Sesquiterpenoids from the Liverwort *Porella canariensis**

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Z. Naturforsch. **54c,** 151–155 (1999); received November 23/December 29, 1998

Porella, Liverwort, Pinguisane, Drimane, Germacrane, Aromadendrane

From the liverwort *Porella canariensis*, collected in Madeira, Portugal, nine pinguisanes, four drimanes, including the pungent polygodial, two germacranes, and two aromadendranes were isolated. The major compounds were the aromadendrane ent-cyclocolorenone and the nor-pinguisane norpinguisone. Three hydrocarbons were isolated by low temperature column chromatography. One germacrane and one pinguisane proved to be new natural products.

Introduction

Liverworts of the genus *Porella* exhibit a great variety of sesquiterpenoids. Besides aromadendranes and drimanes, which are also found in higher plants, they produce pinguisanes and related structures, rearranged terpenoid skeletons, restricted to liverworts (Asakawa, 1995). Some *Porella* species produce the very pungent drimane dialdehyde polygodial up to 10–30 per cent in their crude extracts. This amount is much bigger than in higher plants like e. g. *Polygonum hydropiper*, a spice for grilled fish in Japan (Asakawa, 1990).

In a recent study of *P. canariensis* from the Canary Islands, ent-cyclocolorenone, three drimanes: cinnamolide, cis-didehydrocinnamolide, and isodrimeninol and one pinguisane derivative, an artefact from the methanol extraction, could be isolated (Nagashima *et al.,* 1996). The availability of larger amounts from Madeira led us to reinvestigate the species.

Result and Discussion

A combination of vacuum liquid chromatography, column chromatography, and HPLC of the dichloromethane extract of *P. canariensis* afforded six pinguisanes: α-pinguisene (**1**) (Asakawa *et al.,* 1978), pinguisenol (**2**) (Asakawa *et al.,* 1976), 7-keto-pinguisenol-12-oic acid methyl ester (**3**) (Toyota *et al.,* 1991), deoxopinguisone (**4**) (Krutov *et al.,* 1973), the new deoxopinguisone-12-oic acid methyl ester (**5**), and deoxopinguisone-15-oic acid methyl ester (**6**) (Asakawa and Aratani, 1976), two norpinguisanes: norpinguisone (**7**) (ibid.) and norpinguisone methyl ester (**8**) (Fukuyama *et al.,* 1988) and the spiropinguisane ptychanolide (**9**) (Takeda *et al.,* 1983). From the drimane series isodrimeninol (**10**) (Asakawa *et al.,* 1979), drimenin (**11**) (He and Wu, 1988), cinnamolide (**12**) (Kioy *et al.,* 1990) and the pungent polygodial (**13**) (ibid.) could be obtained. Additional sesquiterpenes were germacrene D (**14**) (Yoshihara *et al.,* 1969), a mimeticum of the sex pheromone of the American cockroach, *Periplaneta americana* (Tahara *et al.,* 1975), the new 1(10),5,11-germacratriene-7α-ol (**15**) and finally the two aromadendranes α-gurjunene (**16**) (Asakawa *et al.,* 1978) and ent-cyclocolorenone (**17**) (Wu and Chen, 1992). Norpinguisone (**7**) and ent-cyclocolorenone (**17**) were the major compounds of this liverwort, with 1.5 g isolated each.

Compound **5** showed in its ^{1}H (Table I) and ^{13}C NMR (Table II) data a close similarity to deoxopinguisone (**4**) except for the presence of a carboxylic carbon atom (δ_C 174.8, s) and a methyl ester (δ_C 51.0, q) and consequently the loss of one of the four methyl carbon atoms. Since both doublet methyl signals of H-13 and H-15 were still present, the carbomethoxy group must be located either on C-8 or C-9 of the pinguisane skeleton. From the 2D NMR data, the structure of the deoxopinguisone-12-oic acid methyl ester could be deduced

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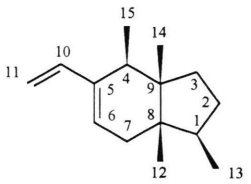
* Publication No. 132 in the series „Arbeitskreis Chemie und Biologie der Moose". For publication No. 131 see Cullmann and Becker.

0939–5075/99/0300–0151 $ 06.00



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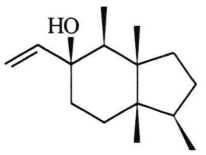

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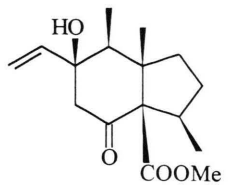

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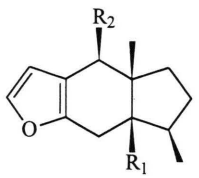
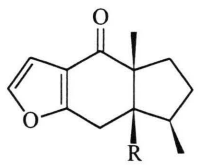


4 $R_1 = R_2 = H$
5 R_1 = COOMe $R_2 = H$
6 $R_1 = H$ R_2 = COOMe



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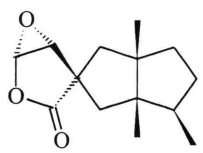
8 R = COOMe



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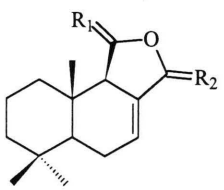
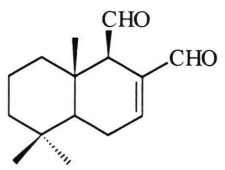


10 R_1 = α-OH, H R_2 = H, H
11 R_1 = O R_2 = H, H
12 R_1 = H, H R_2 = O



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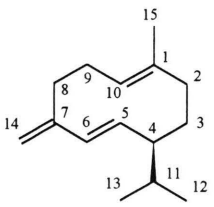

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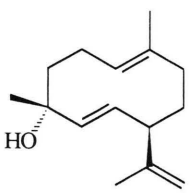

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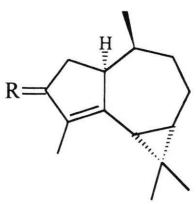

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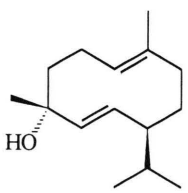
17 R = O



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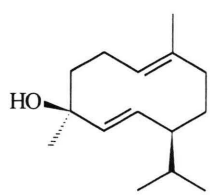

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unambiguously. This is the first report of this structure, but related compounds, 12-acetoxy-deoxopinguisone and deoxopinguisone-12-al had already been described for *Dicranolejeunea yoshinagana* (Toyota *et al.*, 1995).

Table I. ^{1}H NMR spectral data and coupling constants (in Hz in parenthesis) for compounds **5** and **15** ($CDCl_3$).

Proton	Compound **5**	Compound **15**
1	2.12 m	–
2 α	1.58 m	2.27 m
β	1.98 m	
3 α	1.69 m	1.47 m
β	2.02	1.54 m
4	2.53 m	2.77 ddd (10.2, 10.2, 3.2)
5	–	5.16 dd (16.0, 9.7)
6	–	5.34 d (16.0)
7 α	2.70 d (18.0)	–
β	3.20 dd (18.0, 2.4)	
8 α	–	1.63 m
β		1.67 m
9 α	–	2.05 m
β		2.20 m
10	6.16 d (1.9)	4.90 brd (11.3)
11	7.22 d (1.9)	–
12	–	4.64 brs
13	0.86 d (6.8)	1.67 s
14	0.73 s	1.33 s
15	1.12 d (7.1)	1.50 s
16	3.66 s	–

Table II. ^{13}C NMR spectral data for compounds **5** and **15** ($CDCl_3$).

Carbon	Compound **5**	Compound **15**
1	38.5 d	131.8 s
2	29.8 t	41.1 t
3	34.8 t	27.4 t
4	34.5 d	53.3 d
5	117.2 s	130.5 d
6	147.1 s	139.7 d
7	24.2 t	73.0 s
8	62.1 s	40.8 t
9	48.3 s	24.7 t
10	108.9 d	129.5 d
11	140.9 d	149.0 s
12	174.8 s	108.6 t
13	15.1 q	21.2 q
14	16.0 q	23.5 q
15	14.4 q	16.6 q
16	51.0 q	–

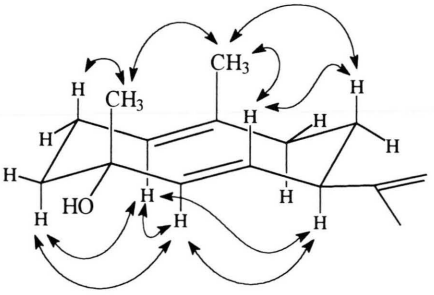

Fig. 1. Significant NOESY correlations of compound **15**.

The mass spectrometry of compound **15** established a molecular formula of $C_{15}H_{24}O$, supported by ^{13}C NMR (Table II). The ^{13}C NMR spectrum of **15** also established that three olefine bonds (one disubstituted, one trisubstituted, and one exomethylene) were present, and that there was a tertiary alcohol (a singlet carbon at δ_C 73.0). Consideration of the unsaturation present in the molecule formula gave a monocyclic compound. The similarity of the ^{1}H NMR data of compound **15** and germacrene D (**14**) led to the assumption of a germacrane-type sesquiterpene, which was confirmed by the 2D NMR data. From these data, the plain structure of a 1(10),5,11-germacratriene-7-ol was derived. The interpretation of the NOESY spectrum, shown in Fig. 1, established the relative configuration of the new 1(10),5,11-germacratrien-7α-ol.

A similar compound, the 1(10),5-germacradien-7α-ol (**18**) had been reported from *Lemnalia africana* (Izac *et al.,* 1982) and the stereochemistry was established by means of an $Eu(fod)_3$ experiment. The data presented herein were the basis for the reports of this compound from *Peucedanum palustre* (Schmaus *et al.,* 1989) and from the liverworts *Marchantia polymorpha*, *Conocephalum conicum*, and *Porella swartziana* (Asakawa, 1995).

However, the NMR spectral data of this compound were not in accordance with our data, especially the chemical shift of C-14 was significantly different (δ_C 21.2, q for **15** compared to δ_C 30.8 in the literature). By carefully checking the literature, we learned that San Feliciano *et al.* (1995) and Kitagawa *et al.* (1987) described a germacradienol from *Juniperus communis* subsp. *hemisphaerica* and *Nephthea* sp., respectively, with data identical to those of Izac *et al.* (1982), but their structure elucidation by means of modern 2D NMR methods established the structure of 1(10),5-germacradiene-7β-ol (**19**). Moreover, Bohlmann *et al.* (1977, 1984) reported both epimers, but published merely identical data (being identical to Izac *et al.*). Thus, to our opinion, all the above reported germacradienols should be 1(10),5-germacradiene-

7β-ol (**19**). Furthermore, this finding is in accordance with the typical range of the ^{13}C NMR shift of a methyl group geminal to an alcohol function as published in literature (δ_C 30.3–32.9 for equatorial, δ_C 22.5–26.1 for axial position, respectively) (Joseph-Nathan *et al.*, 1984).

Consequently, the structures of the liverwort germacranes derived from the data of Izac should be revised to 1(10),5-germacradien-7β-ol, thus having the same stereochemistry as the similar 1(10),5-germacradien-7β,11-diol reported from *Bryopteris filicina* (Nagashima *et al.*, 1994) and *Ptychanthus striatus* (Asakawa, 1995).

Once more, a *Porella* species proved to be a valuable source of sesquiterpenoids, especially the high contents of norpinguisone (**7**) and ent-cyclocolorenone (**17**) are worth mentioning. The occurrence of polygodial (**13**) for *P. canariensis* is reported for the first time. Thus, unlike stated in literature (Müller, 1954), *P. canariensis* belongs to the pungent type *Porella* species. The differences in the chemical constituents of *P. canariensis* from the Canary Islands and Madeira suggests the existence of different chemical races of this liverwort in Europe.

Experimental

Plant material

Porella canariensis (F. Web.) Bryhn.:

a) Madeira, Montado dos Pecegueros. *Coll.* et *det.* S. Sà Fontinha, 04.07.1996, Herbarium SAAR 5451.

b) Madeira, Faja da Nogueira. *Coll.* et *det.* S. Sà Fontinha, 31.07.1996, Herbarium SAAR 5452

Extraction and isolation

General: All HPLC separations were performed isocratic. The composition of the mobile phase is given as v/v (in parenthesis).

Since the liverworts of both collecting sites exhibited identical TLC and HPLC chromatograms, they were combined and the resulting 575 g of air dried gametophytes of *P. canariensis* were ground and successively extracted with a total of 20 l CH_2Cl_2 to yield 18.2 g of lipophilic extract. This extract was subjected to VLC on silicagel in a hexane-ethyl acetate gradient (0–100%) to yield seven main fractions (I – VII). Fraction I, containing the hydrocarbons, was separated by low temperature (–20°) column chromatography on silicagel (hexane) to yield **1** (373 mg), **4** (255 mg), **14** (105 mg) and **16** (50.5 mg). Fraction II was prepurified by HPLC (Si, CH_2Cl_2/hexane 25/75) and gave rise to **5** (112 mg), **7** (240 mg) and a subfraction whose separation by HPLC (Si, EtOAc/hexane 0.5/99.5) resulted in 54.5 mg of compound **2** and 161 mg of compound **6**. Fraction III was almost pure and by recrystallisation from hexane another 1216 mg of compound **7** could be obtained. Fraction IV was separated by HPLC (RP18, MeOH/H_2O 75/25) and gave compounds **8** (59.5 mg), **9** (51.5 mg), and **11** (33.1 mg). Due to its high amount, Fraction V was chromatographed by CC, (Si, $CHCl_3$) to yield compound **17** (993 mg) and a mixture, from which after separation by HPLC (Si, EtOAc/hexane 12/88) another 455 mg of compound **17** could be obtained together with compounds **12** (160 mg) and **15** (11.8 mg). Fraction VI was separated by HPLC (Si, EtOAc/hexane 12/88) into compound **13** (399 mg) and a fraction containing sterols, which were not investigated further. After separation by HPLC, Fraction VII, the last fraction, yielded (Si, EtOAc/hexane 20/80) compounds **3** (144 mg) and **10** (246 mg).

Spectroscopic methods

NMR-spectroscopy: BRUKER, $CDCl_3$, ambient temperature, 400 MHz (^1H), 100 MHz (^{13}C) for one-dimensional, 500 MHz and 125 MHz for two-dimensional techniques, respectively; chemical shifts are given in δ values (ppm) from TMS; mass spectroscopy: VARIAN MAT 311, 70 eV; GC-MS was performed on a HP-1 capillary column with a G 1800A GCD system (HP).

Spectroscopic data

Compound **5**: yellowish oil, $[\alpha]_D^{20} = -50.1°$ (c = 0.696); ^1H NMR: see Table I; ^{13}C NMR: see Table II; EIMS *m/z* (rel. int.) = 262 (3) $[M]^+$, 201 (2), 145 (3), 108 (100), 91 (5), 79 (7)

Compound **15**: colourless oil, $[\alpha]_D^{20} = -20.0°$ (c = 0.280); ^1H NMR: see Table I; ^{13}C NMR: see Table II; EIMS *m/z* (rel. int.) = 220 (1) $[M]^+$, 205 (20), 202 (14), 187 (22), 159 (21), 145 (22), 134 (42), 119 (47), 107 (100), 91 (51), 79 (62), 67 (38), 55(34).

Acknowledgements

We thank Dr. J. Zapp, Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, for the NMR measurements. Financial support of the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF, Bonn) and BAYER AG (Leverkusen) is gratefully acknowledged.

Asakawa Y. (1990), Terpenoids and aromatic compounds with pharmacological activity from bryophytes. In: Bryophytes, Their Chemistry and Chemical Taxonomy (Zinsmeister, H. D. and Mues, R., eds.). Proceedings of the Phytochemical Society of Europe **29**. Clarendon Press Oxford, 369–410.

Asakawa Y (1995), Chemical Constituents of the Bryophytes. In: Progress in the Chemistry of Organic Natural Products (Herz W., Kirby G. W., Moore R. E., Steglich W., and Tamm Ch., eds.). Vol. **65**. Springer-Verlag Wien, New York, 1–618.

Asakawa Y. and Aratani T. (1976), Sesquiterpènes de *Porella vernicosa*. Bull. Soc. Chim. France 1469–1470.

Asakawa Y., Huneck S., Toyota M., Takemoto T., and Suire C. (1979), Mono- and sesquiterpenes from *Porella arboris-vitae*. Journ. Hattori Bot. Lab. **46**, 163–167.

Asakawa Y., Toyota M., and Aratani T. (1976), Un nouvel sesquiterpénique de *Porella vernicosa* et *Porella densifolia* (Hépatiques). Tetrahedron Lett. **40**, 3619–3622.

Asakawa Y., Toyota M., and Takemoto T. (1978), Sesquiterpenes from *Porella* species. Phytochemistry **17**, 457–460.

Bohlmann F., Knoll K.-H., Zdero C., Mahanta P. K., Grenz M., Suwita A., Ehlers D., Le Van N., Abraham W.-R., and Natu A. A. (1977), Terpen-Derivate aus *Senecio*-Arten. Phytochemistry **16**, 965–985.

Bohlmann F., Zdero C., King R. M., Robinson H. (1984), A hydroxygermacrene and other constituents from *Pseudobrickellia brasiliensis*. Phytochemistry **23**, 1798–1799.

Cullmann F. and Becker H. (1999), Prenylated bibenzyls from the liverwort *Radula laxiramea*. Z. Naturforsch. **54c**, 147–150.

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

He J.-F. and Wu Y.-L. (1988), Synthesis of drimane sesquiterpenes. An intramolecular Diels-Alder approach. Tetrahedron **44**, 1933–1940.

Izac R. R., Bandurraga M. M., Wasylyk J. M., Dunn F. W., and Fenical W. (1982), Germacrene derivatives from diverse marine soft-corals (Octocorallia). Tetrahedron **38**, 301–304.

Joseph-Nathan P., Santilan R. L., and Gutiérrez A. (1984), ^{13}C-NMR study of cedrol, 6-isocedrol, and α-cedrene. J. Nat. Prod. **47**, 924–933.

Kitagawa I., Cui Z., Son B. W., Kobayashi M., Kyogoku Y. (1987), Marine natural products. XVII. Nephtheoxydiol, a new cytotoxic hydroperoxy-germacrane sesquiterpene, and related sesquiterpenoids from an Okinawan soft coral of *Nephthea* sp. (Nephtheidae). Chem. Pharm. Bull. **35**, 124–135.

Kioy D., Gray A. I., and Waterman P. G. (1990), A comparative study of the stem-bark drimane sesquiterpenes and leaf volatile oils of *Warburgia ugandensis* and *W. stuhlmannii*. Phytochemistry **29**, 3535–3538.

Krutov S. M., Samek Z., Benešova V., and Herout V. (1973), Isolation and the structure of deoxopinguisone from the liverwort *Ptilidium ciliare*. Phytochemistry **12**, 1405–1407.

Müller K. (1954), Die Lebermoose Europas. In : Dr. L. Rabenhorst's Kryptogamen-Flora VI. Band, Akademische Verlagsgesellschaft Geest & Portig KG, Leipzig.

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.

Nagashima F., Momosaki S., Watanabe Y., Takaoka S., Huneck S., and Asakawa Y. (1996), Sesquiterpenoids from the liverworts *Bazzania trilobata* and *Porella canariensis*. Phytochemistry **42**, 1361–1366.

San Feliciano A., Medarde M., Gordaliza M., and Lucas M. J. (1995), Structure elucidation of germacrane alcohols from *Juniperus communis* subsp. *hemisphaerica*. J. Nat. Prod. **58**, 1059–1064.

Schmaus G., Schultze W., and Kubeczka K.-H. (1989), Volatile constituents of *Peucedanum palustre*. Planta Medica **55**, 482–487.

Tahara S., Yoshida M., Mizutani J., Kitamura C., Takahashi S. (1975), A sex stimulant to the male American cockroach in the Compositae plants. Agr. Biol. Chem. **39**, 1517–1518.

Takeda R., Naoki H., Iwashita T., Mizukawa K., Hirose Y., Isida T., and Inoue M. (1983), Sesquiterpenoid constituents of the liverwort, *Ptychanthus striatus* (Lehm. et Lindenb.) Nees. Bull. Chem. Soc. Jpn. **56**, 1125–1132.

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

Toyota M., Ueda A., and Asakawa Y. (1991), Sesquiterpenoids from the liverwort *Porella acutifolia* subsp. *tosana*. Phytochemistry **30**, 567–573.

Wu C.-L. and Chen C.-L. (1992), Oxygenated sesquiterpenes from the liverwort *Bazzania tridens*. Phytochemistry **31**, 4213–4217.

Yoshihara K., Ohta Y., Sakai T., and Hirose Y. (1969), Germacrene D, a key intermediate of cadinene group compounds and bourbonenes. Tetrahedron Lett. **27**, 2263–2264.