Studies on the Use of Purified CBH I for Oligosaccharide Synthesis

F.M. Gama and M. Mota*
Dept. Engenharia Biológica, Universidade do Minho, Campus de Gualtar, 4709 Braga Codex, Portugal

The importance of biologically active carbohydrates has been recognized over the last decade. The availability of cheap oligosaccharides for biological activity studies is very reduced. The isolation of these compounds from natural sources is almost impossible, because of their very high specific activity, and consequently very low concentration in nature. As chemical synthesis is a difficult and time consuming, the enzymatic synthesis has been regarded over the last years as a very attractive methodology for oligosaccharide production.

The main approach when utilizing glycanases for di- or trisaccharides synthesis has been the transglycosylation reaction. However, the isolation of products is quite complicated. On the other hand, the condensation reaction by reversed hydrolysis activity, which in many cases requires cheaper substrates, has a very low yield.

In this work, a purified exoglucanase CBH I from the fungus *Trichoderma reesei* was analyzed for its reversed hydrolysis activity. The enzyme was purified by conventional methodologies (preparative isoelectric focusing, gel filtration on Sephacryl 100 HR, anionic exchange on a Mono Q column and cationic exchange on a Mono S column), from a commercial cellulase, Cellulast, from Novo. The activity of the purified enzyme on a large set of substrates, such as lichenan, laminarin, filter paper, acid swollen Avicel, xylan and carboxymethylcellulose was characterized, suggesting that it is basically free of contaminant activities.

The enzyme was incubated in aqueous media with high sugar concentrations. Several mono- and disaccharides were used, in order to study the enzyme specificity. The obtained products were analyzed in a Dionex chromatographer using a CarboPac PA-100 column. The separated reaction products were analysed by NMR. The yields of the condensation reaction were in several cases considerably high.

Study of HMW Compounds in Sugar Using Gel Permeation Chromatography with an Avaporative Light Scattering Detector

Luis San Miguel Bento*, Susana Sá
RAR-Refinarias de Açúcar Reunidas, SA, Porto, Portugal

Raw sugars and other sugar process materials are studied by GPC (Gel Permeation Chromatography) using a Superose 12 column. As eluent was used a solution of 30% acetronitrile with 0.005 M ammonium acetate. As detector was used a spectrophotometric Diode Array Detector (DAD) and an Evaporative Light Scattering Detector, in series. By this arrangement both chromophoric and non chromophoric compounds are detected simultaneously. High sensitivity of both detectors allows a rapid detection of high molecular weight compounds without pre concentration of samples.

Synthesis and Reactions of Some Glycosidic Spiroacetal Derivatives of D-fructopyranose – Novel Quasi-di and Trisaccharides

H. Regeling, B. Zwanenburg and G.J.F. Chittenden*
Department of Organic Chemistry, NSR-Center for Molecular Structure, Design and Synthesis, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

In connection with our interests in fructosides, the synthesis of an intramolecular fructopyranosyl spiro-acetal from allyl-β-D-fructopyranoside, and some of its reactions will be described. Little is known about glycosidic acetals from carbohydrates and glycol aldehyde. These derivatives were used to synthesize novel quasi-di and tri-saccharide derivatives.

Synthesis of Glycosyl Phosphatidylinositol Anchors and Phosphatidyl-inositol-(3,4,5)-triphosphate

Peter Konradsson*, Daina Lezdins and Katinka Ruda
Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

myo-Inositol appears widely in Nature, most frequently as phosphorylated or phospholipid derivatives, but *O*-methyl and glycosyl inositols have also been identified and synthesized. The discovery that inositol derivatives containing phosphates, phospholipids, glycans or glycan bound proteins are involved or act as "second messengers" in various cell regulation systems, have dramatically increased the synthesis of these compounds. Most eukaryotic cells utilize glycosyl phosphatidylinositols (GPIs) to anchor proteins to the cell membrane. Partial structural data, accumulated for over 100 GPI membrane-anchored proteins from a variety of organisms, have led to the proposal of the generalized anchor structure Glycan-Manα-4GlcNH$_2$α-6D-*myo*-inositol 1-phosphate. Only three of these structures have thus far been fully characterized, the variant surface glycoprotein (VSG) from *Trypanosoma* and *Leishmania*, and the Thy-1 glycoprotein anchor from rat brain. We are now synthesizing parts of the *Leishmania* structure. Parts of the structures of *Trypanosoma*, *Leishmania* and the Thy-1 glycoprotein anchor, have already been synthesized by others.

Inositol-(4,5)-diphosphate is a well-known precursor for a Ca^{2+}-mobilized "second messenger" inositol-(1,4,5)-triphosphate (IP_3). The metabolism and biological function of IP_3 have been described in detail during the last decade. More recently a phosphatidyl-inositol-(3,4,5)-triphosphate (PIP_3) has been found. PIP_3 is believed to initiate actin polymerisation in neutrophiles, respiratory burst, protein synthesis, secretion and glucose metabolism.

Synthesis of Oligosaccharides of Biomedical Relevance

Per J. Garegg
Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S. 106 91 Stockholm, Sweden

Oligosaccharide structures, usually linked to proteins in glycoproteins and proteoglycans or to lipids in glycolipids participate in a large number of biological processes. These involve, *inter alia*, bacterial and viral recognition of specific cell structures as well as immune system recognition of invasive organisms. They are also involved in autoimmune processes. The enormous structural variability possible in oligosaccharides structures is the probable reason for Nature using them for the purpose of molecular recognition.

Advances in biology and in structural carbohydrate chemistry and emerging knowledge of structure-activity relationships have made necessary access to these structures and to analogues of them. Considerable progress has been made in the last few years in the chemical synthesis of oligosaccharides and glycoconjugates. Examples will be given of ongoing research in methodology as well as of recent syntheses of oligosaccharides of biomedical relevance.

Synthesis of Potential Inhibitors of Carbohydrate Processing Enzymes

Francesco Nicotra
Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, CNR Centro di Studio sulle Sostanze Organiche Naturali, Via Venezian 21, 20133 Milano, Italy

The interest in "glycomimetics" is growing with the knowledge of the multifarious roles that carbohydrates play in the biological events. It is now well proved that glycoconjugates are the main structures responsible for cell–cell and cell–molecule recognition events and host-pathogen interactions. Glycomimetics can, in principle, replace the normal substrate in interactions with receptors and active sites of enzymes, so inhibiting these processes.

We have developed innovative procedures for the synthesis of different glycomimetics of biological relevance. Among them we have synthesized isosteric phosponono analogues of some glycosyl phosphates, looking for inhibitors of glycosyltransferases. The phosphono analogues of α-L-rhamnose 1-phosphate, N-acetyl-α-D-glucosamine 1-phosphate, N-acetyl-α-D-mannosamine 1-phosphate and its GDP-derivative, have been stereoselectively synthesized. Furthermore, C-glycosidic analogues of glycosyl aminoacids, glyceroglycolipids and spacer-connected C-disaccharides, have been synthesized through innovative procedures that exploits ionic and radical chemistry.

The Reductive Cleavage Method for Polysaccharide Structural Analysis

Gary R. Gray
Department of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, Minnesota 55455, USA

My lecture will describe a new procedure for establishing the primary structure of polysaccharides. The salient feature of the method, reductive cleavage of the glycosidic carbon-oxygen bonds in fully methylated polysaccharides, gives rise to partially methylated *anhydroalditols* that are subsequently analyzed as their acetyl derivatives by gas-liquid chromatography combined with mass spectrometry. Comparison of the GLC retention indices and mass spectra of these products with those of authentic standards establishes the composition of the polysaccharide and the ring form and position(s) of linkage of each of its glycosyl residues in a single step. The method has already been shown to be applicable to the analysis of polysaccharides containing pentoses, hexoses, deoxyhexoses, hexuloses, 2-acetamido-2-deoxyhexoses, uronic acids, and sialic acids. In addition, the method is applicable to the analysis of polysaccharides containing a wide variety of covalently-attached, non-carbohydrate substituents such as carboxylic acid esters, ethers (methyl, ethyl, carboxymethyl, hydroxypropyl), and pyruvic acid acetals. Furthermore, through proper choice of reagents, the reductive cleavage of glycosidic linkages can be accomplished *selectively* to generate small oligomers that can be characterized and used to deduce the *sequence* of glycosyl residues in polysaccharides. The current status of development of the method will be illustrated by its application to a variety of polysaccharides of biological and industrial importance.

Utilization of Planar Chromatography in the Sugar Industry

G. Mantovani*, G. Vaccari, G. Lodi and E. Dosi
Chemistry Department, University, Via L. Borsari 46, I-44100 Ferrara, Italy

Analysis of sugar mixture by modern liquid chromatography technique, both column and planar, has gained much prominence. As far as planar chromatography is concerned, the introduction of high performance bounded phases layer and of instrumental development technique has opened new perspectives in TLC analysis of sugars, particularly for samples which need cumbersome clean-up processes. In chromatography, gradient elution is the technique of choice whenever the mixture components span a wide range of polarity (or molecular structure). Also in planar chromatography gradient development is possible using an automated technique (AMD), recently introduced. This technique allows large spot capacities because of the reconcentration effect caused by the multiple development and the accommodation of many spots on the same chromatographic plate because of the gradient development. Thus, complex samples, as beet or cane molasses, can be analyzed (or at least screened) on high performance thin layers using modern scanners which allow much information to be obtained both on underivartized and derivatized analysis.

In the analysis of molasses it is interesting also the Over Pressure Liquid Chromatography (OPLC); although it does not allow to utilize the effect of spot reconcentration constituents.

Details concerning the analysis of both beet and cane molasses are reported and a comparison between the two techniques mentioned above is discussed.

What Is The News in Sucrochemistry

G. Descotes
UMR 143-CNRS/Béghin-Say, 69603-Villeurbanne, France

After the presentation of some economical informations on the wealth and European productions and the non-food applications of sucrose, the recent studies in sucrochemistry mainly developed in France will be presented.

New activation processes (microwaves, ultrasound, thermolysis) were used in reactions of acetylation, etherification, esterification and oxidation of sucrose. Using specific reagents (heterogeneous and homogeneous catalysts, TEMPO) regioselective transformations can be controlled.

Substitutions reactions were also studied in relationship, with the concentrations of sucrose and the subsequent hydrophobic effects of the introduced groups on the sucrose moiety. The applications of sucroethers, sucroesters, sucroacetals in polymer chemistry and detergency will be evoked.