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FIVE LIGNAN DERIVATIVES FROM *IN VITRO* CULTURES OF THE LIVERWORT *JAMESONIELLA AUTUMNALIS**

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Key Word Index—*Jamesoniella autumnalis*; Hepaticae; *in vitro* culture; lignan.

Abstract—Five new lignan derivatives, 2,3,6′-tricarboxy-6,7-dihydroxy-1(3′)-2′-pyranonyl-1,2-dihydronaphthalene, its two monomethyl esters, 2,6′-dicarboxy-6,7-dihydroxy-1(3′)-2′-pyranonyl-1,2-dihydronaphthalene and 2,3-dicarboxy-6,7-dihydroxy-1-(3′,4′-dihydroxy)-phenylnaphthalene, were isolated from the methanolic extract of aseptic cultures of the liverwort *Jamesoniella autumnalis*. Their structures were determined by spectroscopic analysis.

INTRODUCTION

Liverworts are known to be a rich source of terpenoids and phenolic constituents [1–3], including a range of bioactive compounds. Since less than 5% of known species have been phytochemically investigated many interesting substances remain to be isolated. However, analysis is hindered by the difficulty in obtaining sufficient amounts of plant material. The small size and the fact that liverworts grow mixed with other bryophytes make their purification a difficult and time consuming task. Furthermore, numerous species can usually be obtained only in small amounts from their natural habitat. A possible alternative is growing these liverworts in aseptic cultures, which has the advantage of producing sufficient and homogenous amounts of plant material for subsequent analysis. In the course of our investigations on aseptic cultures of liverworts we have isolated several diterpenes from cultures of *Jamesoniella autumnalis*: we reported six *ent*-labdanes, one *cis*-clerodane and the three *seco*-clerodanes Jamesoniellide A, B and C [4, 5]. Recently, we focused our attention on hydrophilic phenolic constituents of liverworts such as phenanthrenes and lignan derivatives [6–8]. Continuing these studies, we now have examined the methanolic extract of *J. autumnalis*. This paper reports the isolation and structure determination of five new lignan derivatives from *in vitro* cultures of this plant.

RESULTS AND DISCUSSION

The *n*-butanol soluble portion of the methanol extract of *in vitro* cultured *J. autumnalis* was fractionated on Sephadex LH-20 using methanol as the eluent. Fractions 4 and 5 were further separated by HPLC (RP18, methanol–H_2O–acetic acid mixtures as solvent systems) to afford compounds **1** (100 mg), **2** (49 mg), **3** (26 mg), **4** (2 mg) and **5** (1 mg).

Compound **1** gave the $[M + H]^+$ ion peak at *m/z* 389 in the FAB^+ mass spectrum corresponding to a molecular formula of $C_{18}H_{12}O_{10}$. Its UV spectrum (in methanol) showed absorption maxima at 225, 249 and 308 nm. The IR spectrum displayed absorption bands characteristic of hydroxyl groups (3430 cm^{-1}), α, β-unsaturated carboxyl groups (1750 and 1645 cm^{-1}) and aromatic ring systems (1590 and 1525 cm^{-1}). The ^1H NMR spectrum (in DMSO-d_6) was similar to that of the known cyclolignan 2,3-dicarboxy-6,7-dihydroxy-1-(3′4′-dihydroxy)-phenyl-1,2-dihydronaphthalene (**9**), recently isolated from the liverwort *Pellia epiphylla* [8]. The multiplicity and proton shift of the three singlets at δ_H 7.10, 6.78 and 6.39 (1H each) and the two signals of the aliphatic methine protons (δ_H4.56 (1H, *br d*, J = 1.2 Hz); 3.57 (1H, *br d*, J = 1.2 Hz)] clearly indicated the presence of a 2,3-dicarboxy-6,7-dihydroxy-dihydronaphthalene moiety, substituted in C-1. Further evidence for this partial structure was given by the similarities of the ^{13}C NMR spectra of **1** and **9**. The two doublets at δ_H 6.69 (1H, *d*, J = 6.5 Hz) and 6.54 (1H, *d*, J = 6.5 Hz) were attributed to an α,β-unsaturated α-pyrone ring, based on the comparison of the ^{13}C NMR data for **1** with those for scapaniapyrone A (**7**), isolated from the liverwort *Scapania undulata* [9]. In order to obtain better resolved NMR spectra and to determine the substitution pattern by extensive NOE studies, **1** was methylated. The formation of a pentamethyl derivative (**6**) confirmed the presence

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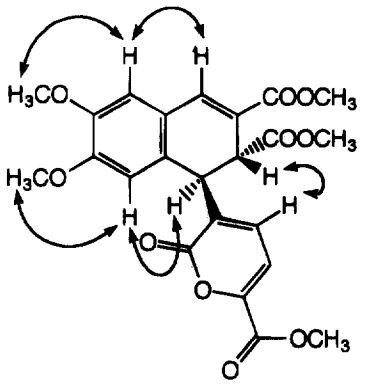
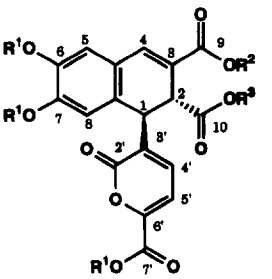
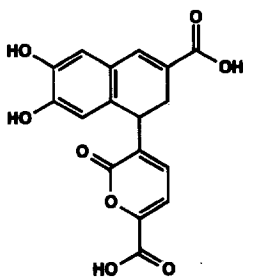


Fig. 1. NOE interaction of **6**.



	R^1	R^2	R^3
1:	H	H	H
2:	H	Me	H
3:	H	H	Me
6:	Me	Me	Me



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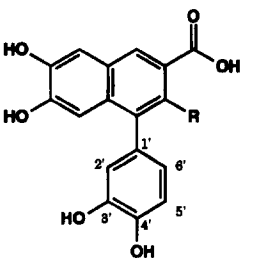
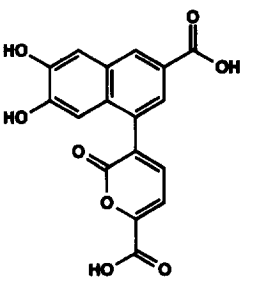


	R
5:	COOH
8:	H



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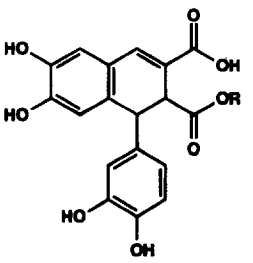


	R
9:	H
10:	Me

of five hydroxyl or carboxyl groups in the molecule. Compound **6** showed the $[M]^+$ ion peak at m/z 458 in the EI-mass spectrum. From the high resolution mass spectrum the molecular formula was calculated to be $C_{23}H_{22}O_{10}$. The connection of the dihydronaphthalene moiety and the α-pyrone ring between C-1 and C-3′ and their substitution pattern could be deduced from the results of the NOE experiments on **6**, as shown in Fig. 1. According to the NOEs observed, the methyl group signals at δ_H 3.89 and 3.85 (3H, *s* each) were attributed to the methoxyls at C-6 and C-7, respectively. This also proves the location of the hydroxyl groups at C-6 and C-7 in the original compound **1**. The signals of the methyl groups at δ_H 3.64, 3.76 and 3.88 (3H, *s* each) did not give any NOEs. The singlet appearing at higher field (δ_H 3.64) was assigned to the non-conjugated methyl ester group at carbon C-10. This is in accordance with data from the literature for compound **10** [8]. The two methyl signals appearing at lower field (δ_H 3.76 and 3.88) were attributed to the methyl esters at C-9 and C-7′, respectively. The conjugated position of these ester groups causes a downfield shift of the methyl signals compared to the methyl ester signals at C-10. The final assignment of both signals was based on a comparison of the electronegatives of the oxygens bonded to the methyl groups: the electronegativity of the oxygen at C-7′ is higher than that at C-9, which led us to assume that the methyl signal of the ester at C-7′ is at lower field than the corresponding signal at C-9. Thus **1** is 2,3,6′-tricarboxy-6,7-dihydroxy-1(3′)-2′-pyranonyl-1,2-dihydronaphthalene and **6** is the appropriate pentamethyl derivative 2,3,6′-tricarboxy-6,7-dimethoxy-1(3′)-2′-pyranonyl-1,2-dihydronaphthalene-9,10-dimethyl ester.

The coupling constant of 1.2 Hz between H-1 (δ_H 4.90, *dd*) and H-2 (δ_H 4.14, *d*) in **6** indicates a dihedral angle of nearby 90° between the bonds C-1–H-1 and C-2–H-2. This result, which is also in accordance with the data for [1*R*,2*S*]-2,3-dicarboxy-6,7-dimethoxy-1-(3′,4′-dimethoxy)-phenyl-1,2-dihydronaphthalene-9,10-dimethyl ester [10–12], proves the *trans* orientation of the α-pyrone ring to the carboxyl group at C-2.

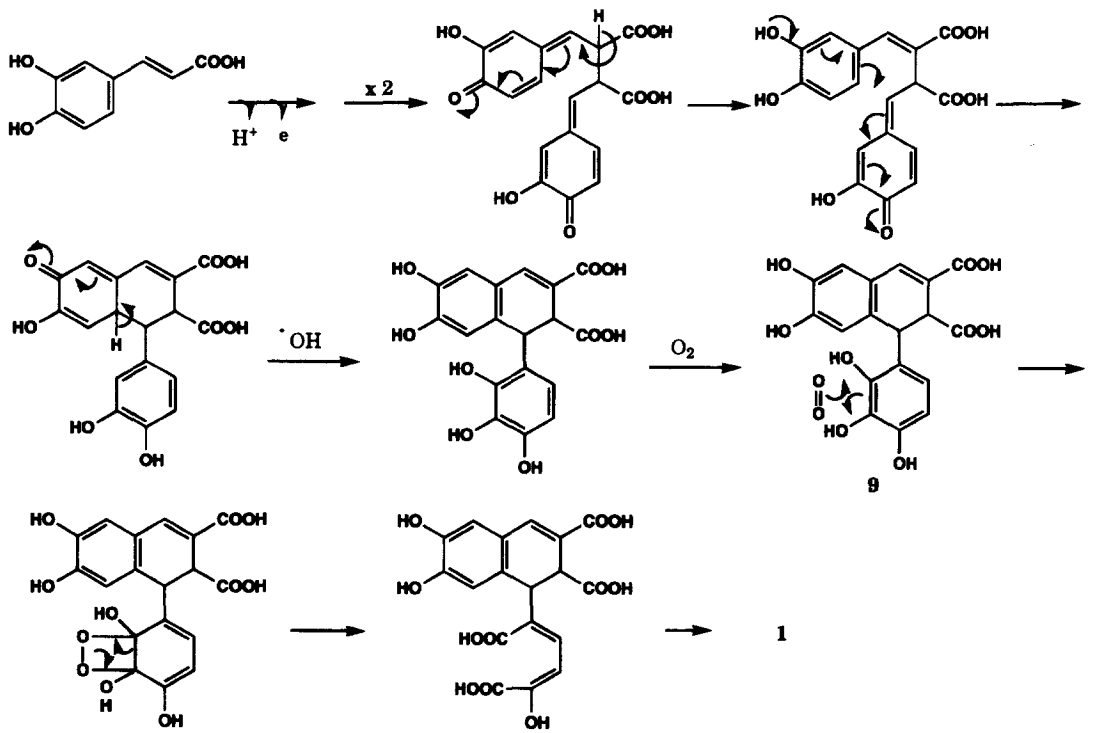
Both **2** and **3** showed $[M + H]^+$ ion peaks at m/z 403 in the FAB^+ mass spectrum. Their UV and IR spectra were similar to those of **1**, again indicating the presence of an α-pyrone ring. The ^1H NMR spectra of **2** and **3** were also close to that of **1**. They display additional methyl group singlets at δ_H 3.73 and 3.61, respectively (Table 1). These results indicated that **2** and **3** are monomethyl esters or ethers of **1**. Comparison of the chemical shift of their methyl signals with that of **6** confirmed the structures of **2** and **3** as 9- and 10-methyl esters of **1**, respectively. Although **1** is stable in methanolic solution, it cannot be excluded that **2** and **3** could have been formed from **1** during extraction of the plant material with methanol.

Compound **4** gave a $[M + H]^+$ ion peak at m/z 345 in the FAB^+ mass spectrum. Its UV spectrum (in methanol) showed absorption bands at 221, 250 and 309 nm, suggesting the presence of an α-pyrone ring. The ^1H NMR of **4** is similar to that of **1** except for the absence of two

Table 1. ^{1}H NMR spectral data for lignans **1–3** and **6**

H	1*	2†	3†	6‡
1	4.56 *br d* (1.2)	4.73 *br d* (1.5)	4.70 *br d* (1.5)	4.90 *dd* (1.1, 1.2)
2	3.57 *br d* (1.2)	4.05 *br d* (1.5)	4.08 *d* (1.5)	4.14 *d* (1.2)
4	7.10 *s*	7.54 *s*	7.55 *s*	7.61 *s*
5	6.78 *s*	6.86 *s*	6.85 *s*	6.84 *s*
8	6.39 *s*	6.62 *s*	6.62 *s*	6.71 *s*
4′	6.54 *d* (6.5)	6.54 *d* (6.8)	6.56 *d* (6.7)	6.43 *dd* (1.1, 7.0)
5′	6.69 *d* (6.5)	6.96 *d* (6.8)	6.95 *d* (6.7)	6.87 *d* (7.0)
6-OMe				3.89 *s*
7-OMe				3.85 *s*
9-OMe		3.73 *s*		3.76 *s*
10-OMe			3.61 *s*	3.64 *s*
7′-OMe				3.88 *s*

*In DMSO-d_6.
†In MeOH-d_4.
‡In $CDCl_3$.



Scheme 1. Proposed biosynthetic pathway of lignans in liverworts.

signals at δ_H4.56 and 3.57 corresponding to H-1 and H-2. According to their multiplicity, the signals at δ_H4.21 (1H, *dd*, J = 3.9, 7.7 Hz), 2.95 (1H, *dd*, J = 3.9, 17.3 Hz) and 2.70 (1H, *ddd*, J = 2.3, 7.7, 17.3 Hz) were assigned to an aliphatic 'ABX' spin system of an CH_2–CH partial structure. The small coupling constant (2.3 Hz) between the signals at δ_H2.70 and 7.45 (1H, *dd*) suggested the methylene group to be in an allylic position. These observations, together with the molecular weight of 344 indicated **4** to be a derivative of **1**, which is decarboxylated at C-2. Therefore, the structure of **4** is established as 2,6′-dicarboxy-6,7-dihydroxy-1(3′)-2′-pyranonyl-1,2-dihydronaphthalene.

Compound **5** gave a $[M + H]^+$ ion peak at m/z 357 in the FAB$^+$ mass spectrum. The IR and ^{1}H NMR spectra were also similar to those of **1**, but strong UV absorption at 252 nm (log ε: 4.62) relative to the peak at 307 nm (log ε: 4.17) suggested that **5** did not have an α-pyrone ring. The ^{1}H NMR showed the signals corresponding to a 3,4-dihydroxyphenyl moiety [δ_H 7.82 (1H, *d*,

J = 6.8 Hz, H-5'), 7.30 (1H, *d*, J = 6.8 Hz, H-6'), 7.07 (1H, *s*, H-2')] and three singlets [δ_H8.38, 7.78 and 7.47 (1H, *s* each)] attributed to a naphthalene ring system substituted at C-1, C-2, C-3, C-6 and C-7. These facts and the molecular weight of **5** (356) indicated **5** is the 1,2-dehydro-derivative of the known compound **9**. Thus, **5** is 2,3-dicarboxy-6,7-dihydroxy-1-(3',4'-dihydroxy)-phenylnaphthalene.

As mentioned, several cyclolignan derivatives, e.g. scapaniapyrone A (**7**), 3-carboxy-6,7-dihydroxy-1-(3',4'-dihydroxy)-phenylnaphthalene (**8**) and 2,3-dicarboxy-6,7-dihydroxy-1-(3',4'-dihydroxy)-phenyl-1,2-dihydronaphthalene (**9**) have been isolated from the liverworts *S. undulata* [9] and *P. epiphylla* [8, 13]. Their structures are closely related to each other. Our results on the liverwort *J. autumnalis* suggested a possible biogenetic pathway for the formation of **1** via **9** from caffeic acid, as shown in Scheme 1.

EXPERIMENTAL

Optical rotations were measured in MeOH. NMR spectra were recorded in $CDCl_3$, MeOH-d_4 and DMSO-d_6 soln, using a 400 MHz instrument (H: 400 MHz; C: 100.5 MHz) relative to $CHCl_3$ at δ_H7.25, MeOH at δ_H3.30, DMSO at δ_H2.50, $CDCl_3$ at δ_C77.0, MeOH-d_4 at δ_C49.0 and DMSO-d_6 at δ_C40.8, respectively. ^{13}C multiplicities were determined using the DEPT pulse sequence.

Plant material. Jamesoniella autumnalis was collected in December 1988 near Orscholz Saar and identified by Prof. Dr R. Mues. Voucher specimens were deposited at the Fachbereich 12.3, Pharmakognosie und Analytische Phytochemie der Universität des Saarlandes, Saarbrücken. An axenic culture of *J. autumnalis* was induced from the surface-sterilized gametophyte of field material. The cultures were grown in 250 ml flasks with 70 ml solid modified Gamborg B5 medium (pH 6.0) [14], containing 20 g l^{-1} sucrose. The flasks were kept under constant illumination (2000 lux) at 20°.

Extraction and isolation. Powdered dry plant material (815 g) was successively extracted with Et_2O and MeOH at room temp. The MeOH (103.5 g) extract was dissolved in H_2O (400 ml), washed with EtOAc (400 ml × 3) and extracted with *n*-BuOH (300 ml × 3). The *n*-BuOH soln was evapd under red. pres. to afford 10.2 g of residue. The concd *n*-BuOH extract was subjected to CC on Sephadex LH-20 (1500 × 25 mm i.d.) with MeOH as eluent to give five frs. Frs 4 (310 mg) and 5 (120 mg) were further sepd by HPLC (RP18 250 × 8 mm i.d., 2.5% HOAc–20% MeOH/H_2O) to afford **1** (100 mg), **2** (49 mg), **3** (26 mg), **4** (2 mg), **5** (1 mg).

Methylation of **1**. To a soln of **1** (5 mg) in Me_2CO (10 ml), MeI (2 ml) and K_2CO_3 (100 mg) were added, and the mixt. was refluxed for 12 hr. After cooling, the solvent was evapd, and the residue passed through a column of silica (500 mg) with *n*-hexane–Et_2O (4:1) as eluent to afford **6** (3 mg).

2,3,6'-*Tricarboxy*-6,7-*dihydroxy*-1(3')-2'-*pyranonyl*-1,2-*dihydronaphthalene* (**1**). Light yellow amorphous powder, $[\alpha]_{546}^{20}$ + 54.88° (MeOH; *c* 0.30). FAB-MS *m/z*: 389 $[M + H]^+$. UV λ_{max} nm (log ε): 225 (4.01), 249 (4.23), 308 (4.22), 322sh. CD: $\Delta\varepsilon_{216}$ + 1.5, $\Delta\varepsilon_{245}$ + 6.0, $\Delta\varepsilon_{278}$ + 1.8, $\Delta\varepsilon_{312}$ + 8.2, $\Delta\varepsilon_{350}$ − 2.8 (MeOH; *c* 8.2 × 10^{-5}). IRν^{KBr} cm^{-1}: 3430, 3200, 1715, 1645, 1620, 1590, 1525, 1200, 780. ^{1}H NMR: see Table 1. ^{13}C NMR (DMSO-d_6): δ 171.8 (*s*), 170.2 (*s*), 161.6 (*s*), 146.9 (*s*), 144.4 (*s*), 139.4 (*d*), 132.5 (*d*), 130.3 (*s*), 126.2 (*s*), 123.7 (*s*), 116.2 (*d*), 115.6 (*d*), 106.0 (*d*) 44.2 (*d*), 38.9 (*d*).

2,3,6'-*Tricarboxy*-6,7-*dihydroxy*-1(3')-2'-*pyranonyl*-1,2-*dihydronaphthalene*-9,10-*dimethyl ester* (**6**). Yellow syrup, $[\alpha]_{546}^{20}$ + 47.23° (MeOH; *c* 0.07). HR-MS $C_{23}H_{22}O_{10}$ (*m/z*: 458.121802 calc. 458.1213), $C_{22}H_{18}O_9$ (*m/z*: 426.0953 calc. 426.0951), $C_{21}H_{18}O_8$ (*m/z*: 398.0989 calc. 398.0976), $C_{20}H_{15}O_7$ (*m/z*: 367.0826 calc. 367.0818), $C_{19}H_{15}O_6$ (*m/z*: 339.0879 calc. 339.0869), $C_{18}H_{11}O_5$ (*m/z*: 307.0589 calc. 307.0607), $C_{15}H_{13}O_5$ (*m/z*: 273.0589 calc. 273.0584). UV λ_{max} nm (log ε): 225 (4.11), 250 (4.24), 309 (4.19), 318 (sh). IRν^{KBr} cm^{-1}: 2920, 1720, 1635, 1605, 1570, 1515, 1440, 1240, 760. ^{1}H NMR: see Table 1. ^{13}C NMR ($CDCl_3$): δ 171.4 (*s*), 166.7 (*s*), 159.9 (*s*), 159.8 (*s*), 149.8 (*s*), 149.4 (*s*), 147.7 (*s*), 138.0 (*d*), 137.1 (*d*), 134.2 (*s*), 127.1 (*s*), 124.6 (*s*), 123.0 (*s*), 112.1 (*d*), 112.1 (*d*), 110.2 (*d*), 56.1 (*q* × 2), 53.0 (*q*), 52.7 (*q*), 52.0 (*q*), 42.3 (*d*), 40.4 (*d*). EI-MS *m/z* (rel. int.): 458 ($[M]^+$, 14), 426 (35), 398 (100), 367 (97), 339 (39), 307 (63), 273 (58), 225 (22), 152 (15), 139 (4), 126 (21), 59 (79), 43 (71).

2,3,6'-*Tricarboxy*-6,7-*dihydroxy*-1(3')-2'-*pyranonyl*-1,2-*dihydronaphthalene*-9-*methyl ester* (**2**). Light yellow amorphous powder, $[\alpha]_{546}^{20}$ + 33.49° (MeOH; *c* 1.70). FAB-MS *m/z*: 403 $[M + H]^+$. UV λ_{max} nm (log ε): 225 (4.03), 250 (4.21), 308 (4.18), 321 (sh). IRν^{KBr} cm^{-1}: 3400, 1715, 1640, 1590, 1525, 1250, 1200, 775. ^{1}H NMR: see Table 1. ^{13}C NMR (MeOH-d_4): δ 173.8 (*s*), 170.0 (*s*), 162.5 (*s*), 149.5 (*s*), 146.5 (*s*), 140.7 (*d*), 139.0 (*d*) 134.3 (*s*), 127.6 (*s*), 125.5 (*s*), 123.5 (*s*), 117.4 (*d*), 117.1 (*d*), 110.9 (*d*), 52.9 (*q*), 43.7 (*d*), 42.0 (*d*).

2,3,6'-*Tricarboxy*-6,7-*dihydroxy*-1(3')-2'-*pyranonyl*-1,2-*dihydronaphthalene*-10-*methyl ester* (**3**). Light yellow amorphous powder, $[\alpha]_{546}^{20}$ + 50.48° (MeOH; *c* 0.95). FAB-MS *m/z*: 403 $[M + H]^+$. UV λ_{max} nm (log ε): 221 (4.14), 250 (4.24), 309 (4.20), 319 (sh). IRν^{KBr} cm^{-1}: 3400, 1715, 1645, 1596, 1525, 1450, 1390, 1270, 1250, 775. ^{1}H NMR: see Table 1. ^{13}C NMR (MeOH-d_4): δ 175.0 (*s*), 169.0 (*s*), 162.6 (*s*), 149.5 (*s*), 146.4 (*s*), 140.5 (*d*), 138.8 (*d*), 134.5 (*s*), 127.7 (*s*), 125.6 (*s*), 123.6 (*s*), 117.4 (*d*), 117.2 (*d*), 110.8 (*d*), 52.3 (*q*), 43.8 (*d*), 42.0 (*d*).

2,6'-*Dicarboxy*-6,7-*dihydroxy*-1(3')-2'-*pyranonyl*-1,2-*dihydronaphthalene* (**4**). Light yellow amorphous powder, $[\alpha]_D^{20}$ + 135.40°, $[\alpha]_{546}^{20}$ + 156.52° (MeOH; *c* 0.08). FAB-MS *m/z*: 345 $[M + H]^+$. UV λ_{max} nm (log ε): 220 (4.23), 245 (4.26), 307 (4.22), 318 (sh). IRν^{KBr} cm^{-1}: 3400, 1700, 1640, 1590, 1520, 1400, 1320, 1250, 770. ^{1}H NMR (MeOH-d_4): δ 7.45 (1H, *d*, J = 2.3 Hz, H-5), 6.98 (1H, *d*, J = 6.8 Hz, H-5'), 6.80 (1H, *s*, H-8), 6.74 (1H, *d*, J = 6.8 Hz, H-6'), 6.56 (1H, *s*, H-2'), 4.21 (1H, *dd*, J = 3.9, 7.7 Hz, H-3). 2.95 (1H, *dd*, J = 3.9, 17.3 Hz, H-4), 2.70 (1H, *ddd*, J = 2.3, 7.7, 17.3 Hz, H-4').

2,3-*Dicarboxy*-6,7-*dihydroxy*-1-(3',4'-*dihydroxy*)-*phenylnaphthalene* (**5**). Light yellow amorphous powder, FAB-MS

m/z: 357 $[M + H]^+$. UV λ_{max} nm (log ε): 224 (4.30), 252 (4.62), 307 (4.17). IR ν^{KBr} cm^{-1}: 3450, 1700, 1670, 1650, 1610, 1560, 1485, 1255, 785. ^1H NMR (MeOH-d_4): δ8.38 (1H, *s*, H-4), 7.82, (1H, *d*, J = 6.8 Hz, H-5'), 7.78 (1H, *s*, H-5), 7.47 (1H, *s*, H-8), 7.30 (1H, *d*, J = 6.8 Hz, H-6'), 7.07 (1H, *s*, H-2').

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