Secondary Metabolites by Chemical Sceening, 26.7-O-#-D-Galactosyl-brefeldin A via Transglycosylation with Penicillium brefeldianum

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SECONDARY METABOLITES BY CHEMICAL SCREENING, 26.[1] 7-*O*-β-D-GALACTOSYL-BREFELDIN A VIA TRANSGLYCOSYLATION WITH *PENICILLIUM BREFELDIANUM*

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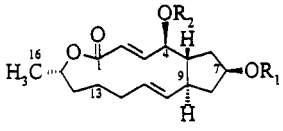
ABSTRACT.—A lactose-containing fermentation medium initiated the formation of 7-*O*-β-D-galactosyl-brefeldin A [**1**] in the brefeldin A-producing organism, *Penicillium brefeldianum*. The detection, isolation, and physicochemical properties of **1**, which has been produced by biological derivatization via transglycosylation, are described. Compound **1** is the first glycosylated brefeldin A derivative to have been prepared.

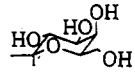
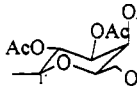
A brefeldin A-producing fungus (strain FH-A 6474, isolated from a soil sample collected in Hawaii) has been discovered in the course of our chemical screening project (2–4), guided by the striking dark blue to violet coloration of brefeldin A [**2**] after staining with anisaldehyde/H_2SO_4 on tlc plates [R_f-values: 0.50 ($CHCl_3$-MeOH, 9:1) and 0.95 (*n*-BuOH-AcOH-H_2O, 4:1:5, upper phase)]. The purified brefeldin A [**2**] corresponded completely with an authentic sample, especially the optical rotation value, thereby excluding 7-*epi*-brefeldin A (5). Brefeldin A [**2**] bears a 13-membered lactone ring and has been found to be an antifungal, nematocidal, antiviral, and cytostatic agent, as well as a fish toxin and mild wheat-germination inhibitor with a distinct toxicity (LD_{50}=275 mg/kg to rats, given orally) (6). Recently, this compound has become prominent because of interesting immunological effects, which may be relevant to tissue transplantation. In this context, **2** has been reported to abolish the ability of a cell to present antigens to both class I- and II-restricted cytotoxic T cells (7,8). Because of the toxicity of **2**, there exists a need for additional derivatives of brefeldin A, which stimulated the studies described in this communication.

Given that a number of brefeldin derivatives are available from a variety of chemical approaches (9), we investigated a biological derivatization approach (10). In preliminary work, *Penicillium brefeldianum* strain FH-A 6474 was subjected to a fermentation media variation program. On a medium containing lactose, corn steep, and $CaCO_3$, a new zone by tlc [R_f=0.05 ($CHCl_3$-MeOH, 9:1) and 0.50 (*n*-BuOH-AcOH-H_2O, 4:1:5, upper phase)] appeared, while production of **2** was not observed. From the nearly identical colorization behavior to various staining reagents (anisaldehyde/H_2SO_4: dark blue to violet; blue tetrazolium reagent: violet (weak); orcinol reagent: brown), a structural analogy between the new metabolite and **2** was apparent. From a 10-liter fermentation vessel using the lactose-containing medium, 20 mg/liter of a colorless amorphous material was isolated by adsorption on Amberlite XAD-16, followed by cc on Si gel and Sephadex LH-20.

The structure was ascertained by detailed analysis of ^1H-, ^{13}C-, and 2D (e.g., ^1H-^1H-COSY, ^1H-^{13}C-HETCOR) nmr spectra, in combination with derivatization reactions and degradation studies. An analysis of the nmr data for the metabolite obtained from the lactose-con-

[1]For Part 25, see Ritzau *et al.* (1).



	R_1	R_2
1		H
2	H	H
3		Ac

taining fermentation medium revealed close similarities to **2**. Besides the sixteen carbon signals attributed to the 13-membered lactone moiety, additional signals were found at δ_C 62.4 (CH_2), 70.2, 72.5, 75.0, 76.5, and 103.6 (all CH) pointing to a carbohydrate substituent. This assumption was verified by hrms of its pentaacetate (*m/z* 652.2731, $C_{32}H_{44}O_{14}$), which was obtained by treatment of **1** with Ac_2O/pyridine. Four of the acetyl groups are located at the OH-groups of the sugar moiety and one belongs to the aglycone, in which H-4 is shifted downfield from δ_H 4.04 to 5.23. The main differences in the ^{13}C-nmr data of **2** and its glycoside were observable in the chemical shifts of C-7 (δ_C 80.0; **2**: 72.7), proving the glycosylation to be located at this position. A 500 MHz ^{1}H-nmr spectrum indicated that the carbohydrate moiety is linked in β-glycosidic manner (1′-H: δ_H 4.25, J=7.5 Hz) (11). Acidic methanolysis of the glycoside resulted in near quantitative isolation of methyl D-galactopyranoside (α:β=4.1), which was identified by nmr analysis as well as by comparison of optical rotation values, and the seco acid methyl ester of **2** (9). Thus, the structure of the new fermentation product was established as 7-*O*-β-D-galactosyl-brefeldin A [**1**].

In proliferation assays (MTT-reduction) with some tumor cell lines [L1210 (IC_{50}=10 μg/ml), A 549 (1.2 μg/ml), and HT 29 (1.3 μg/ml)], **1** exhibited weaker activity in comparison to brefeldin A [**2**] (12), pointing to reduced toxicity.

To our knowledge, the new metabolite **1** is the first glycosylated brefeldin A derivative reported thus far. Because the change of carbon source in the fermentation medium of strain FH-A 6474 initiated its formation, we suggest a transglycosidase activity was apparent, cleaving the glycosidic bond of the disaccharide lactose and transferring the D-galactose moiety to the 7-OH group of the 13-membered brefeldin A aglycone. Further studies concerning this enzyme activity are in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra on KBr discs were recorded on a Perkin-Elmer Model 297 spectrometer, and uv spectra on a Kontron Uvikon 860 instrument. Eims were taken with Varian MAT-731 (70 eV) and Finnigan MAT-311A mass spectrometers, direct insert, and for hrms high-resolution perfluorokerosine was used as standard. Nmr spectra were measured with Varian VXR-200 and VXR-500S instruments. Chemical shifts are expressed in δ values with tetramethylsilane (TMS) as internal standard. Tlc was performed on Si gel plates (Merck, hptlc plates, Si gel 60F_{254} on glass, RP_{18}-plates on glass), and cc on Si gel 60 (Merck, 0.040×0.063 mm) or Sephadex LH-20 (Pharmacia). Fermentation was carried out in a 10-liter fermenter (Biostat E) from Braun Dissel (Melsungen, Germany).

CULTURE MATERIAL.—*Penicillium brefeldianum* (strain FH-A 6474, deposited in the German Culture Collection: DSM 7427) was grown on agar slants containing malt extract 2%, yeast extract 0.2%, glucose 1%, $(NH_4)_2HPO_4$ 0.05%, agar 2%, pH 6.0 prior to sterilization (medium A) or potato extract (aqueous extract from 200 g potatoes) 0.4%, glucose 2%, agar, 1.5%, pH=5.6 prior to sterilization (medium B) for 10 to 14 days at 25°. Storage of the strain was carried out in 50% aqueous glycerol at −20°.

FERMENTATION.—For the production of **2** the glycerol-containing storage mixture (3 ml) was used to inoculate two 300 ml Erlenmeyer flasks containing medium A omitting agar (medium C, 100 ml). In order to obtain 7-*O*-β-D-

galactosyl-brefeldin A [**1**], medium D (lactose 3%, cornsteep 3%, $CaCO_3$ 1%, pH 6.5 prior to sterilization) was used. The flasks were cultivated on a rotary shaker (140 rpm) for 3–6 days at 25°. These cultures were used to inoculate a fermenter (10 liters working volume, inoculation volume 1.5%, 200 rpm, 25°, aeration 5 liters/min) containing medium C for production of **2** and medium D for **1**, respectively. Usually, highest yields (**1**: 20 mg/liter; **2**: 100 mg/liter) were reached after 96 to 120 h. Foaming could be decreased using EtOH polyol solutions (e.g., Niax polyol). Details of the chemical screening method (cultivation, extraction of mycelium and adsorption of the metabolites from the culture filtrate, concentration steps, and the conditions for tlc) have already been reported (1–4).

ISOLATION.—For the isolation and purification of 7-*O*-β-D-galactosyl-brefeldin A [**1**] and brefeldin A [**2**] nearly identical procedures were applied. After filtration, the culture filtrates (about 10 liters) were adsorbed on Amberlite XAD-16 (ca. 1.5 liters of resin) and washed with deionized H_2O (5 liters). The metabolites were eluted with a mixture of MeOH-H_2O (4:1) and evaporated to a watery residue, which then was lyophilized. The dark brown crude products were chromatographed on Si gel (EtOAc-MeOH-H_2O, 7:2:0.2) and Sephadex LH-20 (MeOH). Compound **1** was further purified on RP8-Si gel (MeOH-H_2O, 9:5) to yield 20 mg/liter of pure amorphous product.

7-*O*-β-D-GALACTOSYL-BREFELDIN A [**1**].—Mp 119°; $[\alpha]^{20}D$ +25.7 (c=0.39, MeOH); uv λ max (MeOH) 204 (ε 11600) nm, λ max (MeOH+HCl) 203 (10900) nm, λ max (MeOH+NaOH) 214 (11200) nm; ir (KBr) ν max 3400, 2980, 2940, 2880, 1715, 1645, 1452, 1360, 1260 and 1070 cm^{-1}; ^1H nmr (500 MHz, CD_3OD) δ 0.90 and 1.80 (2H, both m, 13-H_2), 1.23 (3H, d, J=6.5 Hz, Me-16), 1.57 and 2.20 (2H, both m, H_2-14), 1.62 and 2.20 (2H, both m, H_2-8), 1.80 (1H, m, H-5), 1.80 (2H, m, H_2-6), 1.80 and 2.00 (2H, both m, H_2-12), 2.37 (1H, m, H-9), 3.45 (3H, m, H-2′, H-3′, H-5′), 3.71 (2H, m, H_2-6′), 3.82 (1H, dd, J=3.0 and 1.0 Hz, H-4′), 4.04 (1H, ddd, J=10.0, 3.0, and 2.0 Hz, H-4), 4.25 (1H, d, J=7.5 Hz, H-1′), 4.34 (1H, m, H-7), 4.79 (1H, m, H-15), 5.30 (1H, dd, J=15.0 and 9.5 Hz, H-10), 5.74 (1H, ddd, J=15.0, 10.0, and 4.5 Hz, H-11), 5.81 (1H, dd, J=15.5 and 2.0 Hz, H-2), and 7.45 (1H, dd, J=15.5 and 3.0 Hz, H-3); ^{13}C nmr (50.3 MHz, CD_3OD) δ 21.1 (C-16), 28.0 (C-13), 32.9 (C-6), 35.0 (C-12), 38.8 (C-8), 42.4 (C-14), 45.3 (C-9), 52.7 (C-5), 62.4 (C-6′), 70.2 (C-4′), 72.5 (C-2′), 73.1 (C-15), 75.0 (C-3′), 76.5 (C-5′), 76.6 (C-4), 80.0 (C-7), 103.6 (C-1′), 117.7 (C-2), 131.2 (C-11), 138.1 (C-10), 155.1 (C-3), and 168.3 (C-1).

7-*O*-β-D-GALACTOSYL-BREFELDIN A-PENTAACETATE [**3**].—A 13.0 mg (0.029 mmol) quantity of **1** was dissolved in 1 ml of Ac_2O. Pyridine (1 ml) was added and the mixture was stirred for 16 h. After hydrolysis with ice H_2O for 2 h, the mixture was extracted with EtOAc (3×15 ml). The organic layer was dried with Na_2SO_4, evaporated to dryness, and chromatographed on a Si gel column [20×1 cm, $CHCl_3$-MeOH (99:1)] to yield 15.1 mg (0.023 mmol, 80%) of pure amorphous **3**: Mp 89°; $[\alpha]^{20}D$ −9.2° (c=0.49, MeOH); uv λ max (MeOH) 202 (ε 12700) nm, λ max (MeOH+HCl) 203 (13400) nm, λ max (MeOH+NaOH) 213 (7800) nm; ir (KBr) ν max 3450, 2975, 2925, 2860, 1750, 1710, 1430, 1370, 1225, 1075, and 1050 cm^{-1}; ^1H nmr (200 MHz, $CDCl_3$) δ 0.90 and 1.82 (2H, both m, H_2-13), 1.24 (3H, d, J=6.6 Hz, Me-16), 1.60 and 2.10 (2H, both m, H_2-14), 1.60 and 1.70 (2H, both m, H_2-8), 1.60 and 2.10 (2H, both m, H_2-6), 1.90 (1H, m, H-5), 2.00, 2.04, 2.05, 2.14, and 2.15 (3H×5, each s, CH_3CO), 2.02 (2H, m, H_2-12), 2.41 (1H, m, H-9), 3.89 (1H, dt, J=6.5 and 1.0 Hz, H-5′), 4.11 and 4.18 (2H, ABX, J=11.0 and 6.5 Hz, H_2-6′), 4.26 (1H, m, H-7), 4.46 (1H, d, J=7.8 Hz, H-1′), 4.85 (1H, m, H-15), 5.03 (1H, dd, J=10.5 and 3.3 Hz, H-3′), 5.18 (1H, dd, J=10.5 and 7.8 Hz, H-2′), 5.38 (1H, dd, J=3.3 and 1.0 Hz, H-4′), 5.23 (2H, both m, H-4 and H-10), 5.67 (1H, dd, J=15.5 and 1.8 Hz, H-2), 5.67 (1H, ddd, J=15.0, 10.0, and 4.5 Hz, H-11) and 7.24 (1H, dd, J=15.5 and 3.2 Hz, H-3); eims (*m/z* 70 eV) 652.2731 [0.4%, M^+, $C_{32}H_{44}O_{14}$, found as calcd], 73 (100), 43 (76).

METHANOLYSIS OF **1**.—50.7 mg (0.115 mmol) of **1** were stirred in 5 ml of methanolic HCl (1.5 mol/liter) for 48 h at room temperature. The reaction mixture was evaporated to dryness and chromatographed on a Si gel column [25×1.5 cm, $CHCl_3$-MeOH (4:1)] to yield 27.3 mg (0.088 mmol, 76%) of the secoacid methyl ester of **2** (9) and 14.4 mg (0.074 mmol, 65%) of methyl D-galactosylpyranoside (α:β=4:1).

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LITERATURE CITED

1. M. Ritzau, M. Keller, P. Wessels, K.O. Stetter, and A. Zeeck, *Liebigs Ann. Chem.*, 871 (1993).
2. S. Grabley, E. Granzer, K. Hütter, D.

Ludwig, M. Mayer, R. Thiericke, G. Till, and J. Wink, *J. Antibiot.*, **45**, 56 (1992).
3. G. Bach, S. Breiding-Mack, S. Grabley, P. Hammann, K. Hütter, R. Thiericke, H. Uhr, J. Wink, and A. Zeeck, *Liebigs Ann. Chem.*, 241 (1993).
4. S. Grabley, G. Kretzschmar, M. Mayer, S. Philipps, R. Thiericke, J. Wink, and A. Zeeck, *Liebigs Ann. Chem.*, 573 (1993).
5. C.P. Gorst-Allman, P.S. Steyn, and C.J. Rabie, *J. Chem. Soc., Perkin Trans. I*, 2387 (1982).
6. R.J. Cole and R.H. Cox, "Handbook of Toxic Fungal Metabolites," Academic Press, New York, 1981, p. 844.
7. J.G. Nuchtern, J.S. Bonifacino, W.E. Biddison, and R.D. Klausner, *Nature*, **339**, 223 (1989).
8. L. Adorini, S.J. Ullrich, E. Appella, and S. Fuchs, *Nature*, **346**, 63 (1990).
9. B.M. Trost, J. Lynch, P. Renaut, and D.H. Steinman, *J. Am. Chem. Soc.*, **108**, 284 (1986).
10. R. Thiericke and J. Rohr, *Nat. Prod. Rep.*, **10**, 265 (1993).
11. K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, 1037 (1973).
12. S. Ishii, M. Nagasawa, Y. Kariya, and H. Yamamoto, *J. Antibiot.*, **42**, 1877 (1989).

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