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ISOLATION AND SYNTHESIS OF 6,7-DIHYDROXY-4-(3,4-DIHYDROXYPHENYL)NAPHTHALENE-2-CARBOXYLIC ACID FROM *PELLIA EPIPHYLLA*

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Key Word Index—*Pellia epiphylla*; Metzgeriales; Hepaticae; 6,7-dihydroxy-4-(3,4-dihydroxyphenyl)naphthalene-2-carboxylic acid.

Abstract—A new naphthalene derivative, 6,7-dihydroxy-4-(3,4-dihydroxyphenyl)naphthalene-2-carboxylic acid has been isolated from gametophytic tissues of the liverwort *Pellia epiphylla*. The structure of the compound was elucidated by means of spectroscopic methods and by independent synthesis.

INTRODUCTION

Bryophytes have been shown to produce phenolic compounds of various structural types [1, 2]. By far most of those compounds are flavonoids, whereas other phenolics have been identified up to now only occasionally. We wish to report here the first isolation, identification and independent synthesis of a new phenolic product from gametophytic tissues of the liverwort *Pellia epiphylla* (L.) Corda.

RESULTS AND DISCUSSION

An aqueous alcoholic extract of air-dried gametophytes of *Pellia epiphylla* yielded after chromatographic purification *ca* 40 mg of a colourless microcrystalline compound, mp 325–327° (dec., from methanol). On a 2D-TLC the blue fluorescent compound spot is characterized by high R_f values (0.7–0.8) in non-polar solvents and low R_f in 15% aqueous acetic acid (0.12; see Experimental).

A number of structural characteristics can be deduced from the spectroscopic data of the new compound. Thus, the UV spectrum shows an absorption at 312 nm and a shoulder at 293 nm suggesting the presence of an aromatic system as basic structural unit. Both in the FD- and EIMS the molecular ion of the compound is observed at *m/z* 312. The high resolution MS of the molecular ion reveals the molecular formula $C_{17}H_{12}O_6$.

The ^{13}C NMR spectrum shows signals for 17 carbon atoms; 16 of them appear in the region of δ150–105 characteristic for aromatic carbons, one signal appearing at δ167.7 indicates the presence of a carboxyl group. A DEPT-^{13}C NMR spectrum differentiates the aromatic carbon atoms into seven tertiary and nine quaternary carbons, four of them (in the range δ145–149) apparently linked to oxygen.

The ^1H NMR spectrum reveals signals for seven aromatic protons in the range δ8.2–6.6 ppm and for five other protons (four of them in the range δ9.9–9.0 ppm, one of them as a broad signal at δ12.7 ppm), which are exchanged by treatment with D_2O. Since the signal at δ12.7 is indicative for the carboxyl-OH-group, the remaining four signals can be assigned to phenolic OH-functions.

Accordingly, in the IR spectrum intensive absorptions of OH-groups are found in the range of 3500–3400 cm^{-1}, together with a C=O-bond at 1705 cm^{-1}, characteristic for a carboxyl group linked to an aromatic nucleus. The foregoing spectroscopic data reveal the presence of a phenyl residue linked to a naphthalene system of a carboxyl group and four hydroxyl groups as additional substituents of the two aromatic nuclei. The substitution pattern at the phenyl ring and at the naphthalene system can be deduced from the coupling constants of the aromatic proton signals.

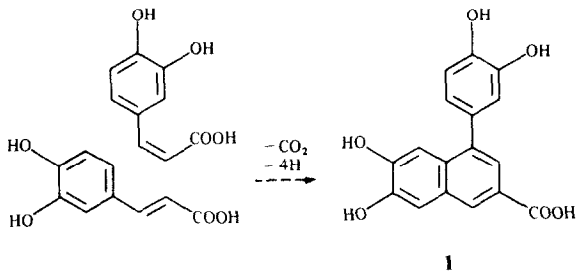
First, in the region δ6.7–6.9 a double-doublet of a 1.2- and 1.3- coupled proton (at δ6.69 with $J \approx 8.0$ and 2.1 Hz) and doublets of two further protons (at δ6.82 with $J = 2.1$ Hz and δ6.86 with $J = 8.0$ Hz) are found, which are generally [3] characteristic for a 1,2,4-trisubstituted benzene ring. Second, in the range of δ8.2–7.25 two uncoupled single protons ($\delta = 7.23$ and 7.30) are found, which should be α-positioned in one of the six-membered rings of the naphthalene system. The remaining two aromatic protons appear at δ8.19 and 7.50 as 1,3-coupled doublets with $J = 1.6$ Hz, and should be consequently α- and β-positioned in the second six-membered ring of the naphthalene system.

The exact position of the 4 OH-groups, the carboxyl group and the phenyl ring at the naphthalene system cannot be determined unequivocally from the data available. Recently Mues *et al.* [7] reported the isolation and structure determination of a similar compound from the liverwort *Scapania undulata*, the naphthalene moiety of which is substituted by hydroxyl groups in 6,7-position and a carboxyl group in 3-position. The ^{13}C NMR spectrum of this *Scapania* compound shows a value (δ167.6) for the carboxy carbon almost identical with the value found for our new compound.

If biosynthesis of a phenyl-substituted naphthalene system—as already discussed for similar systems in the

literature [4]—is considered to start from caffeic acid [which is a common phenolic unit in liverworts (Mues, R., unpublished results)], our new compound is most likely to possess the structure **1** of a 4-(3,4-dihydroxyphenyl)-substituted naphthalene system with a second *ortho*-dihydroxy moiety at 6,7-position and the carboxyl group in 2-position. This structural proposal is confirmed by independent synthesis of **1** as described below.

In a modification and extension of a literature procedure [5] β-(3,4-dimethoxybenzoyl)propionic acid **2** was condensed with 3,4-dimethoxybenzaldehyde **3** in acetic anhydride by means of sodium hydride to give 3*H*-furan-2-one **5** as the product of an aldol condensation and subsequent lactonization of the primarily formed (enolized) keto acid **4**. Basic ring-opening of the butenolide **5** with sodium methoxide in methanol yields the keto acid **4**, which cyclizes readily on treatment with hydrogen chloride in methanol to give the tetramethoxy ester **6** possessing the 2,4,6,7-substitution pattern of the desired naphthalene system. Saponification of the ester **6** yields the acid **7**, which is smoothly demethylated by boron tribromide in dichloromethane. The resulting synthetic product (obtained in an overall-yield of 30% from **2**) is identical in all analytical respects (IR, UV, ^{1}H and ^{13}C NMR, MS, mp, R_f) with the natural product thus proving its structure to be that of 6,7-dihydroxy-4-(3,4-dihydroxyphenyl)-naphthalene-2-carboxylic acid **1**.

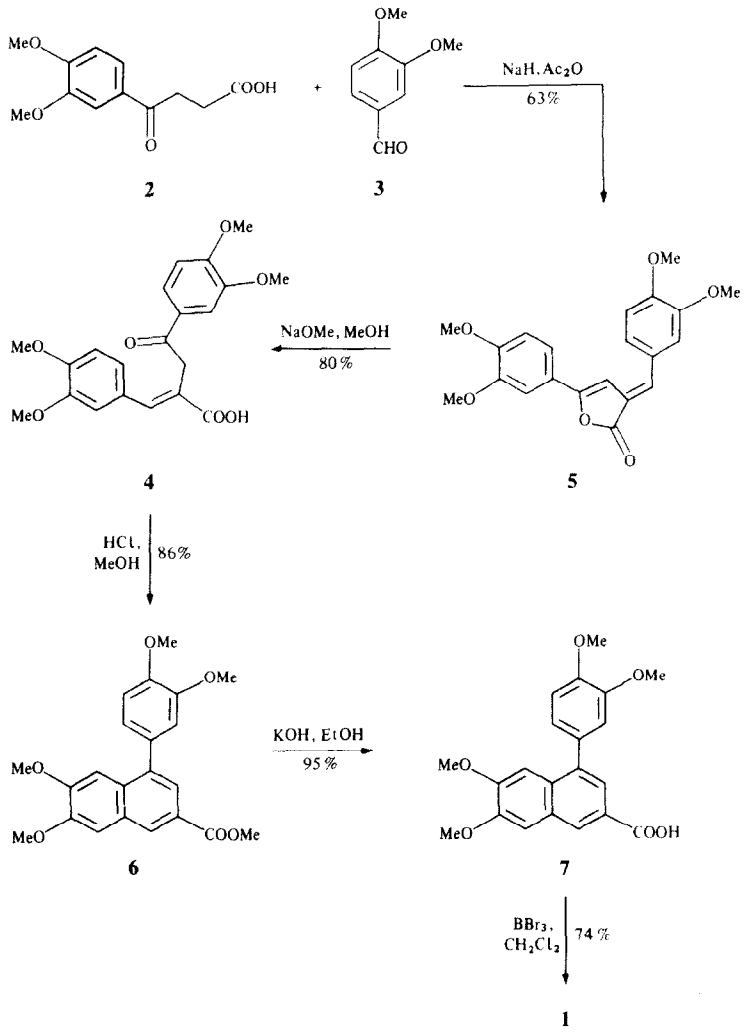


1

Scheme 1.

EXPERIMENTAL

Plant material. Fresh gametophytes of *Pellia epiphylla* were collected in April and May 1986 in the Hunsrück near Züsch-/Börfink, F.R.G. Voucher specimens are deposited in the Her-



2 **3** **4** **5** **6** **7** **1**

Scheme 2.

barium of the Fachrichtung Botanik, Universität des Saarlandes, Saarbrücken. The plants were identified by the second author.

Extraction and isolation. After careful cleaning 1300 g air-dried gametophytes were ground in a Waring blendor and extracted with $CHCl_3$ to remove chlorophyll and lipids, then with 50% Me_2CO, 50% EtOH and finally with 50% MeOH. As the compound was detected in each aq. extract, the extracts were combined, evapd to the H_2O phase and extracted first with $CHCl_3$ to separate the rest of the chlorophyll. The water fraction was cleared with MeOH and this extract was separated by CC on cellulose (microcrystalline, Merck) with 3% HOAc. From the last fraction of this column about 40 mg of the compound crystallized from MeOH.

Chromatography. The purity of the compound was checked in 5 solvents on cellulose (microcrystalline for TLC, Merck): 15% HOAc (R_f 0.12); 40% HOAc (R_f 0.43); BAW (R_f 0.76); PEW (= *n*-pentanol–HOAc–H_2O, 2:1:1; R_f 0.75); TBA (R_f 0.7).

Spectroscopic data of **1**. IR (KBr): 3440, 3300, 1705, 1620, 1530 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 12.71, 9.93, 9.69 (*br s*, 1H), 9.08 (*br s*, 2H), 8.19, 7.50 (*d*, J = 1.6 Hz; 1H), 7.30, 7.23 (*s*, 1H), 6.86 (*d*, J = 8.0 Hz; 1H), 6.82 (*d*, J = 2.1 Hz; 1H), 6.69 (*dd*, J_1 = 2.1 Hz, J_2 = 8.0 Hz; 1H). ^{13}C NMR (400 MHz, DMSO-d_6): δ = 167.8, 149.0, 147.2, 145.0, 144.7, 137.9, 131.4, 129.4, 128.4, 127.4, 124.6, 122.9, 120.4, 116.9, 115.7, 111.4, 108.0. UV (MeOH): λ_{max} (log ε) 243 (4.25), 293 (sh, 3.94), 312 nm (4.00). MS (70 eV): *m/z* (%) 312 (100, M^+), 268 (22), 266 (10), 249 (19), 221 (36), 165 (18), 164 (10), 163 (18), 88 (10), 63 (10), 45 (17), 43 (35).

3-(3,4-*Dimethoxybenzylidene*)-5-(3,4-*dimethoxyphenyl*)-3H-*furan-2-one* **5**. β-(3,4-Dimethoxybenzoyl)propionic acid **2** (3.00 g, 12.5 mmol) [6] and 3,4-dimethoxybenzaldehyde **3** (4.15 g, 25.0 mmol) were dissolved in Ac_2O (20 ml) in N_2. Within 20 min NaH (0.30 g, 12.5 mmol) was added with stirring. After 60 min the reaction mixture was heated under reflux for 2 hr. After 12 hr at 20° the black-brown suspension was treated with H_2O (50 ml) and CH_2Cl_2 (50 ml), the organic phase is separated and the aq. phase extracted twice with CH_2Cl_2 (50 ml). The combined extracts were washed with 2 N Na_2CO_3-solution and dried over Na_2SO_4. Removal of the solvent *in vacuo* yielded a brownish oil, which was purified by chromatography (SiO_2, $CHCl_3$). The product was obtained as an orange-yellow oil, which crystallized by addition of methanol; 2.90 g (63%) orange crystals, mp 150–151° (lit. [5]: m.p. 140–147°).

Calcd. for $C_{21}H_{20}O_6$ (368.4): C 68.47, H 5.47; found: C 68.89, H 5.35. IR ν^{KBr}: 1770, 1630, 1610 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36, 7.28 (*dd*, J_1 = 8.4 Hz, J_2 = 2.0 Hz; 1H), 7.29 (*s*, 1H), 7.22, 7.08 (*d*, J = 2.0 Hz; 1H), 6.95, 6.92 (*d*, J = 8.4 Hz; 1H), 6.76 (*s*, 1H), 3.95, 3.94, 3.93, 3.92 (*s*, 3H). ^{13}C NMR (400 MHz, $CDCl_2$): δ = 169.82, 156.16, 151.17, 149.38. 134.38, 128.53, 124.01, 123.55, 121.26, 118.82, 113.25, 111.62, 111.47, 108.27, 98.30, 56.17, 56.07. UV (MeOH): λ_{max} (log ε) 201 (4.27), 358 (3.94), 282 (sh, 3.77), 413 nm (4.27). MS (70 eV): *m/z* (%) 368 (49, M^+), 165 (100), 137 (11), 79 (15), 77 (13).

β-(3,4-*Dimethoxybenzoyl*)-α-(3,4-*dimethoxybenzylidene*) *propionic acid* **4**. Butenolide **5** (2.00 g, 5.43 mmol) was stirred with NaOMe (0.32 g, 5.92 mmol) in MeOH (15 ml) for 12 hr at 20°. Water (20 ml) was added and MeOH distilled off *in vacuo*. The solution was filtered and acidified with 2 M HCl. The pptd product was filtered by suction and recrystallized from MeOH; 1.68 g (80%) colourless needles, mp 178–179° (ref. [6]: mp 175°). Calcd. for $C_{21}H_{22}O_7$ (386.4): C 65.28, H 5.74; found: C 66.05, H 5.64. IR (KBr): 3400, 1675 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.06 (*s*, 1H), 7.66 (*dd*, J_1 = 1.6 Hz, J_2 = 8.4 Hz; 1H), 7.58 (*d*, J = 1.6 Hz; 1H), 6.9–6.8 (*m*, 4H), 4.23 (*s*, 2H), 3.95, 3.92, 3.86, 3.69 (*s*, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 195.82, 173.10, 153.57, 150.01, 149.15, 148.87, 144.17, 129.85, 127.79, 124.56, 122.97, 122.65, 112.16, 111.13, 110.44. 110.17, 56.11, 56.01, 55.92,55.69, 37.71. UV (MeOH): λ_{max} (log ε) 205 (4.39), 227 (4.44), 275 nm (4.44). MS (70 eV): *m/z* (%) 386 (0.04, M^+), 368 (27), 165 (73), 78 (42), 73 (47), 60 (47), 55 (53), 43 (96), 41 (100), 38 (42).

Methyl-6,7-dimethoxy-4-(3,4-dimethoxyphenyl)naphthalene-2-carboxylate **6**. The acid **4** (1.80 g, 4.65 mmol) was heated under reflux in MeOH satd with HCl (10 ml) for 6 hr. The mixture was cooled to 0°, the ppt. filtered by suction, washed acid-free with H_2O and recrystallized from MeOH; 1.55 g (87%) colourless crystals, mp 178–180° (ref. [5]: mp 177–178°). Calcd. for $C_{22}H_{22}O_6$ (382.4): C 69.10, H 5.80; found: C 69.02, H 5.65. IR ν^{KBr}: 1710 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.44, 7.89 (*d*, J = 1.6 Hz; 1H), 7.28, 7.27 (*s*, 1H), 7.05–7.0 (*m*, 3H), 4.02, 3.97, 3.96, 3.91, 3.85 (*s*, 3H). UV (MeOH): λ_{max} (log ε) = 255 (4.65), 304 nm (sh, 4.07). MS (70 eV): *m/z* (%) = 382 (100, M^+), 292 (10), 59 (16).

6,7-*Dimethoxy*-4-(3,4-*dimethoxyphenyl*)*naphthalene*-2-*carboxylic acid* **7**. The ester **5** (1.20 g, 3.14 mmol) and KOH (0.35 g, 6.23 mmol) were dissolved in EtOH (30 ml) and heated under reflux for 3 hr. The reaction mixture was cooled to 20°, diluted with H_2O (30 ml), extracted three times with CH_2Cl_2 (20 ml), and the aq. phase acidified with 2 M HCl. The pptd acid was collected and dried over P_2O_5 *in vacuo*; 1.10 g (95%) colourless crystals, mp 222–223° (lit. [5]: mp 222–223°). Calcd. for $C_{21}H_{20}O_6$ (368.4): C 68.47, H 5.47; found: C 68.61, H 5.35. IR ν^{KBr}: 3385, 1725/1685 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.53, 7.95 (*s*, 1H), 7.29 (*s*, 2H), 7.65–7.0 (*m*, 3H), 4.04, 3.98, 3.92, 3.85 (*s*, 3H). UV (MeOH): λ_{max} (log ε) 204 (4.24), 254 (4.31), 309 nm (3.69). MS (70 eV): *m/z* (%) 268 (100, M^+).

6,7-*Dihydroxy*-4-(3,4-*dihydroxyphenyl*)*naphthalene*-2-*carboxylic acid* **1**. The methylated acid **7** (0.80 g, 2.17 mmol) was dissolved in CH_2Cl_2 (10 ml) in N_2 and the solution cooled to −78°. BBr_3 (1.90 g, 7.58 mmol) was added at once, the mixture was allowed to come to room temp. and heated under reflux for 3 hr. After cooling to 20° the reaction mixture was hydrolysed by addition of water (5 ml) and made alkaline with 2 M NaOH. The aqueous phase was separated and extracted twice with CH_2Cl_2 (20 ml). The product was pptd by addition of 2 M HCl, filtered by suction, dried and purified by chromatography (SiO_2, EtOAc). Recrystallization from MeOH yielded 0.50 g (74%) microcrystalline colourless powder, mp 325–327° (dec.); mmp with natural product shows no depression; spectroscopic data identified with those of the natural product. Calcd for $C_{17}H_{12}O_6$ (312.2): C 65.39, H 3.87; found: C 65.06, H 3.92.

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