-2-enyl ether* (**7**) (23 mg) had a m.p. identical with that of the foregoing natural product; $[\alpha]_D$ +62° and $[\alpha]_{546}$ +77° (*c* 0.215 in MeOH) (Found: C, 64.7; H, 6.75; N, 9.3. $C_{16}H_{20}N_2O_3 \cdot 0.5H_2O$ requires C, 64.6; H, 7.1; N, 9.4%); $\nu_{max.}$(KBr) 3 215, 3 110, and 1 665 cm^{-1}. The ^1H n.m.r. spectrum [200 MHz; $(CD_3)_2CO$] was identical with that of the natural product.

Gliotoxin E (**1b**) *and Gliotoxin G* (**1c**).—Gliotoxin E was identified by comparison of its mass and ^1H n.m.r. spectra with those of material synthesized as follows.[5] The ^1H n.m.r. spectra of naturally-occurring gliotoxin E[17] and G[13] provided by Dr. Waring were identical with those of synthetic materials.

A suspension of gliotoxin (**1a**) (98 mg, 0.3 mmol) in carbon disulphide (7.5 ml) containing rhombic sulphur (960 mg) (previously recrystallised from toluene), to which had been added lithium phenylmethanethiolate (0.03 mmol) (prepared from phenylmethanethiol and n-butyl-lithium in tetrahydrofuran), was stirred at room temperature. The gliotoxin dissolved as the reaction proceeded. After 17 h the mixture was poured onto a silica column and the excess of sulphur was eluted with dichloromethane. Elution with ether gave successively gliotoxin (**1a**), gliotoxin E (**1b**), and gliotoxin G (**1c**). After further purification on silica plates developed with dichloromethane–ethyl acetate (2:1), (**1a**), (**1b**), and (**1c**) were obtained in yields of 13, 26, and 60%, respectively. *Gliotoxin E* had m.p. 171—173 °C (from Et_2O), $[\alpha]_D$ −376 and $[\alpha]_{546}$ −475° (*c* 0.9 in $CHCl_3$) (Found: C, 43.3; H, 4.0; N, 7.9; S, 27.0. $C_{13}H_{14}N_2O_4S_3$ requires C, 43.6; H, 3.9; N, 7.8; S, 26.8%); $\nu_{max.}$(KBr) 3 300, 1 685, and 1 646 cm^{-1}; the ^1H n.m.r. spectrum (200 MHz; dilute solution in $CDCl_3$) showed sets of signals, of approximately equal intensity, for 2 conformations; OH signals and OH–CH couplings within CH_2OH groups were confirmed by exchange with D_2O; δ 2.56 (dd, *J* 9.1 and 5.7 Hz, 3a-OH), 2.81 (dd, *J* 9.2 and 6.3 Hz, 3a-OH), 2.97 (d, *J* 16.5 Hz, 10-H), 3.00 (d, *J* 16.5 Hz, 10-H), 3.11 (s, NMe), 3.30 (s, NMe), 3.30 (d, *J* 16.5 Hz, 10-H), 3.35 (d, *J* 16.5 Hz, 10-H), 3.83 (dd, *J* 12.8 and 9.1 Hz, 3a-H), 4.01 (dd, *J* 12.8 and 9.2 Hz, 3a-H), 4.28 (dd, *J* 12.8 and 6.3 Hz, 3a-H), 4.64 (dd, *J* 12.8 and 5.7 Hz, 3a-H), 4.85 (d, *J* 14 Hz, 5a- or 6-H), 5.02 (d, *J* 14 Hz, 5a- or 6-H), 5.08 (br s, 5a- and 6-H), 5.58 (s, 6-OH), 5.93 (s, 6-OH), and 5.70—5.95 (m, vinyl-H); *m/z* (CI; isobutane) 359 (M + 1), 327 (M + 1 − S), 263 (M + 1 − S_3), 245 (M + 1 − S_3 − H_2O), 227 (M + 1 − S_3 − $2H_2O$), and 215 (M + 1 − S_3 − H_2O − CH_2O). *Gliotoxin G* had m.p. 163—164 °C (from Me_2CO), $[\alpha]_D$ −499 and $[\alpha]_{546}$ −607° (*c* 1.25 in $CHCl_3$) (Found: C, 39.8; H, 3.4; N, 7.0; S, 33.15. $C_{13}H_{14}N_2O_4S_4$ requires C, 40.0; H, 3.6; N, 7.2; S, 32.8%); $\nu_{max.}$(KBr) 3 425, 1 677, and 1 652 cm^{-1}; δ_H(300 MHz; $CDCl_3$) 3.05 and 3.26 (ABq, *J* 16.6 Hz, 10-CH_2), 3.12 (s, NMe), 4.00 (s, OH), 4.08 and 4.36 (ABq, *J* 12.5 Hz, after D_2O exch., CH_2OD), 4.78 and 5.06 (ABq, *J* 13.0 Hz, 5a- and 6-H), 5.50 (s, 6-OH), and 5.75—5.95 (m, vinyl-H); *m/z* (CI; isobutane) 391 (M + 1), 359 (M + 1 − S), 327 (M + 1 − S_2), 263 (M + 1 − S_4), 245 (M + 1 − S_4 − H_2O), 227 (M + 1 − S_4 − $2H_2O$), and 215 (M + 1 − S_4 − H_2O − CH_2O).

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