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ASTRAGALIN 2″,6″-DI-*O*-GALLATE FROM *LOROPETALUM CHINENSE*

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Key Word Index—*Loropetalum chinense*; Hamamelidaceae; astragalin 2″,6″-di-*O*-gallate; Loropetalin D; kaempferol 3-(2″,6″-digalloylglucoside); astragalin 2″-*O*-gallate and 6″-*O*-gallate; galloylated flavonol glycosides; hydrolysable tannins.

Abstract—A new galloylated flavonol glycoside, astragalin 2″,6″-di-*O*-gallate (loropetalin D), together with two related flavonol glycosides was isolated from the leaves of *Loropetalum chinense*. The two related flavonol glycosides were characterized as astragalin 2″-*O*-gallate and astragalin 6″-*O*-gallate by chemical methods, ^{1}H and ^{13}C NMR spectral analysis. The structure of loropetalin D was established by comparison of ^{1}H and ^{13}C NMR spectral data with those of the monogallates. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

In the course of chemical and pharmacological studies on tannins and other related polyphenols of *Loropetalum chinense* Oliv., we have reported previously four new oligomeric hydrolysable tannins: prostratin B [1] and loropetalin A–C [2], and 18 other known compounds including four oligomeric hydrolysable tannins. Further examination of the tannins and related polyphenols in this plant by HPLC led to the isolation of a new galloylated flavonol glycoside, loropetalin D (**1**) along with two known related compounds, astragalin 2″-*O*-gallate (**2**) and astragalin 6″-*O*-gallate (**3**). Compounds **2** and **3** have been reported for their biological activity in inhibiting xanthine oxidase (XOD) [3], but ^{1}H and ^{13}C NMR data have not been published previously. We now present the isolation and structural elucidation of loropetalin D as well as **2** and **3**, which are reported from this plant for the first time.

RESULTS AND DISCUSSION

The concentrated 70% aqueous acetone extract from a homogenate of the dried leaves was fractionated by column chromatography (CC) over Diaion HP-20 as described previously [2]. The 40 and 60% methanol eluates were further examined by TLC and compared with standard markers. Repeated CC over Toyopearl HW-40 and Sephadex LH-20 using aqueous methanol resulted in the isolation of loropetalin D (**1**), astragalin 2″-*O*-gallate (**2**) and astragalin 6″-*O*-gallate (**3**). Compound **2**, a yellow amorphous powder, gave colour reactions characteristic of a flavonol glycoside. It gave a bright-yellow colour with 2% $ZrOCl_2$ reagent which disappeared on addition of 2% citric acid and water, showing the presence of a free hydroxyl at the 5-position but no free 3-hydroxyl. UV spectral analysis confirmed the presence of free C-4′ and C-7 hydroxyls. The ^{1}H NMR spectrum showed the presence of one galloyl group by a two-proton singlet, a kaempferol moiety by the signals of δ 8.00 (2H, *d*, $J = 8.7$ Hz, H-2′,6′), 6.92 (2H, *d*, $J = 8.7$, Hz, H-3′,5′), 6.42 (1H, *d*, $J = 2.3$ Hz, H-8) and 6.19 (1H, *d*, $J = 2.3$ Hz, H-6) and a sugar moiety. The configuration at C-1 of the glucose was determined to be β, as shown by the coupling constant of the anomeric proton signal ($J = 8.0$ Hz). The linkage of the galloyl group to the glucose was decided to be at C-2 by the triplet (δ 5.11, 1H, *t*, $J = 8.0$ Hz) and spin-decoupling experiment, i.e., when given irradiation to the doublet of glucose H-1, the triplet at 5.11 became a doublet. Thus, compound **2** was identified as astragalin 2″-*O*-gallate. Compound **3** had the same colour reaction as **2**, indicating that it was also a flavonol glycoside and had no free 3 hydroxyl. ^{1}H NMR spectroscopy showed similar proton signals to compound **2** except for the signals of δ 4.25 and δ 4.35 attributable to glucose at H-6 instead of the triplet of δ 5.10 caused by glucose at H-2, indicating that the C-6 hydroxyl of the glucose is acylated by a galloyl

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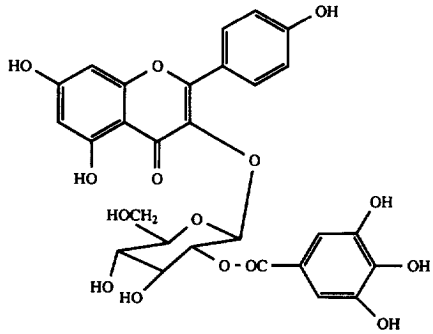

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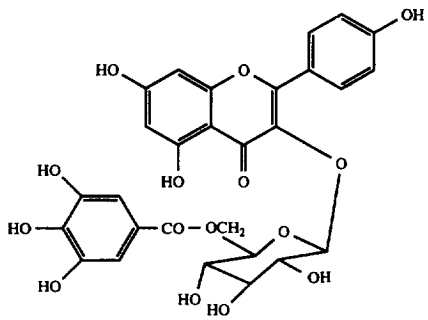

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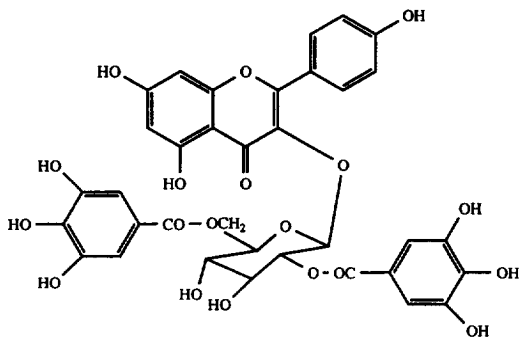

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Table 1. ^{13}C NMR data for loropetalin D (**1**), compound **3** and related compounds **4** and **5**

C no.	4	5	3	1
Aglycone				
C-2	156.3	156.4	156.8	156.3
3	133.0	133.4	133.2	132.7
4	177.4	177.5	177.3	177.2
5	161.1	161.3	160.9	160.7
6	98.2	98.5	98.7	98.6
7	163.9	164.2	164.2	163.7
8	93.6	93.5	93.8	93.8
9	156.3	156.5	156.8	157.0
10	104.7	103.7	104.0	104.1
1′	121.0	120.7	120.7	121.1
2′	130.7	130.0	130.8	130.6
3′	115.0	115.7	115.1	115.0
4′	159.8	159.7	159.8	159.2
5′	115.0	115.7	115.1	115.0
6′	130.7	130.0	130.8	130.8
Glucose				
C-1″	101.4	101.2	101.4	98.6
2′	74.2	74.3	74.1	74.2
3″	76.5	76.3	76.0	74.2
4″	70.1	70.4	69.2	70.3
5″	77.2	74.3	74.0	74.2
6″	61.0	63.0	62.8	62.8
Galloyl				
C-1‴			119.4	119.9, 120.2
2‴, 6‴			108.5	109.0, 109.6
4‴			138.5	137.9, 138.1
3‴, 5‴			145.4	144.6, 144.6
CO			165.7	166.0, 166.3

group. Because no ^1H and ^{13}C NMR data have been published for this compound, we compared its ^{13}C NMR data with those of astragalin (**4**) and kaempferol 3-*O*-(6″-*O*-coumaroyl-β-D-glucoside) (**5**) [4]. As indicated in Table 1, the chemical shift variation of sugar moiety of **3** is consistent with those of **5**. Thus, **3** was identified as astragalin 6″-*O*-gallate.

Compound **1** showed the characteristic colouration and precipitation of hydrolysable tannin with $FeCl_3$ (dark blue) and geratin, indicating that it was a hydrolysable tannin. Its UV spectrum is very similar to that of **2** and **3** except that the absorption at 210 nm and 267 nm attributable to the galloyl group is significantly stronger than in **2** and **3**, indicating the presence of more than one galloyl group. ^1H NMR spectroscopy, in addition to aromatic protons similar to **2** and **3**, displayed two two-proton singlets at δ 7.24 and 7.06 due to two galloyl groups, a triplet at δ 5.20 (J = 8.0 Hz) and a broad singlet at δ 4.39 attributable to H-2 and H-6 of glucose, respectively, which were assigned by a spin-decoupling experiment. The upfield shifts of C-1, C-3 and C-5 due to acylated C-2 and C-6 hydroxyls and downfield shifts of C-2 (unchanged) and C-6 of glucose in ^{13}C NMR compared with **4** and **5** are consistent with those of **3** and **2** (Table 1). Thus, the structure of **1**, named loropetalin D, was established as astragalin 2″,6″-di-*O*-gallate.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were measured on Bruker AM 500 instrument. Normal-phase HPLC was carried out on a Supersphere Si60 (Merck) column (4 × 125 mm) developed with *n*-hexane–MeOH–THF–HCOOH (60:45:15:1) containing oxalic acid (450 mg l^{-1}) (flow rate, 1.5 ml min^{-1}; detection 280 nm) at room temp. Analytical TLC was carried out on silica gel G plate prepd by 0.8% CMC-Na soln and developed with EtOAc–HCOOH–H_2O (7:2.2:3). CC was carried out on Toyopearl HW-40 (Tosoh) and Sephadex LH-20 (Merck).

Plant material. Leaves of *Loropetalum chinense* Oliv., were collected at Xin Xian county, Henan prov-

ince, People's Republic of China in September 1988, and air-dried. The identity of plant material was verified by Mr Wei-Zheng Xiong and a voucher specimen is deposited at the Herbarium of Henan College of Traditional Chinese Medicine.

Isolation of galloylated flavonol glycosides. As described previously [2] the dried leaves (2 kg) were homogenized in 70% Me_2CO and filtered. The concd extract was chromatographed over Diaion HP-20 using H_2O and aq. MeOH in a step gradient (10–100%). A part (24 g) of the 40% MeOH eluate (33 g) was subjected to CC over Toyopearl HW-40 (coarse grade) developing with aq. MeOH and Me_2CO (40% MeOH → 60% MeOH → MeOH–Me_2CO–H_2O 8:1:1 → 70% Me_2CO). Frs 307–319 (15 ml fr^{-1}, 150 mg, 60% MeOH) were combined and further purified by CC over Sephadex LH-20 eluated with H_2O and aq. MeOH (10% MeOH → 20% MeOH → 60% MeOH) to give **2** (80 mg). The 60% MeOH eluate (40 g) from the Diaion HP-20 CC was chromatographed over Toyopearl HW-40 (coarse grade) developing with the same solvent system as **2** to yield two yellow crystalline compounds: **3** (440 mg, 60% MeOH) and **1** (80 mg, 60% MeOH).

Compound **1**, *loropetalin D.* UV λ_{max}^{MeOH} nm: 211(br), 267, 290(sh), 347; (NaOMe) 205, 274, 321, 389; (NaOAc) 210(br), 267, 290(sh), 347; (NaOAc–H_3BO_3) 211(br), 267, 295(sh), 347; ($AlCl_3$) 212, 267, 290(sh), 347. ^{1}H NMR (Me_2CO-d_6, δ): 8.00 (2H, *d*, J = 8.8 Hz, H-2′,6′), 7.24 (2H, *s*, galloyl-H), 7.06 (2H, *s*, galloyl-H), 6.87 (2H, *d*, J = 8.8 Hz, H-3′,5′), 6.40 (1H, *d*, J = 2.0 Hz, H-8), 6.20 (1H, *d*, J = 2.0 Hz, H-6), 5.92 (1H, *d*, J = 8.0 Hz, H-1 Glc), 5.20 (1H, *t*, J = 8.0 Hz, H-2 Glc), 4.39 (2H, *brs*, H-6 Glc), 3.0–4.0 (*m*, Glc-H).

Compound **2**, *astragalin-2″-O-gallate.* UV λ_{max}^{MeOH} nm: 227, 266, 294(sh), 352; (NaOMe) 275, 324, 399; (NaOAc) 227, 270, 299(sh) 360; (NaOAc–H_3BO_3) 210, 265, 293, 351; ($AlCl_3$) 203, 265, 298(sh), 351; ($AlCl_3$–HCl) 204, 266, 299(sh), 349. ^{1}H NMR (Me_2CO-d_6, δ): 8.00 (2H, *d*, J = 8.6 Hz, H-2′,6′), 7.71 (2H, *s*, galloyl-H), 6.91 (2H, *d*, J = 8.6 Hz, H-3′,5′), 6.41 (1H, *d*, J = 2.3 Hz, H-8), 6.19 (1H, *d*, J = 2.3 Hz, H-6), 5.77 (1H, *d*, J = 8.0 Hz, H-1 Glc), 5.10 (1H, *t*, J = 8.0 Hz, H-2 Glc), 4.5–3.0 (*m*, Glc-H).

Compound **3**, *astragalin-6″-O-gallate.* UV λ_{max}^{MeOH} nm: 205, 267 295(sh), 347. ^{1}H NMR (Me_2CO-d_6, δ): 8.01 (2H, *d*, J = 8.5 Hz, H-2′,6′), 7.00 (2H, *s* galloyl-H), 6.85 (2H, *d*, J = 8.5 Hz, H-3′,5′), 6.49 (1H, *d*, J = 1.5 Hz, H-8), 6.29 (1H, *d*, J = 1.5 Hz, H-6), 5.54 (1H, *d*, J = 7.5 Hz, H-1 Glc), 4.35 (1H, *d*, J = 11.0 Hz, H-6 Glc), 4.25 (1H, *dd*, J = 11.0, 3.5 Hz, H-6′ Glc), 3.54 (1H, *m*, H-5 Glc), 3.49, 3.40, 3.34 (each 1H, *t*, J = 8.5 Hz, H-2, 3, 4 Glc).

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