HETEROCYCLES, Vol. 83, No. 7, 2011, pp. 1603 - 1610. © The Japan Institute of Heterocyclic Chemistry
Received, 5th April, 2011, Accepted, 25th April, 2011, Published online, 11th May, 2011
DOI: 10.3987/COM-11-12228

NEW FURANOCOUMARINS FROM THE FRUITS OF *MELICOPE TRIPHYLLA*

Ken-ichi Nakashima,[a] Masayoshi Oyama,*,[a] Tetsuro Ito,[a] Hiroko Murata,[b] and Munekazu Iinuma[a]

a) Laboratory of Pharmacognosy, Gifu Pharmaceutical University, 1-25-4 Daigaku-Nishi, Gifu 501-1196, Japan; e-mail: oyama@gifu-pu.ac.jp

b) Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan

Abstract – Four new furanocoumarins (**1**–**4**) were isolated from the fruits of *Melicope triphylla* (Rutaceae), together with two known coumarins (**5**, **6**), nine flavonoids (**7**–**15**), two alkaloids (**16**, **17**), and methyl *p*-geranyloxy-*trans*-cinnamate (**18**). The structures of the newly identified compounds were determined by extensive 1D- and 2D-NMR spectroscopic analyses to be linear-types of furanocoumarins bearing a hydroxyl or a hydroperoxy group on the geranyloxy side chain.

INTRODUCTION

Melicope triphylla (LAM.) MERR. is a rutaceous shrub that grows to 1.5–15 m high, and widely distributed throughout the Pacific islands including the Ryukyus in Japan.[1] Continuous studies by Higa *et al*. have revealed the presence of polymethoxyflavonoids,[2–4] furoquinoline alkaloids,[5] and *p*-coumaric acid derivatives[5] in the leaves of the plant. On the other hand, Wu *et al*. have worked on the root barks and found sesquiterpene lactones.[6,7] However, no phytochemical studies of the fruits have been achieved. Herein we described on the structure elucidation of four new furanocoumarins, as well as fourteen known compounds, isolated from the fruits of *M. triphylla*.

RESULTS AND DISCUSSION

A new coumarin, named melicotriphyllin A (**1**), was obtained as yellow oil with absorption maxima at 312 and 270 nm in the UV spectrum. The HR-ESIMS gave an $[M+Na]^+$ ion peak at *m/z* 407.1458, which established the quasi-molecular formula as $C_{22}H_{24}O_6Na$ (calc. 407.1465). The ^{1}H-NMR spectrum

showed two pairs of mutually coupled doublets at δ_H 6.26, 8.11 (1H each, d, J = 9.7 Hz) and δ_H 7.01, 7.63 (1H each, d, J = 2.4 Hz); the former protons were assignable to H-3 and H-4 in a coumarin skeleton, while the latter were regarded as H-2' and H-3' on a furan ring. A three-proton singlet resonated at δ_H 4.18 to be identified as a methoxy group that exhibited essential NOEs toward H-4 and H-3' in the NOESY spectrum. Therefore, **1** was considered to be a linear furanocoumarin having a methoxy group at C-5.

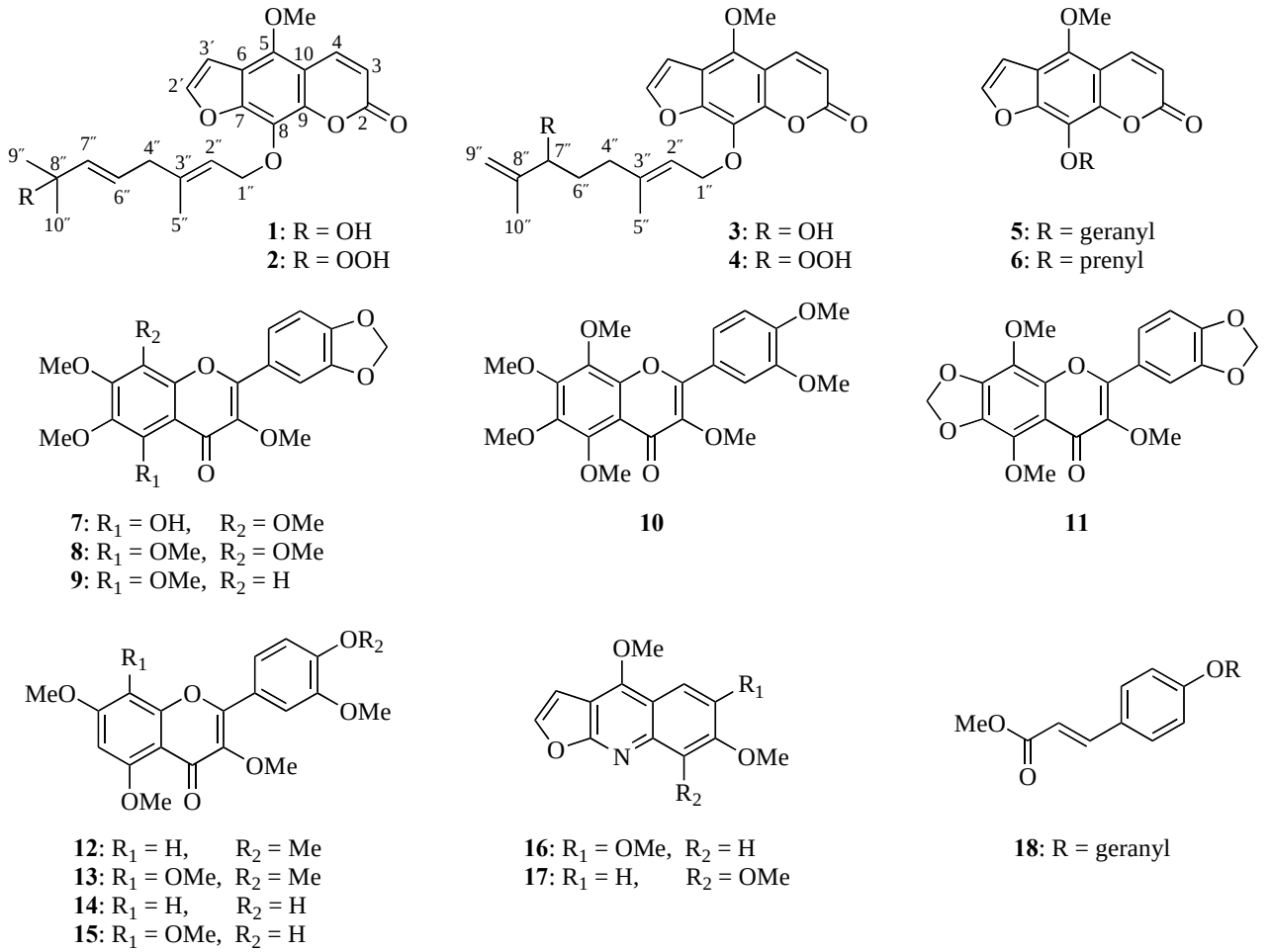

Figure 1. Structure of the compounds **1**–**18** isolated from the fruits of *M. triphylla*

The double quantum filtered COSY (DQF-COSY) spectrum inferred two segments [δ_H 4.86 (2H, d, J = 7.2 Hz), 5.60 (1H, br t, J = 7.2 Hz) for –OCH_2CH=; δ_H 2.68 (2H, d, J = 6.6 Hz), 5.50 (1H, dt, J = 15.9, 6.6 Hz), 5.59 (1H, d, J = 15.9 Hz) for –CH_2CH=CH–] and three methyl groups [δ_H 1.29 (6H, s, overlapping) and 1.64 (3H, s)]. The HMBC spectrum showed a side chain as –OCH_2CH=$C(CH_3)CH_2CH$=$CHC(CH_3)_2OH$ formed from a geranyl or a neryl group by the following correlations: H_2-1"/C-3"; H-2"/C-4", C-5"; H_2-4"/C-7"; H-6"/C-3", C-8"; and H-7"/C-9"(10") (Figure 2). In the EIMS, the base ion peak at *m/z* 233 generated after the loss of $C_{10}H_{16}O$ from the molecular ion also supported the presence of the side chain. In the NOESY spectrum, the key NOEs were observed

between H_2-1"/H_3-5", H-2"/H_2-4" and H_2-4"/H-7", which indicated that the C_{10} side chain was originated from a geranyl group and the configurations were 2"*E* and 6"*E*. The C-8 substitution was demonstrated by a HMBC correlation between H_2-1" (δ_H 4.86) and C-8 (δ_C 126.5). Thus, the structure of melicotriphyllin A was established as **1** (Figure 1).

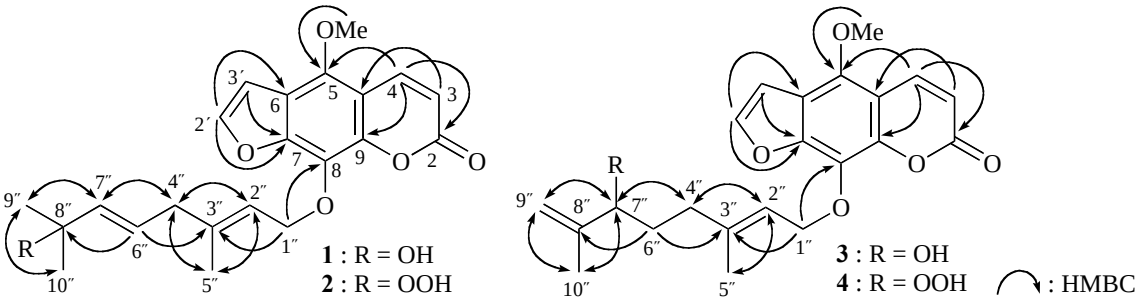

Figure 2. Selected HMBC correlations in **1**–**4**

Table 1. ^{1}H-NMR (400 MHz) data of compounds **1**–**4** in $CDCl_3$

No.	1	2	3	4
3	6.26 (1H, d, 9.7)	6.28 (1H, d, 9.7)	6.27 (1H, d, 9.7)	6.28 (1H, d, 9.7)
4	8.11 (1H, d, 9.7)	8.13 (1H, d, 9.7)	8.12 (1H, d, 9.7)	8.12 (1H, d, 9.7)
2′	7.63 (1H, d, 2.4)	7.63 (1H, d, 2.4)	7.63 (1H, d, 2.4)	7.63 (1H, d, 2.4)
3′	7.01 (1H, d, 2.4)	7.00 (1H, d, 2.4)	7.00 (1H, d, 2.4)	7.00 (1H, d, 2.4)
1″	4.86 (2H, d, 7.2)	4.87 (2H, d, 7.2)	4.87 (2H, m)	4.87 (2H, d, 7.0)
2″	5.60 (1H, br t, 7.2)	5.65 (1H, m)	5.62 (1H, br t, 7.2)	5.62 (1H, br t, 7.0)
4″	2.68 (2H, d, 6.6)	2.73 (2H, d, 5.5)	2.03 (2H, m)	2.04 (2H, br t, 7.2)
5″	1.64 (3H, s)	1.63 (3H, s)	1.67 (3H, s)	1.67 (3H, s)
6″	5.50 (1H, dt, 15.9, 6.6)	5.56 (1H, dt, 15.9, 5.5)	1.58 (2H, m)	1.51 (1H, m)
				1.64 (1H, m)
7″	5.59 (1H, d, 15.9)	5.55 (1H, d, 15.9)	3.93 (1H, t, 6.8)	4.23 (1H, t, 6.8)
9″	1.29 (3H, s)[a)]	1.31 (3H, s)[b)]	4.94 (1H, br s)	4.94 (1H, br s)
			4.99 (1H, br s)	4.99 (1H, t, 1.5)
10″	1.29 (3H, s)[a)]	1.31 (3H, s)[b)]	1.71 (3H, s)	1.71 (3H, s)
5-OMe	4.18 (3H, s)	4.19 (3H, s)	4.15 (3H, s)	4.18 (3H, s)

δ_H in ppm, J in Hz. [a, b)] overlapping signals.

Melicotriphyllin B (**2**), yellow oil, exhibited absorption bands at 313 and 269 nm in the UV spectrum. The quasi-molecular formula of **2** was confirmed to be $C_{22}H_{24}O_7Na$ (calc. 423.1414) based on the $[M+Na]^+$ ion peak at *m/z* 423.1434 in the HR-ESIMS. This compound, then, bears one more oxygen atom than **1**. The ^{1}H-NMR spectrum of **2** was superimposed to that of **1**, which implied that **2** was the similar furanocoumarin as **1** (Table 1). Comparison with both ^{13}C-NMR spectral data, however, showed a significant downfield shift of the carbon signal at C-8" from δ_C 70.4 (**1**) to δ_C 82.0 (**2**) (Table 2), indicating that the hydroxyl group attaching to C-8" in **1** was converted into the hydroperoxy group in **2**.

The reduction of **2** with triphenylphosphine in MeOH at room temperature produced a compound identical to **1**.[8] Accordingly, the structure of melicotriphyllin B was determined as **2**.

Table 2. ^{13}C-NMR (100 MHz) data of compounds **1–4** in $CDCl_3$

No.	1	2	3	4
2	160.5	160.7	160.5	160.7
3	112.5	112.7	112.8	112.7
4	139.4	139.5	139.4	139.5
5	144.3	144.4	144.4[c)]	144.3
6	114.3	114.4	114.5	114.5
7	150.8	150.9	150.9	150.8
8	126.5	126.6	126.7	126.6
9	144.4	144.5	144.4[c)]	144.4
10	107.3	107.4	107.5	107.5
2′	145.0	145.1	145.1	145.1
3′	105.0	105.1	105.1	105.1
1″	70.1	70.2	70.2	70.2
2″	120.2	120.6	119.9	120.3
3″	141.5	141.2	147.4	142.1
4″	41.9	42.2	35.4	35.3
5″	16.4	16.6	16.4	16.3
6″	123.8	128.5	32.7	28.6
7″	140.1	135.6	75.1	88.8
8″	70.4	82.0	142.6	143.6
9″	29.6[a)]	24.2[b)]	111.0	114.2
10″	29.6[a)]	24.2[b)]	17.6	17.0
5-OMe	60.6	60.7	60.7	60.7

δ_C in ppm. [a-c)] overlapping signals.

Melicotriphyllin C (**3**), obtained as optically inactive yellow oil, showed the same quasi-molecular formula for $C_{22}H_{24}O_6Na$ (calc. 407.1465) as **1** in the HR-ESIMS ($[M+Na]^+$ at *m/z* 407.1488). The ^{1}H- and ^{13}C-NMR spectra had identifiable signals for a linear furanocoumarin framework (Tables 1 and 2). The location of a methoxy group was confirmed at C-5 by the NOESY correlations from MeO [δ_H 4.15 (3H, s)] to H-4 [δ_H 8.12 (1H, d, J = 9.7 Hz)] and H-3' [δ_H 7.00 (1H, d, J = 2.4 Hz)]. Therefore, the structural difference between **1** and **3** was due to the geranyl-derived side chain. The ^{1}H-NMR and DQF-COSY spectra exhibited a $-OCH_2CH=$ moiety [δ_H 4.87 (2H, m), 5.62 (1H, br t, J = 7.2 Hz)], a $-CH_2CH_2CH(O)-$ moiety [δ_H 2.03 (2H, m), 1.58 (2H, m), 3.93 (1H, t, J = 6.8 Hz)], two methyl groups [δ_H 1.67, 1.71 (3H each, s)], and an olefinic methylene group [δ_H 4.94, 4.99 (1H each, br s)]. The side chain was established as $-OCH_2CH=C(CH_3)CH_2CH_2CH(OH)C(CH_3)=CH_2$ by the following HMBC correlations: H_2-1"/C-3"; H-2"/C-4", C-5"; H_2-4"/C-7"; H_2-6"/C-3", C-8"; and H-7"/C-9", C-10" (Figure

2). The configuration at C-2" was indicated to be *E* by the NOEs between H_2-1"/H_3-5" and H-2"/H_2-4". Consequently, the structure of melicotriphyllin C was confirmed as **3**.

Melicotriphyllin D (**4**) was obtained as optically inactive yellow oil. The quasi-molecular formula was established as $C_{22}H_{24}O_7Na$ (calc. 423.1414) by an $[M+Na]^+$ ion peak observed at *m/z* 423.1380 in the HR-ESIMS. Then, the structure relationship between **3** and **4** seemed identical to that between **1** and **2**. The significant downfield shifts observed in the proton and carbon signals at C-7" from δ_H 3.93 and δ_C 75.1 (**3**) to δ_H 4.23 and δ_C 88.8 (**4**) demonstrated that a hydroperoxy group was substituted at C-7" in **4** instead of a hydroxyl group in **3**. The idea was corroborated by the chemical conversion of **4** to **3** upon treatment with triphenylphosphine in the same manner as **2**. Thus, the structure of melicotriphyllin D was determined to be **4**. Since **3** and **4** were optically inactive, both were suggested to exist as racemic mixtures.

In addition, 8-geranyloxy-5-methoxypsoralen (**5**),[9] phellopterin (**6**),[10] 5-demethylmelibentin (**7**),[3,11] melibentin (**8**),[11,12] melisimlexin (**9**),[12,13] 3,5,6,7,8,3',4'-heptamethoxyflavone (**10**),[14] 3,5,8-trimethoxy-6,7:3',4'-bis(methylenedioxy)flavone (**11**),[2,12] quercetin pentamethyl ether (**12**),[3] gossypetin hexamethyl ether (**13**),[3] 4'-hydroxy-3,5,7,3'-tetramethoxyflavone (**14**),[2] 4'-hydroxy-3,5,7,8,3'-pentamethoxyflavone (**15**),[3] kokusaginine (**16**),[15] skimmianine (**17**),[16] and methyl *p*-geranyloxy-*trans*-cinnamate (**18**)[5] were isolated and identified by comparisons with the literatures.

All new compounds (**1**–**4**) were oxidative metabolites of **5** that was rarely found in natural sources and a characteristic component in the fruits of *M. triphylla*, although **6** was isolated from several rutaceous and apiaceous plants and common in every parts of this plant. And, geranyloxycoumarins possessing a hydroperoxy group such as **2** and **4** were yet discovered in genera of *Phebalium*[17] and *Clausena*[18] (Rutaceae). We also revealed that the fruits of *M. triphylla* abundantly contained **5**, **8**, **11**, **17** and **18**. Higa *et al*. have reported the presence of **18** in the leaves, however, the compound was apparently major in the fruits rather than the leaves based on our TLC analysis. From the chemotaxonomic standpoint of view,[19–21] the genus *Melicope* may involve two chemical races depending on whether the primary components are polymethoxyflavones or prenyl acetophenones. Considering the previous and the present studies, *M. triphylla* would be a specimen classified into the polymethoxyflavone-rich race in the genus.

EXPERIMENTAL

General. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-AL-400 spectrometer equipped with a field gradient system (^{1}H at 400 MHz and ^{13}C at 100 MHz). Chemical shifts are given in δ values (ppm) relative to tetramethylsilane as an internal standard. EIMS (at 30 eV) and HR-ESIMS were obtained using a JEOL JMS-700T and a Shimadzu LCMS-IT-TOF spectrometers, respectively. UV

spectra were recorded using a Shimadzu UV-3100 spectrophotometer (in MeOH solution). Optical rotations were recorded using a Jasco P-1020 polarimeter (in MeOH solution). Silica gel 60 (70–230 mesh, Merck) and Sephadex LH-20 (GE Healthcare) were used for column chromatography (CC). Vacuum liquid chromatography (VLC) was performed using silica gel 60H (Merck), while TLC analysis was performed using silica gel $60F_{254}$ (Merck) and silica gel RP-$18F_{254S}$ (Merck). Semi-preparative HPLC was performed on a Shimadzu LC-6AD liquid chromatography system equipped with a SCL-10A system controller, SIL-10A autoinjector, CTO-10A column oven, and SPD-10A UV-Vis detector, SPM-10Avp diode array detector, with the aid of Shiseido Capcell Pak C_{18} UG120 5 μm (10 mm i.d. x 250 mm).

Plant Material. The fruits of *M. triphylla* (1.7 Kg, dried weight) were collected at Ishigaki-jima in the Ryukyu Islands, Japan, in August 2009. A voucher specimen was deposited at Gifu Pharmaceutical University, Gifu, Japan.

Extraction and Isolation. The fruits were extracted with a 1:1 mixture of $CHCl_3$ and MeOH at room temperature. The extract (220 g) was subjected to silica gel CC (Si CC) to afford 16 main fractions: Frs. 1, 2 (eluted with *n*-hexane–acetone, 20:1), Frs. 3, 4 (15:1), Fr. 5 (10:1), Fr. 6 (8:1), Frs. 7, 8 (6:1), Frs. 9, 10 (4:1), Frs. 11, 12 (2:1), Frs. 13, 14 (1:1), Frs. 15, 16 (0:1). Fr. 2 was separated by Si CC (*n*-hexane–EtOAc, 40:1 to 20:1) to yield **18** (746.3 mg). Fr. 4 was purified repetitively using Sephadex LH-20 CC ($CHCl_3$–MeOH, 1:2), Sep-Pak tC_{18} cartridge (MeOH–H_2O, 9:1), Si CC ($CHCl_3$), and preparative HPLC (48% MeCN–H_2O) to afford **2** (35.0 mg), **3** (12.9 mg), **4** (27.0 mg), and **5** (1.26 g). Fr. 5 was purified by Si CC (benzene–EtOAc, 7:2) and Sep-Pak tC_{18} cartridge (MeOH–H_2O, 9:1) to yield **1** (23.4 mg), **6** (387.6 mg), and **7** (114.0 mg). Fr. 7 was dissolved in acetone to crystallize **8** (4.29 g). Fr. 9 was separated with the aid of Sephadex LH-20 CC (acetone), Sep-Pak tC_{18} cartridge (MeOH–H_2O, 1:1), Si CC (*c*-hexane–EtOH, 5:1; benzene–EtOAc, 6:1), and VLC (benzene–acetone, 15:1) to provide **9** (143.4 mg), **10** (50.0 mg), and **16** (50.0 mg). Frs. 10 and 12 were dissolved in acetone to give **11** (1.57 g) and **17** (2.07 g) in crystal forms, respectively. Fr. 16 was separated by Si CC ($CHCl_3$) and successive crystallization in EtOH to afford **12** (149.4 mg), **13** (26.2 mg), **14** (390.3 mg), and **15** (71.0 mg).

Melicotriphyllin A (4-methoxy-9-[[(2*E*,5*E*)-7-hydroxy-3,7-dimethyl-2,5-octadien-1-yl]oxy]-7*H*-furo-[3,2-*g*][1]benzopyran-7-one) (**1**): Yellow oil. UV (log ε, MeOH): 270 (4.19), 312 (4.00). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see Tables 1 and 2, respectively. HR-ESIMS (pos.): 407.1458 ($[M+Na]^+$, $C_{22}H_{24}O_6Na^+$; calc. 407.1465). EIMS (%): 233 ($[M+H-C_{10}H_{16}O]^+$, 100), 217 (94), 189 (43), 161 (21), 134 (54), 119 (46).

Melicotriphyllin B (4-methoxy-9-[[(2*E*,5*E*)-7-hydroperoxy-3,7-dimethyl-2,5-octadien-1-yl]oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one) (**2**): Yellow oil. UV (log ε, MeOH): 269 (4.58), 313 (4.39). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see Tables 1 and 2, respectively. HR-ESIMS (pos.): 423.1434 ($[M+Na]^+$, $C_{22}H_{24}O_7Na^+$; calc. 423.1414). EIMS (%): 232 ($[M-C_{10}H_{16}O]^+$, 100), 217 (93), 189 (49), 161 (24), 135 (55), 134 (49), 119 (23).

Melicotriphyllin C (4-methoxy-9-[[(2*E*)-6-hydroxy-3,7-dimethyl-2,7-octadien-1-yl]oxy]-7*H*-furo-[3,2-*g*][1]benzopyran-7-one) (**3**): Yellow oil. $[\alpha]_D$ 0° (*c* 0.1, MeOH). UV (log ε, MeOH): 271 (4.09), 313 (3.91). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see Tables 1 and 2, respectively. HR-ESIMS (pos.): 407.1488 ($[M+Na]^+$, $C_{22}H_{24}O_6Na^+$; calc. 407.1465). EIMS (%): 232 ($[M-C_{10}H_{16}O]^+$, 100), 217 (100), 189 (52), 161 (26).

Melicotriphyllin D (4-methoxy-9-[[(2*E*)-6-hydroperoxy-3,7-dimethyl-2,7-octadien-1-yl]oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one) (**4**): Yellow oil. $[\alpha]_D$ 0° (*c* 0.1, MeOH). UV (log ε, MeOH): 269 (4.11), 313 (3.92). ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz): see Tables 1 and 2, respectively. HR-ESIMS (pos.): 423.1380 ($[M+Na]^+$, $C_{22}H_{24}O_7Na^+$; calc. 423.1414). EIMS (%): 233 ($[M+H-C_{10}H_{16}O]^+$, 100), 217 (100), 189 (60), 161 (28).

Reduction of 2 and 4 with triphenylphosphine. **2** (13.2 mg) was dissolved in MeOH (10 mL), and triphenylphosphine (13.3 mg) was added to the solution. The mixture was stirred for 4 hrs at room temperature, and then the solvent was evaporated off. The residue was purified by Sep-Pak tC_{18} cartridge (75% MeOH–H_2O) to give **1** (7.6 mg). Equally, **4** (11.2 mg) and triphenylphosphine (11.3 mg) was treated as above. After purification, **3** (8.4 mg) was obtained.

REFERENCES

1. T. G. Hartley, *Allertonia*, 2001, **8**, 1.
2. M. Higa, Y. Miyagi, S. Yogi, and K. Hokama, *Yakugaku Zasshi*, 1987, **107**, 954.
3. M. Higa, T. Ohshiro, K. Ogihara, and S. Yogi, *Yakugaku Zasshi*, 1990, **110**, 822.
4. M. Higa, E. Nakadomari, M. Imamura, K. Ogihara, and T. Suzuka, *Chem. Pharm. Bull.*, 2010, **58**, 1339.
5. M. Higa, S. Yogi, and K. Hokama, *Bulletin of the College of Science, University of the Ryukyus*, 1987, **43**, 47.
6. T. S. Wu, T. T. Jong, W. M. Ju, A. T. McPhail, D. R. McPhail, and K. H. Lee, *J. Chem. Soc., Chem. Commun.*, 1988, 956.

7. T. T. Jong and T. S. Wu, *J. Chem. Res. (S)*, 1989, 237.
8. K. Baba, K. Nakata, M. Taniguchi, T. Kido, and M. Kozawa, *Phytochemistry*, 1990, **29**, 3907.
9. K. Franke, A. Porzel, M. Masaoud, G. Adam, and J. Schmidt, *Phytochemistry*, 2001, **56**, 611.
10. O. Bergendorff, K. Dekermendjian, M. Nielsen, R. Shan, R. Witt, J. Ai, and O. Sterner, *Phytochemistry*, 1997, **44**, 1121.
11. E. Ritchie, W. C. Taylor, and S. T. K. Vautin, *Aust. J. Chem.*, 1965, **18**, 2021.
12. T. T. Jong and T. S. Wu, *Phytochemistry*, 1989, **28**, 245.
13. L. H. Briggs and R. H. Locker, *J. Chem. Soc.*, 1950, 2376.
14. T. Kinoshita, *Heterocycles*, 1999, **50**, 269.
15. J. Pusset, J. L. Lopez, M. Pais, M. A. Neirabeyeh, and J. M. Veillon, *Planta Med.*, 1991, **57**, 153.
16. A. Ahond, F. Picot, P. Potler, C. Poupat, and T. Sevenet, *Phytochemistry*, 1978, **17**, 166.
17. M. A. Rashid, A. I. Gray, and P. G. Watermann, *J. Nat. Prod.*, 1992, **55**, 851.
18. C. Ito, S. Katsuno, and H. Furukawa, *Chem. Pharm. Bull.*, 1998, **46**, 341.
19. K. M. Ng, P. P. H. But, A. I. Gray, T. G. Hartley, Y. C. Kong, and P. G. Waterman, *Biochem. Syst. Ecol.*, 1987, **15**, 587.
20. A. Adsersen, U. W. Smitt, H. T. Simonsen, S. B. Christensen, and J. W. Jaroszewski, *Biochem. Syst. Ecol.*, 2007, **35**, 447.
21. A. A. Auzi, T. G. Hartley, and P. G. Waterman, *Biochem. Syst. Ecol.*, 1997, **25**, 611.