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Studies on the Substrate Specificity of Taka-amylase A. III. Syntheses of Some New Derivatives of Phenyl α-Maltoside

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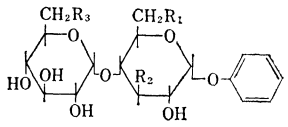
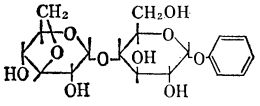
In order to obtain information of the interaction between various portions of the substrate molecule and the enzyme protein, mono-*O*-methyl derivatives of phenyl α-maltoside and its analogues were synthesized and the enzymatic action was investigated. The purity and chemical structure of the compounds were confirmed by chromatographic method, phenol determination, and elemen talanalyses. The anomeric configuration of the glycosidic linkage to phenol was presumed to be of an alpha type on the basis of the high positive value of specific rotation. Some regularities in the enzymatic action on these synthetic substrates were observed.

Elucidation of the interaction through definite atoms or atomic groups between the substrate and the enzyme molecule is an important problem in understanding enzymatic action as a whole, as shown by Blake *et al.*[1] in an X-ray crystallographic investigation of hen egg-white lysozyme. A useful chemical approach to this problem will be an investigation of the enzymatic action on the substrates with different but analogous chemical structures. Helferich and Gruenler[2] and Pigman and Richtmyer[3] investigated the action of β-glucosidase of almond emulsion on phenyl β-glucoside and its *O*-methyl derivatives and analogues. The enzyme preparations at that time were far from being pure. Isemura *et al.*[4] studied the Taka-amylase action on partially *O*-methylated amylose, and obtained some information on the substrate specificity of the enzyme.

Taka-amylase A [EC 3.2.1.1. α-1,4-glucan-4-glucanohydrolase, *Aspergillus oryzae*] crystallized by Akabori *et al.*[5] has been known to liberate phenol from a synthetic substrate, phenyl α-maltoside.[6] The present authors synthesized the compounds shown in the following formulae, and investigated the enzymatic action on them. The structure of the compounds and the ratio of the initial rate of phenol liberation by the enzyme are summarized in Table 1.



[VI, VII, IX, XI, XIX, XXIV, XXVII, XXXI, XXXIV]



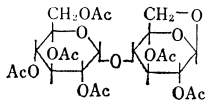
[XXII]

TABLE 1.

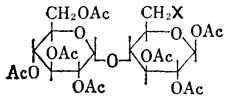
Compound	R_1	R_2	R_3	Ratio of the initial rate of phenol liberation by the enzyme
Phenyl α-maltoside	OH	OH	OH	1.00
XI	OCH_3	OH	OH	0.00
XXXIV	OH	OCH_3	OH	0.00
VI	H	OH	OH	0.02
IX	Cl	OH	OH	0.00
VII	I	OH	OH	0.00
XXVII	OH	OH	OCH_3	2.10
XIX	OH	OH	H	0.05
XXXI	OH	OH	Cl	0.24
XXIV	OH	OH	I	0.29
XXII	—	—	—	0.00

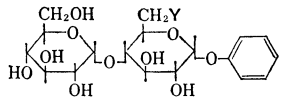
The reaction sequences of the syntheses were represented in Schemes 1 and 2. The basic principle of the syntheses was as follows:

1) C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips and V. R. Sarma, *Proc. Roy. Soc.*, **167B**, 378 (1967).
2) B. Helferich and S. Gruenler, *J. Prakt. Chem.*, [2] **148**, 107 (1937).
3) W. Pigman and N. K. Richtmyer, *J. Amer. Chem. Soc.*, **64**, 374 (1942).
4) M. Isemura, T. Ikenaka and Y. Matsushima, *J. Biochem.*, **64**, 73 (1968).
5) S. Akabori, B. Hagihara and T. Ikenaka, *ibid.*, **41**, 577 (1954).
6) S. Akabori, K. Uehara, Y. Shimazu and K. Nakanishi, Nippon Kwagaku Kwaishi, **64**, 1046 (1943).



I





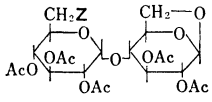
II: X=OH
III: X=OTs
IV: X=I
V: X=H
VIII: X=Cl
X: X=OCH_3

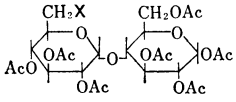
VI: Y=H
VII: Y=I
IX: Y=Cl
XI: Y=OCH_3

(1) I → II(X=OH) → III(X=OTs) → IV(X=I) → V(X=H) → VI(Y=H)
↓
VII(Y=I)

(2) I → II(X=OH) → VIII(X=Cl) → IX(Y=Cl)
↓
X(X=OCH_3) → XI(Y=OCH_3)

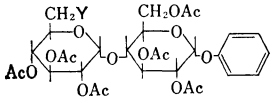
Scheme 1

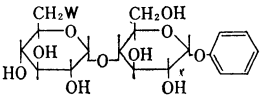




XIII: Z=OTr
XIV: Z=OH
XV: Z=OTs
XVI: Z=I
XVII: Z=H
XXV: Z=OCH_3
XXVIII: Z=Cl

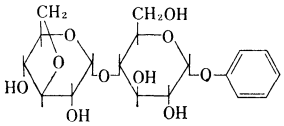
XX: X=OTs
XXIII: X=I
XVIII: X=H
XXVI: X=OCH_3
XXIX: X=Cl





XXI: Y=OTs
XXX: Y=Cl

XIX: W=H
XXIV: W=I
XXVII: W=OCH_3
XXXI W=Cl



XXII

(1) XIII(Z=OTr) → XIV(Z=OH) → XV(Z=OTs) → XVI(Z=I) → XVII(Z=H) → XVIII(X=H) → XIX(W=H)

(2) XV(Z=OTs)
↓
XX(X=OTs) → XXI(Y=OTs) → XXII
↓
XXIII(X=I) → XXIV(W=I)

(3) XIV(Z=OH) → XXV(Z=OCH_3) →
↓ XXVI(X=OCH_3) → XXVII(W=OCH_3)
↓
XXVIII(Z=Cl) → XXIX(X=Cl) →
XXX(Y=Cl) → XXXI(W=Cl)

Scheme 2

Maltosan hexaacetate was obtained by the alkali treatment of phenyl β-maltoside followed by acetylation.[7,8] 1,6-Anhydro-ring of maltosan hexaacetate was cleaved by treatment with titanium tetrachloride in chloroform,[9,10] and the hydroxyl on position-6 was set free. The hydroxyl on the position-6′ was set free according to the method of Dutton and Slessor.[11] Introduction of chlorine into the 6- or 6′-position was performed with the use of sulfuryl chloride in pyridine.[12] *O*-Methyl derivatives were prepared through methylation with diazomethane in the presence of boron trifluoride as a catalyst. We found a convenient method for leading 3,6-anhydro-ring into sugar by treating terminal tosyloxy compound with Dowex 1 (OH^-) at room temperature. Using this method, phenyl 3′,6′-anhydro-α-maltoside was prepared from phenyl 6′-*O*-*p*-toluenesulfonyl α-maltoside in good yield. Dick and co-workers[13] havereported that acetylation of β-maltose monohydrate with acetylpyridinium chloride gave acetylated maltose which had free hydroxy at 3-position. This method was used for preparing 3-*O*-substituted phenyl α-maltoside. Phenyl glycosidation was accomplished by the fusion of *O*-acetylated disaccharides with phenol and zinc chloride,[14] followed by deacetylation with sodium methoxide in anhydrous methanol.

In order to eliminate the ambiguity of the structure of the compounds which might arise from the migration of the acetyl groups during the course of syntheses, confirmation was carried out by means of analytical methods: (1) Position of the substitution on the glucose residue. (2) Moiety of the glucose residues which underwent substitution.

Experimental

Maltosan Hexaacetate (I). Maltosan hexaacetate was prepared from phenyl β-maltoside hexaacetate[7] by alkali treatment according to the method of Karrer and Kamienski.[8] Crystallization in methanol gave colorless needles which melted at 182—184°C. Found: C, 50.23; H, 5.58%. Calcd for $C_{24}H_{32}O_{11}$: C, 50.00; H, 5.56%.

1,2,3,2′,3′,4′,6′-Hepta-*O*-acetyl-β-maltose (II). This compound was prepared from I by cleaving 1,6-anhydro ring with titanium tetrachloride following the method

7) B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, **66**, 378 (1933).
8) P. Karrer and L. Kamienski, *Helv. Chim. Acta*, **15**, 739 (1932).
9) L. Asp and B. Lindberg, *Acta Chem. Scand.*, **6**, 941 (1952).
10) G. Zemplen and Z. Csuros, *Ber.*, **62**, 993 (1929).
11) G. C. S. Dutton and K. N. Slessor, *Canad. J. Chem.*, **44**, 1069 (1966).
12) A. G. Cottrell, E. Buncel and J. K. N. Jones, *ibid.*, **44**, 1483 (1966).
13) W. E. Dick, Jr., B. G. Baker and J. E. Hodge, *Carbohyd. Res.*, **6**, 52 (1968).
14) B. Helferich and S. R. Petersen, *Ber.*, **68**, 790 (1935).

of Asp and Lindberg[9] in principle. Crystallization in cold methanol-water (1 : 5) gave colorless needles which melted at 140—142°C. Found: C, 49.59; H, 5.81%. Calcd for $C_{26}H_{36}O_{18}$: C, 49.65; H, 5.66%.

6-*O*-*p*-Toluenesulfonyl-1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (III). II (4 g) was converted into III by the method of Asp and Lindberg.[9] Recrystallization from methanol gave pure substance (4 g) which melted at 138—141°C. Found: C, 49.06; H, 5.30; S, 3.98%. Calcd for $C_{33}H_{42}O_{20}S$: C, 50.10; H, 5.31; S, 4.03%.

6-Iodo-6-deoxy-1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (IV). This compound was prepared from III (4.0 g) according to the method of Asp and Lindberg.[9] Crystallization in methanol gave colorless crystals (3.2 g) which melted at 129—131°C. Found: C, 41.69; H, 4.27; I, 16.34%. Calcd for $C_{26}H_{35}O_{17}I$: C, 41.82; H, 4.70; I, 17.00%.

6-Deoxy-1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (V). IV (3.0 g) was dissolved in a mixture of dioxane (50 m*l*), ethanol (150 m*l*) and pyridine (2 m*l*). Reduction with hydrogen over 10% palladium-charcoal (3.0 g) at 70°C was repeated twice. The solution was extracted with chloroform, and the extract was washed with 5% aqueous sodium thiosulfate and water, and was dried over anhydrous sodium sulfate. After chloroform had been evaporated, the residue was crystallized in methanol (10 m*l*). Colorless crystals thus obtained (2.1 g) melted at 163—164.5°C. $[\alpha]_D^{19}$ +17° (*c*, 1.00, chloroform), Found: C, 50.24; H, 5.81%. Calcd for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.80%.

Phenyl 6-Deoxy-α-maltoside (VI). Zinc chloride (0.5 g) was dissolved in phenol (3 m*l*), and to this mixture was added V (2.5 g). The mixture was heated for 30 min at 115—120°C under vigorous stirring. Benzene (40 m*l*) was poured into the reaction mixture, which was washed with 1N sodium hydroxide and then with water. The benzene layer was dried over anhydrous sodium sulfate and was evaporated. The residual syrup was dissolved in a solution of 0.1M sodium methoxide in methanol (20 m*l*), and the solution was kept for 20 min at room temperature. The alkoxide was excluded by the addition of Dowex 50×8(H^+ form). The product was purified by passing through a column of Biogel P-2(polyacrylamide gel for chromatographic use, purchased from BIO. RAD Laboratories). The freeze-dried substance gave $[\alpha]_D^{24}$ +176° (*c* 1.00, water). Found: C, 54.05; H, 6.40%. Calcd for $C_{18}H_{26}O_{10}$: C, 53.73; H, 6.47%.

Phenyl 6-Iodo-6-deoxy-α-maltoside (VII). IV (2.0 g) was reacted with phenol (3.5 m*l*) in the presence of zinc chloride (0.35 g) in a manner similar to that described above, followed by deacetylation with sodium methoxide. The product was passed through Biogel P-2 column. The fraction of phenyl 6-iodo-6-deoxy-α-maltoside was lyophilized and crystallized in methanol. The yield of crystals which melted at 174°C (decomp.) was 150 mg. $[\alpha]_D^{24}$ +128° (*c* 0.50, water). Found: C, 40.45; H, 4.81; I, 24.17%. Calcd for $C_{18}H_{25}O_{10}I$; C, 40.92; H, 4.77; I, 24.02%.

6-Chloro-6-deoxy-1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (VIII). II (2.0 g) was dissolved in anhydrous pyridine (20 m*l*). The solution was cooled in ice-water, and then sulfuryl chloride (2.0 m*l*) was added dropwise under stirring, and the mixture was kept at room temperature for 12 hr. A brown solution thus obtained was extracted with chloroform (2×25 m*l*), and the extract was washed several times with water, dried over calcium chloride and concentrated *in vacuo*. The syrupy residue was dissolved in methanol and decolorized with Norit A. Colorless needles were obtained in a methanol solution, which were recrystallized in the same solvent. The yield of the specimen which melted at 138—139°C was 1.38 g. $[\alpha]_D^{19}$ +50° (*c* 1.00, pyridine). Found: C, 47.51; H, 5.36; Cl, 5.39%. Calcd for $C_{26}H_{35}O_{17}Cl$: C, 47.67; H, 5.38; Cl, 5.41%.

Phenyl 6-Chloro-6-deoxy-α-maltoside (IX). VIII (1.2 g) was reacted with phenol (3 m*l*) and zinc chloride (0.2 g) at 115°C for 30 min. After deacetylation with sodium methoxide, the product was purified by passing through a column of Biogel P-2, and was crystallized in methanol. The yield of the colorless crystals which melted at 184—186°C was 240 mg. $[\alpha]_D^{20}$ +142° (*c*, 0.50, water). Found: C, 49.19; H, 5.82; Cl, 7.86%. Calcd for $C_{18}H_{25}O_{10}Cl$: C, 49.49; H, 5.77; Cl, 8.12%.

6-*O*-Methyl-1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose (X). II (2.5 g) was dissolved in anhydrous dichloromethane (20 m*l*) and methylated with diazomethane in the mixture of ether (100 m*l*) and boron trifluoride (5 m*l* of boron trifluoride etherate-dichloromethane, 1 : 25). The reaction was carried out in a dry ice-acetone bath. After 12 hr, the reaction mixture was filtered off from insoluble substance and extracted with chloroform. The extract was washed with water and dried over calcium chloride. Rapid crystallization occurred in methanol. Recrystallization in the same solvent gave in 80% yield 6-*O*-methyl-1,2,3,2′,3′,4′,6′-hepta-*O*-acetyl-β-maltose which melted at 135—136°C. $[\alpha]_D^{20}$ +60.7° (*c*, 1.36, chloroform). Found: C, 49.56; H, 5.87%. Calcd for $C_{27}H_{38}O_{18}$: C, 49.84; H, 5.88%.

Phenyl 6-*O*-Methyl-α-maltoside (XI). X (1.8 g) was reacted with phenol (4 m*l*) in the presence of zinc chloride (0.3 g) in a similar manner as described above, followed by deacetylation with sodium methoxide. The syrup thus obtained was purified by gel filtration using Biogel P-2. The yield of an amorphous product obtained was 390 mg. $[\alpha]_D^{20}$ +168° (*c*, 0.67, water). Found: C, 52.14; H, 6.56%. Calcd for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.53%.

Maltosan (XII).[11] Hexa-*O*-acetyl-maltosan (I) was deacetylated with sodium methoxide. Crystals were obtained in methanol which melted at 149—150°C. Found: C, 43.00; H, 6.23%. Calcd for $C_{12}H_{20}O_{10}$: C, 44.44; H, 6.17%.

6′-*O*-Trityl-penta-*O*-acetyl-maltosan (XIII).[11] Maltosan (XII, 11 g) was dissolved in anhydrous pyridine (125 m*l*). To the solution was added freshly recrystallized trityl chloride (15 g). After standing three days at room temperature, the starting material disappeared on a thin layer chromatogram. The mixture was then acetylated with acetic anhydride (20 m*l*) at room temperature for 12 hr. The solution was extracted with chloroform, washed with sodium bicarbonate and then with water, dried over calcium chloride and evaporated *in vacuo*. The acetylated trityl compound contaminated with triphenyl carbinol was used without purification in the next procedure.

2,3,2′,3′,4′-Penta-*O*-acetyl-maltosan (XIV). The product XIII (5 g) was detritylated in 80% aqueous acetic acid (200 m*l*) by the method of Dutton and Slessor.[11] Crystallization in methanol gave XIV (2.4 g) almost free from triphenyl carbinol, which melted at

82—83°C. Found: C, 49.51; H, 5.71%. Calcd for $C_{22}H_{30}O_{15}$: C, 49.44 H, 5.62%.

6'-*O*-*p*-Toluenesulfonyl-penta-*O*-acetyl-maltosan (XV).[11] XIV (5.5 g) was dissolved in anhydrous pyridine (80 ml) and *p*-toluenesulfonyl chloride (7 g) was added to the solution. After 24 hr, needles appeared on addition of water (30 ml). Recrystallization in methanol gave pure 6'-*O*-*p*-toluenesulfonyl-penta-*O*-acetyl-maltosan (6.0 g) which melted at 170—171°C. Found: C, 50.51; H, 5.23; S, 4.44%. Calcd for $C_{29}H_{36}O_{7}S$: C, 50.58; H, 5.23; S, 4.65%.

6'-Iodo-6'-deoxy-penta-*O*-acetyl-maltosan (XVI).[11] XV (6 g) was dissolved in acetone (120 ml) and sodium iodide (15 g) was added to the solution. The mixture was refluxed for 15 hr and then extracted with chloroform. The extract was evaporated after it had been washed with 5% sodium thiosulfate and then with water. A syrupy residue was crystallized in methanol.

Recrystallization in methanol gave 6'-iodo-6'-deoxy-penta-*O*-acetyl-maltosan (5.5 g) which melted at 193—195°C. Found: C, 40.97; H, 4.57; I, 19.77%. Calcd for $C_{22}H_{29}O_{14}I$: C, 40.99; H, 4.50; I, 19.72%.

6'-Deoxy-penta-*O*-acetyl-maltosan (XVII). XVI (5.5 g) was dissolved in a mixture of ethanol (200 ml), dioxane (100 ml) and pyridine (1 ml), and reduced with hydrogen over 10% palladium-charcoal (6 g) according to the method of Dutton and Slessor.[11] Crystallization in methanol gave XVII (3.2 g) which melted at 140—141°C. $[\alpha]_D^{19}$ +42° (*c*, 1.00, chloroform). Found: C, 50.88; H, 5.88%. Calcd for $C_{22}H_{30}O_{14}$: C, 50.96; H, 5.77%.

6'-Deoxy-hepta-*O*-acetyl-maltose (XVIII). XVII (1.5 g) was dissolved in an acetolysis-mixture (30 ml of sulfuric acid-acetic anhydride-acetic acid, 1 : 70 : 30), and treated according to the method of Dutton and Slessor.[11] Crystallization in ethanol - *n*-hexane (3 : 1) gave XVIII (1.5 g) which was a mixture of α- and β-anomer. Thus the melting point (85—87°C) was different from that (mp 183—185°C) given by Dutton and Slessor.[11] $[\alpha]_D^{19}$ +85° (*c*,1.00, chloroform). Found: C, 49.45; H, 5.83%. Calcd for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.80%.

Phenyl 6'-Deoxy-α-maltoside (XIX). XVIII (2.0 g) was dissolved in phenol (4 ml), and zinc chloride (0.35 g) was added to the solution. The mixture was treated as described above. After deacetylation with sodium methoxide, the product was passed through a column of Biogel P-2. The fraction of the product was freeze-dried, but it did not crystallize. $[\alpha]_D^{19}$ +170° (*c*, 0.96, water). Found: C, 53.42; H, 6.48%. Calcd for $C_{18}H_{26}O_{10}$: C, 53.73; H, 6.47%.

6'-*O*-*p*-Toluenesulfonyl-hepta-*O*-acetyl-maltose (XX). XV (1.5 g) was dissolved in the above acetolysis mixture. After standing for three hours at room temperature, 10% aqueous barium acetate was added to the mixture, which was extracted with chloroform (3×50 ml), after barium sulfate was removed by centrifugation. A white powder (1.5 g) was obtained by evaporation *in vacuo*. Though the product gave a single spot on a thin-layer chromatogram, it did not crystallize. $[\alpha]_D^{24}$ +100° (*c*, 2.00, dioxane). Found: C, 50.63; H, 5.50; S, 3.96%. Calcd for $C_{33}H_{42}O_{20}S$: C, 50.12; H, 5.35; S, 4.05%.

Phenyl 3',6'-Anhydro-α-maltoside (XXII). A mixture of XX (2.0 g), phenol (3.5 ml) and zinc chloride (0.35 g) was heated under stirring on an oil bath at 118—120°C for 30 min. Benzene (50 ml) was added to the hot reaction mixture, which was washed with 1N sodium hydroxide, water, and evaporated *in vacuo*. The syrupy product was dissolved in methanol (30 ml), and Dowex 1×4 (OH^- form) was added to the solution under stirring at room temperature. After two hours, the resin was filtered and the filtrate was concentrated *in vacuo*. The product was purified by passing through a column of Biogel P-2. The fraction of the product was crystallized in methanol. The colorless crystals (0.2 g) melted at 194—195°C. $[\alpha]_D^{24}$ +136° (*c*, 0.90, water). Found: C, 53.85; H, 5.99%. Calcd for $C_{18}H_{24}O_{10}$: C, 54.00; H, 6.00%.

6'-Iodo-6'-deoxy-hepta-*O*-acetyl-maltose (XXIII). XX (2.5 g) was dissolved in acetone (30 ml), and sodium iodide (5.0 g) was added to the solution. The solution was refluxed for 10 hr and was extracted with chloroform. The extract was washed with 5% aqueous sodium thiosulfate and then with water. Evaporation of chloroform gave a syrupy product, which did not crystallize. $[\alpha]_D^{20}$ +102° (*c*, 2.00, dioxane). Found: I, 16.41%. Calcd for $C_{26}H_{35}O_{17}I$: I, 17.00%.

Phenyl 6'-Iodo-6'-deoxy-maltoside (XXIV). XXIII (2.0 g) was treated with phenol (4 ml) and zinc chloride (0.3 g) by the method described above. The product was deacetylated with 0.1M sodium methoxide for five minutes under cooling with ice-water bath. Column chromatography using Biogel P-2 gave a single product, which did not crystallize. $[\alpha]_D^{27}$ +130° (*c*, 1.00, water). Found: C, 40.45; H, 4.81; I, 24.17%. Calcd for $C_{18}H_{25}O_{10}I$: C, 40.92; H, 4.77; I, 24.02%.

6'-*O*-Methyl-penta-*O*-acetyl-maltosan (XXV). XIV (3.0 g) was dissolved in dichloromethane (25 ml). After the solution was cooled in a dry ice-acetone bath, diazomethane in ether (140 ml) and boron trifluoride (7 ml of boron trifluoride etherate-dichloromethane, 1 : 25) were added dropwise during two hours. Insoluble substance was filtered off, and the solution was extracted with chloroform. The extract was washed with water and dried over calcium chloride. The chloroform extract was evaporated to a syrup which crystallized in methanol. Recrystallization in the same solvent gave 6'-*O*-methyl-penta-*O*-acetyl-maltosan (2.5 g) which melted at 164°C. $[\alpha]_D^{20}$ +20° (*c*, 1.00, chloroform). Found: C, 50.06; H, 5.95%. Calcd for $C_{23}H_{32}O_{15}$: C, 50.36; H, 5.88%.

6'-*O*-Methyl-hepta-*O*-acetyl-maltose (XXVI). XXV (2.0 g) was dissolved in the acetolysis mixture (40 ml) and the mixture was kept at room temperature for three hours. Five per cent barium acetate solution was added to the reaction mixture, and the resulting barium sulfate was removed by centrifugation. The mixture was extracted with chloroform, and the extract was washed with sodium bicarbonate and repeatedly with water. Evaporation of chloroform gave a syrupy product, which gave a single spot on a thin-layer chromatogram, though it did not crystallize. Found: C, 49.97; H, 5.93%. Calcd for $C_{27}H_{38}O_{18}$: C, 49.98; H, 5.89%.

Phenyl 6'-*O*-methyl-α-maltoside (XXVII). XXVI (1.5 g) was reacted with phenol (3.5 ml) in the presence of zinc chloride (0.4 g) in a similar manner, and the deacetylated product was purified by gel filtration as described above. $[\alpha]_D^{18}$ +183° (*c*, 0.48, water). Found: C, 52.72; H, 6.57%. Calcd for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.53%.

6′-Chloro-6′-deoxy-penta-*O*-acetyl-maltosan (XXVIII). 2,3,2′,3′,4′-Penta-*O*-acetyl-maltosan (XIV, 3.0 g) was dissolved in freshly distilled pyridine and the solution was cooled in an ice-water bath, and sulfuryl chloride (1 ml) was added dropwise. The reaction was continued for 12 hr at room temperature. Water was poured into the mixture in order to destroy excess sulfuryl chloride, and the mixture was extracted with chloroform. The extract was washed with sodium bicarbonate and water. Evaporation of chloroform *in vacuo* gave a syrup, which crystallized rapidly in methanol. The crystals thus obtained (2.3 g) melted at 129°C. $[\alpha]_D^{18}$ +53.8° (*c*, 1.30, chloroform). Found: C, 47.28; H, 5.60; Cl, 6.07%. Calcd for $C_{22}H_{29}O_{14}Cl$: C, 47.79; H, 5.28; Cl, 6.41%.

6′-Chloro-6′-deoxy-hepta-*O*-acetyl-maltose (XXIX). XXVIII (2.0 g) was dissolved in the acetolysis mixture (40 ml) and the mixture was kept at room temperature for three hours. Aqueous barium acetate was added to the mixture in order to remove sulfuric acid. The chloroform extract was evaporated to a syrup (1.8 g) which did not crystallize.

Phenyl 6′-Chloro-6′-deoxy-hexa-*O*-acetyl-α-maltoside (XXX). XXIX (1.5 g) was converted into a phenyl glycoside in a similar manner, and a syrupy product was obtained which was crystallized in ethanol. Recrystallization from the same solvent gave colorless crystals (0.2 g) which melted at 133—134°C. $[\alpha]_D^{18}$ +186.9° (*c*, 0.57, chloroform). Found: C, 52.55; H, 5.36; Cl, 5.23%. Calcd for $C_{30}H_{37}O_{16}Cl$: C, 52.29; H, 5.41; Cl, 5.14%.

Phenyl 6′-Chloro-6′-deoxy-α-maltoside (XXXI). XXX (0.143 g) was dissolved in chloroform (3 ml) and 0.1 M sodium methoxide in methanol (20 ml) was added to this solution. After 20 min, the solution was treated with Dowex 50(H^+ form), followed by filtration and evaporation. A syrup thus obtained was purified by gel filtration through Biogel P-2 column. Found: C, 48.89; H, 5.89; Cl, 7.68%. Calcd for $C_{18}H_{25}O_{10}Cl$: C, 49.49; H, 5.77; Cl, 8.12%.

1,2,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-maltose (XXXII). Maltose (10 g) was mixed with pyridine (30 ml) and dry benzene (200 ml), and acetylated with acetyl chloride (18 ml) following the method of Dick and coworkers.[13] The syrup (5 g) was chromatographed through a column of silica-gel using an elution solvent (ethyl acetate, benzene, 1 : 1).[15] The syrup obtained (1.4 g) showed a single spot on a thin-layer chromatogram, though it did not crystallize. $[\alpha]_D^{15}$ +77.3° (*c*, 0.97, chloroform). Found: C, 48.82; H, 5.66%. Calcd for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70%.

1,2,6,2′,3′,4′,6′-Hepta-*O*-acetyl-3-*O*-methyl-β-maltose (XXXIII). XXXII (2.5 g) was dissolved in dichloromethane (25 ml) and was methylated with diazomethane and boron trifluoride in a dry ice-acetone bath. Methylation was not complete, and the same procedure was repeated three times. The product showed contamination of a small amount of the starting material on a thin-layer chromatogram, and we carried out the next procedure without further purification.

Phenyl 3-*O*-Methyl-α-maltoside (XXXIV). Crude XXXIII (2.0 g) was reacted with phenol and deacetylated in the similar manner as described above. The product was purified by passing through a column of Biogel-P-2. $[\alpha]_D^{17}$ +199° (*c*, 0.80, water). Found: C, 52.25; H, 6.41%. Calcd for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.53%.

Discussion

The purity of the phenyl glycosides obtained was examined by the determination of phenol content using the colorimetric method.[16] It was shown that all samples contained one mole of phenol per mole of sample. The results, together with those of the elemental analyses show a high degree of

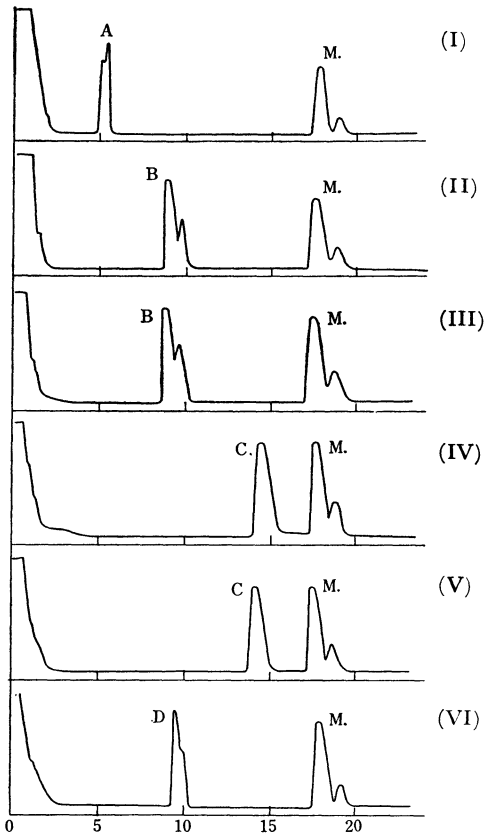


Fig. 1. Gas-liquid chromatograms of methanolyzed and trimethylsilylated compounds.
(I) Phenyl 3′,6′-anhydro-α-maltoside.
(II) Phenyl 6′-deoxy-α-maltoside.
(III) Phenyl 6-deoxy-α-maltoside.
(IV) Phenyl 6′-*O*-methyl-α-maltoside.
(V) Phenyl 6-*O*-methyl-α-maltoside.
(VI) Phenyl 3-*O*-methyl-α-maltoside.
The position of peak (A) is identical with that of authentic methyl 3,6-anhydro-glucoside,[18] (B) with methyl 6-deoxy-glucosides,[19] (C) with methyl 6-*O*-methyl glucoside[20] and (D) with methyl 3-*O*-methyl-glucosides.[21] Peak (M) is of methyl glucosides.[22] The analyses were carried out at 185°C under 1.6 atm pressure of carrier nitrogen.

15) M. L. Wolfrom and R. M. de Lederkremer, *J. Org. Chem.*, **30**, 1560 (1965).

16) T. Ikenaka, *J. Biochem.*, **54**, 328 (1963).

purity of the specimens.

The position of substitution on the glucose residues, which might be an unexpected one due to possible migration of acetyl groups during the course of synthesis, was confirmed by gas-liquid chromatography. Samples were methanolyzed and trimethylsilylated with hexamethyl disilazane and trimethylsilyl chloride.[17] The peaks in the gas-chromatograms were identified with the authentic derivatives of D-glucose.[18-22] The substitution occurred at the expected positions of glucose residues as shown in Fig. 1. As the iodine derivatives were the precursor of the deoxy compounds in the course of synthesis (Schemes 1 and 2), the position of iodine was also confirmed.

Gas-liquid chromatography was also used for determining which of the two glucose residues in the phenyl maltosides was substituted. As shown in Fig. 2, the precursor disaccharides of the phenyl glycosides were reduced with borohydride, after the *O*-acetyl groups had been removed, and the products were methanolyzed and analyzed by means of gas liquid chromatography. The reducing-end groups of the original disaccharides appeared in the chromatogram as corresponding sugar alcohols.

Compound XXXIII gave 3-*O*-methyl-glucitol and the compounds XXVI, XVIII and XXIX gave glucitol. These results confirm the structure of the phenyl glycosides XXXIV, XXVII, XIX and XXXI. As a counterpart of XIX, compound VI had an identical composition, and showed different behavior toward the enzymatic action (Table 1), the structure of VI was confirmed. Similarly XI was the counterpart of XXVII and IX was of XXXI, and structures of VI and IX were also confirmed. A slight ambiguity remained about the position of chlorine in the chloro-derivatives, because gas-chromatographic analysis of the chloro-deoxy-glucose had been unsuccessful.

Phenyl 3′,6′-anhydro-α-maltoside (XXII) was prepared from tosyloxy derivative (XV) which was a precursor of 6′-deoxy derivative (XVII). Thus the position of 3,6-anhydro ring was confirmed.

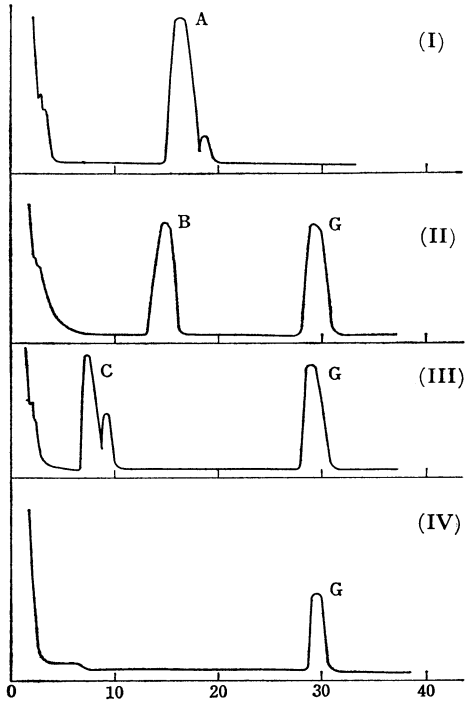


Fig. 2. Reducing-end analyses by gas-liquid chromatography.
(I) 3-*O*-Methyl-maltose derived from XXXIII.
(II) 6′-*O*-Methyl-maltose derived from XXVI.
(III) 6′-Deoxy-maltose derived from XVIII.
(IV) 6′-Chloro-6′-deoxy-maltose derived from XXIX.
The position of peak (A) is identical with that of authentic 3-*O*-methyl-glucitol, (B) with methyl 6-*O*-methyl-glucoside, (C) with methyl 6-deoxy-glucosides and (G) with glucitol. The analyses were performed after borohydride reduction and methanolyzation. All the procedures were carried out at 185°C under a 1.6 atm pressure of carrier nitrogen.

It is of interest that Taka-amylase A showed different hydrolysis rate on each substrate (Table 1). As regards the non reducing-end glucose of substrate, electronegativity at 6-position seems to be very necessary, but the steric factor is not so important for enzymatic action. On the other hand, data for the reducing-end group show that the steric factor has great significance as well as the role of electronegative residues at 6-position. These results are in line with a data presented by Isemura *et al.*[23]

17) C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Amer. Chem. Soc.*, **85**, 2497 (1963).
18) W. N. Haworth, L. N. Owen and F. Smith, *J. Chem. Soc.*, **1941**, 97.
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21) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
22) B. Helferich, "E. Fischers Anleitung zur Darstellung Organischer Präparate," Braunschweig (1939), p. 93.
23) M. Isemura, T. Ikenaka and Y. Matsushima, *J. Biochem.*, **66**, 77 (1969).