the Director Dr. K. Abe and Dr. M. Kawanishi of this Laboratory for their encouragements. Thanks are also due to Mrs. F. Hisamichi, Messrs. T. Yoda, T. Kono, and N. Takeda for microanalytical data, and Miss M. Ninomiya and Messrs. K. Tanikawa, K. Kotera and R. Murata for infrared data.

Summary

A new synthesis of *rac*-nicotine was described. For this end the removal of the benzylidene group of 2-substituted 3-benzylidene-1-pyrroline prepared by Sugasawa and Ushioda was first examined. For the best effect, the starting materials ((I) : 2-methyl; (IXa) : 2-phenyl) were reduced by sodium borohydride and the reduction products were acylated to yield ((IIIa) : 1-acetyl-2-methyl; (IIIb) : 1-benzoyl-2-methyl; (XIIa) : 1-benzoyl-2-phenyl). These were cleaved via the corresponding ozonides to yield 3-pyrrolidinones, which were characterized appropriately.

This method was successfully extended to 2-(3-pyridyl)-3-benzylidene-1-pyrroline to afford 1-benzoyl-2-(3-pyridyl)-3-pyrrolidinone, whose tosylhydrazone was converted into 1-benzoyl-2-(3-pyridyl)-3-pyrroline according to the method of Bamford and Stevens. The latter was reduced catalytically to *rac*-nornicotine, from which *rac*-nicotine was obtained smoothly by Eschweiler-Clark's method.

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129. **Toshio Miyazaki** : Studies on Fungal Polysaccharides. II.[*1] On the Componental Sugars and Partial Hydrolysis of the Capsular Polysaccharide from *Cryptococcus neoformans*.

(*Tokyo College of Pharmacy*[*2])

The preceding paper described some properties of the capsular polysaccharide isolated from *Cryptococcus neoformans* CRD-1 (Duke). In this paper, results on further investigations on the kinds of componental sugars, their molecular ratio, and partial hydrolysis of the capsular polysaccharide are described.

TABLE I. Rf values of the Componental Sugars

Substance \ Solvent system	(1)	(2)
D-Xylose	0.26	0.28
D-Mannose	0.17	0.20
D-Galactose	0.15	0.17
D-Glucuronic acid	0.12	0.14
Monosaccharide from Hydrolysate	0.26	0.28
	0.17	0.20
	0.12	0.14

Solvent system : (1) AcOEt-AcOH-H_2O (3:1:3).
(2) BuOH-AcOH-H_2O (4:1:5).
Detection by 3% *p*-anisidine-hydrochloride (BuOH) spray.

[*1] Part I. This Bulletin, **9**, 715 (1961).

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The componental sugars of the polysaccharide were detected from its acid hydrolysate, obtained by treatment of the polysaccharide with 95% formic acid and followed with N sulfuric acid. On detection by paper chromatography, three sugars, D-xylose, D-mannose, and D-glucuronic acid, were found as components of this polysaccharide, but no D- galactose or other sugars. This result is shown in Table I.

Quantitative estimation of the componental sugars were made by phenol-sulfuric acid reaction,[1] cysteine-sulfuric acid reaction,[2,3] and by carbazole-sulfuric acid reaction.[4] Phenol-sulfuric acid reaction on the hydrolysate revealed the ratio of D-xylose to D-mannose of 1:3 and this is shown in Table II.

Table II. Quantitative Ratio of the Componental Sugars

Method / Sugar	Cysteine-H_2SO_4 [2] (%)	Cysteine-H_2SO_4 [3] (%)	Carbazole-H_2SO_4 [4] (%)
D-Xylose	20.5	—	—
D-Mannose	—	61.2	—
D-Glucuronic acid	—	—	18.9

Molecular ratio : D-Glucuronic acid; D-Xylose; D-Mannose=1:1:3

Estimation of the reducing power (reducing end-group) of the material by both the modified Somogyi's method[5] and hypoiodite oxidation[6] revealed the respective ratio of reducing group to monosaccharide unit of 1:0.024 and 1:0.023, calculated as mannose.

Molecular weight of this polysaccharide was about 6600 by Akiya-Barger's method,[7] and it was almost the same as that calculated from the reducing end-group assay of this polysaccharide.

Hydrolysis of the polysaccharide with 0.1N sufuric acid under relatively mild condition of 100° for 7 hours failed to show any reducing sugars, indicating the absence of furanose sugars.[8] When the polysaccharide was heated with 0.5N sulfuric acid for 12 hours, the reducing power was 1:0.208 calculated as mannose. Fig. 1 shows the increase of reducing power during acid hydrolysis of the polysaccharide.

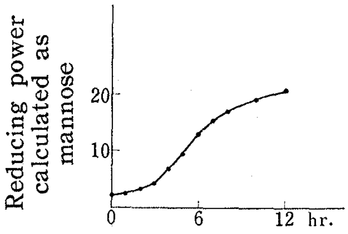

Fig. 1. Change in Reducing Power during Acid Hydrolysis of the Polysaccharide with 0.5N H_2SO_4 at 100°

Paper chromatography of the hydrolysate obtained by treatment with 0.5N sulfuric acid showed the presence of D-xylose and a trace of D-glucuronic acid as the liberated reducing sugars. After hydrolysis with 2N sulfuric acid for 4 hours, D-mannose and D-glucuronic acid, as well as a small amount of D-xylose, were liberated.

From the above results, it was concluded that the polysaccharide was a xyloglucuronomannan, which consisted of D-glucuronic acid, D-xylose, and D-mannose in 1:1:3 ratio, and the molecular weight was about 6600 calculated from the results of its reducing end-

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[1] M. Dubois, *et al.* : Anal. Chem., **28**, 350 (1956).
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[5] M. Somogyi : *Ibid.*, **195**, 19 (1952).
[6] R. Willstätter, G. Schudel : Ber., **51**, 780 (1918).
[7] S. Akiya : Yakugaku Zasshi, **57**, 967 (1937).
[8] W.N. Haworth : Ber., **65**, 43 (1932).

group assay and by Akiya-Barger's method. D-Xylose and a part of D-glucuronic acid in this polysaccharide was weak to hydrolysis compared with that of D-mannose.

Experimental

Componental Sugars of the Polysaccharide——The purified polysaccharide (40 mg.) was hydrolyzed with 95% HCOOH (1.8 cc.) in a sealed tube, placed in a boiling water-bath for 20 hr. HCOOH was distilled off and the residue was further hydrolyzed with N H_2SO_4 (2 cc.) for 2 hr. at 100°. The acid was neutralized with $BaCO_3$, filtered, the filtrate was concentrated to a small volume, and examined by paper chromatography (Table I).

Quantitative Estimation of Xylose and Mannose by Phenol and Sulfuric Acid——A small portion of the above hydrolysate was spotted on a filter paper (Toyo Roshi No. 51, 20×40 cm.) and developed for 8 hr. with the solvent system (1). After air-drying overnight the spots on the chromatogram were quantitatively extracted with 20 cc. of distilled H_2O, filtered through a sintered glass filter, and the amount of the sugar in 2 cc. of the filtrate was estimated colorimetrically by the method of Dubois, *et al.*[1]. The result indicated the presence of xylose and mannose in 1:3 ratio. Found: D-xylose, 18 γ, 19 γ; D-mannose, 63 γ, 66 γ.

Quantitative Estimation of Xylose and Mannose by Cystein and Sulfuric Acid——The polysaccharide solution (40 γ in 0.1 cc.) was estimated by the method of Dische.[2] The molecular ratio of xylose to mannose was 1:3. Found: D-xylose in the polysacchaside, 20.5%; D-mannose, 61.2%.

Quantitative Estimation of Glucuronic Acid with Carbazole and Sulfuric Acid——The polysaccharide solution (40 γ in 0.1 cc.) was estimated by the method of Dische.[3] Found: 18.9%.

Reducing Power of the Polysaccharide——a) Somogy's method[5]: A solution of 5 cc. of 1.02 mg. of the polysaccharide was heated with 5 cc. of the alkaline copper reagent for 40 min. at 100°, cooled in ice, and the mixture was acidified with $2N$ H_2SO_4 (1.5 cc.). The liberated I_2 was titrated with $0.005N$ $Na_2S_2O_3$ and it consumed 0.15 cc. of $0.005N$ $Na_2S_2O_3$. The control experiment was conducted in the same way using 0.104 mg. of mannose and it consumed 0.63 cc. of $0.005N$ $Na_2S_2O_3$. From the above experiments, the reducing power of this polysaccharide was found to be 0.024 calculated with mannose as 1.

b) Hypoiodite oxidation. To the polysaccharide solution (10.2 mg. in 2 cc.), $0.1N$ I_2 (1 cc.) and $0.1N$ NaOH (2 cc.) were added, and this mixture, closed with a glass stopper, was kept at room temperature. After 3 hr., the alkaline solution was acidified with $2N$ H_2SO_4 (0.5 cc.) and the liberated I_2 was titrated with $0.01N$ $Na_2S_2O_3$. The consumption of $0.01N$ $Na_2S_2O_3$ was 0.41 cc. for 10.2 mg. of polysaccharide, and 4.46 cc. for 5.04 mg. of mannose (as control). This result showed the ratio of the reducing power of mannose and the polysaccharide to be 1:0.023.

Attempted Hydrolysis of the Polysaccharide with 0.1*N* Sulfuric Acid——The polysaccharide (26.0 mg.) was dissolved in $0.1N$ H_2SO_4 (25 cc.), heated in a boiling water bath. At suitable intervals, 0.5 cc. of the solution was taken out and its reducing power was estimated by Somogyi's method. The consumption of $0.005N$ $Na_2S_2O_3$ was 0.08 cc. (every time).

Partial Hydrolysis of the Polysaccharide with 0.5*N* Sulfuric Acid——The polysaccharide (25.4 mg.) dissolved in $0.5N$ H_2SO_4 (25 cc.) was heated in a boiling water bath for 12 hr., and treated in the same way described above. The consumption (cc.) of $0.005N$ $Na_2S_2O_3$ was as follows: 0.08 (0 hr.), 0.09 (1 hr.), 0.11 (2 hr.), 0.14 (3 hr.), 0.24 (4 hr.), 0.31 (5 hr.), 0.45 (6 hr.), 0.50 (7 hr.), 0.56 (8 hr.), 0.63 (10 hr.), 0.68 (12 hr.), and 0.36 for 0.28 mg. of mannose (as control) (Fig. 1).

Paper Chromatography of the Hydrolysate from Treatment with 0.5*N* Sulfuric Acid——The above hydrolysate was neutralized with $BaCO_3$, filtered, and the precipitate was washed with H_2O. The filtrate, combined with the washings, was concentrated to dryness *in vacuo*, the syrup obtained was dissolved in MeOH, and centrifuged. The supernatant was concentrated to dryness *in vacuo* and the residual syrup was examined by paper chromatography. Rf: 0.26, 0.12 (trace) (solvent system (1)), 0.28, 0.14 (trace) (solvent system (2)).

Complete Hydrolysis of the Partial Hydrolysate with 2*N* Sulfuric Acid——Since paper chromatography on the MeOH-insoluble fraction showed no reducing sugars, it was further hydrolyzed with $2N$ H_2SO_4 for 4 hr. The hydrolysate was neutralized with $BaCO_3$, concentrated to a syrup *in vacuo*, and examined by paper chromatography. Rf; 0.26 (trace), 0.17 and 0.12 (solvent system (1)), 0.28 (trace), 0.20 and 0.14 (solvent system (2)).

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Summary

Capsular polysaccharide of the *Cryptococcus neoformans* was found to be a xyloglucuronomannan, which consisted of D-glucuronic acid, D-xylose, and D-mannose (in 1:1:3 ratio), and its molecular weight was about 6600 by the Akiya-Barger's method and from results of end-group assay. D-Xylose and a part of D-glucuronic acid in this polysaccharide were liberated more easily than D-mannose.

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130. **Toshio Miyazaki**: Studies on Fungal Polysaccharides. III.[*1]
Chemical Structure of the Capsular Polysaccharide from *Cryptococcus neoformans.*

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In the previous paper of this series,[*1] it was shown that the polysaccharide of *Cryptococcus neoformans* consisted of D-xylose, D-glucuronic acid, and D-mannose in a ratio of 1:1:3, and hydrolysis of this polysaccharide liberated D-xylose and D-glucuronic acid more easily than D-mannose. In this paper, the results of further investigations on the chemical structure of this polysaccharide, based on periodate oxidation and methylation, will be described.

On periodate oxidation of potassium salt of the capsular polysaccharide, the consumption of periodate per 166 g. of the polysaccharide was as follows : 0.99 moles (after 6 hours), 1.24 (24 hours), 1.31 (48 hours), 1.38 (120 hours). The value of formic acid liberated from 166 g. of polysaccharide was 0.47 moles (after 20 hours), 0.55 (144 hours), and that of formaldehyde was 0.02 mole (after 216 hours) respectively.

The periodate-oxidized polysaccharide was reduced with sodium borohydride followed by acid hydrolysis. Paper chromatographic analysis of the hydrolysate revealed the presence of D-mannose as the only reducing sugar in addition to erythritol and a small amount of glycerol, and xylose and glucuronic acid were not detected.

These results, pointed to the following conclusions : (1) D-Xylose and D-glucuronic acid are not present as branching point of the polysaccharide. (2) A part of D-mannose would be the branching point or have 1:3 linkage. (3) Other part of D-mannose would have 1:4 linkage. (4) The value of formaldehyde liberated from the polysaccharide in periodate oxidation was equivalent to one mole per 45 moles hexose units.

It is known[1)] that D-glucuronide consumes periodate in neutral medium more rapidly than other aldoses. In the case of this polysaccharide, in spite of the presence of non-oxidized mannose the rapid consumption of periodate in the initial stage of oxidation suggests that glucuronic acid would be present as the end group of this polysaccharide.

The polysaccharide was methylated several times with dimethyl sulfate and sodium hydroxide, and further repeatedly with methyl iodide and silver oxide. During the course

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