7-*epi*-Brefeldin A, a Co-metabolite of Brefeldin A in *Curvularia lunata*

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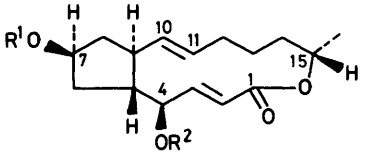
The macrolide antibiotic brefeldin A and its 7-epimer have been isolated from cultures of *Curvularia lunata*. The structure of 7-*epi*-brefeldin A was determined by spectrometric and chemical means.

THE obvious structural resemblance of the sixteen-membered macrolide antibiotic brefeldin A (1) [1-3] to the prostaglandins has led to a wealth of knowledge concerning its synthesis, biosynthesis, chemical reactions, and biological properties.[4-7] This ubiquitous metabolite is produced by a variety of organisms [4] and has been known variously as ascotoxin, cyanein, and decumbin. In this paper we report the isolation of brefeldin A from toxinogenic isolates of *Curvularia lunata* (Wakker) Boedijn,† as well as the natural occurrence of a previously undescribed epimer (5) of brefeldin A,‡ which we have identified on the basis of physical and chemical data.

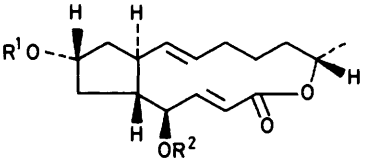
The close structural relationship of the unknown metabolite, $C_{16}H_{24}O_4$, to brefeldin A was suggested by its spectroscopic parameters. The i.r. spectrum indicated the presence of one or more hydroxy-groups (3 400 cm^{-1}) and an αβ-unsaturated lactone (1 700 and 1 261 cm^{-1}), as well as weak C=C stretching frequencies (1 641 and 1 635 cm^{-1}).

The ^1H n.m.r. spectrum of compound (5) was very similar to that of brefeldin A (Table), the main difference, apart from small chemical-shift changes, being in the shape of the overlapping H(4) and H(7) multiplets (Figure) at δ 4.13. The ^{13}C n.m.r. spectrum of compound (5) was also consistent with the assigned structure, showing the presence of five sp^2 and eleven sp^3 carbon atoms. The sp^3 carbon resonances can be further subdivided into three oxygen-bearing atoms, two methine, five methylene, and one methyl atom.

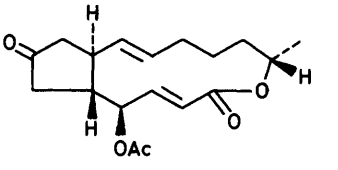
Acetylation of compound (5) with a large excess of acetic anhydride in pyridine smoothly afforded the diacetate (6). The ^1H n.m.r. spectrum of this compound was very similar to that of the corresponding diacetate of brefeldin A, compound (2) (Table). The two monoacetates of brefeldin A, compounds (3) and (4), are easily prepared by varying the acetylating conditions.[5] Accordingly, treatment of compound (5) with 2.2 equiv. of acetic anhydride in pyridine afforded a mixture of unchanged (5), the diacetate (6), and the two monoacetates (7) and (8), from which the 4-*O*-acetyl derivative (7) could be isolated in 37% yield. Comparison of the ^1H n.m.r. spectrum of this compound with that of compound (3) emphasized their close structural similarity (Table).



(1) $R^1 = R^2 = H$ (3) $R^1 = H$, $R^2 = Ac$
(2) $R^1 = R^2 = Ac$ (4) $R^1 = Ac$, $R^2 = H$



(5) $R^1 = R^2 = H$ (7) $R^1 = H$, $R^2 = Ac$
(6) $R^1 = R^2 = Ac$ (8) $R^1 = Ac$, $R^2 = H$



(9)

Oxidation of compound (7) with pyridinium chlorochromate in dichloromethane gave the ketone (9) (82% yield) which was identical in all aspects with the ketone obtained on oxidation of 4-*O*-acetylbrefeldin A (3) under the same conditions.[5] This result unambiguously determines the structure of compound (5), since the only stereochemical centre removed in this oxidation process is that at C(7).

† *C. lunata* (MRC 975) was found to be the major contaminant of grass in a pasture in which deaths in cattle due to unknown causes had occurred.

‡ Mabuni *et al.* reported the isolation of small quantities of what they assumed was 7-*epi*-brefeldin A (5) during the preparation of [7-^{18}O]brefeldin A (ref. 5). However, no physical data were given.

TABLE

^{1}H N.m.r. data for compounds (1)—(3), (5)—(7), and (9) [chemical shift,[a] multiplicity,[b] and coupling constant (Hz)]

	Diol		Diacetate		4-Acetate		Ketone
Proton	(1) [c]	(5) [c]	(2) [d]	(6) [d]	(3) [d]	(7) [d]	(9) [d]
2	5.82 (dd, J = 16,2)	5.78 (dd, J = 16,1.5)	5.72 (dd, J = 16,1.5)	5.58 (dd, J = 16,1.5)	5.77 (dd, J = 16,1.5)	5.73 (dd, J = 16,1.5)	5.80 (dd, J = 16,1.5)
3	7.43 (dd, J = 16,3)	7.45 (dd, J = 16,3)	7.27 (dd, J = 16,3)	7.22 (dd, J = 16,3)	7.32 (dd, J = 16,3)	7.33 (dd, J = 16,3)	7.31 (dd, J = 16,3)
4	4.12 (m)	4.07 (m)	5.23 (m)	5.13 (m)	5.30 (m)	5.36 (m)	5.38 (m)
4-OH	5.15 (d, J = 6)	5.10 (d, J = 6)					
7	4.12 (m)	4.07 (m)	5.23 (m)	5.13 (m)	4.38 (m)	4.40 (m)	
7-OH	4.55 (d, J = 4)	4.45 (d, J = 4)					
10	5.23 (dd, J = 14,9)	5.16 (dd, J = 15,9)	5.23 (m)	5.13 (m)	5.34 (m)	5.30 (m)	5.23 (m)
11	5.75 (obscured)	5.78 (obscured)	5.75 (m)	5.77 (m)	5.77 (m)	5.83 (m)	5.83 (m)
15	4.81 (m)	4.76 (m)	4.88 (m)	4.80 (m)	4.93 (m)	4.92 (m)	4.97 (m)
16	1.23 (d, J = 6)	1.21 (d, J = 6)	1.23 (d, J = 6)	1.14 (d, J = 6)	1.30 (d, J = 6)	1.29 (d, J = 6)	1.28 (d, J = 6)

[a] Relative to internal $(CH_3)_4Si$. [b] d = Doublet, m= multiplet. [c] Solvent $[^2H_6]DMSO$. [d] Solvent $CDCl_3$.

The identity of compound (5) as a true fungal metabolite, and not as an artefact of brefeldin A produced in the isolation process, was confirmed by subjecting a sample of pure brefeldin A to identical extraction and purification procedures. No trace of the epimer (5) was

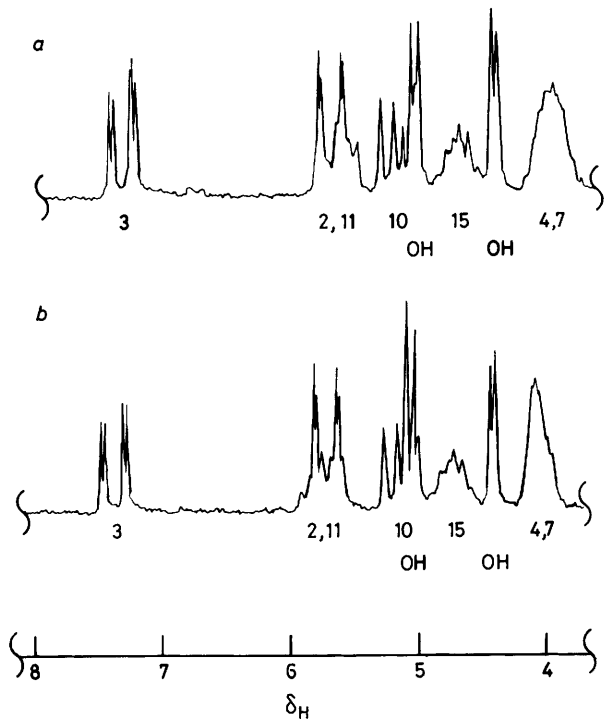


FIGURE Sections from the ^1H n.m.r. spectra of (a) brefeldin A (1) and (b) 7-*epi*-brefeldin A (5) in [$^2H_6$]DMSO. Numbers refer to positions of hydrogen atoms

detected. It is interesting that the fungus should co-produce two metabolites differing only in their stereochemistry at one position. As brefeldin A is produced entirely from acetate and the oxygen atom at C(7) arises from molecular oxygen,[5] this implies a duality in the enzyme system which introduces oxygen at this position.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. U.v. absorptions were measured for solutions in methanol on a Unicam SP8–100 spectrometer. I.r. spectra were recorded on a Perkin-Elmer 237 spectrometer for solutions in chloroform. Mass spectra were taken on a Varian MAT 212 spectrometer. ^1H and ^{13}C N.m.r. spectra were recorded on a Varian EM 390 spectrometer and a Varian CFT-20 spectrometer, respectively. Optical rotations were measured for solutions in methanol on a Perkin-Elmer 241 polarimeter.

Isolation of Brefeldin A (1) *and* 7-epi-*Brefeldin A* (5).—*C. lunata* (MRC 975) was grown on sterilised whole yellow maize for 21 d at 25 °C, and was then dried, milled, and incorporated in the feed of ducklings and rats. The culture material proved to be highly toxic. Rats receiving 25—50% mouldy material died within 3 weeks, and ducklings receiving 50% died after 6 d. Ethyl acetate extracts of maize culture material gave strong positive skin reactions in rabbits. The crude maize meal (5 kg) was exhaustively extracted with chloroform–methanol (1 : 1 v/v) for 48 h, and then with acetone–water (1 : 1 v/v) for 48 h. The extracts were kept separate, but treated identically. The solvent was removed under reduced pressure and the resultant gum was partitioned between hexane and 90% aqueous methanol (1 l, 1 : 1 v/v). The methanol layer was evaporated to dryness and the residue was partitioned between chloroform and water (1 l, 1 : 1 v/v). The chloroform layer, which contained brefeldin A (1) and its 7-epimer (5), was evaporated to dryness. Compounds (1) and (5) were obtained pure by chromatography of the resultant brown gum on silica gel (1 kg) with methanol–chloroform (1 : 19 v/v) as eluant. 7-epi-*Brefeldin A* (5) was eluted first and was crystallized from diethyl ether–n-pentane as fine needles (1.31 g), m.p. 124—125 °C; $[\alpha]_D^{20}$ +108.6° (*c*, 1.03); $\nu_{max.}$ 3 400, 2 925, 1 700, 1 641, 1 635, and 1 261 cm^{-1}; δ_C ($CDCl_3$) 166.7 (s), 152.5 (d), 135.3 (d), 131.1 (d), 117.3 (d), 78.6 (d), 72.9 (d), 71.9 (d), 52.0 (d), 44.4 (d), 43.6 (t), 40.1 (t), 34.0 (t), 32.0 (t), 26.7 (t), and 20.8 (q) p.p.m. (letters in parentheses refer to S.F.O.R.D. multiplicities) (Found: C, 68.6; H, 8.55%; M^+, 280.1670. $C_{16}H_{24}O_4$ requires C, 68.55; H, 8.63%; *M*, 280.1674).

Brefeldin A (1) (1.2 g) was eluted immediately after compound (5) and was crystallized from methanol, m.p. 203 °C (lit.,[1] 204—205 °C). The compound had identical spectral parameters to those cited in the literature.

Acetylation of 7-epi-*Brefeldin A* (5).—A solution of 7-*epi*-brefeldin A (5) (88 mg) in dry pyridine (3 ml) was treated with acetic anhydride (1.5 ml). After 24 h at 20 °C, standard work-up gave a yellow oil which was purified by chromatography on silica gel (30 g) with methanol–chloroform (3 : 97 v/v) as eluant to give the *diacetate* (6) (110 mg, 96%) as an oil; $[\alpha]_D^{20}$ +19.5° (*c*, 1.11); $\nu_{max.}$ 2 930, 1 728, 1 710, 1 649, 1 450, 1 375, and 1 245 cm^{-1}; δ_C ($CDCl_3$) 169.6 (s), 168.9 (s), 164.7 (s), 146.3 (d), 133.6 (d), 131.3 (d), 117.7 (d), 76.0 (d), 75.0 (d), 70.9 (d), 48.4 (d), 44.1 (d), 40.0 (t), 36.5 (t), 33.3 (t), 31.2 (t), 25.7 (t), 20.5 (q), and 20.0 (2 × C, q) (Found: M^+, 364.1881. $C_{20}H_{28}O_6$ requires *M*, 364.1886).

Selective Acetylation of 7-epi-*Brefeldin A* (5).—A solution of 7-*epi*-brefeldin A (5) (207 mg) in dry pyridine (10 ml) was stirred with acetic anhydride (165 mg). After 20 h at 20 °C, standard work-up gave a yellow oil which was purified by chromatography on silica gel (100 g) with methanol–chloroform (2 : 98 v/v) as eluant to give the diacetate (6) (80 mg) identical with the compound prepared above, followed by the *monoacetate* (7) (89 mg, 37%) which was crystallized from aqueous methanol as prisms, m.p. 130—132 °C; $\nu_{max.}$ 3 480, 2 922, 1 705, 1 648, 1 449, 1 371, and 1 248 cm^{-1} (Found: M^+, 322.1783. $C_{18}H_{26}O_5$ requires *M*, 322.1780). Continued elution with the same solvent gave an additional product (7 mg), presumably the monoacetate (8), which was not characterized.

Oxidation of 4-O-*Acetyl*-7-epi-*brefeldin A* (7).—A solution of 4-*O*-acetyl-7-*epi*-brefeldin A (7)(44 mg) in dichloromethane (1.5 ml) was added to a suspension of pyridinium chloromate (52 mg) in dichloromethane (1 ml) and the mixture was stirred for 3 h at 20 °C. After filtration through silica gel (0.5 g) the solvent was removed and the resultant yellow gum was purified by chromatography on silica gel (30 g) with methanol–chloroform (2 : 98 v/v) as eluant to give the ketone (9) which was crystallised from aqueous methanol as needles (36 mg, 82%), m.p. 120—122 °C (lit.,[5] 122—123.5 °C); $[\alpha]_D^{20}$ −35.3° (*c*, 0.90); $\nu_{max.}$ 2 922, 1 740, 1 708, 1 372, 1 267, and 1 244 cm^{-1} (Found: M^+, 320.1617. $C_{18}H_{24}O_5$ requires

M, 320.1624). This compound was identical with the product obtained on oxidation of 4-acetylbrefeldin A (3) using the conditions described by Mabuni *et al.*[5]

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