Chemistry of Natural Compounds, Vol. 45, No. 3, 2009

TRITERPENE GLYCOSIDES FROM THE TUBERS OF *Leontice smirnowii*

N. Tabatadze,[1*] B. Tabidze,[1] V. Mshvildadze,[1] R. Elias,[2] G. Dekanosidze,[1] G. Balansard,[2] and E. Kemertelidze[1]

UDC 547.918.543.422

Leontice smirnowii (Trautv.) (Berberidaceae) is an endemic plant of Georgia [1]. Its tubers have been used in folk medicine as an antitubercular remedy [2]. The occurrence of triterpene saponins and alkaloids in the plant as well as the biological activity of the crude extract and monodesmoside fraction has been previously reported [3–7].

The tubers of *Leontice smirnowii* were collected in Lagodekhi region of Georgia (May, 2005) and dried in the shade. A voucher specimen is deposited in the department of Pharmacobotany, Institute of Pharmacochemistry, Georgia (tubers No. 95343).

Dried and powdered tubers of *L. smirnowii* (1 kg) were extracted with 80% MeOH (7 L). The extract was concentrated under vacuum. After evaporation of solvent the dry extract (298 g) was disolved in MeOH (900 mL) and precipitated in acetone (5 L). The precipitate was filtered and dried to obtain a crude saponin fraction (205 g). Forty grams of crude saponins were subjected to low-pressure liquid chromatography (LPLC) (ChromatoSPAC Prep 100, Lichroprep C-18, 15–25 μm) by using reversed phase RP-18 and eluted with MeOH–H_2O (10% to 80% of MeOH) to afford four fractions. The obtained fractions were subjected to column chromatography on silica gel (0.04–0.063 mm, Merck) and eluted with $CHCl_3$–MeOH–H_2O (26:14:3) to give the individual compounds: **1** (90 mg), **2** (45 mg), **3** (80 mg), **4** (60 mg).

The structures of the glycosides **1–4** were established on the basis of acid hydrolysis and NMR data.

Leonticin D (**1**) was obtained as an amorphous solid with a molecular peak at *m/z* 865 $[M-1]^+$, indicating a mol. wt. of 866 daltons. Acid hydrolysis of **1** yielded arabinose, glucose, and xylose as the sugar components identified on TLC by comparison with authentic samples. The ^1H and ^{13}C NMR data of **1** suggested the presence of α-arabinopyranosyl, β-xylopyranosyl, and β-glucopyranosyl moieties, as shown by three anomeric proton signals at δ 4.37 (d, J = 7.6 Hz), 4.66 (d, J = 7.7 Hz), and 4.58 (d, J = 7.6 Hz), as well as the corresponding anomeric carbons at δ 106.04, 104.86, and 105.05. The ^{13}C NMR spectrum showed that the aglycone of **1** contained 29 carbon atoms out of 63 and consisted of five methyls, eleven methylenes, five methines, and eight quaternary carbons as determined from a DEPT experiment. The ^1H NMR spectrum of **1** showed signals for five tertiary methyl groups at δ 1.18, 1.04, 0.95, 0.84, and 0.79, an exomethylene group at δ 4.63 and 4.62 (each br.s), and an olefinic proton at δ 5.32 (br.t). Compound **1** was clearly shown to have two unsaturations: one endocyclic between C-12 (δ 124.32) and C-13 (δ 144.10), while signals at δ 149.28 and δ 107.58 were assigned to an exocyclic double bond involving C-20 and C-29. The 20(29)-exomethylene group was ascertained by long-range correlations from the exomethylene proton signals to the methylene carbons C-19 and C-21, in the heteronuclear multiple-bond connectivity (HMBC) spectrum of **1**. The above spectral information allowed to us to establish the structure of aglycone **1** as 3β-hydroxy-30-nor-olean-12,20(29)-dien-28-oic acid, in agreement with literature data [8]. The aglycone 3β-hydroxy-30-nor-olean-12,20(29)-dien-28-oic acid was described for the first time in *Leontice* species. The HMBC spectrum showed correlations between C-3 (d 91.10) of aglycone and H-1 (δ 4.37) of the arabinose (Ara), and between C-2 (δ 77.53) and C-3 (δ 84.04) of the arabinose and H-1 (δ 4.66) of the xylose (Xyl) and H-1 (δ 4.58) of the glucose (Glc), respectively.

Based upon the above observations the structure of **1** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-α-L-arabinopyranosyl-3-hydroxy-30-nor-olean-12,20(29)-dien-28-oic acid, which was previously reported as trifoside B [9].

1) Iovel Kutateladze Institute of Pharmacochemistry, 36, St. P. Sarajishvili, 0159, Tbilisi, Georgia, fax: 995 32 52 00 23, e-mail: nino_tabatadze@yahoo.com; 2) Laboratoire de Pharmacognosie, faculte de Pharmacie, Universite de la Mediterranee, 27 Boulevard Jean Moulin, 13385 Marseille, cedex 5, France. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 382–383, May–June, 2009. Original article submitted July 27, 2007.

0009-3130/09/4503-0453

Leonticin F (**2**) was obtained as an amorphous powder. TLC analysis after acid hydrolysis and ^{13}C NMR shifts of C-3 (δ 81.99) and C-28 (δ 177.80) indicated that compound **2** was a bidesmoside. The NMR spectral features suggested that the oleanoic acid was an aglycone. The ^{1}H NMR spectrum of **2** showed four anomeric proton signals at δ 4.45, 5.24, 5.34, and 4.34. The NMR experiments indicated the presence of a terminal α-rhamnopyranose (Rha), a 3-substituted β-xylopyranose (Xyl), 28-substituted β-glucopyranose (Glc1), and 6-substituted β-glucopyranose (Glc2). The HMBC spectrum showed correlations between aglycone C-3 (δ 81.99) and Xyl H-1 (δ 4.45), Xyl C-2 (δ 78.73) and Rha H-1 (δ 5.24), Glc1 C-6 (δ 69.47) and Glc2 H-1 (δ 4.34). It can be concluded that the structure of **2** was determined 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-oleanolic acid. This glycoside was previously described as anhuienoside C [10].

Leonticin H (**3**) showed molecular formula as $C_{65}H_{106}O_{30}$ by HR-ESI-MS (*m/z* 1390.5401 $[M+Na]^+$, theoretical mass for $C_{65}H_{106}NaO_{30}$ 1389.7202). Acid hydrolysis of **3** furnished arabinose, glucose and rhamnose as sugar units. Detailed COSY, HMQC and HMBC correlations revealed that **3** had the aglycone of oleanolic acid. Compound **3** was also a bisdesmosidic glycoside deduced from the chemical shift of C-3 (δ 82.15) and C-28 (δ 177.78). The ^{1}H NMR spectrum of **3** showed six anomeric proton signals at δ 4.83, 4.83, 4.38, 4.58, 5.31 and 4.38. The HSQC-TOCSY correlations were indicative of three β-D-glucose, two α-L-rhamnose, one α-L-arabinose. Moreover, the HMBC spectrum showed correlations between aglycone C-3 (δ 82.15) and Ara H-1 (δ 4.38), Ara C-4 (δ 84.03) and Glc3 H-1 (δ 4.58), Ara C-2 (77.58) and Rha H-1 (4.83), Glc2 C-4 (δ 79.52) and Rha H-1 (δ 4.83), Glc1 C-6 (δ 69.36) and Glc2 H-1 (δ 4.38). Thus the structure of **3** was determined as 3-*O*-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-oleanolic acid, which was previously reported as Hederacolchiside E [11].

Leonticin K (**4**) was obtained as a white amorphous powder. Inspection of the NMR spectral data suggested the presence of oleanolic acid and seven monosaccharides, which were elusidated as arabinose, xylose, rhamnose, galactose, and glucose identified on TLC analysis with authentic samples after acid hydrolysis.

A linear sequence of the tetrasaccharide unit linked at C-3 of the aglycone was indicative of a set of glycosidic cleavage fragments. The identification of the individual spin systems associated with the seven monosaccharides and the assignments of the NMR resonances were accomplished by a combination of TOCSY, COSY, HMBC, and HMQC experiments. The above spectral information allowed us to establish the structure of **4** as 3-*O*-β-D-xylopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-oleanolic acid, which was previously reported as Leonticin G [12].

ACKNOWLEDGMENT

This work was supported by the INTAS program (project No. 01-2043). We thank Mr. Gilbert BOUDON for his technical assistance.

REFERENCES

1. *Sakartvelos Flora*, Tbilisi, Matsniereba, **2**, 1973, p. 173.
2. A. C. Aladashvili and A. D. Saldadze, *Sbornic Trudov TNICHFI*, Tbilisi, 1955, **7**, p. 49.
3. E. G. Tkeshelashvili and K. S. Mudjiri, *Khim. Prir. Soedin*., 807 (1975).
4. G. E. Dekanosidze, N. A. Aneli, and M. S. Loladze, *Bull. Georgian Acad. Sci.*, **70**, 113 (1973).
5. E. G. Tkeshelashvili, S. Iskandarov, K. S. Mudjiri, and S. I. Iunusov, *Khim. Prir. Soedin*, 539 (1971).
6. A. Gepdiremen, V. Mshvildadze, K. Bakuridze, and R. Elias, *Phytomedicine*, **13** (9–10), 728 (2006).
7. I. Gulcin, V. Mshvildadze, A. Gepdiremen, and R. Elias, *Phytomedicine*, **13** (5), 343 (2006).
8. R. E. Hurd and B. K. John, *J. Magn. Reson*., **91**, 648 (1991).
9. A. Ikuta, *J. Nat. Prod.,* **58** (9), 1378 (1995).
10. W. C. Ye, Q. W. Zhang, Sh. X. Zhao, and C. T. Che, *Chem. Pharm. Bull.*, **49**, 632 (2001).
11. G. Dekanosidze, O. Djikia, M. Vugalter, and E. Kemertelidze, *Khim. Prir. Soedin*, 747 (1984).
12. M. Chen, W. W. Wu, D. Nanz, and O. Sticher*, Phytochemistry*, **44** (3), 497 (1997).