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DITERPENES FROM *ARNICA ANGUSTIFOLIA**

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Key Word Index—*Arnica angustifolia* ssp. *attenuata*; *A. montana*; Asteraceae; diterpenes; labdanes; labd-13(*Z*)-ene-8α,15-diol; (13*R*,14*R*)8,13-epoxylabdane-14,15-diol; 8α-hydroxylabd-13(*Z*)-ene-15-al; 8α-hydroxylabd-13(*E*)-ene-15-al; 8,13-epoxylabdane-15-al; 13,14,15,16-tetranorlabdane-12,8-olide (norambreinolide).

Abstract—From flowerheads of *Arnica angustifolia* ssp. *attenuata* the labdane diterpenes labd-13(*Z*)-ene-8α,15-diol (main component) and (13*R*,14*R*)8,13-epoxylabdane-14,15-diol were isolated and their structures established by extensive NMR and MS measurements. Both compounds are reported as natural products for the first time. Oxidative degradation of the main component was observed and the oxidation products 8α-hydroxylabd-13(*Z*)-ene-15-al, 8α-hydroxylabd-13(*E*)-ene-15-al and 8,13-epoxylabdane-15-al were identified. Additionally, the tetranorlabdanolide norambreinolide, most probably also derived from the labdanes, was detected in minor amounts. A search for diterpenes in flowers of *A. montana* revealed the presence of small amounts of labd-13(*Z*)-ene-8α,15-diol.

INTRODUCTION

Arnica angustifolia, formerly referred to as *A. alpina* (L.) Olin (for systematics and nomenclature see Refs [3–5]), is a polymorphous circumpolar aggregate consisting of seven more or less distinct subspecies [3]. Maguire places this group at the phylogenetic base of the subgenus Arctica and considers it to be the most archetypal among the 32 *Arnica* species [3]. Since, in contrast to the other four subgenera, no systematic chemical investigations so far have dealt with plants of the subgenus *Arctica*, *analysis of A. angustifolia* was of interest as part of our phytochemical investigations within the genus *Arnica* (for overview see Ref. [6]). Moreover, the flowerheads of *A. angustifolia* were formerly used in folk medicine in the same range of indications as the officinal 'Arnicae flos' of the German Pharmacopoea from *A. montana* L. and *A. chamissonis* Less. ssp. *foliosa* Nutt. (Maguire) [7, 8].

RESULTS AND DISCUSSION

In the course of our chemical analysis of *A. angustifolia* [1], we isolated from the flowerheads of ssp. *attenuata* the labdane diterpenes labd-13(*Z*)-ene-8α,15-diol (**1**) and (13*R*,14*R*)-8,13-epoxylabdane-14,15-diol (**5**), which, to the best of our knowledge, are described as natural products for the first time. Additionally, three oxidation products of **1**, the aldehydes **2**, **3** and **4**, as well as the tetranorlabdane norambreinolide **6** were found to be present. This is the first report on diterpenoids isolated from an *Arnica* species.

The diterpene **1** (main component), shows the typical mass spectrum of a bicyclic labdene diol with one free hydroxyl at C-8 and the other hydroxyl group as well as the double bond located in the sidechain. Characteristic peaks result from $[M]^+$ at *m/z* 308 and the fragments at *m/z* 192 $[M - C_6H_{12}O_2]^+$ and 191 $[M - (H_2O + C_6H_{11}O)]^+$, due to loss of the entire sidechain and a molecule of water, and 177 $[192 - CH_3]^+$ [9, 10]. The ^{13}CNMR spectra (PND and DEPT 135, see Table 1) show signals for five methyl-, eight methylene-, three methine- and four quaternary carbons. Comparison of the chemical shifts with literature data [11–13] confirmed the presence of a labdane with a Δ13,14-unsaturated sidechain and two free hydroxyls at C-8 and C-15. The stereochemistry at C-8 (β-CH_3, α-OH) follows from the chemical shift of C-6 (δ20.5) which should be shifted upfield to about δ17 as a consequence of a γ-gauche-effect in the case of a β-axially orientated OH-function [13].

The double bond was shown to be *Z*-configurated by comparison of the chemical shifts of C-12 and C-16 (δ35.6 and 23.7, respectively) with the corrresponding carbons in nerol (*Z*) and geraniol (*E*) [14] and with the

*The results presented here are part of a dissertation [1]. Some of them have been presented in abstract form at the 40th Annual Congress of the Society for Medicinal Plant Research 1992 [2].

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Dedicated to Prof. Dr Hans Möhrle on the occasion of his 65th birthday.

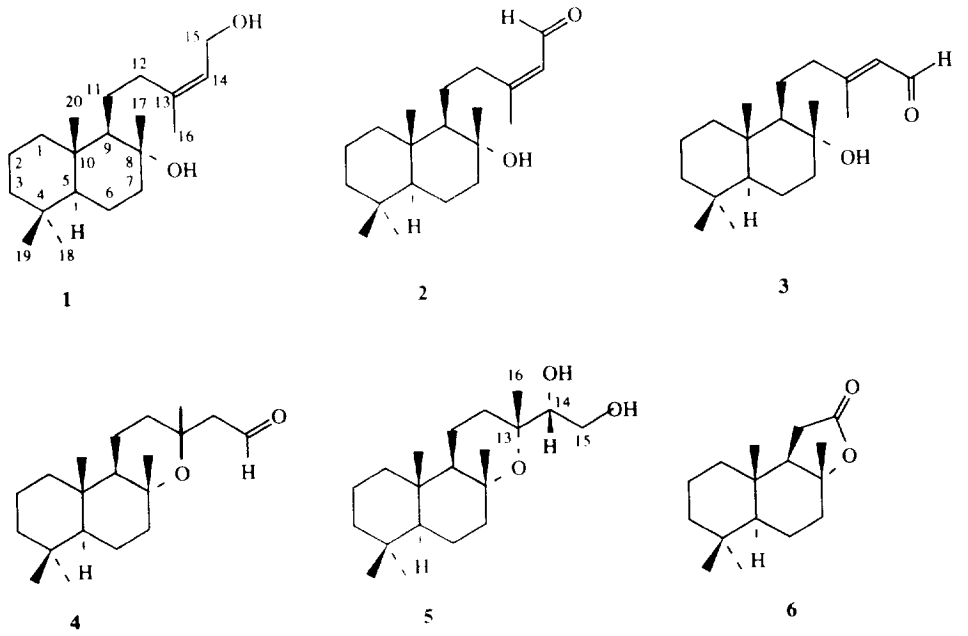


Table 1. ^{13}C NMR spectral data of compounds **1*** and **5** ($CDCl_3$, 125 MHz)

C	1	5
1	39.7	39.0
2	18.5	18.6
3	42.0	42.1
4	33.3	33.3
5	56.1	56.4
6	20.5	19.8
7	44.3	43.0
8	74.4	76.6†
9	61.4	58.0
10	39.0	36.9
11	25.0	14.7
12	35.6	33.3
13	142.6	76.1†
14	123.4	76.6
15	58.4	63.3
16	23.7	24.7
17	24.0	24.6
18	33.4	33.3
19	21.5	21.3
20	15.6	15.8

*Assignments confirmed by ^{13}C ^{1}H-HETCOR.

†Assignments interchangeable.

E-isomer of **1**, which has been isolated repeatedly from plants [15–17] (C-12: δ44.6; C-16: δ16.5 [15]).

The 2D-^{1}H,^{1}H-COSY and ^{13}C ^{1}H-HETCOR spectra allowed unequivocal assignment of all proton signals in the ^{1}H NMR spectrum (Table 2). Interestingly, the spin-system of H15a/b in **1**, unlike that of the *E*-isomer (H-15, *d*, 2H at δ4.15 [16]), displays two distinct *dd* signals at δ4.14 and 4.06, which indicates that in the *Z*-isomer free rotation of the sidechain is hindered, possibly as a consequence of a hydrogen bond between the free hydroxyls.

The absolute stereochemistry of **1**, i.e. its *normal*-labdane nature, was established by measurement of the optical rotation ($[\alpha_D^{20}] = -8.5°$) which is in agreement with the value given by Asselineau *et al.* [18], who obtained the same compound synthetically from sclareol ($[\alpha_D^{20}] = -11°$).

The allyl alcohol moiety of **1** was found to be easily oxidised to the corresponding α,β-unsaturated aldehyde during storage under normal atmosphere at room temperature. TLC analysis of **1** four months after its isolation as a pure compound showed two additional spots with a strong UV absorbance at highly increased R_f values, which were found to be identical with two minor constituents that had already been detected in a preceeding fraction during the isolation of **1**.

Consequently, the ^{1}H NMR measurement was repeated with **1** in admixture with its oxidation products **2** and **3**. In this spectrum, signals of two aldehyde protons, formerly unobserved, occurred at δ9.94 and 9.99. Besides these, the corresponding signals of the olefinic proton and of the methyl group at C-13 as well as the neighboring methylene group (H-12a, 12b) for both of the artefacts could be localized in the spectrum (Table 2). The chemical shifts and coupling patterns in the two sets of signals are in agreement with the presence of the *Z*- and the *E*-configured 8α-hydroxylabd-13-ene-15-al as they are almost identical with those of similar pairs of labdenealdehydes reported in the literature [19, 20]. The ratio of the *Z*- and *E*-isomer in the mixture is about 2:1, as follows from the signal intensities. Since oxidation of **1** should lead to the *Z*-configured aldehyde **2**, the *E*-isomer **3** is likely to be formed by isomerization via an enol-intermediate in which the positive charge at C-13 is

Table 2. ^{1}H NMR spectral data of compounds **1**,*† **2**,‡ **3**‡ and **5*** ($CDCl_3$, 500 MHz)

H	1	2	3	5
1α	0.97 *ddd* [*dt*]			0.86–0.91 *m*§
1β	1.67 *m*§			1.54–1.63 *m*§
2α	1.46 *m*§			1.28–1.33 *m*§
2β	1.61 *ddddd* [*tq*]			1.54–1.63 *m*§
3α	1.16 *ddd* [*dt*]			1.15 *ddd* [*dt*]
3β	1.33 *m*§			1.28–1.33 *m*§
5	0.93 *dd*			0.94 *dd*
6α	1.64 *m*§			1.64–1.66 *m*§
6β	1.26 *dddd* [*dq*]			1.28–1.33 *m*§
7α	1.44 *m*§			1.28–1.33 *m*§
7β	1.85 *ddd* [*dt*]			1.77 *ddd* [*dt*]
9	1.09 *dd* [*t*]			1.12 *dd*
11a	1.47 *m*§			1.54–1.63 *m*§
11b	1.34 *m*§			(β) 1.50 *dddd* [*dq*]
12a	2.28 *ddd*	2.75 *m*	2.75 *m*	(α) 2.05 *ddd* [*dt*]
12b	2.09 *ddd*	2.53 *m*	2.53 *m*	(β) 1.42 *ddd* [*dt*]
14	5.46 *br dd* [*t*]	5.85 *d*	5.92 *d*	3.11 *dd* [*t*]
15a	4.14 *dd*	9.94 *d*	9.99 *d*	3.84 *dd*
15b	4.06 *dd*			3.66 *dd*
16	1.77 *s*¶	2.00 *s*	2.19 *br s*	1.30 *s*¶
17	1.14 *s*¶			1.26 *s*¶
18	0.87 *s*¶			0.86 *s*¶
19	0.79 *s*¶			0.79 *s*¶
20	0.78 *s*¶			0.77 *s*¶

*Assignments confirmed by ^{1}H,^{1}H-COSY.

†Assignments confirmed by ^{13}C,^{1}H-HETCOR.

‡Signals taken from spectrum of a mixture of **2** and **3** with **1**.

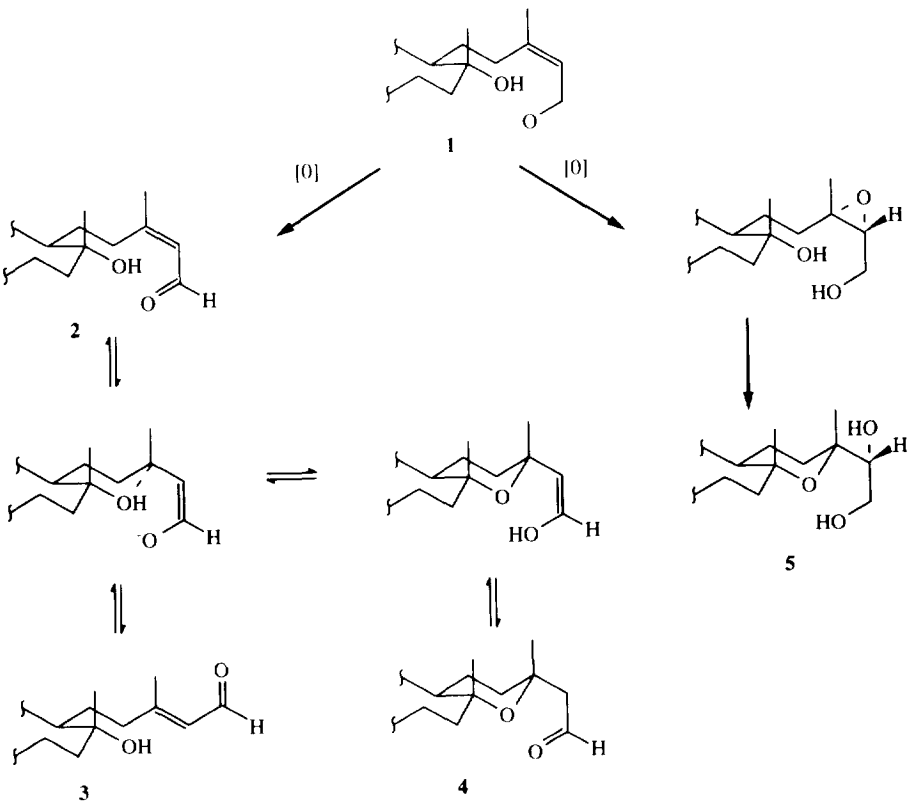
§Overlapping signals, δ-values determined from centre of cross peaks in HETCOR spectrum (compound **1**), or from COSY (compound **5**); multiplicity not determined.

¶Intensity three protons.

J (Hz): compound **1**: 2α,2β = 14; 1α,1β = 1α,2β = 2β,3α = 3α,3β = 6α, 6β = 6β,7α = 12a,12b = 13; 5,6β = 7β,7α = 15a,15b = 12; 11a,12b = 11b,12a = 10; 14,15a = 14,15b = 7.5; 11b,12b = 7; 11a,12a = 6; 1α,2α = 1β,2β = 2α,3α = 2β,3β = 9,11a = 9,11b = 4; 6α,7β = 6β,7β = 3; 5,6α = 2. Compounds **2** and **3**: 14,15 = 8. Compound **5**: 2β,3α = 3α,3β = 11α,11β = 11β,12α = 12α,12β = 13; 5,6β = 7α,7β = 9,11β = 12; 15a,15b = 11; 11α,12α = 14,15a = 5; 2α,3α = 11α,12β = 11β,12β = 4; 6α,7β = 6β,7β = 14,15b = 3; 5,6α = 9,11α = 2.

partly stabilized by the oxygen at C-8 as shown in Scheme 1. From this hypothetic enol, as a third isomer, the tricyclic 8,13-epoxylabdan-15-al **4** might arise (see Scheme 1). The formation of this compound could be proven by mass spectrometry. The GC-MS analysis revealed, besides **1**, the presence of only one compound with a molecular ion at *m/z* 306. The fragmentation pattern of this compound is typical for an 8,13-epoxylabdane showing intense fragments at *m/z* 263 $[M - C_2$ sidechain$]^+$ and 245 $[263 - H_2O]^+$ [9] which should not be formed from the open chain aldehydes **2** and **3**, which would be expected to yield a fragmentation analogous to **1**. Since the presence of **4** could not be observed in the ^{1}H NMR spectrum, it is likely that under the high-temperature GC conditions, the equilibrium between the three isomers is shifted towards **4**, whereas at room temperature **2** and **3** are the most stable forms.

The CI mass spectrum of compound **5** displays quasimolecular ions at *m/z* 342 $[M + NH_4]^+$ and 325 $[M + H]^+$ from which a molecular mass of 324 could be deduced. The EI mass spectrum, again, shows the typical fragments for an 8,13-epoxylabdane [9] (see compound **4**) with , in this case, two hydroxyl functions in the C_2-sidechain. The NMR spectra (^{13}C-PND, DEPT, ^{1}H, ^{1}H/^{1}H-COSY) confirm the structure as an 8,13-epoxylabdane-14,15-diol. As expected, all carbon atoms except for C-14 and C-15 show chemical shifts very similar to those of dihydromanoyloxide [11] (Table 1). The ^{1}H-NMR spectrum displays an ABX system for H15/15′ (AB-part, δ3.84 and 3.66) and H-14 (X-part, δ3.11) with $^2J_{AB} = (-)11.3$, $^3J_{AX} = 4.7$ and $^3J_{BX} = 3.3$ Hz proving the CH(OH)–CH_2–OH structure. According to Rodriguez *et al.* [21], the chemical shifts of the ABX system and of the methyl groups at C-13 and C-8 allow assignment of the relative configurations at C-13 and C-14. Provided that **5**, like **1**, is a regular labdane, which is very likely from the biosynthetic point of view, these centres possess a 13*R*,14*R*-*threo*-configuration.



Scheme 1. Possible formation of compounds **2**, **3**, **4** and **5** from **1**.

Compound **6** is a minor constituent which could be detected by means of TLC and GC-MS in a fraction obtained during an attempt to isolate the aldehydes **2** and **3**. Its CI and EI mass spectral data (see experimental) clearly prove it to be a 13,14,15,16-tetranorlabdane-12,8-olide [9, 22], most likely with the depicted configuration (= norambreinolide), in keeping with its probable origin as a degradation product of compounds **1**–**5**. Norambreinolide has repeatedly been isolated from plant sources and has already been detected in an asteraceous species by Herz *et al.* [23].

From the biosynthetic point of view, the bicyclic labdane type represents the most primitive type of carbocyclic diterpenes [24]. It is interesing to note, that the direct cyclization products of geranylgeraniol (*E*-isomer of **1**) and geranyllinalool (sclareol) are rather widespread in nature, while this appears to be the first report on the corresponding product of geranylnerol. The tricyclic diol **5** is most likely to be biosynthesized from **1** via the 13,14-epoxide as depicted in Scheme 1, since conversions of this type have already been shown to occur *in vitro* [25, 26].

Considering the fairly large amount of **1** present in the flowerheads of *A. angustifolia* ssp. *attenuata*, it is likely that this compound and/or the α,β-unsaturated aldehydes formed after exposure to oxygen are part of the plant's defense system. Antibacterial and antifungal properties of the *E*-isomer of **1** and some of its derivatives have been reported recently [27].

Regarding the increasing number of reports on diterpenes as chemotaxonomic markers [24, 28], the presence of diterpenes within the genus *Arnica* might provide a further source of chemotaxonomic evidence for the systematic placement of this problematic taxon within the Compositae [6]. As part of the search for diterpenes in other *Arnica* species, compound **1** could be detected in minor amounts in the flowerheads of *A. montana* by means of GC-MS and direct GC and TLC comparison with the authentic compound.

EXPERIMENTAL

Plant material. Achenes of *A. angustifolia* Vahl (*A. alpina* (L.) Olin) collected at natural habitats in northwest Canada and Alaska were provided by Prof. K. E. Denford, University of Alberta, Edmonton, Canada as *A. angustifolia* ssp. *angustifolia* Downie [5]. After cultivation at the Botanic Garden of the University of Düsseldorf, they were identified as *A. angustifolia* ssp. *attenuata* (Greene) Maguire according to the literature [3, 29], which had recently been integrated in ssp. *angustifolia* by Downie [5]. Voucher specimens are deposited at the herbarium of the Institut für Pharmazeutische Biologie, University of Düsseldorf. Flowerheads were collected in May–June 1990.

Extraction and isolation. 1500 g of dried and powdered flowerheads were extracted with CH_2Cl_2 (Soxhlet) and the extract (127 g) macerated with 4 × 500 ml of MeOH

at ambient temp to give 38.5 g of a MeOH-soluble fraction. Gel chromatography with Sephadex LH-20/MeOH and subsequently with cyclohexane–CH_2Cl_2–MeOH (7:4:1) gave a fraction containing diterpenes together with sesquiterpene lactones. CC on silica with mixtures of CH_2Cl_2–EtOAc of increasing polarity gave a fraction (1074 mg, CH_2Cl_2–EtOAc 1:1) in which the diterpenes were present in admixture with sesquiterpene lactones. Separation of this fraction by repeated CC on silica with cyclohexane–EtOAc and CH_2Cl_2–Me_2CO mixtures of various polarities gave 69 mg **1**, 3 mg **5** and a mixture (2 mg) of **2**, **3** and **6** along with further impurities.

Arnica montana flowerheads (DAB10) purchased from Fa. CAELO, Hilden, Germany were extracted as described above. Compound **1** was detected (GC-MS, GC, TLC) as a minor component in a crude fraction after CC on Sephadex LH-20/MeOH along with esters of helenalin and dihydrohelenalin and identified by direct comparison with the authentic compound from *A. angustifolia*.

Instrumentation. GC-MS: OV-1-DF capillary (25 m × 0.25 mm i.d.; film: 0.25 μM), carrier N_2 at 65 ml/min (total flow), split 1:50, gradient: 150–270°, 10° min^{-1}, FID; HPLC: RP18 (Hypersil ODS) (5 μM, 125 × 4.6 mm), elution at 1.8 ml/min, gradient: 0–15 min: 55% MeOH–80% MeOH, followed by 80% MeOH isocratic; detection: UV 240 ± 10 nm; UV spectra of **2** and **3** were measured on line with the HPLC multiple wavelength detector: TLC: silica 0.2 mm on alumina sheets, toluene–EtOAc (1:4).

Labd-13(Z)-*ene*-8α,15-diol (**1**). Resin; CIMS (NH_3): *m/z* (rel. int.): 326 $[M + NH_4]^+$ (100), 308 $[M - H_2O + NH_4]^+$ (48), 291 $[M - H_2O + H]^+$ (14), 273 $[M - 2H_2O + H]^+$ (90); EIMS 70 eV, *m/z* (rel. int.): 290 $[M - H_2O]^+$ (1), 275 $[290 - CH_3]^+$ (2), 272 $[290 - H_2O]^+$ (1), 257 $[272 - CH_3]^+$ (2), 192 $[M - C_6H_{12}O_2]^+$ (34), 191 $[290 - C_6H_{11}O]^+$ (30), 177 $[192 - CH_3]^+$ (55), 163 (10), 149 (19), 135 (20), 123 (37), 109 (46), 95 (58), 81 (77), 69 (76), 55 (58), 43 (100); $[\alpha]_D^{20}$: −8.5° (*c* = 0.085, $CHCl_3$); IR: (KBr) ν (cm^{-1}): 3300–3500 (OH); 1650 (C=C); TLC: R_f 0.49; GC: R_t 10.78 min; ^{13}C NMR: Table 1; ^1H NMR: Table 2.

Z- *and* E-8α-*Hydroxylabd*-13-*ene*-15-*al* (**2** *and* **3**). UV: λ_{max} = 240 nm (MeOH); TLC: R_f 0.67 and 0.75; HPLC: R_t 15.03 and 15.45 min; ^1H NMR: Table 2.

8,13-*Epoxylabdan*-15-*al* (**4**). CIMS (NH_3): *m/z* (rel. int.): 324 $[M + NH_4]^+$ (43), 307 $[M + H]^+$ (100), 289 (15), 263 (32); EIMS 70 eV, *m/z* (rel. int.): 306 $[M]^+$ (3), 291 $[M - CH_3]^+$ (47), 278 $[M - CO]^+$ (5), 273 $[291 - H_2O]^+$ (28), 263 $[M - CH_2CHO]^+$ (14), 255 (13), 245 $[263 - H_2O]^+$ (57), 229 (11), 218 (9), 205 (11), 204 (10), 191 (58), 177 (30), 163 (20), 149 (38), 137 (47), 123 (57), 109 (52), 95 (54), 81 (57), 69 (57), 55 (52), 43 (100); GC: R_t 9.83 min.

(13R,14R)-8α,13-*Epoxylabdan*-14,15-*diol* (**5**). CIMS (NH_3): *m/z* (rel. int.): 342 $[M + NH_4]^+$ (52), 325 $[M + H]^+$ (32), 310 $[M - CH_3 + NH_4]^+$ (10), 263 (100); EIMS 70 eV, *m/z* (rel. int.): 309 $[M - CH_3]^+$ (1), 291 $[309 - H_2O]^+$ (1), 263 $[291 - CO]^+$ (17), 245 $[263 - H_2O]^+$ (78), 205 (5), 191 (10), 163 (13), 149 (20), 137 (58), 95 (57), 81 (66), 69 (54), 55 (51), 43 (100); TLC: R_f 0.50; GC: R_t 12.01 min; ^{13}C NMR: Table 1; ^1H NMR: Table 2.

13,14,15,16-*Tetranorlabdan*-12,8-*olide* (*norambreinolide*) (**6**). CIMS (NH_3): *m/z* (rel. int.): 268 $[M + NH_4]^+$ (100), 251 $[M + H]^+$ (7); EIMS 70 eV, *m/z* (rel. int.): 235 $[M - CH_3]^+$ (6), 207 $[235 - CO]^+$ (4), 206 $[M - CO_2]^+$ (4), 191 $[235 - CO_2]^+$ (2), 189 (2), 175 (1), 163 (2), 150 (6), 137 (8), 123 (22), 109 (21), 95 (30), 82 (37), 81 (37), 69 (44), 55 (55), 43 (100); TLC: R_f 53; GC: R_t 7.76 min.

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