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TWO LABDANE DITERPENOIDS FROM *NICOTIANA RAIMONDII*

MASANA NOMA, FUMIYO SUZUKI, KENJI GAMOU and NOBUMARO KAWASHIMA

Central Research Institute, The Japan Tobacco and Salt Public Corporation, 6–2 Umegaoka, Midoriku,
Yokohama, Kanagawa 227, Japan

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Key Word Index—*Nicotiana raimondii*; Solanaceae; new diterpene; labdanoids.

Abstract—Two new labdanoids from *Nicotiana raimondii* have been identified as (12*E*) - 7β - hydroxylabda - 8(20),12,14 - triene and 7β - hydroxy - labda - 8(20),13(16),14 - triene.

INTRODUCTION

The diterpenes in exudates from trichomes of leaves of all species of *Nicotiana* examined so far are species specific and can be used as chemotaxonomic markers [1, 2]. This finding has led us to investigate the diterpenes in exudates among various species of *Nicotiana*. *Nicotiana raimondii* (Paniculatae) exudes especially large amounts of gummy materials which sometime reach up to 0.3% of the dry wt of green leaves. We now report on the main components of this exudate.

RESULTS AND DISCUSSION

The ^{13}C NMR spectra of compounds **1** and **2** (named raimonol and isoraimonol, respectively) and related compounds are shown in Table 1. The presence in **1** and **2** of the A ring moiety including three Me groups found in **3**, obtained as a fungal metabolite of *cis*-abienol [3], is indicated by the fact that their spectra contain the same signals as those assigned to C-1, C-2, C-3, C-4, C-17, C-18 and C-19 of **3**. In **1** three of the signals (δ 11.9, 110.0 and 141.6) are as assigned to C-14, C-15 and C-16 of *trans*-

Table 1. ^{13}C NMR spectra of compounds **1–5** (25.0 MHz, CDCl$_3$, TMS as internal standard)

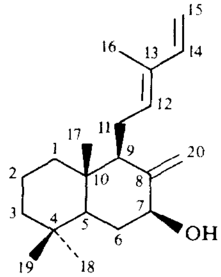
	Raimonol (**1**)	Isoraimonol (**2**)	(**3**)	(**4**)	*t*-Abienol (**5**)
C-1	39.1 *t*	39.2 *t*	39.3 *t*	39.1 *t*	40.1 *t*
C-2	19.4 *t*	19.3 *t*	19.5 *t*	19.3 *t*	18.6 *t*
C-3	42.0 *t*	42.0 *t*	42.2 *t*	42.1 *t*	41.9 *t*
C-4	33.6 *s*	33.9 *s*	33.5 *s*	33.5 *s*	33.2 *s*
C-5	53.1 *d*	53.2 *d*	55.5 *d*	55.3 *d*	56.1 *d*
C-6	33.4 *t*	33.4 *t*	24.3 *t*	24.1 *t*	20.4 *t*
C-7	73.8 *d*	73.8 *d*	38.2 *t*	38.0 *t*	44.1 *t*
C-8	150.5 *s*	150.4 *s*	148.2 *s*	148.5 *s*	73.7 *s*
C-9	55.3 *d*	54.8 *d*	57.4 *d*	57.0 *d*	62.2 *d*
C-10	39.3 *s*	38.8 *s*	39.6 *s*	39.4 *s*	38.9 *s*
C-11	22.9 *t*	21.1 *t*	22.1 *t*	23.0 *t*	24.0 *t*
C-12	133.3 *d*	30.0 *t*	133.9 *d*	134.0 *d*	136.4 *d*
C-13	133.7 *s*	146.8 *s*	131.4 *s*	133.2 *s*	132.1 *s*
C-14	141.6 *d*	139.0 *d*	131.6 *d*	141.6 *d*	141.7 *d*
C-15	110.0 *t*	113.1 *t*	113.0 *t*	109.6 *t*	110.0 *t*
C-16	11.9 *q*	115.5 *t*	19.7 *q*	11.7 *q*	11.8 *q*
C-17	14.5 *q*	14.5 *q*	14.5 *q*	14.3 *q*	15.4 *q*
C-18	33.6 *q*	33.5 *q*	33.6 *q*	33.5 *q*	33.5 *q*
C-19	21.7 *q*	21.6 *q*	21.8 *q*	21.6 *q*	21.6 *q*
C-20	104.2 *t*	103.1 *t*	107.7 *t*	107.3 *t*	24.1 *q*

abienol (**5**), and this together with its UV absorption (230 nm, log 4.36) shows the presence of a *trans* diene moiety. Changing the C-16 Me group (δ 11.9, *q*) of raimonol to a terminal methylene (δ 115.5, *t*) affords isoraimonol. The ^{1}H NMR data of **1** and **2** are shown in Table 2. In double resonance experiments, irradiation at δ 3.99 (1H, *q*, $J = 5.5$, 11 Hz) changed an octet (1H, $J = 2.5$, 5.5 and 12 Hz) at δ 2.09 and a sextet (1H, $J = 12$, 12 and 11 Hz) at δ 1.28 to a broad doublet and a triplet respectively and resulted in the elongation of singlets at δ 4.67 and 5.19. These changes, together with the presence of a quartet (1H, $J = 2.5$, 12 Hz) at δ 1.16, point to the presence of the partial structure **A** in **1** and **2** (Fig. 1). The configurations of the protons Ha, Hb, Hc and Hd which satisfy the Karplus rule are equatorial, axial, equatorial and axial, respectively. Thus the hydroxyl group is in an equatorial position. In a triple resonance experiment with **1**, irradiation at a triplet (1H, $J = 6.5$ Hz) at δ 5.42 and a broad doublet at δ 1.70 changed a septet (1H, $J = 6.5$, 11 and 16 Hz) at δ 2.24 and an octet (1H, $J = 2.5$, 6.5 and 16 Hz) at δ 2.42 to AB doublets ($J = 16$ Hz) affording the partial structure **B**. Tosylation of **1** followed by reduction with $LiAlH_4$ gave **4** which was identical (IR and MS) with the compound obtained by dehydration of **5**. This confirmatory evidence and the positive specific rotation of these hydrocarbons established the structures of raimonol (**1**) and isoraimonol (**2**) as (12*E*) - 7β - hydroxylabda - 8(20),12,14 - triene and 7β - hydroxylabda - 8(20),13(16),14 - triene.

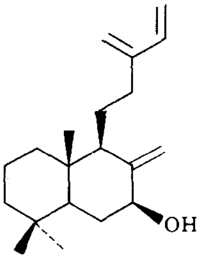
Table 2. ^{1}H NMR spectra of raimonol (**1**) and isoraimonol (**2**) (400 MHz, $CDCl_3$, TMS is internal standard)

	Raimonol	Isoraimonol
H-5	1.16 (1H, *q*)	1.16 (1H, *q*)
H-6 ax	1.28 (1H, *sextet*)	1.28 (1H, *sextet*)
H-6 eq	2.09 (1H, *octet*)	2.12 (1H, *octet*)
H-7	3.99 (1H, *q*)	4.00 (1H, *q*)
H-9	1.70 (1H, *br*, *d*)	1.43 (1H, *br*, *d*)
H-11	2.24 (1H, *septet*)	*
	2.42 (1H, *octet*)	*
H-12	5.42 (1H, *t*)	*
H-14	6.35 (1H, *q*)	6.39 (1H, *q*)
H-15	4.91 (1H, *d*)	5.24 (1H, *d*)
	5.07 (1H, *d*)	5.08 (1H, *d*)
H-16	1.76 (3H, *s*)	5.00 (1H, *s*)
		5.03 (1H, *s*)
H-17	0.92 (3H, *s*)	0.91 (3H, *s*)
H-18	*0.84 (3H, *s*)	*0.81 (3H, *s*)
H-19	0.72 (3H, *s*)	0.68 (3H, *s*)
H-20	4.67 (1H, *s*)	4.76 (1H, *s*)
	5.19 (1H, *s*)	5.23 (1H, *s*)

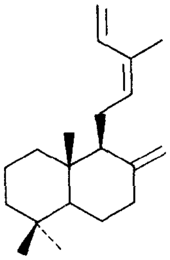
*Unassigned.



1 Raimonol



2 Isoraimonol



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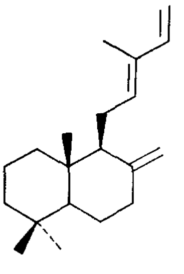

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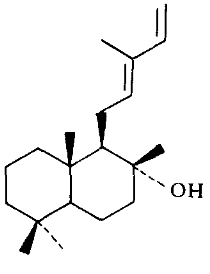
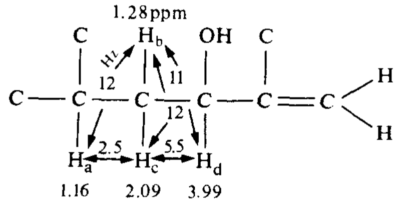


5 *t* - Abienol

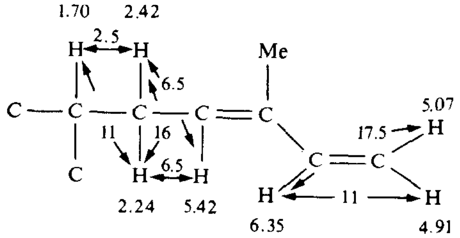
EXPERIMENTAL

Isolation of raimonol (**1**) *and isoraimonol* (**2**). Exudate of field-grown *N. raimondii* was washed out from green leaves (1 kg) by soaking in $CHCl_3$ for 30 sec (×2), and purified by SiO_2 column. The Et_2O–hexane (1:4) fraction (400 mg), after washing with hexane, was subjected to prep. GLC (1 m × 3 mm, packed with 5% OV-101, He 50 ml/min, 240° isothermal). R_ts of **1** and **2** were 5.0 and 4.0 min, respectively. 180 mg **1** and 60 mg **2** could have been obtained from the entire fraction on the basis of GLC analysis, but these compounds were purified by prep. GLC as required, because they are not very stable when purified. Reinjection of pure **1** or **2** gave a single peak at the expected R_t. Therefore, these isomers are not products of thermal isomerization.

Raimonol. Mp 101–103° (fine needles); $[\alpha]_D^{25}$ +24.8° (c = 0.55, EtOH); UV λ_{max}^{EtOH} 230 nm (log ϵ 4.36); IR ν_{max} cm^{-1}: 3250, 3040, 1640, 1604, 1100, 1062, 1038, 990; MS (low resolution) m/z: 288 $[M]^+$, 231, 206, 185, 151, 123.



A



B

Fig. 1. Partial structures A and B. Numbers above or below H are chemical shifts of the proton in ^{1}H NMR. Numbers between arrows are coupling constants between the protons indicated by the arrows.

Isoraimonol. Mp 70–72° (needles); $[\alpha]_D^{25} + 18.1°$ ($c = 0.24$, EtOH); UV λ_{max}^{EtOH} 223 nm (shoulder) (log ϵ 3.52); IR ν_{max} cm^{-1}: 3400, 3100, 1650, 1208, 1100, 1038, 900; MS (low resolution) *m/z*: 288 (M$^+$), 273, 255, 206, 135, 121.

Hydrocarbon (**4**) *from* cis-*abienol.* To a soln of *trans*-abienol (1.3 g) [obtained from *cis*-abienol [4] isolated from Canadian fir balsam (*Abies balsameae*)] in pyridine (26 ml), $POCl_3$ (910 μl) was added dropwise at 4°. After 48 hr at 4°, the reaction mixture was poured into ice H_2O and extracted with CH_2Cl_2. Purification on SiO_2 containing $AgNO_3$ (25%, w/w) (hexane as an eluant) gave **4** (436 mg), $[\alpha]_{365}^{25} + 77.0°$ ($c = 0.693$, CCl_4) $[\alpha]_{546}^{25} + 31.8°$; IR ν_{max} cm^{-1}: 3090, 1640, 1602, 1200, 1085, 985, 890; MS (low resolution) *m/z*: 272 [M]$^+$, 257, 216, 201, 191, 135; ^{1}H NMR (100MHz, CCl_4, TMS as int. standard): δ 6.20 (1H, *q*), 5.30 (1H, *t*), 4.94 (1H, *d*), 4.84 (1H, *d*), 4.76 (1H, *s*), 4.43 (1H, *s*), 1.65 (3H, *s*), 0.87 (3H, *s*), 0.82 (3H, *s*), 0.72 (3H, *s*).

Hydrocarbon **4** *from* **1**. To a soln of **1** (10 mg) in pyridine (0.2 ml), TsCl (67 mg) in pyridine (0.3 ml) was added dropwise with cooling by ice. After 16 hr at room temp., the tosylate (6 mg) was obtained by usual work-up. ^{1}H NMR (100 MHz, CCl_4, TMS as int. standard): δ 7.77 (2H, *d*), 7.28 (2H, *d*), 6.20 (1H, *q*), 5.08 (1H, *d*), 5.04 (*s*), 4.83 (1H, *d*), 4.52 (*s*), 2.44 (3H, *s*), 1.68 (3H, *s*), 0.86 (3H, *s*) 0.80 (3H, *s*), 0.66 (3H, *s*). Without further purification, the tosylate was reduced with $LiAlH_4$ (2.6 mg) in boiling Et_2O (2 ml). After 2 hr, the reaction mixture was passed through a small SiO_2 column (200 mg), and the eluate (2 mg) purified by prep. GLC (1 m × 3 mm, packed with 5% OV-101, temp. programmed 160–250° at 2°/min, He 50 ml/min) to give the hydrocarbon (400 μg): IR and MS spectra identical with those from *cis*-abienol; $[\alpha]_{365}^{25}$ *ca* + 49°, $[\alpha]_{365}^{25}$ *ca* + 12° (c = *ca* 0.016, CCl_4).

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