IRIDOIDS FROM *Symphoricarpos albus*

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Twelve compounds including secologanin, loganin, the aglycon of loganin, and a new iridoid called glucologanin were isolated from fruit of common snowberry Symphoricarpos albus *(L.) Blake. The structure of glucologanin was confirmed using PMR and mass spectroscopy and chemical transformations. 2′,3′,4′,6′,7-Penta-*O*-acetylloganin and 2′,3′,4′,6′-tetra-*O*-acetylloganin were synthesized.*

Key words: iridoids, *Symphoricarpos albus*, common snowberry, loganin, secologanin, aglycon of loganin, glucologanin, lactone.

Common snowberry *Symphoricarpos* L. (Caprifoliaceae, honeysuckle) is a bush up to 1.5 m in height and is widely used as a decorative plant. In nature it is found mainly in North America and in the northern strip of European Russia. In Ukraine, white common snowberry *Syphoricarpos albus* (L.) Blake is most common.

The phytochemistry of common snowberry is insufficiently studied. The contents in fruit of *S. albus* of phenolic acids [1], carbohydrates [2], and iridoids [3, 4] have been reported.

We studied fruit of white common snowberry (*S. albus*) collected in October-November 2005 in the Botanical Garden of Karazin Kharkov National University (Ukraine). Fruit contains a large amount (85-87%) of water, which we took into account during its processing.

Freshly collected berries were ground and extracted with *n*-butanol. The extract was divided into three fractions by polarity and chromatographed over columns using mixtures of $CHCl_3$ and CH_3OH of increasing polarity for elution (see Experimental).

A total of 12 pure compounds was isolated. Three of the isolated compounds were iridoids and were identified as secologanin (**1**), loganin (**2**), and loganigenin (**3**) [3, 5, 6]. A fourth glycoside (**4**) was new. The structures of **1**, **2**, and **3** were confirmed by PMR and mass spectrometry.

Compound **1** was found to be highly unstable during its isolation. The compound was quickly oxidized in solutions by oxygen in air. The product of auto-oxidation was presumably cyclized to form lactone **8**. The carboxyl and vinyl (ethylene) groups were involved in the cyclization. The product of auto-oxidation **8** was isolated during additional purification of **1** by column chromatography. Compound **8** contained two C=O groups according to IR spectra, one of which was an ester conjugated to a C=C bond (absorption band at 1696 cm^{-1}); the second, a carbonyl of a seven-membered lactone ring (absorption band at 1655 cm^{-1}).

The PMR spectrum of **8** exhibited a characteristic resonance for the C-3 proton at 7.3 ppm, for the anomeric C-1′ proton at 4.5 ppm; a characteristic multiplet for the ester OCH_3 group at 3.7 ppm; and for protons H-5 and H-11 at 2.7 ppm. There was no resonance for an aldehyde.

The mass spectrum of the lactone was consistent with the proposed structure of **8** (see Experimental).

Compound **2** can exist as cyclic dimer **5**. The C-7 OH group and ester carbonyl form two intermolecular H-bonds. The mass spectrum of the loganin dimer was consistent with the proposed structure of **5**.

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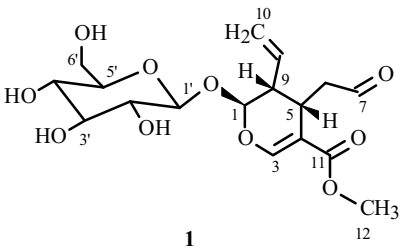
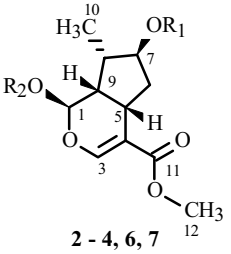


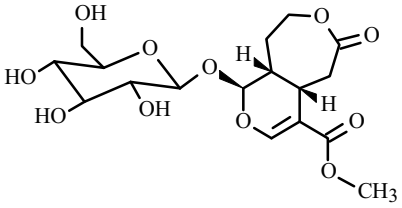
1



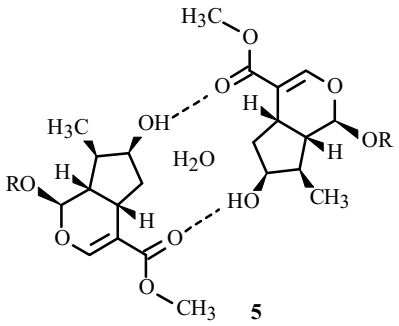
2 - 4, 6, 7

3: $R_1 = R_2 = H$

2, 4, 7: $R_1 = H$; **6:** $R_1 = Ac$



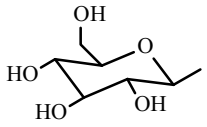
8



5

R = β-D-Glc

2: $R_2 =$



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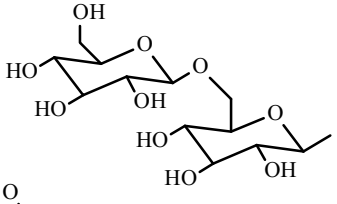
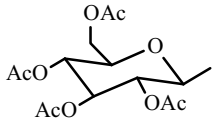


6, 7: $R_2 =$



The dimeric structure of **5** explains the reduced reactivity of the OH group in the aglycon part of **2** (see below). This group gave a broad absorption band in the IR spectrum at 3300-3550 cm^{-1} that belonged to the glycoside alcohols. The broad band was characteristic of an OH group involved in a H-bond with other functional groups. The IR spectrum also had a strong band for C=O stretching vibrations at 1712 cm^{-1} that was shifted to short wavelength compared with ordinary α,β-unsaturated esters. This also confirmed that a H-bond was formed [7].

The chemical transformations of loganin described below, in particular, acetylation of glycoside **2**, provided additional confirmation of the correctness of the structure of **5**.

New iridoid glucologanin (**4**) was water-soluble. Enzymatic hydrolysis of **4** was carried out in order to establish its structure. This formed loganigenin (**3**) and D-glucose. These same products were obtained by hydrolysis of loganin monoglycoside (**2**). Therefore, it was assumed that ths compound was a diglycoside, glucologanin. The large difference in the polarity of glycosides **2** and **4** also confirmed this assumption: R_f(**2**) = 0.52; R_f(**4**) = 0.11 for TLC using $CHCl_3$:CH_3OH (60:40).

PMR spectra of **4** showed two anomeric protons near 4.0 ppm and two CH_2 groups of glucose units (4H resonance as a doublet and multiplet near 4.6 ppm). These data indicate that the two CH_2 groups are nonequivalent. This is possible only with a 1→6 bond between the D-glucose units. This conclusion was confirmed by partial acid hydrolysis. The hydrolysate contained loganin aglycon, D-glucose, and the disaccharide gentiobiose, in which the D-glucose units are found in the pyranose form and are bonded to each other in the 1→6 positions. The detection of gentiobiose indicated that the terminal D-glucose had a β-glycoside bond. The hypothesis was confirmed because the calculated contribution of the terminal D-glucose to the molecular rotation of **4** calculated by the Klyne rule [8] was 180°. Such a value is possible only with a β-glycoside bond.

Thus, the new glycoside was loganigenin-1β-*O*-β-D-glucopyranosyl-6′-*O*-β-D-glucopyranoside. Its structure corresponds to structural formula **4**.

Compound **2** was used to synthesize 2′,3′,4′,6′,7-penta-*O*-acetylloganin (**6**) and 2′,3′,4′,6′-tetra-*O*-acetylloganin (**7**). The pure compounds were prepared. Their structures were confirmed by PMR and IR spectra and chemical transformations.

The PMR spectrum of **6** exhibited characteristic resonances for acetyl protons at 1.85 ppm and 3.65 ppm. The IR spectrum lacked absorption bands for free OH groups and absorption band for OH groups. Three absorption bands were found in the region of C=O stretches for the starting glycoside (1715 cm^{-1}), OCH_3-2′,3′,4′,6′ acetyls (1758 cm^{-1}), and the acetyl on C-7 of the aglycon (1703 cm^{-1}).

The PMR spectrum of **7** had characteristic resonances near 1.9 ppm for four acetyls. The IR spectrum showed an absorption band for free OH on C-7 with a maximum at 3479 cm^{-1}. Acid hydrolysis of **7**, which formed the aglycon of loganin (**3**) and 2,3,4,6-tetra-*O*-acetyl-D-glucose, indicated that the OH group was on C-7.

The IR spectrum of **7** exhibited a strong absorption band with maximum at 1755 cm^{-1} for the acetyl carbonyl (CO-conjugated). A broad symmetric band with maximum at 3479 cm^{-1} was found in the region of OH stretching vibrations.

It was established experimentally that the C-7 OH group of **2** was comparatively difficult to acylate. A kinetic analysis of the acylation of **2** showed that the half-reaction time was 3 h (at 23°C); the time for full acylation, >30 h. The equatorial OH groups in D-glucose (on C-2′, C-3′, C-4′) and the one primary OH (on C-6′) were highly reactive, as expected [9]. The time for complete acylation of the D-glucose unit was about 50 min.

The significant difference in the reactivity of the C-7 OH group and the others in the glycoside enabled loganin to be acylated to form primarily **7**. Thus, the ratio of **7** to **6** was about 3:1 after 50 minutes of acylation.

EXPERIMENTAL

TLC was performed on Sorbfil UV-254 silica-gel plates (10 × 15 cm) (ZAO Sorbpolimer, Krasnodar, Russia) using $CHCl_3$:CH_3OH (from 97:3 to 60:40).

Chromatograms were developed using vanillin—H_2SO_4. The plate was sprayed with vanillin solution in ethanol (1%) and then H_2SO_4 in ethanol (10%). The plate was viewed in daylight after heating to 110°C for 3-5 min [10].

Melting points of isolated compounds were determined on a Kofler block; specific rotation, on a SM-3 circular polarimeter.

Column chromatography used silica gel (particle size 40-60 μm, Aldrich) that was washed with HCl solution (0.5%) beforehand to remove metal ions. Silica gel was dried in air at room temperature and activated at 130-140°C for 3 h in a drying chamber.

PMR spectra in DMSO-d_6 were recorded on a Varian WXR-400 spectrometer (operating frequency 400 MHz) with TMS internal standard; mass spectra, on a PE SCIEX API 165 (150) (Canada) spectrometer.

Extraction of Compounds from Raw Material. Freshly collected fruit of *S. albus* (10 kg) was ground and extracted with *n*-butanol (6 L). The extraction was carried out with shaking in a shuttle apparatus. The extraction was accelerated by adding anhydrous Na_2SO_4 (10% of the fruit weight). The aqueous butanol extract was separated from the pulp and concentrated on a boiling water bath. The water-saturated *n*-butanol obtained by distillation was used for subsequent extractions (11 × 6 L) (separating the water layer beforehand). A solid precipitated from anhydrous *n*-butanol and was dissolved with heating in the minimal amount of water. Highly polar iridoids were extracted again with *n*-butanol. The *n*-butanol extracts obtained from the precipitate dissolved in water were settled at room temperature, filtered, and evaporated in vacuo to afford fraction 3. The decanted anhydrous *n*-butanol solution was mixed with two times the amount of toluene. The toluene-butanol solution was separated from the precipitate and evaporated in vacuo to afford fraction 1. The precipitate was fraction 2 and was chromatographed through a column.

The aglycon of loganin (**3**) was detected in fraction 1 using TLC. Then it was isolated pure by column chromatography over silica gel.

Fraction 2 was chromatographed over silica gel columns. The eluents were mixtures of $CHCl_3$:CH_3OH of increasing polarity (from pure $CHCl_3$ to 40% CH_3OH in $CHCl_3$). Fractions (30-40 mL) were collected and analyzed using TLC. Solutions containing pure compounds were combined and evaporated to dryness. Thus, fraction 2 produced 10 pure compounds that were designated preliminarily as **1**, **2**, **A**, **B**, **C**, **D**, **E**, **F**, **G**, and **H**.

Fraction 2 that was stored in CH_3OH solution (5 d at room temperature) was used to obtain **8**. TLC established preliminarily that the iridoid composition had changed substantially during this time. This mixture was separated analogously as described above (see the separation of fraction 2). This isolated several compounds that were obtained earlier and new compound **8**, the spectral characteristics of which are given below.

Fraction 3 was chromatographed over a column and preparatively on paper to isolate new iridoid glucologanin (**4**). The eluents were EtOAc:MeOH mixtures with 30 to 50% of the latter.

Compound **A** was crystallized from diethylether and CH_2Cl_2, mp 75-77°C (CH_2Cl_2), $[\alpha]_D^{20}$ +31.1 ± 5° (*c* 0.1, $CHCl_3$).

Compound **B** was crystallized from CH_2Cl_2, mp 73-75°C (CH_2Cl_2), $[\alpha]_D^{20}$ +7.1 ± 3° (*c* 0.84, $CHCl_3$:MeOH 85:15).

Compound **C** was amorphous, $[\alpha]_D^{20}$ +118.7 ± 4° (*c* 0.16, $CHCl_3$).

Compound **D** was amorphous, $[\alpha]_D^{20}$ +47.5 ± 4° (*c* 0.16, $CHCl_3$:CH_3OH 85:15).

Compound **E** was amorphous, $[\alpha]_D^{20}$ +87.3 ± 4° (*c* 0.25, $CHCl_3$:CH_3OH 85:15).

Compound **F** was crystallized from EtOAc, mp 221-224°C (EtOAc), $[\alpha]_D^{20}$ +22.3 ± 2° (*c* 1.17, $CHCl_3$:CH_3OH 2:1).

Compound **G** was amorphous, $[\alpha]_D^{20}$ +66.7 ± 3° (*c* 1.12, CH_3OH:H_2O 70:30).

Compound **H** was amorphous, $[\alpha]_D^{20}$ –30.0 ± 3° (*c* 1.16, CH_3OH).

Compound **1** (secologanin) was crystallized from isopropanol:hexane, $[\alpha]_D^{20}$ –84.8 ± 3° (*c* 0.5, CH_3OH).

Mass spectrum (*m*/*z*, I_{rel}, %): 389.4 (100) $[M]^+$, 227 (17.4), 177.4 (43.5), 149.5 (56.5).

Compound **2** (loganin) was crystallized from hot isopropanol, mp 213-218°C, $[\alpha]_D^{20}$ –109 ± 2° (*c* 1.0, CH_3OH).

Mass spectrum (*m*/*z*, I_{rel}, %): 408.5 (80), 391.7 (22.5), 229.6 (100), 197.3 (10), 179.4 (65).

Compound **3** (loganigenin) was amorphous, $[\alpha]_D^{20}$ –27.0 ± 3° (*c* 1.12, $CHCl_3$), R_f 0.23 ($CHCl_3$:CH_3OH 97:3).

PMR spectrum of loganin aglycon (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.05 (3H, d, J = 8.0, CH_3), 1.35 (1H, m, H-9), 1.65 (2H, m, H-6), 2.05 (1H, dd, J = 6.0, 6.0, H-8), 2.95 (1H, m, H-5), 3.65 (3H, m, OCH_3), 3.90 (1H, d, J = 2.0, H-7), 4.45 (1H, d, J = 3.0, OH-7), 4.75 (1H, t, H-1), 7.2 (1H, d, J = 7.0, OH-1), 7.4 (1H, s, H-3).

Compound **4** (glucologanin) was amorphous, $[\alpha]_D^{20}$ –80.0 ± 3° (*c* 0.2, CH_3OH).

Mass spectrum (*m*/*z*, I_{rel}, %): 591.6 (10), 575.3 (4), 391.5 (20), 229 (33), 212 (30), 193 (20), 179 (10), 149.3 (100).

PMR spectrum of glucologanin (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.2 (3H, s, CH_3-10), 1.75 (2H, m, H-6), 2.20 (1H, m, H-9), 2.65 (1H, m, H-5), 4.0 (2H, s, CH-1′, CH-1″), 4.6 (4H, dm, J = 7.8, CH_2-6′, CH_2-6″), 7.1 (1H, s, H-3).

Compound **5**. Mass spectrum of loganin dimer (**5**) (*m*/*z*, I_{rel}, %): 798.3 (18), 781.4 (6) $[M]^+$, 766.2 (6), 601.4 (2), 408.3 (43), 391.1 (10), 229.2 (100), 211.1 (9), 197.2 (7), 179.1 (16), 149.1 (5).

Compound **8**. Mass spectrum of **8** (*m*/*z*, I_{rel}, %): 422.2 (45), 405.4 (35) $[M]^+$, 389.2 (90), 243.2 (100), 225.2 (86), 211.0 (29), 165.3 (49), 151.0 (40).

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm): 1.2 (3H, s, CH_3-10), 2.7 (2H, m, H-5, H-11), 3.7 (3H, m, OCH_3), 7.3 (1H, s, H-3).

IR spectrum (KBr, ν, cm^{-1}): 1696 (C=O), 1655 (C=O).

Partial Acid Hydrolysis of 4. Glycoside (15 mg) was dissolved in HCl solution (0.2 mL, 9%), and stored for 3 d at room temperature. Then the hydrolysate was analyzed using TLC and $CHCl_3$:CH_3OH (60:40) with detection by anilinium phthalate [11]. Reference samples were D-glucose, cellobiose, and gentiobiose. Sugars in the hydrolysate were D-glucose and gentiobiose (5:1 ratio). This was a convenient method for analyzing sugars using TLC because it did not require preliminary removal of acid.

Partial Enzymatic Hydrolysis of Glucologanin (4). Glycoside (50 mg) was dissolved in water (0.5 mL), treated with enzyme preparation (30 mg) obtained from grapevine snail [12], and stored at room temperature (25°C) for 2 h. The enzyme was precipitated with ethanol (see above). The filtrate was evaporated to dryness in vacuo. The solid was analyzed by TLC using $CHCl_3$:CH_3OH (97:3) (detection of loganin aglycon) and $CHCl_3$:CH_3OH (84:16) (detection of loganin). Thus, **2** and **3** were identified.

Hydrolysis of Loganin (2). Loganin (**2**, 0.2 g) was dissolved in water (1.5 mL), treated with enzyme preparation (0.15 g) obtained from grapevine snail [12], stored in a thermostat for 22 h at 38-40°C, treated with ethanol (9 mL), heated until clear precipitation of the enzyme, and filtered. The filtrate was evaporated in vacuo. The solid was extracted with hot EtOAc (2 × 5 mL). The EtOAc solution was cooled to room temperature (+20°C), filtered, concentraed to a volume of about 0.1 mL, and left overnight for crystallization. The resulting crystals of the aglycon were separated, washed with EtOAc, and dried at room temperature (+20°C) to afford loganin aglycon (0.1 g).

The solid that was insoluble in EtOAc was analyzed using PC and *n*-BuOH:HOAc:H_2O (4:1:2). The chromatographic reference was an authentic sample of D-glucose. Detection used anilinium phthalate and heating [11]. Sugars appeared as colored spots. It was found that this part of the hydrolysate contained D-glucose.

Synthesis of 6 and 7. Loganin (0.2 g) was dissolved in anhydrous pyridine (1.5 mL), treated with acetic anhydride (1 mL), stored at room temperature (22-24°C) for 23 h, treated with icewater (10-fold amount relative to the reaction volume), and stirred with cooling for 2 h. The resulting thick mass was ground with a glass rod until it converted to a crystalline mass that was stored in the cold (+5°C) for 20 h. The precipitate was separated, thoroughly washed with cold water, and dried. The resulting mixture of **6** and **7** (0.19 g) was chromatographed over a column (L = 47 cm, D = 1.2 cm) packed with silica gel in a 1:80 ratio (mixture:silica gel). The eluent was a $CHCl_3$:CCl_4 mixture (1:1, 200 mL), then $CHCl_3$:CCl_4 + CH_3OH (1%). Fractions containing pure compounds were combined and evaporated to dryness to afford **6** (0.097 g) and **7** (0.035 g).

Compound **6**, mp 124-125°C (CCl_4), $[\alpha]_D^{20}$ –60.0 ± 4° (*c* 0.4, $CHCl_3$:CH_3OH 83:17). PMR spectrum (400 MHz, DMSO-d_6, δ, ppm): 1.15 (1H, m, H-9), 1.9 (15H, m, OCH_3-2′,3′,4′,6′,7), 3.65 (3H, m, OCH_3-12), 7.3 (1H, s, H-3). IR spectrum (KBr, ν, cm^{-1}): 1715 (C=O), 1758 (C=O), 1703 (C=O).

Compound **7** was amorphous, $[\alpha]_D^{20}$ –59.9 ± 4° (*c* 0.5, $CHCl_3$:CH_3OH 83:17).

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