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SESQUITERPENES FROM *PORELLA* SPECIES*

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Key Word Index—*Porella vernicosa*; *P. macroloba*; *P. gracillima*; *P. perrottetiana*; Hepaticae; sesquiterpenes; ent-bicyclogermacrene; ent-aromadendranes; drimanes; pinguisanes; monoterpenes.

Abstract—The *Porella* liverworts contain abundant sesquiterpenes. ent-Biocyclogermacrene, three ent-aromadendrenes, a unique hydrocarbon, α-pinguisene and two drimane type sesquiterpenes were obtained together with the intensly pungent component, tadeonal, from *P. vernicosa* and *P. gracillima*. *P. macroloba* contained the same sesquiterpenes except for the absence of ent-bicyclogermacrene and the ent-aromadendrenes. The fragrant odor of *P. perrottetiana* was composed of α-pinene and camphor.

INTRODUCTION

The liverworts show some interesting biological activities, such as allergenic contact dermatitis [1], anticancer [2], antimicrobial [3, 4] and antifeedant effects [5]. As part of our programme on such biologically active substances of the Hepaticae and as an extension of a chemosystematic investigation based on their terpenes, we have recently reported the isolation and structure of a unique pungent sesquiterpene dialdehyde and other sesquiterpenes from *P. vernicosa* and its complex (*P. macroloba* and *P. gracillima*) [6, 7]. Further investigations of the hydrocarbon and the more polar sesquiterpene fractions of *P. vernicosa* and its complex are now reported.

RESULTS AND DISCUSSION

Each *Porella* species was extracted with ether. The crude oil was chromatographed on Si gel using a *n*-hexane–EtOAc gradient. The structures of the sesquiterpenes and monoterpenes isolated by preparative GLC and TLC were deduced from their spectral data and confirmed by comparison of the physical constants and spectra with the published information, for the substances themselves and for their derivatives.

ent-Bicyclogermacrene (**1**) *and ent-α-gurjunene* (**2**)

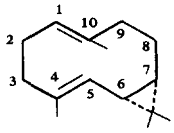
The structures of **1** and **2** deduced from IR, NMR and MS were confirmed by the identity of physical constants and spectra with those of (−)-bicyclogermacrene [8] and (−)-α-gurjunene [9], but both isolated hydrocarbons had opposite optical rotations to those found in the higher plants. Treatment of (+)-cyclocolorenone (**5**) with LiAlH$_4$–AlCl$_3$ [10] gave α-gurjunene together with a minor amount of cyclolorenol (**7**) [6]. The former was identical to the natural (+)-α-gurjunene (**2**).

ent-β-Gurjunene (**3**)

$C_{15}H_{24}$ (M^+ 204). The structure of the minor hydrocarbon was deduced from the spectral evidence. The NMR spectrum included signals for two tertiary methyls (δ 0.96 and 1.10), a secondary methyl (0.94, *d*, $J = 7$ Hz), two protons on a cyclopropane ring (~ 0.3) and an exocyclic methylene group (4.70, *bs*; IR: 890 cm^{-1}). The MS was similar to that of α-gurjunene, suggesting the presence of an aromadendrane skeleton. Hydrogenation of **3** gave the dihydroderivative which had an IR spectrum and R_t on GLC identical to those of authentic aromadendrane (**4**) [11]. The aromadendrane hydrocarbons have been isolated from various plants and their optical isomers have been synthesized [12]. However, the present aromadendrenes were not identical to any reported ones. The spectral data, biogenetic considerations and coexistence of the other aromadendrane type sesquiterpenes in the same *Porella* species led us to the structure **3**, named β-gurjunene. Further chemical reactions have not been carried out, because of the small amount of the original substance.

α-Pinguisene (**8**)

$C_{15}H_{24}$ (M^+ 204). The hydrocarbon indicated the presence of a conjugated double bond (λ_{max} 237 nm), a



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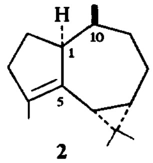

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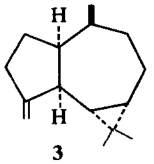

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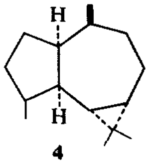

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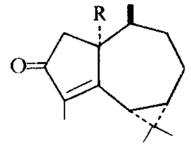
*A part of this work was presented at the *20th Symposium of Chemistry of Terpenes, Essential Oils and Aromatics of Japan*, Akita, Symposium paper p. 39 (1976).



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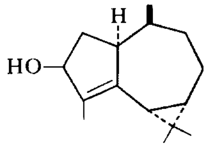
6 R = OH



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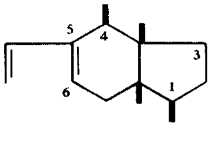

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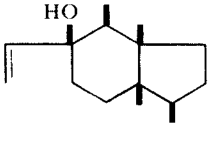

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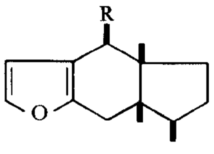

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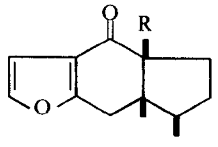

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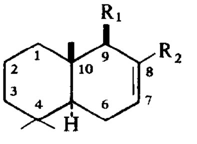
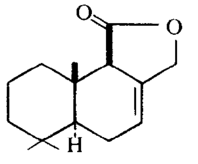
13 R = COOMe



14 $R_1 = CH_2OH$ R_2 = Me
15 R_1 = CHO R_2 = CHO
16 $R_1 = CH_2OH$ $R_2 = CH_2OH$



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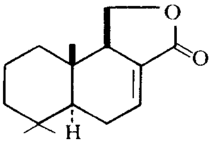

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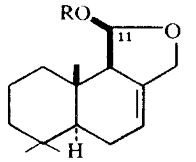

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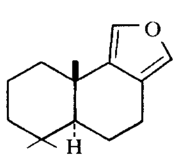

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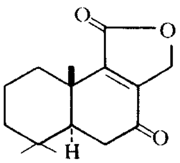

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vinyl group (δ 4.6–5.2, 2H, 6.30, *dd*, J = 17, 10 Hz, 1H; IR: 990, 910 cm^{-1}) and a trisubstituted double bond (δ 5.66, *t*, J = 7 Hz; IR: 840 cm^{-1}). The NMR spectrum also included two singlets at 0.76 (3H) and 0.91 ppm (3H) due to two tertiary methyl groups and two doublets at 0.90 (3H) and 1.00 ppm (3H) assignable to two secondary methyl groups. The IR spectrum was similar to that of pinguisene mixtures derived from pinguisenol (**9**) [13], showing the presence of the pinguisene skeleton. The GC–MS was completely identical to that of the C-5, C-6 double bond isomer of pinguisene. Thus the structure of α-pinguisene was determined to be **8**.

Drimenol (**14**)

The structure of **14** deduced from the NMR and IR spectra was confirmed by the identity of physical constants and spectra with those of (+)-drimenol [14].

1-Hydroxycyclocolorenone (**6**)

$C_{15}H_{22}O_2$ (M^+-18, 216), mp 126–128°. The compound showed the IR band at 1670 cm^{-1} and a UV band at 247 nm, indicating the presence of an α,β-unsaturated cyclopentenone. The NMR spectrum indicated the presence of a secondary methyl group (δ 1.18 ppm, J = 6 Hz), two tertiary methyl groups (1.03 and 1.25 ppm) and a vinylic methyl group (1.77 ppm, J = 2 Hz). The IR and NMR spectra were similar to those of cyclocolorenone (**5**), indicating **6** to have the same skeleton as cyclocolorenone. The IR spectrum also included an absorption band at 3210 cm^{-1}, attributable to a hydroxyl group, which resisted acetylation, showing it to be tertiary. The NMR spectrum further indicated the presence of a methylene group appearing as a typical AB doublet at δ 2.44 and 2.82 ppm (J = 14 Hz, each), but with no signals downfield of the latter signal. Hence, the location of the hydroxyl group was proved to be at C-1 in cyclocolorenone. The absolute configuration of the hydroxyl group was not determined. However, it was tentatively assumed to be α, on the basis of the weak paramagnetic shift of the secondary methyl group in the NMR spectrum after increasing the amount of $Eu(FOD)_3$.

Drimeninol (**19**)

$C_{15}H_{24}O_2$ (M^+-18, 218). This compound showed the presence of a hydroxyl group (3400 cm^{-1}), three tertiary methyl groups, an isolated methylene group in an asymmetrical environment attached to oxygen (δ 4.09 and 4.45 ppm, *d*, J = 11 Hz) and an olefinic proton (5.55 ppm, *m*). Furthermore, the NMR spectrum showed a broad doublet at 3.12 ppm (J = 5 Hz), assignable to a methine proton which might be linked to unsaturated carbon and a broad signal at 5.26 ppm due to a methine proton adjacent to a hydroxyl group. Treatment of **19** with $LiAlH_4$ gave a diol, identical with drimanediol (**16**) [6]. This fact indicated that drimeninol possessed the drimane skeleton with the same absolute configuration as tadeonal (**15**). Drimeninol was acetylated to give a labile monoacetate (**20**) (1740 cm^{-1}; δ 2.01 ppm), which was easily hydrolysed by oxalic acid to generate the original alcohol. During TLC the monoacetate (**20**) was converted into drimeninol. GLC of **19** at 220° produced an Ehrlich positive substance with a β,β-substituted furane ring (**21**). The reactions described above showed that drimeninol was an acetal in which a hydroxyl group was placed at C-11. Treatment of **19** with Collins' reagent gave oxoisodrimenine (**22**) [15], conclusively establishing the structure **19** for dimeninol. The absolute configuration of the hydroxyl group was assumed to be β on the basis of the facile dehydration of **19**.

All the sesquiterpenes described above were identified in *P. gracillima*. Except for the bicyclogermacrene and aromadendrane type sesquiterpenes, all the sesquiterpenes found in *P. vernicosa* were also obtained from *P. macroloba*. The major sesquiterpenes were (+)-

cyclocolorenone (**5**) in *P. gracillima* and (−)-tadeonal (**15**) in *P. macroloba*.

P. perrottetiana, one of the large liverworts, had a fragrant odor like pine-needles. The odorous substance was in the hydrocarbon fraction and repeated chromatography on Si gel showed that the odor was due to monoterpenes, mainly α-pinene and camphor.

The liverworts often elaborate the optical isomers of the components found in the higher plants [16–18]. It is interesting to note that bicyclogermacrene (**1**) and its derivatives (**2**, **3**, **5**, **6**) found in the present two species are the enantiomers of those found in the higher plants, whereas all the drimanes have the usual 9β,10β configuration.

EXPERIMENTAL

All mps are uncorr. The solvent used for spectral determinations were: $CDCl_3$–TMS (NMR); $CHCl_3$ (IR); 95% EtOH (UV); dioxane (CD), unless otherwise stated. GLC. analytical GLC (a) 5% SE-30, 3 m × 2 mm glass column, temp. programme: 100–240° at 5°/min, N_2 30 ml/min. (b) 10% DEGS, 3 m × 2 mm, operation conditions were the same as in (a). (c) 10% OV-17, 45 m × 0.25 mm, temp. programme: 100–240° at 5°/min, N_2 40 ml/min. (d) 10% PEG 20M, 45 m × 0.25 mm, temp. programme: 100–200° at 5°/min, N_2 40 ml/min. Preparative GLC: 10% SE-30, 3 m × 2 mm, temp. 180°, He 30 ml/min. TLC: precoated Si gel (0.25 mesh) F_{254}, *n*-hexane–C_6H_6–EtOAc (5.14.6). Spots were detected by UV light (254 and 360 nm) and by spraying with Ehrlich reagent, 50% H_2SO_4 or 2,4-DNP.

Extraction and isolation. Porella vernicosa (collected in Tokushima Prefecture, Kamikatsu-cho, 550m, Jan. 1977), *P. macroloba* (in Yamaguchi Prefec. Mito-cho, 230m), *P. gracillima* (in Yamaguchi Prefec. Akiyoshi-cho, 160 m, Dec. 1974), and *P. perrottetiana* (in Tokushima Prefec. Kaminaka-cho, 500 m, Dec. 1976) were washed with H_2O several times. After being air-dried for 1 week, the ground material (*P. vernicosa* 230 g), (*P. macroloba* 87 g), (*P. gracillima* 6.5 g) and (*P. perrottetiana* 180 g) were extracted with Et_2O for 2 weeks and the crude extracts (3.80 g, 1.84 g, 670 mg and 4.10 g respectively) were chromatographed on Si gel using a *n*-hexane–EtOAc gradient. In the case of *P. vernicosa*, the first fraction eluted by *n*-hexane contained sesquiterpene hydrocarbons, which were purified by the combination of column chromatography on Si gel impregnated $AgNO_3$ (5%) and prep GLC. (+)-Bicyclogermacrene (**1**): 12 mg; $[\alpha]_D$ −56° (lit. [8], +61°); ν_{max} 1655, 830 cm^{-1}; NMR and R_t on GLC were identical to authentic (−)-bicyclogermacrene. (+)-α-Gurjunene (**2**). 21 mg; $[\alpha]_D$ + 176° (lit. [9], −180°); δ 0.83 (*s*, 3H), 0.90 (*d*, *J* = 6 Hz, 3H), 1.06 (*s*, 3H), 1.67 (*bs*, 3H). (+)-β-Gurjunene (**3**): 12 mg; $[\alpha]_D$ +53°; 1640, 890 cm^{-1} (=CH_2). α-Pinguisene (**8**): 24 mg; $[\alpha]_D$ −43°; λ_{max} 237 nm (ε, 7031); 1640, 990, 910, 840 cm^{-1}. The second fraction eluted by *n*-hexane–AcOEt (4:1) gave deoxopinguisone (**10**, 85 mg), pinguisenol (**9**, 70 mg), norpinguisone (**12**, 155 mg), deoxopinguisone methyl ester (**11**, 35 mg), norpinguisone methyl ester (**13** 25 mg), drimenine (**17**, 32 mg) and cinnamolide (**18**, 85 mg). The third fraction eluted by *n*-hexane–EtOAc (7:3) was rechromatographed on Si gel using the same solvent system described above to afford cinnamolide (**18**, 30 mg), cyclocolorenone (**5**, 540 mg) and tadeonal (**15**, 320 mg). The fourth fraction (*n*-hexane–EtOAc, 3.2) was rechromatographed on Si gel to give a mixture of alcohols, which by PLC yielded drimenol (**14**, 8 mg). 1-hydroxycyclocolorenone (**6**, 35 mg) and drimeninol (**19**, 143 mg). Drimenol: mp 95–96° (lit. [14], 96°); $[\alpha]_D$ −20° (lit. [14], −18°). 1-Hydroxycyclocolorenone: mp 126–128°; $[\alpha]_D$ +58°; λ_{max} 247 nm (ε, 6573); ν_{max}^{liq} 1670, (=C—C=O), 1635 (C=C), 1380, 1365 (*gem* dimethyl), 1100, 960 cm^{-1}; CD $[\phi]_{326\,nm}$ −1652; NMR-[equimolar Eu$(FOD)_3$]: δ 1.63 (*d*, *J* = 6 Hz, 3H, secondary Me), 2.01, 2.20 (each *s*, 3H, tertiary Me's), 5.41 (*bs*, 3H, Me—C=). Drimeninol. $[\alpha]_D$ −26°; ν_{max}^{liq} 3400 (OH), 1390, 1370 (*gem* dimethyl); δ 0.81, 0.85, 0.95 ppm (each *s*, tertiary Me's). From *P. macroloba*, the sesquiterpenes **5**, **6**, **9**, **12**, **15**, **18**, **19** have been isolated and **2**, **3**, **8**, **10**, **11**, **13**, **17** detected by capillary GLC. From *P. gracillima*, **5**, **6**, **9**, **10**, **12**, **15**, **18**, **19** have been isolated and **1**, **2**, **8**, **11**, **13**, **17** detected by GLC.

Reduction of (+)-*cyclocolorenone* (**5**) *to* (+)-*α-gurjunene* (**2**). To cyclocolorenone (100 mg) in dry THF (10 ml) was added $LiAlH_4$ (50 mg) and $AlCl_3$ (20 mg) at 0°. The reaction mixture was then refluxed for 1 hr. The product was filtered and the solvent evapd to give the mixture of a hydrocarbon and an alcohol. Chromatography on Si gel afforded α-gurjunene (**2**, 40 mg) and cyclocolorenol (**7**, 16 mg).

Hydrogenation of β-gurjunene (**3**). β-Gurjunene (10 mg) was hydrogenated under prereduced Pd-C in EtOH. The catalyst was filtered off and recovery of the product in the usual way afforded the dihydro derivative **4**, identical to authentic aromadendrane derived from cyclocolorenone (**5**) [11].

Dehydration of pinguisenol (**9**). To pinguisenol (60 mg) in dry C_6H_6 (3 ml) was added a catalytic amount of *p*-TsOH and the soln was stirred at 40° for 3 hr. The evapn of solvent gave a brown oil which was chromatographed on Si gel to afford hydrocarbons. Rechromatography on Si gel impregnated with $AgNO_3$ (5%) using *n*-hexane gave α-pinguisene (**8**, C-5, C-6 double bond) and β-pinguisene (C-4, C-5 double bond) in the ratio 7:3 on GLC [13]. α-Pinguisene. GC–MS *m/e* (rel. int.). M^+ 204 (67), 189 (100), 175 (33), 161 (50), 147 (67), 133 (85), 119 (52), 120 (52), 109 (48), 104 (53), 93 (68), 79 (30), 77 (32), 59 (50), 53 (70), 41 (47). β-Pinguisene: GC-MS *m/e* (rel. int.): M^+ 204 (58), 189 (67), 175 (23), 161 (39), 147 (52), 133 (100), 119 (79), 109 (68), 95 (43), 93 (48), 91 (52), 79 (43), 55 (43), 41 (67).

Acetylation of drimeninol (**19**). Drimeninol (60 mg) was acetylated by Ac_2O–Py for 18 hr. Recovery of the product in the usual way gave the monoacetate: $C_{17}H_{26}O_3$ (M^+ 278); $[\alpha]_D$ −38°; ν_{max} 1740 (acetyl), 1370, 1230 cm^{-1}; δ 2.01 (*s*, MeCO), 0.80, 0.85, 0.88 (each *s*, 3H, tertiary Me's), 2.40 (*m*, 1H), 4.26 (*m*, 1H), 5.53 (*m*, 1H), 6.13 ppm (*d*, *J* = 4 Hz).

Dehydration of drimeninol (**19**). Drimeninol was injected on a 10% SE-30 column at 220° to show one peak. The pure material was preparatively obtained and filtered through a short Si gel column to give a furanosesquiterpene (**21**). Ehrlich test. positive; δ 0.96, 0.98, 1.26 (each *s*, tertiary Me's), 7.1 (*bs*, 2H on β,β-disubstituted furan ring).

Reduction of drimeninol (**19**). To $LiAlH_4$ (10 mg) in dry Et_2O was added drimeninol (20 mg) at 0° and stirred for 1 hr to give a diol (18 mg), identical to drimanediol (**16**) [6].

Hydrolysis of drimeninol monoacetate (**20**). To 25% EtOH soln of **20** (30 mg) was added oxalic acid (10 mg) and the reaction mixture was stirred for 2 hr at room temp. A few drops of H_2O were added, the mixture extracted with Et_2O and dried over $MgSO_4$. Recovery of the product gave dimeninol (18 mg).

Oxidation of drimeninol (**19**) *with Collin's reagent.* To Collins' reagent prepared from CrO_3 (160 mg) and Py (2 ml) was added drimeninol (30 mg) in CH_2Cl_2 (4 ml) and stirred for 10 hr at room temp. The recovered brown oil was purified by PLC to afford an α,β-unsaturated ketone (12 mg) identical with oxoisodrimenine (**22**) [15].

Isolation of monoterpenes from P. perrottetiana. The fraction (190 mg) eluted by *n*-hexane–EtOAc (19:1) included the mixture of mono- and sesquiterpene hydrocarbons and oxigenated monoterpenes and had an intense pine-needle like odor. On repeated chromatography on Si gel, the fragrant odor transfered into the monoterpene fraction (40 mg), in which two peaks (9:1) appeared on GLC. Prep GLC afforded α-pinene (major) and camphor (minor).

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