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MODIFIED COUMARINS. 17. SYNTHESIS AND ANTICOAGULANT ACTIVITY OF 3,4-CYCLOANNELATED COUMARIN D-GLYCOPYRANOSIDES

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3,4-Cyclocondensed coumarin O-glycopyranosides containing glucose, galactose, xylose, and arabinose were synthesized by condensation of potassium salts of hydroxycoumarins and acetobromosugars. 7-(β-D-Galactopyranosyloxy)-2,3-dihydrocyclopenta[c]chromen-4-one, 3-(β-D-xylopyranosyloxy)-7,8,9,10-tetrahydrobenzo[c]chromen-6-one, and 3-(α-D-arabinopyranosyloxy)benzo[c]chromen-6-one exhibited distinct anticoagulant activity.

Key words: coumarins, O-glycosides, O-glycosylation, anticoagulants.

Coumarins in both the free state and as O-glycosides are known to be widely distributed in nature [1]. The carbohydrate unit occurs at various positions of the benzopyran ring. The sugar component of coumarin O-glycosides can be monosaccharides (D-glucopyranose, L-rhamnopyranose, D-galactopyranose, D- and L-arabinopyranose, D-xylopyranose, etc.) and also certain disaccharides. Compared with the coumarins themselves, their glycosides are very soluble in water. Considering the high biological activity of natural and synthetic coumarins and the broad spectrum of their pharmacological activity, the synthesis and investigation of biologically active glycosides of compounds based on the benzopyran-2-one skeleton is very interesting. Therefore, the present study examined the synthesis of 5-hydroxy- and 7-hydroxy-3,4-cycloannelated coumarin D-glycopyranosides and their anticoagulant activity.

Hydroxycoumarins **1**-**3** that were required for further transformations were prepared by Pechmann condensation of polyphenols (resorcinol and orcinol) with ethyl-2-oxocyclopentanecarboxylate or ethyl-2-oxocyclohexanecarboxylate in the presence of conc. H_2SO_4 at 0°C [2, 3]; 3-hydroxybenzo[*c*]chromen-6-one (**4**), by Hartley condensation in NaOH solution of 2-bromobenzoic acid and resorcinol with a catalyst of copper sulfate solution (10%) [4].

3,4-Cyclocondensed coumarin O-glycosides were synthesized using the most convenient method based on condensation of a glycosyl donor and potassium salts of hydroxycoumarins in aqueous acetone with cooling (0°C) (Modified Michael method) and were used successfully to synthesize a similar type of compounds [5-7]. Solutions of these salts were prepared using equivalent amounts of the respective hydroxycoumarin and KOH solution (10%) and double (relative to the volume of base) the amount of acetone. The glycosyl donors in this synthesis were acetobromosugars (D-isomers): α-acetobromoglucose (Ac_4Glu*p*Br), β-acetobromogalactose (Ac_4Gal*p*Br), α-acetobromoxylose (Ac_3Xyl*p*Br), and β-acetobromoarabinose (Ac_3Ara*p*Br), which were prepared by known methods [8, 9]. The synthesis gave 42-51% yields of the O-peracetates of glucopyranosides **5** and **6**, galactopyranoside **7**, xylopyranoside **8**, and arabinopyranosides **9** and **10**, which contain a carbohydrate in the 5- or 7-position of the coumarin ring.

The structures of the resulting glycosides and the configurations of their anomeric centers were unambiguously confirmed using PMR spectroscopy. The PMR spectra of **5**-**10** contain signals for four (for glucosides and galactosides) or three (for arabinosides and xylosides) acetyls at 2.00-2.20 ppm and signals for the carbohydrate and aglycon moieties.

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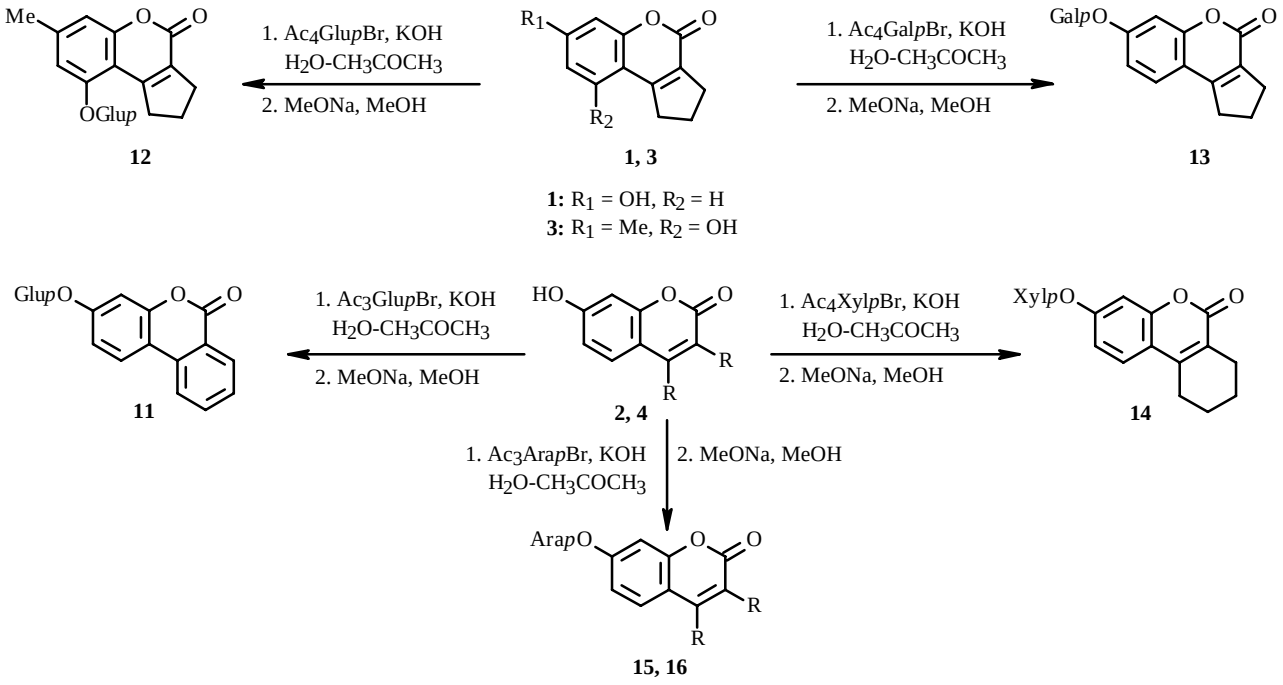

2, 9, 15: RR = $(CH_2)_4$; **4, 10, 16:** RR = -CH=CH-CH=CH-

The PMR spectra of tetraacetylglucosides **5** and **6** exhibit doublets at 5.20 ppm with SSCC J = 7.2-7.8 Hz that belong to H-1 of the carbohydrate ring. Such a SSCC for H-1 and H-2 in carbohydrates corresponds to their *trans*-diaxial arrangement in the ring [10], which together with the chemical shift of H-1 confirms the β-configuration of the glucopyranosides. Methylene protons CH_2-6 of the glucosides are chemically nonequivalent and together with H-5 form a separate group of signals near 4 ppm. The methylene protons resonate as two doublets of doublets at 4.17-4.22 and 4.26-4.32 ppm with SSCC J = 12.0, J = 2.0 and J = 12.0, J = 5.6 Hz, respectively.

The PMR spectrum of tetraacetylgalactoside **7** contains a doublet for carbohydrate H-1 at 5.12 ppm with SSCC J = 8.0 Hz. This is consistent with the β-configuration of the resulting galactopyranosides. In contrast with the glucopyranosides, the CH_2-6 methylene protons of the galactopyranosides appear as a complex multiplet at 4.19-4.24 ppm.

The presence in the PMR spectrum of triacetylxyloside **8** of a doublet for carbohydrate H-1 at 5.20 ppm with SSCC J = 6.9 Hz is consistent with the β-configuration of the anomeric center. The CH_2-5 methylene protons resonate as two doublets of doublets at 3.58 and 4.23 ppm with SSCC J = 12.0, J = 7.2 and J = 12.0, J = 4.5 Hz, respectively.

The presence in the PMR spectra of **9** and **10** of doublets for carbohydrate H-1 near 5.10-5.20 ppm with SSCC J = 6.0 Hz confirms that H-1 and H-2 are 1,2-*trans*-diaxial. For the D-triacetylarabinoside, this occurs exclusively for the α-anomer, i.e., the resulting arabinosides have the α-configuration. The methylene CH_2-5 protons resonate as two doublets of doublets at 3.80 and 4.10 ppm with SSCC J = 12.9, J = 2.4 and J = 12.9, J = 4.2 Hz, respectively.

The IR spectra of **5-10** exhibit two bands at 1750-1760 and 1700-1735 cm^{-1} that are typical stretching vibrations of acetyl C=O and the coumarin ring, respectively.

Zemplen deacetylation (sodium methoxide in absolute methanol) of tetra-O-acetylglycopyranosides **5-10** produced D-glycopyranosides **11-16** with free hydroxyls in high (83-92%) yields. The PMR spectra of the synthesized glycosides contain signals for the carbohydrate and aglycon fragments. However, in contrast with the starting peracetates, they lack signals for acetyls. The presence in the PMR spectra of doublets for anomeric H-1 with a characteristic SSCC confirms that the 1,2-*trans*-diaxial arrangement of H-1 and H-2 is retained. The IR spectra of the glycopyranosides contain two bands at 3300-3400 and 1690-1720 cm^{-1} that are typical of hydroxyl and coumarin ring C=O stretching vibrations, respectively.

Table 1 lists the results from tests for anticoagulant and hemostatic activities in synthesized D-glycopyranosides **11-16**.

Judging from the results, compounds **11** and **15** at doses of 0.30 mg/kg have the highest hemostatic activities whereas **13**, **14**, and **16** exhibit anticoagulant activity. The results indicate that **13** has anticoagulant activity at the same level as heparin and that **14** and **16** are better anticoagulants than heparin.

TABLE 1. Effect of Coumarin O-D-Glycopyranosides **11-16** on Blood Clotting

Compound	Blood clotting time, s		
	Dose 0.1 mg/kg	Dose 0.25 mg/kg	Dose 0.30 mg/kg
11	425.00	188.50	152.00
12	349.00	268.00	279.00
13	512.00	494.00	183.50
14	496.00	374.00	547.00
15	294.50	301.00	127.00
16	506.00	652.50	283.50

Note. Blood clotting time for physiological solution, 91.00 s; ε-aminocaproic acid, 40.00 s; heparin, 514.00 s.

EXPERIMENTAL

The course of reactions and purity of products were monitored using TLC on Merck 60 F254 plates with elution by $CHCl_3$:CH_3OH (9:1). Melting points were determined on a Kofler block. IR and UV spectra were measured on a Nicolet FTIR Nexus 475 spectrometer and Specord M40 spectrophotometer, respectively. PMR spectra were recorded on Varian VXR-300 and Mercury-400 spectrometers at 300 and 400 MHz, respectively, with TMS internal standard. Elemental analyses of all compounds agreed with those calculated.

The syntheses of **1-4** have been reported [2-4]. Peracetylglycopyranosylbromides were prepared as before [8, 9]. The syntheses and physicochemical properties of **5**, **6**, **11**, and **12** have been published [7].

General Method of O-Glycosylation. A solution of **1-4** (10 mmol) in acetone (10 mL) and KOH solution (5.6 mL, 10%, 10 mmol) were stirred vigorously and cooled (0°C) for 30 min. The resulting mixture was treated in portions with stirring over 1 h with the respective acetobromoglycopyranose (10 mmol). The resulting solution was stirred for 4 h at 0°C, left overnight at room temperature, treated with $CHCl_3$ (50 mL), and treated successively in a separatory funnel with KOH solution (1 N, 2×50 mL) and water (50 mL). Acidification of the combined basic extracts regenerated unreacted hydroxycoumarin. The organic phase was dried over anhydrous $MgSO_4$. Solvent was removed in vacuo in a rotary evaporator. The oily residue was crystallized from propan-2-ol.

7-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyloxy)-2,3-dihydrocyclopenta[*c*]-chromen-4-one (7). Yield 51%, $C_{26}H_{28}O_{12}$, mp 171-172°C.

IR spectrum (KBr, cm^{-1}): 1756, 1700, 1616, 1432, 1372, 1228, 1156, 1078.

UV spectrum (EtOH, λ_{max}, nm, log ε): 205 (4.67), 218 (4.24), 319 (4.17).

PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.03, 2.08, 2.11, 2.19 (12H, 4s, 4×CH_3COO), 2.23 (2H, m, CH_2-2), 2.89 (2H, m, CH_2-1), 3.06 (2H, m, CH_2-3), 4.12 (1H, m, H-5′), 4.20 (2H, m, CH_2-6′), 5.12 (1H, d, J = 8.0, H-1′), 5.14 (1H, dd, J = 10.8, J = 3.2, H-3′), 5.49 (1H, d, J = 3.2, H-4′), 5.52 (1H, dd, J = 10.8, J = 8.0, H-4′), 6.93 (1H, dd, J = 2.4, J = 8.8, H-8), 7.02 (1H, d, J = 2.4, H-6), 7.37 (1H, d, J = 8.8, H-9).

3-(2,3,4-Tri-O-acetyl-β-D-xylopyranosyloxy)-7,8,9,10-tetrahydrobenzo[*c*]chromen-6-one (8). Yield 44%, $C_{24}H_{26}O_{10}$, mp 182-183°C.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.76-1.90 (4H, m, CH_2-8, CH_2-9), 2.10 (9H, s, 3×CH_3COO), 2.56 (2H, m, CH_2-7), 2.75 (2H, m, CH_2-10), 3.58 (1H, dd, J = 12.0, J = 7.2, H-5′a), 4.23 (1H, dd, J = 12.0, J = 4.5, H-5′e), 5.03 (1H, m, H-4′), 5.20 (1H, d, J = 6.9, H-1′), 5.22-5.30 (2H, m, H-2′, H-3′), 6.91 (1H, dd, J = 8.7, J = 2.4, H-2), 6.94 (1H, d, J = 2.4, H-4), 7.48 (1H, d, J = 8.7, H-1).

3-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyloxy)-7,8,9,10-tetrahydrobenzo[*c*]chromen-6-one (9). Yield 49%, $C_{24}H_{26}O_{10}$, oil.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.75-1.90 (4H, m, CH_2-8, CH_2-9), 2.10, 2.11, 2.16 (9H, 3s, 3×CH_3COO), 2.55 (2H, m, CH_2-7), 2.75 (2H, m, CH_2-10), 3.80 (1H, dd, J = 12.9, J = 2.4, H-5′a), 4.12 (1H, dd, J = 12.9,

J = 4.2, H-5′e), 5.17 (1H, d, J = 6.0, H-1′), 5.19 (1H, dd, J = 6.6, J = 3.3, H-2′), 5.35 (1H, m, H-4′), 5.44 (1H, dd, J = 8.4, J = 6.6, H-2′), 6.92 (1H, dd, J = 8.7, J = 2.4, H-2), 6.96 (1H, d, J = 2.4, H-4), 7.45 (1H, d, J = 8.7, H-1).

3-(2,3,4-Tri-O-acetyl-α-D-arabinopyranosyloxy)benzo[*c*]chromen-6-one (10). Yield 42%, $C_{24}H_{22}O_{10}$, oil.

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.09, 2.12, 2.18 (9H, 3s, 3×CH_3COO), 3.78 (1H, dd, J = 12.9, J = 2.4, H-5′a), 4.13 (1H, dd, J = 12.9, J = 4.2, H-5′e), 5.10 (1H, d, J = 6.0, H-1′), 5.17 (1H, dd, J = 6.6, J = 3.3, H-2′), 5.32 (1H, m, H-4′), 5.44 (1H, dd, J = 8.4, J = 6.6, H-2′), 6.99 (1H, dd, J = 8.7, J = 2.4, H-2), 7.01 (1H, d, J = 2.4, H-4), 7.55 (1H, t, J = 6.9, H-8), 7.81 (1H, t, J = 6.9, H-9), 7.97 (1H, d, J = 8.7, H-1), 8.02 (1H, d, J = 8.4, H-10), 8.37 (1H, d, J = 8.0, H-7).

General Deacetylation Method of Peracetylglycopyranosides 7-10. A solution of **7-10** (5 mmol) in absolute CH_3OH (20 mL) was treated with sodium methoxide (50 mg). The reaction mixture was boiled for 10-30 min (end of reaction determined by TLC). The precipitate that formed on cooling (0°C) was filtered off and washed with cold CH_3OH.

7-(β-D-Galactopyranosyloxy)-2,3-dihydrocyclopenta[*c*]chromen-4-one (13). Yield 84%, $C_{18}H_{20}O_8$, mp 253-254.5°C.

IR spectrum (KBr, cm^{-1}): 3476, 2936, 1702, 1616, 1390, 1284, 1242, 1168, 1088, 1068, 1030.

UV spectrum (EtOH, λ_{max}, nm, log ε): 205 (4.63), 219 (4.19), 321 (4.22).

PMR spectrum (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.11 (2H, m, CH_2-2), 2.74 (2H, m, CH_2-1), 3.06 (2H, m, CH_2-3), 3.37-3.74 (6H, H-2′, H-3′, H-4′, H-5′, CH_2-6′), 4.54 (1H, d, J = 4.8, OH), 4.68 (1H, t, J= 5.6, OH-6), 4.90 (1H, d, J = 5.2, OH), 4.97 (1H, d, J = 7.2, H-1′), 5.23 (1H, d, J = 5.2, OH), 7.02 (1H, dd, J = 2.0, J = 8.8, H-8), 7.08 (1H, d, J = 2.0, H-6), 7.54 (1H, d, J = 8.8, H-9).

3-(β-D-Xylopyranosyloxy)-7,8,9,10-tetrahydrobenzo[*c*]chromen-6-one (14). Yield 88%, $C_{18}H_{20}O_7$, mp 214-215°C.

PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.70-1.85 (4H, m, CH_2-8, CH_2-9), 2.41 (2H, m, CH_2-7), 2.75 (2H, m, CH_2-10), 3.10-3.40 (4H, m, H-2′, H-3′, H-4′, H-5′a), 3.76 (1H, m, H-5′e), 4.95 (1H, d, J = 6.9, H-1′), 5.03 (1H, d, J = 4.5, OH), 5.08 (1H, d, J = 3.9, OH), 5.34 (1H, d, J = 4.8, OH), 6.94 (1H, d, J = 2.7, H-4), 6.96 (1H, dd, J = 8.7, J = 2.7, H-2), 7.57 (1H, d, J = 8.7, H-1).

3-(α-D-Arrabinopyranosyloxy)-7,8,9,10-tetrahydrobenzo[*c*]chromen-6-one (15). Yield 89%, $C_{18}H_{20}O_7$, mp 219-220.5°C.

PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.70-1.85 (4H, m, CH_2-8, CH_2-9), 2.41 (2H, m, CH_2-7), 2.75 (2H, m, CH_2-10), 3.49 (1H, m, H-4′), 3.60-3.80 (4H, H-2′, H-3′, CH_2-5′), 4.57 (1H, d, J = 3.9, OH), 4.75 (1H, d, J = 5.4, OH), 4.93 (1H, d, J = 7.2, H-1′), 5.19 (1H, d, J = 4.5, OH), 6.94 (1H, d, J = 2.4, H-4), 6.96 (1H, dd, J = 8.1, J = 2.4, H-2), 7.56 (1H, d, J = 8.1, H-1).

3-(α-D-Arabinopyranosyloxy)benzo[*c*]chromen-6-one (16). Yield 92%, $C_{18}H_{16}O_7$, mp 236-237°C.

PMR spectrum (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.50 (1H, m, H-4′), 3.62-3.82 (4H, H-2′, H-3′, CH_2-5′), 4.59 (1H, d, J = 4.2, OH), 4.77 (1H, d, J = 5.4, OH), 4.98 (1H, d, J = 6.9, H-1′), 5.22 (1H, d, J = 4.8, OH), 7.02 (1H, d, J = 2.4, H-4), 7.04 (1H, dd, J = 8.1, J = 2.4, H-2), 7.58 (1H, t, J = 7.8, H-8), 7.88 (1H, t, J = 7.8, H-9), 8.20 (1H, d, J = 8.4, H-1, H-10), 8.36 (1H, d, J = 7.8, H-7).

Biological Experiments. The anticoagulant and hemostatic activities of the compounds were determined *in vitro*. The anticoagulant and hemostatic effects were estimated from the blood clotting time of the investigated compounds compared with a control (physiological solution). The activities of the compounds were studied at doses of 0.1, 0.25, and 0.30 mg/kg. The rate of blood clotting was determined by the Althausen method based on determining the time for spontaneous appearance of the first fibrin fibers in whole blood. For this, a drop of blood from the ear vein of a guinea pig and a drop of a solution of the investigated compound were placed on a thoroughly wahsed and dried glass slide heated to 37°C. A scarificator was passed through the drop of blood every 15-20 s until the first fibrin fiber appeared [11, 12]. The controls were heparin (natural anticoagulant) and ε-aminocaproic acid (synthetic hemostatic).

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