# Pinguisane-Type Sesquiterpenes from the South American Liverwort *Porella recurva* (Taylor) Kuhnemann

John W. van Klink[a,b] Josef Zapp[a] and Hans Becker[a,*]

[a] FR 12.3, Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, D-66041 Saarbrücken, Germany. Fax +49-681-302-2476.
E-mail: hans.becker@mx.uni-saarland.de

[b] On leave from: Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research Limited, Department of Chemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand

* Author for correspondence and reprint requests

Z. Naturforsch. **57c,** 413–417 (2002); received February 5/February 21, 2002

Bryophyte, Jungermanniales, Norsesquiterpenes

The chemical composition of a dichloromethane extract of the South American liverwort *Porella recurva* has been examined. Two new pinguisane-type norsesquiterpenoid natural products were isolated: 6,11-epoxy-15-nor-3,4-dioxo-5,10-pinguisadien-12-acetate and 6,11-epoxy-15-nor-4-oxo-5,10-pinguisadien-12-acetate. In addition two known pinguisane-type sesquiterpenes were also isolated: norpinguisone and norpinguisone methyl ester. All structures were elucidated by means of NMR spectroscopic techniques and mass spectrometry.

## Introduction

Of the large number of bryophytes reported in the literature, it is the chemical diversity of the liverworts that has been most extensively studied and this has been acknowledged in numerous literature reviews (Asakawa, 1995; Becker, 2001). The *Porella* (Jungermanniales) species are a rich source of terpenoid natural products including a range of pinguisanes (Asakawa *et al*., 2001). In continuing our investigations on the constituents of liverworts (Geis and Becker, 2000; Barlow *et al.,* 2001) we now report the identification of pinguisane-type sesquiterpenoids from a dichloromethane extract of a South American liverwort, *Porella recurva* Kuhnemann, which has not previously been investigated phytochemically.

## Results

Chromatography on silica gel, of a dichloromethane extract of *Porella recurva,* resulted in the isolation of four pinguisone-type sesquiterpenes (see Fig. 1): two new natural products **1** and **2**, and two known substances, norpinguisone **3** and norpinguisone methyl ester **4**. Except for the small sample of **4** (obtained by HPLC), all compounds were obtained from crystalline material that formed in column fractions from silica-gel chromatography. Subsequent recrystallisations enabled these substances to be obtained pure and their structures to be fully chemically characterised. Assignment of the $^{1}H$ and $^{13}C$ NMR data for **1**, **2** and **3** were carried out with the aid of DEPT, COSY, HSQC, HMBC and NOESY experiments. The 1D NMR data obtained for **4** matched that reported in the literature (Fukuyama *et al*., 1988).

GC-MS analysis of **1** gave a parent ion of 290 Da. and supported the molecular formula $C_{16}H_{18}O_5$. The strong mass fragmentation ion at *m/z* 108 and the following spectroscopic data suggested that this compound was a norpingiusone-type sesquiterpenoid. The optical rotation of **1** was strongly positive and the CD spectrum showed a positive cotton effect. The $^{1}H$ NMR spectrum showed distinctive signals at δ 6.66 and δ 7.38 (see Table I) typical of an α,β-disubstituted fused furan ring. The $^{13}C$ NMR spectrum for **1** displayed sixteen carbon resonances, assigned by DEPT to seven quaternary carbons, three methines, three methylenes and three methyls which together accounted for the expected 18 protons (Table II). An HSQC NMR experiment established the links between protons and the carbons they were attached to, while an HMBC experiment established 2- and 3-bond connectivities (Table I). Key HMBC correlations included: H-14 to C-4, C-3 and C-9; H-12 to C-7, C-8 and C-1; H-2 to C-1, C-3, and C-13. The NOESY interactions were used to as-

0939–5075/2002/0500–0413 $ 06.00  

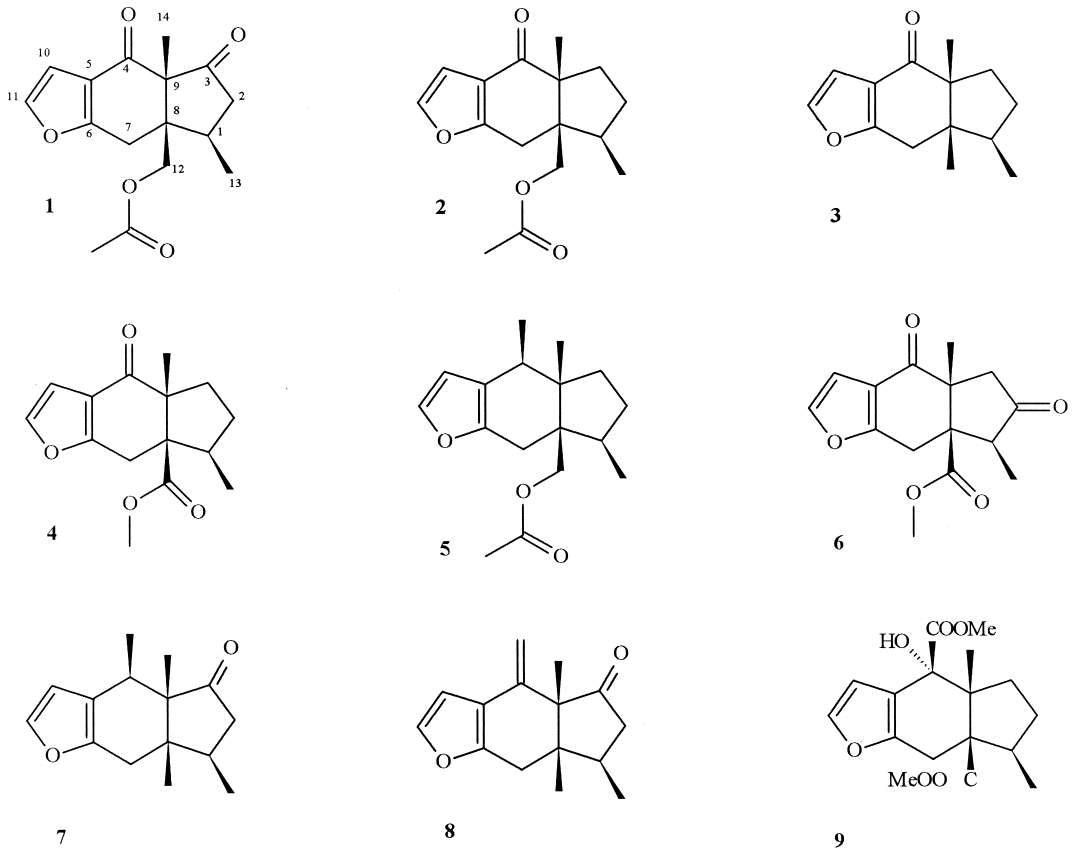


Fig. 1. Pinguisane sesquiterpenes isolated from *Porella recurva*[a] and other liverworts[b].

[a] **1** and **2** new natural products; **3**, norpinguisone; **4**, norpinguisone methyl ester.
[b] **6**, bryopterin D; **7**, pinguisone; **8**, dehydropinguisone; **9**, bryopterin C.

sign the relative stereochemistry at the three chiral centres; the C-8 to C-9 ring junction was assigned as *cis* because of a 1,3-diaxial interaction between H-12 and Me-14, and the strong interaction between Me-13 and H-12. This was expected based on literature reports of other compounds with the same carbon skeleton.

The structure of **1** represents a new pinguisane-type sesquiterpene and is only the second occurrence (including that of **2**, see below) of a norpinguisane natural product with an acetate group at position 12. The previously isolated compound **5** has an acetate group in position 12 and was isolated from the liverwort *Dircranolejeunea yoshinagana,* also from the Jungermanniales (Toyota *et al.*, 1995). Compounds **1** and **2** do not seem to be artefacts, since their $^1$H NMR signals could be seen in fresh dichloromethane extracts of *P. recurva*. Oxidation of the 'C ring' of pinguisanes is not without precedence since it occurs in several other pinguisane-type sequiterpenoids: those oxidised in position 2 such as **6**, bryopterin D (Nagashima *et al.*, 1994); and those oxidised in position 3, including pinguisone **7** and dehydropinguisone **8** (Asakawa, 1995).

GC-MS analysis gave a parent ion of 276 Da. (major fragment at 108) for **2**, which suggested a molecular formula of $C_{16}H_{20}O_4$. The spectroscopic (1- and 2D NMR data) evidence led to structure **2**. Key HMBC correlations showed the same patterns of connectivity as that for **1**. A previous publication by Nagishima *et al.* (1994) reported **2** as a semi-synthetic product derived from a sequence of reduction and acetylation reactions starting with **9**, bryopterin C. The NMR data of our sample of **2** in deuterobenzene (data not shown) matched that

Table I. $^{1}$H NMR data[a] for compounds **1**, **2** and **3**.

| Proton | **1** | HMBC for **1** | **2** | **3** |
|---|---|---|---|---|
| 1 | 2.40, ddq (11, 9.5, 7) | C2, C7, C8, C12, C13 | 1.95, ddq (10, 10, 7) | 1.82, ddq (10, 10, 7) |
| 2a | 2.52, dd (18.5,11) | C1, C3, C8, C13 (w) | 1.82, dddd (13.5, 10,10, 6.5) | 1.74, dddd (13, 10, 10, 6) |
| 2b | 1.99, dd[b] (18.5, 9.5) | C1, C2, C13 | 1.30, dddd (13.5, 11.5, 10, 4) | 1.20, dddd (13, 12, 9.5, 4) |
| 3a | – | – | 2.72, ddd (13.5, 10, 4) | 2.67, m[b] |
| 3b | | | 1.54, ddd (13.5, 11.5, 6.5) | 1.44, ddd (13.5, 12, 6) |
| 7a | 2.94, d (18) | C1, C4 (w), C5, C6, C8, C9 | 2.91 d (18) | 2.85, d (18) |
| 7b | 2.64, d (18) | C1, C4(w), C5, C6, C8, C10(w), C12 | 2,87 d (18) | 2.67, d[b] (18) |
| 10 | 6.66, d (2) | C5, C6, C11 | 6.63, d (2) | 6.63, d (2) |
| 11 | 7.38, d (2) | C5, C6, C10 | 7.33, d (2) | 7.30, d (2) |
| 12a | 4.17, d (12) | C1, C7, C8, C9, *C*OMe | 4.13 s | 0.92, s |
| 12b | 4.06, d (12) | C1, C7, C8, C9 | | |
| 13 | 1.10, d (7) | C1, C2, C8 | 0.93, d (7) | 0.85, d (7) |
| 14 | 1.20, s | C3, C4, C8, C9 | 1.13, s | 1.07, s |
| CO*Me* | 1.98, s[b] | *C*OMe | 2.08, s | – |

[a] Measured at 500 MHz in $CDCl_3$, referenced to 7.26 ppm; coupling constants ($J$ in Hz) given in parentheses and rounded to the nearest 0.5 Hz.
[b] = overlapped signals within column, resolved by 2D NMR; w = weak.

Table II. $^{13}$C NMR data[a] for compounds **1**, **2** and **3**.

| Carbon | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 33.08 | 38.49 | 38.08 |
| 2 | 42.34 | 28.85 | 28.50 |
| 3 | 209.80 | 31.59 | 30.68 |
| 4 | 190.04 | 198.32 | 199.93 |
| 5 | 117.94 | 117.05 | 117.28 |
| 6 | 162.49 | 162.46 | 163.08 |
| 7 | 24.88 | 24.97 | 29.38 |
| 8 | 48.87 | 50.17 | 47.49 |
| 9 | 65.44 | 57.61 | 57.88 |
| 10 | 107.16 | 107.15 | 107.15 |
| 11 | 143.83 | 143.21 | 142.76 |
| 12 | 65.06 | 65.50 | 17.79 |
| 13 | 14.27 | 15.19 | 14.52 |
| 14 | 15.00 | 20.18 | 19.66 |
| CO*Me* | 20.58 | 21.02 | – |
| *C*OMe | 169.84 | 170.79 | – |

[a] Measured at 125 MHz in $CDCl_3$, referenced to 77.0 ppm.

reported previously. Whereas our sample of **2** was a crystalline solid, that reported by Nagishima was an oil. However, this may have been due to the small amount obtained since despite this difference the remaining spectroscopic data was in good agreement with that for our sample of **2**. We now report for the first time the fully assigned $^{13}$C NMR data (Table II) for **2** and include $^{1}$H NMR data for the sample run in deuterochloroform (Table I).

Again as with the mass spectral analyses of **1** and **2**, the strong mass fragmentation ion at *m/z* 108 for the analysis of **3** suggested another norpinguisone-type sesquiterpenoid. The parent ion of 218 Da. supported the molecular formula of $C_{14}H_{18}O_2$. By comparison of the spectroscopic data obtained for **1** and **2** with 1D $^{1}$H- and $^{13}$C NMR data for **3** we were able to elucidate the structure as norpinguisone (Asakawa and Aratani, 1976). Furthermore we decided to undertake a series of 2D NMR experiments to fully assign the $^{13}$C NMR spectra (Table II), which had not previously been reported. HMBC correlations showed the same patterns of connectivity as measured for compounds **1** and **2**.

The fourth pinguisone that was isolated from *Porella recurva* was substance **4**, norpinguisone methyl ester. Only small amount was isolated by silica-gel HPLC, since the spectroscopic data was in aggreement with that of previously published data for **4** (Nagashima *et al*., 1994).

## Experimental

### *General experimental procedures*

All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 35° C. Silica-gel 60 TLC grade was used for vacuum liquid chromatography (VLC). HPLC was performed using a Rheodyne injector connected to a Bischoff HPLC pump and 8110 RI detector. Melting points were measured without correction using a Leitz hot-stage microscope. Optical rota-

tions were measured on a Perkin-Elmer 241 polarimeter. GC-MS was performed with a Hewlett Packard G1800A GCD system using helium (60 Kpa, 1 ml/min) as the carrier gas. Samples were injected (250° C) and analysed on an HP-5 column (15 m × 0.25 mm ID, 0.25 μm film). CD and IR spectra were recorded on Jasco J-715 and Zeiss IMR instruments, respectively. NMR spectra, at 298 K, were recorded at 500 MHz for $^{1}$H NMR and 125 MHz for $^{13}$C NMR on a Bruker DRX500 spectrometer. Chemical shifts are given in parts per million (ppm) on the δ scale referenced to the solvent peak $CHCl_3$, at 7.26 and $CDCl_3$ at 77.0. $^{13}$C multiplicities were determined using the DEPT pulse sequence. 2D spectra were recorded as COSY, HSQC, HMBC and NOESY experiments.

*Plant material*

*Porella recurva* was collected in Rio Negro, Argentina in 1997 by Prof. R. Mues. A voucher specimen (collection Nr. 970001, herbarium Nr. 3216) is retained in the Herbarium Drehwald, Göttingen, Germany.

*Extraction and isolation*

Dried plant material (500 g) was ground in liquid nitrogen to a fine powder and extracted in a Soxhlet apparatus using $CH_2Cl_2$ (2 l). After removal of the solvent, the crude extract (16 g) was chromatographed on silica gel (150 g, VLC) employing an *n*-hexane–EtOAc gradient. Column fractions were stored at –20° C from which several crystalline deposits formed, subsequently the corresponding compounds were isolated as follows: Crystalline material from the fraction that eluted between 90:10 and 85:15 hexane:EtOAc was recrystallised from hexane to yield **3** (CAS RN 62121-27-1) colourless prisms (93 mg): $^{1}$H and $^{13}$C NMR, see Tables I and II; spectroscopic data matched that of literature values. Crystalline material from the fraction that eluted between 80:20 hexane:EtOAc was recrystallised from DCM/hexane to yield 180 mg of **2** (CAS RN 158921-71-2). In addition another compound was isolated from this VLC fraction using HPLC (250 × 4 mm, Si 60 Lichrospher Merck, 5 μm, hexane–EtOAc, 85:15, 1.0 ml/min, RI detection). Several injections were performed and the peak that eluted at approximately 6 minutes (pooled total of 5 mg) was isolated and its structure determined to be **4** (CAS RN 119285-56-2); spectral data matched that reported in the literature (Fukuyama *et al*., 1988). Crystalline material from the fraction that eluted between 65:35 and 55:45 hexane: EtOAc was recrystallised from DCM/hexane to yield **1** (360 mg).

6,11-Epoxy-15-nor-3,4-dioxo-5,10-pinguisadien-12-acetate (**1**) was obtained as pale yellow prisms; sublimation point = 180° C; anal. C 66.22%, H 6.23%, calcd for $C_{16}H_{18}O_5$, C 66.20%, H 6.25%; Si-gel TLC $R_F$ = 0.56 (1:1 Hex:EtOAc, UV visualisation); $[\alpha]_{589}^{20}$ = +188°, $[\alpha]_{578}^{20}$ = +205°, $[\alpha]_{546}^{20}$ = +248°, $[\alpha]_{436}^{20}$ = +603°, $[\alpha]_{365}^{20}$ = +1950° (*c:* 1.0, $CHCl_3$); CD (DCM/MeOH) λ(Δε) 230 (0), 267 (–11.2), 280 (0), 305 (+12.2) nm; IR (KBr disk) $\nu_{max}$ 3122, 3112, 2962, 2920, 1743 (br), 1669, 1611, 1440, 1358, 1230, 1040, 944 cm$^{-1}$; GC-MS *m/z* (rel. int.) $[M]^+$ 262 (10), 207 (19), 203 (20), 161 (5), 108 (100), 80 (22); $^{1}$H and $^{13}$C NMR data are presented in Tables I and II.

6,11-Epoxy-15-nor-4-oxo-5,10-pinguisadien-12-acetate (**2**) was obtained as pale yellow prisms; melting point = 102–103° C; anal. C 69.35%, H 7.41%, calcd for $C_{16}H_{20}O_4$, C 69.55%, H 7.29%; Si-gel TLC $R_F$ = 0.83 (1:1 Hex:EtOAc, UV visualisation); $[\alpha]_{589}^{20}$ = +2° $[\alpha]_{578}^{20}$ = +2°, $[\alpha]_{546}^{20}$ = +5°, $[\alpha]_{436}^{20}$ = +46°, $[\alpha]_{365}^{20}$ = +47° (*c:*c 1.0, $CHCl_3$); CD (DCM/MeOH) λ(Δε) 254 (0), 273 (–0.8), 284 (0), 304 (+2.1) nm; IR (KBr disk) $\nu_{max}$ 3110, 2940, 2812, 1735, 1662, 1618, 1361, 1231, 1049, 945 cm$^{-1}$; GC-MS m/z (rel. int.) $[M]^+$ 276 (10), 203 (13), 161 (32), 160, (29), 109 (30), 108 (100), 80 (31); $^{1}$H- and $^{13}$C NMR data are presented in Tables I and II.

*Acknowlegements*

We are grateful to Prof. R. Mues, Universität des Saarlandes, for collection and identification of the sample of *P. recurva,* and to the Alexander von Humboldt Foundation for a research fellowship awarded to J. V.

Asakawa Y. (1995), Chemical constituents of the bryophytes. In: Herz W., Kirby G. W., Moore R. W., Steglich W. and Tamm Ch. (Eds.), Progress in the Chemistry of Organic Natural Products, Vol 65. Springer Verlag, Wien, New York.

Asakawa Y. and Aratani T. (1976), Sesquiterpene of *Porella vernicosa* (Hepaticae). Bull. Soc. Chim. France (Chim. Mol.) 1469–1470.

Asakawa Y., Toyota M., Nagashima F., Hashimoto T. and El Hassane L. (2001), Sesquiterpene lactones and acetogenin lactones from the hepaticae and chemosystematics of the liverworts *Frullania, Plagiochila* and *Porella*. Heterocycles **54**, 1057–1093.

Barlow A. J., Becker H. and Adam K. P. (2001), Biosynthesis of the hemi- and monoterpene moieties of isoprenyl phenyl ethers from the liverwort *Trichocolea tomentella*. Phytochemistry **57**, 7–14.

Becker H. (2001), Moose und ihre biologisch aktiven Naturstoffe. Z. Phytotherapie **22**, 152–158.

Geis W. and Becker H. (2000), Sesquiterpenoids and diterpenoids from the Chilean liverwort *Lepicolea ochroleuca*. Phytochemistry **53**, 247–252.

Fukuyama Y., Tori M., Wakamatsu M. and Asakawa Y. (1988), Norpinguisone methyl ester and norpinguisonolide pinguisane-type norsesquiterpenoids from *Porella elegantula*. Phytochemistry **27**, 3557–3561.

Nagashima F., Izumo H., Takaoka S., Tori M. and Asakawa Y. (1994), Sesqui- and diterpenoids from the Panamanian liverwort *Bryopteris filicina*. Phytochemistry **37**, 433–439.

Toyota M., Koyama H., Hashimoto T. and Asakawa Y. (1995), Sesquiterpenoids from the liverwort *Dicranolejeunea yoshinagana* (Hatt.) Mizut. Chem. Pharm. Bull. **43**, 714–716.