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ISOALANTOLACTONE DERIVATIVES AND GERMACRANOLIDES FROM *BLUMEA DENSIFLORA*

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Key Word Index—*Blumea densiflora*; Inuleae; Compositae; sesquiterpene lactones; isoalantolactone derivatives; germacranolides

Abstract—The germacranolides tagitinin A and tirotundin ethyl ether and a series of new ring A-hydroxylated isoalantolactone derivatives were isolated from *Blumea densiflora*. Structures were established by spectroscopic methods and chemical transformations.

INTRODUCTION

Phytochemical studies of the large genus *Blumea* have been spasmodic [1]; recent reports describe mainly monoterpenoids or flavonoids [2–7]†. We now report on the isolation from *Blumea densiflora* (Wall.) DC. of the sesquiterpene lactones tirotundin ethyl ether (**1**) [9, 10], tagitinin A (**2**) [11–13] and a family of new hydroxylated isoalantolactones derivatives **3a, b**, **4a, c**, **5a, b** and **6a, b**. Compounds **2**, **4a** and **6a** were the major lactone constituents.

RESULTS AND DISCUSSION

That **3a** and **3b** were the 3-(α-methylbutyryl) and the 3-isobutyryl esters of granilin (**3d**) [14–16] was evident from a comparison of their ^{1}H NMR spectra (Table 1) with the NMR spectrum of granilin itself [16], the H-3 signal appearing at considerably lower field. All assignments were confirmed by spin decoupling. The NMR spectrum of the monoacetate **3c** was comparable with that of diacetylgranilin (**3e**) [16]. Lactone **4a**, on NMR spectral evidence and the facile formation of a 1,2-acetonide **7** (Table 2), carried an additional hydroxyl group on C-2; the α-orientation assigned to the latter was based on the coupling constants observed more readily in the derived diacetate **4b** ($J_{1,2} = 3.5$, $J_{2,3} = 4$ Hz). In lactone **4c** the hydroxyl group on C-1 was acetylated as shown by the shift of H-1 to considerably lower field.

Comparison of the NMR spectra also showed that lactone **5a** was an isomer of **4a** in which the C-2 hydroxyl carried the 2-methylbutyryl ester side chain and that **5b** was the corresponding isomer of **4c**. The H-1β, H-2β, H-3β stereochemistry assigned to **4a, c** and **5a, c** was confirmed by the NOE difference spectrum of **5b** (Table 3): saturation of equatorial H-3β produced only enhancement of the H-2β and H-15a resonances whereas saturation of axial H-2β resulted in enhancement of the H-14 as well as the H-3β resonance. Conversely, irradiation at the frequency of H-14 increased in the intensity of H-2β and of H-1β.

Lactone **6a**, which could not be freed completely from **5a**, and its acetate **6b**, which also occurred naturally, were α,β-unsaturated ketones with an α-methylbutyrate ester on C-3 (NMR spectrum). A chemical correlation was effected by silver carbonate–celite oxidation of **4a** to **6a**. The selective oxidation by this reagent of the equatorial and possibly less hindered C-2 hydroxyl group of vicinal glycol **4a** with concomitant migration of the double bond is of interest. That the C-1 hydroxyl group of **6a** retained its axial orientation was shown by the NOE difference spectrum of **6b**, saturation of the H-1β resonance producing a 5% enhancement of the H-14 signal and saturation of the H-14 resonance producing a 4.2% intensity increase in the H-1 resonance. Reduction of **6a** with zinc–acetic acid resulted in deoxygenation to **9** whose NMR spectrum is listed in Table 2.

The following additional transformations of **4a**, the lactone present in largest amount, were carried out. Oxidation with *m*-chloroperbenzoic acid produced approximately equal amounts of two epimeric epoxides **8a** and **8b** which were easily distinguishable by the chemical shift of H-2 and H-3 (Table 2), the isomer exhibiting the H-2 signal at much lower field being assigned formula **8b** Hydrolysis (KOH–MeOH) afforded a mixture of **4d** and two methanol adducts, mainly the epimer **10a**, which after acetylation was separated into **4e** and **10b**. Catalytic hydrogenation of **4a** gave **11a** characterized as the diacetate **11b**; the C-11 stereochemistry assigned to these compounds, as well as to **10a** and **10b**, is based on the magnitude of $J_{7,11}$ (7 Hz), the result of reagent approach from the least hindered side. Sodium borohydride reduction of a mixture of **5a** and **6a** gave **13** (from **6a**) and a mixture of **11a** (also from **6a**) and **12a** (from **5a**), which was separated after acetylation into **11b** and **12b**, thus providing a second correlation between **4a** and **6a**.

Sesquiterpene lactones have not been found previously in *Blumea* species nor are they common in other rep-

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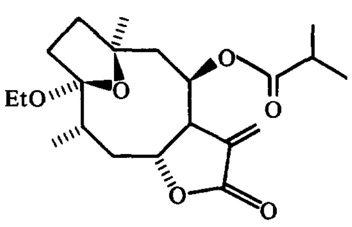
† A collection originally described [5] as *Inula cappa* was later [8] found to represent *Blumea balsamifera* DC. The last-named taxon was also studied by the authors of ref. [7] who isolated similar but not identical dihydroflavonols.



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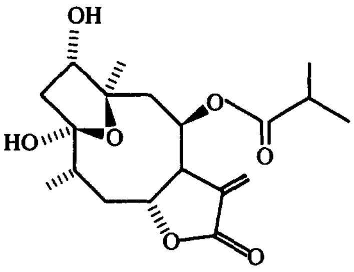

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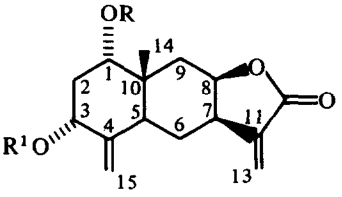
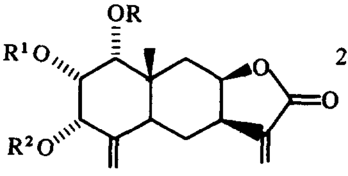
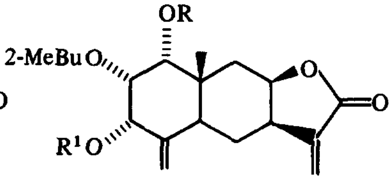


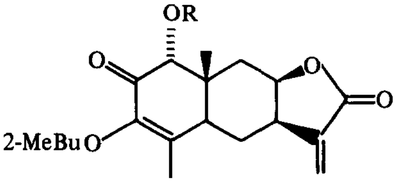
3a R = H, R^1 = 2-MeBu
3b R = H, R^1 = iBu
3c R = Ac, R^1 = 2-MeBu
3d R, R^1 = H
3e R, R^1 = Ac



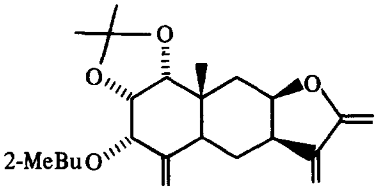
4a R, R^1 = H, R^2 = 2-MeBu
4b R, R^1 = Ac, R^2 = 2-MeBu
4c R = Ac, R^1 = H, R^2 = 2-MeBu
4d R, R^1, R^2 = H
4e R, R^1, R^2 = Ac



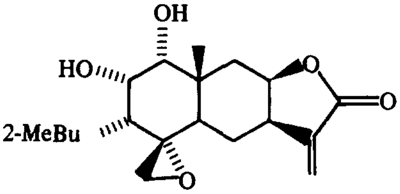
5a R, R^1 = H
5b R, R^1 = Ac



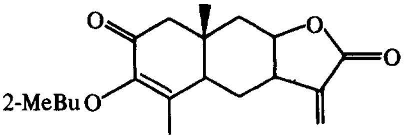
6a R = H
6b R = Ac



7



8a
8b C-4 epimer



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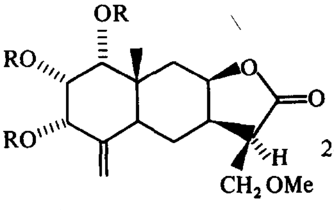
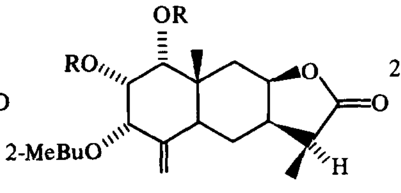
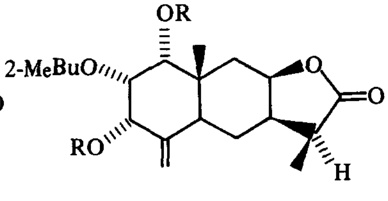


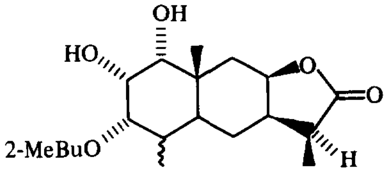
10a R = H
10b R = Ac



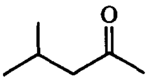
11a R = H
11b R = Ac



12a R = H
12b R = Ac



13

2-MeBu =


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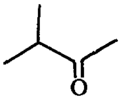

Table 1. ^{1}H NMR spectra of compounds 3–6 (270 MHz, $CDCl_3$)*

H	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	**4d**	**4e**	**5a**	**5b**	**6a**	**6b**
1	3.44 *m*	3.44 *m*	4.67 *d* (*br*) (3)	3.52 *m*	5.05 *d* (*br*) (3.5)	5.04 *d* (*br*) (4)	3.50 *m*	5.05 *d* (*br*) (3.5)	3.61 *m*	5.14 *d* (*br*) (3)	3.62 *br*	5.04 *br*
2a	2.18 *dt* (15.5, 3)	2.17 *dt*	2.11¶	4.00 *m*	5.18 *dd* (4, 3.5)	4.08 *t* (4)	3.80 *m*	5.15 *dd* (4, 3.5)	4.94 *t*	5.07 *t*	—	—
2b	2.04 *dt* (15.5, 3)	2.04 *dt*		—	—	—	—	—	—	—	—	—
3	5.55 *t* (*br*) (3)	5.54 *t* (*br*)	5.44 *t* (*br*)	5.60 *dd* (4, 1)	5.68 *dd*	5.55 *d* (*br*)	4.38 *m*	5.67 *d* (*br*)	4.50 *d* (*br*)	4.39 *d* (*br*)	—	—
5	2.52 *dd* (*br*) (12, 3)	2.54 *dd* (*br*)	2.68 *dd* (*br*)	2.50 (obsc.)	2.62 *dd* (*br*) (12, 3)	2.50 *dd* (*br*)	‖	2.67 *dd* (*br*)	2.70 *dd* (*br*)	2.67 *dd* (*br*)	2.96 *dd* (*br*)	2.86 *dd* (*br*)
6a	1.81 *ddd* (13, 7, 3)	1.81 *ddd*	1.81 *ddd*	1.84 *ddd*	1.82 *ddd*	1.82 *ddd*	‖	1.86 *ddd*	2.11 *ddd*	1.85 *ddd*	2.12 *ddd*	2.10 *ddd*
6b	1.50 *m*	1.45 *q* (12.5)	1.46 *dt* (13, 12)	1.42 *dt*	1.42 *dt*	1.44 *dt*	‖	**	1.47 *dt*	1.45 *dt*	1.46 *ddd*	1.46 *ddd*
7	3.02 *dd* (*br*) (12, 7, 5)	3.03 *ddd* (*br*)	3.06 *ddd* (*br*)	3.04 *ddd* (*br*)	3.06 *ddd* (*br*)	3.03 *ddd* (*br*)	3.03 *m*	3.07 *ddd* (*br*)	3.07 *ddd* (*br*)	3.02 *ddd*	3.10 *ddd* (*br*)	3.10 *ddd* (*br*)
8	4.63 *dt* (2.5, 5)	4.64 *dt*	4.59 *dt*	4.64 *dt*	4.58 *dt*	4.56 *dt*	‖	4.59 *dt*	4.64 *dt*	4.65 *dt*	4.64 *dt*	4.60 *dt*
9a	2.35 *dd* (16, 5)	2.49 *dd*	1.98¶	2.44 *m*	2.02 *dd* (16, 2)	2.06 *dd*	‖	**	2.44 *dd*	2.04 *dd*	2.37 *dd*	2.10 *dd*
9b	1.94 *dd* (16, 2)	1.95 *d* (*br*) (16)		2.02 *dd* (16, 2)	1.82 *dd* (16, 5)	**	‖	1.86 *dd*	1.96 *dd*	1.71 *dd*	1.98 *dd*	**
13a	6.16 *d* (1)	6.16 *br*	6.16 *d*	6.18 *d*	6.18 *d*	6.18 *d*	6.18 *br*	6.20 *br*	6.18 *d* (1)	6.18 *d*	6.21 *d* (1)	6.22 *d*
13b	5.62 *d* (1)	5.62 *br*	5.62 *d*	5.64 *d*	5.64 *d*	5.63 *d*	5.65 *br*	5.64 *br*	5.63 *d* (1)	5.64 *br*	5.69 *d* (1)	5.70 *d*
14†	0.84	0.84	0.93	0.86	1.02	0.97	0.86	1.04	0.91	1.00	1.05	1.15
15a	5.26 *br*	5.26 *br*	5.28 *br*	5.34 *d* (1)	5.39 *d*	5.39 *br*	5.22 *br*	5.38 *br*	5.22 *br*	5.25 *br*		
15b	4.85 *d* (*br*) (1.5)	4.86 *br*	4.86 *d* (*br*)	4.90 *d* (1.5)	4.95 *d*	4.93 *d* (*br*)	4.83 *br*	4.96 *d* (*br*)	4.84 *d*	4.85 *d* (2)	1.82 *d*† (1.5)	1.84 *d*†
Misc	‡	§	2.13 (Ac)†	2.70 *m* (OH)	2.16 (Ac)†	2.19 (Ac)†		2.17 (Ac)†	‡	2.17 (Ac)†		
			†	2.62 *d* (6) (OH)	1.96 (Ac)†	‡		2.10 (Ac)†		‡	‡	‡

*Unmarked signals are singlets. Figures in parentheses are coupling constants in Hz and are not listed if they correspond to those in the preceding column.

†Intensity three protons.

‡Signals of 2-MeBu side chain—H-2′ near 2.40 *sex* (7), H-3′, near 1.65 *m* and 1.50 *m*, H-4′ near 0.9 *t*†, H-5′ near 1.15 *d* (7)†.

§Signals of *i*-Bu side chain—H-2′ at 2.50 *m*, H-3′ and H-4′ at 1.19 *d* (7)† and 1.18 *d* (7)†.

‖Overlapping signal.

¶Centre of AB system.

**Obscured signal.

Table 2 ^{1}H NMR spectra of **7**–**13** (270 MHz, $CDCl_3$)*

H	**7**	**8a**	**8b**	**9**	**10a**	**10b**	**11a**	**11b**	**11b**‖	**12a**	**12b**	**13**
1	3.94 *d* (6)	3 63 *m*	3.59 *m*	¶	3.50 *m*	5.02 *d* (*br*) (3.5)	3 49 *m*	5 03 *d* (*br*)	5.17 *d* (*br*)	3.60 *m*	5.04 *d* (*br*)	3.68 *m*
2	4.41 *t* (6)	3.69 *dd* (3.5, 1.5)	4.18 *m*	—	3.80 *m*	5.16 *dd* (4, 3.5)	3.99 *t* (4)	5.18 *t*	5 20 *t*	4.94 *t*	5.16 *t*	4 24 *m*
3	5.59 *d* (*br*) (5.5)	5.11 *t* (3.5)	4.88 *dd* (4.1)	—	4.38 *m*	5.66 *d* (*br*) (7)	5.58 *dd* (4.1)	5.67 *d* (*br*)	5.82 *d* (*br*)	4.50 *m*	5.69 *d* (*br*)	5.15 *t*
5	2.62 *dd* (*br*) (12.3)	1.85 *c*	¶	¶	¶	2.64 *dd* (*br*)	2.63 *dd* (*br*)	2.56 *dd* (*br*)	2.40 *dd* (*br*)	2.63 *dd* (*br*)	2.60 *dd* (*br*)	¶
6a	1.02 *ddd*	1.40 *c*	¶	2.12 *ddd*	¶	1 79 *ddd*	1.84 *ddd*	1.80 *m*	**	¶	1.60 *m*	¶
6b	1.519 (12.5)		¶			**	**	1.50 *m*	**	¶	1.60 *m*	¶
7	3.04 *ddd* (*br*) (12, 7, 5)	2.96 *ddd* (*br*)	2.99 *m*	3.07 *m*	¶	2.64 (obsc.)	2.40 *m*	2.47 *m*	1.30 *m*	¶	2.48 *m*	¶
8	4.61 *dt* (2, 5)	4.62 *dt*	4.62 *dt*	4.54 *dt*	4.64 *dt*	4.58 *dt*	4.60 *dt*	4.55 *dt*	3.71 *dt*	4.61 *dt*	4.55 *dt*	4.57 *dt*
9a	2.26 *dd* (16, 5)		¶	¶	¶	**	2.40 *m*	2.02 *dd*	1.65 *dd*	¶	2.02 *dd*	¶
9b	2.02 *dd* (16, 2)	1.95 *dd*	¶	¶	¶	1.84 *dd*	1.97 *dd*	1.80	1.44 *dd*	¶	1.78 *dd*	1.75 *dd*
11	—	—	—	—	3.14 *ddd* (10, 6, 5 5)	3.14 *ddd* (10, 6.5, 5)	2.86 *quint* (7)	2.85 *quint*	1.95 *quint*	2.86 *quint*	2.86 *quint*	2.87 *quint*
13a	6.18 *d* (1)	6.15 *d*	6.15 *d*	6.21 *br*	3.80 *m*	3.80 *dd* (10, 5)	1.23 *d*† (7)	1.25 *d*†	0.87 *d*†	1.24 *d*†	1.25 *d*†	1.23 *d*†
13b	5.63 *d* (1)	5.60 *d*	5.60 *d*	5.68 *br*	3.63 *t* (10)	3.61 *t* (10)						
14†	0.95	1.07	1.02	1.10	0.85	1.02	0.83	1.01	0.75	0.88	1.01	1.14
15a	5.26 *br*	2.89 *d* (4)	2.86 *d*	1.80 *br*†	5.22 *br*	5.39 *br*	5.33 *br*	5 39 *br*	5.00 *br*	5.23 *br*	5.39 *br*	1.07 *d*† (7)
15b	4.97 *br*	2.76 *d* (4)	2.76 *d*		4.86 *br*	4.99 *d* (*br*) (1.5)	4.94 *d* (*br*)	4.98 *d* (*br*)	4.43 *d* (*br*)	4.87 *br*	4.98 *br*	
Misc	1.54†§ 1.37†§ ‡	‡	‡	‡	3.40 (OMe)	3.41 (OMe)† 2.17 (Ac)† 2.09 (Ac)† 1.99 (Ac)†	‡	2.17 (Ac)† 1.96 (Ac)† ‡	1.92 (Ac)† 1.76 (Ac)† ‡	‡	2.08 (Ac)† ‡	‡

*Comments in Table 1 apply.

†Intensity three protons.

‡Signals of Me Bu side chain as in Table 1.

§Methyls of acetonide.

¶Overlapping signals.

‖In C_6D_6

**Obscured signals.

Table 3. NOE difference spectrum of **5b**

Saturation	Observed NOE (%)
H-3	H-2 (8.7) H-15a (8)
H-2	H-3 (7.3) H-14 (9)
H-14	H-1 (3.6) H-2 (5)

resentatives of the *Pluchea* group of subtribe Inulinae *s.l.* to which *Blumea* has been assigned [17, 18]. However, only about 15 taxons have been examined thoroughly. Among these *Pluchea dioscorides* furnished two eudesmanolides [19, 20] and *Pechuel-loeschia leibnitziae* a guaianolide [21]. Further work on other members of the group is clearly desirable.

EXPERIMENTAL

Isolation of constituents of B. densiflora. Aerial parts of *B. densiflora* (Wall) DC. (2 kg), collected in Doinukh, Itanagar, Arunachal Pradesh, India, in December 1982 (voucher on deposit in herbarium of RRL, Jorhat) were extracted with $CHCl_3$ in a Soxhlet apparatus. The crude extract was concentrated (reduced pressure), dissolved in 200 ml MeOH containing 10% H_2O, allowed to stand overnight and filtered. The filtrate was extracted with petrol (60–80°), concentrated (reduced pressure) and extracted with $CHCl_3$ (5 × 100 ml). The combined $CHCl_3$ extracts were dried and evaporated (reduced pressure); the crude residue (8 g) was chromatographed over 200 g silica gel packed in C_6H_6, 200 ml fractions being collected as follows: Fr. 1–12 (C_6H_6), 13–15 (C_6H_6–$CHCl_3$, 21:1), 16–20 (C_6H_6–$CHCl_3$, 9:1), 21–24 (C_6H_6–$CHCl_3$, 5:1), 25–29 (C_6H_6–$CHCl_3$, 2.1), 30–35 (C_6H_6–$CHCl_3$, 1:1), 36–41 (C_6H_6–$CHCl_3$, 1:2), 42–51 ($CHCl_3$), 52–54 ($CHCl_3$–MeOH, 50·1), 55–63 ($CHCl_3$–MeOH, 20:1), 64–70 ($CHCl_3$–MeOH, 9:1), 71–73 ($CHCl_3$–MeOH, 4:1), 74–75 ($CHCl_3$–MeOH, 1:1).

Fr 48–55 which exhibited the same five spots on TLC (EtOAc–petrol, 1:2) were combined (0 45 g) and separated by preparative TLC using the same solvent system (3 developments). In order of increasing polarity, the constituents were tirotundin ethyl ether (**1**, 15 mg), identified by comparison with authentic material [9], **3a** (25 mg), **6b** (45 mg), **5b** (70 mg) and **3b** (8 mg). Fr. 56–59 (2.6 g) which contained two major and two minor spots were combined and purified by preparative TLC (EtOAc–petrol, 1:2, three developments). The constituents were **4a** (800 mg), **6a** (600 mg), **4c** (12 mg) and **5a** (18 mg). Preparative TLC (C_6H_6–EtOAc, 1:1) of fr. 68 and 69 (wt. 825 mg) which exhibited one major spot gave tagitinin A (**2**) identified by comparison with authentic material [13].

1α-Hydroxy-3α(2-methylbutanoyloxy)-isoalantolactone (**3a**). Gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 2900, 1775, 1750, 1650, 1385, 1335, 1275, 1140, 1075, 1060, 975, 810; MS *m/z* (rel. int.): 349 $[M+1]^+$ (4), 246 (82), 228 (83.6), 85 (100). [Calc. for $C_{20}H_{28}O_5$: M_r, 348.1935. Found: M_r (MS), 348.1950].

Acetylation of 10 mg **3a** (Ac_2O–pyridine) followed by the usual work-up gave 8 mg **3c** as a gum whose ^1H NMR spectrum is listed in Table 1; IR 1775, 1745, 1735, 1675, 1555, 1470, 1380, 1350, 1270, 1250, 1225, 1180, 1160, 1125, 1050, 975 and 825 cm^{-1}; MS *m/z* (rel. int.): 391 $[M+1]^+$, 348 (0.2), 330 (0.5), 288 (3.2), 246 (7.9), 228 (100), 85 (83). [Calc. for $C_{22}H_{31}O_6$ and $C_{22}H_{30}O_6$: M_r+H and M_r; 391.2118 and 399.2040. Found: M_r+H and M_r (MS), 391.2110 and 399.2052].

1α-Hydroxy-3α-isobutyryloxyisoalantolactone (**3b**). Gum; IR ν_{max} cm^{-1}: 3500, 1765, 1750, 1645, 1535, 1475, 1390, 1270, 1225, 1165, 1080, 1040, 1015, 1000, 970, 925, 890, 875; MS *m/z* (rel. int.): 334 $[M]^+$ (0.5), 246 (38), 228 (34.6), 71 (100).

1α,2α-Dihydroxy-3α(2-methylbutanoyloxy)-isoalantolactone (**4a**). Gum; IR ν_{max} cm^{-1}: 3500 (br), 1765, 1730, 1655, 1455, 1375, 1340, 915. The low resolution MS gave no $[M]^+$ but had significant peaks at *m/z* (rel. int.): 280 $[M-84]^+$ (0.4), 262 (2.5), 244 (5.6), 226 (2.5), 85 (40), 57 (100); MS (CI, isobutane) *m/z* (rel. int.): 365 $[M+1]^+$ (35.6), 349 (10.1), 347 (19.2), 263 (77.8), 261 (23.0), 245 (20.8), 227 (14.2), 225 (10.9), 223 (23.6), 211 (11.4), 207 (15.3), 197 (100), ^{13}C NMR spectrum (67.89 MHz, $CDCl_3$): 77.89 *d*, 77 32 *d*, 77.01 *d*, 67.64 (C-1, C-2, C-3 and C-8), 143.41 *s* (C-4), 34.07 *d* (C-5), 26.77 *t* (C-6 or C-3′), 40.16 *d* (C-7), 33.53 *t* (C-9), 37 70 *s* (C-10), 141.79 *s* (C-11), 120.58 *t* (C-13), 17 69 *q* (C-14), 116.26 *t* (C-15), 176.10 *s* (C-1′), 41.37 *d* (C-2′), 26.45 *t* (C-3′ or C-8), 11.43 *q* (C-4′), 16.40 *q* (C-5′). The C-12 signal was not detected.

Acetylation of 10 mg **4a** followed by the usual work-up and TLC (petrol–EtOAc, 2:1) gave 7 mg **4b**, gum; IR ν_{max} cm^{-1}: 1765, 1740, 1730, 1655, 1460, 1375, 1250, 1150, 1040, 970, 815; MS *m/z* (rel. int.): 449 $[M+1]^+$ (0 04), 338 (0.09), 346 (1.6), 304 (17 4), 286 (9.5), 262 (8.3), 244 (56.5), 226 (16.4), 85 (36), 57 (100).

A soln of 17 mg **4a** in 1 ml Me_2CO containing a crystal of *p*-toluenesulfonic acid was allowed to stand at room temp. overnight. Purification by prep. TLC (petrol–EtOAc, 2:1) afforded 3 mg starting material and 6 mg crystalline acetonide 7, mp 141–143° (EtOAc); IR ν_{max} cm^{-1} 1765, 1730, 1465, 1380, 1275, 1215, 1150, 1075, 1040, 965; MS *m/z* (rel. int.): 404 (0.5), 389 (4.1), 346 (1.2), 328 (0.7), 320 (1.1), 302 (2.4), 262 (17.2), 244 (13.4), 227 (9.9), 215 (7.2), 85 (100). [Calc for $C_{33}H_{32}O_6$. M_r, 404 2196. Found: M_r (MS), 404.2203]

A soln of 42 mg **4a** in 5 ml MeOH and 5 drops 5% aq. KOH was stirred at room temp. (N_2 atmosphere) for 30 min, acidified with dil. HOAc, diluted with H_2O and extracted with EtOAc. Evaporation of the washed and dried extract followed by prep TLC (petrol–EtOAc, 1:1, 3 developments) gave 26 mg of a 1:3 mixture of **4d** and **10a** whose NMR spectra are listed in Table 1 Acetylation of 10 mg of the mixture (Ac_2O–pyridine) followed by the usual work-up and prep TLC (petrol–EtOAc, 2:1) furnished as the less polar product 4 mg **4e**; gum; IR ν_{max} cm^{-1}· 1770, 1740 (br), 1660, 1465, 1430, 1375, 1255, 1230, 1215, 1160, 1000, 1000, 965, 930; MS *m/z* (rel. int): 304 $[M-102]^+$ (43.4), 286 (16), 262 (16.8), 244 (100), 226 (30.7); MS (CI, isobutane) 407. The more polar gummy material (4 mg), IR ν_{max} cm^{-1}: 1765, 1745 (br), 1660, 1375, 1260, 1150, 1035, 925, was identified as a 9:1 mixture of epimers; the NMR spectrum of the major epimer **10b** is listed in Table 2.

A soln of 8 mg **4a** and 20 mg *m*-chloroperbenzoic acid in 3 ml CH_2Cl_2 was stirred at room temp for 24 hr, diluted with CH_2Cl_2, washed with dil KI, $Na_2S_2O_3$ soln and H_2O, dried and evaporated (reduced pressure) Analytical TLC of the residue revealed two spots different from the starting material Purification by prep. TLC (petrol–EtOAc, 2:1) gave 3 mg of less polar **8a**, 3 mg of more polar **8b** containing a little **8a** and 5 mg of starting material Epoxide **8a**, a gum, had IR ν_{max} cm^{-1}: 3200–3600, 1765, 1730, 1655, 1465, 1375, 1265, 1150, 1050, 1035. The low resolution MS gave no $[M]^+$, but had peaks at *m/z* (rel int.): 279 $[M-101]^+$ (0.8), 232 (3 4), 219 (5.1), 201 (2.5), 190 (5.6), 175 (2 3), 173 (4.7), 165 (3.0), 162 (2.2), 147 (4 8), 145 (4.2); MS (CI, isobutane): 381 $[M+1]^+$ (100), 365 (33.6), 364 (21.2), 363 (91), 349 (10.8), 347 (10 9), 279 (39.9), 263 (14 2), 262 (11 5), 261 (57.4), 243 (20.9), 237 (23.0), 235 (10), 219 (23.8), 197 (22 3). The more polar epoxide **8b** was also a gum; IR ν_{max} cm^{-1}: 3200–3600, 1765, 1730, 1660, 1540, 1475, 1375, 1260, 1165, 1150, 1075; the MS (CI) spectrum was similar to that of **8a**.

A soln of 50 mg **4a** in 25 ml EtOAc was hydrogenated with 100 mg 5% Pd–C for 2 hr, filtered, washed and evaporated. The residue was purified by prep. TLC (petrol–EtOAc, 3 developments) to give 39 mg **11a**, gum; IR ν_{max} cm^{-1}: 3200–3600, 1765, 1730, 1650, 1455, 1375, 1335, 1150, 1055, 1025, 1000, 950, 930, 910. The MS gave no $[M]^+$, but had significant peaks at m/z (rel. int.): 264 $[M-102]^+$ (5.3), 246 (10.1), and 85 (100). Acetylation of 10 mg **11a** and purification of the crude product by prep. TLC (petrol–EtOAc, 2:1, 2 developments) afforded 10 mg **11b** as a gum, IR ν_{max} cm^{-1}: 1775, 1740 (br), 1665, 1465, 1385, 1260, 1220, 1165, 1050, 975, 955, 930. The MS gave no $[M]^+$ but had significant peaks at m/z (rel. int): 348 $[M-102]^+$, (3), 306 (38.7), 288 (14.8), 264 (17.2), 246 (95.2), 228 (40.7), 85 (100).

C_6H_6 was distilled azeotropically from a mixture of 100 mg Ag_2CO_3 on celite in 50 ml C_6H_6 until the vol was reduced to 5 ml After addition of a soln of 5 mg **4a** the mixture was refluxed for several hours, cooled, filtered, washed and evaporated. TLC of the residue afforded 3 mg **6a** identical with material from the plant

1α-Acetoxy-2-hydroxy-3α(2-methylbutanoyloxy)-isoalantolactone (**4c**). Gum; IR ν_{max} cm^{-1}: 3200–3600, 1760, 1735, 1735, 1725, 1650, 1460, 1375, 1360, 1230, 1215, 1140, 1025, 960, 915; MS m/z (rel int.): 407 $[M+1]^+$ (3.6), 346 (1.3), 304 (5), 268 (10), 262 (19 5), 244 (42), 226 (39 9), 85 (100). [Calc. for $C_{22}H_{31}O_7$: M_r, 407 2067. Found· M_r (MS), 407 2039].

1α,3α-Dihydroxy-2α(2-methylbutanoyloxy)-isoalantolactone (**5a**). Gum; IR ν_{max} cm^{-1}: 3200–3600, 1765, 1735, 1450, 1255, 1200, 1135, 950; MS m/z (rel. int.): 365 $[M+1]^+$ (7), 347 (7.7), 329 (6 4), 279 (1.2), 280 (0.6), 262 (22.2), 244 (14), 85 (100). [Calc. for $C_{20}H_{28}O_6$; M_r, 364.1884. Found: M_r (MS), 364 1875].

1α-Acetoxy-3α-hydroxy-2α(2-methylbutanoyloxy)-isoalantolactone (**5b**). Gum; IR ν_{max} cm^{-1}: 2925, 1775, 1750, 1735, 1385, 1275, 1250, 1185, 1160, 1060, 955, 910. The low resolution MS gave no $[M]^+$, but had significant peaks at m/z (rel. int): 346 $[M-60]^+$ (0.8), 262 (6.6), 244 (8.3), 85 (41) and 57 (100); MS (CI, isobutane): 407 $[M+1]^+$ (100), 389 (15 1), 329 (50.6), 305 (75.7), 245 (80.6).

1α-Hydroxy-3-(2-methylbutanoyloxy)-pinnatifidin (**6a**). Gum which always contained some **5a**, the MS is that of semi-synthetic **6a** from oxidation of **4a**; IR ν_{max} cm^{-1}: 3200–3600, 1765, 1745, 1690, 1650, 1460, 1375, 1200, 1015, 920; MS m/z (rel int.): 362, 260 (1 7), 85 (45.9), 57 (100). [Calc. for $C_{20}H_{26}O_6$. M_r, 362.1727. Found: M_r (MS), 362.1737]. Acetylation of 10 mg of this substance afforded 8 mg **6b** identical with material from the plant.

To a solution of 20 mg **6a** in 5 ml of HOAc and 1 ml H_2O was added 200 mg Zn dust in small portions over a 15 min period, the temperature being kept at 100–110°. The reaction was monitored by TLC. After 1 hr, the mixture was cooled, diluted with H_2O and extracted with EtOAc The washed and dried extract was evaporated, prep. TLC of the residue (petrol–EtOAc, 2:1, 2 developments) furnished 8 mg **9** as a gum; IR ν_{max} cm^{-1} 1765, 1735, 1695, 1665, 1210, 1150, 1015 [Calc. for $C_{20}H_{26}O_5$ M_r, 346.1778. Found· M_r (MS), 346.1785].

To a soln of a 1·2 mixture of **5a** and **6a** (50 mg) in 5 ml MeOH cooled to 0° was added 50 mg $NaBH_4$ with stirring. After 1 hr the soln was acidified with dil HOAc, diluted with H_2O and extracted with $CHCl_3$. Evaporation of the washed and dried extract and purification of the residue by prep. TLC (petrol–EtOAc, 2·1, 2 developments) gave as the less polar fraction 20 mg of a 1:2 mixture of **11a** and **12a**. The NMR spectrum of **12a** is listed in Table 2. Acetylation of the mixture and purification of the product by prep. TLC (petrol–EtOAc, 2.1, 3 developments) afforded 2 mg **11b** and 9 mg **12b** as a gum; IR ν_{max} cm^{-1}: 1770, 1740 (br), 1660, 1460, 1375, 1245, 1210, 1155, 1035, 975, 925. The MS gave no $[M]^+$ but had significant peaks at m/z (rel. int): 366 $[M-84]^+$ (0.7), 348 (0.5), 330 (1.7), 306 (41.5), 288 (2.9), 264 (13.7), 246 (67.9), 228 (16.8), 85 (100).

The more polar fraction (5 mg) from the $NaBH_4$ reduction was identified as **13**, gum: IR ν_{max} cm^{-1}: 3200–3600, 1760, 1730, 1550, 1460, 1380, 1335, 1185, 1155, 1075, 1025, 965, 910; CIMS (isobutane): 347 $[M+1]^+$

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