Saccharide constituents of horse chestnut (*Aesculus hippocastanum* L.) seeds I. Monosaccharides and their isolation

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Starch, arabinans and eventually glucoarabinans are the main constituents of horse chestnut seeds. Episperm contains xylans and eventually glucoxylans. D-Glucose, L-arabinose, D-glucuronic acid, D-xylose, D-galactose, and fucose were found in the hydrolyzed seeds. A simple procedure for isolation of L-arabinose and D-glucuronic acid is described.

Horse chestnut seeds are a valuable source for preparation of remarkable pharmaceuticals [1], as *e.g.* β-escin — haemolytically active mixture of acylated triterpenic glycosides [2]. Further diterpenes were isolated from the horse chestnut seed oil; in addition to flavonoid glycosides purine derivatives and various other substances, proteins and starch were found in this plant material [3].

To make use of the debris remaining after extraction of β-escin on large scale we investigated its chemical composition. The greatest portion in the horse chestnut seeds is formed by saccharides (starch more than 50 %, pentoglycans almost 7 %, fibrous material above 8 %, Ref. [4]), and therefore, we paid attention to the preparation of selected monosaccharides obtained from the hydrolyzate of polysaccharides. Isolation and characterization of the starch itself will be presented separately.

Experimental

Investigated were three various samples of horse chestnut seeds:

Sample A: fresh ground seeds (particle size up to 0.76 mm), w(dry matter) = 54.9 % (105 °C).

Sample B: peeled and sprout-removed seeds mixed in methanol, w(dry matter) = = 86.85 %, w(N) = 1.24 % (this squash was later used for isolation and characterization of starch).

Sample C: crushed seeds after extraction of β-escin with 50 vol. % methanol obtained from Slovakofarma, Pharmaceutical Works, Hlohovec; w(dry matter) = 88.2 %.

Following reference substances were employed for chromatographic identification of saccharides: D-glucose, D-galactose, and L-rhamnose (Lachema, Brno), L-arabinose, D-xylose, D-ribose, L-fucose, D-glucuronic and D-galacturonic acids (Koch-Light, England).

Starch was enzymically hydrolyzed by a mixture of α-amylase *ex Bacillus subtilis* (Koch-Light) and amyloglucosidase (γ-amylase for biochemical use, Merck, Darmstadt). All chemicals were anal. grade (Lachema, Brno). Ion exchangers for preparative isolation of saccharides were Dowex 50 W × 8 (grain size 0.07—0.15 mm, Fluka, Buchs), Ostion KS 0210 and AT 0809 (Spolek pro chemickou a hutní výrobu, CSFR).

Melting points were determined on a Kofler micro-hot stage, optical rotations were measured with a polarimeter, model 141 A (Perkin—Elmer). Solutions were evaporated under reduced pressure at a temperature not exceeding 50 °C (bath temperature). Saccharides in hydrolyzates of ground seeds were qualitatively monitored by paper chromatography on Whatman No. 1 paper in the following solvent systems [5]: S_1 butanol—ethanol—water ($\varphi_r = 5:1:4$), S_2 acetone—butanol—water ($\varphi_r = 7:2:1$), S_3 ethyl acetate—acetic acid—water ($\varphi_r = 3:1\ 1$). Anilinium hydrogen phthalate [6], or diphenylamine [7] were the visualization reagents after a 2—3 min heating of chromatograms at 105 °C. For quantitative determination the spots were clipped out, extracted with water and spectrophotometrically measured.

Methanolic extract of seeds

Fresh seeds were crushed, ground, sieved and extracted with a triple amount of methanol at 65 °C for 8 h. The filtered solution was concentrated to 1/4 of its volume and the sirupy residue was hydrolyzed with 4 % sulfuric acid at 90 °C for 6 h. The solution was diluted with water and sulfuric acid was removed by adding barium carbonate. The precipitate was filtered off, washed with water ($2 \times 50\,cm^3$) and the filtrate was concentrated, acidified with acetic acid to pH = 2—3; according to chromatography, only D-glucose and D-glucuronic acid were in the filtrate.

Acid hydrolysis of sample C

a) Hydrolysis with sulfuric acid: The material was hydrolyzed with 0.3 M-H_2SO_4 at 90 °C for 6 h, filtered, washed with water and the filtrate was concentrated to a small volume. Sulfuric acid was removed by addition of aqueous suspension of calcium hydroxide and the precipitate was filtered off. An equal amount of methanol was added to the concentrated filtrate, the precipitated dextrins were centrifuged 4000 *g* (20 min), the supernatant was evaporated to dryness and dissolved in tap water. Baker's yeast was added and the mixture was allowed to ferment till all D-glucose was consumed (3 d). L-Arabinose, D-glucuronic acid, traces of fucose and oligosaccharides were seen on the chromatogram.

b) Hydrolysis with formic acid: The sample was heated with 10, 20 or 30 vol. % formic acid at 90 °C while the samples were withdrawn in 1 h intervals for monitoring the time course. The chromatograms were developed in S_2 and sprayed with anilinium hydrogen phthalate; hydrolysis was similar to that of method *a*.

Enzymic hydrolysis of sample B

The squash was filtered off, washed with methanol and let to dry in air. A 50-fold amount of distilled water was added and the stirred suspension was brought to boiling. The starch paste being formed was cooled to room temperature and an equal amount of acetate buffer of pH = 5.8 was added. To this mixture α-amylase and amyloglucosidase in a mass ratio enzyme to substrate 1 : 250 and 1 1000, respectively were introduced and the mixture was left standing at an ambient temperature for 55 h. After this time the enzymes were deactivated by heating, the mixture was concentrated and ethanol was added to 60 vol. %. The precipitate was filtered off and hydrolyzed with 10 vol. % formic acid at 90 °C for 8 h. The filtrate showed the presence of glucose and maltooligosaccharides; arabinose, glucuronic acid, and a small amount of glucose were chromatographically detected in the hydrolyzate of the residue.

Hydrolysis of the episperm

The episperm from fresh seeds was ground and hydrolyzed with sulfuric acid as described with seeds — procedure *a*. D-Glucose, D-xylose, and D-glucuronic acid were chromatographically estimated in the hydrolyzate.

Isolation of L-*arabinose*, D-*glucuronic acid and ethanol*

The samples *A* and *C* (300 g of dry matter), respectively were hydrolyzed with 1 dm^3 of 10, 15, and 20 mass % sulfuric acid at 90 °C for 6 h. The insoluble portion was filtered off, the filtrate was neutralized with calcium carbonate, the precipitated solid was removed, washed with water (3 × 100 cm^3), the combined filtrates were concentrated to 400—500 cm^3 and filtered once more. To the filtrate diluted with tap and distilled water (500 cm^3 each) baker's yeast (10 g) was added. The solution was fermented till all D-glucose (3 d) and D-galactose (another 4 d) were consumed. The filtrate was distilled under atmospheric pressure. Ethanol was estimated in the distillate (500—600 cm^3) (Table 1).

The distillation residue was concentrated to its half, filtered and deionized (Ostion KS in H^+ cycle and AT in HCO_3^- cycle). The ion exchangers were washed with distilled water and the eluates containing L-arabinose were evaporated to dryness, whilst D-glucuronic acid remained trapped on the Ostion AT ion resin.

The distillation residue was extracted with methanol (600 cm^3) under reflux for 1 h, hot-filtered, the cooled filtrate was filtered once more and concentrated to a sirupy consistence. The sirup was diluted with a small amount of water and fractionated on a column (0.3 cm × 125 cm) packed with Dowex 50 W × 8 (Ba^{2+} cycle, elution with water, flow rate 45 $cm^3 h^{-1}$). The first fraction (350—530 cm^3) contained disaccharides, a small amount of D-glucose and D-galactose, the second one (530—810 cm^3) L-arabinose.

The combined fractions *2* containing L-arabinose (27.4 g) from the respective samples *A* (Table 1) were dissolved in water (30 cm^3) and heated. Methanol (90 cm^3) was in-

Table 1

Products isolated from horse chestnut seeds (300 g of dry material) after hydrolysis (6 h, 95 °C)

Sample	$\varrho(H_2SO_4)/(g\,dm^{-3})$	Ethanol Yield/cm^3	D-Glucuronic acid Yield/g	L-Arabinose Yield/g
A	100	55.5	6.0	9.5
A	150	60.2	6.0	9.0
A	200	67.5	6.5	8.9
C	100	58.5	5.0	9.7
C	150	64.5	5.5	9.3
C	200	69.0	5.5	9.0

troduced and the solution was hot-filtered and left to crystallize. Like procedure was applied for combined samples *C* (28 g, Table 1). Yield 17.5 g and 18.2 g, respectively, $[\alpha]$(D, 23 °C, $\varrho = 20\,mg\,cm^{-3}$, water) = +180° (3 min) → + 104°; Ref. [8] gives $[\alpha]$(D, water) = +190° → +105°

The mother liquors were concentrated to sirupy consistence and diluted with methanol (40 cm^3); after addition of acetic acid (2 cm^3) and 4-nitroaniline (9 g) the mixture was heated at 65 °C for 3 h and allowed to stand at room temperature for 24 h. The yield from samples *A* and *C* was 7.3 g and 8.7 g of *N*-(4-nitrophenyl)-L-arabinosylamine, respectively (this corresponds to 4 g and 4.8 g of L-arabinose, respectively), m.p. = 191—194 °C. (Ref. [9] gives m.p. = 202 °C), $[\alpha]$(D, 23 °C, $\varrho = 20\,mg\,cm^{-3}$, pyridine) = −132° (5 min—24 h); Ref. [9] gives $[\alpha]$(D, pyridine) = −140.6°.

The ion exchanger Ostion AT was washed out with 7 vol. % formic acid (2 × 500 cm^3) and water (2 × 500 cm^3), the evaporated washings containing D-glucuronic acid were purified *via* the above-described ion exchanger procedure followed by chromatography to afford D-glucurono-6,3-lactone as amorphous compound, $[\alpha]$(D, 23 °C, $\varrho = 20\,mg\,cm^{-3}$, water) = +20°; Ref. [8] gives $[\alpha]$(D, water) = +19.2°.

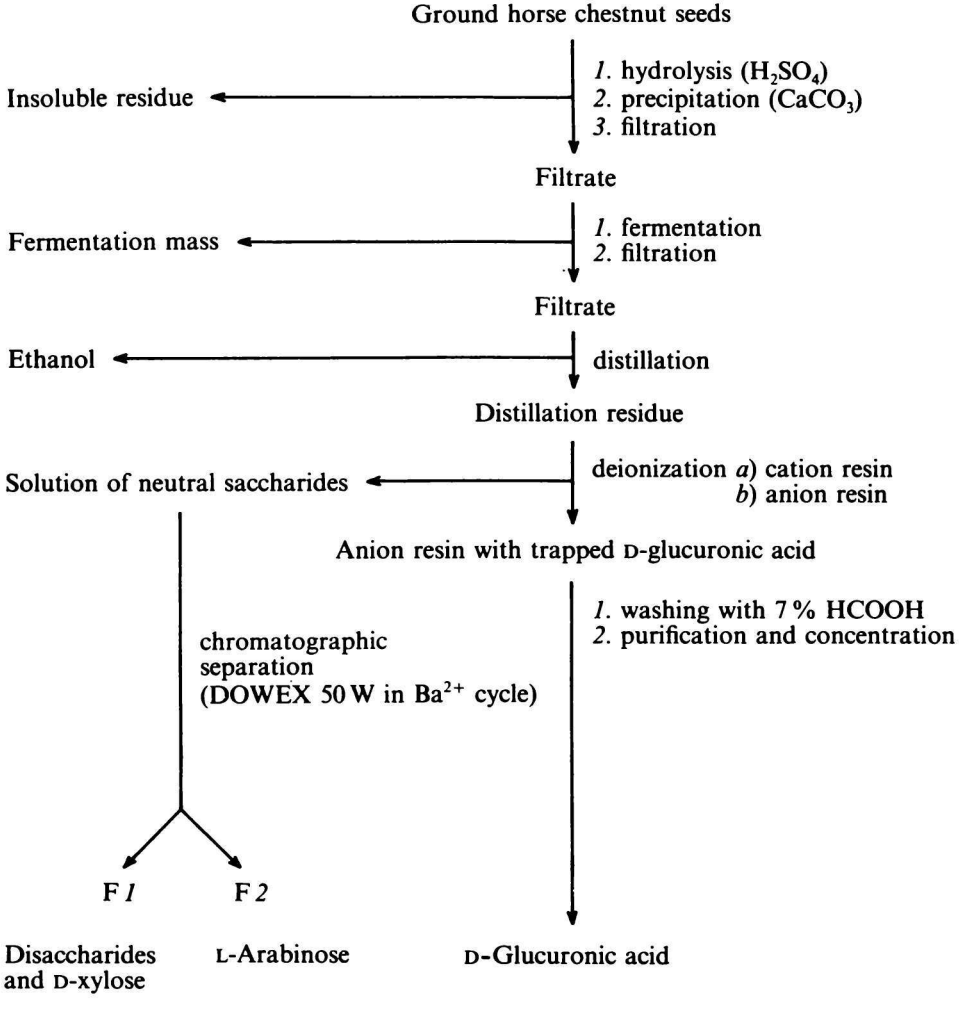
Discussion

The horse chestnut seeds were pre-treated according to the aim. Fresh seeds (55 % dry matter) consisted of kernel (78 %), episperm (18.2 %), and sprout (3.6 %). The seeds were extracted prior to work-up to remove the unwanted compounds as *e.g.* saponins and fats both complicating the isolation of saccharides; another advantage of this procedure is that through extraction the material becomes dry. Methanol extracted *ca.* 10 mass % of material from the ground seeds.

It has been found that the episperm has another composition of saccharides and removal of sprouts is also important because they contain proteins. Nevertheless, the material obtained as debris from the industry contained these components.

Monosaccharides were absent in the seeds, the dominant components of the hydrolyzate after removal of β-escin are D-glucose, L-arabinose, D-glucuronic acid, D-xylose, D-galactose, and fucose (6-deoxygalactose). Hydrolyzate of the methanolic extract of seeds contained D-glucose and D-glucuronic acid, that of the episperm D-glucose, D-xylose, and D-glucuronic acid. It could be, therefore concluded that the kernel contains, in addition to the main component — starch — arabinans, glucoarabinans, whilst the episperm xylans and glucoxylans.

Various solutions of formic acid (10, 20, and 30 vol. %) liberated successively L-arabinose and D-glucose from the seeds at 90 °C. According to the acid



Scheme 1

concentration the limit concentration of L-arabinose in the solution reached sooner or later 60—70 g dm^{-3} Similarly, the freed amount of D-glucose successively increased, but the limit concentration was not achieved within 10 h of hydrolysis; D-xylose was freed only in slight amounts. The soluble portion — D-glucose, maltooligosaccharides and dextrins became liberated also at room temperature with respect to concentration of formic acid, but on the other hand, arabinans were not liberated. A great disadvantage of hydrolysis with formic acid at 90 °C is that only a part of starch was fully hydrolyzed to D-glucose, the greater part remained in the form of maltooligosaccharides and starch paste. Due to this fact the liberated L-arabinose could be very difficultly isolated even after removal of D-glucose by fermentation because filtration could not be applied and the insoluble part had to be removed by centrifugation or precipitation with alcohols. Moreover, distillation was accompanied with unpleasant foaming.

The excess of starch could be removed on laboratory scale by enzymic hydrolysis with α-amylase combined with glucoamylase, followed by acid hydrolysis; thus, the respective monosaccharides can be obtained from the remaining polysaccharides.

For isolation of L-arabinose from the seeds of horse chestnut the material (samples *A*—*C*) was hydrolyzed with 20 mass % sulfuric acid, and the freed saccharides were fermented to ethanol. The nonfermentable saccharides obtained from 100 g of dry sample yielded approximately 3 g of L-arabinose and 2 g of D-glucuronic acid (Scheme 1); at the same time 20—23 cm^3 of ethanol can be produced. So, the debris after removal of β-escin can be utilized in the proposed way; L-arabinose is also a suitable material for preparation of further, less common saccharides [10, 11].

References

1. Kresánek, J. and Krejča, J., *Atlas liečivých rastlín a lesných plodov.* (Atlas of Medicinal Herbs and Wood Fruits.) P. 202. Osveta Publishing House, Martin, 1982.
2. Reppel, J., *Planta Med. 3,* 55 (1955).
3. Bogs, U. and Bremer, D., *Pharmazie 26,* 410 (1971); *Chem. Abstr. 75,* 126573 (1971).
4. Lűers, H., Diemair, W., and Gergs, W., *Z. Lebensm.-Unters. Forsch. 88,* 24 (1948).
5. Charlsson, B. and Samuelson, O., *Acta Chem. Scand. 23,* 261 (1969).
6. Partridge, S. M., *Nature 164,* 443 (1949).
7. Buchan, J. L. and Savage, R. I., *Analyst* (London) *77,* 401 (1952).
8. Staněk, J., Černý, M., Kocourek, J., and Pacák, J., *Monosacharidy.* (Monosaccharides.) P. 414. Publishing House of the Czechoslovak Academy of Sciences, Prague, 1960.
9. Weygand, F., Perkov, W., and Kuhnen, P., *Chem. Ber. 84,* 594 (1951).
10. Bílik, V *Collect. Czechoslov. Chem. Commun. 39,* 1621 (1974).
11. Bílik, V *Chem. Zvesti 26,* 187 (1972).

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