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METHYLATION OF QUERCETIN BY DIAZOMETHANE AND HYPOGLYCEMIC ACTIVITY OF ITS TETRA-*O*-METHYL ETHER

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*The tetra-*O*-methyl ether of quercetin (QU)* ***3*** *(54%), 3,7,4′-tri-*O*-methyl ether* ***4*** *(30%), and a previously unreported 3,7,3-tri-*O*-methyl ether of QU* ***5*** *(7%) were obtained via methylation of QU by an excess of diazomethane in dioxane. Their structures were established using 2D NMR (^1H–^1H COSY, ^1H–^1H NOESY, ^1H–^{13}C HSQC, ^1H–^{13}C HMBC). Tetra-*O*-methyl ether of QU* ***3*** *exhibited pronounced hypoglycemic activity, reduced alloxan-induced hyperglycemia in rats by 44.5% compared to a control, and was 2.7 times more active than QU.*

Keywords: quercetin, diazomethane, methyl ethers, hypoglycemic activity.

Quercetin (QU, **1**) is a well-known bioflavonoid with P-vitamin activity [1] combined with antioxidant [2], hepatoprotective [3], antibacterial [4], and antiviral properties [5, 6] and anticancer activity [7, 8]. QU can detoxify carcinogens, cause DNA to deform, and protect cellular membranes from lipid peroxidation [9]. It is used in medicine as a therapeutic and prophylactic agent for diseases associated with disturbed capillary-wall permeability and damage, for liver hypertonia, ischemic cardiac disease and atherosclerosis, myocardial infarct, diabetes mellitus, and other diseases [10–12]. However, the use of QU in medicine and pharmacy is limited by its poor solubility, low bioavailability and permeability through biomembranes, and rapid metabolism in the gastrointestinal tract [13].

Chemical modification of QU is a modern approach to producing new biologically active compounds with improved bioavailability and pharmacological activity. Ether synthesis is a widely used chemical transformation of QU and other bioflavonoids that allows the production of polyphenolic prodrugs capable of penetrating membranes and regenerating the native compounds during *in vivo* circulation [14]. Addition of lipophilic or mitochondria-seeking groups to the QU phenolic hydroxyls was shown to enhance the cell permeability and membrane affinity, resulting in the improvement of the biochemical and pharmacological parameters [15, 16]. Therefore, methoxylated QU derivatives that were metabolically more stable than the native flavonoid and possessed various types of pharmacological activity seemed especially interesting. Thus, QU penta-*O*-methyl ether could regulate production of adiponectin, a hormone playing a key role in regulating lipid and glucose metabolism and the destruction of which leads to type 2 diabetes, obesity, atherosclerosis, etc. [15]. Multi-step syntheses of several QU monomethyl ethers, e.g., natural polyphenolic antioxidants 7-*O*-methyl-QU (rhamnetin), 3′-*O*-methyl-QU (isorhamnetin), 4′-*O*-methyl-QU (tamarixetin), 5-*O*-methyl-QU (azaleatin) [16, 17], and QU 3,7,4′-tri-*O*-methyl ether (ayanin) [18], using various protecting groups have been proposed.

Previously, one-step methods for preparing penta-*O*-methyl (**2**), 3,7,3′,4′-tetra-*O*-methyl (**3**), and 3,7,4′-*O*-methyl ethers (**4**) by methylation of QU using methyl iodide in DMSO in the presence of KOH or in DMF in the presence of K_2CO_3 were proposed by us [19]. QU tetra-*O*-methyl ether **3** was also produced via methylation of QU by an excess of diazomethane in MeOH in 39% yield [20].

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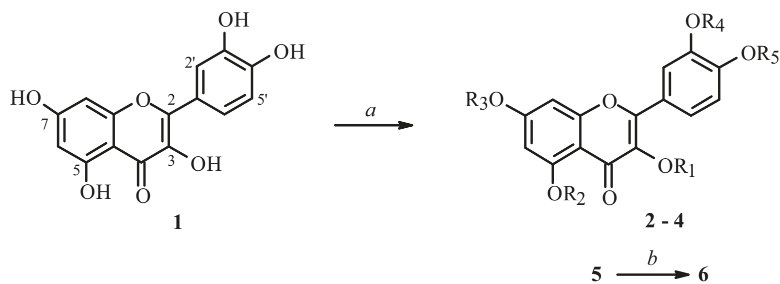

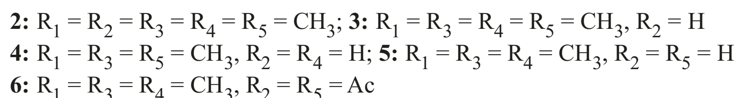

a. CH_2N_2, dioxane, 48 h, 0 – 5°C; *b*. Ac_2O, AcONa, 2 h, 140°C

Herein, the methylation of QU by diazomethane in dioxane and studies of the hypoglycemic activity of QU tetra-*O*-methyl ether **3** in an alloxan-induced hyperglycemia model in rats are reported.

Methylation of QU by an excess of diazomethane in dioxane in a 1:(~10) eq. ratio gave a mixture of methylation products that was separated by column chromatography (CC). The obtained compounds were identified using TLC with labels and NMR spectra as QU tetra-*O*-methyl ether **3** (54.4% yield) and 3,7,4′-tri-*O*-methyl ether **4** (30%) and the previously unreported QU 3,7,3′-tri-*O*-methyl **5** (7%).

The structure of ether **5** was established using high-resolution NMR and 2D experiments (^1H–^1H COSY, ^1H–^1H NOESY, ^1H–^{13}C HSQC, ^1H–^{13}C HMBC). NMR spectra recorded with full proton suppression and in DEPT90 mode revealed the chemical shifts (CS) of methyl and quaternary C atom resonances. Thus, two doublets at δ 7.05 (J = 8.5 Hz) and 7.53 (J = 8.5 Hz) the PMR spectrum of **5** corresponded to protons on C-5′ (δ 111.8 ppm) and C-6′ (δ 120.4 ppm), respectively. A proton with CS 7.56 ppm was located on C-2′ (δ 115.1 ppm). Two protons with strong-field resonances and a small SSCC between them at δ 6.30 ppm (J = 1.8 Hz) and 6.61 ppm (J = 1.8 Hz) were assigned to H-8 and H-6, respectively. This was confirmed by cross-peaks for their coupling in the COSY spectrum.

The HMBC correlation spectrum allowed resonances of quaternary C atoms and the CS of C atoms in ring A to be assigned from coupling to the corresponding protons. Thus, H-8 had cross-peaks for coupling with resonances of quaternary C atoms with CS 165.1 (C-7), 160.9 (C-8a), 105.2 (C-4a), and 97.7 ppm (C-6). CS of C atoms in ring C were established analogously. Thus, the resonance of the proton with CS 7.05 ppm in the HMBC spectrum coupled to C atoms with CS 150.3 (C-3′), 146.4 (C-4′), and 122.2 ppm (C-1′); the proton with CS 7.53 ppm, to C atoms with CS 150.3, 146.4, 115.1 (C-2′), and 155.5 ppm (C-5). Resonances with CS 178.0 and 138.2 ppm were attributed to C-4 and C-3, respectively. Furthermore, the HMBC spectrum showed a methoxy resonance at δ 3.85 ppm that gave a cross-peak with the resonance of a C atom with CS 150.3 ppm (C-3′); at δ 3.82, 165.1 ppm (C-7); and at δ 3.78, 138.2 ppm (C-3). This correlation was also confirmed by the NOESY spectrum. The resonance for the OCH_3 protons at δ 3.82 coupled with protons H-8 (δ 6.30) and H-6 (δ 6.61); the resonance for the C-3 OCH_3 protons gave cross-peaks for coupling with H-6′ (δ 7.53).

The structure of ether **5** and the positions of the CH_3O groups were also established using 2D correlation NMR spectra of the diacetate (**6**), which was obtained via acetylation of **5** by acetic anhydride in the presence of AcONa under reflux (78% yield).

The PMR spectrum of diacetate **6** showed two singlets for CH_3CO protons at 2.36 and 2.46 ppm and three singlets for CH_3O protons with δ 3.85, 3.88, and 3.90 ppm. Five resonances of aromatic protons were observed at 8.05 (dd, J = 8.5 and 2.0 Hz) (H-6′) and 7.06 ppm (d, J = 8.5 Hz). A proton with δ 7.80 ppm (J = 2.0 Hz) was assigned to C-2′; two resonances with J = 2.2 Hz (δ 6.80 and 6.58 ppm), to H-6 and H-8. The HSQC spectrum showed these protons correlated with C atoms with δ 112.1 (C-5′), 127.6 (C-6′), 122.8 (C-2′), 98.6 (C-6), and 108.2 ppm (C-8). The HMBC spectrum showed the proton with δ 6.58 ppm had cross-peaks with geminal and vicinal C atoms with δ 163.3 (C-7), 150.7 (C-5), 111.3 (C-4a), and 98.6 ppm (C-6); the proton with δ 6.81 ppm, C atoms with δ 163.3, 111.3, and 108.2 ppm (C-8). Proton H-5′ in ring C with δ 7.06 ppm correlated with C atoms with δ 139.6 (C-4′), 153.0 (C-3′), and 123.2 (C-1′); H-6′, with C atoms with δ 122.8 (C-2′) and 153.0 (C-3′). In turn, H-2′ coupled with C atoms with δ 153.0 (C-3′), 139.6 (C-4′), and 127.6 (C-6′).

The resonance with δ 141.1 ppm was assigned to C-3 because the HMBC had coupling to the CH_3O group with δ 3.85 ppm. The resonance at δ 153.0 ppm was assigned to C-2. The two remaining CH_3O groups in the HMBC spectrum gave cross-peaks at 3.88 ppm with a C atom with δ 163.3 (C-7); at 3.90 ppm, a C atom with δ 153.0 ppm (C-3′) (Fig. 1).

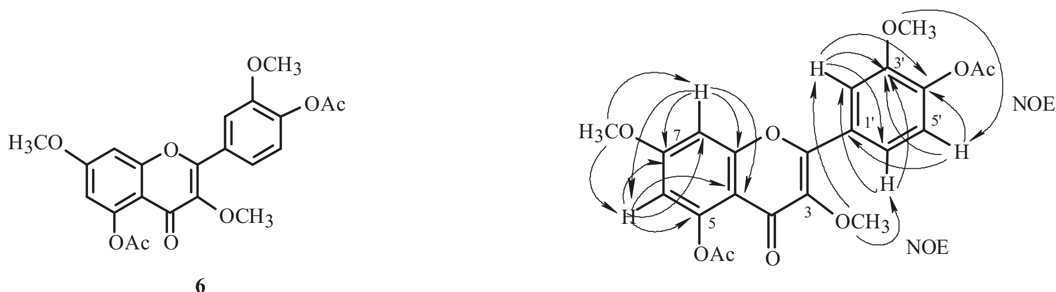

Fig. 1. Structure and CH correlations in the HMBC spectrum of quercetin-3,7,3′-tri-*O*-methyl ether 5,4′-di-*O*-acetate (**6**).

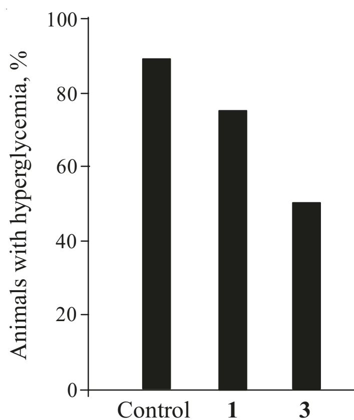

Fig. 2. Effect of quercetin tetra-*O*-methyl ether (4-MeQU, **3**) and quercetin (**1**) on alloxan-induced hyperglycemia (n = 8).

Thus, methylation of QU by an excess of diazomethane in dioxane gave QU tetra-*O*-methyl ether **3** (54% yield), 3,7,4′-tri-*O*-methyl ether **4** (30%), and new 3,7,3′-tri-*O*-methyl ether **5** (7%).

The hypoglycemic activity of **3** was studied and compared to QU in male Wistar rats (200–270 g) using an experimental early hyperglycemia model induced by i.p. injection of alloxan solution (5%) at a dose of 170 mg/kg as before [21]. Animals with hyperglycemia (>15 mM) in the groups were counted. The results were expressed in percent of the control.

The studied compounds were administered perorally at a dose of 150 mg/kg one hour before alloxan injection. The glucose concentration in blood from a tail vein was determined using a glucose-oxidase biosensor and a hand-held blood glucose monitoring device (Onetouch® Ultra, Johnson & Johnson, USA) 120 min after alloxan injection. The hypoglycemic activity of the compounds was gauged from the reduction of blood glucose level as compared to that of control animals (100%). Figure 2 illustrates the experimental results.

QU tetra-*O*-methyl ether **3** exhibited pronounced hypoglycemic activity, causing hyperglycemia suppression by 44.5% as compared to the control 120 min after alloxan injection and was 2.7 times more active than QU (16.7%).

EXPERIMENTAL

General Comments. PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS internal standard on a Bruker Avance-III pulsed spectrometer at operating frequency 500 (^{1}H) and 125 MHz (^{13}C). IR spectra were recorded from mineral-oil mulls on an IR Prestige-21 spectrophotometer (Shimadzu). Molecular ions were determined by LC-MS on a LCMS-2010 (Shimadzu) using chemical ionization at atmospheric pressure. Melting points were measured on a Boetius apparatus. TLC used Sorbfil plates (Sorbpolimer, Russia) and solvent systems $CHCl_3$–EtOH (20:1, A) and C_6H_6–EtOH (20:1, B). Spots were detected using H_2SO_4 solution (5%) in EtOH followed by heating at 110–120°C for 2–3 min. Compounds were separated by column chromatography using KSK silica gel (50–150 mesh, Sorbpolimer). QU pharmacopoeial drug substance (Pharmstandard-UfaVITA) was used in the work.

Methylation of QU by Diazomethane. QU (1.57 g, 5.2 mmol) was suspended in dioxane (22 mL), cooled in an ice bath, and treated with a solution of diazomethane in dioxane that was prepared from nitrosomethylurea (5.5 g, 55 mmol). The mixture was stirred for 2 h at 20–22°C and left for 3 d in a refrigerator (+4–8°C). The solvent was distilled off. The residue was separated by CC to give three pure compounds that were identified by TLC with labels and NMR spectra as QU tetra-*O*-methyl ether **3** (0.89 g, 54.4%), tri-*O*-methyl ether **4** (0.54 g, 30%), and 3.7.3′-tri-*O*-methyl ether **5** (0.12 g, 7.0%).

Quercetin 3,7,3′,4′-Tetra-*O*-methyl Ether (3). R_f 0.73 (system A); R_f 0.66 (B); mp 158–159°C (MeOH–Et_2O) (yellow needles). Lit. [19]: 157–159°C (anhydr. EtOH). IR spectrum (ν_{max}, cm^{-1}): 3215 (OH), 1650, 1603, 1589, 1512. MS, *m/z*: 359 $[M + H]^+$, 357 $[M – H]^-$. $C_{19}H_{22}O_7$. MM 358.4.

Quercetin 3,7,4′-Tri-*O*-methyl Ether (4). Mp 169–170°C (EtOH) (yellow needles). Lit. [19]: 168–170°C (Me_2CO–MeOH). R_f 0.47 (B). IR spectrum (ν_{max}, cm^{-1}): 3398 (OH), 1651, 1625, 1517. MS, *m/z*: 345 $[M + H]^+$, 343 $[M – H]^-$. $C_{18}H_{16}O_7$. MM 344.3.

Quercetin 3,7,3′-Tri-*O*-methyl Ether (5). Mp 172–174°C. R_f 0.42 (B). ^1H NMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.78, 3.82, 3.85 (15H, all s, $3OC\underline{H}_3$), 6.30 (1H, d, J = 1.8, H-8), 6.61 (1H, d, J = 1.8, H-6), 7.05 (1H, d, J = 8.5, H-5′), 7.53 (1H, d, J = 8.5, H-6′), 7.56 (1H, s, H-2′), 8.29. ^{13}C NMR spectrum (125 MHz, DMSO-d_6, δ, ppm): 178.1 (C-4), 165.1 (C-7), 160.9 (C-8a), 156.2 (C-2), 155.5 (C-5), 150.3 (C-3′), 146.4 (C-4′), 138.2 (C-3), 122.2 (C-1′), 120.4 (C-6′), 115.1 (C-2′), 111.8 (C-5′), 105.2 (C-4a), 97.7 (C-6), 92.1 (C-8); $3OCH_3$: 55.6, 56.0, 59.7. MS, *m/z*: 345 $[M + H]^+$, 343 $[M – H]^-$. $C_{18}H_{16}O_7$. MM 344.3.

Quercetin 5,4′-Di-*O*-acetyl-3,7,3′-tri-*O*-methyl Ether (6). Compound **5** (0.1 g) was dissolved in acetic anhydride (6 mL), treated with anhydrous NaOAc (0.1 g), refluxed without access of moisture for 3 h, and diluted with cold H_2O. The precipitate was filtered off, rinsed with H_2O, dried, and recrystallized from $CHCl_3$–MeOH. Yield 78%. ^1H NMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.05 (1H, dd, J = 8.5, 2.0, H-6′), 7.80 (1H, d, J = 2.0, H-2′), 7.06 (1H, d, J = 8.5, H-5′), 6.81 (1H, d, J = 2.2, H-6), 6.58 (1H, d, J = 2.2, H-8), 3.90, 3.88, 3.85 (9H, all s, $3OCH_3$), 2.46, 2.36 (6H, both s, 2 Ac). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 173.1 (C-4), 169.7 (C=O Ac), 168.9 (C=O Ac), 163.3 (C-7), 153.0 (C-3′, C-2), 150.7 (C-5), 150.4 (C-9), 141.1 (C-3), 139.6 (C-4′), 127.6 (C-6′), 123.2 (C-1′), 122.8 (C-2′), 112.1 (C-5′), 111.3 (C-4a), 108.2 (C-8), 98.6 (C-6), 59.8 (OCH_3), 56.0 (2 OCH_3), 21.2 (CH_3 Ac), 20.7 (CH_3 Ac).

Hypoglycemic activity was studied using male Wistar rats (200–270 g). The hyperglycemia model was induced by i.p. injection of alloxan solution (5%) at a dose of 170 mg/kg as before [21, 22]. QU ether **3** and QU were administered perorally at a dose of 150 mg/kg 1 h before alloxan injection. The glucose concentration in tail-vein blood was determined by a biosensor glucose-oxidase method using a hand-held Onetouch® Ultra blood glucose monitoring device (Johnson & Johnson, USA) 120 min after alloxan injection. Animals with hyperglycemia (>15 mM) were counted. The results were expressed in percent of the control. Hypoglycemic activity of the compounds was gauged from the reduced number of animals with hyperglycemia and the reduction of the blood glucose level as compared with control animals (100%).

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