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MEDICINAL PLANTS OF TUNISIA. THE STRUCTURE OF PERIPLOCADIOL, A NEW ELEMANE-TYPE SESQUITERPENE ISOLATED FROM THE ROOTS OF *PERIPLOCA LAEVIGATA*

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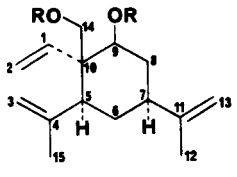
ABSTRACT.—A new elemane-type sesquiterpene has been isolated from the roots of *Periploca laevigata* and its structure elucidated by chemical and spectroscopic methods, principally ^{1}H and ^{13}C nmr.

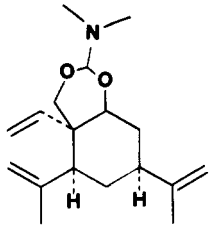
Roots of *Periploca laevigata* Ait. (Asclepiadaceae), a species widespread in the Mediterranean Sahara, have been indicated as a traditional plant medicine useful in the treatment of diabetes in Tunisia (1–3). A range of cardiotonic glycosides have been isolated from *Periploca sepium* (4), *Periploca graeca* (5), and *Periploca nigrescens* (6). Previous work on *P. laevigata* has shown the stems to yield tetracyclic and pentacyclic triterpenes and a sterol (7) and, in 1982, some of us published a preliminary account of the structure of a new sesquiterpene (8). The present work describes the confirmation of the latter and, for the first time, its stereochemistry.

RESULTS AND DISCUSSION

Dried and ground roots of *P. laevigata* were continuously extracted successively with hexane, Me_2CO, MeOH, and H_2O. Repeated purification by chromatography of the Me_2CO extract allowed the isolation of α- and β-amyrin, lupeol, and β-sitosterol [previously isolated from the stems (7)], as well as the acetate of β-amyrin. These known substances were identical in every respect with authentic samples. In addition, a colorless, odorless viscous liquid (named periplocadiol [**1**]) was isolated, the ir spectrum of which showed broad, intense absorption at 3400 cm^{-1} (-OH) and a strong, narrow band at 1640 cm^{-1} (C=C). The ms gave a molecular ion peak at *m/z* 236 with



1 R=H
2 R=Ac



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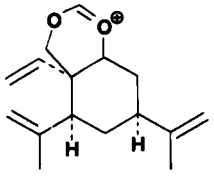

4 (*m/z* 247)

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TABLE 1. Nmr Signals for Periplocadiol [**1**] (δ, $CDCl_3$).[a]

Position	^1H (*J* in Hz)	^{13}C
C-1	5.78, dd (18.0, 11.0)	143.2
C-2	5.42, dd (18.0, 1.5)	113.7
	5.35, dd (11.0, 1.5)	
C-3	4.62, 2H, s	109.3
C-4	—	148.7[b]
C-5	1.98, dd (12.0, 3.0)	51.5
C-6	1.56, 2H, br dd (12.0, 3.0)	32.5
C-7	2.10, dd (12.5, 3.8)	43.3
C-8	α: 1.77, ddd (12.5, 3.5, 3.8)	35.2
	β: 2.02, ddd (12.0, 12.5, 12.0)	
C-9	3.79, dd (12.0, 3.5)	77.9
C-10	—	48.0
C-11	—	145.7[b]
C-12	1.77, s	25.3
C-13	4.88, 2H, br s	115.9
C-14	4.11, d (11.5)	62.5
C-15	1.72, s	21.0
OH	3.12, br	—

[a]Some of the reported locations and coupling constants were only visible after the use of a shift reagent, $Eu(fod)_3$.

[b]These signals may be reversed.

intense peaks at *m/z* 218 and 203. Acetylation yielded a single product **2**, showing a molecular ion peak in the ms at *m/z* 320, corresponding with a diacetate.

The ^{13}C-nmr spectrum of **1** gave signals for 15 carbons, which leads to a molecular formula of $C_{15}H_{24}O_2$. The off-resonance ^{13}C-nmr spectral data are shown in Table 1 and indicate the presence of two primary carbons (δ 25.3 and 21.0), two secondary carbons (type CH_2, δ 35.2 and 32.5), one CH_2 carbon resonating at the low field of 62.5 ppm (probably of the type $-CH_2OH$), three vinylic carbons of the type $=CH_2$ (δ 109.3, 113.8, and 115.9), four tertiary carbons resonating at δ 143.2 (characteristic of a trisubstituted vinyl carbon), δ 77.9 (likely to be for a carbon bearing a secondary hydroxyl group), δ 51.5, and 43.3, and finally for three quarternary carbons at δ 148.7, 145.7 (as would be expected for fully substituted olefinic carbons), and δ 48.0.

These data are in accord with a hydroxyl-substituted cyclohexane further substituted with two 2-propylene residues and one ethene. Biogenetically, the most reasonable is an elemane-type skeleton, as in **1**, the only problems remaining being the location of the two hydroxyl groups and the stereochemistry. Further insight into the former problem was gained by reaction of **1** with the dimethylacetal of *N,N*-dimethylformamide in CH_2Cl_2, which yielded a single product **3**, the ms of which showed a molecular ion peak at *m/z* 291 and a characteristic fragment at *m/z* 247 $[M-44]^+$, attributable to an ion of type **4**. The formation of the derivative **3** clearly shows that the hydroxyl groups are likely to be disposed 1,3 with respect to each other, and this, coupled with the ^{13}C- and ^1H-nmr data (Table 1), allows only one formula, as shown in **1**. Moreover, from an examination of Dreiding models, it is clear that only certain configurations are possible.

Assignment of the ^1H and ^{13}C spectra of **1** was straightforward except for certain signals, but the placement of the latter was facilitated by a detailed consideration of $^1H/^{13}C$ correlated data. Thus, for example, it was possible to distinguish clearly between the two methylene carbon signals at δ 32.5 and 35.2 because the latter was correlated with hydrogen signals δ 1.77 and 2.02; these had been unequivocally assigned to the hydrogens at C-8 because they were coupled with the low field signal for a methine hy-

drogen due to H-9. The carbon at δ 32.5 could therefore be assigned with certainty to C-6. Further, the large H-9/H-8 coupling (12 Hz) showed that the hydroxyl at C-9 was equatorial. Similarly, the observation of a large (12.5 Hz) coupling between H-8 and H-7 showed that the substituent at C-7 was equatorial. The sole problems remaining were the stereochemistry at C-5 and C-10, solutions to which were provided by nmr studies.

Convincing evidence about the stereochemistry at C-5 was obtained by the use of a shift reagent. Progressive addition of $Eu(fod)_3$ to **2** caused shifts in the nmr signals totally in accordance with the proposed assignments. One signal, however, entirely obscured by resonances of H-8α and the H-12s in the unshifted spectrum, gradually appeared at lower field. At a molar ratio of $Eu(fod)_3$: **2** of 0.448, this signal showed at δ 2.73. Extrapolation to a molar ratio of 0 showed that this signal originated at about δ 1.54, very close to the value for the C-6 protons found by INDOR experiments and 2D heteronuclear spectroscopy. The appearance of this signal is shown in Figure 1, together with calculated spectra for H-6β (axial) in both the 5α- and 5β-isomers using a

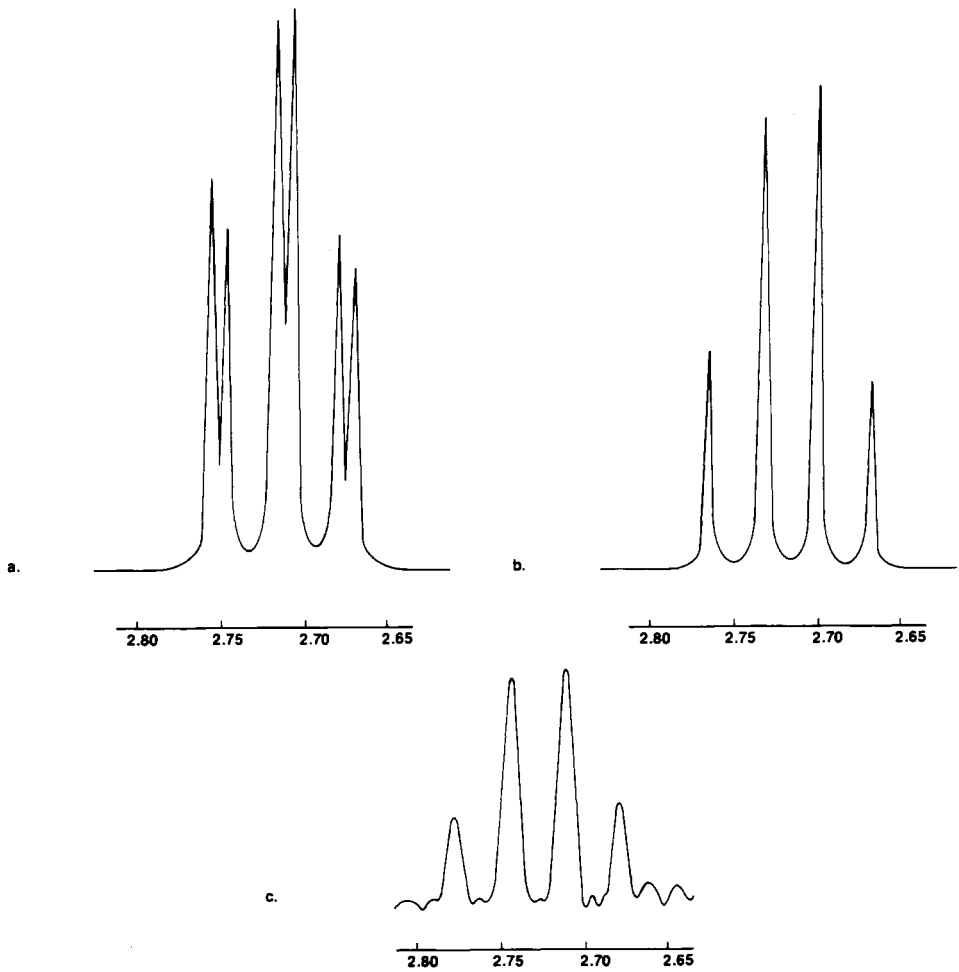
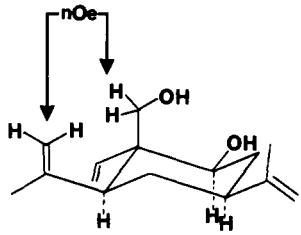


FIGURE 1. Calculated (PC-SIG 590NMR) and observed nmr absorptions for H-6β in the spectrum ($CDCl_3$) of **2**, δ: (**a**) calculated for H-6 in 5β-isomer, (**b**) calculated for H-6 in 5α-isomer, (**c**) observed for signal shifted downfield to δ 2.73 [apparently from δ 1.54 after addition of $Eu(fod)_3$ to a ratio of $Eu(fod)_3$: **2** of 0.448]. Coupling constant assumptions are given in the text.

computer nmr calculation program (PC-SIG 590NMR), the data for true shifts and coupling constants where they were known, and the estimates $J_{5,6\beta} = 12$ Hz for the 5α-isomer and $J_{5,6\beta} = 3$ Hz for the 5β-isomer. As is readily seen from Figure 1, there is complete agreement between the calculated spectrum for the 5α-isomer and the observed shifted spectrum, showing that **2** is the 5α-isomer.

Further assignments were made possible by 2D NOESY spectra. In the 2D spectra, an nOe correlation was observed between the signals due to the C-14 protons and those giving a signal at δ 4.62. These had previously been assigned to the vinyl hydrogens at either C-3 or C-13 (the other set of corresponding signals being at δ 4.88) but now, because they were shown to be in proximity with the C-14 protons, there was no doubt about their assignment, i.e., that they were due to the C-3 protons because C-13 is distant from C-14. (Interpretation of the $^1H/^{13}C$ correlated data also allowed unequivocal assignment of the three vinyl ^{13}C-nmr signals at δ 109.3, 113.7 and 115.9.) Furthermore, as there was an nOe correlation between the C-3 protons and C-14 protons (as shown in **5**), and the isopropylene substituent at C-5 was known to be β, but no nOe correlation between C-2 protons and H-6β or between C-2 protons and H-8β, it is likely that the $-CH_2OH$ function at C-10 is axial, ensuring that the C-2 protons are as distant from H-6β and H-8β as possible. The stereochemistry of periplocadiol is thus best illustrated by **1**.



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Studies on the potential antidiabetic activity of *Periploca laevigata* are underway in collaboration with the Faculté de Pharmacie, Université de Monastir.

EXPERIMENTAL

Ms were taken using an A.E.I. MS902 instrument and nmr spectra on a Bruker WH400. Roots of *P. laevigata* were collected in June 1980, from the mountains in Oueslatia, Kairouan, Tunisia, and a sample is deposited in the Department of Botany, University of Tunis. Extraction was carried out on a pilot scale using successive quantities of hexane, Me_2CO, MeOH, and H_2O. Dried, powdered roots (1 kg) yielded 35 g of Me_2CO extract.

Si gel cc [elution with hexane-Et_2O (1:1) followed by Et_2O-MeOH (1:1)] allowed separation of the crude extract into nonpolar and polar fractions, each of 15 g. The nonpolar fraction was rechromatographed on Si gel (elution with hexane and mixtures of hexane/Et_2O of increasing polarity) to provide eight fractions. The fraction eluted with hexane-Et_2O (1:4) weighed 2.77 g and provided compound **1**, periplocadiol, as an odorless, colorless liquid, $[\alpha]_D -19°$ ($c = 12.4$, $CHCl_3$); ir ν (film) 3400 (broad), 1640 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 236 (9), 218 (10), 203 (8), 105 (100); ^1H and ^{13}C nmr see Table 1.

ACETYLATION OF PERIPLOCADIOL.—Compound **1** (10 mg) was dissolved in the minimum of pyridine, and Ac_2O (1 ml) was added. After 12 h, the reaction was stopped by the addition of H_2O, and the mixture was extracted into Et_2O. Purification by cc (elution with CH_2Cl_2) gave **2** as a colorless, odorless liquid: η^{20} 1.5169, $[\alpha]_D -28°$ ($c = 12.1$, $CHCl_3$), ir ν (film) 2980–3000, 1740, 1640 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 320 (4), 260 (4), 218 (12), 203 (4), 200 (100), 105 (100).

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