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FLAVONOIDS OF THE LEAVES OF *Stevia rebaudiana*

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Stevia rebaudiana (Bertoni) (fam. Compositae) is widely known as a rich source of sweet-tasting components, the main ones being the diterpene glycosides stevioside and rebaudioside [1]. We have studied the chemical composition of an ethyl acetate extract of an ethanolic extract of the leaves of this plant cultivated in the Tashkent oblast.

The dry comminuted leaves (1 kg) were extracted with ethanol (5 liters). After evaporation, the extract was dissolved in water (0.5 liter) and extracted first with chloroform and then with ethyl acetate. This gave 32.5 g of ethyl acetate extract, 16 g of which was chromatographed on a column of KSK silica gel. Using the chloroform–methanol (4:1) system as eluent, we isolated the flavonoid compound (**1**) (0.3 g) with the composition $C_{21}H_{20}O_{10}$, mp 203-205° (MeOH), $[\alpha]_D^{17}$ −52.8° (*c* 0.91; DMFA).

UV spectrum $\nu_{max}^{C_2H_5OH}$ 269, 338, $\nu_{max}^{CH_3COONa}$ 268, 338, 400, $\nu_{max}^{AlCl_3}$ 279, 301, 343, $\nu_{max}^{CH_3ONa}$ 268, 398 nm.

IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3520 (OH), 2910, 1660 (C=O), 1595, 1500 (C_6H_5–), 1460, 1420, 1078, 1050, 1035, 1000, 910, 750, 680.

Mass spectrum *m/z*: 270 (M^+ of the genin), 242, 153, 144, 124, 119, 96.

PMR spectrum (Py-d_5, δ, ppm): 4.12 (6H, m, H-6 of glucose), 5.73 (1H, d, J=8 Hz, H-1 of glucose), 6.65 (1H, d, J=2.5 Hz, H-6), 6.8 (1H, H-3), 6.98 (1H, d, J=2.5 Hz, H-8), 7.13 (2H, d, J=8 Hz, H-3′, H-5′), 7.8 (2H, d, J=8 Hz, H-2′, H-6′) [2].

The magnitude of the absorption in UV light showed that substance (**1**) belonged to the flavone group [3]. Acid hydrolysis of the compound with 5% sulfuric acid (90°C, 5 h) led to an aglycon with mp 339-341°C, which was identified by TLC in the chloroform–methanol (4:1) and (10:1) systems in comparison with an authentic sample as apigenin. The molecular mass of the aglycon (M^+ 270) showed that its molecule contained three hydroxy groups.

PC in the butan-1-ol–pyridine–water (6:4:3) system revealed glucose as the carbohydrate component. The absence of a difference in the UV spectra of apigenin and compound (**1**) showed the position of the glucose residue at the C-7 hydroxyl of the genin. This was confirmed by the spectra taken with diagnostic additives [3]

The presence of three absorption bands in the IR spectrum of the glycoside (1078, 1050, and 1035 cm^{-1}) presupposed the pyranose form of the ring of the glucose residue, while a doublet at δ 5.73 ppm with the SSCC J = 8 Hz in the PMR spectrum showed the β-configuration of the glycosidic bond.

It followed from the facts presented that flavonoid (**1**) was a known glycoside — cosmosiin — apigenin 7-O-β-*D*-glucopyranoside [4-6].

Elution of the column with the chloroform–methanol (3:1) system, followed by rechromatography with the chloroform–methanol–water (15:6:1) system, yielded compound (**2**) (0.58 g), having the composition $C_{21}H_{20}O_{11}$, mp 189-191° (MeOH), $[\alpha]_D^{20}$ −151.7° (*c* 0.83; MeOH).

UV spectrum: $\nu_{max}^{C_2H_5OH}$ 254, 265 sh., 354; $\nu_{max}^{CH_3COONa}$ 273, 400 split, $\nu_{max}^{AlCl_3}$ 263, 300 sh., 362, 405 sh., $\nu_{max}^{CH_3ONa}$ 273, 330, 400 nm.

IR spectrum (ν_{max}^{KBr}, nm^{-1}): 3320, 2960, 1660, 1620, 1580, 1510, 1450, 1390, 1110, 1100, 1070, 1010, 1000, 965, 920, 895, 850, 720.

Mass spectrum: *m/z*: 302 (M^+ of the genin), 273, 262, 153, 141, 137, 128, 110, 95, 69, 57.

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PMR spectrum (Py-d_5, δ, ppm): 1.26 (3H, d, J=6 Hz, CH_3 of rhamnose), 4.12 (2H, m, H-4, H-5 of rhamnose), 4.45 (1H, m, H-3 of rhamnose), 4.82 (1H, br.s, H-2 of rhamnose), 6.15 (1H, H-1 of rhamnose), 6.52 (2H, d, J=2.0 Hz, H-6), 6.60 (1H, d, J=2.0 Hz, H-8), 7.20 (1H, d, J=8.0 Hz, H-5′), 7.57 (1H, d, J=2.0 Hz, H-6′), 7.92 (1H, d, J=2.0 Hz, H-2′), 13.81 (1H, br.m, OH-5).

The UV spectrum of compound (2) corresponded to a flavonol glycoside and, in particular, to a quercetin rhamnoside [3]. Its acid hydrolysis gave an aglycon with mp 302-304°C, $[\alpha]_D^{20}$ +70.4° (*c* 0.69; chlf–MeOH (1:1)).

PMR spectrum (Py-d_5, δ, ppm): 6.62 (2H, dd, J=1.5 and 2.5 Hz, H-6, H-8), 7.30 (1H, d, J=7.5 Hz, H-5′), 8.2 (1H, dd, J=2.0 and 2.5 Hz, H-6′), 8.5 (1H, d, J=2.0 Hz, H-2′), 11.75 (1H, br.m, OH-3), 13.81 (1H, br.m, OH-5).

The characteristics given corresponded to those for quercetin [2]. A TLC comparison with an authentic specimen showed their identity. Rhamnose was detected in a hydrolysate.

The results of UV spectroscopy with diagnostic additives and also a comparison of the PMR spectra of compound (2), quercetin, and rutin showed that the rhamnose was attached to the C-3 OH group of the aglycon through an α-glycosidic bond. Consequently, the flavonoid glycoside (2) could be quercetin 3-α-*L*-rhamnoside.

Both flavonoids have been isolated previously from *Stevia serrata, St. geleopsidifolia*, and *St. soratensis* [7].

REFERENCES

1. H. Kohda, R. Kasai, et al., Phytochemistry, **15**, No. 6, 981 (1976).
2. T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, New York (1970).
3. T. A. Geissman, The Chemistry of Flavonoid Compounds, Pergamon, New York (1962), p. 107.
4. M. I. Borisov, Khim. Prir. Soedin., 662 (1974).
5. T. I. Plekhanova, V. A. Bamdyukova, and G. A. Mikhailova, Khim. Prir. Soedin., 862 (1977).
6. Ts. Dashbalyn and V. I. Glyzin, Khim. Prir. Soedin., 807 (1978).
7. A. Rajbhandari and M. F. Roberts, J. Nat. Prod., **48**, No. 5, 858 (1985).