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Furanoflavonoids from *Pongamia pinnata* fruits☆

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Abstract

Fruits of *Pongamia pinnata* afforded four new furanoflavonoids, pongapinnol A–D (**1**–**4**), and a new coumestan, pongacoumestan (**5**) along with thirteen known compounds **6**–**18**. Compounds **16** and **17** are isolated for the first time from this plant. The structures of isolated compounds were elucidated on the basis of spectroscopic data interpretation.

Keywords: *Pongamia pinnata*; Leguminosae; Furanoflavones; Furanoflavonols; Coumestan

1. Introduction

Pongamia pinnata Pierre (Leguminosae) is a medicinal plant native to Western Ghats and chiefly found in tidal forests of India (Krishnamurthi, 1969). Different parts of the plant have been used in traditional system of medicine for bronchitis, whooping cough, rheumatic joints and to quench dipsia in diabetes (Kirtikar and Basu, 1995). Previous phytochemical examination of this plant indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, flavones, and furanodiketones (Talapatra et al., 1980; 1982a, Murty and Seshadri, 1944; Rangaswami et al., 1942; Sharma et al., 1973; Pathak et al., 1983; Toshiyuki et al., 1992). In the present communication, we describe the isolation and characterization of four new furanoflavonoids named pongapinnols A–D (**1**–**4**) and a new coumestan named pongacoumestan (**5**).

2. Results and discussion

The $CHCl_3$ soluble fraction of the EtOH extract of *Pongamia pinnata* fruits afforded five new compounds **1**–**5** together with 13 known compounds **6**–**18**, after repeated CC purifications (Fig. 1).

The molecular formula of pongapinnol-A (**1**) was determined as $C_{19}H_{14}O_6$ by FAB-MS (*m*/*z* 339 $[M+H]^+$); this was confirmed by elemental analysis and NMR spectra. The UV spectrum of compound **1** showed the absorption maxima (λ_{max} 309, 248 nm), and positive Shinoda test that are characteristic of a flavonoid nucleus (Mabry et al., 1970). ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed the presence of characteristic signals for furan ring as δ_H/δ_C at δ 8.27 (*d*, $J=2.1$ Hz, H-2″)/δ 147.5 (C-2″) and at δ 7.43 (*br d*, $J=1.8$ Hz, H-3″)/δ 107.7 (C-3″). In ^1H-^1H COSY the proton at C-3″ showed a long range correlation with the H-6 proton at δ 7.78, indicating that the anellation of the furan ring was at the C-7/C-8 positions. Three *meta*-coupled aromatic protons at δ 7.18 (*br t*, $J=1.5$ Hz, H-2′), δ 7.13 (*br t*, $J=1.8$ Hz, H-6′) and δ 6.56 (*t*, $J=2.1$ Hz, H-4′) indicated a ring-B, disubstituted at the C-3′ and C-5′ positions. The location of the two methoxyl groups at C-3 (δ 3.85) and C-5′ (δ 3.82) and that of hydroxy group at C-3′ (δ 3.88) were established from nOe (Fig. 2); irradiation of the C-3 methoxyl group signal at δ 3.85 resulted in an enhancement of the H-2′ and H-6′ signals, observed due to free rotation of phenyl at C-2 position; while irradiation of the C-5′ methoxyl group signal at δ 3.82 showed an enhancement of the H-2′ and H-4′ signals. This confirmed the structure of **1** as 3′-hydroxy,3,5′-dimethoxy furo [8,7:4″,5″] flavone; a new naturally occurring furanoflavonoid designated with the common name pongapinnol-A.

Pongapinnol-B (**2**) was assigned molecular formula $C_{20}H_{16}O_6$ based on FAB-MS (*m*/*z* 353 $[M+H]^+$);

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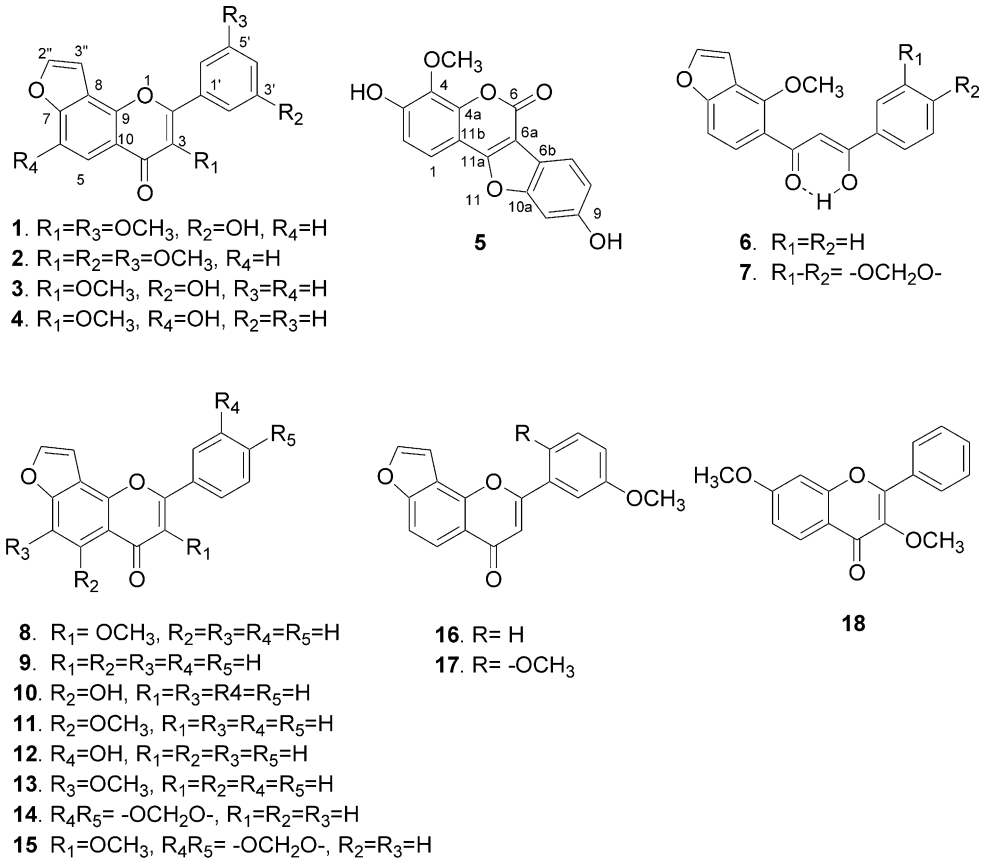

Fig. 1. Structure of compounds isolated.

Table 1
^{1}H NMR spectra of compounds **1**–**4**

Proton No.	**1**	**2**	**3**	**4**
	DMSO-d_6, δ_H (J in Hz)	$CDCl_3$, δ_H (J in Hz)	DMSO-d_6, δ_H (J in Hz)	DMSO-d_6, δ_H (J in Hz)
5	8.01 (*d*, J = 8.7 Hz)	8.19 (*d*, J = 8.8 Hz)	7.94 (*d*, J = 8.8 Hz)	7.46 (*s*)
6	7.78 (*d*, J = 8.7 Hz)	7.55 (d, J = 8.8 Hz)	7.65 (*d*, J = 8.8 Hz)	–
2′	7.18 (*br t*, J = 1.5 Hz)	7.32 (*d*, J = 2.2 Hz)	7.53 (*m*)	8.21–8.26 (*m*)
3′	–	–	–	7.70–7.74 (*m*)
4′	6.56 (*t*, J = 2.1 Hz)	6.63 (*t*, J = 2.2 Hz)	6.98 (*dd*, J = 8.1 and 1.0 Hz)	7.70–7.74 (*m*)
5′	–	–	7.41 (*t*, 8.1 Hz)	7.70–7.74 (*m*)
6′	7.13 (*br t*, J = 1.8 Hz)	7.32 (*d*, J = 2.2 Hz)	7.53(*m*)	8.21–8.26 (*m*)
2″	8.27 (*d*, J = 2.1 Hz)	7.76 (*d*, J = 2.1 Hz)	8.13 (*d*, J = 1.9 Hz)	8.31 (*d*, J = 2.0 Hz)
3″	7.43 (*br d*, J = 1.8 Hz)	7.17 (*br d*, J = 1.2 Hz)	7.32 (*br d*, J = 1.3 Hz)	7.57 (*d*, J = 2.0 Hz)
3-OCH_3	3.85 (*s*)	3.93 (*s*)	3.85 (*s*)	3.84 (*s*)
3′-OCH_3	–	3.89 (*s*)	–	–
5′-OCH_3	3.82 (*s*)	3.89 (*s*)	–	–
6-OCH_3	–	–	–	–
3′-OH	9.88 (*s*)	–	10.0 (br s)	–
6-OH	–	–	–	10.7 (*br s*)

elemental analysis and NMR spectra. Compound **2** was also characterised as furanoflavonoid with anellated furan ring at C-7/C-8 positions, as described for **1**. NMR spectrum of **2** (Tables 1 and 2) showed it to be the 3′-OMe derivative of pongapinnol-A (**1**). Apart from the results discussed for compound 1, ^{1}H NMR spectrum showed the presence of two identical methoxyl groups at δ 3.89 (6H, *s*) and two symmetrical protons at δ 7.32 (2H, *d*, J = 2.2 Hz, H-2′ and H-6′), *meta*-coupled to H-4′ at δ 6.63 (1H, *t*, J = 2.2 Hz) indicated presence of 3′, 5′-disubstituted B-ring as supported from ^{1}H–^{1}H COSY experiment. Thus cumulative experimental results showed **2** as 3,3′,5′-trimethoxy furo [8,7:4″,5″] flavone designated with common name pongapinnol-B.

Table 2
^{13}C NMR spectra of compounds **1**–**4**

Carbon	**1**	**2**	**3**	**4**
	DMSO-d_6, δ_C	$CDCl_3$, δ_C	DMSO-d_6, δ_C	DMSO-d_6, δ_C
2	157.4	158.5	157.7	152.3
3	141.1	142.3	141.3	140.0
4	173.7	175.3	174.2	172.0
5	121.0	122.2	130.2	100.9
6	110.2	110.3	110.4	140.1
7	154.2	154.9	154.6	141.6
8	116.7	117.3	119.2	118.4
9	149.1	150.2	149.4	145.9
10	119.0	120.0	117.0	117.1
1′	131.8	132.9	131.6	139.0
2′	104.7	107.0	121.3	127.2
3′	160.4	161.1	119.3	126.5
4′	103.5	103.0	118.3	129.1
5′	158.6	161.1	157.6	126.5
6′	107.7	107.0	115.1	127.2
2″	147.5	146.1	147.5	145.6
3″	104.3	104.6	104.4	103.3
3-OCH_3	59.7	60.6	60.0	60.0
3′-OCH_3	–	55.9	–	–
5′-OCH_3	55.1	55.9	–	–

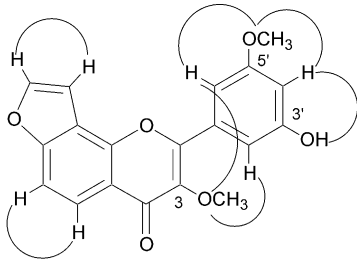

Fig. 2. nOe correlation of **1**.

The molecular formula of pongapinnol-C (**3**) was determined as $C_{18}H_{12}O_5$ by its FAB-MS (*m*/*z* 309 $[M+H]^+$), elemental analysis and NMR spectra (Tables 1 and 2). Nature of ring-A and its anellation pattern was similar to that of 1. Ring-B was mono-substituted at C-3′ as indicated by a triplet at δ 7.41 (1H, $J=8.1$ Hz, H-5′), a double doublet at δ 6.98 (1H, $J=8.1$, 1.0 Hz, H-4′) and a broad multiplet at δ 7.53 (2H, H-2′ and 6′), confirmed by ^1H–^1H COSY experiment. UV spectrum of **3** showed no bathochromic shift on addition of $AlCl_3$, indicating presence of methoxyl group at C-3 (δ 3.85, 3H, *s*) and free hydroxyl group at C-3′ (δ 10.0, 1H, *br s*, exchangeable with D_2O). Thus compound **3** was characterized as 3′-hydroxy,3-methoxy furo[8,7:4″,5″] flavone, a new furanoflavonoid which is 3′-demethyl derivative of previously isolated pachycarin D (Weiyan et al., 2001).

The molecular formula for pongapinnol-D **4** was determined as $C_{18}H_{12}O_5$ by FAB-MS (*m*/*z* 309 $[M+H]^+$), elemental analysis and NMR spectra (Tables 1 and 2). This compound showed characteristic

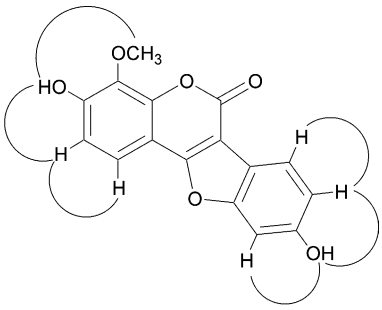

Fig. 3. nOe correlation of **5**.

signals for C-7/C-8 anellated furanoflavonoid nucleus as discussed for **1**. Absence of long-range correlation of H-3″ with H-6 in ^1H–^1H COSY experiment and appearance of a sharp singlet assigned for H-5 (δ 7.46) indicated a substitution at C-6 position. Unsubstituted ring-B was supported by ^1H NMR signals at δ 7.70–7.74 (3H, *m*, H-3′, 4′, 5′) and δ 8.21–8.26 (2H, *m*, H-2′, 6′). Positions of methoxyl group at C-3 (δ 3.84) and D_2O exchangeable hydroxyl group at C-6 (δ 10.7, 1H, *br s*) were established from nOe and UV experiments. Irradiation of the methoxyl group signal (δ 3.84) resulted in enhancement of signals for H-2′ and H-6′ (δ 8.21–8.26); while irradiation of the hydroxyl group signal (δ 10.7) resulted in enhancement of signals for H-5 (δ 7.46). Thus on the basis of above spectral analysis compound **4** was established as 6-hydroxy,3-methoxy furo [8,7:4″,5″] flavone, a new naturally occurring furanoflavonoid assigned the name pongapinnol-D. Literature survey showed that **4** was 6-methyl ether of previously reported compound (Kamperdick et al., 1998).

The molecular formula of coumestan (**5**), designated common name pongacoumestan, was determined by FAB-MS (*m*/*z* 299 $[M+H]^+$), elemental analysis and NMR spectra. It showed UV pattern (λ_{max} 343, 302, 253 nm) characteristic of coumestan chromophore (Dewick, 1983) and strong IR absorption band at 1709 cm^{-1}, indicating presence of unsaturated lactone ring. The ^1H NMR spectrum displayed an ABX system; most downfield doublet at δ 7.83 ($J=8.6$ Hz) was assigned to H-7 which was coupled to double doublet at δ 6.93 ($J=8.6$ Hz, 2.0 Hz, H-8) and further H-8 showed *meta*-coupling with H-10 at δ 6.99 (*d*, $J=2.0$ Hz). UV spectrum of **5** showed no bathochromic shift on addition of shift reagents ($AlCl_3$, H_3BO_3), thus indicating absence of *ortho*-dihydroxy system. Presence of two hydroxyl groups at C-3 (δ 9.91) and C-9 (δ 8.12), and that of methoxyl group at C-4 (δ 4.24) was confirmed by nOe. (Fig. 3). Irradiation of C-3 hydroxyl showed enhancement of signal for H-2 (δ 7.03) and methoxyl signal at δ 4.24, while irradiation of C-9 hydroxyl showed enhancement of signal for H-8 (δ 6.93) and H-10 (δ 6.99). Thus based on these observations, structure of 5 was characterized as 3,9-dihydroxy-4-methoxy-benzo[4,5] furo[3,2-c] chromen-6-one. Sativol isolated from *Alfalfa*

plant (Spencer et al., 1966) has the same basic nucleus and oxidation pattern, but sativol has methoxyl group at C-3 whereas compound **5** has methoxyl at C-4 position. This is the first reported isolation of pongacoumestan (**5**).

Additionally 13 known flavonoids 1-(4-methoxy-5-benzofuranyl)-3-phenyl-1,3-propanodione (**6**) (Paramar et al., 1989), 1-(4-methoxy-5-benzofuranyl)-3-(3′,4′-methylenedioxy-phenyl)-1,3-propanedione (**7**) (Garg et al., 1978), 3-methoxy furo[8,7:4″,5″] flavone (**8**) (Manjunath et al., 1939), furo[8,7:4″,5″] flavone (**9**) (Talapatra et al., 1982b), 5′-hydroxy furo[8,7:4″,5″] flavone (**10**), 5-methoxy furo[8,7:4″,5″] flavone (**11**), (Talapatra et al., 1980), 3′-hydroxy furo[8,7:4″,5″] flavone (**12**) (Roy and Khanna, 1979), 6′-methoxy furo[8,7:4″,5″] flavone (**13**) (Talapatra et al., 1982b), 3′,4′-methylenedioxy furo[8,7:4″,5″] flavone (**14**) (Khanna and Seshadri, 1963), 3-methoxy,3′,4′-methylenedioxy furo[8,7:4″,5″] flavone (**15**) (Mahey et al., 1972), 3′-methoxy furo[8,7:4″,5″] flavone (**16**) (Sritularak et al., 2002), 2′,5′-dimethoxy furo[8,7:4″,5″] flavone (**17**) (Sritularak et al., 2002), and 3,7-dimethoxy,2(3′,4′-methylenedioxy-phenyl)-chromen-4-one (**18**) (Mittal and Seshadri, 1956), were isolated from different parts of this plant and their structures elucidated by comparing their spectroscopic data with those reported in the literature.

3. Experimental

3.1. General

Melting points were recorded on a Complab melting point apparatus and are uncorrected. IR spectra (KBr) were recorded on a Perkin-Elmer RX-1 spectrophotometer. UV spectra were obtained on a Perkin-Elmer λ-15 UV spectrophotometer. NMR spectra were run on an AVANCE DPX 200 and Brüker DRX 300 spectrometer, FAB-MS were carried out on Jeol SX 102/DA-6000 mass spectrometer. Elemental analyses were obtained in a Carlo-Erba-1108 CHN elemental analyzer. Column chromatography was performed using flash silica gel (230–400 mesh).

3.2. Plant material

The fruits of *Pongamia pinnata* were collected from Lucknow in the month of May 2000, and identified by Botany Division of Central Drug Research Institute. Voucher specimen (No. 6331) is kept in the herbarium of the institute.

3.3. Extraction and isolation

Air dried and powdered fruits of *Pongamia pinnata* (6 kg) were extracted at room temperature with EtOH. The EtOH extract (750 g) was then fractionated successively into four fractions: *n*-hexane (360 g), $CHCl_3$ (70 g), *n*-BuOH (50 g), and aqueous (240 g). The $CHCl_3$ fraction was subjected to column chromatography over flash silica gel (230–400 mesh), eluting with a gradient of C_6H_6–EtOAc (1:0 to 1:1) to afford 60 fractions. These fractions were pooled into 9 fractions (F-1 to F-9) according to their similarity on TLC. Flash CC of F-1 using hexane–EtOAc (98:2) afforded **6** (300 mg), **7** (20 mg), and **10** (15 mg); similar purification of F-2 using C_6H_6 as eluent yielded **8** (5 g) and **15** (200 mg), while a CC of F-3, eluting with gradient of C_6H_6–EtOAc, afforded **18** (100 mg), **9** (80 mg), and **1** (15 mg). CC purification of F-4, performed with gradient of C_6H_6–EtOAc, afforded **16** (120 mg) and **14** (50 mg); CC of F-5 with C_6H_6–EtOAc (95:5) afforded **13** (30 mg). Fraction F-6 was purified by CC using a gradient elution with C_6H_6–EtOAc to give **3** (25 mg). Successive purification of F-7 using gradient of C_6H_6–EtOAc, yielded **2** (6 mg), **4** (20 mg), **5** (8 mg), and **17** (8 mg). Finally CC of F-8 using C_6H_6–EtOAc (9:1) eluted **12** (50 mg) and CC of F-9 with C_6H_6–EtOAc (9:1) afforded **11** (20 mg).

3.4. 3′-Hydroxy,3,5′-dimethoxy furo[8,7:4″,5″] flavone (*1*)

White crystals from MeOH; mp 230 °C; UV (MeOH) λ_{max} nm: 309, 248; IR ν_{max} (KBr) cm^{-1}: 2928, 1620, 1581, 1383, 1216, 1058, 764, 675. ^1H NMR (DMSO-d_6, 300 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 50.32 MHz) see Table 2; FAB MS (pos.): m/z 339 $[M+H]^+$, 273; Elemental analysis: calc. for $C_{19}H_{14}O_6$: C, 67.45%, H; 4.17%. Found: C, 67.52%, H, 4.03%.

3.5. 3,3′,5′-Trimethoxy furo[8,7:4″,5″] flavone (*2*)

White crystals from MeOH; mp 184 °C; UV (MeOH) λ_{max} nm: 310, 241; IR ν_{max} (KBr) cm^{-1}: 2938, 2839, 1618, 1461, 1366, 1210, 1158, 1055. ^1H NMR ($CDCl_3$, 200 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 50.32 MHz) see Table 2; FAB MS (pos.): m/z 353 $[M+H]^+$, 705 $[2M+H]^+$; Elemental analysis: calc. for $C_{20}H_{16}O_6$: C, 68.17%, H; 4.57%. Found: C, 67.98%, H, 4.68%.

3.6. 3′-Hydroxy,3-methoxy furo[8,7:4″,5″] flavone (*3*)

White crystals from DMSO; mp 188 °C; UV (MeOH) λ_{max} nm: 309, 250; (+$AlCl_3$): no change; (+NaOMe): 313, 248; IR ν_{max} (KBr) cm^{-1}: 3406, 2831, 1630, 1476, 1362, 1197, 1074, 1029; ^1H NMR (DMSO-d_6, 200 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 50.32 MHz) see Table 2; FAB MS (pos.): m/z 309 $[M+H]^+$, 617 $[2M+H]^+$; Elemental analysis: calc. for $C_{18}H_{12}O_5$: C, 70.12%, H; 3.92%. Found: C, 69.95%, H, 3.81%.

3.7. 6-Hydroxy,3-methoxy furo[8,7:4″,5″] flavone (4)

Yellow crystals from DMSO; mp 283 °C; UV (MeOH) λ_{max} nm: 309,272; (+$AlCl_3$): no change; (+NaOMe): 390, 282; IR ν_{max} (KBr) cm^{-1}: 3271, 2830, 1628, 1593, 1478, 1359, 1175, 1035. ^1H NMR (DMSO-d_6, 200 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 50.32 MHz) see Table 2; FAB MS (pos.): m/z 309 $[M+H]^+$, 617 $[2M+H]^+$; Elemental analysis: calc. for $C_{18}H_{12}O_5$: C, 70.12%, H; 3.92%. Found: C, 69.94%, H, 3.85%.

3.8. 3, 9-Dihydroxy-4-methoxy-benzo[4,5] furo[3,2-c] chromen-6-one (5)

Brown amorphous solid; UV (MeOH) λ_{max} nm: 343, 302, 253; (+$AlCl_3$): no change; (+ H_3BO_3): no change; IR ν_{max} (KBr) cm^{-1}: 3379, 2934, 1709, 1625, 1506, 1435, 1345, 1299, 1244, 1169, 1049, 984, 806, 610. ^1H NMR ($CDCl_3$+DMSO-d_6, 9:1, 200 MHz): δ 9.91 (1H, *s*, 3-OH), 8.12 (1H, s, 9-OH), 7.83 (1H, d, $J=8.6$ Hz, H-7), 7.55 (1H, d, $J=8.3$ Hz, H-1), 7.03 (1H, d, $J=8.3$ Hz, H-2), 6.99 (1H, br d, $J=2.0$ Hz, H-10), 6.93 (1H, dd, $J=8.6$ Hz, 2.0 Hz, H-8), 4.24 (3H, s, 4-OCH_3); ^{13}C NMR (DMSO-d_6, 50.32 MHz): δ 123.2 (C-1), 114.4 (C-2), 147.7 (C-3), 148.7 (C-4), 133.4 (C-4a), 161.8 (C-6), 104.3 (C-6a), 116.7 (C-6b), 115.6 (C-7), 114.1 (C-8), 160.1 (C-9), 103.3 (C-10), 158.3 (C-10a), 155 (C-11a), 102.5 (C-11b), 60.9 (−OCH_3) FAB MS (pos.): m/z 299 $[M+H]^+$, 597 $[2M+H]^+$; Elemental analysis: calc. for $C_{18}H_{10}O_6$: C, 64.43%, H; 3.37%. Found: C, 64.35%, H, 3.49%.

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