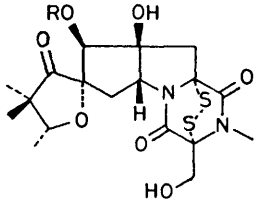
Structure and Synthesis of Phomamide, a New Piperazine-2,5-dione related to the Sirodesmins, isolated from the Culture Medium of *Phoma lingam* Tode

By **Jean-Pierre Ferezou, Annik Quesneau-Thierry,** and **Michel Barbier,*** Institut de Chimie des Substances Naturelles, C.N.R.S., 91190 Gif-sur-Yvette, France
Albert Kollmann and **Jean-François Bousquet,** Station Centrale de Pathologie Végétale, I.N.R.A., 78000 Versailles, France

Several active substances are produced by the fungus *Phoma lingam* Tode, among which are the previously reported sirodesmins PL (1) and (2), sulphur-containing piperazinediones. A new metabolite, phomamide, also a piperazine-2,5-dione, has now been isolated from the culture filtrate of the same fungus. On the basis of physicochemical and spectroscopical data, the structure (3) of cyclo-*O*-(γγ-dimethylallyl)-L-tyrosyl-L-serine has been demonstrated for this compound and confirmed through subsequent synthesis. This metabolite is assumed to be an intermediate in the biosynthesis of the sirodesmin group of antibiotics.

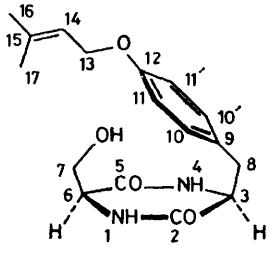
DURING the course of investigations on active metabolites produced by the fungus *Phoma lingam* Tode, we reported [1,2] the isolation and structures of sirodesmin PL (1) and deacetylsirodesmin PL (2), two epidithiopiperazinediones. A careful investigation of the organic extracts from the culture filtrate has led to the isolation of a new compound for which the name phomamide is proposed, the structure (3) having been confirmed through synthesis.



(1) Sirodesmin PL : R = —CO—CH_3

(2) Deacetylsirodesmin PL : R = H

The isolation was performed under the same conditions [1] as for the sirodesmins, two silicic acid column chromatographies giving the non-toxic compounds (3) as well as the two sulphur-containing phytotoxins. The new metabolite (3), $C_{17}H_{22}N_2O_4$ (*M* 318), exhibits u.v. bands at 209 (ε 12.4 × 10^3), 229 (13.5 × 10^3), and 227 nm (1.45 × 10^3), and i.r. absorptions at 1 650—1 680



(3)

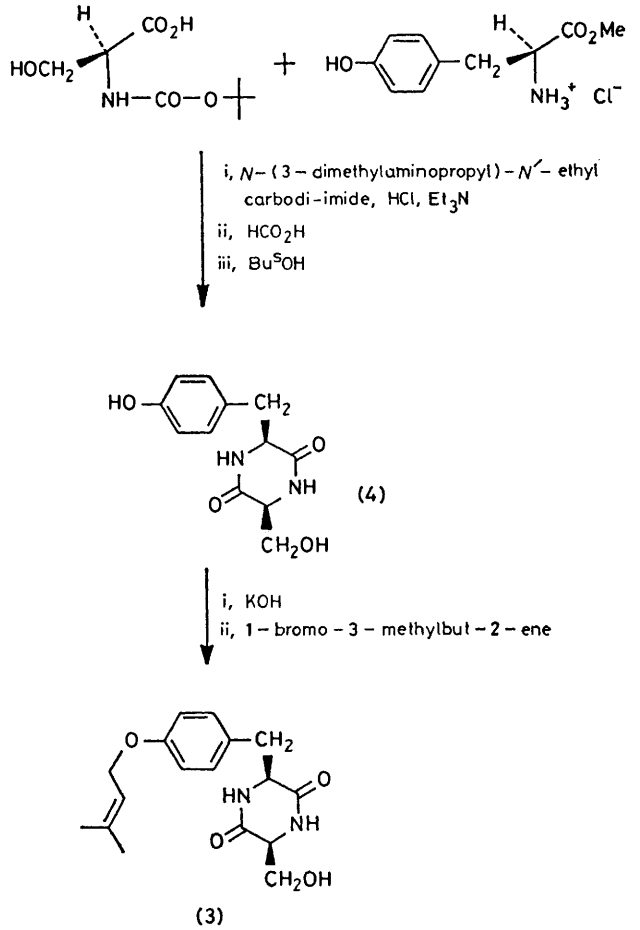
cm^{-1} (amide C=O) and 3 320—3 200 (NH). In the ^1H n.m.r. spectrum the two NH protons appeared at δ 7.90 and 7.92 as exchangeable signals. The structure (3) of a piperazine-2,5-dione is consistent [3] with these results. I.r. bands at 1 620 (shoulder), 1 585, and 1 515 cm^{-1} as well as the 277 nm u.v. absorption, indicate an aromatic ring, the i.r. band at 820 cm^{-1} (CH) and the AA'BB' ^1H n.m.r. pattern centred on δ 6.95 (tyrosyl residue) signifying a 1,4-disubstituted system. Furthermore, signals at δ 1.69 (3 H, s), 1.73 (2 H, s), 5.41 (1 H, m), and 4.48 (2 H, d, *J* 6.8Hz) are in agreement with the *O*-isoprenoid unit [4] as shown. Two more complex ABX systems, 3-H,8-H_2 and 6-H,7-H_2, were assigned after double irradiation experiments. The first one (δ_A 2.91, δ_B 3.01, δ_X 3.99, J_{AB} 14, J_{AX} 4.5, J_{BX} 6.1 Hz) is related to the CH_α–$CH_{2\beta}$ of the tyrosyl unit, and the second overlapped ABX system is consistent with the CH_α–$CH_{2\beta}$ part of the seryl unit [5] (6-H,7-H_2). The δ 2.91 and 3.35 AB signals of this last system are simplified after exchange with D_2O of one proton at δ 4.89 (t), in agreement with a primary hydroxy-group [$\nu_{max.}$ 3 500 cm^{-1}]. The acetylation of this hydroxy-group results in a 0.45 p.p.m. deshielding of these AB signals. On the basis of these data and also of ^{13}C n.m.r. spectra of the product and its monoacetylated derivative, (3) was shown to be cyclo-*O*-(γγ-dimethylallyl)tyrosylserine.

Information on the stereochemistry can be obtained from the ^1H n.m.r. spectrum as follows: the C_β methylene protons of the seryl residue resonate at higher field than that expected from the free amino-acid, and this is consistent with previous findings [6] concerning piperazine-2,5-diones derived from aromatic amino-acids. These protons undergo a strong anisotropic shielding effect due to the neighbouring aromatic ring, indicating a *cis* configuration of the two amino-acid side-chains. The C_α methine proton of the seryl residue (6-H) is not shielded, due to its situation [6] *trans* to the tyrosyl residue. Furthermore, the $^3J_{H_\alpha-H_\beta}$ coupling constants in the two amino-acids (6-H,7-H_2 and 3-H,8-H_2) agree with a folded conformation in solution, so that the aromatic ring assumes the maximum interaction with the nearly planar piperazinedione cycle as already reported [7] for cyclo-L-tyrosyl-L-serine.

A more difficult problem deals with the determination of the absolute configuration at the two asymmetric centres C-3 and C-6 of the piperazinedione. Because of

some inconsistency in the literature,[8,9,10] the $[\alpha]_D^{20}$ value of (3) (−76° in methanol) was of no help for these assignments. However, the c.d. curve [$\Delta\varepsilon$ (217 nm) −10.4; $\Delta\varepsilon$ (227 nm) −9.2; $\Delta\varepsilon$ (275 nm) +1] indicates an LL (*SS*) configuration, comparing well with some reported [11] values for cyclodipeptides.

The structure (3) was confirmed by a stereospecific synthesis (as shown on the Scheme) and direct comparison of the natural and synthetic samples. Condensation of t-BOC-L-serine with L-tyrosine methyl ester



SCHEME

gave the known [9] dipeptide methyl ester, the formate salt of which was cyclised to cyclo-L-tyrosyl-L-serine (4), and since no diastereoisomer was detected (t.l.c., n.m.r.) it was presumed that no racemisation had occurred during the process. The sodium salt of (4) was then treated with 1-bromo-3-methylbut-2-ene in DMF to give the expected product (3), pure after crystallisation (u.v., i.r., n.m.r., and mass spectra, and R_F values).

EXPERIMENTAL

M.p.s were determined with a Reichert apparatus and are corrected. I.r. spectra were recorded for KBr discs on a Perkin-Elmer 257 spectrophotometer and electron impact mass spectra on an A.E.I. MS 50 spectrometer. ^{1}H N.m.r. spectra were obtained for solutions in $(CD_3)_2SO$ (Me_4Si as internal standard) on a Varian T60 or, for double irradiation experiments, a Cameca 250 MHz instrument). P.n.d. and s.f.o.r.d. ^{13}C n.m.r. spectra were recorded at 22.63 Hz on a Bruker HX f.t. spectrometer. Optical rotations were determined with a Jouan Quick polarimeter and c.d. curves with a Jouan Dichrograph apparatus.

Isolation of the Phomamide (3).—The fungus *Phoma lingam* Tode was grown as surface cultures in Roux flasks under the previously reported [1] conditions. Ethyl acetate extracts of the culture filtrate were submitted to chromatography on silica gel using a gradient of methanol in ethyl acetate as eluant, the more polar fraction being combined. A second chromatography, eluting with chloroform–ethyl acetate–methanol 12 : 4 : 1, afforded the crude product (amorphous). The final purification was achieved on a Sephadex LH 20 column using methanol for elution whilst monitoring by t.l.c. (R_F 0.50; ethyl acetate–methanol 4 : 1; Schleicher–Schüll F_{254} SiO_2 films). The pure (3) was obtained after crystallisation from ethyl acetate (8 mg l^{-1} culture medium) as white prisms, m.p. 213—215°, $[\alpha]_D^{20}$ −76° (methanol); $\nu_{max.}$ 3 500, 3 320, 3 200, 1 680, 1 650, 1 620, 1 585, 1 515, and 825 cm^{-1}; *m/e* 318 (M^+, 2%), 250 (30), 144 (95), 107 (97), and 69 (100); $\lambda_{max.}$ (methanol, 0.1 mmol l^{-1}) 209 (ε 12.4 × 10^3), 229 (13.5 × 10^3), and 277 nm (1.45 × 10^3); c.d. (methanol, 0.1 mmol l^{-1}) $\Delta\varepsilon$ (217 nm) −10.4, (227) −0.2, and (275) +1; δ 1.69 (3 H, s, 17-H$_3$), 1.73 (3 H, s, 16-H), 2.91 (1 H, m, A part of $A_1B_1X_1$ system, *J* 4.5 and 14 Hz, 8-H$_a$), 2.91 (1 H, m, A part of $A_2B_2X_2$ system, *J* 6 and 11 Hz, 7-H$_a$), 3.01 (1 H, dd, B part of $A_1B_1X_1$, *J* 6.1 and 14 Hz, 8-H$_b$), 3.35 (1 H, m, B part of $A_2B_2X_2$, *J* 3 and 11 Hz, 7-H$_b$), 3.67 (1 H, m, X part of $A_2B_2X_2$, *J* 6 and 3 Hz, 6-H), 3.99 (1 H, m, X part of $A_1B_1X_1$, *J* 4.5 and 6.1 Hz, 3-H), 4.48 (2 H, d, *J* 6.8 Hz, 13-H$_2$), 4.89 (1 H, t, exchangeable with D_2O, 7-OH), 5.41 (1 H, m, 14-H), 6.95 (4 H, m, aromatic), and 7.90 and 7.92 (2 H, m, exchangeable with D_2O, 1- and 4-NH); δ_c 17.9 (q, C-17), 25.3 (q, C-16), 38.9 (t, C-8), 55.6 (d, C-3 or -6), 57.1 (d, C-3 or -6), 63.1 (t, C-7), 64.2 (t, C-13), 114.3 (d, C-11 and -11′), 120.1 (d, C-1 and -10′), 128.3 (s, C-9), 130.9 (d, C-14), 136.7 (s, C-15), 157.2 (s, C-12), 165.8 (s, C-2 or -5), and 166.7 (s, C-2 or 5, C-carbonyl atoms) (Found: C, 64.3; H, 7; N, 8.5; O, 20.2. Calc. for $C_{17}H_{22}N_2O_4$: C, 64.1; H, 7; N, 8.8; O, 20.1%), *m/e* 318 (M^+).

Synthesis of Cyclo-O-(γγ-dimethylallyl)-L-tyrosyl-L-serine.—The cyclic dipeptide cyclo-L-tyrosyl-L-serine (4) was prepared from t-BOC-L-serine (Serva; 12.5 mM) and L-tyrosine methyl ester hydrochloride (Serva; 12.5 mM) in 70% yield, m.p. 268—272°, using a previously reported [9] synthesis. The product had $[\alpha]_D^{20}$ −90° (methanol), $\lambda_{max.}$ (methanol, 0.15 mmol l^{-1}) 212 (ε 7.2 × 10^3), 226 (8.7 × 10^3), and 280 nm (1.45 × 10^3); *m/e* 250 (15%), 173 (41), 158 (46), 144 (100), 130 (46), 107 (100), 96 (54), and 69 (66), ^{1}H n.m.r. in agreement with previously reported [6] data for cyclotyrosylserine (Found: C, 57.5; H, 5.6; N, 11.2. Calc. for $C_{12}H_{14}N_2O_4$: C, 57.6; H, 5.6; N, 11.2%), *m/e* 250 (M^+).

This product (4) was treated with 1 equivalent of potassium hydroxide in 40 ml H_2O at 5 °C for 2 h. After lyophilisation, the cyclopeptide potassium salt was dissolved in DMF (25 ml), 1-bromo-3-methylbut-2-ene (Lancaster Synthesis Ltd., 4 mM) was added, and the solution stored at 30 °C for 6 h. After removing the bulk of the DMF under reduced pressure at 40 °C, the product was crystallised twice from H_2O–methanol (yield 76%) and was shown [n.m.r. spectrum, t.l.c. (SiO_2, R_F 0.50, ethyl acetate–methanol 4 : 1), m.p. 214—216°] to be free of diastereoisomers; *m/e* 318 (2%), 250 (40), 144 (96), 107 (100), and 69 (80); $[\alpha]_D^{20}$ −77°

(methanol); $\lambda_{max.}$ (methanol, 0.1 mol l^{-1}) 209 (ε 12.3 $\times$ 10^3), 229 (13.2 $\times$ 10^3), and 277 nm (1.42 $\times$ 10^3); c.d. (218 nm) −9.9, (229) −8.9, and (275) +0.8; i.r. and ^{1}H n.m.r. spectra of natural (3) identical to those of synthetic cyclo-*O*-($\gamma\gamma$-dimethylallyl-L-tyrosyl-L-serine (Found: C, 63.9; H, 6.7; N, 8.6%); *m/e* 318 (M^+).

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