8TH BRATISLAVA
SYMPOSIUM
ON SACCHARIDES

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PROGRAMME
AND
ABSTRACTS

September 1-5, 1997
Smolenice, Slovakia

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8TH BRATISLAVA SYMPOSIUM ON SACCHARIDES

organized by the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia in collaboration with the Slovak Chemical Society and the Slovak Society for Biochemistry and Molecular Biology.

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Scientific Programme

Monday, September 1

14.00 Opening ceremony

Plenary (PL) and invited (IL) lectures

Chairpersons: Crescenzi V. and Tvaroška I.

14.20 PL Peters T. (Germany):
Protein carbohydrate interactions as studied by NMR experiments

15.05 PL Paoletti S. (Italy):
Ionic polysaccharides: Experiments, theory and structural information

15.50 Coffee break

16.20 PL Belton P.S. (United Kingdom):
Spectroscopic studies of dynamics and structure in oligo- and polysaccharides

17.05 IL Paradossi G., Miano A., Chiessi E., Malovíková A., and Crescenzi V. (Italy, Slovakia):
A study on the interaction of D- and L-poly(lysine) enantiomers with potassium pectate

17.25 IL Pätoprstý V. and Kováčik V. (Slovakia):
Pyridine - an useful reagent in chemical ionization mass spectrometry of carbohydrate derivatives

18.15 Concert and mixer

Tuesday, September 2

Plenary and invited lectures

Chairpersons: Kováč P. and Kováčik V.

9.00 PL Schmidt R.R. (Germany):
New aspects of glycoside bond formation

9.45 PL Beau J.M. (France):
***C*-glycoside construction from anionic *C*-glycosyl donors: Sugar mimics for sugar receptors**

10.30 Coffee break

11.00 PL Descotes G. (France):
Anomeric radicals, carbenes and nitrenes in monosaccharide chemistry

11.45 IL Casenave B., Deléris G., Latxague L., Cassel S., and Rollin P. (France):
Synthesis of arylalkyl and indolylmethyl glucosinolates: A new approach

12.05 IL Eastwick-Field V. (United Kingdom):
The role of carbohydrates in today's commercial world

12.25 Lunch break

Plenary and invited lectures

Chairpersons: Schmidt R.R. and Hirsch J.

14.00 PL Kováč P. (USA):
Recent studies towards a vaccine for cholera based on a synthetic oligosaccharide antigen

14.45 PL Nilsson K. (Sweden):
Recent developments in the field of use of glycosidases for synthesis of complex saccharides and derivatives

15.30 IL Tvaroška I., André I., and Carver J.P. (Slovakia, Canada):
Toward an understanding of the catalytic mechanism of glycosyltransferases. An *ab initio* MO study

15.50 IL Petruš L., Hricovíniová Z., Petrušová M., and Matulová M. (Slovakia):
Application of the Bílik reaction to the stereoselective synthesis of 2-ketoses

16.10 Coffee break and Poster session I

Wednesday, September 3

Excursion day

All day trip to Bratislava with sightseeings, boat trip on the Danube river to the Devin Castle with lunch, followed by wine-tasting session, and dinner in the typical wine-grover's town Pezinok in the Small Carpathian area near Bratislava.

Thursday, September 4

Plenary and invited lectures

Chairpersons: Paoletti S. and Biely P.

9.00 PL Jennings H.J. (Canada):
Extended helical polysaccharide epitopes and their role in glycoconjugate vaccines against meningitis

9.45 PL Kosma P. (Austria):
Synthesis of bacterial lipopolysaccharide antigens

10.30 Coffee break

11.00 PL Withers S. (Canada):
Understanding and exploiting glycosidases

11.45 IL Vetere A. and Paoletti S. (Italy):
All-transglycolytic synthesis of branched oligosaccharides belonging to the blood group determinants

12.05 IL Farkaš V., Sulová Z., Takáčová M., and Fry S.C. (Slovakia, United Kingdom):
Xyloglucan endotransglycosylase: Existence of a stable glycosyl-enzyme intermediate

12.25 Lunch break

Plenary and invited lectures

Chairpersons: Kosma P. and Vršanská M.

14.00 PL Durán A., Pérez P., Roncero C., Valdivieso M.H., Arellano M., Santos B., De Cos T., Trilla J.A., Castro C., Sánchez Y., and Ribas J.C. (Spain):
Characterization of genes involved in the regulation of cell wall biosynthesis in yeast

14.45 IL Kollár R., Reinhold B., Petráková E., Ashwell G., Yeh H., and Cabib E. (Slovakia, USA):
Crosslinkings among the yeast cell wall components

15.05 IL Wende G. (United Kingdom)
Cross-linking of plant cell wall polysaccharides: Ferulate incorporation and dimerization

15.25 IL Cote G.L., Willet J.L., and Payne-Wahl K. (USA):
Breaking chains: A comparison of methods for the production of low-viscosity dextrans and alternan

15.45 IL Biely P., Cote G.L., Kremnický L., and Greene R.V. (Slovakia, USA):
Mechanism of action of acetylxylan esterases: A study on acetylated methyl glycosides as substrates

16.05 Coffee break and Poster session II

19.30 Barbecue picnic

Friday, September 5

Plenary and invited lectures

Chairpersons: Withers S. and Farkaš V.

9.00 PL Hoson T. (Japan):
A two-step breakdown of xyloglucans and cell wall loosening

9.45 PL Zabotina O., Gurjanov O., Ibragimova N., Ayupova D., Larskaya I., Nikolaeva O., Lozovaya V., Zabotin A., Beldman G., and Voragen A. (Russia, The Netherlands):
Effect of oligosaccharins on morphogenesis and adaptation

10.30 Coffee break

11.00	PL	Cervone F., De Lorenzo G., Aracri B., Bellincampi D., Capone I., Cappari C., Devoto A., Leckie F., Mattei B., Nuss L., and Salvi G. (Italy): **Oligogalacturonides, polygalacturonates and inhibitor proteins (PGIPs) in cell-cell signalling**
11.45	IL	Markovič O. and Stratilová E. (Slovakia): **The primary structure homologies of pectinesterases and of polygalacturonases**
12.05		Closing ceremony
12.30		Lunch
14.00		Departure of buses to Bratislava

Programme for Accompanying Persons

Tuesday, September 2

8.30 Excursion to the Červený Kameň Castle (Red Stone) and to the manufacture of the typical local pottery (maiolic) in Modra

Thursday, September 4

8.30 Excursion to the thermal Piešťany Spa and the medieval town of Trnava

List of Posters

POSTER SESSION I

1. Tvaroška I. and Carver J.P.
   The gauche effect and conformations of the hydroxymethyl and methoxymethyl groups in saccharides

2. Gajdoš J., Ragazzi M., and Ferro D.R.
   Molecular mechanics and solvation energy calculations of heparin epoxide

3. Petrušová M., Pham-Huu D.-P., Petruš L., and BeMiller J.N.
   Application of *C*-glycosylnitroalkanes in the synthesis of *C*-glycosyl amino acids

4. Pham-Huu D.-P., Petrušová M., Petruš L., and BeMiller J.N.
   Michael additions of fully functionalized β-glycosylnitromethanes and 1,2-dideoxy-1-nitrohex-1-enitols

5. Pribulová B., Petrušová M., Matulová M., and Petruš L.
   Preparation of some *N*-acetyl-D-galactosamine- and L-rhamnose-derived *C*-glycosyl synthons

6. Turjan J., Petrušová M., Petruš L., and BeMiller J.N.
   Synthesis of *N*-acetyl-D-galactosamine *via* both ozonolysis and peroxomolybdate oxidation of a nitronate derivative

7. Poláková M. and Joniak D.
   Synthesis of glucopyranosiduronic acids by oxidation from derivates of glucose

8. Joniak D. and Poláková M.

   Synthesis of 4-methoxybenzyl (methyl α-D-glucopyranosid)-uronate

9. Linek K., Alföldi, Stach T., and Pätoprstý V.

   Preparation of some *N*-acetylglycosylamines and *N*-acetyldiglycosylamines

10. Linek K., Stach T., Alföldi J., Pätoprstý V., Sulová Z., and Farkaš V.

    Syntheses of some glycosylamines and diglycosylamines

11. Lorin C. and Rollin P.

    Synthesis from thiosugars of chiral *THF-THF* or *THF-THP* bicyclic building blocks

12. Gueyrard D., Lorin C., Moravcová J., and Rollin P.

    A novel intramolecular migration in saccharidic sulfones: Benzothiazol-2-yl group transfer through an *ipso*-substitution

13. Moravcová J., Bláhová R., Střížová P., Lorin C., and Rollin P.

    Synthetic studies of a pentofuranose with a fused pyrazolidine ring

14. Kefurt K., Hamerníková M., Havlíček J., Votavová H., and Raich I.

    Preparation and structure of aminodeoxyhexonolactams

15. Steiner B. and Koóš M.

    Preparation of some *C*-glycosylated α-amino acids *via* corresponding hydantoins

16. Koóš M. and Steiner B.

A convenient synthesis of 2,3-unsaturated sugar derivatives

17. Kováčik V., Hirsch J., and Heerma W.

Linkage characterization of alditol disaccharides by fast atom bombardment collision induced dissociation tandem mass spectrometry of sodium cationized ions.

18. Königstein J.

On mechanism of simple saccharide transformations

19. Backinowsky L.V., Nepogodiev S.A., and Abronina P.I.

Efficient synthesis of branched oligomannosides

20. Jančová I. and Kolarova N.

Transglycosylase activity of α-galactosidase isolated from the yeast *Aureobasidium pullulans*

21. Sabová D., Kolarova N., Bhat M.K., and Biely P.

Enzymic synthesis of α-D-Gal*p*- (1→6)-α-D-Gal*p*-(1→6)-D-Gal*p*

22. Sulová Z., Nahálka J., and Farkaš V.

Affinity purification of XET based on the entrappment of productive XET: xyloglucan complex on cellulose

23. Kremnický Ľ. and Biely P.

Xylobiose and mannobiose permease of *Aureobasidium pullulans*

24. Vršanská M., Nerinckx W., Biely P., and Claeyssens M.

Fluorogenic substrates for endo-β-1,4-xylanases

25. Mislovičová D., Vikartovská A., and Gemeiner P.

The glycoenzyme-binding assay for the study of interaction of concanavalin A with mannosaccharides

26. Stratilová E. and Dzúrová M.

Exopolygalacturonases prefering oligogalacturonides with low degree of polymerization as substrates

27. Stratilová E., Markovič O., Dzúrová M., Malovíková A., and Capek P.

The pectic enzymes complex of carrots

28. Breierová E., Stratilová E., and Sláviková E.

The occurrence of yeasts and their extracellular glycoproteins produced in stress environments

POSTER SESSION II

1. Toman R., Škultéty Ľ., Kováčová E., and Pätoprstý V.:

Serological activity of a smooth lipopolysaccharide of *Chlamydia psittaci*

2. Ftáček P., Škultéty Ľ., Pätoprstý V., and Toman R.

Composition study of lipopolysaccharides in the phase transition of *Coxiella burnetii*

3. Sinitsya A., Čopíková J., and Matějka P.

FT Raman spectra of polygalacturonic acid and pectins

4. Sinitsya A., Čopíková J., Machovič V., and Pavliková H.
   Curve-fitting analysis in diffuse reflectance FTIR and ^{13}C CP/MAS NMR spectroscopv of pectins

5. Čopíková J., Sinitsya, and Tarkošová J.
   Use of near infrared spectroscopy in the chocolate analysis
6. Wellner N., Belton P.S., Kačuráková M., and Malovíková A.
   FTIR study of intermolecular interactions in pectin gels

7. Sasinková V., Šandula J., and Kačuráková M.
   FTIR spectroscopy for the analysis of β-glucans

8. Kačuráková M., Hromádková Z., Ebringerová A., and Hříbalová V.
   Modification of heteroxylans by ultrasonic treatment in aqueous neutral and alkaline medium

9. Malovíková A., Ebringerová A., Hromádková Z., Kačuráková M., Machová E., Pavlov G.M., and Harding S.E.
   Some physicochemical properties of beech wood 4-*O*-methyl-D-glucuronoxylan

10. Hromádková Z. and Ebringerová A.
    The use of ultrasound to remove the UV-absorbing component of beechwood xylan

11. Hromádková Z., Ebringerová A., and Machová E.
    Application of ultrasound in isolation of the xylan component of annual plants

12. Talába P., Sroková I., Ebringerová A., and Hodul P.
    Preparation and study of mesogeneous properties of aromatic and aliphatic esters of cellulose

13. Sroková I., Talába P., Hromádková Z., Ebringerová A., and Alföldi J.
Structure and properties of partially hydrophobised D-xylan type polysaccharides

14. Kardošová A., Matulová M., Capek P., Nosáľová G., and Fraňová S.
Polysaccharide complex with antitussive activity from the roots of *Rudbeckia fulgida*, var. *Sullivantii* (Boynton et Beadle): structure of the fructofuranan component

15. Kardošová A. and Matulová M.
4-*O*-methyl-D-glucurono-D-xylan from *Rudbeckia fulgida*, var. *Sullivantii* (Boynton et Beadle)

16. Capek P. and Kardošová A.
A neutral heteropolysaccharide from the flowers of *Malva mauritiana* L.

17. Kolarova N., Capek P., and Matulová M.
A neutral extracellular polysaccharide from the yeast *Cryptococcus lautentii*

18. Machová E., Kogan G., Matulová M., Pätoprstý V., Šandula J., and Kvapilová K.
Carboxymethylated chitin-glucan complex from *Aspergillus niger* - degradation with laminarinase and chitinase

19. Mislovičová D., Masárová J., Bendžálová K., Šoltés L., and Machová E.
Preparation and characterization of water soluble chitin-glucan from *Aspergillus niger*

20. Korolenko T.A., Vereschagin E.I., and Šandula J.

Carboxymethyl-β-1,3-glucan fractions as new perspective immunostimulators and macrophage stimulators

21. Košíková B. and Ebringerová A.

Binding sites of carbohydrate moieties towards residual lignin in spruce soda pulp

22. Košíková B., Hricovíni M., and Cosentino C.

Interaction of lignin and polysaccharides in beech wood *Fagus sylvatica* during drying processes

23. Šimkovic I., Hricovíni M., Ďuriš P., Mendichi R., and Šoltés L.

Preparation of hydroxypropyl derivatives of hyaluronic acid in water/NaOH solution with epichlorohydrin in the presence of NH_4OH

24. Slováková Ľ., Karácsonyi Š., Kubačková M., Kákoniová D., Bilisics L., and Lišková D.

Effect of galactoglucomannan-derived oligosaccharides on hypersensitive reaction of cucumber TNV

25. Karácsonyi Š., Pätoprstý V., Kubačková M., Lišková D., Kákoniová D., and Bilisićs L.

Arabinogalactan-protein from spruce callus cells: Structure and biological activity

Plenary Lectures

PROTEIN CARBOHYDRATE INTERACTIONS AS STUDIED BY NMR EXPERIMENTS

Thomas Peters
Medical University of Luebeck, Institute of Chemistry, Ratzeburger Allee 160,
23538 Luebeck, Germany

Transfer-NOE (trNOE) experiments and related NMR-experiments are well suited to study the bioactive conformations of oligosaccharide ligands as this has been shown in many cases during the past years. Oligosaccharides bound to antibodies, lectins, selectins and glycosidases have been investigated so far [1]. Experimental techniques that allow the unambigious determination of the bioactive conformation of an oligosaccharide will be discussed, and examples will be given. Recently, we found that trNOE experiments are also valuable tools for the identification and structural characterization of biologically active molecules from mixtures [2]. Examples for the screening of mixtures for biological activity using NMR will be presented.

References:

[1] T. Peters and B. M. Pinto, *Curr. Opin. Struct. Biol.*, **6** (1996) 710-720.

[2] B. Meyer, T. Weimar and T. Peters, *Eur. J. Biochem.*, **246** (1997) 705-709; T. Peters and B. Meyer, German patent application 196 49 359.5-52.

IONIC POLYSACCHARIDES:
EXPERIMENTS, THEORY AND STRUCTURAL INFORMATION

Sergio Paoletti

Department of Biochemistry, Biophysics and Macromolecular Chemistry,
University of Trieste, I-34127 Trieste, Italy, and
POLY-biòs Research Centre, AREA Science Park, I-34012 Trieste, Italy

Whereas the most widespread polysaccharides, as to mass, are non-ionic (namely cellulose, starch, chitin) still the largest majority of polysaccharides of "active" biological significance are ionic, i.e. *polyelectrolytes*. In particular, with the outstanding exception of chitosan, they are polyanionic. In the past decades ionic polysaccharides have undergone a tremendous increase of interest for the most various applications, from biomedicine to enhanced oil recovery, not to say the food and biotechnological ones. In the past decades, they have given rise to one of the most extensive set of studies in the polymer area aimed at the deeper understanding of the "*structure / properties*" correlations.

It is self-evident that ionic polysaccharides bear ionic groups in their primary structure. Therefore it is not unexpected that ionic polysaccharides have been the target of experimental methods typical of polyelectrolytes, still along with all kinds of "classical" experiments on polymers, and on polymer solutions in particular. Anyway, even all "classical" experimental methods are profoundly influenced by the presence of ionic polymeric charges. This fact is at the same time a nuisance and a valuable source of information. For example, the value of the average dimensions of an ionic polysaccharide, either as manifested through its intrinsic viscosity or by the angular dependence of the scattered light (radius of gyration), will be strongly influenced by the amount of the fixed charges on the polymer, the type of counterions and the overall amount of mobile charged species present in solution (*ionic strength*). It will be absolutely mandatory for the experimentalist to carefully take into account all these variables to get a sound set of physical parameters out of the data. Not unrelated, the problem of chain association (and the related kinetically "irreversible" aggregation) has been too often and too easily overlooked, lending space to misleading conclusions on polysaccharide structures. The delicate job of proper sample and solution handling will be rewarded by, e.g., the possibility of evaluating the "intrinsic" stiffness of the chain from the ionic-strength dependence of the measured properties.

A very important aspect arises from what said above. The polyelectrolytic properties of ionic polysaccharides become an effective investigational tool in themselves able to shed light onto the structure and the behavior of those polymers. This effect has been called "contrast effect" (1) , inasmuch as the knowledge (or, better, the quantitative description) of the polyelectrolytic component of a given measurable property can enable one to assess the "intrinsic", *i.e.* non-polyelectrolytic, properties of the saccharidic polymer chain. This in turn implies the availability of a good polyelectrolyte theory.

A good theory should be conceptually simple, give expressions (better so if analytical expressions) for physically measurable properties and all this should be function of some structural parameter of the polymer chain. This is the case of the Counterion Condensation (CC) theory, mainly due to G.S.Manning (2), which has been amply demonstrated to account for avery large number of experimental properties of natural and synthetic polyelectrolytes (3). The theory has been developed to include the effect of the presence of counterions of mixed valency (4), of the chain stiffness (5), and of specific interactions between polyion and counterions.

The combination of experimental data and theoretical approach is shown to be able to give valuable information in the case of carboxyl- and sulphate-bearing polysaccharide, namely poly(galacturonic acid), alginic acid and carrageenans. Ion-(and/or temperature) induced conformational transition, interchain association and, eventually, gel formation, are phenomena which have been neatly and consistently interpreted in terms of the polyelectrolytic properties.

References

1. S.Paoletti, A.Cesàro, F.Delben, V.Crescenzi, R.Rizzo, in "Microdomains in Polymer Solutions", P.Dubin Ed., Plenum Press, N.Y., 1985, pp.159-189.
2. G.S.Manning, J.Chem.Phys., 51, 924 (1969)
3. G.S.Manning and S.Paoletti, in "Industrial Polysaccharides: The impact of Biotechnology and Advanced Methodologies", S.S.Stivala, V.Crescenzi and I.C.M.Dea Eds., Gordon & Breach, N.Y., 1987, pp.305-324.
4. S.Paoletti, J.Benegas, A.Cesàro, G.Manzini, F.Fogolari, V.Crescenzi, Biophys.Chem., 41, 73 (1991).
5. A.Cesàro, S.Paoletti, R.Urbani, J.Benegas, Int.J.Biol.Macromol., 11, 66, (1989).

SPECTROSCOPIC STUDIES OF DYNAMICS AND STRUCTURE IN OLIGO- AND POLYSACCHARIDES

P S Belton
Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK.

Vibration spectroscopy and NMR offer very sensitive tools for the investigation of structure in solid and gelled oligo- and polysaccharides. FTIR may be used to investigate structural changes in gels and crystalline saccharides and may be illustrated by reference to the gelling of carrageenans(1) and the hydration and dehydration of trehalose(2), xylooligosaccharides and pectin. Whilst changes in structure may be readily observed, it is much more difficult to relate NMR and FTIR spectra to the absolute structures of solids and gels. Our work on the prediction of both infrared and NMR spectra have shown that very careful calculation is required if useful results are to be obtained. In particular(2) solid state ^{13}C NMR can give misleading results about the number and nature of magnetically non-equivalent carbon atoms in crystalline materials.

NMR not only yields information about structure it is also very sensitive to dynamical behaviour of molecules. In the case of plant cell walls this sensitivity can be used to edit spectra to obtain chemical information about the types of molecules that exist in different motional regimes and to examine the overall changes in molecular dynamics at different temperatures and hydration levels. In potato cell walls proton NMR relaxation time studies show that side chain motions are not much affected by hydration whereas backbone motions are much more affected. When magic angle spinning methods are applied to observe the proton spectrum, two groups of hydroxyl protons associated with the mobile molecules are distinguishable. When solid state carbon spectra are edited by exploiting the proton relaxation times reflecting the different mobilities of the molecules, it is possible to identify those chemical species associated with particular levels of mobility(3).

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2. A M Gil, P S Belton and V Felix, Spectrocim Acta A, 52, 164 (1996).
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NEW ASPECTS OF GLYCOSIDE BOND FORMATION

Richard R. Schmidt
Fakultät Chemie, Universität Konstanz, Fach M 725,
D-78457 Konstanz, Germany

The chemical synthesis of oligosaccharides has seen years of dynamic progress, mainly based on highly reactive glycosyl donors, versatile building block strategies, and advanced protective-group design during the last two decades (1). The endeavour was stimulated by the eminent role of carbohydrates and especially of glycoconjugates in various fields of modern biology (2). A prominent area in this regard is glycoconjugate synthesis which is essentially based on suitable monosaccharide building blocks, their regio- and diastereoselective glycosidic linkage, and finally removal of the protective groups. A central role plays glycoside bond formation because of its difficulty in arriving at high chemical yields and diastereocontrol. Major progress has been made for aldoses in recent years. However, improvement in sialylation, e.g. ketoside formation, arriving at gangliosides, is an important task, because further knowledge of the biological function of gangliosides should result from such endeavours. In chemical ganglioside synthesis, sialylation has been carried out by various methodologies (3). Consideration of various leaving groups led us to phosphite (and phosphate, the in vivo glycosyl donors) moieties and their derivatives. Thus, a readily available O-acetylated neuraminic acid derivative is transformed into a sialyl donor which with alcohols as acceptors and in nitriles as solvent provides the target molecules. Recent results and extensions of this approach will be discussed.

Despite of these synthetic achievements, the synthesis of large and complex oligosaccharides in solution remains a demanding task. A well established method for the preparation of oligosaccharides (homo- and heteroglycans) at a solid phase could be superior to solution trechniques in respect to efficiency, future automatization, and application in combinatorial synthesis. Our efforts on the investigation of solid phase glycosylations (4) will be reported.

1. R.R. Schmidt, *Angew. Chem.*, *98*, 213-236 **(1986)**; *Angew. Chem. Int. Ed. Engl.*, *25*, 212-235 **(1986)**; R.R. Schmidt, in Glycosciences: Status and Perspectives (Eds. H.J. Gabius, S. Gabius), Chapman and Hall, Weinheim, 1997.
2. R.A. Dwek, *Chem. Rev.*, *96*, 683 **(1996)**.
3. T.J. Martin, R.R. Schmidt, *Tetrahedron Lett.*, *33*, 6123-6126 **(1992)**; G. Hummel, R.R. Schmidt, *Tetrahedron Lett.*, *38*, 1173-1176 **(1997)**.
4. J. Rademann, R.R. Schmidt, *Tetrahedron Lett.*, *37*, 3889-3990 **(1996)**; *J. Org. Chem.* in print.

C-GLYCOSIDE CONSTRUCTION FROM ANIONIC *C*-GLYCOSYL DONORS: SUGAR MIMICS FOR SUGAR RECEPTORS

Jean-Marie Beau
Laboratoire de Synthèse de Biomolécules, Institut de Chimie Moléculaire d'Orsay, 91405 Orsay, France

During the last twenty years, carbohydrate chemists have been deeply involved in the evaluation of strategies for the efficient contruction of natural or modified oligosaccharides in quantities large enough for biological studies. Potential therapeutic applications now require new molecular assemblies that are good mimics of the three-dimensional characteristics of natural substances and stable enough to survive metabolic degradations. *C*-Glycosidic analogues fulfill these major criteria. We will show how glycosyl samarium(III) species derived from neutral or 2-amino sugars are unique among nucleophilic *C*-glycosyl donors for *C*-oligosaccharide constructions as they provide stereospecifically the corresponding 1,2-*trans* or 1,2-*cis*-*C*-glycosides with appropriate acceptors in simple experimental procedures.

ANOMERIC RADICALS, CARBENES AND NITRENES IN MONOSACCHARIDE CHEMISTRY

G. DESCOTES

University Lyon I - France-

Starting from photochemical intramolecular cyclizations obtained by NORRISH-type II reactions at the anomeric centre of functionalized glycosides, more recent processes involving radicals, carbenes and nitrenes will be described.

Anomeric gem-bromochlorosugars are the starting material for inducing substitutions, eliminations and rearrangement reactions leading to glycoheterocyclic compounds.

Anomeric gem-diazido sugars are transformed into fused carbohydrate tetrazoles and the mechanisms of these reactions will be discussed. The last results obtained with Hungarian groups for the use of the Staudinger reaction are also summarised.

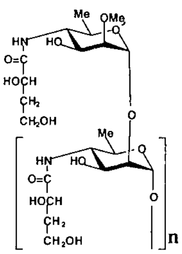
Metallocarbenic catalysts are also used for intermolecular or intramolecular metathesis reactions to yield amphiphilic « bolaforms » or macrocyclic « pseudocyclodextrines ».

RECENT STUDIES TOWARDS A VACCINE FOR CHOLERA BASED ON A SYNTHETIC OLIGOSACCHARIDE ANTIGEN

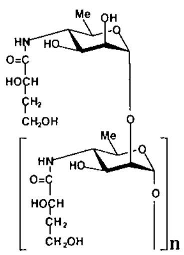
Pavol Kováč, *National Institutes of Health, Bethesda, MD 20892-0815, USA.*

The intracatenary part of the O-PS of *Vibrio cholerae* O1, serotypes Inaba and Ogawa, consists of (1→2)-linked moieties of 4-amino-4,6-dideoxy-α-D-mannopyranose (D-perosamine), the amino groups of which are acylated with 3-deoxy-L-*glycero*-tetronic acid. Only the Ogawa O-PS has the *O*-2 at its nonreducing D-perosaminyl group methylated. Serologically, three antigen factors have been associated with the O-antigenic structure of *V. cholerae* O1: Major group antigen factor A, antigen factor B (Ogawa factor), and antigen factor C (Inaba factor). The chemical nature of these factors has not been clarified. Fragments of O-PS of *V. cholerae* O1, serotypes Inaba and Ogawa, mono- through hexasaccharides, were prepared using various synthetic strategies for construction of oligosaccharide chains composed of D-perosamine, and for introduction of the 3-deoxy-L-*glycero*-teronamido side chain. Oligosaccharides were obtained in the form of methyl α-glycosides, and their interaction with two monoclonal anti *V. cholerae* O1 IgGs directed against the LPS of *V. cholerae* O1, serotype Ogawa, was studied. The antibodies exhibited similar affinities for fragments of Ogawa O-PS, but did not react with the corresponding fragments of Inaba O-PS which do not carry the 2-OCH_3 group. The methyl α-glycoside of the residue that forms the non reducing terminal residue of the Ogawa O-PS contributed ~90% of the maximal binding energy, suggesting that the 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranosyl group in the Ogawa LPS is the immunodominant epitope. Binding studies indicate that the latter structural entity constitutes the Ogawa-specific antigen factor B. On the other hand, the lack of reaction of the anti-Ogawa antibodies with oligosaccharide fragments of the Inaba O-PS indicates that the *internal* part of the O-PS, common to both O-PSs, is probably *not* the group-specific antigen factor A.

Ogawa



Inaba



Hexa- and dodecasaccharide fragments of the O-PS of *Vibrio cholerae* O1, serotype Ogawa were also prepared in the form of α-glycosides bearing aglycons suitable for linking to proteins.

RECENT DEVELOPMENTS IN THE FIELD OF USE OF GLYCOSIDASES FOR SYNTHESIS OF COMPLEX SACCHARIDES AND DERIVATIVES

Kurt G.I. Nilsson
Glycorex AB, Sölveg. 41, S-223 70 Lund, Sweden.

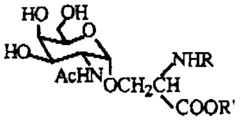
A number of glycoconjugate saccharide sequences have been prepared with glycosidases (see ref. 1 for a recent review). Glycosidases are of interest since they are stereospecific, abundant catalysts and allow the use of simple glycosyl donors (e.g. mono- or disaccharides). Glycosidases often give mixtures of regioisomers. However, a number of compounds have been prepared in highly regioselective reactions. An important factor in achieving this is the possibility to manipulate the regioselectivity of glycosidase-catalysed reactions via the use of different acceptor glycosides (2). Here we would like to focus on some recent examples of stereospecific and highly regioselective synthesis of compounds of central current interest to the field of glycobiology.

Structures **I** - **V** below were prepared in multi-gramme scale employing abundant glycosidases. Thus, structure **I** (T-antigen) was obtained in a stereospecific, α-D-galactosaminidase-catalysed reaction. Upon reaction of structure **I** with lactose as glycosyl donor and β-D-galactosidase as catalyst, structure **II** (TF-antigen) was obtained in a regio- and stereospecific manner (Biotechnology Lett. 1996, 18, 791-794).

Structure **III** represents one of several useful partially protected amino-deoxy disaccharides prepared in our laboratory employing different glycosidases (see e.g. J. Carbohydr. Chem. 1997, in press). These reactions were also stereo- and regiospecific.

Structure **IV** represents one of several lactosamine derivatives prepared directly from lactose as glycosyl donor using different glycosidases and partially modified glucosamine derivatives as acceptors (Biotechnology Letters, 1995 and J. Carbohydr. Chem. 1997, in press). The trisaccharide derivative **V**, (a precursor to e.g. tri- and pentasaccharide antigens of the Galili-type), was obtained directly from compound **IV** in a stereospecific and highly regioselective manner, employing p-nitrophenyl α-D-galactopyranoside and α-D-galactosidase as catalyst (Tetrahedron Lett. 1997, 38, 133-166).

We believe these type of reactions with abundant glycosidases and donor substrates are advantageous, especially for large-scale preparation.



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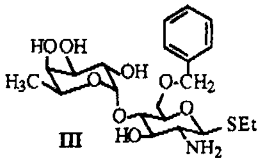

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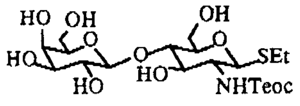

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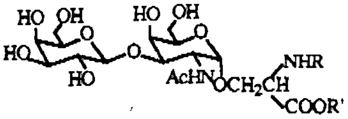
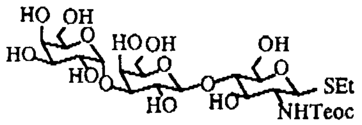


II



V

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EXTENDED HELICAL POLYSACCHARIDE EPITOPES AND THEIR ROLE IN GLYCOCONJUGATE VACCINES AGAINST MENINGITIS

Harry J. Jennings

National Research Council of Canada, 100 Sussex Drive, Ottawa, Ontario
K1A 0R6 Canada

The poor immunogenicity of some sialic acid containing polysaccharides is attributable to the fact that they contain structural features which mimic human tissue antigens, and when immunogenicity can be achieved the antibodies only recognize length-dependent epitopes expressed on extended helical domains of the polysaccharides. These length-dependent epitopes are of vital importance to the production of human protective antibodies and their presence was established by immunological studies and confirmed by means of NMR and potential energy calculations. This lack of immunogenicity can be overcome by chemical modification of the polysaccharides and two examples will be discussed which involve the polysaccharides of type III group B Streptococcus and group B Neisseria meningitis; both significant causes of meningitis in humans. The immunogenicity of the type III group B streptococcal polysaccharide can be improved by coupling it to a protein carrier to form a conjugate vaccine. However, the above conjugate technology does not appreciably improve the immunogenicity of the group B meningococcal polysialic acid capsule. In this case, the problem can be resolved by replacing the N-acetyl groups of the polymer with more hydrophobic N-acyl groups to form a synthetic antigen, which mimics unique bactericidal epitopes on the surface of the bacteria.

Synthesis of Bacterial Lipopolysaccharide Antigens

Paul Kosma
Universität für Bodenkultur -Wien, University of Agricultural Sciences - Vienna
Muthgasse 18, A-1190 Wien, Austria
e-mail:pkosma@edv2.boku.ac.at

Lipopolysaccharides (LPS) are essential components of the outer membrane of Gram-negative bacteria acting as potent stimulators of the immune system but also leading to severe pathological disorders such as septic shock, which despite antibiotic treatment, results in the death of 25-30% of patients with septicaemia. The chemical synthesis of LPS structures is presently focussed on the development of endotoxin antagonists, the establishment of structure-activity relationships and the interactions of these biomedically important molecules with proteins of the host cells.

Chlamydiae, which are intracellular parasites of increasing biomedical and clinical relevance, contain in their outer membrane a genus-specific epitope of a trisaccharide of 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) of the sequence α-Kdo-(2→8)-α-Kdo-(2→4)-α-Kdo which is assembled by a single, trifunctional Kdo-transferase (1). The trisaccharide and several analogs containing inverted anomeric configurations, deoxy-derivatives and carboxyl-reduced Kdo moieties, respectively, have been synthesized and converted into neoglycoconjugates. Serological analyses using natural as well as synthetic antigens have identified antibodies recognizing cross-reactive epitopes as well as the *Chlamydia* specific, 2→8- linked Kdo disaccharide and the trisaccharide antigens. Furthermore, the conformations of the carbohydrate epitopes have been investigated by molecular modelling, transfer-NOE experiments with monoclonal antibodies (2) and the crystal structure determination of a synthetic disaccharide part structure.

The deep-rough mutant *Haemophilus influenzae* I-69 Rd-/b+ contains the smallest LPS-structure known thus far, the lipid A part being linked to single phosphorylated Kdo residue. In order to investigate the substitution site of the phosphate group, Kdo-

4- and 5-phosphates attached to a ß-linked GlcNAc moiety have been synthesized using the phosphoamidite procedure. By using neoglycoconjugates derived from the synthetic allyl glycosides, two antibody specificities could be found being specific for a single Kdo-4-phosphate and Kdo-5-phosphate residue, respectively, thereby proving that both epitopes are present in natural LPS and not the result of artefacts during analytical work-up procedures. In addition, the synthesis of the trisaccharide determinant L-*gro*-α-D-*man*Hep*p*-(1→5)-α-Kdo*p*-4-phosphate-(2→6)-ß-D-GlcNAc has been carried out employing the trichloroacetimidate procedure for the elaboration of the disaccharide donor L-*gro*-α-D-*man*Hep*p*-(1→5)-α-Kdo*p*, which was subsequently coupled to the GlcNAc acceptor followed by efficient phosphorylation of *O*-4 of the Kdo unit (3,4). The trisaccharide constitutes an important epitope of the inner core in *Haemophilus, Vibrio* and *Bordetella* species.

Human-pathogenic strains of *Pseudomonas aeruginosa* of the RNA group I contain a highly phosphorylated heptose region with one 7-*O*-carbamoyl L-*glycero*-D-*manno* heptose moiety which may be exploited as immunochemical marker for the diagnosis of pathogenic *Pseudomonas* species. The carbamoyl-subsituted heptose as well as the disaccharide 7-*O*-carbamoyl-L-*gro*-α-D-*man*Hep*p*-(1→3)-L-*gro*-α-D-*man*Hep*p* was synthesized by regioselective formation of a 6,7-*O*-carbonate group followed by ring opening with ammonia/ammonium bicarbonate in excellent yields.

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UNDERSTANDING AND EXPLOITING GLYCOSIDASES

Steve Withers

Department of Chemistry, University of British Columbia, Vancouver, B.C.
Canada, V6T 1Z1

The hydrolysis of glycosidic bonds by glycosidases can take place in two fundamentally different ways: with net retention or net inversion of anomeric configuration. Accordingly there are two fundamentally different mechanisms which are followed. We have studied mechanisms of retaining glycosidases which carry out the reaction by a two step mechanism in an active site containing two carboxylic acids separated by approximately 5=C5. In the first step a glycosyl- enzyme intermediate is formed on one of the carboxyl groups with general acid catalysis from the other group. In the second step this intermediate is hydrolysed by general base-catalysed attack of water at the anomeric centre. Both steps occur via transition states with substantial oxocarbenium ion character. We have developed methods for trapping this glycosyl-enzyme intermediate by use of C-2 or C-5 fluorinated glycosides and have employed these reagents in the identification of the catalytic nucleophile. We have also stably trapped the intermediate using "normal" glycoside substrates in conjunction with mutant enzymes, and have obtained 3-dimensional structures of the reaction intermediates which provide unprecedented insight into enzyme/substrate interactions important for catalysis. We have also developed an understanding of the way in which the general acid/base catalyst can function effectively in both roles through 13C-NMR measurement of the active site pKa values of the two carboxylic acids, both in the free enzyme and in the glycosyl-enzyme intermediate. Finally we have developed new methods for the synthesis of oligosaccharides using engineered mutant glycosidases in combination with artificial substrates. These new enzymes have been named "Glycosynthases".

CHARACTERIZATION OF GENES INVOLVED IN THE REGULATION OF CELL WALL BIOSYNTHESIS IN YEAST.

A. Durán, P. Pérez, C. Roncero, M. H. Valdivieso, M. Arellano, B. Santos, T. de Cos, J. A. Trilla, C. Castro, Y. Sánchez and J.C. Ribas.
Instituto de Microbiología Bioquímica. CSIC / Univ. Salamanca. Spain

Chitin and (1-3)ß-D-glucan are the main structural polysaccharide components of the fungal cell wall. Taking advance of the antifungal effect of the fluorochrome Calcofluor, we have identified 5 complementation groups that defined 5 different genes (*CHS3* to *CHS7*) involved in *Saccharomyces cerevisiae* cell wall chitin synthesis mediated by chitin synthase III activity (CSIII) (1). CSIII activity is responsible for most of the "in vivo" synthesis of chitin during the yeast life cycle and *CHS3* encodes most probably the structural component of the synthase (2-3). However, not much is known about the role of the remaining genes. In this contribution we will present some information about a partial characterisation of *CHS3* and *CHS4* genes (4,5). *CHS5* has been also cloned and characterised. It is not essential for yeast cell growth but it is important for mating. Deletion of *CHS3* results in a modest decrease in mating efficiency, whereas *chs5*Δ cells exhibit a much stronger mating defect. However, *chs5* cells produce more chitin than *chs3* mutants, indicating that *CHS5* play a role in other processes besides chitin synthesis. Analysis of mating mixtures of *chs5* cells reveals that cells fail to undergo cell fusion. This mating defect can be partially rescued by *FUS1* and/or *FUS2*, two genes which have been implicated previously in cell fusion. Our results indicate that Chs5p plays an important role in the cell fusion step of mating (6) and further studies indicated that Chs5p is involved in the proper localisation of Chs3p (7).

Taking advantage of the antifungal effect of papulacandin B, we have cloned and partial characterised *PBR1* (*FKS1*) (8) a structural subunit of one of the (1-3)ß-D-glucan synthase activities described *in S.cerevisiae.* Recently, we have also advanced in the isolation and characterisation of the homologous gene in *Schizosaccharomyces pombe* (9).

We have also progressed in the characterisation of the GTP binding component of the (1-3)ß-D-glucan synthase activity in *S.pombe* as the Rho1 GTPase (10). Overexpression of the *rho1*$^+$ gene in wild type *S.pombe* cells causes aberrant morphology with loss of polarity and cells with several septa. Under this condition (1-3)β-D-glucan synthase activity is increased four times but is still dependent on GTP. When *S.pombe* is transformed with constitutively active *rho1* mutant alleles *(rho1-G15V* or *rho1-Q64L),* cells stop growing and show a very thick cell wall with hardly any septum. Under this condition the level of (1-3)β-D-glucan synthase activity is at least twenty times higher than wild type and is independent of GTP. Cells overproducing Rho1 are hypersensitive to inhibitors of cell wall biosynthesis or to cell wall degrading enzymes. We conclude that Rho1 directly activates (1-3)β-D-glucan synthase and regulates *S.pombe* morphogenesis (10).

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A TWO-STEP BREAKDOWN OF XYLOGLUCANS AND CELL WALL LOOSENING

Takayuki Hoson
Department of Biology, Faculty of Science, Osaka City University,
Sumiyoshi-ku, Osaka 558, Japan

Xyloglucans are the principal matrix polysaccharides of the primary cell walls of higher plants. They have a backbone of β-(1→4)-linked D-glucosyl residues and are hydrogen bonded to cellulose microfibrils, cross-linking the fibrils to form the macromolecular network of the cell walls (1). Because of such features, xyloglucans are supposed to be related to various functions of the cell walls. The important role of the breakdown of xyloglucans in the cell wall loosening induced by a plant hormone auxin has been confirmed with xyloglucan-specific antibodies and lectins (2,3). Nevertheless, the processes of xyloglucan breakdown still remain unclear. We will here propose a novel breakdown mechanism of xyloglucans.

The analysis of the sugar composition of xyloglucans, extracted from the cell walls of azuki bean epicotyls and fractionated on a gel filtration column, showed that xyloglucans with a molecular mass of about 50 kDa and higher exhibited a constant composition, whereas for those smaller than 50 kDa, the lower the molecular mass was, the lower was the level of fucose. The results as well as the effects of auxin and fucose-binding lectins on a molecular mass of xyloglucans lead to the hypothesis that auxin-induced breakdown of xyloglucans in azuki epicotyl cell walls consists of two major processes, the first step being the partial cleavage of the glucan backbone of xyloglucans into 50 kDa products without any stripping of side chains and the second step being the degradation of overall molecule, which is triggered by the removal of the terminal fucose from side chains (4). In relation to the hypothesis, it is noteworthy that fucose-rich, high molecular mass xyloglucans are localized at the cell walls of the outer, inextensible portion of azuki epicotyls (3,4).

An enzyme involved in xyloglucan breakdown was extracted with 1 M NaCl from the cell wall preparation of azuki epicotyls and purified by chromatography on several different columns. The enzyme hydrolyzed the xyloglucans of high molecular mass from azuki epicotyl cell walls to yield fragments of about 50 kDa without producing any oligo- or monosaccharide but it did not have an effect on those less than 60 kDa (5). Thus, the enzyme appears to be involved in the first step of the above-mentioned mechanism of xyloglucan breakdown.

The purified enzyme gave a single band with a molecular mass of about 32 kDa after SDS-PAGE and this size is similar to that of endotransferase (6). So, we examined whether the present enzyme had such an activity. When xyloglucans with a molecular mass of 64 kDa were used as substrates and the activity was determined by HPLC in terms of the width of the peak at 50% of its maximum height, no activity was detected, unlike the endotransferase (6). However, in the presence of pyridylamino-labeled xyloglucan oligosaccharides as acceptors, the enzyme catalyzed the transfer of cleaved products of about 50 kDa to the oligosaccharides (5). Thus, the purified enzyme is a kind of endotransferase with novel acceptor specificity: it prefers smaller acceptors, such as water. The partial amino acid sequence of the enzyme resembled that of endotransferase but it showed a clear difference in some regions, which may be related to the difference in acceptor specificity. Farkaš et al. (7) purified a similar enzyme from germinating seeds of nasturtium. However, the present enzyme is unique that it yields only the products of 50 kDa and above.

The results obtained in the present study indicate that xyloglucans are degraded via a two-step reaction in the cell walls of azuki bean epicotyls. Similar mechanism has been proposed for the breakdown of (1→3),(1→4)-β-D-glucans in Poaceae (2,3). On the other hand, the molecular size of xyloglucans and (1→3),(1→4)-β-D-glucans, when synthesized in the Golgi apparatus, is by far smaller than their final size which is about 1 MDa. Thus, there is a possibility that matrix polysaccharides in general are synthesized and degraded via intermediate products of the above size in the cell wall architecture.

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FFECT OF OLIGOSACCHARINS ON MORPHOGENESIS AND ADAPTATION

. Zabotina. O. Gurjanov, N. Ibragimova, D. Ayupova, I. Larskaya, O. Nikolaeva, V. Lozovaya, A .Zabotin, G. Beldman*, A. Voragen*
Institute of Biology of Russian Academy of Sciences. P. O. Box 30, 420503 Kazan, Russia;
*Wageningen Agricultural University. Bomenweg 2, 6703 HD. The Netherlands

Oligosaccharide fragments of cell walls that can, at very low concentrations, show biological effects on higher plants have been named "oligosaccharins" [1]. These effector molecules have diverse roles in plant's life. According their signaling effects, oligosaccharins can be divided into two main classes. Some of them play important role in the process of plant resistance against diseases. An other group of active oligosaccharides participate in different processes of plant growth, development and morphogenesis. All the oligosaccharins of the last group known to date were isolated artificially by cleavage of cell wall polysaccharides or as extracellular fragments secreted from cell wall into the spent medium of cultured cells. Thus, the question about the existence of such molecules in vivo in their active forms, showing their functions, is still open.

The attempts to separate active oligosaccharide fragments from boththe cell wall pectins and cell sap from 10-day-old pea stems (Pisum sativum L.) were done [2,3]. Several oligosaccharides soluble in cell sap showed different effects on the process of root formation in thin-cell-layer explants and on the process of stem elongation. Stimulation or inhibition of different stages of root development (initiation and growth) were obtained. Very similar fragments with the same effects have been found as the products of acid hydrolysis of pectins from pea cell walls. Monosaccharide composition of fragments separated was completely different from that of known to date oligosaccharins. It was found that fragments promoted the rhizogenesis can restore or effect the action of endogenic hormones.

An other type of active oligosaccharides was found in winter wheat root tissues. These fragments have been shown to be accumulated in the cells during the low temperature acclimation of wheat. Two types of oligosaccharides with opposite effects on the capability of adaptation of wheat seedlings were separated. Results obtained support the following concepts:

1. The oligosaccharins are really exist in their active forms in the plant tissues.

2. Similarity between the compositions of active fragments from pectins and soluble one in cell sap can testify, that the wall polysaccharides are the source of oligosaccharins.

3. The existence of very broad group of oligosaccharins with different structure and signaling properties.

4. At least some of oligosaccharins can be considered as the chemical messages processed by phytohormones, which have delineated set of biochemical processes.

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OLIGOGALACTURONIDES, POLYGALACTURONASES AND INHIBITOR PROTEINS (PGIPs) IN CELL-CELL SIGNALLING.

F. Cervone, G. De Lorenzo, B. Aracri, D. Bellincampi, I. Capone, C. Caprari, A. Devoto, F. Leckie, B. Mattei, L. Nuss, G. Salvi. Dipartimento di Biologia Vegetale, Università "La Sapienza", Piazzale Aldo Moro, 00185 Rome, Italy.

Endopolygalacturonases (PGs) produced by phytopathogenic fungi catalyze the fragmentation and solubilization of plant cell wall homogalacturonans, thereby assisting in the colonization of the plant tissue and providing nourishment to the invading fungi. On the other hand, PGs are potential avirulence factors, as they can generate oligogalacturonides that elicit accumulation of phytoalexins and other defense responses. Oligogalacturonides not only elicit defense responses but also regulate different aspects of growth and development in plants. Many of the development-related effects of oligogalacturonides appear to be amenable to an auxin-antagonist activity of these oligosaccharins (1,2). To clarify the role of oligogalacturonides in antagonizing auxin, we have recently analyzed their effect on root formation in leaf explants of tobacco harboring the plant oncogene *rolB*. We have shown that oligogalacturonides are capable of inhibiting root morphogenesis driven by *rolB* in transgenic leaf explants when this process requires exogenous auxin. We have also shown that oligogalacturonides antagonize auxin primarily because they strongly inhibit auxin-regulated transcriptional activation of a *rolB*-β-glucuronidase (GUS) gene fusion, in both leaf explants and cultured leaf protoplasts (3). All dicots thus far examined contain cell wall-associated proteins (PGIPs = Polygalacturonase Inhibiting Proteins) that inhibit fungal PGs. PGIPs are modular leucine-rich repeat (LRR) proteins evolutionarily related to several plant resistance genes and regulate the action of fungal PGs favouring the formation of elicitor-active oligogalacturonides. At present PGIP is the only plant LRR protein for which the interaction with a pathogen-derived ligand has been demonstrated and characterized (4). In *Phaseolus vulgaris* L. (bean), PGIP is encoded by a gene family (5). We have undertaken a systematic analysis of the specificity and regulation of the different members of the *pgip* gene family in the bean cultivar Pinto (6,7). We have isolated cDNA clones corresponding to three different *pgip* genes; one is identical to the gene, now called *pgip-1*, previously characterized from a genomic library of the cultivar Saxa. *Pgip-1* and *pgip-2* have been transiently expressed in *Nicotiana benthamiana* using the potato virus X (PVX) as a vector. PGIP-1 and PGIP-2 in crude protein extracts of PVX-infected *N. benthamiana*, and a PGIP-1 purified from transgenic tomato plants carrying the *pgip-1* coding sequence under the control of the CaMV 35S promoter, have been assayed for specificity of interaction with several fungal PGs (8). PGIP-1 from both plant sources exhibited a similar specificity, which was different from that of the bulk PGIP purified from bean. Notably, both PGIP-1 were unable to interact with homogeneous PG from *Fusarium moniliforme,* as determined by surface plasmon resonance analysis, while the bulk bean PGIP interacted with this enzyme; also, the tomato- and *N. benthamiana*-expressed PGIP-1 had only a limited capacity to inhibit crude PG preparations from *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea* and *Alternaria solani.* Instead, PGIP-2 was able to interact with both *A. niger* and *F. moniliforme* PGs. The presence of different PGIP proteins in bean extracts has been analysed by differential affinity chromatography. A PGIP (PGIP-A) with specificity similar to that of PGIP-1 was separated from a PGIP (PGIP-B) able to interact with both *A. niger* and *F. moniliforme* PGs. Our data indicate that PGIPs with different specificities are expressed in *P. vulgaris.*

Upon expression of a *pgip-1*-b-glucuronidase (GUS) gene fusion in *Nicotiana tabacum*, GUS activity was detected mainly in the stigma and, to a lesser extent, in the anthers and in the conductive vascular tissue. The promoter responded to wounding but not to oligogalacturonides, fungal glucan, salicylic acid, cryptogein or pathogen infection. This expression pattern does not mirror that of the whole *pgip* gene family. Using specific sense and antisense primers which differentially amplify *pgip-1, pgip-2 and pgip-3*, RT-PCR experiments have been performed to individually analyze the expression of these genes in response to several treatments and stimuli. Indeed, *pgip-1* transcripts accumulated in response to wounding, but not to elicitors, salicylic acid or infection, while, for example, *pgip-2* transcripts accumulated in response to the stimuli examined. These observations indicate that *pgip* members are also differentially regulated in *P. vulgaris.*

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Invited Lectures

A STUDY ON THE INTERACTION OF D- AND L-POLY(LYSINE) ENANTIOMERS WITH POTASSIUM PECTATE

G. Paradossi, A. Miano, E. Chiessi, A. Malovikova* and V. Crescenzi¶.

Department of Chemistry, Universitá di Roma "Tor Vergata", 00133 Roma, Italy. *) Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovakia. ¶) Department of Chemistry, Universitá di Roma "La Sapienza". 00181 Rome, Italy.

Macromolecular interactions are ubiquitous in living systems and enjoy a continuing interest in many fields of macromolecular science. New areas such as molecular imprinting and formulation of novel chemical networks could have not been developed at the present state without the knowledge of the interactions governing the macromolecular assemblies. Among these systems, oppositely charged polyelectrolytes are known to undergo the formation of macromolecular complexes. In this process the driving force is electrostatic.

In the present study, we report some new experimental findings concerning the formation of the diastereomeric complexes between pectate, a carboxylated polysaccharide, and D-, and L- enantiomers of polylysine. Upon binding, poly L-lysine undergoes a coil to α-helix conformational transition at neutral pH whereas poly D-lysine remains in the disordered state. In this context we have studied different aspects of such polyanionic - polycationic interaction. In particular, the thermal stability and the energetics of the binding are analyzed comparing opposite chirality and different size of polylysine samples. Measurements of the molecular weight of the macromolecular complexes by osmometry and of the competive binding of the cationic dye Methylene Blue with pectate in the presence of polylysine will be also reported.

PYRIDINE - AN USEFUL REAGENT IN CHEMICAL IONIZATION MASS SPECTROMETRY OF CARBOHYDRATE DERIVATIVES

V.Pätoprstý and V.Kováčik
Institute of Chemistry, Slovak Academy of Sciences,
842 38 Bratislava, Slovak Republic

The electron ionization mass spectra of carbohydrates and their derivatives result from complex fragmentation paths and show very low molecular ion intensities. Molecular weight information is an ideal complement to characteristic fragmentation patterns observed under EI in the elucidation of the structure of unknowns.

Chemical ionization with protonated pyridine as a reagent due to high value of proton affinity of pyridine (924 $kJmol^{-1}$) produces very stable $[M + PyrH]^+$ ions. The pyridine CI mass spectra give unambiguous information about the molecular weight also of minor components in the mixture.

The utility of pyridine mass spectra is demonstrated in the following problems solution in carbohydrate chemistry:

- methylation analysis of complex polysaccharides
- methylated alditol oligosaccharides as products of partial hydrolysis of polysaccharides
- per-O-acetylated aldononitriles of monosaccharides
- trimethylsilyl derivatives of monosaccharides
- acetylated N-glycosides of monosaccharides
- experiments using labeling with stable isotopes

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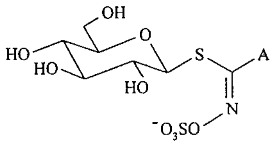
SYNTHESIS OF ARYLALKYL AND INDOLYLMETHYL GLUCOSINOLATES : A NEW APPROACH

Brigitte Casenave[a], Gérard Deléris[a], Laurent Latxague[a]
Stéphanie Cassel[b] and Patrick Rollin[b]*

a) Laboratoire de Chimie Bio-Organique, Université Victor Segalen Bordeaux 2
146, Rue Léo Saignat, F-33076 Bordeaux, France.
b) Institut de Chimie Organique et Analytique, UPRES-A 6005, Université d'Orléans
B.P. 6759, F-45067 Orléans Cedex 2, France

Glucosinolates (GSL) constitute a structurally homogeneous family of phytomolecules to which are associated strong and diversified physiological properties.

Whereas a limited number of pure GSL could be isolated from Crucifers by extraction,[1] the synthetic approach opens convenient ways for the elaboration of many naturally occurring GSL.[2] Moreover, it gives an easy access to structurally modified or totally artificial GSL.[3]



A = alkyl, alkenyl, arylalkyl, hydroxyalkenyl, indol-3-ylmethyl, thiofunctionalized

The synthetic pathways which have been developed for GSL within the last decades are invariably based on a key coupling step between a partially protected 1-thio-β-D-glucopyranose and a highly reactive hydroximoyl chloride. This electrophilic partner can be obtained:

- by direct chlorination of the corresponding aldoximes; in this case, unwanted side-reactions cannot always be avoided

- through transformation of a primary nitro-derivative into its nitronate anion, which in turn is reacted with an electrophilic activator / halogen-donor.

Kulkarni et al.[4] recently developed a method which allows a direct access to arylalkyl hydroximoyl chlorides from nitrovinyl precusors.[5]

In our laboratory, we have tested this method for the synthesis of arylalkyl GSL and furthermore an extension to the case of indolylmethyl GSL has been considered.

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THE ROLE OF CARBOHYDRATES IN TODAY'S COMMERCIAL WORLD

Dr Vanessa Eastwick-Field
Holliday Chemical Holdings PLC, The Quorum, Oxford Business Park, Oxford,
OX4 2JZ, UK

Carbohydrates are attractive to the food, chemical and pharmaceutical industries because many carbohydrates can be isolated, purified and modified from natural sources and are renewable on an annual basis. Carbohydrates are indeed the most abundant biological compounds and sucrose is considered to be the biggest single organic bulk chemical produced.

Carbohydrates are also of fundamental importance in the structural components of plants, as a source of metabolic energy, in the regulation of nearly all interactions between cells, in cell ageing and destruction and they also play a significant role in the control and resistance to disease.

These factors have lead to carbohydrates being the topic of intense research in recent years. As our understanding of their role in biological systems improves, expectations are that carbohydrates and carbohydrate-based products will play a pivotal role in many emerging commercial products.

The talk will address, although not exhaustively, areas of commercial application of carbohydrate and carbohydrate-based products.

TOWARD AN UNDERSTANDING OF THE CATALYTIC MECHANISM OF GLYCOSYLTRANSFERASES. AN *AB INITIO* MO STUDY.

Igor Tvaroška* Isabelle Andre and Jeremy P. Carver
GlycoDesign Inc, Toronto, Ontario, Canada

The conformational properties of the sugar-phosphate linkage have been studied with *ab initio* methods using the 2-*O*-methylphosphono-tetrahydropyran anion (**1**) and sodium 2-*O*-methylphosphono-tetrahydropyran (**2**) as models. The geometry of the conformers around the C1-O1 and O-P bonds was determined by gradient optimization at the SCF level and for some minima using the adiabatic connection method (ACM) of density functional theory (DFT) using standard 6-31G* basis set. The optimized geometries were used to calculate the energy of the conformers with the 6-31G* and tzp basis sets at SCF level and with the cc-pvtz basis set using the ACM method. Vibrational frequencies were calculated at the 6-31G* level and used to evaluate zero-point energies, thermal energies, and entropies for minima. From 27 staggered conformers assumed for the three bonds in the O5-C1-O1-P-O4-C sequence of atoms; the optimization led to 16 minima for **1** and to 14 minima for **2**, respectively. At all levels of theory, unexpectedly and contrary to the prediction based on the *exo*-anomeric effect, **1** prefers the trans to the gauche conformer around the C1-O1 bond. The repulsive electrostatic interactions between the ring oxygen and the PO_2^- group are assumed to be responsible for this phenomenon, which has been termed the reverse *exo*-anomeric effect. The presence of the sodium counter-ion completely reversed the relative energy of conformers, such that in the ion-pair complex **2**, the gauche conformer is favored. Based on these results, it is suggested that metal cofactor plays a crucial role in the catalytic mechanism of transferases. The complexation by metal cofactor changes the structure of the sugar donor into proper conformation, activates the C1-O1 glycosidic linkage, and finally, enables the protonation of the glycosidic oxygen to begin the cleavage of glycosidic bond. The conformers of **1** and **2** were also optimized by the quantum semi-empirical method, MNDO, and by molecular mechanics methods using the CVFF and AMBER force fields and the results were compared with those from *ab initio* calculations.

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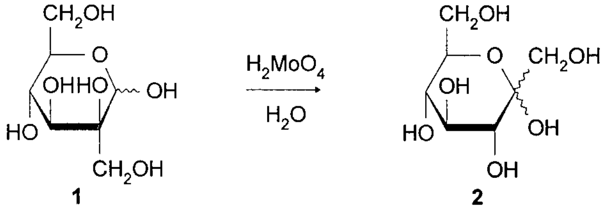
*On the leave of absence from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic.

APPLICATION OF THE BÍLIK REACTION TO THE STEREOSELECTIVE SYNTHESIS OF 2-KETOSES

L. Petruš, Z. Hricovíniová, M. Petrušová, and M. Matulová

Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia

The contribution introduces an extrapolation of the Bílik reaction - molybdic acid-catalyzed epimerization of aldoses [1] to 2-*C*-hydroxymethyl branched-chain aldoses. While the carbon skeleton rearrangement occurring during the transformation of aldoses [2] formally causes only the configurational change at the carbon atom C-2 resulting in the epimerization, at the same conditions the 2-*C*-hydroxymethyl branched-chain aldoses irreversibly turn into the straight-chain 2-ketoses. Thus, *e.g.*, 2-*C*-hydroxymethyl-D-mannose (**1**) on treatment with molybdic acid gives D-*gluco*-2-heptulose (**2**) as the only product of the transformation [3].



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ALL-TRANSGLYCOLYTIC SYNTHESIS OF BRANCHED OLIGOSACCHARIDES BELONGING TO THE BLOOD GROUP DETERMINANTS

Amedeo Vetere and Sergio Paoletti
Dept. Biochemistry, Biophysics and Macromolecular Chemistry, University of Trieste, Via L. Giorgieri 1, I-34127 Trieste (Italy); e-mail vetere@univ.trieste.it

The blood group antigens are expressed in mucins and one possible function of these molecules is the binding of microorganisms to be removed. Some tumors are associated with an increase of expression of blood groups antigens. These and other events make these molecules very interesting as modulators of biological (normal and pathological) phenomena

The genes encoding the glycosyltransferases related to blood group antigens are difficult to clone due to the low levels of protein expressed. Furtheremore their high specificity and the low avaibility of their substrates (i.e. NDP-sugars) cannot allow a extensive use of these enzymatic approach for large-scale preparation of biologically important oligosaccharides, in general, and of blood-group antigens, in particular. We have chosen the alternative enzymatic way based on the use of transglycosylation reactions, catalyzed by glycosidases, for the synthesis of biologically important oligosaccharides. In particular here we report the results concerning the synthesis of the branched oligosaccharide Lewis^x (Galβ 1-4[Fucα1-3]GlcNAc) and of its sialylated derivative Sialyl-Lewis^x (NeuAcα2-3Galβ1-4[Fucα1-3]GlcNAc). Both are antigenic determinants belonging to the Lewis group. The synthesis of the Lewis^X trisaccharide was performed using a crude preparation of an α-fucosidase from *Aspergillus niger* that allowed for the regiospecific synthesis of the disaccharide Fucα1-3GlcNAc using Fuc-PNP as the donor and GlcNAc as the acceptor. The product obtained after purification was used as the acceptor for a further transglycosylation reaction catalyzed by *Bacillus circulans* β-galactosidase affording the synthesis of the desired product. The sialylation to obtain Sialyl-Lewis^x was carried out by use of the catalytic activity of *Trypanosoma cruzi* trans-sialidase and NeuAc-MU as donor. The chemical identity of products was demonstrated by HPLC, NMR and ion spray mass spectrometry.

XYLOGLUCAN ENDOTRANSGLYCOSYLASE: EXISTENCE OF A STABLE GLYCOSYL–ENZYME INTERMEDIATE

Vladimír Farkaš*, Zdena Sulová*, Miriam Takáčová*, and Stephen C. Fry†
*Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia, and
†The Edinburgh Cell Wall Group, ICMB, University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Edinburgh EH9 3JH, U.K.

Xyloglucan endotransglycosylases (XET's) catalyse cleavage of xyloglucan molecules predominantly by transglycosylation (1,2). In this process, fragments of cleaved polysaccharide are preferentially transferred to other xyloglucan molecules or their oligosaccharide subunits, with retention of the anomericity of glycosidic bond. Previously, we proposed (1) that the cleavage and reformation of the glycosidic bond involves the formation of a glycosyl–enzyme intermediate that decomposes by transfer of the glycosyl moiety to a suitable carbohydrate acceptor. XET from nasturtium seed cotyledons, interacted with xyloglucan to form complexes of high molecular weight as judged by gel-permeation chromatography. The enzyme also showed evidence of XET:xyloglucan complex formation according to anion-exchange chromatography. The intermediate complex could be trapped on the basis of selective hydrogen-binding of its xyloglucan moiety to cellulose. The native complex was stable in water and in acidic and alkaline buffers (pH 2.5 and 9.5), but readily decomposed when incubated with xyloglucan-derived oligosaccharides. These results support the assumption that the XET from nasturtium cotyledons catalyses transglycosylation by the conventional double-displacement mechanism involving the formation of a glycosyl–enzyme intermediate characteristic of retaining glycanases.

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CROSSLINKINGS AMONG THE YEAST CELL WALL COMPONENTS

Roman Kollar[1], Bruce Reinhold[2], Eva Petrakova[3], Gilbert Ashwell[4], Herman Yeh[5] and Enrico Cabib[6]
[1]Department of Biochemical Technology, Slovak Technical University, Radlinskeho 9, 812 37 Bratislava, Slovak Republic
[2]Mass Spectrometry Resource, Boston University School of Medicine, Boston, Massachusetts 02118, U.S.A.
[3]Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland 20892, U.S.A.
[4]Laboratory of Cell Biochemistry, [5]Laboratory of Bioorganic Chemistry, [6]Laboratory of Biochemistry and Metabolism, NIDDK, Bethesda, Maryland 20892, U.S.A.

In the yeast *Saccharomyces cerevisiae,* the cell wall contains β(1-3)-D-glucan, β(1-6)-D-glucan, chitin, and mannoproteins. Cross-linkages among these cell wall components are important in endowing the wall with mechanical strength. We have previously isolated and identified the linkage region between chitin and β(1-3)-D-glucan in the form of oligosaccharide, after digestion of yeast cell wall with β(1-3)-D-glucanase, reduction with borotritide and subsequent incubation with chitinase (1). Another product isolated in the same operation had a much higher molecular weight, and we found that it represents areas in which all four structural components of the cell wall are linked together. Mannoprotein is attached to β(1-6)-D-glucan through a remnant of GPI-anchor. β(1-6)-D-glucan contains β(1-3)-linked branches and it is to these branches that chitin chains are attached. Finally, the reducing terminus of β(1-6)-D-glucan is connected to the nonreducing terminal glucose of β(1-3)-D-glucan. It is concluded that β(1-6)-D-glucan has a central role in the organization of the yeast cell wall.

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CROSS-LINKING OF PLANT CELL WALL POLYSACCHARIDES: FERULATE INCORPORATION AND DIMERIZATION

Gundolf Wende
IBLS Division of Biochemistry and Molecular Biology, Glasgow University, Glasgow G12 8QQ and Institute of Food Research, Norwich Research Park, Colney Lane, Norwich NR4 7UA, UK

Ferulic acid is an important hydroxycinnamic acid in primary cell walls. In grasses it is linked to the O-5 position of a-L-arabinofuranosides ofarabinoxylans (1,2) and in beet to O-2 of arabinans in addition to the O-6 position of galactose residues of type 1 galactans (3). Sugar beet, highly relevant to the food industry, has been shown to contain high levels of ferulate (4). Numerous roles have been suggested for feruloylated oligo-and polysaccharides. It was demonstrated (5,6) that the esterification of ferulic acid to matrix polysaccharides in an intracellular process. However, it is still in question whether or not further feruloylation and also diferuloylation occurs within the wall.

(A) The first step to investigate this process was to monitor the uptake of 14C-labelled cinnamate through sugar beet leaves and also through roots. The subsequent transformation into ferulic acid will be discussed together with information obtained from red beet suspension cultures (fed with and without 14C-cinnamate). The O-feruloyl groups are of particular interest because of their high reactivity in vivo to form diferuloyl cross-linkages. Such peroxidase-mediated coupling-reactions (7) are thought to restrict cell wall extensibility (8). Recently it has been suggested (9) that diferulates inhibit elongation in rice coleoptiles when exposed to white light.

(B) The diferulic acid referred to in the past was the 5,5-coupled dehydrodiferulate product. However, it has been shown (10) that a wide range of coupled dehydrodiferulates are possible. Work will be presented to qualify and quantify the types of diferulate bridges involved and to address their role in the process of primary growth. It is argued that dimerization is NOT the important mechanism for cessation of growth.

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BREAKING CHAINS: A COMPARISON OF METHODS FOR THE PRODUCTION OF LOW-VISCOSITY DEXTRANS AND ALTERNAN.

G.L. Cote[1], J.L. Willett[2], and K. Payne-Wahl[1]

[1]Biopolymer Research Unit and [2]Plant Polymer Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, 1815 N. University St., Peoria, Illinois, 61604, USA

Low-molecular-weight dextrans are used for such clinical applications as blood plasma extenders and starting materials for dextran sulfates. Related low-molecular weight glucans have been proposed as food ingredients. Normally, dextrans are synthesized from sucrose as high-molecular weight polymers, which are unsuitable for many applications. Strategies for producing materials of lower molecular size include acid hydrolysis, enzymolysis, peroxide-catalyzed depolymerization, ultrasonication, gamma-irradiation, and shear degradation. Synthesis in the presence of acceptors (polymerization terminators) is also an option. Each of these methods has its advantages and drawbacks. We will compare some of these methods and their suitability for various end-uses. Since our main interest is the cost-efficient production of low-viscosity alternan and branched dextrans as bulk chemicals, we have focused mainly on physical depolymerization methods. Our results suggest that the most effective method on a laboratory scale is ultrasonication, whereas twin-screw extrusion shows promise for larger-scale production. Properties of materials processed by both methods will be described.

MECHANISM OF ACTION OF ACETYLXYLAN ESTERASES: A STUDY ON ACETYLATED METHYL GLYCOSIDES AS SUBSTRATES

P. Biely[a], G.L. Coté[b], L. Kremnický[a] and R.V. Greene[b], [a]Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia; [b]Biopolymer Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL, USA

Substrate specificities of purified acetylxylan esterases (AcXEs) from several microorganisms were investigated on partially and fully acetylated methyl glycopyranosides. AcXE from *Schizophyllum commune* preferentially deacetylated the 3-position of methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (2,3,4-tri-*O*-Ac-Me-β-Xyl*p*) and methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (2,3,4,6-tetra-*O*-Ac-Me-β-Glc*p*). Removal of the 3-acetyl group was followed by slow deacetylation at positions 2 and 4 in the xyloside and position 2 in the glucoside. *S. commune* AcXE exhibited high regioselectivity of double deacetylation at positions 2 and 3 with 2,3,4,6-tetra-*O*-Ac-Me-β-Man*p*. Thus, 4,6-di-*O*-Ac-Me-β-Man*p*, a new mannose derivative, could be obtained in 80 % yield. The straightforward performance of *S. commune* AcXE on the mannoside and the liberation of acetic acid from acetygalactomannan suggests a wider specificity for the esterase. In contrast, AcXE from *Streptomyces lividans* deacetylated 2,3,4-tri-*O*-Ac-Me-β-Xyl*p* and 2,3,4,6-tetra-*O*-Ac-Me-β-Glc*p*, but not 2,3,4,6-tetra-*O*-Ac-Me-β-Man*p*. The enzyme catalyzed double deacetylation of both substrates at positions 2 and 3 to give high yields of 4-mono-*O*-Ac-Me-β-Xyl*p* and 4,6-di-*O*-Ac-Me-β-Glc*p*. AcXE from *S. lividans* exhibited an unusually high affinity for acetylxylan. Both studied AcXEs preferred partially deacetylated methyl glycosides with special arrangements of acetylated and free hydroxyl groups versus fully acetylated ones, which is in contrast to the behaviour of other types of acetylesterases or lipases. The initial rate of deacetylation of 2,4-di- and 3,4-di-*O*-Ac-Me-β-Xyl*p* by AcXE from *S. lividans* was 100-fold faster than that of 2,3,4-tri-*O*-Ac-Me-β-Xyl*p* and 2,3-di-*O*-Ac-Me-β-Xyl*p*. These observations explained the double deacetylation. The second acetyl group at position 2 or 3 is released immediately after the first acetyl group is removed from fully acetylated derivative. Therefore, regioselectivity of the deacetylation of carbohydrates by AcXEs is complementary to that of lipases and acetylesterases investigated so far in this regard. The results provided insight pertaining to the function and the action mechanism of AcXEs in deacetylation of mono- or doubly acetylated aldopyranosyl residues in acetylated polysaccharides. In addition, they suggested certain applications of these enzymes in synthetic carbohydrate chemistry.

THE PRIMARY STRUCTURE HOMOLOGIES OF PECTINESTERASES AND OF POLYGALACTURONASES

Markovič O., Stratilová E.
Institute of Chemistry of SAS, Dúbravská cesta 9, 842 38 Bratislava, Slovakia

Pectinesterase (EC 3.1.1.11) and polygalacturonases (EC 3.2.1.15, EC 3.2.1.67 and EC 3.2.1.82) belong to the group of pectic enzymes. They are present in all parts of higher plants or are produced by microorganisms. Their natural substrate is pectin, acidic heteropolysaccharide of plants cell walls.

Pectinesterase catalyzes the deesterification of pectin without changing the lenght of chain of pectin molecule. Sequence comparisons of ten primary structures of different pectinesterases suggest a special importance for three segments of the entire protein. One segment is at the N-terminal part, another in the C-terminal portion and the third is located in the central part between the two disulfide bridges of tomato pectinesterase and appears to highlight the functional site of the enzyme. Most of the ca 8% strictly conserved residues are confined to essentially four separate five-residue segments present in all ten pectinesterases in identical positions.

Polygalacturonases split hydrolytically the deesterified galacturonate chain of pectin releasing D-galactopyranuronic acid (EC 3.2.1.67), di-(D-galactosiduronic) acid (EC 3.2.1.82) from nonreducing end, or they split it in random manner (EC 3.2.1.15). The other difference is the action pattern on oligomeric substrates (EC 3.2.1.15) or the dependence of the affinity of enzymes to the substrates with various degree of polymerization. In spite of the extensive variability of the primary structure of these enzymes, functional replacements are apparently not frequent, compatible with the largely conserved substrate specifity. Possible candidates for the essential amino acids in the hydrolysis reaction of these enzymes are in a segment with localized homology to other forms, and in the C-terminal half of each molecule. Strictly conserved residues of polygalacturonases are in agreement with the findings obtained by chemical modifications of essential amino acids. The role of one histidine residue in catalytic action was also proved by its replacement to lysine. All polygalacturonases are glycoproteins, however the extent and localization of potential N-glycosylation sites differ. Some sites are quite similar and immediately adjacent to strictly conserved residues at the potential active site.

Posters

THE GAUCHE EFFECT AND CONFORMATIONS OF THE HYDROXYMETHYL AND METHOXYMETHYL GROUPS IN SACCHARIDES.

Igor Tvaroška* and Jeremy P. Carver
GlycoDesign Inc, Toronto, Ontario, Canada

Conformational properties and the gauche effect of the two functional groups, hydroxymethyl and methoxymethyl, in hexopyranosides have been studied[1] with *ab initio* methods using the methyl 2,3,4-trideoxy-α-D-*glycero*-hexopyranoside (**1**), methyl 2,3,4-trideoxy-β-D-*glycero*-hexopyranoside (**2**), methyl 6-*O*-methyl-2,3,4-trideoxy-α-D-*glycero*-hexopyranoside (**3**), and methyl 6-*O*-methyl-2,3,4-trideoxy-β-D-*glycero*-hexopyranoside (**4**) as models. The geometry of the conformers around the C5-C6 bond for the methyl 2,3,4-trideoxy-D-*glycero*-hexopyranosides was determined by gradient optimization at the SCF level using the 6-31G*, 6-31+G* and 6-311++G** basis sets. The optimized geometries were used to calculate the energy of the gauche-trans (*gt*), trans-gauche (*tg*), and gauche-gauche (*gg*) conformers with the 6-31G*, 6-31+G* 6-31+G**, 6-31G**, 6-311G*, and 6-311++G** basis sets. Electron correlation effects were accounted for at the second-order Moller-Plesset perturbation (MP2) level using the 6-31G* basis set and using the adiabatic connection method (ACM) of density functional theory (DFT) using standard 6-31G*, dzvp and cc-vpzt basis sets. Solvent effects on the stability of conformers were evaluated using a continuum model. At all levels of theory, **1** and **2** prefer the gauche over the trans conformers around the C5-C6 bond. This preference is due to internal hydrogen bonding which is possible in the *gg*(*sc*) and *gt*(-*sc*) conformers. Solvent effects decrease this preference by ~0.9 kcal/mol. Methylation of the oxygen of the C6 hydroxyl completely reversed the relative energy of conformers, such that in **3** and **4**, the trans conformer is favored. The trans preference is decreased by solvent which stabilized the gauche conformers by 0.7 - 1.3 kcal/mol with respect to the trans. These results suggest that the gauche preference of the hydroxymethyl group in **1** and **2**, is due to the presence of hydrogen bonding and not due to the gauche effect.

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*On the leave of absence from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic.

MOLECULAR MECHANICS AND SOLVATION ENERGY CALCULATIONS OF HEPARIN EPOXIDE

Ján Gajdoš, Massimo Ragazzi, and Dino R. Ferro

Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava, Slovakia
Istituto di Chimica delle Macromolecole del CNR, I-20133 Milano, Italy

Glycosaminoglycan heparin and its derivatives are known as biologically active compounds. In this paper we continue the conformational analysis [1] of heparin epoxide. The molecular mechanics program (MM) CHAMP and the interactive molecular modeling system MacroModel have been used for the computations. Net atomic charges, recently derived by "*ab initio*" calculations, were included. Solvent effect calculations have been done in a continuum model approach.

In our previous paper [1] we assumed the preferred $^5H_0$ ring form of 2,3-anhydro-α-L-guluronic unit (U). However, even if the $^5H_0$ form of the ring is computed to be more stable than $^0H_5$, both in MM (also taking solvent effect into account), and in "ab initio" (up to level 6-31+G**) calculations, we found that in higher homologs there are also $^0H_5$ structures possible. In a nonamer model of heparin epoxide introduction of "perturbation" (one $^0H_5$ conformation of the ring of U unit in seventh position in the chain) doesn't show increase in energy. Since the interconversion between structures is hindered, that intermediate form (found as saddle point about 3-4 kcal/mol) may be present and even stabilized by interaction with the solvent. This could explain the discrepancies observed in interproton distances in comparison with our former results and dynamic NMR studies on heparin epoxide in solution. We also assume that in highly polar envinronment significant distortions of U ring are possible. The present paper is taking into account also the presence of charged and neutral forms of carboxyl group of the epoxide unit U. Since the solvent effect calculations have been done only for monomers and dimers we expect to investigate the influence of solvent effect also on higher oligomers.

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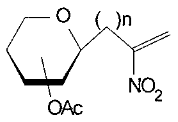
APPLICATION OF *C*-GLYCOSYLNITROALKENES IN THE SYNTHESIS OF *C*-GLYCOSYL AMINO ACIDS

M. Petrušová [a], D.-P. Pham-Huu [a], L. Petruš [a], and J. N. BeMiller [b]

[a] *Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia,*

[b] *The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907-1160, USA*

Two general routes based on the nitromethane methodology and leading to *C*-glycosyl amino acids have been elaborated. The first one as Michael acceptors utilizes 1-glycosyl-1-nitroethenes (**1**) or 3-glycosyl-1-nitropropenes (**2**), available by one-molar addition of a glycosylnitro(m)ethane to formaldehyde [1], and a Schiff base glycine ester nucleophile, and after the nitro group removal gives γ- and δ-*C*-glycosyl-α-amino acids. The second method is based on the addition of ammonia to 1-glycosyl-2-nitroethenes (**3**), available from glycosylnitromethanes through ozonolysis and nitromethane addition [2], and by the subsequent transformation of the terminal nitromethyl to carbonyl group gives α-*C*-glycosyl-α-amino acids.



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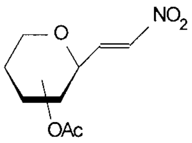

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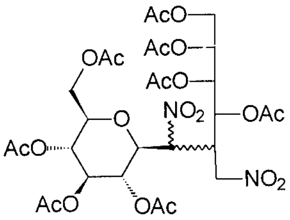
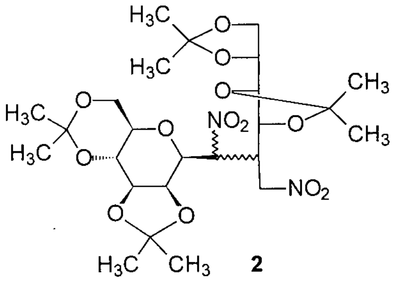
MICHAEL ADDITIONS OF FULLY FUNCTIONALIZED β-GLYCOSYL-NITROMETHANES AND 1,2-DIDEOXY-1-NITROHEX-1-ENITOLS

D.-P. Pham-Huu [a], M. Petrušová [a], L. Petruš [a], and J. N. BeMiller [b]
[a] *Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia*
[b] *The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907-1160, USA*

In recent years, *C*-disaccharide chemistry has been one of the main topics in carbohydrate chemistry, not only because of the synthetic challenges posed, but also because *C*-disaccharides have the potential to serve as carbohydrate analogues resistant to metabolic processes. In 1990, as the technology surrounding the preparation of *C*-disaccharides progressed [1], also β-glycopyranosyl-nitromethanes began to be used [2]. Now, we wish to introduce the utility of fully acetalated and acetylated β-glycopyranosylnitromethanes and 1,2-dideoxy-1-nitro-D-*arabino*-hex-1-enitols [3] in Michael additions. The additions were catalyzed with DBU utilizing THF as solvent and afforded high yields of the desired aducts **1** and **2**, which are convenient precursors of *C*-1,2-linked disaccharides.



1



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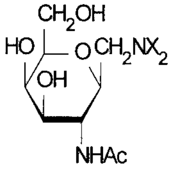
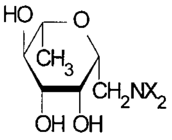
PREPARATION OF SOME *N*-ACETYL-D-GALACTOSAMINE- AND L-RHAMNOSE-DERIVED *C*-GLYCOSYL SYNTHONS

B. Pribulová, M. Petrušová, M. Matulová, and L. Petruš
Institute of Chemistry, Slovak Academy of Sciences,
84238 Bratislava, Slovakia

Epimeric 3-acetamido-1,3-dideoxy-1-nitroheptitols formed in the nitromethane synthesis with *N*-acetyl-D-galactosamine (**1**) were converted by the intramolecular cyclodehydration to an equilibrium mixture of 2-acetamido-2-deoxy-D-galactosylnitromethanes. The compounds were efficiently separated from starting **1** by chromatography on a strongly basic anion-exchange resin in the HCO_3^- form. The prevailing β-pyrano isomer **2** was converted to 2-acetamido-2-deoxy-β-D-galactopyranosylmethylamine (**3**) by reduction with iron(II) hydroxide *in situ* [1].



2: X = O
3: X = H



4: X = O
5: X = H

A similar sequence with starting L-rhamnose gave rise to a mixture of 6-deoxy-L-mannosylnitromethanes from which crystalline 6-deoxy-β-L-mannopyranosylnitromethane (**4**) obtained was analogically transformed to 6-deoxy-β-L-mannopyranosylmethylamine (**5**).

Reference:

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SYNTHESIS OF *N*-ACETYL-D-GALACTOSAMINE *VIA* BOTH OZONOLYSIS AND PEROXOMOLYBDATE OXIDATION OF A NITRONATE DERIVATIVE

J. Turjan [a], M. Petrušová [a], L. Petruš [a], and J. N. BeMiller [b]

[a] *Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia,*

[b] *The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, IN 47907-1160, USA*

N-Acetyl-D-galactosamine (**4**) is rather a common amino sugar. In spite of its relatively high abundance the amino sugar is difficult to isolate from natural sources since its polymers are not easy to purify; synthesis is a simplier way to obtain it. Here we report on two alternative possibilities of the conversion of intermediate 5-acetamido-5,6-dideoxy-6-nitro-D-altritol (**1**) and 2-acetamido-1,2-dideoxy-1-nitro-D-galactitol (**2**), readily available by the nitromethane route from D-lyxose [1], to the respective 2-acetamido-2-deoxy-D-talose (**3**) and **4**.

Both ozonolysis [2] and peroxomolybdate oxidation [3] of sodium nitronate forms of **1** and **2** gave good yields of respective acetamido sugars **3** and **4**. From their 1:4 equilibrium mixture resulting from the alkali-catalyzed izomerization the latter sugar is then obtained crystalline. Comparing the two newly introduced methods with the earlier known Nef method [1], the ozonolysis is the most advantageous as it is least laborous and affords the best yield of **4**.

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SYNTHESIS OF GLUCOPYRANOSIDURONIC ACIDS BY OXIDATION FROM DERIVATES OF GLUCOSE

M.Poláková, D. Joniak
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava

Last time for oxidation the primary hydroxyl group of saccharides Pt/C, O_2 or nitrogen dioxide were used. These methods gave low yields of products [1,2,3]. As a new method for selective oxidation of primary hydroxyl group of saccharide is used sodium hypochlorite in the presence of catalytic amounts of 2,2,6,6-tetramethyl-1-piperidinyl oxy, free radical (TEMPO) at pH 10-11 and 0 ° C. This method described [4,5] was applied in carbohydrate chemistry [6].

In our paper methyl 4-O-methyl-α-D-glucopyranosiduronic acid and methyl 2,3-di-O-substituted α-D-glucopyranosiduronic acids were prepared by oxidation of the substituted derivates of D-glucose As starting material was choosen acetyl, benzyl and acyl- protection derivates of monosaccharides and protecting groups were unaffected during the oxidation. The water insoluble monosaccharides were reacted in water in a heterogeneous suspension in presence of phase transfer catalyst (Bu_4NCl). ^{13}C NMR spectra of the oxidized saccharides gave chemical shift at 175 ppm indicating that primary alcohol group had been oxidized to a carboxylic group.

The oxidation products are starting material for preparing ester type lignin-saccharide model compounds.

Scheme1

$$RCH_2OH \xrightarrow[NaHCO_3,\ CH_2Cl_2,\ KBr,\ Bu_4NCl]{NaOCl,\ TEMPO} RCOOH$$

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SYNTHESIS OF 4-METHOXYBENZYL (METHYL α-D-GLUCOPYRANOSID)URONATE

Joniak D. and Poláková M.,
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava

The nature of interactions between lignin and polysaccharides in plant tissues has been examined extensively in our recent papers by the isolation of soluble lignin-carbohydrate complexes as well as by using of model compounds representing acetal, glycosidic ether and ester types of linkage (1,2). The subject of the present work is synthesis of 4-methoxybenzyl ester of methyl α-D-glucopyranosiduronic acid from methyl α-D-glucopyranoside.

Primary hydroxyl groups in methyl α-D-glucopyranoside was rapidly oxidised to carboxylic acid with the selectively method to oxidation of primary hydroxyl grup (3,4) using catalytic amounts of 2,2,6,6,-tetramethyl-1-piperidinyloxy (TEMPO) and sodium hypochlorite/sodium bromide as the regenerating oxidant in water medium. in 60 % yields. The less protected methyl α-D-glucopyranoside where three secondary hydroxyl groups were competing with the primary hydroxyl group still led to the carboxylate as the main products. The selectivity for primary hydroxyl groups towards oxydation in this sugar is evident.

4-Methoxybenzyl (methyl α-D-glucopyranosid)uronate was prepared by electrofilic reaction of N,N-dimethylformamide dineopentyl acetal and 4-methoxybenzyl alcohol with methyl α-D-glucopyranosiduronic acid. Neopentyl acetal would not esterify acid (5,6), however it enters into acetal exchange reaction with 4-methoxybenzyl alcohol, resulting in esterification of glucuronic acid..

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PREPARATION OF SOME N - ACETYLGLYCOSYLAMINES AND N - ACETYLDIGLYCOSYLAMINES

K. LINEK, J. ALFŐLDI, T. STACH, and V. PÄTOPRSTÝ

Institute of Chemistry, Slovak Academy of Sciences
SK-842 38 Bratislava

N-Acetylation of (2,3,4,6-tetra-*O*-acetyl-α-ᴅ-glucopyranosyl)(2,3,4,6-tetra-*O*-acetyl-β-ᴅ-glucopyranosyl)amine or bis(2,3,4,6-tetra-*O*-acetyl-β-ᴅ-glucopyranosyl)amine afforded *N*-acetyl-bis(2,3,4,6-tetra-*O*-acetyl-β-ᴅ-glucopyranosyl)amine; deacetylation of the latter gave *N*-acetyl-di-β-ᴅ-glucopyranosylamine. Similarly, N-acetylation of bis(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)amine led to *N*-acetyl-bis(2,3,4-tri-*O*-acetyl-β-ʟ-rhamnopyranosyl)amine and its deacetylation to di-β-ʟ-rhamnopyranosylamine. The structure, anomeric configuration and conformation of the above-mentioned compounds and also of *N*-acetyl-β-ᴅ-xylopyranosylamine,*N*-acetyl-2,3,4-tri-*O*-acetyl-β-ᴅ-xylopyranosylamine, *N*-acetyl-β-ᴅ-ribopyranosylamine, and *N*-acetyl-2,3,4-tri-*O*-acetyl-β-ᴅ-ribopyranosylamine were determined by ^{1}H, ^{13}C NMR,and mass spectrometric methods.

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SYNTHESES OF SOME GLYCOSYLAMINES AND DIGLYCOSYLAMINES

K. LINEK, T. STACH, J. ALFÖLDI, V. PÄTOPRSTÝ, Z. SULOVÁ and V.FARKAŠ

Institute of Chemistry, Slovak Academy of Sciences
SK-842 38 Bratislava

Recently we were engaged in the synthesis, structure and conformation elucidations of glycosylamines and diglycosylamines of D-glucose, D-xylose /1/, L-rhamnose, D-mannose, D-arabinose /2/, D-galactose /3/, and their N-acetylderivatives /4/. As found, the preffered anomeric configuration of both glycosylamines and diglycosylamines was that having the amino group in equatorial position /1-3/.

Glycosylamines and diglycosylamines are compounds of interest to enzymologists, because these amines are reported to inhibit some glycosidases /5/; diglycosylamines, viz. /α-D-glucopyranosyl//β-D-glucopyranosyl/-amine and di-β-D-glucopyranosylamine are effective inhibitors of β-glucosidase of Trichoderma resei /5/.

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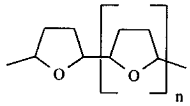
SYNTHESIS FROM THIOSUGARS OF CHIRAL *THF-THF* OR *THF-THP* BICYCLIC BUILDING BLOCKS

C. Lorin, P. Rollin

Institut de Chimie Organique et Analytique, Université d'Orléans,
B.P. 6759, F-45067 Orléans Cedex 2, France.

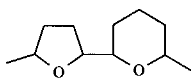
Chiral THF units are frequently encountered in the structure of many bioactive naturally-occurring products[1]. 2,5-Connected oligotetrahydrofurans of type **I** are the most common structural key elements of the Annonaceous acetogenins[2], a growing class of phytomolecules which present outstanding pesticidal and cytotoxic properties.

Furthermore, some acetogenins recently isolated from *Rollinia mucosa*[3] display new oxacyclic sub-structures of the muconin- (**II**) and the mucoxin-type (**III**).

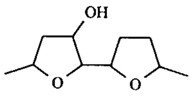


I

n = 0, 1 or 2



II



III

Starting from novel azaheterocycle / thiosugar hybrids[4], we have developed original regio- and stereocontrolled ring-closure approaches to deliver chiral bicyclic building blocks based either on the THF-THF or on the THF-THP podand templates.

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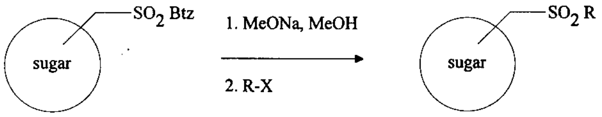
A NOVEL INTRAMOLECULAR MIGRATION IN SACCHARIDIC SULFONES : BENZOTHIAZOL-2-YL GROUP TRANSFER THROUGH AN *IPSO*-SUBSTITUTION

D. Gueyrard[a], C. Lorin[a], J. Moravcova[b], P. Rollin[a]

a/ Institut de Chimie Organique et Analytique, Université d'Orléans, B.P. 6759, F-45067 Orléans Cedex 2, France.
b/ Institute of Chemical Technology, Technicka 5, 166 28 Praha, Czech Republic.

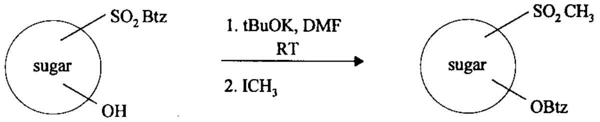
In the course of ongoing projects involving sugar-derived heteroarylsulfones and their use in stereocontrolled synthesis, we have checked that the benzothiazol-2-ylsulfonyl moiety is prone to undergo nucleophilic *ipso*-substitutions.[1, 2, 3]

We have already taken advantage of this property for synthesizing alkyl sulfones derived from D-galactose[4] and the method could further be extended to other sugar series.[5]



Btz : benzothiazol-2-yl R : alkyl or functionalized chain

In the case when free hydroxyl groups are available in sugar-derived sulfones, an internal S→O-transfer of the benzothiazol-2-yl group can be observed.



This new type of intramolecular group migration was shown to occur in miscellaneous structural situations of several sugar series.

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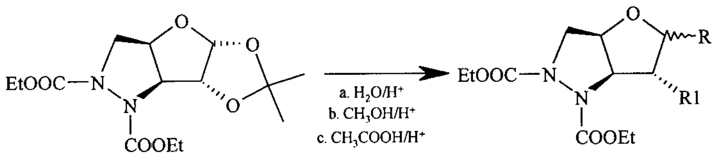
SYNTHETIC STUDIES OF A PENTOFURANOSE WITH A FUSED PYRAZOLIDINE RING

J. Moravcová[a], R. Bláhová[a], P. Střížová[a], C. Lorin[b], P. Rollin[b]

[a]Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Technická 5, 166 28 Praha, Czech Republic
[b] ICOA, Université d'Orléans, B.P. 6759, F-45067 Orléans Cedex 2, France

Pyrazolidine **1** represents a new type of uncommon saccharide/heterocycle hybrid which could be an attractive target for the drug design. Starting from 1,2-*O*-isopropylidene-α-D-ribofuranose, pyrazolidine **1** is readily obtained (1) through a one-step procedure involving optimized Mitsunobu conditions. Precipitation of by-products of the reaction (2) allowed minimization of chromatographic separations : this method can therefore be adapted for a large scale application.

Hydrolysis, methanolysis, and acetolysis of the title compound **1** were investigated using ^{1}H-NMR, ^{13}C-NMR or GC(LC)/MS techniques. In all cases, the formation of a complex mixture of products was observed.



1

a. $R = R_1 = OH$
b. $R = OCH_3, R_1 = OH$
c. $R = R_1 = OCOCH_3$

Among those products, the proportion of isomers arising from the acyclic form was surprisingly high : such a fused ring arrangement apparently introduces strain into the furanose form.

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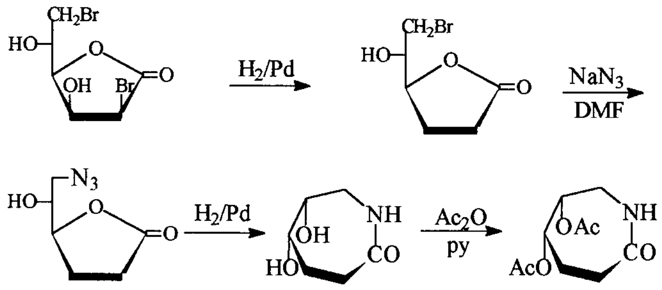
PREPARATION AND STRUCTURE OF AMINODEOXYHEXONOLACTAMS

K. Kefurt [a], M. Hamerníková[a], J. Havlíček[a], H.Votavová [b], I. Raich[a]

a/ Institut of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic
b/ Institut of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 16610 Praha 6, Czech Republic

Following the previous publication concerning the structure of seven-membered lactams derived from 6-amino-6-deoxyhexonic acids (1) the series of lactams of 6-amino-2,6-dideoxy-, 6-amino-2,3,6-trideoxy- and 6-amino-3,6-dideoxyhexonic acids is studied. The scheme presents the last steps in the synthesis of 6-amino-2,3,6-trideoxy-D-*erythro*-hexonolactam as an example.



The prevailing conformations of the lactams and their *O*-acetylderivatives in solutions were determined from ^{1}H-NMR data using the modified Karplus equation (2).

References :

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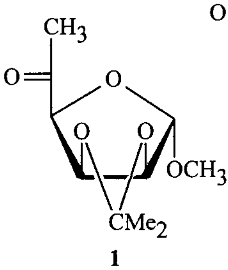
PREPARATION OF SOME *C*-GLYCOSYLATED α-AMINO ACIDS *VIA* CORRESPONDING HYDANTOINS

Bohumil STEINER, Miroslav KOÓŠ

Institute of Chemistry, Slovak Academy of Sciences,
Dúbravská cesta 9, SK-842 38 Bratislava, Slovak Republic

Sugar amino acids represent a very important class of organic compounds which in diversity and ubiquity has few parallels in the biological field. Their biological and medical importance with regard to distribution, metabolism, interactions, biological activity, *etc*. has inspired rapid increase of synthetically prepared amino sugar-containing molecules.

Starting from methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-hexofuranosid-5-ulose (**1**) (prepared in five steps from D-mannose), we have recently prepared corresponding *C*-5 epimeric amino nitriles using the classical Strecker synthesis. Because of difficulties observed during basic hydrolysis of these amino nitriles to amino acids, we have used an alternative procedure - preparation of hydantoin **2** which is, after removal of 2,3-*O*-isopropylidene group under mild acid conditions to produce 2,3-*O*-deprotected sugar **3**, much more susceptible to basic hydrolysis affording sugar α-amino acid **4**.



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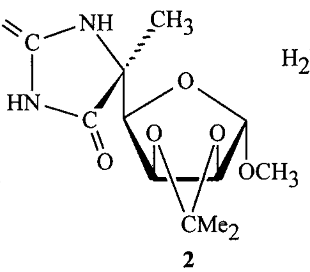

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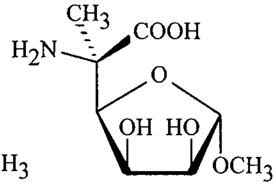

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Structure of all synthesized compounds was confirmed by ^{1}H and ^{13}C NMR spectra using 2D techniques and by the EI and CI mass spectral data. 6-Deoxy-D-*manno* configuration was confirmed by X-ray analysis.

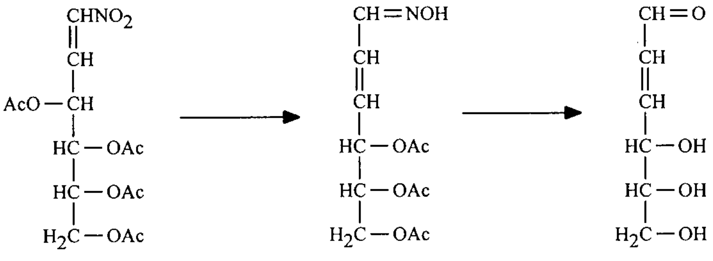
A CONVENIENT SYNTHESIS OF 2,3-UNSATURATED SUGAR DERIVATIVES

Miroslav KOÓŠ, Bohumil STEINER

Institute of Chemistry, Slovak Academy of Sciences,
Dúbravská cesta 9, SK-842 38 Bratislava, Slovak Republic

Zinc in acetic acid has proven to be a very effective reducing agent for converting nitroalkenes into oximinoalkanes. This reducing agent was successfully applied to aliphatic nitroalkenes, to produce saturated ketoximes.

When zinc in acetic acid is applied to D-*arabino*-3,4,5,6-tetraacetoxy-1-nitro-1-hexene (**1**), corresponding 2,3-dideoxy-4,5,6-tri-*O*-acetyl-D-*erythro*-hex-2-enose oxime (**2**) is formed directly in high yield. Deacetylation and deoximation gave known 2,3-dideoxy-D-*erythro*-hex-2-enose (**3**).



1 **2** **3**

Analogous L-*erythro*- and D-*threo*-hex-2-enose oximes and D-*arabino*-hept-2-enose oxime were obtained by this method in about 90 % yields starting from corresponding peracetoxy-1-nitro-1-alkenes prepared from L-arabinose, D-xylose, and D-mannose.

Since the oxime group and double bond in the products like **2** may be modified through a variety of reactions, this method may be of use for preparing further interesting and not so readily available sugar derivatives. The limiting factor is, of course, the availability of starting 3-*O*-acetylated sugar 1-nitroalkenes.

LINKAGE CHARACTERISATION OF ALDITOL DISACCHARIDES BY FAST ATOM BOMBARDMENT COLISION INDUCED DISSOCIATION TANDEM MASS SPECTROMETRY OF SODIUM CATIONIZED IONS.

Vladimír Kováčik, Ján Hirsch
Institute of Chemistry, Slovak Academy of Sciences,
84238 Bratislava, Slovak Republic

Wigger Heerma
Bijvoet Center for Biomolecular Research, Faculty of Chemistry,
University Utrecht, 3508 Utrecht, The Netherlands

The oligosaccharide alditols, labelled in C-1 position by deuterium possessing various types of the linkage between cyclic (pyranoid or furanoid) and alditol units have been examined by fast atom bombardment and collision induced tandem mass spectrometry (FAB CID MS/MS) of protonated and sodium cationized molecular ions. The CID MS/MS spectra of $[M+H]^+$ species give the information about molecular weight and the masses of monomeric units. The CID spectra of sodium-cationized compounds show additional fragment ions resulting from cross-ring cleavages.

The criteria for linkage analysis of alditol disaccharides have been proposed following the eliminations of water, methanol, formaldehyde and ethylene glycol molecules from FAB CID MS/MS spectra of sodium cationized molecules.

References: V. Kováčik, J.Hirsch, W. Heerma, Rapid Commun.in Mass Spectrom. (in press).

ON MECHANISM OF SIMPLE SACCHARIDE TRANSFORMATIONS

J.Königstein

Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovakia

The enolization of glycolaldehyde is a general-acid-base-catalyzed reaction. Glyoxal obtained from glycolaldehyde after oxidation in DCL and NaOD in D_2O was isolated as quinoxaline. This contains incorporated D during enolization, which proves the validity of the endiol mechanism. Abstraction of proton in the 1,2-position with respect to the carbonyl group in the glycolaldehyde is the rate-determining step.

When the oxido-reduction disproportionation and aldolization of methylglyoxal in alkaline solution are carried out in the presence of NaOD(D_2O), D-containing lactic acid is not formed as product of oxido-reduction disproportionation. This proves that the intramolecular reaction mechanism involves a transfer of the hydride anion within the hydrated methylglyoxal molecule. In agreement with the general mechanism of aldolization reactions, aldolization of methylglyoxal yields hexan--4-ol-2,3-dione-1-al. The primary product undergoes further conversions through a system of consecutive reactions. Products of these processes were converted into silylated oximes or quinoxaline derivatives and studied by GC-MS technique.

Open dimers of ketoforms, keto and enol forms and open trimer have been identified among the products of aldolization of methylglyoxal. The formation of the dimer of two enol forms, or a higher than trimeric product or cyclic forms has not been observed.

The transformations of trioses are general-acid-base-catalyzed reactions. By incorporation of D into reaction components also in acid medium the endiol mechanism was confirmed. A good agreement between yield of D incorporation and kinetic data indicates the similarity of reaction mechanismus involved (1).

References:

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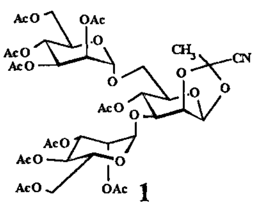
EFFICIENT SYNTHESIS OF BRANCHED OLIGOMANNOSIDES

Leon V. Backinowsky,[1] *Sergey A. Nepogodiev,*[2] *Polina I. Abronina*[1]

[1]N D Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences,
Leninsky pr. 47, Moscow, Russia

[2]School of Chemistry, University of Birmingham, Edgbaston,
Birmingham B15 2TT, UK

Synthesis of highly branched (dendritic type) oligosaccharides requires the use of unambiguous and efficient glycosylation procedures. A new approach to the preparation of oligomannosides bearing a 3,6-(Man)$_2$-Man fragment is proposed. This is exemplified in the TrClO$_4$-catalysed condensation of an easily accessible, trisaccharide 1,2-O-cyanoethylidene derivative (**1**) as a glycosyl donor with mono- or bis-trityl ethers (**2** and **3**) as glycosyl acceptors, which results in the resprective higher oligosaccharides (**4** and **5**). The Helferich glycosylation of mannose 1,2-O-cyanoethylidene derivative was employed for the preparation of **1**, and selective 3- or 3,6-bis-glycosylation of the corresponding 3,6-di-O-trityl-α-D-mannosides (the Bredereck reaction) was the key step in the synthesis of **2** and **3**.



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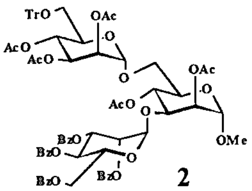

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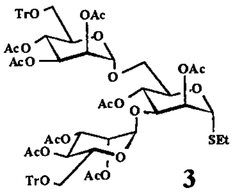

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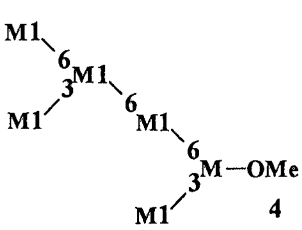

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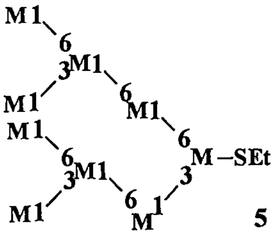


5

Transglycosylase activity of α-galactosidase isolated from the yeast *Aureobasidium pullulans*

I. Jančová and N.Kolarova
Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia

The natural function of glycosidases is to catalyse the hydrolysis of glycosidic linkages. Hydrolysis reactions are transfer reactions involving water as the glycosyl acceptor. In a reaction system containing only small amount of water, it is possible to displace the equilibrium of the reaction towards the synthesis of glycosides.

The inducible extracellular α-galactosidase from the yeast *Aureobasidium pullulans* was purified by gel chromatography. The α-galactosidase activity was highest at pH 4.8 and at temperature 60°C. The kinetic constants K_m (measured for hydrolysis of 4-nitophenyl-α-D-galactopyranoside) was 0.57 mM. Transglycosylation activity of this enzyme will be discussed.

Enzymic Synthesis of α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-D-Gal*p*

D. Sabová*, N. Kolarova*, M.K. Bhat** and P. Biely*; *Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia; **Institute of Food Research, Reading Laboratory, Early Gate, Reading, RG6 6BZ, UK

Condensation of monosaccharides and glycosyl transfer reactions catalyzed by glycosyl hydrolases under special reaction conditions, such as high substrate concentration, represent a simplier alternative to multistep organic syntheses of oligosaccharides. In enzyme-catalyzed reactions, a high positional selectivity is achieved without protecting hydroxyl groups and without use of hazardous chemicals [1].

An extracellular α-galactosidase (EC 3.2.1.22) of a thermophilic fungus *Thermomyces lanuginosus* produced during growth on locust bean galactomannan was partially purified from a culture fluid and tested for ability to catalyze condensation and glycosyl transfer reactions leading to oligosaccharides. The enzyme was found to catalyze the formation of a trisaccharide from D-galactose at high substrate concentration. The trisaccharide, obtained in about 10% yield, was isolated from the reaction mixture by gel filtration on Biogel P2. On the basis NMR spectroscopy its structure appears to be identical with α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-D-Gal*p*. An interesting feature of the reaction is that it does not lead to other oligosaccharides. The trisaccharide appears to be the only reaction product. The formation of the trisaccharide showed an optimum at pH 4.8, a temperature of 60-65°C and D-galactose concentration of 45% (w/w). The reason for the formation of a single reaction product will be investigated.

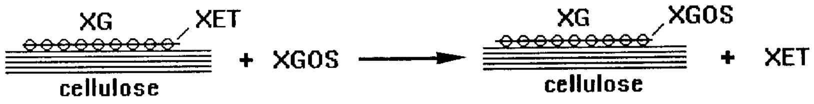
[1] Nilsson K.G. (1988) Trends Biotechnol. 6:256-264.

Acknowledgement. This work was supported by the Commission of the European Communities through the INCO Copernicus programme and by the Slovak Grant Agency VEGA (2/4147/97).

AFFINITY PURIFICATION OF XET BASED ON THE ENTRAPPMENT OF PRODUCTIVE XET:XYLOGLUCAN COMPLEX ON CELLULOSE

Zdena Sulová, Jozef Nahálka and Vladimír Farkaš
Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia

Xyloglucan endotransglycosylase (XET) catalyses the cleavage of xyloglucan (XG) molecules by a transglycosylation mechanism involving two steps: (a) endo-cleavage of the β-(1-4)-linked polyglucosyl backbone of xyloglucan molecule under formation of a glycosyl-enzyme intermediate; (b) transfer of the glycosyl residue from the intermediate to C-4 position of the nonreducing-end glucosyl unit of another XG molecule or XG-derived oligosaccharide. A method for the affinity purification of XET has been devised, based on the known tendency of xyloglucan to bind tightly via hydrogen bonds to cellulose (1,2). The crude extract containing the enzyme was mixed with xyloglucan and applied to cellulose. After drying, the cellulose with adsorbed XET:XG complex was washed thoroughly with buffer containing 0.5 M NaCl to remove unadsorbed material and the XET was released from the complex into solution by incubation with XG-derived oligosaccharides (XGOS) according to scheme:



Based on this principle, combined with gel-permeation chromatography, about 120-fold purification of XET from nasturtium cotyledons and from the supernatant of suspension-cultured *Catharanthus* cells was achieved.

References:
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(2) Farkaš, V., Sulová, Z., Takáčová, M., Fry, S. C. (1997). This symposium, p....

XYLOBIOSE AND MANNOBIOSE PERMEASE OF *AUREOBASIDIUM PULLULANS.*

Kremnický L. and Biely P., Institute of Chemistry, Slovak Academy of Sciences,
Dúbravská cesta 9, 842 38 Bratislava, Slovak Republic

Yeast-like microorganism *Aureobasidium pullulans* is known as a very good producer of inducible enzymes hydrolyzing major plant hemicelluloses, xylans and β-mannans (1,2).

When washed glucose-grown cells of *A. pullulans* were supplied with the natural inducer of the xylanolytic enzyme system, β-1,4-xylobiose, initialy low rate of disaccharide uptake increased considerably after 1-2 hour lag phase. When β-1,4-xylobiose was added to xylan grown cells, disaccharide dissapeared from the medium rapidly without any lag phase. Disaccharide transport to the cells could be blocked by inhibitors of energy metabolism, 2,4-dinitrophenol or sodium azide. Increase in the rate of β-1,4-xylobiose uptake was prevented by addition of cycloheximide, an inhibitor of proteosynthesis. When glucose-grown cells were supplied with β-1,4-mannobiose, the inducer of the mannanolytic enzyme system, for transport of β-1,4-mannobiose simillar results were obtained as for transport of β-1,4-xylobiose during induction of xylanolytic system. Both transport systems were specific for the corresponding disaccharides. These results indicate the existence of two inducible *de novo* synthesized active transport systems associated with hemicellulose utilisation in investigated yeast. They were named xylobiose permease and mannobiose permease.

Both permeases were induced by the same inducers as corresponding hemicellulolytic systems - xylobiose permease was induced by xylan, xylobiose, xylose and by methyl-β-*D*- xylopyranoside (inducers of xylanolytic system), mannobiose permease was induced by galactomannan, mannobiose and by methyl-β-*D*-mannopyranoside (inducers of mannanolytic system). Synthetic glycosides methyl-β-*D*-xylopyranoside and methyl-β-*D*-mannopyranoside were slowlier and less effective inducers than xylose, β-1,4-xylobiose and β-1,4-mannobiose. Both xylobiose permease and mannobiose permease activities were inhibited by glucose. Substrate specificity of both permeases will be discussed.

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1. Leathers T.D., Detroy R.W. and Bothast R.J., Biotechnol. Lett., 8, 867-872 (1986)
2. Kremnický L. and Biely P., Arch. Microbiol., 167, 350-355 (1997)

Fluorogenic substrates for endo-β-1,4-xylanases

M. Vršanská*, W. Nerinckx**, Peter Biely* and M. Claeyssens**
*Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia
**Universiteit Gent, Laboratorium voor Biochemie, Gent, Belgium

Endo-β-1,4-xylanases (EC 3.2.1.8) represent the main enzyme components of microbial xylanolytic systems which play the major role in xylan depolymerization. Endoxylanases usually differ in physico-chemical properties, such as molecular mass and isoelectric point. Based on hydrophobic cluster analysis and amino acid sequence homologies, endoxylanases were classified into two glycanase families, family 10 and 11 (formerly F and G) [1]. How to identify, detect and differentiate different types of endoxylanases? For this purpos, new fluorogenic substrates, 4-methylumbelliferyl-β-D-glycosides of xylobiose and xylotriose were synthesized. Hexa-*O*-acetylxylobiose and octa-*O*-acetylxylotriose were treated with benzylamine to deacetylate the C-1 position. The resulting penta-*O*-acetylxylobiose and hepta-*O*-acetylxylotriose reacted with trichloroacetonitrile to give the corresponding 1-α-trichloroacetimidates. These were condensed with 4-methylumbelliferone to give 4-methylumbelliferyl-per-*O*-acetyl-β-xylobioside and β-xylotrioside. On deacetylation the compounds afforded 4-methylumbelliferyl-β-xylobioside (Umb-Xyl_2) and 4-methylumbelliferyl-β-xylotrioside (Umb-Xyl_3). Both glycosides were found to be suitable substrates for endoxylanases, particularly for extremely sensitive detection of the enzymes in gels and for fluorometric determination of their activities. Several endoxylanases of family 10 and 11 were tested on both substrates. Umb-Xyl_2 was hydrolyzed at the aglyconic bond practically by all examinated enzymes. However, not all endoxylanase forms were so well detectable by Umb-Xyl_3, apparently due to different type of the cleavage, namely to xylobiose and and Umb-Xyl. The detection of endoxylanase with the fluorogenic glycosides is very fast in comparison with other methods so far used and, moreover, the liberation of 4-methylumbelliferone can be followed under UV light from very early stages of hydrolysis.

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THE GLYCOENZYME-BINDING ASSAY FOR THE STUDY OF INTERACTION OF CONCANAVALIN A WITH MANNO-SACCHARIDES

Mislovičová, D., Vikartovská, A. and Gemeiner, P.
Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava,
Slovak Republic

The glycoenzymes (invertase and glucose oxidase) were used as the competitive markers for a simple and rapid determination of the lectin-saccharide interactions. The method is based on the formation of the conjugate of an appropriate glycoenzyme with specific carbohydrate-binding lectins and the inhibition of the conjugate formation with a monosaccharide. This method was used to estimate the relative carbohydrate specificity of Concanavalin A for manno-saccharides. The inhibition effect of the saccharides on the formation of Concanavalin A - glycoenzyme precipitate was compared with their influence on the enzyme sorption on conjugate Concanavalin A-bead cellulose support.

The amount of the interacting enzyme was estimated either indirectly from its concentration in a supernatant that was determined spectrophotometrically (Con A was in a free or immobilized form) or directly in the immobilized form linked to Con A-sorbent using the flow microcalorimetric method. The results obtained, using different methods, agreed in general.

EXOPOLYGALACTURONASES PREFERING OLIGOGALACTURONIDES WITH LOW DEGREE OF POLYMERIZATION AS SUBSTRATES

Stratilová E., Dzúrová M.

Institute of Chemistry of SAS, Dúbravská cesta 9, 842 38 Bratislava, Slovakia

There are described exopolygalacturonases produced by *Aspergillus tubigensis* (1) and carrots (2) with active site consisting from five subunits with the resulting increase of reaction rate with the increasing degree of polymerization of the substrate (DP) to DP corresponding five. The polymeric substrate is equally or a little bit slowly degraded as the pentasaccharide.

An other form of exopolygalacturonase clearly prefering oligomeric substrates (DP 3-4) was found in juice from carrot roots, pectic enzymes preparation Leozyme (byproduct by the manufacturing of citric acid by *Aspergillus niger* in Slovak Canning Factory and Destilleries, Leopoldov, Slovakia) and the cultivation media after *Aureobasidium pullulans* growth. The polymeric substrate is degraded by the same rate as the dimer, which is degraded with the sooner described exopolygalacturonases extremely slowly.

All these exopolygalacturonases showed an acidic isoelectric point and pH optima of their action. They were able to degrade Ostazin Brilliant Red - Galacturonate DP 10, a substrate, which was held to be a specific substrate for distinquishing of polygalacturonases and exopolygalacturonases based on the unability of exopolygalacturonases to degrade it (3).

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THE PECTIC ENZYMES COMPLEX OF CARROTS

Stratilová E., Markovič O., Dzúrová M., Malovíková A., Capek P.
Institute of Chemistry of SAS, Dúbravská cesta 9, 842 38 Bratislava, Slovakia

The pectic enzymes complex of late spring and autum carrots (carrot roots juice, extract with 1 M and 2 M NaCl, and 1 M NaCl extract of carrot leaves) was evaluated. Pectinesterase, the main enzyme of the fraction extracted with 2 M NaCl, was purified to homogeneity combining gel-permeation and ion-exchange chromatographies. This enzyme had an extremely basic isoelectric point and blocked N-terminus. No significant difference between late spring and autum form was found.

Five forms of exopolygalacturonase were observed in the carrot juice and the 1 M NaCl extract. They differed in isoelectric points, molecular ratio, pH optima and substrate preference.

The carrot roots juice contained pectin and pectate lyase activities, while rhamnogalacturonase activity was found in the 1 M NaCl extract of roots. Rhamnogalacturonate lyase activity was present in all fractions of carrot roots, mainly in the 1 M NaCl extract. No polygalacturonase activity was detected in roots. The extract of carrot leaves was free of rhamnogalacturonate lyase activity and there were found only traces of pectinesterase activity; other pectic enzymes were present. Pectin acetylesterase was present in all fractions.

THE OCCURRENCE OF YEASTS AND THEIR EXTRACELLULAR GLYCOPROTEINS PRODUCED IN STRESS ENVIRONMENTS

E. BREIEROVÁ, E. STRATILOVÁ AND E. SLÁVIKOVÁ
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava

The ability to adapt to stress conditions was studied on the eight yeast species isolated from water enviroment (river, lake and fish-pound). The adaptation of isolated strains to an extreme environmental situation (higher water activity - a_w) correlated with qualitative and quantitative differences in mannoproteins and glucans of their extracellular glycoproteins.

Hansenula anomala CCY 38-1-22 a *Rhodotorula rubra* CCY 20-7-29 belonged to the most resistant strains. Phosphomannans were produced the as extracellular glycoproteins of predominance by these two species. On the contrary sensitive species such as *Saccharomyces cerevisiae* CCY 21-4-100 and *Candida maltosa* CCY 29-97-15 produced extracellular glycoproteins with higher content of glucans. The pullulans produced by *Aureobasidium pullulan* CCY 27-1-111 in control conditions were not produced under stress environments. Separation of the glycoproteins fraction on Superose 12 column yielded fractions with M_r ranging from 13 to 2 000 kDa.

The yeast cultures ability to resist stress conditions enables them to occur in locality with unfavourable influence of environment.

SEROLOGICAL ACTIVITY OF A SMOOTH LIPOPOLYSACCHARIDE OF *CHLAMYDIA PSITTACI*

R. TOMAN[1], Ľ. ŠKULTÉTY[1], E. KOVÁČOVÁ[1], and V. PÄTOPRSTÝ[2]
[1]*Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Slovakia*
[2]*Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovakia*

Chlamydia psittaci is a widely distributed obligatory phagosomal intracellular bacterium, which exhibits a broad pathogenic potential for birds, ruminants, other animals and man. It has been shown that the chlamydial lipopolysaccharide (LPS) is phenotypically of the rough (R) type. In two strains of *C. psittaci* and in *C. trachomatis* serotype L_1, the presence of a smooth (S) LPS, in addition to the known (R) LPS, has been suggested. However, the (S) LPS could not be isolated in order to determine its chemical composition and structure.

Most recently, we have isolated a (S) LPS from the supernatant of yolk sacs of embryonated chicken eggs infected with *C. psittaci* strain PK5082 that is associated with enzootic abortion in ewes. The structure/function relationship studies of this LPS may offer some intriguing possibilities in terms of the mechanisms of infection and immunopathology. In these initial studies, serological reactions of the LPS with polyclonal rabbit sera as revealed by ELISA and immunoblot will be reported.

COMPOSITION STUDY OF LIPOPOLYSACCHARIDES IN THE PHASE TRANSITION OF *COXIELLA BURNETII*

Ftáček P.[1], Škultéty Ľ.[1], Pätoprstý V.[2], and Toman R.[1]
[1]*Institute of Virology,Slovak Academy of Sciences, 842 46 Bratislava, Slovakia*
[2]*Institute of Chemistry,Slovak Academy of Sciences, 842 38 Bratislava, Slovakia*

The rickettsial agent of Q fever, *Coxiella burnetii,* undergoes a transition from virulent (phase I) to avirulent (phase II). This phase transition correlates with some of the biological characteristics of the smooth-to-rough lipopolysaccharide (LPS) variation observed the Gram-negative bacteria. *Coxiella burnetii* in phase I may be converted to phase II by serial passage in the yolk sacs of embryonated eggs. Phase I organisms express a smooth (S) LPS with O-polysaccharide chain, whereas phase II organisms express a rough (R) LPS.

The transition of phases was monitored by serological methods but it was not investigated with respect to both composition and structure of *Coxiella burnetii* LPS. The LPSs isolated from the cells of various egg passages (EPs) were fractionated by steric exclusion chromatography (Biogel 60) and the individual LPS fractions analyzed by the respective physico-chemical methods.

The results have shown both the quantitative and qualitative changes of LPSs in dependence on EP. With the progress in EP, a decrease of yields of the high molecular weight fractions was observed (EP3 50% yield, EP40 23% yield) together with an increase of yields of the low molecular weigh fractions (EP3 2% yield, EP40 23% yield). Similarly, the changes in saccharide compositions of the high molecular weight fractions were observed. Thus, an increase of major constituent sugars and a decrease of minor constituent sugars in dependence on a progress in EP were found.

FT RAMAN SPECTRA OF POLYGALACTURONIC ACID AND PECTINS

Andriy Sinitsya, Jana Čopíková and Pavel Matějka
Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic

Raman spectroscopy is not a wide-spread method in analysis of pectins, but it is very useful in structural investigation of mono-, oligo- and some polysaccharides (1-4). Raman spectra of polygalacturonic acid, citrus and sugar beer pectins were measured and interpreted compared with the IR results. The most intensive band of this spectra (2950-2940 cm^{-1}) is assidned to the vibrations of C-H bonds. In IR spectra this band is overlapped by the broad and intense absorption band of O-H bonds not observed in Raman spectra. The strong Raman band at 853 cm^{-1} indicates α-glycosidic bond in pectins. The corresponding IR band is very weak and overlapped by the intense absorption at 833cm^{-1}. Hydrolysis of methyl ester groups leads to decreasing at 2960-2940 (νCH_3), 1746 ($\nu C{=}O_{ester}$), 1458 ($\delta_{as}CH_3$), 1380 ($\delta_s CH_3$) and 925 (ρCH_3) cm^{-1}. Forming of potassium salts of polygalacturonic acid and pectins is followed by decreasing at 1736 cm^{-1} ($\nu C{=}O_{COOH}$). Weak band at 1612 ($\nu_{as}COO^-$) and strong band at 1405($\nu_s COO^-$) cm^{-1} appear in the spectra of pectin salts. In IR spectra the first band is much more intensive than the second. The weak bands at ca. 1672 (Amid I) and 1594 cm^{-1} (Amid II) appear in the spectra of amidated pectins. Raman spectra of sugar beet pectin have peaks at 1633 cm^{-1} and 1602 cm^{-1}. This bands belong to aromatic system of feruloil groups linked to the pectin macromolecules.

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CURVE-FITTING ANALYSIS IN DIFFUSE REFLECTANCE FTIR AND ^{13}C CP/MAS NMR SPECTROSCOPY OF PECTINS

Andriy Sinitsya, Jana Čopíková, Vladimir Machovič and Helena Pavliková*
Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic
*Institute of Macromolecular Chemistry, Academy of Sciences of Czech Republic,
Heyrovského nám. 2, 162 06 Prague 6, Czech Republic

Diffuse reflectance FTIR and ^{13}C CP/MAS NMR spectra of citrus and sugar beet pectins were measured and used for the curve-fitting analysis. The IR spectra were studied in the region of 1850-1500 cm^{-1} by the curve-fitting program. This region containes ν(C=O), Amid I and ν_{as}(COO$^-$) vibration bands useful in quantitative analysis of pectins (1-4). The products of Gaussian and Lorentzian curves and the initial half width of 35-40 were used in the analysis. ^{13}C CP/MAS NMR spectra were studied in C-6 region (180-165 ppm). The chemical shift and the shape of peaks in this region are strongly influenced by the relation between different forms of uronic carboxyl groups in pectin (5). Multiple Gaussian fitting procedure with initial half width of 0.5-1.0 was used in analysis of NMR spectra. The relative content of different forms of uronic carboxyl groups and in case of sugar beet pectins acetyl groups was calculated on the basis of the areas of separated peaks. Results of curve-fitting were applied to calculation of the degrees of methylation (DMe), amidation (DAm) and acetylation (DAc).

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USE OF NEAR INFRARED SPECTROSCOPY IN THE CHOCOLATE ANALYSIS

Jana Čopíková, Andrej Sinica, Jolana Tarkošová
Institute of Chemical Technology
Technická 3, 166 28 Praha 6
the Czech Republic

The presented study indicates that near-infrared spectroscopy in the reflectance mode can be applied in analysis of sugars in food products. Preliminary studies were performed on dark and milk chocolate with different composition in order to establish how parameters like the fat, sugars and the moisture content can be determined by near-infrared reflectance spectroscopy (NIR). The correlation for fat and saccharose in dark and the same procedure for fat, saccharose and lactose in milk chocolate resulted in the calibration equations with the correlation coefficients $r>0,9$. Opposite to it, the correlation for moisture in dark and in milk chocolate was not satisfied with the correlation coefficient $r<0,7$. Conventional methods (NMR and GC) were used for the determination of values to calibrate two types of the NIR instrument: Inframatic 8620 and Nicolet 740.

FTIR STUDY OF INTERMOLECULAR INTERACTIONS IN PECTIN GELS

Nikolaus Wellner, Peter S. Belton (Institute of Food Research, Norwich,UK)
Marta Kačuráková, Anna Malovíková (Slovak Acad. of Sciences, Bratislava, SK)

Pectin is a major plant cell wall constituent and commercially important as as a gelling agent in the food industry. Infrared spectroscopy is one way to study its structures in situ (1).

In plant cell walls the intermolecular cohesion of pectin chains is brought about mainly by Ca^{2+} ions. The organisation of the junction zones in pectate gels has been described with the 'egg-box' model (2), whereby strands of polygalacturonate are linked by calcium ions. The same ion complexes exist in plant cell walls. However, a very high local concentration does render the gel almost solid. Polygalacturonate gels have been shown to undergo a conformation change upon drying (3). The pectin in cell walls may behave similarly (4). Also, the pectin in cell walls is variably esterified, enabling hydrophobic interactions similar to those observed in high methoxyl pectin gels. Our IR spectra have shown characteristic band shifts due to dehydration and intermolecular interactions in concentrated gels.

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FTIR SPECTROSCOPY FOR THE ANALYSIS OF β-GLUCANS

Vlasta Sasinková, Jozef Šandula, Marta Kačuráková
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava

Beta-glucans isolated from yeasts, fungi, bacteria and lichens belong to the class of drugs known as biological response modifiers, which modify the host's biological response by stimulation of the immune system. Fibrillar, water-insoluble glucan is a typical cell-wall skeletal constituent. Glucans have a common structure with the main chain consisting of (1→3)- linked β-D-glycopyranosyl units along which randomly dispersed single β-D-glycopyranosyl units are attached by (1→6)-linkages. In the case of yeast glucan the ratio of (1→3) to (1→6) linkages is 8:1.

Beta-glucan isolated from yeast cells by alkaline treatment contains also a small amount of impurities (mostly α-glucan and α-mannan) which show no immunomodulatory activity, therefore determination of β- and α-bonds of glucan in samples is necessary .

FT-IR spectroscopy analysis in the (C-O) and ring vibrations range (1) at 1200-950 cm^{-1} as well as in the "anomeric region" (1) at 950-750 cm^{-1} shows differences in the α- and β-glucan spectra. The characteristic bands for (1→3)- and/or (1→6)-linked β-D-glucans (laminarin, curdlan) are at 1160, 1041, 889 cm^{-1}. In the case of α-(1→4, 1→6)-linked the characteristic bands are at 1155, 1023, 930, 850, and 765 cm^{-1}. The intensity ratios at 1160/1155, 1041/1023, 1160/1023, and 889/930, 889/765 are suitable for β/α ratio determination. The application of FT-IR spectroscopic techniques can give quite quick and valuable information about quality of the glucan preparations.

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MODIFICATION OF HETEROXYLANS BY ULTRASONIC TREATMENT IN AQUEOUS NEUTRAL AND ALKALINE MEDIUM

Kačuráková, M., Hromádková, Z., Ebringerová, A., and [a]Hříbalová, V.
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic
[a]National Institute of Public Health, 100 42 Prague, Czech Republic

As a preliminary study for ultrasound-assisted extraction of xylans from annual plant waste materials, the immunological active corn cob xylan (CCX)[1] and the highly viscous corn hull xylan (CHX)[2] were subjected to ultrasonication at a frequency of 20 kHz in water, 1%, and 5% NaOH at various sonic powers, and irradiation time. The ultrasound-induced changes were followed in view of composition, structure and molecular mass distribution (MMD) of the xylans using sugar analysis, FT-IR and ^{13}C NMR spectroscopic methods, and HPGPC. The main functional properties of CCX and CHX were investigated using mitogenic and comitogenic tests, and viscoelastic properties, respectively. The irradiation was shown to affect the MMD as well as the distribution of the UV-absorbing component in both xylans in dependence on the sonic power and time as well as alkali concentration. Substantial chemical changes and a decrease of the biological activity of CCX occured in neutral and alkaline medium only at high sonic power and long irradiation time, particularly, with increasing intensity at the higher alkali concentration. A these conditions, expect of a viscosity decrease, no changes in the rheological behaviour of CHX were observed.

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SOME PHYSICOCHEMICAL PROPERTIES OF BEECH WOOD 4-O-METHYL-D-GLUCURONOXYLAN

Malovíková A.[1], Ebringerová A.[1], Hromádková Z.[1], Kačuráková M.[1], Machová E.[1]
Pavlov G.M.[2], and Harding S.E.[3]
[1]Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic
[2]Institute of Physics, St. Petersburg State University, 198904 St. Petersburg, Russia
[3]Department of Applied Biochemistry & Food Science, University of Nottingham, Sutton Bonington LE12 5RD, UK

Hardwood xylans are biopolymers with various potential applications (1,2). For these purposes the knowledge about the chemical composition and primary structure of xylans is not sufficient and more detailed information on physicochemical properties of these natural polysacharides has to be obtained. It concerns the molecular properties, secondary structure, interaction with oppositely charged ions, etc. The 4-O-methyl-D-glucuronoxylan isolated from beech wood has been fractionated by ultracentrifuge and treated with ultrasound. Both original xylan and fractions obtained have been characterized by chemical and spectroscopic methods (FTIR, UV). The molecular properties have been studied by means of viscometry, HPGPC, and analytical ultracentrifuge. The results of interaction of original and fractionated xylan with calcium cations using spectrophotometry will be presented as well.

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THE USE OF ULTRASOUND TO REMOVE THE UV-ABSORBNG COMPONENT OF BEECHWOOD XYLAN

Hromádková, Z. and Ebringerová, A.
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic

Xylans isolated from woody materials are usually contaminated with residual lignin and/or lignin degradation products. Oxidation with hypochlorite or sodium chlorite used to remove these components is connected with a more or less severe xylan chain degradation. Ultrasound treatment of unbleached cellulose pulp was found[1] to decrease the chlorination time, i.e. it attacked the residual lignin. Recently, ultrasonic irradiation was reported[2] to facilitate the oxidation of lignin. In the presented study we attempted to use ultrasonication at different reaction conditions to remove the UV-absorbing component of an beechwood xylan. The effects of irradiation was studied by characterizing the treated xylan using RI- and UV_{254}-detected HPGPC chromatograms as well as the separated low molecular compounds using UV-spectroscopy in acidic and alkaline medium.

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APPLICATION OF ULTRASOUND IN ISOLATION OF THE XYLAN COMPONENT OF ANNUAL PLANTS

Hromádková, Z., Ebringerová, A., and Machová, E.
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic

In the last decade, hemicelluloses of the D-xylan type, have more and more been recognized as a valuable and abundant raw material for the production of biodegradable polymers [1,2]. The cell walls of corn cobs[3]and corn hulls[4] are particularly rich in heteroxylans of different structures. In order to commercialize the xylan production, ecologically feasible conditions have to be found. In this work, ultrasound was applied at various conditions during the extraction of both the waste residue of the production of corn grain (cobs) and the by-product of starch production (hulls). The yield, composition, structure and molecular properties of xylan preparations obtained by classical and ultrasound-assisted extraction procedures were compared. Short ultrasonic treatment in the first extracion step was found to increase the yield of both xylan types at lower extraction temperature and time when compared to classical extraction procedures.

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PREPARATION AND STUDY OF MESOGENEOUS PROPERTIES OF AROMATIC AND ALIPHATIC ESTERS OF CELLULOSE

[a]Talába P., [b]Sroková I., [c]Ebringerová, A. and [b]Hodul P.
[a] Faculty of Industrial Technologies ŽU, ul. T. Vansovej 1054/45, 020 32 Púchov, Slovak Republic
[b] Faculty of Chemical Technology, Slovak Technical University, Radlinského 9, 812 37 Bratislava, Slovak Republic
[c] Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic

In this work the esterification of cellulose with both substituted or unsubstituted benzoic[1] and higher aliphatic acids in the new acylation system of pyridine containing methanesulfonyl chloride at various reaction conditions was investigated. Cellulose could be readily acylated with 2-nitro-, 3-nitro-, 4-nitro-, and 4-azidobenzoic acids to form: *O*-(2-nitrobenzoyl)cellulose (DS=3), *O*-(3-nitrobenzoyl)cellulose (DS=3), *O*-(4-nitrobenzoyl)cellulose (DS=2), and *O*-(4-azidobenzoyl)cellulose (DS=1), respectively. In the case of benzoic and higher aliphatic acids, the mixed esters *O*-benzoyl-*O*-(methanesulfonyl)cellulose (DS≈0.9), *O*-dodecanoyl-*O*-(methanesulfonyl)cellulose (DS≈2.4), *O*-tetradecanoyl-*O*-(methanesulfonyl)cellulose (DS≈1.2), and *O*-hexadecanoyl-*O*-(methanesulfonyl)cellulose (DS≈1.2) were prepared. All products were characterized by elemental and FTIR analyses. ^{13}C NMR spectroscopy analysis was performed on hydrolyzed samples in dimethyl sulfoxide (DMSO). The prepared derivatives are partially soluble in usual organic solvents, such as DMSO and chloroform. Some of them were studied from the mesogeneous behaviour point of view.

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STRUCTURE AND PROPERTIES OF PARTIALLY HYDROPHOBISED D-XYLAN TYPE POLYSACCHARIDES

[a]Sroková, I., [b]Talába, P., [c]Hromádková, Z., [c]Ebringerová, A., and [c]Alföldi, J.
[a]Faculty of Chemical Technology, Slovak Technical University, 812 37 Bratislava, Slovak Republic
[b]Faculty of Industrial Technologies, ŽU, 020 32 Púchov, Slovak Republic
[c]Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic

The growing demand for natural and biodegradable surfactants had evoked a renewed interest in new polysaccharide raw materials and amphiphilic derivatives based on commercial type polysaccharides. As a continuation of our studies on xylan derivatives[1,2], we report on the partial hydrophobisation of structurally different water-soluble heteroxylans using modification with long (C_{10}-C_{18}) alkyl chains. In the case of the water-insoluble heteroxylans, p-carboxybenzyl substituents were introduced to increase the solublity of the xylan derivatives as well. The derivatives were characterized by chemical analysis, FT-IR, UV, and NMR spectroscopic techniques. The functional properties were analysed by surface activity tests, including surface tension, critical micelle concentration, foamability, protein foam stabilising effect, and emulsifying efficiency.

Water-soluble derivatives were prepared with important surface active properties from all xylans tested indicating the industrial potential of this new class of biopolymer surfactants.

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POLYSACCHARIDE COMPLEX WITH ANTITUSSIVE ACTIVITY FROM THE ROOTS OF *RUDBECKIA FULGIDA*, VAR. *SULLIVANTII* (BOYNTON ET BEADLE): STRUCTURE OF THE FRUCTOFURANAN COMPONENT

A. Kardošová[1], M. Matulová[1], P. Capek[1], G. Nosáľová[2], S. Fraňová[2]
[1]Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava
[2]Department of Pharmacology, Jesenius Medical School, Comenius University,
SK-037 53 Martin

The present work is a part of our continuing program on isolation of new, potentially active polysaccharides of plant origin. A crude mixture of polysaccharides has been isolated from the roots of the title herb and tested for antitussive activity on mechanically induced cough reflex in nonanaesthetized cats. It was found that the number of cough efforts in the irritated laryngopharyngeal and tracheobronchial mucose membranes of the airways was reduced significantly after peroral administration of the polysaccharide complex and that the cough-suppressing activity was comparable to that of drugs generally used in clinical practice (dropropizine and prenoxdiazine).

Ion-exchange chromatography and gel filtration were applied to separate and purify the dominant polysaccharide component from the crude mixture. The obtained homogeneous polymer had Mn = 4 600 and its molar composition determined by NMR and HPLC analyses was found to be D-fructose and D-glucose in the ratio of 26:1. The results of methylation analysis and ^{1}H and ^{13}C NMR measurements showed that the polysaccharide is a (2--1)-β-linked fructofuranan of the inulin type.

4-O-METHYL-D-GLUCURONO-D-XYLAN FROM *RUDBECKIA FULGIDA*, VAR. *SULLIVANTII* (BOYNTON ET BEADLE)

A. Kardošová and M. Matulová
Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava

From the aerial parts of the title herb a low-molecular-weight 4-O-methylglucuronoxylan has been isolated by alkaline extraction, followed by ethanol precipitation, ion-exchange chromatography, and gel filtration. The results of compositional and linkage analyses, supported by those of ^{1}H and ^{13}C NMR measuremtns of oligomers generated on partial acid hydrolysis, showed the (1--4)-linked β-D-xylopyranosyl backbone with about 18 % 4-O-methyl-D-glucuronic acid attached to O-2 of the xylose residues.

A NEUTRAL HETEROPOLYSACCHARIDE FROM THE FLOWERS OF *MALVA MAURITIANA* L.

P. Capek and A. Kardošová
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic

The flowers and leaves of the medicinal plant *Malva mauritiana* L., containing mucous material, have been used as crude drugs in treatment of catarrhs of the respiratory system and various inflammations of the nasal and oral cavities. Recently, we have found that the mucilage isolated from the flowers of this herb and especially its acidic heteropolysaccharide component exhibited antitussive activity on mechanically induced cough reflex in non-anaesthetized cats (1). Besides the acidic, branched rhamnogalacturonan, we have isolated the linear α-1,6-D-glucan and the highly branched α-1,5-L-arabino-3,6-β-D-galactan components of the mucilage (2-4).

The present work reports on isolation and structure determination of a high molecular-weight arabinogalactorhamnoglycan from the same source. The polysaccharide was composed of D-galactose, L-arabinose, and L-rhamnose in the mole ratio 1.0 : 1.8. : 2.0. The results of chemical and spectroscopic investigations indicated a branched structure with 3,6-linked D-galactopyranose, 5-linked L-arabinofuranose, 4- linked and terminal L-rhamnopyranose residues as the main building units.

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A NEUTRAL EXTRACELLULAR POLYSACCHARIDE FROM THE YEAST *Cryptococcus laurentii*

N. Kolarova, P. Capek, and M. Matulová
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovak Republic

Cryptococcus laurentii is a zoopathogenic yeast-like organism and is closely related to the human pathogen *Cryptococcus neoformans* that causes opportunistic infection affecting AIDS patients and transplant recipients. Extracellular carbohydrates play several roles in this infection (1). Until now, it has been reported that *C. laurentii* produced an acidic extracellular polysaccharide composed of D-Xyl, D-Man, and D-GlcA containing (1→3) mannan backbone and a neutral one composed of D-Xyl, D-Gal, and D-Man in which mannose units are linked by (1→3) and (1→2) linkages (2,3). Recently, we have found that *C. laurentii* produces also extracellular glucomannan with (1→6) mannan backbone (4).

The present work reports on isolation and characterization of a neutral homogeneous extracellular polysaccharide, a galactoglucoxylomannan. The polymer was composed of galactose, glucose, xylose, and mannose in the 3.0 : 0.8 : 1.0 : 9.0 mole proportions. Compositional and methylation analyses, acetolysis, ^{1}H and ^{13}C NMR spectroscopy pointed to highly branched comb-like structure of the polysaccharide. The backbone consisted of α-(1→6)-linked mannopyranose units, about 66% of which were substituted at position O-3 by side short branched (1→2)-linked mannooligomeric chains.

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CARBOXYMETHYLATED CHITIN-GLUCAN COMPLEX FROM *ASPERGILLUS NIGER* - DEGRADATION WITH LAMINARINASE AND CHITINASE

[1] E. Machová, [1] G. Kogan, [1] M. Matulová, [1] V. Pätoprstý, [1] J. Šandula, and [2] K. Kvapilová

[1] *Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 842 38 Bratislava, Slovak Republic*

[2] *Faculty of Natural Sciences, Comenius University, Mlynská dolina, 842 15 Bratislava, Slovak Republic*

Cell walls of Ascomycetes contain a mixture of the polysaccharides that include neutral glucans and chitin. The alkali-insoluble cell wall fraction of Aspergilli is represented by a covalently linked chitin-glucan complex that plays an important role in the fungal morphogenesis.

A water-soluble carboxymethylated derivative was prepared from the chitin-glucan complex isolated from *Aspergillus niger* which possessed pronounced antimutagenic activity towards cyclophosphamide effect in mice.

In order to investigate the structure of the chitin-glucan complex, we subjected it to the action of the following enzymes: laminarinase (1,3-β-D-glucan hydrolase) and chitinase (poly[1,4-β-(2-acetamido-2-deoxy-D-glucoside)] glycanhydrolase. The process of degradation was followed by HPLC analysis. After 72 h of enzymatic action, the oligomers produced were separated from the non-degraded polymeric molecule by gel filtration on BioGel P-2. Monosaccharide composition and structure of the oligosaccharides were characterized by means of ^{13}C NMR spectroscopy and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The oligosaccharides were hydrolyzed with 2 M trifluoroacetic acid and the monosaccharide mixture was analyzed by thin-layer and gas chromatography and mass spectrometry.

The relative molecular weight of the polymeric fractions left after the enzymatic treatment was determined by means of HPLC and their chitin content was estimated by means of the elemental analysis. The results obtained were compared with the respective data for the initial carboxymethyl chitin-glucan.

PREPARATION AND CHARACTERIZATION OF WATER SOLUBLE CHITIN-GLUCAN FROM *ASPERGILLUS NIGER*

Mislovičová, D.[1] Masárová, J.[1]Bendžálová, K.[2] Šoltés, L.[3]and Machová.E.[1]
[1]Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava, Slovak Republic
[2]Faculty of Natural Sciences of Comenius University, Department of Analytical Chemistry, SK-842 15 Bratislava, Slovak Republic
[3]Institute of Experimental Pharmacology, Slovak Academy of Sciences, SK-842 16 Bratislava, Slovak Republic

The insoluble chitin-glucan complex, prepared from *Aspergillus niger,* was swollen in various aqueous media and subsequently treated by means of ultrasound. The influence of swelling conditions such as stirring, time duration and kind of media on solubility of native chitin-glucan were investigated. The degradation of insoluble chitin-glucan by means of ultrasonication was dependent on the media used for swelling, on the duration of effect of ultrasound and on the concentration of chitin-glucan in the suspension. The yield of water soluble fractions and the distribution of their molecular weights were determined. The results of within the range of 7-33 % from native chitin-glucan were obtained, according to the media used and the time of ultrasonication. The soluble chitin-glucan fragments were separated and divided by ultrafiltration method into 4 fractions according to their molecular weight (I. up to 3 KDa, II. 3 - 10 KDa, III. 10 - 30 KDa, IV. above 30 KDa). The main part of the fragments were situated in the fraction of the molecular weight above 30 KDa (77 %). The fractions were characterized by FPLC. By HPLC analysis, the changes of polydispersity of water soluble chitin-glucan were investigated during ultrasound action. HEMA BIO 1000 (10 μm) column, calibrated by pullulans of molecular weight within the range of 5 KDa - 800 KDa, was used. The results demonstrated that under the action of ultrasound on swelled chitin-glucan the fragments of the three main molecular weights were released : Mw ≅ 570 KDa, 132 KDa and 9.5 KDa.

CARBOXYMETHYL-β-1,3-GLUCAN FRACTIONS AS NEW PERSPECTIVE IMMUNOSTIMULATORS AND MACROPHAGE STIMULATORS

Korolenko T.A., Vereschagin E.I., and Sandula J.*
Institute of Physiology RAMS, Novosibirsk 630117, Russia;
Institute of Chemistry SAN, Bratislava, SK-842 38, Slovakia*

Carboxymethylated glucans (CMG) are important group of immunomodulators (Sandula et al., 1994). The aim of this study was to compare the biological effect of CMG three fractions with mol.weight 1 000 000 and 120 000 as macrophage (Mp) stimulators and study their effect in vivo during acute shock induced by haemorrhage and LPS. In vitro according to NBT test all CMG fractions studied was shown to stimulate murine peritoneal Mps in concentration 150 μg/ml: CMG 3rd fr. > CMG 1st fr. > CMG 2nd fr. Single i.v. or i.p. CMG administration to rats or mice in a dose of 25 mg/kg increased the rate of carbon particles phagocytosis (at 1st-14th days). A protective effect of CMG 2nd fraction (but not the 3rd) was shown in acute blood loss in mice. CMG 3rd fraction had also protective effect against LPS-induced shock in rats (Vereschagin et al., 1997). In all experiments CMG fractions were administered to the animals before acute shock (24 h). Preliminary administration of CMG 2nd fraction normalized Mps depression induced by gadolinium chloride or acute cold in rats and mice. One can conclude that CMG 2nd fraction is effective Mp stimulator and can be used in pathology followed by Mp depression (Korolenko et al., 1996). In general the effect of CMG 2nd fraction in vivo was more potent compared to the same dose of common Mp stimulator zymosan. Obviously, chemically modified glucan fractions exerted their effect as Mp stimulators and 2nd and 3rd fractions of CMG were the most perspective for clinical usage.

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BINDING SITES OF CARBOHYDRATE MOIETIES TOWARDS RESIDUAL LIGNIN IN SPRUCE SODA PULP

B. Košíková and A. Ebringerová
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava

Lignin-carbohydrate complexes (LCC) have been isolated by HCl/dioxane/water extraction of soda spruce pulps and subsequent alkaline extraction of the pulp residues. Sugar analysis revealed that all fractions obtained consist of lignin and carbohydrates in the ratios of 10.8-6.3 : 1, 0.5-0.3 : 1 and 1.0-0.4 : 10, respectively. The carbohydrate components of LCC contain hemicelluloses and cellulose fragments in the ratio 0.3-3.3 : 1. The lignin-carbohydrate complexes isolated by dioxane extraction from the pulps were subjected to acetylation, followed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Methylation analysis of the liberated sugar acetates showed that predominantly cellulose fragments are bound to residual lignin in soda pulps at C-6 position of glucose by p-alkoxy benzyl ether linkage.

INTERACTION OF LIGNIN AND POLYSACCHARIDES IN BEECH WOOD FAGUS SYLVATICA DURING DRYING PROCESSES

B. Košíková,[1]* M. Hricovíni[1] and C. Cosentino[2]

[1]Institute of Chemistry, Slovak Academy of Sciences, SK-842 38 Bratislava,
Slovakia
[2]Institute of Chemistry and Biochemistry "G.Ronzoni", I-201 33 Milano, Italy

^{13}C CP MAS NMR spectroscopy was used to characterize the structural changes of cell wall polymers in beech wood Fagus sylvatica during drying processes. The analysis of five wood samples, namely, untreated, untreated dried, pre-treated by steam and/or NaOH subjected to drying showed partial depolymerization of lignin component as well as the change of the ratio of the crystalline and of the amorphous parts of cellulose as the consequence of wood pre-treatment. In addition, $T_{1\rho}(^1H)$ relaxtion times were determined in beech wood sample pre-treated with steam at 135°C and the lignin isolated from this sample. The magnitudes of the relaxation times were found comparable in both samples as well as in the lignin-cellulose model compound. These unique $T_{1\rho}(^1H)$ values indicate that spin diffusion is complete and homogeneous due to spatial proximity of spins and confirmed the formation of lignin-cellulose complex during thermal treatment of wood.

Preparation of hydroxypropyl derivetives of hyaluronic acid in water/NaOH solution with epichlorohydrin in the presence of NH_4OH

Ivan Šimkovic[a], Miloš Hricovíni[a], Pavol Ďuriš[a], Raniero Mendichi[b] & Ladislav Šoltés[c]
[a]Institute of Chemistry, Slovak Academy of Sciences, 84238 Bratislava, Slovakia
[b]CNR, Instituto di Chimica delle Macromolecole, Via Bassini 15, 20133 Milan, Italy
[c]Institute of Experimental Pharmacology, Slovak Academy of Sciences, 84216 Bartislava, Slovakia

Hyaluronic acid (HA) at 0.1 and 1 mmol quantities was crosslinked in water (1 and 5 mol) with epichlorohydrin (E; 0.01 to 0.5 mol) in the presence of NaOH (0.005 to 0.5 mol) and optionally with or without NH_4OH (0.005 and 0.01 mol). On the basis of elemental analysis and yields it is evident that HA is optimally crosslinked with or without NH_4OH at E/NaOH = 1. There are indications that the hydroxypropyl groups are predominantly linked to deacetylated amine groups of glucosamine units. At the presence of NH_4OH poly(hydroxypropylamine) groups are incorporated into the material as could be concluded from yields and elemental analysis. The deacetylation of N-acetyl-D-glucosamine units and degradation due to the presence of NaOH was confirmed by NMR in solution and by the light scattering analysis. Also the degradation of HA due to bacterial treatment in blank experiment was observed. The material was less degraded with NaOH prior to crosslinking than on the blank experiment during the dialysis process. The products were water soluble when 0.005 to 0.1 mols of NaOH and NH_4OH and 0.01 mol of E were used for modification and turned gradually water-insoluble although they could be analysed before with NMR in solution and by light scattering technique. The cross-linked water-insoluble products were analysed with solid-state ^{13}C CP/MAS NMR spectroscopy.

EFFECT OF GALACTOGLUCOMANNAN-DERIVED OLIGOSACCHARIDES ON HYPERSENSITIVE REACTION OF CUCUMBER TNV

Slováková, Ľ.[1], Karácsonyi, Š., Kubačková, M., Kákoniová, D., Bilisics, L., Lišková, D.

Institute of Experimental Phytopatology, Slovak Academy of Sciences, 900 28 Ivánka pri Bratislave[1], Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 842 38 Bratislava, Slovakia

Effect of (GGMOs) galactoglucomannan-derived oligosaccharides, obtained from galactoglucomannan isolated from the wood of poplar (*Populus monilifera* AIT) (3, 4, 5, 6-7, and a mixture of 4-8 DP), on the hypersensitive reaction of cucumber (*Cucumis sativus* L. cv. Laura) to TNV (tobacco necrosis virus) was tested. Cotyledons were inoculated with TNV (500 mg/ml) 24 h before treatment with GGMOs in concentrations between 0.005 and 5.0 mg/ml. Inhibition of infection (IP) was calculated using the formula: IP=100(1-nt/nc), where nt and nc are the average number of lesions on treated and control cotyledons, respectively. Differences between the number of lesions were statistically analyzed using Student's t-test. It was found that investigated GGMOs have antiviral properties. The percentage of disease symptoms inhibition ranged in all variations of tested polymerization degrees from 20 - 50%. However, the dispersion of repeated experiments was the lowest at the application of higher DP (6 - 7) and the mixture of oligosaccharides samples.

ARABINOGALACTAN-PROTEIN FROM SPRUCE CALLUS CELLS: STRUCTURE AND BIOLOGICAL ACTIVITY

Karácsonyi, Š., Pätoprstý, V., Kubačková, M., Lišková, D., Kákoniová, D., Bilisics, L.

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 842 38 Bratislava

Two homogeneous arabinogalactan-proteins (AGPs) have been purified from spruce (*Picea abies* L. Karst) callus cells. The apparent molecular masses of these highly glycosylated proteoglycans **AGP-1** and **AGP-2** were 43 and 19 kD, respectively. Both macromolecules contained predominately terminal α-L-arabinofuranosyl residues; t-, 3-, 6- and 3-6-linked β-D-galactopyranosyl residues and t- glucuronopyranosyl residues. Small amounts of 4- and 4-6-linked galactose residues were also present. The AGPs exhibited 1-carboxyethylidene substituents (pyruvic acid groups) at O-4 and O-6 some of the 3-linked β-D-galactopyranosyl residues as a characteristic structural feature of spruce proteoglycans. The protein content was reach in hydroxyproline, serine, threonine and alanine. Reductive alkaline degradation of AGPs indicated that serine and threonine were not involved in carbohydrate-protein linkage. While sharing general structural similarity these endoplasmic AGPs were distinguishable by composition, size and shape of the macromolecules.

AGP-1 was tested on the biological activity. Induction of various morphological responses in different plant material "in vitro" has been observed.

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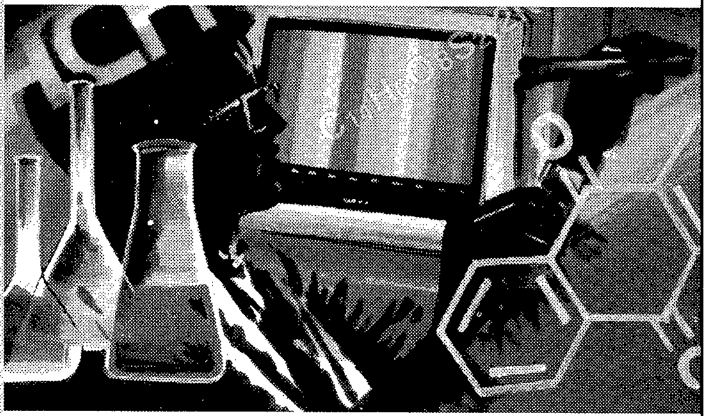

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