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NEW FARNESANE SESQUITERPENES FROM *LACTARIUS PORNINSIS*[1]

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ABSTRACT.—Intact fruiting-bodies of *Lactarius porninsis* contain a mixture of new fatty acid esters **1a–1c**. If the fruiting bodies are injured, these esters are converted rapidly to the two new farnesane sesquiterpenes porninsal [**2**] and porninsol [**3**]. This enzymatic conversion of sesquiterpenes may be part of a new variant of the chemical defense system that protects pungent or bitter Russulaceae species against parasites and predators.

Guaiane, drimane, lactarane, marasmane, and secolactarane sesquiterpenes (1,2), and chromene and benzofuran derivatives (3) have been isolated from Basidiomycetes of several genera, including *Lactarius* and *Russula*. Some of these compounds have been suggested to take part in a complicated chemical defense system in which tasteless and biologically inactive fatty acid esters [for example, guaiane sesquiterpenoids in *Lactarius deliciosus* and *Lactarius deterrimus* (4), velutinal esters in *Lactarius vellereus* (5), *Lactarius scrobiculatus*, and *Lactarius chrysorrheus* (6), a phenol stearate in *Lactarius fuliginosus* (3)] are present in intact fruiting-bodies and can be isolated when mushrooms are worked up under carefully controlled conditions. These compounds are inactive forms. If the fruiting-bodies are injured, the esters are enzymatically converted into unsaturated aldehydes or dialdehydes or a simple free phenol, depending on the species and the compound originally present. The latter compounds impart an intense pungent or bitter taste to the latex and flesh of the mushrooms and possess potent antibiotic, antifungal, and antifeedant activities; therefore, they are considered to function as active forms. The possibility that a similar biochemical mechanism also has evolved in *Lactarius porninsis* Roll. motivated our investigation of the initial chemical content of the fruiting-bodies of this species, as well as of the nature of any new compounds formed in damaged specimens.

L. porninsis grows in European larch woods during late summer and early autumn (7). Ingestion of the mushroom causes slight disorders of the digestive system, ascribed by the mycologists to the so-called "acrid-resinous principles." When the fruiting-bodies are broken, the white latex and flesh are initially tasteless but slowly turn bitter and astringent, never pungent, and smell like oranges. Nothing is known of the nature of the molecules involved in these transformations. This is also the first investigation of a *Lactarius* species belonging to the section Zonarii Quel. (8).

RESULTS AND DISCUSSION

In order to compare our results with those reported for similar studies on other *Lactarius* species (3–6, 9–11), we adopted the procedure previously followed for *L. vellereus* (5) and *L. chrysorrheus* (6). Only young specimens of *L. porninsis* that appeared undamaged by parasites were collected. To simulate injury, the mushrooms were minced without the addition of solvent and extracted with hexane at room temperature. A series of extractions was made at different times (1 min, 3 min, 15 min, 30 min, 60 min) after injury, and the extracts were analyzed by tlc and uv spectroscopy. In addition, a few drops of the milky juice were collected with a capillary tube, suspended in CH_2Cl_2, and

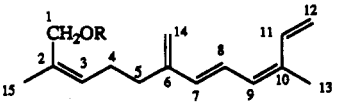
[1]Communication 30 in the series "Fungal Metabolites." For part 29 see De Bernardi *et al.* (6).

immediately analyzed by tlc. A single compound, as the fatty acid esters **1a–1c**, was found in significant amount in a specimen of tasteless latex and in extracts made 1–2 min after breakage of the fruiting-bodies. About 15 min after injury, a tlc of the hexane extract still showed large quantities of **1a–1c**, while revealing minor and comparable amounts of two new sesquiterpenes **2** and **3**. After 1 h the spot of **2** was the most intense, while those of **1a–1c** had ca. half of their initial intensity. Interestingly, while the content of the extract varied with time, the uv absorption spectra remained almost the same. Moreover, no traces of the sesquiterpenes previously isolated from the pungent *Lactarius* species, in particular acyl velutinals and their enzymatic transformation products (5, 6, 9–11), were detected in this investigation.

In order to isolate and characterize the products formed in the ground mushrooms on a preparative scale, extraction with hexane was performed about 15–20 min after the tissues of the fruiting bodies had been disrupted. The thermal and photochemical lability of compounds **1a–1c**, **2**, and **3** required special conditions for performing preparative cc on Si gel and for recording the spectral data (see the Experimental section). Particularly noteworthy was the rapid polymerization of esters **1a–1c** in solution when taken to dryness, and aldehyde **2** decomposed within two days when left in $CDCl_3$ solution in an nmr tube. Structures **1a–1c**, **2**, and **3** were assigned mainly on the basis of ^{1}H- and ^{13}C-nmr measurements, including DEPT, nOe, and selective decoupling experiments and 2D techniques (homo- and heteronuclear COSY).

The uv absorption curves of the three sesquiterpenes were almost superimposable indicating the same chromophore with three maxima at λ max 285, 297, and 311 nm. In addition, most of the ^{1}H- and ^{13}C nmr spectral data (Table 1) were very similar, suggesting the same parent structure. Therefore, the assignment of structure **2** to porninsal will be discussed in detail, while the spectral data of porninsol [**3**] and its esters **1a–1c** will be considered only in comparison with those of compound **2**.

Sesquiterpene **2** had a molecular formula $C_{15}H_{20}O$ (ms and ^{13}C nmr) that required six double-bond equivalents. From the ^{13}C-nmr data and ir spectrum of **2**, which exhibited a strong band for an unsaturated carbonyl group at 1677 cm^{-1}, the six sites were assigned to an aldehyde group and five double bonds. From the ^{1}H-nmr spectrum it was evident that the latter comprised two trisubstituted double bonds, each carrying a methyl group (δ 1.79 and 1.92, respectively), a vinyl group (ABX pattern at δ 5.20, 5.29, and 6.95), one terminal methylene group (broad doublet at δ 4.99 and broad singlet at δ 5.10), and one disubstituted double bond (doublet at δ 6.22 and double doublet at δ 6.72). One trisubstituted double bond was assigned to a methacrylaldehyde moiety because of the characteristic chemical shift of a deshielded vinylic proton (δ 6.53). The latter is further coupled to the methylene hydrogens of a -CH_2CH_2- system placed between two double bonds. The vinylic proton of the other trisubstituted double bond is further coupled with one vicinal proton of the disubstituted double bond. These features coupled with the uv absorption data, comparable with those previously reported for polyenes (12), indicated a conjugated tetraene structure. The latter was fully consistent with a farnesane skeleton containing the methacrylaldehyde unit at one end



1a R=CO(CH$_2$)$_n$Me; n=12, 13, 14, 16
1b R=CO(CH$_2$)$_7$CH=CH(CH$_2$)$_n$Me; n=5, 7
1c R=CO(CH$_2$)$_7$(CH=CHCH$_2$)$_2$(CH$_2$)$_3$Me
3 R=H

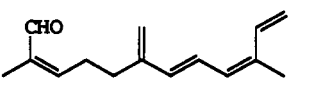


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TABLE 1. ^{1}H-nmr[a] and ^{13}C-nmr[b] Spectral Data for Compounds **1a–1c**, **2**, and **3**.

Proton	Compound			Carbon	Compound		
	1a–1c[c,e]	**2**[d]	**3**[c,f]		**1a–1c**[c,g]	**2**[d]	**3**[c]
H-1	4.55 s	10.07 s	3.90 d	C-1	61.0 (2)	191.0 (1)	59.6 (2)
				C-2	128.8 (0)	134.8[h] (0)	134.0[h] (0)
H-3	5.43 m	6.53 tq	5.22 m	C-3	131.7[h] (1)	148.2 (1)	133.6[i] (1)
H_2-4	2.25–2.30 m	2.80 qq	2.10–2.30 m	C-4	25.0 (2)	25.1 (2)	26.1 (2)
H_2-5	2.25–2.30 m	2.45 bt	2.10–2.30 m	C-5	30.4 (2)	31.7 (2)	31.9 (2)
				C-6	143.9 (0)	144.3 (0)	145.8 (0)
H-7	6.22 d	6.22 d	6.26 d	C-7	133.0[h] (1)	133.7 (1)	134.7[i] (1)
H-8	6.72 dd	6.72 dd	6.79 dd	C-8	121.9 (1)	123.7 (1)	123.7 (1)
H-9	6.03 bd	6.05 bd	6.09 bd	C-9	128.2[i] (1)	129.7 (1)	125.5 (1)
				C-10	132.6 (0)	136.4[h] (0)	135.9[h] (0)
H-11	6.98 dd	6.95 ddd	7.08 dd	C-11	128.6[i] (1)	132.9 (1)	130.4 (1)
H_a-12	5.26 bd	5.29 dt	5.27 bd	C-12	113.2 (2)	114.8 (2)	115.0 (2)
H_b-12	5.17 dt	5.20 dt	5.19 dt				
H_3-13	1.90 bs	1.92 bs	1.86 bs	C-13	19.9 (3)	19.9 (3)	19.7 (3)
H_a-14	5.05 bd	5.10 bs	5.07 bd	C-14	114.8 (2)	116.7 (2)	116.3 (2)
H_b-14	4.99 bs	4.99 bd	5.01 bs				
H_3-15	1.74 bs	1.79 bs	1.70 bs	C-15	18.4 (3)	16.3 (3)	21.3 (3)

[a]J(Hz) **1**: 11,12a=17.5; 11,12b=10.5; 7,8=15.5; 8,9=11.5; 12a,12b=1.5; 9,12b=1.5; 3,15=1.2. **2**: 11,12a=17; 11,12b=10.5; 11,9=1; 7,8=15; 8,9=11; 3,4=8; 3,15=1.5; 7,14a<1; 9,13=1; 9,12b=1.5; 9,12a=1; 9,11=1; 9,14a=1; 12a,12b=1.5; 14a,13<1; 14a,14b=1.2; 4,5=7.5; 4,15=1.2; 5,14b=1. **3**: 11,12a=17.2; 11,12b=10.5, 7,8=15.5; 8,9=11.5; 12a,12b=1.5; 3,15=1.2; 1, OH=5; 9,12b=1.5.

[b]The number in parentheses indicates the number of hydrogens attached to the corresponding carbon and was determined from DEPT experiment.

[c]In DMSO-d_6 solution.

[d]In $CDCl_3$ solution.

[e]RCOO-=0.88 (t, J=6.5, ω-H_3), 1.15–1.40 [bs, -$(CH_2)_n$-], 1.52–1.66 (m, H_2-3′), 2.03 (bq, J=7.0, H_2-8′ and H_2-14′ linoleate, H_2-8′ and H_2-11′ oleate and palmitoleate), 2.29 (t, J=7.5, H_2-2′), 2.75 (bt, J=7.0, H_2-11′ linoleate), 5.30–5.35 (m, H-9′, H-10′, H-12′, H-13′ linoleate, H-9′ and H-10′ oleate and palmitoleate).

[f]OH=4.55 t.

[g]RCOO- (italic figures indicate low intensity signals of minor esters) 12.6 (Me), 20.9 (CH_2), 21.0 (CH_2), 23.3 (CH_2), 24.0 (CH_2), 25.5 (CH_2), 27.4 (CH_2), 27.5 (CH_2), 27.6 (CH_2), 27.7 (CH_2), 27.9 (CH_2), 28.0 (CH_2), 29.8 (CH_2), 30.2 (CH_2), 32.5 (CH_2), 126.3 (CH=), 126.4 (CH=), 128.3 (=CH), 128.4 (=CH), 171.7 (CO).

[h,i]Assignments in the same vertical column may be interchanged.

and the extended system of conjugated double bonds at the other as indicated in structure **2**. The latter received further support by the allylic and long range coupling constants between the olefinic protons (e.g., H-7 with H_a-14; H-9 with H-11, H_a-12, H_b-12, and H_a-14) and the allylic coupling between H_b-14 and the methylene group at C-5. Moreover, the simultaneous presence of an unsaturated aldehyde and a tetraene system in the same molecule accounts for the great lability of porninsal [**2**].

The stereochemistry of the double bonds was established to be 2*Z*, 7*E*, 9*Z* from the chemical shift of H-3 (δ 6.53), the coupling constant between H-7 and H-8 (15.0 Hz), and NOEDS studies. NOEDS results (Figure 1) indicated interactions between H-8 (δ 6.72) and H-11 (δ 6.95) and H_2-5 (δ 2.45), between H_3-13 (δ 1.92) and H-9 (δ 6.05) and H_a-12 (δ 5.29), and between H-3 (δ 6.53) and H_2-4 (δ 2.80) and H_3-15 (δ 1.79). These results also indicated that all conjugated double bonds of the tetraene system exist in the *S* trans conformation, as shown in Figure 1. Porninsal [**2**] was thus established to be (2*Z*, 7*E*, 9*Z*)-farnesa-2,6(14),7,9,11-pentaen-1-al.

A major difference in the ^{1}H-nmr spectrum of porninsol [**3**] compared with that of compound **2** was that the olefinic proton H-3 was upshifted, in accordance with the absence of a carbonyl function at C-1. In addition, the signal for H-1 of the aldehyde **2** (δ 10.05) had been replaced by a doublet (2H) at δ 3.90, coupled with an OH group (5.0 Hz), indicating that the aldehyde had been reduced to a hydroxymethyl group. The ms, ir, and ^{13}C-nmr data also support the suggested structure **3**, and reduction of porninsal [**2**] with DIBALH in THF yielded an alcohol identical in all respects to porninsol [**3**]. Porninsal [**2**] and porninsol [**3**] both have orange-like flavors, but both also have unpleasant tastes.

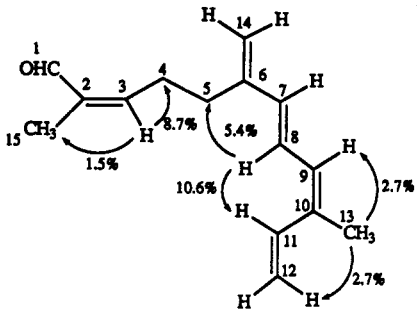

FIGURE 1. Stereochemistry of **2** based on nOe studies.

No attempt was made to separate the nonpolar esters **1a–1c** because of their similar chromatographic properties. However, inspection of the ^{1}H-nmr spectrum of a purified fraction of **1a–1c**, in comparison with the spectrum of **3**, strongly suggested that it was a mixture of porninsol fatty acid esters. In fact, the only difference (except for signals of the fatty acid protons) was that the signal for H_2-1 was shifted downfield from δ 3.90 in the ^{1}H-nmr spectrum of porninsol [**3**] to δ 4.55 in the spectrum of its esters **1a–1c**. Moreover, comparison of the ^{1}H-nmr and ^{13}C-nmr data (chemical shift, multiplicity, and integral) of the fatty acid moiety with those of various free fatty acids suggested that the major components are saturated esters **1a** (about 86%) and the minor components are unsaturated esters **1b** and **1c** (about 14%).

The eims spectrum of the mixture showed a cluster of molecular ions, in which the most prominent peaks were at *m/z* 484, 480, and 456, corresponding to the mol wt of **1a** (n=16), **1c**, and **1a** (n=14), respectively. In gc-ms, individual esters **1a–1c** could not be separated very well from each other. However, saponification of the ester mixture gave porninsol [**3**] and a mixture of acids that, after exposure to CH_2N_2 in Et_2O, was again analyzed by high resolution gc and gc-ms. On comparison with the retention times and mass spectra of standard fatty acid methyl esters and the reference mass spectra of commercial libraries (13,14) we could detect (the approximate relative percentage of each ester in parentheses) the methyl derivatives of the following acids: tetradecanoic acid (3%), pentadecanoic acid (2%), (9*Z*)-hexadecenoic acid (1%), hexadecanoic acid (10%), (9*Z*,12*Z*)-octadecadienoic acid (8%), (9*Z*)-octadecenoic acid (2%), octadecanoic acid (64%), and minor unidentified acids (10%). Therefore, the mixture of porninsol esters is mainly composed of compounds **1a–1c**.

Compounds **1a–1c**, **2**, and **3** are new farnesane sesquiterpenes and represent the first examples of this class of compounds found in any *Lactarius* species. So far, among Basidiomycetes, farnesane sesquiterpenes have been found only in a nonrelated species, *Hebeloma senescens* (15).

In conclusion, it appears that the fruiting-bodies of *L. porninsis* originally contain fatty acid esters of one farnesane sequiterpene (i.e., compounds **1a–1c**) and that these esters are converted enzymatically into the aldehyde **2** and the alcohol **3** when the fruiting-bodies are injured. This behavior is similar to that of other pungent and/or bitter *Lactarius* species; however, the tasteless substrate originally present can vary from species to species (see above) and this is of some chemotaxonomic interest. Moreover, in some *Lactarius* species such as in *L. deliciosus* (4) and *L. porninsis,* two enzymes, an esterase and an alcohol dehydrogenase, are necessary for converting the fatty acid esters into the corresponding aldehydes, while for other species such as *L. vellereus* (5) and *L. chrysorrheus* (6), the original ester is a masked form of the free aldehyde. The latter is liberated directly during hydrolysis of the ester (6). This hydrolytic step is very rapid for one group of

mushrooms [*L. vellereus* (5), *L. chrysorrheus* and *L. scrobiculatus* (6), *L. fuliginosus* and *L. picinus* (3)], while it is much slower for another group [*L. porninsis, L. deliciosus,* and *L. deterrimus* (4), *Lactarius sanguifluus* (9)], reflecting either a different intrinsic reactivity of the original substrate or enzymatic differences in the different species.

The instability of sesquiterpenes **1a–1c**, **2**, and **3** makes any quantitative test of antimicrobial and antifeedant activity unreliable. However, in spite of this lack of evidence, the kind of enzymatic transformations occurring in *L. porninsis* may suggest that, for this mushroom, compounds **1a–1c**, **2**, and **3** constitute a new variant of the chemical defense system that protects *Lactarius* species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded as films with a Perkin-Elmer Model 881 spectrophotometer; uv spectra were obtained in hexane solutions with a Perkin-Elmer Lambda 5 uv/vis spectrophotometer. ^{1}H-nmr (300 MHz, δ_H values in ppm, relative to δ_H=0.00 for TMS) and ^{13}C-nmr spectra (75.5 MHz, δ_C values in ppm, relative to δ_C=76.9 for $CDCl_3$) were recorded on a Bruker ACE 300 instrument. Ms spectra were determined with a Finnigan MAT 8222 mass spectrometer at 70 eV using a direct inlet system. Cc was performed at atmospheric pressure on prewashed [2.5% NEt_3 in hexane-EtOAc (40:1)] Kieselgel 60 (Merck) 0.040–0.063 mm, slurry packed. Solvents for elution were degassed by sonication just before use. Tlc was carried out on Si gel plates (GF_{254}, Merck, 0.25 mm). The spots were visualized by spraying the plates with 0.5% vanillin solution in H_2SO_4-EtOH (4:1) and then heating at 120° for ca. 1 min. Preparative centrifugal circular chromatography was performed on a commercial apparatus (Chromatotron) using Si gel plates (PF_{254}, Merck), 1 mm layer thickness.

Glc capillary analysis was performed with an HRGC-5160 Mega Series Carlo Erba apparatus (SupelCowax TM10 capillary column, 30 m long, 0.32 mm i.d., 0.25 μ film thickness) in the following conditions: injection "on column," FID detector at 300°; H_2 as a carrier gas at 0.3 kg/cm^2 pressure; temperature of the column oven increased from 90° to 180° at 40° per min, then from 180° to 270° at 5° per min, finally kept at 270° for 20 min. Gc-ms analysis was performed with a Finnigan-MAT ITS-40 instrument equipped with a DB-5MS capillary column, 30 m long, 0.25 mm i.d., 0.25 μ film thickness. Chromatographic conditions: injector 250°, detector 270°; temperature of the column oven increased from 40° to 300° at 8° per min, then kept at 300° for 20 min; He as a carrier gas. Standard fatty acids were purchased from Sigma and were methylated with CH_2N_2 in Et_2O.

EXTRACTION AND ISOLATION OF THE SESQUITERPENES.—*L. porninsis* (2.4 kg) was collected on the hills near Imperia, Italy, in October 1992 and was identified by Prof. Giorgio Baiano (Mycological group "G. Bresadola," Asti). A voucher specimen has been deposited in the herbarium of the Mycological group "G. Bresadola," Fara Novarese, Italy (No. GMFN 1847). The mushrooms were brought to the laboratory in Pavia and analyzed a few hours after collection. The fruiting bodies, apparently not invaded by parasites, were minced and left at room temperature for ca. 20 min without addition of solvent. They were then extracted two times at 20° with degassed hexane (2.5 liters), leaving the solvent in contact with the mushrooms for about 5–10 min each time. The combined extracts were dried rapidly ($MgSO_4$) and concentrated to ca. 300 ml below 30° under reduced pressure and under diffuse light. It was very important not to evaporate the solution completely to dryness at any time to prevent decomposition of the sesquiterpenes. For isolation work, successive aliquots (ca. 20 ml) of the mother extracts were concentrated to ca. 2.5 ml and adsorbed on the top of a Si gel column, eluted with a gradient of EtOAc in hexane (from 2.5 to 100% EtOAc) to give, in order, compounds **1a–1c**, **2**, and **3**. All operations were performed under diffuse light. For a rough estimate of the content of each sesquiterpene, homogenous fractions from one standard separation were pooled and taken to dryness to give **1a–1c** (135 mg), **2** (35 mg), and **3** (20 mg). The ir spectra were recorded immediately after evaporating a concentrated solution of each compound directly on the NaCl disks with an ir lamp. Ms spectra were determined by introducing a concentrated hexane solution of each compound into the probe of the direct inlet system and pumping off the solvent by the pre-high vacuum pump system of the instrument. For obtaining the nmr spectra of **1a–1c** and **2**, the corresponding chromatographic fractions, homogeneous by tlc, were evaporated to ca. 5 ml; then DMSO-d_6 (1 ml) was added, and residual low-boiling solvents were removed at water aspirator pressure and then under 0.1 mm Hg vacuum.

(2Z,7E,9Z)-2,10-Dimethyl-6-methylene-2,7,9,11-dodecatetraen-1-ol esters (porninsol esters) [**1a–1c**].—R_f 0.87 in cyclohexane-EtOAc (9:1); ir ν max 2927, 2857, 1734, 1655, 1559, 1458, 1435, 1380, 1235, 1161, 964 cm^{-1}; uv λ max 311.2 nm, 297.2 nm, 284.8 nm; ^{1}H and ^{13}C nmr see Table 1; eims *m/z* (% rel. int.) [stearate M]$^+$ 484 (15), [linoleate M]$^+$ 480 (2), [palmitate M]$^+$ 470 (2), 267 (8), [M+H−RCO]$^+$ 218 (10), [M−RCO]$^+$ 217 (13), 201 (28), [M−RCOOH]$^+$ 200 (100), 185 (41), 172 (16), 171 (15), 159 (71), 157

(21), 145 (28), 134 (27), 133 (62), 132 (88), 131 (25), 121 (31), 120 (41), 117 (25), 109 (16), 108 (31), 107 (34), 105 (45), 95 (23), 93 (48), 91 (38), 81 (49), 79 (33), 71 (24), 67 (46), 57 (53), 55 (68), 43 (77), 41 (58).

(2Z,7E,9Z)-2,10-Dimethyl-6-methylene-2,7,9,11-dodecatetraen-1-al (porninsal) [**2**].—R_f 0.58 in cyclohexane-EtOAc (9:1); ir ν max 3090, 3045, 2925, 2850, 1677, 1616, 1562, 1453, 1376, 1155, 1082, 980, 960, 902, 850 cm^{-1}; uv λ max (log ϵ) 311.0 nm (4.20), 297.0 nm (4.28), 284.8 (4.11); ^1H and ^{13}C nmr see Table 1; eims *m/z* (% rel. int.) $[M]^+$ 216 (7), 188 (7), 183 (7), 173 (7), 157 (10), 145 (28), 133 (24), 132 (27), 119 (87), 117 (41), 115 (23), 105 (61), 91 (100), 79 (33), 77 (52), 65 (25), 55 (22), 53 (24), 41 (43); hreims *m/z* $[M]^+$ 216.1531 ($C_{15}H_{20}O$) (calcd 216.1514).

(2Z,7E,9Z)-2,10-Dimethyl-6-methylene-2,7,9,11-dodecatetraen-1-ol (porninsol) [**3**].—R_f 0.22 in cyclohexane-EtOAc (9:1); ir ν max 3341, 3093, 3040, 2930, 2850, 1616, 1564, 1450, 1436, 1376, 1003, 983, 961, 897, 848 cm^{-1}; uv λ max (log ϵ) 310.8 nm (4.19), 297.2 nm (4.25), 284.6 nm (4.11); ^1H and ^{13}C nmr see Table 1; eims *m/z* (% rel. int.) $[M]^+$ 218 (6), 200 (3), 159 (26), 145 (12), 131 (18), 119 (33), 117 (25), 105 (49), 93 (41), 91 (67), 81 (34), 79 (42), 77 (34), 67 (61), 57 (53), 55 (80), 43 (57), 41 (100); hreims *m/z* $[M]^+$ 218.1682 ($C_{15}H_{22}O$) (calcd 218.1671).

DIBALH REDUCTION OF ALDEHYDE **2** TO ALCOHOL **3**.—DIBALH (1.0 M) in THF (192 µl, 0.192 mmol) was added by syringe to compound **2** (35 mg, 0.16 mmol) in THF (2 ml) at 0° under an Ar atmosphere. The reaction mixture was stirred for 30 min, then quenched with H_2O (50 µl) and MeOH (200 µl), diluted with CH_2Cl_2, and filtered through a short celite pad. The organic solution was dried ($MgSO_4$) and evaporated. The residue was immediately redissolved in hexane-CH_2Cl_2 (9:1) and separated by centrifugal circular chromatography. Elution with hexane-EtOAc (7:1) gave alcohol **3** (20 mg), identical (R_f, ir, ^1H nmr) with the natural compound.

NaOH HYDROLYSIS OF COMPOUNDS **1a–1c**.—A mixture of **1a–1c** (25 mg) was stirred in 2 ml EtOH-15% aqueous NaOH (1:2) at 40° overnight. The mixture was diluted with H_2O (10 ml) and extracted with Et_2O (4×15 ml). The organic layer was dried ($MgSO_4$), concentrated, and analyzed by tlc, ir, and ^1H-nmr spectroscopy following the procedure described above. The spectra were identical to those of alcohol **3**. The aqueous phase was acidified with 10% HCl and extracted with Et_2O (3×10 ml). After addition of an ethereal CH_2N_2 solution, the solvent was evaporated and the residue (ir band at 1735 cm^{-1}) analyzed by gc and gc-ms. Individual components of the mixture were identified by comparison of retention times and mass spectra with authentic reference compounds.

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