Biochemical Studies on Carbohydrates.

CXLIII. Mucosin.

Second Report: A Few Derivatives.

By

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Cinchonidine salt of mucosin, N-acetylmucosin-methylester and cinchonidine salt of desaminomucosin have been separated in crystalline form. The properties of them are noted here.

As will be described, acetylation according to Zückerkandl and Messiner-Klebermass gave rise to only partial N-acylation of mucosin-methyl-ester. To obtain N-acetyl mucosin the product was treated with a dilute baryta at low temperature. But hydrolysis of the ester linkage was apparently confined in the non-acylated mucosinester, because the cinchonidine salt which was prepared from the ethereal precipitates of the reaction mixture proved that of free mucosin. Under the conditions of hydrolysis preferred the acetyl group combined with nitrogen is not thought to have been split off. From the mother liquid of the ethereal precipitates of the acetylation mixture above settled out crystals of N-acetylmucosin-methylether on addition of petroleum ether. For preparation of desaminomucosin free mucosin was deaminated and for its isolation as crystals cinchonidine was employed.

EXPERIMENTAL.

1) *A mixture of N-acetylmucosin-methylester and mucosin-methylester.* 750 mg. of mucosin-methylester hydrochloride¹⁾ were taken up in 6 cc. of abs. methanol, shaken with 0.3 g. of silver acetate and 0.8 cc. of acetic anhydride for 10 hours (room temp., 18°C) and filtered with suction with 8 cc. of methanol. To the filtrate were added 10 volumes of ether. The deposits (The mother liquid will be referred to as **Fluid A**) were washed with ether and after drying, again subjected to a similar procedure. 430 mg. of a hygroscopic yellowish powder were given. It gave the positive indirect-Osaki-Turumi reaction, whereas its acetyl content did not reach beyond half the calculated for N-acetylmucosinmethylester.

2) *Mucosin cinchonidine salt*. The balance (400 mg.) of the product above was dissolved in 20 cc. of water, the saturated $\text{Ba}(\text{OH})_2$ added till a pH of 9.5 and stood overnight in an ice chest. The mixture was then freed from barium with an equivalent quantity of sulfuric acid and distilled in vacuo to about 3 cc., with following dilution to 9 cc. with methanol and precipitation with 4 volumes of ether (No precipitation happened on addition of additional ether). The precipitates, washed with ether and dried, weighed 320 mg. Next, the substance was dissolved in 20 cc. of water and after addition of 500 mg. cinchonidine as dissolved in 10 cc. methanol and standing several hours, distilled almost to dryness. 15 cc. of water were added to the residue and the insoluble part filtered off. The filtrate was exhausted with chloroform, concentrated by vacuum distillation to a thick solution and placed in an ice-chest. Crystals in white needles came out slowly, which were collected, after 1 week, on a suction funnel and washed with a small quantity of ice-cooled water. The amount yielded was 110 mg. F.P. 193–193.5°C. Once recrystallized from water (The crude product was dissolved in 10 cc. of water and distilled to a small volume under diminished pressure at 35°C), the substance showed the constant melting point of 194–194.5°C. Yield 80 mg. Fehling, Goldschmiedt and Neuberg-Saneyoshi, all positive. With Ehrlich reagent it reacted neither immediately nor after treatment with hot alkali (Osaki-Turumi method). It left no ash on ignition.

Crystal water.

30.15 mg. substance, 1.88 mg. of water lost at 90–100°C under reduced pressure.

$\text{C}_{12}\text{H}_{20}\text{O}_{11}\text{N}\cdot\text{C}_{19}\text{H}_{22}\text{ON}_2 + 2\frac{1}{2}\text{H}_2\text{O}$. Found, 6.24%; calc., 6.36%.

N (Friedrich).

7.02 mg. substance, 2.95 cc. 0.01 N acid neutralized by distilled NH_3 .

$\text{C}_{31}\text{H}_{42}\text{O}_{12}\text{N}_3 + 2\frac{1}{2}\text{H}_2\text{O}$. Found, 5.88%; calc., 6.07%.

$\text{NH}_2\text{-N}$ (micro Van Slyke).

4.42 mg. substance, 0.16 cc. N_2 (756 mm. Hg, 27°C).

$\text{C}_{12}\text{H}_{18}\text{O}_{11}(\text{NH}_2)\cdot\text{C}_{19}\text{H}_{22}\text{ON}_2 + 2\frac{1}{2}\text{H}_2\text{O}$. Found, 1.98%; calc., 2.02%.

CH_3CO (Suzuki's modification²⁾ of Friedrich, Rapoport and Sternberg method).

8.45 mg. substance, 0.205 cc. 0.01 N thiosulfate used. Found, 1.04%.

The figure obtained indicates that all NH_2 -group is in the free form, because the acetyl content of cinchonidine salt of N-acetylmucosin containing 6.36% crystal water would reach 5.85% (Cf. Suzuki²⁾ regarding the yield from non-acetylated sugars).

The filtrate of the crude product above gave the positive indirect-Osaki-Turumi test.

3) *N-Acetylmucosin-methylester*. To Fluid A in 1) was added petroleum ether in small portions till permanent turbidity. On standing crystals came out gradually in white thin needles. Petroleum ether was supplemented from time to time with cautions against occurrence of flocculent precipitates and when no more cloudiness appeared, the deposits

were separated, washed with a mixture of alcohol and ether (1:2 by volume) and dried in air. From 2.8 g. of the material (mucosin-methylester hydrochloride) were thus obtained 150 mg. of the crude substance. It melted at 124–124.5°C. Recrystallization was processed from methanol by the aid of ether and petroleum ether as above. 81 mg. of a substance with the constant melting point of 126°C were produced. It left no ash on burning. It dissolved readily in water, giving a neutral solution (proved by alkaline titration). Indirect-Osaki-Turumi, Fehling, Tollens, aniline acetate (for glucuronic acid), Goldschmiedt and Neuberg-Saneyoshi test resulted all in positive.

Crystal water.

21.45 mg. substance lost 0.88 mg. of water at 100–110°C under reduced pressure.

$C_{15}H_{25}O_{12}N + H_2O$. Found, 4.10%; calc., 4.20%.

N (micro Kjeldahl).

8.931 mg. substance, 2.10 cc. 0.01 N acid neutralized by distilled NH_3 .

$C_{15}H_{25}O_{12}N + H_2O$. Found, 3.29%; calc., 3.26%.

No free amino nitrogen was detected (micro Van Slyke method).

Glucosamine (Hamasato and Akakura).

11.32 mg. substance was taken for analysis. 2 cc. of the hydrolysate, neutralized, filled up to 25 cc. and filtered, contained 0.452 mg. glucosamine as hydrochloride.

Found, 41.50%; calc., 41.72%.

Glucuronic acid.

14.88 mg. substance was dissolved in 10 cc. of water and 1 and 0.5 cc. respectively of the solution were taken for the colorimetric techniques.

Found, 45.15% by the indole method and 44.89% by the orcinol method; calc., 45.22%.

CH_3CO .

5.996 mg. substance, 1.39 cc. 0.01 N thiosulfate used.

$C_{13}H_{22}O_{11}N(CH_3CO) + H_2O$. Found, 9.96%; calc., 10.0%.

Mol. wt. from iodine value (Bergmann and Machemer).

6.18 mg. substance, 1.41 cc. of 0.02 N iodine solution used.

$C_{15}H_{25}O_{12}N + H_2O$. Found, 438; calc., 429.

Optical rotation in water.

19.65 mg. substance was dissolved in 1 cc. of water. Temp., 9°C.

Time min.	α deg.	$[\alpha]_D = \frac{\alpha \times 100}{1.965 \times l}$
5	−0.13	− 6.66
10	−0.30	−15.27
15	−0.40	−20.36
20	−0.42	−21.37
30	−0.43	−21.88
40	−0.43	−21.88
60	−0.44	−22.39
60×2	−0.47	−23.92
60×3	−0.50	−24.45
60×4	−0.54	−27.48
60×6	−0.57	−29.01

60×8	—0.57	—29.01
60×24	—0.57	—29.01
The extrapolated initial $[\alpha]_D^{25}$ was +3.82°.		

4) *Desaminomucosin cinchonidine salt*. 3.8 g. of free mucosin¹⁾ were taken up in 95 cc. of 0.1 N HCl, shaken with 1.52 g. of AgNO₃ for 2 hours and filtered, then the filtrate was refluxed for 2 hours on a boiling water-bath (Some precipitates appeared) and after again filtering, stood overnight at room temperature. The reaction mixture was again boiled for the same duration of time with 0.76 g. of AgNO₃, removed from the merest trace of an insoluble substance and bubbled with H₂S. The deposits were rejected. The fluid was distilled to about 5 cc. under diminished pressure and precipitated with abs. alcohol and ether. The precipitates were washed with ether and dried. 1.5 g. of crude desaminomucosin were given as a dark brown powder. It scarcely contained nitrogen and hardly showed Elson-Morgan reaction, Molisch, slightly positive. Goldschmiedt and Neuberg-Saneyoshi both positive.

1 g. of the amorphous product in 50 cc. of water and 2 g. of cinchonidine in 20 cc. methanol were mixed together and boiled on a water-bath for 1.5 hours. Thereby the brown mixture became deeper colored, which was freed from some precipitates, when cold, and distilled to a syrup. Excess of cinchonidine was separated off as above by dissolving it in water, filtering and chloroform-extraction. The clear watery solution was then concentrated and stood in the room and in an ice-chest. Since, however, the amount of crystalline deposits was small, the mixture of the crystals and mother liquid was diluted up to about 30 cc. and to the solution obtained the saturated Ba(OH)₂ added till a pH of 9.5. A voluminous dark mucilaginous substance settled out together with cinchonidine. After standing overnight, the precipitates were filtered off and the filtrate, which was only light colored, was liberated from barium with H₂SO₄ just sufficient. Hereupon addition of cinchonidine and following operations was repeated (The mixture after addition of cinchonidine was heated only slightly). Crystals separated soon on cooling in ice of the condensate. Stood overnight. The deposits were separated and dried. They amounted to 0.6 g. and possessed F.P. of 181.5–182°C. Recrystallized once from water, the substance melted constant at 183°C and ignition gave no ash. Schiff, Goldschmiedt and Neuberg-Saneyoshi, positive. Elson-Morgan and Molisch, negative. Analysis:—

Crystal water.

30.15 mg. substance lost 1.55 mg. of water at 95–105°C under reduced pressure.

C₁₂H₁₉O₁₁·C₁₉H₂₂ON₂+2H₂O. Found, 5.14%; calc., 5.38%.

N (Friedrich).

8.30 mg. substance, 2.40 cc. 0.01 N acid neutralized by distilled NH₃.

$C_{12}H_{19}O_{11} \cdot C_{19}H_{22}ON_2 + 2H_2O$. Found, 4.06%; calc., 4.19%.
Iodine value (Macleod-Robison).

8.118 mg. substance, 1.17 cc. of 0.02 N iodine solution used.

$C_{11}H_{18}O_{10}(CHO) \cdot C_{19}H_{22}ON_2 + 2H_2O$. Found, 28.83; calc., 29.90.

Optical rotation.

$$[\alpha]_D^{25} = \frac{-0.61 \times 100}{1.256 \times 1} = -48.6^\circ \text{ (in water, no mutarotation).}$$

This owes entirely to cinchonidine in the molecule, because a cinchonidine sulfate solution containing the same quantity of the alkaloid as the solution examined above had just the same rotatory power ($\alpha = -0.61^\circ$).

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References.

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 - 2) Suzuki, J. Bioch., 1938, **27**, 367.
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