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N-Lactosylation of Amino Benzoic Acids

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ABSTRACT. The N-lactosylation of isomeric amino benzoic acids by D-lactose is studied. N-m-Carboxyphenyl-β-D-lactosyl amine and N-p-Carboxyphenyl-β-D-lactosyl amine are synthesized and characterized. *© 2012 Bull. Georg. Natl. Acad. Sci.*

***Key words**: N-lactosylation, amino benzoic acids, D-lactose.*

O-, m-, and p-amino benzoic acids carry out important physiological functions. P-amino benzoic acid is vitamin for many microorganisms, and the necessary precursor for biosynthesis of folic acid. P-amino benzoic acid is applied to treatment of such diseases, as scleroderma [1], Personae's disease [2]. As a component, it is widely used in protective anti-sun creams [3]. All three isomers of amino benzoic acids are antimut agens [4]. Such derivatives of amino benzoic acids which, after penetration into the action area, purposefully decompose with liberation of an active ingredient are successfully applied. [5]. It is necessary to note that such derivatives of amino benzoic acids frequently cause specific physiological effects which considerably differ from the physiological effects of initial amino benzoic acids [6], and for this reason, researchers carry out intensive investigations with the purpose of revealing such derivatives. One of the ways of synthesis of such derivatives is N-glycosylation of amino benzoic acids.

One of the prospective ways of preparation of N-glycosides of amino benzoic acids is direct interaction of aldoses and amino benzoic acids in aqueous or alcoholic or aqueous-alcoholic solutions, in cold or at heating, in the presence of the catalyst (the base or acid) or without it. This way of synthesis is intensively investigated, and so has not developed into a preparative method [7]. The basic barrier in the process of this synthesis is the accompanying melanoidin reaction, as a result of which N-glycosides, formed by N-glycosylation of amino benzoic acids, are transformed into a mix of melanoidine products. N-glycosylation of amino benzoic acids by reducing disaccharides is investigated only for maltose [8]; In this work we investigated the reaction of N-lactosylation of amino benzoic acids.

The reaction between o-, m-, p-amino benzoic acids and D-lactose was carried out in 96% ethanol medium, under the reflux, in the presence of small quantities of water and catalyst (glacial acetic acid).

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Table 1. Formation of N-lactosides by reaction of D-lactose with o-, m-, p-amino benzoic acids

No.	N-Lactosyl amine	Yield, (%)	M. P., ^{o}C
I	N-o-Carboxyphenyl-D-lactosyl amine	0	—
II	N-m-Carboxyphenyl-β-D-lactosyl amine	30.0	138-140
III	N-p-Carboxyphenyl-β-D-lactosyl amine	50.0	105-106

The synthesized N-lactosides were purified by means of recrystallization (ethanol, diethyl ether), and their purity was checked by the method of TLC and paper chromatography. The identification of synthesized N-lactosides was carried out by a method of elementary analysis, by infrared spectra (UR-20, in KBr), ^{13}C-nuclear magnetic resonance spectra (Bruker NM-250 MGH, standard $(CD_3)_2SO$), and melting points. The results are shown in Table 1.

The data of Table 1 show that from m- and ï-isomers of amino benzoic acid the corresponding N-lactosides are formed; however, despite the change of the key parameters of reaction, we did not manage to obtain the desirable N-lactoside from o-amino benzoic acid. It is known that the process of N-glycosylation is significantly influenced by the basic nature of the reacting amine, and those factors which define the stability of sugar conformation, the more the basicity of the amine, the more actively it participates in the reaction of N-glycosylation; however subsequent transformations of the formed N-glycosides also proceed actively (Amadori rearrangement, Maillard reaction, deamination-decarboxylation, etc [9]).

By interaction of lactose with p-amino benzoic acid, the carboxylic group - because of its negative inductive and negative mesomeric effects - reduces the density of the electron cloud of the nitrogen atom, thereby reducing its basic nature. In this case, the N-lactoside of p-amino benzoic acid, because of its high stability, is formed with higher yield than the N-lactoside of m-amino benzoic acid. At reaction of the o-amino benzoic acid with D-lactose it is not possible to isolate the desirable product – appropriate lactoside; it is possible to assume that because of spatial effects, reaction between them does not occur. The most specific reaction typical of all oligosaccharides is their hydrolysis with cleavage of glycoside bonds and formation of monosaccharides. The infra-red and ^{13}C PMR spectra of synthesized N-lactosides (II, III) show that in these compounds the 1→4 β-glycoside bond is preserved between A and B carbohydrate rings.

The following were characteristic regions of infra-red absorption spectra of synthesized N-carboxyphenyl-D-lactosyl amines: 3380-2600 cm^{-1} (valence vibrations of O-H bond of carbohydrate); 2850-2900 cm^{-1} (valence vibrations of C-H bonds of carbohydrate); 1490-1510 cm-1 (absorption of N-glycoside bond of N-lactosylamines); 1450-1200 cm^{-1} (deformation vibrations of C-H and C-O-H); 1130-1150 cm^{-1} (vibrations of carbohydrate ring: C-O, C-C, C-O-C); 1020-1100 cm^{-1} (valence vibrations of C_1-N bond af anomeric centre at C_1); 700-1000 cm^{-1} (vibrations of carbohydrate ring); 750-900 cm-1 (deformation vibrations of C_1-H bonds of anomeric centre at C_1); 900-930 and 740-770 cm^{-1} (absorption of cyclic pyranose forms of N-glycosylamines) [10].

The ^{13}C PMR spectra of compounds II and III are divided into three basic ranges: the carbon atoms of both carbohydrate rings A and B are located in the range of 60-100 ppm respectively. From them the shift in the weakest field at 60.47 ppm and 60.47 ppm assigns to carbon atoms 6 and 6 of A and B ring. Chemical shifts at 103.85 and 103.82 correspond to C1 carbon atoms of ring A, which through β-glycoside bond are linked to C4 carbon atoms of ring B [11, 12]. C1 atoms of a rings B, which by amino-glycoside bond are linked to a benzene ring, resonate at 84.82 and 83.68 ppm; according to our data the

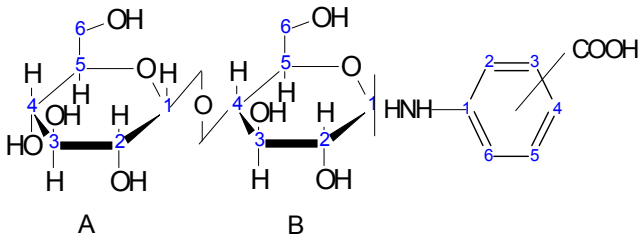

Fig. 1. Carboxyphenyl-D-lactosyl amines

Table 2. ^{13}**C PMR spectra of synthesized N-carboxyphenyl lactosylamines. Chemical shifts of C atoms, ppm.**

Number of C atom	Carbohydrate ring A	Carbohydrate ring B	Aromatic ring
N-m-carboxyphenyl-β-D-lactosylamine			
C1	103.82	84.82	147.29
C2	73.27	68.20	114.10
C3	75.83	75.56	131.34
C4	70.65	80.75	118.06
C5	75.28	72.79	128.93
C6	60.47	60.47	117.04
N-p-carboxyphenyl-β-D-lactosylamine			
C1	103.85	83.68	151.30
C2	73.26	68.20	112.27
C3	75.84	75.55	130.93
C4	70.61	80.73	118.68
C5	75.36	72.70	130.93
C6	60.46	60.46	112.27

appropriate glycoside bond has a β-configuration.

Synthesis of N-carboxyphenyl lactosylamines. A mixture of 3.42 g (0.01 M) of D-lactose, 1.47 g (0.01 M) of o-, m-, or p-amino benzoic acid, 15 ml of 96% ethanol, 0.5 ml of water, and 0.3 ml of a glacial acetic acid was heated up in a boiling water bath to full dissolution of initial products. The mixture cooled up to room temperature 50 ml of diethyl ether was added and after blending left for the night at room temperature. The precipitated crystals were filtered, ground with 96% ethanol, diethyl ether added to the mixture and after careful blending the precipitate was filtered. The purity of the synthesized N-carboxyphenyl lactosylamines was checked by TLC.

ორგანული ქიმია

ამინობენზოის მჟავების N-ლაქტოზილირება

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შესწავლილია იზომერული ამინობენზოის მჟავების N-ლაქტოზილირება D-ლაქტოზით. სინთეზირებულია და დახასიათებულია N-M-კარბოქსიფენილ-β-D-ლაქტოზილამინი და N-P კარბოქსიფენილ-β-D-ლაქტოზილამინი.

REFERENCES

1. *C.J. Zarafonetis, L. Dabich, J.J. Skovronski, et al.* (1998), Clin. Exp. Rheumatol., **6**: 261-268.
2. *C.C. Carson* (1997), Tech. Urol., **3**: 135-139.
3. *B.R. Premachandra* (1989), Biochem. Med. Metabol. Biol., **41**: 1-17.
4. *T. Gichner, G. Voutsinas, A. Patrineli, et al.* (1994), J. Mutat. Res., **309**: 201-210.
5. *M. Sittig* (1988), Pharmaceutical Manufacturing Encyclopedia. New Jersey, USA, 177.
6. *F. Staud, Z. Fendrich, J. Hartl, et al.* (1998), J. Drug Target, **5**: 207-213.
7 *R. Kublashvili (2003),* Chem. Natural Compounds, **39**: 586-588.
8. *R. Kublashvili, M. Labartkava, Sh. Samsoniya* (2004), Bull. Georgian Acad. Sci., **169**: 306-308.
9. *R. Ikan* (1996), The Maillard Reaction. Chichester, p. 7-29.
10. *N.I. Kaletina* (1988), N-glikozilaminy i mikroelementy. Erevan, p.11-36 (in Russian)
11. *E.Breitmeier, C. Voelter* (1978), ^{13}C NMR Spectroscopy. New York, p. 183-189, 212-215, 248-263.
12. *E. Pretsch, I. Siebl, W. Siivon, T. Clerc* (1986), Tabellen zur Strukturaufklärung organischer Verbindungen mit spektroskopischen Methoden. Berlin, 125-130.

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