Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1045–1049

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Anastatins A and B, New Skeletal Flavonoids with Hepatoprotective Activities from the Desert Plant *Anastatica hierochuntica*

Masayuki Yoshikawa,* Fengming Xu, Toshio Morikawa, Kiyofumi Ninomiya and Hisashi Matsuda

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan

Received 2 December 2002; accepted 16 January 2003

Abstract—New skeletal flavonoids, anastatins A and B, were isolated from the methanolic extract of an Egyptian medicinal herb, the whole plants of *Anastatica hierochuntica*. Their flavanone structures having a benzofuran moiety were determined on the basis of chemical and physicochemical evidence. Anastatins A and B were found to show hepatoprotective effects on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes and their activities were stronger than those of related flavonoids and commercial silybin.

Introduction

The whole plants of *Anastatica hierochuntica* (Cruciferae), which is a winter annual plant of the Sahara-Arabian deserts, are prescribed in Egyptian folk medicine for fatigue and uterine haemorrhage and are used by women as a charm for child birth.[1] In the course of our characterization studies on bioactive constituents from Egyptian medicinal herbs,[2] the methanolic extract from the whole plants of *A. hierochuntica* was found to show potent hepatoprotective effect on D-galactosamine (D-GalN)-induced cytotoxicity in primary cultured mouse hepatocytes.[3] By bioassay-guided separation, two novel skeletal flavanones, anastatins A (**1**) and B (**2**), were isolated from the ethyl acetate (EtOAc)-soluble fraction with the hepatoprotective effect together with seven flavonoids, 11 aromatic compounds, three phenylpropanoids, 12 lignans, and four flavonolignans. This communication deals with the absolute stereostructure elucidation of anastatins (**1**, **2**) as well as the hepatoprotective activities of anastatins (**1**, **2**) and the principal constituents on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes.

Isolation of Anastatins A (1) and B (2) from *A. hierochuntica*

The whole plants of *A. hierochuntica* (3.5 kg, collected in Egypt) were extracted with methanol three times under reflux for 3 h. The methanolic extract (5.2% from this natural medicine) was partitioned into an EtOAc and water mixture to give an EtOAc-soluble fraction (2.4%) and an aqueous phase. The aqueous phase was further extracted with *n*-butanol (*n*-BuOH) to give an *n*-BuOH-soluble fraction (0.6%) and an H_2O-soluble fraction (2.2%). As shown in Table 1, the methanolic extract and the EtOAc-soluble fraction showed inhibitory effects on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes. The EtOAc-soluble fraction was subjected to ordinary-phase silica-gel (SiO_2) [*n*-hexane–EtOAc (20:1 → 10:1 → 5:1 → 2:1 → 1:1) → $CHCl_3$–MeOH–H_2O (10:3:1, lower layer) → MeOH] and reversed-phase silica-gel (ODS) column chromatography [MeOH–H_2O], and finally HPLC [YMC-Pack ODS-5-A, 250×20 mm i.d., MeOH–H_2O or CH_3CN–H_2O] to give anastatins A (**1**, 0.0010% from the natural medicine) and B (**2**, 0.00098%) together with seven flavonoids, naringenin (**3**,[4] 0.0038%), eriodictyol (**4**,[5] 0.0027%), aromadendrin (**5**,[6] 0.00081%), (+)-taxifolin (**6**,[7] 0.044%), 3′-*O*-methyltaxifolin (**7**,[8] 0.00038%), (+)-epitaxifolin (**8**,[7] 0.0035%), and quercetin[9] (0.0010%), 11 aromatic compounds, three phenylpropanoids, 12 lignans, and four flavonolignans (Chart 1).[10]

*Corresponding author. Tel.: +81-75-595-4633; fax: +81-75-595-4768; e-mail: shoyaku@mb.kyoto-phu.ac.jp

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00088-X

Table 1. Inhibitory effects of the MeOH extract and EtOAc-soluble fraction from *A. hierochuntica* on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes

	Inhibition (%)				
	0 μg/mL	3 μg/mL	10 μg/mL	30 μg/mL	100 μg/mL
MeOH extract	0.0±3.4	20.0±1.7**	39.2±4.5**	52.2±2.5**	68.7±4.3**
EtOAc-soluble fraction	0.0±1.5	33.4±1.5**	33.8±4.9**	48.1±2.5**	80.9±1.1**

Each value represents the mean±SEM ($N=4$). Significantly different from the control, $^{**}p<0.01$.

Table 2. ^{13}C NMR data of anastatins A (**1**) and B (**2**) and their acetates (**1a**, **2a**)

	1[a]	**1a**[b]	**2**[a]	**2a**[b]
C-2	80.4	79.2	80.8	79.9
C-3	43.9	45.3	43.8	45.6
C-4	199.6	189.7	198.5	188.5
C-5	158.4	145.5	162.5	150.4
C-6	108.0	113.0	92.9	101.8
C-7	163.6	161.6	163.8	160.8
C-8	92.4	98.8	106.8	111.2
C-9	161.6	162.3	157.3	158.0
C-10	104.9	110.5	104.9	109.7
C-1′	130.7	135.7	130.6	135.8
C-2′	129.1	127.4	129.1	127.3
C-3′	116.2	122.1	116.4	122.3
C-4′	158.8	151.0	158.9	151.0
C-5′	116.2	122.1	116.4	122.3
C-6′	129.1	127.4	129.1	127.3
C-1″	114.9	120.0	114.8	120.4
C-2″	150.9	153.6	150.9	153.3
C-3″	99.2	107.2	99.3	107.1
C-4″	143.3	141.6	145.9	141.4
C-5″	145.9	138.9	143.3	139.0
C-6″	107.8	115.8	107.6	116.4
*C*H$_3$CO–		20.6		20.7
		20.7		20.7
		21.1		21.1
		21.2		21.2
CH$_3$*C*O–		168.1		168.1
		168.4		168.6
		168.5		169.4
		169.3		169.7

[a]Measured in acetone-d_6 at 125 MHz.
[b]Measured in $CDCl_3$ at 125 MHz.

Absolute Stereostructures of Anastatins A (1) and B (2)

Anastatin A (**1**) was isolated as a yellow powder with positive optical rotation {$[\alpha]_D^{24}$ +121.3° (*c* 0.63, MeOH)}. The electron impact (EI)-MS of **1** showed molecular ion peak at *m/z* (%) 378 (72) and the molecular formula $C_{21}H_{14}O_7$ of **1** was determined by high resolution MS measurement [calcd for $C_{21}H_{14}O_7$ (M^+): 378.0739. Found: 378.0741]. The IR spectrum of **1** showed absorption bands at 3677, 3432, 3282, 1655, 1647, 1569, 1509, 1458, 1154, 1088, and 831 cm^{-1} ascribable to hydroxyl, carbonyl, chelated carbonyl, aromatic ring, and ether functions. In the UV spectrum of **1** (measured in MeOH), absorption maxima were observed at 247 (log ε 4.1), 268 (4.3), 297 (4.2), and 371 (3.3) nm, suggestive of the flavanone structure.[5b] The ^{1}H NMR (acetone-d_6) and ^{13}C NMR (Table 2) spectra of **1** showed signals assignable to a dihydropyron moiety in the flavanone structure by a characteristic ABX type coupling pattern {[δ 2.87 (1H, dd, $J=2.7$, 17.1 Hz), 3.34 (1H, dd, $J=13.1$, 17.1 Hz), 3-H$_2$], 5.56 (1H, dd, $J=2.7$, 13.1 Hz, 2-H)}, three singlet aromatic protons [δ 6.63 (1H, s, 8-H), 7.07, 7.46 (1H each, both s, 3″, 6″-H)], *ortho*-coupled A_2B_2-type aromatic protons [δ 6.92, 7.44 (2H each, both d, $J=8.6$ Hz, 3′, 5′-H and 2′, 6′-H)], and a chelated hydroxyl proton [δ 12.93 (1H, br s, 5-OH)]. Acetylation of **1** with acetic anhydride–pyridine gave the tetraacetate (**1a**).[11] The proton and carbon signals due to the flavanone structure in the ^{1}H and ^{13}C NMR spectra of **1** were similar to those of naringenin (**3**), except for the signals assignable to the benzofuran moiety. The new skeletal flavanone structure of **1** with a benzofuran moiety at the 6- and 7-positions was characterized by the heteronuclear multiple bond connectivity (HMBC) experiment on **1** and **1a**, which showed long-range correlations between the 2-proton and 4, 1′, 2′ (6′)-carbons, between the 5-hydroxyl proton and 5, 6, 10-carbons, between the 8-proton and 6, 7, 9, 10-carbons, between the 3″-proton and 1″, 2″, 4″, 5″-carbons, and between the 6″-proton and 6, 1″, 2″, 4″, 5″-carbons (Fig. 1).[12] Furthermore, the new flavanone structure of **1** with a benzofuran ring was confirmed by EI-MS fragmentation pattern of **1** and **1a**. Namely, the EI-MS of **1** exhibited characteristic fragment ion peaks (**i**, **ii**) at *m/z* (%) 258 (100) and 120 (3), which were derived by retro Diels–Alder type cleavage of the dihydropyron ring, together with fragment ion peaks (**iii**, **iv**, **v**) at *m/z* (%) 230 (4), 202 (9), and 147 (1) (Fig. 2). Finally, the circular dichroic (CD) spectrum of **1** showed negative Cotton effects [MeOH, λ_{max} 287 nm ($\Delta\varepsilon=-0.32$)], which indicated the absolute configuration of the 2-position to be *S*.[5b, 13] On the basis of this evidence and comparison of the physicochemical data for **1** with those for **1a**, the absolute stereostructure of anastatin A (**1**) was elucidated as shown.

Anastatin B (**2**) was also obtained as a yellow powder with positive optical rotation {$[\alpha]_D^{24}$ +149.0° (*c* 0.52, MeOH)} and its IR and UV spectra were very similar to those of **1**.[14] The proton and carbon signals in the ^{1}H NMR (acetone-d_6) and ^{13}C NMR (Table 2) spectra[12] of **2** indicated the presence of the same functional groups as **1**: a dihydropyron moiety in flavanone structure {[δ 2.93 (1H, dd, $J=2.7$, 17.1 Hz), 3.41 (1H, dd, $J=13.1$, 17.1 Hz), 3-H$_2$], 5.77 (1H, dd, $J=2.7$, 13.1 Hz, 2-H)}, three singlet aromatic protons [δ 6.59, 7.05, 7.26 (1H each, all s, 6, 3″, 6″-H)], *ortho*-coupled A_2B_2-type aromatic protons [δ 6.98, 7.53 (2H each, both d, $J=8.6$ Hz, 3′, 5′-H and 2′, 6′-H)], and a chelated hydroxyl proton [δ 12.19 (1H, br s, 5-OH)]. Acetylation of **2** yielded the tetraacetate (**2a**).[15] In the mass fragmentation of **2**, the

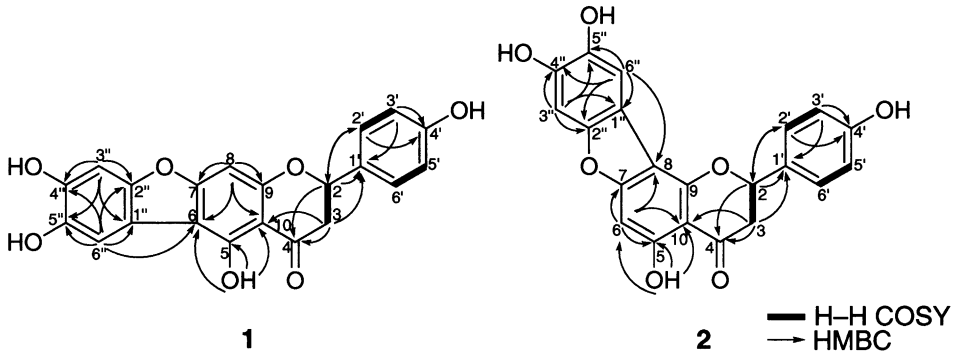

Chart 1.

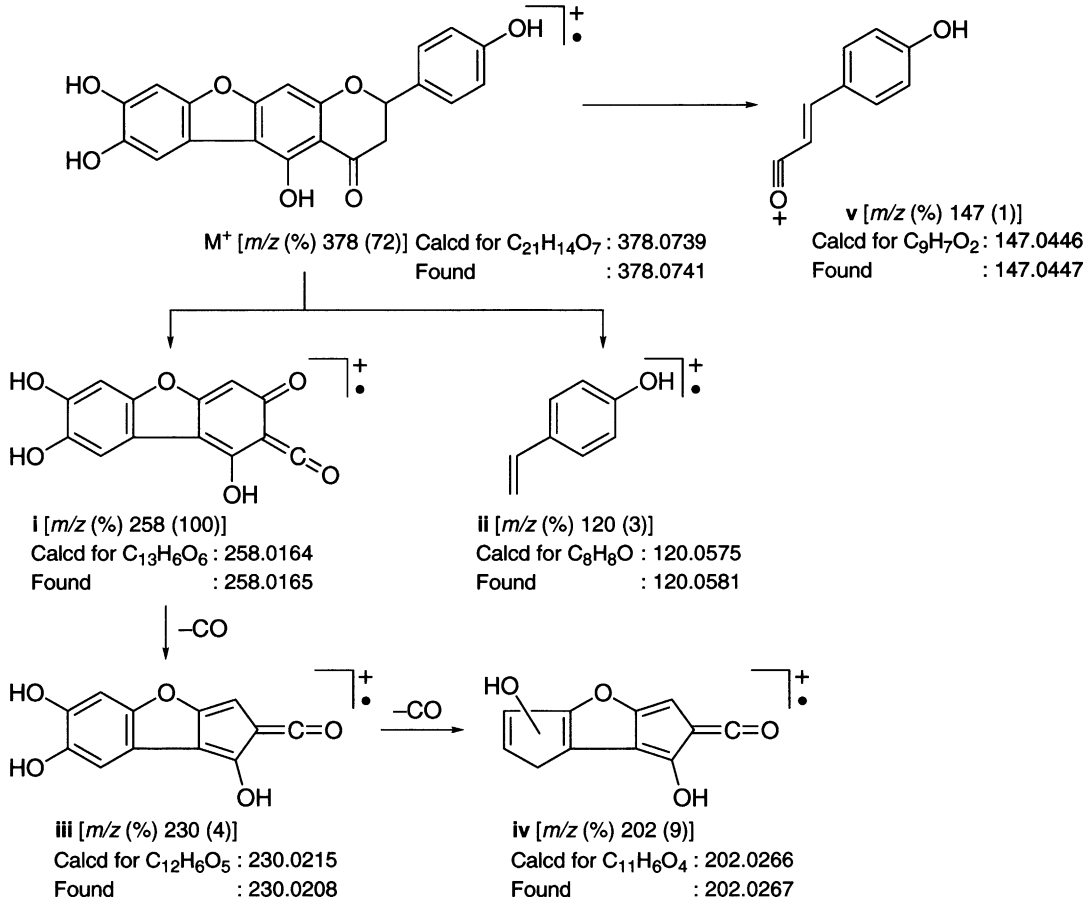

Figure 1. MS fragmentation pattern of anastatin A (**1**).

fragment ion peaks were observed at *m/z* (%) 258 ($M^+-C_8H_8O$, 100), 230 ($M^+-C_9H_8O_2$, 5), 202 ($M^+-C_{10}H_8O_3$, 7), 174 ($M^+-C_{11}H_8O_4$, 8), 147 ($M^+-C_{12}H_7O_5$, 1), 120 ($M^+-C_{13}H_6O_6$, 8). The position of the benzofuran moiety in **2** was clarified by the HMBC experiment on **2** and **2a**. Namely, long-range correlations were observed between the 5-hydroxyl proton and 5, 6, 10-carbons, between the 6-proton and the 5, 7, 8, 10-carbons, between the 3″-proton and 1″, 2″, 4″, 5″-carbons, and between the 6″-proton and 8, 1″, 2″, 4″, 5″-carbons in the HMBC experiment (Fig. 1). The CD spectrum of **2** exhibited negative Cotton effects [MeOH, λ_{max} 291 nm ($\Delta\varepsilon = -0.25$)]. Those findings and comparison of the ^1H and ^{13}C NMR data for **2** with those for **2a** led us to formulate the absolute stereostructure of anastatin B (**2**) as shown.

Protective Effects of Anastatins and Flavonoid Constituents from *A. hierochuntica* on D-GalN-induced Cytotoxicity in Primary Cultured Mouse Hepatocytes

The inhibitory effects of anastatins (**1**, **2**) and isolated flavonoids (**3**–**8**) on D-GalN-induced cytotoxicity in pri-

Table 3. Inhibitory effects of constituents from *A. hierochuntica* on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes

	Inhibition (%)			
	0 μM	3 μM	10 μM	30 μM
Anastatin A (**1**)	0.0±0.2	17.2±6.7*	31.5±2.9**	46.2±3.9**
Anastatin B (**2**)	0.0±0.6	28.6±8.6*	40.5±8.8**	55.0±0.5**
Naringenin (**3**)	0.0±0.9	2.9±0.4	9.3±2.1	43.1±0.4**
Eriodictyol (**4**)	0.0±1.0	5.9±1.8	8.3±0.9**	27.3±1.0**
Aromadendrin (**5**)	0.0±2.0	7.8±2.0	14.2±1.0	33.2±3.4**
(+)-Taxifolin (**6**)	0.0±1.7	9.6±1.5**	11.7±1.0**	13.0±2.1**
3′-*O*-Methyltaxifolin (**7**)	0.0±2.1	4.0±1.6	7.3±1.9	36.7±3.3**
(+)-Epitaxifolin (**8**)	0.0±0.7	2.1±1.2	11.0±0.7**	21.7±1.1**
Silybin[a]	0.0±0.3	4.8±1.1	7.7±0.7	45.2±8.8**

Each value represents the mean±SEM ($N=4$). Significantly different from the control, *$p<0.05$, **$p<0.01$.
[a]Commercial silybin was purchased from Funakoshi Co., Ltd. (Tokyo, Japan).

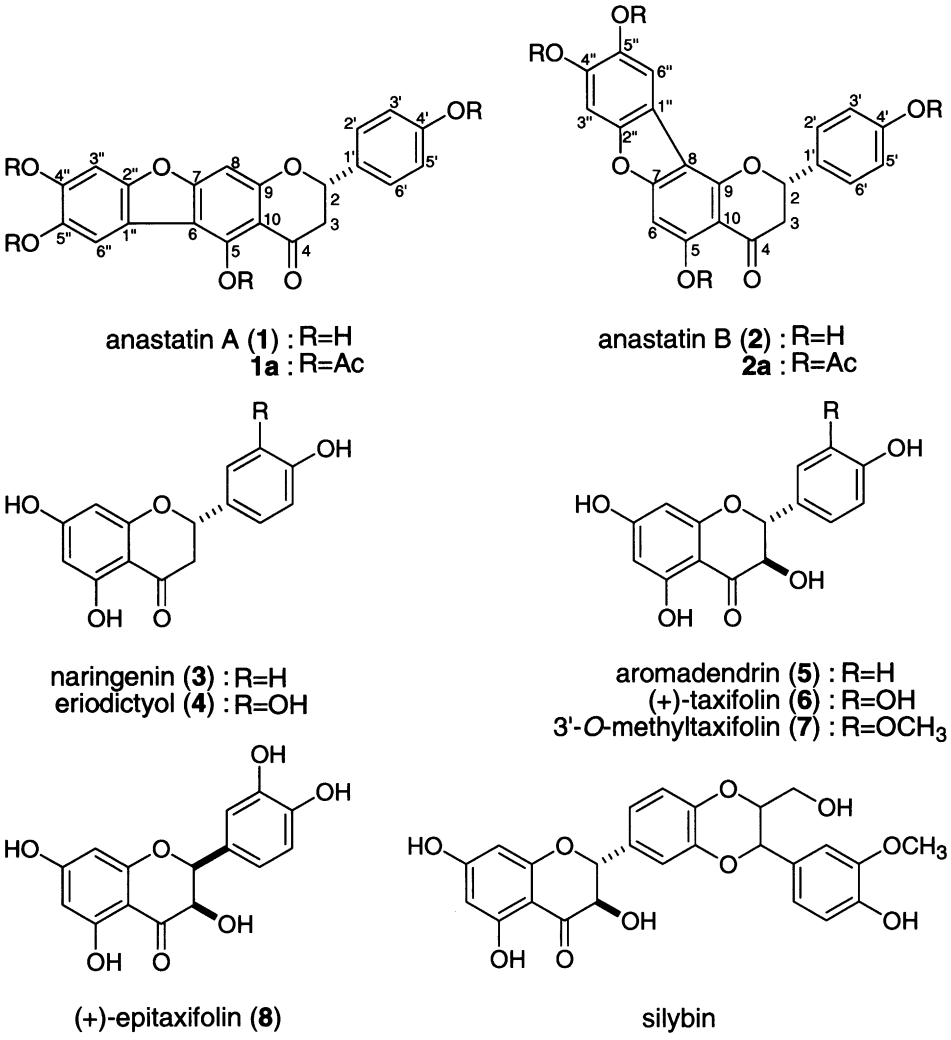

Figure 2.

mary cultured mouse hepatocytes were examined. As shown in Table 3, two new flavanones, anastatins A (**1**) and B (**2**), were found to show potent inhibitory activities. Thus, the hepatoprotective activities of **1** and **2** were stronger than those of other flavonoids and commercial silybin, which is well known to show potent hepatoprotective activity.[16]

References and Notes

1. (a) Rizk, A. M.; Hammouda, F. M.; Ismail, S. I.; Hassan, N. M.; Ahmed, F. A. *Int. J. Pharmacog.* **1993**, *31*, 327. (b) Abou-Mandour, A.; Hartung, W. *Plant Cell Reports* **1995**, *14*, 657.
2. (a) Yoshikawa, M.; Murakami, T.; Kishi, A.; Kageura, T.; Matsuda, H. *Chem. Pharm. Bull.* **2001**, *49*, 863. (b) Murakami, T.; Kishi, A.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 974. (c) Morikawa, T.; Xu, F.; Matsuda, H.; Yoshikawa, M. *Heterocycles* **2002**, *57*, 1983.
3. (a) Matsuda, H.; Morikawa, T.; Ninomiya, K.; Yoshikawa, M. *Bioorg. Med. Chem.* **2001**, *9*, 909. (b) Murakami, T.; Kohno, K.; Ninomiya, K.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 1003. (c) Matsuda, H.; Ninomiya, K.; Shimoda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2002**, *10*, 707. (d) Morikawa, T.; Matsuda, H.; Ninomiya, K.; Yoshikawa, M. *Biol. Pharm. Bull.* **2002**, *25*, 627.
4. Niwa, M.; Otsuji, S.; Tatematsu, H.; Liu, G.; Chen, X.; Hirata, Y. *Chem. Pharm. Bull.* **1986**, *34*, 3249.
5. (a) Eriodictyol (**4**) was identified by comparison of its physical date (IR, UV, MS, and ^{1}H and ^{13}C NMR) with those of authentic sample.[5b] (b) Matsuda, H.; Morikawa, T.; Toguchida, I.; Harima, S.; Yoshikawa, M. *Chem. Pharm. Bull.* **2002**, *50*, 972.
6. Bellakhdar, J.; Passannanti, S.; Paternostro, M. P.; Piozzi, F. *Planta Med.* **1988**, *54*, 94.
7. Nonaka, G.; Goto, Y.; Kinjo, J.; Nohara, T.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, *35*, 1105.
8. Marco, J. A.; Sanz-Cervera, J. F.; Yuste, A.; Oriola, M. C. *Phytochemistry* **1994**, *36*, 725.
9. (a) Matsuda, H.; Morikawa, T.; Toguchida, I.; Yoshikawa, M. *Chem. Pharm. Bull.* **2002**, *50*, 788. (b) Matsuda, H.; Morikawa, T.; Ueda, K.; Managi, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2002**, *10*, 3123.
10. From the EtOAc-soluble fraction, 11 aromatic compounds; *p*-hydroxybenzoic acid (0.0012%), *p*-methoxybenzoic acid (0.00075%), 3,4-dihydroxybenzoic acid (0.0025%), 3-methoxy-4-hydroxybenzoic acid (0.0068%), *p*-hydroxybenzaldehyde (0.00093%), 3,4-dihydroxybenzaldehyde (0.0016%), vanillin (0.0036%), acetovanillone (0.00066%), 2,4′-dihydroxy-3′-methoxyacetophenone (0.0011%), ω-hydroxypropioguaiacone (0.0015%), and (+)-2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (0.0015%), three phenylpropanoids; *trans*-cinnamic acid (0.00059%), *trans*-ferulic acid (0.00079%), and coniferaldehyde (0.0013%), 12 lignans; 1,2-bis-(4-hydroxy-3-methoxyphenyl)-propane-1,3-diols [*erythro* form (0.0029%) and *threo* form (0.0011%)], evofolin B (0.00093%), (+)-isolariciresinol (0.0013%), (+)-pinoresinol (0.00047%), ficusal (0.0011%), balanophonin (0.00045%), (+)-dehydrodiconiferyl alcohol (0.0011%), 2,3-dihydro-2-(3,4-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-7-hydroxybenzofuran (0.0061%), 4-[2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethoxy]-3-methoxybenzaldehyde (0.00060%), 1-(4-hydroxy-3-methoxyphenyl)-2-{4-[2-formyl-(*E*)-vinyl]-2-methoxyphenoxy}-propane-1,3-diol(0.00019%) and 3-hydroxy-1-{4-[2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethoxy]-3-methoxyphenyl}-1-propanone (0.00024%) and 4 flavonolignans; (+)- and (−)-silychristins [(0.0011%), (0.00073%)], silybin (0.0025%), and isosilybin (0.0024%) were isolated.
11. **1a**: a pale yellow powder, $[\alpha]_D^{25}$ +10.4° (*c* 0.35, $CHCl_3$). High resolution EI–MS: calcd for $C_{29}H_{22}O_{11}$ (M^+): 546.1162. Found: 546.1171. UV (MeOH, log ε): 220 (4.3), 244 (4.3), 268 (4.5), 308 (4.0), 329 (3.6) nm. IR (KBr): 1771, 1684, 1653, 1617, 1509, 1148, 1073, 1017 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.33, 2.34, 2.36, 2.57 (3H all, each s, –CO*C*H$_3$), [2.85 (1H, dd, *J* = 2.6, 16.8 Hz), 3.12 (1H, dd, *J* = 13.1, 16.8 Hz), 3-H$_2$], 5.56 (1H, dd, *J* = 2.6, 13.1 Hz, 2-H), 7.09, 7.43, 7.64 (1H all, each s, 8, 3″, 6″-H), 7.18, 7.51 (2H each, both d, *J* = 8.6 Hz, 3′, 5′-H and 2′, 6′-H). EI–MS (%): *m*/*z* 546 (M^+, 9), 504 ($M^+ - C_2H_2O$, 40), 462 ($M^+ - 2C_2H_2O$, 44), 420 ($M^+ - 3C_2H_2O$, 100), 378 ($M^+ - 4C_2H_2O$, 3), 258 ($M^+ - 4C_2H_2O - C_8H_8O$, 52), 120 ($M^+ - 4C_2H_2O - C_{13}H_6O_6$, 9).
12. The ^{1}H and ^{13}C NMR spectra of **1**, **2**, **1a**, and **2a** were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.
13. Gaffield, W. *Tetrahedron* **1970**, *26*, 4093.
14. **2**: High resolution EI–MS: calcd for $C_{21}H_{14}O_7$ (M^+): 378.0739. Found: 378.0741. UV (MeOH, log ε) 243 (4.2), 263 (4.3), 295 (4.2), 365 (3.4) nm. IR (KBr): 3630, 3590, 1630, 1605, 1518, 1509, 1152, 1016 cm^{-1}.
15. **2a**: a pale yellow powder, $[\alpha]_D^{25}$ −5.2° (*c* 0.10, $CHCl_3$). High resolution EI–MS: calcd for $C_{29}H_{22}O_{11}$ (M^+): 546.1162. Found: 546.1166. UV (MeOH, log ε) 221 (4.4), 258 (4.5), 296 (4.0), 329 (3.7) nm. IR (KBr): 1773, 1734, 1684, 1653, 1636, 1509, 1136 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.33, 2.33, 2.35, 2.44 (3H all, each s, –CO*C*H$_3$), [2.87 (1H, dd, *J* = 2.7, 16.5 Hz), 3.16 (1H, dd, *J* = 13.4, 16.5 Hz), 3-H$_2$], 5.77 (1H, dd, *J* = 2.7, 13.4 Hz, 2-H), 6.97, 7.48, 7.73 (1H all, each s, 6, 3″, 6″-H), 7.23, 7.56 (2H each, both d, *J* = 8.6 Hz, 3′, 5′-H and 2′, 6′-H). EI–MS (%): *m*/*z* 546 (M^+, 4), 504 ($M^+ - C_2H_2O$, 28), 462 ($M^+ - 2C_2H_2O$, 44), 420 ($M^+ - 3C_2H_2O$, 100), 378 ($M^+ - 4C_2H_2O$, 2), 258 ($M^+ - 4C_2H_2O - C_8H_8O$, 62), 120 ($M^+ - 4C_2H_2O - C_{13}H_6O_6$, 10).
16. (a) Feher, J.; Deak, G.; Muzes, G.; Lang, I.; Niederland, V.; Nekam, K.; Karteszi, M. *Orv. Hetil.* **1989**, *130*, 2723. (b) Skottova, N.; Krecman, V. *Physiol. Res.* **1998**, *47*, 1.