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Identification of Chromatographically Separated Arabinose, Galactose, and Mannose by a Simple X-Ray Method

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By means of powder X-ray diffraction it is possible to identify 15 μg arabinose, 12 μg galactose, and 12 μg mannose. The sugars were first converted into hydrazones.

In chromatography of monosaccharides an accurate identification method may be desirable. Minute amounts of crystalline sugars may be identified by powder X-ray diffraction.[1] It is, however, often difficult to obtain sugars, extracted from chromatograms, in a crystalline state. On extracting arabinose, galactose, and mannose from paper chromatograms using water, no crystalline evaporation residue could be obtained for any of these sugars. As for fructose, it has been shown [2] that it is possible to obtain a good crystalline osazone derivative from a fructose syrup, even with such small amounts of the sugar as 18 μg. The osazone prepared from this amount of fructose was sufficient to give a characteristic X-ray pattern.[2]

An attempt was therefore made to convert the sugars arabinose, galactose, and mannose into derivatives with good crystalline properties suitable for an X-ray analysis. A number of crystalline derivatives of arabinose, galactose, and mannose has been described; most of these derivatives, however, do not crystallize well enough when prepared from the minute amounts of sugars available from a paper chromatogram. The following derivatives were found to satisfy the requirements: Arabinose-benzoylhydrazone, galactose-phenylmethylhydrazone, and mannose-phenylhydrazone.

EXPERIMENTAL

Arabinose-benzoylhydrazone.[3] 2.5 g benzoylhydrazine was dissolved in 50 ml 96 % ethanol. To approximately 1.5 ml of a 1 % arabinose solution was added 1 ml of the reagent. This was left to stand for 24 h at a temperature of 20°C and then for 22 h at −3°C. Ice-like, leaf-shaped crystals, m.p. 188–198°C, $d = 1.44$.

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Table 1. Unit cells for (I) arabinose-benzoylhydrazone and (II) galactose-phenylmethylhydrazone.

	a, Å	*b*, Å	*c*, Å	*β*	Molecules per unit cell
I Orthorhombic	8.42	29.38	5.08		4
II Monoclinic	4.93	32.11	4.79	90°	2

Table 2. X-Ray powder data for arabinose-benzoylhydrazone.

hkl	Spacing, Å		Relative intensity	*hkl*	Spacing, Å		Relative intensity
	Obs.	Calc.			Obs.	Calc.	
120	7.28	7.30	0.13	280	2.74	2.76	0.20
140	5.49	5.52	0.20	340	2.63	2.62	0.13
060	4.86	4.90	0.16	301	2.45	2.46	0.15
150		4.82		331	2.39	2.38	0.15
200	4.21	4.21	0.27	042	2.34	2.35	0.15
220	4.06	4.05	1.00	152	2.25	2.25	0.07
240	3.63	3.65	0.57	361	2.21	2.20	0.16
161	3.31	3.29	0.13	162	2.16	2.17	0.16
221	3.12	3.16	0.20	400	2.12	2.11	0.07
231		3.08		440	2.01	2.00	0.10
190	3.04	3.04	0.10	092	1.98	2.00	0.10
251	2.85	2.85	0.13	1,15,0		1.96	

Galactose-phenylmethylhydrazone.[3] 2.5 g freshly distilled 1-phenyl-1-methylhydrazine [3] was dissolved in 10 ml abs. ethanol and 0.3 ml glacial acetic acid. To approximately 1.5 ml of a 0.5 % galactose solution was added 0.5 ml of the reagent. This was left to stand at 33°C for 12 h and at 0°C for 8 h. Long prisms, m.p. 179—187°C, $d = 1.35$.

Mannose-phenylhydrazone.[3] To 2.5 g of freshly distilled phenylhydrazine was added 10 ml abs. ethanol and 0.3 ml glacial acetic acid. 1.5 ml of a 0.5 % mannose solution was mixed with 0.5 ml of the reagent. This was left to stand for 15 h at 33°C and 12 h at 0°C. Rhombic crystals, m.p. 190—195°C, $d = 1.44$.

The sugars were separated circle-chromatographically on Whatman No. 1 paper with ethyl acetate:pyridine:water (40:11:6), and were localized by staining very small sectors of the chromatogram with a suitable reagent. The arcs containing the sugars were cut out of the circular paper, and extracted with water. The following set-up was found useful: Round the rim of a microscope slide with one or two ground cavities a 1—2 mm thick layer of plastic glue was placed. After drying overnight this ring of glue was ground plane by means of carborundum and a plane surface. As a cover acted a microscope slide fastened with paper clips, and made air-tight with a thin layer of vaseline. The eluate from the chromatogram (two drops) was allowed to drop into one of the cavities, evaporated, and redissolved in approximately 1.5 µl of water, which was found to give the desirable concentration when the sugar quantity spotted was about 70 µg. The calculated amount of reagent was then added, and the reaction carried out as described above. A small piece of filter paper, moistened with one drop of ethanol and placed between the cavities, counter-acted evaporation from the reaction mixtures. It was neither necessary nor did it serve the purpose to recrystallize or in any other way purify the product. The sticky, non-crystalline material in which the crystals are embedded served

Table 3. X-Ray powder data for galactose-phenylmethylhydrazone.

hkl	Spacing, Å Obs.	Calc.	Relative intensity	*hkl*	Spacing, Å Obs.	Calc.	Relative intensity
060	5.36	5.35	0.08	181	2.61	2.61	0.20
110	4.85	4.87	1.00	200	2.44	2.46	0.14
070	4.61	4.59	0.70	220		2.44	
031	4.40	4.38	0.50	002	2.40	2.40	0.12
080	4.03	4.01	0.25	230			
051	3.81	3.84	0.04	042	2.30	2.30	0.04
160	3.64	3.63	0.02	250			
101	3.45	3.44	0.95	052	2.25	2.24	0.04
121	3.34	3.35	0.80	201	2.18	2.19	0.60
141	3.15	3.16	0.08	221		2.17	
151	3.08	3.07	0.22	241	2.12	2.11	0.20
161	2.93	2.90	0.10	215	2.07	2.07	0.16
091	2.83	2.86	0.08	132		2.06	

Table 4. X-Ray powder data for mannose-phenylhydrazone.

Spacing, Å Obs.	Relative intensity	Spacing, Å Obs.	Relative intensity
6.01	0.50	2.60	0.15
5.26	0.88	2.53	0.05
4.48	0.88	2.48	0.05
4.36	0.50	2.41	0.63
4.11	0.15	2.33	0.15
3.96	0.88	2.28	0.15
3.81	0.15	2.23	0.20
3.65	0.10	2.18	0.25
3.48	0.15	2.07	0.28
3.31	1.00	2.04	0.15
3.10	0.37	2.01	0.18
2.99	0.63	1.98	0.20
2.83	0.15	1.91	0.13
2.78	0.25	1.87	0.10
2.68	0.20	1.82	0.10

as an excellent adhesive for mounting of the specimens on the glass fiber in the powder camera. The mounting was easily accomplished by shaping the reaction mixture into a globe by means of a spatula, and then transferring it to the glass fiber.[2] Sometimes it was necessary, however, to employ a little extra (non-crystalline) adhesive. The X-ray powder diffraction data for the three sugar derivatives are given in Tables 2, 3, and 4. They were derived from photographs taken with Ni-filtered Cu*K* radiation in a Debye-Scherrer camera with diameter 11.46 cm. The intensities were estimated visually.

The powder photographs of two of the derivatives could be indexed on the bases of the unit cells, first determined from single-crystal oscillation and Weissenberg photographs (Table 1). The spacings calculated from these unit cell dimensions are included in Tables 2 and 3.

DISCUSSION

The described X-ray powder method was found suitable and reliable for identification of as little as 15 μg of arabinose, and 12 μg of galactose and mannose. An attempt to identify 8 μg by this method was unsuccessful. However, when the method is applied to sugars separated on and eluated from ordinary Whatman No. 1 chromatographic paper, larger amounts, about 72 μg, of each of the sugars had to be spotted on the chromatogram in order to give a sufficient quantity for an identification. This is believed to be due to traces of disturbing substances present in the paper. Duff [4] has shown that chromatographic paper treated with sodium hydroxide may result in an aldose ⇌ ketose transformation of the sugars. Braun [5] observed that sodium hydrogen sulphite completely prevents the formation of hydrazones of aldoses. It is also possible that tannins and lignins may have some disturbing effects. When the chromatographic paper was washed with water before use, it was found to be sufficient for an identification by the present method to spot 24 μg of each of the sugars.

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