JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Novel Derivatives of *ent*-17,19-Dihydroxy-16β*H*-kaurane Obtained by Biotransformation with *Verticillium lecanii*

HENRIETE S. VIEIRA, JACQUELINE A. TAKAHASHI, AND MARIA AMÉLIA D. BOAVENTURA*

Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, Pampulha 31270-901, Belo Horizonte, MG, Brazil

In the search for new routes in the synthesis of hydroxylated kaurane diterpenoids, *Verticillium lecanii*, grown in a mineral liquid medium for 48 h, was fed with *ent*-17,19-dihydroxy-16β*H*-kaurane, obtained by hydroboration/oxidation of kaurenoic acid, a natural product easily isolated from plants of the genera *Xylopia* (Annonaceae) and *Wedelia* (Asteraceae). After 14 days, the culture medium was extracted with ethyl acetate, and the metabolites were purified by column chromatography on silica gel. The results show that *V. lecanii* biotransformed the starting material into three novel compounds: *ent*-11α,17,19-trihydroxy-16β*H*-kaurane; *ent*-7α,17,19-trihydroxy-16β*H*-kaurane; and *ent*-7β,17,19-trihydroxy-16β*H*-kaurane, the structures of which were fully elucidated by using classical and modern two-dimensional NMR techniques.

KEYWORDS: *ent*-7α,17,19-Trihydroxy-16β*H*-kaurane; *ent*-11α,17,19-trihydroxy-16β*H*-kaurane; *ent*-7β,17,19-trihydroxy-16β*H*-kaurane; *ent*-17,19-dihydroxy-16β*H*-kaurane; *Verticillium lecanii*; microbial hydroxylation

INTRODUCTION

Kaurane diterpenoids are a very important class of natural products widespread in the plant kingdom. These compounds are the biosynthetic precursors of gibberellins, the well-known fungal plant growth hormones. A considerable number of important biological activities have been associated with kaurane diterpenoids, such as antimicrobial, antiparasitic, insect antifeedant, cytotoxic, anti-HIV, antifertility, hypotensive, and antiinflammatory (*1*) activities, among others. Kaurenoic acid (**1**) is a typical kaurane representative possessing several biological activities including the ability to promote leaf sheath elongation in some *Zea mays* dwarf mutants (*2*). Kaurenoic acid can be easily isolated from plants of *Xylopia* (Annonaceae) and *Wedelia* (Asteraceae) genera in good yields (*3*, *4*). Other known sources of **1** are seedless sunflower heads, discarded as wastes in the edible sunflower oil industry (*5*). The ready availability of kaurenoic acid from natural sources makes this compound a suitable starting material for the synthesis of its novel derivatives for biological screening. The synthesis of hydroxylated kaurane derivatives is of special interest because highly oxygenated kaurane diterpenoids, scarcely available from natural resources, usually have higher levels of biological activity than their less hydroxylated precursors (*1*). However, the introduction of functional groups into the inactive backbone of kaurenoic acid is a nearly impossible task. Alternatively, fungal biotransformation is a method of considerable value for the partial synthesis of highly hydroxylated compounds, which are potentially bioactive (*6*). This approach is safer and, in most cases, more efficient for the production of many different novel compounds (*7*).

Verticillium lecanii (former *Cephalosporium aphidicola*) is a very versatile fungus, having the ability to transform several diterpenoids into hydroxylated derivatives (*8*). This ability seems to be related to its endogenous production of the antiviral and antitumoral tetrahydroxylated diterpenoid aphidicolin (**2**) (*9*).

In the search for an efficient microbial route to prepare novel polyhydroxylated kaurane diterpenoids to be evaluated in biological screenings, the biotransformation of *ent*-17,19-dihydroxy-16β*H*-kaurane (**3**) by whole cells of the fungus *V. lecanii* was performed. Compound **3** was prepared from kaurenoic acid (**1**) via hydroboration, followed by oxidation with hydrogen peroxide. We report herein the successful biotransformation of **3** by *V. lecanii* into three novel compounds: *ent*-11α,17,19-trihydroxy-16β*H*-kaurane (**4**); *ent*-7α,17,19-trihydroxy-16β*H*-kaurane (**5**); and *ent*-7β,17,19-trihydroxy-16β*H*-kaurane (**6**).

MATERIALS AND METHODS

General. Melting points were determined with a Kofler hot plate apparatus and are uncorrected.

Infrared (IR) spectra were recorded with a Shimadzu/IR-408 spectrophotometer.

Nuclear magnetic resonance (NMR) spectra were recorded, in pyridine, at room temperature, on a Bruker Avance DRX 400 MHz spectrometer (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz). Two-

* Author to whom correspondence should be addressed (telephone +55 31 34995754; fax +55 31 3499 5700; e-mail dianadb@dedalus.lcc.ufmg.br).

10.1021/jf020009f CCC: $22.00
Published on Web 05/23/2002

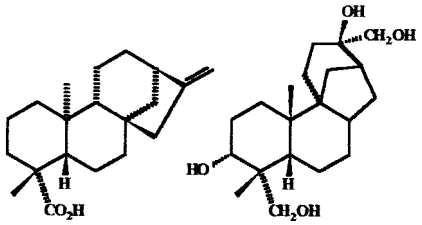


Figure 1. Structures of kaurenoic acid (**1**) and aphidicolin (**2**).

dimensional (2D) NMR experiments used were heteronuclear multiple-quantum correlation (HMQC) and nuclear Overhauser effect spectroscopy (NOESY). Chemical shifts are reported in parts per million (ppm), with respect to tetramethylsilane as the internal standard. Multiplicities were determined by DEPT pulse sequence.

Elemental analyses were performed on a Perkin-Elmer 2400, CHN elemental analyzer instrument. The optical rotation was determined with a Perkin-Elmer (model 341) polarimeter.

Starting material for this work, kaurenoic acid (**1**), was previously isolated from green fruits of *Xylopia frutescens* (*3*).

Hydroboration and Oxidation of Kaurenoic Acid (1; Figure 1). Diborane was generated in situ by the addition of $NaBH_4$ (0.75 g, 19.8 mmol) in dry tetrahydrofuran (THF, 10 mL) dropwise over 15 min to $BF_3{\cdot}Et_2O$ (5.0 mL, 41.0 mmol). To this solution was slowly added compound **1** (0.6 g, 1.9 mmol) in dry THF (10 mL), with stirring. After an additional 0.5 h of stirring, 10 mL of a 2 M NaOH solution and 7.0 mL of 30% H_2O_2 were added at 0 °C to the resulting reaction mixture and stirred for 0.5 h. THF was then removed with a rotatory evaporator, and the residue was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The recovered residue was submitted to flash chromatography with 30% EtOAc in petroleum ether to yield *ent*-17,-19-dihydroxy-16β*H*-kaurane (**3**) (0.516 g, 85% yield): mp 178 °C; $[\alpha]_D^{25} = -30°$ (*c* 0.007, in CH_3OH). Elem Anal. Calcd for $C_{20}H_{34}O_2$: C, 78.6%; H, 11.4%. Found: C, 78.4%; H, 11.2%. IR (KBr) ν_{max} 3400, 2900 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz), see **Table 1**; ^{13}C NMR (pyridine-d_5, 100 MHz), see **Table 2**. {Lit. mp 180−181 °C; $[\alpha]_D25 = -66°$ (*c* 1.8, in EtOH). Elem. Anal.: C, 78.2%; H, 11.2% (*10*).}

Fermentation Conditions. A culture of *V. lecanii* (IMI 68689), obtained from International Mycological Institute, Egham, Surrey, U.K., was grown at 25 °C on malt agar slopes (3 days) to furnish stock cultures. After this period, they were stored at 7−10 °C. The liquid culture used for biotransformation consisted of the following: glucose (100 g/L), $MnSO_4$ (2.0 g/L), KCl (1.0 g/L), KH_2PO_4 (5.0 g/L), glycine (2.0 g/L), and an aqueous solution of trace elements (2.0 mL) that contained (per liter) $Co(NO_3)_2{\cdot}6H_2O$ (0.1 g), $FeSO_4{\cdot}7H_2O$ (1.0 g), $CuSO_4{\cdot}5H_2O$ (0.15 g), $ZnSO_4{\cdot}7H_2O$ (1.61 g), $MnSO_4{\cdot}4H_2O$ (0.1 g), and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (1.0 g). Sterilization of malt agar slopes and liquid medium was carried in an autoclave for 15 min.

Biotransformation of *ent*-17,19-Dihydroxy-16β*H*-kaurane (3). Small sections of the stock cultures were cut under sterile conditions and transferred to a 500 mL Erlenmeyer flask containing 200 mL of liquid medium, which was incubated for 48 h at 25 °C on an orbital shaker (150 rpm). The resulting culture was used to inoculate 10 250-mL conical flasks containing the same culture medium (110 mL/flask). All of the cultures were incubated for an additional 48 h (two-stage culture), and then the substrate, *ent*-17,19-dihydroxy-16β*H*-kaurane (**3**) (450 mg) in DMSO (5 mL), was evenly distributed among the cultures, except one that was used as a cell control. The same culture medium in a flask containing only the substrate was prepared as a substrate control. All culture media were shaken for 14 days at 25 °C. The resulting mycelium was separated from the broth by filtration. The broth was extracted with EtOAc (3 × 500 mL), and the resulting EtOAc solution was dried over anhydrous Na_2SO_4. After filtration, the solvent was removed by evaporation under reduced pressure to furnish a yellowish residue (470 mg). The residue was purified by silica gel flash chromatography (hexane/EtOAc, 3:7 v/v) to give products **4** (10 mg, 2.1%), **5** (12 mg, 2.5%), and **6** (15 mg, 3.2%).

ent-11α*,17,19-Trihydroxy-16*β*H-kaurane* (**4**): oil; $[\alpha]_D^{25} = -23°$ (*c* 0.004, in CH_3OH); IR (KBr) ν_{max} 3300, 2900 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz), see **Table 1**; ^{13}C NMR (pyridine-d_5, 100 MHz), see **Table 2**. Elem Anal. Calcd for $C_{20}H_{34}O_3$: C, 74.7%; H, 10.3%. Found: C, 74.5%; H, 10.6%.

ent-7α*,17,19-Trihydroxy-16*β*H-kaurane* (**5**): oil; $[\alpha]_D^{25} = -21°$ (*c* 0.002, in CH_3OH); IR (KBr) ν_{max} 3400, 2900 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz), see **Table 1**; ^{13}C NMR (pyridine-d_5, 100 MHz), see **Table 2**. Elem Anal. Calcd for $C_{20}H_{34}O_3$: C, 74.7%; H, 10.3%. Found: C, 74.6%; H, 10.6%.

ent-7β*,17,19-Trihydroxy-16*β*H-kaurane* (**6**): oil; $[\alpha]_D^{25} = -17°$ (*c* 0.002, in CH_3OH); IR (KBr) ν_{max} 3300, 2900 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz), see **Table 1**; ^{13}C NMR (pyridine-d_5, 100 MHz), see **Table 2**. Elem Anal. Calcd for $C_{20}H_{34}O_3$: C, 74.7%; H, 10.3%. Found: C, 74.4%; H, 10.6%.

RESULTS AND DISCUSSION

In this experiment, *V. lecanii* metabolized diol **3** into three novel compounds hydroxylated in the remote methylene carbons C-11 and C-7. On the basis of the formal similarity between the structure of **3** and the steroidal skeleton, the formation of compound **4** is of special interest, as the presence of an oxygen function at C-11 position of the steroidal nucleus is an obligatory structural requirement for steroidal carbohydrate-regulating hormonal activity (*11*). The formation of 11-hydroxylated steroidal derivatives by the biotransformation using *V. lecanii* was previously reported by Boynton et al. (*12*). Hydroxylation of C-7 (compounds **5** and **6**) is also relevant as some antitumor kaurane diterpenoids bear a C-7-hydroxyl function (*13*). The oxidation in this position makes easier the preparation of novel kaurane diterpenoids modified in ring B.

The preparation of **3** by the hydroboration of **1** was carried out with a mixture of sodium borohydride and boron trifluoride diethyl etherate (in situ borane generation) in tetrahydrofuran under reflux, followed by oxidation with 30% hydrogen peroxide (*14*). The reaction produced, after flash chromatography, *ent*-17,19-dihydroxy-16β*H*-kaurane (**3**) (**Figure 2**), used as substrate for the biotransformation by *V. lecanii* that furnished the novel compounds **4**−**6**. We show here their structures were completely elucidated by using modern NMR techniques.

The ^1H NMR spectra of metabolites **4**−**6** showed that the two compounds contain methyl groups at C-4 and C-10 and two hydroxymethyl (CH_2OH) groups at C-4 and C-16 as in starting material **3**. Moreover, the presence of a signal at δ 2.04 (d, $J = 6.8$ Hz) in the ^1H NMR spectrum of **4** together with the absence of a signal corresponding to the C-11 methylene carbon, the appearance of a signal at δ 64.6 corresponding to a hydroxylated methine carbon, and the downfield shift of signals corresponding to C-9 (from δ 56.7 to 68.2) and C-12 (from δ 25.9 to 36.1) observed in the ^{13}C NMR spectrum suggested the presence of a hydroxyl group on C-11 in **4**. Two-dimensional NMR experiments were used to establish stereochemistry at C-11. On the basis of the ^1J correlation among ^1H and ^{13}C nuclei observed in the 2D-HMQC spectrum of **4**, the signals at δ 1.14 and 1.97 were assigned to H-1 protons, whereas signals at δ 0.92 and 2.04 were assigned to H-20 and H-11, respectively. Moreover, the corresponding 2D-NOESY−NMR spectrum (see **Table 3** and **Figure 3**) showed that spatial correlation among H-11, H-20, and the signal at δ 1.97, corresponding to H-1α, is compatible only with β-stereochemistry for hydroxyl group at C-11. In addition, the multiplicity for the signals of H-17 in the CH_2OH group at C-16 in the ^1H NMR spectrum of **4** differed with that of **3**, indicating that the resonance of ^1H nuclei in the CH_2 group at C-16 is partially affected by the neighboring hydroxyl group at C-11 via long-range coupling. This together with the absence of γ-gauche effect over C-10 relative to **3** is in good agreement with the proposed stereochemistry.

Table 1. ^{1}H NMR (Pyridine-d_5, 400 MHz, δ) Chemical Shift[a] Values for Diterpenes **3**–**6**

	3		**4**		**5**		**6**	
H	δ H$_\alpha$	δ H$_\beta$	δ H$_\alpha$	δ H$_\beta$	δ H$_\alpha$	δ H$_\beta$	δ H$_\alpha$	δ H$_\beta$
1	1.74 br d (12.8)	0.75 dt (3.7; 12.8)	1.97 br d (12.8)	1.13–1.15 m	1.70–1.80 m	0.87–0.97 m	1.74–1.76 m	0.73–0.75 m
2	1.45–1.60 m	1.65–1.75 m	1.35–1.37 m	1.65–1.67 m	1.35–1.45 m	1.70–1.80 m	1.37–1.39 m	1.59–1.61 m
3	2.15 br d (12.8)	0.93–0.99 m	2.15 br d (13.1)	0.97 dt (4.0; 13.1)	2.10–2.20 m	1.00–1.08 m	2.14–2.16 m	0.92–1.05 m
5		0.95–1.05 m		1.03–1.05 m		2.10–2.20 m		1.12–1.14 m
6	1.65–1.75 m	1.32–1.45 m	1.68–1.70 m	1.30–1.50 m	1.83–1.85 m	2.07–2.08 m	2.14–2.16 m	2.25 dd (3.4; 11.9)
7	1.65 dd (1.2; 12.0)	1.20 ddd (1.2; 7.0; 12.0)	2.27–2.29 m	1.68–1.70 m	3.80–3.82 m			3.70–3.72 m
9		0.85–0.95 m		1.55–1.60 m		1.74–1.76 m		1.12–1.14 m
11	1.65–1.75 m	1.32–1.40 m	2.04 d (6.8)		1.60–1.70 m	1.55–1.65 m	1.74–1.76 m	1.59–1.61 m
12	1.45–1.60 m	1.75–1.85 m	1.86–1.88 m	2.40–2.42 m	1.60–1.62 m	1.83–1.84 m	1.59–1.61 m	1.89–1.91 m
13	2.36 br s		2.48 br s		2.43 br s		2.45 br s	
14	1.97 d (11.3)	0.94 br d (11.3)	1.93 d (11.2)	1.03 br d (11.2)	1.93 d (11.3)	1.14–1.16 m	2.14–2.16 m	1.74–1.76 m
15	1.45–1.55 m		1.55–1.57 m		1.51–1.53 m	2.42–2.43 m	2.74 t (12.3)	1.10–1.12 m
16	2.35–2.47 m		2.46 br s		2.49–2.51 m		2.55 br s	
17	3.95 d (7.4)		4.35 dd (7.6; 10.4); 4.58 dd (7.6; 10.4)		3.99 d (7.5)		4.06 d (7.6)	
18	1.18 s		1.18 s		1.27 s		1.20 s	
19	3.63 d (10.4); 3.97 d (10.4)		3.63 d (10.8); 3.97 d (10.8)		3.71 d (10.6); 4.09 d (10.6)		3.70 d (10.4); 4.00 d (10.4)	
20	0.99 s		0.92 s		1.08 s		1.08 s	

[a] Chemical shift values are in parts per million, and *J* values in hertz are presented in parentheses. All signals were assigned by COSY, HMQC, and NOESY spectra.

Table 2. ^{13}C NMR (Pyridine-d_5, 100 MHz, δ) Chemical Shift Values for Diterpenes **3**–**6**

C	**3**	**4**	**5**	**6**	C	**3**	**4**	**5**	**6**
1	40.5	40.4	40.9	40.8	11	18.2	64.6	18.9	18.8
2	18.9	18.7	19.1	19.2	12	25.9	36.1	26.9	27.0
3	35.6	36.3	37.8	36.3	13	37.1	38.3	39.5	37.5
4	38.6	38.5	36.5	39.2	14	40.3	40.2	39.9	32.0
5	57.6	56.9	49.0	53.9	15	43.7	43.3	41.2	39.6
6	20.8	20.9	28.9	30.7	16	43.2	44.6	46.8	44.4
7	42.5	42.0	76.7	75.6	17	64.3	63.6	63.4	63.6
8	44.2	43.3	44.2	50.7	18	27.0	27.9	27.9	28.0
9	56.7	68.2	52.5	57.5	19	65.5	64.1	64.6	64.3
10	39.2	39.3	38.9	39.2	20	18.2	18.3	18.5	18.7

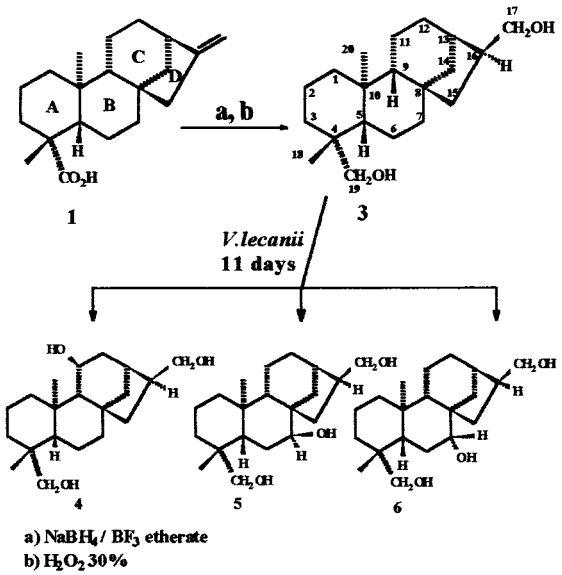


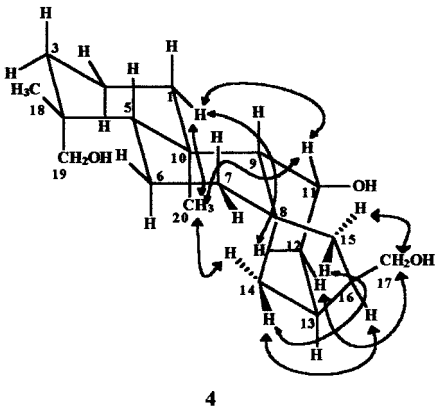
Figure 2. Synthesis of *ent*-17,19-dihydroxy-16β*H*-kaurane (**3**) and its biotransformations by *V. lecanii*.

The ^{1}H spectrum of **5** showed a new one-proton signal centered at δ 3.80 (in the ^{13}C NMR spectrum at δ 76.7), whereas the ^{13}C NMR spectrum of **5** showed the absence of a signal for C-7 at δ 42.5 and the downfield shift of signal for C-6 from δ 20.8 to 28.9, as compared to that of the starting material **3**. These indicate the occurrence of hydroxylation at C-7 in the biotransformation of **3** to **5**. On the basis of ^1J correlation among ^{1}H and ^{13}C nuclei observed in the HMQC spectrum of **5**, signals centered at δ 1.15 and 1.93 were assigned to H-14 protons, whereas those at δ 1.08 and 3.80−3.82 were assigned to H-20 and H-7, respectively. Furthermore, the corresponding 2D-NOESY−NMR spectrum (see **Table 3** and **Figure 4**) showed that signals at δ 1.93 and 1.15 are correlated to those of H-20

Table 3. Significant Long-Range[a] NOE Peaks Observed for NOESY Spectra of **4**–**6**

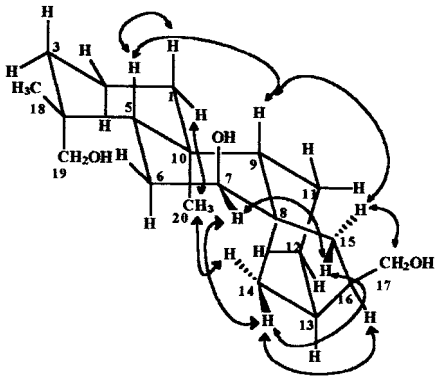
4	**5**	**6**
H-1α/H-20; H-12α; H-11α	H-1α/H-20	H-1α/H-20
H-1β/H-3β; H-5β; H-9β	H-1β/H-5β	H-1β/H-3β; H-5β; H-9β
H-3α/H-18;	H-3α/H-18;	H-3α/H-18;
H-3β/H-5β	H-3β/H-5β	H-3β/H-5β
H-5β/H-9β; H-7β	H-5β/H-9β	H-5β/ H-7β
H-6α/H-20	H-6α/H-20	H-6β/H-18
H-7α/H-15α; H-14β	H-6β/H-18	H-7β/H-9β; H-15β
H-7β/H-9β; H-15β	H-7α/H-15α; H-14β	H-11β/H-17
H-11α/H-20	H-9β/H-15β	H-12α/H-20
H-12β/H-17	H-11β/H-17	H-12β/H-17
H-14α/H-20	H-12β/H-17	H-13α/H-16β
H-14β/H-16α; H-15α	H-14α/H-20	H-14α/H-20
H-15β/H-17	H-14β/H-16α; H-15α	H-14β/H-16α; H-15α
H-19α/H-20	H-15β/ H-17	H-15β/H-17
	H-19α/ H-20	H-19α/H-20

[a] Excluding NOE cross-peaks between geminal or vicinal pairs of protons.



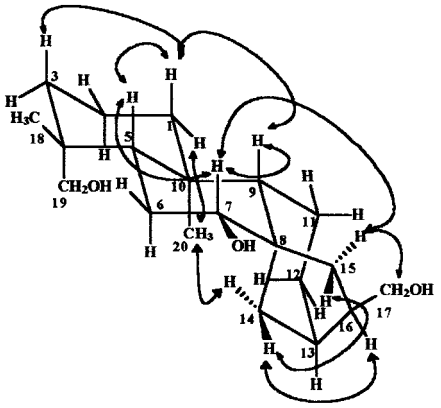
4

Figure 3. Phase-sensitive NOESY responses of *ent*-11α,17,19-trihydroxy-16β*H*-kaurane (**4**).



5

Figure 4. Phase-sensitive NOESY responses of *ent*-7α,17,19-trihydroxy-16β*H*-kaurane (**5**).



6

Figure 5. Phase-sensitive NOESY responses of *ent*-7β,17,19-trihydroxy-16β*H*-kaurane (**6**).

at δ 1.08 and H-7 at δ 3.80−3.82, respectively. Therefore, the signals at δ 1.93 and 1.15 correspond to H-14α and H-14β. These data are compatible only with a β-stereochemistry for the hydroxyl group at C-7. Furthermore, the upfield shift of signals assigned to C-5, C-9, and C-15, in the ^{13}C NMR spectrum, the γ-gauche effect relative to **3**, is additional evidence supporting the proposed stereochemistry.

In the ^{1}H NMR spectrum of **6**, an overlapped one-proton signal centered at δ 3.70 (in the ^{13}C NMR spectrum at δ 75.6) indicated that compound **6** contains an additional hydroxyl group as compared to the starting compound **3**. Furthermore, the presence of a hydroxyl group at C-7 in **6** is evidenced by the absence of a signal at δ 42.5 corresponding C-7 in the starting material **3** and the downfield shifts observed in the ^{13}C NMR spectrum of **6**. The signals for C-6 and C-8 of **6** are shifted from δ 20.8 to 30.7 and from δ 44.2 to 50.7 relative to those of **3**, respectively. Here again, on the basis of ^{1}J correlation among ^{1}H and ^{13}C nuclei observed in the HMQC spectrum of **6**, signals at δ 1.75 and 2.15 were assigned to H-14 protons, whereas those at δ 1.08 and 3.70 were assigned to H-20 and H-7, respectively. Furthermore, the corresponding 2D-NOESY−NMR spectrum (see **Table 3** and **Figure 5**) showed that the signal at δ 2.15 is correlated with H-20. Consequently, the signals at δ 2.15 and 1.75 correspond to H-14α and H-14β. Because the latter did not correlate to H-7, it is evident that **6** has a 7α-hydroxyl group at C-7. In addition, an overlapped signal at δ 1.12−1.14 was correlated to the signal at δ 3.70 (H-7β), and then the signal is assigned to H-5β and H-9β. The spatial correlation elucidated leads to the proposal of an α-stereochemistry for the 7-hydroxyl group in **6**, which is also confirmed by the upfield shift of C-14 and C-15 signals, the γ-gauche effect relative to **3**.

Compounds **4**−**6** are new compounds with no known natural sources. Despite the low yields, the biotransformation of **3** into its hydroxylated derivatives by *V. lecanii* is a very useful tool for the preparation of such compounds and related ones for biological testing or for submission to further chemical or biological modifications. Improvement in the yields may be achieved by evaluation of different biotransformation conditions as well as by using immobilized cells or genetic engineering tools. From a natural products chemistry point of view, this route is of high interest because the chemical synthesis of **4**−**6** is considerably difficult. The results so far are encouraging because this methodology for the preparation of the same kind of novel compounds is not only rapid and inexpensive but also ecologically compatible.

LITERATURE CITED

(1) Ghisalberti, E. L. The biological activity of naturally occurring kaurane diterpenes. *Fitoterapia* **1997**, *68*, 303−325.

(2) Katsumi, M.; Phinney, B. O.; Jefferies, P. C.; Henrick, C. A. Growth Response of the d-5 and an-1 Mutants of Maize to Some Kaurene Derivatives. *Science* **1964**, *144*, 849−850.

(3) Takahashi, J. A.; Boaventura, M. A. D.; Bayma, J. C.; Oliveira, A. B. Frutoic acid, a dimeric kaurane diterpene from *Xylopia frutescens. Phytochemistry* **1995**, *40*, 607−609.

(4) Vieira, H. S.; Takahashi, J. A.; Boaventura, M. A. D. Constituents of aereal parts of *Wedelia paludosa*. *Fitoterapia* **2001**, *72*, 854−856.

(5) Panizo, M. F.; Rodriguez, B. Algunos componentes diterpenicos del girasol (*Helianthus annuus* L.). *An. Quim.* **1979**, *75*, 428−431.

(6) Boaventura, M. A. D.; Hanson, J. R.; Hitchcock, P. B.; Takahashi, J. A. The biotransformation of *ent*-19-hydroxykaur-16-en-15-one by *Cephalosporium aphidicola*. *Phytochemistry* **1994**, *37*, 387−389.

(7) Ramachandra, Rao S.; Tripathi, U.; Ravishankar, G. A. Biotransformation of codeine to morphine in freely suspended cells and immobilized cultures of *Spirulina platensis*. *World J. Microbiol. Biotechnol.* **1999**, *15*, 465−469.

(8) Hanson, J. R. Diterpenoids. *Nat. Prod. Rep*. **1995**, *12*, 207−218.

(9) Bucknall, R. A.; Moores, H.; Simms, R.; Hesp, B. Antiviral effects of aphdicolin, a new antibiotic produced by *Cephalosporium aphidicola*. *Antimicrob. Agents Chemother.* **1973**, *4*, 294−298.

(10) Henrick, C. A.; Jefferies, P. R. The chemistry of the Euphorbiaceae VII. The diterpenes of *Ricinocarpus stylosus* Diels. *Aust. J. Chem.* **1964**, *17*, 915−933.

(11) Dutta, T. K.; Samanta, T. B. Bioconversion of progesterone by activated immobilized conidia of *Aspergillus ochraceus* TS. *Curr. Microbiol.* **1999**, *39*, 309−312.

(12) Boynton, J.; Hanson, J. R.; Hunter, A. C. The hydroxylation of some 13α-methylsteroids by *Cephalosporium aphidicola*. *Phytochemistry* **1997**, *45*, 951−956.

(13) Nagao, Y.; Ito, N.; Kohno, T.; Kuroda, H.; Fujita, E. Antitumor activity of *Rabdosia* and *Teucrium* diterpenoids against P 388 lymphocytic leukemia in mice. *Chem. Pharm. Bull.* **1982**, *30*, 727−729.

(14) Castellaro, S. J.; Dolan, S. C.; Macmillan: J.; Willis, C. L. Deuterium labeling of kaur-16−19-oic acid at carbon-6 and -7. *Phytochemistry* **1990**, *29*, 1823−1831.

Received for review January 3, 2002. Revised manuscript received March 29, 2002. Accepted March 29, 2002. We acknowledge CNPq, FAPEMIG, CAPES, and FINEP for grants and financial support.

JF020009F