Journal of Natural Products
Vol. 51, No. 1, pp. 125-128, Jan-Feb 1988

BIOCONVERSION OF GRINDELIC ACID INTO 3α-HYDROXYGRINDELIC ACID

JOSEPH J. HOFFMANN,* HUNSA PUNNAPAYAK,

Bioresources Research Facility, 250 East Valencia Road, Tucson, Arizona 85706

SHIVANAND D. JOLAD,

College of Pharmacy, University of Arizona, Tucson, Arizona 85721

ROBERT B. BATES, and FERNANDO A. CAMOU

Chemistry Department, University of Arizona, Tucson, Arizona 85721

ABSTRACT.—*Aspergillus niger, Aspergillus carbonarius, Cunninghamella echinulata, Cunninghamella elegans, Penicillium brevicompactum,* and *Sordaria bombioidee* were used to microbially transform grindelic acid [**1**] into 3α-hydroxygrindelic acid [**2**].

Hydroxylated grindelic acid derivatives, such as 6-hydroxygrindelic acid, are known to possess antifeedant or potential insecticidal properties (1,2). However, facile chemical transformation of grindelic acid [**1**] into its 6-hydroxy derivative proved impossible (3). Furthermore, there exists no direct chemical synthesis of 3-hydroxygrindelic acid [**2**], a potential starting material for a new class of biorationally designed insecticides (Hirosuke Yoshioka, Riken, private communication, Nov. 28, 1985). Therefore, we decided to attempt to produce this novel group of hydroxygrindelane derivatives by microbial transformation. This report describes the successful production of **2** from **1** by cultures of *Aspergillus niger, Aspergillus carbonarius, Cunninghamella echinulata, Cunninghamella elegans, Penicillium brevicompactum,* and *Sordaria bombioidee.*

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—A description of the analytical procedures has been produced by Jolad *et al.* (4); a sample of grindelic acid [**1**] was afforded by previously published procedures (5). Gc analysis was conducted according to our previously reported procedures (6) after methylation with MeI/K_2CO_3/Me_2CO (7).

MICROORGANISMS.—The microbial cultures were obtained from Dr. J.P.N. Rosazza of the College of Pharmacy, University of Iowa, Iowa City. They were *Aspergillus alliaceus* NRRL 315, *A. carbonarius* ATCC 6277, *Aspergillus flavus* ATCC 24741, *A. niger* UI X-172, *Bacillus cereus* UI 1477, *Cunninghamella bainieri* UI 3065, *C. echinulata* ATCC 9244, *C. elegans* UI 1393, *Hansenula anomala* ATCC 20144, *Helicostylum piriforme* QM 6945, *P. brevicompactum* ATCC 10418, *Sepedonium chrysospermum* ATCC 13378, *S. bombioidee* UI 183, *Streptomyces griseus* UI L-103, *Streptomyces griseus* UI 8090, *Streptomyces punipalus* UI 3529, *Streptomyces rutgersensis* NRRL B-1256, and *Stysanus stemonites* UI 2831. The cultures were maintained on Sabouraud maltose agar.

FERMENTATION.—The fermentations were carried out according to a standard two-stage fermentation protocol (8) in a soybean meal-glucose medium (9). The screening experiment was conducted in 125-ml Delong flasks containing 25 ml of medium. Cultures were incubated at room temperature with moderate shaking in a Fisher Scientific Versa reciprocating-shake bath. Grindelic acid [**1**] (10 mg dissolved in 0.1 ml DMF) was added to the 24-h-old stage II cultures and 4-ml samples were taken at 16-, 72-, and 168-h intervals, acidified with 6 N HCl to pH 3.0 and extracted with 1 ml of EtOAc-*n*-BuOH (9:1). The extracts were spotted onto tlc plates (Si gel GF) and developed in *n*-hexane-EtOAc-HOAc (70:30:2) for analysis and to follow the course of biotransformation. The tlc spots were visualized by spraying with *p*-anisaldehyde-HOAc-H_2SO_4 (1:120:1) followed by warming with a heat gun.

The preparative scale experiments were conducted in 1-liter Delong or Erlenmeyer flasks and a 2-liter fermentor (BioFlo, New Brunswick Scientific). Grindelic acid [**1**] was used as a substrate at a final concentration of 0.5 mg/ml. The fermentation was terminated when tlc indicated a complete biotransformation reaction, usually between 96 and 168 h.

The transformed products were recovered by filtering the fermentation slurry through a cheesecloth to

remove the cells followed by partitioning the resultant clear filtrate with CH_2Cl_2 (3 × 500 ml). The combined CH_2Cl_2 extracts were dried in vacuo and analyzed by gc.

ISOLATION OF 3α-HYDROXYGRINDELIC ACID [**2**].—The reddish oil (1.45 g) obtained from the *A. niger* preparative scale fermentation (1.5 g of **1**) contained only one major compound according to tlc and gc. This material was triturated with Et_2O and filtered, and the concentrated Et_2O-soluble fraction was submitted to preparative tlc on Si gel PF-254 (*n*-hexane-Et_2O-MeOH-HOAc, 20:60:6:1, single development). This gave 535 mg of yellow oil, which on tlc, exhibited a major spot with a minor component partially resolved and some other impurities. This was resubmitted to preparative tlc on Si gel PF-254 (*n*-hexane-Et_2O-MeOH-HOAc, 35:15:1:1, four developments) and divided into two fractions. Tlc showed the first fraction still to be contaminated with the minor component. When the second fraction, single spot on tlc, was dissolved in Et_2O and treated with petroleum ether, 3α-hydroxygrindelic acid [**2**] precipitated out as a colorless solid (130 mg), homogeneous by tlc, ill-defined mp, $[\alpha]^{24}D$ −111° ($CHCl_3$, c=4.3); ir ν max (KBr) cm^{-1} 3440 (OH), 3000–2500 and 1710 (COOH), 855 (C=C); ms *m/z* 336 $[M]^+$, 196 [B], 178 $[B-H_2O]^+$, 163 $[B-(H_2O+Me)]^+$, 150 $[B-HCO_2H]^+$, 137 $[B-CH_2CO_2H]^+$, 136 $[B-HOAc]^+$, 119, 109, 96, 82; ^1H nmr (250 MHz, $CDCl_3$, TMS, δ) 10.45 (br s, OH), 5.65 (br s, H-7), 3.27 (dd, 10.8, 4.3 Hz, H-3), 2.68 (br d, 15.4 Hz, H-14), 2.57 (d, 15.4 Hz, H′-14), 1.79 (d, 1.4 Hz, H-17), 1.39 (s, H-16), 0.99 (s, H-18), 0.89 (s, H-19), 0.84 (s, H-20); ^{13}C nmr see Table 1.

METHYL 3α-HYDROXYGRINDELATE [**2a**].—Methylation of **2** (25 mg) with MeI in Me_2CO and K_2CO_3 (5) gave **2a** (27 mg, oil), homogeneous by tlc, $[\alpha]^{24}D$ −113.6° ($CHCl_3$, c=0.09); ir ν max (neat) cm^{-1} 3500 (OH), 1730 (COOMe); ^1H nmr and ms identical to data reported by Bohlmann *et al.* (10).

RESULTS AND DISCUSSION

Of the microorganisms screened for the biotransformation of grindelic acid [**1**], *A. niger* gave one major compound (70% by gc) that was isolated and identified as 3α-hydroxygrindelic acid [**2**] as described below.

The eims of **2** displayed all the diagnostic peaks of grindelic acid [**1**] except *m/z* 320, the molecular ion peak of **1**, which shifted to *m/z* 336 in **2**. This increment of 16 mass units indicated an additional oxygen atom in **2** and suggested that grindelic acid [**1**] was hydroxylated or epoxidized. The former possibility was supported by the strong hydroxyl absorption at 3440 cm^{-1} in the ir (KBr) spectrum of **2** and the base peak for **2**, as in **1**, being at *m/z* 196, for the right-hand half of the retro-Diels-Alder (RDA) fragment, characteristic of 7-*en* labdanes. The presence of this fragment as well as another peak at *m/z* 136 (*m/z* 196 − HOAc) required the hydroxyl group to be in ring A. The appearance of a strong peak at *m/z* 96 in **2**, as in **1**, derived from the left-hand half of the RDA fragment by a second RDA breakdown before or after the loss of H_2O, showed the hydroxyl to be at C-2 or C-3 (Figure 1).

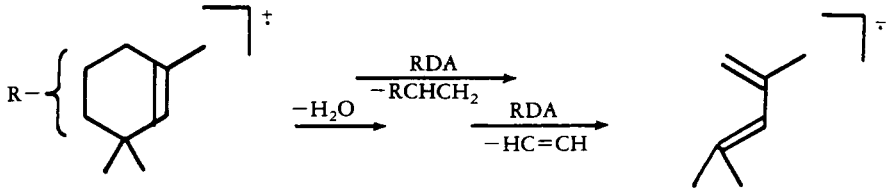


FIGURE 1. Fragmentation of ring A after an initial retro-Diels-Alder reaction.

The choice of C-3 over C-2 as the site for the hydroxyl group was made by examining the ^1H-nmr spectrum that contained a double doublet at δ 3.27 (J= 10.8, 4.3 Hz) characteristic of an equatorial 3-hydroxyl group in a diterpene, such as the methyl ester of natural 3α-hydroxygrindelic acid (δ 3.28, dd, J= 11.0, 4.0 Hz), a constituent of *Grindelia stricta* reported by Bohlmann *et al.* (10). Methylation of **2** with MeI gave methyl 3α-hydroxygrindelate [**2a**], identical in all respects with Bohlmann's com-

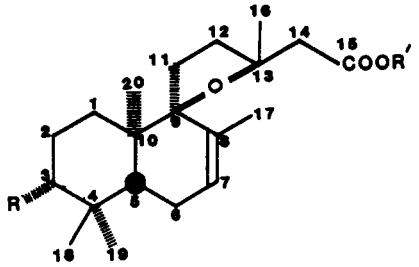
pound, which is the enantiomer shown, since grindelic acid [**1**] and its derivatives have recently been shown to be *ent*-labdanes (11).

The ^{13}C-nmr spectral parameters of **2** (Table 1), assigned on the basis of assign-

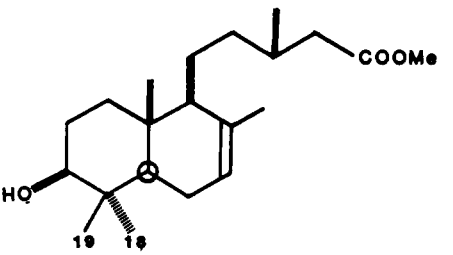
TABLE 1. ^{13}C-nmr (22.63 MHz) Chemical Shifts (δ, $CDCl_3$) for **1a**, **2**, and **3**.

Carbon	Compound		
	1a	**2**	**3**
1	32.7	30.8	39.4
2	18.7	27.0	27.4
3	41.9	78.5	79.2
4	33.1	38.8	38.7
5	42.6	42.3	49.7
6	24.1	23.8	24.5
7	126.5	128.4	122.1
8	134.8	133.2	135.2
9	90.5	92.1	55.2
10	40.6	40.5	36.5
11	28.4	27.7	23.5
12	38.1	39.2	37.3
13	81.5	81.2	31.3
14	47.9	47.4	41.4
15	171.8	172.4	173.7
16	27.3	26.8	19.9
17	22.3	21.0	21.9
18	32.9	27.6	27.9
19	21.2	15.1	15.1
20	16.7	16.8	13.6
OMe	51.3	—	51.4

ments made for **1a** and **3** (12), were in full accord with the structure shown. We have reassigned the peaks for carbons 17–19 in **1a**, **2**, and **3** as shown in Table 1. It is also possible now to assign C-17 at δ 21.1 and C-19 at δ 17.8 in methyl 18-isobutyroxy- and 18-isovaleroxy-grindelates (13).



1 R=H, R′=H
1a R=H, R′=Me
2 R=OH, R′=H
2a R=OH, R′=Me



3

Several of the other microorganisms were also successful in converting **1** into **2**. Because the mixtures obtained from the fermentation broths were similar according to gc, the identification with previously characterized components was made by comparison of retention times and by peak enhancement with pure standards (6). The result with *S.*

bombioidee (83% **2**) was comparable to *A. niger*. However, *A. carbonarius* was relatively inefficient in transforming **1**; after 168 h 43% of **1** remained unchanged with only 24.6% of the mixture being **2**. *C. echinulata* and *C. elegans* produced complex mixtures of at least 10 major compounds. Nevertheless, gc determinations did indicate the presence of **2** equal to about 18% of the mixture with 6-hydroxy- and 17-hydroxy-grindelic acid accounting for another 15–28%. *P. brevicompactum* also produced **2** but only equal to 12.6% of the mixture.

In conclusion, *A. niger* and *S. bombioidee* appear to be very effective organisms for the production of 3α-hydroxygrindelic acid [**2**] from grindelic acid [**1**]. However, *A. niger* is more readily available and easier to culture than *S. bombioidee*. In order to provide sufficient material for extensive biological testing a preparative scale fermentation in our 2-liter fermentor was repeated using cultures of *A. niger* that furnished 556 mg of **2** from 900 mg of **1**. Inasmuch as Asaki and Sakan (14) have shown that a 3-hydroxy group enhances antifungal activity of 15-carboxy-labdanes, we plan on expanding this biotransformation screen to include other labdanes isolated in our laboratory.

ACKNOWLEDGMENTS

We wish to express our sincere gratitude to Dr. J.P.N. Rosazza for his invaluable assistance and recommendations in this project as well as starter cultures for the microorganisms used. Our appreciation is also extended to L.K. Hutter for running the gc throughout the course of this project.

LITERATURE CITED

1. A.F. Rose, *Phytochemistry*, **19**, 2689 (1980).
2. A.F. Rose, K.C. Jones, W.F. Haddon,, and D.L. Dreyer, *Phytochemistry*, **20**, 2249 (1981).
3. M.G. Sierra, M.I. Colombo, A.C. Olivieri, M.E. Zudenigo, and E.A. Ruveda, *J. Org. Chem.*, **49**, 4984 (1984).
4. S.D. Jolad, J.J. Hoffmann, K.H. Schram, J.R. Cole, M.S. Tempesta, and R.B. Bates, *J. Org. Chem.*, **46**, 4267 (1981).
5. B.N. Timmermann, D.J. Luzbetak, J.J. Hoffmann, S.D. Jolad, K.H. Schram, R.E. Klenck, and R.B. Bates, *Phytochemistry*, **22**, 523 (1983).
6. B.N. Timmermann, S.P. McLaughlin, and J.J. Hoffmann, *Biochem. Syst. Ecol.*, **15**, 401 (1987).
7. B.N. Timmermann, J.J. Hoffmann, S.D. Jolad, K.H. Schram, R.E. Klenck, and R.B. Bates, *J. Org. Chem.*, **47**, 4114 (1982).
8. R.B. Betts, D.E. Walters, and J.P. Rosazza, *J. Med. Chem.*, **17**, 599 (1974).
9. J. Williamson, D. Van Orden, and J.P. Rosazza, *Appl. Environ. Microbiol.*, **49**, 563 (1985).
10. F. Bohlmann, M. Ahmed, N. Borthakur, M. Wallmeyer, J. Jakupovic, R.M. King, and H. Robinson, *Phytochemistry*, **21**, 167 (1982).
11. J. Jakupovic, R.N. Baruah, C. Zdero, F. Eid, V.P. Pathak, T.V. Chau-Thi, F. Bohlmann, R.M. King, and H. Robinson, *Phytochemistry*, **25**, 1873 (1986).
12. B.N. Timmermann, J.J. Hoffmann, S.D. Jolad, R.B. Bates, and T.J. Siahaan, *Phytochemistry*, **25**, 723 (1986).
13. B.N. Timmermann, J.J. Hoffmann, S.D. Jolad, R.B. Bates, and T.J. Siahaan, *Phytochemistry*, **26**, 467 (1987).
14. M. Asaki and F. Sakan, *Nippon Nogei Kagaku Kaishi*, **58**, 887 (1984).

Received 4 September 1987