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SYNTHESIS OF THE FIRST DIHYDROQUERCETIN–CYTISINE CONJUGATES

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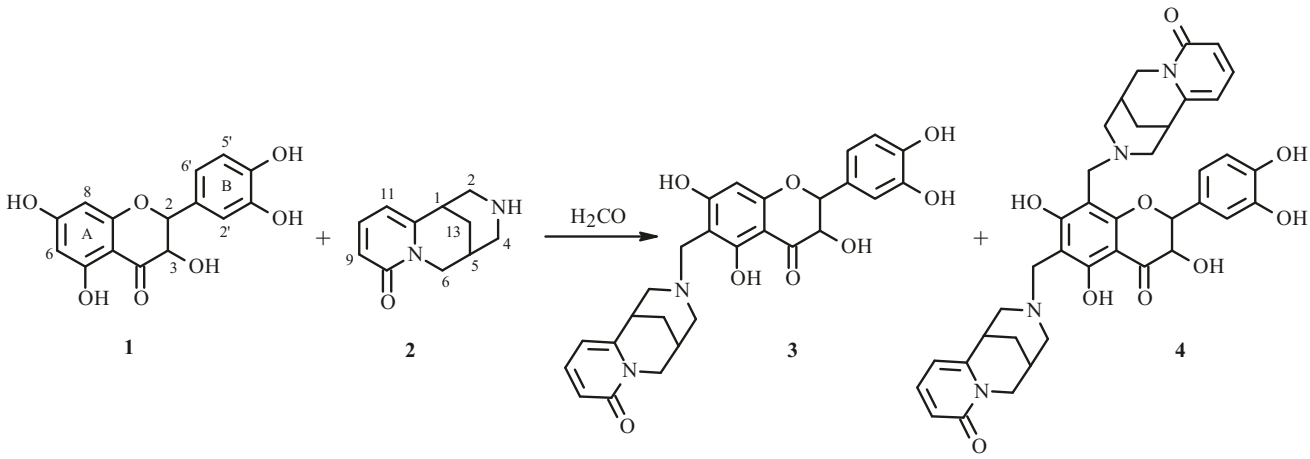
The first mono- and disubstituted conjugates of the natural biologically active flavonoid dihydroquercetin and alkaloid cytisine were synthesized using a Mannich reaction.

Keywords: dihydroquercetin, cytisine, Mannich reaction.

Chemical modification of natural biologically active compounds can expand their spectrum of activity and is a promising direction in new drug discovery [1]. Flavonoids are a large group of natural biologically active compounds, among which dihydroquercetin (DHQ) occupies a special place. It is isolated from *Larix sibirica* wood and exhibits powerful antioxidant, hepatoprotective, antitumor, immunomodulating, and other properties [2]. The broad spectrum of biological activity and low toxicity [2] classify DHQ as a lead compound for chemical modification in order to synthesize new hybrid polyfunctional pharmacologically active compounds.

Alkaloids such as cytisine occupy a special place among numerous heterocyclic natural compounds. The pharmacological properties of cytisine, like nicotine, are typical of ganglionic toxins that excite the CNS and autonomic nervous system ganglia, reflexively strengthen breathing [3], and exhibit hypolipidemic activity [4]. Cytisine derivatives with isoflavones and coumarins were obtained earlier [5]. The reaction of DHQ and its close structural analogs with primary and secondary amines was used as an example to demonstrate [6, 7] that the Mannich reaction occurred at the 6-position of ring A and produced primarily the mono-substituted derivative if equimolar amounts of the reagents were used; the disubstituted derivative at the 6- and 8-positions, with a two-fold excess of amine.

The goal of the present work was to synthesize conjugates of DHQ (**1**) and cytisine (**2**) that were promising for discovering new pharmacologically active polyfunctional drugs.



1 **2** **3** **4**

Scheme 1

1) N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, 630090, Novosibirsk, Prosp. Akad. Lavrent′eva, 9, e-mail: kosheleva@nioch.nsc.ru; 2) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, 100170, Tashkent. Translated from *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2014, pp. 383–385. Original article submitted December 29, 2013.

The reaction of **1**, **2**, and formaldehyde in a 1:1.4:1.4 mol ratio was carried out by adding a mixture of the reagents to the substrate to produce a mixture of mono- and disubstituted **3** and **4** (Scheme 1) in an ~2:1 ratio (with an equimolar ratio of reagents and ~10–15% DHQ). The product ratio was found using HPLC. A mixture of mono- and disubstituted DHQ derivatives in an ~1:3 ratio was obtained if a two-fold excess of the reagents was used and the order of addition was reversed. Apparently, disubstituted **4** formed because of the greater basicity of **2** compared with the previously used amines [6].

Compounds **3** and **4** were isolated from the reaction mixtures and characterized by IR, UV, PMR, and ^{13}C NMR spectroscopy and mass spectrometry.

IR spectra of the synthesized compounds showed a strong broad band at 1643 cm^{-1} that was due to cytisine and DHQ carbonyls. The UV spectrum of monosubstituted **3** retained the absorption maximum (λ_{max}) at 290 nm that was characteristic of DHQ. The characteristic DHQ band for disubstituted **4** shifted by 24 nm to longer wavelength. Mass spectra of **3** and **4** exhibited molecular ions $[M + H]^+$ with *m*/*z* 507.185 and 709.285 and fragments $[M + H - 190]^+$ with *m*/*z* 317.076 and 519.176 that corresponded to loss of cytisine. We observed earlier an analogous fragmentation of spin-labeled aminomethyl DHQ derivatives [7]. Resonances in PMR and ^{13}C NMR spectra were assigned taking into account the corresponding literature data [6–8].

Thus, we synthesized for the first time mono- and disubstituted aminomethyl derivatives of DHQ with the alkaloid cytisine.

EXPERIMENTAL

NMR spectra of DMSO-d_6 solutions were recorded (relative to TMS) on a Bruker AV-400 spectrometer. The course of reactions was monitored by TLC on Sorbfil 254 plates using EtOAc–MeOH–H_2O (10:2:1). IR spectra were recorded in KBr pellets on a Vector-22 instrument. UV spectra were obtained during HPLC analysis. HPLC-UV analysis of the products was performed on an Agilent 1100 chromatograph with a diode-matrix detector and a Zorbax XDB-C8 column (4.6 × 150 mm) using MeOH–TFA (0.1%) mobile phase and a MeOH gradient from 10% to 90% over 30 min. HPLC-MS analysis of the products was performed on an Agilent 1200 LC with a micrOTOF-Q hybrid quadrupole-TOF mass spectrometer (Bruker) and a Zorbax XDB-C8 column (2.1 × 50 mm) using MeOH–HCOOH (2%) mobile phase and a MeOH gradient from 20% to 100% over 20 min. The mass detector used electrospray at atmospheric pressure (API-ES) and scanning of negative and positive ions in the range *m*/*z* 100–1200. The drying gas was N_2.

DHQ of purity 96–97% was purchased (Sibirskii Kedr Ltd.).

Preparation of 3 and 4. Formaldehyde (as paraformaldehyde, 3.8 mg, 1.2×10^{-4} mol) and $ZnCl_2$ (1.4 mg, 8.5×10^{-6} mol) were refluxed in EtOH (1 mL) for 4–6 h until the paraformaldehyde dissolved, cooled to ~20–25°C, treated with cytisine (22.5 mg, 1.2×10^{-4} mol), and stirred until the cytisine dissolved. The resulting mixture was added dropwise with stirring over 15 min to a solution of DHQ (25 mg, 8.2×10^{-5} mol) in EtOH (0.6 mL) and stirred for 3.5 h. The solvent was distilled off to afford a mixture of **3** and **4** in an ~2:1 ratio according to HPLC. The yields of **3** and **4** after separation and purification were 55 and 35%, respectively.

Formaldehyde (as paraformaldehyde, 5.3 mg, 1.7×10^{-4} mol) and $ZnCl_2$ (1.4 mg, 8.5×10^{-6} mol) were refluxed in EtOH (1 mL) for 4–6 h until the paraformaldehyde dissolved, cooled to ~20–25°C, treated with cytisine (32 mg, 1.7×10^{-4} mol), and stirred until the cytisine dissolved. The resulting mixture was treated dropwise with stirring over 15 min with a solution of DHQ (25 mg, 8.2×10^{-5} mol) in EtOH (0.6 mL) and stirred for 3.5 h. The solvent was distilled off. The residue was dried to afford a mixture of **3** and **4** in an ~1:3 ratio according to HPLC. The yields of **3** and **4** after separation and purification were 25 and 65%, respectively.

Separation and Purification of 3 and 4. A mixture of **3** and **4** (10 mg) was treated with EtOAc (5 mL), stirred at ~20–25°C, treated dropwise with HCl-saturated MeOH (0.2 mL, pH 2), stirred for 10 min, and centrifuged. The solid was separated, dried, dissolved in H_2O (4 mL), treated with $NaHCO_3$ solution (1%) until the pH was 7–8, treated with EtOAc (4 mL), and shaken. The organic layer was separated. The aqueous layer was extracted with EtOAc (2 × 4 mL). The organic extracts were combined and dried over $MgSO_4$ (anhydr.). The solvent was distilled off to afford **3**. The aqueous layer was extracted with $CHCl_3$ (2 × 4 mL). The organic layer was separated, dried over $MgSO_4$ (anhydr.), and evaporated to afford **4**.

3-{[3,5,7-Trihydroxy-2-(3,4-dihydroxyphenyl)chroman-4-on-6-yl]methyl}-1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-α]diazocin-8-one (3). Pale-yellow powder, R_f 0.5 (EtOAc–MeOH–H_2O, 10:2:1), mp 199–202°C. Mass spectrum *m/z* 507.185 $[M + H]^+$, calcd for $C_{27}H_{27}N_2O_8^+$, 507.176 $[M + H]^+$. UV spectrum (λ_{max}, nm): 290. IR spectrum

(KBr, ν, cm^{-1}): 3406, 3256, 2939, 1643, 1547, 1479, 1450, 1391, 1358, 1283, 1163, 1126, 1090, 966, 804, 737, 685. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): *a*) cytisine: 1.72–1.83 (2H, m, H-13), 2.46 (1H, br.s, H-5), 2.42, 2.47, 2.90, 2.99 (each 1H, d, J = 10.8, H_2-2, 4), 3.08 (1H, br.s, H-1), 3.68–3.80 (2H, m, H-6), 6.09 (1H, dd, J = 7.0, 1.0, H-11), 6.22 (1H, dd, J = 8.7, 1.2, H-9), 7.32 (1H, dd, J = 8.9, 7.1, H-10); *b*) 3.57 (2H, br.s) – CH_2-6 methylene protons); *c*) DHQ: 4.50 (1H, dd, J = 11.6, 4.0, H-3), 4.94 (1H, d, J = 11.4, H-2), 5.72 (1H, s, H-8), 5.75 (1H, d, J = 5.6, 3-OH), 6.73 (2H, s, H-5′, 6′), 6.85 (1H, s, H-2′), 8.98, 9.05 (each 1H, s, 3′, 4′-OH), 11.92 (1H, s, 7-OH), 12.34 (1H, s, 5-OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): *a*) cytisine: 24.68 (C-13), 26.99 (C-5), 34.12 (C-1), 49.23 (C-6), 58.59, 59.59 (C-2, 4), 104.17 (C-11), 115.91 (C-9), 138.80 (C-10), 150.90 (C-12), 162.19 (C-8); *b*) 50.87 – C-6 methylene C atom; *c*) DHQ: 71.49 (C-3), 83.03 (C-2), 94.83 (C-8), 99.97, 100.91 (C-4a, 6), 115.07 (C-2′), 115.40 (C-5′), 119.42 (C-6′), 128.02 (C-1′), 144.91 (C-3′), 145.78 (C-4′), 160.68 (C-8a), 161.41 (C-5), 167.18 (C-7), 198.14 (C-4).

3-{3-[(3,5,7-Trihydroxy)-2-(3,4-dihydroxyphenyl)chroman-4-on-8-ylmethyl]-(1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-α]diazocin-8-one)-6-yl}methyl-1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-α]diazocin-8-one (4). White powder, R_f 0.25 (EtOAc–MeOH–H_2O, 10:2:1), mp 205–208°C. Mass spectrum *m/z* 709.285 $[M + H]^+$, calcd for $C_{39}H_{41}N_4O_9^+$, 709.287 $[M + H]^+$. UV spectrum (λ_{max}, nm): 314. IR spectrum (KBr, ν, cm^{-1}): 3408, 3233, 2928, 1643, 1547, 1480, 1450, 1358, 1285, 1136, 1124, 1084, 993, 802, 752, 665. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): *a*) two cytisines: 1.64–1.81 (4H, m, H-13, 13′), 2.39 (2H, br.s, H-5, 5′), 2.25–2.33, 2.78–2.86 (each 4H, br.m, H_2-2, 2′, 4, 4′), 3.10 (2H, br.s, H-1, 1′), 3.73–3.83 (4H, m, H-6, 6′), 6.15, 6.20 (each 1H, dd, J = 7.0, 1.0, H-11, 11′), 6.24 (2H, m, H-9, 9′), 7.34 (1H, dd, J = 9, 6.5), 7.35 (2H, dd, J = 9, 6.5) – (2H, H-10, 10′); *b*) 3.44 (2H, br.s), 3.48 (2H, br.s) – CH_2-6, CH_2-8 methylene protons; *c*) DHQ: 4.36 (1H, dd, J =11.3, 6.1, H-3), 4.86 (1H, d, J = 11.4, H-2), 5.73 (1H, d, J = 6.2, 3-OH), 6.69 (1H, dd, J = 7.4, 1.8, H-5′), 6.74 (1H, d, J = 8.1, H-6′), 6.81 (1H, d, J = 1.8, H-2′), 9.01, 9.11 (each 1H, br.s, 3′, 4′-OH), 12.36 (1H, s, 5-OH). ^{13}C NMR spectrum (100 MHz, DMSO-d_6, δ, ppm): *a*) two sets of cytisine resonances: 24.96, 25.02 (C-13, 13′), 27.32, 27.40 (C-5, 5′), 34.42, 34.47 (C-1, 1′), 49.66, 49.73 (C-6, 6′), 58.68, 58.87, 59.50, 59.69 (C-2, 2′, 4, 4′), 104.44, 104.61 (C-11, 11′), 115.66, 115.90 (C-9, 9′), 139.16, 139.31 (C-10, 10′), 151.63, 152.04 (C-12, 12′), 162.57, 162.62 (C-8, 8′); *б*) 49.77, 49.87 – C-6 and C-8 methylene C atoms; *c*) DHQ: 71.90 (C-3), 83.28 (C-2), 99.71, 100.92, 101.02 (C-6, 4a, 8), 115.09 (C-2′), 115.36 (C-5′), 119.66 (C-6′), 128.39 (C-1′), 145.27 (C-3′), 146.04 (C-4′), 159.50 (C-8a), 160.25 (C-5), 167.22 (C-7), 198.57 (C-4).

The spectral characteristics of the synthesized compounds were obtained at the Chemical Service, CCU, SB, RAS.

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