Chemistry of Natural Compounds, Vol. 48, No. 1, March, 2012 [Russian original No. 1, January-February, 2012]

ISOLATION OF CHONDROITIN-6-SULFATE FROM A MIXTURE WITH DERMATAN SULFATE SELECTIVELY OXIDIZED BY NaOCl–NaBr–2,2,6,6-TETRAMETHYLPIPERIDINE-1-OXYL

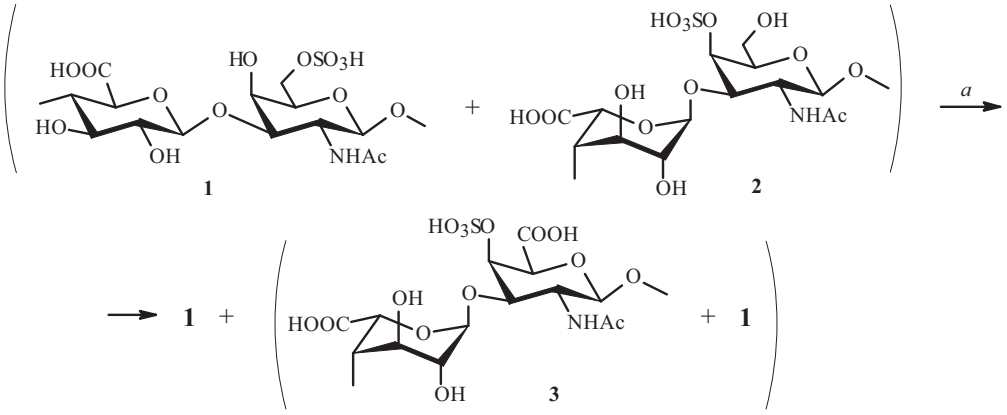
I. Yu. Ponedel′kina,* E. A. Khaibrakhmanova, and V. N. Odinokov

UDC 547.995.13

Chondroitin-6-sulfate (CS-6) is a linear polysaccharide of the acidic glycosaminoglycan (GAG) class and consists of repeating units of D-glucuronic acid and *N*-acetyl-D-galactosamine sulfated at the C6-OH group. CS-6 is found in connective tissues (cartilage, tendons, ligaments, spinal disks) as a mixture with other GAGs such as hyaluronic acid (HA), chondroitin-4-sulfate (CS-4), dermatan sulfate (DS), and heparin sulfates and/or heparin. Its tissue content is often less than those of the aforementioned GAGs [1, 2]. Depending on the type of biomaterial and purification method, CS-6 preparations contain various amounts of impurities of other chondroitin sulfates (CSs), most often CS-4, the predominant component of CS mixtures. An effective method for purifying CS-6 from CS-4 is an enzymatic method. An enzyme from the tail fin of tadpoles selectively cleaves CS-4 into low-molecular-weight fragments, from which CS-6 is readily separated [3].

CS-6 (**1**) is found in umbilical cords of newborns as a mixture with DS (**2**) in an ~2:1 ratio [4]. It can be released from most proteins by treatment with proteolytic enzymes and then isolated by fractional precipitation by alcohol as a proteoglycan complex [5]. The CS-6/DS mixture is extracted by base, which is most often used for preparative production of GAGs, and then easily purified of accompanying HA and proteins by anion-exchange column chromatography over DEAE-cellulose using aqueous NaCl solutions of various concentrations as eluents [4]. However, CS-6 is not separated from DS by this method because of the same charges on the molecules and the similarity of their molecular weights (average ~16.9 kDa whereas the molecular weights of natural CSs not treated with base are in the range 50–100 kDa [6]). By increasing the content of charged groups in DS using selective oxidation of the primary hydroxyls in the *N*-acetyl-4-sulfo-D-galactosamine (GalNAc4S) chain to carboxylic acids, it is expected that CS-6 and oxidized DS (carboxy-DS [7]) will be eluted from the chromatography column at different NaCl concentrations.

For this, the CS-6/DS mixture [(**1**)/(**2**) 2:1] from newborn umbilical cords was oxidized in aqueous base by NaOCl–NaBr–2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) (Scheme 1). We used this reagent previously to oxidize DS (from pig skin) to carboxy-DS (**3**) [7].



a. [CS-6/DS] = 13.6 μmol/mL (6.8 mg/mL); ratio of reagents (CS-6/DS):NaOCl:NaBr:TEMPO 1:4.4:0.2:0.01; H_2O (20 mL), pH 10.2, 1.5 h.

Scheme 1

Institute of Petrochemistry and Catalysis, Russian Academy of Sciences, 450075, Ufa, fax: (347) 284 27 50, e-mail: ink@anrb.ru, ponedelkina@rambler.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2012, pp. 102–103. Original article submitted June 14, 2011.

 0009-3130/12/4801-0112

The oxidized products were separated by column (150 × 50 mm) chromatography over DEAE-cellulose using NaCl solutions of increasing concentration (from 0.3 to 1.0 M in 0.05 steps). Effluents with 0.40–0.50 M NaCl contained **3**/**1** mixtures with various proportions of carboxy-DS and CS-6 (or their hybrids). Macromolecules with heterogeneous monosaccharide compositions are common among GAGs. In addition to GalNAc4S chains, DS from human umbilical cords contain, like CS-6, GalNAc6S chains. Such molecules are called hybrids [8]). Pure CS-6 (35% yield) was obtained unexpectedly from effluent with a higher NaCl concentration (0.55–0.60 M) than for the **3**/**1** mixture. Apparently DS was highly degraded by hypochlorite during the oxidation [9]. The charge density on carboxy-DS was reduced because of the decrease of molecular weight so that the product was eluted by NaCl solutions of lower ionic strength.

The purity and identity of the isolated **1** (sodium salt) was confirmed by PMR and ^{13}C NMR spectra [10, 11], which were missing resonances of both DS [4, 12] and carboxy-DS [7]. The specific rotation $\{[\alpha]_D^{20}$ –25° (*c* 0.33, $H_2O)\}$ and contents of S (5.73%), and D-glucuronic acid (38 mass%, determined by Dische's reaction [13]) agreed with the characteristic values for CS-6 [1].

Chondroitin-6-sulfate (1). PMR spectrum (400 MHz, D_2O, δ, ppm): 2.03 (s, MeCON), 3.31 (H-2, GlcA), 3.55–4.25 (H-2-6 GalNAc6S and H-3-6 GlcA), 4.45 (H-1, GlcA), 4.52 (H-1 GalNAc6S). ^{13}C NMR spectrum (100 MHz, D_2O, δ, ppm): 25.0 ($\underline{C}H_3CON$), 53.3 (C-2 GalNAc6S), 70.0 (C-4, C-6 GalNAc6S), 75.0 (C-2GlcA, C-5 GalNAc6S), 76.3 (C-3 GlcA), 78.8 (C-5 GlcA), 82.5 (C-3 GalNAc6S), 83.7 (C-4 GlcA), 103.8 (C-1 GalNAc6S), 106.7 (C-1 GlcA), 176.7 (COOH), 177.4 (MeCON).

REFERENCES

1. M. Stacey and S. A. Barker, *Carbohydrates of Living Tissues*, Van Nostrand, New York, 1962.
2. L.-C. F. Silva, *Adv. Pharmacol. (San Diego, CA, U.S.)*, **53**, 21 (2006).
3. J. E. Silbert and S. DeLuka, *J. Biol. Chem.*, **245**, 1506 (1970).
4. I. Yu. Ponedel′kina, V. N. Odinokov, E. S. Lukina, and U. M. Dzhemilev, *Bioorg. Khim.*, **34**, 704 (2008).
5. I. Danishefsky and G. Abraham, *Prep. Biochem.*, **1**, 133 (1971).
6. Summary of Data for Chemical Selection. Chondroitin Sulfate, available at the website: http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumpdf/chondroitin.pdf
7. I. Yu. Ponedel′kina, E. A. Khaibrakhmanova, V. N. Odinokov, L. M. Khalilov, and U. M. Dzhemilev, *Bioorg. Khim.*, **36**, 387 (2010).
8. L.-A. Fransson, *J. Biol. Chem.*, **243**, 1504 (1968).
9. B. Jiang, E. Drouet, M. Milas, and M. Rinaudo, *Carbohydr. Res.*, **327**, 455 (2000).
10. D. Welti, D. A. Rees, and E. J. Welsh, *Eur. J. Biochem.*, **94**, 505 (1979).
11. S. M. Bociek, A. H. Darke, D. Welti, and D. A. Rees, *Eur. J. Biochem.*, **109**, 447 (1980).
12. P. N. Sanderson, T. N. Huckerby, and I. A. Nieduszynski, *Biochem. J.*, **257**, 347 (1989).
13. T. Bitter and H. M. Muir, *Anal. Biochem.*, **4**, 330 (1962).