Phytochemistry, 1979, Vol. 18, pp. 1349–1353. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0801-1349 $02.00/0

PINGUISANIN, PINGUISANOLIDE AND β-PINGUISENEDIOL, THREE NEW PINGUISANE-TYPE SESQUITERPENES FROM *PORELLA PLATYPHYLLA*

YOSHINORI ASAKAWA*, MASAO TOYOTA*, TSUNEMATSU TAKEMOTO* and CLAUDE SUIRE†

*Institute of Pharmacognosy, Tokushima-Bunri University, Yamashiro-cho, 770 Tokushima, Japan; †Laboratoire de Botanique, Université de Bordeaux 1, Avenue des Facultés, 33405 Talence, France

(*Revised received* 22 *January* 1979)

Key Word Index—*Porella platyphylla*; Jungermanniales; Hepaticae; pinguisanin; pinguisanolide; β-pinguisenediol; pinguisane-type sesquiterpenes.

Abstract—Three new pinguisane-type sesquiterpenes, pinguisanin, pinguisanolide and β-pinguisenediol, together with the previously known deoxopinguisone, have been isolated from a European liverwort, *Porella platyphylla*, and their structures have been established by the spectral evidence and some chemical transformations.

INTRODUCTION

The *Porella* species of liverworts produce various sesquiterpenes and in particular, *P. vernicosa* complex contains a sharp pungent substance. Recently, we have reported the isolation and structures of several drimane-, pinguisane- and aromadendrane-type sesquiterpenes from *P. vernicosa* complex and the pungency of these species was due to a sesquiterpene dialdehyde, polygodial [1–4]. It is known that *Porella platyphylla* contains monoterpene hydrocarbons [5] and the crude extract of this species shows antimicrobial activity against gram-positive bacteria [6]. The active compounds, however, remained to be discovered. As part of our systematic investigation of the biologically active substances of bryophytes, we have reinvestigated the chemical constituents of *P. platyphylla*. The present paper reports on the isolation and structures of the three new pinguisane-type sesquiterpenes.

RESULTS AND DISCUSSION

Extraction of air-dried and ground material with ether and fractionation on a Si gel column and PLC gave three new pinguisane-type sesquiterpenes, named as pinguisanin (**1a**), pinguisanolide (**2a**) and β-pinguisenediol (**3**), together with the previously known deoxopinguisone (**13**).

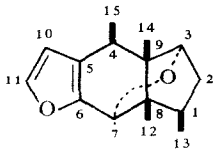
Pinguisanin (**1a**)

The major component (**1a**), mp 50–51°, $C_{15}H_{20}O_2$, was very unstable in dilute acids. The UV and IR spectra showed the presence of a furane ring (1503, 1470 cm^{-1}; λ_{max} 224 nm). 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 ^1H NMR and double resonance spectra (NMDR) (Table 1) contained the signals attributable to two protons of the typical α,β-disubstituted furane ring, two secondary methyl and two tertiary methyl groups, one allylic proton which was coupled with one of the secondary methyl groups and two methine groups attached to the ether oxygen, one of which was placed between a quaternary sp^3 carbon atom and a double bond. The ^{13}C NMR spectrum also showed the presence of a tetrasubstituted double bond [122.5 (*s*), 149.3 (*s*)], a 1,2-disubstituted double bond [110.2 (*d*), 142.8 (*d*)] characteristic of an α,β-disubstituted furane ring, two methine groups [76.1 (*d*), 79.5 (*d*)], linked to the ether oxygen, two quaternary sp^3 carbon atoms [40.1 (*s*), 51.1 (*s*)], together with two secondary methyl, two quaternary methyl and one methylene groups. The above spectral evidence along with the molecular formula indicated that **1a** was a bicyclic sesquiterpene ether with the α,β-disubstituted furane ring and the partial structure was represented as **1c**. This partial structure has been found in deoxopinguisone (**13**) and pinguisone (**14**) isolated from the liverworts, *Aneura pinguis* [7], *Ptilidium ciliare* [8] and *Porella vernicosa* complex [1–4]. In fact, the ^1H NMR signal pattern of **1a** strikingly resembled those of deoxopinguisone (**13**) (Table 1) and pinguisone (**14**) [7] except for the presence of the two methine protons at δ 3.96 and 4.38 indicating that the structure of pinguisanin might be deoxopinguisone with a C-3/C-7 (**1a**) or C-2/C-7 ether linkage (**1b**). Hydrogenation of **1a** gave two compounds (GC–MS: M^+ 236 and 238), which on acetylation with Ac_2O–Py, followed by column chromatography afforded the tetrahydro derivative (**4**) possessing an ether linkage and a monoacetate (**6**) (1735 cm^{-1}; δ 2.11). Oxidation of the hydrogenated mixture (**4** and **5**) with CrO_3–Py gave a γ-lactone (**7**) (1770 cm^{-1}; M^+ 250) and a six-membered ketone (**8**) (1700 cm^{-1}; M^+ 236], respectively. The formation of the γ-lactone (**7**) is unusual. The crystalline pinguisanin used for the reduction reaction was pure (GLC, TLC and spectral data). The hydrogenated mixture derived from **1a** contained no oxidative product corresponding to the γ-lactone (**7**) on GLC, TLC and GC–MS. Reproducibility of the formation of **7** from the hydrogenated mixture treated with CrO_3–Py was observed. Since the

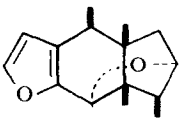
Table 1. ^{1}H NMR data* of the new pinguisanes and their derivatives

	1a	2a	3	10	11	13
H-1	1.9 (*m*)		1.95 (*m*)		2.95 (*q*, J = 7)	
H-2	2.3 (*m*)		3.78 (*m*)	4.70 (*sextet*, J = 7, 7, 4)		
H-3	3.96 (*bs*, $W_{\frac{1}{2}}$ = 7)	−1.17† 4.06 (*bs*)			2.15 (*d*, J = 18) 2.90 (*d*, J = 18)	
H-4	2.60 (*q*, J = 8)	−0.18† 2.77 (*q*, J = 8)				2.54 (*q*, J = 7)
H-6		4.22 (*d*, J = 2)	2.65 (*dd*, J = 15, 6)	2.26 (*dd*, J = 16, 6)	3.15 (*bs*)	
H-7	4.38 (*s*)	−0.64† 3.91 (*s*)	3.78 (*m*)	5.01 (*m*)		2.22 (*dd*, J = 17, 3) 2.15 (*d*, J = 17)
H-10	6.29 (*bd*, J = 2)	−0.13† 5.57 (*d*, J = 2)	6.71 (*dd*, J = 15, 9)	6.73 (*dd*, J = 18, 12)	6.80 (*dd*, J = 17, 12)	6.16 (*d*, J = 3)
H-11	7.34 (*d*, J = 2)	0†	5.01 (*d*, J = 9) 5.15 (*d*, J = 15)	5.05 (*d*, J = 12) 5.13 (*d*, J = 18)	5.15 (*d*, J = 17) 5.18 (*d*, J = 1)	7.20 (*bs*, $W_{\frac{1}{2}}$ = 3.5)
H-12	1.22 (*s*)	1.25 (*s*)	0.95 (*s*)‡	0.88 (*s*)	1.01 (*s*)	0.73 (*s*)‡
H-13	1.16 (*d*, J = 8)	1.00 (*d*, J = 8)	1.13 (*d*, J = 8)	1.08 (*d*, J = 7)	0.91 (*d*, J = 7)	0.85 (*d*, J = 7)
H-14	1.10 (*s*)	1.05 (*s*)	0.97 (*s*)‡	1.04 (*s*)	1.16 (*s*)	0.82 (*s*)‡
H-15	1.16 (*d*, J = 8)	1.03 (*d*, J = 8)	1.80 (*bs*)	1.77 (*bs*)	1.91 (*bs*)	1.12 (*d*, J = 7)
OAc				1.98 (*s*) 2.07 (*s*)		

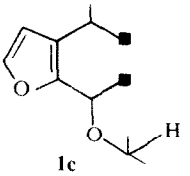
* All assignments were confirmed by the double resonance experiments.
† $\Delta Eu = \delta_{CDCl_3} - \delta_{Eu(fod)_3}$, pinguisanin (12 mg, 5.17×10^{-5} mol) containing 6 mg of $Eu(fod)_3$.
‡ The signals may be interchanged.



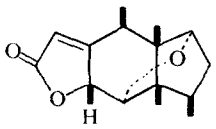
1a



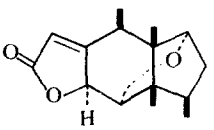
1b



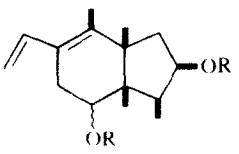
1c



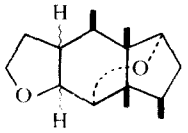
2a



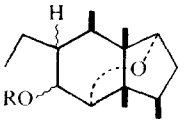
2b



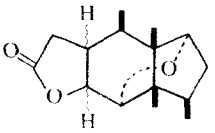
3 R = H
10 R = Ac



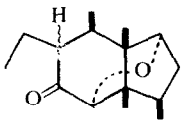
4



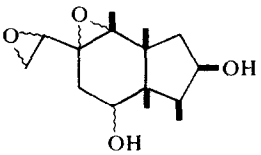
5 R = H
6 R = Ac



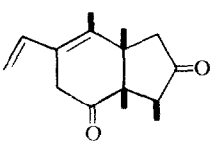
7



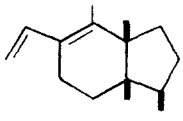
8



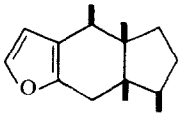
9



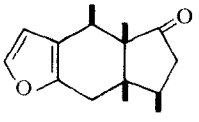
11



12



13



14

oxidation of the tetrahydro derivative of deoxopinguisone (**13**) with the same CrO_3–Py complex gave no γ-lactone, it is considered that the ether linkage of **1a** may have some influence upon the formation of the γ-lactone. The reaction mechanism, however, remains to be confirmed. The above chemical transformation further supported the structure of **1a** or **1b** for pinguisanin. The confirmation of structure **1a** for pinguisanin is apparent from the following arguments. Examination of the Dreiding model of **1a** shows that the dihedral angles between H_e-3 and $H_{quasi\text{-}e}$-2 and between H_e-3 and $H_{quasi\text{-}a}$-2 are *ca* 60°, respectively and the ^1H NMR signal of H-3 may appear as a broad singlet, like the carbinyl proton of a 4,4-dimethyl-3-hydroxyl triterpene. On the other hand, in the struture **1b** the dihedral angles of the vicinal protons (H_e-C_2-C_1-H_e, H_e-C_2-C_3-H_e and H_e-C_2-C_3-H_a) are 60, 20 and 90°, respectively and the H-2 may appear at least as a doublet signal with a coupling constant *ca* 6 Hz as predicted from the Karplus equation [9]. The observed signal of the proton in question appeared at δ 3.96 ($W_{\frac{1}{2}}$ = 7 Hz) as a broad singlet, which supported the ether linkage at the C-3/C-7 position. In the ^1H NMR spectrum of deoxopinguisone, the long range coupling (J = 1.5 Hz) has been observed with the α-proton of the furane ring. On the other hand, a similar long range coupling has been seen between the β-proton and H-7 in pinguisanin (**1a**). This long range coupling and the presence of the lower field signal of one tertiary methyl group (δ 1.22) on C-8, which was considerably strained by the ether linkage, gave additional evidence for structure **1a**. On the basis of all the facts discussed above, we propose the structure **1a** for pinguisanin.

Pinguisanolide (**2a**)

The second pinguisane-type sesquiterpene, $C_{15}H_{20}O_3$ (M^+ 248), exhibited an intense IR band at 1790 cm^{-1}, attributable to the characteristic butenolide group. The ^1H NMR and NMDR spectra indicated the presence of two tertiary methyl and two secondary methyl groups and a doublet signal of one proton assigned to the α-proton of a butenolide. In the IR spectrum, absorption bands corresponding to hydroxyl and carbonyl groups other than that for the presence of the butenolide, could not be observed, indicating that the third oxygen was ether. This assumption was further confirmed by the ^1H NMR signals at δ 3.91 (1H, *s*) and 4.06 (1H, *bs*) assignable to two protons on a carbon bearing an ether oxygen. The above spectral evidence coupled with the molecular formula showed that pinguisanolide was a bicyclic sesquiterpene ether with a butenolide group. The partial structure of **2a** was established by double resonance. Irradiation at the center of the doublet (δ 4.22) assigned to H-6 caused the doublet at 5.57 (H-10) to collapse to a sharp singlet. The reverse irradiation at 5.57 caused the doublet at 4.22 to collapse to a singlet. Irradiation at the center of the quartet at 2.77, assigned to one allylic proton (H-4), caused the doublet at 1.03, attributable to the secondary methyl group at C-4, to collapse to a singlet. Reverse irradiation caused the quartet at 2.77 to collapse to a singlet. The ^1H NMR signal pattern was strikingly similar to that of pinguisanin (**1a**) except for the presence of the pair of doublet signals (H-6 and H-10) assigned above, indicating that pinguisanolide was unambiguously the oxidation product of the furane ring in pinguisanin. Thus, the structure of pinguisanolide has been represented as **2a**, which would have the six-membered ring in the half chair conformation with 6β-H, or **2b**, possessing the boat conformation with 6α-H. In the latter conformation, the tertiary methyl group at C-8 is located over the shielding zone of the double bond of the butenolide and the H-12 would be shielded and allylic long range coupling between H-4 and H-10 might be observed. In the ^1H NMR spectrum of pinguisanolide, the strongly shielded signal of the tertiary methyl group and the long range coupling between H-4 and H-10 could not be observed. These results, coupled with the absence of vicinal coupling between H-6 and H-7 (the dihedral angle is H-C_6-C_7-H, *ca* 85°), were only compatible with the preferred half chair conformation in the six-membered ring of **2a**. The above spectral data showed that the new butenolide was most favourably represented by the formula **2a**.

β-Pinguisenediol (**3**)

The most polar compound (**3**), $C_{15}H_{20}O_2$ (M^+ 236), contained a conjugated double bond (λ_{max} 241 nm), vinyl group (990, 910 cm^{-1}) and hydroxyl group (3400 cm^{-1}). Treatment of **3** with *m*-chloroperbenzoic acid gave a diepoxide (**9**), $C_{15}H_{24}O_4$ (M^+ 268), indicating the presence of two double bonds. Acetylation of **3** with Ac_2O–Py gave a diacetate (**10**) (1740 cm^{-1}; δ 1.98 and 2.07, each, 3H), confirming two hydroxyl groups. Oxidation of **3** with Collins' reagent afforded a diketone (**11**), $C_{15}H_{20}O_2$ (M^+ 232), whose IR spectrum showed bands at 1710 and 1745 cm^{-1}, indicating the presence of non-conjugated six- or more than six-membered and five-membered ketones. Wolff–Kishner reduction of **11** resulted in the formation of a sesquiterpene hydrocarbon (M^+ 204) whose spectral and chromatographic behaviour were completely identical to β-pinguisene (**12**) [3]. Thus, the location of the six-membered ketone of **11** was at C-7 and the five-membered one might be placed at the C-2 or C-3 position. Hence, one of the original two secondary hydroxyls of **3** was placed at C-7 and the other one at the C-2 or C-3 position of β-pinguisene. The exact position of the secondary hydroxyl group in the five-membered ring was confirmed by the AB doublet signals at δ 2.15 and 2.90 (J = 18 Hz) assigned to the methylene protons at C-3 of **11** and by the well separated sextet at 4.70 (J = 7, 7 and 4 Hz), attributable to H-2 of **10**. The latter signal pattern was not anticipated when the acetoxyl group was placed at C-3 in place of C-2 in structure **10**.

The stereochemistry of the secondary hydroxyl group at C-2 was suggested to be β by the sextet signal of H-2 of **10**, discussed above. The configuration at C-7 of **3** remains to be confirmed since the signals of H-7 of **3** and **10** were superimposed by the other signals. The above results, along with the co-existence of pinguisanin and pinguisanolide, led to structure **3** for β-pinguisenediol.

Pinguisane-type sesquiterpenes, usually rare in nature, have frequently been encountered in liverworts such as *Aneura* [7], *Ptilidium* [8], *Trichocoleopsis* [10] and *Porella* species [1–4]. There are two types of *Porella* species, one of which contains the intense pungent sesquiterpene dialdehyde and one containing no pungent substance. The former species, for example *P. vernicosa* complex and *P. alboris-vitae* [11], commonly elaborate

drimane-, aromadendrane- and pinguisane-type sesquiterpenes. On the other hand, the latter species, for example *P. densifolia* [2, 3] and *P. japonica* [12], do not contain drimane- and aromadendrane-type sesquiterpenes, but they produce a large amount of pinguisanes. The present European species belongs to the latter type and it elaborates a large amount of pinguisanes. The eremophilane-type sesquiterpenes containing a butenolide have been found together with furanosesquiterpenes [13–17] and the former lactone compounds are often considered as the auto-oxidation product [13]. The ether extract of *P. vernicosa* complex and the other *Porella* species so far examined contain various pinguisane-type furano sesqui- and furanonorsesquiterpenes. However, no butenolides have been detected. The TLC and GC–MS analysis of the extract of *P. platyphylla* immersed for two days in ether showed the presence of pinguisanolide (**2a**), indicating that compound **2a** might be a natural product.

EXPERIMENTAL

The solvents used for spectral determinations were: TMS–$CDCl_3$ (^1H NMR and ^{13}C NMR, 90 MHz); $CHCl_3$ (IR and $[\alpha]_D$); 95% EtOH (UV); MeOH (CD). TLC and PLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–EtOAc (4:1) and C_6H_6–EtOAc (4:1 and 1:1). Spots were detected by 50% H_2SO_4 or 2,4-DNP and UV light (254 and 360 nm). GC–MS: 70 eV, 5% OV-17, 3 m × 2 mm glass column, temp. programme, 50–250° at 5°/min, He 30 ml/min. CI-MS: 500 eV, reaction gas, *iso*-butane

Extraction and isolation. Porella platyphylla collected in Eyzies, Dordogne, France, Nov., 1977 was air-dried for 5 days. The ground material (240 g) was extracted with Et_2O for 2 weeks. The green viscous oil (5.740 g) was directly chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane 100%) gave a pale yellow oil (500 mg) which contained the complex mono- and sesquiterpene hydrocarbon mixtures not identified. The second fraction (n-hexane–EtOAc, 19:1) gave a yellow oil (1.400 g) which was rechromatographed on Si gel to afford pinguisanin (**1a**) (900 mg) as colourless needles, mp 50–51°. $[\alpha]_D$ +18° (*c*, 1.7); $C_{15}H_{20}O_2$ (MS anal. 232.1486; calc. 232.1463); UV λ_{max} nm 224: (log ε, 3.71); IR ν_{max} cm^{-1}: 1503, 1470, 1392, 1377, 1354, 1167, 1134, 1118, 1062, 1028, 983, 970, 949, 910, 900, 843, 695, 516; ^{13}C NMR: 12.5(*q*), 12.9 (*q*), 20.1 (*q*), 35.6 (*q*), 39.1 (*d*), 40.1 (*s*), 48.1 (*t*), 51.1 (*s*), 53.2 (*d*), 76.1 (*d*), 79.5 (*d*), 110.2 (*d*), 122.5 (*s*), 142.8 (*d*), 149.3 (*s*); MS *m/e* (rel. int.): 232 (M^+ 38), 173 (11), 124 (15), 109 [base, C_7H_{12} anal. 109.0982; calc. 109.0972], 41 (10). The third fraction (19:2) contained pinguisanin (**1a**) and an unknown lactone which was purified by PLC to afford pinguisanolide (**2a**) (10 mg). $C_{15}H_{20}O_3$; IR ν_{max} cm^{-1}: 1790, 1403, 1393, 1380, 1318, 1239, 1228, 1174, 1158, 1119, 1091, 1072, 1055, 1014, 991, 965, 945, 925, 852, 807, 780, 763, 738, 720, 670; MS *m/e* (rel. int.): 248 (M^+, 2), 109 (76), 108 (33), 97 (base), 41 (26). The fourth fraction (7:3) gave a viscous oil (480 mg) which was rechromatographed on Si gel to afford β-pinguisenediol (**3**) (210 mg). $[\alpha]_D$ −42° (*c*, 0.4); $C_{15}H_{24}O_2$; UV λ_{max} nm: 241 (log ε, 3.21); IR ν_{max} cm^{-1} 3400(OH), 1630(C=C), 1380, 1090, 1030, 990, 910(CH_2=CH—), 973, 682; MS *m/e* (rel. int.): 236 (M^+, 29), 218 (M^+ − 18, 30), 185 (63), 163 (20), 161 (34), 159 (45), 157 (27), 147 (79), 146 (35), 141 (31), 135 (51), 133 (66), 123 (89), 121 (57), 119 (83), 109 (base), 108 (57), 107 (71), 105 (76), 95 (74), 91 (52), 79 (55), 41 (56).

Hydrogenation of **1a**. EtOAc soln of **1a** (100 mg) was hydrogenated in the presence of pre-reduced PtO_2 (30 mg) for 5 hr. Work-up as usual gave a viscous oil in which the tetrahydro derivative (**4**) and hydrogenolysed product (**5**) were detected. Compound (**5**): $C_{15}H_{26}O_2$ [GC–MS: M^+ 238 (1)], 220 (M^+ − 18, 12), 137 (33), 121 (32), 109 (base), 97 (87), 96 (55), 95 (30), 69 (43), 55 (46), 43 (54), 39 (69).

Acetylation of the hydrogenated mixtures of **1a**. The hydrogenated mixtures (30 mg) of **1a** was acetylated with Ac_2O–Py, followed by PLC to give the tetrahydro derivative (**4**) (20 mg) and an acetate (**6**) (5 mg). Compound **4**: $[\alpha]_D$ −43.6° (*c*, 2.0); $C_{15}H_{24}O_2$; IR ν_{max} cm^{-1}: 1385, 1375, 1130, 1108, 1090, 1060, 1030, 1005, 985, 950, 906, 875; ^1H NMR: δ 1.03 (3H, *d*, *J* = 7), 1.08 (3H, *d*, *J* = 7), 1.08 (6H, *s*), 3.83 (2H, *m*), 4.10 (3H, *m*); MS *m/e* (rel. int.): 236 (M^+, 2), 121 (27), 109 (56), 97 (base), 96 (37), 95 (25), 69 (32), 41 (59). Compound **6**: $C_{17}H_{28}O_3$; IR ν_{max} cm^{-1}: 1735, 1248 (OAc), 1380, 1370, 1110, 1030, 988, 975, 918; ^1H NMR: δ 0.99 (3H, *t*, *J* = 6, H-11), 1.12 (3H, *s*), 1.08–1.12 (superimposed signals of H-13 and H-15), 2.11 (3H, OAc), 3.73 (*d*, *J* = 2, H-7), 4.05 (*bs*, $W_{\frac{1}{2}}$ = 4 Hz, H-3), 5.19 (*dd*, *J* = 5, 2, H-6). MS *m/e* (rel. int.): M^+ (no parent peak), 220 (M^+ − 60, 4), 151 (34), 136 (74), 109 (33), 108 (33), 97 (20), 55 (29), 43 (base).

Oxidation of the hydrogenated mixtures of **1a**. The hydrogenated mixture (50 mg) of **1a** in CH_2Cl_2 (2 ml) was treated with CrO_3–Py complex (50 mg) in CH_2Cl_2 (2 ml) at 0° for 24 hr. The reaction mixture was filtered through a short column packed with Si gel to give a pale yellow oil which was purified by PLC to afford the γ-lactone (**7**) (8 mg) and a saturated ketone (**8**) (5 mg). Compound **7**: $C_{15}H_{22}O_3$; IR ν_{max} cm^{-1}: 1770(γ-lactone), 1170, 1025, 1005, 1000, 970, 963, 875; ^1H NMR: δ 1.11 (6H, *d*, *J* = 8, H-13 and H-15), 1.13 (6H, *s*, H-12 and H-14), 3.96 (*bd*, *J* = 3, H-7), 4.15 (*bs*, H-3), 4.66 (*m*, H-6); MS *m/e* (rel. int.): 250(M^+ 5), 235 (5), 109 (96), 97 (base), 55 (75), 41 (68). Compound **8**: mp 76–77°; $[\alpha]_D$ −49° (*c*, 0.2); Δε, 316 nm (−1.54); IR ν_{max} cm^{-1}: 1700 (C=O), 1395, 1380, 1235, 1100, 1055, 1000, 940; ^1H NMR: δ 0.93 (3H, *t*, *J* = 7, H-11), 0.97 (3H, *d*, *J* = 7), 1.09 (3H, *d*, *J* = 7), 1.07 (3H, *s*), 1.13 (3H, *s*), 3.63 (*s*, H-7), 4.20 (*bs*, $W_{\frac{1}{2}}$ = 6 Hz, H-3); MS *m/e* (rel. int.): 236 (M^+, 16), 136 (31), 109 (63), 97 (base), 96 (31), 69 (25), 55 (25), 41 (24).

Epoxidation of β-pinguisenediol (**3**). The alcohol **3** (20 mg) was treated with *m*-chloroperbenzoic acid (10 mg) for 12 hr at 0°. Work-up as usual afforded epoxide **9** (5 mg). $C_{15}H_{24}O_4$; IR ν_{max} cm^{-1}: 3420 (OH), 1295, 1265, 1138, 1080, 1050, 1025, 885, 753; ^1H NMR: δ 1.61 (3H, *bs*, H-15), 1.1 (9H, complex signal); MS *m/e* (rel. int.): 268 (M^+, 2), 250 (M^+ − 18, 2), 181 (76), 139 (43), 109 (64), 97 (base).

Acetylation of **3**. To Py soln of **3** (20 mg) was added Ac_2O and the soln allowed to stand overnight. Work-up as usual gave diacetate **10** (21 mg). $[\alpha]_D$ + 13° (*c*, 1.0); UV λ_{max} nm: 237.5 (log ε, 3.00); IR ν_{max} cm^{-1}: 1740, 1243 (OAc), 1634, 1600 (C=C), 1380, 1365, 1155, 1115, 1085, 945, 905, 830, 754; MS *m/e* (rel. int.): 260 (M^+ − 60, 25), 201 (19), 200 (M^+ − 60 − 60, 62), 185 (81), 157 (23), 146 (50), 145 (29), 43 (base); CI-MS: 321 (M^+).

Oxidation of **3**. To CH_2Cl_2 soln of CrO_3–Py complex was added compound **3** (80 mg) in CH_2Cl_2 soln. Work-up as usual afforded a pale yellow oil, which was purified by PLC to give diketone **11** (60 mg). $[\alpha]_D$ +53° (*c*, 0.37); IR ν_{max} cm^{-1}: 1745 (C=O), 1710 (C=O), 1410, 1385, 1315, 1240, 1185, 1138, 1100, 1070, 990, 908, 820, 760, 650; Δε, 300 nm (−3.41); MS *m/e* (rel. int.) 232 (M^+, base), 189 (29), 174 (25), 161 (35), 151 (53), 150 (27), 147 (47), 137 (50), 136 (34), 133 (32), 124 (30), 123 (25), 109 (54), 103 (36), 91 (37).

Wolff–Kishner reduction of **11**. A mixture of diketone **11** (60 mg), hydrazin hydrate (1.5 ml), ethylene glycol (7 ml) and *n*-BuOH (5 ml) was heated at 110° in N_2 stream for 3 hr and then excess solvent was distilled off. To the reaction mixture was added NaOH (0.4 g) and the mixture heated again at 110° for 7 hr. H_2O was added to the reaction mixture which was ex-

tracted with Et_2O, the extract was dried over Na_2SO_4 and evapd, leaving a pale yellow oil (10 mg), whose spectral data and chromatographic behavior were identical to those of β-pinguisene [3].

Acknowledgements—The authors are indebted to all participants at the International Congress of Bryology (at Bordeaux) for the collection and identification of the species. The plant was dried and milled at the Institut de Chimie, Université Louis Pasteur, Strasbourg. The authors thank Prof. G. Ourisson (Univ. L.P.) for his helpful suggestions.

REFERENCES

1. Asakawa, Y. and Aratani, T. (1976) *Bull. Soc. Chim. Fr.* 1469.
2. Asakawa, Y., Toyota, M., Uemoto, M. and Aratani, T. (1976) *Phytochemistry* **15**, 1929.
3. Asakawa, Y., Toyota, M. and Aratani, T. (1976) *Tetrahedron Letters* 3619.
4. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Phytochemistry* **17**, 457.
5. Suire, C. and Bourgeois, G. (1977) *Phytochemistry* **16**, 284.
6. Pavietic, Z. and Stilinovic, B. (1963) *Acta Bot. Croat.* **22**, 133.
7. Benešová, V., Samek, Z., Herout, V. and Šorm, F. (1968) *Collect. Czech. Chem. Commun.* **34**, 582.
8. Krutov, S. M., Samek, Z., Benešová, V. and Herout, V. (1973) *Phytochemistry* **12**, 1405.
9. Jackman, L. M. and Sternhell, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*. Pergamon Press, Oxford.
10. Asakawa, Y., Takemoto, T., Toyota, M. and Aratani, T. (1977) *Tetrahedron Letters* 1407.
11. Asakawa, Y., Huneck, S., Toyota, M., Takemoto, T. and Suire, C. (1979) *J. Hattori Bot. Lab.* **46** (in press)
12. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T. and Suire, C. (1979) *J. Hattori Bot. Lab.* **45**, 395.
13. Novotný, L., Herout, V. and Šorm, F. (1964) *Collect. Czech. Chem. Commun.* **29**, 2182.
14. Ishii, H., Tozyo, T. and Minato, H. (1966). *J. Chem. Soc. C* 1545.
15. Moriyama, Y. and Takahashi, T. (1976) *Chem. Pharm. Bull.* **24**, 360.
16. Bohlmann, F. and Zdero, C. (1978) *Phytochemistry* **17**, 1135.
17. Jizba, J., Samek, Z., Novotný, L., Najidenova, E and Boeva, A. (1978) *Collect. Czech. Chem. Commun.* **43**, 1113.