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A Rapid Permethylation of Glycolipid, and Polysaccharide Catalyzed by Methylsulfinyl Carbanion in Dimethyl Sulfoxide

An effective, and rapid permethylation of complex carbohydrates noted in the heading is to be reported in this letter. Methylation of a complex carbohydrate is a difficult and painstaking task, but if successful, it offers good confirmation of the chemical structure (*e.g. 1—3*). With more recognition of the significance of complex carbohydrates, roles played in determination of specificities of hormones, immunity, biological transport, and in various pathological phenomena, it has become more urgent to learn the chemical structure. For this purpose, permethylation on a micro scale applicable to the complex carbohydrates is desired. The alcoxide formation, greatly catalyzed by methylsulfinyl carbanion (I) in dimethyl sulfoxide, followed by methylation with methyl iodide was found to be applicable, in some degree, for this purpose.

Since Whistler and BeMiller described an extraction of glycogen from tissue by dimethyl sulfoxide (*4*), 'non-aqueous' chemical reactions might be carried out in this extremely polar, but stable solvent. Not only glycogen but also 'Fr. 4-urinary glycoprotein' (*5, 6*), ovomucoid, a certain subfraction of the Wilson gastric mucin, and various glycolipids were found to the more or less soluble in this solvent. Therefore, it was undertaken to demonstrate the presence of various kinds of acyl residue in this solvent by 'hydroxylaminolysis' of the urinary glycoprotein.* While studying along this line, the author learned that Corey and his group developed a new reaction, using methylsulfinyl carbanion, the conjugate base of dimethyl sulfoxide (*7*). The Witting type synthesis was achieved with great ease (*8, 9*) by a strong nucleophilic property of the carbanion on one hand, and on the other the alcoxide formation of hydroxyl group of the aromatic, oxo-cyclic compound*, and of cyclitol** was greatly accelerated by virtue of a strong de-protonation activity of this carbanion. It was considered that the principle of the reaction could be applied to the alcoxide formation of carbohydrates, and hence to the methylation of carbohydrates of even a complex structure. The proposed reaction may be comparable to reacting carbohydrates with sodium in liquid ammonia, followed by methylation (*10—13*). The present method, however, has obvious advantages in that the reaction is more rapid and complete when catalyzed by the carbanion, can be controlled by the amount of the reagent added, and can be carried out at room temperature in one continuous process without the use of complicated apparatus. Furthermore the co-presence of sodium ion and the carbanion possibly prohibit various undesirable effects of sodium. Two steps of reaction shown below were carried out in one process as described later;

1) $R\text{-}O\text{-}H + CH_3\text{-}SO\text{-}CH_2^-$ (I) $Na^+ \longrightarrow R\text{-}O^- Na^+ + CH_3\text{-}SO\text{-}CH_3$,

2) $R\text{-}O^- Na^+ + CH_3I \longrightarrow R\text{-}O\text{-}CH_3 + NaI$

As a model experiment, cerebroside, glycogen, and ovomucoid were methylated by the following procedures: 1) *Cerebroside*; 20—50 mg. of cerebroside, dissolved in a few ml. of dimethyl sulfoxide, were mixed in a small flask, with a magnetic stirrer, under a nitrogen stream, with an amount of methylsulfinyl carbanion equivalent to the hydroxyl content

* Ishimoda and Hakomori, unpublished.

* Private communication from Dr. Uda, Department of Chemistry, Faculty of Science, Tohoku University, the work being in the press.

** Unpublished observation of Dr. Uda, made in formerly professor Dr. Fujise's laboratory, Tohoku University.

of the cerebroside. The carbanion was generated according to the method of Chaykovsky and Corey (*7*) by dissolving a weighed amount of sodium hydride in dimethyl sulfoxide. The amount of sodium hydride was roughly equivalent to the hydroxyl content of the cerebroside. The mixture was stirred under a nitrogen stream for ten minutes at room temperature, then an excess of methyl iodide was added and further stirred for twenty minutes. The reaction mixture was diluted with water and was followed by extraction of the methylated products with chloroform, washing with water, and evaporation *in vacuo*. The residue was taken up in a few ml. of ether-petroleum ether mixture, and washed with water to remove traces of dimethyl sulfoxide. The solution was then evaporated under nitrogen leaving a crystalline residue. The infra-red spectra of this product (Fig. 1. I) had no absorption at 3200—3700 cm.$^{-1}$ and was almost identical with the pattern of permethylated cerebroside (Fig. 1. II), which was obtained by three repetitions of the Purdie methylation in dimethylformamide (*2*). $[\alpha]_D^{24} = -16.5°$, c=2, in chloroform. Fatty acid methyl ester, and the sphingosine fraction were obtained from the methanolysate (in 10% HCl-methanol). Hydrolysis of the methanolysate in 0.5 *N* H_2SO_4 gave only one spot corresponding to tetramethyl-galactose (pyranose) on paper chromatography. Thus, it is evident that galactosidic, and acid amide linkages had not been attacked by the sodium carbanion, and only the hydroxyl groups had been methylated. Permethylation of cerebroside is more difficult to do than was predicted; the author observed that it was necessary to repeat the Purdie type methylation four times in chloroform, or even two times in dimethylformamide. The present method was completed with only one treatment within one hour, including isolation. 2) *Glycogen*; Glycogen (80—100 mg.) was methylated by the same reaction as described above, except that the duration of time for reaction with the carbanion was prolonged to six hours, and that with methyl iodide was prolonged 'overnight'. Isolation of the product was done by dialysis in running water, and lyophilization. Although one treatment gave an almost permethylated product with a methoxyl content of 39%, which was freely soluble in chloroform and carbon tetrachloride, but still showed an absorption band for free or associated OH-

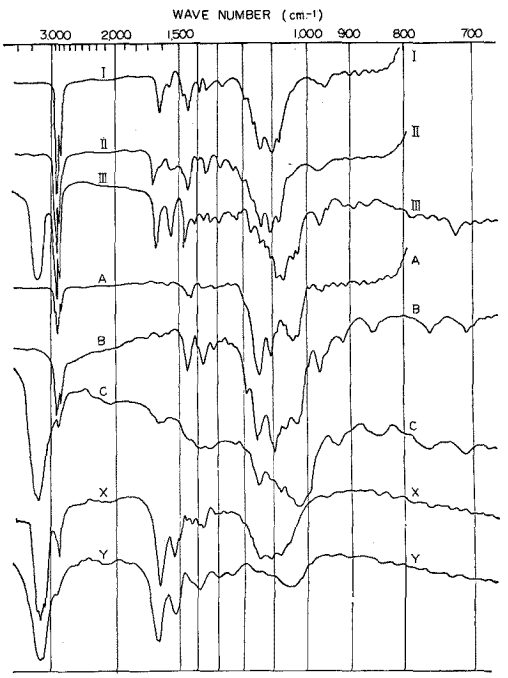

FIG. 1. Infra-red absorption patterns of complex carbohydrates and their methylated products, taken in the Perkin-Elmer 'Infracord' apparatus.
I: permethylated cerebroside by the present procedure, II: permethylated cerebroside by three repetition of the Purdie methylation in dimethylformamide, both I and II were taken as the solution in carbon tetrachloride in the NaCl-cell.
III: cerebroside as KBr-tablet.
A: permethylated glycogen by the present procedure, taken as a solution in carbon tetrachloride.
B: the same sample as in A, taken as KBr-tablet.
C: glycogen as KBr-tablet.
X: methylated ovomucoid by the present procedure, taken as KBr-tablet.
Y: ovomucoid as KBr-tablet.

groups in an infra-red pattern. One more repetition of the methylation gave a product without OH-absorption, a methoxyl content of 43.5% and $[\alpha]_D^{24} = +185°$, c=2, in chloroform. The yield was 85 mg. from 100 mg. of the glycogen. In the infrared pattern (Fig. 1 A and B), bands for CH_3-stretching, CH_3-deformation, and stretching vibrations for ether bond, particularly for CH_3-O-C (near 1150 cm.$^{-1}$) were pronounced. Bands with maximum absorption at 710, 750, 850 cm.$^{-1}$ were not changed, which indicated that glycosidic links or the configuration of carbohydrate chain were unchanged. The total procedure was finished within several days, requiring less than 100 mg. of the glycogen, which is in striking contrast to the conventional methylation of glycogen (*14*, *15*) which required months to complete, grams of glycogen. 3) *Ovomucoid*; Ovomucoid was methylated by the same procedure as used for the methylation of glycogen. About one third of the total amount remained insoluble even after the methylation, and this was eliminated by centrifugation. The soluble part was dialyzed, isolated, and again methylated. The product had a methoxyl content of 9.5%. On the third methylation, the methoxyl content was unchanged (9.5%). The product showed very pronounced absorption bands at about 1150, 1100, and 1050 cm.$^{-1}$, due to ether bonds (Fig. 1 X), but the bands at 3300—3500 cm.$^{-1}$ (due to −N-H·· O=C), 1650 and 1550 cm.$^{-1}$ (due to −NH-CO-), those characteristic for protein structure, were almost unchanged*. The yield of the final product was 25 mg. from 100 mg. Another experiment of methylation of the urinary Fr. 4-glycoprotein yielded only 15 mg. of the methylated product from 100 mg. Majority of the glycoprotein became insoluble during the methylation procedure, and a part became dialysable, thus the method is only a limited value for the methylation of glycoprotein class. Improvement of the method applicable to the glycoprotein is being under way.

* The bands at 3200—3500 cm.$^{-1}$ became sharper than that of before methylation, this is possibly due to disapperance of absorption by OH-vibration. The disappearance of the band at this region as the criteria of permethylation is not valid in the glycoprotein class.

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REFERENCES

(*1*) Stoffyn, P.J., and Jeanloz, R.W., *J. Biol. Chem.*, **235**, 2507, (1960); Cf. Jeanloz, R.W., "Polysaccharide in Biology", edited by G.F. Springer, The Josiah Macy Jr. Foundation, New York, fourth conference, p. 23—35 (1958)

(*2*) Kuhn, R., Trischmann, M., and Löw, I., *Angew, Chem.*, **67**, 32 (1955); Kuhn, R., Egge, H., Brossmer, R., Gauhe, R., Klesse, W., Böhm, H., Trischmann, H., and Tschampel, D., *Angew. Chem.*, **72**, 805 (1960)

(*3*) Yamakawa, T., Yokoyama, S., and Handa, N., *J. Biochem.*, **53**, 28 (1963)

(*4*) Whistler, R.L., and BeMiller, J.N., *Arch. Biochem. Biophys.*, **98**, 126, (1962)

(*5*) Hakomori, S., Ishimoda, T., Kawauchi, H., and Eidoh, F., *J. Biochem.*, **49**, 307 (1961)

(*6*) Hakomori, S., Kawauchi, T., and Ishimoda, T., *Nature*, **190**, 265 (1961)

(*7*) Corey, E.J., and Chaykovsky, M., *J. Am. Chem. Soc.*, **84**, 866 (1962)

(*8*) Chaykovsky, M., and Corey, E.J., *J. Org. Chem.*, **28**, 254 (1962)

(*9*) Greenwald, R., Chaykovsky, M., and Corey, E.J., *J. Org. Chem.*, **28**, 1128 (1962)

(*10*) Freudenberg, K., Hixon, R.M., *Ber. deut. chem. Ges.*, **56**, 2119 (1923); **69**, 2041 (1936)

(*11*) Schmid, L., and Becker, B., *Ber. deut. chem. Ges.*, **58**, 1966 (1925)

(*12*) Muskat, I. E., *J. Am. Chem. Soc.*, **56**, 693 (1934); **56**, 2449 (1934)

(*13*) Isbell, H.S., Frush, H.L., Bruckner, B.H., Kowkabany, G.N., and Wampler, G., *Anal. Chem.*, **29**, 1523 (1957)

(*14*) Haworth, W.N., Hirst, E.L., and Isherwood, F.A., *J. Chem. Soc.*, 577 (1937)

(*15*) Bacon, T.S.D., Baldwin, E., Bell, D.J., *Biochem. J.*, **38**, 198 (1944)

Department of Biochemistry, Institute for Cancer Research, Tohoku Pharmaceutical School, Odawara-Nankozawa, Sendai

SEN-ITIROH HAKOMORI

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