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ISOTACHIN A AND ISOTACHIN B, TWO SULPHUR-CONTAINING ACRYLATES FROM THE LIVERWORT *ISOTACHIS JAPONICA**

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Key Word Index—*Isotachis japonica*; Jungermanniales; Hepaticae; isotachin A; isotachin B, benzyl *trans*-β-methylthioacrylate; β-phenylethyl *trans*-β-methylthioacrylate; benzoates; cinnamates; isotachioside; 2-methoxy-4-hydroxyphenyl-1β-glucoside; stigmasteryl 3β-glucoside; chemosystematics.

Abstract—Isotachin A and isotachin B, two new sulphur-containing acrylates, were isolated from the liverwort *Isotachis japonica*. Their structures were established to be benzyl *trans*-β-methylthioacrylate and β-phenylethyl *trans*-β-methylthioacrylate by chemical and spectral methods. This is the first report of sulphur-containing substances from bryophytes. Some previously known benzoates and cinnamates as well as 2-methoxy-4-hydroxyphenyl 1β-glucoside and stigmasteryl 3β-glucoside were also isolated.

INTRODUCTION

Most liverworts contain terpenoids and lipophilic aromatic compounds which constitute the oil bodies and are responsible for the biological activity of the Hepaticae [1–4]. The classification of liverworts is often difficult since their gametophytes are small. Terpenoids and aromatic compounds found in the Hepaticae are major components and, thus, these compounds are of value in taxonomic investigations [4]. *Isotachis* sp. belonging to Isotachidaceae are taxonomically primitive liverworts found in Japan, New Zealand and South America. Three aromatic esters, benzyl *trans*-cinnamate (**3**), β-phenylethyl *trans*-cinnamate (**5**) and benzyl benzoate (**7**) have previously been isolated from *I. japonica* [5, 6]. As part of a chemosystematic study of the Hepaticae, we have re-examined the chemical constituents of *I. japonica* and isolated two new sulphur-containing acrylates, isotachin A and isotachin B, which may be of value when considering the evolution of the Hepaticae.

In this paper, we report the chemical structures of isotachins A and B and discuss the chemosystematics of *Isotachis* sp.

RESULTS AND DISCUSSION

The benzene soluble portion of a methanol extract of fresh *I. japonica* was examined by GC, TLC and GC/MS; benzyl *trans*-cinnamate (**3**), benzyl *cis*-cinnamate (**4**), β-phenylethyl *trans*-cinnamate (**5**), benzyl dihydrocinnamate (**6**), benzyl benzoate (**7**) and β-phenylethyl benzoate (**8**) as well as stigmasterol (**12**) were detected. The remaining material was chromatographed on silica gel and Sephadex LH-20 to give isotachin A (**1**) and isotachin B (**2**), and the previously known benzoate **7** and the cinnamates **3** and **5**. The water soluble portion was partitioned with *n*-butanol and the crude extract chromatographed on silica gel to afford isotachioside, 2-methoxy-4-hydroxyphenyl 1β-glucoside (**10**) and stigmasteryl 3β-glucoside (**13**).

Isotachin A (**1**)

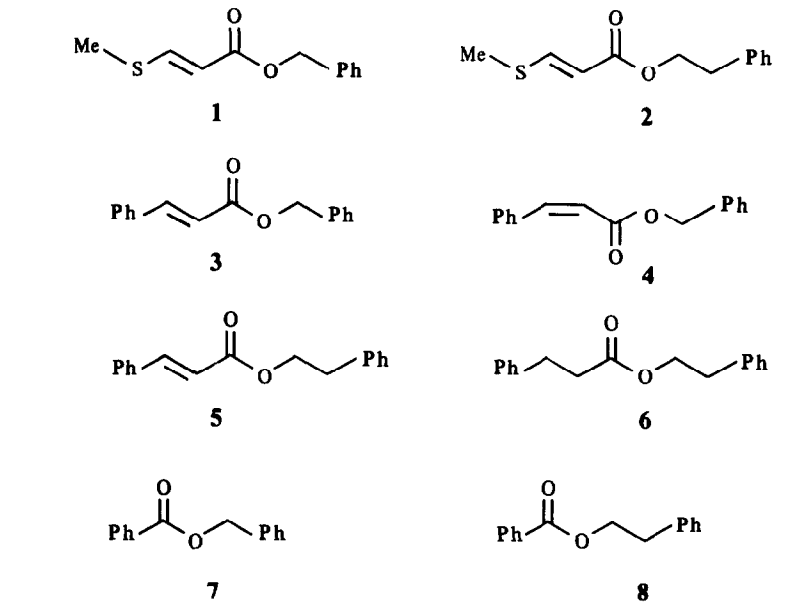
High resolution mass spectrometry indicated that the molecular formula of **1** was $C_{11}H_{12}O_2S$. The spectral data indicated the presence of a benzyl group [*m/z* 91 (base) and δ_H 7.30–7.40, (5H, *m*)], an ester group (1715 cm^{-1}; δ_C 164.9), a methyl mercapto group [δ_H 2.33 (*s*)] and a *trans*-ethylenic olefin [δ_H 5.71 and 7.80 (each *d*, J = 15 Hz)]. These data, coupled with the molecular formula, led to structure **1**, benzyl *trans*-β-methylthioacrylate. Further evidence for this structure was obtained from lithium aluminium hydride reduction of **1** which gave benzyl alcohol and *trans*-β-methylthioallyl alcohol (**9**).

Isotachin B (**2**)

This compound also contained a *trans*-β-methylthioacrylate moiety. The presence of two vicinal methylene groups [δ_H 2.96 and 4.35 (both 2H, *t*, J = 7.3 Hz); δ_C 35.2 (*t*) and 64.6 (*t*)] indicated that the acidic function was esterified with β-phenylethyl alcohol and, thus, isotachin B was β-phenylethyl *trans*-β-methylthioacrylate (**2**). This structure was supported by the presence in the high resolution mass spectrum of intense fragment ions at *m/z* 101.0055 (C_4H_5OS, 60%) and *m/z* 104.0626 (C_8H_8, 100%) resulting from a McLafferty rearrangement (Fig. 1). In addition, lithium aluminium hydride reduction of **2** gave the expected products, β-phenylethyl alcohol and *trans*-β-methylthioallyl alcohol (**9**).

In addition to the new acrylates and the aromatic esters, *I. japonica* also contained two glucosides, isotachioside (**10**) and stigmasteryl 3β-glucoside (**13**). The former compound possessed a benzene ring with a methoxy group [δ3.68 (*s*)] and a phenolic hydroxyl group (3400 cm^{-1}), and could be methylated to give a dimethoxy derivative (**11**). The presence of a glucose moiety was

*Part 18 in the series "Chemosystematics of Bryophytes". For Part 17, see Asakawa, Y, Harrison, L. J and Toyota, M. (1985) *Phytochemistry* **24**, 262.



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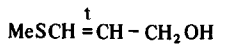

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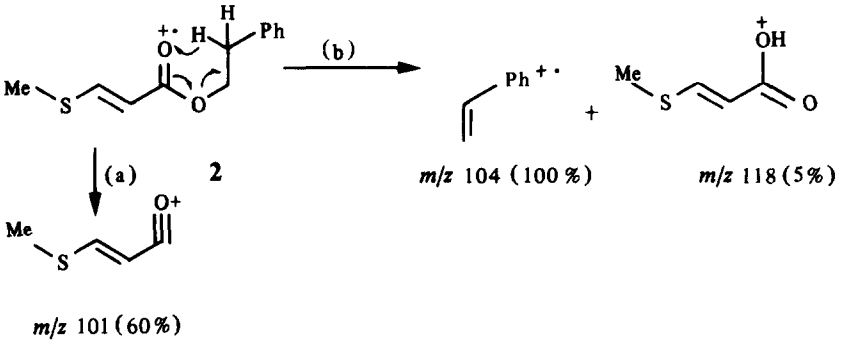


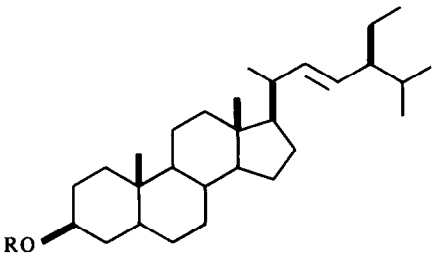
Fig. 1.

supported by the ^{1}H NMR and ^{13}C NMR spectra (see Experimental). The 1,2,4-substitution of the benzene ring was established by analysis of the 400 MHz ^{1}H NMR spectra of **10** and **11** (see Experimental). The arrangement of the functional groups on the benzene ring was established by the presence of NOEs between the anomeric proton and H-6 and between the methoxy group and H-3 in **10** (Fig. 2). Compound **11** showed NOEs between: (i) the anomeric proton and H-6; (ii) H-3 and the methoxy group at C-2; (iii) H-3 and the methoxy group at C-4; and (iv) the methoxy group at C-4 and H-5 (Fig. 2). The β-configuration of the anomeric carbon was deduced from the magnitude of the coupling constant (J = 7.3 Hz) of the anomeric proton. Thus, the structure of isotachioside was assigned as 2-methoxy-4-hydroxyphenyl 1β-glucoside (**10**).

The structure of stigmasteryl 3β-glucoside (**13**) was established by comparison of its 400 MHz ^{1}H NMR and 100 MHz ^{13}C NMR spectra with those of a co-metabolite, stigmasterol (**12**) (see Experimental).

Although β-methylthioacrylates have been found in the Compositae [7], sulphur-containing compounds have not previously been found in bryophytes. However, propiothethine (**14**) is commonly found in algae [8] and is responsible for the characteristic dimethyl sulphide odour obtained upon drying (Fig. 3). We have noticed that many of the 500-plus species of liverworts, in particular the Metzgeriales, studies in our laboratory emit a similar smell when dried. It is probable that the present sulphur-containing acrylates are important chemical markers for *Isotachis* sp. They may also be of relevance when considering the evolution of the lower terrestrial green plants.

This is the first instance that the phenyl glucoside and sterol glucoside have been isolated from the bryophytes, although a number of flavonoid glucosides have been detected in the bryophytes [4]. *Isotachis japonica* is



12 R=H

13 R=Glc

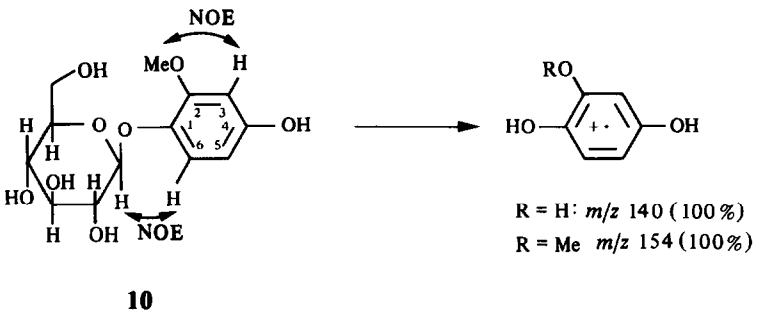
unusual in that, unlike other species of Jungermanniales, no terpenoids have been detected even by GC/MS. It would, therefore, appear that *I. japonica* is unique in the Isotachidaceae.

EXPERIMENTAL

The solvents used for spectral determination were. TMS-$CDCl_3$ [^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz)]; MeOH (UV); and CCl_4 (IR) unless otherwise stated. TLC: pre-coated silica gel (0.25 mm) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1) as solvents. Spots were visualized in UV light (254 nm) and by spraying with 30% H_2SO_4 and then heating at 120°. GC. 1% SE-30, 3 m × 2 mm glass column, temp. programme from 50° to 270° at 5°/min, inject temp 260°, N_2 30 ml/min. GC/MS: 70 eV, 1% SE-30, 3 m × 2 mm, glass column, temp. programme from 50° to 270° at 5°/min, injector temp 260°, He 30 ml/min. MS (direct inlet) 20 eV

Plant material. Isotachis japonica Steph. identified by Dr. M. Mizutani was deposited in the Herbarium of Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and isolation. Isotachis japonica collected on Yaku Island, Japan, December 1983 was homogenized twice with MeOH and the homogenate filtered under red pres The crude extract was partitioned between C_6H_6 and H_2O. On removal of the solvent a viscous oil (18 g) was obtained from the C_6H_6 layer. The aq. layer was extracted with *n*-BuOH and the solvent removed to give crude material (9.9 g). A small amount of C_6H_6 extract was directly checked by TLC, GC and GC/MS. The components obtained by GC/MS were identified by direct comparison of their MS with those of authentic samples or by analysis of their MS The presence of benzyl *trans*-cinnamate (**3**), benzyl *cis*-cinnamate (**4**), β-phenyl *trans*-cinnamate (**5**), β-phenylethyl dihydrocinnamate (**6**), benzyl benzoate (**7**) and β-



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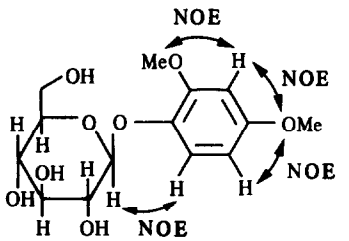

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Fig 2.

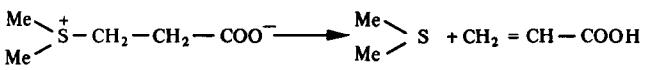

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Fig. 3

phenylethyl benzoate (**8**) was, thus, confirmed. The remaining C_6H_6 extract was chromatographed on silica gel using a C_6H_6–EtOAc gradient to give six fractions From fraction 3 (20% EtOAc), stigmasterol (**12**) (30 mg) was isolated. Stigmasterol (**12**): ^{1}H NMR (400 MHz, C_5H_5N) δ0.72 (3H, *s*), 0.87 (3H, *d*, *J* = 6.8 Hz), 0.90 (3H, *t*, *J* = 7.3 Hz), 0 92 (3H, *d*, *J* = 6 8 Hz), 1.07 (3H, *s*), 1 10 (3H, *d*, *J* = 6.8 Hz), 2.63 (2H, *br s*), 3.85 (1H, *m*), 5 10 (1H, *dd*, *J* = 15.1, 9 3 Hz), 5 23 (1H, *dd*, *J* = 15 1, 8 8 Hz) and 5.43 (1H, *br s*); ^{13}C NMR (400 MHz, C_5H_5N): δ12 1, 12 5, 19.2, 19 6, 21.3, 21 4, 21 5, 24.6, 25 7, 29 3, 32.3 (three signals overlapped), 32.5, 36.9, 37.8, 39.9, 40.8, 42 4, 43 2, 50.5, 51 5, 56 1, 57.0, 71.2, 121.2, 129 5, 138.8 and 142 0; MS *m/z* (rel int.): 412 $[M]^+$ (48), 55 (100).

The second fraction (10% EtOAc) was chromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) as eluant and then further chromatographed on silica gel using an *n*-hexane–EtOAc gradient to give five fractions. The second fraction was chromatographed on silica gel using the same solvents described above to afford benzyl benzoate (**7**) (4.3 g), β-phenylethyl benzoate (**8**) (170 mg) and isotachin A (**1**) (250 mg). Isotachin A (**1**): $C_{11}H_{12}O_2S$ [high resolution MS found 208 0555; calc. 208 0558], UV λ_{max} nm (log ε): 204 (3.51), 229 (3.52), 271 (2.51) and 281 sh (2.44), IR ν_{max} cm^{-1} 1715, 1585, 1325, 1295, 1255 and 1160; ^{1}H NMR: δ2 33 (3H, *s*), 5 18 (2H, *s*), 5 71 (1H, *d*, *J* = 15.1 Hz), 7.30–7.40 (5H, *m*) and 7.80 (1H, *d*, *J* = 15 1 Hz); ^{13}C NMR δ14.2 (Me, *q*), 66 0 (CH_2, *t*), 112.5 (CH=, *d*), 147 6 (CH=, *d*), 128 1 (Ph–C, *d*), 128 2 (Ph–C, *d*, two signals overlapped), 128 5 (Ph–C, *d*, two signals overlapped), 136 3 (Ph–C, *s*) and 164 9 (–COO, *s*), MS *m/z* (rel int) 208 $[M]^+$ (12), 161 0598 (calc 161 0602, $C_{10}H_9O$, 39), 133 (12), 107 (6), 105 (10), 101 0059 (calc. 101 0061, C_4H_5O, 60), 91.0550 (calc. 91 0548, C_7H_7, 100), 65 (12)

Fraction 3 was also chromatographed on silica gel using the same solvent system as described above to give benzyl *trans*-cinnamate (**3**) (690 mg), β-phenylethyl *trans*-cinnamate (**5**) (270 mg) and isotachin B, (**2**) (200 mg) Isotachin B (**2**) $C_{12}H_{14}O_2S$; UV λ_{max} nm (log ε) 205 (3 63) and 275 (3 82); IR ν_{max} cm^{-1} 1715, 1635, 1495, 1448, 1323, 1305 and 1165, ^{1}H NMR δ2.31 (3H, *s*), 2 96 (2H, *t*, *J* = 7 3 Hz), 4.35 (2H, *t*, *J* = 7 3 Hz), 5 65 (1H, *d*, *J* = 15 1 Hz), 7 2–7.3 (5H, *m*) and 7 72 (1H, *d*, *J* = 15 1 Hz); ^{13}C NMR δ14.2 (Me, *q*), 35 2 (CH_2, *t*), 64 6 (CH_2, *t*), 113 (CH=, *d*), 147 1 (CH=, *d*), 126.5 (Ph–C, *d*, two signals overlapped), 128 4 (Ph–C, *d*, two signals overlapped), 128 8 (Ph–C, *d*, two signals overlapped), 137.9 (Ph–C, *s*) and 164 9 (–COO, *s*), MS *m/z* (rel int) $[M]^+$ (absent), 173 (3), 118 (5), 105 (13), 104 0609 (calc 104 0626, C_8H_8, 100), 103 (8), 101.0055 (calc 101 0061, C_4H_5OS, 60), 91 (10) and 77 (5)

The *n*-BuOH extract was chromatographed on silica gel using a $CHCl_3$–MeOH gradient to afford isotachioside (**10**) (100 mg) and stigmasteryl 3β-glucoside (**13**) (80 mg) together with sucrose (20 mg) Isotachioside (2-methoxy-4-hydroxyphenyl 1β-glucoside, **10**) UV λ_{max} nm (log ε) 204 (4 34), 218 sh (3 95) and 285 (3 56); IR ν_{max}^{KBr} 3400, 1600, 1515, 1265, 1220, 1210, 1160, 1075, 1040, 1010 and 800, ^{1}H NMR (C_5D_5N) δ3 98 (1H, *br s*, Glc H-5), 4 21–4 28 (3H, *m*, Glc H-2–H-4), 4 45 (1H, *d*, *J* = 11 7 Hz, Glc H-6), 4.32 (1H, *dd*, *J* = 11 7, 5 Hz, Glc H-6), 5 44 (1H, *d*, *J* = 7 3 Hz, Glc H-1), 3 68 (3H, *s*, OMe), 6.68 (1H, *br d*, *J* = 8.3 Hz, Ph-H-5), 6 88 (1H, *br s*, Ph-H-3) and 7 45 (1H, *d*, *J* = 8 3 Hz, Ph-H-6), MS *m/z* (rel. int). $[M]^+$ (absent), 140 (100), 125 (23), 97 (16) and 60 (5). Stigmasteryl 3β-glucoside (**13**) IR ν_{max}^{KBr} cm^{-1}: 3430, 1070 and 1020, ^{1}H NMR (C_5D_5N) δ0 68 (3H, *s*), 0 86 (3H, *d*, *J* = 6.8 Hz), 0.89 (3H, *d*, *J* = 6.8 Hz), 0.91 (3H, *t*, *J* = 7.3 Hz), 0.93 (3H, *s*), 1 08 (3H, *d*, *J* = 6.8 Hz), 3.95 (1H, *m*, H-3), 3.95 (1H, Glc H-5), 4.08 (1H, Glc H-2), 4 29 (1H, Glc H-3, H-4), 4.42 (1H, Glc H-6), 4.57 (1H, Glc H-6), 5.05 (1H, *d*, *J* = 8 Hz, Glc H-1), 5.05 (1H, *d*, *J* = 8 Hz), 5.05 (1H, *m*), 5.23 (1H, *m*) and 5.35 (1H, *br s*); ^{13}C NMR (C_5D_5N): δ12.2, 12.6, 19.5, 21.3, 21.5, 24.6, 25.7, 29.4, 30.3, 32 1, 32.2 (two signals overlapped), 36.5, 37.0, 37.5, 39.4, 39 9, 40.8, 42 4, 50 4, 51.5, 56.1, 57.0, 62.9, 71.7, 75.3, 78 1, 78.5, 78 6, 102 6 (Glc C-1), 121.9, 129.5, 138 9 and 140.9, MS *m/z* (rel. int.): $[M]^+$ (absent), 394 (83), 380 (24), 255 (53), 145 (23), 139 (26), 97 (21), 85 (24), 83 (100), 81 (22), 69 (50) and 57 (29).

Reduction of isotachin A (**1**). Compound **1** (10 mg) was added to $LiAlH_4$ (50 mg) in dry Et_2O (2 ml). The mixture was then stirred for 30 min at 0°. Work-up as usual gave benzyl alcohol and *trans*-β-methylthioallyl alcohol (**9**): ^{1}H NMR: δ2 27 (3H, *s*), 4.17 (2H, *d*, *J* = 6.4 Hz), 5.58 (1H, *dt*, *J* = 14.7, 6.4 Hz) and 6.35 (1H, *d*, *J* = 14 7 Hz); MS *m/z* (rel int). 104 $[M]^+$ (70), 91 (100), 75 (40) and 57 (60).

Reduction of isotachin B (**2**). Compound **2** (10 mg) was treated with $LiAlH_4$ (50 mg) as described above to afford β-phenylethyl alcohol and *trans*-β-methylthioallyl alcohol (**9**).

Methylation of isotachioside (**10**) Compound **2** (20 mg) in MeOH (2 ml) was treated with an excess of an ethereal soln of CH_2N_2. Work-up gave the dimethyl ether, **11** (10 mg), UV λ_{max} nm (log ε): 207 (3.87), 223 sh (3.65) and 283 (3.21); IR ν_{max}^{KBr} cm^{-1}: 3370, 1615, 1515, 1380, 1220, 1200, 1130, 1080, 1030, 985, 950 and 800; ^{1}H NMR (C_5D_5N): δ4.08 (1H, *br s*, Glc H-5), 4 29–4 36 (3H, *m*, Glc H-2–H-4), 4 38 (1H, *dd*, *J* = 11 7, 5 3 Hz, Glc H-6), 4.54 (1H, *dd*, *J* = 11.7, 2.5 Hz, Glc H-6), 5.55 (1H, *d*, *J* = 6.8 Hz, Glc H-1), 3.65 (3H, *s*, OMe), 3.70 (3H, *s*, OMe), 6 47 (1H, *dd*, *J* = 8.8, 2 9 Hz, Ph-H-5), 6 73 (1H, *d*, *J* = 2 9 Hz, Ph-H-3) and 7 53 (1H, *d*, *J* = 8.8 Hz, Ph-H-6); MS *m/z* (rel int.) $[M]^+$ (absent), 155 (9), 154 (100) and 139 (13).

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