Prenylated Bibenzyls from the Liverwort *Radula laxiramea**

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Dedicated to Professor Hans-Jörg Kallmayer on behalf of his 60th birthday

Radula, Liverwort, Bryophytes, Prenyl Bibenzyls, Chromene, Cannabinoid

From *Radula laxiramea*, 12 bibenzyl derivatives and the common bisbibenzyl perrottetin E were isolated. Two compounds are described for the first time as natural products, and one, previously only known after derivatisation, was found in genuine form. Perrottetinene, a cannabinoid from a liverwort is worth mentioning.

Introduction

Radula Dum. is an isolated genus in the Jungermanniales and is chemically characterized by prenylated bibenzyls and the almost total lack of terpenoid compounds. From the *Radula* species examined so far, a great number of bibenzyls have been described with great structure variety (Asakawa, 1995). Some of these substances serve as chemical markers for the three subgenera *Radula*, *Cladoradula*, and *Odontoradula* (Yamada, 1979). Here, we want to present the results of our phytochemical investigation on *Radula laxiramea*, a species not examined so far, belonging to the subgenus *Radula*, there representing the type of the Sect. Dichotomae.

Result and Discussion

The dichloromethane extract of *R. laxiramea* from Costa Rica was examined and yielded the new 3,5-dihydroxy-4-(3-hydroxy-3-methylbutyl) bibenzyl **5**, and the new chromene **11**, whose existence had already been predicted (Crombie *et al.*, 1988). The salicylic acid derivative **3**, known so far only after derivatisation, had been isolated for the first time. Additional compounds were the prenyl bibenzyls **1** (Asakawa *et al.*, 1978, 1982, 1991, Mitscher *et al.*, 1983), **2** (Asakawa *et al.*, 1978, 1981, 1982), **6** (Asakawa *et al.* 1991, 1991a, Toyota *et al.* 1994, Asakawa and Inoue, 1984, Kraut *et al.* 1997), **7** (Asakawa *et al.*, 1982, Kraut *et al.* 1997), and **8** (Asakawa and Inoue, 1984), the salicylic acid derivative amorfrutin A (**4**) (Mitscher *et al.* 1981, Ghisalberti *et al.* 1981), the chromene **9** (Asakawa *et al.*, 1991a), the benzofurane **10** (Asakawa *et al.*, 1991a), the bibenzyl cannabinoid perrottetinene **12** (Toyota *et al.*, 1994), and the common bisbibenzyl perrottetin E **13** (Asakawa, 1995). The known compounds were identified by spectral evidence and comparison of their spectroscopic properties with published data.

Compound **5** showed the $[M]^+$ ion at $m/z = 300$ in the EI mass spectrum in agreement with a molecular formula of $C_{19}H_{24}O_3$. Since the ^{13}C NMR spectrum showed only 11 different signals, a symmetric molecule was assumed. The ^1H NMR spectrum supported this assumption: At higher field there was the typical signal pattern of a monosubstituted benzene ring (δ_H 7.22 (2H, m); 7.12 (3H, m), H-10–H-14) and a singlet at δ_H 6.21 integrating for two protons (H-2, H-6). Additional signals at lower field were the signals of the bibenzyl bridge (δ_H 2.80 (2H, m, H-7); 2.69 (2H, m, H-8)), two additional methylene groups (δ_H 2.66 (2H, t, 7.6 Hz, H-1′); 1.69 (2H, t, 6.7 Hz, H-2′)), coupling with each other, and two isochrone methyl groups at δ_H 1.20 (H-4′, H-5′), suggesting a heteronuclear shift. The interpretation of the ^{13}C NMR and DEPT spectra showed close similarities to those of compound **1**, the only difference being the lack of the double bond in the prenyl side chain. Instead, their was a signal of an additional methylene carbon at δ_C 41.8 (C-2′) and a quar-

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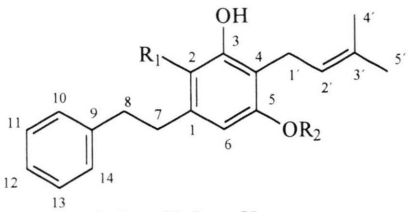
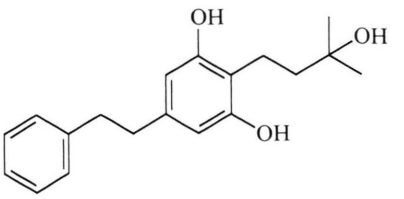


1: $R_1 = H$ $R_2 = H$
2: $R_1 = H$ $R_2 = Me$
3: $R_1 = COOH$ $R_2 = H$
4: $R_1 = COOH$ $R_2 = Me$



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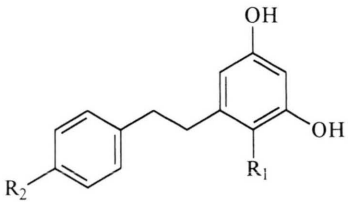
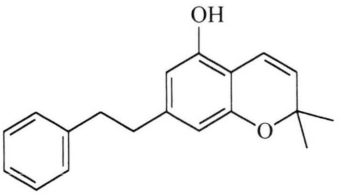


6: R_1 = prenyl $R_2 = H$
7: R_1 = geranyl $R_2 = H$
8: R_1 = geranyl $R_2 = OH$



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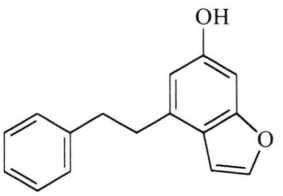

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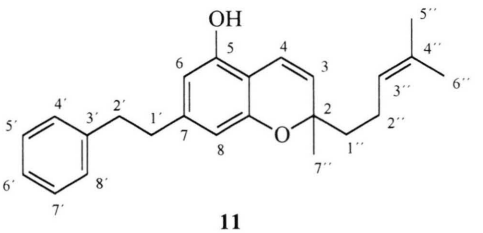

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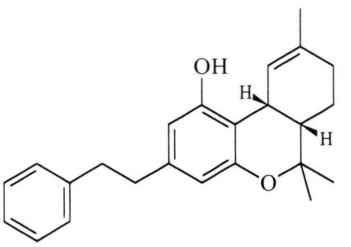

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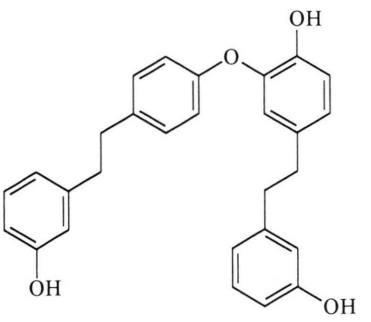

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ternary carbon atom at δ_C 71.5 (C-3′) of an oxygen bearing nucleus. Thus, compound **5** is the new 3,5-dihydroxy-4-(3-hydroxy-3-methylbutyl) bibenzyl. The assignment of the ^{13}C NMR data were made on the basis of compound **1** and a similar compound, 3-hydroxy-5-methoxy-2-(3-hydroxy-3-methyl-butyl) bibenzyl (Asakawa *et al.*, 1991a).

The spectral data of compound **11** revealed the structure of a geranylated bibenzyl, where the side chain is part of a chromene moiety. Although this

is the first report of **11** as a natural product, this substance had been synthesised in the course of the search for bibenzyl cannabinoids from *Cannabis sativa* (Ghisalberti *et al.*, 1981). The ^{1}H NMR data of the natural compound are identical to those reported for the synthetic product.

The ^{1}H NMR spectrum of compound **3** showed the very characteristic signal of a chelate proton at δ_H 11.87 (C-3-OH). Such a signal had been observed in the ^{1}H NMR spectrum of compound **4**, amorfrutin A, a salicyclic acid derivative. Further characteristic signals were a singlet proton at δ_H 6.26 (H-6) together with the already known patterns for the monosubstituted benzene ring and an isoprene side chain. The significant difference between the spectra of **3** and **4** was the lack of the methyl ether signal in the spectrum of compound **3**, corresponding to a lower molecular ion peak in the mass spectra (326 amu for **3** compared to 340 amu for **4**). Further proof of the structure gave a NOESY spectrum. There were correlations between the aliphatic bridge and the singlet proton at δ_H 6.26 proving the proton to be in 2-position and therefore, the isoprene side chain to be in 4-position. Thus, compound **3** is the 2-carboxy-3,5-dihydroxy-4-prenyl bibenzyl, a substance already described for *R. complanata* (Asakawa *et al.*, 1978) and *Helichrysum umbraculigerum* (Bohlmann and Hoffmann, 1979) after derivatisation and therefore not fully characterized yet. This is the first report on the isolation of **3** as genuine natural product.

Due to their secondary metabolites, *R. laxiramea* should belong to the subgenus *Odontoradula*, which produces 2-prenyl-3,5-dihydroxy bibenzyls and chromenes. Since *R. laxiramea* belongs to the subgenus *Radula,* the chemosystematic classification given by ASAKAWA (1995), in this case, does not support the classification of YAMADA (1979) for the Radulaceae. The occurrence of bibenzyl derived cannabinoids as for perrottetinene **12** and chromene **11** is surprising. Similarly, the occurrence of such typical liverwort constituents in higher plants as *Glycyrrhiza* species (Fabaceae) and *Helichrysum umbraculigerum* (Asteraceae) is noteworthy.

Experimental

Plant material

Radula laxiramea Steph.: Monte Verde Cloud Forest, Costa Rica. *Coll.*. R. Mues Sept. 1994; *det.* Yamada 1996; Deposited at Herbarium Mues 3344, FR Botanik der Universität des Saarlandes.

Extraction and isolation

General: Until stated otherwise, all HPLC separations were made on silicagel with a mixture of hexane/ethyl acetate. The composition of the mobile phase is given as v/v (in parenthesis).

The air dried gametophytes of *R. laxiramea* (63 g) were ground and successively extracted with a total of 5 l CH_2Cl_2 to yield 5.1 g of lipophilic extract. This extract was subjected to a Vacuum Liquid Chromatography (VLC) on silicagel in a hexane-ethyl acetate gradient to yield seven main factions. Fraction 1, containing the hydrocarbons, was not further separated. Fraction 2 was purified by HPLC (92/8) to yield compound **2** (88.0 mg). Fraction 3 was prepurified in the same mobile phase and gave compound **1** (45.6 mg) and three subfractions, whose purification by HPLC with a mixture of hexane/*tert.*-butyl methyl ether gave rise to compound **12** (11.7 mg) (85/15), compound **11** (21.9 mg) (85/15), and compound **9** (9.0 mg) (83/17). Fraction 4 was nearly pure and by recristallisation from hexane another 230 mg of compound **1** could be obtained. As fractions 5–7 contained higher amounts of chlorophyll and other by-products, they were prepurified by CC on Sephadex LH20 with MeOH/CH_2Cl_2 (50/50) as mobile phase. After this CC, fraction 5 was further purified by VLC on diol modified silicagel with a hexane-ethyl acetate gradient and finally by HPLC (75/25) and gave rise to compound **7** (312 mg). Fraction 6, after CC, was purified by HPLC (77/23) to yield compound **10** (4.8 mg), another 290 mg of compound **7**, and compound **6** (84.9 mg). CC of the last fraction, fraction 7, gave two subfractions, whose separation succeeded with the same HPLC system (65/35). Fraction 7.1 yielded compound **4** (39.5 mg) and compound **5** (73.9 mg). Fraction 7.2 contained compound **3** (7.4 mg), compound **13** (23.2 mg) and compound **8** (29.0 mg).

Spectroscopic methods

NMR-spectroscopy: BRUKER, $CDCl_3$, ambient temperature, 400 MHz (^{1}H), 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 **3**: ^{1}H NMR: Table I; EIMS *m/z* (rel. int.): 326 $[M]^+$ (2), 282 (47), 267 (7), 227 (100), 191 (57), 147 (19), 123 (13), 105 (21), 91 (91)

Compound **5**: ^{1}H NMR: Table I; ^{13}C NMR: δ 154.9 (2xs, C-3, C.-5), 141.9 (2xs, C-1, C-9), 128.2 (4xd, C-10, C-11, C-13, C-14), 125.6 (d, C-12), 113.6 (s, C-4), 107.5 (2xd, C-2, C-6), 71.5 (s, C-3′), 41.8 (t, C-2′), 37.3 (2xt, C-7, C-8), 28.9 (2xq, C-4′, C-5′), 17.3 (t, C-1′) ; EIMS *m/z* (rel. int.): 300 $[M]^+$ (3), 282 (19), 267 (4), 227 (90), 191 (32), 91 (100)

Compound **11**: $[\alpha]_D^{20}$ = -27,1° (c = 0.240); ^{1}H NMR: δ 7.26 – 7.18 (5H, H-4′, H-5′, H-6′, H-7′, H-8′), 6.61 (d, *J* = 10.1 Hz, H-4), 6.28 (s, H-6), 6.09 (s, H-8), 5.50 (d, *J* = 10.1 Hz, H-3), 5.09 (t, *J* = 7.1 Hz, H-3′′), 4.79 (s, C-5-OH), 2.86 (2H, m, H-2′), 2.75 (2H, m, H-1′), 2.10 (2H, m, H-2′′), 1.73 (2H, m, H-1′′), 1.66 (3H, s, H-6′′), 1.57 (3H, s, H-5′′), 1.37 (3H, s, H-7′′); EIMS *m/z* (rel. int.) 348 $[M]^+$ (3), 333 (3), 265 (100), 174 (32), 91 (11).

Table I. ^{1}H NMR spectral data and coupling constants (in Hz in parentheses) for compounds **3** and **5** ($CDCl_3$).

Proton	Compound **3**	Compound **5**
H-2	–	6,21 s
H-6	6.62 s	6.21 s
H-7	3.19 m	2.80 m
H-8	2.88 m	2.69 m
H-10/14	7.20 m	7.12 m
H-11/13	7.29 m	7.22 m
H-12	7.20 m	7.12 m
H-1′	3.42 d (7.2)	2.66 t (7.6)
H-2′	5.27 t (7.2)	1.69 t (7.6)
H-4′	1.82 s	1.20 s
H-5′	1.75 s	1.20 s
Chelate	11.87 s	–

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