A New Highly Oxygenated Flavone from *Vernonia saligna*

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A new highly oxygenated flavone, namely 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone (**1**), together with other five known flavonoids were isolated from the tropical plant *Vernonia saligna.* Their structures were established on the basis of spectral (MS, IR, UV, 1D & 2D NMR) measurement and chemical evidence.

Key words: Vernonia saligna, Highly Oxygenated Flavone, 8, 3′-Dihydroxy-5, 6, 7, 4′-tetramethoxyflavone

Introduction

Vernonia saligna. (Wall.) DC (Compositae) is a shrub widely distributed over hillside, moisture pasture, and wetland, in tropical and subtropical Asian region, and has been used by the people of Yi nationality for the treatment of sore throat, cough, tuberculosis, and uterus prolapse (Edit. Committee of "Zhong Hua Ben Cao", 1999), in Yunnan province, southwest of China. In the course of our screening of biologically active substances, it was found that the crude extract of *V. cinerea* exhibits potent anti-infective activity. By literature search for the phytochemical constituents of plants of the genus *Vernonia*, we found these have been intensively investigated. As reported they are very rich in secondary metabolites, such as sesquiterpenoids, flavonoids, and triterpenoids. In this study, we report the presence of a novel highly oxygenated flavone, named 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxy-flavone (**1**), together with other five flavonoids including 5, 3′-dihydroxy-6, 7, 4-trimethoxy flavone (**2**), 6, 7-dimethoxy kaempferol-3-β-O-glucoside (**3**), 6-hydroxy kaempferol-7-β-O-glucoside (**4**), quertagetin-5-β-O-glucoside (**5**), luteolin-7-β-O-glucoside (**6**) in the chloroform extract of dried leaves of *V. saligna.* This report describes the structure elucidation of the new flavone compound (**1**).

Results and Discussion

8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone (**1**) was obtained as pale yellow needles, m. p. 183–185 °C. Its molecular formula ($C_{19}H_{18}O_8$) was determined by high resolution EI-MS ($[M]^+$ 374.1005, calc. for $C_{19}H_{18}O_8$: 374.1002). EI-MS exhibited the molecular ion peak at *m/z* 374, and characteristic fragmentation ion peaks due to loss of methyl units at *m/z* 359 (M-CH_3), 344 (M-2 × CH_3), 329 (M-3 × CH_3), 314 (M-4 × CH_3). Spectral data of **1** revealed the presence of a flavone skeleton with 2 OH and 4 OMe groups. Evidence for the existence of four methoxy groups in the structure was provided by the presence of four methoxyl signals (δ 56.3q, 61.1q, 61.6q, 62.1q) in the ^{13}C-NMR, and (δ 3.98, 4.09, 3.93, 3.95, each 3H, s) in the ^{1}H-NMR.

Besides the four methoxyl signals, there were other 15 carbon signals in the ^{13}C-NMR, and 14 of them were aromatic carbons, another carbonyl signal appeared at δ 182.9 (s). It is indicated that compound **1** was a four methoxy-substituted and two hydroxy-substituted flavone.

UV spectrum of compound **1** at 341 nm (band I) showed that it was substituted at 3′, 4′, but its band II at 272, 244 nm (shoulder) were different from those of the congeneric known six-substituted flavonoids (Bernard *et al.*, 1983). Its ^{1}H-NMR spectrum indicated that 3-, 2′-, 5′-, 6′-positions in the flavone skeleton were unsubstituted (Table I). The ^{1}H-^{1}H COSY showed that H-5′ proton signal at δ 7.52 correlated with the H-6′ signal at δ 7.03. In the HMBC the proton at δ 6.57 (H-3, s) was correlated with the carbons at δ 164.0 (s, C-2), 123.2 (s, C-1′) and 120.8 (d, C-6′); the protons at δ 7.52 (H-5′), 7.39 (H-2′) and 7.03 (H-6′) with the carbon at δ 164.0 (s, C-2), respectively. It supported that the B-ring was connected to C-2

Table I. ^{1}H and ^{13}C NMR data of **1**, $CDCl_3$, δ in ppm, *J* in Hz.

	δ_H	δ_C	HMBC selected		δ_H	δ_C	HMBC selected
C (2)		164.0	H-3,H-2′,H-6′	C (1′)		123.2	
H-C (3)	6.57 (1H, s)	108.3		H-C (2′)	7.39 (1H, d, 1.7)	103.8	
C (4)		182.9		C (3′)		135.7	
C (5)		152.9		C (4′)		146.9	4′-OMe
C (6)		136.9		H-C (5′)	7.03 (1H, d, 8.5)	115.1	
C (7)		132.9		H-C (6′)	7.52(1H, dd, 1.7, 8.5)	120.8	
C (8)		106.9		4′-OMe	3.98 (3H, s)	56.3	
C (9)		149.0		5-OMe	4.09 (3H, s)	61.7	
C (10)		95.7		6-OMe	3.93 (3H, s)	62.1	
				7-OMe	3.9 (3H, s)	61.6	

* Data of C-6, 7 and the methoxyl groups can be interconverted.

with 3′, 4′ substituted by methoxy or hydroxy groups (Fig. 1). The ^{13}C-NMR spectrum (Table I) showed three hindered methoxy groups δ 61.1q, 61.6q, 62.1q, and one non-hindered at δ 56.3q, which indicated that the carbon of the methoxyl at δ 56.3q is not bordered by the other methoxy groups (Lima *et al.*, 1996). ^{1}H-^{13}C correlations between δ 3.98 (s, H-OMe) and δ 56.3 (q, C-OMe) and the long-range correlations between δ 3.98 (s, H-OMe) and δ 146.9 (s, C-4′) indicated that C-4′ at the B-ring was substituted by the only unhindered methoxy group. It was also ensured that one of the hydroxy group was located at 3′-position of the B-ring. Therefore, the left three methoxy groups were bordered to each other, *i.e.*, the left hydroxy group was located at C-5 or C-8 position of the A-ring.

The UV spectrum of compound **1** exhibited a main band at 358 nm, and also in the presence of hydrochloric acid plus $AlCl_3$. Compared with known 5-OH flavones, the bathochromic shift (+ 17 nm) of compound **1**'s band I and its intensity were markedly less (Huang *et al.*, 1988) than those of 5-OH flavones (+ 42 ~ 53 nm) in the presence of the above mentioned shift reagents (Table II). It indicates that the left hydroxy group is located on C-8 position at A-ring as 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone. In the ^{1}H-^{1}H COSY correlations between H-5′ and H-6′ are observed. HMBC, and HMQC experiments confirmed the proposed structure (Fig. 1).

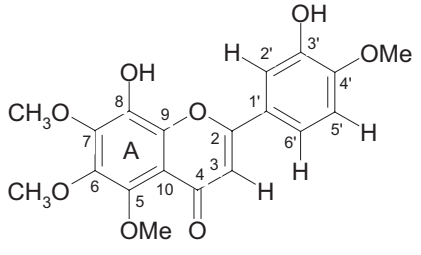

Fig. 1. The structure of 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone (**1**).

Experimental

General

Mp: on a VEB Wägetechnik (PHMK) apparatus and uncorrected. UV spectral: UV-210 spectrometer, λ_{max} in nm. Infrared: Bio-Rad (Richmond, C. A.) FTS135 spectrophotometer, KBr pellets. ^{1}H and ^{13}C-NMR: Bruker AM-400; 2D-NMR: DRX-500 spectrometer (Rheinstetten, Germany), δ in ppm, *J* in Hz. MS: VG Autospec-3000 spectrometer (Manchester, England), *m/z* (rel. %). Silica gel (200–300 mesh) for column chromatography (CC), GF_{254} for thin-layer chromatography

Table II. UV spectral diversification of **1** in the Presence of shift reagents.

	Band I [nm]	Band II [nm]	
Methanol	341	272	244 (sh.)
$AlCl_3$(anhydrous)	358	278	256 (sh.)
Hydrochloric acid-$AlCl_3$	358	305, 280	258 (sh.)

(TLC) and preparative thin-layer chromatography (PTLC) were obtained from Qingdao Marine Chemical Ltd. (Qingdao, P. R. China). Reversed-phase chromatography was carried out on LiChroprep® RP-8 (40–63 μm, Merck, Darmstadt, Germany).

Plant material

The plants of *Vernonia saligna.* (Wall.) DC were collected at Xi Shuang Ban Na of Yunnan province, P. R. China, in 1992. The plants were air-dried. The voucher specimen (Kun 0485578) was deposited at Kunming Institute of Botany, Chinese Academy of Science.

Extraction and isolation

The powder of air-dried aerial parts of *Vernonia saligna* (1.8 kg) were extracted with chloroform (1 l × 3), followed by extraction with methanol (1 l× 3) at room temperature. The combined extract was concentrated *in vacuo* to give a crude residue, which was partitioned between chloroform/water, ethyl acetate/water, respectively, to provide chloroform extract (78 g) and an ethylacetate extract (56 g). The chloroform extract was subjected to CC gradient elution with petroleum ether/acetone (99/1 ~ 5/5, v/v) and chloroform/methanol (99/1 ~ 5/5, v/v) to give 22 fractions. Fraction 12 (chloroform/methanol 98/2 ~ 95/5, v/v) was subjected to repeated CC elution with chloroform/methanol (98/2 ~ 92/8, v/v), and after purification by PTLC (chloroform/methanol, 95/5, v/v) to afford 8, 3′-dihydroxy-5, 6, 7, 4′-tetramethoxyflavone (**1**, 20mg) and 5, 3′-dihydroxy-6, 7, 4-trimethoxy flavone (**2**, 22 mg). The ethyl acetate extract was subjected to repeated CC and elution with chloroform/methanol (99/1 ~ 5/5, v/v) to give 16 fractions. Fraction 12 (chloroform/methanol, 9/1 ~ 82/18, v/v) was submitted to repeated CC (chloroform/methanol, 9/1 ~8/2, v/v), with further purification by LiChroprep RP-8 CC (gradiently eluted by acetonitrile/water, 7/3 ~ 9/1, v/v), to afford 6, 7-dimethoxy kaempferol-3-β-O-glucoside (**3**, 7 mg), luteolin-7-β-O-glucoside (**6**, 8 mg), 6-hydroxy kaempferol-7-β-O-glucoside (**4**, 12 mg), quertagetin-5-β-O-glucoside (**5**, 15 mg), respectively.

8, 3′-Dihydroxy-5, 6, 7, 4′-tetramethoxyflavone (**1**), was obtained as pale yellow needles, m.p. 183–185 °C; $[\alpha]_D^{24}$ – 16.8° (*c* 0.39, MeOH); IRν_{max} (KBr) cm^{-1}: 3415, 2928, 2859, 1747,1651, 1603, 1577, 1514, 1479, 1434, 1371, 1284, 1224, 1034, 969, 841, 816; UV (MeOH) λ_{max}nm: 344.5, 262, 241; EI-MS *m/z* 374 ($[M]^+$, 95), 359 (100), 344 (12), 329 (20), 314 (7), 211 (12), 183 (22), 91 (10), HREI-MS *m/z* 374.1005 $[M]^+$ (calcd. for $C_{19}H_{18}O_8$, 374.1002).

5, 3′-Dihydroxy-6, 7, 4-trimethoxy flavone (**2**), pale yellow needles, m.p. 175–178 °C, IRν_{max} (KBr) cm^{-1}: 3428, 2925, 2854, 1750, 1656, 1589, 1515, 1492, 1457, 1348, 1204, 1124, 837; UV (MeOH) λ_{max}nm: 342, 275, 205; EI-MS *m/z* 344 ($[M]^+$, 100), 329 (90), 315 (22), 391 (20), 181 (38), 153 (70); ^{1}HNMR (CD_3COCD_3, 500 MHz), δ 6.73 (1H, s, H-3), 6.84 (1H, s, H-8), 7.61(1H, d, *J* =1.85, H-2′), 6.99 (1H, d, *J* = 8.26, H-5′), 7.63 (1H, dd, *J* = 1.85, 8.26, H-6′), 8.55 (1H, s, OH-5), 3.77 (3H, s, CH_3O-4′), 3.97 (3H, s, CH_3O-6), 3.93 (3H, s, CH_3O-7); ^{13}CNMR (CD_3COCD_3, 125 MHz) δ, 148.8 (s, C-2), 113.4 (d, C-3), 183.2 (s, C-4), 149.7 (s, C-5), 140.2 (s, C-6), 151.4 (s, C-7), 94.9 (d, C-8), 157.4 (s, C-9), 104.8 (s, C-10), 125.2 (s, C-1′), 105.8(d, C-2′), 155.2 (s, C-3′), 142.2(s, C-4′), 124.5 (d, C-5′), 104.7 (d, C-6′). All spectroscopic data were consistent with the literature (Iinuma *et al.*, 1980).

6, 7-Dimethoxy kaempferol-3-β-O-glucoside (**3**), pale yellow powders, m.p. 208–212 °C, IRν_{max} (KBr) cm^{-1}: 3500, 3100, 2900, 1700, 1615, 1560, 1500, 1385, 1200, 1185, 1100, 1020, 820; UV (MeOH) λ_{max}nm: 371, 345; EI-MS *m/z* 330 ($[M\text{-}C_6H_{11}O_5]^+$), 100, 315 (12), 312 (8), 287 (18), 269 (32), 244 (20), 181 (45), 121 (32), 117 (55), FAB$^-$-MS *m/z*: 491 [$(M\text{-}1)^-$, 100], 328 (28), $C_{23}H_{24}O_{12}$; ^{1}HNMR (DMSO, 400 MHz): δ 6.89 (1H, s, H-8), 8.15 (1H, d, *J* = 8.8, H-2′), 6.88 (1H, d, *J* = 7.1, H-3′), 6.88 (1H, d, *J* = 7.1, H-5′), 8.12 (1H, d, *J* = 7.1, H-6′), 5.45 (1H, d, *J* = 7.2, H-1″), 3.92 (3H, s, OCH_3-6), 3.75 (3H, s, OCH_3-7), 12.6 (1H, s, OH-5); ^{13}CNMR (DMSO, 100 MHz) δ: 157.6 (s, C-2), 133.4 (s, C-3), 178.2 (s, C-4), 152.2 (s, C-5), 158.9 (s, C-6), 160.4 (s, C-7), 91.9 (d, C-8), 132.5 (s, C-9), 105.8 (s, C-10), 123.5 (s, C-1′), 132.8 (d, C-2′), 115.9 (d, C-3′), 152.2 (s, C-4′), 116.4 (d, C-5′), 132.2 (d, C-6′), 107.2 (d, C-1″), 72.4 (d, C-2″), 74.1 (d, C-3″), 68.5 (d, C-4″), 76.5 (d, C-5″), 60.9 (t, C-6″). The above spectral data were identical with those reported (Bacon *et al.*, 1978).

6-Hydroxy kaempferol-7-β-O-glucoside (**4**), pale yellow powder, m.p. 242–245 °C; IRν_{max}

(KBr) cm^{-1}: 3600, 3200, 1700, 1615, 1560, 1400, 1300, 1200; UV (MeOH) λ_{max}nm: 329, 275, 258; EI-MS *m/z* 302 ([M-$C_6H_{11}O_5$]$^+$, 100), 273 (18), 245 (12), 200 (25), 169 (32), 134 (15), 105 (35), 77 (55), FAB$^-$-MS *m/z*: 463 [(M-1)$^-$, 100], 301 (8), 283 (15), 255 (28), 167 (45); ^{1}HNMR (DMSO, 400 MHz): δ 6.95 (1H, s, H-8), 8.15 (1H, d, *J* = 8.7, H-2′), 6.88 (1H, d, *J* = 8.7, H-3′), 6.97 (1H, d, *J* = 8.7, H-5′), 8.15 (1H, d, *J* = 8.7, H-6′), 5.01 (1H, d, *J* = 7.3, H-1″); ^{13}CNMR (DMSO, 100 MHz) δ: 147.6 (s, C-2), 135.4 (s, C-3), 176.2 (s, C-4), 150.2 (s, C-5), 130.2 (s, C-6), 145.4 (s, C-7), 93.9 (d, C-8), 151.7 (s, C-9), 105.8 (s, C-10), 122.5 (s, C-1′), 130.8 (d, C-2′), 116.2 (d, C-3′), 159.2 (s, C-4′), 116.1 (d, C-5′), 130.2 (d, C-6′), 101.0 (d, C-1″), 73.8 (d, C-2″), 76.2 (d, C-3″), 70.5 (d, C-4″), 77.5 (d, C-5″), 61.0, (t, C-6″). The above spectral data were in agreement with those reported (Bacon *et al.*, 1978).

Quertagetin-5-β-O-glucoside (**5**), pale yellow needles, m.p. 212–215 °C, IRν_{max} (KBr) cm^{-1}: 3600, 3150, 1680, 1545, 1480, 1020; UV (MeOH) λ_{max}nm: 280, 257, 240; EI-MS *m/z* 318 ([M-$C_6H_{11}O_5$]$^+$, 100), 302 (5), 289 (15), 261 (22), 218 (28), 169 (12), 137 (35), 109 (55), FAB$^-$-MS *m/z*: 479 [(M-1)$^-$, 100], 317 (12), 189 (55); ^{1}HNMR (DMSO, 400 MHz): δ 6.92 (1H, s, H-8), 7.85 (1H, d, *J* = 2.0, H-2′), 6.85 (1H, d, *J* = 8.4, H-5′), 7.65 (1H, dd, *J* = 8.0, 2.0, H-6′), 4.98 (1H, d, *J* = 7.2, H-1″), 9.18 (1H, s, OH-3), 8.21 (1H, s, OH-6), 9.56 (1H, s, OH-7); ^{13}CNMR (DMSO, 100 MHz) δ: 148.8 (s, C-2), 133.4 (s, C-3), 176.2 (s, C-4), 151.8 (s, C-5), 145.2 (s, C-6), 149.4 (s, C-7), 92.9 (d, C-8), 126.7 (s, C-9), 105.6 (s, C-10), 122.2 (s, C-1′), 115.8 (d, C-2′), 145.8 (s, C-3′), 149.2 (s, C-4′), 116.5 (d, C-5′), 120.72 (d, C-6′), 101.2 (d, C-1″), 74.2 (d, C-2″), 76.4 (d, C-3″), 70.2 (d, C-4″), 77.9 (d, C-5″), 61.3 (t, C-6″). The above spectral data were in accordance with those reported (Hattori *et al.*, 1994).

Luteolin-7-β-O-glucoside (**6**), pale yellow square crystals, m.p. 232–237 °C, IRν_{max} (KBr) cm^{-1}: 3600, 3150, 1650, 1085, 1035, 1020; UV (MeOH) λ_{max} nm: 301, 226, 258; EI-MS *m/z* 286 (100), 258 (7), 229 (12), 153 (26), 134 (12), 105 (18), FAB$^-$-MS *m/z*: 447 ([M-1]$^-$, 100), 325 (32), 285 (35); ^{1}HNMR (DMSO, 400 MHz): δ 6.65 (1H, s, H-3), 6.27 (1H, d, *J* = 2.0, H-6), 6.72 (1H, d, *J* = 2.0, H-8), 7.45 (1H, d, *J* = 2.0, H-2′), 6.85 (1H, d, *J* = 8.2, H-5′), 7.42 (1H, dd, *J* = 8.0, 2.0, H-6′), 5.04 (1H, d, *J* = 7.2, H-1″), 2.91-3.81 (5X1 H, m, H-2″ ~ H-6″); ^{13}CNMR (DMSO, 100 MHz) δ: 164.8(s, C-2), 103.4 (d, C-3), 179.2 (s, C-4), 161.2 (s, C-5), 99.2 (d, C-6), 163.4 (s, C-7), 94.9 (d, C-8), 156.7 (s, C-9), 105.7 (s, C-10), 120.8 (s, C-1′), 116.3 (d, C-2′), 145.6 (s, C-3′), 149.6 (s, C-4′), 113.5 (d, C-5′), 118.7 (d, C-6′), 100.1 (d, C-1″), 73.2 (d, C-2″), 76.4 (d, C-3″), 69.6 (d, C-4″), 77.1 (d, C-5″), 60.3 (t, C-6″). The above spectral data agreed with those reported (Igile *et al.*, 1994).

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