tenths of a ml. of 2 *M* potassium hydroxide and 2 mg. of iodine were added to the hydrolysate. The glucose in the solution was completely oxidized to gluconic acid in a 6-hr. reaction period. Next, the reaction mixture was acidified with hydrochloric acid and a Ruff degradation of the gluconate was effected according to the procedure of Hockett and Hudson.[20] Five mg. of barium acetate, 3 mg. of ferric sulfate and 0.5 ml. of hydrogen peroxide were employed in the degradation. The carbon dioxide produced in the reaction was recovered as barium carbonate. Total counts per minute in the oven dried sample of barium carbonate were 686. The reaction mixture from the degradation was chromatographed with arabinose and glucose standards. The finished strip showed that a reducing compound with the same R_f value as arabinose had been produced. Radioactivity was not detected in this compound.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Maltopentaose and Crystalline Octadeca-*O*-acetylmaltopentaitol[1]

BY ROY L. WHISTLER AND JOHN H. DUFFY

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A pentasaccharide isolated from corn sirup by chromatography on charcoal and cellulose columns is characterized as maltopentaose by periodate and hypoiodite oxidations and by hydrolysis with β-amylase. Hydrogenation to maltopentaitol followed by acetylation yields a crystalline octadeca-*O*-acetyl derivative. $R_{glucose}$ values at 25° are given for members of the malto-homologous series up to seven D-glucose units using as eluting agent butanol–ethanol–water (3:2:1 v./v.).

Aside from their chemical significance the maltooligosaccharides are of particular interest to enzymologists. Members of this series are produced by hydrolytic enzymes acting on starches and glycogens and are used as primers and substrates for enzymatic syntheses. This importance of the series in general metabolic investigations provides impetus to the isolation and characterization of the individual members. The richest and most available source of maltoöligosaccharides is commercial corn sirup. From this source individual members can be isolated by chromatographic separations on successive carbon[2] and cellulose[3] columns. It is unfortunate that the maltoöligosaccharides are poor crystallizers. Presumably the presence of the repeating α-D-1 → 4 linkages connecting the D-glucopyranose units coupled with the activity of the potentially reducing group cause the molecules to assume shapes which do not readily fit into crystalline lattices. As a consequence the oligosaccharides are obtained in amorphous form. The acetylated glycitols crystallize from ethanol and hence constitute excellent reference points.

Several members of the maltoöligosaccharides have been isolated, from corn sirups[4,5] and from an acid hydrolyzate of potato amylose.[6] The structures of maltose,[7] maltotriose[8,9] and maltotetraose[5] have been determined and evidence for the structures of still higher homologous has been given.[6] Here is described the isolation and characterization of maltopentaose.

Maltopentaose is present to an extent[4] of about 8% in a standard commercial corn sirup of 42 dextrose equivalent. A concentrate of maltopentaose mixed with small amounts of its homologous neighbors can be obtained by chromatography from a carbon column. Final purification is effected by subsequent separation on a cellulose column. The specific optical rotation of the pure amorphous powder, $[\alpha]^{25}D+179.4°$ agrees with the calculated value[10,11] $[\alpha]D +178°$. A fivefold increase in reducing power upon acid hydrolysis demonstrates that five monosaccharide units are present in the oligosaccharide. On periodate oxidation a mole of pentasaccharide consumes 7.8 moles of periodate and produces 3.0 moles of formic acid and 1.4 moles of formaldehyde.

Enzymic hydrolysis illustrates the nature of the glycosidic linkages present. β-Amylase is known[12] to hydrolyze specifically α-D-(1 → 4)-glucosidic linkages if three or more such linkages occur successively. Hydrolysis of the pentasaccharide with crystalline sweet potato β-amylase[13] gives maltose and maltotriose in isolated yields of 34.3 and 52.9%, respectively. Similar results are obtained by Whelan, Bailey and Roberts.[6] The known structure of the isolated maltose and maltotriose aids in establishing that the pentasaccharide is composed of five D-glucopyranose units linked by four α-D-1 → 4 bonds in a linear chain.

Low pressure hydrogenation converts maltopentaose to maltopentaitol which can be isolated as a crystalline octadecaacetate. The optical rotation of the maltopentaitol (+158.5°) agrees with the value (+160°) calculated by means of the Freudenberg, Friedrich and Baumann equation.[10] Likewise the rotation of the maltopentaitol acetate agrees with the calculated value. Thus the optical rotation data also support the view that maltopentaose is a linear molecule of D-glucopyranose units connected by α-D-1 → 4 links.

The $R_{glucose}$ values of chromatographically pure

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maltoöligosaccharides from D-glucose to maltoheptaose are given in Table I.

TABLE I

$R_{glucose}$ VALUES IN BUTANOL-ETHANOL-WATER (3:2:1 V./V.) OF THE MALTOÖLIGOSACCHARIDES FROM CORN SIRUP

Substance	$R_{glucose}$ values, 25°	log R_G
D-Glucose	100.0	2.00
Maltose	54.0	1.73
Maltotriose	29.0	1.46
Maltotetraose	14.0	1.15
Maltopentaose	6.5	0.81
Maltohexaose	3.8	0.58
Maltoheptaose	2.3	0.36

Maltotetraose isolated in the course of this work has a specific optical rotation, $[\alpha]^{25}D$ +176.4°, somewhat higher than previously reported.

Experimental

Isolation of Maltopentaose.—Removal from corn sirup[14] of the lower molecular weight oligosaccharides was accomplished by successive elution from a carbon column[2] with water, 3.5, 7.5 and 15% aqueous ethanol. The 15% aqueous ethanol eluate contained maltotetraose[5,6] which on cellulose column purification gave chromatographically pure maltotetraose, $[\alpha]^{25}D$ +176.4° (*c* 1.0, water). A crude concentrate of pentasaccharide was obtained by elution with 20% aqueous ethanol and evaporation of the eluate at 50° *in vacuo*. A composite of a number of these fractions was further refined by cellulose[15] column chromatography[3] using successively 85% and 80% aqueous ethanol as eluting agents. The pentasaccharide fraction was removed by elution with 80% ethanol. Final purification was attained by chromatography on a second cellulose column[3] with butanol-ethanol-water (3:2:1 v./v.) as the eluent. Fractions were collected on an automatic fraction collector.[16] Maltopentaose was obtained on evaporation of a portion of eluate. The sugar was stirred with activated charcoal in 28% aqueous ethanol, filtered and evaporated *in vacuo* to a white amorphous powder $[\alpha]^{25}D$ +179.4° (*c* 1.0, water). To detect the presence of the carbohydrate in eluate portions, 1.0-ml. aliquots were each mixed with 1.0 ml. of 1% orcinol in sulfuric acid. The sulfuric acid concentration varied with the irrigating solvent used. Fractions in aqueous ethanol required 115% sulfuric acid (three parts 100%[5] acid and two parts fuming sulfuric acid containing 30% SO_3) while 97% sulfuric acid was sufficient for fractions in butanol-ethanol-water eluents. A red to brown color is obtained with as little as 2 μg. of sugar. The purity of the oligosaccharide was determined by paper chromatography.

Reducing Power.—Reducing power was determined by the iodine method[5] previously described. When maltopentaose was hydrolyzed by refluxing with 1 *N* sulfuric acid the reducing power increased by a factor of 4.85. Maltose under identical conditions increased in reducing power by a factor of 1.86.

Periodate Oxidation.—In a volumetric flask a 200-mg. sample of pentasaccharide in 10.00 ml. of 0.3 *M* sodium metaperiodate was diluted to 25.00 ml. with water. The solution was held at room temperature in the dark. Aliquots were removed at intervals to follow the progress of oxidation by estimation of periodate consumption and formic acid and formaldehyde production. An apparent end point was reached in ten hours (Table II).

TABLE II

PERIODATE OXIDATION OF MALTOPENTAOSE

Determination	Mole equiv. Calcd.	Found
Periodate consumed	8	7.8
Formic acid produced	3	3.0
Formaldehyde produced	1	1.4

(a) **Formic Acid Produced.**—A mixture of 1.00 ml. each of the oxidation mixture and of ethylene glycol was mixed and allowed to stand for 30 minutes in the dark to destroy excess periodate. Formic acid produced was titrated with 0.02 *N* sodium hydroxide to a phenolphthalein end-point (persistent pink color for ten seconds[17,18]).

(b) **Unreacted Periodate.**—The procedure was previously described.[5] Difference between the total oxidant introduced and the unreacted periodate was a measure of periodate consumed.

(c) **Formaldehyde Produced.**[19]—Periodate oxidation in acid media may yield low results because of a failure of the formate ester to saponify.[20] Therefore samples were oxidized in bicarbonate buffered solution.[19]

β-Amylolysis of Maltopentaose.—The enzymic hydrolysis of 0.67 g. of maltopentaose was conducted as previously described.[5] After deionization with Amberlite resins,[21] IR 120 followed by IR 4B, the hydrolysate was evaporated *in vacuo*, and chromatographed on a cellulose column (2.5 × 50 cm.) with a butanol-ethanol-water (3:2:1) developer. The orcinol-sulfuric test indicated three fractions. Yields of these are shown in Table III. The maltose fraction was acetylated with 0.10 g. of freshly fused sodium acetate and 4.0 ml. of acetic anhydride at 120° until solution was complete (about 30 min.), the mixture poured into 50 ml. of ice and water, stirred vigorously for 30 minutes and extracted with chloroform. The chloroform extract was washed free of acetic acid and evaporated to dryness. The octa-*O*-acetyl-β-maltose was obtained from absolute ethanol as a white crystalline product, m.p. 158–159° (uncor.). An authentic sample[22] has a m.p. of 159–160°. The maltotriose, $[\alpha]^{25}D$ +152.8° (*c* 1.2, water); reported value +160°[8,9] was acetylated as described above. The specific optical rotation of the acetone was $[\alpha]^{22}D$ +85.6° (*c* 0.4, chloroform). This compares with the reported value[8,9] of +86°.

TABLE III

CHROMATOGRAPHY OF ENZYME HYDROLYZED MALTOPENTAOSE

Fraction	Yield Gram	Yield %	Substance by paper chromatography
A	0.130	34.3	Maltose
B	.202	53.4	Maltotriose
C	.292	..	Maltopentaose

Maltopentaitol Octadecaacetate.—A maltopentaose sample of 1.75 g. was catalytically reduced by the procedure described previously.[5] When the solution gave a negative Fehling reducing power test, the catalyst was filtered off and the filtrate evaporated at 50° under vacuum, producing a white amorphous powder, yield 1.78 g. Approximately 1.0 g. of this material was chromatographed on a cellulose column (5.5 × 50 cm.) using the butanol-ethanol-water eluting agent (3:2:1). On testing separate portions of the eluate with the orcinol-sulfuric acid test three different fractions were found to contain carbohydrate. Paper chromatograms of these components sprayed with aniline hydrogen phthalate[23] showed no color, but colored areas were produced when sprayed with ammoniacal silver nitrate. The major fraction obtained in 88% yield had a $[\alpha]^{25}D$ +158.5° (*c* 0.6, water). This value agrees with that calculated (+160) for maltopentaitol using the Freudenberg, Friedrich and Baumann equation.[19] One-half gram of the major fraction was acetylated as described above with 0.3 g. of freshly fused sodium acetate and 5 ml. of acetic anhydride. Upon evaporation the washed chloroform extract gave a light yellow colored sirup; yield 1.014 g. It was purified by dissolving in 30 ml. of benzene and chromatographing the solution on a 70 × 230 mm. column of Magnesol[24] and

(14) Corn Sirup, Unmixed, 42 D. E., Corn Products Refining Company, Argo, Illinois.

(15) Whatman Cellulose Powder, Standard Grade, W. R. Balston, Ltd., England.

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Celite[25] (5:1 by weight) using as developer four liters of a solution of *t*-butyl alcohol in benzene (1:75 v./v.). On extrusion the column was streaked with alkaline potassium permanganate.[7] The principal zone was eluted with acetone and evaporated to yield 0.538 g. of crude sirup. Decolorization with activated charcoal in absolute ethanol and evaporation produced crystalline octadeca-*O*-acetylmaltopentaitol. It was recrystallized from absolute ethanol; m.p. 197–197.5°, $[\alpha]^{25}D$ +132.7° (*c* 0.9, chloroform). An X-ray diffraction pattern confirmed the crystalline nature of the acetate.

Anal. Calcd. for $C_{66}H_{90}O_{44}$: C, 49.99; H, 5.67. Found: C, 49.63; H, 5.77.

$R_{glucose}$ Values of Pure Corn Sirup Components.—Approximately equal amounts of the pure corn sirup oligosaccharides of degree of polymerization one through seven were placed on Whatman No. 4 grade paper at a distance of two centimeters from each other. The chromatograms eluted at 25° for 44 or 144 hours with butanol–ethanol–water (3:2:1) and developed with aniline hydrogen phthalate.[23] The paper eluted the shorter time was used to calculate the $R_{glucose}$ values of maltose, maltotriose and maltotetraose. The $R_{glucose}$ value of the higher oligosaccharides on the paper eluted longer were calculated from the $R_{glucose}$ value of maltotriose. The $R_{glucose}$ value of maltotetraose in both cases was used as a secondary reference point. The values are recorded in Table I.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE, CAIRO UNIVERSITY, AND THE LABORATORIES OF THE MEMPHIS CHEMICAL COMPANY]

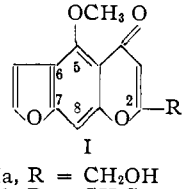
Furo-chromones and -Coumarins. IX. Reactions of Khellol Glucoside, Visnagin and Bergapten

BY ALEXANDER SCHÖNBERG, NASRY BADRAN AND NICOLAS A. STARKOWSKY

RECEIVED JUNE 28, 1954

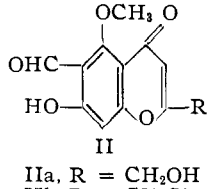
A method is described for the hydrolysis of khellol glucoside and the subsequent transformation of khellol to visnagin. The oxidation of khellol with chromic acid yields 6-formyl-7-hydroxy-2-hydroxymethyl-5-methoxychromone (IIa) and that of bergapten 6-formyl-7-hydroxy-5-methoxycoumarin (apoxanthoxyletin) (IV). 5,6,7-Trimethoxy-2-methylchromone (VIId) can be obtained from visnagin through the intermediate 6-formyl-7-hydroxy-5-methoxy-2-methylchromone (IIc).

An improvement of the original method of Geissman[1] is described for the conversion of khellol glucoside (Id), a natural product extracted from the seeds of *Ammi visnaga* L., to another constituent of this plant, visnagin (Ic). The first step: acid hydrolysis of the glucoside, of a few minutes duration, yielded khellol (Ia) of high purity. Under more drastic conditions, khellol glucoside was not only hydrolyzed, but also demethylated to 5-norkhellol.[2] Khellol reacted with thionyl chloride to give (Ib) which was converted into visnagin by reduction with zinc and acetic acid. These reactions are analogous to the synthesis of allomaltol from kojic acid[3].



I

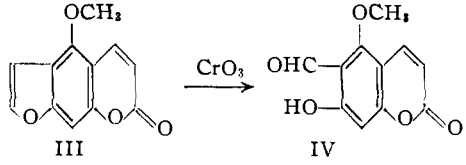
Ia, R = CH_2OH
Ib, R = CH_2Cl
Ic, R = CH_3
Id, R = $CH_2OC_6H_{11}O_5$



II

IIa, R = CH_2OH
IIb, R = CH_2Cl
IIc, R = CH_3

The oxidation of visnagin (Ic) with chromic acid to 6-formyl-7-hydroxy-5-methoxy-2-methylchromone[4] (IIc) has already been reported. Khellol (Ia) reacted with chromic acid in the same manner, with the destruction of the furan ring and the production of a formylchromone (IIa). The alcoholic group of khellol is probably protected from oxidation by the formation of an ester of chromic acid. The chloro derivative of khellol (Ib), when subjected to the same reaction, yielded 2-chloromethyl-6-formyl-7-hydroxy-5-methoxychromone (IIb). The structure of these derivatives of salicylaldehyde was proved by the fact that, when IIb was treated with alcoholic silver nitrate, the chlorine atom was replaced by a hydroxyl group; the product was identical with the IIa[5] produced by the oxidation of khellol with chromic acid. Furthermore, IIb on treatment with zinc and acetic acid yielded IIc which has already been obtained by the oxidation of visnagin with chromic acid.[4]



III IV

Like visnagin (Ic)[4] and khellol (Ia), bergapten (III) was oxidized with chromic acid with the destruction of the furan ring; it yielded the aldehydocoumarin, 6-formyl-7-hydroxy-5-methoxycoumarin (IV), which was shown to be identical with apoxanthoxyletin, the ozonolysis product of the natural coumarin xanthoxyletin.[6]

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(5) IIa is probably identical with the ozonolysis product of khellol obtained by M. K. Hassan, M.Sc. Thesis, Faculty of Science, Egyptian University, Cairo, 1932; m.p. 213°, m.p. of IIa 217°. He tentatively identified his product as $C_9H_8O_5$, but his analytical data are closer to the values calculated for $C_{12}H_{10}O_6$ (IIa). *Anal.* Calcd. for $C_9H_8O_5$: C, 55.10; H, 4.08. Calcd. for $C_{12}H_{10}O_6$: C, 57.60; H, 4.00. Found: C, 56.76; H, 4.01.

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