Original Research Paper

Dihydrobenzofurans and Propynylthiophenes From the Roots of *Eupatorium heterophyllum*

Yiming Hu[1], **Yoshinori Saito**[1] iD, **Xun Gong**[2], **Yosuke Matsuo**[1]
and **Takashi Tanaka**[1] iD

Abstract

Seven new dihydrobenzofurans and 2 new propynyl thiophenes were isolated from the roots of *Eupatorium heterophyllum* together with 13 known compounds. The compounds were characterized using spectroscopic methods including 2D NMR, infrared, and mass spectrometric techniques. Aerial parts of this plant have been known to contain various sesquiterpenoids and displayed high chemical diversity (several compounds isolated and/or identified) among their chemical constituents depending on the collection site. Nevertheless, we found that the chemical diversity in the roots was lower than in the aerial parts.

Eupatorium species (Asteraceae) are herbaceous perennials, which are widely distributed worldwide, especially in East Asia.[1] Sesquiterpenoids with germacrane, guaiane, eudesmane skeletons have been isolated from this genus. Interestingly, our studies demonstrated that plant samples of *Eupatorium* species, which were collected from different locations in Japan showed significant variations in their chemical compositions.[2–4] The differences in chemical compositions were primarily observed in the above-ground parts.[5] Additionally, an investigation on the roots (underground part) of *E. heterophyllum*, which were collected from five districts in China showed only small differences in chemical compositions.[6] In this study, we further examined the roots of *E. heterophyllum*, which were collected from Sichuan, China. The isolation, determination of structures, and identification of new dihydrobenzofurans and propynylthiophenes were described in this paper.

Results and Discussion

E. heterophyllum plant samples were collected from Sichuan, China. The dried roots of the plant were extracted with methanol (MeOH), and the extracts were fractionated by silica gel column chromatography. The separation of the fractions via preparative high-performance liquid chromatography (HPLC) using various types of columns afforded seven new compounds (**1 – 7**), an inseparable mixture of diastereomeric isomers of new compounds (**8** and **9**), along with 13 known compounds. By comparison of spectroscopic data with data in literatures, the known compounds were identified as six dihydrobenzofurans (**10**,[7] **11**,[8] **12**,[9] **13**,[6] **14**,[10,11] and **15**[12]), two benzofurans (**16**[7,13] and **17**[14]), one enyne-type compound (**18**[6]), one dihydroxy-formyl-acetophenone (**19**[15]), and three propynyl thiophenes (**20**,[16] **21**,[16] and **22**[6]) (Figure 1).

Compound **1** was isolated as a yellow amorphous powder, and its molecular formula $C_{13}H_{14}O_4$ was determined based on the molecular ion (M$^+$) peak at m/z 234.0891 in the high-resolution chemical ionization mass spectrometry (HR-CI-MS) (calculated molecular mass for $C_{13}H_{14}O_4 = 234.0892$). The infrared (IR) spectrum showed the presence of hydroxyl (3390 cm^{-1}) and conjugated carbonyl groups (1643 cm^{-1}). The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) showed signals attributable to an acetyl [δ_H 2.84 (s, H$_3$-2′), δ_C 204.2 (C-1′)], an allylic methyl [δ_H 1.75 (s, H$_3$-10), δ_C 17.7 (C-10)], mutually *ortho*-coupled aromatic protons [δ_H

[1]Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
[2]Kunming Institute of Botany, Chinese Academy of Science, Kunming, China

Corresponding Authors:
Takashi Tanaka, Department of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.
Email: t-tanaka@nagasaki-u.ac.jp
Yoshinori Saito, Department of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan.
Email: saiyoshi@nagasaki-u.ac.jp
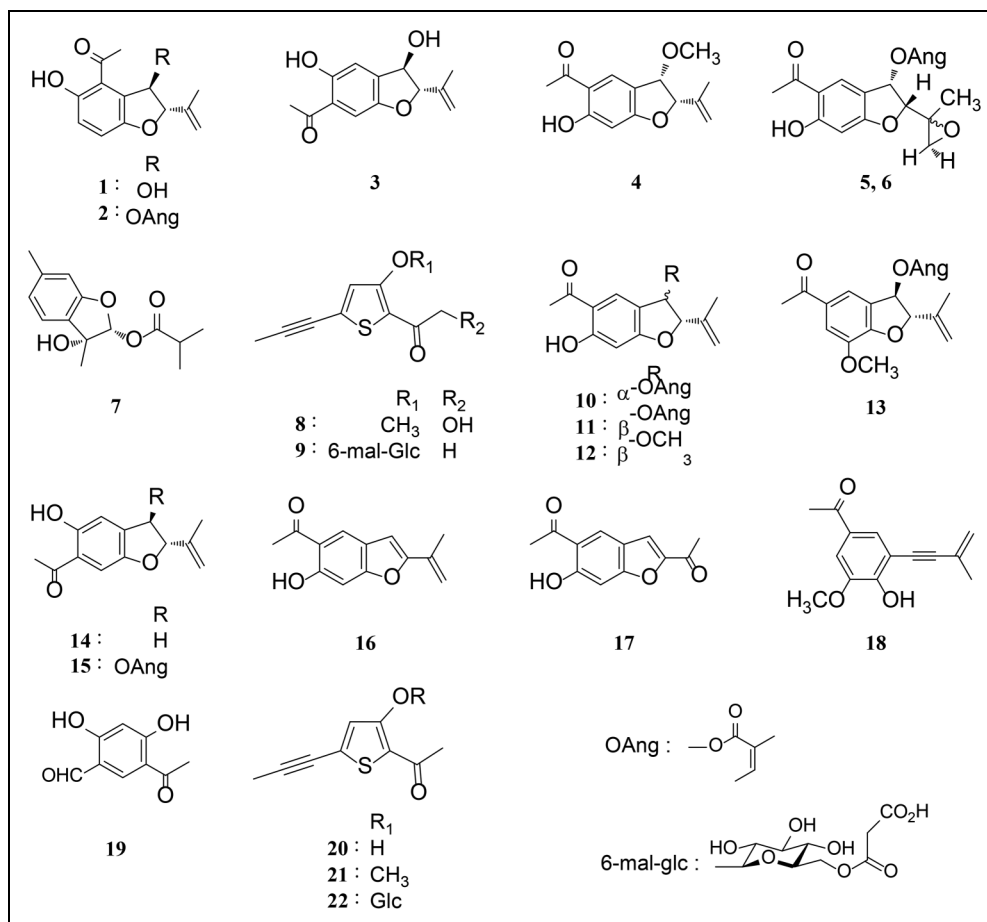
Figure 1. Structures of new (**1**-**9**) and known (**10**-**22**) compounds isolated from the dried roots of *E. heterophyllum* (Sichuan, China).

7.10 (d, J = 8.8 Hz, H-7); 6.97 (d, J = 8.8 Hz, H-6)], and an exo-methylene group [δ_H 5.06 (d, J = 0.7 Hz, H-9a), 4.92 (d, J = 0.7 Hz, H-9b); δ_C 112.7 (C-9)]. Additionally, the hydroxyl proton signal at δ_H 12.25 (s, 5-OH) suggested the formation of a hydrogen bond between a hydroxyl group and a carbonyl group. The ^{1}H–^{1}H homonuclear correlated spectroscopy (COSY) and proton-detected heteronuclear multiple bond correlation (HMBC) spectra (Figure 2) indicated that **1** is a benzofuran-type compound with two hydroxyl groups at C-3 and C-5 and an acetyl group at C-4. The relative stereochemistry of C-2 and C-3 was found to have a *trans* geometry on the basis of the nuclear overhauser effect between C-3-OH and H-2 and between H-3 and allylic methyl (H-10). This geometry was supported by the small $J_{H-2-H-3}$ (1.7 Hz) observed.[17,18] Based on these spectroscopic results, **1** was determined to be 4-acetyl-3β,5-dihydroxy-2α-(propen-2-yl)-2,3-dihydrobenzofuran. The absolute configuration of **1** was not determined.

Compound **2** was obtained as a yellow amorphous powder. The molecular formula was determined to be $C_{18}H_{20}O_5$ via HR-CI-MS. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) resembled those of **1**, suggesting that **2** is also a benzofuran derivative that is related to **1**. Comparison of the NMR signals of **1** and **2** showed that the spectra of **2** showed additional signals arising from

an angeloyl ester group [δ_H 6.18 (qq, J = 7.1,1.5 Hz, H-3″); 1.96 (dq, J = 7.1,1.5 Hz, H-4″); 1.87 (quint, J = 1.5 Hz, H-5″), δ_C 167.2 (C-1″), 126.8 (C-2″), 140.6 (C-3″), 16.0 (C-4″), 20.5 (C-5″)]. This was accompanied by a large low field shift of H-3 to δ_H 6.38 (Δδ 1.07) in the ^{1}H NMR spectrum. Relative stereochemistry was the same for **1** and **2** based on the NOESY correlations (Figure 3). These observations indicated that **2** is 4-acetyl-3β-angeloyloxy-5-hydroxy-2α-(propen-2-yl)-2,3-dihydrobenzo-furan. This compound is a positional isomer of the known compound **11** (Figure 1).

The molecular formula of **3** was found to be the same as that of **1** by HR-CI-MS. The ^{1}H and ^{13}C NMR results (Table 1) obtained for **3** and **1** suggested a benzofuran skeleton for both molecules; thus, we concluded that **3** and **1** are isomers. However, two aromatic protons in **3** appeared as singlet signals [δ_H 7.18 (s, H-7); 7.01 (s, H-4)], and ^{1}H–^{1}H COSY, HMBC, and NOESY correlations (Figure 4) indicated that the location of the hydroxyl and acetyl groups in **3** were in C-5 and C-6, respectively. The HMBC and NOESY correlations also confirmed that the structure of the furan ring of **3** is the same as that of **1**. Accordingly, **3** was identified as 6-acetyl-3β,5-dihydroxy-2α-(propen-2-yl)-2,3-dihydrobenzofuran.

Table 1. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR Data of Compounds **1-3**.

Position	**1** δ_H	(mult., J in Hz)	δ_C	**2** δ_H	(mult., J in Hz)	δ_C	**3** δ_H	(mult., J in Hz)	δ_C
2	4.95	(1H, br s)	93.2	4.99	(1H, d, 1.2)	90.7	4.85	(1H, d, 4.6)	93.5
3	5.31	(1H, dd, 9.6, 1.7)	78.0	6.38	(1H, d, 1.2)	78.8	5.13	(1H, dd, 7.6, 4.6)	76.6
3a	–	–	124.9	–	–	120.3	–	–	137.4
4	–	–	116.6	–	–	116.7	7.01	(1H, s)	114.9
5	–	–	158.1	–	–	158.5	–	–	157.7
6	6.97	(1H, d, 8.8)	122.0	7.05	(1H, d, 9.0)	122.6	–	–	120.0
7	7.10	(1H, d, 8.8)	119.3	7.17	(1H, d, 9.0)	119.3	7.18	(1H, s)	109.6
7a	–	–	153.2	–	–	154.8	–	–	151.9
8	–	–	141.0	–	–	140.2	–	–	141.2
9	5.06	(1H, d, 0.7)	112.7	5.07	(1H, d, 1.0)	113.5	5.12	(1H, d, 0.5)	113.0
	4.92	(1H, d, 0.7)		4.98	(1H, d, 1.0)		4.96	(1H, d, 0.5)	
10	1.75	(3H, s)	17.7	1.87	(3H, s)	18.4	1.77	(3H, s)	17.5
1'	–	–	204.2	–	–	203.2	–	–	204.0
2'	2.84	(3H, s)	30.4	2.55	(3H, s)	30.1	2.61	(3H, s)	27.0
3-OH	2.32	(1H, d, 9.6)	–				2.25	(1H, d, 7.6)	–
3-OCH$_3$									
5-OH	12.25	(1H, s)	–	12.42	(1H, s)	–	12.05	(1H, s)	–
1"				–	–	167.2			
2"				–	–	126.8			
3"				6.18	(1H, qq, 7.1, 1.5)	140.6			
4"				1.96	(3H, dq, 7.1, 1.5)	16.0			
5"				1.87	(3H, quint, 1.5)	20.5			

Abbreviation: Measured in CDCl$_3$.
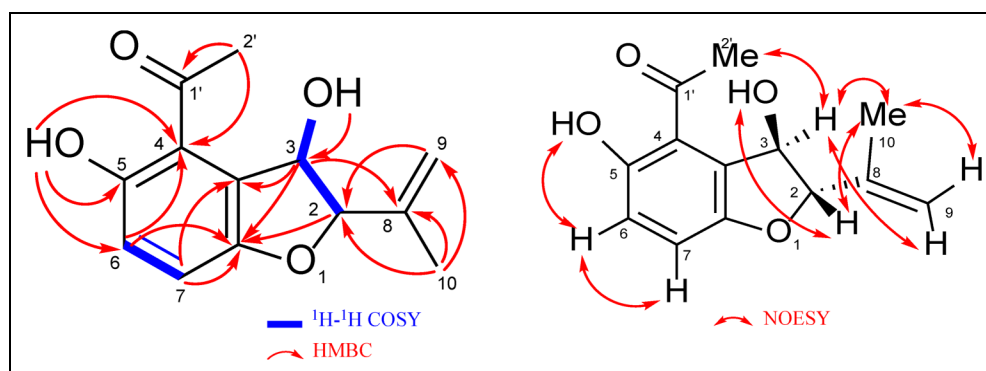
Figure 2. Key 2D NMR correlations for compound **1**.

The ^{1}H and ^{13}C NMR spectra of **4** were closely related to known compound **12** (Figure 1). The HR-CI-MS analyses of **4** and **12** suggested a molecular weight of 248 amu for both compounds. The difference was found in the ^{1}H NMR signals of their furan ring part, where the $J_{H-2-H-3}$ values of **4** and **12** were 6.1 and 2 Hz,[9] respectively (Table 2). After considering the HMBC and NOESY correlations shown in Figure 5, **4** was determined to be 5-acetyl-6-hydroxy-3α-methoxyl-2α-(propen-2-yl)-2,3-dihydrobenzofuran.

Compounds **5** and **6** were obtained as an inseparable mixture (detected in a ratio of 3:1 [**5**:**6**] based on the integration of ^{1}H NMR signals). The CI-MS of the mixture exhibited one [M + H]$^+$ peak at m/z 333, and the HR-CI-MS confirmed a molecular formula of $C_{18}H_{20}O_6$, which was 16 amu larger than that found for **10** and **2**. The ^{1}H NMR signals obtained from **5** and **6** were distinguishable (Table 2), and their chemical shifts and HMBC correlations (Figure 6) suggested structural similarities of these compounds to **10**. Nevertheless, instead of the olefinic carbons of C-8 and C-9 found in **10**, signals attributable to oxymethylene protons were observed [**5**: δ_H 2.90 (d, J = 4.6 Hz, H-9a); 2.71 (d, J = 4.6 Hz, H-9b), **6**: δ_H 3.12 (d, J = 4.6 Hz, H-9a); 2.77 (d, J = 4.6 Hz, H-9b)]. Additionally, the C-10 methyl protons were shifted to the upper field (Δδ - 0.4 compared with that of **10**). Considering the unsaturation index of 9 for both **5** and **6**, these compounds were decided to be a pair of diastereomeric isomers of epoxides
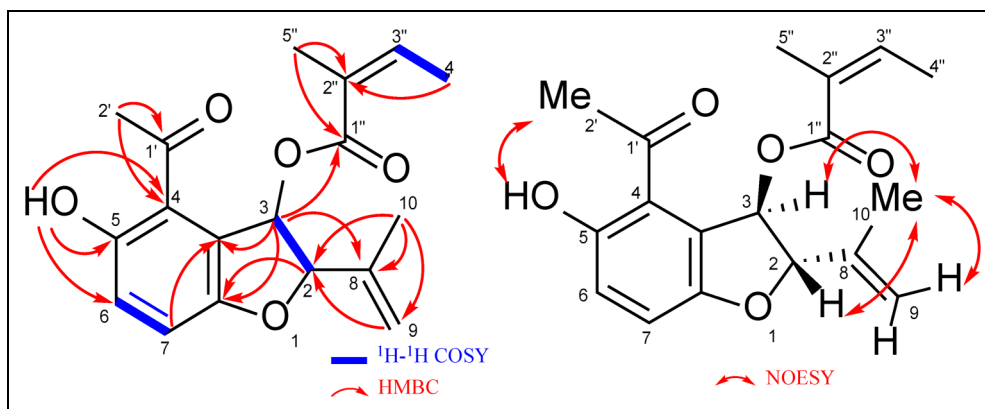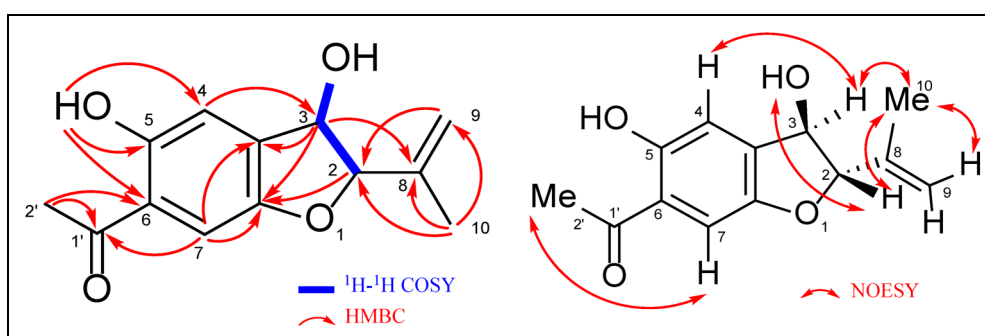
Figure 3. Key 2D NMR correlations for compound **2**.

Figure 4. Key 2D NMR correlations for compound **3**.

of **10** as shown in Figure 6. The chemical shifts of C-8 and C-9 supported the formation of epoxides at these positions (Table 2). Thus, **5** and **6** were characterized as C-8 epimers of 5-acetyl-3α-angeloyloxy-6-hydroxy-2α-(2-methyloxiran-2-yl)-2,3-dihydrobenzofuran.

Compound **7** was isolated as a colorless amorphous powder. The molecular formula corresponded to $C_{14}H_{18}O_4$ based on HR-CI-MS. The ^1H NMR spectrum (Table 3) showed signals due to a 12,4-trisubstituted aromatic ring [δ_H 6.76 (s, H-2), 7.22 (d, $J = 7.6$ Hz, H-5), 6.85 (dd, $J = 7.6$, 0.5 Hz, H-6)] and an isobutanoyl group [δ_H 2.59 (sept, $J = 7.1$ Hz, H-2'), 1.19 (d, $J = 7.1$ Hz, H_3-3'), 1.18 (d, $J = 7.1$ Hz, H_3-4')]. The presence of an ester moiety was supported by the strong absorption at 1747 cm^{-1} in the IR spectrum. The presence of an acetal methine carbon [δ_H 6.50 (s, H-9), δ_c 104.9 (C-9)] was shown by ^{13}C NMR and heteronuclear single quantum coherence spectra. The large chemical shift value of the C-9 methine proton indicated the esterification of an acetal hydroxy group. In the HMBC spectrum, correlations from H_3 to 7 (δ_H 2.35, s) to C-1 (δ_c 141.6), C-2 (δ_c 111.7) and C-6 (δ_c 123.1), and from H_3 to 10 (δ_H 1.62, s) to C-4 (δ_c 127.6), C-8 (δ_c 79.8) and C-9 (δ_c 104.9) revealed the presence of a menthane skeleton in **7**. Furthermore, the HMBC correlation of H-9 acetal proton with C-3 oxygenated aromatic carbon (δ_c 158.5) and C-1' ester carbonyl carbon (δ_c 175.7) suggested a planar structure of **7**

(Figure 7). As for relative stereochemistry, the NOESY correlation between H-9 and H_3 to 10 indicated that these protons were located at the same side of the molecule. Based on these results, **7** was identified as 3,9β-epoxy-9α-isobutanoyloxymentha-13,5-trien-8α-ol.

^1H and ^{13}C NMR data of **8** and **9** were closely related to those of known compounds **21** and **22** (Figure 1), respectively. This suggests that **8** and **9** are propyn-1-yl thiophene derivatives. The presence of a triple bond in each compound was confirmed by absorptions (**8**: 2229 cm^{-1}; **9**: 2229 cm^{-1}) in the IR spectra and quaternary carbon signals [**8**: δ_C 73.1 (C-6), 96.2 (C-7); **9**: δ_C 73.2 (C-6), 94.8 (C-7)] in the ^{13}C NMR spectra (Table 4). The differences in the NMR spectra of **8** and **21** were the appearance of the signals attributable to a hydroxymethyl group in **8** [δ_H 4.63 (d, $J = 4.7$ Hz, H_2-2'), 3.55 (t, $J = 4.7$ Hz, 2'-OH); δ_C 67.8 (C-2')] instead of the signals of C-2' methyl group in **21**. Thus, **8** was given the structure shown in Figure 8 and was identified as 2-(hydroxyacetyl)-3-methoxy-5-(propyn-1-yl)thiophene. The HMBC and NOESY correlations illustrated in Figure 8 were consistent with the proposed structure.

Compared with the NMR spectra of **22**, **9** showed additional signals from a malonate group [δ_H 3.87 (d, $J = 15.7$ Hz, H-2‴a), 3.82 (d, $J = 15.7$ Hz, H-2‴b); δ_C 42.0 (C-2‴), 167.3 (C-1‴), 169.0 (C-3‴)] and large low field shifts of glucose H_2 to 6

Table 2. ^1H (500 MHz) and ^{13}C (126 MHz) NMR Data of Compounds **4**-**6**.

	4			**5**			**6**		
Position	δ_H	(mult., J in Hz)	δ_C	δ_H	(mult., J in Hz)	δ_C	δ_H	(mult., J in Hz)	δ_C
2	5.01	(1H, d, 6.1)	90.6	4.56	(1H, d, 7.0)	89.0	4.79	(1H, d, 5.9)	85.8
3	4.82	(1H, d, 6.1)	79.5	6.40	(1H, d, 7.0)	72.0	6.31	(1H, d, 5.9)	71.9
3a	–	–	118.8	–	–	117.5	–	–	117.5
4	7.74	(1H, s)	128.8	7.85	(1H, s)	130.1	7.94	(1H, s)	130.7
5	–	–	114.2	–	–	114.9	–	–	114.9
6	–	–	167.0	–	–	167.1	–	–	167.1
7	6.46	(1H, s)	99.1	6.46	(1H, s)	99.0	6.46	(1H, s)	99.2
7a	–	–	166.5	–	–	166.3	–	–	166.9
8	–	–	139.3	–	–	54.9	–	–	54.9
9	5.26	(1H, d, 0.7)	113.9	2.90	(1H, d, 4.6)	50.4	3.12	(1H, d, 4.6)	51.2
	5.13	(1H, q, 1.5)		2.71	(1H, d, 4.6)		2.77	(1H, d, 4.6)	
10	1.89	(3H, s)	19.3	1.45	(3H, s)	17.5	1.48	(3H, s)	18.8
1'	–	–	202.4	–	–	202.9	–	–	202.9
2'	2.59	(3H, s)	26.3	2.56	(3H, s)	26.4	2.57	(3H, s)	26.5
3-OH									
3-OCH$_3$	3.35	(3H, s)	56.5						
5-OH									
6-OH	13.01	(1H, s)	–	13.01	(1H, s)	–	13.01	(1H, s)	–
1"				–	–	167.2	–	–	167.2
2"				–	–	126.8	–	–	126.8
3"				6.18	(1H, qq, 7.1, 1.4)	140.7	6.18	(1H, qq, 7.1, 1.4)	140.7
4"				2.04	(3H, dq, 7.1, 1.4)	15.9	2.01	(3H, dq, 7.1, 1.4)	15.9
5"				1.90	(3H, m)	20.5	1.84	(3H, m)	20.4

Abbreviation: Measured in CDCl$_3$.
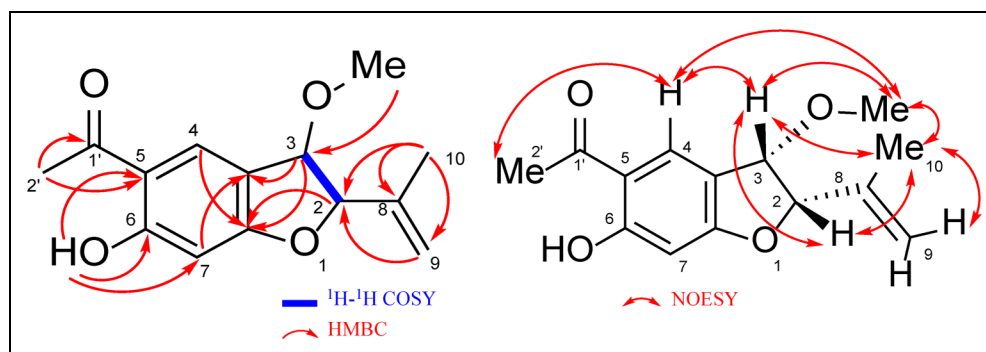
Figure 5. Key 2D NMR correlations for compound **4**.

[δ_H 5.16 (dd, $J = 11.6$, 1.9 Hz, H-6a''), 4.80 (dd, $J = 11.6$, 7.1 Hz, H-6b'')] (Table 4). HMBC correlations between glucose H$_2$ to 6 and malonate H$_2$ to 2''' to the same ester carbonyl carbon at δ_C 167.3 (C-1''') confirmed the esterification of the malonyl group to glucose C-6 position (Figure 9). Based on these observations, **9** was identified as 2-acetyl-3-hydroxy-5-(propyn-1-yl)thiophene 3-O-(6-O-malonyl)-β-glucoside.

This study isolated and identified nine new and 13 known compounds in the roots of *E. heterophyllum*. These compounds mainly belonged to benzofurans, dihydrobenzofurans, and propynyl thiophenes. The major constituents of the sample were a benzofuran (**16**) and two dihydrobenzofurans (**10** and **12**). This finding was consistent with a similar previous study.[6] Interestingly, the aerial parts of this plant have been shown to contain mainly sesquiterpenoids and displayed considerable chemical diversity, which was dependent on the collection site.[5] However, our results clearly suggested a lower chemical diversity in the roots.[6] The difference in the chemical diversity between the aerial parts and roots may be important for plant physiology and chemical ecology. For example, biosynthesis in the aerial part was most likely sensitive to changes in the environmental conditions, whereas biosynthesis in the roots was generally not affected by environmental factors. Our results also raise the question of why the root and aerial parts of the studied plant show different biosynthetic systems.
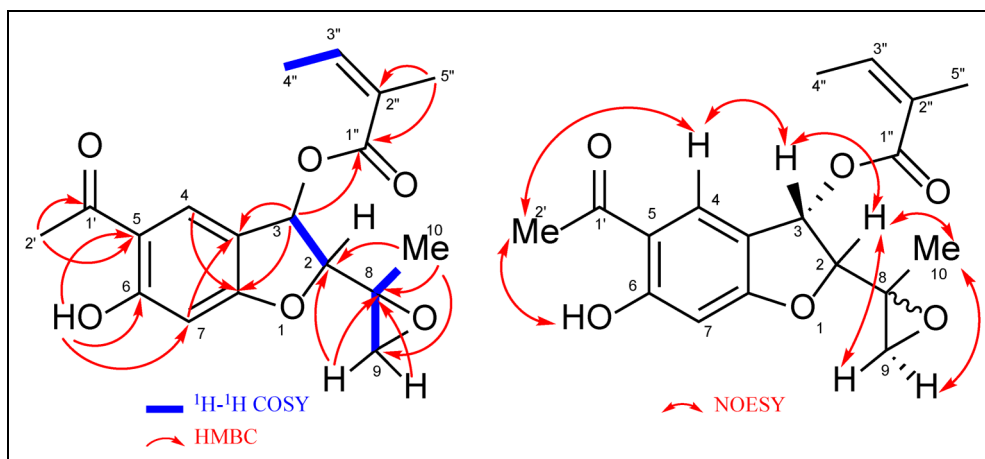
Figure 6. Key 2D correlations for compounds **5** and **6**.

Table 3. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR Data of 7.

	δ_H	(*mult., J* in Hz)	δ_C
1	–	–	141.6
2	6.76	(1H, s)	111.7
3	–	–	158.5
4	–	–	127.6
5	7.22	(1H, d, 7.6)	122.7
6	6.85	(1H, dd, 7.6, 0.5)	123.1
7	2.35	(3H, s)	21.7
8	–	–	79.8
9	6.50	(1H, s)	104.9
10	1.62	(3H, s)	20.1
1'	–	–	175.7
2'	2.59	(1H, sept, 7.1)	34.1
3'	1.19	(3H, d, 7.1)	18.8
4'	1.18	(3H, d, 7.1)	18.5
8-OH	2.03	(1H, s)	–

Abbreviation: Measured in CDCl$_3$.

Experimental

General

The IR spectra were obtained using an FT/IR-400 spectrophotometer (JASCO, Tokyo, Japan). The ^{1}H, ^{13}C, and 2D NMR spectra were measured using a Varian Unity *plus* 500 spectrometer (^{1}H: 500 MHz; ^{13}C: 126 MHz), JEOL JNM-AL 400 spectrometer (^{1}H: 400 MHz; ^{13}C: 100 MHz). The mass spectra were obtained with JEOL JMS-700N mass spectrometer. Preparative HPLC columns used were COSMOSIL 5C$_{18}$-PAQ (20 mm i.d. × 250 mm), COSMOSIL 5SL-II (4.6 mm i.d. × 250 mm), and COSMOSIL 5SL-II (10 mm i.d. × 250 mm) from Nacalai Tesque Inc. (Kyoto, Japan); Inertsil CN-3 (4.6 mm i.d. × 250 mm) from GL Science (Tokyo, Japan); and YMC-Pack Diol-120-NP (4.6 mm i.d. × 250 mm) from YMC Co., Ltd (Kyoto, Japan). Preparative HPLC was with a JASCO PU2080 pump system. Silica gel 60N (100-210 μm) (Kanto Chemical Co., Inc., Tokyo, Japan) was used for column chromatography. TLC was with silica gel 60 F254 plates (Merck).

Plant Materials

The plant materials were collected in Maoxian, Sichuan, China during August 2015. A voucher specimen (#2015-25) was deposited at the herbarium of Kunming Institute of Botany. The plant was identified by Dr Takayuki Kawahara, Japan Forest Technology Association, General Incorporated Association, Japan.

Extraction and Isolation

The dried root (122.5 g) of *E. heterophyllum* was extracted twice with MeOH at room temperature. The extract (16.0 g) was separated by silica gel column chromatography (50 mm i.d. × 250 mm, *n*-hexane:CHCl$_3$ = 9:1, 7:3, 1:1, CHCl$_3$:MeOH = 1:0, 98:2, 95:5, 9:1, 7:3, 0:1) into nine fractions: Fr. A–I. Fr. C (344 mg) was separated by preparative HPLC using COSMOSIL 5SL-II (10 mm i.d. × 250 mm, 6 ml/min, *n*-hexane:EtOAc = 95:5) to yield **20** (44.7 mg) and **16** (166.2 mg). Fr. D (389 mg) was separated into six subfractions by silica gel column chromatography (20 mm i.d. × 150 mm, *n*-hexane:EtOAc = 1:0, 99:1, 98:2, 96:4, 94:6, 9:1, 8:2, EtOAc, MeOH). Fr. D-3 (259 mg) was identified as **10**. Fr. D-2 (82.7 mg) was separated by COSMOSIL 5SL-II (10 mm i.d. × 250 mm, 6 ml/min, *n*-hexane:EtOAc = 9:1) to give Fr. D-2 to 1 (26.0 mg), **11** (33.1 mg), and **16** (13.2 mg). Separations of the Fr. D-2 to 1 were done via successive preparative HPLC using YMC-Pack Diol-120-NP (4.6 mm i.d. × 250 mm, 2 ml/min, *n*-hexane:EtOAc = 95:5), YMC-Pack Diol-120-NP (4.6 × 250 mm, 1 ml/min, *n*-hexane:EtOAc = 99:1), and Inertsil CN-3 (4.6 mm i.d. × 250 mm, 1 ml/min, *n*-hexane:EtOAc = 99.5:0.5). These afforded **14** (0.8 mg), **15** (1.9 mg), and **2** (0.6 mg). Fr. D-4 (19.1 mg) was separated by preparative HPLC using COSMOSIL 5SL-II (4.6 mm i.d. × 250 mm, 2 ml/min, *n*-hexane:EtOAc = 9:1) and yielded **21** (0.2 mg), **13** (0.5 mg), and a mixture (5:1) of **12** and **4** (7.9 mg). Fr. E (1.39 g) was separated by silica gel column chromatography (30 mm i.d. × 150 mm, *n*-hexane:EtOAc = 1:0, 98:2, 96:4, 94:6, 92:8, 9:1, 85:15, 8:2, 7:3, EtOAc, MeOH) and gave seven fractions. Fr. E-2 (637 mg) was identified as **10**. Fr. E-3 (246 mg) was a mixture (14:1) of **12** and
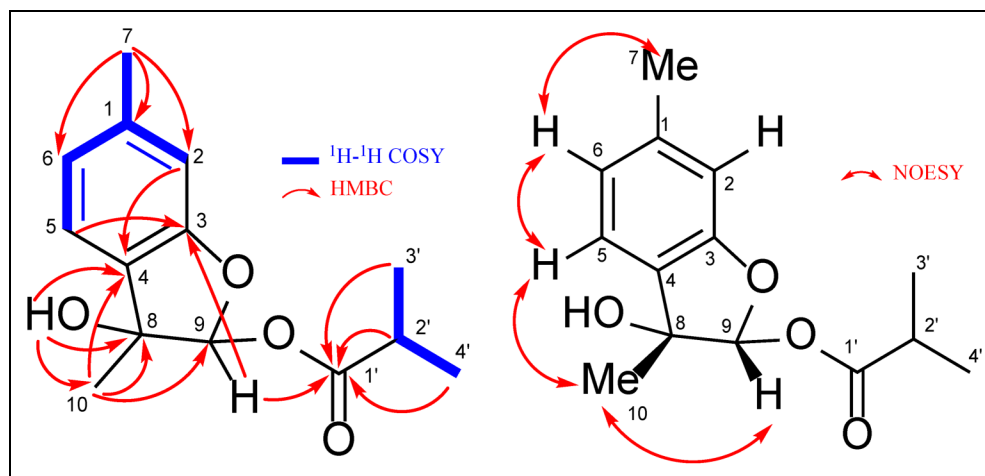
Figure 7. Key 2D NMR correlations for compound **7**.

Table 4. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR Data of Compounds **8** and **9**.

No	**8**[a] δ_H	(*mult., J* in Hz)	δ_C	**9**[b] δ_H	(*mult., J* in Hz)	δ_C
2	–	–	118.0	–	–	124.7
3	–	–	161.2	–	–	156.3
4	6.83	(1H, s)	118.7	7.57	(1H, s)	123.8
5	–	–	132.9	–	–	129.3
6	–	–	73.1	–	–	73.2
7	–	–	96.2	–	–	94.8
8	2.11	(3H, s)	4.9	1.97	(3H, s)	3.7
1'	–	–	190.6	–	–	189.3
2'	4.63	(2H, d, 4.7)	67.8	2.70	(3H, s)	29.1
3-OCH$_3$	3.96	(3H, s)	59.0			
2'-OH	3.55	(1H, t, 4.7)	–			
1"				5.54	(1H, d, 7.3)	103.0
2"				4.28	(1H, m)	73.9
3"				4.28	(1H, m)	77.9
4"				4.14	(1H, dd, 9.6, 8.5)	70.5
5"				4.28	(1H, m)	75.1
6"				5.16	(1H, dd, 11.6, 1.9)	64.5
				4.80	(1H, dd, 11.6, 7.1)	
1'''				–	–	167.3
2'''				3.87	(1H, d, 15.7)	42.0
				3.82	(1H, d, 15.7)	
3'''				–	–	169.0

[a]Measured in CDCl$_3$.
[b]Measured in pyridine-d_5.

4. Preparative HPLC of Fr. E-4 (41.8 mg) was with COSMOSIL 5SL-II (10 mm i.d. × 250 mm, 6 ml/min, *n*-hexane:EtOAc = 85:15) and gave **19** (0.9 mg), a mixture (2:1) of **12** and **4**, and fractions E-4 to 3 and E-4 to 4. Preparative HPLC of Fr. E-4 to 3 (4.2 mg) was with YMC-Pack Diol-120-NP (4.6 mm i.d. × 250 mm, 2 ml/min, *n*-hexane:EtOAc = 85:15) and afforded **7** (1.0 mg). Fr. E-4 to 4 (4.5 mg) was subjected to preparative HPLC using YMC-Pack Diol-120-NP (4.6 mm i.d. × 250 mm, 2 ml/min, *n*-hexane:EtOAc = 85:15) and gave **4** (0.8 mg), **1** (1.3 mg), and a mixture (3:1) of **5** and **6** (1.9 mg). Preparative HPLC of Fr. E-5 (54.9 mg) and Fr. E-6 (62.9 mg) using COSMOSIL 5SL-II (10 mm i.d. × 250 mm, 6 ml/min, *n*-hexane:EtOAc = 85:15 for Fr. E-5; 8:2 for Fr. E-6) yielded **1** (8.5 mg) and **3** (4.6 mg) from Fr. E-5 and **18** (4.6 mg), **8** (5.4 mg), and **17** (7.3 mg) from Fr. E-6. Fractionation of Fr. H (1.10 g) via silica gel column chromatography (30 mm i.d. × 150 mm, CHCl$_3$:MeOH = 95:5, 92:8, 9:1, 88:12, 85:15, 4:1, 7:3, 0:1) gave five subfractions. The Fr. H-3 (539 mg) was identified as **22**. Further separation of Fr. H-5 (108 mg) via silica gel column chromatography (10 mm i.d. × 150 mm, CHCl$_3$:MeOH = 4:1, 3:1, 7:3, 1:1,0:1) gave **22** (28.4 mg) and **9** (10.5 mg). Fr. H-5 to 2 (40.8 mg) was separated via preparative HPLC using COSMOSIL 5C$_{18}$-PAQ (20 mm i.d. × 250 mm, 6 ml/min, H$_2$O:MeOH = 4:6) and yielded **22** (3.3 mg) and **9** (10.0 mg).

4-Acetyl-3β,5-dihydroxy-2α-(propen-2-yl)-2,3-dihydro-benzofuran (1)

A yellow powder, and ^{1}H and ^{13}C NMR data: in Table 1. FT-IR cm^{-1}: 3390, 1643. CI-MS *m/z*: 234.0891 (calcd for C$_{13}$H$_{14}$O$_4$: 234.0892). MS (CI) *m/z*: 234 (M) $^+$. [α]27 D + 1.0 (*c* = 0.9, CHCl$_3$).

4-Acetyl-3β-angeloyloxy-5-hydroxy-2α-(propen-2-yl)-2,3-dihydrobenzofuran (2)

A yellow powder, ^{1}H and ^{13}C NMR data: in Table 1. FT-IR cm^{-1}: 3500 to 2600, 1715, 1643, 1473, 1442, 1144. CI-MS *m/z*: 316.1313 (calcd for C$_{18}$H$_{20}$O$_5$: 316.1311). MS (CI) *m/z*: 316 (M) $^+$. [α]29 D + 12.0 (*c* = 0.05, CHCl$_3$).

6-Acetyl-3β,5-dihydroxy-2α-(propen-2-yl)-2,3-dihydro-benzofuran (3)

A yellow powder, ^{1}H and ^{13}C NMR data: in Table 1. FT-IR cm^{-1}: 3400, 1643, 1237. CI-MS *m/z*: 234.0890 (calcd for C$_{13}$H$_{14}$O$_4$: 234.0892). MS (CI) *m/z*: 234 (M) $^+$. [α]28 D − 20.3 (*c* = 0.5, CHCl$_3$).
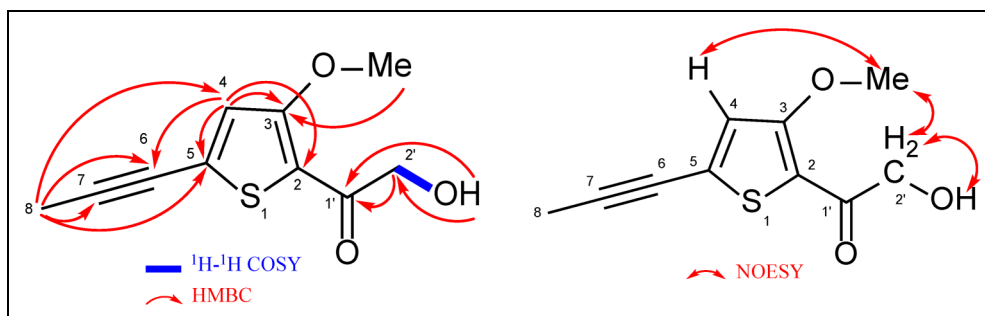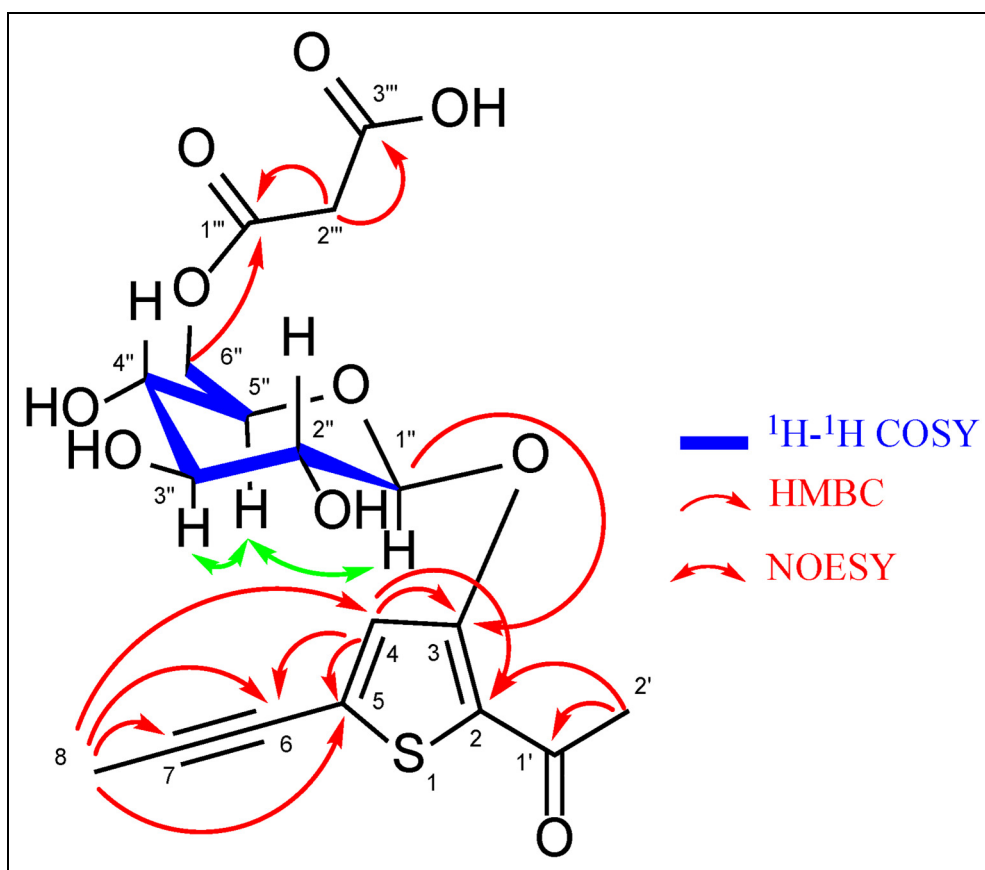
Figure 8. Key 2D NMR correlations for compound **8**.

Figure 9. Key 2D NMR correlations for compound **9**.

5-Acetyl-6-hydroxy-3α-methoxyl-2α-(propen-2-yl)-2,3-dihydrobenzofuran (4)

A yellow powder, ^{1}H and ^{13}C NMR data: in Table 2. FT-IR cm^{-1}: 3200 to 2600, 1632, 1147. CI-MS m/z: 248.1053 (calcd for $C_{14}H_{16}O_4$: 248.1049). MS (CI) m/z: 248 (M)$^+$. [α]30 D + 12.6 (*c* = 0.06, CHCl$_3$).

5-Acetyl-3α-angeloyloxy-6-hydroxy-2α-(2-methyloxiran-2-yl)-2,3-dihydrobenzofuran (5 and 6)

A yellow powder, ^{1}H and ^{13}C NMR data: in Table 2. FT-IR cm^{-1}: 3600 to 2500, 1715, 1651, 1158, 1047. CI-MS m/z: 333.1340 (calcd for $C_{18}H_{21}O_6$: 333.1338). MS (CI) m/z: 333 (M + H)$^+$. [α]30 D + 83.8 (*c* = 0.1, CHCl$_3$).

3,9β-Epoxy-9α-isobutanoyloxymentha-13,5-trien-8α-ol (7)

A colorless amorphous powder, ^{1}H and ^{13}C NMR data: in Table 3. FT-IR cm^{-1}: 3306, 1747, 1187, 1155, 1126. CI-MS m/z: 250.1202 (calcd for $C_{14}H_{18}O_4$: 250.1205). MS (CI) m/z: 250 (M)$^+$. [α]29 D + 2.3 (*c* = 0.1, CHCl$_3$).

2-(Hydroxyacetyl)-3-methoxy-5-(propyn-1-yl)thiophene (8)

A yellow powder, ^{1}H and ^{13}C NMR data: in Table 4. FT-IR cm^{-1}: 3456, 2229, 1638, 1541, 1108. CI-MS m/z: 211.0428 (calcd for $C_{10}H_{11}O_3S$: 211.0429). MS (CI) m/z: 211 (M + H)$^+$.

2-Acetyl-3-hydroxy-5-(propyn-1-yl)thiophene   3-**O**-(6-**O**-malonyl)-β-glucoside (9)

A yellow powder, ^{1}H and ^{13}C NMR data: in Table 4. FT-IR cm^{-1}: 3412, 2229, 1746, 1632, 1455, 1405, 1076. FAB-MS *m/z*: 427.0687 (calcd for $C_{18}H_{19}O_{10}S$: 427.0699). MS (FAB, negative mode) *m/z*: 427 (M-H)$^-$. [α]29 D + 2.3 (*c* = 0.9, MeOH).

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

ORCID iDs

Yoshinori Saito https://orcid.org/0000-0002-3587-9743
Takashi Tanaka https://orcid.org/0000-0001-7762-7432

References

1. Schmidt GJ, Schilling EE. Phylogeny and biogeography of Eupatorium (asteraceae: eupatorieae) based on nuclear ITS sequence data. *Am. J. Bot.* 2000;87(5):716-726.

2. Tori M, Takeichi Y, Kuga H, Nakashima K, Sono M. Two new chlorine-containing germacranolides, eupaglehnins E and F from *Eupatorium glehni. Heterocycles.* 2000;52(3):1075-1078.

3. Tori M, Takeichi Y, Kuga H, Nakashima K, Sono M. Seven germacranolides, eupaglehnins A, B, C, D, E, and F, and 2α-acetoxyepitulipinolide from *Eupatorium glehni. Chem. Pharm. Bull.,* 2002;50(9):1250-1254.

4. Tori M, Morishita N, Hirota N, et al. Sesquiterpenoids isolated from *Eupatorium glehnii.* Isolation of guaiaglehnin A, structure revision of hiyodorilactone B, and genetic comparison. *Chem. Pharm. Bull. (Tokyo).* 2008;56(5):677-681.

5. Saito Y, Mukai T, Iwamoto Y, et al. Germacranolides and their diversity of *Eupatorium heterophyllum* collected in P.R. China. *Chem. Pharm. Bull. (Tokyo).* 2014;62(11):1092-1099.

6. Saito Y, Takiguchi K, Gong X, Kuroda C, Tori M. Thiophene, furans, and related aromatic compounds from *Eupatorium heterophyllum. Nat. Prod. Commun.* 2011;6(3):361-366.

7. Bohlmann F, Zdero C. Notiz über die inhaltsstoffe von *Carelia cistifolia* less. *Chem. Ber.* 1971;104(3):964-966.

8. Bohlmann F, Zdero C, Grenz M. Natürlich vorkommende terpenderivate, 81. Weitere inhaltsstoffe aus vertretern der Eupatoriumgruppe. *Chem. Ber.* 1977;110(3):1034-1041.

9. Zdero C, Bohlmann F, King RM. Diterpenes and norditerpenes from the *Aristeguetia* group. *Phytochemistry.* 1991;30(9):2991-3000.

10. Alarcon SR, Fuente JRDL, Novara L, Sosa VE. Benzofuran and coumarins in *Trichocline reptans* wedd. *An. des la Asoc. Quimica Argentina.* 1998;86(3-6):248-251.

11. Yamaguchi S, Nitta T, Hosaka T, Nishino Y, Kawase Y. The synthesis of two 6-acetyl-2,3-dihydro-5-benzofuranols naturally obtained from *Baccharis conferta. Bull. Chem. Soc. Jpn.* 1990;63(11):3230-3232.

12. Bohlmann F, Zdero C. Stevisalicinon. ein neuer diterpentyp, sowie weitere inhaltsstoffe aus *Stevia*-arten. *Liebigs Ann. Chem.* 1985(9):1764-1783.

13. Kamthong B, Robertson A, Furano compounds V. The synthesis of tetrahydroeuparin and the structure of euparin. *J. Chem. Soc.* 1939;925:933-936.

14. Elsohly MA, Doorenbos NJ, Quimby MW, Knapp JE, Slatkin DJ, Schiff PL. Euparone, a new benzofuran from Ruscus aculeatus L. *J. Pharm. Sci.* 1974;63(10):1623-1624.

15. Gupta SR, Seshadri TR, Sood GR. The structure and synthesis of neobavachalcone, a new component of *Psoralea corylifolia. Phytochemistry.* 1977;16(12):1995-1997.

16. Bohlmann F, Kleine KM, Bornowski H. Polyacetylenverbindungen, XLI. Über zwei thiophenketone aus *Artemisia arborescens* L. *Chem. Ber.* 1962;95(12):2934-2938.

17. Zalkow LH, Keinan E, Steindel S, Kalyanaraman AR, Bertrand JA. On the absolute configuration of toxol at C-3. Vicinal H-H coupling constants in 2-alkyl-3-hydroxydihydrobenzofurans. *Tetrahedron Lett.* 1972;13(28):2873-2876.

18. Mertes MP, Powers LJ, Shefter E. Isolation and identification of the cis-trans stereoisomers of substituted 3-hydroxy-(or acetoxy) 2-methyl-2,3-dihydrobenzofurans. Dihydrobenzofurans which obey the Karplus equation. *J. Org. Chem.* 1971;36(13):1805-1807.