Structural Analysis. The structural analysis of polysaccharides is exceedingly complex and requires the combined use of physical (spectroscopy and spectrometry) and chemical methods (hydrolysis, methanolysis, partial hydrolysis, formation of derivatives, controlled degradation of the polymer and its derivatives, and so forth). Elaborating on the methodologies in use would exceed the scope of this text. Specialized books and publications should be consulted for a description of the techniques for the determination of the monosaccharide composition, of the linkage types, of the molecular weight, how to estimate chain length, how to discover and locate branching points, and so on.

4. MONOGRAPHS

Any attempt at classification turns out to be somewhat arbitrary: the diversity of structures and of uses of polysaccharides and related drugs leads us to adopt a classification based on botanical origin:

- polysaccharides from microorganisms and fungi;
- polysaccharides from algae;
- polysaccharides from higher plants (homogeneous and heterogeneous).

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Polysaccharides from Lower Plants

Polysaccharides from microorganisms and fungi

To date, sugar polymers used by man are obtained chiefly from higher plants, or are semisynthesized from natural polymers: many have been known and utilized for centuries. Their plant origin is not without drawbacks, such as irregular supply in unusual climatic conditions and resulting price fluctuations, uneven quality, and at times, lack of reproducibility of the physical properties due to the variability inherent to living matter.

Polymers from biotechnology alleviate these inconveniences: they are produced under controlled conditions, and with remarkably constant quality and physical properties.

Although for the time being the number of polysaccharides produced by microorganisms and approved for sale is very limited, it might increase in the future, based on the number of products that are published or under study.

• DEXTRANS, dextran (INN)

Dextrans are glucose polymers or glucans made of α-D-glucopyranosyl residues linked 1—>6. These molecules are more or less branched, of high molecular weight

bacteria of the genera *Leuconostoc*, *Lactobacillus*, and *Streptococcus*: the enzyme, dextransucrase, accomplishes the polymerization of the α-glucopyranosyl moieties by transfer from sucrose.

The very general term dextran actually applies to the group of exocellular polymers excreted by the different strains of these species. Each polymer is specific to the strain that produces it, and it may also contain 1—>2, 1—>3, or 1—>4 linkages, but always 1—>6 linkages dominate. The degree of branching varies from 5 to 33% and in most cases, the lateral chains are very short (1 or 2 glucose molecules) and linked to the principal chain by a 1—>3 or 1—>2 bond.

In the case of the products for injectable preparations — dextrans 40, 60, and 70 — that are the subject of a monograph in the European Pharmacopoeia (3rd edition), it is specified that they are "a mixture of polysaccharides [...] obtained [...] using *Leuconostoc mesenteroides* strain NRRL-B-512 or substrains thereof (for example, *L. mesenteroides* B-512F = NCTC 10817)".

Production. Commercial dextran is a polymer containing about 95% α-D-(1—>6) and 5% α-D-(1—>3) linkages involved exclusively in lateral branching. Its production involves selected strains of *Leuconostoc mesenteroides*, cultivated on sucrose-rich media. Upon completion of the culture, ethanol is added to precipitate the polymer. Because the molecular weight is still quite high, a partial hydrolysis follows to dispose of polymers of 40,000 to 75,000 molecular weight. This partial depolymerization can be done in acidic medium, by fungal enzymes, or by ultrasonic treatment. After deionization, precipitation with acetone, and recrystallization, "medicinal dextran" is obtained. The tests for the official products are rigorous and their goal, among others, is to evaluate residual solvents (GC), heavy metals, contamination, and bacterial endotoxins. The tests also include establishing the molecular mass distribution by size-exclusion chromatography (Eur. Ph., 2.2.39).

Uses. Dextrans (of average molecular weight 60,000 [Dextran 60] in 6% solution or of molecular weight 40,000 [Dextran 60] at 3.5 or 10%) are administered intravenously (infusion). The viscosity and osmolarity of these solutions are close to those of plasma. Dextran is non toxic, serologically neutral, of prolonged action and completely eliminated. It is a plasma substitute used for the following indications: for plasma volume expansion in shock due to hemorrhage, trauma, and toxiinfection; for preoperative hemodilution. Because it interferes with hemostasis, the maximum dose is set at 1.5 g/kg/day of dextran, or 20 mL/kg. Dextran 40 has similar indications; it is also indicated for dehydration and extensive burns; in combination with sorbitol, it is proposed for use in the treatment of the initial edema of serious cerebral infarctions. Hypersensitivity reactions are rather rare but always possible, thus the infusion must begin very slowly. To prevent or alleviate the anaphylactic reaction triggered by high molecular weight dextran, it is preferable to first inject (IV) a very low molecular weight dextran (MW 1,000 = Dextran 1) which blocks the antigen sites on antibodies, thereby precluding the formation of antigen-antibody

designed to improve the comfort of contact lens bearers, by maintaining a lubricating film on the cornea. Dextran sulfate enters into the formulation of anti-inflammatory combinations utilized, among other applications, in traumatology (sprains, dislocations, contusions), phlebology (mild phlebitis), and rheumatology (tendinitis, small joint arthropathy). Dextranomer (INN) is used for mechanical cleansing of wounds through absorption of exudates and tissue debris, for example from wet wounds and with or without infection, such as decubitus eschars or leg ulcers due to venous stasis.

Other Uses of Dextrans. Treatment of the polymer by epichlorohydrin leads to cross-linking and yields phases for gel filtration chromatography. Pore size is determined by the distance between cross-links, and allows the molecules undergoing separation to either enter the pores or be excluded as a function of their molecular weight. There are numerous applications of this technique in biochemistry, in aqueous phases, as well as in organic chemistry and phytochemistry, and some gels can be used in non-aqueous medium.

• Xanthan Gum

Origin and preparation. *Xanthomonas campestris* is a bacterium which commonly develops on certain species of Brassicaceae where, by using the vegetable substrate, it produces a gummy exudate: xanthan "gum", a "high-molecular-mass anionic polysaccharide [...] of approximately 1 x 10^6 [...] It contains not less than 1.5 per cent of pyruvoyl groups [...] (it) exists as the sodium, potassium or calcium salt" (Eur. Ph., 3rd Ed., 1998 add.).

Industrially, this "gum" is produced by bacterial culture on correctly buffered and aerated media containing carbohydrates, a source of nitrogen, and minerals. Upon completion of fermentation, the polymer is recovered by precipitation with isopropanol, filtered, dried, and crushed. The tests for the official product must, among other goals, verify the absence of residual solvents (GC), the absence of other polysaccharides (by TLC of a hydrolysate), and the absence of microbial contamination. The tests must also include the spectrophotometric quantitation of pyruvic acid (dinitrophenylhydrazine).

Structure. On a backbone similar to that of cellulose (D-glucopyranoses linked β-(1—>4)), trisaccharides form branches from the 3-position of the glucose units. Each trisaccharide comprises one molecule of D-glucuronic acid salt and two molecules of D-mannose, one of which (the one attached onto the main chain) is acetylated in the 6-position, and the other, which is terminal, is combined to a molecule of pyruvic acid *via* an acetal involving its hydroxyl groups in the 4- and 6-position. About half of these terminal mannose units form a cyclic ketal with

conformation, and this explains the great resistance to enzymes and the physical properties specific to this gum.

...—>)-β-D-Glc*p*-(1—>4)-β-D-Glc*p*-(1—>4)-β-D-Glc*p*-(1—>...
1 —>3
α-D-Man*p*-6-O-Ac
1 —>2
β-D-Glc*p*-*A*
1 —>4
β-D-Man*p*
4( )6
H_3C-C-COOH

Properties. Soluble in hot or cold water, xanthan gum forms aqueous solutions of which the viscosity remains practically unchanged by temperature changes, as well as pH changes. The behavior of these solutions is of the pseudoplastic-type: decrease in viscosity proportional to shearing and instant recovery of the initial viscosity upon discontinuation of shearing *. Incompatibilities are rare (borates, hypochlorites, peroxides, free radical generators).

The gum is compatible with most salts, with moderate surfactant concentrations, and with most preservatives; it tolerates alcohol concentrations up to 50%. Compatible with most vegetable hydrocolloids, it does not form gels by itself, but forms thermally reversible gels in the presence of galactomannans from the family Fabaceae (carob). It is devoid of toxicity.

Uses. A first-choice stabilizer for the formulation of suspensions and emulsions, xanthan gum is highly prized for the pseudoplasticity of its solutions* and its global market is growing rapidly. Food technology makes extensive use of it, since it is authorized (Eur. id. code E415) at concentrations ranging from 0.1% (e.g., in instant soups) to 0.5% (e.g., dessert puddings). A stabilizing and gelating agent, it is a common ingredient of sauces (salad dressings, seasonings, gravies, and the like represent 50% of its market), soups, jellies, milk-containing and jellied desserts, canned products, fruit-based preparations (combined with pectin, it prevents syneresis), bakery products (sandwich loaf bread [USA]), and so forth. Its multiple industrial and household applications include paints, cleaning products, polishes, explosives, pesticides, photography products, printing aids, and textiles.

● Lentinan

Lentinan is a homogeneous polymer isolated from a fungus, *Lentinus edodes* (Berk.) Sing. Structurally, it is a glucan containing a principal chain with β-(1—>3)

* Hence the numerous (extra pharmaceutical!) applications: emulsions that *flow* out the bottle

linkages, substituted by (1—>6)-linked glucoses, and of molecular weight around 500,000. The antitumor properties of lentinan, demonstrated on several experimental models, seem to be due not to any cytotoxic properties, but to an immunogenic activity. The polymer stimulates the proliferation of T lymphocytes in the presence of interleukin-2, macrophage activity, and the production of interleukin-1. Japanese studies in humans treated (IV) with a combination of an antitumor drug and lentinan have shown that the efficacy of the combination was superior to that of the antitumor drug alone (stomach cancer).

Many other fungi, particularly basidiomycetes, manufacture polysaccharides with properties similar to those of lentinan. Generally, they are linear (1—>3) glucans (pachymaran), aremore or less branched (schizophyllan), and are sometimes linked to protein (krestin, active fraction isolated from *Coriolus versicolor*).

Since the present text is focussed on higher plant products, we shall not dwell on other bacterial of fungal polysaccharides: gellan (Gelrite®, produced by *Pseudomonas elodea*), welan (Biozan®, produced by an *Alcaligenes*), rhamsan, curdlan, pullulan (secreted by *Aureobasidium pullulans*), and scleroglucan (synthesized by *Sclerotium rolfsii*). The uses of these products are essentially industrial. The use of gellan as a gelifier and texturizing agent in food products is authorized in Europe (E418). The use of curdlan in foods is authorized in several Asian countries and it may be approved soon in the United States. The reader interested in these fermentation products may refer to chapters 13 to 17 of "Industrial Gums - Polysaccharides and their Derivatives", 3rd edition, Whistler, R.L. and BeMiller, J.N., Eds., 1993, Academic Press, San Diego.

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