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ISOTACHIN C AND BALANTIOLIDE, TWO AROMATIC COMPOUNDS FROM THE NEW ZEALAND LIVERWORT *BALANTIOPSIS ROSEA**

YOSHINORI ASAKAWA, KEIKO TAKIKAWA, MOTOO TORI and ELLA O. CAMPBELL†

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan; †Department of Botany and Zoology, Massey University, Palmerston North, New Zealand

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Key Word Index—*Balantiopsis rosea*; Balantiopsidaceae; Hepaticae; isotachin C; balantiolide; 2-methoxybenzyl *trans*-β-methylthioacrylate; 3-[3′,4′-dimethoxybenzyl]-7-hydroxy-5-methoxyphthalide; isotachin A; isotachin B; benzoates; cinnamates; sesquiterpenoids; chemosystematics.

Abstract—Isotachin C and balantiolide, two new aromatic compounds, were isolated from the New Zealand liverwort *Balantiopsis rosea*. Their structures were established to be 2-methoxybenzyl *trans*-β-methylthioacrylate and 3-[3′,4′-dimethoxybenzyl]-7-hydroxy-5-methoxyphthalide, respectively, by spectral methods. The previously known isotachin A, isotachin B, benzoates, cinnamates and sesquiterpenoids were also found. *B. rosea* is chemically quite close to *Isotachis japonica*.

INTRODUCTION

Liverworts are rich sources of terpenoids and aromatic compounds which often show interesting biological activity, such as allergenic contact dermatitis, antifeedant, antifungal, cytotoxic, piscicidal and plant growth regulatory activity [1–3]. These chemical components are also valuable chemosystematic markers of the Hepaticae [2, 4–7]. As part of a chemosystematic study of the Hepaticae, we have examined the lipophilic components of a New Zealand liverwort, *Balantiopsis rosea* Berggr. belonging to the Balantiopsidaceae and have isolated a new sulphur-containing acrylate and a new phthalide, named isotachin C and balantiolide, respectively. This paper deals with the chemical structures of these new compounds and includes a discussion of the chemosystematics of *Balantiopsis* and *Isotachis* species.

RESULTS AND DISCUSSION

The methanol extract of dried *B. rosea* was examined by TLC, GC and GC/MS; benzyl benzoate (**6**), β-phenylethyl benzoate (**8**), benzyl *trans*-cinnamate (**9**), β-phenylethyl *cis*-cinnamate (**10**), β-phenylethyl *trans*-cinnamate (**11**) and β-phenylethyl dihydrocinnamate (**12**), two sulphur-containing acrylates, isotachin A (**1**) and isotachin B (**3**), which were found in the liverwort *Isotachis japonica* [8–10], were detected together with the previously known four sesquiterpenoids β-caryophyllene (**13**), bicyclogermacrene (**14**), spathulenol (**15**) and β-eudesmol (**16**). The remaining extract was chromatographed further on silica gel, followed by preparative TLC to afford isotachin C (**2**), balantiolide (**4**) and 2-methoxybenzyl benzoate (**7**), along with mixtures of the above aromatic esters, which were also confirmed by analysis of 400 MHz ^{1}H NMR spectra. The chemical structures of the new compounds, **2** and **4**, were established by analysis of the spectral data, particularly those of ^{1}H NMR and ^{13}C NMR spectra, since only limited amounts of the fresh material were available.

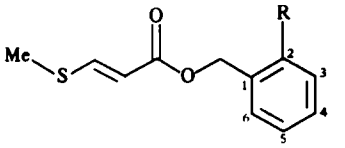
Isotachin C (2)

High-resolution mass spectrometry showed that the molecular formula of **2** was $C_{12}H_{14}O_3S$. The spectral data indicated the presence of a methoxyl group [δ_H 3.85 (*s*)], a methyl mercapto group [δ_H 2.33 (*s*)] and a *trans*-ethylenic olefin [δ_H 5.72 and 7.79 (each *d*, J = 14.5 Hz)] conjugated with an ester carbonyl group [1700 cm^{-1}; 275 nm; δ_C 165.2 (*s*)]. The presence of a methoxybenzyl group was confirmed by the base peak at m/z 121 and the NMR signals δ_H 5.23 (*s*) and δ_C 61.5 (*t*). These spectral data were very similar to those of isotachin A (**1**) isolated from the liverwort *Isotachis japonica* [10], except for the presence or absence of the methoxyl group. The position of the methoxyl group on the benzene ring was established by the 400 MHz NMR spectra (see Experimental) and the presence of NOE only between H-3 and the methoxyl group. These data coupled with the molecular formula and the co-occurrence with 2-methoxybenzyl benzoate (**7**) [11] led to the structure **2**, 2-methoxybenzyl *trans*-β-methylthioacrylate.

Balantiolide (4)

Compound **4**, $C_{18}H_{18}O_6$, was obtained as a white powder. The spectral data indicated the presence of a benzene ring (1624, 1598 and 1518 cm^{-1}), three methoxyl groups [δ_H 3.80, 3.84 and 3.86 (each *s*)], a chelated hydroxyl group [3400 cm^{-1}; δ_H 7.66 (*br s*)] which was confirmed by acetylation to afford a monoacetate (**5**) [1760 cm^{-1}; δ_H 2.28, 3H (*s*)] and benzylic protons [δ_H 3.09 and 3.20 (each *dd*, J = 13.7 and 6.2 Hz)] coupled with a proton [δ_H 5.59 (*t*, J = 6.2 Hz)] on a carbon [δ_C 82.3 (*d*)] bearing oxygen atom. The presence of a 3-substituted

*Part 19 in the series "Chemosystematics of Bryophytes". For Part 18, see ref. [10].



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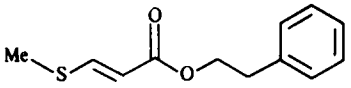

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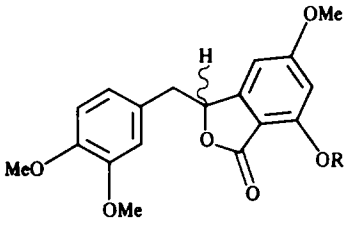

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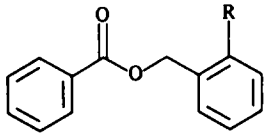

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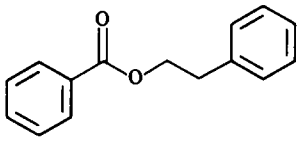

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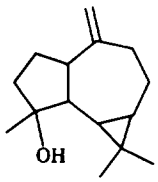

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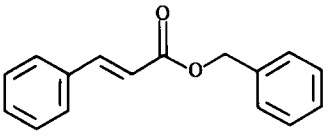

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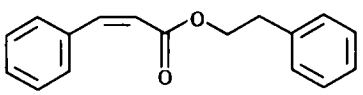

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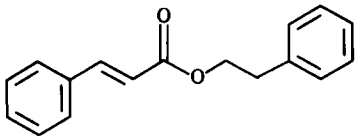

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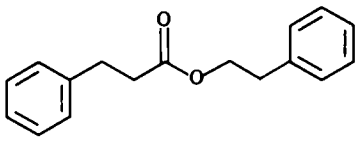

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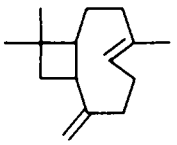

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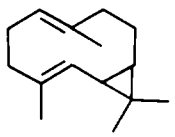

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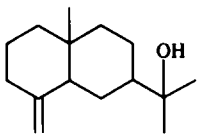

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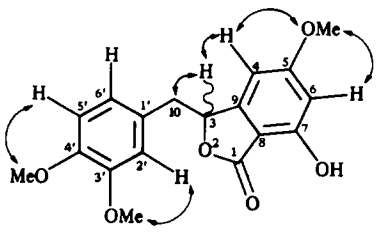

4 NOE

7-hydroxy-5-methoxyphthalide was confirmed by the intense IR and UV absorption bands (1735 cm^{-1}; 252 nm) [12, 13], the ^{13}C NMR spectrum [δ_C 174.4 (*s*); 82.3 (*d*)] and *meta*-coupled protons [δ_H 6.20 and 6.40 (each *d*, J = 1.4 Hz)]. The mass spectrum showed the base peak at *m/z* 151, indicating the presence of a dimethoxybenzyl group. Thus, two of the methoxyl groups in **4** were located on the benzyl moiety. The arrangement of three methoxyl groups on the two benzene rings was established by analysis of the ^1H NMR spectrum (see Experimental), a spin decoupling experiment and the NOE difference spectra of **4**. Irradiation of the triplet at δ 5.59 (H-3) caused (i) the double doublet at 3.09 and 3.20 (H-10) to

collapse to a doublet (J = 13.7), and (ii) the collapse of two broad doublets at 6.20 (H-4) and 6.40 (H-6) to sharp doublets (J = 1.4 Hz). Reverse irradiation at the benzylic protons (H-10) caused the triplet at 5.59 (H-3) to collapse to a singlet. Irradiation of the broad doublet at 6.20 (H-4) sharpened the broad triplet at 5.59 (H-3) and the broad doublet at 6.40 (H-6). Irradiation of the broad doublet at 6.40 (H-6) caused the broad doublet at 6.20 (H-4) to collapse to a broad singlet. Compound **4** showed NOEs between (i) H-5′ and a methoxyl group (C-4′), (ii) H-2′ and a methoxyl group (C-3′), (iii) H-4 and a methoxyl group (C-5), (iv) H-6 and a methoxyl group (C-5), (v) H-3 and H-4, and (vi) H-3 and H-10, respectively. From the above spectral data together with the molecular formula, the structure of balantiolide was established to be 3-[3′,4′-dimethoxybenzyl]-7-hydroxy-5-methoxyphthalide (**4**).

This is the second record of the isolation of a sulphur-containing substance and a phthalide [10, 13]. The distribution of 2-methoxybenzyl benzoate (**7**) is very rare; it has been found in the higher plant *Uvaria purpurea* (Annonaceae) [11].

B. rosea is morphologically close to *Isotachis* species, although the former is classified in the Balantiopsidaceae (Jungermannianeae) and the latter in the Isotachidaceae (Herbertinae) [14]. Recently, we reported the chemical constituents of the primitive liverwort *Isotachis japonica* [9, 10]. As shown in Table 1, the constituents of *B. rosea* are chemically quite close to those of *I. japonica*, except for the presence or absence of sesquiterpenoids. Thus, it is suggested that both genera are very close and they might share a common ancestor.

EXPERIMENTAL

TLC, prep. TLC, GC and GC/MS were carried out as previously reported [10]. The solvents used for spectral determination were: TMS–$CDCl_3$ [^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz)]; EtOH (UV) and $CHCl_3$ (IR) unless otherwise stated.

Plant material. Balantiopsis rosea Bergger. identified by E.O.C. has been deposited at the Herbarium of the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Dried *B. rosea* (3.01 g), collected in New Zealand in August 1983, was extracted with MeOH for 3 weeks. On removal of the solvent, a green extract (124.4 mg) was obtained. A small amount of the extract was analysed directly by TLC, GC and GC/MS equipped with a computer. Each component was identified by direct comparison of their MS with those of authentic samples. The presence of isotachin A (**1**), isotachin B (**3**), benzyl benzoate (**6**), β-phenylethyl benzoate (**8**), benzyl *trans*-cinnamate (**9**), β-phenylethyl *cis*-cinnamate (**10**), β-phenylethyl *trans*-cinnamate (**11**), β-phenylethyl dihydrocinnamate (**12**) [9, 10] and four sesquiterpenoids, β-caryophyllene (**13**), bicyclogermacrene (**14**), spathulenol (**15**) and β-eudesmol (**16**), was thus detected. The remaining extract (122 mg) was chromatographed on silica gel using an *n*-hexane–EtOAc gradient to give four fractions. The first fraction (5.7 mg) (0–5% EtOAc–*n*-hexane) contained a mixture of sesquiterpene hydrocarbons and *n*-paraffins in which the sesquiterpenes (**13**, **14**) were detected by GC/MS. The second fraction (49.1 mg) (10% EtOAc–*n*-hexane) was rechromatographed on silica gel using the same solvent system as described above, followed by prep. TLC (*n*-hexane–Et_2O, 4:1) to afford isotachin C (**2**) (1.1 mg), 2-methoxybenzyl benzoate (**7**) (5 mg), a mixture (17 mg) of benzyl benzoate (**6**) and β-phenylethyl benzoate (**8**), a mixture (4 mg) of β-phenylethyl *cis*-cinnamate (**10**) and β-phenylethyl *trans*-cinnamate (**11**), and a mixture (1.3 mg) of isotachin A (**1**) and isotachin B (**3**). *2-Methoxybenzyl benzoate* (**7**): ^{1}H NMR: δ3.86 (3H, *s*, OMe), 5.42 (2H, *s*, OCH_2), 6.92 (1H, *br d*, J = 8.3 Hz, H-3), 6.97 (1H, *dd*, J = 8.3, 7.6 Hz, H-5), 7.32 (2H, *t*, J = 7.5 Hz, H-3′,5′), 7.55 (1H, *t*, J = 7.5 Hz, H-4′) and 8.08 (2H, *d*, J = 7.5 Hz, H-2′,6′); MS *m/z* (rel. int.): 243 $[M]^+$ (10), 137 (100), 121 (25), 105 (28) and 91 (37) [9]. *Isotachin* C (**2**): $C_{12}H_{14}O_3S$ (high-resolution MS: found 238.0670; calc. 238.0663); UV λ_{max} nm (log ε): 207 (3.87), 271 (3.83), and 275 (4.03); IR ν_{max}^{liq} cm^{-1}: 1700, 1575, 1488, 1458, 1315, 1285, 1240, 1150, 1025, 970, 950, 820 and 748; ^{1}H NMR: δ2.33 (3H, *s*, SMe), 3.85 (3H, *s*, OMe), 5.23 (2H, *s*, OCH_2Ph), 5.72 (1H, *d*, J = 14.9 Hz, CH=), 7.79 (1H, *d*, J = 14.9 Hz, CH=), 6.89 (1H, *d*, J = 8.0 Hz, H-3), 6.95 (1H, *dd*, J = 8.0, 7.6 Hz, H-5), 7.30 (1H, *t*, J = 7.6 Hz, H-4) and 7.33 (1H, *d*, J = 7.6 Hz, H-6). ^{13}C NMR: δ14.3 (Me, *q*), 55.4 (OMe, *q*), 61.5 (CH_2–O, *t*), 113.1 (CH=, *d*), 147.2 (CH=, *d*), 110.5 (Ph–C, *d*), 120.4 (Ph–C, *d*), 128.5 (Ph–C, *s*), 129.5 (Ph–C, *d*), 129.7 (Ph–C, *d*), 157.5 (Ph–C, *s*) and 165.2 (COO, *s*); MS *m/z* (rel. int.): 238 $[M]^+$ (12), 191.0709 (calc. 191.0724, $C_{11}H_{11}O_3$) (50), 137.0603 (calc. 137.0607, $C_8H_9O_2$) 121.0653 (calc. 121.0661, C_8H_9O) (100), 91 (94). Fraction 3 (17.5 mg) (15–20% EtOAc–*n*-hexane) contained spathulenol (**15**), β-eudesmol (**16**), two unidentified sesquiterpene

Table 1. Distribution of aromatic compounds and sesquiterpenoids in *Balantiopsis rosea* and *Isotachis japonica*

Compounds	*B. rosea*	*I. japonica* [9, 10]
Isotachin A (**1**)	+ + *	+
Isotachin B (**3**)	+	+
Isotachin C (**2**)	+	−
Balantiolide (**4**)	+ + +	−
2-Methoxybenzyl benzoate (**6**)	+ + + +	−
Benzyl benzoate (**7**)	+ + + + +	+ + + + +
β-Phenylethyl benzoate (**8**)	+	+
Benzyl *trans*-cinnamate (**9**)	+	+ +
β-Phenylethyl *cis*-cinnamate (**10**)	+	+
β-Phenylethyl *trans*-cinnamate (**11**)	+ + +	+
β-Phenylethyl dihydrocinnamate (**12**)	+	+
Sesquiterpenoids (**13–16**)	+	−

* The symbols, +, + +, + + +, etc. are relative concentrations estimated by GC and GC/MS.

alcohols and phytadiene. Fraction 4 (48.5 mg) (30–100% EtOAc–*n*-hexane) was rechromatographed on silica gel using a C_6H_6–EtOAc gradient to give balantiolide (**4**) (7.5 mg): mp 131–132°; $C_{18}H_{18}O_6$ (high-resolution MS: found 330.1092; calc. 330.1203); UV λ_{max} nm (log ε): 204.5 (4.79), 214 (4.85), 252 (4.40), 295 sh (3.95) and 281 (4.08); UV $\lambda_{max}^{+AlCl_3}$ nm (log ε): 218 (5.08); IR ν_{max} cm^{-1}: 3440, 1735, 1637, 1624, 1598, 1518, 1468, 1440, 1378, 1330, 1262, 1160, 1073, 1030, 990, 854 and 686; ^{1}H NMR: δ3.09 (1H, *dd*, J = 13.7, 6.2 Hz, H-10), 3.20 (1H, *dd*, J = 13.7, 6.2 Hz, H-10), 3.80 (3H, *s*, OMe-5), 3.84 (3H, *s*, OMe-3'), 3.86 (3H, *s*, OMe-4'), 5.59 (1H, *t*, J = 6.2 Hz, H-3), 6.20 (1H, *br d*, J = 1.4 Hz, H-4), 6.40 (1H, *br d*, J = 1.4 Hz, H-6), 6.72 (1H, *d*, J = 1.7 Hz, H-2'), 6.76 (1H, *dd*, J = 8.3, 1.7 Hz, H-6'), 6.80 (1H, *d*, J = 8.3 Hz, H-5') and 7.66 (1H, *br s*, HO-7), ^{13}C NMR: δ40.2 (CH_2, *t*), 56.0 (Me × 3, *q*), 82.3 (CH–O, *d*), 100.7 (Ph–C, *d*), 100.8 (Ph–C, *d*), 104.6 (Ph–C, *s*), 111.2 (Ph–C, *d*), 112.8 (Ph–C, *d*), 121.9 (Ph–C, *d*), 127.2 (Ph–C, *s*),148.2 (Ph–C, *s*), 157.2 (Ph–C, *s*), 157.7 (Ph–C, *s*), 167.4 (Ph–C, *s*) and 174.4 (COO, *s*); MS *m/z* (rel. int): 330 $[M]^+$ (5), 179.0349 (calc. 179.0345, $C_9H_7O_4$) (13), 151.0780 (calc. 151.0759, $C_9H_{11}O_2$) (100).

Acetylation of **4**. Compound **4** (5 mg) was acetylated by Ac_2O–pyridine at room temp. for 24 hr. Work-up as usual gave a monoacetate (**5**) (5.2 mg): $C_{20}H_{20}O_7$; UV λ_{max} nm (log ε): 208 (4.34), 217 (4.22) and 254 (3.92) IR ν_{max} cm^{-1}: 1760, 1619, 1518, 1490, 1468, 1370, 1360, 1345, 1262, 1190, 1150, 1062, 1030 and 895; ^{1}H NMR: δ2.38 (3H, *s*, AcO), 3.04 (1H, *dd*, J = 14.2, 6.6 Hz), 3.23 (1H, *dd*, J = 14.2, 6.6 Hz), 3.80, 3.85, 3.87 (each 3H, *s*, OMe × 3), 5.53 (1H, *t*, J = 6.6 Hz), 6.43 (1H, *br d*, J = 1.9 Hz), 6.66 (1H, *d*, J = 1.9 Hz), 6.74 (1H, *br d*, J = 1.7 Hz), 6.76 (1H, *dd*, J = 8.0, 1.7 Hz) and 6.81 (1H, *d*, J = 8.0 Hz); MS *m/z* (rel. int.): 372 $[M]^+$ (9), 179 (20), 151 (100) and 43 (9).

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