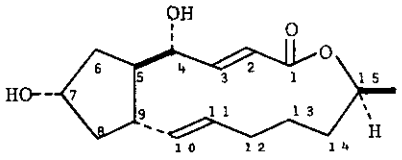
ISOLATION AND STRUCTURE OF BREFELDIN C

Masayuki Sunagawa, Tomihisa Ohta, and Shigeo Nozoe*
Pharmaceutical Institute, Tohoku University,
Aobayama, Sendai 980, Japan

The title macrolide has been isolated from Eupenicillium brefeldianum. This communication describes the isolation and the structural elucidation of brefeldin C which might give a clue to the biogenetic study of brefeldin A.

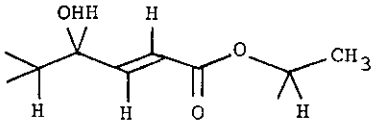
The biogenesis of brefeldin A (2)[1], a fungal metabolite belonged to a C_{15} macrolide antibiotic, has been interested in view point of a structural similarity to prostaglandins.[2,3] Since the biosynthetic pathway of 2 has not been confirmed yet, we have searched its analogs in Eupenicillium brefeldianum, in hope to find a clue for the biogenetic mechanism of cyclopentanol in brefeldin A (2).

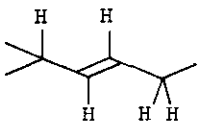


2

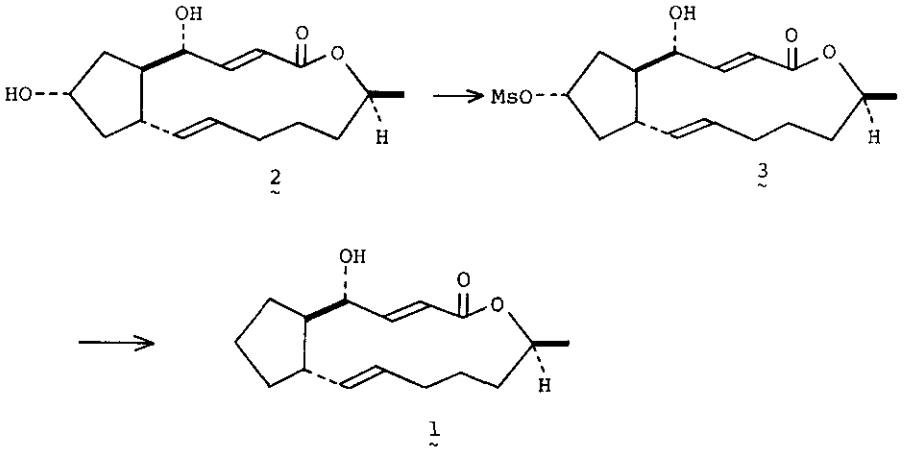
We wish to report here the isolation and the structural elucidation of brefeldin C (1). Brefeldin C has been isolated from Eupenicillium brefeldianum (IFO 8945) which was fermentated in malt-glucose medium (45 l) at 27° for 3 days. The culture broth and the mycelia were extracted with EtOAc and $CHCl_3$-MeOH (1:1) respectively. The latter was fractionated into water and EtOAc soluble parts and both the EtOAc extracts were independently subjected to chromatography over silica gel. n-Hexane-EtOAc (3:2) eluate was then applied to Lobar column (25 mm id X 310 mm) packed with LiChroprep Si 60. Elution with n-hexane-EtOAc (3:2) gave a crystalline mass which was recrystallized from MeOH to yield a new macrolide, brefeldin C. Brefeldin C was given in this manner, 13 mg from the culture broth extract and 2 mg from the mycelia extract. Brefeldin C, mp 160.5-161°, $C_{16}H_{24}O_3$ (m/e 264 as M^+),

shows $[\alpha]_D$ +130.6° (C 0.07, $CHCl_3$). The IR and ^{1}H-NMR spectra of brefeldin C (1) indicate the presence of an unsaturated lactone [ν 1670, 1635, 1260, δ 4.82 (1H, m), 5.86 (1H, dd, J=2, 16 Hz), 7.34 (1H, dd, J=3, 16 Hz)], an allylic hydroxyl [ν 3410, δ 4.04 (1H, ddd, J=2, 3, 9 Hz)], a disubstituted double bond with trans geometry [δ 5.14 (1H, dd, J=9, 15.5 Hz), 5.66 (1H, ddd, J=6, 9, 15.5 Hz)], and a secondary methyl group having α-oxygen linkage [δ 1.26 (3H, d, J=7 Hz)]. The allylic carbinol was shown to be adjacent to the double bond in the conjugated lactone by means of double resonance technique of ^{1}H-NMR (^{1}H-NMDR). Further analysis of the ^{1}H-NMR along with the above properties verified the following partial structures. The displayed partial structures and the spectral properties of brefeldin C, which resemble well to those of brefeldin A (2), implies that brefeldin C is similar in structure to brefeldin A (2)[1].





Brefeldin C differs from brefeldin A (2) in showing sixteen mass units less than the M^+ of 2 and only one carbinol hydrogen signal (δ 4.04) in the ^{1}H-NMR spectrum while 2 shows two [(δ 4.4 (2X1H, m's)]. The chemical correlation of both the substances was performed as follows. Brefeldin A (2) was first transformed into 7-mesylate 3 in 79% on the direct treatment of 2 with metanesulfonyl chloride and triethylamine in pyridine at -10°.[4] Thus one of the two hydroxyls



was mesylated selectively and the possition of mesyl group was confirmed by the ^{1}H-NMDR experiment for the compound 3 demonstrating that unchanged hydroxyl is adjacent to the 2-ene moiety. The mesylate (3) was then reduced with NaI-Zn in dimethoxy ethane to afford 7-deoxy brefeldin A (1) which was identified with brefeldin C.[5] The overall yield was 37%.

It has been reported several biosynthetic experiments[2] for brefeldin A (2) which was thought to be derived from an unsaturated fatty acid precursor. Hutchinson et al.[3], subsequently, have demonstrated that C-15 oxygen of 2 is not brought from O_2, indicating a non-fatty-acid-polyketide origin for brefeldin A (2). They also have proved that the oxygens at C-4 and C-7 are originated from distinct oxygen molecules respectively. Although contribution of oxygens at C-4 and C-7 to the cyclization of the cyclopentane ring in 2 has not been verified, the exsistence of the 7-deoxy analog (1) in Eupenicillium brefeldianum may suggest at least that C-7 oxgen may not take a part in the cyclopentane formation through the biogenesis of brefeldin A (2). Intermediary role of brefeldin C in the brefeldin biosynthesis is under investigation.

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4. Brefeldin A (2) 60 mg in pyridine 1 ml and Et_3N 90 µl was added CH_3SO_2Cl 54 µl dropwise at -10° in the period of 7 min, and the mixture was stirred at -10°

for 13 min. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc layer was washed with ice-water, cold 10% HCl, sat. $NaHCO_3$ aq. and sat. brine, dried over Na_2SO_4 and evaporated in vacuo. The crude product 66 mg was chromatographed on silica gel and elution with n-hexane-EtOAc (1:1) gave a homogeneous oil 36 mg. 3, m/e 359 (M^++1), 358 (M^+). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1710, 1645, 1260. ^{1}H-NMR ($CDCl_3$, 100 MHz) ppm: δ 1.22 (3H, d, J=6.5 Hz), 3.23 (3H, s), 4.32 (1H, m), 4.94 (2H, m), 5.28 (1H, m), 5.71 (1H, m), 6.44 (1H, dd, J=1.5, 16 Hz), 7.55 (1H, dd, J=3, 16 Hz).

5. 7-Mesylate (3) 31 mg in dimethoxy ethane 4 ml was added NaI 67 mg and powdered Zn 58 mg, and the mixture was refluxed for 40 min. Zn powder was filtered off and the filtrate was evaporated to dryness. The residue 30 mg was chromatographed on silica gel and elution with n-hexane-EtOAc (3:2) afforded a crystalline mass. Recrystallization from $CHCl_3$-MeOH gave 7-deoxy brefeldin A (1), 18 mg, mp 160-161°, which was identified with brefeldin C.