Chem. Pharm. Bull. 26(10)3215—3218(1978)

UDC 547.458.02.03 : 581.192

Plant Mucilages. XX.[1] The Location of *O*-Acetyl Groups in Paniculatan and the Influence of Deacetylation on Its Physical Properties

Masashi Tomoda,[2a,c] Noriko Satoh,[2a] and Gō Matsumura[2b]

Kyoritsu College of Pharmacy[2a] and School of Pharmaceutical Sciences, Showa University[2b]

(Received April 1, 1978)

The *O*-acetyl groups in paniculatan, the mucous polysaccharide isolated from the bark of *Hydrangea paniculata* Sieb., molecular weight of 545900, were located in position 3 of about 40% of L-rhamnopyranosyl residues. A completely deacetylated polysaccharide possessing significant lower molecular weight, 41950, was obtained by the action of 0.1 N sodium hydroxide at room temperature for 10 min, and the influence of this treatment on the partial degradation of a macromolecule was considered.

Keywords——location of *O*-acetyl groups; influence of deacetylation; paniculatan; mucous polysaccharide; *Hydrangea paniculata*; intrinsic viscosity; osmotic pressure; molecular weight; sedimentation equilibrium

The isolation and the structural feature of the mucous polysaccharide, named paniculatan, from the inner bark of *Hydrangea paniculata* Sieb. have been reported in the previous papers of this series.[3–5] The polysaccharide contains 2.0% of *O*-acetyl groups.[3] The present work was undertaken to elucidate the location of *O*-acetyl groups. This paper is also concerned with the influence of its deacetylation on the physical properties of the polysaccharide as a native polymer.

The polysaccharide was exhaustively treated with methyl vinyl ether in the presence of *p*-toluenesulfonic acid in dimethyl sulfoxide.[6] After conversion of the free hydroxyl groups to 1-methoxyethyl ethers, the derivative was deacetylated, then methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide.[7] The resultant product was subjected to acid hydrolysis, and the final products were analyzed by gas–liquid chromatography–mass spectrometry (GLC–MS) after conversion into alditol acetates.[8] 1,2,4,5-Tetra-*O*-acetyl-3-mono-*O*-methyl L-rhamnitol was detected and identified. The result of GLC showed that the molar ratio of 3-mono-*O*-methyl-L-rhamnose and L-rhamnose was 1.0:1.4. Basing on this result, it can be concluded that approximately 40% of L-rhamnopyranosyl residues are present as 3-*O*-acetyl derivative in the molecule of paniculatan.

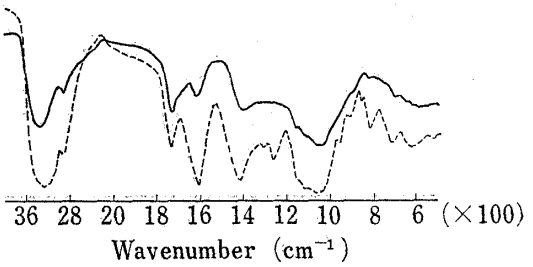


Fig. 1. IR Spectra of Paniculatan and the Deacetylated Paniculatan

——: the deacetylated paniculatan,
-----: paniculatan.

1) Part XIX: M. Tomoda, N. Satoh, and C. Ohmori, *Chem. Pharm. Bull.* (Tokyo), **26**, 2768 (1978).
2) Location: *a*) *1-5-30, Shibakōen, Minato-ku, Tokyo, 105, Japan*; *b*) *1-5-8, Hatanodai, Shinagawa-ku, Tokyo, 142, Japan*; *c*) To whom inquiries should be addressed.
3) M. Tomoda and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **24**, 230 (1970).
4) M. Tomoda, N. Satoh, and Y. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **25**, 968 (1977).
5) M. Tomoda and N. Satoh, *Chem. Pharm. Bull.* (Tokyo), **25**, 2910 (1977).
6) A.N. DeBelder and B. Norrman, *Carbohyd. Res.*, **8**, 1 (1968).
7) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
8) H. Björndal, B. Lindberg, and S. Svensson, *Carbohyd. Res.*, **5**, 433 (1967).

Paniculatan was treated with 0.1 N sodium hydroxide for 10 min at room temperature. The solution was neutralized, dialyzed, then lyophilized. As shown in Fig. 1, the disappearance of the absorption band at 1250 cm^{-1} in the infrared (IR) spectrum of the product proved that it was the deacetylated derivative of the original polysaccharide.

The deacetylated paniculatan was shown to be homogeneous in both ultracentrifugal analysis (Fig. 2) and gel chromatography with Sephadex G-200 (Fig. 3). Its solubility in water increased more than two times that of paniculatan, in contrast to the case of the deacetylated lily glucomannans.[9,10] Its aqueous solution gave the intrinsic viscosity value of 14.2 at 30°. This value and that of the original polysaccharide correspond to a ratio of 1: 3.8.[3]

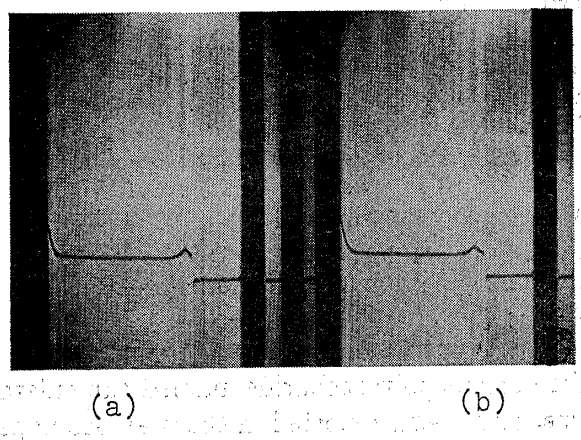


Fig. 2. Ultracentrifugal Pattern of the Deacetylated Paniculatan

a: 0.2% in H_2O, 25°, 18 min, 60000 rpm,
b: 0.2% in H_2O, 25°, 24 min, 60000 rpm.
Hitachi model UCA-1A ultracentrifuge.

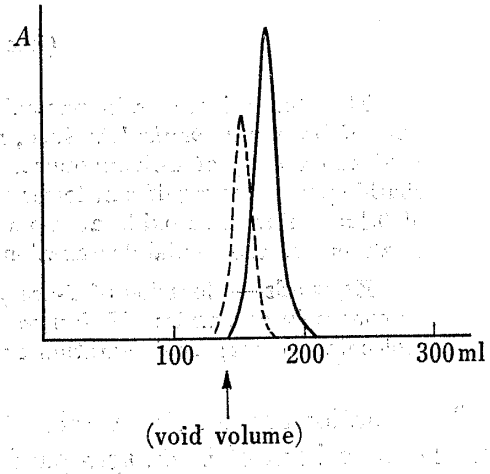


Fig. 3. Chromatogram of Paniculatan and the Deacetylated Paniculatan on Sephadex G-200

——: the deacetylated paniculatan,
------: paniculatan.

The measurement of osmotic pressure gave the value of 14260 as the molecular weight of the ammonium salt of the deacetylation product. The value and that of the ammonium salt of paniculatan[3] correspond to an approximate ratio of 1: 7.6. However, we now found that the ammonium salt of paniculatan contained only 0.72% of *O*-acetyl groups. Thus even the treatment with very dilute ammonium hydroxide resulted in the partial deacetylation of the original polysaccharide. Therefore, the molecular weights of the original and the deacetylated polysaccharides were reinvestigated by the measurement of sedimentation equilibrium. The results gave the values of 545900 and 41950 as the molecular weights of paniculatan and its deacetylation product, respectively. In this case the value of the deacetylation product and that of paniculatan correspond to a ratio of 1: 13.

The deacetylation of paniculatan caused the remarkable lowering of the values in both the molecular weight and the viscosity of aqueous solution. From this fact, it is possible to derive the following two sorts of inference. First, in the case of the treatment with dilute sodium hydroxide, the possibility of the direct degradation of a polymer with alkali hydroxide is undeniable. However, as the second possibility, the result that even the treatment with very dilute ammonium hydroxide caused the release of about two thirds of acetyl groups in the native polysaccharide and the lowering of the molecular weight suggests a significant role of *O*-acetyl groups in the polysaccharide in the association of a macromolecule. Further investigation of these problems is now under progress.

As described above, the original substances were used for the estimation of molecular weights by the sedimentation equilibrium method. On the other hand, the measurement

9) T. Matsuo and T. Mizuno, *Agr. Biol. Chem.*, 38, 465 (1974).
10) M. Tomoda, S. Kaneko, and S. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), 23, 430 (1975).

of osmotic pressure was carried out with the ammonium salts of paniculatan and the deacetylation product. It is possible to conclude that one of the causes of the considerable difference in both values of molecular weight of paniculatan by the each method is due to the partial dissociation of a macromolecule owing to the release of the majority of acetyl groups. And all the results also provide the evidence that paniculatan belongs to a polydispersed polysaccharide.

Experimental

Solutions were concentrated at or below 40° with rotary evaporators under reduced pressure. Optical rotation was measured with JASCO model DIP-SL automatic polarimeter. Viscosity was determined with an Ubbelohde-type viscosimeter. IR spectra were recorded on JASCO model IRA-2 infrared spectrophotometer. GLC–MS was carried out by the use of JEOL model JGC-20K gas chromatograph and JEOL model JMS-D100 mass spectrometer.

Treatment with Methyl Vinyl Ether——The polysaccharide (106 mg) was suspended in dimethyl sulfoxide (12 ml) and then *p*-toluenesulfonic acid (20 mg) was added. The mixture was stirred at 15°, then methyl vinyl ether (5 ml), condensed at −10°, was added in portions under stirring. The reaction mixture was stirred at 15° for 3.5 hr, then dialyzed against running water overnight. The non-dialyzable fraction was concentrated to dryness, then the reaction was similarly repeated three times. The final solution was applied to a column (4×19 cm) of Sephadex LH-20. The column was eluted with acetone, and the eluate of the first yellow band was collected and concentrated. The product was further treated three times under the same condition. The IR spectrum of the final residue had no absorption near 3400 cm^{-1}.

Deacetylation of the *O*-Acetyl-*O*-(1-methoxyethyl)-derivative——The product (150 mg) was dissolved in methanol (5 ml), then 0.2 M methanolic sodium methoxide (5 ml) was added under stirring. The solution was refluxed at 80° for 4 hr, then concentrated and applied to a column (4×26 cm) of Sephadex LH-20, and the column was eluted with methanol. The eluate of the first red band was collected and concentrated.

Methylation of the *O*-(1-Methoxyethyl)-derivative——The product (145 mg) was dissolved in dimethyl formamide (5 ml), then methyl iodide (1 ml) and silver oxide (0.4 g) were added successively under stirring. The reaction mixture was stirred at room temperature for 24 hr in a dark. After filtration, methyl iodide (1 ml) and silver oxide (0.4 g) were added again into the filtrate, then the reaction was similarly repeated. The reaction mixture was filtered, then benzene (45 ml) was carefully added into the filtrate. The precipitate was filtered off, and benzene was evaporated. The residual solution was applied to a column (4×25 cm) of Sephadex LH-20. The column was eluted with methanol, and the eluate of the first yellow band was collected and concentrated. The product was further methylated three times under the same condition. The IR spectrum of the final product had no absorption near 3400 cm^{-1}.

Hydrolysis and Analysis of the *O*-Methyl-derivative——*O*-Methyl-*O*-(1-methoxyethyl)-derivative (20 mg) was hydrolyzed with 2 N sulfuric acid (2 ml) at 100° for 5 hr, then neutralized with barium carbonate. The filtrate was treated with Dowex 50W (H^+), then reduced with sodium borohydride (10 mg) at room temperature for 2 hr. After neutralization with Dowex 50W (H^+), the filtrate was evaporated and boric acid was removed by repeated addition and evaporation of methanol. Then the products were acetylated with acetic anhydride–pyridine mixture (1:1) at 100° for 40 min. After evaporation of the solution, the residue was dissolved in chloroform–methanol mixture (1:1) and applied to GLC–MS. GLC was carried out by the use of a column (0.3 cm×2 m long spiral glass) packed with 3% OV 225 on Gaschrom Q (100 to 120 mesh) at 210° with a flow (1.5 kg/cm^2) of herium. Relative retention times of rhamnose derivatives to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol were as follows: 1,2,4,5-tetra-*O*-acetyl-3-mono-*O*-methyl-L-rhamnitol, 1.62 (main fragments in MS, *m/e* 43, 87, 101, 129, 143, 189, 203); 1,2,3,4,5-penta-*O*-acetyl-L-rhamnitol, 1.82.

Determination of *O*-Acetyl Groups——The determination was performed in the same manner as a former report[1] of this series.

Deacetylation of Paniculatan——The polysaccharide was dissolved in 0.1 N sodium hydroxide, and the solution (0.1% concentration) was left for 10 min at room temperature. After neutralization with 0.1 N hydrochloric acid, the solution was dialyzed against running water overnight. The non-dialyzable fraction was concentrated and lyophilized. The product showed a positive specific rotation ($[\alpha]_D^{26}$ +82.1° in 0.05% NH_4OH, $c=0.1$).

Gel Chromatography——The sample solution was applied to a column (2.6×96.4 cm) of Sephadex G-200 and the elution was carried out by ascending method with 0.1 M ammonium formate as an eluant. Fractions were collected at 5 ml and analyzed by phenol–sulfuric acid method.[11]

11) J.E. Hodge and B.T. Hofreiter, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York and London, 1962, p. 388.

Measurement of Osmotic Pressure——Osmotic pressure was measured at 60° by the use of Knauer Electronic Membrane Osmometer. The sample was dissolved in water, and 0.4, 0.3, 0.2, and 0.1% solution were used.

Measurement of Sedimentation Equilibrium——Sedimentation equilibrium was measured at 23° by the use of Hitachi model UCA-1A ultracentrifuge. The original polysaccharide was dissolved in water at 0.179%. The solutions of the original, 3/4, 1/2, and 1/4 concentrations were centrifuged at 6520 rpm for 20 hr. The deacetylated product was dissolved in water at 0.665%. The solutions of the original, 3/4, 1/2, and 1/4 concentrations were centrifuged at 10490 rpm for 3 hr.

Chem. Pharm. Bull. 26(10)3218—3222(1978)

UDC 547.572.1.04 : 546.14.04

Preparation of Some Ring-Oxygenated Phenacyl Bromides[1]

Tozo Fujii, Shigeyuki Yoshifuji, and Masashi Ohba

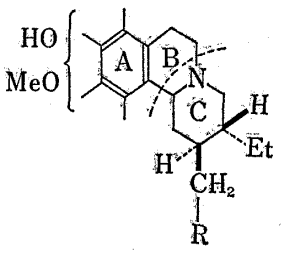
Faculty of Pharmaceutical Sciences, Kanazawa University[2]

(Received April 10, 1978)

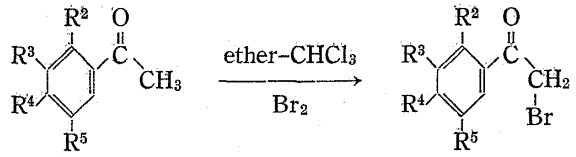
3,4-Dimethoxy, 3-benzyloxy-4-methoxy, 3,4,5-trimethoxy, 2-hydroxy-3,4-dimethoxy, and 2-benzyloxy-3,4-dimethoxy derivatives (**3a**—**e**) of phenacyl bromide have been prepared in 44—84% yields from the corresponding acetophenones by bromination with bromine in a mixture of ether and chloroform.

Keywords——oxygenated phenacyl bromide; oxygenated acetophenone; Friedel-Crafts reaction; bromination; bromine; IR; NMR

The synthetic strategy we have adopted for our syntheses in the Ipecac[3–5] and *Alangium* alkaloids[5–9] (type **1**) and related areas[10] has featured the connection of an adequate phenethyl synthon (fragment A and B in **1**) with a piperidine synthon (*e.g.*, fragment C). This operation has been feasible by the "lactim ether method"[11] in the racemic series[3–5,7,9,10] and by the



1: R=CH_2OH or a heterocyclic moiety



2a—e **3a—e**

a : R^2=H, R^3=R^4=OMe, R^5=H
b : R^2=H, R^3=OCH_2Ph, R^4=OMe, R^5=H
c : R^2=H, R^3=R^4=R^5=OMe
d : R^2=OH, R^3=R^4=OMe, R^5=H
e : R^2=OCH_2Ph, R^3=R^4=OMe, R^5=H

Chart 1

1) Part of this work was reported in a preliminary form.[5]
2) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*
3) T. Fujii and S. Yoshifuji, *Tetrahedron Lett.*, **1975**, 731.
4) S. Yoshifuji and T. Fujii, Abstracts of Papers, 40th Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Kanazawa, June 21, 1975, p. 3.
5) T. Fujii, S. Yoshifuji, and K. Yamada, *Tetrahedron Lett.*, **1975**, 1527.
6) S. Yoshifuji and T. Fujii, *Tetrahedron Lett.*, **1975**, 1965.
7) T. Fujii, K. Yamada, S. Yoshifuji, S. C. Pakrashi, and E. Ali, *Tetrahedron Lett.*, **1976**, 2553.
8) T. Fujii, S. Yoshifuji, S. Minami, S. C. Pakrashi, and E. Ali, *Heterocycles*, **8**, 175 (1977).
9) T. Fujii, S. Yoshifuji, and H. Kogen, *Tetrahedron Lett.*, **1977**, 3477.
10) T. Fujii, S. Yoshifuji, and H. Ito, *Heterocycles*, **7**, 149 (1977).
11) *a*) T. Fujii, S. Yoshifuji, and K. Yamada, *Chem. Ind.* (London), **1975**, 177; *b*) *Idem*, *Chem. Pharm. Bull.* (Tokyo), **26**, 2071 (1978).