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HIGHLY OXYGENATED FLAVONOIDS FROM *MELICOPE TRIPHYLLA*

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Key Word Index—*Melicope triphylla*; Rutaceae; flavonoid; furoquinoline; melicophyllin; anti-platelet aggregation activity.

Abstract—The investigation of the leaves of *Melicope triphylla* resulted in the isolation of two furoquinoline alkaloids, skimmianine, kokusagenine and five known flavones, meliternin, melisimplexin, melibentin, meliternatin, 3,5,8-trimethoxy-6,7; 3′,4′-dimethylenedioxyflavone and a new flavone, melicophyllin. The latter was shown to be 3,5,8,3′,4′-pentamethoxy-6,7-methylenedioxyflavone from its spectroscopic properties. Three of these flavones were found to possess anti-platelet aggregation activity at 100 μg/ml (*in vitro*).

INTRODUCTION

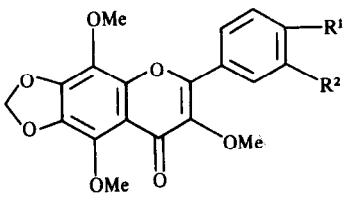
Melicope triphylla, a small evergreen tree, is the only species of *Melicope* native to Taiwan. Furoquinoline alkaloids and polymethoxyflavonoids–including very rare methylenedioxyflavones–have been reported to be the major components in this genus. The antifeedant activity of the leaves extract of this plant [1] and anti-platelet aggregation activity of polymethoxyflavonoids [2] aroused our interest in this plant. Higa *et al.* have reported some flavonoids and furoquinoline alkaloids from this plant [1]. In our study, two sesquiterpene lactones with novel skeleta were obtained from the root bark [3]. From the leaves we have now obtained six polymethoxyflavonoids, each containing one or two methylenedioxy substituents. They are meliternin (**2**) [4], melisimplexin (**3**) [5], melibentin (**4**) [6], meliternatin (**5**) [7], 3,5,8-trimethoxy-6,7; 3′4′-dimethylenedioxyflavone (**6**) [8] and a new flavone-melicophyllin (**1**), as well as two furoquinoline alkaloids, skimmianine (**7**) and kokusagenine (**8**). We now report the isolation and structural elucidation of **1** and anti-platelet aggregation activity of these flavonoids.

RESULTS AND DISCUSSION

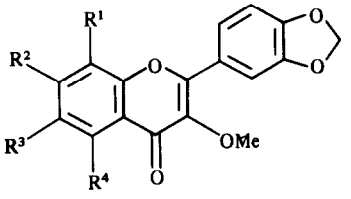
Known compounds, meliternin (**2**), melisimplexin (**3**), melibentin (**4**), meliternatin (**5**), 3,5,8-trimethoxy-6,7; 3′,4′-dimethylenedioxyflavone (**6**) and skimmianine (**7**), kokusagenine (**8**) were characterized based on the spectroscopic analyses and comparisons with the authentic samples.

Melicophyllin (**1**), mp 175–177°, $C_{21}H_{20}O_9$ (M^+,416) was shown to be a flavonoid from UV absorption bands at λ_{max} 255, 275 and 339 nm, together with IR spectrum showing a peak at 1640 cm^{-1} (α, β-unsaturated carbonyl). ^1H NMR spectrum of **1** showed five methoxy groups at δ4.06 (6H, *s*), 3.96 (6H, *s*), 3.89 (3H, *s*) and methylenedioxy protons at δ6.08 (2H, *s*). At the downfield region, there is one ABX type protons at δ7.00 (1H, *d*, $J=9$ Hz, H-5′); 7.79 (1H, *d*, $J=2$ Hz, H-2′) and 7.81 (1H, *dd*, $J=2$ and 9 Hz, H-6′), suggesting that 3′,4′-positions in ring B are substituted. From the above spectroscopic data, we concluded that melicophyllin (**1**) is an isomer of melibentin (**4**), for which there are three possible structures.

From the mass spectrum of **1**, through the presence of a peak at *m/z* 209, the A ring has one methylenedioxy group



1 $R^1 = R^2 =$ OMe
6 $R^1, R^2 =$ ——OCH_2O——



2 $R^1 = R^2 = R^4 =$ OMe, $R^3 =$ H
3 $R^1 =$ H, $R^2 = R^3 = R^4 =$ OMe
4 $R^1 = R^2 = R^3 = R^4 =$ OMe
5 $R^1 =$ H, $R^2, R^3 =$ ——OCH_2O——, $R^4 =$ OMe

Table 1. ^{1}H NMR spectra of flavonoids **1**–**6**.

	1		**2**		**3**		**4**		**5**		**6**	
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6
3-OMe	3.89	3.88	3.88	3.79	3.86	3.80	3.89	3.80	3.86	3.78	3.89	3.78
5-OMe	4.06*	4.04	3.99*	3.47	4.00	4.06	4.09	4.02	4.12	4.00	4.07*	4.01
6-OMe (or H)			6.41	5.91	3.96*	3.76	3.97*	3.73				
7-OMe			3.92*	3.22	3.91*	3.18	3.94*	3.75				
8-OMe (or H)	4.05*	3.73	3.99*	3.70	6.73	6.23	3.99*	3.62			4.05*	3.66
6,7-O-CH_2-O-	6.08	5.08							6.04	5.05	6.08	5.03
2′-H	7.79	7.85	7.69	7.88	7.59	7.72	7.67	7.84	7.56	7.66	7.70	7.87
3′,4′-O-CH_2-O-			6.05	5.23	6.06	5.26	6.07	5.26	6.04	5.25	6.06	5.23
3′-OMe	3.96	3.54										
4′-OMe	3.96	3.36										
5′-H	7.00	6.58	6.94	6.68	6.93	6.70	6.95	6.68	6.90	6.67	6.94	6.67
6′-H	7.81	7.94	7.77	7.83	7.67	7.66	7.76	7.80	7.63	7.58	7.79	7.82

Values are in ppm.
*Values with same superscript may be interchanged.

and two methoxy groups and there appeared fragmentation ions at m/z $[M-1]^+$, and m/z $[M-19]^+$, indicating the C-3 and C-5 were substituted with methoxy groups [9]. However, the peak at m/z 401 $[M-15]^+$ is not the base peak; therefore, the presence of a C-6 methoxy group can be excluded [9]. In the ^{1}H NMR spectrum in C_6D_6, the shifts observed for the methoxy groups of the A ring excluded the presence of a methoxy group at C-7 [10]. Consequently, the methylenedioxy group is located at C-6, C-7 position and **1** is 3,5,8,3′4′-pentamethoxy-6,7-methylenedioxyflavone.

This structure is further confirmed by the comparison of the ^{1}H NMR spectrum of **1** with other five known flavones isolated from this plant (Table 1). Thus the chemical shift for the methylenedioxy protons of **1** in C_6D_6 is similar to that of compound (**5**) and (**6**) with 6,7-substituted methylenedioxy groups. Second, the chemical shifts of H-2′ and H-6′ on ring-B shifted downfield (>0.1 ppm), whenever H-8 was substituted by methoxy group in ring-A both in $CDCl_3$ and C_6D_6. This is an effective means to decide if H-8 is substituted by a methoxyl group in the flavonoid series.

The highly oxygenated flavonoids (**2**–**6**) were subjected to both ADP and collagen induced platelet aggregation tests and results shown meliternin (**2**), meliternatin (**5**) and 3,5,8-trimethoxy-3′,4′; 6,7-dimethylenedioxyflavone (**6**) at 100 μg/ml (*in vitro*) were found to possess medium inhibitory activity toward collagen-induced platelet aggregation.

EXPERIMENTAL

UV spectra were run in MeOH and IR spectra as KBr discs. ^{1}H NMR spectra were run at 100 MHz in $CDCl_3$ using TMS as internal standard. MS were obtained at 70 eV direct inlet system. Mps: uncorr.

Plant material. Melicope triphylla was collected from Orchid Island (Lan-Yu) in Taiwan on September, 1985 and verified by Prof. C. S. Kuoh. The specimen is deposited in the Herbarium of Cheng-Kung University, Taiwan, Republic of China.

Extraction and separation. Air-dried and powdered leaves (645 g) of *Melicope triphylla* were extracted with hot Me_2CO (11 × 7). The Me_2CO extract was concd and the ppt was identified as skimmianine (1.2 g). The filtrate was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was extracted with 5% HCl soln. The acidic layer was neutralized with NH_4OH and extracted with $CHCl_3$. After concn and chromatography over silica gel ($CHCl_3$–Me_2CO 19:1),-skimmianine (120 mg), kokusagenine (25 mg) and two unknown compounds were obtained. The $CHCl_3$ layer after removal of the basic portions was chromatographed on silica gel and eluted exhaustively with $CHCl_3$–Me_2CO (19:1) to afford successively, melisimplexin (**3**) (23 mg), melibentin (**4**) (35 mg), 3,5,8-trimethoxy-3′,4′; 6,7-dimethylenedioxyflavone (**6**) (10 mg), meliternatin (**5**) (23 mg), melicophyllin (**1**) (20 mg), and meliternin (**2**) (28 mg).

Melicophyllin (**1**). Colourless crystals, mp 175–177° (Me_2CO). UV λ_{max} nm: 255, 275, 339; IR ν_{max} cm^{-1} 1640, 1620, 1600. MS m/z (%): 416 (M^+, 100), 415 (66), 397 (22), 387 (25), 385 (25), 383 (20), 373 (19), 355 (18), 209 (20), 194 (22), 165 (30), 149 (30).

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