Triterpene caffeoyl esters and diterpenes from *Celastrus stephanotifolius*

Bei Chen, Hongquan Duan, Yoshihisa Takaishi*

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770-8505, Japan

Abstract

The methanol extract of the dried stems of *Celastrus stehanotifolius* afforded two new triterpenes: lup-20(29)en-28-al-3β-yl caffeate (betulinaldehyde-3β-yl-caffeate) and olean-28-al-3β-yl caffeate, two new diterpenes: celaphanol A and celaphanol B, three known triterpenes: lup-20(29)en-28-ol-3β-yl caffeate (betulin-3β-yl-caffeate), lup-20(29)en-28-oic-3β-yl caffeate (betulinic acid-3β-yl-caffeate) and olean-3β-yl-caffeate, and one known diterpene: nimbidiol. Their structures were established on the basis of spectroscopic evidence. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The Celastraceae comprises 55 genera and over 850 plant species. They have been the subject of continued and growing interest, due to the range of biological activities shown by many members of this family (Bruning & Wagner, 1978; Tu, 1990; Tu, 1991), with some having been used in traditional medicine (Shizuri, Wada, Sugiura, Yamada, & Hirata, 1973) or as a stimulant (Geutaharn & Krikorian, 1973) from ancient times. We are interested in the constituents of *Celastrus stephanotifolius* and have elucidated the structures of eight new dihydroagarofuran sesquiterpenepolyol esters from the seeds (Takaishi, Oshima, Nakano, & Tomimatsu, 1993). We now report on the structural elucidation of the new triterpene esters **1** and **4**, and the new diterpenes, celaphanol A (**6**) and celaphanol B (**7**), together with the four known compounds **2**, **3**, **5** and **8** from the stems of *C. stephanotifolius*.

2. Results and discussion

The methanol extract of dried stems of *C. stephanotifolius* was partitioned between H_2O and EtOAc; the EtOAc extracts were subjected to CC to separate compounds **1**–**8**.

Compound **1**, obtained as an amorphous powder, showed an M^+ peak at m/z 602 $[M]^+$ in its EIMS. Its IR spectrum contained absorption bands due to ketone (1708 cm^{-1}) and hydroxy (3413 cm^{-1}) groups, and its UV spectrum showed absorption bands at 216, 242, 298 and 328 mn. The ^1H NMR spectrum revealed the presence of six tertiary methyls (δ_H 0.78, 0.79, 0.82, 0.83, 0.90, 1.63 (each 3H, s)), one aldehyde (δ_H 9.60 (1H, s)) and three aromatic protons; the coupling relationship established a 1,3,4-substituted ring. In addition, the coupling constant of the proton signals at δ_H 7.47 and 6.16 is 15.8 Hz, indicating the presence of two *trans*-olefinic protons. Thus, it was presumed there is an *O*-caffeoyl group in **1**. The ^{13}C NMR spectrum of **1** showed one ester carbonyl carbon at δ_C 168.0, four double bond carbons at δ_C 114.3, 115.4, 115.9, 122.3, 127.4, 144.1, 144.9, 146.6, an aldehyde resonance at δ_C 207.1 and a terminal double bond at δ_C 110.3. In addition, there are six methyl, 10 methylene,

* Corresponding author.

R

1 CHO
2 CH$_2$OH
3 COOH

R

4 CHO
5 COOH
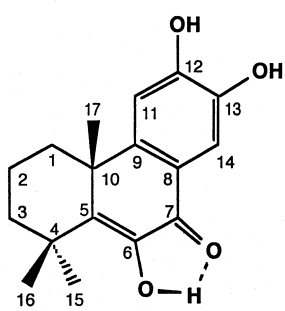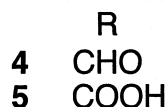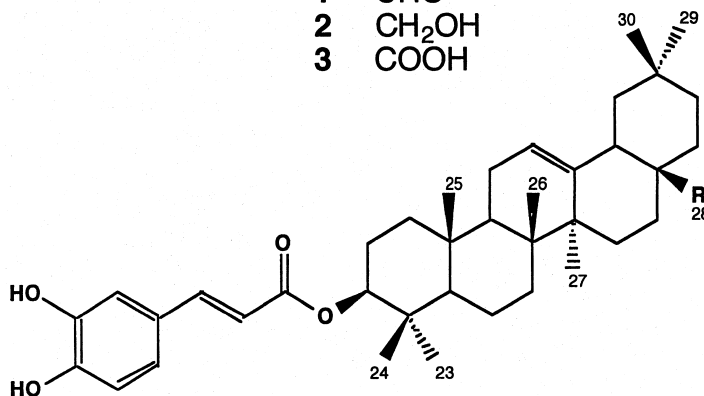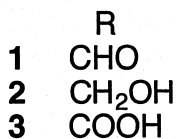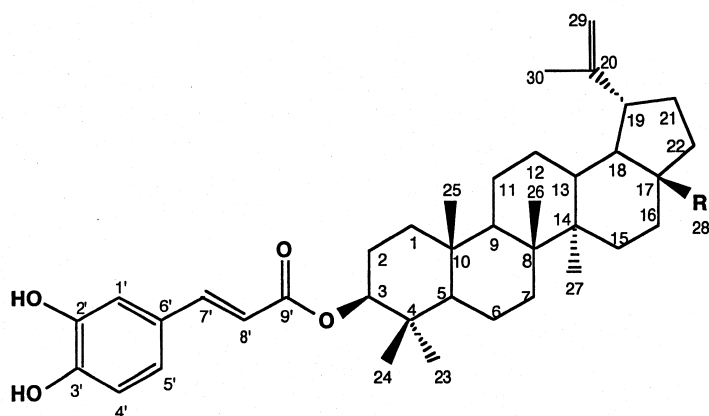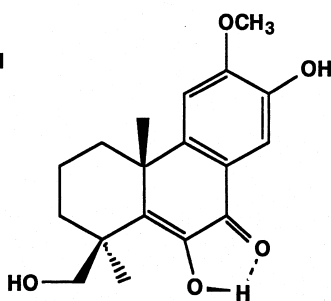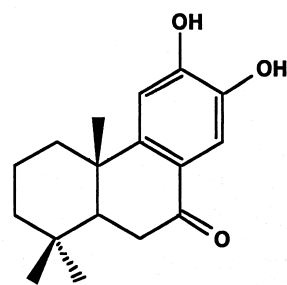
6   **7**   **8**

11 methine and six quaternary carbon signals. The HR-EIMS gave a molecular formula of C$_{39}$H$_{54}$O$_5$, in agreement with the NMR spectral data. Thus, **1** has an *O*-caffeoyl group, and a C-skeleton containing 30 carbons, and was deduced to be a lupene ester from the number of remaining carbons and the presence of six tertiary-linked methyl groups and a terminal methylene. The ^{13}C NMR spectral data (Table 1) of **1** were

similar to those of lup-20(29)-en-28-ol-3β-yl caffeate (**2**) and betulin (**3**) except for the chemical shifts of C-17 and C-28. To confirm the location of the *O*-caffeoyl and aldehyde groups, the 2D NMR spectra of **1** were obtained. In the ^{13}C–^{1}H long-range correlation spectrum, the proton signal at δ_H 4.51 (1H, br t, $J = 7.2$ Hz, C-3) showed long-range correlations with the carbon signals at δ_C 38.4 (C-1), 23.8 (C-2), 16.7 (C-24)

Table 1
^{13}C NMR spectral data for compounds **1–5**[a]

	1[b]	**2**	**3**	**4**[c]	**5**
1	38.4	38.4	38.9	38.3	38.1
2	23.8	23.8	24.6	23.7	23.6
3	81.4	81.3	80.8	80.4	78.8
4	38.1	38.1	38.6	38.1	38.2
5	55.4	55.5	56.0	55.5	55.1
6	18.2	18.2	18.8	18.4	18.5
7	34.3	34.2	34.9	33.3	33.2
8	40.9	41.0	41.4	39.8	39.7
9	50.4	50.3	51.0	47.7	47.6
10	37.1	37.1	37.6	37.1	37.2
11	28.8	27.0	30.5	24.1	24.1
12	25.5	25.2	26.3	123.3	122.4
13	38.8	37.3	38.9	143.4	144.8
14	42.6	42.7	43.2	41.9	42.1
15	20.8	20.9	21.5	27.0	28.2
16	29.2	29.2	33.1	22.3	23.8
17	59.4	47.8	56.9	49.2	46.4
18	48.1	48.8	50.5	40.7	41.9
19	47.6	47.8	48.1	45.7	46.6
20	149.6	150.4	151.6	30.7	31.0
21	29.9	29.7	31.5	28.1	34.2
22	33.3	34.0	37.9	32.9	33.0
23	28.0	28.0	28.4	28.2	28.2
24	16.7	16.7	16.6	17.3	17.4
25	16.2	16.2	17.2	15.4	15.4
26	15.9	16.0	16.7	17.2	17.1
27	14.3	14.8	15.2	25.7	26.2
28	207.1	60.7	179.2	207.4	180.2
29	110.3	109.8	110.3	33.1	33.3
30	19.0	19.1	19.8	23.5	23.8

[a] Compounds **1** and **2** were measured in CDCl$_3$, and compounds **3**, **4** and **5** were measured in C$_5$D$_5$N.

[b] Additional signals: 114.3 (C-4′), 115.4 (C-1′), 115.9 (C-8′), 122.3 (C-5′), 127.4 (C-6′), 144.1 (C-2′)q, 144.9 (C-7′), 146.6 (C-3′), 168.0 (C-9′).

[c] Additional signals: 115.5 (C-8′), 115.9 (C-1′), 116.8 (C-4′), 122.1 (C-5′), 126.9 (C-6′), 145.7 (C-7′), 147.7 (C-2′), 150.5 (C-3′), 167.4 (C-9′).

and 168.0 (C-9′), indicating that the *O*-caffeoyl group was located at C-3. Due to the correlation of the 3-H and 5-H shown in the NOESY spectrum, the relative stereochemistry of the *O*-caffeoyl group was determined to be 3β. The 18-H proton signal showed a long-range correlation with the carbon signals at δ_C 59.4 (C-17), 47.6 (C-19) and 207.1, and the aldehyde proton (δ_H 9.60) showed a long-range correlation with the carbon signals at δ_C 29.2 (C-16), 59.4 (C-17) and 48.1 (C-18). Thus, the aldehyde group was considered to be located at C-28. This assignment was in agreement with the NOESY spectrum, in which the 28-H was correlated with 13-H and 19-H. Thus, the structure of compound **1** was determined.

Compound **4**, showed a hydroxy (3425 cm^{-1}) and ketone bands (1713 and 1607 cm^{-1}) in its IR spectrum. Its UV spectrum showed absorption at 245, 301 and 327 nm. The HR-EIMS gave a molecular formula of

C$_{39}$H$_{54}$O$_5$. The ^{1}H NMR spectrum revealed the presence of seven tertiary methyls (δ_H 0.80, 0.92, 0.94, 0.96, 0.98 and 1.21 (each 3H, s)), an aldehyde (δ_H 9.60 (1H, s)) and an *O*-caffeoyl group, the same as that of **1**. The ^{13}C NMR spectrum of **4** showed a carbonyl carbon at δ_C 167.4, five double bond carbons at δ_C 115.5, 115.9, 116.8, 122.1, 123.3, 126.9, 143.4, 145.7, 147.7 and 150.5 and an aldehyde signal at δ_C 207.4. In addition, there are seven methyl, 10 methylene, 10 methine and five quaternary carbon signals. Thus, this compound has one *O*-caffeoyl group and its C-skeleton contains 30 carbons. The framework of this compound was deduced from the carbon number and the presence of seven tertiary-linked methyls and a double bond. The ^{13}C NMR spectrum data of **4** was similar to those of oleanolic acid 3β-caffeate (**5**). To confirm the position of the *O*-caffeoyl and aldehyde groups, the 2D NMR spectra were measured. In the HMBC spectrum, the proton signal at δ_H 4.85 (1H, dd, *J* = 4.9, 11.7 Hz, C-3) showed long-range correlations with the carbon signals at δ_C 38.3 (C-1), 23.7 (C-2), 38.1 (C-4), 17.3 (C-24) and 167.4 (C-9′); the aldehyde proton showed long-range correlations with the carbon signals at δ_C 22.3 (C-16), 49.2 (C-17) and 40.7 (C-18). Thus, the *O*-caffeoyl group was confirmed to be at C-3, and the aldehyde group was at C-28. Due to the correlation of the 3-H and 5-H shown in the NOESY spectrum, the relative stereochemistry of the *O*-caffeoyl group was indicated to be 3β. Therefore, the structure of **4** was determined to be as shown. The known triterpenoids **2**, **3** and **5** were identified by comparison of their physicochemical and spectral data with literature values (Patra, Chaudhuri, & Panda, 1988; Pan, Lundgren, & Andersson, 1994).

Celaphanol A (**6**) showed hydroxy (3413 cm^{-1}) and ketone (1702 cm^{-1} bands in its IR spectrum, and its UV spectrum showed aromatic absorption at 254, 279 and 308 nm. The ^{1}H NMR spectrum revealed the presence of three tertiary-linked methyl groups (δ_H 1.51, 1.55, 1.60 (each 3, s)) and two *para* protons on the benzene ring. The ^{13}C NMR spectrum of **6** showed a carbonyl carbon at δ_C 180.1 and four double bond carbons at δ_C 112.4, 113.0, 121.0, 140.2, 145.1, 146.3, 149.4 and 153.5. In addition, there are three methylene, two methine and two quaternary carbon signals. The HR-EIMS gave a molecular formula of C$_{17}$H$_{20}$O$_4$, and showed that the degree of unsaturation of **6** was eight with four degrees of the aromatic ring, one degree from the remaining double bond carbon and one degree from the carbonyl carbon; there are two further degrees, suggesting the presence of two more rings.

The nature of the aromatic ring (C-ring) was affirmed by the 2D NMR spectral data. From the ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY spectra, the carbon signals at δ_C 33.8, 17.8 and 38.1 could be assigned to C-

Table 2
^{13}C NMR spectral data for compounds 6–8[a]

	6	7	8
1	33.8	33.7	37.9
2	17.8	17.1	18.9
3	38.1	31.8	41.3
4	36.1	43.3	33.2
5	140.2	140.1	49.8
6	145.1	145.5	35.9
7	180.1	181.1	200.0
8	121.0	122.4	123.8
9	149.4	150.2	152.2
10	40.4	41.6	37.8
11	113.0	109.1	110.1
12	153.5	154.2	151.1
13	146.3	146.7	142.1
14	112.4	111.9	113.5
15	27.9	22.7	32.5
16	28.4	68.9	21.3
17	35.4	33.5	23.2
OCH$_3$	–	56.5	–

[a] Compound 6 was measured in C_5D_5N, 7 was measured in CD_3OD and 8 was measured in $CDCl_3$.

1, C-2 and C-3, respectively. In the HMBC spectrum, the proton of methyl (16-H) showed long-range correlations with the carbon signals at δ_C 38.1 (C-3), 36.1 (C-4), 140.2 (C-5) and 27.9 (C-15), and the protons of the methyl (17-H$_3$) showed long-range correlations with the carbon at δ_C 36.1 (C-4), 40.4 (C-10) and 140.2 (C-5). Thus the structure of ring A was formulated. Moreover, the 17-H$_3$ signal showed a long-range correlation with the carbon at δ_C 149.4 (C-9) and the 11-H on the C ring signal showed a long-range correlation with the carbon at δ_C 40.4 (C-10). On the other hand, the proton at δ_H 8.24 (1H, s) showed long-range correlations with the carbons at δ_C 121.0 (C-8), 149.4 (C-9), 153.5 (C-12) and 180.1 (C-7), suggesting that this proton was related to the carbonyl carbon. In this way, the structure of ring B was deduced to be as shown. In addition, the structure of this compound was further supported by the result of a NOESY experiment and was in accord with the result of HR-EIMS. The ^{13}C NMR spectral data (Table 2) of 6 were very similar to that of nimbidiol (8) (Majumder, Maiti, Kraus, & Bokel, 1987), except for the resonances of C-5, 6 and 7. It was assumed that compounds 6 and 8 have the same framework, and thus, the structure of 6 was determined to be as shown.

Celaphanol B (7) exhibited a molecular ion at m/z 318.1456 (HR-EIMS), indicating a molecular formula of $C_{18}H_{22}O_5$. It showed hydroxy (3426 cm^{-1}) and ketone (1719 cm^{-1}) bands in its IR spectrum. The ^{1}H NMR spectrum revealed the presence of two tertiary methyls (δ_H 1.46, 1.62 (each 3H, s)), a methoxy (δ_H 4.04 (3H, s)) and two *para* protons on an aromatic ring (δ_H 7.16, 7.51 (each 1H, s)). The ^{13}C NMR spec-

trum of 7 showed a carbonyl carbon and four double-bond carbons. In addition, there are four methylene, two methine and two quaternary carbon signals. The structure of 7 is very similar to that of 6, except for having a methoxy instead of a hydroxyl, and a hydroxy ethylene instead of a methyl. Thus 7 was deduced to be 12- or 13-methoxy-, 15- or 16-hydroxy celaphanol A (6). The methyl protons (17-H$_3$) showed long-range correlations with the carbons at δ_C 33.7 (C-1), 41.6 (C-10), 140.1 (C-5) and 150.2 (C-9), and the other methyl protons (15-H$_3$) showed long-range correlations with the carbons at δ_C 31.8 (C-3), 43.3 (C-4), 140.1 (C-5) and 68.9 (C-16). The 16-H showed long-range correlations with the carbons at δ_C 22.7 (C-15), 31.8 (C-3) and 43.3 (C-4), and the following HMBC cross peaks were also observed: C-10/11-H, C-12/11-H, C-13/11-H, C-8/11-H, C-7/14-H, C-9/14-H and C-12/14-H. Moreover, the proton of methoxy group (δ_H 4.04) showed a long-range correlation with the resonance at δ_C 154.2 (C-12). In the NOESY spectrum, the proton group at δ_H 7.16 (11-H) was correlated with 17-H and the methoxy group. Due to the NOE effects observed between 17-H and 16-H in the NOE experiment, the relative stereochemistry of the hydroxy-methylene group was determined to be 4β. Thus, the methoxy was attributed to C-12 and the hydroxy methyl to C-16, affording the structure of the isolate as 7.

3. Experimental

^{1}H NMR: 400 MHz with TMS as int.stand; ^{13}C NMR: 400 MHz; CC: silica gel 60 (Merck); Sephadex LH-20 (Pharmacia) and Toyo Pearl HW-40 (Tosho); HPLC: GPC H-2002 (Shodex); HPLC silica60 (YMC-Pack SIL-06).

3.1. Isolation of compounds 1–8

Stems of *C. stephanotifolius* were collected in October 1995 in Yamakawa, Tokushima Prefecture and dried. Herbarium specimens are deposited in the herbarium of University of Tokushima. The dried stems (8.2 kg) were extracted with MeOH at 60°C. The MeOH extracts were concd. in vacuo to give a residue, which was partitioned between EtOAc and H_2O. The EtOAc layer was concd. to give a residue (238 g), which was chromatographed on a silica gel column. The column was eluted with solvents of increasing polarity (hexane–EtOAc; CHCl$_3$–MeOH) to give 32 frs. Fr. 7 (2.2 g) was CC on silica gel with CHCl$_3$–MeOH to give 6 frs. (7.1–7.6). Fr. 7.3 (0.75 g) was chromatographed on Toyo Pearl HW-40 with CHCl$_3$–MeOH (2:1) to give 16.7 mg of 6. Fr. 7.3.3 and Fr. 7.3.4 were CC on silica gel with CHCl$_3$–

MeOH to give two frs (7.3.3.1–7.3.3.2). Fr. 7.3.3.1 (0.36 g) was sepd. by HPLC (GPC, CHCl$_3$; silica 60 Hex:EtOAc) to give 62.0 mg of **1**, 42.4 mg of **2** and 34.2 mg of **4**. Fr.9 (2.8 g) was CC on silica gel with CHCl$_3$–MeOH to give 13 frs. (9.1–9.13). Fr. 9.9 was CC on Sephadex LH-20 with MeOH to give six frs. (9.9.1–9.9.6). Fr. 9.9.5 (62.4 mg) was crystallized from MeOH to give 10.1 mg of **3**, the mother liquid was concd. in vacuo and sepd. by HPLC (GPC, MeOH) to give 12.2 mg of **3** and 12.5 mg of **6**. Fr. 9.9.4 was sepd. by GPC (MeOH) to give five frs. (9.9.4.1–9.9.4.5). Fr. 9.9.4.2 (33.3 mg) was sepd. by preparative TLC to give 8.9 mg of **8**. Fr. 10 and Fr. 11 were CC on silica gel with CHCl$_3$–MeOH to give 11 frs. (10.1–10.11). Fr. 10.7 were CC on Sephadex LH-20 with MeOH to give seven frs. (10.7.1–10.7.7). Fr. 10.7.5 (1.2 g) were crystallized from CHCl$_3$ to give 176 mg of **5**. Fr. 15 (5.9 g) was CC on silica gel with CHCl$_3$–MeOH to give 12 frs. (15.1–15.12). Fr. 15.6 (1.1 g) was CC on Toyo Pearl HW-40 with CHCl$_3$–MeOH (2:1) to give seven frs. (15.6.1–15.6.7). Fr. 15.6.6 was sepd. by HPLC (GPC, MeOH) to give 9.8 mg of **7**.

3.2. Compound **1**

Amorphous powder. $[\alpha]_D^{25}$ +48° (MeOH c 1.0). IR ν_{max}^{KBr} cm^{-1}: 3413, 1708, 1605, 1274, 1180, 979; UV λ nm (log ε): 216 (4.2), 242 (4.0), 298 (4.1), 328 (4.2); ^{1}H NMR δ (CDCl$_3$): 0.78, 0.79, 0.82, 0.83, 0.90, 1.63 (each 3H, s), 2.77 (1H, m, 19-H), 4.51 (1H, br t, J = 7.2 Hz, 3-H), 4.56 (1H, s, 30-H), 4.68 (1H, s, 30-H), 6.16 (1H, d, J = 15.8 Hz, 8′-H), 7.47 (1H, d, J = 15.8 Hz, 7′-H), 6.88 (1H, d, J = 8.1 Hz, 6′-H), 6.79 (1H, d, J = 8.1 Hz, 5′-H), 7.02 (qH, s, 2′-H), 9.60 (1H, s, 28-H); ^{13}C NMR (CDCl$_3$): Table 1; EI-MS m/z (rel. int.): 602 [M]$^+$ (14), 190 (33), 189 (54), 180 (27), 163 (100), 135 (26), 121 (24), 107 (25), 95 (26), 81 (24), 69 (24); HR-MS m/z: 602.3987 [M]$^+$ C$_{39}$H$_{54}$O$_5$ requires 602.3971.

3.3. Compound **4**

Brown amorphous powder. $[\alpha]_D^{25}$ +25° (MeOH c 1.0). IR ν_{max}^{KBr} cm^{-1}: 3425, 2926, 1713, 1607, 1262, 1170; UV λ nm (log ε): 245 (4.0), 301 (4.0), 327 (4.1); ^{1}H NMR δ (C$_5$D$_5$N): 0.80, 0.92, 0.94, 0.96, 0.98, 1.21 (each 3H, s), 2.78 (1H, br d, J = 9.8 Hz), 4.85 (1H, dd, J = 4.9, 11.7 Hz), 5.38 (1H, s), 6.69 (d, J = 16.1 Hz), 7.22 (d, J = 7.8 Hz), 7.32 (dd, J = 1.9, 7.8 Hz), 7.73 (d, J = 1.9 Hz), 8.03 (d, J = 16.1 Hz), 9.60 (1H, s); ^{13}C NMR (C$_5$D$_5$N$_5$): Table 2; HR-MS m/z: 602.3948 [M]$^+$ C$_{39}$H$_{54}$O$_5$ requires 602.3971.

3.4. Celaphanol A (**6**)

Red amorphous powder. $[\alpha]_D^{25}$ +13° (CDCl$_3$ c 0.9). IR ν_{max}^{KBr} cm^{-1}: 3413, 1702, 1655, 1619, 1297, 1024, 670; UV λ nm (log ε): 254 (3.7), 279 (3.7), 308 (3.7); ^{1}H NMR δ (C$_5$D$_5$N): 1.36 (1H, m), 1.51, 1.55, 1.60 (each 3H, s), 1.59 (1H, m), 1.75 (1H, m), 1.76 (1H, m), 1.89 (1H, m), 2.30 (1H, m), 7.41 (1H, s), 8.24 (1H, s); ^{13}C NMR (C$_5$D$_5$N): Table 2; EI-MS m/z (rel. int.): 288 [M]$^+$ (91), 273 (13), 246 (9), 245 (34), 232 (11), 229 (14); HR-MS m/z: 288.1363 [M]$^+$ C$_{17}$H$_{20}$O$_4$ requires 288.1362.

3.5. Celaphanol B (**7**)

Yellow amorphous powder. $[\alpha]_D^{25}$ +15° (MeOH c 0.6). IR ν_{max}^{KBr} cm^{-1}: 3856, 3651, 3426, 1719, 1610, 1544, 1510, 1459, 1301, 1030; UV λ nm (log ε): 217 (3.9), 245 (3.8), 280 (4.08), 310 (3.8), 339 (3.7); ^{1}H NMR δ (CD$_3$OD): 1.46, 1.62 (each 3H, s), 1.47, 1.81, 1.95, 2.10, 2.48, 2.57 (each 1H, m), 3.72 (1H, d, J = 10.8 Hz), 4.04 (3H, s), 4.24 (1H, d, J = 10.8 Hz), 7.16 (1H, s), 7.51 (1H, s); ^{13}C NMR (CD$_3$OD): Table 2; HR-MS m/z: 318.1456 [M]$^+$ C$_{18}$H$_{22}$O$_5$ requires 318.1467.

References

Bruning, R., & Wagner, H. (1978). *Phytochemistry*, 17, 1821.

Geutaharn, A., & Krikorian, A. D. (1973). *Economic Botany*, 27, 353.

Majumder, P. L., Maiti, D. C., Kraus, W., & Bokel, M. (1987). *Phytochemistry*, 26, 3021.

Pan, H., Lundgren, L. N., & Andersson, R. (1994). *Phytochemistry*, 37, 795.

Patra, A., Chaudhuri, S. K., & Panda, S. K. (1988). *Journal of Natural Products*, 51, 217.

Shizuri, Y., Wada, H., Sugiura, K., Yamada, K., & Hirata, Y. (1973). *Tetrahedron*, 29, 1173.

Takaishi, Y., Oshima, S., Nakano, K., & Tomimatsu, T. (1993). *Journal of Natural Products*, 56, 815.

Tu, Y. Q. (1991). *Journal of the Chemical Society, Perkin Transactions I*, 425.

Tu, Y. Q. (1990). *Journal of Natural Products*, 53, 915.