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CONSTITUENTS FROM LEAVES OF *Crataegus sanguinea*

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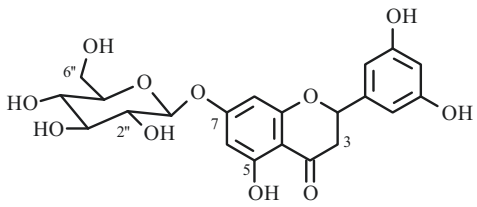
Leaves of Crataegus sanguinea *Pall. afforded for the first time ergosterol 3*-O-β-*D-glucopyranoside,* p-*coumaric acid 4*-O-β-*D-glucopyranoside, trifolin, quercitrin, the new compound sanguineoside (5,7,3′,5′-tetrahydroxyflavanone 7*-O-β-*D-glucopyranoside), and compounds known from this species, e.g. oleanolic acid, caffeic acid, hyperoside, vitexin, and vitexin 2″*-O-*rhamnoside, which was the dominant flavonoid.*

Keywords: hawthorn, *Crataegus sanguinea*, leaves, flavonoids, phenylpropanoids, sterols, triterpene saponins.

Fruit and flowers of pharmacopoeial species of hawthorn (*Crataegus* L.) are widely used as cardiotonics and antiarrhythmics to treat cardiovascular conditions [1, 2]. *C. sanguinea* Pall. (Rosaceae) is a widely used pharmacopoeial species [1–3].

Leaves of *C. sanguinea* are known to contain flavonoids (hyperoside, vitexin, 2″-*O*-rhamnoside, apigenin, cosmosiin, luteolin, cynaroside, quercetin, rutin, fisetin, naringin, hesperidin, dihydroquercetin, catechin, and procyanidin); phenylpropanoids (caffeic acid, chlorogenic acid); saponins; vitamins; and many other secondary and primary metabolites [1–3]. Further studies of the chemical composition of *C. sanguinea* leaves as a promising domestic medicinal raw material are critical despite published data on their constituent composition. The goal of the present work was to study the constituent composition of *C. sanguinea* leaves.

Flavonoids **1**–**6**, phenylpropanoids **7** and **8**, saponin **9**, and sterol **10** were isolated from hawthorn leaves during the studies.



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The PMR spectrum of **1** exhibited resonances for flavonoid aromatic protons at δ 6.15 (2H, d, J = 2.5 Hz, H-6,8), 6.88 (1H, s, H-4′), and 6.77 ppm (2H, s, H-2′,6′). The resonances for protons H-2ax (1H, dd, J = 4, 12 Hz), H-3ax (1H, dd, J = 12, 17 Hz), and H-3eq (1H, dd, J = 4, 17 Hz) at 5.43, 3.15, and 2.76 ppm, respectively, and UV spectral data characterized **1** as a flavanone [4]. Furthermore, the PMR spectrum of **1** had a 1H singlet at 12.07 ppm for the 5-OH group and a doublet for the glucose anomeric proton at 4.98 ppm with spin–spin coupling constant 7 Hz. The 2H doublet for H-6 and H-8 at 6.15 ppm (2H, d, J = 2.5 Hz) led to the conclusion that the 7-OH was glycosylated. Acid and enzymatic (*β*-glucosidase) hydrolysis of **1** formed glucose and the aglycon (5,7,3′,5′-tetrahydroxyflavanone), the structure of which was confirmed by mass-spectral data (M^+ 288, 100%) and the ^{13}C NMR spectrum.

Thus, **1** was called by us sanguineoside and had the structure 5,7,3′,5′-tetrahydroxyflavanone 7-*O*-*β*-D-glucopyranoside and was a new natural compound.

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Flavonoids **2–6** were identified using UV, PMR, ^{13}C NMR, and mass spectral data as vitexin (**2**), vitexin 2″-*O*-rhamnoside (**3**), trifolin (**4**), quercitrin (**5**), and hyperoside (**6**) [3, 5–9]. Flavonoids **4** and **5** were isolated from *C. sanguinea* leaves for the first time.

Phenylpropanoids **7** and **8** were identified as caffeic acid (**7**), which was reported earlier from *C. sanguinea* leaves [3], and *p*-coumaric acid 4-*O*-*β*-D-glucopyranoside, which was isolated earlier from *Rhodiola rosea* biomass [10].

Compound **9** was identified as oleanolic acid [11], which is known for *Crataegus* L. species [2, 3]. Compound **10** (ergosterol 3-*O*-*β*-D-glucopyranoside) was isolated for the first time from *C. sanguinea* leaves and other *Crataegus* species.

EXPERIMENTAL

PMR spectra were recorded on a Bruker AM 300 (300 MHz) instrument; ^{13}C NMR spectra, on a Bruker DRX 500 (126.76 MHz) instrument. Mass spectra were taken on a Kratos MS-30 mass spectrometer. UV spectra were recorded using a Specord 40 spectrophotometer (Analytik Jena).

Extraction and Separation. Air-dried *C. sanguinea* leaves (100 g) that were collected in June 2016 in Samara Oblast were extracted with EtOH (70%) first with two extractions at room temperature for 24 h and then with heating on a boiling-water bath for 30 min. The combined aqueous EtOH extract was evaporated in vacuo to 50 mL, mixed with L 40/100 silica gel (30 g), and dried. The dried powder (dried extract + silica gel) was placed onto a layer of silica gel (8 cm diameter × 5 cm height) that was formed from a suspension in $CHCl_3$. The chromatography column was eluted with $CHCl_3$ and $CHCl_3$–EtOH in various ratios (99:1, 98:2, 97:3, 95:5, 93:7, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70).

The separation was monitored using TLC on Sorbfil PTSKh-AF-A-UF plates and $CHCl_3$–EtOH (9:1), $CHCl_3$–EtOH–H_2O (26:16:3), and *n*-BuOH–AcOH (glacial) –H_2O (4:1:2). Fractions containing **1** as the dominant compound were combined. The precipitate that formed in them was separated and recrystallized from an H_2O–Me_2CO mixture to afford **1** in 0.6 mass% of air-dried raw material. Fractions containing **2** were combined. The precipitate that formed in them was separated and recrystallized from aqueous EtOH to afford **2** in 0.1 mass% of air-dried raw material. Fractions containing **3** were combined. The precipitate that formed in them was separated and recrystallized from aqueous EtOH to afford **3** in 0.05 mass% of air-dried raw material.

Fractions containing **4**–**6** were placed onto a Woelm polyamide column for further purification. Dried powder (extract + polyamide) was placed onto a chromatographic column (sorbent height 5.0 cm, diameter 4 cm) and eluted by H_2O and aqueous EtOH (20, 40, 70, 96%) to produce **4** (40% EtOH) and **5** and **6** (70% EtOH), which were further purified by recrystallization from aqueous EtOH.

Fractions containing **7**–**10** were also purified over a column of silica gel with elution by $CHCl_3$ and $CHCl_3$–EtOH in various ratios (99:1, 98:2, 97:3, 95:5, 93:7, 90:10, 85:15). Compound **7** was finally purified by recrystallization from H_2O; **8**–**10**, from EtOH.

Sanguineoside (5,7,3′,5′-tetrahydroxyflavanone 7-*O*-*β*-D-glucopyranoside) (1). Light-yellow amorphous compound, $C_{21}H_{22}O_{11}$, mass spectrum (70 eV, 200°C, *m/z*, %): aglycon M^+ 288 (100%), 153 (67), 137 (8). UV spectrum (EtOH, λ_{max}, nm): 290, 336 (sh); +A1C1$_3$ 285, 304, 344, 394 (sh). ^1H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.07 (1H, s, 5-OH), 9.17 (1H, s, 7-OH), 9.05 (2H, br.s, 3′, 5′-OH), 6.88 (1H, s, H-4′), 6,77 (2H, s, H-2′, 6′), 6.15 (2H, d, J = 2.5, H-6, 8), 5.43 (1H, dd, J = 4, 12, H-2ax), 3.15 (1H, dd, J =12, 17, H-3ax), 2.76 (1H, dd, J = 4, 17, H-3eq), 4.98 (1H, d, J = 7, Glc H-1″), 3.1–5.2 (6H, m, Glc). ^{13}C NMR spectrum (126 MHz, DMSO-d_6, δ, ppm): 197.17 (C-4), 78.71 (C-2), 165.30 (C-7), 145.81 (C-3′), 145.81 (C-5′), 118.07 (C-4′), 162.94 (C-5), 162.71 (C-9), 115.36, 94 (C-2′), 115.16 (C-6′), 129.22 (C-1′), 103.28 (C-10), 96.43 (C-8), 95.44 (C-6), 99.60 (Glc C-1″), 78.71 (C-3″), 77.09 (C-5″), 73.03 (C-2″), 70.06 (C-4″), 60.78 (C-6″), 42.19 (C-3).

Vitexin (5,7,4′-trihydroxyflavone 8-*C*-*β*-D-glucopyranoside) (2). Light-yellow crystalline powder, $C_{21}H_{20}O_{10}$, mp 273–275°C (aqueous EtOH). Mass spectrum (ESI-MS, 180°C, *m/z*): M^+ 433 (432 + H). ^1H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.18 (1H, s, 5-OH), 10.37 (1H, br.s, 7-OH), 9.04 (1H, br.s, 4′-OH), 8.04 (2H, d, J = 9, H-2′, 6′), 6.89 (2H, d, J = 9, H-3′, 5′), 6.78 (1H, s, H-3), 6.27 (1H, s, H-6), 4.84 (1H, d, J = 9.9, Glc H-1″), 3.1–4.6 (6H, m, Glc). ^{13}C NMR spectrum (126 MHz, DMSO-d_6, δ, ppm): 182.07 (C-4), 165.27 (C-2), 162.68 (C-7), 162.08 (C-4′), 161.11 (C-5), 160.36 (C-9), 128.94 (C-2′), 128.94 (C-6′), 121.59 (C-1′), 115.78 (C-3′), 115.32 (C-5′), 104.01 (C-10), 104.59 (C-8), 98.12 (C-6), 81.02 (C-3″), 81.02 (C-5″), 73.35 (Glc C-1″), 70.82 (C-2″), 70.52 (C-4″), 61.27 (C-6″).

Vitexin 2″-*O*-Rhamnoside (5,7,4′-trihydroxyflavone 2″-*O*-α-L-rhamnopyranosyl-8-*C*-β-D-glucopyranoside) (3). Light-yellow crystalline powder, $C_{27}H_{30}O_{14}$, mp 213–215°C (aqueous Me_2CO). Mass spectrum (ESI-MS, 180°C, *m/z*): 579 $(M + H)^+$, 601 $(M + Na)^+$, 617 $(M + K)^+$. ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.21 (1H, s, 5-OH), 11.03 (1H, br.s, 7-OH), 10.37 (1H, br.s, 4′-OH), 8.21 (2H, d, J = 9, H-2′, 6′), 6.92 (2H, d, J = 9, H-3′, 5′), 6.83 (1H, s, H-3), 6.25 (1H, s, H-6), 5.33 (1H, br.s, Rha H-1‴), 4.84 (1H, d, J = 9.9, Glc H-1″), 3.2–5.25 (10H, m, Glc-H), 1.70 (3H, s, Rha CH_3). ^{13}C NMR spectrum (126 MHz, DMSO-d_6, δ, ppm): 182.05 (C-4), C-2 (169.22), 164.05 (C-7), 162.08 (C-4′), 161.23 (C-5), 156.44 (C-9), 129.04 (C-2′), 129.04 (C-6′), 121.58 (C-1′), 116.21 (C-3′), 115.80 (C-5′), 104.01 (C-10), 103.98 (C-8), 97.81 (C-6), 102.45 (Rha C-1‴), 97.81 (Glc C-2″), 82.02 (C-5″), 75.68 (C-3‴), 75.68 (C-5″), 72.52 (Glc C-1″), 70.93 (C-2‴), 70.93 (C-4″), 70.46 (C-5‴), 60.99 (C-6″), 20.44 (CH_3).

Trifolin (3,5,7,4′-tetrahydroxyflavone 3-*O*-β-D-galactopyranoside) (4). Light-yellow crystalline compound, mp 240–242°C (aqueous EtOH), Mass spectrum (70 eV, 200°C, *m/z*, %): 286 (aglycon M^+, 100 %), 153 (11), 152 (20), 121 (10). UV spectrum (EtOH, λ_{max}, nm): 270, 338; + NaOAc 280, 371; + NaOAc + H_3BO_3 280, 371; +A1C1$_3$ и +A1C1$_3$ + HCl 275, 305, 396.

Quercitrin (3,5,7,3′,4′-pentahydroxyflavone 3-*O*-α-L-rhamnopyranoside) (5). Light-yellow crystalline compound, mp 224–226°C (aqueous EtOH), Mass spectrum (70 eV, 200°C, *m/z*, %): 302 (aglycon M^+, 100%), 153 (12), 152 (43), 137 (8). ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 12.65 (1H, s, 5-OH), 9.82 (1H, s, 7-OH), 9.27 (1H, s, 4′-OH), 7.65 (1H, d, J = 2.5, H-2′), 7.61 (1H, dd, J = 2.5, 9, H-6′), 6.88 (1H, d, J = 9, H-5′), 6.82 (1H, d, J = 2.5, H-8), 6.42 (1H, d, J = 2.5, H-6), 5.58 (1H, d, J = 1.5, Rha H-1″), 3.1–5.2 (4H, m, Rha-H), 1.25 (3H, d, J = 6, Rha CH_3). ^{13}C NMR spectrum (126 MHz, DMSO-d_6, δ, ppm): 177.60 (C-4), 161.57 (C-7), 160.86 (C-5), 160.12 (C-9), 156.66 (C-2), 149.44 (C-4′), 148.65 (C-3′), 131.00 (C-3), 122.13 (C-6′), 121.71 (C-1′), 116.21 (C-2′), 115.23 (C-5′), 101.62 (C-10), 100.86 (Rha C-1″), 98.41 (C-8), 94.30 (C-6), 116.21 (C-2′), 74.07 (C-3″), 73.16 (C-5″), 70.24 (C-4″), 70.07 (C-2″), 17.90 (C-6″, CH_3).

Hyperoside (3,5,7,3′,4′-pentahydroxyflavone 3-*O*-β-D-galactopyranoside) (6). Light-yellow amorphous compound, mp 230–232°C (aqueous Me_2CO). Mass spectrum (70 eV, 200°C, *m/z*, %): 302 (aglycon M^+, 100%), 153 (23), 137 (64). UV spectrum (EtOH, λ_{max}, nm): 258, 266 sh, 363; + NaOAc 274, 381; + NaOAc + H_3BO_3 262, 378; +A1C1$_3$ 275, 414; +A1C1$_3$ + HCl 271, 403.

Caffeic Acid (7). Light-yellow crystalline compound, $C_9H_8O_4$, mp 200–202°C (H_2O). Mass spectrum (70 eV, 200°C, *m/z*, %): 180 (M^+, 100%).

***p*-Coumaric Acid 4-*O*-β-D-Glucopyranoside (8)**. White crystals, mp 206–208°C (EtOH). Mass spectrum (EI-MS, 180°C, *m/z*): aglycon M^+ 164 (54%). UV spectrum (EtOH, λ_{max}, nm): 285, 316 (sh). ^{1}H NMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.68 (2H, d, J = 9, H-2, 6), 7.01 (2H, d, J = 9, H-3, 5), 6.85 (1H, d, J = 16, H-7), 5.83 (1H, d, J = 16, H-8), 4.88 (1H, d, J = 7, Glc H-1′), 3.1–4.6 (6H, m, Glc-H). ^{13}C NMR spectrum (126 MHz, DMSO-d_6, δ, ppm): 176.00 (C-9), 167.82 (C-4), 131.97 (C-2), 131.97 (C-6), 128.00 (C-3), 128.00 (C-5), 118.95 (C-7), 115.63 (C-8), 100.10 (Glc C-1′), 77.89 (C-3′), 77.15 (C-5′), 76.61 (C-2′), 72.53 (C-4′), 60.71 (C-6′).

Oleanolic Acid (9). White crystals (EtOH), $C_{30}H_{48}O_3$, mp 303–305°C (EtOH). Mass spectrum (70 eV, 200°C, *m/z*, %): 456 (M^+, 23), 341 (9), 248 (100), 219 (14), 208 (44), 203 (68), 135 (33), 121 (37), 107 (30), 81 (30), 55 (25).

Ergosterol 3-*O*-β-D-Glucopyranoside (10). White amorphous compound, UV spectrum (EtOH, λ_{max}, nm): 271. Mass spectrum (70 eV, 200°C, *m/z*, %): 396 (M^+, 18), 215 (5), 164 (23), 149 (19), 120 (31), 95 (24), 85 (17), 81 (33), 73 (58), 60 (100), 43 (84). ^{1}H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.34 (2H, m, H-6, 7), 4.87 (2H, d, J = 7.7, H-22, 23), 4.22 (1H, d, J = 7, Glc H-1′), 3.65 (1H, m, H-3), 1.0–3.5 (26H, m, incl. Glc 6H), 0.65–0.95 (18H, m, CH_3). ^{13}C NMR spectrum (126 MHz, DMSO-d_6, δ, ppm): 140.52 (C-5), 140.52 (C-8), 133.59 (C-22), 129.26 (C-23), 121.26 (C-6), 121.26 (C-7), 76.81 (C-3), 100.86 (Glc C-1′), 76.99 (C-3′), 76.84 (C-5′), 73.59 (C-2′), 70.19 (C-4′), 61.17 (C-6′), 56.24 (C-17), 54.50 (C-14), 49.67 (C-9), 45.21 (C-24), 41.92 (C-13), 40.51 (C-4), 40.51 (C-20), 38.38 (C-12), 36.28 (C-1), 35.53 (C-10), 33.42 (C-2), 31.49 (C-25), 28.78 (C-16), 22.68 (C-11), 20.66 (C-21), 19.77 (C-26), 19.16 (C-27), 19.00 (C-19), 18.68 (C-28), 11.50 (C-18).

REFERENCES

1. *Plant Resources of the USSR. Flowering Plants, Their Chemical Composition and Use. Families Hydrangeaceae–Haloragaceae* [in Russian], Nauka, Leningrad, 1987, 326 pp.
2. A. L. Budantsev (chief ed.), *Plant Resources of Russia: Wild Flowering Plants, Their Constituent Composition and Biological Activity*, Vol. 2, *Families Actinidiaceae, Malvaceae–Euphorbiaceae, Haloragaceae*, Tovarishchestvo Nauchnykh Izdanii KMK, St. Petersburg, Moscow, 2009, 513 pp.

3. S. R. Khasanova, Author's Abstract of a Doctoral Dissertation, Samara, 2016, 46 pp.
4. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer Verlag, Berlin–Heidelberg–New York, 1970, 354 pp.
5. M. G. Raghu and A. Pushpa, *IOSR J. Pharm. Biol. Sci.*, **11** (6), 73 (2016).
6. J. Gupta and A. Gupta, *Int. J. Chem. Stud.*, **4** (1), 14 (2016).
7. S. Thenmozhi and U. Subasini, *Int. J. Res. Pharmacol. Pharmacother.*, **1**, 84 (2016).
8. K. F. Taha and Z. T. Abd El Shakour, *Int. J. Pharm. Sci. Res.*, **8** (3), 1081 (2017).
9. N. B. Begmatov, Kh. M. Bobakulov, X. Xin, and H. A. Aisa, *Chem. Nat. Compd.*, **50**, 1116 (2014).
10. V. A. Kurkin, G. G. Zapesochnaya, A. G. Dubichev, E. D. Vorontsov, I. V. Aleksandrova, and R. V. Panova, *Chem. Nat. Compd.*, **27**, 419 (1991).
11. H. C. Kwon, K. R. Lee, and O. P. Zee, *Arch. Pharm. Res.*, **20** (2), 180 (1999).