RESULTS AND DISCUSSION

A polysaccharide was obtained in 4-4.5 % yield from the ground seeds of lupinus termis (variety Giza 2) by extraction with solution of 0.2 % sodium hydroxide and warm water respectively. It was purified via its copper complex (2 gm. polysaccharide gave 1.8743 gm. cuprammonium complex). Its specific optical rotation was Ca + 75 regardless the method of isolation employed i.e. hot water, hot alkali and/or thorough its copper complex.

The pure product was a pale yellow powder which did not reduce Fehling's solution and was free of Starch.

The pure polysaccharide of L.termis was hydrolysed using N sulphuric acid and paper chromatographic examination was carried out. The results indicated the presence of four monosaccharides in the hydrolysate as shown in Fig. (XII).

One of these components was similar to authentic D-galactose in color reactions and R_F value whatever the solvent used. The second monosaccharide was identified as with L-arabinose, the third as D-xylose, and the fourth as L-rhamnose. The presence of the latter

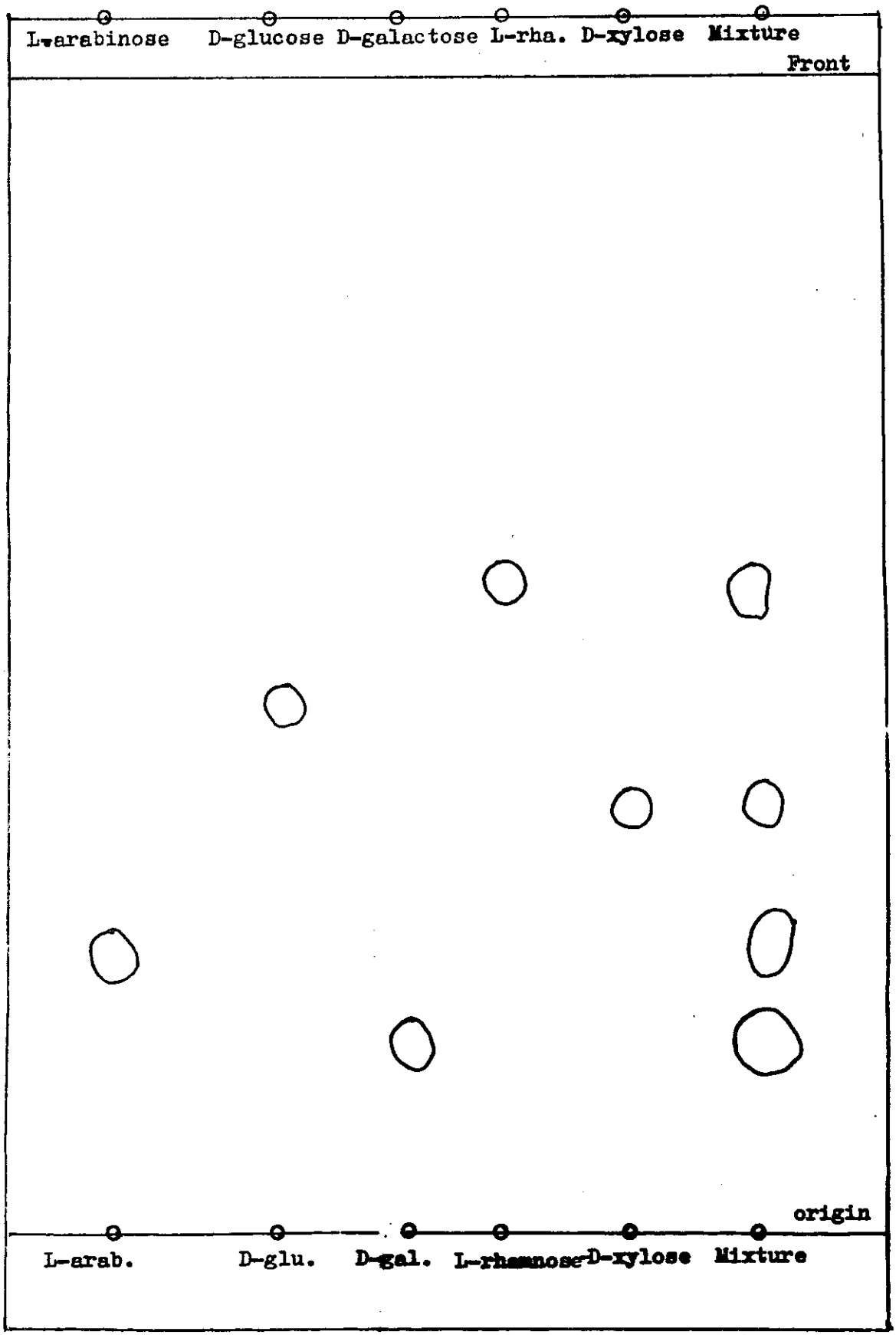


Fig. (XII) paper chromatogram.

deoxy sugar was supported by the presence of a strong band at Ca 2960 cm^{-1} in the infrared absorption spectrum of the polysaccharide (Fig. XIII), which is characteristic of the strech band of C —— H bonds in methyl groups, Wolfrom (1957).

A quantitative colorimetric determination of each of the four components of the hydrolysate of the polysaccharide extracted from L. termis was carried out and the results showed that the mentioned monosaccharides are present in the ratio D-galactose, L-arabinose, D-xylose, L-rhamnose as 5 : 3 : 1 : 1.

Such results are in complete agreement with that stated before by El-Shafee (1977) on a similar study on L.termis.

It has to be mentioned in this aspect that three monosaccharides D-galactose, D-xylose and L-rhamnose invariably exist in the pyranose ring structures in polysaccharide, while L-arabinose has been always found to occur in the furanose from in arabinoglycans and other polysaccharides.

Some of the monosaccharides present in the hydrolysate of the polysaccharide present in lupinus termis (variety Giza 2) were also reported to exist in polysaccharides present of other species of lupinus. Hirst

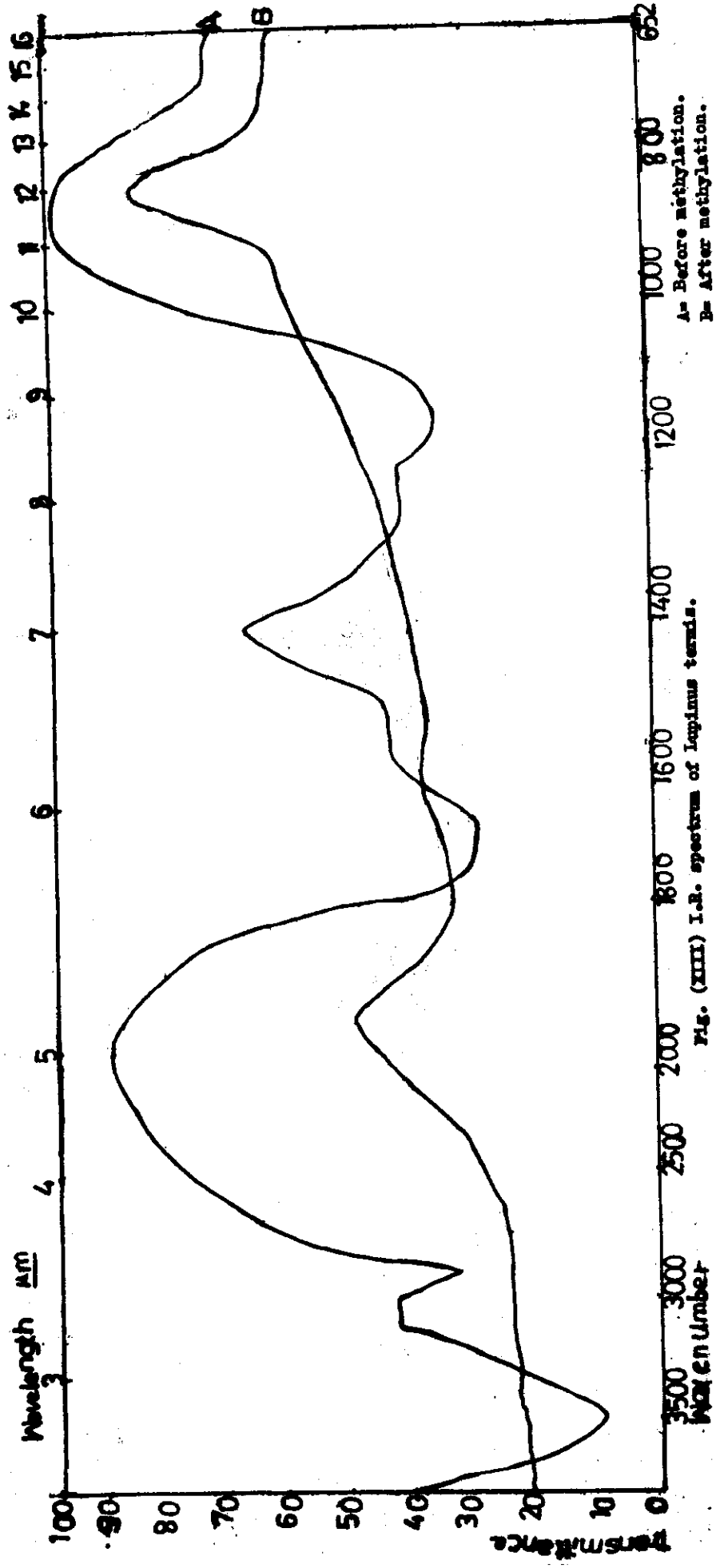


Fig. (XIII) I.R. spectrum of lupinus termis.

et al., (1947), for instance obtained D-galactose and L-arabinose from the hydrolysate of a polysaccharide which was isolated from the seeds of Lupinus albus. Hay and Gray (1966), on the other hand studied the polysaccharide present in the latter species and reported the presence of a backbone which is essentially composed of D-galactose units. A third species, viz. Lupinus luteus had been reported by Tomoda and Kimie (1965) to contain a polysaccharide which contains the four monosaccharides present in the polysaccharide under investigation galactouronic acid units.

In another study of the polysaccharide isolated from lupinus termis and reported by Tadros and Kamel (1952) D-xylose and L-rhamnose were not detected, while D-galactouronic acid was reported to exist. However, the difference in the values presented in the present work and those reported in the literature for other varieties may be attributed to interspecific variations and the use of different methods for monosaccharides determinations.

Formation of polysaccharide copper complex :

The polysaccharide of L-termis on reaction with copperammonium solution formed a precipitate copper complex with the polysaccharide. The latter when washed

with N HCl produce a polysaccharide which was considered in a pure form.Comparison of the specific rotation of the polysaccharide in dilute alkali after purification (+75) with that of copperammonium complex in water (- 468.75) showed that a levorotatory complex was formed in copperammonium. This has been shown by Reeves (1949) to occur in reactions involving the hydroxyl groups at C_2 and C_3 of type D-galactopyranoside units as well as at C_2 and C_3 and C_4 of D-xylopyranoside units. Also the complex formation depends on the angle between hydroxyl groups on the adjacent carbon atoms. However such shift in the sign of rotation was also noticed in the cases of starch and glycogen, where the reaction involved at the hydroxyl groups at C_2 and C_3 were shifted from (+ 375) to (- 715) and from (+ 366) to (- 797) respectively, (Reeves, 1951).

Since the polysaccharide of L. termis was not soluble in water as neutral aqueous solution, it was dissolved in 1 % solution of sodium hydroxide. Consequently this may not be the correct specific optical rotation of this polysaccharide. This incorrectness may be due to hydrolysis or to degradation reactions of the polysaccharide in the alkaline media. Also the possibility is considered that these alkali-sensitive substances might undergo an alteration in the fine structure(conformation of the molecules arising as a result of a

tendency of axially oriented hydroxyl groups to shift toward an equatorial orientation under strongly, alkaline conditions, Reeves and Blouin (1957). However the rotation taking in the present investigation is comparative.

Results of methylation :

The polysaccharide was exhaustively methylated as mentioned before. One gm. of the pure polysaccharide gave 0.7 gm of methylated polysaccharide after complete methylation. Of course some of the original polysaccharide were lost during methylation due to degradation and other reactions.

I.R. spectra was applied on the resulted methylated polysaccharide and the region spectra indicated the OH groups in the original polysaccharide almost disappeared this is an indication that the free hydroxyl groups were blocked and turned to methoxyl groups(Fig. XIII).

Examination and separation of the fission products of the methylated polysaccharide were carried out using preparative T.L.C. on silca gel G.

The results indicated cleary the presence of six fractions on the chromoplates as hydrolysate products of the methylated polysaccharide (Fig. XIV). After separation, purification each fraction weight was carefully determined and was subjected to carbon and hydrogen determination also the determination of the specific optical rotation and the melting point of each fraction were carefully accomplished. The results are tabulated in table (3) and the molar ratios of the methylated monosaccharides is reported in table (4).

Fraction I:

The determined constants are in agreement with the compound 2,3 di-O-methyl-D-galactopyranose (14.1 mg), sirup and $(\alpha)_D^{20}$ + 80 (water), Pacsu and Trister (1940) stated that $(\alpha)_D$ in water was + 80.9 for this sugar while Robertson and Lamb (1934) estimated its specific optical rotation in chloroform $(\alpha)_D$ + 11 and the sugar is in a liquid state. Oldman and Bell (1938) noticed that when the sugar dissolved in methanolic hydrogen chloride, it displayed change in rotation from positive to negative indicating the presence of a free hydroxyl group in a position 4.

Calculated analysis for $C_8H_{16}O_6$: C % 46.16, H % = 7.68

Experimental analysis C % = 45.77, H% = 7.55

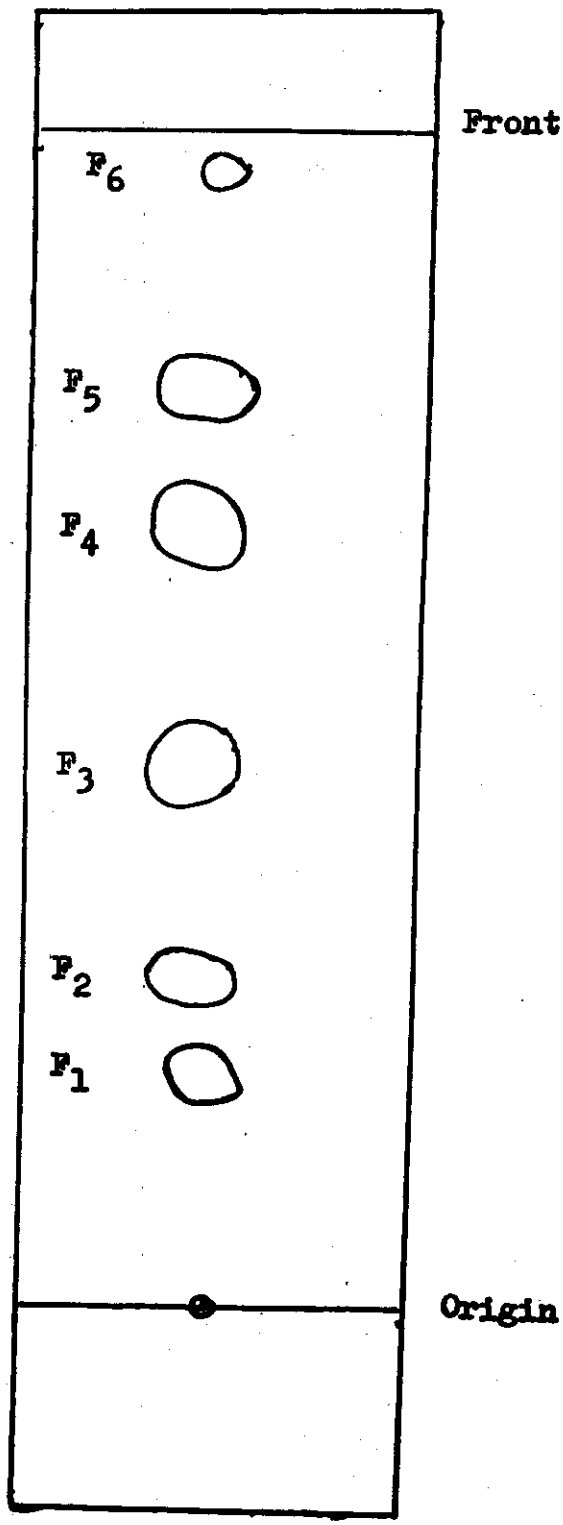


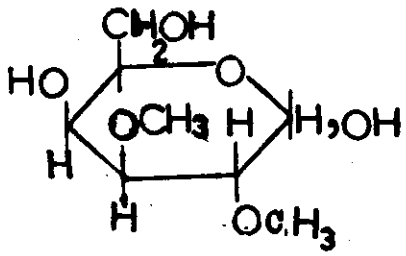
Fig. (XIV). T.L.C. Chromatogram of the polysaccharide after methylation and hydrolysis.

Table (3): Determinations of weight and analysis of the hydrolysis products of methylated polysaccharide.

Fractions	Weight fraction (mg.)	C %	H %	Melting point C°	$(\alpha)_D^{20}$ distilled water
I	14.1	45.77	7.55	Liquid	+ 80
II	13.2	46.76	7.59	81-83	+ 30 → 60 %
III	37.8	46.09	7.53	Liquid	+ 105
IV	58.2	48.20	8.04	Liquid	+ 84
V	13.8	54.37	9.23	Liquid	—
VI	2.9	47.02	7.87	Liquid	- 100
Total	139.9				

Table (4): Methylated monosaccharides after acid hydrolysis

Fractions	Name	W/mg.	M/W	mol./Ratio
I	2,3 di-O-methyl-D-galactopyranose	14.1	206	0.06
II	2,3, di-O-methyl-D-xylopyranose	13.2	178	0.07
III	2,3, di-O-methyl-L-arabinofuranose	37.8	178	0.21
IV	2,3, 6,tri-O-methyl-D-galacto-pyranose	58.2	221	0.26
V	2,3,4 tri-O-methyl-L-rhamnopyran-ose	13.8	206	0.06
VI	2,3,4,6 tetra-O-methyl-D-gala-ctopyranose	2.9	235	0.012

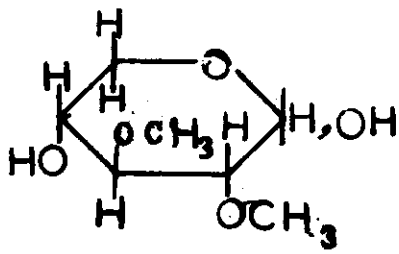


Structure (XX): 2,3 Di-O-methyl-D-galactopyranose.

Fraction II :

This fraction yielded crystaline 2,3 di-O-methyl-D-xylopyranose (13.2 mg.), which after racrystallization from aqueous ethanol, had m.p. 81-83, $(\alpha)_D^{20}$ + 30 → 60 (water). Chanda and Percival (1950) and Hampton et al. (1929) stated the value 79 → 80°C for the melting point and $(\alpha)_D$ +70 → 23. Chanda et al. (1952) mentioned that the original sugar derivative mostly obtained as a sirup but the carefully purification of 2,3 di-O-methyl-D-xylose prepared from pear-cell-wall xylan has now been obtained is crystalline form

Calculated for $C_7H_{14}O_5$: C % = 47.19, H % = 7.86
Experimental analysis C % = 46.76, H % = 7.59

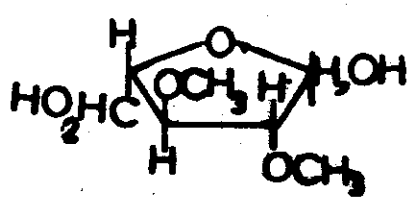


Structure (XXI): 2,3-di-O-methyl-D-xylopyranose.

Fraction III :

Gave 2,3-di-O-methyl arabinofouranose (37.8 mg) as sirup, $(\alpha)_D^{20}$ + 105 (water). Such results are almost in complete agreement with that stated before by Hirst et al. (1947) and Smith (1939) since they recorded $(\alpha)_D$ + 107 and sugar is in a liquid state.

Calculated analysis for $C_7H_{14}O_5$ C % : 47.19, H % = 7.86
Experimental C % : 47.09, H % = 7.59

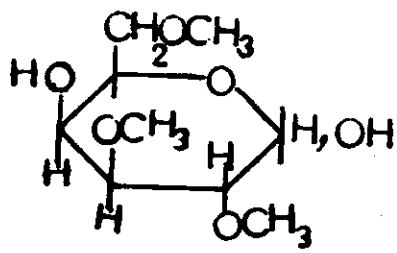


Structure (XXII): 2,3-di-O-methyl-L-arabinofouranose.

Fraction IV :

Yield liquid 2,3,6-tri-O-methyl-D-galactopyranose, which after evaporation of ethanol amounted (58.37 mg.) and its specific optical rotation was $(\alpha)_D^{20}$ + 84 (water). Haworth et al. (1932) stated that this sugar is a liquid and had $(\alpha)_D$ + 87 (water). Also it was postulated that this sugar had not yet been synthesized, but its constitution was established by oxidation with HoBr followed by partial hydrolysis (Haworth et al., 1937). In another study for Haworth et al. (1935) they showed that the hydroxyl group of position 4 was unsubstituted in the sugar using conventional methods.

Calculated analysis for $C_9H_{18}O_6$ C % : 48.64, H : 8.10
Experimental analysis C % : 48.20, H : 8.04

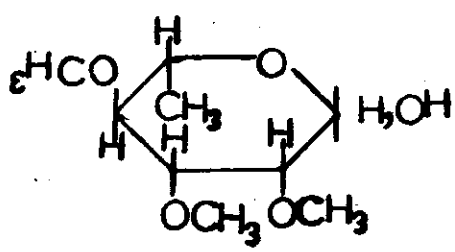


Structure (XXIII);2,3,6 tri-O-methyl-D-galactopyranose.

Fractions V :

This fraction weight was (13.8 mg.) and was found to be 2,3,4-tri-O-methyl-L-rhamnopyranose as sirup and its specific optical rotation was not determined since it changed within short time from + 0.8 to 0.2 with concentration 10 mg./2 ml. (water) Hisrt and Macbeth (1926) and Haworth et al. (1948) did not mention any specific optical rotation nor melting point for this sugar. However Smith (1940) had pointed that the anilide derivative of this sugar is solid with melting point 111°C, also the same author was not able to determine the specific optical rotation of this sugar.

Calculated analysis for $C_5H_{11}O_5$, C % = 52.42 , H % = 8.73
Experimental analysis C % = 54.37 , H % = 9.23



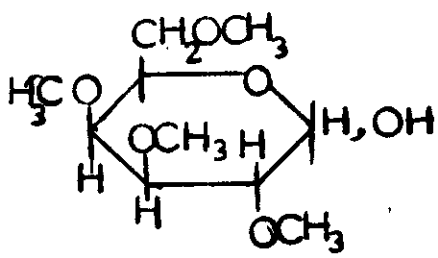
Structure (XXIV): 2,3,4-tri-O-methyl-L-rhamnopyranose.

Fraction VI :

This fraction was found to be 2,3,4,6-tetra-O-methyl-D-galactopyranose (2.9 mg) which formed the terminal units of the polysaccharide polymer. The separated methylated monosaccharide was sirup and had $(\alpha)_D^{20}$- 100 (water). Ivrine and Comerson (1904) and Bourne and Peat (1950) stated that the sugar derivative was in liquid state and had $(\alpha)_D$ - 109.5. However this sugar is conveniently prepared by complete methylation of either α -or B-form of methyl - D-galactopyrnose followed by acid hydrolysis, Haworth et al. (1927).

On the other hand the methylated polysaccharide when yields after hydrolysation this tetra-methyl-D-galactose as one of the products, indicating terminal D-galactopyranose moities in this polysaccharide, Jones and Smith (1949).

Calculated analysis for $C_{10}H_{20}O_6$ C % = 50.85, H % = 8.47
Experimental C % = 47.02, H % = 7.87



Structure (XXV): 2,3,4,6-tetra-O-methyl D-galactopyranose.

The obtained results of carbon and hydrogen showed some little deviations than the calculated ones, this may be due to two main factors, first is the incomplete removal of the solvent used i.e. ethanol since a very small residual traces of CH_3CH_2OH would by change the percentage of carbon and hydrogen. Also the small weight obtained (2.9 mg.) might led to some experimental error within the range 5 to 6 %.

The same arguement could be applied to some extent on fraction V.

It has to be mentioned in this aspect that the results in table (3) indicate that the methylated sugars were successfully hydroysed using N sulfuric acid, with only loss of Ca 10 % of the started material polysaccharide (155 mg.). Since the total weight of the resulted degraded monosaccharides was Ca 140 mg.

The separation of the fission products of the methylated polysaccharide by T.L.C. yielded 2,3,6, tri-O-methyl-D-galactopyranose (58.2 mg.) which represents 37.55 % of the methylated sugar before hydrolysis. 2,3,Di-O-methyl-D-galactopyranose (14.1 mg.) represents 9.1 % of the original methylated sample, 2,3,4,6-tetra-O-methyl-D-galactopyranose with small amount (2.9 mg.)

equivalent to 1.87 %. Fraction III was identified as 2,3, Di-O-methyl-L-arabinofouranose (37.8 mg.) which represents 24.39 %, also 2,3, Di-O-methyl-D-xylopyranose (13.1 mg.) represents 8.52 %, and 2,3,4, Tri-O-methyl-L-rhamnopyranose (13.8 mg.) which represents 8.9 % of the total methylated sugar.

From the results cited in table (4) it could be concluded that 2,3,4,6 tetra-O-methyl-D-galactopyranose and 2,3,4 tri-O-methyl-L-rhamnopyranose formed the terminal nor reducing ends of the polysaccharide molecule.

The isolated polysaccharide probably constituted of a backbone (main chain) formed of anhydro galactopyranose units linked through $1 \longrightarrow 4$ glycosidic linkage. This hypothesis is supported by the isolation of 2,3,6 tri-O-methyl-D-galactopyranose as a predominant product (0.26 mole), 2,3 di-O-methyl D-galactopyranose (0.06 mole) and a minor product of 2,3,4,6 tetra-O-methy-D-galactopyranose (0.012 mole) which form the terminal non reducing end of the molecule. The average number of anhydro galactose units in the chain could be calculated on the basis that one chain yields one mole tetra-O-methyl derivative whatever the length of this chain. Therefore the average number of galactose units in the chain $= \frac{0.26 \times I}{0.012} = 21$ units.

But to this number of anhydro galactose units in the main chain, it must be added the number of mole corresponding to 2,3, di-O-methyl D-galactose which are included in the chain.

$$\frac{0.06 \times 1}{0.012} = 5 \text{ units.}$$

Therefore the total number is = 21 + 5 = 26 units :

This main chain of 26 A.gal.u.(e.g. anhydro galactopyranose units)is ramified through 1 → 6 linkages as proved by the isolation of 2,3 di-O-methyl-D-galactopyranose (0.06) mole.

There are probably 5 branches which derived from the backbone chain. Such hypothesis based on molar ratios of 2,3,6-tri-O-methyl-D-galactose (0.26 mole) to 2,3, di-O-methyl-D-galactose (0.06 mole). Therefore the number of branches $\frac{(0.26 + 0.06) \times 1}{0.06} = 5.2$

It could be concluded that for every 5 units in the main chain ($\frac{26}{5}$ =5.2)a branch is formed as shown in Fig. (XV).

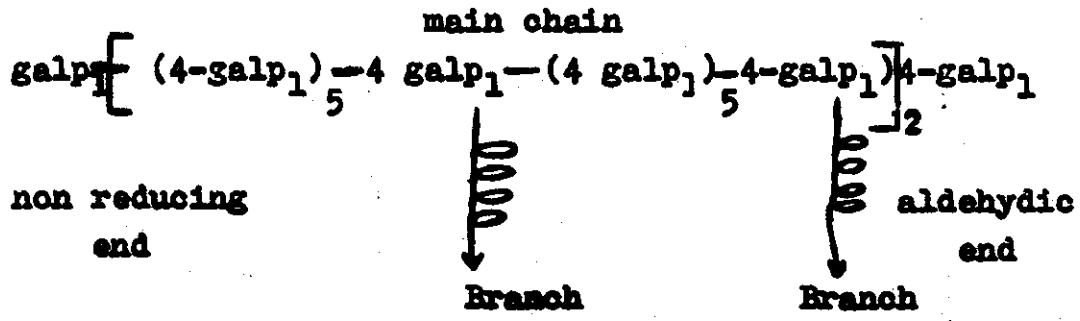


Fig. (XV).

The xylose units in the polysaccharide are linked together through 1 → 4 linkages as proved by the isolation of 2,3 di-O-methyl-xylopyranose which amounted to 0.07 mole, such amount is quite close to that of 2,3 di-O-methyl-D-galactose (0.06 mole). It could be concluded that for every 2,3-di-O-methyl-D-galactose there is a 2,3 di-O-methyl-xylose and thus in the case that xylose occuppied all the branches and each branch is formed of one xylose units and rhamnose occupied the terminal end of the branch as shown in (Fig.XVI).

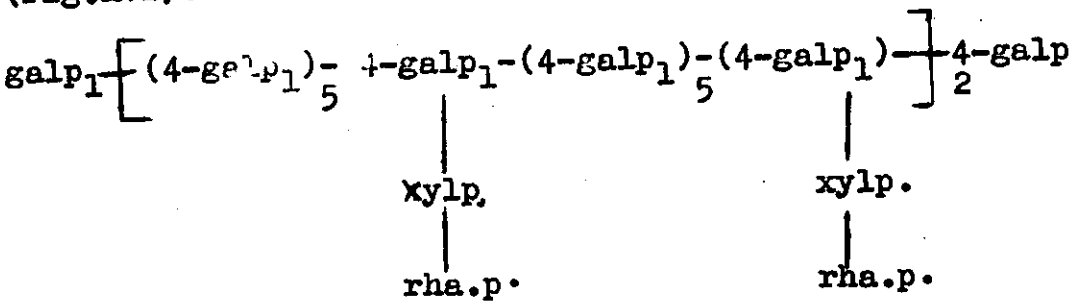


Fig. (XVI)

This hypothesis was established on the basis that the terminal nnits of rhamnose (2,3,4 tri-O-methyl-rhamnose) are equal in amount (0.06 mole) to the 2,3 di-o-methyl-galactose (0.06 mole).

If the structure of the polysaccharide is reported as shown in Fig. (XVI). The arabinofuranose is not incorperated in this polysaccharide backbone and was co-precipitated with it.

Such hypothesis was discarded on the basis that 2,3,5 tri-O-methyl arabinofouranose was not detected as trimethyl arabinofouranose derived from the terminal non reducing end of the arabinan chain. Therefore it could be concluded that arabinose units are most probably linked to the main chain as a single branch of 18 units of arabinose ($\frac{0.21\ X1}{0.012}$ = 18 units) or as two branches of 9 units and each branch is terminated by a rhamnose units.

The structural formula as shown in Fig.(XVII) and Structure (XXVI) for the polysaccharide fit will with the molar ratio of the methylated monosaccharides given after acid hydrolysis of the methylated polysaccharides.

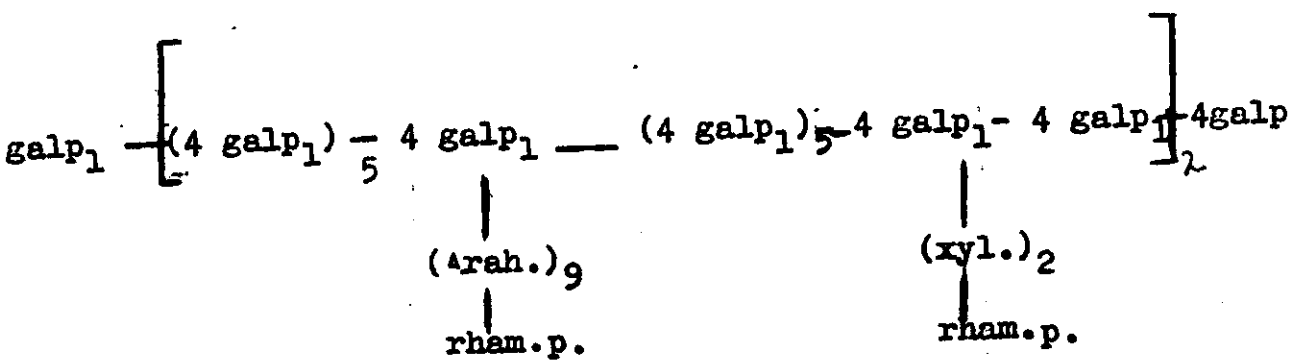
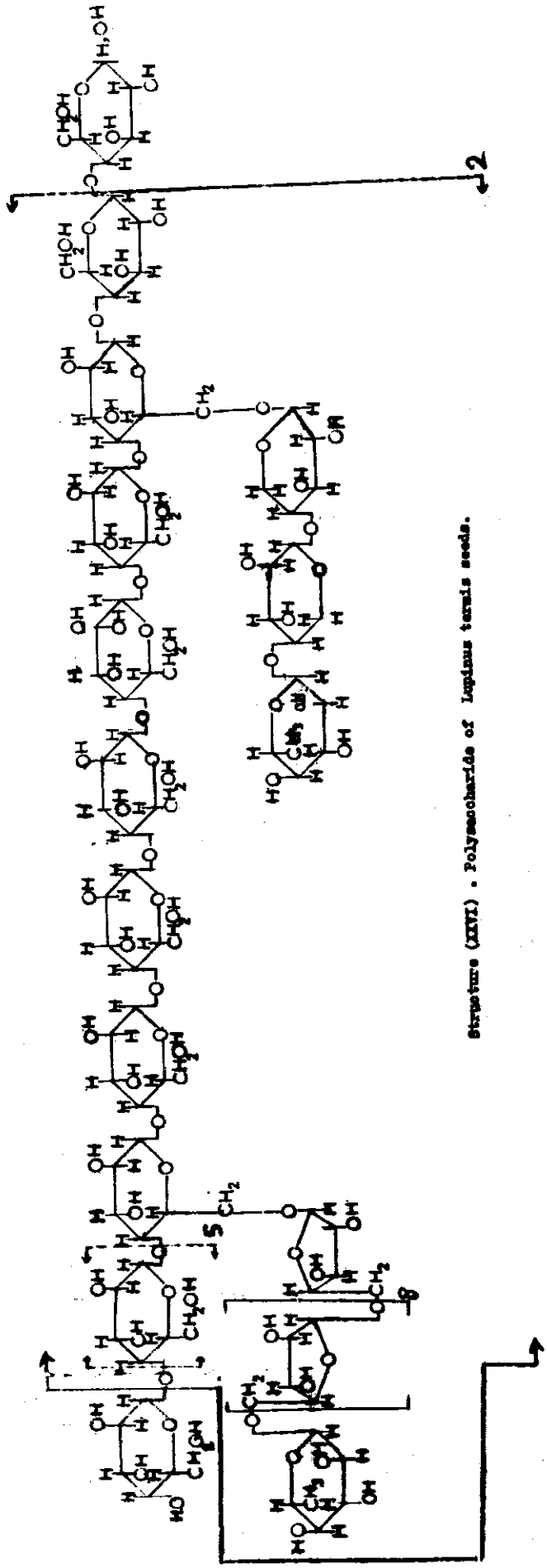


Fig. (XVIII).

A formula based on the incorcoporation of the arabinofuouranose units in the main chain may be also proposed, but it is well known that the enzymes working in the biosynthesis of a heteropolysaccharides tend



Structure (XXVI) - Polysaccharide of Lupinus termis seeds.

in general to arrange the building units in a certain symmetry but not at random.

It is important to mention here that the examination of the infrared absorption spectrum of the polysaccharide revealed some information about the type linkage present in the polymer. Thus the appearance of the characteristic vibrational band at 885 cm^{-1} of B-glycosidic linkage (Whistler and House, 1953, and Barker et al., 1954) and the absence of that the - linkage at 810-840 cm^{-1} give some support to the assumption that all linkages in the polysaccharide are of the B-type.

In similar investigations of the polysaccharides present in L. lutus and L. albus it was shown by methylation technique that D-galactose units are connected through C_1 and C_4. Hay and Gray (1966) identified a galactan backbone in the case of L. albus which is connected through C_1 and C_4. Tomoda and Kimie (1965) pointed out an arabinogalactan backbone in the case of Lupinus leutus, the two monosaccharides in Lupinus luteus arabinofouranose and galactopyranose being in a ration 2 : 3, but the polysaccharide which was isolated in this investigation has the two monosaccharides, arabinofouranose and galactopyranose in a ratio 3 : 5.

D-galacturonic acid could not be detected during the course of this investigation as one of the constitution of the polysaccharide of L. termis. However the only investigators who established the presence of this compound in L.termis were Tadros and Kamel (1952) by a slight precipitate of mucic acid, but the periodate and methylation technique were not used.

However the obtained results are in accordance with the findings of other workers who were able to obtain O-B-D-galactopyranosyl (1→4) D-galactose after partial hydrolysis of the galactan of Lupinus albus (Hirst and Jones, 1947).

In order to propose a definite structural structural formula for the polysaccharide isolated from Lupinus termis beside methylation technique also periodate oxidation followed by Smith degradation technique must be carried out. The partial acid hydrolysis of the polysaccharide and isolation of different oligosaccharides will give also very usefull information about the structure of the polysaccharide under investigation.