Chemistry of Heterocyclic Compounds, Vol. 46, No. 2, 2010

SYNTHESIS OF N-AMINOGLYCOSIDES DERIVED FROM ALKALOID CYTISINE, THEIR BIOLOGICAL ACTIVITY AND CRYSTAL STRUCTURE OF N-(β-D-GALACTOPYRANOSYL)CYTISINE

I. V. Kulakov[1]*, O. A. Nurkenov[1], D. M. Turdybekov[2], A. S. Mahmutova[2], S. B. Ahmetova[2], R. B. Sejdahmetova[2], and K. M. Turdybekov[2]

Optimal conditions were found for the synthesis of a number of new N-aminoglycosides of the alkaloid cytisine, using commercially available monosaccharides, namely, D-glucose, D-galactose, D-xylose, and L-arabinose. X-ray structural analysis indicated an absolute β-anomeric configuration of N-(β-D-galactopyranosyl)cytisine in the crystalline state. The comparative cytotoxicity of cytisine and some of its N-aminoglycosides was determined.

Keywords: alkaloid cytisine, N-aminoglycosides, N-(β-D-galactopyranosyl)cytisine, monosaccharides of D-glucose, D-galactose, D-xylose, and L-arabinose, X-ray structural analysis, cytotoxic activity.

N-Glycosylation of many amino compounds, including heterocyclic and natural physiologically active compounds is considered a new approach for creating promising and efficient drugs due to the active transport of the carbohydrate fragments [1-3]. The introduction of carbohydrate fragments into the structure of physiologically active compounds not only improves their water solubility but also significantly reduces toxicity, which leads us to recommend the glycosylation of physiologically active compounds (or their individual fragments) at the glycoside site of sugars as a possible pathway for the preparation of drugs with low toxicity [4].

1-Glycopyranosylamines **1-4** were prepared by the condensation of D-galactose, D-glucose, D-xylose, and L-arabinose with alkaloid cytisine in a small amount of absolute ethanol [5].

The condensation and, most importantly, the subsequent separation of the desired products is significantly facilitated by using absolute ethanol since the synthesized glycosides dissolve well in water and even small amounts of water hinder crystallization. We also found that the initial use of catalytic amounts of acetic acid significantly affects the rate of aminoglycoside formation but markedly lowers the yields and hinders separation of the final reaction products. Hence, we subsequently did not use acetic acid as a catalyst.

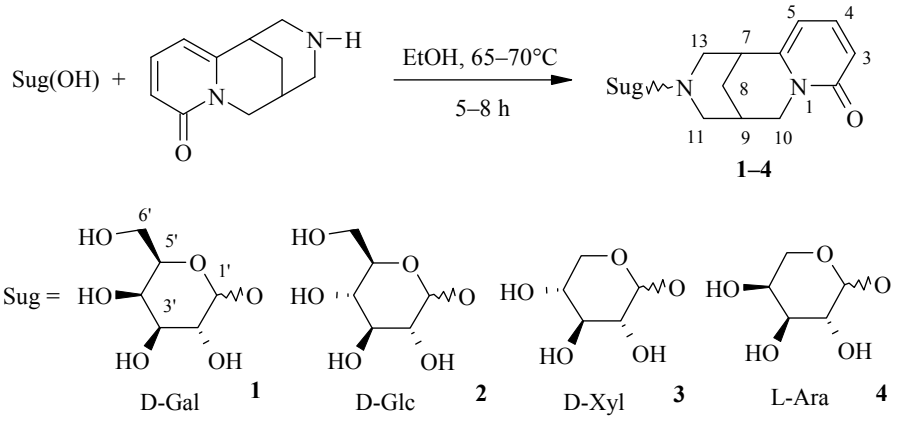
* To whom correspondence should be addressed, e-mail: kulakov_iv@mail.ru.

[1]Institute of Organic Synthesis and Coal Chemistry of the Republic of Kazakhstan, Karaganda 100008, Kazakhstan.

[2]Science and Production Center "Phytochemistry", Ministry of Education and Science of the Republic of Kazakhstan, Karaganda 100009, Kazakhstan.

Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 2, pp. 300-305, February, 2010. Original article submitted June 5, 2009.

The N-cytisinylglycosides may be extremely promising substitutes for pharmaceuticals already in use derived from alkaloid cytisine (respiratory analeptic *cytiton* and *lobesil*, which is an anti-smoking agent) since these derivatives will undoubtedly have much less toxicity and a longer duration due to their gradual hydrolysis in the body.



The conformation of the cytisine aglycone at the glycoside atom C(1) may be established unequivocally relative to the position of the ^{1}H NMR signal of the anomeric proton. A downfield signal is characteristic for the α-anomer at ~4.5-5.5 ppm with a small coupling constant (2.5-2.0 Hz) [1]. For β-anomers, the signal for the *trans*-axial proton is seen at higher field with $J \sim 6.0$-10.0 Hz. Analysis of the ^{1}H NMR spectra of the N-cytisinylglycosides synthesized showed that despite the bulky cytisine moiety and stability of the β-anomers, glycosides **1-4** in DMSO solution exist as 1:1 mixtures of the α- and β-anomers, as indicated by the corresponding integral intensities and specific location of the doublets for the anomeric proton, for example, in the case of derivative **1**, at 4.08 ppm for H(1'β) with $J = 8.8$ and at 4.25 ppm for H(1'α) with $J = 4.5$ Hz.

We found this result somewhat unexpected since many N-aminoglycosides derived from aniline, 2-aminopyridine, and the alkaloid *d*-pseudoephedrine, previously synthesized in our laboratories, even under conditions of possible mutarotation in DMSO solution exist in the more stable and energetically-favored β-form [6-8]. In order to determine the absolute configuration of the N-cytisinylglycosides synthesized, we attempted to grow crystals necessary for an X-ray structural analysis. Of all the N-cytisinylglycosides synthesized, crystals most suitable for analysis were obtained for N-(D-galactopyranosyl)cytisine (**1**) upon repeated recrystallization from 1:1 ethanol–2-propanol and subsequent natural evaporation. Transparent needles with a blue tinge were isolated.

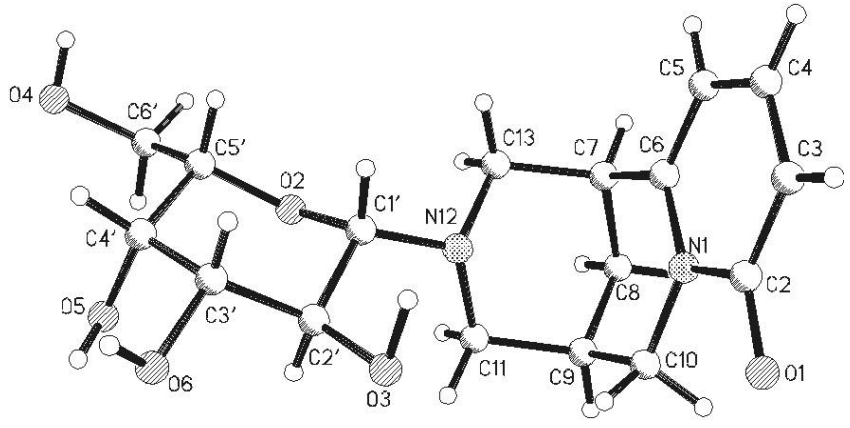


Fig. 1. Molecular structure of N-(β-D-galactopyranosyl)cytisine (**1**).

We then carried out an X-ray structural analysis of derivative **1** (Fig. 1).

The bond lengths and angles of this compound were found to be close to standard values [9]. The cytisine unit is rather rigid and virtually unaltered, which has been in previously studied molecules [10-12]. The dihidropyridine ring is planar to within ±0.009 Å. The carbonyl atom O(1) lies virtually in the plane of the other molecules with deviation of 0.06 Å. The tetrahydropyridine ring N(1)C(6)C(7)C(8)C(9)C(10) has *ideal sofa* conformation (ΔC_s^8 = 1.01 Å) with extrusion of the bridging atom C(8) from the mean plane of the other ring atoms by 0.73 Å(I). The piperidine ring is in *almost ideal chair* conformation ${}^{1'}C_{4'}$ ($\Delta C_s^{C1'}$ = 3.0 Å). Analysis of the three-dimensional structure of derivative **1** unequivocally showed that in the crystal, N-(β-D-galacto-pyranosyl)cytisine (**1**) exists in the more stable β-anomeric configuration, as indicated by the *trans*-axial arrangement of glycoside proton H(1') and pyranosyl ring proton H(2').

Since, as already noted, ^{1}H NMR spectroscopy shows that derivative **1** exists in DMSO solution in an equilibrium between the α- and β-anomeric forms, we carried out MINDO/3, MNDO, and AM1 quantum-chemical calculations (from the MOPAC version 6.0 program package) for a possible determination of the configuration of the anomeric hydrogen atom H(1') and H(2'). The calculation results indicate favored *cis* orientation of these atoms. The difference in enthalpies between the α- and β-configurations is 2 kcal/mole in favor of the α-form. Thus, derivative **1** may take the α-anomeric configuration upon crystallization. Stepanenko [1] has noted that a definite equilibrium of both exists for carbohydrates and aminoglycosides in solution in polar solvents. The energies of interaction of the protons and hydroxyl groups at C(1') and C(2') in D-galactose calculated according to Reeves [13] showed that the β-anomeric configuration should be favored due to the sterically-advantageous equatorial arrangement of the substituent at C(1'), which is actually noted in the molecular structure of derivative **1**.

In order to determine the comparative toxicity of some of the N-cytisinylglycosides synthesized and of the starting alkaloid cytisine, we tested samples of N-(β-L-arabinopyranosyl)cytisine (**4**) and N-(β-D-gluco-pyranosyl)cytisine (**2**) relative to brine shrimp larvae *Artemia salina* (*Leach*) for cytotoxic activity under *in vivo* cultivation conditions. The cytotoxic activity testing results for half toxic dose LD_{50}, μg/ml are as follows: 189.36 for N-(β-L-arabinopyranosyl)cytisine, 172.55 for N-(β-D-glucopyranosyl)cytisine, and 84.56 for cytisine alkaloid.

In summation, we have found that N-(β-L-arabinopyranosyl)cytisine and N-(β-D-glucopyranosyl)-cytisine display weak cytotoxic activity relative to brine shrimp larvae *Artemia salina* (*Leach*) and are less than half as cytotoxic as the reference sample, namely, cytisine, which displays moderate cytotoxic activity.

EXPERIMENTAL

The IR spectra were taken on an Avatar-320 Fourier transform spectrometer for KBr pellets. The ^{1}H NMR spectra were taken on a Bruker DRX500 spectrometer at 500 MHz in DMSO-d_6 with TMS as the internal standard. The reaction course and purity of the products were monitored by thin-layer chromatography on Sorbfil plates using 10:5:2 2-propanol–benzene–25% ammonium hydroxide as the eluent with development by iodine vapor.

X-ray Structural Analysis. The unit cell parameters and intensities of 1731 independent reflections of derivative **1** were measured at 20°C on a Bruker P4 automatic four-circle diffractometer with a graphite monochromator using MoKα radiation, θ/2θ scanning, 2θ < 52.7°. The unit cell parameters of the orthorhombic crystals are as follows: a = 7.158, b = 10.753, c = 21.855 Å, V = 1682.2 Å^3, d_{calc} = 1.391 g/cm^3, Z = 4 ($C_{17}H_{24}N_2O_6$). Space group $P2(1)2(1)2(1)$.

A total of 1035 reflections with intensity $I > 2\sigma(I)$ were used in the calculations. The structure was solved by the direct method using the Sir-2002 program [14] and refined by the full-matrix method of least squares anisotropically for the non-hydrogen atoms. The hydrogen atoms were given geometrically and

determined by the "horse and rider" model except for the hydroxyl group hydrogens, which were found from the electron density difference map. The final deviation factors $R = 0.0614$ and $wR_2 = 0.1337$. The refinement of the geometry was carried out using the SHEXL-97 program [15]. The data on the geometrical structure of **1** were deposited at the Cambridge Crystallographic Data Center (CCDC 692503).

N-(β-D-Galactopyranosyl)cytisine (1). Cytisine (1.90 g, 0.01 mol) was added with stirring to a solution of D-galactose (1.80 g, 0.01 mol) in absolute methanol (15 ml) and stirring was continued for 8 h at 65-70°C. Intensive cooling to -10°C gave a white crystalline precipitate, which was filtered off and washed with acetone to give 2.62 g (74%) compound **1**; mp 188-189°C (2-propanol–ethanol). IR spectrum, ν, cm^{-1}: 3410 (OH), 1642 (N–C=O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.75 (2H, m, H-8); 2.40 (1H, br. d, H-9); 2.65 (2H, m, H-11); 2.80 (2H, m, H-2'); 3.02 (2H, m, H-13); 3.09 (1H, br. d, H-7); 3.20 (1H, m, H-4'); 3.39 (1H, m, H-5'); 3.48 (2H, m, H-6'); 3.72 (1H, m, H-3'); 3.80 (1H, m, H-10*a*); 3.85 (1H, d, $J_{10a,10e} = 15.5$, H-10*e*); 4.08 (1H, d, $J_{1',2'} = 8.8$, H-1'β); 4.25 (1H, d, $J_{1',2'} = 4.5$, H-1'α); 6.06 (1H, d, $J_{5,4} = 6.8$, H-5); 6.18 (1H, d, $J_{3,4} = 9.0$, H-3); 7.30 (1H, dd, $J_{4,5} = 6.8$, $J_{4,3} = 9.0$, H-4). Found, %: C 58.29; H 6.51; N 8.18. $C_{17}H_{24}N_2O_6$. Calculated, %: C 57.94; H 6.86; N 7.95.

N-(β-D-Glucopyranosyl)cytisine (2) was obtained analogously to compound **1** but with reaction time 5 h. The separation and crystallization of compound **2** was carried out after drying the crude product for 72 h in a desiccator over P_2O_5. The residue was crystallized from acetonitrile to give compound **2** in 90% yield; mp 183-184°C. IR spectrum, ν, cm^{-1}: 3406 (OH), 1648 (N–C=O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.75 (2H, m, H-8); 2.36 (1H, br. s, H-9); 2.65 (2H, m, H-11); 2.95 (2H, m, H-4', H-2'); 3.02 (2H, m, H-13); 3.06 (1H, m, H-7); 3.14 (2H, m, H-6'); 3.38 (1H, m, H-5'); 3.51 (1H, d, $J_{1',2'} = 9.0$, H-1'β); 3.62 (1H, dd, $J_{3',2'} = 6.0$, $J_{3',4'} = 5.8$, H-3'); 3.72 (1H, dd, $J_{10a,10e} = 15.0$, $J_{10a,9} = 7.2$, H-10*a*); 3.85 (1H, d, $J_{10a,10e} = 15.0$, H-10*e*); 3.98 (1H, d, $J_{1',2'} = 5.1$, H-1'α); 6.07 (1H, d, $J_{5,4} = 6.8$, H-5); 6.18 (1H, d, $J_{3,4} = 9.0$, H-3); 7.30 (1H, dd, $J_{4,5} = 6.8$, $J_{4,3} = 9.0$, H-4). Found, %: C 58.12; H 6.47; N 8.24. $C_{17}H_{24}N_2O_6$. Calculated, %: C 57.94; H 6.86; N 7.95.

N-(β-D-Xylopyranosyl)cytisine (3) was obtained in 77% yield analogously to derivative **2**; mp 162-164°C. IR spectrum, ν, cm^{-1}: 3390 (OH), 1642 (N–C=O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.73 (2H, m, H-8); 2.35 (1H, br. s, H-9); 2.55 (2H, m, H-11); 2.72 (1H, br. d, H-7); 2.89 (1H, t, $J_{2,3} = 10.9$, H-2'); 3.02 (3H, m, H-4', H-13); 3.16 (2H, m, H-5'); 3.46 (1H, d, $J_{1',2'} = 9.1$, H-1'β); 3.64 (1H, dd, ($J_{3',2'} = 5.5$, $J_{3',4'} = 5.4$, H-3'); 3.71 (1H, dd, $J_{10a,10e} = 15.3$, $J_{10a,9} = 6.9$, H-10*a*); 3.84 (1H, d, $J_{10a,10e} = 15.3$, H-10*e*); 3.93 (1H, br. s, H-1'α); 6.04 (1H, d, $J_{5,4} = 6.8$, H-5); 6.17 (1H, d, $J_{3,4} = 8.9$, H-3); 7.30 (1H, dd, $J_{4,5} = 6.8$, $J_{4,3} = 8.9$, H-4). Found, %: C 59.92; H, 6.60; N 9.04. $C_{16}H_{27}N_2O_3$. Calculated, %: C 59.61; H 6.88; N 8.69.

N-(β-L-Arabinopyranosyl)cytisine (4) was obtained in 62% yield analogously to derivative **2**; mp 175-177°C. IR spectrum, ν, cm^{-1}: 3390 (OH), 1646 (N–C=O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.75 (2H, m, H-8); 2.40 (1H, br. d, H-9); 2.66 (2H, m, H-11); 2.80 (1H, t, $J_{2',3'} = 9.7$, H-2'); 3.04 (2H, m, H-13); 3.21 (1H, m, H-7); 3.30 (1H, m, H-4'); 3.50 (2H, m, H-5'); 3.63 (1H, d, $J_{1',2'} = 7.2$, H-1'β); 3.73 (1H, m, H-3'); 3.81 (1H, dd, $J_{10a,10e} = 15.2$, $J_{10a,9} = 6.4$, H-10*a*); 3.85 (1H, d, $J_{10a,10e} = 15.2$, H-10*e*); 4.05 (1H, d, $J_{1',2'} = 5.6$, H-1'α); 6.07 (1H, d, $J_{5,4} = 6.7$, H-5); 6.18 (1H, d, $J_{3,4} = 8.5$, H-3); 7.31 (1H, dd, $J_{4,5} = 6.7$, $J_{4,3} = 8.5$, H-4). Found, %: C 60.02; H 7.19; N 9.11. $C_{16}H_{22}N_2O_5$. Calculated, %: C 59.61; H 6.88; N, 8.69.

Biological testing. The cytotoxicity of N-(β-L-arabinopyranosyl)cytisine, N-(β-D-glucopyranosyl)-cytisine, and cytisine were tested for the survival of two-day-old brine shrimp larvae *Artemia saline (Leach)* cultivated *in vitro*. The larvae were grown by immersion of brine shrimp eggs in artificial seawater and incubation for 48 h at 37°C. A sample of each sample studied was dissolved in 2 ml methanol and, then, 500 (three parallels), 50 (three parallels), and 5 μl (three parallels) were taken of this solution. After evaporation of methanol, 5 ml artificial seawater was added to each flask. Thus, if the initial sample mass was 2 mg, the final sample concentration would be 100, 10, and 1 μg/ml, respectively, of each concentration in three repetitions. Then, 10 two-day-old brine shrimp larvae *Artemia salina (Leach)* were introduced into each flask with sample using a Pasteur pipette. Then, all the flasks were left at room temperature in the light for 24 h. At the end of 24 h, the surviving and dead larvae were counted. Thus, the half toxic dose of each sample was calculated using the data obtained for the upper and lower toxic limit.

REFERENCES

1. B. N. Stepanenko, *Chemistry and Biochemistry of Carbohydrates (Monosaccharides)* [in Russian], Vysshaya Shkola, Moscow (1977).
2. L. S. Krasavina, R. P. Turchina, M. M. Vigdorchik, K. F. Turchin, R. N. Akhvlediani, and N. N. Suvorov, *Zh. Org. Khim.*, **24**, 1960 (1988).
3. A. M. Gazaliev, O. A. Nurkenov, I. V. Kulakov, A. A. Ainabaev, and D. V. Bessonov, *Zh. Prikl. Khim.*, **79**, 517 (2006).
4. R. K. Sarymzakova, Yu. A. Abdurashitova, and Zh. A. Dzhamanbaev, *Vestn. Moskovsk. Gos. Univ.*, *Ser. 2, Khim.*, **47**, 242 (2006).
5. I. V. Kulakov, *Khim. Prirod. Soed.*, **44**, 596 (2008).
6. I. V. Kulakov, A. I. Il'in, Zh. A. Kabyl, and A. M. Gazaliev, *Izv. Akad. Nauk, Ser. Khim.*, 2393 (2008).
7. D. V. Bessonov, I. V. Kulakov, A. M. Gazaliev, and O. A. Nurkenov, *Zh. Prikl. Khim.*, **80**, 510 (2007).
8. I. V. Kulakov, *Zh. Obshch. Khim.*, **79**, 147 (2009).
9. F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen, and R. Taylor, *J. Chem. Soc., Perkin Trans. 2*, No. 12, S1 (1987).
10. A. A. Freer, D. J. Robins, and G. N. Sheldrake, *Acta Crystallogr.*, **C43**, 1119 (1987).
11. A. M. Gazaliev, K. M. Turdybekov, S. V. Lindeman, Yu. T. Struchkov, B. I. Tuleuov, and M. Zh. Zhurinov, *Zh. Obshch. Khim.*, **62**, 456 (1992).
12. O. A. Nurkenov, A. M. Gazaliev, A. B. Shalbaeva, K. M. Turdybekov, M. Zh. Zhurinov, and A. Aubakirova, *Zh. Obshch. Khim.*, **69**, 675 (1999).
13. R. E. Reeves, in: C. S. Hudson and S. M. Cantor (editors), *Advances in Carbohydrate Chemistry*, vol. 6 (1951), p. 107.
14. M. C. Burla, M. Camalli, B. Carrozzini, G. L. Cascarano, G. Giacovazzo, G. Polidori, and R. Spagna, "SIR2002: The Program," *J. Appl. Crystallogr.*, **36**, 1003, Pt. 4 (2003).
15. G. M. Sheldrick, SHELXL-97—Crystal Structure Refinement, dos/win95/nt version + 1993-1997, Release 97-2.