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SYNTHESIS OF ACETYLATED GLYCOSYL-CONTAINING THIOUREA DERIVATIVES BASED ON THE ALKALOIDS CYTISINE AND ANABASINE AND THE MOLECULAR STRUCTURE OF *N*-CYTISINO-*N′*-(2,3,4,6-TETRA-*O*-ACETYL-*β*-D-GLUCOPYRANOSYL)THIOCARBAMIDE

I. V. Kulakov,[1*] O. A. Nurkenov,[1] A. E. Arinova,[1] D. M. Turdybekov,[2] S. A. Talipov,[3] and B. T. Ibragimov[3]

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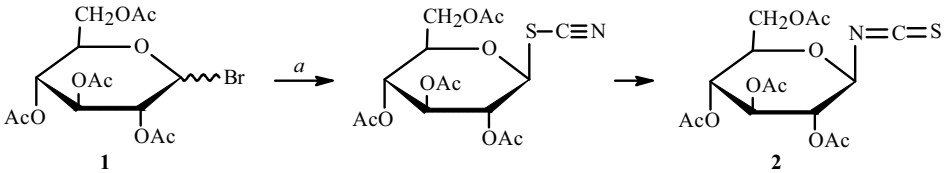
*Glycosylthiourea derivatives were synthesized from the alkaloids cytisine and anabasine and 1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylisothiocyanate. The structures of the synthesized compounds were proved using PMR spectroscopy, mass spectrometry, and an x-ray crystal structure analysis.*

Keywords: cytisine, anabasine, glycosylisothiocyanate, x-ray crystal structure analysis.

Most thiourea derivatives are known to exhibit valuable pharmacological properties and are used as antituberculosis, antimicrobial, anti-ulcer, and other therapeutically active agents [1, 2]. Thiourea derivatives containing natural biologically active compounds in their structure are especially interesting.

In continuation of our research on the synthesis of various polyfunctional thiourea derivatives based on the alkaloids cytisine and anabasine [3–6], the preparation of glycosyl-containing thiourea derivatives of these alkaloids was planned because it is known that introducing biologically active compounds into the structure reduces sharply their toxicity, increases the water solubility, and prolongs the action of the drugs [7]. One of the oldest methods for synthesizing *N*-glycosylthioureas is the Fischer isothiocyanate method [8] based on the reaction of acetyl-substituted glycosylisothiocyanates with amines. A convenient preparative method for synthesizing the starting glycosylisothiocyanates, in addition to the method developed at the start of the last century by Fischer (from the corresponding glycosylbromides and silver thiocyanate), involves nucleophilic substitution of glycosylhalides by potassium thiocyanate under phase-transfer catalysis conditions in the presence of quaternary ammonium salts as proposed by Tashpulatov et al. [9]. Because the starting silver thiocyanate was expensive, the cheaper and more available lead thiocyanate was used to synthesize glycosylisothiocyanates [10]. Also, glycosylisothiocyanates were prepared in a melt of the starting glycosylbromides and potassium thiocyanate [11].

We synthesized tetra-*O*-acetyl-*α*-D-glucopyranosylbromide (acetobromoglucose, ABG) by a simplified method developed by us that differed markedly from that described before [12] in order to prepare glycosylthiourea derivatives of several alkaloids. The method is classical for its simplicity and higher yields and purities. The resulting ABG (**1**) underwent a substitution reaction with a 1.5-fold excess of lead thiocyanate.



1 **2**

a. $Pb(SCN)_2$, 130 – 140°C

1) Institute of Organic Synthesis and Carbon Chemistry of the Republic of Kazakhstan, Kazakhstan, 100008, Karaganda, fax: (87212) 41 38 66, e-mail: kulakov_iv@mail.ru; 2) Multi-discipline Humanitarian-Technical University, Kazakhstan, 100000, Karaganda, Bukhar-Jyrau St., 12; 3) A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, September–October, 2011, pp. 682–685. Original article submitted January 20, 2011.

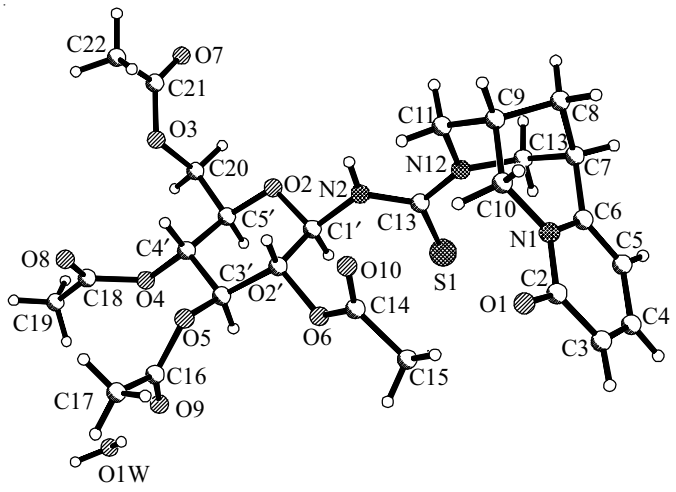
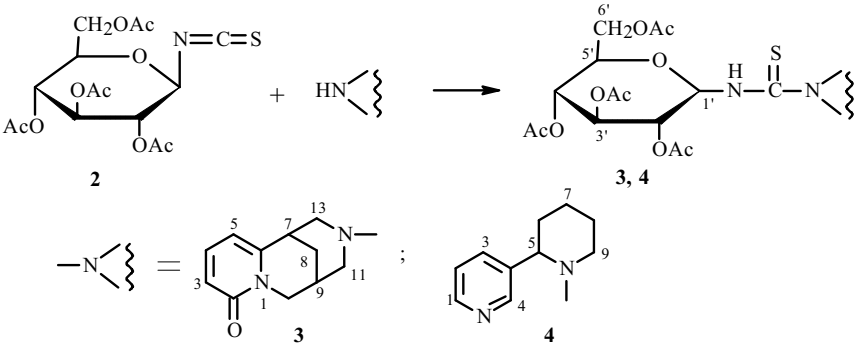


Fig. 1. Molecular structure of *N*-cytisino-*N′*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)thiocarbamide (**3**).

The reaction was carried out in refluxing *o*-xylene for 8–10 h for a more complete conversion of **1** into isothiocyanate **2**. Then, the resulting *o*-xylene solution of 1-isothiocyanato-1-deoxy-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose (**2**) was used without isolation (*in situ*) in nucleophilic addition reactions with cytisine and anabasine.

It was found that **2** reacted rather readily with these alkaloids at room temperature in 3–5 h. The synthesized compounds (**3** and **4**) were obtained after distillation of solvent in about 90% yield. They were slightly yellowish powdery compounds that were very soluble in many organic solvents except saturated hydrocarbons. The isolated compounds **3** and **4** were rather easily purified by recrystallization to transparent colorless crystals with sharp melting points.



The structures of **3** and **4** were elucidated using IR and PMR spectral data in addition to mass spectrometry. An analysis of the PMR spectra of *N*-anabasino-*N′*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)thiocarbamide (**4**) showed that four singlets for protons of acetate methyls appeared at strong field of ~2 ppm. The four pyranose protons $H_{1'}$–$H_{4'}$ appeared as sharp triplets at 4.91, 5.14, 5.37, and 6.12 ppm with SSCC J = 9.84, 9.31, 9.58, and 9.00 Hz, indicating the equivalence of the resonating proton with its neighbors on the pyranose ring. The *gem*-acyl methine protons of the pyranose ring with acetylated hydroxyls were recorded at much weaker field than protons of free non-acetylated saccharides. The anomeric $H_{1'}$ proton appeared at weaker field at 6.12 ppm because of the double shielding effect of the neighboring electronegative O and N atoms. The protons of the pyridine and piperidine rings appeared in the characteristic spectral region. Methine proton H_5 appeared as a triplet not in the characteristic region (~3.00 ppm) but at stronger field of 2.71 ppm.

It was interesting to study the molecular structure of acetylated glucosylthiourea cytisine derivative **3** because we performed earlier an x-ray crystal structure analysis of *N*-(*β*-D-galactopyranosyl)cytisine and established the absolute configuration of this unacetylated glycoside analog [13]. Figure 1 shows the molecular structure of **3**.

It was found that the bond lengths and angles in **3** were close to the standard values [14]. The cytisine framework was rather rigid and practically unchanged. This was observed in previously studied molecules [15–17]. The dihydropyridine ring was planar within ±0.008 Å. Carbonyl atom O1 lay almost in the plane of the other atoms, deviating by 0.02 Å. The tetrahydropyridine ring N1C6C7C8C9C10 adopted an ideal boat conformation (ΔC_S^8 = 5.31 Å) with bridging atom C8

deviating from the average plane of the other atoms by 0.73 Å (I). The piperidine ring had the chair conformation (ΔC_S^{11} = 2.99 Å). The pyranose ring adopted an ideal chair conformation $^{3'}C_{O2'}$ ($\Delta C_S^{C3'}$ = 1.29 Å). The molecule studied earlier by us, *N*-(β-D-glucopyranosyl)cytisine, adopted the same conformation in the more stable β-anomeric configuration as indicated by the *trans*-axial disposition of the glycoside C1′ and pyranose ring C2′ protons. Here the C1′ proton had the β-orientation because of the sterically favorable axial disposition relative to the bulky C2′ substituent and the N2 proton. The acetyl groups on C2′, C3′, C4′, and C5′ were oriented equatorially relative to the pyranose ring. An intermolecular H-bond was observed through a water of hydration O9-O1W (x,y,z)…N2(–1+x,y,z) (O9…H–O1W 2.02 Å, O1W…H–N2 2.07 Å). This formed an infinite chain along the *a* axis.

EXPERIMENTAL

PMR spectra were recorded in DMSO-d_6 with TMS internal standard on a Bruker DRX 500 spectrometer at 500 MHz operating frequency. Mass spectra were recorded on a Finnigan Mat.Incos 50 instrument by direct sample introduction at 70 eV ionizing-electron energy. TLC was carried out on Sorbfil plates using 2-propanol:benzene:NH_4OH (25%) (10:5:2) with detection by I_2 vapor.

X-ray Crystal Structure Analysis (XSA). A colorless prismatic crystal (0.20 × 0.30 × 0.45 mm) that was obtained by natural evaporation of a saturated EtOH solution of **3** was selected for the XSA. The unit-cell constants and intensities of 5343 independent reflections of **3** were measured on an Xcalibur CCD diffractometer (Oxford Diffraction) using Cu K_α-radiation (λ 1.5418 Å) from an Enhance X-ray Source sharp-focus (Cu) tube and a graphite monochromator at room temperature (θ/2θ-scanning, 2θ < 75°). The crystals were orthorhombic, a = 9.4542(4), b = 15.9815(7), c = 19.5170(6), V = 2948.9(2) Å^3, d_{calc} = 1.346 g/cm^3, Z = 4 ($C_{26}H_{35}N_3O_{11}S$), space group $P2_12_12_1$. The experimental dataset was collected using the CrysAlisPro program [18]. Integrated intensities were measured by ω-scanning with monochromatic radiation reflected from the graphite crystal. Equivalent reflections were averaged and weak ones were removed [$I < 2\sigma(I)$] to produce a working dataset of 4070 reflections. Absorption corrections were applied by the multi-scan method in the CrysAlisPro program set. A total of 4070 reflections with $I > 2\sigma(I)$ was used in the calculations. The structure was solved by direct methods and refined by anisotropic full-matrix least-squares methods for nonhydrogen atoms using the SHELXS-97 program set. H atoms were found geometrically and fixed in a rider model. The final agreement factors were R = 0.0478 and wR_2 = 0.1299. The structure was refined using the SHELXL-97 program [19]. Data for the XSA were deposited in the Cambridge Crystallographic Data Centre (CCDC 783967).

***N*-Cytisino-*N′*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiocarbamide (3).** A solution of **1** (2.05 g, 5 mmol) in *o*-xylene (10 mL) was treated with Pb(II) thiocyanate (2.26 g, 7 mmol), stirred vigorously, and heated to about 145°C for 8 h. The resulting solution of **2** was filtered through filter paper to remove precipitated inorganic salts that were washed twice with anhydrous benzene (5 mL each). The combined solutions were added dropwise with stirring to a solution of cytisine (0.86 g, 4.5 mmol) in benzene (10 mL). The mixture was stirred at 30°C for 5 h. Then the solvent was evaporated. The solid was ground with hexane to afford a yellow finely crystalline product. Yield 1.42 g (93%) of crude product. Several recrystallizations from hexane:benzene (2:1 and 1:1) produced white transparent crystals, mp 133–135°C. Elemental analysis of **3** agreed with that calculated, $C_{26}H_{33}N_3O_{10}S$. Mass spectrum (EI, 70 eV, m/z, I_{rel}, %): 331 (11.3), 169 (48), 146 (25), 109 (43), 43(100).

PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.87 (1H, d, J = 12.6, H-8a), 1.98 (1H, d, J = 12.6, H-8b), 1.94 [3H, s, $CH_2OC(O)C\underline{H}_3$], 1.97 [9H, s, 3CHOC(O)C$\underline{H}_3$], 2.502 (1H, br.d, H-9), 3.166 (1H, dd, $J_{11a,11e}$ = 12.5, $J_{11a,9}$ = 2.0, H-11a), 3.178 (1H, br.d, H-7), 3.223 (1H, br.d, $J_{13a,13e}$ = 11.4, H-13a), 3.655 (2H, br.d, J = 3.4, H-10), 3.894 (1H, d, $J_{6'b,6'a}$ = 11.3, H-6′b), 3.898 (1H, m, H-5′), 4.175 (1H, dd, $J_{6'a,5'}$ = 4.9, $J_{6'a,6'b}$ = 12.4, H-6′a), 4.376 (1H, br.d, J = 12.4, H-13e), 4.857 (1H, t, J = 9.7, H-4′), 5.060 (1H, t, J = 9.4, H-2′), 5.235 (1H, br.d, J = 12.4, H-11e), 5.278 (1H, t, J = 9.56, H-3′), 5.813 (1H, t, J = 9.1, H-1′), 6.153 (1H, dd, $J_{5,4}$ = 6.9, $J_{5,3}$ = 1.3, H-5), 6.166 (1H, dd, $J_{3,4}$ = 9.0, $J_{3,5}$ = 1.3, H-3), 7.280 (1H, dd, $J_{4,5}$ = 6.88, $J_{4,3}$ = 9.01, H-4), 8.121 (1H, d, J = 9.03, N–H).

***N*-Anabasino-*N′*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiocarbamide (4)** was prepared analogously to **3** from **1** (1.32 g, 3.2 mmol), Pb(II) thiocyanate (1.61 g, 5 mmol), and anabasine (0.48 g, 3 mmol). Yield of crude product 1.44 g (87%). Several recrystallizations from hexane:benzene (2:1 and 1:1) afforded white transparent crystals, mp 154–155°C. Elemental analysis of **4** agreed with that calculated, $C_{25}H_{33}N_3O_9S$. Mass spectrum (EI, 70 eV, m/z, I_{rel}, %): 552 (0.5) $[M]^+$, 331 (18), 205 (16), 169 (95), 161 (56), 133 (35), 127 (34), 109 (87), 106 (39), 105 (45), 84 (83), 44 (50), 43 (100).

PMR spectrum (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.35–1.80 (6H, m, H-6, H-7, H-8), 1.96–2.00 [12H, 4s, 4C(O)C$\underline{H}_3$], 2.71 (1H, t, $J_{5,6}$ = 12.04, H-5), 2.47 (1H, br.d, H-9a), 4.03 (2H, m, H-6′), 4.24 (1H, dd, J = 4.48, 12.42, H-5′), 4.26 (1H, br.d, H-9e), 4.91 (1H, t, J = 9.84, H-4′), 5.14 (1H, t, J = 9.31, H-2′), 5.37 (1H, t, J = 9.58, H-3′), 6.12 (1H, t, J = 9.00, H-1′), 7.40 (1H, dd, $J_{2,1}$ = 4.75, $J_{2,3}$ = 7.96, H-2), 7.53 (1H, d, $J_{3,2}$ = 7.97, H-3), 8.33 (1H, s, H-4), 8.44 (1H, d, J = 8.9, N–H), 8.46 (1H, d, $J_{1,2}$ = 4.60, H-1).

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