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DEHYDROPINGUISANIN, DEHYDROPINGUISENOL AND PINGUISENAL, THREE PINGUISANE-TYPE SESQUITERPENES FROM *TROCHOLEJEUNEA SANDVICENSIS*

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Key Word Index—*Trocholejeunea sandvicensis*; Lejeunaeceae; Hepaticae; dehydropinguisanin; dehydropinguisenol; pinguisenal; pinguisane-type sesquiterpenes.

Abstract—Three new pinguisane-type sesquiterpenes, dehydropinguisanin, dehydropinguisenol and pinguisenal together with the previously known deoxopinguisone, pinguisanin, pinguisanolide and (−)-bicyclogermacrene, have been isolated from the liverwort, *Trocholejeunea sandvicensis*, and their structures have been established from spectral evidence.

INTRODUCTION

As a part of our systematic investigation of the biologically active substances of Hepaticae, we are continuing to study their chemical constituents. Recently, we have reported the isolation and structures of several sesqui- and diterpenoids which show allergenic contact dermatitis, plant growth inhibition, anticancer and antifeedant effects [1–12]. The species belonging to Lejeuneaceae also elaborate interesting sesqui- and diterpenoids [13]. In the present communication, we wish to report the isolation and the structures of three new unstable pinguisane-type sesquiterpenes named dehydropinguisanin, dehydropinguisenol and pinguisenal from the small liverwort, *Trocholejeunea sandvicensis*.

RESULTS AND DISCUSSION

Column chromatography and prep. TLC on Si gel of the combined ether extract of the air-dried material resulted in the isolation of three new pinguisane-type sesquiterpenes, dehydropinguisanin (**1**), dehydropinguisenol (**2**) and pinguisenal (**3**), together with the previously known pinguisane-type sesquiterpenes, pinguisanin (**4**), pinguisanolide (**5**) [14], deoxopinguisone (**6**) [15–17], and a sesquiterpene hydrocarbon, (−)-bicyclogermacrene (**9**) [18, 19]. The new pinguisane-type sesquiterpenes were very unstable in dilute acids and decomposed gradually by exposure to air.

Table 1. ^{1}H NMR spectral data of the new pinguisanes*

	1†	2†	3‡
H-1			1.7–2.4§
H-3	3.90 (*bs*, $W\frac{1}{2} = 4.5$)		5.36 (*s*)
H-4			2.36 (*q*, $J = 7$)
H-6			1.7–2.4§
H-7	4.20 (*bs*, $W\frac{1}{2} = 3$)	4.30 (*bs*, $W\frac{1}{2} = 4$)	4.27 (*bs*, $W\frac{1}{2} = 6$)
H-10	6.36 (*bd*, $J = 2$)	6.36 (*bd*, $J = 2$)	6.10 (*d*, $J = 8$)
H-11	7.20 (*d*, $J = 2$)	7.27 (*d*, $J = 2$)	9.90 (*d*, $J = 8$)
H-12	0.95 (*s*)	1.07 (*s*)	1.23 (*s*)
H-13	1.10 (*d*, $J = 8$)	0.87 (*d*, $J = 7$)	1.07 (*d*, $J = 7$)
H-14	1.30 (*s*)	1.25 (*s*)	1.30 (*s*)
H-15	4.95 (*s*) 5.10 (*s*)	5.05 (*s*) 5.16 (*s*)	1.30 (*d*, $J = 7$)

* All assignments were confirmed by the double resonance experiments.

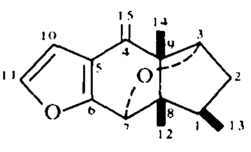
† The spectra were run in CCl_4.

‡ The spectrum was run in $CDCl_3$.

§ The signals of H-1 and H-6 overlapped.

Dehydropinguisanin (**1**)

The major component (**1**), $C_{15}H_{18}O_2$ (M^+ 230), gave the positive Ehrlich test. The UV and IR spectra showed the presence of a furane ring (1595 and 1510 cm^{-1}) and an exomethylene (1650 cm^{-1}; λ_{max} 212, 217 and 237 nm) conjugated with the furane ring. In the IR spectrum, the absorption bands of carbonyl and hydroxyl groups could not be observed, indicating the additional oxygen to be an ether. The ^{1}H NMR and double resonance spectra (Table 1) contained the signals of two protons of a typical α,β-disubstituted furane ring, two tertiary methyl groups and a secondary methyl group, two protons of an exomethylene and two methine groups attached to the ether oxygen, one of which was located between a double bond and a quaternary sp^3 carbon atom. The above spectral data coupled with the molecular formula showed that **1** was a bicyclic sesquiterpene ether with an α,β-disubstituted furane ring conjugated with a double bond. The ^{1}H NMR spectrum of **1** resembled that of pinguisanin (**4**) recently isolated from the European liverwort, *Porella platyphylla* [14], except for the presence of two protons of an exomethylene group and the absence of one allylic secondary methyl group, indicating that dehydropinguisanin had the same structure as **4** in which one secondary methyl group at C-4 was replaced by the exomethylene group. Hydrogenation of **1** in the presence



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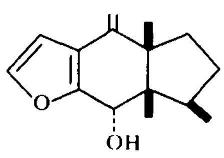

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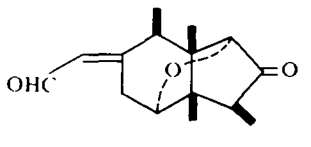

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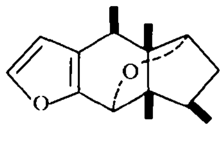

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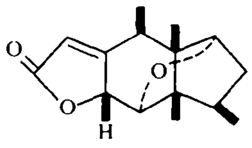

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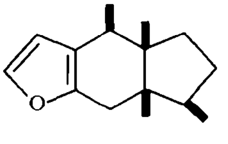

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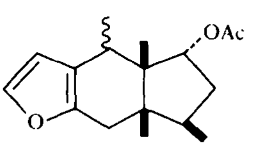

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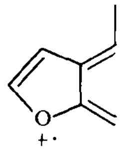

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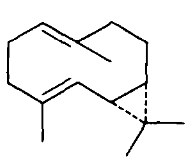

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of Pd–C, followed by acetylation gave the C-4 epimeric monoacetates (7), $C_{17}H_{24}O_3$ (M^+ 276). The mass spectrum of 7 indicated the base peak at *m/e* 108 which resulted from the *retro*-Diels–Alder reaction between the furane ring and cyclohexane ring as shown in the formula **8** [20]. Thus, the above results together with the co-occurrence of pinguisanin (**4**) in the same plant, established the structure of dehydropinguisanin to be **1**.

Dehydropinguisenol (**2**).

The IR spectrum of the minor component (**2**), $C_{15}H_{20}O_2$ (M^+ 232), exhibited the presence of a hydroxyl group (3450 cm^{-1}), a double bond (1645 cm^{-1}) and a furane ring (1595 and 1510 cm^{-1}). The UV spectrum was almost identical to that of dehydropinguisanin (**1**) indicating that **2** has an exomethylene group conjugated with a furane ring. The ^{1}H NMR spectrum (Table 1) was also quite similar to that of **1**, except for the presence of one broad singlet signal at δ 4.30 (1H), assignable to one allylic proton on a carbon bearing a secondary hydroxyl group and the absence of two methine groups attached to the ether oxygen. The above spectral evidence along with the molecular formula showed that the second new pinguisane-type sesquiterpene alcohol might be represented by the formula **2**. The position and the stereochemistry of the secondary hydroxyl group of **2** were further confirmed to be C-7α by the presence of a long range coupling between H-7 and H-10 [14]. Irradiation at the centre of the β-proton, which appeared as a broad doublet at δ 6.36, caused the broad singlet at 4.30 to collapse to a sharp singlet; in the reverse irradiation at 4.30, the signal at 6.36 collapsed to a sharp doublet.

Pinguisenal (**3**)

The third minor pinguisane (**3**), $C_{15}H_{20}O_3$ (M^+ + 1, 249), showed the intense positive 2,4-DNP test. The UV and IR spectra indicated the presence of an enal group (λ_{max} 232.5 nm; 1680 cm^{-1}) and a five-membered ketone (1745 cm^{-1}). In the IR spectrum, the absorption bands of a hydroxyl group and another carbonyl group could not be observed, meaning that the additional oxygen might be an ether. The ^{1}H NMR spectrum contained the signals of two tertiary methyl groups, two secondary methyl groups, one allylic methine proton coupled with secondary methyl protons, a 2,2-disubstituted ethenal group, two protons on a carbon bearing an ether oxygen, two allylic methylene protons and an additional methine proton coupled with another secondary methyl group. Irradiation of the allylic methylene protons, which appeared at δ 1.7–2.4 as a multiplet, caused a proton on a carbon bearing an ether oxygen to collapse to a sharp singlet. The above spectral evidence coupled with the molecular formula and the co-occurrence of the other pinguisanes in the same plants led us to the structure **3** for pinguisenal. The positive Cotton effect further supported this structure. It is suggested that pinguisenal **3** may be an important precursor of the pinguisane-type sesquiterpenes having an ether linkage between C-3 and C-7, and a hydroxyl group at C-7.

Pinguisanin (**4**), *pinguisanolide* (**5**), *deoxopinguisone* (**6**) *and* (−)*-bicyclogermacrene* (**9**)

The structures of three pinguisanes (**4**–**6**) and a sesquiterpene hydrocarbon (**9**) were established by the complete identity of the physical constants and spectral data with those of authentic samples[14,16,17,19]. Furthermore, β-caryophyllene was detected in the hydrocarbon fraction by GC–MS analysis.

It is interesting from the taxonomic view-point that *T. sandvicensis* (Lejeuneaceae) and *Porella platyphylla* (Porellaceae) produce the same pinguisane-type sesquiterpenes (**4**–**6**), although the two species are morphologically quite different.

EXPERIMENTAL

The solvents used for spectral determination were: CCl_4(^{1}H NMR, 60 or 90 MHz); MeOH ($[\alpha]_D$ and CD); 95% EtOH (UV). IR spectra were measured in liquid film. TLC and prep. TLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–EtOAc (4:1), C_6H_6–EtOAc (4:1) and *n*-hexane–$CHCl_3$–MeOH (14:19:1). Spots were detected by 2,4-DNP, Ehrlich reagent, 30% H_2SO_4 and UV light (254 nm). MS: EI–MS (DI method), 70 eV; GC–MS: 70 eV, SE-30 1%, glass column, 3m × 2mm, temp. programme, 50–270° at 5°/min., inject. temp. 260°, He 30 ml/min.

Extraction and isolation. *Trocholejeunea sandvicensis* collected in Tokushima and in Miyazaki prefectures, was air-dried and ground mechanically. The ground material (53.77 g) was extracted with Et_2O for 10 days. The crude extract (2.45 g) was directly chromatographed on Si gel (70–270 mesh) using *n*-hexane–EtOAc gradient. The first fraction eluted with *n*-hexane contained the mixture of sesquiterpenes (180 mg) in which β-caryophyllene, bicyclogermacrene (**9**), an unidentified sesquiterpene hydrocarbon [M^+ 204 (base 161)] and a furanosesquiterpene [M^+ 218 (base 108)] have been detected by GC–MS. The above fraction was rechromatographed on Si gel using C_6H_6 to afford (–)-bicyclogermacrene (**9**) (80 mg) [18, 19] and deoxopinguisone (**6**) (20 mg). The second fraction (*n*-hexane–EtOAc, 19:1) contained carotenoids (50 mg). The third fraction (9:1) gave a mixture of triglycerides (390 mg). The fourth fraction (7:3) contained a mixture of furanosesquiterpenes (750 mg) which was rechromatographed on Si gel using C_6H_6–EtOAc gradient to afford pinguisanin (**4**) (180 mg), pinguisanolide (**5**) (50 mg)[14] and dehydropinguisanin (**1**) (250 mg), which was purified by prep. TLC using *n*-hexane–$CHCl_3$–MeOH (14:19:1) to give a colourless oil.

Dehydropinguisanin (**1**): $[\alpha]_D + 4.4$ (*c*, 5.8); MS (high resolution) (Found: 230.1316. Calc.: $C_{15}H_{18}O_2$, 230.1307): IR ν_{max} cm^{-1}: 1650, 880 (C=C), 1595, 1510, (furane ring), 1400, 1390, 1375, 1300, 1265, 1240, 1215, 1145, 1120, 1090, 1065, 1050, 1035, 1010, 980, 950, 920, 900, 850, 845, 785, 755, 750, 730, 650; UV λ_{max} nm(ε): 212 (2431), 217.5 (2277), 237.5 (2258); MS *m/e* (rel. int.): 230 (M^+, 100), 215 (13), 201 (36), 187 (60), 186 (69), 178 (95), 177 (30), 176 (32), 159 (76), 145 (65), 131 (33), 115 (28), 109 (70), 91 (30), 77 (25). The yellow oil (80 mg) obtained from the fifth fraction (4:1) showed one Ehrlich positive, one 2,4-DNP positive and one Liebermann–Burchardt positive spots on TLC. Rechromatography of the fraction on Si gel using C_6H_6–EtOAc gradient gave dehydropinguisenol (**2**) (10 mg), pinguisenal (**3**) (17 mg) and a sterol mixture (campesterol, stigmasterol and sitosterol, 2:3:1 by GC–MS).

Dehydropinguisenol (**2**): $[\alpha]_D + 5.6$ (*c*, 2.5); MS (high resolution) Found: 232.1467, Calc.: $C_{15}H_{20}O_2$ 232.1463; IR ν_{max} cm^{-1}: 3450, 1000 (OH), 1645 (C=C), 1595, 1510, (furane ring), 1200, 1140, 1070, 940, 915, 900, 875, 840, 800, 775, 750, 730; UV λ_{max} nm(ε): 213 (2910), 218 (2889), 236 (2869); MS *m/e* (rel. int.): 232 (M^+, 81), 217 (M^+ – 15, 50), 214 (M^+ – H_2O), 199 (48), 189 (22), 175 (24), 161 (39), 159 (27), 145 (24), 122 (45), 121 (36), 109 (100), 108 (21), 91 (23).

Pinguisenal (**3**): $[\alpha]\frac{589}{0}\frac{370}{-0.7}\frac{276\,\mathrm{nm}}{+15.0}$ (*c*, 0.18); Anal.: C, 77.59; H, 8.18, Calc. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.10%; IR ν_{max} cm^{-1}: 1745 (C=O), 1680 (C=C–CHO), 1625 (C=C), 1395, 1380, 1360, 1320, 1295, 1275, 1235, 1200, 1175, 1155, 1140, 1120, 1095, 1060, 1000, 945, 865, 745, 700, 665, 640; UV λ_{max} nm(ε): 202.5 (3569), 233.5 (6510); CD: $\Delta\varepsilon\frac{336}{-0.66}\frac{264\,\mathrm{nm}}{+2.03}$; MS *m/e* (rel. int.): 249 (M^+ + 1, 1), 137 (10), 110 (19), 109 (48), 108 (100), 97 (39), 93 (35), 67 (10), 55 (10), 43 (11), 41 (41).

Acetylation of the hydrogenated mixtures of **1**. The compound (**1**) (150 mg) in EtOH was hydrogenated in the presence of prereduced 5% Pd–C (20 mg) for 2 hr. The reaction mixture was filtered off and the solvent was evaporated to give a viscous oil (133 mg) whose IR spectrum showed the presence of a hydroxyl group (3400 cm^{-1}) and a furane ring (1595 and 1510 cm^{-1}). The product showed at least eight spots on TLC and it was acetylated by Ac_2O–Py, without purification, followed by chromatography on Si gel using a C_6H_6–EtOAc gradient to afford the C-4 epimeric 3-acetoxypinguisones (**7**) (1:1 on GLC) (20 mg). $C_{17}H_{24}O_3$; IR ν_{max} cm^{-1}: 1740, 1240 (OAc), 1505 (furane), 1375, 1360, 1160, 1130, 1075, 1020, 940, 890, 835, 790, 730; ^{1}H NMR: δ 0.85–1.16 (complex signals), 2.00 (3H, *s*), 4.57 (1H, *bs*), 6.00 (1H, *bd*, *J* = 2), 7.10 (*d*, *J* = 2); MS *m/e* (rel. int.): The component of R_t = 14.7: 276 (M^+, 4), 201 (8), 161 (30), 147 (30), 133 (8), 109 (21), 108 (100), 43 (17). The component of R_t = 15: 276 (M^+, 4), 216 (7), 201 (4), 161 (17), 147 (10), 133 (5), 109 (20), 108 (100), 43 (12).

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