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FLAVONOIDS FROM *ARTABOTRYS HEXAPETALUS*

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Key Word Index—*Artabotrys hexapetalus*; Annonaceae; flavonoids; arapetaloside A, B; taxifolin; apigenin-7-*O*-apiosyl (1→2) glucoside; glucoluteolin.

Abstract—Two novel flavonol glycosides named arapetaloside A and B were isolated from the leaves of *Artabotrys hexapetalus*, together with three known flavonoids, taxifolin, apigenin-7-*O*-apiosyl (1→2) glucoside and glucoluteolin. They were identified as quercetin and kaempferol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinofuranosides.

INTRODUCTION

Artabotrys hexapetalus (Annonaceae) is widely distributed throughout the southern part of China. As a Chinese traditional folk medicine, its roots and fruits are used for treating malaria and scrofula, respectively. Earlier chemical studies showed its sesquiterpenes [1, 2] and alkaloids [3, 4] possess antimalaria and antitumour activities, respectively, but information on its chemistry is limited to the root, stembark and pulp. In the course of our ethnopharmacological investigations of the genus *Artabotrys*, some cardiovascular principles have been isolated from the leaves of *Artabotrys hexapetalus*. In this paper we report the isolation and structural elucidation of two novel flavonol glycosides, arapetaloside A and B, along with three known flavonoids from the same species.

RESULTS AND DISCUSSION

Compound **1**, yellow amorphous powder, mp 175–177° (MeOH), gave *m/z* 579 $[M-H]^-$, $C_{26}H_{28}O_{15}$ in FABMS. Its IR spectrum showed a strong absorption band at 1650 cm^{-1} for a chelated carbonyl group. The UV spectrum of **1** in methanol showed absorptions at 254 (band II) and 350 (band I) nm, indicating a 3-*O*-substituted flavonol [5]. The bathochromic shift (60 nm) and then hypsochromic shift of band I (40 nm) with aluminum chloride and hydrochloride acid, respectively, is a characteristic feature of 5-hydroxy-3-*O*-substituted flavonol with *ortho*-dihydroxyl groups in its B ring [5], which was supported by the presence of the bathochromic shift (20 nm) of band I with boric acid. The bathochromic shift of band II (14 nm) with sodium acetate indicated the presence of a free hydroxy group at C-7 [5]. In the EIMS spectrum of **1**, the presence of fragment ions at *m/z* 302 (aglycone), 273 (aglycone−CO−H), 153 (A^++H) and 137 (B_2^+) suggested that the aglycone was quercetin, which was further confirmed by the presence of signals at δ 6.41 and 6.20 (each 1H, *d*, *J* = 1.8 Hz) assignable to the protons at C-8 and C-6 positions in the A ring, and signals at δ 7.55 (1H, *d*, *d*, *J* = 8.5 Hz, 2.2 Hz), 7.48 (1H, *d*, *J* = 2.2 Hz) and 6.86 (1H, *d*, *J* = 8.5 Hz) assignable to the protons at C-6′, 2′ and 5′ position, respectively, in the B ring. The negative ion FAB mass spectroscopy of **1** showed peaks at *m/z* 579, 433 and 301, which was ascribed, respectively, to $[M-H]^-$, $[M-146-H]^-$ and $[M-146-132-H]^-$ ions, clearly suggesting the presence of a deoxyhexose and a pentose in the molecule. The sugars were identified to be L-rhamnose and L-arabinose by co-TLC with authentic specimens [6].

The NMR evidence revealed the α-L-arabinofuranosyl and α-L-rhamnopyranosyl structures by the appearance of aromeric protons at δ 5.73 (ara) as a broad singlet and δ 4.80 (rha) as a doublet (*J* = 0.88 Hz) in the ^1H NMR spectrum, and the appearance of aromeric carbons at δ 106.2 (ara) and δ 99.2 (rha) in the ^{13}C NMR spectrum. In the ^1H-^1H COSY spectrum of **1**, the signals at δ 4.28 (1H, *m*), 3.83 (1H, *m*), 3.60 (1H, *m*) and 3.30 (2H, *m*) were reasonably assigned to the protons at arabinose C-2, 3, 4, and 5 position. Those proton signals showed cross-peaks with carbon signals at δ 86.5, 75.5, 85.7 and 60.4, respectively, in the ^1H-^{13}C COSY spectrum. The C-2 signal (86.5 ppm) of arabinose showed a downfield shift of 4.4 ppm upon comparison with the corresponding C-2 signal (82.1 ppm) of a quercetin-3-*O*-arabinofuranoside [7], revealing a 1→2 linkage between the 3-*O*-arabinosyl and the terminal rham-

nosyl moieties. Thus **1** was elucidated as quercetin 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinofuranoside and was named as arapetaloside A. This is a new glycoside of quercetin, although the isomeric 3-rhamnosyl (1→2)-L-arabinopyranoside was reported by Geiger *et al.* in *Brassica nigra* seed [8].

Compound **2**, pale yellow amorphous powder, mp 262–265° (MeOH–Me_2CO), gave *m/z* 563 $[M-H]^-$, $C_{26}H_{28}O_{14}$, and 417 $[M-146-H]^-$ and 285 $[M-146-132-H]^-$ in FAB mass spectroscopy. Its UV spectrum in methanol showed absorptions at 262 (band II) and 346 (band I) nm. The bathochromic shift (48 nm) of band I with aluminium chloride–HCl and bathochromic shift (6 nm) of band II with sodium acetate indicated **2** was a 5-hydroxy-3-*O*-substituted flavonol with the presence of free hydroxy groups at C-7 and 4′ [5]. The EIMS spectrum of **2** gave peaks at *m/z* 286 (aglycone), 257 (aglycone−CO−H), 153 ($A^{+\cdot}+1$) and 121 (B_2^+), suggesting that the aglycone was kaempferol, which was confirmed by the presence of signals [δ 6.21 and 6.44 (each 1H, *d*, *J* = 1.9 Hz) assignable to A ring protons; δ 6.90 and 8.00 (each 2H, *d*, *J* = 8.8 Hz) assignable to B ring protons]. The products of acid hydrolysis of **2** produced kaempferol, rhamnose and arabinose. In the ^1H and ^{13}C NMR spectra, the chemical shifts of the protons and carbons in the sugar moiety were the same with that of **1**. Thus, the structure of **2** was concluded to be kaempferol-3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinofuranoside and named as arapetaloside B.

Compound **3** (mp 218–220°), 4 (mp 206–209°) and 5 (mp 252–256°) were obtained as colourless or yellow amorphous powders. The IR, UV, mass, ^1H and ^{13}C NMR spectral data suggested that **3–5** were the known flavonoids, taxifolin [9], apigenin 7-*O*-apiosyl (1→2) glucoside [7] and glucoluteolin [7], respectively.

EXPERIMENTAL

All mps are uncorr. MS were obtained at 70 eV. Chemical shifts are given in δ values (ppm) with TMS as internal standard.

Plant material. The leaves of *A. hexapetalus* were collected in Guangdong province, China, in June, 1995, and a voucher specimen has been deposited in the Herbarium of Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Extraction and isolation. The leaves of *A. hexapetalus* (5 kg) were extracted with 95% EtOH (40 l × 3, each 3 hr) under reflux. The exacts were concd and the residue (480 g) suspended in H_2O and extracted with petrol, $CHCl_3$ and EtOAc. The EtOAc extract (23.3 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (in gradient) as eluent to give 11 frs. Fr. 9 (1.7 g) was subjected to a Sephadex LH-20 column eluted with MeOH to yield subfr. 17. Subfr. 14 was rechromatographed on a polyamide column with $CHCl_3$–MeOH (9:1) as eluent to give **1** (52 mg). Fr. 7 (2.1 g) was rechromatographed on a silica gel column with $CHCl_3$–MeOH (5:1) as eluent to give subfr. 12. Subfr. 11 was subjected to a Sephadex LH-20 column eluted with MeOH to afford **2** (26 mg). Compound **3** (26 mg) from fr. **5**, 4 (12 mg) from fr. 9 and **5** (21 mg) from fr. 8 were obtained by Sephadex LH-20 chromatography or crystallization.

Compound **1** (*arapetaloside A*). Yellow amorphous powder (MeOH, 52 mg), mp 173–175°, $C_{26}H_{28}O_{15}$. UV λ_{max}^{MeOH} nm: 254, 350, + NaOMe: 270, 400, + $AlCl_3$: 272, 410, + $AlCl_3$-HCl: 266, 354, 390, + NaOAc: 268, 318 (sh), 370, + NaOAc-H_3BO_3: 258, 370. EIMS *m/z* (%): 302 (100), 273 (10), 245 (10), 153 (15), 137 (22). ^1H NMR (500 MHz, DMSO-d_6) δ ppm: 12.65 (1H, *s*, 5-OH), 10.93 (1H, *br s*, 7-OH), 9.79 (1H, *br s*, 4′-OH), 9.33 (1H, *br s*, 3′-OH), 7.55 (1H, *d*, *d*, *J* = 8.5 Hz, 2.2 Hz, H-6′), 7.48 (1H, *d*, *J* = 2.2 Hz, H-2′), 6.86 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.41 (1H, *d*, *J* = 1.8 Hz, H-8), 6.20 (1H, *d*, *J* = 1.8 Hz, H-6), 5.73 (1H, *br s*, Ara-H-1), 4.28 (1H, *m*, Ara-H-2), 3.83 (1H, *m*, Ara-H-3), 3.60 (1H, *m*, Ara-H-4), 3.30 (2H, *m*, Ara-H-5), 4.82 (1H, *d*, *J* = 0.88 Hz, Rha-H-1), 3.60 (1H, *m*, Rha-H-2), 3.39 (1H, *m*, Rha-H-3), 3.23 (1H, *m*, Rha-H-4), 3.51 (1H, *m*, Rha-H-5), 1.12 (3H,*d*,*J* = 6.0 Hz, Rha-H-6). For ^{13}C NMR, see Table 1.

Compound **2** (*arapetaloside B*). Pale yellow amorphous powder (MeOH–Me_2CO, 26 mg). $C_{26}H_{28}O_{14}$. UV λ_{max}^{MeOH} nm: 262, 346, + NaOMe: 272, 320, 392, + $AlCl_3$: 272, 302, 350, 394, + $AlCl_3$-HCl: 270, 342, 390, + NaOAc: 268, 300, 354, + NaOAc–H_3BO_3: 262, 346. EIMS *m/z* (%): 286 (100), 257 (7), 229 (7), 153 (5), 143 (5), 121 (20), 111 (15), 99 (46), 81 (35), 69 (7), 57 (5), 45 (17). ^1H NMR (500 MHz, DMSO-d_6) δ ppm: 10.21 (1H, *s*, 7-OH), 10.09 (1H, *s*, 4′-OH), 8.00 (2H, *d*, *J* = 8.8 Hz, H-2′, 6′), 6.90 (2H, *d*, *J* = 8.8 Hz, H-3′, 5′), 6.44 (1H, *d*, *J* = 9 Hz, H-8), 6.21 (1H, *d*, *J* = 1.9 Hz, H-6),5.73 (1H, *br s*, Ara-H-1), 4.28 (1H, *d*, *J* = 3 Hz, Ara-H-2), 3.83 (1H, *m*, Ara-H-3), 3.60 (1H, *m*, Ara-H-4), 3.30 (2H, *m*, Ara-H-5), 4.82 (1H, 2d, *J* = 1.0 Hz, Rha-H-1), 3.60 (1H, *m*, Rha-H-2),

Table 1. ^{13}C NMR spectral data of **1** and **2** (DMSO-d_6)*

Position	1	2	Position	1	2
2	156.3	156.5	Ara-1	106.2	106.4
3	133.3	133.3	2	86.5	86.3
4	177.6	177.5	3	75.5	75.4
5	161.2	160.9	4	85.7	86.1
6	98.6	98.6	5	60.4	60.5
7	164.1	163.9	Rha-1	99.2	99.1
8	93.5	93.6	2	70.4	70.3
9	156.7	156.3	3	70.5	70.5
10	103.9	104.0	4	71.9	71.8
1′	121.6	120.6	5	68.8	68.9
2′	115.5	130.6	6	17.6	17.6
3′	145.0	115.3			
4′	148.4	159.8			
5′	115.5	115.3			
6′	120.8	130.6			

* Chemical shifts are given as ppm values.

3.39 (1H, *m*, Rha-H-3), 3.20 (1H, *m*, Rha-H-4), 3.52 (1H, *m*, Rha-H-4), 1.13 (3H, *d*, $J = 6.2$ Hz, Rha-H-6). For ^{13}C NMR, see Table 1. Compounds **3–5**, spectra identical to those reported earlier.

Acid hydrolysis of **1** *and* **2**. The solns of **1** and **2** were applied to 1 cm from the bottom edge of an HPTLC silica gel plate (10 × 10 cm) and hydrolysed with HCl vapour for 40 min at 50 ~ 60° and the plate developed in 9 ml of the lower layer of a mixt. of $CHCl_3$–MeOH–H_2O (15:6:2) to which 1 ml of HOAc was added. The identification of sugar was carried out by comparison with authentic sugar marker on the same plate.

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