

SYNTHESIS OF 2,4-DI-O-METHYL-L-ERYTHROSE

by

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(ii)

ABSTRACT

2,4-Di-O-methyl-L-erythrose is thought to occur in the hydrolysis and periodate oxidation products of polysaccharides. This sugar has now been synthesized from methyl-L-arabofuranoside by tosylation, methylation, detosylation and periodate oxidation. The free sugar was obtained as a sirup and was characterized by the preparation of a crystalline phenylhydrazone and a crystalline p nitrobenzoate.

ACKNOWLEDGEMENT

I wish to express my sincere thanks to Dr. G.G.S. Dutton for his stimulating and patient guidance throughout the course of this work.

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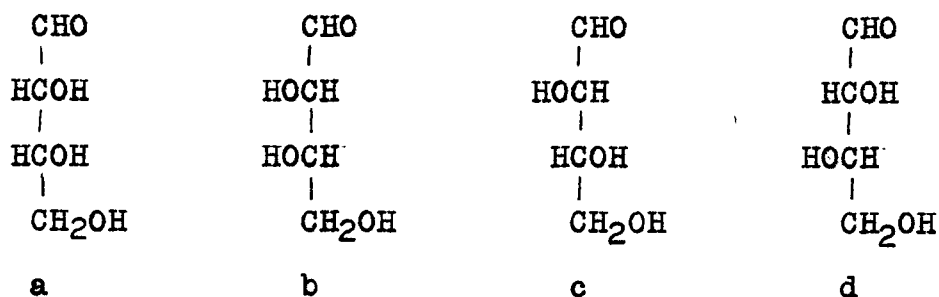
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HISTORICAL INTRODUCTION

Within recent years so many sugars previously prepared by synthetic methods in the laboratory have been found playing an important role in proof of structure of polysaccharides and in natural processes, that new attention has been focused upon the necessity of filling those gaps which still remain in this field of carbohydrate chemistry. Particularly does this necessity apply to sugars of lower carbon atom number which could be encountered as intermediates of degradation of polysaccharides. The tetroses and their derivatives are still among the least known members of the sugar group, despite the fact that the first efforts toward their preparation were made seventy years ago (1).

These are two parent tetroses, erythrose and threose, both have two asymmetric carbon atoms therefore four possible structures exist.



a : D-erythrose

b : L-erythrose

c : D-threose

d : L-threose



There are two general methods of synthesizing tetroses

(a) by degradation of sugars with greater numbers of carbon atoms.

(b) by building up from simple organic compounds.

The latter method is not used very often, as the product of the synthesis is usually a mixture of the optically active sugars, which are difficult to separate.

The methods of degrading sugars devised by Wohl (2,3,4) and Ruff seemed at first to furnish a very promising means of preparing tetroses and from 1899 to 1902 several attempts were made to synthesize them, (5,6,7,8,9) but the results proved disappointing and very few crystalline compounds were prepared and few data recorded. Except for the later application of Weerman's (10) method, which resulted in little actual new success the field was abandoned until 1935.

In 1935 Hockett (11) synthesized D-threose by using Ruff's degradation. A crystalline derivative, D-threose triacetate (11) was obtained.

In 1930 a new method of degradation, periodate oxidation, advanced the synthesis of unknown sugars, but even with this new method the number of crystalline tetroses was very small. As a preparatory method oxidation with periodic acid is of particular importance for the preparation of short chain sugars. The mild conditions of the oxidation are well suited for sensitive carbohydrate structures and the selectivity (12) of the oxidation gives the desired oxidation product.

As mentioned before, the number of crystalline tetrose derivatives is relatively small and especially the number of

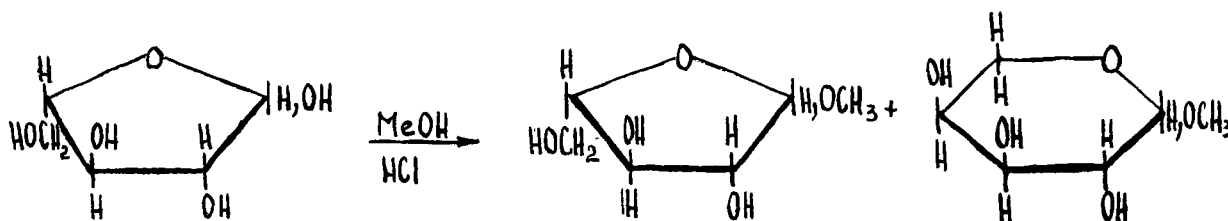
methylated tetrose derivatives (13,14) is very small.

The purpose of this research was to synthesize a di-O-methyl tetrose in the L series and to identify it by preparation of crystalline derivatives. The choice fell upon 2,4-di-O-methyl-L-erythrose.

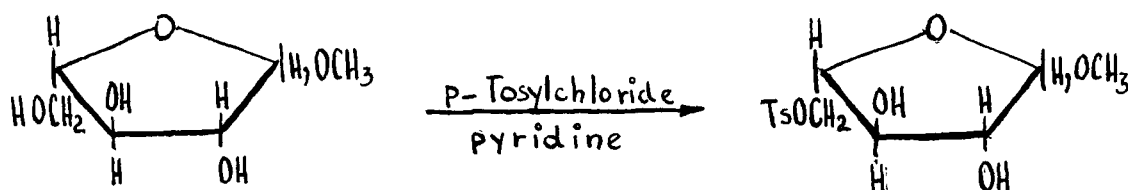
DESCRIPTION AND RESULTS OF PRESENT RESEARCH

To synthesize 2,4-di-O-methyl-L-erythrose, L-arabofuranose was chosen as starting material. The main problem was to achieve selective methylation at the 3 and 5 position and leaving the 1 and 2 position unsubstituted, vulnerable to periodate oxidation, which preferably attacks 1,2 glycols. The most useful path for blocking the 5 position of the L-arabofuranose was to prepare at first the methyl L-arabofuranoside and then react it with p-toluenesulphonyl chloride. p-Toluenesulphonyl chloride selectively esterifies carbohydrates under proper conditions and this property was used to block the primary hydroxyl at the 5 position (14).

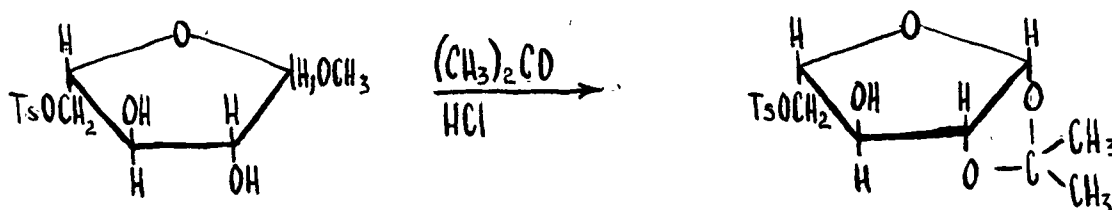
The preparation of methyl L-arabofuranoside yields not only the required product, but as a byproduct methyl L-arabopyranoside forms too in significant amounts. Some unchanged L-arabofuranose can also be found in the reaction mixture.



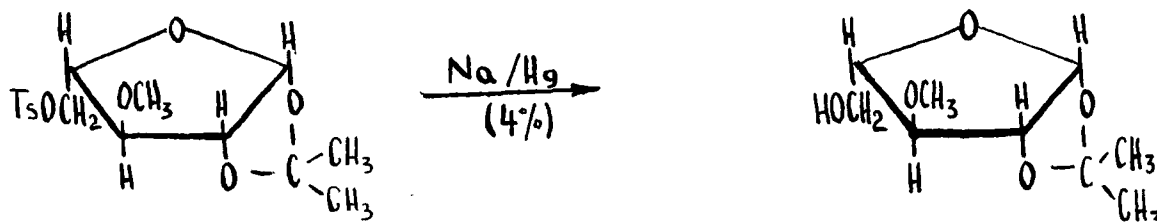
The mixture of L-arabofuranoside and L-arabopyranoside can be tosylated directly as only L-arabofuranoside has a free primary hydroxyl group.



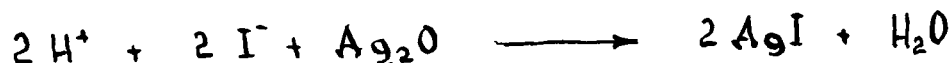
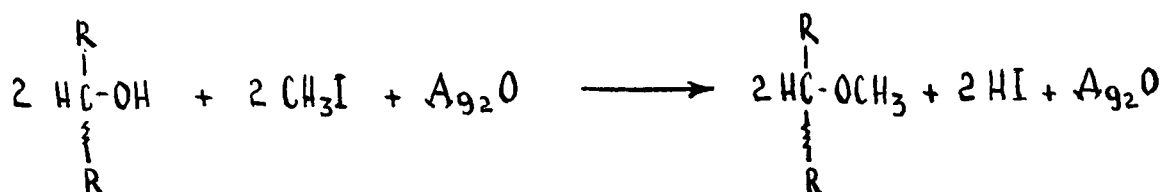
When methyl 5-p-toluenesulphonyl-L-arabofuranoside is reacted with absolute acetone in the presence of hydrogen chloride, the glycosidic methoxyl group is hydrolysed and the free hydroxyl groups at the 1 and 2 positions will form a 1,2 isopropylidene derivative. The product of this reaction is crystalline and easily identified.



As the 1 and 2 positions are blocked, the methylation of the only free hydroxyl group at the 3 position can proceed. In this research Purdie's method was used for methylation, which employs methyl iodide and silver oxide, as this method is mild, and sensitive carbohydrates do not suffer decomposition. The product of this methylation is crystalline 1,2 isopropylidene-3-O-methyl-5-p-toluenesulphonyl-L-arabofuranose (17). To obtain a free hydroxyl group at the 5 position for further methylation, the p-toluenesulphonyl group was removed by sodium-amalgam reduction (17). The progress of the reaction can be visually followed as the starting material is only sparingly soluble in aqueous methanol and the reduced 1,2-isopropylidene-3-O-methyl-L-arabofuranose is soluble.



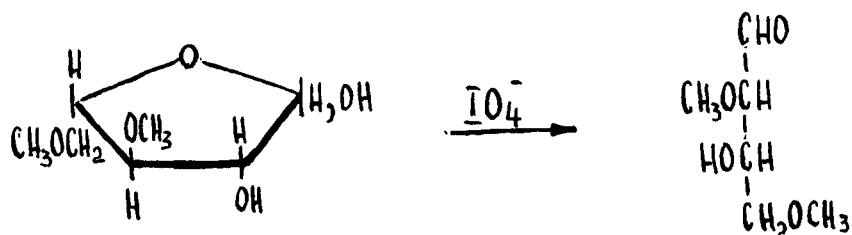
The 1,2 isopropylidene-3-O-methyl-L-arabofuranose was methylated by Purdie's method. During the methylation some side reactions occurred and the isolated product was not chromatographically pure, the presence of a tri-O-methyl-L-arabofuranose was detected. The presence of this component was unexpected as the methylation was done in a non-acidic medium and the hydrolysis of the isopropylidene group should not have occurred. To explain this hydrolysis the following reaction can be postulated:



where the momentary formation of hydrogen iodide can induce some hydrolysis. No experimental work was done to establish the feasibility of this theory.

After acid hydrolysis of 1,2-isopropylidene-3,5-di-O-L-arabinose, the hydrolysate which contained mainly 3-5-di-O-methyl-L-arabinose and traces of a tri-O-methyl-L-arabinose was put on a cellulose hydrocellulose column and the impurities separated from the required product. The chromatographically pure 3,5-di-O-methyl-L-arabinose, which had two hydroxyl groups adjacent was then oxidised with sodium metaperiodate. The oxidation of this compound yields one mole of 2,4-di-O-methyl-L-erythrose and one mole of formic acid. Before a large scale

periodate oxidation was done, several pilot scale oxidations were completed to find the necessary experimental conditions. After finding suitable experimental conditions a larger amount of 3,5-di-O-methyl-L-arabinose was oxidised and the reaction product was isolated.



The oxidation product 2,4-di-O-methyl-L-erythrose was purified by column chromatography, but could not be obtained in crystalline form. To identify this compound and characterize it, a portion was reduced with sodium borohydride and the 2,4-di-O-methyl-L-erythritol was prepared. The 2,4-di-O-methyl-L-erythritol was not obtained in crystalline form, but by reacting it with p-nitro-benzoyl chloride a crystalline derivative was obtained and this was characterized.

To another portion of 2,4-di-O-methyl-L-erythrose, 2,4-dinitrophenylhydrazine was added and the reaction resulted in a crystalline derivative, which was also characterized.

EXPERIMENTALMelting points.

All melting points were taken by means of a Leitz electrically heated melting point block and are corrected.

Optical rotations.

Optical rotations were taken on a O.C. Rudolph & Sons, Caldwell N.Y. polarimeter using a sodium lamp (General Electric) and the specific rotations were calculated according to the formula (15).

$$\left[ \alpha \right]_D^{20} = \frac{100 \times \alpha}{c \times l}$$

where  $[\alpha]$  is the specific rotation at 20°C measured with the D line of sodium,  $\alpha$  the observed rotation,  $c$  the concentration expressed in g per 100 ml. solution, and  $l$  the length of the polarimeter tube expressed in decimeters.

Chromatography.

## 1. Paper partition chromatography

All paper partition chromatography was done on Whatman No.1 chromatography paper by means of the descending method. All chromatograms were air dried after development and after spraying the chromatograms were oven dried at 110°C.

## 2. Column chromatography

(a) Column chromatography was done on a cellulose-hydro-cellulose (1:1) column (2.8 x 42 cm), which was kept at a

constant temperature (40°C) by means of a thermostat. Fractions were collected on an automatic fraction collector (Time/Flow).

(b) Florisil columns were also used for separation and purifications.

#### Electrophoresis.

A high voltage apparatus (made at the Dept. of Chem. U.B.C) was used, aqueous sodium borate (0.05 M.) served as electrolyte.

#### Evaporations.

All evaporations were done in vacuo at 40°C.

#### Methoxyl group determinations.

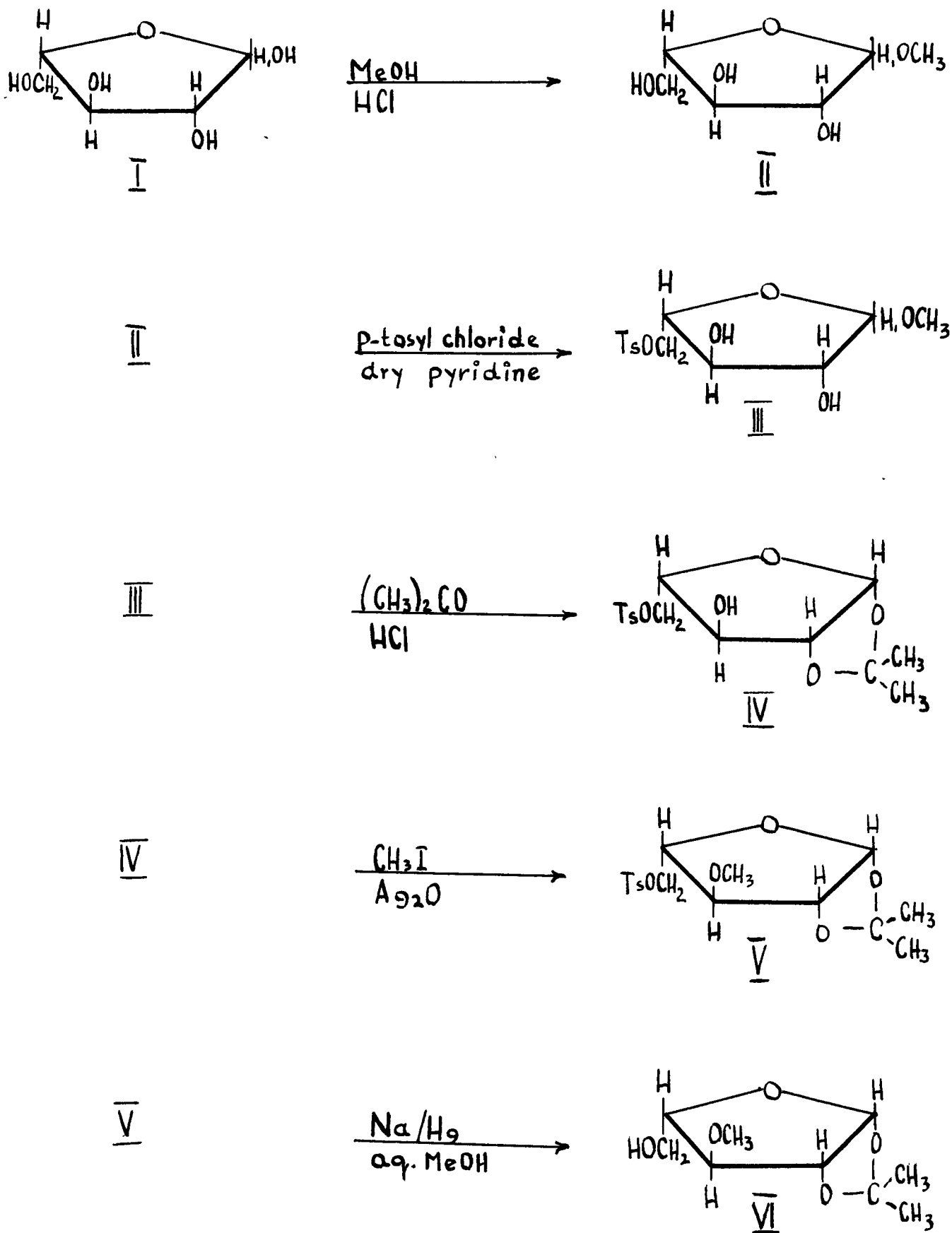
All methoxyl analyses were carried out by the method of Viebock and Schwappach as described by Clark (16).

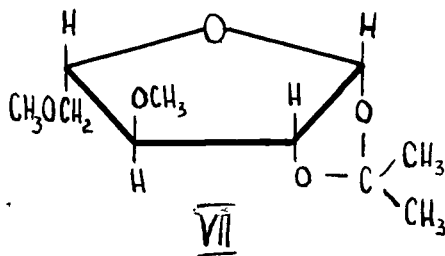
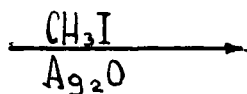
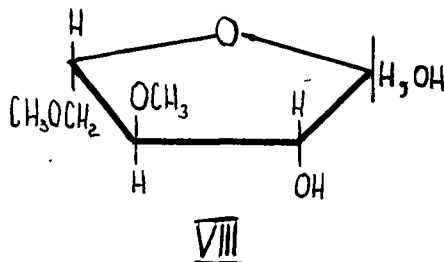
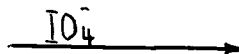
Nitrogen analysis by Mrs. A. Aldrich of this department.



Figure 1.

Flow sheet showing synthesis of 2,4-di-O-methyl-L-erythrose



VIVIIVIIII: L-arabofuranoseII: methyl L-arabofuranosideIII: methyl 5-p-tosyl-L-arabofuranosideIV: 1,2 isopropylidene-5-tosyl-L-arabofuranoseV: 1,2 isopropylidene-5-tosyl-3-O-methyl-L-arabofuranoseVI: 1,2 isopropylidene-3-O-methyl-L-arabofuranoseVII: 1,2 isopropylidene-3,5-di-O-methyl-L-arabinoseVIII: 3,5-di-O-methyl-L-arabinoseIX: 2,4-di-O-methyl-L-erythrose

Preparation of methyl L-arabofuranoside (17).

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To commercial L-arabinose (20 g, m.p. 159-60°C,  $[\alpha]_D^{20} -105^\circ$ ) absolute methanol (600 ml) was added, containing hydrogen chloride (6 g). The mixture was shaken by a mechanical shaker and the reaction followed by polarimeter after all the L-arabinose was dissolved. When the maximum negative rotation was obtained, the hydrogen chloride was neutralised with lead carbonate. After filtration the precipitate was extracted several times with absolute methanol and the fractions united with the filtrate. The combined solutions were evaporated in vacuo and yielded a colourless sirup. Theoretical yield 21.8 g, actual yield 19.7 g (90.3%).

Paper partition chromatography was carried out on the sirup and after developing with butanone-water azeotrope and detecting with p-anisidine spray three spots were found having:

$R_F$  0.04 corresponding to L-arabinose

$R_F$  0.09 corresponding to methyl L-arabopyranoside

$R_F$  0.33 corresponding to methyl L-arabofuranoside

No attempt was made to separate the methyl L-arabofuranoside from the mixture.

Table I

PREPARATION OF METHYL L-ARABOFURANOSIDE

---

<u>Time in minutes</u>	$\alpha$	$[\alpha]_D^{20}$
120	-0.450	-15.0°
150	-1.000	-33.3°
180	-1.340	-44.7°
210	-1.450	-48.3°
240	-1.532	-51.0°
300	-1.550	-51.7°
360	-1.550	-51.7°

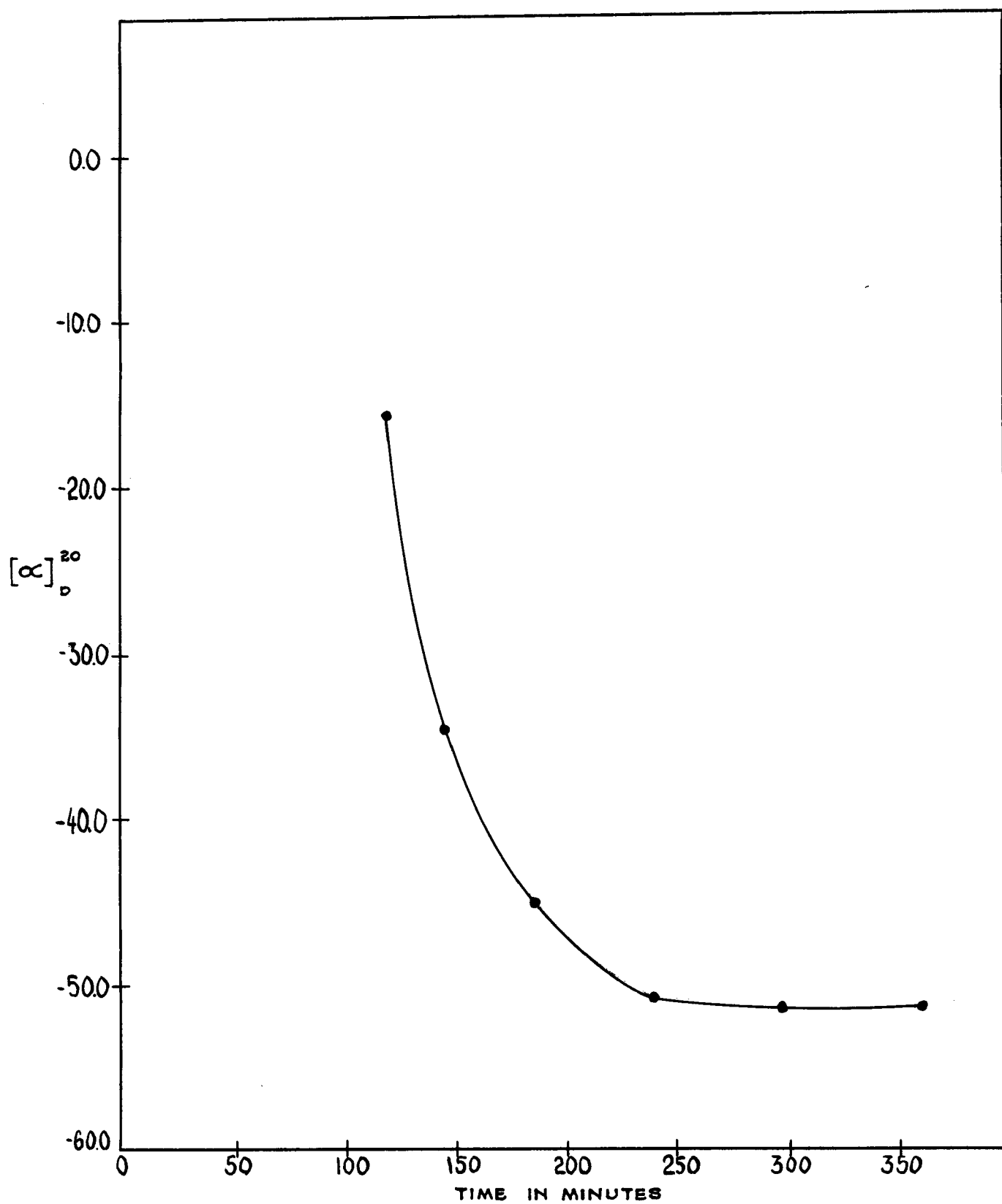


FIG. 2 - PREPARATION OF methyl-L-arabofuranoside  
SPECIFIC ROTATION vs. TIME

Preparation of Methyl 5-p-toluene-sulphonyl-L-arabofuranoside (18).

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The mixture of glycosides (19.7 g) was dissolved in dried and distilled pyridine (180 ml) by shaking at room temperature. Recrystallised p-toluene sulphonyl chloride (27 g, m.p. 67.5-68.5° lit. m.p. 68-69°C) was dissolved in dry pyridine (50 ml) and was added to the above solution. The reaction mixture was left standing for 20 hours at room temperature in the dark. After 20 hours the excess pyridine was distilled off in vacuo and to the yellow sirupy residue chloroform (50 ml) was added. The chloroform solution was washed in a separatory funnel with hydrochloric acid (1 N, 30 ml), then with saturated sodium hydrogen carbonate solution (30 ml), finally with water (3 times 30 ml). The purified chloroform solution was dried with anhydrous sodium sulphate for 10 hours then filtered and evaporated in vacuo. A yellow sirup methyl 5-p-toluene-sulphonyl-L-arabofuranoside resulted. Theoretical yield 36.2 g, actual yield 16.8 g (46.4%).

Preparation of 1,2 isopropylidene-5-p-toluene-sulphonyl-L-arabofuranose.

---

The sirupy methyl 5-p-toluene-sulphonyl-L-arabofuranoside (16.8 g) was dissolved in absolute acetone (300 ml) containing hydrogen chloride (3 g), anhydrous calcium sulphate was added and the reaction mixture was left standing for 70 hours at room temperature. After 70 hours the hydrogen chloride was

was neutralised with silver carbonate. The precipitate was extracted several times with acetone and the extracts were combined with the filtrate. The combined solution was evaporated in vacuo and to the sirupy residue a small amount of methanol (3 ml) was added. Crystals formed immediately. The crystals were recrystallised from methanol-light petroleum ether mixture. Theoretical yield 18.2 g, actual yield 8.4 g (46.2%). M.p. 129-130°C, lit. m.p. 129.5-130°C (17),  $[\alpha]_D^{20} -34.5^\circ$  (c 0.7, CHCl<sub>3</sub>).

Preparation of 1,2 isopropylidene-3-Q-methyl-5-p-toluenesulphonyl-L-arabofuranose.

---

1,2 Isopropylidene-5-p-toluenesulphonyl-L-arabofuranose (5.6 g) was dissolved in acetone (30 ml) and methyl iodide (24.1 g) was added. The solution was kept gently boiling and by constant mechanical stirring freshly prepared silver oxide (20 g) was added in ten portions at half hour intervals. The heating and stirring was continued for 15 more hours. After a reaction time of 20 hours the excess methyl iodide was distilled and the residue extracted several times with boiling chloroform. The extracts were combined and evaporated in vacuo. After evaporation the sirupy residue was dissolved in a small amount of acetone (3 ml) and light petroleum ether was added (2 ml). The solution was left standing overnight and white needle like crystals formed. Theoretical yield 5.8 g, actual yield 5.5 g (94.1%). M.p. 99-100°C, lit. m.p. 99-100°C (17). Methoxyl group determination showed 15.3% OCH<sub>3</sub>, theoretical OCH<sub>3</sub> content 15.2%.

Preparation of 1,2 isopropylidene-3-O-methyl-L-arabofuranose.

---

To 1,2 isopropylidene-3-O-methyl-5-p-toluenesulphonyl-L-arabofuranose (5 g) methanol (30 ml) and water (15 ml) were added. The suspension was mechanically stirred at high speed and in four portions at half hour intervals sodium amalgam (4%, 60 g) was added. The reaction mixture was kept under constant stirring at room temperature until the suspension cleared (5 hours) and the reduced 1,2 isopropylidene-3-O-methyl-L-arabofuranose went into solution. The excess sodium amalgam was destroyed by adding water and the solution was decanted. The mercury was washed several times with water and then with methanol. The washings were united with the solution and to the combined solution solid carbon dioxide was added to lower the highly alkaline pH. When pH 7.5 was reached the aqueous solution was evaporated in vacuo. From the residue the oily 1,2 isopropylidene-3-O-methyl-L-arabofuranose was extracted with cold chloroform. The chloroform extract was evaporated in vacuo and a light yellow oil was obtained. Theoretical yield 2.98 g, actual yield 2.71 g (91.2%).

Preparation of 1,2 isopropylidene-3,5-di-O-methyl-L-arabinose.

---

1,2 Isopropylidene-3-O-methyl-L-arabofuranose (2.7 g) was dissolved in acetone (25 ml) and methyl iodide (19 g) was added. The solution was kept gently boiling and silver oxide (15 g) was added in ten portions at half hour intervals with constant mechanical stirring. After the last portion of silver oxide was



added, the heating and stirring were continued for an additional forty hours. After forty-five hours the methylation was complete and the excess methyl iodide was distilled. The residue was extracted several times with hot chloroform and the extracts were combined, and evaporated in vacuo. A yellow oil resulted. Theoretical yield 2.90 g, actual yield 2.45 g (84.5%). Methoxyl determination showed 28.7% OCH<sub>3</sub>, theoretical OCH<sub>3</sub> content 28.4%.

Preparation of 3,5-Di-O-methyl-L-arabinose.

1,2 Isopropylidene-3,5-di-O-methyl-L-arabinose (2.4 g) was dissolved in water (10 ml) and sulphuric acid (2 N, 10 ml) was added. The reaction mixture was boiled for three hours and the hydrolysis followed by polarimeter. After three hours the specific rotation was constant and equal to the reported value of  $[\alpha]_D^{20} - 49^\circ$  (18) and the reaction was stopped. After cooling the sulphuric acid was neutralised with barium carbonate and the solution filtered. The precipitate was washed several times with methanol and the washings combined with the filtrate, and evaporated in vacuo. A yellow oil was obtained. Theoretical yield 1.96 g, actual yield 1.75 g (89.3%). Methoxyl determination showed 35.4% OCH<sub>3</sub>, theoretical OCH<sub>3</sub> content 34.8%.

Paper partition chromatography was carried out using as developer butanone-water azeotrope and p-anisidine as spray reagent. After developing and spraying two spots were detected with R<sub>F</sub> 0.86 corresponding to 2,3,5-tri-O-methyl-L-arabinose and R<sub>F</sub> 0.64 corresponding to 3,5-di-O-methyl-L-arabinose.

The oil was investigated by electrophoresis using 1000 volt and 40 mA current for forty minutes. After spraying with p-anisidine, two spots were detected. A migrating component with  $M_G$  value 0.74 correspond to 3,5-di-0-methyl-L-arabinose and a non-migrating component with  $M_G$  value of 0.0 corresponding to 2,3,5-tri-0-methyl-L-arabinose.

Separation of 3,5-di-0-methyl-L-arabinose from 2,3,5-tri-0-  

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methyl-L-arabinose.  

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A mixture of 3,5-di-0-methyl-L-arabinose and 2,3,5-tri-0-methyl-L-arabinose (0.600 g) in the minimum amount of butanone-water azeotrope. Fractions were collected at seven minute intervals at a flow rate of forty ml per hour. After 150 fractions were collected no further carbohydrate material could be detected in the eluate by application of the Molisch test and fractionation was terminated. The fractions were investigated systematically by paper chromatography. Two fractions were found to be chromatographically pure, tubes 8-35 inclusive contained the pure 2,3,5-tri-0-methyl-L-arabinose and tubes 55-120 inclusive contained the pure 3,5-tri-0-methyl-L-arabinose while an overlap of the two components was found in tubes 36-54 inclusive. The separation yielded 3,5-di-0-methyl-L-arabinose (0.334 g) and 2,3,5-tri-0-methyl-L-arabinose (0.130 g) in chromatographically pure state.

Table II

SEPARATION OF METHYLATED L-ARABINOSE FRACTIONS ONCELLULOSE-HYDROCELLULOSE COLUMN

<u>Classification</u>	<u>Tube number</u>	<u>Weight mg</u>	<u>R<sub>F</sub></u>
2,3,5-tri- <u>O</u> -methyl- <u>L</u> - arabinose	8-35	130	0.84
3,5-di- <u>O</u> -methyl- <u>L</u> - arabinose	55-120	334	0.64
mixture	36-54	40	0.64 & 0.64
total recovery		504 (84%)	

## Characterization of 3,5-di-O-methyl-L-arabinose.

---

### 1. Preparation of 3,5-di-O-methyl-L-arabonolactone

3,5-Di-O-methyl-L-arabinose (113 mg) was dissolved in water (2 ml) and excess bromine was added. The reaction mixture was kept in the dark at room temperature for 48 hours. After 48 hours the excess bromine was removed by means of aeration. When the solution turned colourless excess silver carbonate was added to remove all bromide ion present. After filtration the precipitate was washed several times with methanol and the washings combined with the filtrate. This solution was saturated with hydrogen sulphide to remove all silver ion present. The reaction mixture was evaporated in vacuo and the 3,5-di-O-methyl-L-arabonolactone was extracted with hot chloroform. The extract was evaporated in vacuo and a yellow sirup resulted. By treatment with small amounts of ethyl acetate and light petroleum ether long needle like crystals formed from the sirup. The crystals were recrystallized from a mixture of ethyl acetate and light petroleum ether. Theoretical yield 122 mg, actual yield 86 mg (70.6%). M.p. 75-76°C, lit. m.p. 75°C (17), 78°C (19).  $[\alpha]_D^{20} -85^\circ$  (c 0.5, water).

### 2. Preparation of 3,5-di-O-methyl-L-arabonamide

3,5-Di-O-methyl-L-arabonolactone (48 mg) was dissolved in methanol (15 ml) containing ammonia and was kept at -5°C for 24 hours. After 24 hours the solution was evaporated in vacuo. A yellow sirup was obtained from which upon addition of ethyl

acetate and light petroleum ether crystals formed. The crystals were recrystallized from ethyl acetate-light petroleum ether. Theoretical yield 47 mg, actual yield 45 mg (95.7%). M.p. 132-132.5°C, lit. m.p. 132.5°C (17).  $[\alpha]_D^{20}$  10° (c 0.5, water).

Preparation of 2,4-di-O-methyl-L-erythrose.

1. Pilot scale periodate oxidation of 3,5-di-O-methyl-L-arabinose

Aqueous solutions containing 3,5-di-O-methyl-L-arabinose (20 mg, 0.112 mmole) and sodium metaperiodate (48 mg, 0.224 mmole) were mixed and immediately made up to 20 ml with distilled water and maintained at 20°C in the dark. Aliquots (1 ml) were withdrawn at intervals and excess sodium hydrogen carbonate and a measured excess of 0.1 N sodium arsenite solution were added and the solutions were allowed to stand for 15 minutes to allow complete reduction of the excess sodium metaperiodate. Consumption of periodate was then determined by back titration with 0.1 N iodine solution. When the periodate consumption had reached a constant value the solution was neutralized with barium carbonate and the precipitate was extracted several times with cold chloroform. The extracts and the filtrate were combined and evaporated in vacuo. A yellow oily residue was obtained. Paper partition chromatography was carried out. As developer butanone-water azeotrope was used and the chromatogram was sprayed with p-anisidine. One spot was detected with  $R_F$  value 0.71. The periodate oxidation was followed by polarimetry, and optical rotations were taken at the time when aliquots

were withdrawn from the solution. The periodate oxidation was repeated by using a fourfold excess of sodium metaperiodate. The results were tabulated and graphically evaluated and are shown in Tables III and IV.

Table III

PILOT SCALE PERIODATE OXIDATION OF  
3,5-DI-O-METHYL-L-ARABINOSE

Time minutes	Volume of I <sub>2</sub> ml	Net volume of I <sub>2</sub> ml	IO <sub>4</sub> <sup>-</sup> uptake/mole	[α] <sub>D</sub> <sup>20</sup>
0	-----	3.900	-----	-47.0°
30	3.970	0.070	0.618	-32.0°
60	4.000	0.100	0.883	-10.0°
90	4.010	0.110	0.972	- 6.2°
120	4.010	0.110	0.972	- 6.0°
150	4.010	0.110	0.972	- 6.0°
180	4.010	0.110	0.972	- 6.0°
900	4.013	0.113	1.003	- 6.0°

2 Mole excess of sodium periodate was used.

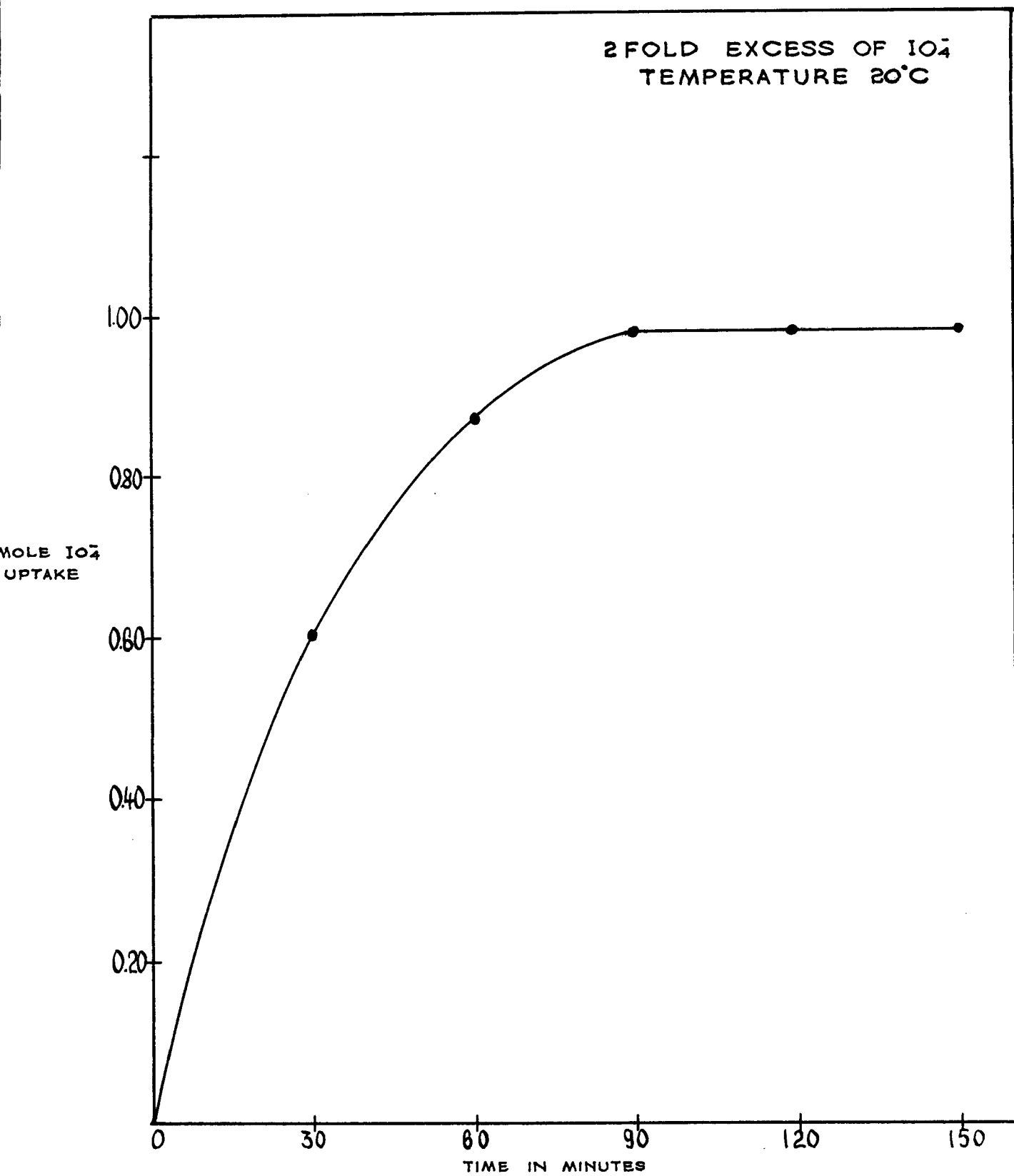


FIG. 3 - PERIODATE OXIDATION OF  
3,5 - di - O - methyl - L - arabinose  
 $\text{IO}_4^-$  UPTAKE vs. TIME



Table IV

PILOT SCALE PERIODATE OXIDATION OF  
3,5-DI-O-METHYL-L-ARABINOSE

<u>Time</u> <u>minutes</u>	<u>Volume</u> <u>of I<sub>2</sub> ml</u>	<u>Net volume</u> <u>of I<sub>2</sub> ml</u>	<u>IO<sub>4</sub><sup>-</sup></u> <u>uptake/mole</u>	<u>[α]<sub>D</sub><sup>20</sup></u>
0	-----	4.360	-----	-48.0°
30	4.450	0.090	0.795	-30.0°
60	4.460	0.100	0.883	-10.0°
90	4.480	0.120	1.000	- 6.0°
120	4.480	0.120	1.000	-6.0°
150	4.480	0.120	1.000	- 6.0°
900	4.485	0.125	1.040	- 6.0°

4 mole excess of sodium periodate used.

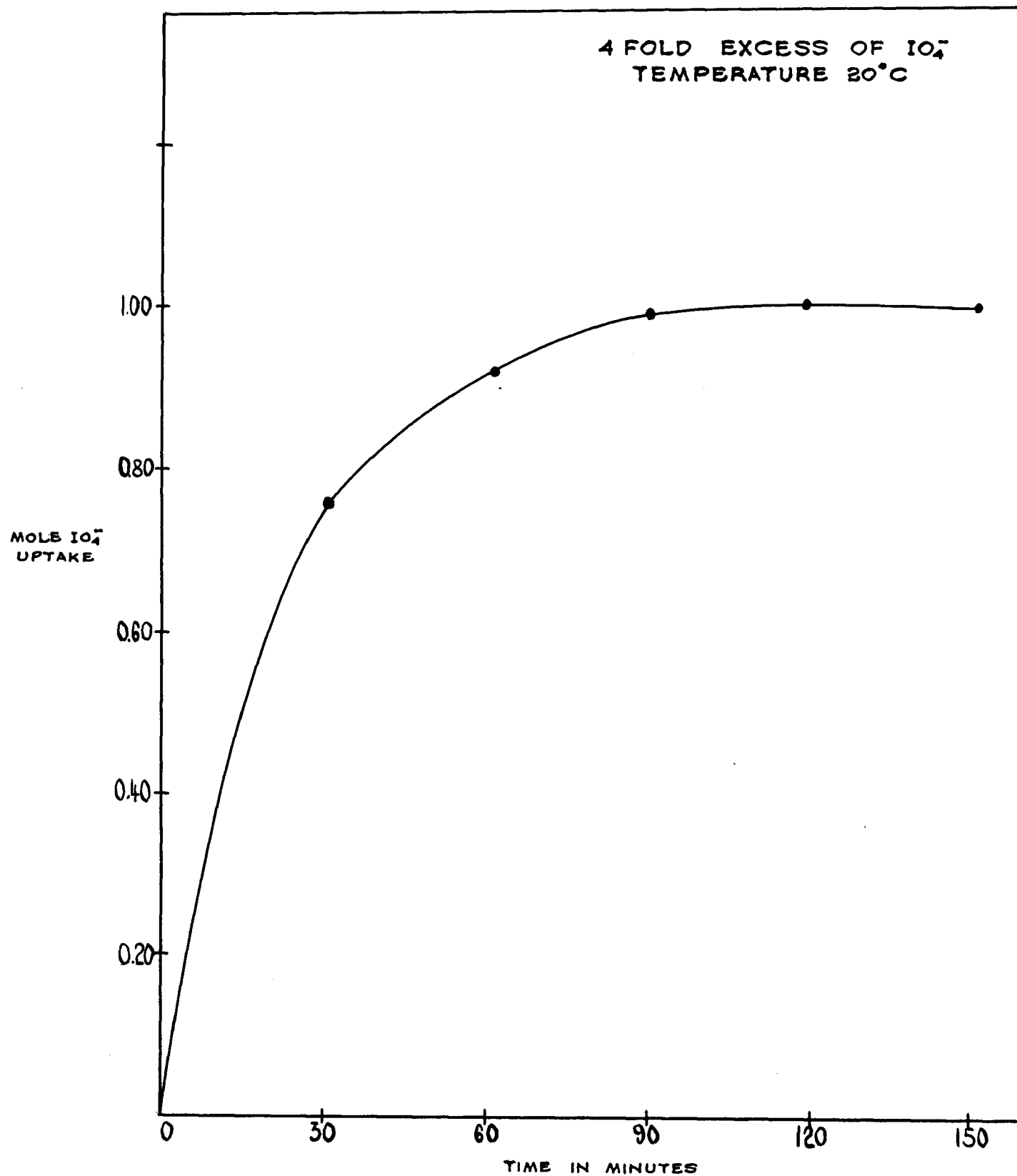


FIG. 4 - PERIODATE OXIDATION OF  
3,5 - di - O - methyl - L - arabinose  
 $\text{IO}_4^-$  UPTAKE vs TIME

## 2. Large scale periodate oxidation of 3,5-di-O-methyl-L-arabinose

Chromatographically pure 3,5,-di-O-methyl-L-arabinose (2.0 g, 0.0112M) was dissolved in distilled water (100 ml) and a solution of 0.5 N sodium metaperiodate (99.6 ml, 0.0448 M) was added. The reaction mixture was kept at room temperature in the dark for 2 hours and then barium carbonate was added to precipitate periodate and iodate and to neutralize formic acid formed. The reaction mixture was filtered and the precipitate extracted several times with cold chloroform. The extracts and the filtrate were combined and evaporated in vacuo. A yellow oil was obtained. Paper partition chromatography was carried out using as developer 1. butanone-water azeotrope, 2. butan-1-ol-ethanol-water-ammonia (4:1:4:1). The chromatograms were sprayed with p-anisidine and two spots were detected on each chromatogram. When developer 1. was used spots with  $R_F$  value of 0.73 corresponding to the oxidised product and  $R_F$  value 0.0 corresponding to impurities were found. In developer 2. spots with  $R_F$  value 0.70 corresponding to the oxidised product and  $R_F$  value 0.0 corresponding to impurities were found. Theoretical yield 1.66 g, actual yield 1.43 g (86.0%).  $[\alpha]_D^{20} -30.2^\circ$  (c 1.3, water).

## Purification of 2,4-di-O-methyl-L-erythrose by column elution chromatography.

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### 1. Pilot scale purification

In a minimum amount of benzene, impure 2,4-di-O-methyl-L-erythrose (50 mg) was placed on a Florisil column (10.5 x 1.4 cm) which was previously wetted with petroleum ether (b.p 60-110°C).

The column was developed with 50 ml fractions of petroleum ether (b.p. 60-110°C), petroleum ether-benzene (4:1, b.p. of benzene 79-80°C), petroleum ether-benzene (1:1), benzene, benzene-chloroform (4:1, b.p. of chloroform 59-61°C), benzene-chloroform (1:1) and chloroform. Fractions, each containing 25 ml were collected and individually evaporated in vacuo. The individual fractions were chromatographically investigated by means of paper partition chromatography. Chromatographically pure 2,4-di-O-methyl-L-erythrose was found in fractions 8-10 inclusive. The results are given in Table V.

Table V

PILOT SCALE PURIFICATION OF 2,4-DI-O-METHYL-L-ERYTHROSE

BY ELUTION CHROMATOGRAPHY ON A FLORISIL COLUMN

<u>Fraction</u>	<u>Volume of fraction in ml</u>	<u>Composition of developer</u>	<u>Recovery in mg</u>
1	25	petroleum ether	0.0
2	25	petroleum ether	0.0
3	25	petroleum ether-benzene (4:1)	0.0
4	25	petroleum ether-benzene (4:1)	0.0
5	25	petroleum ether-benzene (1:1)	0.0
6	25	petroleum ether-benzene (1:1)	0.0
7	25	benzene	0.0
8	25	benzene	7.1
9	25	benzene-chloroform (4:1)	14.2
10	25	benzene-chloroform (4:1)	22.5
11	25	benzene-chloroform (1:1)	2.2
12	25	benzene-chloroform (1:1)	0.0
13	25	chloroform	0.0
Total recovery			46.0 (92.0%)

## 2. Large scale purification of 2,4-di-O-methyl-L-erythrose

Impure 2,4-di-O-methyl-L-erythrose (1.50 g) was dissolved in minimum amount of benzene and was placed on a Florisil column (28 x 3.3 cm) which was previously wetted with petroleum ether. The column was developed with 200 ml fractions of petroleum ether, petroleum ether-benzene (4:1) petroleum ether-benzene (1:1), benzene, benzene-chloroform (4:1), benzene-chloroform (1:1) and chloroform.\* Fractions, each containing 100 ml were collected and individually evaporated in vacuo. The individual fractions were investigated by means of paper partition chromatography. Chromatographically pure 2,4-di-O-methyl-L-erythrose was found in fractions 6-10 inclusive, see table VI. These fractions were combined and checked for methoxyl content. Theoretical methoxyl content 41.9%, actual methoxyl content 41.8%.  $[\alpha]_D^{20}$   $-32.7^\circ$  (c 0.5, water). The chromatographically pure 2,4-di-O-methyl-L-erythrose gave positive Schiff and Benedict tests.

\*Boiling points of developing solvents were identical to those, which were used in the pilot scale purification.

Table VI

## LARGE SCALE PURIFICATION OF 2,4-DI-O-METHYL-L-ERYTHROSE

BY ELUTION CHROMATOGRAPHY ON A FLORISIL COLUMN

<u>Fraction</u>	<u>Volume of fraction in ml</u>	<u>Composition of developer</u>	<u>Recovery in mg</u>
1	100	petroleum ether	0.0
2	100	petroleum ether	0.0
3	100	petroleum ether-benzene (4:1)	0.0
4	100	petroleum ether-benzene (4:1)	0.0
5	100	petroleum ether-benzene (1:1)	0.0
6	100	petroleum ether-benzene (1:1)	110.0
7	100	benzene	424.5
8	100	benzene	676.4
9	100	benzene-chloroform (4:1)	63.0
10	100	benzene-chloroform (4:1)	21.2
11	100	benzene-chloroform (1:1)	5.0
12	100	benzene-chloroform (1:1)	0.0
13	100	chloroform	0.0
Total recovery in mg			1300.1 (86.6 %)

Optical rotation of fractions

6-10 inclusive was  $-32.7^{\circ}$

Preparation of 2,4-di-O-methyl-L-erythritol.

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Chromatographically pure 2,4-di-O-methyl-L-erythrose (200 mg) was dissolved in water (20 ml) and sodium borohydride (600 mg) was added. The reaction mixture was kept at room temperature for twenty hours. After twenty hours methanol containing hydrogen chloride was added and the precipitated sodium chloride was filtered. The precipitate was washed several times with absolute methanol and the washings were united with the filtrate, and evaporated in vacuo. To the residue methanol was added, the evaporation was continued until no more boric acid was present in the residue. The oily residue was kept under vacuum until constant weight was obtained. Theoretical yield 202 mg, actual yield 160 mg (78.9%),

Preparation of 1,3-di-p-nitrobenzoyl, 2,4-di-O-methyl-L-erythritol.

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2,4 Di-O-methyl-L-erythritol (150 mg) was dissolved in dry pyridine (20 ml) and p-nitrobenzoyl chloride (400 mg) in pyridine (20 ml) was added. The reaction mixture was kept at 75°C for three hours and then a few drops of water and a saturated solution of sodium hydrogen carbonate (25 ml) were added. The reaction product, 1,3-di-p-nitrobenzoyl, 2,4-di-O-methyl-L-erythritol precipitated and was isolated by filtration. The precipitate was washed several times with water and then it was recrystallised from methanol-water and dried



in vacuo. Theoretical yield 448 mg, actual yield 376 mg (83.9%). M.p. 217-219°C. Methoxyl determination showed 13.0% OCH<sub>3</sub>, theoretical OCH<sub>3</sub> content 13.8%. Theoretical nitrogen content 6.3 % found 7.8%. These results show that the derivative is a mixture, though the melting point was sharp.

Preparation of 2,4 dinitrophenylhydrazone of 2,4-di-O-methyl-L-erythrose.

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2,4-Di-O-methyl-L-erythrose (100 mg) was dissolved in ethanol (10 ml) and 2,4 dinitrophenylhydrazine (200 mg) in ethanol (10 ml) was added. The reaction mixture was kept at room temperature overnight. During this time a crystalline product separated which was filtered and washed with cold methanol. After drying in vacuo the crystals were dissolved in chloroform (2 ml) and were put on a Florisil column (10.5 x 1.4 cm), which was prewashed with chloroform. The column was eluted with chloroform and two bands were obtained by the elution. Fractions were collected and were evaporated in vacuo. Each fraction was checked by melting point determination and the identical fractions were united. Two main fractions were found, one which appeared to contain 2,4 dinitrophenylhydrazine the other fraction contained light yellow crystals which were assumed to be the 2,4 dinitrophenylhydrazone of 2,4-di-O-methyl-L-erythrose. Theoretical yield 221 mg, actual yield 168 mg (76 %). M.p. 148-150°C. Methoxyl determination showed 18.9% OCH<sub>3</sub>, theoretical OCH<sub>3</sub> content 18.1%. Theoretical nitrogen content 17.1%, found 18.1%.

The higher nitrogen content and the lower methoxy content indicates that some of the methoxy groups have been replaced by 2,4 dinitrophenyl hydrazone.

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