Biochemical Studies on Carbohydrates.

LXXVII. The Non-Amino Sugars in the Carbohydrate of Pseudomucin.

Preliminary Note.

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The non-amino sugar constituent in the polysaccharide of pseudomucin has not been yet identified, although Zängerle¹ established, as early as 1900, the glucosamine nature of the amino sugar in this glycoprotein. Fürth and his coworkers² pointed to its presence, but they worked on non-heat-coagulable and alcohol-precipitable proteins or protein-mixtures from ovarian cysts, and not on pseudomucin in the strict sense of Hammarsten. The substances showed Dische's colour reactions of sugar diverging in intensity as expressed as any of ordinary aldohexoses, and from their hydrolysates neither mannose phenylhydrazone nor galactose tolylhydrazone could be separated. Two phenylosazones were obtained, but the melting points of them did not coincide not only with each other but also with those of known phenylhexosazones.

As a large cyst containing typical pseudomucin came to hand, we took up the problem again, which will be described in this communication. The mucin was isolated according to Hammarsten. It gave a similar analytical figure of nitrogen as his preparations. The grades of coloration by the reactions of Dische (the indole and the diphenylamine reaction) and that of Tillmans and Philippi (the orcinol reaction with a minor modification) did not agree as expressed as a single hexose, unexpectedly in accord with what had been reported by Fürth and others. Here, however, notice-

able was that the colour developed by diphenylamine was very faint in comparison with those by the other reagents. On the other hand, it did not give the naphthoresorcinol reaction for hexuronic acid. These findings appeared to indicate that such a non-amino and non-uronic sugar as rhamnose,³ which is almost inert to diphenylamine while quite reactive to orcinol and indole, is existent besides a hexose. Therefore, fractionation of the non-amino sugars as hydrazones was resorted to in the next trial. The mentioned predecessors used mucin hydrolysates, containing glucosamine, as such or after mere treatment with the Hopkins reagent to prepare an osazone or a hydrazone to which be due their unsuccessfulness in obtaining a well-characterized compound or compounds. We applied to a hydrolysate preliminarily the Hg-Ba procedure of Masamune and Tanabe⁴ in order to remove not only the amino sugar but also most of the amino acids. Of phenyl⁵-, *p*-bromophenyl-, *p*-nitrophenyl-, benzylphenyl- and methylphenyl-hydrazine, which were employed, the last hydrazine proved most available. After treatment with this reagent, crystals came out from the solution in two shapes. They could be separated by fractional crystallization from 95% alcohol. One of them, that was less soluble in alcohol and took the shape of plate, melted at 190–191°C, and was identified with *d*-galactose methylphenylhydrazone, whereas the other hydrazone in thin needle form melted at 181–182°C. The latter evolved so much N gas (micro Dumas) as corresponds to methylpentose methylphenylhydrazone, and further evidences for its being this hydrazone were afforded by tests of the non-amino sugar-fraction of another hydrolysate:—The positive acetone (Rosenthaler), phloroglucinol (Oshima and Tollens) (colours with characteristic absorption bands), vanilline (Schäffer and Arbenz) and resorcinol (red colour, faint) reaction for methylpentose were given, but the aniline test for pentose resulted in negative.

We undertook to synthesize rhodose methylphenylhydrazone which melts at the same temperature as the methylpentosehydrazone from pseudomucin. Although the product was ascertained to resemble the unknown in crystalline form very closely, its yield was too small for complete identification. But, apart from this respect, methylpentose has never before been found in the animal body.

EXPERIMENTAL.

Preparation and Analysis of Pseudomucin.

Transparent fluids, some yellowish colored and viscous and some uncolored and serous and all of pH 8.8–9.0, in a big multilocular ovarian cyst⁶, were combined (in total 1870 cc.) and subjected to the procedure of Hammarsten⁷ for preparation of pseudomucin. Nitrogen of the crude

product (55 gms.) amounted to 12.36%. By reprecipitation from 1000 cc. of water (the water insoluble part was removed) with 3 volumes of 95% alcohol and 1 gm. of sodium chloride, nitrogen was reduced to an amount resembling those of Hammarsten's preparations. (A portion of the product was purified further on but nitrogen remained constant). The yield was 30 gms. It gave an opalescent solution in water, which was not precipitated by acidification, and behaved towards precipitants as Hammarsten described. It analyzed:

Analyses	In per cent	Analyses	In per cent
Nitrogen*	10.1	„ mannose	26.0
Total reducing force, as glucose† on ferricyanide after hydro- lysis	37.6	„ glucose	37.4
Glucosamine‡	16.0	Indole r. as galactose	40.8
Non-amino sugar§ Orcinol r.		„ mannose	43.8
as galactose	25.6	„ glucose	31.4
		Diphenylamine I r.	unmeasurable
		Ash//	5.3

* Micro Dumas method. Nitrogen of Hammarsten's preparations, 10.30 and 10.26%. † Masamune and Tanabe method (First procedure). ‡ Hamasato and Akakura method. § The solution of the mucin was subjected to the colour reactions without preliminary fractionation. // Micro analysis.

*Fractionation of the Non-Amino Sugars of Pseudomucin as
Methylphenylhydrazones.*

5 mgs. of the pseudomucin were heated on a boiling water-bath with 300 cc. of 1 n. H_2SO_4 solution for $3\frac{1}{2}$ hours. The hydrolysate was removed in the cold from sulfuric acid by the aid of a saturated baryta water and evaporated under a reduced pressure to about 300 cc. and 160 cc. of 25% solution of mercuric acetate were added. The deposit that was small in quantity was centrifuged off and the liquid was neutralized accurately with a saturated baryta water. Precipitates appearing hereupon were separated on a filter and barium and mercury precipitated out with just a sufficient quantity of 1 n. H_2SO_4 and with H_2S gas. The filtrate from the sulfide was condensed at 40°C to 100 cc. at first and, after passing ether in a continuous extractor to remove acetic acid, to about 30 cc. 1 cc. of methylphenylhydrazine and then 95% alcohol enough to obtain a homogeneous solution were added, and the solution was stood for 10 minutes on a boiling water-bath and followingly in a dark room for several hours. Crystals, which came out meanwhile, were collected and recrystallized from 95% alcohol. The product was a mixture of crystals in a plate form and those in a thin needle form. Fortunately, however, we soon noticed that the former crystallize out from

hot alcohol more quickly than the latter, and fractional crystallization gave substances with constant melting points. The whole processes were carried out over again with another 5 gms. portion of pseudomucin. The total yields of the hydrazones were 120 mgms. each.

Crystals in the plate form melted at 190–191° and analyzed:

$C_6H_{12}O_5NN(CH_3)C_6H_5$:	Calc.,	9.85% N.
	Found,	10.00% N.

No depression of melting point occurred, when this substance was mixed with galactose methylphenylhydrazone.

The crystals in the thin needle form melted at 181–182° and analyzed:

$C_6H_{12}O_4NN(CH_3)C_6H_5$:	Calc.,	10.44% N.
	Found,	10.38% N.

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Bibliography and Notes.

- 1 Zängerle, Münch. med. Wochens. 1900, No. 13, 2.
- 2 Fürth, Herrmann and Scholl, Bioch. Zeits., 1934, **271**, 395. A detailed historical presentation is seen in this publication.
- 3 Rhamnose gives only a faint greenish orange colour with diphenylamine.
- 4 Masamune and Tanabe, J. Bioch., 1938, **28**, 19.
- 5 Mannose did not come out of the solution, when the latter was treated with this hydrazine and glacial acetic acid in the cold.
- 6 Delivered from Prof. S. Ohno of the Institute of Obstetrics and Gynecology, Sapporo.
- 7 Hammarsten, Zeits. physiol. Chem., 1882, **6**, 194.