Structure and Synthesis of *cis*-3,6-Dibenzyl-3,6-bis(methylthio)-piperazine-2,5-dione, a New Metabolite of *Aspergillus terreus*

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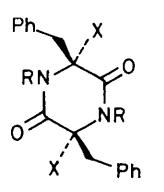
The titled compound (**1b**), the first naturally occurring, sulphur-containing dioxopiperazine unsubstituted on nitrogen, has been isolated from *Aspergillus terreus* and synthesised *via* the corresponding epidisulphide (**2d**), which has been tested as a biosynthetic precursor of bisdethiobis(methylthio)acetylaranotin (**3**).

Cyclo-(L-Phe-L-Phe) (**1a**) is incorporated into bisdethiobis-(methylthio)acetylaranotin (BDA) (**3**) in *Aspergillus terreus*.[1] However, the steps involved in this transformation remain undefined as do those for the biosynthetic pathways leading to sulphur-containing dioxopiperazines in other fungi.[2] We report here two lines of evidence suggesting early introduction of sulphur in the biosynthesis of BDA (**3**).

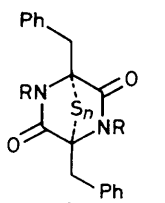
In various feeding experiments, with *A. terreus* (NRRL 3319), using radio-labelled precursors, thin-layer chromatograms of metabolite mixtures were routinely examined by radioscanning and autoradiography. A faint, fast-running spot was commonly observed in experiments with [^{14}C]-phenylalanine, [^{14}C]-*cyclo*-(L-Phe-L-Phe), or sodium [^{35}S]-sulphate. Preparative t.l.c. led to the isolation of a new, minor metabolite, *cis*-3,6-dibenzyl-3,6-bis(methylthio)piperazine-2,5-dione (**1b**), m.p. 291—293 °C, $[\alpha]_{549}$ −122° (*c*, 0.019 in $CHCl_3$). The gross structure (**1b**) was deduced from the ^{1}H n.m.r. spectrum (see below); the observed optical activity demanded a *cis*-configuration. The absolute configuration is provisionally drawn [as (**1b**)] to accord with that of the major metabolites. Since the available quantities of (**1b**) were too small to allow full characterisation the structure was confirmed by the following synthesis of the corresponding racemate.

Schmidt *et al.*[3] achieved direct attachment of sulphur at the 3- and 6-positions of *cyclo*-(L-Pro-L-Pro) using sodamide and elemental sulphur in liquid ammonia. Although the yields are low, this approach was adapted for our purposes since *cyclo*-(Phe-Phe) is readily prepared[4] from phenylalanine. Accordingly (±)-*cis*-*cyclo*-(Phe-Phe) [racemate of (**1a**)] was converted into the derivative (**1c**) by successive treatment with potassium t-butoxide and methoxymethyl chloride in dimethyl sulphoxide. The oily product (**1c**) was treated with lithium di-isopropylamide (2.2 mol. equiv.) and monoclinic sulphur (8 atom equiv.) in tetrahydrofuran (THF) containing *N*,*N*-dimethylformamide (0.025 mol. equiv.) initially at −78 °C and then at 0 °C. Crystallisation of the viscous product from acetone gave (35%) the epitetrasulphide (**2a**),†



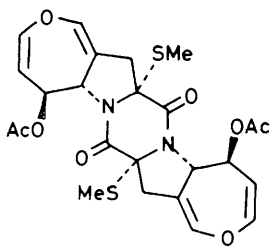
(1)

a; R = X = H
b; R = H, X = MeS
c; R = $MeOCH_2$, X = H
d; R = $MeOCH_2$, X = HS
e; R = $MeOCH_2$, X = MeS



(2)

a; R = $MeOCH_2$, *n* = 4
b; R = $MeOCH_2$, *n* = 2
c; R = $BrCH_2$, *n* = 2
d; R = H, *n* = 2



(3)

m.p. 165—167 °C. Reduction of (**2a**) with sodium borohydride in THF–ethanol at 0 °C gave the unstable dithiol (**1d**) which was converted with ethanolic ferric chloride into the epidisulphide (**2b**) [90% from (**2a**)], m.p. 108—110 °C. Methylation of (**1d**) with iodomethane *in situ* gave (88%) the bis(methylthio)-derivative (**1e**). Treatment of (**2b**) with boron tribromide (2.2 mol. equiv.) in dichloromethane at −78 °C afforded (96%) the bis(bromomethyl) compound (**2c**), m.p. 167—168 °C, which, with a suspension of ammonium acetate in dichloromethane, gave (89%) the epidisulphide (**2d**), m.p. 246—249 °C (decomp.). Reductive methylation of (**2d**), as before, then gave (49%) the desired racemate of (**1b**), m.p. 302—304 °C. Alternatively, (**1b**) was obtained (31%) from

† The structure (**2a**) has been confirmed by Dr. A. A. Freer using *X*-ray methods.

(**1e**) by deprotection using boron tribromide and then sodium hydrogen carbonate.[5] The ^{1}H n.m.r. spectrum of the synthetic, racemic (**1b**) [δ($CDCl_3$, 100 MHz) 2.25 (s, 2 SMe), 2.70 and 3.00 (ABq, *J* 14 Hz, 2 CH_2), 5.80 (br. s, 2 NH, exchangeable with D_2O), and 7.0—7.5 (m, 2 aryl-H)] corresponded exactly with that of the natural product.

The metabolite (**1b**) is the first natural dioxopiperazine having sulphur attached to the 3- and 6-positions without changes elsewhere in the molecule. This shows that, at least in *A. terreus*, biosynthetic introduction of sulphur can occur immediately following construction of a cyclodipeptide. The same conclusion was drawn from experiments using an unnatural cyclodipeptide precursor in *Gliocladium deliquescens*.[5] It appeared possible, therefore, that the simple epidisulphide (**2d**), or the related dithiol, might be a precursor for BDA (**3**) in *A. terreus*. To test this idea, doubly labelled, racemic (**2d**) was synthesised from [4-^{3}H]phenylalanine and [^{35}S]-sulphur. The labelled (**2d**) (45 mg) (^{3}H : ^{35}S ratio, 5.16) was fed in dimethyl sulphoxide (1.2 ml) to cultures (400 ml) of *A. terreus* in the usual way.[1] After 4 days the resulting BDA (**3**) (10 mg)‡ was crystallised to constant specific activity (^{3}H : ^{35}S ratio, 3.24; incorporation of ^{3}H, 2.7%;‡ dilution of ^{3}H, 5.4). The incorporation of (**2d**) into (**3**) with low dilution suggests a biosynthetic precursor–product relationship, but the change in isotope ratio requires that this conclusion be treated with caution. In a second experiment using a shorter (1 day) incubation time, incorporation of (**2d**) into (**3**) was again accompanied by a significant, though smaller, change in the relative isotope ratios of the precursor (4.86) and product (3.82).

Further studies with the epidisulphide (**2d**), for example using separate enantiomers or the related dithiols, are needed to clarify its status as an intermediate in the biosynthesis of BDA (**3**).

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‡ Losses of BDA occur during isolation and purification; the yield (10 mg) and incorporation of ^{3}H [2.7% from (±)-(**2d**)] are consequently conservative estimates.

References

1 M. I. Pita Boente, G. W. Kirby, and D. J. Robins, *J. Chem. Soc., Chem. Commun.*, 1981, 619.
2 G. W. Kirby and D. J. Robins, 'The Biosynthesis of Mycotoxins,' ed. P. S. Steyn, Academic Press, New York, 1980, ch. 9.
3 E. Öhler, H. Poisel, F. Tataruch, and U. Schmidt, *Chem. Ber.*, 1972, **105**, 635. See also R. M. Williams and W. H. Rastetter, *J. Org. Chem.*, 1980, **45**, 2625.
4 C. Sannié, *Bull. Soc. Chim. Fr.*, 1942, **9**, 487.
5 G. W. Kirby, W. Lösel, P. S. Rao, D. J. Robins, M. A. Sefton, and R. R. Talekar, *J. Chem. Soc., Chem. Commun.*, preceding communication.