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DITERPENE FATTY ACID ESTER FROM *LEUCAS NUTANS*

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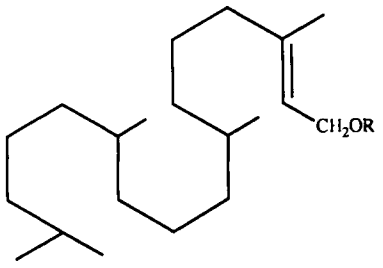
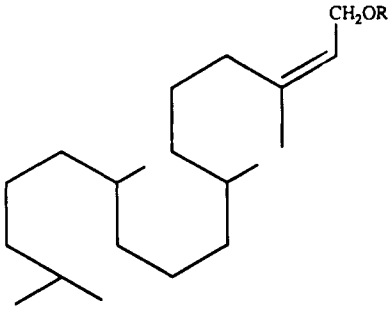
ABSTRACT.—A new diterpene fatty acid ester, *trans*-phytyl palmitate [**1**], has been isolated from *Leucas nutans* and characterized on the basis of chemical investigation and spectroscopic studies. *n*-Hentriacontane, 1-dotriacontanol, and phytol [**2**] were also isolated for the first time from this plant.

Different species of *Leucas* have been reported to possess interesting pharmacological properties (1,2). However, limited chemical work on the genus induced us to investigate the chemical constituents of *Leucas nutans* Spreng. (Syn. *Leucas decurva* Bth.). The plant belongs to the Labiatae family and is found in Pakistan and India (1). No work on the chemical constituents of this species is reported in the literature. This paper describes the isolation of a new diterpene fatty acid ester which is characterized as *trans*-phytyl palmitate [**1**]. The plant also yielded *n*-hentriacontane, 1-dotriacontanol, and phytol [**2**].



1 R=$Me(CH_2)_{14}CO$
2 R=H



3 R=H
4 R=$Me(CH_2)_{14}CO$

Compound **1** was obtained as colorless waxy material. Its molecular formula was established as $C_{36}H_{70}O_2$ by its high resolution mass spectrum. The ir spectrum indicated the absorption of an ester group at 1713 and 1155 cm^{-1}. Its ^{1}H-nmr spectrum exhibited a triplet at δ 5.32 (J = 6.9 Hz) assigned to an olefinic proton and a doublet at δ 4.57 (J = 7.0 Hz) consistent with a methylene group adjacent to an ester oxygen. The other observed signals were a singlet at δ 1.68, representing a methyl group attached to a quaternary carbon, a doublet at δ 0.86 (J = 6.6 Hz) representing the four methyl groups attached to tertiary carbon atoms at positions 7, 11, 15, and 16, and a triplet at δ 0.87 (J = 6.7 Hz) due to the Me-16′ protons. The ^{13}C-nmr spectrum showed 36 carbon atoms. The DEPT experiments (3) revealed the presence of six methyl, four methine, and twenty-four methylene carbon atoms.

The structure of **1** was elucidated by comparison of its spectral data to those of phytol (4,5). The terpenoid moiety of the former seemed to be structurally related to the latter. The high resolution mass spectrum of **1** showed a fragment ion peak at *m/z* 239.2410 corresponding to a $C_{16}H_{31}O$ moiety from the molecular

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ion. The other fragment ion peaks at *m/z* 123.1194 (C_9H_{15}), 97.1014 (C_7H_{13}), 83.0846 (C_6H_{11}), and 81.0723 (C_6H_9) provided strong evidence for the long chain fatty acid attached to the phytol skeleton. The ^{13}C-nmr spectrum of **1** (Table 1), which showed a downfield shift of the signal for C-1 as compared to the values of the relevant carbon for phytol (5), as well as signals for the palmitate moiety (6), also indicated the presence of phytol palmitate (Table 1). On alkaline hydrolysis, **1** yielded palmitic acid and the acyclic diterpene *trans*-phytol (4,5).

TABLE 1. ^{13}C-nmr Chemical Shift[a] Data of Compounds **1** and **2**.

Carbon	Compound	
	1	**2**
C-1	61.22	59.47
C-2	122.63	123.18
C-3	139.82	140.37
C-4	39.88	39.90
C-5	24.81	25.16
C-6	37.04	36.69
C-7	32.69	32.72
C-8	37.46	37.39
C-9	24.48	24.48
C-10	38.20	37.46
C-11	32.82	32.81
C-12	37.32	37.32
C-13	24.74	24.81
C-14	39.40	39.40
C-15	27.99	27.99
C-16	22.70	22.72
3-Me	16.37	16.18
7-Me	19.75	19.73
11-Me	19.82	19.76
15-Me	22.63	22.63
C-1′	173.42	
C-2′	34.44	
C-3′	26.15	
C-4′–C-13′	29.19	
	29.28	
	29.38	
	29.48	
	29.62	
C-14′	31.94	
C-15′	23.10	
C-16′	14.11	

[a]Chemical shifts are in ppm for TMS.

The identification of the hydrolyzed product as trans rather than the cis isomer of phytol is based on the published (4) ^{1}H-nmr spectra in which significant spectroscopic differences between these two isomers have been observed. The main difference lies with the 1-methylene group where the values of *trans*-phytol and *cis*-phytol [**3**] are reported as doublets at δ 4.05 and 4.48, respectively. The 1-methylene group of the hydrolysate from **1** appeared as a doublet (Table 2) at δ 4.14 (J = 6.9 Hz), which coincides with that of *trans*-phytol. The palmitate moiety was identified by gc-ms, thus establishing that **1** is *trans*-phytyl palmitate. The isolation of **2** from *L. nutans* further supports the established structure. One of the researchers in our group has recently reported (7) a cis isomer of phytyl palmitate [**4**] from *Pentatropis spiralis*. However, a literature survey revealed that **1** is a new compound.

In addition to the above-mentioned diterpene fatty acid ester **1**, several known compounds were isolated for the first time from the plant and identified through their spectral data as hentriacontane, 1-dotriacontanol, and phytol [**2**].

EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *L. nutans* were collected from Quaid-i-Azam University campus, Islamabad, Pakistan in the fall of 1988. A voucher specimen is deposited at the Herbarium of Department of Biology, Quaid-i-Azam University. The sample was dried in a cool, dark place and coarsely powdered.

EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: ir, JASCO A-302 spectrophotometer; nmr, Bruker AM-400 MHz, operating at 400 MHz for ^{1}H-nmr and at 75 MHz for ^{13}C-nmr; ms, Finnigan MAT-112S for eims and JEOL JMX HX-110 for hrms and gc-ms; tlc on Si gel PF_{254}; cc on Si gel 60. The spots were visualized with ceric sulphate reagent and iodine vapors.

ISOLATION AND IDENTIFICATION OF COMPOUNDS.—The EtOAc extract (220 g) obtained from partitioning of an EtOH extract was chromatographed on a column of silica and eluted

TABLE 2. Comparison of ^{1}H-nmr Chemical Shifts[a] and Coupling Constants for Compounds **2** and **3**.

Carbon	Compound	
	2	**3**
C-1	4.14 (d, J = 6.9 Hz, 2H)	4.48 (d, J = 6.4 Hz, 2H)
C-2	5.32 (t, J = 6.9 Hz, 1H)	5.33 (t, J = 6.4 Hz, 1H)
C-4	1.97 (m, 2H)	2.00 (m, 2H)
3-Me	1.66 (s, 3H)	1.71 (s, 3H)
7-, 11-, 15-, 16-Me	0.86 (d, J = 6.5 Hz, 12H)	0.91 (d, J = 6.0 Hz, 12H)

[a]Chemical shifts are in ppm from TMS.

with n-C_6H_{14} using $CHCl_3$ as gradient. The fraction obtained by n-C_6H_{14}–$CHCl_3$ (17:3) was rechromatographed on a Si gel column. The eluate obtained in n-C_6H_{14}–Et_2O (99:1) was evaporated and resulted in a waxy mass (0.065 g) which was further purified through preparative layer chromatography using n-C_6H_{14}–$CHCl_3$ (4:1) as mobile phase to yield **1**: $[\alpha]^{22}D$ −6.6° (c = 0.24, $CHCl_3$); ir ν max ($CHCl_3$) 2860, 1713, 1155 cm^{-1}; hrms $[M]^+$ 534.5425 ($C_{36}H_{70}O_2$), $[C_{20}H_{39}]^+$ 279.2992, $[C_{16}H_{32}O_2]^+$ 256.2463, $[C_{16}H_{31}O]^+$ 239.2410, $[C_9H_{15}]^+$ 123.1194, $[C_7H_{13}]^+$ 97.1014, $[C_7H_{11}]^+$ 95.0864, $[C_5H_9]^+$ 69.0702; ^{1}H-nmr ($CDCl_3$) δ 0.86 (12H, d, J = 6.6 Hz), 0.87 (3H, t, J = 6.7 Hz, H-16′), 1.68 (3H, br s), 1.98 (2H, m), 2.34 (2H, t, J = 7.4 Hz, H-2′), 4.57 (2H, d, J = 7.0 Hz, H-1), 5.32 (1H, t, J = 6.9 Hz, H-2). Found $[M]^+$ 534.5425; calcd for $C_{36}H_{70}O_2$, 534.5358.

ALKALINE HYDROLYSIS OF **1**.—The ester **1** was hydrolyzed with alcoholic 5% NaOH. The hydrolysate was extracted with $CHCl_3$, washed with H_2O, and evaporated. The colorless viscous substance obtained was identified as **2**: ms molecular ion m/z 296 (corresponds to $C_{20}H_{40}O$); ^{1}H-nmr ($CDCl_3$) δ 0.86 (12H, d, J = 6.5 Hz, H-7a, -11a, -15a, -16), 1.66 (3H, s, H-3a), 1.97 (2H, m, H-4), 4.14 (2H, d, J = 6.9, H-1), 5.40 (1H, t, J = 6.9 Hz, H-2).

METHYLATION OF PALMITIC ACID.—The aqueous layer was acidified with 5% HCl and extracted with Et_2O. The organic layer was washed with H_2O and evaporated. The residue obtained was methylated with CH_2N_2 and found to be identical with palmitic acid methyl ester (8): gc-ms $[M]^+$ 270 ($C_{17}H_{34}O_2$), $[M-31]^+$ 239, $[M-43]^+$ 227, 227, 185, 157, 143, 129, 87, 74 (100%).

n-Hentriacontane (0.5 g) was obtained from the EtOAc extract fractions (preceding those from which **1** was eluted) using n-C_6H_{14} as mobile phase. 1-Dotriacontanol and **2** were obtained in mixture by subsequent elution of the EtOAc extract with n-C_6H_{14}–$CHCl_3$ (1:1). The mixture was resolved by flash cc; 1-dotriacontanol (0.007 g) was eluted with n-C_6H_{14} and **2** (0.039 g) with n-C_6H_{14}–$CHCl_3$ (99:1). These compounds were identified by comparison of their spectroscopic data with those published (5,6,9,10).

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