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DITERPENES FROM THE LEAVES OF *LEONOTIS OCYMIFOLIA* VAR. *RAINERIANA*

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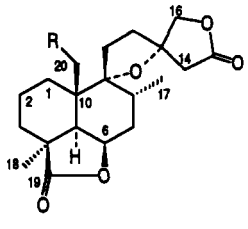
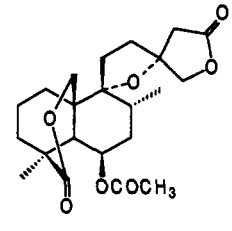
ABSTRACT.—Two known diterpenes, leonitin [**1**] and compound X [**2**], and two new diterpenes, 6β-acetoxy-9α,13α-epoxylabda-20(19),16(15)-diol dilactone [**3**] and 20-acetoxy-9α,13ξ-dihydroxy-15(16)-epoxylabd-14-en-6β(19)-lactone [**4**], have been isolated from the leaves of *Leonotis ocymifolia* var. *raineriana*. Their structures were established on the basis of spectral evidence.

Several species of *Leonotis* are known for their medicinal value and are sometimes regarded as narcotic and habit forming (1). *Leonotis ocymifolia* (Burm. f.) Iwarsson var. *raineriana* (Visiani) Iwarsson (Labiatae) is indigenous to Eastern and Southern Africa (2) and is occasionally cultivated for its medicinal uses which include acting as an ascaricide, an anticancer drug, and as a treatment for ulcers and wounds (3,4). We wish to report the isolation and structural elucidation of two new diterpenes and unambiguous ^{13}C-nmr assignments of the known compounds, leonitin [**1**] (5,6) and compound X [**2**] (7–9), all obtained from the leaves of this plant collected in the flowering stage. The structures of the new compounds were established as 6β-acetoxy-9α,13α-epoxylabda-20(19),16(15)-diol dilactone [**3**] and 20-acetoxy-9α,13ξ-dihydroxy-15(16)-epoxylabd-14-en-6β(19)-lactone [**4**].

The cold EtOH extract was subjected to vlc over Si gel eluting with solvents of increasing polarity. Four diterpenes were isolated from the 8:2 to 1:1 hexane-EtOAc eluents by cc (Sephadex LH-20) followed by prep. tlc. The structures of the compounds were elucidated



1 R=$OCO^{21}CH_3^{22}$
2 R=H



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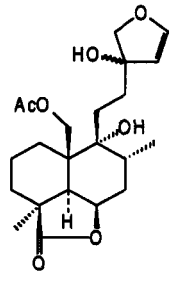

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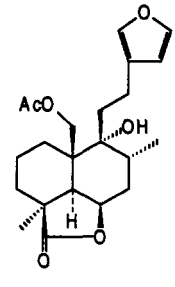

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by a combination of eims, ^{1}H-nmr, ^{13}C-nmr, and 2D nmr (^{1}H-^{1}H COSY, NOESY, and HMBC) techniques.

Leonitin [**1**] was isolated as needles, and hreims allowed assignment of the molecular formula $C_{22}H_{30}O_7$. The ir spectrum revealed absorption bands for γ-lactone carbonyls at 1770 and 1780 cm^{-1} and an ester carbonyl at 1730 cm^{-1}. The structure of leonitin was established primarily by X-ray crystallography (5,6) and consequently there is a paucity of published spectral data. In this paper we report the results of a comprehensive nmr study of this compound. The ^{1}H-nmr spectrum revealed three ABq signals for H_2-14, H_2-16, and H_2-20, a methyl singlet (Me-18), an acetyl methyl (Me-22), a methyl doublet (H_3-17) and an oxymethine (H-6). One of the C-20 oxymethylene protons (δ 4.33) exhibited *W*-bond coupling (J=2.0 Hz) to the H-1 axial proton (Figure 1). Further assignments of ^{1}H-nmr resonances were based on the ^{1}H-^{1}H COSY spectrum which revealed the C-17 methyl doublet coupling to H-8, the H-6 oxymethine coupling with a doublet (H-5) and a multiplet (H-7_{ax}), and H-7_{ax} exhibited further coupling to a multiplet, which must be H-7_{eq}.

The ^{13}C-nmr chemical shift values of **1** (Table 1) were obtained from a JMOD experiment and assigned by means of HC-COBI (^1J) and HMBC (10) (^2J and ^3J) heteronuclear coupling experiments. Important observations from the HMBC experiment (Table 2) were the ^3J coupling of H-5 and H_3-18 to the carbonyl δ_C 182.3 H_2-16 to the carbonyl δ_C 174.6,

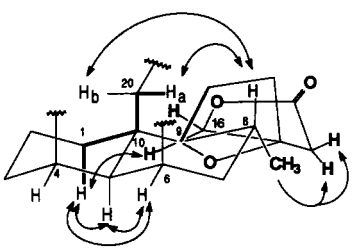

FIGURE 1

TABLE 1. ^{13}C-Nmr Chemical Shift Data for Compounds **1–3**.[a]

Carbon	Compound		
	1	**2**	**3**
1	23.3	28.2	34.7
2	18.2	18.1	20.6
3	28.5	28.9	39.8
4	43.6	46.1	41.1
5	48.1	46.1	47.2
6	75.5	76.1	68.4
7	32.0	29.2	34.1
8	32.9	31.8	31.2
9	90.9	92.2	90.1
10	42.7	39.1	40.5
11	31.2	31.8	29.4
12	37.4	37.1	37.8
13	85.2	88.2	86.6
14	41.5	43.2	42.8
15	174.6	174.7	174.3
16	78.9	78.8	78.1
17	17.8	17.6	17.2
18	21.2	23.5	20.6
19	182.3	183.6	175.7
20	65.9	23.2	75.7
21	170.1		170.7
22	23.7		22.5

[a]δ values in $CDCl_3$, 100 MHz.

and H_2-20 to the acetyl carbonyl at δ_C 170.1. A^2J coupling was observed between the δ_C 174.6 carbonyl and H_2-14 and between the acetyl carbonyl and H_3-22. The two quaternary oxygen-bearing carbons (C-9 and C-13) could also be differentiated as C-9 showed ^3J coupling to the H_2-20 and H_3-17 protons while C-13 showed the expected ^2J coupling to the H_2-14 and H_2-16 protons. Thus, except for C-2 and C-11, all the ^{13}C-nmr resonances could be assigned directly from the HMBC spectrum.

The relative stereochemistry of **1** was established from a ^{1}H-^{1}H NOESY spectrum. Interactions between H-6 and H-5 and H-1_{ax} indicated that these are on the same face of the molecule (Figure 1). Similar interactions between H-8 and H_2-20 supported their close proximity. The NOESY spectrum further revealed an interaction between H_3-17 and H_2-14 and thus supported their relative stereochemistry as shown in Figure 1. This

TABLE 2. ^{1}H-^{13}C Correlations (^{2}J and ^{3}J Interactions) of Compound **1**, Obtained from an HMBC Experiment.

Proton	Carbon resonance	
	^{2}J	^{3}J
H-5	42.7 (C-10)	18.1 (C-2), 65.9 (C-20), 90.9 (C-9), 182.3 (C-19)
H-6	48.1 (C-5)	32.9 (C-8)
H-7	32.9 (C-8)	
H-11		32.9 (C-8), 90.9 (C-9)
H-12	31.2 (C-11), 85.2 (C-13)	41.5 (C-14), 78.9 (C-16), 90.9 (C-9)
H-14	85.2 (C-13), 174.6 (C-15)	37.4 (C-12), 78.9 (C-16)
H-20		23.3 (C-1), 90.9 (C-13), 170.1 (C-21)
Me-17	32.9 (C-8)	32.0 (C-7), 90.9 (C-13)
Me-18	43.6 (C-4)	28.5 (C-3), 48.1 (C-5), 182.3 (C-19)
Me-22	170.1 (C-21)	

assignment was further substantiated by the observation of a strong nOe interaction between H_2-16 and the H-1 axial proton. Unambiguous assignment of absolute stereochemistry of the isolated material was established by direct comparison of its cd curve with that of the original material used in the X-ray analysis (5,6).

Compound **2**, $C_{20}H_{28}O_5$ by hreims, showed absorption bands at 1780 and 1760 cm^{-1} (γ lactones) in the ir spectrum. The ^{1}H- and ^{13}C-nmr spectra (Table 1) were very similar to those of **1** except that the acetate resonances were absent and the C-20 oxymethylene was replaced by a methyl resonance. Similar NOESY interactions were also found to those observed for **1**; e.g., H-5/H-6, H-5/18-Me, H-6/18-Me, and H-14/17-Me. These observations suggested that the compound had structure **2**, which is the compound X previously isolated from *L. leonurus* (7–9). The occurrence of identical cd curves for the isolated compound and an authentic sample of compound X confirmed the identity of the isolated compound.

The same series of spectroscopic experiments were performed for compound **3** as described for **1**. These revealed that **3** also possessed an acetoxy substituent, two γ lactone rings (one 15→16 as in **1** and **2**), an oxymethine and a 9,13-epoxy function. Differences from **1** and **2** were the downfield shift of the resonance of C-20 in the ^{13}C-nmr spectrum (Table 1) and H-6 in the ^{1}H-nmr spectrum. These data suggested that C-20 was part of a lactone ring system and that the acetate ester was situated at C-6. These structural assignments were substantiated by an HMBC experiment for which the important ^{2}J and ^{3}J correlations are listed in Table 3. Key observations in this study were a ^{3}J coupling of the H_2-20 methylene protons to the C-19 carbonyl carbon (δ 175.7), which thus supported the 19→20 lactone ring in **3**. The relative stereochemistry of **3** was deduced from a NOESY study. This revealed similar interactions with those of **1** and **2** except for the loss of the nOe between H_2-14 and H_3-17 and the appearance of an interaction between H_3-17 and H_2-16. This suggested the relative stereochemistry shown in structure **3**. Compound **3** is a new natural product but was previously obtained by saponification of leonitin from *Leonotis leonotis* followed by acetylation (5).

Compound **4**, $C_{22}H_{32}O_7$, showed characteristic bands for ester (1740 cm^{-1}), γ-lactone carbonyl (1770 cm^{-1}) and hy-

TABLE 3. ^{1}H-^{13}C Correlations (^{2}J and ^{3}J Interactions) of Compound **3**, Obtained from an HMBC Experiment.

Proton	Carbon resonance	
	^{2}J	^{3}J
H-14	86.6 (C-13), 90.1 (C-9), 174.3 (C-15)	37.8 (C-12), 78.1 (C-16)
H-16	86.6 (C-13)	86.6 (C-13), 37.8 (C-12), 174.3 (C-15)
H-20	40.5 (C-10)	34.7 (C-1), 47.2 (C-5), 175.7 (C-19)
Me-17	31.2 (C-8)	34.1 (C-7), 90.1 (C-9)
Me-18	41.1 (C-4)	47.2 (C-5), 175.7 (C-19)
Me-22	170.7 (C-21)	

droxyl (3450 cm^{-1}, broad) functional groups in the ir spectrum. Comparison of the ^{1}H-nmr spectrum with those of **1–3** revealed that **4** had 19→6 lactone, C-20 acetoxy and C-16 oxymethylene functional groups but not a 15→6 lactone. Coupled olefinic proton signals at δ 6.47 and δ 5.16 (J=2.7 Hz) were typical for a five-membered ring system and must be placed at C-14 and C-15. As H_2-16 showed no further coupling, C-13 must be blocked, which required placement of a hydroxyl in that position. This still required another hydroxyl which was assigned to C-9, so that the dihydroxylation pattern is that for a normal *Leonotis* diterpene prior to the formation of the 9,13-epoxide. On this basis the structure **4**, which is novel, was proposed. The low optical activity ([α]D +1°) and doubling of signals in some of the ^{1}H-nmr spectrum were indicative of presence of both C-13 epimers.

Compound **4** was unstable and changed to **5** on warming in $CHCl_3$. The transformation was characterized by disappearance of the ^{1}H-nmr signals due to the vinyl ether (H-14, H-15) and oxymethylene protons (H_2-16) and the appearance of those typical for a monosubstituted furan [**5**]. This transformation was further substantiated by the eims which showed a fragment at *m/z* 81 for the furan ring.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were uncorrected. Ir spectra were run as KBr discs. Nmr spectra were recorded on a Bruker AMX-400 instrument in $CDCl_3$. Eims spectra were recorded on an AEI-MS902 double-focusing spectrometer with direct probe insert (110–130°, 70 eV). Si gel (Merck 7749) for vlc and Si gel 60 PF_{254} for prep. tlc were used.

PLANT MATERIAL.—The leaves of *L. ocymifolia* var. *raineriana* were collected in September 1989 from beside the Ginfile River (ca. 2450 m), Addis Ababa, Ethiopia. A voucher specimen (SHM-20) was deposited at the National Herbarium of Ethiopia, Addis Ababa University.

EXTRACTION AND ISOLATION.—Dried leaves of *L. ocymifolia* var. *raineriana* (500 g) were placed in a glass percolator and continually extracted with EtOH for five days. Removal of the solvent under reduced pressure yielded 10 g of extract residue, which was subsequently fractionated by vlc over Si gel eluting with solvents of increasing polarity, i.e., hexane and then hexane containing increasing amounts of EtOAc. The hexane-EtOAc (4:1) fractions were bulked and subjected to separation over a short Sephadex LH-20 column (solvent, $CHCl_3$-MeOH, 1:1). The chlorophyll-free column eluate was subjected to prep. tlc [hexane-$CHCl_3$-EtOAc (2:3:2)] to give 4 mg of **4** and 550 mg of **2**. The hexane-EtOAc (7:3) vlc eluate, after removal of chlorophyll (Sephadex LH-20 column, $CHCl_3$-MeOH, 1:1), gave a fraction containing one major compound. This compound was purified by prep. tlc (Si gel, hexane-$CHCl_3$-EtOAc, 1:2:3) to give **1** (30 mg). Similar treatment of the hexane-EtOAc (1:1) vlc eluent gave **3** (9 mg).

(13S)-20-Acetoxy-9α,13α-epoxylabda-6β(19)-16(15)-diol dilactone (leonitin) [**1**].—

Needles from hexane; mp 240°, $[\alpha]_D$ −9° (c=0.5, $CHCl_3$); cd $\Delta\epsilon_{235}$ max +7.1, MeOH; ir ν max (KBr) 2920, 1780, 1770, 1730, 1390, 1365, 1240, 1235, 1200, 1170, 1030 cm^{-1}; ^1H nmr δ 4.67 (1H, dd, J=6.4 and 4.4 Hz, H-6), 4.33 (1H, dd, J=12.5 and 2.0 Hz, H-20α), 4.22 and 4.10 (2H, ABq, J=8.9 Hz, H_2-16), 4.19 (1H, dd, J=12.5 Hz, H-20β), 2.90 and 2.36 (2H, ABq, J=17.3 Hz, H_2-14), 2.36 (1H, d, J=4.4 Hz, H-5), 2.23 and 1.82 (2H, m, H_2-11), 2.17 (1H, m, H-7_{ax}), 2.15 (2H, m, H_2-12), 2.08 (1H, m, H-8), 2.04 (3H, s, Me-22), 1.65 (1H, m, H-7_{eq}), 1.28 (3H, s, Me-18), 1.15 (1H, br dd, H-1_{ax}), 0.89 (3H, d, J=6.4 Hz, Me-17); ^{13}C nmr, see Table 1; eims m/z $[M]^+$ 406.2003, calcd for $C_{22}H_{30}O_7$ 406.1992 (22), 347 (4), 247 (16), 183 (50), 182 (22), 181 (100), 168 (16), 137 (13), 135 (15), 109 (10), 107 (144), 97 (14), 93 (10), 91 (12), 69 (16).

(13S)-9α,13α-Epoxylabda-6β(19),16(15)-diol dilactone (compound X) [**2**].—Needles from hexane, mp 236°, $[\alpha]_D$ −27° (c=0.1, $CHCl_3$); cd $\Delta\epsilon_{233}$ max +16.9, MeOH; ir ν max (KBr) 2970, 1780, 1760, 1470, 1200, 1170, 1090, 1070, 1030 cm^{-1}; ^1H nmr δ 4.68 (1H, t, J=4.8 Hz, H-6), 4.24 and 4.12 (2H, ABq, J=8.9 Hz, H_2-16), 2.96 and 2.56 (2H, ABq, J=17.2 Hz, H_2-14), 2.07 (1H, d, J=4.5 Hz, H-5), 1.28 (3H, s, Me-18), 1.02 (3H, s, Me-20), 0.87 (3H, d, J=6.3 Hz, Me-17); ^{13}C nmr, see Table 1; eims m/z $[M]^+$ 348.1964, calcd for $C_{20}H_{28}O_5$ 348.1937 (19), 182 (12), 181 (100), 168 (15), 167 (12), 139 (16), 109 (19), 69 (9).

6β-Acetoxy-9α,13α-epoxylabda-20(19)-16(15)-diol dilactone [**3**].—Needles from hexane; mp 240°; $[\alpha]_D$ +8° (c=0.1, $CHCl_3$); ir ν max (KBr) 2920, 1780, 1730, 1715, 1460, 1370, 1240, 1160, 1140, 1020 cm^{-1}; ^1H nmr δ 5.16 (1H, q, J=3.0 Hz, H-6), 4.58 (1H, dd, J=11.5 and 2.8 Hz, H-20a), 4.39 and 4.23 (2H, ABq, J=9.2 Hz, H_2-16), 4.15 (1H, d, J=11.5 Hz, H-20b), 2.92 and 2.51 (2H, ABq, J=17.0 Hz, H_2-14), 2.00 (3H, s, Me-22), 1.14 (3H, s, Me-18), 0.87 (3H, d, J=6.6 Hz, Me-17); ^{13}C nmr, see Table 1; eims $[M]^+$ not found but $[M-OCOCH_3]^+$ occurred at m/z 346.1770, calcd for $C_{20}H_{26}O_5$ 346.1780 (100), 318 (6), 302 (14), 194 (29), 182 (5), 181 (27), 16 (7), 161 (5), 133 (5), 119 (55), 109 (7), 107 (5), 105 (6), 91 (8), 79 (6).

20-Acetoxy-9α,13ξ-dihydroxy-15(16)-epoxylabd-14-en-6β(19)-lactone [**4**].—Gum, $[\alpha]_D$ +1° (c=0.1, $CHCl_3$); uv λ max (EtOH) 274 (log ε 5.09) nm; ir ν max (KBr) 3450 br, 2910, 1770, 1740, 1600, 1460, 1220, 1030 cm^{-1}; ^1H nmr δ 6.47 (1H, d, J=2.7 Hz, H-15), 5.16 (1H, d, J=2.7 Hz, H-14), 4.67 (1H, dd, J=7 and 4.9 Hz, H-6), 4.43 and 4.08 (2H, ABq, J=10.4 Hz, H_2-16), 4.35 (1H, dd, J=12.3 and 2.0 Hz, H-20a), 4.22 (1H, d, J=12.3 Hz, H-20b), 2.43 (1H, d, J=4 Hz, H-5), 2.04 (3H, s, Me-22), 1.22 (3H, s, Me-18), 0.87 (3H, d, J=6.2 Hz, Me-17); eims m/z $[M]^+$ 408.2141, calcd for $C_{22}H_{32}O_7$, 408.2148 (8), 390 $[M-H_2O]^+$ (10), 350 (14), 331 (26), 330 (98), 217 (10), 183 (100), 181 (18), 165 (14), 163 (26), 161 (14), 159 (9), 153 (14), 152 (11), 139 (11), 137 (18), 133 (16), 125 (18), 108 (50), 95 (29), 93 (23), 91 (20), 81 (35), 79 (19), 69 (15), 67 (17).

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