Phytochemistry, 1981, Vol. 20, pp. 257–261. © Pergamon Press Ltd. Printed in England. 0031-9422/81/0201-0257 $02.00/0

TWO GUAIANE-TYPE SESQUITERPENE LACTONES AND THEIR RELATED SESQUITERPENE LACTONES FROM *PORELLA JAPONICA*

YOSHINORI ASAKAWA, MASAO TOYOTA and TSUNEMATSU TAKEMOTO

Institute of Pharmacognosy, Tokushima-Bunri University, Yamashiro-cho, 770 Tokushima, Japan

(*Received* 8 *May* 1980)

Key Word Index—*Porella japonica*; Jungermanniales; Hepaticae; new germacranolide; new guaianolides; porelladiolide; (1*R*, 2*S*, 5*R*, 6*S*, 7*S*, 11*S*)-guaian-3,9-dien-10,2-12,6-diolide; 3α,4α-epoxyporelladiolide; eregoyazidin; isoeremanthin; plant growth inhibitory activity.

Abstract—Two new guaiane-type sesquiterpene dilactones, porelladiolide and 3α,4α-epoxyporelladiolide and a new germacranolide, 3β-hydroxycostunolide, together with the previously known germacranolides, guaianolides, pinguisane-type sesquiterpenes, and a sacculatane-type diterpene dialdehyde have been isolated from the liverwort, *Porella japonica* and their structures have been established by the spectral evidence and some chemical transformations. Porelladiolide, 3α,4α-epoxyporelladiolide and the sesquiterpene with α-methylene γ-lactone group showed inhibitory activity toward the germination and growth of roots of rice in the husk.

INTRODUCTION

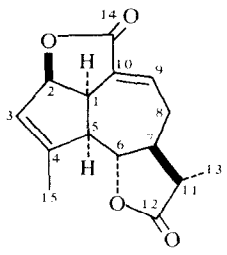
The *Porella* species of the liverworts are a rich source of sesqui- and diterpenoids which show some interesting biological properties such as antimicrobial activity and inhibition of germination of higher plants. Recently we have reported the isolation of various types of sesqui-and diterpenoids from *Porella* species [1–12]. In the continuation of our studies of terpenoids and lipophilic aromatic compounds of the liverworts, two pinguisanes (**12**, **14**) and a sacculatane-type diterpene dial (**15**) together with various mono- and sesquiterpene hydrocarbons have been found in *Porella japonica* [13]. We reinvestigated *P. japonica*, which proved to be a rich source of guaianolides and germacranolides showing the properties of plant-growth inhibitory activity. We now report the isolation and structures of two new guaiane-type sesquiterpene dilactones, named porelladiolide (**1**) and 3α,4α-epoxyporelladiolide (**2**), a new germacranolide, 3β-hydroxycostunolide (**8**) and their related sesquiterpene lactones.

RESULTS AND DISCUSSION

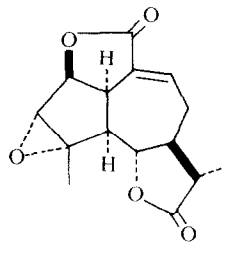
Column chromatography and PLC on silica gel of the green fragrant oil resulted in the isolation of two new guaianolides, porelladiolide (**1**), 3α,4α-epoxyporelladiolide (**2**), a new germacranolide, 3β-hydroxycostunolide (=3-*epi*-tamaulipin B) (**8**), together with the previously known isoeremanthin (**3**), eregoyazidin (**5**), costunolide (**9**) and dihydrocostunolide (**10**), the pinguisane-type sesquiterpenes deoxopinguisone (**12**), norpinguisone (**13**) and norpinguisone methyl ester (**14**) and a sacculatane-type diterpene dial, perrottetianal A (**15**).

Porelladiolide (**1**)

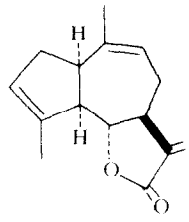
The major component (**1**), mp 194–195°, $[\alpha]_D$ − 64.6°, showed the characteristic intense blue spot on TLC obtained by spraying with 30% H_2SO_4 and then heating at 100°. The high resolution MS indicated that the molecular formula was $C_{15}H_{16}O_4$. The presence of a γ-lactone group was confirmed by the intense absorption band in the IR spectrum at 1765 cm^{-1}. The UV and CD spectra showed bands at 222 nm (log ε, 4.23) and 272 nm (Δε, +0.77), assignable to an α,β-unsaturated γ-lactone [14]. The ^{1}HNMR and ^{1}HNMDR spectra (Table 1) contained the signals of one vinylic methyl group, a typical α-methyl group of a γ-lactone, two vinylic protons, one of which was located on a carbon conjugated with a lactone carbonyl group, two protons on an oxygen bearing carbon and two methine groups located between double bonds and oxygen bearing carbons. The IR spectrum displayed an intense absorption band at 1740 cm^{-1}, indicating the additional two oxygens to be in a lactone. This assumption was further confirmed by the following spectral and chemical evidence: the ^{13}C NMR spectrum (Table 2) of **1** indicated the presence of two singlet signals (δ 170.3 and 177.9), attributable to lactone carbon atoms and two doublet signals (81.5 and 84.5) for the carbons bearing lactone oxygens. Hydrogenation of **1** in the presence of PtO_2 easily gave a mono carboxylic acid (**6**), followed by methylation with CH_2N_2 to afford a monomethyl ester (**7**), $C_{16}H_{24}O_4$ (M^+ at *m/e* 280) [1765 (γ-lactone), 1735 cm^{-1} (COOMe)]. Treatment of **1** with *m*-chloroperbenzoic acid gave a monoepoxide (**2**), mp 225–226°, $C_{15}H_{16}O_5$ (M^+ at *m/e* 276). The above spectral and chemical data coupled with the molecular formula showed that porelladiolide was a bicyclic sesquiterpene with two γ-lactones and two trisubstituted double bonds, one of which was conjugated with a γ-lactone. The ^{13}C NMR spectrum of **1** also showed the presence of the signals of two trisubstituted ethylenic carbons, four methine carbons, one methylene and two methyl groups. In the ^{1}H NMR spectrum of **1** the assignment of the low field multiplet (δ 6.81, 1H) to the vinylic proton (H-9) which was located over the deshielding zone of the lactone carbonyl group was obvious. This proton was also coupled with the methylene protons and with one of the allylic protons (H-1) which was also coupled with one



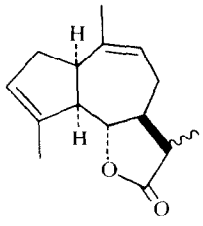
1



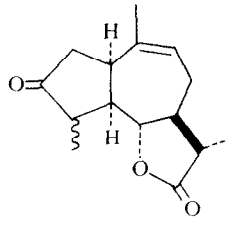
2



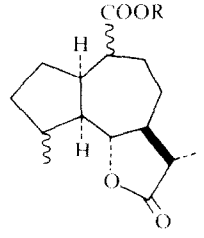
3



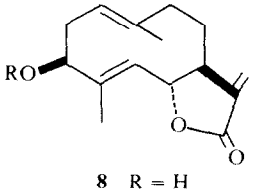
4



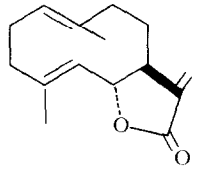
5



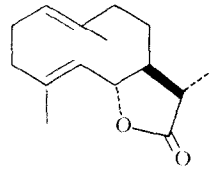
6 R = H
7 R = Me



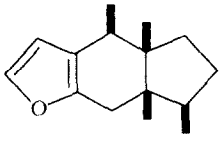
8 R = H
11 R = Ac



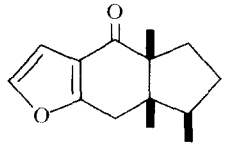
9



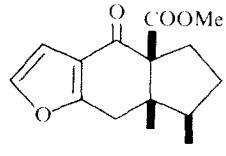
10



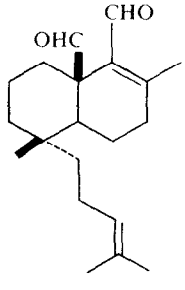
12



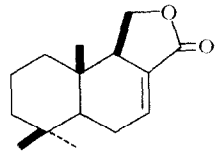
13



14



15



16

proton (H-2) on a carbon bearing a lactone oxygen. The UV spectrum, and the chemical shift of the vinylic proton (H-9) and its splitting pattern (δ 6.81, *dd*, J = 8, 3 Hz) of **1** were quite similar to those of cinnamolide (**16**) (λ_{max} 224 nm; δ 6.86; H-7, *dd*, J = 8, 3 Hz) isolated from other *Porella* species [2–4, 6, 7, 10]. Furthermore, the CD spectrum of **1** was identical to that of cinnamolide (272 nm, $\Delta\varepsilon$, +1.16). The arrangement of the α-methyl γ-lactone of **1** was confirmed as *trans* by the double doublet signal (δ 4.12, J = 9.8 Hz) of the proton (H-6) on a carbon bearing a lactone oxygen. In the ^{1}H NMR one allylic proton (H-5) appeared as a triplet at 3.17 (J = 8 Hz) coupled with another allylic proton (H-1). Irradiation at a broad singlet of the vinylic proton (H-3) caused the broad

Table 1. ^{1}H NMR spectral data* of porelladiolide (**1**), 3α,4α-epoxyporelladiolide (**2**) and 3β-hydroxycostunolide (**8**)

	1	2	8
H-1	3.92 *m*	3.60 *m*	4.93 *bt* (J = 8 Hz)
H-2	5.47 *bd* (J = 9 Hz)	4.96 *dd* (J = 9, 2 Hz)	1.9 2.4 *m*
H-3	5.58 *bs*	3.51 *d* (J = 2 Hz)	4.28 *dd* (J = 10, 6 Hz)
H-5	3.17 *t* (J = 8 Hz)	2.75 *t* (J = 9 Hz)	4.85 *bd* (J = 9 Hz)
H-6	4.12 *dd* (J = 9, 8 Hz)	4.63 *dd* (J = 9, 8 Hz)	4.63 *t* (J = 9 Hz)
H-7	3.00 *m*	2.2–2.5 *m*	1.9–2.4 *m*
H-8	2.35 *m*	2.2–2.5 *m*	1.9–2.4 *m*
H-9	6.81 *dd* (J = 8, 3 Hz)	6.53 *dd* (J = 8, 3 Hz)	1.9–2.4 *m*
H-11	2.35 *m*	2.2–2.5 *m*	
H-13	1.32 *d* (J = 8 Hz)	1.28 *d* (J = 8 Hz)	5.56 *d* (J = 3 Hz) 6.30 *d* (J = 3 Hz)
H-14			1.46 *bs*
H-15	1.87 *bs*	1.53 *s*	1.76 *bs*

* All assignments were confirmed by double resonance experiments.

doublet at 5.47 (H-2) and the broad singlet at 1.87 (H-14) to collapse to a sharp doublet and a sharp singlet, respectively. Thus, the above mentioned facts permit the formation of the gross structure (**1**) for porelladiolide. The stereochemistries of C-1, C-2, C-5, C-6 and C-7 were confirmed by the splitting patterns (Table 1) of each proton in the ^{1}H NMR spectra of **1** and **2**. The β (axial) configuration of the lactone oxygen at C-2 was further confirmed by the facile hydrogenolysis of allylic γ-lactones [15]. The remaining question was the stereochemistry at C-11. We could not confirm the configuration of the secondary methyl group by the ^{1}H NMR solvent shift method [16] because **1** hardly dissolved in C_6H_6 or C_5H_5N. We assigned the quasi-equatorial methyl group at C-11 by the co-occurrence of eregoyazidin (**5**) and dihydrocostunolide (**10**) and by the higher chemical shift (δ 13.5) of C-13 in the ^{13}C NMR spectrum [17, 18]. On the basis of the above evidence, the structure of porelladiolide was established to be (1*R*, 2*S*, 5*R*, 6*S*, 7*S*, 11*S*)-guaian-3,9-dien-10,2-12,6-diolide (**1**).

Table 2. ^{13}C NMR chemical shifts of porelladiolide (**1**) and 3α,4α-epoxyporelladiolide (**2**) (ppm from internal TMS)*

	1	2
C-1	42.3 *d*	42.3 *d*
C-2	81.5 *d*	79.0 *d*†
C-3	125.9 *d*	64.5 *d*
C-4	147.0 *s*	68.0 *s*
C-5	44.0 *d*	38.6 *d*
C-6	84.5 *d*	79.3 *d*†
C-7	56.9 *d*	53.2 *d*
C-8	34.2 *t*	34.3 *t*
C-9	137.8 *d*	133.6 *d*
C-10	127.9 *s*	127.8 *s*
C-11	40.5 *d*	44.3 *d*
C-12	177.9 *s*	177.7 *s*
C-13	13.5 *q*	13.5 *q*
C-14	170.3 *s*	170.3 *s*
C-15	15.1 *q*	16.2 *q*

* The spectra were obtained at 22.6 MHz in Fourier transform mode in $CDCl_3$ solutions.

† Values within the vertical column may be reversed.

3α, 4α-Epoxyporelladiolide (**2**)

The second dilactone (**2**), mp 225–226°, $C_{15}H_{16}O_5$ (high MS: 276.1001), also showed the intense blue spot on TLC by the same treatment as described in porelladiolide (**1**). The ^{1}H NMR spectrum (Table 1) was quite similar to that of **1**, except for the presence of an additional signal of a tertiary methyl group and of signal which would be that of an epoxide proton, and the absence of one vinylic methyl group and one vinylic proton, indicating that compound **2** might be the 3, 4-epoxide of porelladiolide (**1**). In fact, all spectral data and chromatographic behavior were completely identical to those of mono-epoxide **2** derived from **1**. The stereochemistry of the epoxide was established to be α by the small coupling constant (J = 2 Hz) of H-3 in the ^{1}H NMR spectrum. Thus, the structure of the second new sesquiterpene dilactone was established to be (3*R*, 4*S*)-epoxy-porelladiolide (**2**).

3β-Hydroxycostunolide (**8**) (= 3-epi-*tamaulipin-B*)

The compound **8**, $C_{15}H_{20}O_3$ (M^+ at *m/e* 248) was obtained as an unstable oil. It exhibited the presence of a hydroxyl group (3450 cm^{-1}) and a γ-lactone (1760 cm^{-1}). The ^{1}H NMR spectrum (Table 1) contained the signals of two vinylic methyl groups, two vinylic protons, one proton on a carbon bearing a lactone oxygen, one carbinyl proton and typical α-methylene protons of a γ-lactone. The ^{1}H NMR spectrum was in good agreement with that of costunolide (**9**) [19], except for the presence of a double doublet at δ 4.28 corresponding to one proton, indicating that **8** was costunolide with an allylic secondary hydroxyl group. The ^{1}H NMR spectrum of **8** also resembled that of tamaulipin B having a 3α-hydroxyl group, isolated from *Ambrosia* species (Compositae) [19, 20], except for the lower chemical shifts of H-1, H-3 and H-5, and their splitting modes, meaning

that **8** might be the C-3 epimer of tamaulipin-B. The above assumption was further confirmed by the following evidence. Acetylation of **8** gave a monoacetate whose spectral data and physical constants were completely identical to those of novanin (**11**) and the acetate was further converted into cyclonovanin A by $SOCl_2$ [21]. Recently, hanphyllin was isolated from *Handelia trichophylla* and its structure was assigned to be 3β-hydroxycostunolide (**8**) only by the ^1H NMR spectrum [22]. However, the IR and ^1H NMR spectral data of hanphyllin were different from those of 3β-hydroxycostunolide isolated from *P. japonica*. It may be necessary to convert hanphyllin into novanin (**11**), since the presence of a secondary hydroxyl group at C-9 in place of C-3 in hanphyllin is not excluded by ^1H NMR spectrum.

In addition to the new guaiane-type dilactones (**1** and **2**), germacranolide (**8**) and two guaianolides (**3** and **5**) have been isolated. Isoeremanthin (**3**) has recently been derived from naturally occurring eremanthin [23]. As far as we are aware this is the first report of the isolation of isoeremanthin from the plants. Eregoyazidin (**5**) has been isolated from *Eremanthus* species (Compositae) [24]. In addition to the two guaianolides, the presence of dihydroeremanthin (**4**), which was prepared from isoeremanthin by $NaBH_4$ reduction, was confirmed by GC–MS analysis. Furthermore, two germacranolides (**9** and **10**), three pinguisane-type sesquiterpenes (**12**–**14**) and a sacculatane-type diterpene dialdehyde (**15**) have been isolated and their structures were established by the identity of physical constants and spectral data with those of deoxopinguisone, norpinguisone and norpinguisone methyl ester [3], costunolide and dihydrocostunolide [19, 25] and perrottetianal A [11], respectively.

The liverworts generally elaborate the optical isomeric sesquiterpenoids to those found in higher plants. This is a most significant biochemical property in the Hepaticae. However, there are several exceptions. In Jungermanniales, some *Porella* [1–4, 6–10] and *Frullania* species [26–29] and in Marchantiales, *Wiesnerella* [25], *Marchantia* [30] and *Conocephalum* [31] produce sesquiterpenoids with the same configurations as those found in the higher plants. The present guaianolides and germacranolides isolated from *Porella japonica* have the same 6α, 7β configurations as those found in the higher plants. Porellaceae is classified between Frullaniaceae and Lejeuneaceae. Some *Frullania* species elaborate large amounts of eudesmanolides and eremophilanolides, and their precursor, costunolide (**9**) [26–29, 32]. *P. japonica* also produced costunolide together with its cyclized products, the guaianolides. However, neither eudesmanolides nor eremophilanolides have been detected in the present experiment. The same situation has been observed in *Conocephalum* and *Wiesnerella* species [30, 31] although these species are morphologically quite different from *Porella* species. Porellaceae in general elaborate pinguisane-type sesquiterpenes, such as **12**–**14** [1–13] but these unique sesquiterpenes have not yet been detected in Frullaniaceae. Thus, chemically speaking, Porellaceae is far from Frullaniaceae. The above chemical results may be applied to study the complicated systematics of Porellaceae and Frullaniaceae [8, 9, 13].

The crude extract of *Porella japonica* showed the inhibitory activity against the germination and growth of rice in the husk. The natural guaianolides (**1**–**3**) and 3β-hydroxycostunolide (**8**) showed the above mentioned activity at a concentration of 100–200 ppm [31].

EXPERIMENTAL

All mps are uncorr. The solvents used for spectral determinations were: TMS and $CDCl_3$ (^1H NMR and ^{13}C NMR); 95% EtOH (UV); MeOH (CD); $CHCl_3$ ($[\alpha]_D$). The IR spectra were obtained by the KBr pellet method, unless otherwise stated. TLC: precoated Si gel (0.25 mesh) F_{254}, solvent system: *n*-hexane–EtOAc (4:1), C_6H_6–EtOAc (4:1 and 1:1). Spots were detected in UV light (254 nm) and by spraying with 30% H_2SO_4 and then heating at 100°. GC–MS: 70 eV, column, 1% SE-30, 3 m × 2 mm, temp. programme, 50–270° at 5°/min, He 30 ml/min.

Plant material. P. japonica identified by Dr. S. Hattori is deposited in the Herbarium, Inst. of Pharmacognosy, Tokushima Bunri Univ.

Bioassay with rice in the husk. Bioassay of each sesquiterpene lactone isolated from *P. japonica* was carried out by Kato's method [33].

Extraction and isolation. P. japonica collected in Kamikatsucho, Tokushima, Japan in May 1979 was ground, after being air-dried for 1 week. The ground material (480.5 g) was extracted with Et_2O for 3 weeks. The crude extract (14.50 g) was directly chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The first fraction (*n*-hexane 100%) contained the mixtures of mono- and sesquiterpene hydrocarbons (400 mg) in which α- and β-pinenes, camphene, limonene, β-phellandrene, *p*-cymene, β-elemene and calamenene have been detected by GC–MS analysis [13]. The second fraction (*n*-hexane–EtOAc, 19:1) gave the mixtures of triglycerides, furanosesquiterpenes and sesquiterpene lactones (3.50 g) which were rechromatographed on Si gel using a *n*-hexane–EtOAc gradient to afford deoxopinguisone (**12**) (10 mg), norpinguisone (**13**) (200 mg), norpinguisone methyl ester (**14**) (50 mg) [3, 4, 13], (+)-costunolide (**9**) (50 mg), (+)-dihydrocostunolide (**11**) (10 mg) and isoeremanthin mixture (250 mg). The latter fraction was recrystallized from C_6H_6–EtOAc to afford isoeremanthin (**3**) (200 mg), $[\alpha]_D$ + 5.8 (*c*, 0.72); CD 256 nm, $\Delta\varepsilon$, −0.70, whose mp and spectral data were identical to those of the synthetic compound [23]. GC–MS analysis of the mother liquor of isoeremanthin confirmed the presence of dihydroisoeremanthin (**4**). GC–MS of **4** was identical to that of dihydroisoeremanthin derived from isoeremanthin by $NaBH_4$ reduction. The third fraction (9:1) gave the aldehyde mixture (250 mg) which was rechromatographed on Si gel using a C_6H_6–EtOAc gradient, followed by PLC to afford perrottetianal A (25 mg) (**15**) [11, 13] and phytol (20 mg). The fourth fraction (1:1) contained the sesquiterpene lactones (826 mg) which were rechromatographed on Si gel using a $CHCl_3$–MeOH gradient to give porelladiolide (**1**) (100 mg), 3α, 4α-epoxyporelladiolide (**2**) (80 mg), 3β-hydroxycostunolide (= 3-*epi*-tamaulipin B) (**3**) (50 mg) and eregoyazidin (**5**) (60 mg). $[\alpha]_D$ − 1.05° (*c*, 0.90). The mp and spectral data of **5** were completely identical to those of eregoyazidin isolated from Compositae [24].

Porelladiolide (**1**). Mp 194–195°. $[\alpha]_D$ − 64.5° (*c*, 1.80); $C_{15}H_{16}O_4$ (high resolution MS: anal. 260.1032; calcd. 260.1044); UV λ_{max} nm (log ε): 210 sh (4.14), 222 (4.23); IR ν_{max} cm^{-1}: 1765, 1740 (γ-lactone), 1685 (C=C), 1210, 1180, 1010, 990; CD: 272 nm, $\Delta\varepsilon$, +0.77; MS *m/e* (rel. int.): 260 (M^+, 100), 216 (45), 215 (32), 187 (34), 159 (22), 134 (20), 119 (30), 118 (59), 117 (48), 115 (21), 105 (29), 91 (40), 80 (23), 77 (23).

3α, 4α-Epoxyporelladiolide (**2**). Mp 225–226°, $[\alpha]_D$ − 6.3 (*c*, 4.4); $C_{15}H_{16}O_5$ (high resolution MS: anal. 276.1001; calcd. 276.0993); UV λ_{max} nm (log ε): 211 sh (4.14), 226 (4.42); IR ν_{max} cm^{-1}: 1760 (γ-lactone), 1680 (C=C), 1415, 1200, 1020, 995; CD:

260 nm, $\Delta\varepsilon$, +0.67; MS *m/e* (rel. int.): 276 (M^+ 53), 217 (95), 189 (24), 176 (48), 175 (100), 162 (29), 161 (45), 159 (21), 148 (25), 147 (34), 135 (24), 134 (63), 133 (42), 122 (23), 121 (48), 119 (23), 117 (22), 105 (26), 95 (47), 94 (33), 91 (54), 79 (33), 77 (41), 69 (29), 65 (20), 55 (35), 53 (25), 43 (52), 41 (22).

3β-Hydroxycostunolide (**8**). Oil; $C_{15}H_{20}O_3$; $[\alpha]_D$ + 103.8° (*c*, 2.60); UV λ_{max} nm (log ε): 210 (4.01); IR ν^{liq}_{max} cm^{-1}: 3450 (OH), 1760 (γ-lactone), 1665 (C=C), 1290, 1250, 1140, 1055, 980, 940; MS *m/e* (rel. int.): 248 (M^+, 0.5), 85 (77), 83 (100).

Hydrogenation of **1**. A soln of **1** (55 mg) in EtOAc (5 ml) was hydrogenated in the presence of prereduced PtO_2 (18 mg) for 1 hr. Work up as usual gave carboxylic acid **6** (50 mg). IR ν^{liq}_{max} cm^{-1}: 3150, 1705 (COOH), 1765 (γ-lactone), 1455, 1187, 985; ^1H NMR: δ 7.63 (1H, COOH), 4.43 (H-6, *t*, *J* = 9 Hz), 1.12 (3H, *d*, *J* = 7 Hz), 1.17 (3H, *d*, *J* = 7 Hz). Compound **6** was treated with CH_2N_2 in Et_2O to afford a methyl ester (**7**) (40 mg), mp 88–90°, $[\alpha]_D$ + 11.0° (*c*, 1.6); $C_{16}H_{24}O_4$; IR ν^{liq}_{max} cm^{-1}: 1765 (γ-lactone), 1735 (ester), 1450, 1383, 1190; ^1H NMR: δ 1.05 (3H, *d*, *J* = 7 Hz), 1.20 (3H, *d*, *J* = 7 Hz), 3.66 (3H, *s*), 4.52 (H-6, *t*, *J* = 9 Hz); MS *m/e* (rel. int.): 280 (M^+, 0.2), 170 (38), 165 (24), 138 (27), 134 (29), 133 (27), 121 (26), 119 (24), 111 (20), 110 (31), 109 (27), 107 (35), 105 (33), 95 (45), 94 (34), 93 (54), 91 (51), 87 (56), 82 (20), 81 (100), 79 (56), 77 (28), 69 (28), 68 (29), 67 (56), 59 (30), 55 (90), 53 (26), 41 (48).

Epoxidation of porelladiolide (**1**). To a soln of **1** (30 mg) in $CHCl_3$ (3 ml) was added *m*-chloroperbenzoic acid (20 mg) and the mixture was stirred for 24 hr at 0°. Work up as usual gave an epoxide which was purified by PLC to afford 3α,4α-epoxyporelladiolide (**2**) (20 mg) whose spectral data and chromatographic behavior were completely identical to those of the natural 3α, 4α-epoxyporelladiolide.

Acetylation of 3β-hydroxycostunolide (**8**). Compound **8** (30 mg) was acetylated with 0.6 ml Ac_2O–pyridine (1:1) at 0°. Work up as usual gave a monoacetate (28 mg) whose spectral data and physical constants were identical to novanin (**11**) [20].

Cyclization of 3β-acetoxycostunolide (**11**). Compound **11** (28 mg) in $CHCl_3$ (3 ml) was treated with $SOCl_2$ (0.2 ml) with stirring for 20 min. Evaporation of the solvent gave the cyclonovanins which were purified by PLC to afford cyclonovanin A (10 mg) as the major product [21].

Reduction of **3** *with* $NaBH_4$. Compound **3** (8 mg) in EtOAc (2 ml) was treated with $NaBH_4$ (10 mg) at room temp. Work up as usual gave dihydroisoeremanthin (**4**) (3 mg). GC–MS *m/e* (rel. int.): 232 (M^+, 7), 152 (100), 119 (30), 107 (32), 91 (25).

Acknowledgements—The authors thank Dr. S. Hattori of Hattori Botanical Laboratory, Miyazaki, Japan for his identification of the species and his useful suggestion. Thanks are also due to Prof. T. Fujita of Tokushima University for much help in measuring high resolution MS.

REFERENCES

1. Asakawa, Y., Aratani, T. and Ourisson, G. (1976) *Misc. Bryol. Lichenol.* **7**, 99.
2. Asakawa, Y., Toyota, M., Uemoto, M. and Aratani, T. (1976) *Misc. Bryol. Lichenol.* **7**, 124.
3. Asakawa, Y. and Aratani, T. (1976) *Bull. Soc. Chim. Fr.* 1469.
4. Asakawa, Y., Toyota, M., Uemoto, M. and Aratani, T. (1976) *Phytochemistry* **15**, 1929.
5. Asakawa, Y., Toyota, M. and Aratani, T. (1976) *Tetrahedron Letters* 3619.
6. Asakawa, Y., Toyota, M. and Takemoto, T. (1978) *Phytochemistry* **17**, 457.
7. Asakawa, Y. and Takemoto, T. (1978) *Bryophythorum Bibl.* **13**, 335.
8. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T. and Suire, C. (1979) *J. Hattori Bot. Lab.* **45**, 395.
9. Asakawa, Y., Tokunaga, N., Toyota, M., Takemoto, T., Hattori, S., Mizutani, M. and Suire, C. (1979) *J. Hattori Bot. Lab.* **46**, 67.
10. Asakawa, Y., Huneck, S., Toyota, M., Takemoto, T. and Suire, C. (1979) *J. Hattori Bot. Lab.* **46**, 163.
11. Asakawa, Y., Toyota, M. and Takemoto, T. (1979) *Phytochemistry* **18**, 1681.
12. Asakawa, Y., Tokunaga, N., Takemoto, T., Hattori, S., Mizutani, M. and Suire, C. (1980) *J. Hattori Bot. Lab.* **47**, 153.
13. Asakawa, Y., Yamamura, A., Waki, T. and Takemoto, T. (1980) *Phytochemistry* **19**, 603.
14. Canonica, L., Corbella, A., Gariboldi, P., Jommi, G., Křepinský, J., Ferrari, G. and Casagrande, C. (1969) *Tetrahedron* **25**, 3895.
15. House, H. O. (1965) *Modern Synthetic Reactions*, p. 12. Benjamin, New York.
16. Narayanan, C. R. and Venkatasubramanian (1968) *J. Org. Chem.* **33**, 3156.
17. Pregosin, P. S., Randall, E. W. and McMurry, T. B. H. (1972) *J. Chem. Soc. Perkin Trans.* 1, 1299.
18. González, A. G., Bermejo, J., Mansilla, H., Galindo, A., Amaro, J. M. and Massanet, G. M. (1978) *J. Chem. Soc. Perkin Trans.* 1, 1243.
19. Yoshioka, H., Mabry, T. J. and Timmermanne, B. N. (1973) *Sesquiterpene Lactones, Chemistry, NMR and Plant Distribution*, pp. 142–143. University of Tokyo Press, Tokyo.
20. Yoshioka, H., Renold, W., Fischer, N. H., Higo, A. and Mabry, T. J. (1970) *Phytochemistry* **9**, 823.
21. Irwin, M. A. and Geissman, T. A. (1973) *Phytochemistry* **12**, 875.
22. Tarasov, V. A., Abdullaev, N. D., Kasymov, Sh. Z. and Sidyakin, G. P. (1976) *Khim. Prir. Soedin.* 263.
23. Macaira, L. A., Garcia, M. and Rabi, J. A. (1977) *J. Org. Chem.* **42**, 4207.
24. Vichnewski, W., Machado, F. W. L., Rabi, J. A., Murari, R. and Herz, W. (1977) *J. Org. Chem.* **42**, 3910.
25. Asakawa, Y., Matsuda, R. and Takemoto, T. (1980) *Phytochemistry* **19**, 567.
26. Knoche, H., Ourisson, G., Perold, G. W., Foussereau, J. and Maleville, J. (1969) *Science* **166**, 239.
27. Perold, G. W., Muller, J.-C. and Ourisson, G. (1972) *Tetrahedron* **28**, 5797.
28. Connolly, J. D. and Thornton, I. M. S. (1973) *Phytochemistry* **12**, 631.
29. Asakawa, Y., Ourisson, G. and Aratani, T. (1975) *Tetrahedron Letters* 3957.
30. Kanasaki, T. and Ohta, K. (1976) *Agric. Biol. Chem.* **40**, 1239.
31. Asakawa, Y. and Takemoto, T. (1979) *Phytochemistry* **18**, 285.
32. Asakawa, Y., Muller, J.-C., Ourisson, G., Foussereau, J. and Ducombs, G. (1976) *Bull. Soc. Chim. Fr.* 1465.
33. Kato, T., Tsunakawa, M., Sasaki, N., Aizawa, H., Fujita, K., Kitahara, Y. and Takahashi, N. (1977) *Phytochemistry* **16**, 45.