Chemistry of Natural Compounds, Vol. 33, No. 6, 1997

ISOPRENOIDS OF *Sambucus nigra*

O. V. Makarova and M. I. Isaev

UDC 547.914.4.4+547.918:547.926.2

European elder, *Sambucus nigra* L. (Caprifoliaceae), is a medicinal plant. The flowers and fruit and, sometimes, the bark are used as the medicinal raw material. We have studied the flowers of this plant for their isoprenoid content.

Air-dry flowers of *S. nigra* (8 kg) gathered in 1988 in the Beshtau and Mashuk region of the Northern Caucasus were exhaustively extracted with methanol (3 × 50 liters). The methanolic extracts were evaporated to a syrupy consistency, and the precipitate that had deposited (205.34 g) was filtered off. TLC in various solvent systems showed that the qualitative compositions of the desired substances in the precipitate and in the mother solution were identical and included no fewer than six substances of triterpenoid and sterol natures. They were designated in order of increasing polarity as substances (**1**)-(**6**).

The precipitate (135 g) was chromatographed on a column of type KSKG silica gel, with elution successively by petroleum ether, petroleum ether–chloroform (1:1), chloroform, and chloroform–methanol (20:1) with a gradient increase in the methanol content to a ratio of (5:1). The fractions eluted by petroleum ether contained no steroids or triterpenoids. On continuing elution of the column with petroleum ether–chloroform (1:1), we collected fractions containing (**1**) and (**2**) together and (**3**) as an individual substance (0.58 g). Rechromatography of the mixture of (**1**) and (**2**) on a column in the benzene–chloroform–ethyl acetate (30:1:1) system led to the isolation of 8.33 g of substance (**1**) in the form of a clear viscous oil with yellow tinge and 0.54 g of the crystalline substance (**2**). The oily substance (**1**) has not been identified.

On TLC in various solvent systems, substance (**2**) was revealed in the form of a homogeneous spot. Consideration of its PMR and mass spectra showed a nonindividuality of substance (**2**) and the triterpene nature of its components. In the PMR spectrum ($CDCl_3$) of substance (**2**), we observed at 0.26 and 0.50 ppm one-proton doublets of an *AB* system (^2J = 4 Hz) characteristic for an isolated methylene of a cyclopropane ring. This fact permitted the assumption that one of the components of the mixture belonged to the cycloartane series [1, 2].

In the mass spectrum of the mixture (**2**), two groups of peaks were distinguished: 1) *m/z* (%): M^+ 440 (34.4), 425 (25.0), 422 (37.5), 397 (9.4), 379 (15.6), 353 (9.4), 315 (12.5), 300 (31.3), 175 (59.4), 107 (100), and 2) M^+ 426 (22.2), 411 (4.7), 408 (1.6), 393 (1.1), 218 (100), 207 (13.3), 203 (11.1), 189 (8.9). The first group of ions was characteristic for 24-methylenecycloartanol [3], and the second for α- and β-amyrins [4]. We showed by the GLC method (Chrom-41, glass column 4 mm × 2.0 m, stationary phase 3% of SE-30 on Chromaton N-AW; carrier gas helium; temperature of the evaporator 310°C, temperature of the thermostat 280°C, internal standard β-sitosterol) that mixture (**2**) consisted of 10.79% of β-amyrin (relative retention time T_{rel} 1.06), 74.10% of α-amyrin (T_{rel} 1.16), and 15.10% of 24-methylenecycloartanol (T_{rel} 1.29) [5].

Substance (**3**), with mp 130-132°C (from MeOH), $[\alpha]_D^{30}$ −43.7±2° (*c* 0.8; $CHCl_3$) was identified as β-sitosterol, also with the aid of PMR characteristics, mass spectra, and GLC [6].

The further elution of the column with chloroform and chloroform–methanol (20:1) led to 54.85 g of substance (**4**). To free it from pigments, it was chromatographed repeatedly on a column with elution by various solvent systems. After recrystallization from ethanol, substance (**4**) had mp 284-286°C $[\alpha]_D^{30}$ +72±2° (*c* 1.4; $CHCl_3$–MeOH (1:1). These characteristics and also those of its PMR and IR and mass spectra coincided with those of ursolic acid [7].

On continuing elution of the column with chloroform–methanol (10:1 and 5:1) systems, we isolated fractions containing the individual substances (**5**) and (**6**).

After repeated rechromatography of the fraction containing substance (**5**) on a column in various solvent systems, we obtained 1.16 g of a compound with mp 213-214°C (from $CHCl_3$–MeOH (25:1)), which hardened again at 228-232°C and remelted at 277-282°C, $[\alpha]_D^{30}$ +90±2° (*c* 0.9; C_5H_5N). From its PMR, IR, and mass spectra, substance (**5**) was identified as 20β-hydroxyursolic acid [7].

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 891-892, November-December, 1997. Original article submitted June 16, 1997.

 0009-3130/97/3306-0702$18.00

The fraction containing substance (**6**) was recrystallized from methanol, to give 30 mg of a glycoside with mp 278-280°C, $[\alpha]_D^{30}$ $-36\pm2°$ (*c* 0.45; C_5H_5N), identified as β-sitosterol β-*D*-glucopyranoside [6], again from its chromatographic behavior on TLC in comparison with an authentic specimen and by PMR and IR spectroscopy.

REFERENCES

1. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 431 (1985).
2. M. I. Isaev, M. B. Gorovits, and N. K. Abubakirov, Khim. Prir. Soedin., 156 (1989).
3. R. T. Aplin and G. M. Hornby, J. Chem. Soc. (B), 1078 (1966).
4. Eight-peak Index of Mass Spectra, 3rd ed., Royal Society of Chemistry, Nottingham, Vol. 1, Part 2 (1983), p. 1152.
5. V. L. Salenko, V. N. Sidel'nikov, M. L. Troshkov, V. A. Raldugin, and V. A. Pentegova, Khim. Prir. Soedin., 328 (1982).
6. L. J. Swift, J. Am. Chem. Soc., **74**, 1099 (1052).
7. R. Haensel and M. Kussmaul, Arch. Pharm., **308**, 790 (1975).