Three New Agarofuran Sesquiterpenes Reissantins F – H from *Reissantia buchananii*

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Abstract

Three new agarofuran sesquiterpenes, reissantins F – H (**1 – 3**), were isolated from a methanol extract of *Reissantia buchananii*. Their structures as well as their relative stereochemistry were established on the basis of modern mass and spectroscopic methods. Compounds **1 – 3** were assayed for cytotoxicity against HepG2 and Hep3B human liver cancer cell lines.

Sesquiterpene polyol esters from the Celestraceae family have attracted much interest due to their structural diversities and broad spectrum of biological activities, including stimulant, appetite suppressive, sedative, emetic, purgative, memory-restorative, male contraceptive, antitumor, antileukemic, antibacterial, insecticidal and insect repellent activities [1]. During our search for anticancer compounds from traditional medicines, we previously reported five new agarofuran sesquiterpenes and nine known triterpenoids from *Reissantia buchananii* (Celastraceae) [2]. Among the isolated compounds, reissantin B was found to be moderately active (EC_{50} = 11 – 17 μg/mL) against six different cell lines. Based on the prior reports [1], [2], we have further investigated the constituents of this plant and isolated three new minor dihydroagarofuran sesquiterpenes, reissantins F – H (**1 – 3**). In this paper, we present the isolation and structure elucidation of these three compounds.

The MeOH extract of the root bark of *Reissantia buchananii* (8.0 g) was eluted on a Celite 545 column to obtain a $CHCl_3$ eluate (3.9 g). The extract exhibited significant cytotoxicity against A-549 (human lung carcinoma) and HOS cell lines. Further chromatographic fractionation and purification led to the isolation of compounds **1 – 3**.

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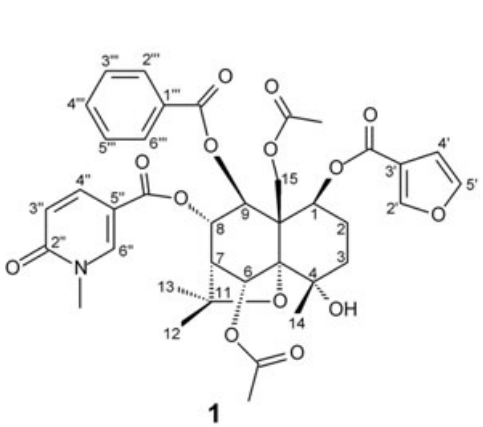
Received: May 13, 2005 · **Accepted:** June 20, 2005

Bibliography: Planta Med 2006; 72: 92–96 · © Georg Thieme Verlag KG Stuttgart · New York · DOI 10.1055/s-2005-873186 · Published online November 10, 2005 · ISSN 0032-0943



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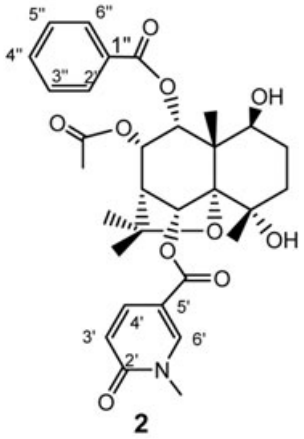

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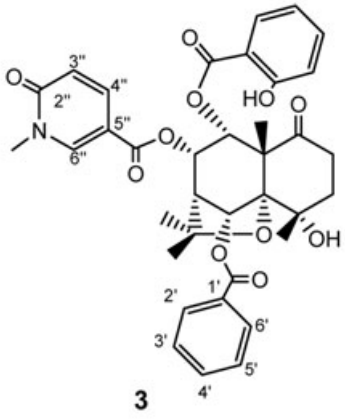

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Compound **1** was assigned the molecular formula $C_{38}H_{41}O_{14}N$ based on a HR-FAB-MS peak at m/z = 736.2612 $[M + H]^+$. Its IR spectrum showed hydroxy and ester carbonyl absorptions (3428 and 1721 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table **1**) indicated the presence of two acetyl singlets (δ = 2.43 and 2.16), one benzoate group [δ = 7.55 (2H, br d, J = 7.5 Hz), 7.27 (2H, br t, J = 7.5 Hz), and 7.48 (1H, br t, J = 7.5 Hz)], one 5-carboxy-*N*-methyl-2-pyridone (CNMP) [1] [δ = 6.41 (1H, d, J = 9.5 Hz), 7.66 (1H, dd, J = 9.5, 2.5 Hz), 7.98 (1H, d, J = 2.5 Hz), and 3.49 (3H, s, *N*-methyl)], and one furancarboxylate ester [δ = 6.97 (1H, br t, J = 2.0 Hz), 6.34 (1H, br d, J = 1.0 Hz), and 7.38 (1H, br d, J = 1.0 Hz)]. The ^{13}C-NMR spectral data (Table **2**) of **1** revealed the presence of three methyl carbons at δ = 25.79 (C-12), 29.62 (C-13), and 23.41 (C-14), two methylene carbons at δ = 25.06 (C-2) and 37.99 (C-3), one oxygenated methylene carbon at δ = 61.03 (C-15), one methine carbon at δ = 52.07 (C-7), four oxygenated methine carbons at δ = 76.39 (C-1), 75.11 (C-6), 75.50 (C-8), and 75.04 (C-9), and four quaternary carbons at δ = 70.22 (C-4), 92.51 (C-5), 50.71 (C-10), and 83.97 (C-11). From the above information, the structure of compound **1** was a sesquiterpene polyol ester with a dihydroagarofuran skeleton, which has already been found in the *Reissantia* genus and other plants of the Celastraceous family [1], [2], [3], [4]. The structure of **1** and the positions of the functional groups were determined completely by a study of its HMBC spectrum (Fig. **1**). The key HMBC correlations were found between δ_C = 161.64/δ_H = 5.66 (H-1), δ_C = 163.27/δ_H = 5.61 (H-8) and 7.66 (CNMP-8, H-4″), δ_C = 165.62/δ_H = 6.25 (H-9) and 7.55 (Bz-9, H-2‴ and 6‴), δ_C = 169.82/δ_H = 6.63 (H-6) and 2.16 (acetyl methyl), as well as δ_C = 170.47/δ_H = 4.95 (H-15b) and 2.43 (acetyl methyl). The relative stereochemistry was determined by a NOESY experiment (Fig. **2**). From the NOESY spectrum of **1** and previous reports [2], [3], [4], [5] for this compound type, both H-6 and H-1 were assigned axial stereochemistries. The correlations of H-6 with H-8, Me-14, and H_2–15 indicated that H-8, C-14, and C-15 also have axial orientations. A NOESY correlation was found between H-1 and H-9 while no correlation was found between H-6 and H-9, suggesting that H-9 is axial in compound **1**. Thus, the structure of **1** was determined as illustrated and named reissantin F.

Compound **2** has a molecular formula of $C_{31}H_{38}O_{10}N$ as determined by HR-FAB-MS, m/z = 584.2488 ($[M + H]^+$, calcd.: 584.2495). The IR spectrum indicated the presence of hydroxy (3466 cm^{-1}) and carbonyl (1717 cm^{-1}) groups. The ^{1}H-NMR spectrum of **2** (Table **1**) showed signals for one acetyl methyl (δ = 1.94), one benzoate group [δ = 8.14 (2H, br d, J = 7.6 Hz), 7.26 (2H, br t, J = 7.6 HZ), and 7.51 (1H, br t, J = 7.6 Hz)], and one 5-carboxy-*N*-methyl-2-pyridone (CNMP) [1] [δ = 6.56 (1H, d, J = 9.6 Hz), 7.97 (1H, dd, J = 9.6, 2.4 Hz), 8.44 (1H, d, J = 2.4 Hz), and 3.60 (3H, s, *N*-methyl)]. The ^{13}C-NMR and DEPT spectra (Table **2**) indicated that **2** has an agarofuran skeleton [2], [3], [4], [5], [6] with 15 carbons, including four methyl carbons at δ = 30.41 (C-12), 26.51 (C-13), 23.78 (C-14), and 17.95 (C-15), two methylene carbons at δ = 27.49 (C-2) and 39.11 (C-3), five methine carbons at δ = 77.32 (C-1), 78.54 (C-6), 53.71 (C-7), 68.74 (C-8), and 72.68 (C-9), and four quaternary carbons at δ = 70.95 (C-4), 91.32 (C-5), 51.08 (C-10), and 84.47 (C-11). The HMBC spectrum of **2** (Fig. **3**) exhibited cross-peaks due to long-range correlations between δ_C = 163.39/δ_H = 5.52 (H-6), 7.97 and 8.44 (CNMP-6, H-4′ and 6′), δ_C = 165.69/δ_H = 5.39 (H-9) and 8.14 (Bz-9, H-2″ and 6″), and δ_C = 169.24/δ_H = 1.94 (acetyl methyl). Therefore, the positions of the benzyl and CNMP moi-

Table **1** ^{1}H-NMR chemical shifts for compounds **1 – 3** (in $CDCl_3$, 500 MHz)

Proton	*1*	*2*	*3*
1	5.66 (dd, 12.5, 4.5)	4.36 (m)	
2	1.84 (m, H-2α), 1.51 (m, H-2β)	1.73 (m), 1.62 (m)	2.28 (d, 14.0), 2.78 (td, 14.0, 6.0)
3	1.99 (m, H-3α), 1.79 (m, H-3β)	1.82 (m, H-3α), 1.66 (m, H-3β)	2.05 (m), 2.16 (m)
6	6.63 (s)	5.52 (s)	6.53 (s)
7	2.68 (d, 3.5)	2.61 (d, 3.2)	2.62 (d, 3.5)
8	5.61 (dd, 10.0, 3.5)	5.70 (dd, 6.0, 3.2)	5.97 (t, 3.5)
9	6.25 (d, 10.0)	5.39 (d, 6.0)	6.12 (d, 3.5)
12	1.58 (s)	1.52 (s)	1.74 (s)
13	1.76 (s)	1.70 (s)	1.63 (s)
14	1.39 (s)	1.31 (s)	1.65 (s)
15	4.61 (d, 13.0), 4.95 (d, 13.0)	1.34 (s)	1.75 (s)
Bz			
2 and 6	7.55 (br d, 7.5)	8.14 (br. d, 7.6)	8.22 (d, 7.5)
3 and 5	7.27 (br t, 7.5)	7.26 (br. t, 7.6)	7.52 (t, 7.5)
4	7.48 (br. t, 7.5)	7.51 (br. t, 7.6)	7.64 (t, 7.5)
2-hydroxy-Bz			
2-OH			12.19
3			7.73 (d, 7.5)
4			7.62 (t, 7.5)
5			7.07 (t, 7.5)
6			8.85 (d, 7.5)
CNMP			
N-methyl	3.49 (s)	3.60 (s)	3.70 (s)
3	6.41 (d, 9.5)	6.56 (d, 9.6)	6.56 (d, 9.5)
4	7.66 (dd, 9.5, 2.5)	7.97 (dd, 9.6, 2.4)	7.78 (dd, 9.0, 2.0)
6	7.98 (d, 2.5)	8.44 (d, 2.4)	8.56 (d, 2.0)
Ac-1			
CH_3	2.16 (s)	1.94 (s)	
Ac-2			
CH_3	2.43 (s)		
Fu			
2	6.97 (br. t, 1.0)		
4	6.34 (br. d, 1.0)		
5	7.38 (br. d, 1.0)		

eties were confirmed. Based on the NOESY correlation (Fig. **4**) and the ^{13}C-NMR data (around δ = 68 – 69 for C-8), the acetoxy group could be assigned at C-8. The relative stereochemistry of compound **2** was also determined on the basis of NOESY experiments (Fig. **4**). In conclusion, the structure of **2** was determined as shown and named reissantin G.

Compound **3** was obtained as an oil with the molecular formula $C_{36}H_{37}O_{11}N$. The IR and UV absorptions were similar to those of **2**, indicating the presence of hydroxy, ester, and phenyl groups. ^{1}H- and ^{13}C-NMR spectra showed signals corresponding to a β-agarofuran skeleton with a ketone carbonyl function, along with two different benzoate esters and one 5-carboxy-*N*-methyl-2-pyridone (CNMP) moiety. Based on the analysis of chemical shifts and coupling constants, one benzoate was unsubstituted and the other benzoate was *o*-hydroxylated. In the HMBC spectrum (Fig. **5**), the CNMP carbonyl at δ_C = 163.20 was correlated with δ_H = 5.97 (H-8), and the benzoate carbonyl carbons (1-benzoate at δ_C = 166.27 and 2-hydroxy-1-benzoate at δ_C = 167.43) were correlated with δ_H = 6.53 (H-6) and 6.12 (H-9), respectively. Furthermore, the keto carbonyl signal of the agarofuran showed long-range correlations with ^{1}H signals at δ = 2.28, 2.78 (H-2) and 2.05, 2.16 (H-3). The relative stereochemistry of **3** was found to be similar to that of **1** except for the configuration of H-9, which was confirmed to be equatorial based on the correlations between H-9 and H-15 in the NOESY spectrum. Thus, the structure of compound **3** was established as reissantin H.

Based on NMR data of dihydroagarofuran sesquiterpenes isolated from *Reissantia* spp., we have identified some typical features of ^{13}C-NMR chemical shifts: δ = 83 – 85 for C-11, δ = 91 – 94 for C-5, δ = 161.5 – 162.9 for furancarboxylate carbonyls, δ = 163.2 – 163.5 for CNMP carbonyls, δ = 165.5 – 167.5 for benzoate carbonyls, and δ = 169.0 – 170.5 for acetyl carbonyls. In a cytotoxicity evaluation, reissantins F – H had no significant effect against HepG2 and Hep3B human liver cancer cell lines at concentrations below 20 μg/mL.

Table **2** ^{13}C-NMR chemical shifts for compounds **1 – 3** (in $CDCl_3$, 125 MHz)

Carbon	*1*	*2*	*3*
1	76.39	77.32	208.35
2	25.06	27.49	34.11
3	37.99	39.11	39.84
4	70.22	70.95	70.16
5	92.51	91.32	93.87
6	75.11	78.54	74.87
7	52.07	53.71	53.74
8	75.50	68.74	70.88
9	75.04	72.68	69.38
10	50.71	51.08	58.25
11	83.97	84.47	83.82
12	25.79	30.41	24.00
13	29.62	26.51	29.37
14	23.41	23.78	23.29
15	61.03	17.95	17.75
Bz			
C = O	165.62	165.69	166.27
1	128.38	128.83	129.28
2 and 6	129.02	129.90	130.16
3 and 5	128.38	128.67	128.82
4	133.35	133.31	133.82
2-hydroxy-Bz			
C = O			167.43
1			115.91
2			140.84
3			120.79
4			134.94
5			123.52
6			130.30
CNMP			
C = O	163.27	163.39	163.20
N-methyl	38.29	38.55	38.40
2	162.88	162.96	162.99
3	119.55	119.67	119.73
4	138.28	138.74	138.43
5	108.63	109.26	108.90
6	144.06	144.92	144.81
Ac-1			
C = O	169.82	169.24	
CH_3	21.43	20.80	
Ac-2			
C = O	170.47		
CH_3	21.27		
Fu			
C = O	161.64		
2	143.09		
3	118.45		
4	109.15		
5	147.49		

Materials and Methods

General: Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. UV spectra were obtained on a Hitachi 200 – 20 spectrophotometer. IR spectra were measured on a Mattson Genesis II spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on Varian Inova 500, Varian Unity Plus 400 MHz, or Varian Gemini 200 MHz spectrometers using TMS as internal standard. Chemical shifts are reported in parts per million (δ), and coupling constants (J) are expressed in Hertz. Low-resolution EI-MS were collected on a Bruker APEX II mass spectrometer or a Quattro GC/MS spectrometer having a direct inlet system. High-resolution ESI-MS was obtained on a Bruker APEX II mass spectrometer or Quattro GC/MS spectrometer. Silica gel 60 (Merck, 230 – 400 mesh) and Celite 545 (Merck, 0.02 – 0.1 nm) were used for column chromatography. TLC analysis was carried out on silica gel GF_{254} pre-coated plates with detection using 50 % H_2SO_4 followed by heating on a hot plate. HPLC was performed on a Shimadzu LC-10AT apparatus equipped with a Shimadzu SPD-10A UV-vis detector. Hypersil ODS-5 (250 × 4.6 mm i.d.) and preparative ODS-5 (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

Plant material: The plant extract (8 g) was prepared in MeOH from the root bark of *Reissantia buchananii* (Loes.) N. Hellé collected in January 1989, in the Iringa District of Tanzania by Drs. Roy Gereau and John Lovertt of the Missouri Botanical Garden. Dr. Gereau identified the plant specimen. A voucher specimen (NIH-N031145-T-31) is deposited in the Botany Department, Museum of Natural History, Smithsonian Institution.

Extraction and isolation: Reissantia buchananii extract (8.0 g) was eluted on a Celite 545 column chromatography to provide a $CHCl_3$ (2 L) eluate (3.9 g). The $CHCl_3$ extract exhibited significant cytotoxicity against A-549 (human lung carcinoma) and HOS cell lines. Therefore, the extract was mixed with silica gel 10 g, and then dried-packed on a silica gel column (100 g), which was eluted with *n*-hexane/$CHCl_3$ (1 : 1, 2 L), $CHCl_3$ (2.5 L), and $CHCl_3$/MeOH (4 : 1, 1 L) to give 17 fractions. Fraction 10 (680 mg) was separated by silica gel chromatography eluting with *n*-hexane/EtOAc (20 : 1, 10 : 1, 5 : 1, 1 : 1, each 1.5 L) and $CHCl_3$/MeOH (20 : 1 → 4 : 1, each 1.0 L) to give 12 fractions. Fr. 10 – 4 was purified using RP-HPLC (63 % MeOH, 3 L) to give compound **1** (1.5 mg, *n*-hexane/EtOAc/acetone, 10 : 1 : 7, R_f = 0.4). Fr. 11 (210 mg) was purified using RP-HPLC (57 % MeOH, 2.5 L) to give compound **2** (1.3 mg, *n*-hexane/EtOAc/acetone, 10 : 1 : 7, R_f = 0.3). Fr. 12 (125 mg) was purified using RP-HPLC (57 % MeOH, 2.0 L) to give compound **3** (2.5 mg, *n*-hexane/EtOAc/acetone, 10 : 1 : 7, R_f = 0.3).

Reissantin F (**1**): White powder; m. p. 138 – 139 °C; $[\alpha]_D^{24}$: + 35.70° (*c* 0.23, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 219 (4.35), 270 (4.07) nm; IR (neat): ν_{max} = 3428, 1721 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): see Table **1**; ^{13}C-NMR (125 MHz, $CDCl_3$): see Table **2**; FAB-MS: *m/z* = 736 $[M + H]^+$; HR-FAB-MS: *m/z* = 736.2612 $[M + H]^+$ (calcd. for $C_{38}H_{42}NO_{14}$: 736.2605).

Reissantin G (**2**): White powder; m. p. 168 – 169 °C; $[\alpha]_D^{24}$: + 33.56° (*c* 0.55, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 277 (3.66) nm; IR (neat): ν_{max} = 3428, 1721 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): see Table **1**; ^{13}C-NMR (125 MHz, $CDCl_3$): see Table **2**; HR-FAB-MS: *m/z* = 584.2488 $[M + H]^+$ (calcd. for $C_{31}H_{38}NO_{10}$: 584.2496).

Reissantin H (**3**): Oil; $[\alpha]_D^{21}$: –50.10° (*c* 0.36, MeOH); UV (MeOH): λ_{max} (log ε) = 268 (3.52) nm; IR (neat): ν_{max} = 3273, 1718, 1669 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): see Table **1**; ^{13}C-NMR (125

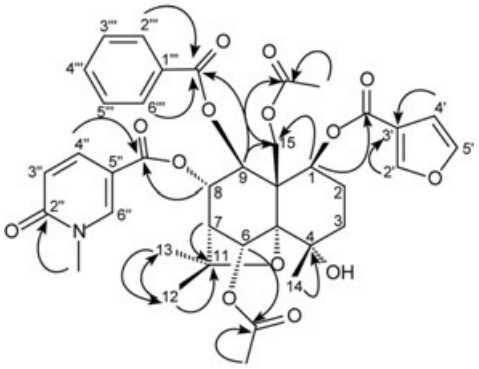

Fig. **1** Key HMBC correlations of **1**.

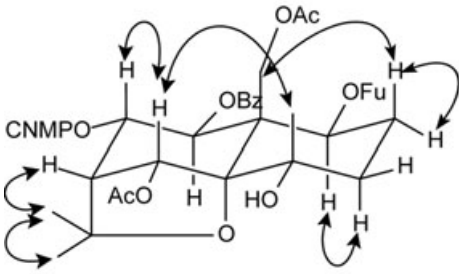

Fig. **2** Key NOESY correlations of **1**.

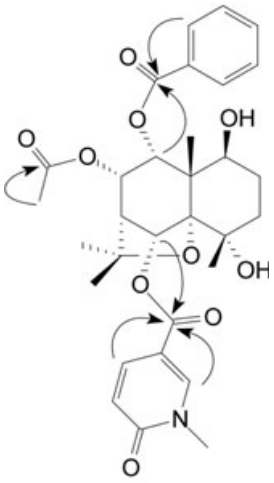

Fig. **3** Key HMBC correlations of **2**.

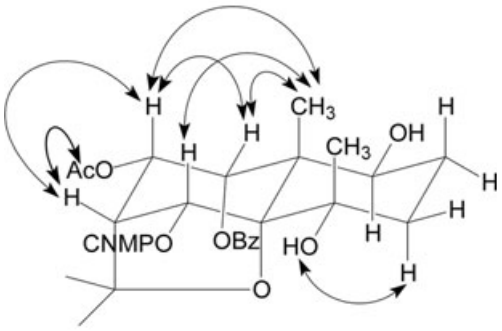

Fig. **4** Key NOESY correlations of **2**.

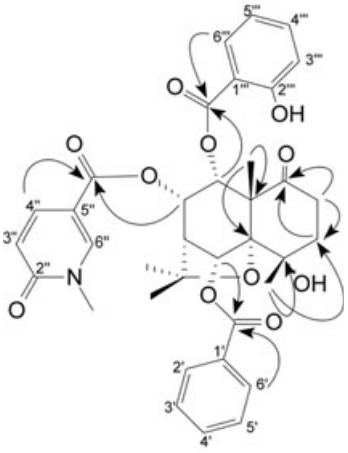

Fig. **5** Key HMBC correlations of **3**.

MHz, $CDCl_3$): see Table **2**; FAB-MS: m/z = 660 $[M + H]^+$. HR-FAB-MS: m/z = 660.2453 $[M + H]^+$ (calcd. for $C_{36}H_{38}O_{11}N$: 660.2445).

The bioassays for measurement of cytotoxicity in Hep G2 and Hep 3B cell lines were carried out according to procedures described in the literature [7].

Acknowledgements

This investigation was supported by grant CA-17625 from the National Cancer Institute, NIH, awarded to K. H. Lee and the financial support from the National Science Council awarded to Y. C. Wu.

References

[1] Spivey AC, Weston M, Woodhead S. Celastraceae sesquiterpenoids: biological activity and synthesis. Chem Soc Rev 2002; 31: 43 – 59

[2] Chang FR, Hayashi K, Chen IH, Liaw CC, Bastow KF, Nakanishi Y et al. Antitumor agents. 228. Five new agarofurans, reissantins A – E, and cytotoxic principles from *Reissantia buchananii*. J Nat Prod 2003; 66: 1416 – 20

[3] Gamlath CB, Gunatilaka AAL, Schlemper EO. Reissantioloxide: a novel epoxytriterpenoid from *Reissantia indica*; X-ray crystal structure. J Chem Soc Chem Commun, 1988: 249 – 50

[4] Gamlath CB, Gunatilaka AAL, Tezuka Y, Kikuchi T. The structure of celastranhydride: a novel triterpene anhydride of Celastraceae. Tetrahedron Lett 1988; 29: 109 – 12

[5] Kuo YH, Chou CJ, Kuo LMY, Hu YY, Chen YC, Chen CF et al. A sesquiterpene ester from *Celastrus hindsii*. Phytochemistry 1996; 41: 549 – 51

[6] Furukawa M, Makino M, Uchiyama T, Ishimi K, Ichinohe Y, Fujimoto Y. Sesquiterpene pyridine alkaloids from *Hippocratea excelsa*. Phytochemistry 2002; 59: 767 – 77

[7] Lan YH, Chang FR, Liaw CC, Wu CC, Chiang MY, Wu YC. Digoniodiol, deoxygoniopypyrone A, and goniofupyrone A: three new styryllactones from *Goniothalamus amuyon*. Planta Med 2005; 71: 153 – 9