Pergamon

PII: S0031-9422(97)00047-2

Phytochemistry, Vol. 45, No. 4, pp. 797–800, 1997

0031-9422/97 $17.00+0.00

9-*EPI*-LABDANE DITERPENOIDS FROM *NOLANA ROSTRATA* VAR. *ROSTRATA*

MARÍA CRISTINA CHAMY, JUAN A. GARBARINO*, MARISA PIOVANO, JOSÉ L. LÓPEZ-PÉREZ†, MARCELLO NICOLETTI‡, ROSSANA GANDOLFO and ARTURO SAN FELICIANO†

Departamento de Química, Universidad Técnica Federico Santa María, Casilla 110-V, Valparaíso, Chile; † Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain; ‡ Dipartimento di Biologia Vegetale, Università La Sapienza, Rome, Italy

(*Received* 30 *October* 1996)

Key Word Index—*Nolana rostrata* var. *rostrata*; Nolanaceae; diterpenes; 9-*epi*-labdane derivatives.

Abstract—Two new 9-*epi*-labdane diterpenes 2α,3α,9β-trihydroxy-9-*epi*-labd-13(*E*)-ene-15-oic acid and 2α,3α,9β-trihydroxy-9-*epi*-labd-13(*Z*)-ene-15-oic acid and the known flavonoid 5,3′,4′-trihydroxy-3,7-dimetoxy-quercetin were isolated from the aerial parts of *Nolana rostrata* var. *rostrata*.

INTRODUCTION

The genus *Nolana* has been reported to produce sesquiterpenes [1] and a series of highly oxygenated labdane diterpenoids [2, 3]. *Nolana rostrata* var. *rostrata*, a taxon of this genus, belonging to the Alona section, which is characterized for having resinous specimens [4], is an endemic shrub from the semidesertic zone of Chile. This paper describes the isolation and structural elucidation of two new 9-*epi*-labdane derivatives **1** and **2** from this species. Elucidation of the structures of these compounds was achieved by one and two dimensional NMR, with particular emphasis on heteronuclear multiple quantum coherence (HMQC) [5] and heteronuclear multiple bond coherence (HMBC) [6] spectra.

RESULTS AND DISCUSSION

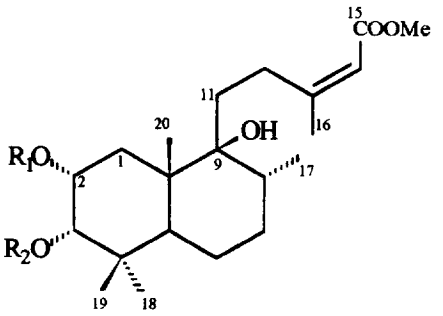
The aerial parts of *Nolana rostrata* var. *rostrata* were extracted successively with petrol and methylene chloride. After work up of the extracts by CC on silica gel, using increasing proportions of ethyl acetate in petrol as eluent, 2α,3α,9β-trihydroxy-9-*epi*-labd-13(*E*)-ene-15-oic acid (**1**) and 2α,3α,9β-trihydroxy-9-*epi*-labd-13(*Z*)-ene-15 oic acid (**2**) were obtained.

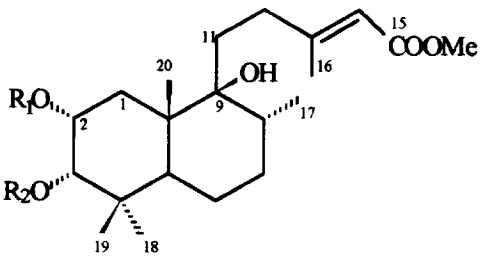
Compound **1** was studied as the methyl ester derivative **1a** obtained after treatment of **1** with ethereal diazomethane, and its acetyl derivative **1b** after treatment of **1a** with acetic anhydride–pyridine. Compound **1a** showed IR absorptions for an ester carbonyl at 1710 cm^{-1}, hydroxyl absorption at 3300–3500 cm^{-1} and a double bond absorption at 1650 cm^{-1}. Its ^1H NMR spectrum showed signals for a vinylic proton at δ 5.68 (1H, *bs*, J = 1Hz, H-14), a vinylic methyl group at δ 2.19; two methines at δ 4.03 (1H, *ddd*, J = 3.7, 6.5, 11.3 Hz) and 3.58 (1H, *d*, J = 3.7 Hz), indicative of the existence of two secondary alcohols, three quaternary methyl groups at δ 1.28, 1.09 and 0.72 and one tertiary methyl group at δ 0.79 (J = 5.7 Hz).

Comparison of the ^1H NMR spectra of **1b** and **1a** showed that one of the signals due to a hydrogen atom geminal to one of the OH groups was shifted from δ 4.03 to 5.12, while the other remained practically unshifted. This could be indicative of the *vicinal* nature of both hydroxyl groups and was confirmed by a COSY experiment, which clearly showed the coupling between the two afore mentioned protons [7].

The molecular formula $C_{23}H_{38}O_6$ was deduced for **1b** from a combined evaluation of the ^1H and ^{13}C NMR spectra, since in its mass spectrum, the highest peak was observed at m/z 378 [M^+ – MeOH]. The loss of MeOH is typical for the presence of an unsaturated methyl ester [8]. The ^{13}C NMR spectrum also provided cogent evidence for the α,β-unsaturated ester, through the presence of signals for the carboxyl group at δ 167.3, a non protonated sp^2 carbon at δ 161.6 a sp^2 CH group at δ 115.1 and for a methoxyl group at δ 50.7. Furthermore, a vinylic methyl group was evidenced by a signal in the ^1H NMR at δ 2.16 (δ_C 19.1) which was long-range coupled (J = 1.6 Hz) to a broad singlet for a vinylic proton at δ 5.63, both charac-

* Author to whom correspondence should be addressed.





	R_1	R_2
1a	H	H
1b	Ac	H
1c	Ac	Ac

	R_1	R_2
2a	H	H
2b	Ac	H

teristic of an unsaturated side chain of a bicyclic diterpenoid with the *E*-configuration for the olefinic bond [9, 10]. The peak at *m/z* 223 [$M^+ - C_7H_{11}O_2 - CH_3$ COOH] in its mass spectrum, confirmed this feature. An ion at *m/z* 318 [M − MeOH − AcOH] along with signals in the NMR spectra at δ_H 2.07 (3H, *s*) and δ_C 170.0 and 21.2 confirmed the presence of only one acetate group. The remaining 17 signals in the ^{13}C NMR spectrum corresponded to one OH and one OAc bearing methines at δ 76.8 and 73.6, respectively, a non-protonated oxygen bearing carbon, in the zone of the $CDCl_3$ signals, two quaternary carbons at δ 40.9 and 38.9, two methines at δ 38.8 and 36.1, five methylenes at δ 37.0, 34.7, 32.0, 26.5, 22.0 and five methyls at δ 21.8, 19.1, 18.3, 17.1 and 15.9.

Particularly informative was the high field ^1H NMR spectrum of **1b**, which showed the five methyl signals of a labdane molecule [9–12], as a vinyl methyl singlet δ 2.16, a secondary methyl δ 0.78 (J = 5.7 Hz) and three tertiary methyl groups at δ 0.72, 1.09 and 1.27.

The two-dimensional COSY ^1H NMR spectrum showed that the signal of the vinyl proton at δ 5.63 was coupled with the vinyl methyl signal at δ 2.16 as well as the secondary methyl signal at δ 0.78 (J = 5.7 Hz) which was coupled to a high field methine signal at δ 1.45. Other informative couplings were those between the signals at δ 3.61 (1H, *d*, J = 3.5 Hz) and 5.12 (1H, *ddd*, J = 3.5, 6.8, 11.5 Hz). Furthermore, the signal at δ 5.12 was coupled to those of the methylene group at δ 1.7. These couplings were indicative of a -CH_2-CH(OAc)-CH(OH)-C- arrangement, in which the proton vicinal to the methylene group is axial and the other methine proton is equatorial [13].

Further determination of the structure was performed through the analysis of its HMQC and HMBC spectra, which confirmed the previous findings and permitted us to observe, respectively, all the expected one-bond H—C correlations and a great number of the possible two- and three-bond H—C connectivities of the molecule. These NMR experiments served not only to confirm the molecular constitution of **1b**, as that of a polyfunctionalised labdane derivative, but also to assign all ^1H and ^{13}C signals unequivocally, with the exception of that corresponding to C-9, which should be coincident with the signal of another carbon or with the solvent signals. Attempts to induce a selective shift of that signal by using different solvents were unsuccessful but, finally, a SEFT experiment [14, 15] permitted the location of the absorption for the non-protonated C-9 at the same frequency as for the hydroxylated methine C-3. The assigment of the signals corresponding to positions C-18 and C-19 was based on the positive NOE observed for the methyl signal at δ 1.09 (H-19) on irradiation of the proton signal corresponding to the angular methyl group (H-20) at δ 0.72 and reciprocally.

The configuration of the C-17 methyl group on C-8 would be equatorial as reflected by the coupling constant of the doublet (J = 5.7 Hz), because an axial methyl group should have a larger value [16]. To try to decide the relative configurations at C-8 and C-9, several ^1H NMR spectra in $CDCl_3$ were made with increasing quantities of pyridine-d_6. In the spectrum with pyridine-d_6 (100%), one could observe that the angular methyl group had surpassed the doublet of 17-Me (δ_H Me-20 0.82, δ_H Me-17 0.78). This result could only be interpreted if the OH group at C-9 was *cis*-oriented with respect to the angular methyl group on C-10 [17]. Therefore, compound **1b** must be methyl-2α-acetoxy-3α,9β-dihydroxy-9-*epi*-labd-13(*E*-en-15-oate.

Compound **2** was characterized as its methyl ester derivative **2a** obtained after treatment of **2** with ethereal diazomethane which had the molecular formula $C_{21}H_{36}O_5$. Comparison of the ^1H NMR spectrum of **2a** with that of **1a** showed only minor differences for the skeletal proton signals and differed only in the signals due to the side chain. Clearly **2a** differed from **1a** in the geometry of the $\Delta^{13,14}$-double bond. As it could be deduced from the ^1H NMR spectrum, **2a** was

a labdane with the *Z*-configuration of the side-chain double bond (δ_H 1.97, δ_C 25.30, Me-16) [18, 19]. Compound **2a** was treated with aceticanhydride–pyridine giving the monoacetate **2b**. The ^{13}C NMR spectrum of **2b** confirmed this point; the C-12, C-13, C-14, C-15 and C-16 carbon atom resonances of **2b** were almost identical to those of the corresponding carbon atom reported for labdanes with the *Z*-configuration of the double bond [18, 19]. The other carbon resonances remained almost unshifted compared with those of **1b**, leading to the assignment of the structure of **2b** as methyl-2α-acetoxy-3α,9β-dihydroxy-9-*epi*-labd-13 (*Z*)-en-15-oate.

It should be noted that the relative stereochemistry is assigned as shown in the figure but unfortunately, the absolute configurations of compounds **1** and **2** could not be determined because of the small quantity of material isolated.

EXPERIMENTAL

Mps uncorr; NMR spectra (^1H 200, 360, 400 MHz; ^{13}C at 50, 90 and 100 MHz) were recorded for $CDCl_3$, with TMS as int. standard. IR were measured for $CHCl_3$ sols. EIMS /CG-MS at 70 eV.

Plant material and isolation. Nolana rostrata var. *rostrata* was collected near Vallenar, IV Region, Chile, in September 1993. A voucher specimen was deposited at the herbarium at Universidad Técnica Federico Santa María. The air dried aerial parts (2 kg) of the plant were extracted at room temp with petrol and CH_2Cl_2 successively for 48 hr each, affording 40 g of a syrup. A portion of this syrup (20 g) was chromatographed on a silica gel column (400 g) eluting with mixtures of petrol and EtOAc of increasing polarity. Frs of 100 ml were taken and combined based upon TLC and ^1H NMR monitoring. The combined frs were purified by repeated CC on silica gel or by treatment with ethereal diazomethane and/or Ac_2O in pyridine.

Methyl-2α,3α,9β-trihydroxy-9-epi-*labd*-13(*E*)-*en*-15-*oate* (**1a**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3300, 2950–2850, 1710, 1650, 1450, 1390, 1235, 1150, 1050. ^1H NMR: δ 5.68 (1H, *bs*, J = 1.0 Hz, H-14); 4.03 (1H, *ddd*, J = 3.7, 6.5, 11.3 Hz, H-2); 3.7 (3H, *s*, OMe); 3.62 (1H, *d*, J = 3.7 Hz, H-3); 2.19 (3H, *d*, J = 1 Hz, Me-16); 1.28, 1.09, 0.72 (3H each, *s*, Me-18, Me-19 and Me-20, respectively); 0.79 (3H, *d*, J = 5.7 Hz, Me-17). ^{13}C NMR see Table 1. MS *m/z* (rel. int.): 368 [M^+, $C_{21}H_{36}O_5$]; 350 [$M^+ - H_2O$] (2); 336 [$M^+ - CH_3OH$] (4); 318 [$M^+ - CH_3OH - H_2O$] (5); 275 (8); 241 [$M^+ - C_7H_{11}O_2$] (3); 223 [$350 - C_7H_{11}O_2$] (6); 205 [$241 - 2H_2O$] (9); 191 (11); 167 (32); 137 (23); 123 (52); 114 (33); 107 (25); 95 (62); 83 (40); 81 (42); 79 (23); 69 (45); 67 (37); 55 (51), 43 (100).

Methyl-2α-acetoxy-3α,9β-*dihydroxy*-9-epi-*labd*-13(*E*)-*en*-15-*oate* (**1b**): IR: $\nu_{max}^{CHCl_3}$ cm^{-1}. 3220, 2980–2760, 1680, 1430–1310, 1250, 1160, 1030, 990. ^1H NMR (400 MHz), ($CDCl_3$): δ 5.70 (1H, *bs*, H-14); 5.14 (1H, *ddd*, J = 3.5, 6.8, 11.5 Hz, H-2); 3.66 (3H, *s*, OMe); 3.66 (1H, overlapped signal); 2.16 (3H, *bs*, Me-16); 2.07 (3H, *s*, $OCOCH_3$), 1.27, 1.09, 0.72 (3H each, *s*, Me-18, Me-19 and Me-20, respectively); 0.78 (3H, *d*, J = 5.7 Hz, Me-17). ^{13}C NMR see Table 1. MS *m/z* (rel. int.): 378 [$M^+ - CH_3OH$] (6); 350 (1); 318 [$M^+ - CH_3OH - HCOOCH_3$] (25); 300 (2); 275 (8), 223 (48); 205 (27); 149 (17); 191 (15); 163 (14); 137 (32); 123 (100); 95 (50); 82 (25); 67 (15); 43 (35).

Table 1. ^{13}C NMR spectral data of compounds **1a–2b**; $CDCl_3$ with TMS as internal standard, chemical shifts in δ values (ppm)

C	1a	1b	1c	2a	2b
1	25.8	22.0	22.8	25.8	22.0
2	69.4	73.6	70.8	69.8	73.7
3	78.3	76.8	75.8	78.6	76.8
4	40.7	40.9	41.2	41.0	40.9
5	38.7	38.8	38.8	38.9	38.7
6	32.1	32.0	31.5	32.4	31.9
7	26.5	26.5	26.5	26.8	26.5
8	36.2	36.1	36.0	36.3	36.0
9	77.1*	76.8	76.7	77.3	77.0*
10	38.6	38.9	38.8	39.0	39.0
11	36.6	37.0	36.8	37.0	36.8
12	34.7	34.7	34.5	27.6	27.5
13	161.4	161.6	161.2	161.3	161.8
14	115.0	115.1	115.1	115.7	115.2
15	167.3	167.3	167.3	166.9	166.6
16	19.2	19.1	19.1	25.2	25.3
17	15.9	15.9	15.9	16.1	15.9
18	22.0	21.8	21.3	21.3	21.6
19	17.1	17.1	17.1	17.2	17.1
20	18.4	18.3	18.3	18.3	18.2
OMe	50.8	50.7	50.7	50.8	50.7
$\underline{C}OCH_3$		170.0	170.4		169.8
$CO\underline{C}H_3$		21.1	21.1		21.3

All the signals for compound **1b** were assigned with the aid of HMQC and HMBC spectra.

* Signals overlapped with the signals of $CDCl_3$.

Acetate carbons at δ 170.0, 21.3.

Methyl-2α,3α-diacetoxy-9β-hydroxylabd-13(*E*)-*en*-15-*oate* (**1c**): $[\alpha]_D^{25}$ 0° ($CHCl_3$, *c* 1.2); IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3510–3400, 2980–2930, 1720, 1630, 1450, 1380, 1280–1230, 1150, 1030, 990. ^1H NMR (300 MHz), ($CDCl_3$): δ 5.70 (1H, *bs*, H-14); 5.22 (1H, *ddd*, J = 3.7, 5.4, 11.4 Hz, H-2); 5.08 (1H, *d*, J = 3.7 Hz, H-3); 3.68 (3H, *s*, OMe); 2.18 (3H, *bs*, Me-16); 2.10, 1.98 (3H each, *s*, $OCOCH_3$); 1.15, 1.08, 0.75 (3H each, *s*, Me-18, Me-19 and Me-20, respectively); 0.82 (3H, *d*, J = 5.5 Hz, Me-17). ^{13}C NMR see Table 1. MS *m/z* (rel. int.): 452 [M, $C_{25}H_{40}O_7$]; 378 [$M^+ - 74$] (4); 318 [$378 - CH_3COOH$] (3); 275 [$318 - 43$] (5); 258 [$M^+ - 2CH_3COOH$] (2); 205 [$M^+ - 2CH_3COOH - C_7H_{11}O_2$] (12); 233 (15), 191 (5); 163 (7); 149 (8), 137 (12); 127 (10); 123 (25); 95 (30); 82 (15); 69 (20) 55 (32); 43 (100).

Methyl-2α,3α,9β-trihydroxy-9-epi-*labd*-13(*Z*)-*en*-15-*oate* (**2a**): IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–3300, 2980–2930, 1690, 1650, 1450, 1380, 1280, 1250, 1200, 1150, 1095, 1035, 980. ^1H NMR (400 MHz), ($CDCl_3$): δ 5.64 (1H, *bs*, H-14); 4.15 (1H, *ddd*, J = 3.7, 4.3, 9 Hz, H-2); 3.68

(3H, *s*, OMe); 3.62 (1H, *d*, $J = 3.7$ Hz, H-3); 1.90 (3H, *bs*, Me-16); 1.30, 1.10, 0.74 (3H each, *s*, Me-18, Me-19 and Me-20, respectively); 0.84 (3H, *d*, $J = 5.8$ Hz, Me-17). ^{13}C NMR see Table 1.

Methyl-2α-acetoxy-3α,9β-dihydroxy-9-epi-labd-13(Z)-en-15-oate (**2b**): $[\alpha]_D^{25} + 27.88$ ($CHCl_3$, *c* 1.2); IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–3100, 2920–2840, 1720–1680, 1620, 1440–1420, 1350, 1240, 1180, 1140, 1050, 1020, 980. ^{1}H NMR (300 MHz), ($CDCl_3$): δ 5.62 (1H, *bs*, H-14); 5.20 (1H, *ddd*, $J = 3.0$, 4.5, 11.2 Hz, H-2); 3.70 (1H, *d*, $J = 3.0$ Hz, H-3); 3.65 (3H, *s*, OMe); 2.76 (1H, *dt*, $J = 4.5$, 11.2 Hz, H-12); 2.29 (1H, *dt*, $J = 4.5$, 11.2 Hz, H-12′); 2.08 (3H, *s* OAc); 1.91 (3H, *bs*, Me-16); 1.28, 1.10, 0.72 (3H each, *s*, Me-18, Me-19 and Me-20, respectively); 0.84 (3H, *d*, $J = 5.7$ Hz, Me-17). ^{13}C NMR see Table 1. MS *m/z* (rel. int.):392 [$M^+ - H_2O$] (1); 378 [$M^+ - CH_3OH$] (3); 350 [$M^+ - CH_3COOH$] (14); 318 [378 − CH_3COOH] (8); 300 [318 − H_2O] (7); 285 [300 − Me] (5); 276 [392 − $C_6H_{10}O_2$] (56); 223 [350 − $C_7H_{11}O_2$] (32); 205 (45); 191 (25); 163 (27); 149 (26); 137 (57); 135 (35); 127 (58); 125 (35); 123 (100); 122 (33); 119 (24); 114 (43); 109 (48); 107 (47); 105 (26); 97 (35); 95 (76); 83 (52); 81 (57); 79 (29); 69 (55).

Acknowledgements—We are grateful to Dr Aldo Mesa, Universidad de Talca, Chile for identification of the plant material; to Dr A. San Martin (Universidad de Chile) we express our appreciation for helpful discussions. This research was supported by a grant from FONDECYT (no. 194408).

REFERENCES

1. Garbarino, J. A., Chamy, M. C., Montagna, M. P. and Gambaro, V., *Phytochemistry*, 1993, **32**, 987.
2. Garbarino, J. A., Chamy, M. C. and Gambaro, V., *Phytochemistry*, 1986, **25**, 2833.
3. Garbarino, J. A., Chamy, M. C., Piovano, M. and Gambaro, V., *Phytochemistry*, 1988, **27**, 1795.
4. Mesa, A., *Flora Neotrópica*, Monograph 26, ed. C. T. Rogerson. New York Botanical Garden.
5. Agrawal, P. K., *Journal of Scientific and Industrial Research*, 1994, **53**, 329.
6. Summers, M. F., Marzilli, L. G. and Bax, A., *Journal of the American Chemical Society*, 1986, **108**, 4285.
7. Gianello, J. C., Pestchauker, M. J., Tonn, C. E., Guo, M. and Giordano, O. S., *Phytochemistry*, 1990, **29**, 656.
8. Audier, H., Bory, S., Fetizon, M. and Nguyen-Trong, *Bolletin de la Société de la Chimie France*, 1966, 4002.
9. Avila, D. and Medina, J. D., *Journal of Natural Products*, 1993, **56**, 1586.
10. San Feliciano, A., Medarde, M., Lopez, J. L., Miguel del Corral, J. M., Puebla, P. and Barrero, A. F., *Phytochemistry*, 1988, **27**, 2241.
11. Buckwalter, B. L., Burfitt, I. R., Nagel, A. A., Wenkert, E. and Näf, W., *Helvetia Chimica Acta*, 1975, **58**, 1567.
12. Bartard, J., Do Khac Manh, D., Fetizon, M., Francis, M., Grant, P., Weavers, R., Kancko, C., Baddeley, G., Bernassau, J. M., Burfitt, I., Workulich, I. and Wenkert, E., *Journal of Natural Products*, 1984, **47**, 592.
13. Rossomando, P. C., Giordano, O. S., Espiñeira, J. and Joseph-Nathan, P., *Phytochemistry*, 1985, **24**, 787.
14. D. W. Brown, T. T. Nakashima and D. L. Rabenstein, *Journal of Magnetic Resonance*, 1981, **45**, 302.
15. LeCocq, C. and Lallemand, J. Y., *Journal of the Chemical Society, Chemical Communications*, 1981, 150.
16. Savona, G., Piozzi, F. and Rodriguez, B., *Heterocycles*, 1978, **9**, 257.
17. Demarco, P. V., Farkas E., Doddrell, D., Mylari, B. L. and Wenkert, E., *Journal of the American Chemical Society*, 1968, **90**, 5480.
18. Tonn, C. E., Rossomando, P. C. and Giordano, O. S., *Phytochemistry*, 1982, **21**, 2599.
19. Bory, S., Fetizon, M. and Laszlo, P., *Bolletin de la Société de la Chimie France*, 1963, 2310.