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# PELLIATIN, A MACROCYCLIC LIGNAN DERIVATIVE FROM *PELLIA EPIPHYLLA**†

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**Key Word Index**—*Pellia epiphylla*; Hepaticae; liverwort; macrocyclic lignan; caffeic acid; biosynthesis; dioxygenase.

**Abstract**—A new macrocycle consisting of a lignan, caffeic acid and an aliphatic $C_8$ moiety has been isolated from the liverwort, *Pellia epiphylla*. Its structure has been elucidated based on extensive NMR spectral evidence and by chemical derivatization. A possible biosynthetic pathway for the $C_8$ moiety is discussed.

## INTRODUCTION

In a previous paper [1], we reported the isolation of several phenolic compounds from *Pellia epiphylla*. In a continuation of this study, we now report the isolation and identification of a new type of compound, pelliatin, from this liverwort.

## RESULTS AND DISCUSSION

From the ethyl acetate-soluble fraction of the methanol extract, obtained as previously reported [1], pelliatin (**1**) was isolated, as a yellow oil. Positive and negative FAB spectra ($[M + H]^+$ *m/z* 721; $[M - H]^-$ *m/z* 719) were in accordance with a molecular formula of $C_{35}H_{28}O_{17}$. $^1H$ and $^{13}C$ NMR spectra showed the presence of three partial structures. The first was a phenyldihydronaphthalene moiety ($C_{18}H_{14}O_8$), which we reported from *P. epiphylla* earlier (Fig. 1). The second moiety was characterized by a set of doublets at $\delta_H$5.95 (H-8″) and $\delta_H$7.34 (H-7″) with a coupling constant of 15.6 Hz, typical for a *trans*-double bond of a substituted cinnamic acid. Additionally, there was a set of two protons with *ortho*-coupling ($\delta_H$6.60 (H-5″) and $\delta_H$6.97 (H-6″), $J = 8.5$ Hz each). The $^1H$–$^{13}C$ COSY and the HMBC revealed the structure of a 2-hydroxycaffeic acid moiety (Fig. 2). The remaining moiety consists of a carboxyl group ($\delta_C$164.8 (*s*) C-1‴), a trisubstituted double bond ($\delta_C$148.5 (*s*) C-2‴ and $\delta_C$109.0 (*d*) C-3‴) and five oxygen-bearing aliphatic carbons, ($\delta_C$67.9 (*d*) C-4‴, $\delta_C$62.1 (*d*) C-5‴, $\delta_C$76.9 (*d*) C-6‴, $\delta_C$70.0 (*d*) C-7‴ and $\delta_C$63.9 (*t*) C-8‴). From the $^1H$–$^1H$ COSY, $^1H$–$^{13}C$ COSY and the HMBC data a linear chain of eight carbon atoms could be deduced. The alignment of these carbon atoms was also proved by decoupling experiments.

Since there are no further carbon atoms with a free valency, the fourth substituent of the double bond must be one of the oxygens of the chain. An ether-bearing carbon should resonate at a lower field than a free alcohol [2]; this can be seen for C-6‴ ($\delta_C$76.9). The neighbouring carbon atoms should resonate at higher field, as for C-5‴ ($\delta_C$62.1) and C-7‴ ($\delta_C$70.0). Therefore, a bond between the oxygen at C-6‴ and C-2‴ was assumed, furnishing a six-membered ring. The chemical shifts of the double bond of this 3,4-dihydro-2*H*-pyran [C-2‴ $\delta_C$148.5 (*s*) and C-3‴ $\delta_C$109.0 (*d*)] are in good agreement with the shifts of a 6-carboxy-3,4-dihydroxy-2*H*-pyran ($\delta_C$144.2 and $\delta_C$111.5) [3] and a 6-carboxy-3,4-dihydroxy-2,2-dimethyl-2*H*-pyran ($\delta_C$142.3 and $\delta_C$110.6) [4] obtained by synthesis. A long-range $^1H$–$^{13}C$ correlation between H-6‴ and C-2‴ was not found. A possible explanation could be the electronegative substituents and the dihedral angle of *ca* 90°, both minimizing the $^3J$-coupling constant [5].

The linkages of the subunits were obtained from HMBC results. H-4‴ ($\delta_H$5.52, *m*) showed a correlation with carbon C-9 of the lignan and H-5‴ ($\delta_H$6.06, *m*) correlated with C-9″ of the substituted caffeic acid moiety (Fig. 3).

From the mass spectrometry results (molecular formula $C_{35}H_{28}O_{17}$) a third linkage must also be present in the molecule. Because no further useful spectroscopic data could be obtained from the original compound, permethylation of the phenolic and acidic hydroxyls was performed to give compound **2** ($[M + H]^+$ *m/z* 819). The $^1H$ NMR spectrum showed the presence of seven methyl signals ($\delta_H$4.06 $R^4$, $\delta_H$3.86 $R^6$, $\delta_H$3.84 $R^2$, $\delta_H$3.81 $R^7$,

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†Dedicated to Prof. Rimpler on the occasion of his sixtieth birthday.

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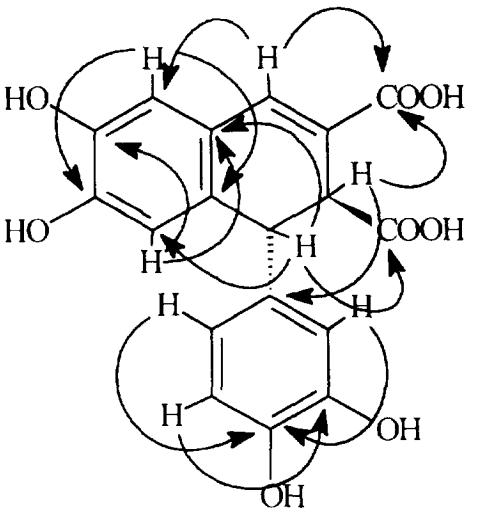



Fig. 1. Significant HMBC couplings of lignan moiety.

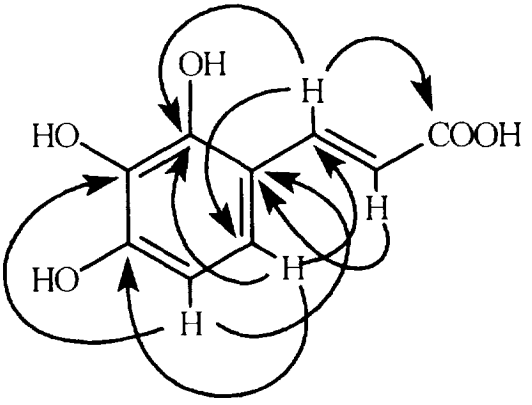



Fig. 2. Significant HMBC couplings of 2-hydroxycaffeic acid moiety.

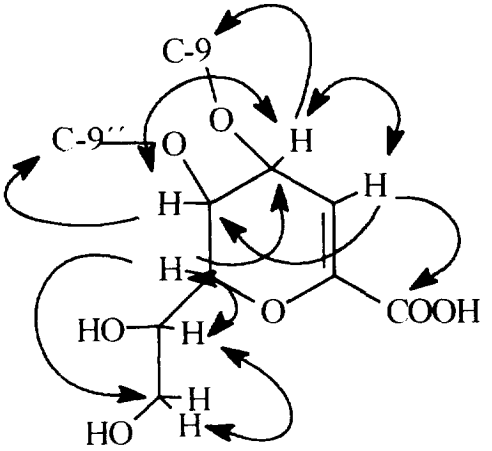



Fig. 3. Significant $^1H$–$^1H$ COSY and HMBC couplings of $C_8$ moiety.

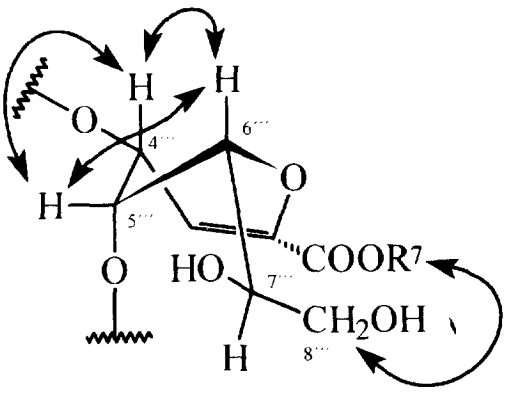
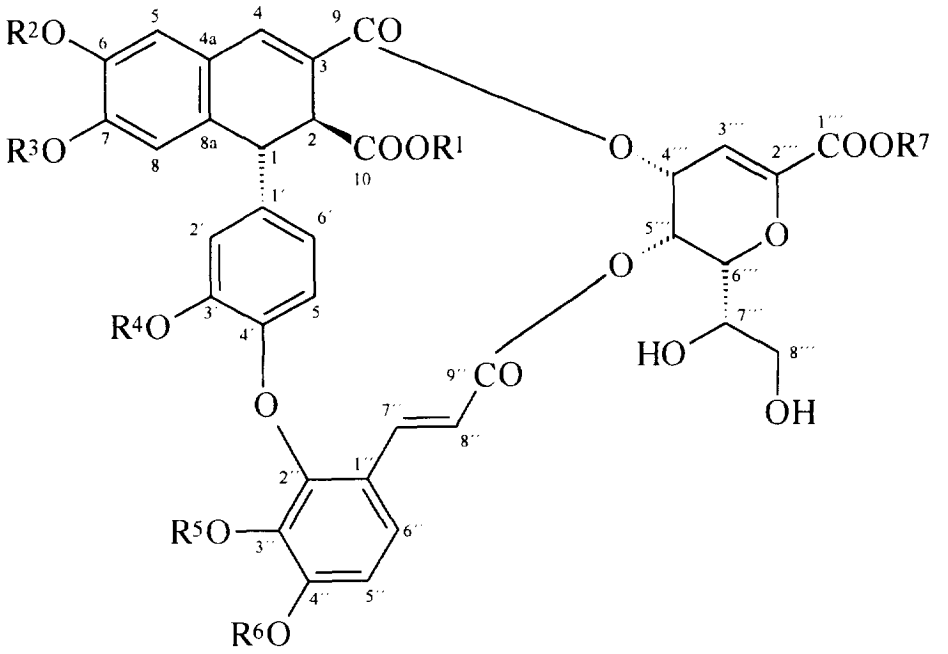



Fig. 4. Stereochemistry of third substructure with ROESY couplings.

Table 1. $^1H$ NMR spectral data for compounds **1** and **2**

| H | 1 | 2 |
|---|---|---|
| Lignan moiety | | |
| H-1 | 4.46 *s* | 4.66 *s* |
| H-2 | 3.74 *s* | 3.86 *s* |
| H-4 | 7.40 *s* | 7.52 *s* |
| H-5 | 6.83 *s* | 7.04 *s* |
| H-8 | 6.58 *s* | 6.82 *s* |
| H-2′ | 7.03 *d* (2.1) | 7.20 *d* (2.1) |
| H-5′ | 5.98 *d* (8.5) | 5.97 *d* (8.5) |
| H-6′ | 5.47 *dd* (8.5, 2.1) | 5.48 *dd* (8.5, 2.1) |
| 2-Hydroxycaffeic acid | | |
| H-5″ | 6.60 *d* (8.5) | 6.85 *d* (8.5) |
| H-6″ | 6.97 *d* (8.5) | 7.30 *d* (8.5) |
| H-7″ | 7.34 *d* (15.6) | 7.27 *d* (15.6) |
| H-8″ | 5.95 *d* (15.6) | 5.98 *d* (15.6) |
| $C_8$ moiety | | |
| H-3‴ | 5.99 *s* (*br*) | 6.01 *s* (*br*) |
| H-4‴ | 5.52 *m* | 5.56 *m* |
| H-5‴ | 6.06 *m* | 6.07 *m* |
| H-6‴ | 4.13 *d* (9.0) | 4.15 *d* (9.0) |
| H-7‴ | 3.63 *m* | 3.64 *m* |
| H-8‴ | 3.76 *m* | 3.72 *m* |
| Methoxyl signals | | |
| $R^1$ | — | 3.63 *s* (3H) |
| $R^2$ | — | 3.84 *s* (3H) |
| $R^3$ | — | 3.79 *s* (3H) |
| $R^4$ | — | 4.06 *s* (3H) |
| $R^5$ | — | 3.79 *s* (3H) |
| $R^6$ | — | 3.86 *s* (3H) |
| $R^7$ | — | 3.81 *s* (3H) |

$\delta_H$3.79 $R^3$, $\delta_H$3.79 $R^5$, $\delta_H$3.63 $R^1$, 3H (*s*) each). From the HMBC spectrum, a cross-peak between $R^1$ and C-10 could be observed, as well as between $R^7$ and C-1‴. Therefore, the third linkage is an ether bond. Correlations between methyl signals to C-6, C-7, C-3′, C-3″ and C-4″ indicated the presence of free phenolic hydroxyl groups at these positions in **1**. Therefore, only a linkage between C-4′ and C-2″ via an oxygen was possible, thus establishing the complete constitution **1**.



| | $R^1$ - $R^7$ |
|---|---|
| **1** | H |
| **2** | Me |

Table 2. $^{13}C$ NMR spectral data for compounds **1** and **2**

| Carbon | 1 | 2 | Carbon | 1 | 2 |
|---|---|---|---|---|---|
| Lignan moiety | | | 2-Hydroxycffeic acid | | |
| C-1 | 46.5 *d* | 46.6 *d* | C-1″ | 119.7 *s* | 121.4 *s* |
| C-2 | 49.6 *d* | 49.0 *d* | C-2″ | 144.8 *s* | 150.1 *s* |
| C-3 | 122.2 *s* | 122.7 *s* | C-3″ | 139.4 *s* | 143.4 *s* |
| C-4 | 140.0 *d* | 139.6 *d* | C-4″ | 150.0 *s* | 156.7 *s* |
| C-4a | 126.0 *s* | 126.7 *s* | C-5″ | 113.0 *d* | 109.7 *d* |
| C-5 | 117.1 *d* | 113.5 *d* | C-6″ | 119.3 *d* | 123.9 *d* |
| C-6 | 145.6 *s* | 149.8 *s* | C-7″ | 143.4 *d* | 142.4 *d* |
| C-7 | 148.9 *s* | 152.4 *s* | C-8″ | 114.9 *d* | 116.0 *d* |
| C-8 | 117.2 *d* | 113.5 *d* | C-9″ | 167.4 *d* | 167.4 *d* |
| C-8a | 129.8 *s* | 130.4 *s* | $C_8$ moiety | | |
| C-9 | 167.9 *s* | 167.5 *s* | C-1‴ | 164.8 *s* | 163.4 *s* |
| C-10 | 175.5 *s* | 173.7 *s* | C-2‴ | 148.5 *s* | n.d. |
| C-1′ | 138.1 *s* | 137.5 *s* | C-3‴ | 109.0 *d* | 108.9 *d* |
| C-2′ | 116.8 *d* | 113.2 *d* | C-4‴ | 67.9 *d* | 67.8 *d* |
| C-3′ | 146.7 *s* | 150.3 *s* | C-5‴ | 62.1 *d* | 61.7 *d* |
| C-4′ | 147.1 *s* | 150.0 *s* | C-6‴ | 76.9 *d* | 76.8 *d* |
| C-5′ | 116.5 *d* | 116.5 *d* | C-7‴ | 70.0 *d* | 69.8 *d* |
| C-6′ | 119.5 *d* | 120.2 *d* | C-8‴ | 63.9 *t* | 63.8 *t* |
| Methoxyl signals | | | $R^4$ | — | 56.4 *q* |
| $R^1$ | — | 52.7 *q* | $R^5$ | — | 61.7 *q* |
| $R^2$ | — | 56.4 *q* | $R^6$ | — | 56.4 *q* |
| $R^3$ | — | 56.4 *q* | $R^7$ | — | 52.7 *q* |

$^{13}C$ NMR data taken from the $^{13}C$ projection of the HMBC spectrum.
n.d. = not detectable.

Additionally, a ROESY experiment was carried out. A cross-peak between $R^4$ and H-2′ gave further evidence for the structure of **1**. This experiment also revealed the stereochemistry of the $C_8$ subunit. As there are cross-peaks between H-6‴ and H-4‴, both protons have to be in an axial position. Furthermore, there are correlations between H-5‴ and both H-4‴ and H-6‴. Therefore, H-5‴ is in an equatorial position. Finally, a small cross-peak between H-8‴ and $R^7$, together with the absence of a cross-peak between H-6‴ and H-7‴ ($^3J$H-6‴/H-7‴ = 9.0 Hz), reveals the stereochemistry at C-7‴ (Fig. 4).

A possible biosynthetic pathway for the $C_8$ moiety is shown in Fig. 5. The aromatic ring of the 3,4-dihydroxy-cinnamic alcohol could be opened by an extradiol-dioxygenase and the formyl group then cleaved after oxygenation. The two remaining double bonds would then undergo epoxidation and hydration to give the diol groups. Finally, the ring closes to a semi-acetal and the double bond formed by elimination of water. This scheme is analogous to the formation of muscaflavin in *Amanita muscaria* and other fungi [6]. A similar pathway can be assumed for the formation of scapaniapyrone A from *Scapania undulata* [7] and *Jamesoniella autumnalis* [8], which are other 2*H*-pyran structures found in Bryophytes.

## EXPERIMENTAL

Solvents used for spectral measurements: MeOH-$d_4$ $^1$H NMR: 400 MHz, $^{13}$C NMR: 100 MHz for 1D

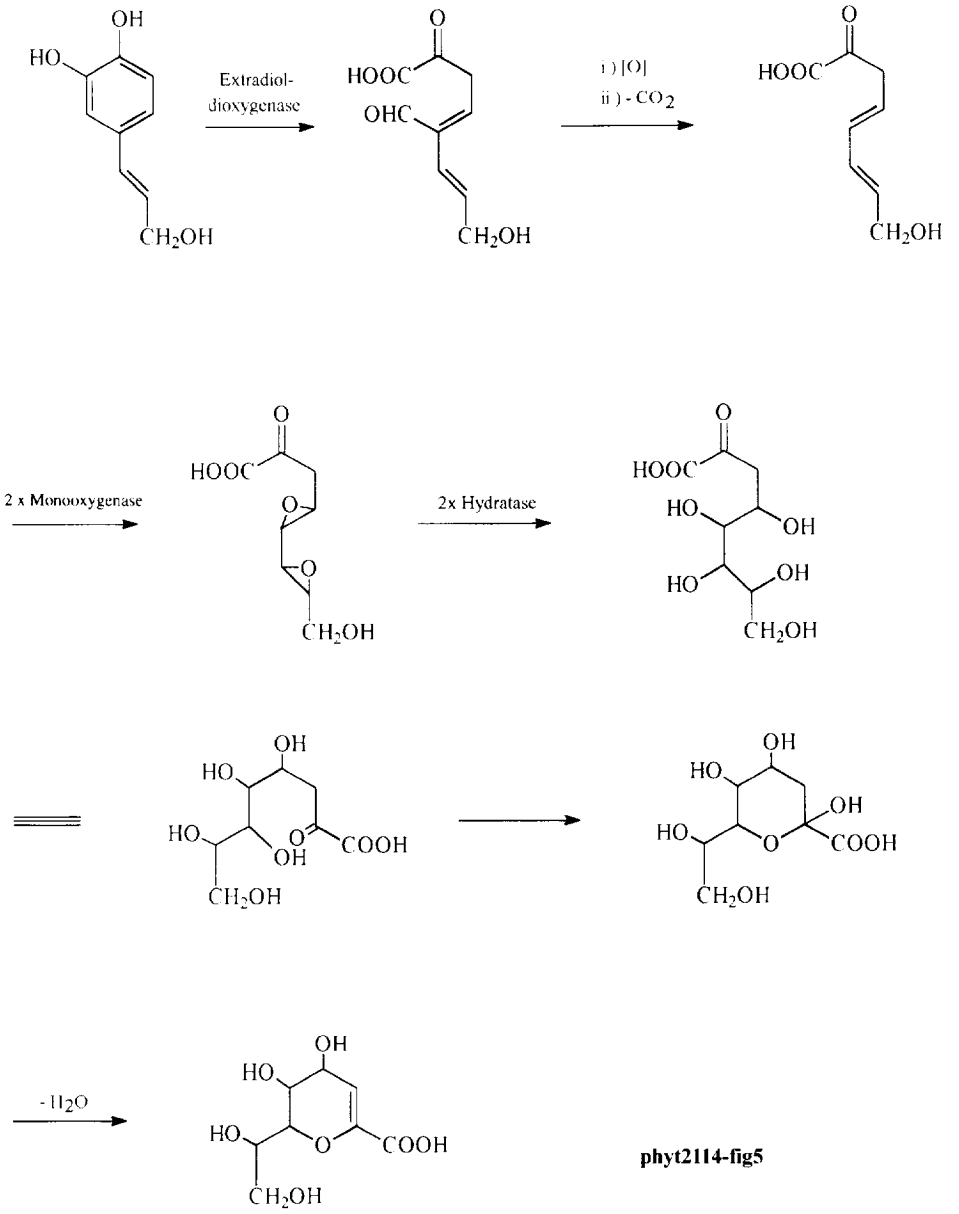



Fig. 5. Possible biosynthetic pathway of $C_8$ moiety.

spectra, 500 and 125 MHz for 2D-spectra, respectively, chemical shifts are given in $\delta$ values (ppm) from TMS] and MeOH (UV, rotation).

*Extraction and isolation.* Freeze-dried, powdered gametophytes of *P. epiphylla* (L.) corda [1] (750 g) were sequentially extracted with $Et_2O$ and MeOH. The MeOH extract was evapd *in vacuo* and distributed between EtOAc and $H_2O$. The organic layer was evapd *in vacuo* and chromatographed on Sephadex LH-20 using MeOH–$CH_2Cl_2$ (4:1) to yield 3 frs. Fr. 2 was chromatographed on diol silica gel *via* VLC with an *n*-hexane–EtOAc–MeOH gradient and then purified by HPLC on diol silica gel with EtOAc–MeOH (47:3) to afford **1** (134.8 mg).

*Methylation of compound* **1**. Methylation of **1** (5 mg) in $Me_2CO$ with $K_2CO_3$ and MeI gave **2** (2 mg).

*Compound* **1**. $[\alpha]_D^{20} - 106.68°$. IR $\nu$ $cm^{-1}$: 3400, 2910, 1700, 1610, 1500, 1450, 1370, 1260, 1110, 1070, 980. UV $\lambda$ nm: 318.0br, 293.6sh, 239.2. $^1H$ NMR: Table 1. $^{13}C$ NMR: Table 2. FABMS: $[M + H]^+$ *m/z* 721, $[M - H]^-$ *m/z* 719.

*Compound* **2**. $^1H$ NMR: Table 1. $^{13}C$ NMR: Table 2. CIMS: $[M + H]^+$ *m/z* 819.

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