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SHORT COMMUNICATION

Preparation of an adduct of levoglucosenone and resorcinol and its *in vitro* antiplatelet and anticoagulant activity

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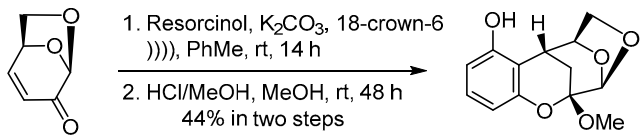

A procedure for the preparation of an adduct of levoglucosenone and resorcinol was developed involving ultrasonic irradiation in the presence of K_2CO_3 and 18-crown-6 ether in toluene. *In vitro* anticoagulant and antiplatelet activity of the adduct was studied.

Keywords: Michael adducts, levoglucosenone, mixed ketal, resorcinol, antiplatelet activity, anticoagulant activity.

The Michael reaction of levoglucosenone with many known nucleophiles leads to the formation of chiral C(4)-adducts.[1] Reactions with 1,3-bifunctional compounds, such as, for example, diketones or di(tri)azoles, often do not stop at the step of Michael adduct formation, and, as a result of subsequent intramolecular transformations, the products of the reaction of the second functional group with the keto group of the carbohydrate fragment are formed.[2] It was found that levoglucosenone itself is capable of such transformations leading to oligomeric products.[3] Thus, in contrast to the reaction with cyclohexanone, dimedone reacts with levoglucosenone with the formation of a hexahydrochromene derivative as a result of the intramolecular formation of a ketal in the reaction of the keto group of levoglucosenone with the enol form of the dimedone fragment.[4] It is known[5] that the chromane system is part of the molecules of such classes of biologically active natural compounds as flavonoids and tocopherols. In the preparation of levoglucosenone derivatives containing the chromane moiety by the reaction with dimedone, the aromatization of hexahydrochromene is prevented by the presence of a *gem*-dimethyl group.

As for aromatic derivatives of levoglucosenone, its Michael adduct with β-naphthol, which was employed in the synthesis of 9-membered lactone annulated to the benzene ring, as well as the fact of the direct addition of the 2-hydroxybenzaldehyde by the oxa-Michael–aldol reaction with the formation of a chiral chromene derivative are known.[6]

Taking into account the importance of the chromane block for elucidating certain aspects of the structure–activity relationship, we studied the possibility of accessing levoglucosenone and resorcinol adducts. Despite the fact that this *meta*-diphenol is not widely found in nature, its fragment, especially the carboxyl derivative, is incorporated into a number of different biologically active compounds. Antibacterial, antiseptic preparations and disinfectants have been created on its basis.[7]

Resorcinol reacts with electrophiles such as aldehydes; therefore, the possibility of its addition to levoglucosenone at the C(4) atom cannot be ruled out. Unfortunately, levoglucosenone turned out to be unreactive under the typical conditions of the reaction of resorcinol with aldehydes in the presence of pyridine. The reaction of levoglucosenone with resorcinol was realized under more severe conditions using ultrasonic irradiation. Thus, the addition reaction of resorcinol (**2**) to levoglucosenone (**1**) in a PhMe solution in the presence of K_2CO_3 and 18-crown-6 ether catalyst proceeded with the formation of a Michael adduct and its subsequent spontaneous cyclization to chromane **3**. Compound **3** turned out to be unstable; therefore, hemiketal **3** was transformed into ketal **4** (Scheme 1).

Tetracyclic compound **3** is the product of a "rear" tandem transformation initiated by the 1,4-nucleophilic

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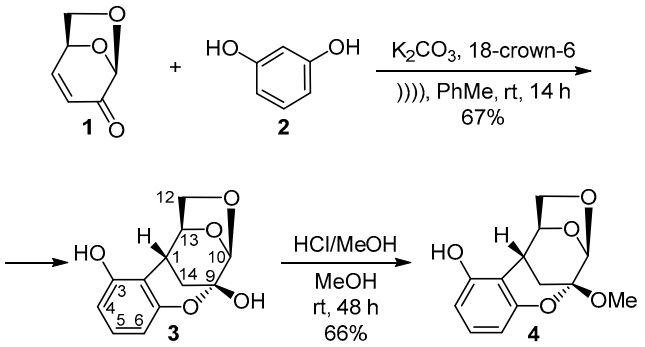
Scheme 1

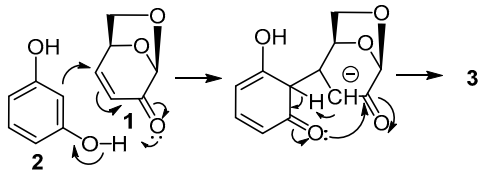


addition of resorcinol (**2**) to levoglucosenone (**1**) and subsequent intramolecular ketalization due to the proximity of the hydroxy group and the keto group. This is possible only with the chair conformation of the six-membered ring and upon an intramolecular attack of the keto group by a hydroxyl group from the α-region of the resorcinol fragment. Thus, the evidence in favor of an *R*-configuration of the C(1) and C(9) centers is the stereochemical feature of the reactivity of levoglucosenone (**1**): the first step takes place under strict control by the 1,6-anhydro-bridge, whereas the second step is made possible by steric assistance. The stereochemistry of the C(10) and C(13) centers remains unaffected during the transformation.

As in all Michael reactions, the addition of the nucleophile to the double bond of levoglucosenone (**1**) occurs stereospecifically from the side opposite to the 1,6-anhydro-bridge; the transformation is completed by the closure of the dihydropyran ring "from below" according to the proposed mechanism (Scheme 2).

The formation of the pyran ring is evidenced by the signal of the C(9) quaternary atom in the ^{13}C NMR spectrum of compound **4** at 101.2 ppm and the presence of correlation peaks in the $^{1}H–^{13}C$ HMBC spectrum between the C(9)/1-CH, C(3)/1-CH, and C(9)/OCH_3 atoms.

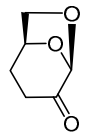
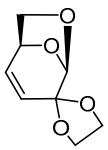
Scheme 2



Considering the supposed biogenetic relationship of resorcinol with coumarins, we studied the *in vitro* anticoagulant and antiplatelet activity of all compounds used in the synthesis as well as of some derivatives of levoglucosenone.

It was established from the results of the studies that in terms of the maximum amplitude levoglucosenone **1** and ketal **4** exhibit antiplatelet activity exceeding the indicators of the reference drug, acetylsalicylic acid, while the monodioxolane derivative of levoglucosenone **6**, obtained by us earlier,[8] and resorcinol (**2**) exhibit an antiplatelet effect comparable to that of acetylsalicylic acid (Table 1). At the same time, compounds **1**–**6** lengthened the latent period of aggregation, which characterizes the intensity of the platelet release reaction, more effectively than acetylsalicylic acid. The rate of platelet aggregation, as in the case of acetylsalicylic acid, decreased for all the indicated derivatives. It should be noted that all compounds caused hypocoagulation, increasing the aPTT by 1.7–4.4%

Table 1. The influence of compounds **1**–**6** and reference drugs on the indicators of platelet aggregation and aPTT, Me (0.25–0.75)

Compound	Latent period, * % to control	Maximum amplitude, % to control	Aggregation rate, % to control	Increase of aPTT, % to control
1	+13.7 (10.6–15.9)**	–18.9 (17.4–21.1)**	–21.6 (18.3–23.1)**	+1.7 (1.5–3.7)
2	+9.9 (6.8–12.3)**	–11.1 (7.8–13.2)**	–13.1 (6.5–15.7)**	+4.4 (3.3–5.1)
3	+7.2 (5.4–8.6)**	–10.2 (7.6–12.3)**	–14.9 (12.4–17.3)**	+2.5 (1.4–4.2)
4	+20.4 (18.7–24.5) **·***	–17.5 (17.1–18.9)**	–13.5 (11.7–14.7)**	+3.8 (3.1–5.3)
 **5**	–10.5 (8.6–11.3)**	–4.7 (3.2–6.7)**	+2.7 (2.1–3.3) ***	+3.4 (3.2–4.6)
 **6**	+11.3 (8.5–14.7)**	–17.6 (15.3–20.1)**	–19.2 (17.4–22.9)**·***	+2.7 (1.9–3.5)
Acetylsalicylic acid	–2.1 (1.1–2.6)	–13.7 (10.8–16.4)**	–10.5 (7.6–12.3)**	–
Heparin sodium	–	–	–	+20.3 (19.7–21.4)**

* Collagen-induced platelet aggregation latent period.
** $p \le 0.05$.
*** $p \le 0.001$, in comparison with acetylsalicylic acid.

compared to the control, and did not affect the fibrinogen concentration and prothrombin time. The studied compounds were significantly inferior to heparin in the severity of action, which increased aPTT by 20.3%.

To conclude, a method for the synthesis of a chiral compound containing the chromane pharmacophore was developed on the basis of levoglucosenone. The first results of a study of the anticoagulant and antiplatelet activity of levoglucosenone were obtained. The introduction of the chroman fragment leads to an increase in these types of biological activity.

Experimental

^{1}H and ^{13}C NMR spectra (500 and 126 MHz, respectively), as well as COSY, NOESY, ^{1}H–^{13}C HMBC, ^{1}H–^{13}C HSQC spectra were recorded on a Bruker Avance III 500 MHz spectrometer in CD_3OD. Mass spectra were recorded on a LCMS-2010 EV (Shimadzu) single quadrupole system, in the positive and negative ion registration mode at a capillary potential of 4.5 and –3.5 kV, respectively, electrospray ionization, eluent MeCN–H_2O. Elemental analysis was performed on a Thermo Scientific Flash 2000 CHNS-analyzer. Optical rotation angles were measured with a PerkinElmer 341 polarimeter. Melting points were determined on a Boetius heating bench equipped with PHMK 05 visual device. Sorbfil PTSKh-AF-A plates (ZAO Sorbpolimer, Krasnodar, Russia) were used for analytical TLC. Macherey-Nagel 60 (0.063–0.2 mm) silica gel was used for column chromatography.

The following reagents were used in the study: levoglucosenone (**1**) ((1*S*,5*R*)-6,8-dioxabicyclo[3.2.1]oct-2-en-4-one, CAS № 37112-31-5) supplied by Circa Group (Australia), assay 87.9% (HPLC), assay after distillation of the technical grade product was 96% (GLC), $[\alpha]_D^{24}$ –498.7° (*c* 1.0, $CHCl_3$); Cyrene (**5**) (dihydrolevoglucosenone, 6,8-dioxabicyclo[3.2.1]octan-4-one, CAS 53716-82-8) was supplied by Circa Group (Australia), bp 231.6°C, *d* 1.25 g/ml.

(1*R*,9*R*,10*R*,13*S*)-8,11,15-Trioxatetracyclo[7.4.1.1^{10,13}.0^{2,7}]-pentadeca-2,4,6-triene-3,9-diol (3). Resorcinol (**2**) (0.88 g, 8.0 mmol), K_2CO_3 (6.9 g, 50.0 mmol), and a catalytic amount of 18-crown-6 ether were added at room temperature to a solution of levoglucosenone (**1**) (1.00 g, 8.0 mmol) in PhMe (30.0 ml). The reaction mixture was irradiated on an UZDN-2T ultrasonic generator (44 Hz range, 80 mA current) for 14 h (TLC control). The solvent was evaporated, and the residue was chromatographed on a SiO_2 column. Yield 1.25 g (67%). R_f 0.3 (petroleum ether – EtOAc, 1:1). White crystals. Mp 250–253°C. $[\alpha]_D^{20}$ –53.7° (*c* 1.0, $CHCl_3$). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.62 (1H, dd, *J* = 12.6, *J* = 0.9, 14-CH_2); 2.40 (1H, dd, *J* = 12.6, *J* = 3.4, 14-CH_2); 2.91 (1H, d, *J* = 2.9, 1-CH); 3.81 (1H, dd, *J* = 6.7, *J* = 4.7, 12-CH_2); 4.15 (1H, d, *J* = 6.7, 12-CH_2); 4.30–4.35 (1H, m, 13-CH); 4.83 (1H, s, 10-CH); 6.23–6.29 (2H, m, H-4,6); 6.85 (1H, d, *J* = 7.8, H-5). ^{13}C NMR spectrum, δ, ppm: 28.8 (C-14); 38.1 (C-1); 67.8 (C-12); 78.0 (C-13); 96.6 (C-9); 102.1 (C-10); 104.0 (C-4); 106.5 (C-6); 116.7 (C-2); 126.9 (C-5); 157.0 (C-3); 157.5 (C-7). Mass spectrum, *m*/*z* (I_{rel}, %): 237 $[M+H]^+$ (100). Found, %: C 60.90; H 5.15. $C_{12}H_{12}O_5$. Calculated, %: C 61.02; H 5.12.

(1*R*,9*R*,10*R*,13*S*)-9-Methoxy-8,11,15-trioxatetracyclo-[7.4.1.1^{10,13}.0^{2,7}]pentadeca-2,4,6-trien-3-ol (4). 20% HCl in MeOH (3.0 ml) was added to a solution of adduct **3** (0.95 g, 4.0 mmol) in MeOH (10.0 ml) at 0°C. The reaction mixture was stirred at room temperature for 48 h (TLC control). The reaction mixture was then neutralized with saturated aqueous $NaHCO_3$ (pH 6), and the reaction products were extracted with EtOAc (3×15.0 ml). The extract was dried over $MgSO_4$, the solvent was evaporated, and the residue was chromatographed on a SiO_2 column. Yield 66 mg (66%). R_f 0.5 (petroleum ether – EtOAc, 1:1). White crystals. Mp 235–237°C. $[\alpha]_D^{20}$ –150° (*c* 1.35, MeOH). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.95–1.98 (1H, m, 14-CH_2); 2.28 (1H, dd, *J* =12.4, *J* = 3.5, 14-CH_2); 2.96 (1H, ddd, *J* = 6.0, *J* = 3.5, *J* = 3.0, 1-CH); 3.35 (3H, s, OCH_3); 3.80 (1H, ddd, *J* = 12.0, *J* = 7.4, *J* = 4.6, 12-CH_2); 4.14 (1H, d, *J* = 7.4, 12-CH_2); 4.34–4.38 (1H, m, 13-CH); 4.62 (1H, br. s, OH); 5.03 (1H, d, *J* = 1.7, 10-CH); 6.27–6.31 (2H, m, H-4,6); 6.86 (1H, d, *J* = 8.8, H-5). ^{13}C NMR spectrum, δ, ppm: 26.5 (C-14); 39.3 (C-1); 49.8 (OCH_3); 69.1 (C-12); 79.7 (C-13); 101.2 (C-9); 103.5 (C-6); 104.8 (C-10); 108.2 (C-4); 118.1 (C-2); 128.4 (C-5); 158.5 (C-3); 159.0 (C-7). Mass spectrum, *m*/*z* (I_{rel}, %): 251 $[M+H]^+$ (100). Found, %: C 62.42; H 5.61. $C_{13}H_{14}O_5$. Calculated, %: C 62.39; H 5.64.

The study of antiplatelet and anticoagulant activity of compounds 1–6. The experiments were performed in accordance with the requirements of the Rules of Good Laboratory Practice of the Eurasian Economic Union in the field of drug circulation. The study of antiplatelet and anticoagulant activity was carried out *in vitro* on the blood of 38 healthy male donors aged 18–24 years. The study was approved by the Ethics Committee of the Bashkir State Medical University of the Ministry of Health of Russia (Protocol No. 1 of 20.02.2019). Informed consent was obtained from all participants of the study prior to blood sampling.

The effect of compounds on platelet aggregation was studied using the Born method[9] on an AT-02 aggregometer (NPF Medtekh, Russia). The antiplatelet activity of the compounds and the reference drug was investigated at a final concentration of $2\cdot10^{-3}$ mol/l with incubation for 5 min. Adenosine diphosphate (ADP) at a concentration of 20 μg/ml and collagen at a concentration of 5 mg/ml (Tekhnologiya-Standard, Russia) were used as inducers of aggregation. The effect of the compounds on the maximum aggregation amplitude (MA), the rate of aggregation, and the time to reach MA during ADP-induced platelet aggregation were studied. The latent period of aggregation, which characterizes the intensity of the platelet release reaction, was estimated in the test of collagen-induced platelet aggregation. Acetylsalicylic acid (substance-powder, Shandong Xinhua Pharmaceutical Co., China) was used as the reference drug.

Determination of anticoagulant activity was carried out by clotting tests[10] on a Solar CGL 2110 (ZAO SOLAR, Belarus) turbidimetric hemocoagulometer, the final concentrations of the studied compounds and the reference drug were $5\cdot10^{-4}$ g/ml. The parameters of activated partial

thromboplastin time (aPTT), prothrombin time (PT), and fibrinogen concentration were studied by the Clauss method. The reference drug was sodium heparin (5000 IU/ml solution for injection, 1 ml ampoules, OAO Sintez, Russia).

Statistical analysis was performed using the Statistica 10.0 software package (StatSoft, Inc., USA). The normal distribution was checked using the Shapiro–Wilk test. To describe the variation series, the median, 25th and 75th percentiles, minimums and maximums of values were calculated. A univariate analysis of variance (if the population of data obeys the laws of normal distribution and variance of all samples, F-value) or the Kruskal–Wallis test (if the population of data does not obey the laws of normal distribution, H-value). The critical level of significance p for statistical tests was assumed to be ≤ 0.05.

Supplementary information file containing ^{1}H and ^{13}C NMR spectra of the synthesized compounds, as well as COSY, NOESY, ^{1}H–^{13}C HMBC, ^{1}H–^{13}C HSQC spectra of compound **4** is available at the journal website at http://link.springer.com/journal/10593.

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The NMR spectra were recorded on the equipment of the Collective Use Center "Khimiya", Ufa Institute of Chemistry, Ufa Federal Research Center of the Russian Academy of Sciences and the Regional Center for Collective Use "Agidel" of the Ufa Federal Research Center of the Russian Academy of Sciences.

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