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DITERPENES AND A FLAVONE FROM *LEUCAS NEUFLISEANA*

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Key Word Index—*Leucas neufliseana*; Lamiaceae; diterpenoid; labdane derivative; acylated flavone.

Abstract—Three labdane-type diterpenes; 3-oxo-marrubiin and a mixture of two related C-15 epimeric diterpenes based on 9α,13α,15,16-bisepoxy-15-hydroxy-3-oxo-labdan-6β,19-olide, in addition to the acylated flavone apigenin 7-*O*-[6″-*O*-(*p*-hydroxy-*trans*-cinnamoyl)glucoside] were isolated and identified from the aerial parts of *Leucas neufliseana*. Their structures were established through physical and chemical methods in addition to standard spectroscopic techniques. This is the first report of the isolation of the two epimeric diterpenes from a natural source.

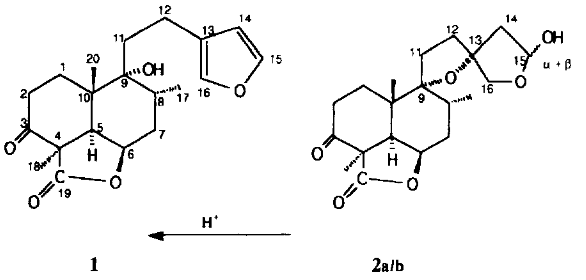
INTRODUCTION

Some species of *Leucas* have been demonstrated to show interesting biological activities [1–3], although very few species have been chemically investigated. However, an isopimarane derivative [4], a diterpene fatty acid ester [5] and several triterpenoids [6–8] have been characterized. *Leucas neufliseana* Courb. (Lamiaceae) is a rare annual herb that grows wild in southeast Egypt [9]. The present investigation represents the first chemical study on this species. A known labdane diterpene, peregrinone **1** a mixture of two new epimeric diterpenes (**2**$_a$, **2**$_b$) and a known acylated flavone (**3**) were isolated from the aerial parts.

RESULTS AND DISCUSSION

Column chromatography of the ether fraction of ethanolic extract on silica gel and gradient elution with $CHCl_3$–MeOH afforded three substances designated **1**, **2** and **3**. Compound **1** was identified as 3-oxomarrubiin (peregrinone) by matching the reported mp, IR, EI-mass spectrum, ^1H NMR [10], ^{13}C NMR [11] (Table 1). This was further substantiated by the 2D NMR experiments as well as the FAB-mass spectrum which revealed a quasimolecular ion $[M + 1]^+$ at *m*/*z* 347 suggesting a molecular formula of $C_{20}H_{26}O_5$. This is the first time **1** has been isolated from the genus *Leucas*. Previous isolation of **1** from members of *Marrubium* [12, 13] and *Ballota* [11] may indicate the close taxonomic relationship of these genera and *Leucas*.

The LC-mass spectrum of **2** revealed a quasimolecular ion at *m*/*z* 382 $[M + NH_4]^+$ indicating a molecular weight 364 corresponding to a molecular formula of $C_{20}H_{28}O_6$. However, the ^{13}C NMR data revealed 20 pairs of signals. For each pair, one signal is more intense than the other. Therefore, **2** is formed of a mixture of two closely related diterpenes; a major **2**$_a$ and a minor **2**$_b$. These could not be resolved using TLC or HPLC. The IR spectrum of **2** revealed strong absorption bands at (cm^{-1}): 3500 (OH stretching vibration), 1780 (a γ-lactone), 1710 (saturated ketone). These data are similar to those of **1** except for the absence of the band at 860 cm^{-1} characteristic for the furan ring. This was further confirmed by the total absence of any signals in the range δ 6.28–7.37 or 110–143 corresponding to those of the furan ring protons and carbons, respectively [14]. ^{13}C NMR signals of rings 1 and 2, and the lactone of either **2**$_a$ or **2**$_b$ (Table 1) were closely similar to those of **1** except for C-9. The molecular formula gave a DBE value of 7 of which those of rings 1 and 2 and the lactone counted for 5 while the rest could adequately be represented by the two spiro-tetrahydrofuran rings. The ^1H NMR (see Experimental) and ^{13}C NMR (Table 1) signals assigned to this part of the molecule are nearly the same as those published for the same moiety in analogous compounds with a hemiacetal hydroxyl at C-15 [15]. The significant differences in the ^1H NMR data of the two isomers could be explained, based on the difference in the configuration at C-15. The multiplet at δ 5.46 and the triplet at δ 5.63 (J = 5.5 Hz) were assigned to the H-15β(equatorial) proton of **2**$_a$ and H-15α(axial) proton of **2**$_b$, respectively,



Scheme 1.

Table 1. ^{13}C NMR data of **1**, 3-oxomarrubiin, **2**$_a$ and **2**$_b$

C	1	3-Oxomarrubiin*	2$_a$	2$_b$
1	34.6	34.6	34.7	37.6
2	34.0	34.1	34.1	34.2
3	206.8	207.1	206.4	205.9
4	53.1	53.2	53.51	53.52
5	46.6	46.5	47.9	48.1
6	74.8	75.1	74.6	74.9
7	31.0	31.0	31.1	31.1
8	31.5	31.5	31.4	31.5
9	75.2	75.1	90.4	90.7
10	40.0	40.0	39.4	39.2
11	28.7	28.7	29.35	29.36
12	20.9	20.1	29.3	29.2
13	124.7	124.8	89.3	90.2
14	110.5	110.6	47.4	46.1
15	143.2	143.1	99.3	99.1
16	138.6	138.5	77.6	77.6
17	15.9	16.0	17.01	16.9
18	18.0	18.1	19.0	19.0
19	174.7	174.8	174.3	174.5
20	20.5	20.4	20.4	20.5

*As reported in reference [11].

indicating an α(axial)-OH at C-15 of **2**$_a$ and a β(equatorial)-OH at C-15 of **2**$_b$. Furthermore, the *gem* protons on C-16 were affected in chemical shift and multiplicity by the orientation of the C-15 hydroxyl group of the isomers. In **2**$_a$, the H-16 axial proton at δ 4.23 (d, $J = 11$ Hz) was deshielded by the additive 1,3-diaxial effects of the C-15 hydroxyl and the inner tetrahydrofuran ring oxygen, while the H-16 equatorial proton appeared at δ 3.75 (d, $J = 11$ Hz). On the other hand, the singlet at δ 4.0 (2H) was simultaneously assigned to both H-16 α and H-16 β of **2**$_b$. The latter *gem* protons possessed the same chemical shift as they are equally affected by the α-oxygen atom of the inner tetrahydrofuran ring and C-15β hydroxyl group.

It was concluded that **2**$_a$ and **2**$_b$ are present as a C-15 epimeric mixture of 9α,13α,15,16-bisepoxy-15-hydroxy-3-oxo-labdan-6β,19-olide. To the best of the authors' knowledge, this is the first report indicating the isolation of either epimer from a natural source. It is noteworthy that related compounds are present as epimeric mixtures and were isolated and identified as such [15, 16]. Final confirmation of the proposed structure of **2** was achieved by its transformation to **1** on treatment with a trace of acid in chloroform [15] (Scheme 1).

Compound **3** was proved to be apigenin 7-*O*-[6″-*O*-(*p*-hyroxy-*trans*-cinnamoyl)-β-D-glucoside] on the basis of its physical and chemical characters, acid and alkaline hydrolysis [17], in addition to spectral data as compared with reported literature values [18, 19]. Compound **3** and related glucosylated acylflavones are of common occurrence in the Lamiaceae [20, 21].

EXPERIMENTAL

Instruments. Mps were uncorr. FAB-MS were recorded on AEI-MS 50 in NOBA as matrix and xenon as bombardment gas. EI-MS were obtained at 70 eV. A Vestec 201 Thermospray MS system was used for LC-MS. NMR was performed at 300 MHz for ^1H and 75 MHz for ^{13}C.

Plant material. The plant material was collected from the Gebel Elba region in southeast Egypt in January 1988 and verified by Dr. N. El-Husseiny, Faculty of Science, Cairo University. A voucher specimen is deposited at the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University.

Extraction and isolation. Dried powdered aerial parts of *L. neufliseana* Courb. (1.8 kg) were exhaustively percolated with EtOH. The extract was concentrated *in vacuo* and defatted with petrol (60–80°). The defatted aq. phase was then extracted with Et_2O. The dried residue (28 g.) was chromatographed on a silica gel column (600 g.) with gradient elution using *n*-hexane, $CHCl_3$ and $CHCl_3$–MeOH mixt. The eluent was collected in 100 ml frs and each fraction monitored by TLC using different $CHCl_3$–MeOH mixtures and vanillin/H_2SO_4 as a spray reagent. Similar frs were pooled, concentrated to small vol. *in vacuo* and left to crystallize. Compound **1** was eluted with 0.5% MeOH in $CHCl_3$, **2** with 5% MeOH in $CHCl_3$ and **3** with 12% MeOH in $CHCl_3$. Compounds **1**–**3** were tested for purity by TLC using silica gel GF_{254} precoated plates and the respective solvent used for elution in CC. Each compound was separately purified on a silica gel column using gradient mixtures of $CHCl_3$–MeOH for elution of **1** and **2**, and of EtOAc–MeOH for **3** to afford 135, 40 and 17 mg of pure **1**, **2** and **3**, respectively.

Transformation of **2** *to* **1**. Compound **2a**/**b** (2 mg) was dissolved in $CHCl_3$ (2 ml) containing a trace of HCl. Complete transformation was reached within 2 hr. The reaction was monitored by TLC on precoated Si gel plates GF_{254} using 0.5% MeOH in $CHCl_3$ for development and vanillin–H_2SO_4 as a spray reagent. The R_f values were 0.09 and 0.67 for **2a**/**b** and **1**, respectively.

Alkaline hydrolysis of **3**. This was carried out with 2 M NaOH *in vacuo* for 2 hr at room temp and worked up in the usual way to give the deacylated product and the liberated acylating acid. The deacylated product dissolved in MeOH was purified by prep TLC on cellulose with 30% HOAc as solvent. The latter was identified as *p*-coumaric acid by TLC on silica gel GF_{254} compared with an authentic marker in $CHCl_3$–MeOH (9:1) (R_f 0.66) and EtOAc–MeOH/H_2O (100:16.5:13.5) (R_f 0.52) as solvents and visualization with $FeCl_3$ as an orange brown spot.

Acid hydrolysis of deacylated **3**. This gave apigenin and glucose by standard procedures.

The structure of **3** was confirmed by EI-MS, IR, UV, and ^1H NMR spectral analysis.

3-Oxomarrubiin (*peregrinone*) (**1**). White plate-like crystals; mp 179–182°; IR ν^{KBr} cm^{-1}: 3500, 3150, 1770, 1700, 1610, 1460, 1410, 1180, 990, 860, 790; UVλ_{max}

(MeOH): 212, 270 nm; EI-MS *m/z* (rel. int.): 346 $[M]^+$ (30), $C_{20}H_{26}O_5$, 328 $[M - H_2O]^+$ (7), 265 (24), 205 (23), 177 (17), 149 (24), 123 (58), 109 (25), 95 (88), 81 (100), 67 (36), 41 (67); FAB-MS: $[M + 1]^+ = 347$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ2.80 (1H, *d*, $J = 4.8$ Hz, H-5), 4.63 (1H, *dd*, $J = 4.8, 5.2$ Hz, H-6), 6.28 (1H, *dd*, $J = 1.8, 0.8$ Hz, H-14), 7.26 (1H, *t*, $J = 1.8$ Hz, H-15), 7.37 (1H, *dd*, $J = 1.8, 0.8$ Hz, H-16), 1.02 (3H, *d*, $J = 6.3$ Hz, H-17), 1.47 (3H, *s*, H-19), 0.94 (3H, *s*, H-20); ^{13}C NMR: Table 1.

9α,13α,15,16-*Bisepoxy*-15-*hydroxy*-3-*oxo*-*labdan*-6β, 19-*olide* ($\mathbf{2_a}$, $\mathbf{2_b}$). White amorphous powder, mp 190–194°; LC-MS, $[M + NH_4]^+ = 382$, $C_{20}H_{28}O_6$; IR ν^{KBr} cm^{-1}: 3500, 2990, 1780, 1710, 1490, 1080, 1010; ^{1}H NMR (300 MHz, $CDCl_3$): δ4.59 (1H, *dd*, $J = 4.8, 5.2$ Hz, H-6 in $\mathbf{2_a}$ or $\mathbf{2_b}$), 5.46 (1H, *m*, H-15 in $\mathbf{2_a}$), 5.63 (1H, *t*, $J = 5.5$ Hz, H-15 in $\mathbf{2_b}$), 3.75 (1H, *d*, $J = 11$ Hz, βH-16 in $\mathbf{2_a}$), 4.23 (1H, *d*, $J = 11$, αH-16 in $\mathbf{2_a}$), 4.0 (2H, *s*, α, β H-16 in $\mathbf{2_b}$), 0.95 (3H, *d*, $J = 6.3$ Hz, H-17 in $\mathbf{2_a}$ or $\mathbf{2_b}$), 1.47 (3H, *s*, H-19 in $\mathbf{2_a}$ or $\mathbf{2_b}$), 0.92 (3H, *s*, H-20 in $\mathbf{2_a}$ or $\mathbf{2_b}$); ^{13}C NMR: Table 1.

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REFERENCES

1. Khaleque, A., Huq, M. E., Huq, M. S. and Mansour, M. H. (1970) *Sci. Res.* **7**, 125.
2. Thakur, D. K., Misra, S. K. and Choudhuri, P. C. (1987) *Indian J. Anim. Health* **26**, 31.
3. Reddy, M. K., Viswanathan, S., Thirugnanasambantham, P. and Kameswaran, L. (1993) *Fitoterapia* **64**, 151.
4. Mahato, S. B. and Pal, B. C. (1986) *Phytochemistry* **25**, 909.
5. Hasan, M., Burdi, D. K. and Ahmed, V. U. (1991) *J. Nat. Prod.* **54**, 1444.
6. Dinda, B. and Jana, U. K. (1987) *J. Indian. Chem. Soc.* **64**, 582.
7. Pradhan, B. P., Chakraborty, D. K. and Subba, G. C. (1990) *Phytochemistry* **29**, 1693.
8. Mashooda, H., Dadu Khan, B. and Ahmed, V. U. (1991) *Phytochemistry* **30**, 4181.
9. Tackholm, V. (1974) *Students' Flora of Egypt*, 2nd Edn. Cairo University, Cooperative Printing Company, Beirut.
10. Canonica, L., Rindone, B., Scolastico, C., Ferrari, G. and Casagrande, C. (1968) *Tetrahedron Letters* **27**, 3149.
11. Savona, G. and Piozzi, F. (1976) *J. Chem. Soc. Perkin Trans. I* 1607.
12. *Fulke, J. W. B., Henderson, M. S. and McCrindle, R.* (1968) *J. Chem. Soc.* (**C**), 807.
13. Amer, M. (1993) *Mansoura J. Pharm. Sci.* **9**, 92.
14. Pretsch, E., Seibl, J., Simon, W. and Clerc, T. (1983) *Spectral Data For Structure Determination of Organic Compounds*, Springer, Berlin.
15. Malakov, P., Papanov, G., Jakupovic, J., Grenz, M. and Bohlmann, F. (1985) *Phytochemistry* **24**, 2341.
16. Henderson, M. S. and McCrindle, R. (1969) *J. Chem. Soc.* (**C**), 2015.
17. Markham, K. R. (1982) *Techniques of Flavonoid Identification*, Academic Press, London.
18. Chari, V. M., Jordan, M., Wagner, H. and Thies, P. W. (1977) *Phytochemistry* **16**, 1110.
19. Itokawa, H. Suto, K. and Takeya, K. (1981) *Chem. Pharm. Bull.* **29**, 254.
20. Harborne, J. B., Mabry, T. J. and Mabry, H. (1975) *The Flavonoids* Chapman and Hall, London.
21. Harborne, J. B. and Williams, C. A. (1988) Flavone and Flavonol Glycosides in *The Flavonoids* (1988) (Harborne, J. B., ed.), p. 312. Chapman and Hall, London.