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Chemical Constituents of Leaves and Barks of *Melicope hookeri* T.G. Hartley

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ABSTRAK

Kajian fitokimia terhadap daun dan kulit batang *Melicope hookeri* T.G. Hartley (Rutaceae) telah berjaya memencilkan dan mengenali tiga flavonoid, ayanin, ombium dan kumatakenin; dua koumarin, umbelliferone dan scopoletin; dan sitosterol. Struktur sebatian ini telah ditentukan dengan kajian mendalam menggunakan kaedah spektroskopi.

ABSTRACT

Phytochemical investigation on the leaves and bark of *Melicope hookeri* T.G. Hartley (Rutaceae) has resulted in the isolation and identification of three flavonoids, ayanin, ombuin and kumatakenin, two coumarins, umbelliferone and scopoletin and β-sitosterol. The structures of these compounds were determined by detailed spectroscopic methods.

Keywords: *Melicope hookeri*, ayanin, ombium, kumatakenin, umbelliferone scopoletin

INTRODUCTION

The genus *Melicope* is usually a shrub or small tree of the family Rutaceae. The plants are widely distributed throughout South East Asia right up to India, Madagascar, Polynesia and Northern Australia. Some members of the genus are traditionally used for the treatment of various ailments such as treatment of cold, rheumatism and spleen inflammation (Jones 1995). Many classes of chemical constituents have been reported to occur in these plants such as alkaloids, flavonoids, lignans, phloroglucinols, benzopyrans, coumarins and essential oils (Fauvel *et al.* 1981; Jong and Wu 1989; Kamperdick *et al.* 1997; Latip *et al.* 1999; Chan *et al.* 1989; Simonsen *et al.* 2002). In this work we wish

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to report the isolation of three flavonoids, two coumarins and β-sitosterol from the leaves and bark extracts of *Melicope* cf *hookeri* collected from Sandakan, Sabah.

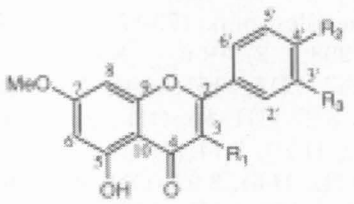
RESULTS AND DISCUSSION

Ayanin (1) was obtained as yellowish needle-shaped crystals, m.p. 173-174°C with the typical UV absorptions of a flavonone skeleton at 356, 255 and 208 nm. The IR spectrum gave a strong and broad absorption at 3423 cm^{-1} which indicated the presence of hydroxyl group and another strong absorption at 1657 cm^{-1} for a carbonyl group. The mass spectrum gave a molecular ion peak at m/e 344 which corresponds to the molecular formula $C_{18}H_{16}O_7$. The aromatic region of the ^{1}H-NMR indicated the presence of five protons in which two occurred as mutually *meta*-coupled doublets at δ 6.33 and 6.69 (J = 1.5 Hz) assigned to H-6 and H-8 protons, respectively. The other three aromatic protons exhibited an ABX system with the appearance of a doublet of doublet at δ 7.71 (J = 8.0 Hz, 1.3 Hz) and two doublets at δ 7.67 (J = 1.3 Hz) and 7.14 (J = 8.0 Hz) due to protons H-6', H-2' and H-5', respectively. The spectrum also indicated the presence of three methoxyl groups with the occurrence of three proton singlets at δ 3.90, 3.95 and 3.97. A very low field broad singlet at δ 12.74 clearly indicated the presence of a chelated hydroxyl group and another hydroxyl group was also observed at δ 8.27 as a broad singlet. The ^{13}C-NMR spectrum indicated the presence of 18 carbon atoms. This further supported the molecular formula suggested of which three occurred as methoxyl groups, five as methine and nine as quarternary carbon atoms. The connectivity between carbons and the directly attached protons were shown by the HSQC spectrum. These spectral data together with the HMBC spectra and comparison with literature values, the compound was unambiguously elucidated as 3',5-dihydoxy-3',4',7-trimethoxyflavone or commonly called ayanin (1) (Wang *et al.* 1989).

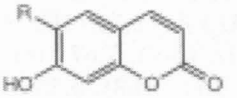
Ombuin (2) was also isolated as white solid, m.p. 231-232°C with the typical UV absorptions at 368, 255 and 208 nm due to the flavonone skeleton. The IR spectrum absorptions are very similar to the above compound. The mass spectrum gave a molecular ion peak at m/e 330 which corresponds to the molecular formula $C_{17}H_{15}O_7$. The aromatic region of the ^{1}H-NMR showed a similar pattern of substitution as in ayanin (1) but differ in their chemical shifts and coupling constants. The spectrum revealed the presence of two methoxyl groups (δ 4.02 and 3.90) and three phenolic protons one of which was chelated occurring at δ 11.73. The ^{13}C-NMR spectrum further supported the presence of 17 carbon atoms with the occurrence of two methoxyl, five methine and ten quarternary carbons. The positions of the hydroxyl and methoxyl groups were established by heteronuclear correlations observed in the HSQC and HMBC spectra. Comparison of these spectral data with literature values indicated the compound is identical to ombuin (2), previously isolated from *Cassia laevigata* (Singh *et al.* 1980).

The third flavonoid, kumatakenin (3), was also isolated as yellowish needle-shaped crystals, m.p 248-249°C with similar UV spectrum absorptions at 347, 257 and 208 nm^{-1} as the above compound. The IR spectrum also revealed the presence of a highly chelated hydroxyl group with a strong and broad band at 3421 cm^{-1} and carbonyl absorption at 1663 cm^{-1}. The ^{1}H-NMR spectrum showed the presence of two methoxyl groups at δ 3.78 and 3.87. It also exhibited two *meta* coupled doublets at δ 6.33 and 6.49 with a coupling constant value of 2.0 Hz. The other aromatic protons occurred as an AA'BB' system giving the typical pair of doublets at δ 6.93 and 7.98 each with a coupling constant value of 8.0 Hz assigned to protons at H-2'/H-6' and H-3'/H-5', respectively. Hence, the compound was elucidated as 5,4'-dihydroxy-3,7-dimethoxyflavone or kumatakenin (3), previously isolated from *Heliotropium chenopodiaceum* var. *chenopodiaceum* (Urzua *et al.* 1998).

The two simple coumarins, unbelliferone (4) and scopoletin (5), were isolated from the ethyl acetate bark extract as white needles with m.p. 244-246°C and 204-206°C, respectively. The UV spectra of the two compounds gave similar absorptions at 324 and 205 nm, typical of coumarin nucleus. The mass spectrum of scopoletin showed a molecular ion peak m/e at 192, 30 mass unit higher than for umbelliferone due to the presence of a methoxyl group. The two compounds were previously isolated from *Peucedanum praeruptorum* (Kong *et al.* 1996).



(1). R_1 = OMe, R_2 = OMe, R_3 = OH
(2). R_1 = OH, R_2 = OMe, R_3 = OH
(3). R_1 = OMe, R_2 = OH, R_3 = H



(4). R = H
(5). R = OMe

EXPERIMENTAL

General Experimental Procedures

Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were recorded using KBr discs on Perkin Elmer FTIR spectrophotometer model 1275X. The UV spectrum was recorded on a Shimadzu UV 160A spectrophotometer in MeOH. The ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian 500 MHz or JOEL FTNMR 400 MHz spectrometer. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal standard.

Plant Material

The leaves and stem bark of *Melicope hookeri* T.G. Hartley were obtained from Sepilok, Sabah, Malaysia and a voucher specimen was deposited at the Forest Research Centre, Sepilok, Sabah (accession numbers FRCES 398).

Extraction and isolation

The ground dried leaves and stem bark were separately and sequentially extracted in soxhlet extractor with petroleum ether, ethyl acetate and methanol each for 16 hours. The extracts were concentrated under reduced pressure to give dark viscous semi-solids. The petroleum ether leaves extract (30 g) was subjected to VLC and eluted with solvent gradient of petroleum ether, EtOAc and MeOH to afford 6 major fractions. Fractions 4 and 6 were subjected to a series of silica gel CC to give β-sitosterol (34 mg) and ayanin (1) (28 mg). The EtOAc extract (25 g) was separated by VLC eluted with hexane and gradual increase in $CHCl_3$ and EtOAc to give 8 fractions. Fractions 4-6 were further purified by a series of CC to give another sample of ayanin (1) (20 mg), ombuin (2) (11 mg) and kumatakenin (3) (16 mg). Chromatographic separation of the MeOH extract also afforded the three above compounds. The EtOAc extract of the bark (10 g) was also subjected to VLC and eluted with mixtures of hexane and EtOAc of increasing polarity to give 30 fractions of 100 ml each. Further CC separation of fractions 22-26 gave umbelliferone (4) (23 mg), scopoletin (5) (18 mg) and another sample of ayanin (1) (25 mg).

Ayanin (1), Isolated as yellowish needles, m.p. 173-174°C; UV λ_{max}, MeOH (log ε) nm: 356 (0.6), 255 (0.76) and 208 (1.2); IR n_{max} (KBr) cm^{-1}: 3554, 3423, 1657, 1620, 1590, 1505, 1367, 1328, 1257, 1215, 1161, 1094; ^{1}H-NMR (500 MHz, CD_3COCD_3) δ: 12.74 (1H, *brs*, OH-5), 8.27 (1H, *brs*, OH-3'), 7.71 (1H, *dd*, 1.3 Hz, 8.0 Hz, H-6'), 7.67 (1H, *d*, 1.3 Hz, H-2'), 7.14 (1H, *d*, 8.0 Hz, H-5'), 6.69 (1H, *d*, 1.5 Hz, H-8), 6.33 (1H, *d*, 1.5 Hz, H-6), 3.97 (OMe-4'), 3.95 (OMe-7), 3.90 (OMe-3); ^{13}C-NMR (125 MHz, CD_3COCD_3) *ppm*: 178.9 (C-4), 165.0 (C-7), 162.1 (C-9), 157.1 (C-5), 156.2 (C-3'), 150.4 (C-4'), 146.8 (C-2), 139.0 (C-3), 123.4 (C-1'), 121.1 (C-6'), 115.2 (C-2'), 111.5 (C-5'), 105.9 (C-10), 97.9 (C-6), 92.2 (C-8), 59.6 (C-3), 55.8 (C-7), 55.6 (C-4'); MS m/e (%): 344 (M^+, 100), 330 (12.5), 301 (32.9), 258 (10.3), 217 (13.6), 158 (22.9), 135 14.9), 120 (8.2), 69 (12.3).

Ombuin (2). Obtained as a white solid, m.p. 231-232°C; UV λ_{max}, MeOH (log ε) nm: 368 (0.85), 255 (0.96), 208 (1.30); IR ν_{max} (KBr) cm^{-1}: 3878, 3251, 3011, 2949, 1842, 1664, 1601, 1586, 1555, 1499, 1460, 1410, 1376, 1228 1098; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 11.73 (1H, *brs*, OH-5), 7.85 (1H, *d*, 8.5 Hz, H-5'), 7.81 (1H, *brs*, H-2'), 7.00 (1H, *d*, 8.5 Hz, H-6'), 6.62 (1H, *brs*, OH-3), 6.52 (1H, *brs*, H-8), 6.40 (1H, *brs*, H-6), 5.72 (1H, *brs*, OH-3'), 4.02 (3H, *s*, OMe-4'), 3.90 (3H, *s*, OMe-7); ^{13}C-NMR (125 MHz, $CDCl_3$) *ppm*: 176.6 (C-4), 166.2 (C-7), 161.7 (C-5), 156.6 (C-9), 156.0 (C-3'), 148.9 (C-4'), 146.6 (C-2), 137.8 (C-3), 121.2 (C-6'), 113.8 (C-2'), 110.7 (C-5'), 102.8 (C-10), 98.2 (C-6), 92.4 (C-8), 94.6 (C-1'), 56.3 (OMe-4'), 56.1 (OMe-7); MS m/e (%): 330 (M^+, 100), 315 (30), 301 (14), 287 (13), 259 (10), 231 (5), 151 (10), 149 (18), 135 (16).

Kumatakenin (3). Isolated as yellowish needles, m.p 248-249°C: UV λ_{max}, MeOH (log ε) nm: 347 (0.8), 257 (0.9), 208 (1.3); IR ν_{max} (KBr) cm^{-1}: 3421, 3257, 1663, 1601, 1499, 1459, 1432, 1376, 1347, 1286, 1228, 1139, 1098; ^{1}H-NMR (500 MHz, CD_3COCD_3) δ: 12.64 (1H, *s*, OH-4), 7.98 (2H, *d*, 8.0 Hz, H-3'/H-5'), 6.93 (2H, *d*, 8.0 Hz, H-2'/H-6'), 6.49 (1H, *d*, 2.0 Hz, H-8), 6.33 (1H, *d*, 2.0 Hz, H-6), 3.87 (3H, *s*, OMe-7), 3.78 (3H, *s*, OMe-3); ^{13}C-NMR (125 MHz, CD_3COCD_3) *ppm*: 179.0 (C-4), 165.8 (C-7), 161.5 (C-9), 159.2 (C-2), 157.5 (C-5), 157.1 (C-4'), 138.7 (C-3), 130.5 (C-3'/C-5'), 121.5 (C-1'), 115.8 (C-2'/C-6'), 106.0 (C-10), 98.1 (C-6) 92.4 (C-8), 61.3 (OMe-3), 55.7 (OMe-7'); MS m/e (%): 314 (M^+, 100), 295 (23), 271 (40), 167 (15), 143 (29), 131 (10), 121 (36).

Umbelliferone (4). Isolated as white needles, m.p. 244-246°C; UV λ_{max} MeOH (log ε) nm: 324 (0.71), 205 (0.97); IR ν_{max} (KBr) cm^{-1}: 3649, 3443, 3346, 3182, 1893, 1683, 1606, 1565, 1510, 1464, 1319, 1133; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.88 (1H, *d*, 8.5 Hz, H-4), 7.52 (1H, *d*, 8.5 Hz, H-5), 6.87 (1H, *dd*, 1.5 Hz, 8.4 Hz, H-6), 6.77 (1H, *d*, 1.5 Hz, H-8), 6.18 (1H, *d*, 8.5 Hz, H-3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.1 (C-7), 160.6 (C-2), 156.5 (C-9), 144.2 (C-4), 129.9 (C-5), 113.3 (C-6), 112.3 (C-10), 102.8 (C-8); MS m/e (%): 162 (M^+, 91), 134 (100), 105 (42), 78 (57), 63 (24).

Scopoletin (5). Isolated as white needles, m.p. 204-206°C; ^{1}H-NMR (500 MHz, CD_3COCD_3) δ: 8.03 (1H, *s*, OH-7), 7.86 (1H, *d*, 9.5 Hz, H-4), 7.22 (1H, *s*, H-5), 6.82 (1H, *s*, H-8), 6.19 (1H, *d*, 9.5 Hz, H-3), 3.93 (3H, *s*, OMe-6); MS m/e (%): 192 (M^+, 100), 177 (48), 164 (38), 149 (56), 121 (27).

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