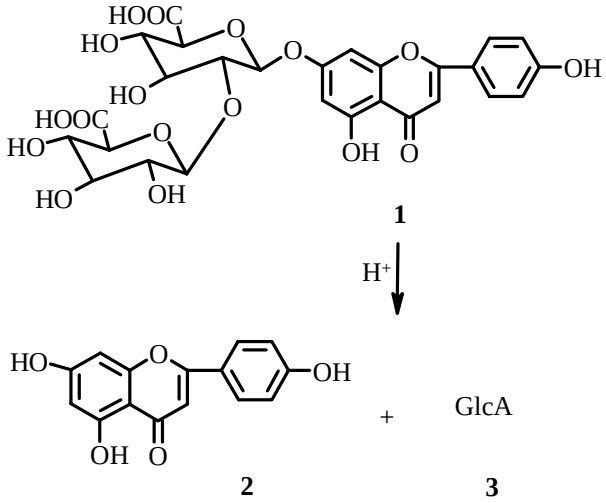
APIGENIN GLUCURONIDE FROM *Perilla nankinensis* LEAVES

D. T. Berashvili,[1] M. D. Alaniya,[2] A. D. Bakuridze,[2] L. N. Gvazava,[2] G. Balansard,[3] and R. Elias[3]

UDC 615.322.547.814.5

Species of the *Perilla* genus (Lamiaceae L.) are decorative plants with essential oils [1, 2]. Data on phenolic compounds and pharmacological properties of representatives of this genus have been reported [3-6]. *P. nankinensis* decne., seu *Dentidia nankinensis* Lour grows prolifically in western Georgia. Its chemical composition has not previously been investigated [1].

Ground air-dried leaves (250 g) that were collected during flowering were exhaustively extracted with MeOH (80%, 1:10). The alcohol extracts were evaporated in vacuo to a water residue that was treated with $CHCl_3$ to remove lipophilic compounds. The purified water extract was condensed to a dry solid containing polar compounds (33.52 g) that was fractionated over ion-exchanger Diaion HP 20 (Mitsubishi Chemical Co., Tokyo, Japan, 2.5 × 50 cm) with elution by water, 50% MeOH, and MeOH. The water fraction (1.0 L) was lyophilized. The solid (2 g total) was separated over a reversed-phase Rp-18 column with elution by water and water:MeOH mixtures of increasing MeOH content to give **1**.



Compound **1** was identified using chemical transformations and UV, IR, PMR, ^{13}C NMR, and mass spectra.

Compound **1** (scheme 1) is a light yellow crystalline powder, $C_{27}H_{26}O_{17}$, mp 216°C (dec.), $[\alpha]_D$ -55.5° (*c* 0.05, MeOH). Mass spectrum (*m*/*z*, %): 622 (4.9) $[M]^+$. λ_{max}^{MeOH} (nm): 336, 268. The IR spectrum contains absorption bands for hydroxyl (3560-3220 cm^{-1}), glucuronic acid carboxyl (1740), γ-pyrone carbonyl (1650), aromatic C=C (1635, 1587, 1510), and glycoside C–O (1100, 1078, 1050) [7].

The absorption band at 1740 cm^{-1} in the IR spectrum is indicative of glucuronic acid in **1** because doublets at 4.62 and 4.61 ppm (J = 9.2 and 8.7) (Table 1) are characteristic of protons H-5″and H-5‴ of a D-glucuronide [5].

Acid hydrolysis of **1** produced the aglycon **2** (~52%), $C_{15}H_{10}O_5$, mp 345°C (dec.), λ_{max}^{MeOH} (nm): 336, 269. IR spectrum (ν_{max}, KBr, cm^{-1}): 3400, 1665, 1561 (characteristic of apigenin) [7, 8]. D-Glucuronic acid (**3**) was detected in the hydrolysate.

Table 1 shows that the carbohydrate unit is bonded to C7 of the aglycon because the signal for C7 shifts to strong field on going from **2** to **1** by 1.13 ppm whereas the signals for C6 and C8 undergo paramagnetic shifts of 5.40 and 0.28 ppm,

1) Tbilisi State Medical University; 2) I. G. Kutateladze Institute of Pharmaceutical Chemistry, Academy of Sciences of Georgia; 3) Mediterranean University, Marseille, fax (99532) 25 00 26. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 78-79, January-February, 2005. Original article submitted August 20, 2004.

0009-3130/05/4101-0097

TABLE 1. PMR and ^{13}C NMR Data for **1** and **2** (δ, ppm, J/Hz, C_5D_5N)

C atom	**1**	**2**	Protons	**1**	**2** [9]
2	164.86	165.50	H-3	6.81 s	6.35 s
3	103.90	104.33	H-6	7.29 d	6.29
4	182.82	183.20	H-8	7.29 d	6.50
5	157.77	158.70	H-2′	7.85 d (J = 7.5)	7.7 d
6	101.00	95.60	H-6′	7.85 d (J = 7.5)	7.7 d
7	163.77	164.90	H-3′	7.12 d (J = 7.5)	6.85 d
8	95.85	95.57	H-5′	7.12 d (J = 7.5)	6.85 d
9	162.70	162.0	H-1″ (anomer.)	5.59 d (J = 6.5)	
10	106.79	105.1	H-1‴ (anomer.)	6.08 d (J = 6.5)	
1′	122.10	122.7	H-2″	4.25	
2′	128.96	129.8	H-2‴		
3′	116.77	117.0	H-3″		
4′	162.66	161.8	H-3‴		
5′	116.80	117.3	H-4″		
6′	128.96	129.8	H-4‴	4.50	
1″	100.31		H-5″	4.62 d (J = 9.2)	
2″	84.19		H-5‴	4.61 d (J = 8.7)	
3″	77.00		5-OH	12.3 s	
4″	72.61				
5″	77.58				
6″	171.91				
1‴	106.97				
2‴	76.20				
3‴	77.81				
4‴	73.32				
5‴	78.19				
6‴	172.48				

respectively. The nature of the bonding between the sugar units was found using ^{13}C NMR data. The chemical shifts of the 1″, 2″, 3′, and 3‴ C atoms are consistent with substitution at C2″of the terminal glucuronic acid (Δδ C2″ is 7.99 ppm). Therefore, the carbohydrate units are bonded to each other through a 2→1-bond. The signals for the anomeric protons of glucuronic acid appear at 5.59 and 6.08 ppm as 1H doublets with SSCC 6.5 Hz. This corresponds with a β-glycoside bond for D-glucuronic acid [9].

Thus, **1** is 5,4′-dihydroxy-7-O-β-D-glucuronyl-(2→1)-glucuronopyranosylflavone.

A compound of analogous structure was isolated from *P. ocimoides* and *P. frutescens* [5, 10].

REFERENCES

1. *Flora of Georgia* [in Russian], Metsniereba, Tbilisi (1987), Vol. II.
2. *Plant Resources of the USSR* [in Russian], Nauka, St. Petersburg (1991), Vol. 6.
3. T. Fujita and M. Nakayama, *Phytochemistry*, **31**, No. 9, 3265 (1992).
4. L. N. Misra and A. Husain, *Planta Med.*, 379 (1987).
5. K. Joshida, K. Kameda, and T. Kondo, *Phytochemistry*, **33**, No. 4, 917 (1993).
6. T. Makino, M. Ito, F. Kiuchiu, T. Ono, E. Muso, and G. Honda, *Planta Med.*, 67 (2001).
7. M. D. Alaniya, E. P. Kemertelidze, and N. F. Komissarenko, *Flavonoids of Certain Species of the Astragalus L. Genus. Flora of Georgia* [in Russian], Metsniereba, Tbilisi (2002).
8. B. Ternai and K. R. Markham, *Tetrahedron*, **32**, No. 5, 565 (1976).
9. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids* Springer-Verlag, New York (1970).
10. T. Makino, Y. Furuta, H. Fum, T. Nakagawa, H. Wakushima, K. Saito, and Y. Kano, *Biol. Pharm. Bull.*, **24**, No. 10, 1206 (2001).