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TRICYCLIC SESQUITERPENES FROM *RUDBECKIA LACINIATA*

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

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Key Word Index—*Rudbeckia laciniata*; Asteraceae; phytotoxic; tricyclic sesquiterpene; sesquiterpene lactone

Abstract—Three phytotoxic sesquiterpenes were isolated from the roots of *Rudbeckia laciniata*, of which one of these was designated as lacinan-8-ol. Its absolute configuration was established by the MNCB [2-(2′-methoxy-1′-naphthyl)-3,5-dichlorobenzoic acid] method. It was chemically transformed into the corresponding secondary allylic alcohol, whose absolute configuration was also determined by MNCB and CD (circular dichroism) allylic benzoate methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The terpenoids and polyacetylenic compounds of *Rudbeckia laciniata* (Asteraceae) have previously been studied in detail [1–3]. This plant is naturalized in Japan and grows along roadsides and fields of Hokkaido island. Our allelopathic interest in this plant resulted in the isolation of three phytotoxic sesquiterpenes (**1**–**3**). Compounds **1** and **3** have been isolated from the same plant previously, and the chemical characterization of **1** has been presented elsewhere [4]. In this paper, the absolute configuration of a new sesquiterpene (**2**) was determined by ^{1}H NMR spectroscopy using an axially chiral reagent (MNCB) [4–7]. The chemical transformation of **2** into the corresponding secondary allylic alcohol, and application of the MNCB method and CD allylic benzoate method [8] to the resulting product are also described.

RESULTS AND DISCUSSION

During screening of higher plants for phytotoxic secondary metabolites, the EtOAc-soluble extract of *R. laciniata* roots was found to be inhibitory against germination and growth of some plants. Through the bioassay-guided fractionation method of Stevens and Merrill [9], three phytotoxic sesquiterpenes (**1**–**3**) were obtained from the extract. Prelacinan-7-ol (**1**) [4] and igalan (**3**) [3] have been reported as constituents of this plant. The structure of **3** was determined by ^{1}H and ^{13}C NMR spectral data comparison to that published [10–14]. Compound **2** was new and named as lacinan-8-ol.

Lacinan-8-ol (**2**, $C_{15}H_{26}O$) exhibited an IR absorption at 3380 cm^{-1}. Its mass spectrum gave a dehydration ion at *m/z* 204, and the corresponding tertiary carbinyl carbon appeared at δ 74.20 (s) in pyridine-d_5 indicated **2** to be a tertiary alcohol. The DEPT spectra showed the presence of fifteen aliphatic carbons consisting of four methyls, five methylenes, three methines and three quaternary carbons. All the carbon signals had chemical shifts less than 80 ppm, indicating no double bond and therefore a tricyclic structure. The ^{1}H–^{1}H COSY spectrum established the correlations of i) H-12 to H-5 and ii) H-7 to H-10. The HMBC spectrum showed correlations of i) H-15 and Cs-7, 8, 9; ii) H-14 and Cs-5, 6, 7, 13; iii) H-13 and Cs-5, 6, 7, 14; iv) H-12 and Cs-1, 2, 3; v) H-3 and Cs-1, 2, 4, 5, 12 and vi) C-1 and Hs- 2, 3β, 4$\alpha\beta$, 5, 9$\alpha\beta$, 10$\alpha\beta$, 12 (Table 1). Taken together, the above data led to the assignment of **2** as 2, 6, 6, 8-tetramethyltricyclo[5.2.5.0^{1,5}]undecan-8-ol and the other HMBC and ^{1}H-^{1}H COSY spectral correlations for **2** supported this structure.

The relative stereochemistry of **2** was concluded from several lines of data: i) W-type long-range couplings between H-9β and H-10α (J = 3 Hz), and H-10β and H-5 (J = 2 Hz); ii) the arrangement of the large pyridine-induced solvent shifts [4, 15] of signals around the hydroxyl group in the ^{1}H NMR spectra (Figure 1A); and iii) the observation of NOEs detected

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Table 1. NMR spectral data ($CDCl_3$ and C_5D_5N*) for lacinan-8-ol (2) and its MNCB esters (**4a** and **4b**)

	2							MNCB esters	
C	δ ^{13}C*	δ ^{1}H* (*J* Hz)	^{1}H-^{1}H COSY*	HMBC*	NOE*	δ ^{13}C	δ 1H	δ ^{1}H **4a** (a*S*)	**4b** (a*R*)
1	45.09			2,3β,4$\alpha\beta$,5,9$\alpha\beta$,10$\alpha\beta$,12		44.69			
2	42.61	1.50 *m*	3$\alpha\beta$,12	3$\alpha\beta$,4α,9β,12	9β	42.05	1.50	0.76	1.25
3	32.31	α 1.23 *dddd* (7,8.5,11,13)	2,3β,4$\alpha\beta$	2,4$\alpha\beta$,12	4α,12	31.90	1.23	1.01	1.14
		β 1.81 *dddd* (6.5,9,9,13)	2,3α,4$\alpha\beta$		4β		1.82	1.59	1.77
4	22.08	α 1.59 *m*	3$\alpha\beta$,4β,5	3$\alpha\beta$,5	3α,10α,14	21.76	ca 1.58	ca 1.28	1.42
		β 1.57 *m*	3$\alpha\beta$,4α,5		3β,5		ca 1.58	ca 1.28	1.45
5	52.27	1.97 *ddd* (2,10,10.5)	4$\alpha\beta$,10β	3β,4$\alpha\beta$,7,9α,10β13,14	4β,13	52.28	1.70	0.56	1.10
6	34.66			4$\alpha\beta$,5,7,11β,13,14		34.23			
7	49.51	1.26 *dd* (2,4)	11$\alpha\beta$	11$\alpha\beta$,13,14,15	14	49.31		1.84	1.72
							1.10		
8	74.20			7,9$\alpha\beta$,11$\alpha\beta$,15		75.70			
9	51.25	α 1.72 *d* (13)	9β	2,5,10α,15	10β,12,15	50.27	1.65	0.79	0.97
		β 1.45 *dd* (3,13)	9α,10α		2		1.13	−0.37	0.31
10	18.42	α 1.08 *dddd* (3,6,11,13)	9β,10β,11$\alpha\beta$	2,5,7,9$\alpha\beta$,11β	4α	17.86	1.13	0.89	0.97
		β 0.89 *dddd* (2,2.5,4,13)	1,10α,11$\alpha\beta$		9α,12		0.92	0.63	0.70
11	20.76	α 1.70 *dddd* (2.5,4,11,14)	7,10$\alpha\beta$,11β	7,10$\alpha\beta$	14	20.56	1.73	1.55	1.55
		β 1.39 *ddd* (2,2.5,11,14)	7,10$\alpha\beta$,11α		15		1.35	1.10	1.09
12	14.70	0.80 *d* (7)	2	2,3α	3α,9α,10β	14.37	0.77	0.55	0.67
13	34.20	1.62 *s*		5,7,14	5,7,14	34.23	1.28	0.83	0.87
14	28.14	0.96 *s*		5,13	4α,11α,13	27.72	0.92	0.77	0.79
15	32.60	1.53 *s*		7,9β	9α,11β	32.53	1.37	1.17	0.87
OH		5.15 *s*					1.08		

Assignments were based on DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY and difference NOE experiments.

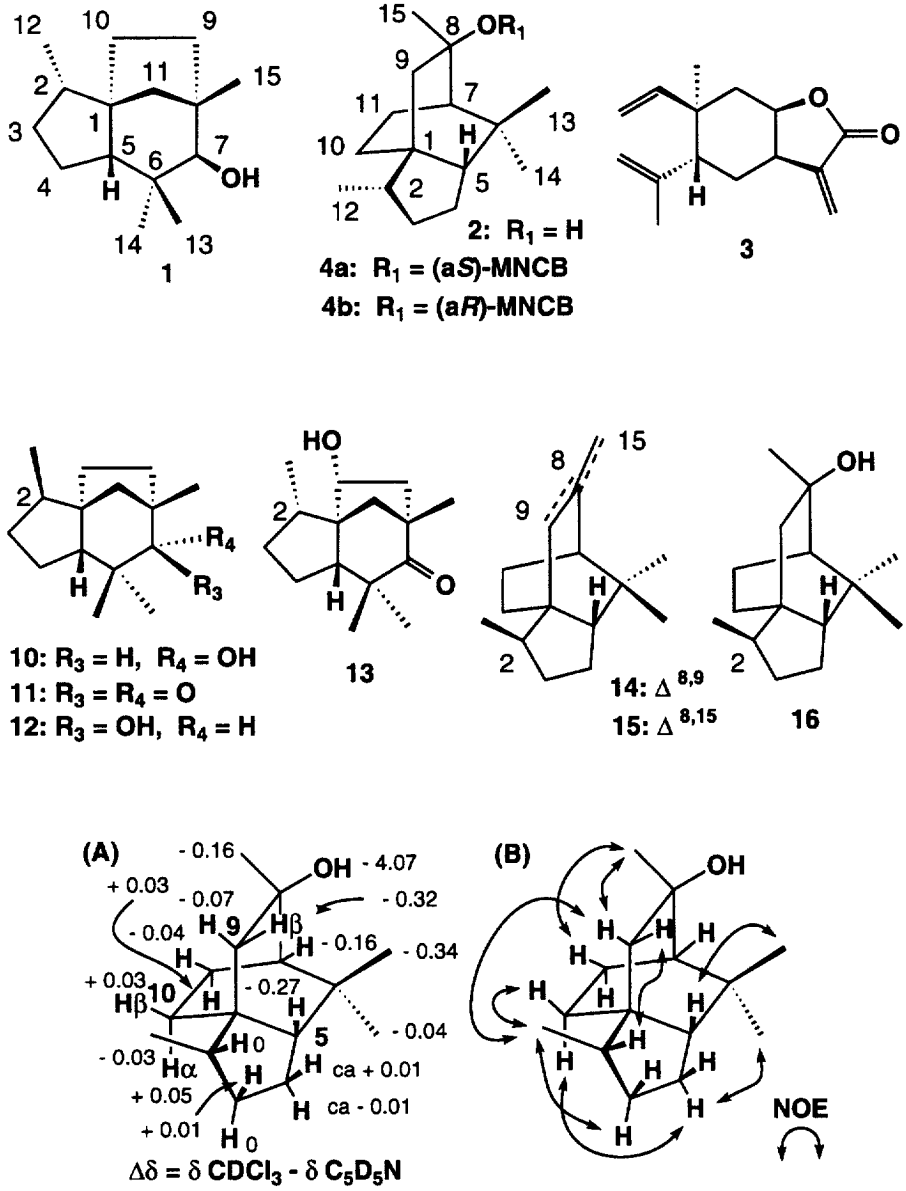


Fig. 1. (A) Pyridine-induced solvent shifts: $\Delta\delta$ ($=\delta CDCl_3$-δC_5D_5N) in ^1H NMR obtained for prelacinan-8-ol (**2**). (B) Key NOEs observed in **2**.

by NOESY and NOE difference spectra in Fig. 1B and Table 1.

To establish the absolute configuration of **2**, it was esterified with (a*S*)- and (a*R*)- [5–7, 16] MNCB in methylene chloride containing DCC and 4-pyrrolidinopyridine [17] to yield **4a** and **4b**, respectively. The chemical shift (δ −0.37) of H-9β in the (a*S*)-MNCB ester (**4a**) indicates that H-9β faces to the centre of the naphthalene ring. The (a*S*)- and (a*R*)-MNCB shifts [4, 18] $\delta\Delta$ ($=\delta$**2**–δ**4a** and δ**4b**) in the ^1H NMR spectra were positive values except that of H-7 (Figure 2A and Fig. 2B). The negative $\Delta\delta$ values for H-7 indicate that H-7 locates close to the carbonyl group and is anisotropically affected more intensively by the carbonyl group than by the naphthalene ring in both esters. The presumable conformations of the esters are depicted in Fig. 2A and Fig. 2B. The values of the MNCB shifts were arranged according to the conformation in each ester. NOE correlations in the (a*R*)-MNCB ester (**4b**) also supported the conformations mentioned above and revealed the absolute configuration of **2** (Figure 2B). The $\Delta\delta$ ($=\delta$**4a**–δ**4b**) values were systematically arranged (Figure 2C). It was concluded that the MNCB method is applicable to tertiary alcohols, when the conformation of esters are reasonably determined.

As an additional line of proof, **2** was transformed to a secondary allylic alcohol (Scheme 1). Dehydration of **2** with thionyl chloride in pyridine [19] gave a mixture of two alkenes **5** and **6** (Table 2). Oxidation

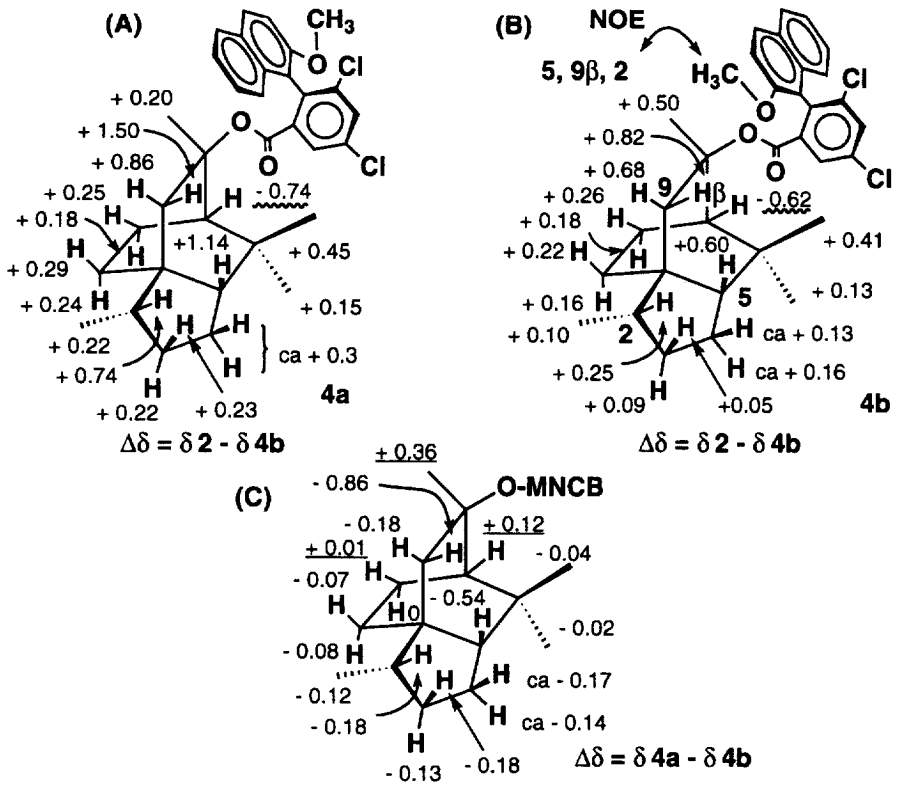
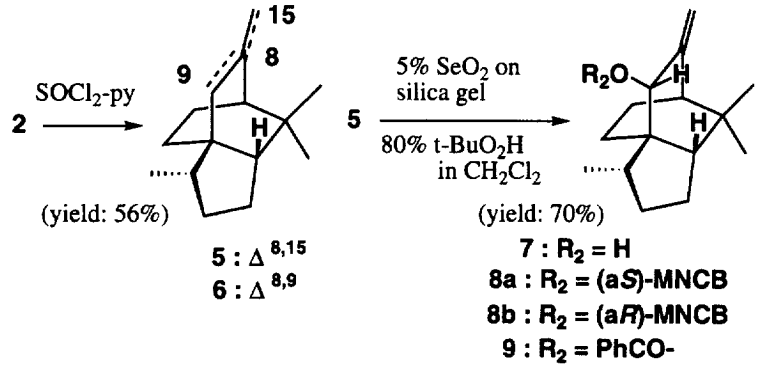


Fig. 2. Shift effects in ^{1}H NMR ($CDCl_3$) caused by MNCB esterification. (A) (a*S*)-MNCB shifts: $\Delta\delta = \delta$**2**-δ**4a**. (B) (a*R*)-MNCB shifts: $\Delta\delta = \delta$**2**-δ**4b**. NOE correlations are also indicated. (C) Chemical shift differences for the MNCB esters of **2**: $\Delta\delta = \delta$**4a**-δ**4b**.



Scheme 1. Chemical conversion of lacinan-8-ol (**2**) to an allylic alcohol (**7**) and its esters (8a, **8b**, **9**).

of **5** with SeO_2 on silica gel and *t*-butylhydroperoxide [20] stereoselectively afforded an allylic alcohol **7**. Esterification of compound **7** with (a*S*)- and (a*R*)-MNCB, and benzoyl chloride yielded **8a**, **8b** and **9**, respectively.

The relative configuration at C-9 in **7** was determined by NOESY and NOE difference spectra (Figure 3A). The *R*-configuration at C-9 in **7** was determined by the application of MNCB method for **8a** and **8b** (Figure 3B and Table 3), and CD allylic benzoate method [8] for **9** (228 nm, $\Delta\epsilon$ +2.40, MeOH: Fig. 3C), respectively. Hence, the absolute configuration of lacinan-8-ol was established as depicted in formula **2**. This finding is in accordance with the conclusion of the ^{1}H NMR MNCB method applied to the tertiary alcohol (**2**).

Four sesquiterpenes with the same skeleton of prelacinan-7-ol (**1**) have been found, such as **10** and **11** in *Eremophila georgei* [21, 22], **10** in *E. metallicorum* [23] (Myoporaceae), **12** in *Juniperus thurifera* (Cupressaceae) [24], **10** in *Fabiana imbricata* (Solanaceae) [25] and **13** in *Acorus calamus* (Araceae) [26]. Three

Table 2. NMR spectral data ($CDCl_3$) for compounds **5** and **6**.

	5		**6**	
C	δ ^{13}C	δ ^{1}H (*J* Hz)	δ ^{13}C	δ ^{1}H
1	44.40		49.06	
2	41.60	1.47 m	38.38	1.86
3	31.75	α 1.24 m	31.75	1.27
		β 1.82 m		1.89
4	22.10	α ca 1.54 m	22.52	1.51
		β ca 1.54 m		1.45
5	54.56	1.25 m	56.01	1.17
6	32.81		32.63	
7	48.88	1.65 dd (2,3.5)	49.45	1.78
8	151.86		143.47	
9	40.63	α 2.34 ddd (2.5,2.5,14)	128.66	5.71
		β 1.72 dddd (2.5,2.5,11.5,14)		
10	18.15	α 1.23 m	16.80	1.16
		β 1.11 dddd (2.5,2.5,11.5,13.5)		0.85
11	22.17	α 1.88 dddd (2.5,3.5,8,12)	22.66	1.79
		β 1.34 ddd (2,7,12,13.5)		1.03
12	14.43	0.79 d (7)	15.13	0.89
13	32.08	0.91 s	33.03	0.81
14	24.04	0.91 s	24.55	0.88
15	106.79	*E* 4.68 ddd (2,2.5,2.5)	21.90	1.80
		Z 4.71 ddd (2,2.5,2.5)		[2]

Assignments were based on DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY and difference NOE experiments.

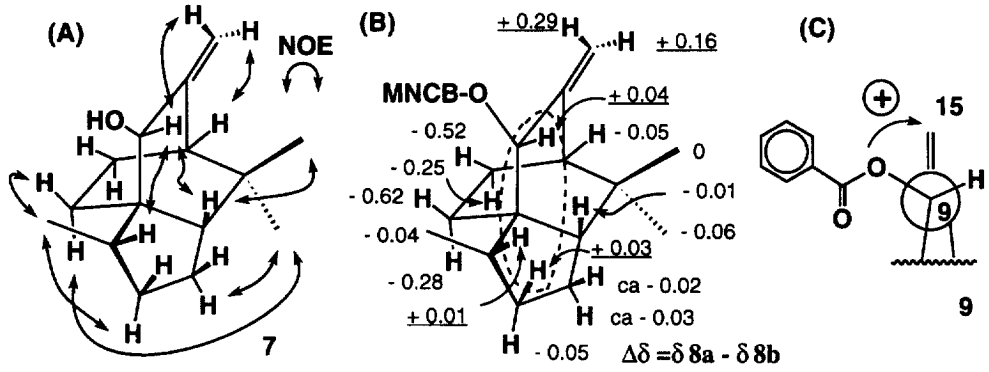


Fig. 3. (A) Key NOEs detected for compound **7**. (B) $\Delta\delta$ ($=\delta$**8a**-δ**8b**) values in ^{1}H NMR ($CDcl_3$). Protons encircled by dotted line are under the border place [5]. (C) Newman projection across the C-9-C-8 bond in compound **9**.

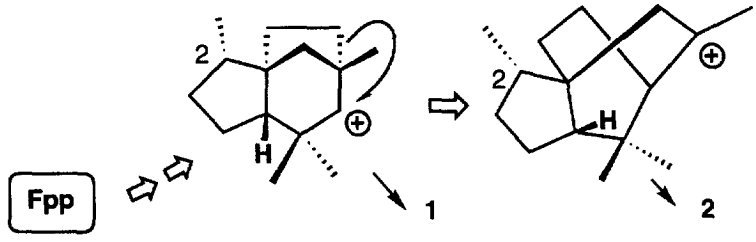
sesquiterpenes (**14-16**) with the same skeleton of **2** have also been known like **14** in *Cupressus dupreziana* [27], **14** and **15** in *Juniperus thurifera* [24], and **16** in *J. chinensis* [28] (Cupressaceae). Among the compounds, only **13** possesses a methyl group of α-configuration at C-2 position. The occurrence of **1** and **2** in the same plant is interesting in the light of their biogenesis (Scheme 2) [24, 27, 29].

Compounds **1** and **2** showed inhibition in growth of seedlings of *Nasturtium officinale* and *Phleum pratense* at 50 ppm, while compound **3** showed inhibition in seed germination and seedling growth at 50 ppm

Table 3. NMR spectral data ($CDCl_3$) for compound **7** and its MNCB esters (**8a** and **8b**).

	7		MNCB esters	
C	δ ^{13}C	δ ^{1}H (J Hz)	δ ^{1}H **8a** (a*S*)	**8b** (a*R*)
1	48.01			
2	41.33	1.76 m	1.59	1.58
3	32.63	α 1.24 dddd (5.5,9,10,13)	1.05	1.10
		β 1.87 dddd (7,9.5,12,13)	1.73	1.70
4	22.28	α 1.59 m	ca 1.45	1.48
		β 1.60 m	ca 1.45	1.47
5	52.47	1.11 ddd (2,9,11)	1.06	1.07
6	32.27			
7	48.72	1.75 dd (2,3.5)	1.40	1.45
8	156.76			
9	79.62	3.76 br s	5.14	5.10
10	12.53	α 1.16 dddd (1.5,7,11.5,13)	0.71	0.99
		β 1.29 dd (11,13)	0.39	0.91
11	22.10	α 1.90 dddd (2.5,3,11,13.5)	1.41	1.66
		β 1.47 dddd (2,7,11.5,13.5)	0.39	0.91
12	16.57	0.98 d (6.5)	0.55	0.59
13	31.95	0.85 s	0.71	0.71
14	24.08	0.94 s	0.77	0.83
15	110.67	*Z* 5.08 dd (1.5,3)	4.56	4.40
		E 4.92 dd (1.5,3)	4.55	4.26
OH		1.39 br		

Assignments were based on DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, NOESY and difference NOE experiments.



Scheme 2. Hypothetical biogenesis scheme for sesquiterpenes **1** and **2** in *R. laciniata*. Fpp = farnesyl pyrophosphate.

and 10-20 ppm, respectively (data not shown). Hence it was found that igalan (**3**) is major phytotoxic constituent of this plant. Moreover igalan (**3**) exhibited clear inhibitory zone on the TLC bioautogram using *Cladosporium herbarum* as the test fungus (data not shown).

EXPERIMENTAL

General

Mps are uncorr. EIMS: direct inlet, 70 eV. IR: KBr pellet or NaCl plate. ^{1}H NMR (500 MHz) and ^{13}C NMR (67.8 and 125 MHz) spectra were taken in

$CDCl_3$ or C_5D_5N at room temp., with TMS as int. standard. Silica gel (Wakogel C-200) was used for CC and silica gel 60 F_{254} (Merck) for TLC. Compounds on TLC plates were detected under UV light (254 nm) or by colour produced using phosphomolybdic acid reagent.

Growth-regulation assay for N. officinale *and* P. pratense

The assay method employed was originally described by Stevens and Merrill [29] using *N. officinale* and *P. pratense* seeds purchased from a local market. To 1 ml of molten agar (0.5%) in a test tube (12 mm i.d. × 105 mm) was added 10 μl of a DMSO soln containing an appropriate amount of the test compound. The contents in the tube were mixed thoroughly, and the final concentration of each test compound was adjusted to 5, 10, 20, 50 and 100 ppm. For the control experiments, 10 μl of DMSO was added to the agar medium. Ten to fifteen seeds of each plant were sown on the medium and cultivated at 25° with a day length of 14 hr under fluorescent light. The percentage seed germination of and root length of the hypocoyl (*N. officinale*) or coleoptyl (*P. pratense*) were measured 7 days after sowing, with the mean of duplicate experimental results being calculated thereafter.

Extraction and isolation

Roots of *R. laciniata* were collected in Sapporo in September in 1991. The fresh roots were sliced into pieces and extracted twice with EtOAc at room temp. This EtOAc extract showed phytotoxic activity, and was subjected on prep. TLC plates developed with hexane: EtOAc (4:1). This separation gave active bands at R_f 0.22–0.43. Rechromatography of these bands on silica gel plates, eluted with CH_2Cl_2 also gave active bands at R_f 0.25–0.40. From these, three active compounds (**1**–**3**) were obtained in small amount. This was then repeated at a larger scale as follows. Thus the EtOAc extract (7.2 g) from roots (fr. wt 2.0 kg) was fractionated into 16 sub-fractions by CC on 100 g of silica gel to give frs 1–5 (hexane), frs 6–8 (hexane:EtOAc, 9:1), frs 9–11 (hexane:EtOAc, 4:1), frs 12–14 (hexane:EtOAc, 2:1) each 100 ml, fr. 15 (EtOAc, 300 ml) and fr. 16 (MeOH, 200 ml). Frs containing **1**, **2** and **3** were further purified to give **1** (60 mg), **2** (150 mg) and **3** (430 mg), respectively.

Lacinan-8-ol (**2**). Colourless plates from hexane; mp 75.0–76.0°; $[\alpha]_D^{25}$ +98.7° ($CHCl_3$; *c* 0.60). EIHR-MS: *m/z* 222.1989 $[M]^+$, $C_{15}H_{26}O$ requires 222.1985. EIMS, *m/z* (rel. int): 222 $[M]^+$ (10), 207 $[M\text{-}Me]^+$ (18), 204 $[M\text{-}H_2O]^+$ (21), 189 (17), 161 (44), 149 (56), 137 (27), 119 (94), 43 (100). IR $\nu_{max}^{KBr}cm^{-1}$: 3380 (OH), 2970, 2930, 2870, 1445, 1360, 1160, 1140, 1100. ^1H and ^{13}C NMR: Table 1.

Igalan (**3**). Colourless prisms from hexane-Et_2O; mp 79.0–80.0°, lit. [10], 79.0–79.5°; $[\alpha]^{25}{}_D$ +102.3° ($CHCl_3$; *c* 0.17, lit. [10], +105°, lit. [11], +44.9°). EIHR-MS: *m/z* 232.1457 $[M]^+$, $C_{15}H_{20}O_2$ requires 232.1464. The physicochemical properties of **3** coincided well with those of igalan, secoeudesmanolid or elemasteiractinolide [10–14].

Esterification of compound **2** *with* (*a*S)- *and* (*a*R)-*MNCB*

To a soln of **2** (21.0 mg, 95 μmol), (a*S*)-MNCB (19.0 mg, 55 μmol) and 4-pyrrolidinopyridine (2.0 mg, 13 μmol) in CH_2Cl_2 (300 μl) was added DCC (15.0 mg, 73 μmol), and the solution was stirred at room temp. for 5 days. The reaction mixt was directly applied to prep. TLC (hexane:EtOAc, 10:1) to give the (a*S*)-ester (**4a**) (4.8 mg, 16%). Under essentially the same conditions, **4b** was obtained in 6% yield. Compounds **4a** and **4b**; ^1H NMR: for spectral analysis, see Table 1. ^{13}C NMR (67.8 Hz, $CDCl_3$) of **4a**: δ 14.02 (C-12), 17.36 (C-10), 19.35 (C-11), 21.62 (C-4), 26.94 (C-15), 27.21 (C-14), 31.41 (C-3), 32.52 (C-13), 33.60 (C-6), 40.81 (C-1), 43.25 (C-2), 44.38 (C-7), 47.53 (C-9), 51.81 (C-5), 87.73 (C-8), (δs of carbons in MNCB moiety 56.41, 113.10, 123.56, 124.60, 126.68, 128.03, 128.89, 128.93, 129.81, 132.06, 133.06, 133.94, 136.82, 137.50, 153.42, 165.35). EIMS of **4a**, *m/z* (rel. int): 550 $[M]^+$ (0.9), 348 (64), 346 $[M\text{-}C_{15}H_{24}]^+$ (100), 329 (8), 252 (7), 69 (7).

Dehydration of compound **2** *to alkene* **5** *and* **6**

To a stirred soln of **2** (123.2 mg, 554 μmol) in CH_2Cl_2-pyridine (6 ml, 5:1) was added dropwise a soln $SOCl_2$ (200 μl) in CH_2Cl_2 (1 ml) at 0°. The reaction soln was stirred for 1 hr at 0° followed by dilution with hexane (5 ml), and directly chromatographed on 10 g of silica gel with hexane to afford a mixt of alkenes (93.1 mg). These were separated by CC on 15 g of $AgNO_3$-silica gel (1:4), eluted with hexane, to give **5** (27.8 mg) and **6** (34.7 mg), respectively.

Compound **5**. Colourless oil; $[\alpha]_D^{25}$ +46.9° ($CHCl_3$; c 1.10). EIMS, *m/z* (rel. int): 204 $[M]^+$ (31), 161 (24), 149 (58), 138 (75), 119 (100), 71 (52), 57 (79), 43 (69). IR $\nu_{max}^{neat}cm^{-1}$: 3065 (C=CH_2), 2830, 2725, 1360, 1401, 1320, 1280, 780. ^1H and ^{13}C NMR see Table 2.

Compound **6**. Colourless oil; $[\alpha]_D^{25}$ +64.2° ($CHCl_3$; c 0.80). EIMS, *m/z* (rel. int): 204 $[M]^+$ (50), 161 (27), 133 (25), 119 (100), 105 (36), 93 (33), 91 (27), 77 (16), 55 (16), 41 (29). IR $\nu_{max}^{neat}cm^{-1}$: 3000, 2940, 2860, 1435, 1365, 1355, 785 for ^1H and ^{13}C NMR spectral analyses: Table 2.

Oxidation of compound **5** *to allylic alcohol* **7**

Selenium dioxide supported on silica gel (5%, 30 mg) and 80% *t*-butylhydroperoxide (20 mg, 177 μmol) were mixed in CH_2Cl_2 (300 μl) and stirred for 15 min at room temp. To this soln was added dropwise a soln of compound **5** (11.7 mg, 57 μmol) in CH_2Cl_2 (50 μl) with stirring. After 1 hr, the reaction

mixt was directly applied to prep. silica gel TLC (H-EA 10:1) to yield the allylic alcohol (**7**) (8.8 mg, 70%).

Compound **7**. Colourless prisms from hexane-EtOAc; mp 73.5–75.0°; $[\alpha]_D^{25}$ +71.6° ($CHCl_3$; *c* 0.74). EIMS, m/z (rel. int): 220 $[M]^+$ (30), 202 $[M-H_2O]^+$ (49), 187 (62), 159 (89), 145 (77), 69 (92), 41 (100). For ^1H and ^{13}C NMR spectral analysis, see: Table 3.

Esterification of compound **7** *with* (*a*S)-*and* (*a*R)-*MNCB*

To a soln of compound **7** (2.2 mg, 10 μmol), (a*S*)-MNCB (5.0 mg, 55 μmol) and 4-pyrrolidinopyridine (1.0 mg, 7 μmol) in CH_2Cl_2 (300 μl) was added DCC (5.0 mg, 24 μmol), and the solution was stirred at room temp. for 8 hr. The reaction mixt was directly applied to prep. TLC (hexane:EtOAc, 5:1) to give the (a*S*)-ester (**8a**) (3.7 mg, 64%). Under essentially the same conditions, **8b** was obtained in 87% yield. Compounds **8a** and **8b**. For ^1H NMR: spectral analysis, see: Table 3. EIMS of **8a**, *m/z* (rel. int): 550 $[M+2]^+$ (10), 548 $[M]^+$ (14), 348 (68), 346 $[M-C_{15}H_{22}]^+$ (100), 252 (8), 91 (7).

Esterification of compound **7** *with benzoyl chloride*

To a soln of **7** (1.7 mg, 8 μmol), Et_3N (1.5 mg, 15 μmol) and 4-dimethylaminopyridine (1.0 mg, 8 μmol) in CH_2Cl_2 (200 μl) was added benzoyl chloride (2.1 mg, 15 μmol), and the solution was stirred at room temp for 3 hr. The reaction mixt was directly applied to prep. TLC (hexane:CH_2Cl_2, 1:5) to give the benzoate **9** (0.9 mg, 50%)as a colourless semi-solid. UV λ_{max} (MeOH) nm (log ε): 229.4 (3.93). CD: $\Delta\varepsilon_{228.0}$ +2.40 (MeOH, *c* 3.94×10^{-4}M, 25°). EIMS, *m/z* (rel. int): 324 $[M]^+$ (0.5), 202 (26), 187 (26), 159 (34), 145 (32), 122 (43), 105 (100), 91 (24), 77 (51), 69 (30), 43 (68), ^1H NMR ($CDCl_3$): 0.78 (3H, *d*, *J*=6.4 Hz), 0.92 (3H, *s*), 0.98 (3H, *s*), 1.1–2.1 (11H), 4.95 (1H, *d*, *J*=0.9 Hz), 5.18 (1H, *d*, *J*=0.9 Hz), 5.47 (1H, *s*), 7.44 (2H, br *dd*, *J*=7.2, 8.2 Hz), 7.55 (1H, *t*, *J*=7.2 Hz), 8.05 (2H, *d*, *J*=8.2 Hz).

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