A Chemical Investigation Of The Galactoaraban Prepared From The Seeds Of The Peanut

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A CHEMICAL INVESTIGATION OF THE GALACTOARABAN PREPARED FROM THE SEEDS OF THE PEANUT

Ryosuke MIYAMA

CONTENTS

INTRODUCTION

Arabans, galactans and galactoarabans are said to be contained in the hemicelluloses of Leguminosae, but they are not yet determined to be either one compound or a mixture of the polysaccharides.

Especially concerning the hemicelluloses in the seeds of the peanut, as far as we know there are very few reports and it is hard to judge them to be a compound by the determination of composition only.

In respect to the polysaccharide, moreover, its colloidal property makes it hard to separate from impurities.

No form of hemicellulose can be said to be absolutely pure if it is not changed to a derivative from the natural state.

The author tried to separate galactoaraban from the seeds of the peanut and sought to show that it might be constructed in

the ratio of one molecule of galactose to two molecules of arabinose, as in the case of the galactoaraban of the pea.

Then it was thought that the next problem to solve was how the components are arranged. If by acetolysis the galactoaraban could be decomposed into a derivative of galactoarabotriose, which contains one molecule of galactose and two molecules of arabinose, it would be ascertained that the galactoaraban should be constructed of this triose, and that galactose and arabinose should be combined in the same molecular ratio, (1 : 2) in the galactoaraban as in this trisaccharide. Then the author wanted to settle the arrangement of every sugar radical according to the assumption of polysaccharide.

The remaining problem for investigation seemed to be to determine the configurations of these sugars. The author tried to methylate the galactoaraban and decompose it to the scission product, which was then distilled to several fractions, and they were investigated for the purpose of seeking the connecting points of every sugar radical.

After they were found, the author intended to insert each of them as free sugar radicals into the arrangement of the galactoaraban to make the constitutional formulae.

CHAPTER I

Preparation of Galactoaraban and its Compositions.

It is said that galactoaraban is distributed widely in the botanical kingdom, especially in seeds, plant substances, mucilages and gummy substances such as seeds of Leguminosae[1, 2, 3, 4, 5], pollens of the timothy grass[6], fruits of *Grevia robusta*[7], "wasser fenchels[8]," *Khaya senegalensis*[9], young beet[10], mucilage of *Acasia arabica*[11], the lichen[12], *Sterculea plarifolea*, *Oenothera jacquinin*, *Kadsura japonica*[13] and gum of *Acasia decurrens*[14], etc.

Concerning the hemicelluloses in Leguminosae, several kinds of galactan with different properties were separated into the following groups as α-galactan in the seeds of the common wicken[15] and the bean[16], β-galactan in the seeds of the lupin[17, 18, 19], γ-galactan in the seeds of *Lupinus angustifolius*[20] and paragalactan in seeds[21], bodies[22] and shells[23] of the lupin.

Earlier investigators, after hydrolising the hemicelluloses and

detecting only the constituental monosaccharides because of the difficulty and incompleteness of the treatments of polysaccharide, customarily reported that the hemicelluloses were xylan, araban and galactan, etc.

It is hard to determine the kind of polysaccharide merely by the detection of cleavage product, owing to the difficulties of isolation of the polysaccharide and to constitutional difference of the components.

Paragalactan came to be called paragalactoaraban after arabinose was identified as one of its constituents, but its composition varied according to the observer owing to the incompleteness of the treatment, and it may be said that it is still unsettled as to whether it is a compound or a mixture.

Concerning the separation of the hemicelluloses contained in the seeds of Leguminosae, MIKI[24] separated the alkali extract of the seeds of *Phaseolus radiatus* into two fractions by fractional precipitation with aceton, and purified each of the hemicellulose with FEHLING's solution.

HEIDUSCHUKA and TETTENBORN[25] precipitated the copper salt of galactoaraban by addition of copper sulphate to the water solution of its alkali compound which was separated from the pea.

Though the purifications of the polysaccharide were repeated, it remained still colloid and the hemicellulose which had two components could not be determined to be either one compound or a mixture of compounds by its colloidal nature and disturbances of analogous substances.

The author tried to make further investigations of the galactoaraban which was studied by HEIDUSCHUKA and TETTENBORN, but the source of the galactoaraban used was the seeds of the peanut and not the pea.

It was extracted with hot 0.2% caustic potash solution from the fat free peanut powder and prepared as copper free substance after being precipitated as copper salt, and it was purified until it gave no iodin nor zinc chloriodin reaction.

The galactan which was prepared from the shells of the lupin by SCHULZE[26] was a yellowish powder and could be dissolved in 2% caustic potash solution converting it into potassium salt, the salt being soluble in water making a colloidal solution.

The paragalactoaraban which was prepared from the seeds of

the lupin[26], was discovered to be constructed of 53.34 % galactose and 14.02 % arabinose, and it gave zinc chloriodin reaction.

The two hemicelluloses which were isolated from the seeds of *Phaseolus radiatus* by MAKI[24], were proved to be constructed of arabinose, galactose, uronic acid and xylose, but one of the hemicelluloses seemed to be galactoaraban of considerable purity, though a part of galactose was found to be oxidised to uronic acid according to the data of MIKI.

Yellowish powder which was prepared from peanuts by the author was insoluble in water and organic solvents but dissolved in alkaline solution making salt.

On the addition of alcohol to the alkaline solution, the alkali salt was precipitated, which was dispersed in water as colloidal solution and again precipitated with the addition of alcohol.

The copper salt was precipitated by adding FEHLING's solution or copper sulphate solution to the colloidal solution of galactoaraban.

The galactoaraban which was separated by the author contained not only galactose and arabinose, but also uronic acid, the oxidation product of combined galactose. But it contained neither methoxyl nor xylose, and, moreover, was negative for both iodin and zincchloriodin reactions.

All these findings might be explained by assuming that it contained no polysaccharide such as pectin, xylan, starch, and cellulose.

But it was found to be constructed chiefly of galactoaraban, though a part of the galactose was found to be oxidised to uronic acid as the result of the experiment.

	Galactose %	Uronic acid %	Arabinose %	Moisture %	Ash %	Total %
No. 1	25.52	7.76	57.34	10.59	0.88	102.90
No. 2	25.11	7.52	56.58	10.98	1.21	101.40
No. 3	25.10	7.61	56.43	10.36	1.06	100.56
Calculated value	40.50	—	67.57	—	—	108.07

On the above mentioned table, the total quantity of uronic acid might be derived from the quantity of galactose, by the reason of the fact that not only xylose was not detected in the product of hydrolysis and therefore the glucuronic acid might be concluded to be absent, but also that brucin salt of 5 keto-galactonic acid, the

derivative of galacturonic acid, was isolated, and, moreover, that the quantity of uronic acid increased because of the different methods of extractions for the preparation.

The ratio of arabinose to galactose was compared with the theoretical ratio after having calculated the substance to be free from ash and moisture and also uronic acid was added to galactose multiplying the theoretical factor.

	No. 1	No. 2	No. 3	Mean	Theoretical value
Arabinose	57.37	56.58	56.43	56.79	67.57
Galactose	32.59	31.91	32.02	32.19	40.50

It was hard to compare them, and so the mean value was calculated to compare easily to the theoretical value, and the result of hemicellulose II which was reported by MIKI was also calculated as follows :

	Arabinose	:	Galactose
Galactoaraban prepared from the peanut	67.57	:	38.36
Hemicellulose II prepared from *Phaseolus radiatus* .	67.57	:	37.93
Theoretical value	67.57	:	40.50

From these results it may be concluded that the galactoaraban was constructed in the ratio of two molecules of arabinose to one molecule of galactose, though a part of the latter was oxidised.

EXPERIMENTS

(1) Preparation of galactoaraban.

The oil free peanut powder was extracted with cold 0.2 % caustic soda solution repeatedly to remove protein, and the residue was again extracted with hot 0.2 % caustic potash solution.

To the clear solution which was decanted from the filtrate, filtered with hemp clothes and settled for several days, three volumes of 95 % alcohol was added, and the galactoaraban was precipitated out as potassium salt.

After having dissolved the precipitate in water, the solution was decanted several days later to separate from the insoluble substances.

The potassium salt of galactoaraban was precipitated again by adding two volumes of 95 % alcohol to the solution.

By further purifications, repeated treatments of dissolution and precipitation, the precipitate was converted into potassium salt of polysaccharide free from nitrogen and excess of alkali.

The copper sulphate solution was added drop by drop to the dilute aqueous solution of the potassium salt of polysaccharide stirring vigorously, then a bluish copper salt of galactoaraban was precipitated, the other polysaccharides being left in the mother liquor. After the precipitate was filtered and washed well, it was decomposed into free galactoaraban and alcoholic copper chloride solution, by adding alcohol containing two volume percent of conc. hydrochloric acid.

After having decanted the solution, the precipitate of galactoaraban was washed successively with 95 % alochol, absolute alcohol and ether, and then dried.

When it was positive with iodin reaction, it had to be dissolved to alkaline solution and then the above mentioned reaction had to be repeated.

The yield of galactoaraban was 0.6 % of the seeds of the peanut.

(2) The properties of galactoaraban.

The galactoaraban was insoluble in water and organic solvents, but soluble in alkaline solution converting it into salt, which was precipitated with sufficient addition of alcohol and aceton.

But when FEHLING's solution or copper sulphate solution was added to the aqueous solution of the salt, copper salt was separated. It gave neither iodin reaction nor zinc chloriodin reaction.

(3) Hydrolysis of galactoaraban.

It was hard to hydrolyse the galactoaraban quantitatively like xylan.

The galactoaraban is hydrolysed with 3 % sulphuric acid on the water bath for 28 hours into 73.12 % reducing sugar.

After being neutralised and bleached, the hydrolysed solution was condensed to syrup by vacuum evoporation, extracted with 95 % alcohol, and was condensed to syrup again.

The residue which was not extracted with alcohol, was dried and was used for the detection of galacturonic acid.

(4) Detection of sugar.

a) Arabinose.

2 g. of diphenylhydrazin was added to the 50 % alcoholic solution containing 2.1791 g. of the syrup and was warmed for 30 min. on the water bath and cooled, then crystals of diphenylhydrazon occurred. Accicular crystals of pink colour were separated after being recrystallised twice and they melted at 203°C. and showed no depression of melting point on admixture with an authentic specimen of *l*-arabinose-diphenylhydrazon.

Nitrogen content corresponded to the calculation.

Anal. subst. 3.61 mg. N = 0.240 cc. (11°C 768 mm.) . . N = 8.53 %
Calc. for $C_{17}H_{20}O_4N_2$ . . . . . N = 8.86 %

b) Galactose.

The syrup was oxidised with nitric acid to get mucic acid according to the usual method and crystals of mucic acid were separated.

They melted at 213°C. and after being dissolved in ammonia, calcium chloride was added to it, then calcium mucate was precipitated.

Anal. value . . . . . 19.97 % CaO 19.93 % CaO
Calc. for $CaC_6H_8O_8 + 1\frac{1}{2}\ H_2O$ . . 20.93 % CaO

c) Galacturonic acid.

Having added a calculated amount of dilute sulphuric acid to the residue, the barium salt of uronic acid, for the purpose of making free uronic acid, and filtered off the barium sulphate, the filtrate was condensed to syrup and it was detected to have been galacturonic acid by the method reported by EHRLICH[27], as follows :

Clear solution of calcium hydroxide was added to the syrup, calcium salt of 5-keto-galactonic acid precipitated and filtered it, a calculated quantity of oxalic acid was added to the filtrate to precipitate the calcium as calcium oxalate and filtered, the filtrate was then condensed to syrup and dissolved into aceton, brucin was added

to the solution and warmed and then cooled over night, the brucin salt of 5-keto-galactonic acid crystallised out, it was recrystallised. It decomposed at 146°C.

Anal. subst. 6.398 mg. N = 0.398 cc. (18.5°C 771.8 mm.) N = 5.07 %
Calc. for $C_{22}H_{26}N_2O_4C_6H_{10}O_7H_2O$ . . . . . N = 4.62 %

Nitrogen content corresponded to the calculation.

d) Xylose.

Xylose could not be found in the syrup after treating the syrup with BERTRAND's xylose reaction.

e) Mannose.

Mannose was not detected in the syrup with phenylhydrazin, as it is usually detected.

f) Fructose.

The syrup gave the ketose reactions, both of SELIWANOFF's and PINOFF's ones, then the quantity of ketose was calculated by the difference of the quantity of total sugar and that of the aldose.

4 g. of air dried sample was hydrolised with the 1 % solution of sulphuric acid for 5 hrs. on the water bath. 5 c.c. of neutralised solutions corresponding 4/30 g. of sample were used for determinations of total sugar and aldose.

Total sugar:

It reduced 136.2 mg. Cu, then it corresponded 76.9 mg. of galactose or 69.3 mg. of arabinose.

Then each sugar was calculated by using the quantitative ratio of galactose to arabinose as follows:

$$\text{Galactose} = \text{Total sugar (calc. as galactose)} \times \frac{\text{Galactose}}{\text{1 glactose + 2 arabinose}}$$

$$= 76.9 \times \frac{40.50}{40.50 + 67.57} = 28.822 \text{ mg.}$$

$$\text{Arabinose} = \text{Total sugar (calc. as arabinose)} \times \frac{\text{2 arabinose}}{\text{1 galactose + 2 arabinose}}$$

$$= 69.3 \times \frac{67.57}{40.50 + 67.57} = 43.326 \text{ mg.}$$

Total sugar = 0.028822 + 0.043326 = 0.072148 g.

$$\text{Per cent. of total sugur} = \frac{4 \times 0.072148}{30 \times 100} = 54.11\ \%$$

Aldose:

It consumed 8.5 c.c. of thiosulphate solution.

Every molecule of sugar, even if it was pentose or hexose, consumed 2 atoms of iodin. The factors then were 0.5912 for pentose and 0.7094 for hexose.

If the sample contained as the ratio of 2 mol. of arabinose and one mol. of galactose, the consumption of iodin of every sugar should be shown as follows:

$$0.012658 \times 8.5 \times \frac{1}{3} = 0.03586 \text{ g. I for galactose.}$$

$$0.012658 \times 8.5 \times \frac{2}{3} = 0.07172 \text{ g. I for arabinose.}$$

Then the quantity of every sugar was calculated as follows:

$0.03586 \times 0.7094 = 0.02543$ g. galactose.

$0.07172 \times 0.5915 = 0.04242$ g. arabinose.

$0.02543 + 0.04242 = 0.006785$ g.

$$\text{Per cent of aldose} = \frac{30 \times 0.06785}{4 \times 100} = 50.89\,\%$$

$\therefore$ Ketose $= 54.11 - 50.89 = 3.22\,\%$

Though the quantity of ketose in the syrup was determined, it was not more than that of uronic acid.

This finding might be explained by assuming that the galacturonic acid was transformed into 5-keto-galactonic acid, even if the small quantity of fructose might have been contained as the mixture in the sample.

g) Methyl pentose.

An attempt was made to detect methyl pentose by the different yields of phloroglucid, in one case directly obtained and in the other extracted with alcohol according to the method of the determination of methyl pentose.

But the difference between the results was too small to think the existence of the methyl pentose in the sample as one constituent.

	1	2	3
Yield of phloroglucid directly obtained . . . . %	57.34	56.58	56.43
Yield of phloroglucid extracted with alcohol . . %	56.05	56.13	55.88

(5) Quantitative analysis.

It has already been shown that the polysaccharide contained

chiefly arabinose, galactose and its oxidised form, the uronic acid.

Then the next problem was to investigate the quantitative ratio of all its components. Galactose was determined as mucic acid, arabinose as furfurol, and uronic acid[28] was calculated from the carbon dioxide which was evolved by decarboxylation.

Galactose was determined as follows:

Sample used	Mucic acid determined	Galactose calculated	Galactose
g	g	g	%
3.8456	0.8179	0.9815	25.52
3.0876	0.6461	0.7753	25.11
3.3073	0.6918	0.8316	25.10

Uronic acid was determined as follows and was calculated as galactose.

Sample used	CO_2 determined	Uronic acid calculated	Uronic acid	Uronic acid culculated as galactose
g	g	g	%	%
2.3152	0.0448	0.1796	7.76	7.07
3.2651	0.0682	0.2728	7.52	6.85
2.9712	0.0565	0.2265	7.60	6.92

The arabinose was calculated[29] from the difference between the total yield of furfurol and the yield of furfurol caused by the uronic acid.

Sample used	Total furfurol determined	Furfurol caused by uronic acid calculated	Furfurol caused by arabinose calculated	Arabinose calculated	Arabinose
g	g	g	g	g	%
0.4677	0.1329	0.0060	0.1269	0.2683	57.34
0.3627	0.1012	0.0045	0.0967	0.2053	56.58
0.4053	0.1135	0.0051	0.1084	0.2287	56.43

Moisture and ash were determined and the above mentioned results were also arranged as follows:

	Galactose	Uronic acid	Arabinose	Moisture	Ash	Total
	%	%	%	%	%	%
No. 1	25.52	7.76	57.34	10.59	0.88	102.09
No. 2	25.11	7.52	56.58	10.98	1.21	101.40
No. 3	25.10	7.61	56.43	10.36	1.06	100.56

The ratio of arabinose to galactose was calculated, the quantity

of galactose including that calculated from the quantity of uronic acid.

	No. 1	No. 2	No. 3	Mean
Arabinose	57.37	56.58	56.43	56.79
Galactose	32.39	31.96	32.02	32.19

The mean values of these were calculated to compare easily to the theoretical values as in the following table.

	Arabinose	:	Galactose
Theoretical ratio	67.57	:	40.50
Determined ratio	67.57	:	38.30

The results of the determinations of uronic acid in galactoaraban have been already mentioned, but they were the nearest content of them selected from all of the preparations.

In respect to the content of uronic acid, though the results corresponded closely in the above mentioned table, they fluctuated more or less in many other specimens prepared as in the usual manner, as shown in the next table.

	Sample	CO_2 evolved	Uronic acid calculated	Uronic acid
	g	g	g	%
1	1.0894	0.0155	0.0620	5.69
2	0.9795	0.0227	0.0908	9.27
3	0.8204	0.0224	0.0896	10.92

They were well known matters that the many carbohydrates were oxydised by the treatment with alkali.

By all of these factors—the fluctuating content of uronic acid, the hot extraction of the peanut with alkali, and the connection between the carbon atoms which were described in Chapter III, in the every componental sugar,—the author came to assume that the galactose radical might be oxydised to galacturonic acid.

Then the several examples which have been effected upon in content of uronic acid caused by the repeated treatments of the different concentration of alkaline solution are mentioned as follows;

	Sample	CO_2 evolved	Uronic acid calculated	Uronic acid
	g	g	g	%
Galactoaraban used	1.3371	0.0279	0.1116	8.34
After extracted with hot 0.2% KOH	1.0184	0.0366	0.1098	10.78
After extracted with 4% KOH	0.9476	0.0405	0.1620	17.1
After extracted with 30% KOH	0.5536	0.0387	0.1548	27.96

All of these results corresponded to the assumption that the galactose might have been oxydised to galacturonic acid.

The ratio of arabinose to galactose corresponded almost to the theoretical ratio, but the reduction of few percent of galactose might be the reason that the uronic acid was decarboxylated[30] by the treatment of hot extraction of alkali.

CHAPTER II

Acetolysis of Galactoaraban.

As already described, a hemicellulose usually contains impurities caused by imperfect purification and though the method of purification was improved, the impurities could not be removed completely.

In a polysaccharide, it was uncertain whether the properties and connecting points of the componental sugars are the same as each other or not, though they were found to be the same monosaccharide as scission products.

In the case of a polysaccharide which contains two or more components, such as glucomannan[31], it cannot be decided whether it is one compound, or a mixture, by the determination of the componental ratio obtained from the scission products.

But a crystal of glucomannotriose was separated as an intermediate product of decomposition, and the conclusion was reached that this was the sugar which constructed the glucomannan.

The galactoaraban prepared by the author from the peanut was proved to be constructed in the ratio of two molecules of arabinose to one molecule of galactose, though a small part of it was oxidised, but it is doubtful whether it is either a compound with two components, or only a mixture of compounds, and also it is still un-

certain if it can be decomposed as an intermediate product, though it might have been a compound.

Then the author tried to determine these problems after decomposing the galactoaraban by acetolysis, but the acetolysis was restricted in order to produce a trisaccharide derivative or multiplied trisaccharide derivatives, on the supposition that was a homogeneous polysaccharide.

For this purpose, the author used the methods applied by SKRAUP and KÖNIG[32], WISE and RUSSEL[33], HAWORTH[34], HESS[35], OST and KNOTH[36], and NISHIDA[31], it was made clear that the methods of OST and KNOTH, HESS and NISHIDA were suitable to separate the trisaccharide derivative from its products.

When it was thought to be homogeneous substance, it would seem that the galactoaraban was acetylated and it was then decomposed into simpler substances in the course of acetolysis as shown in the next table.

	Mol. formulae	Mol. weight	C %	H %	Acetic acid %
Galactoaraban	$(C_5H_8O_4)_{XY}$	(416.2028) $_{XY}$	46.13	6.30	0
Heptacetyl-galactoaraban	$(C_5H_8O_4(CH_3CO_2)_2)_{XY}$	(713.2574) $_{XY}$	50.47	4.66	58.92
Acetyl-higher-polymeride	$(C_5H_8O_4(CH_3CO_2)_2)_X$	(713.2574) $_X$	50.47	4.66	58.92
Nonacetyl-galactoarabotriose	$C_{15}H_{12}O_6(CH_3CO_2)_9$	822.3588	49.61	5.64	65.70
Heptacetyl-galactoarabotriose	$C_{11}H_{12}O_5(CH_3CO_2)_7$	605.2574	49.60	5.30	49.43
Hexacetyl-arabobiose	$C_{16}H_{18}O_9(CH_3CO_2)_6$	532.2184	49.57	5.49	67.68
Pentacetyl-galactose	$C_6H_7O(CH_3CO_2)_5$	390.1716	49.20	5.68	67.93
Tetracetyl-arabinose	$C_5H_6O(CH_3CO_2)_4$	318.1404	49.31	5.70	75.47

The author tried to discover the condition which produced much more nonacetyl-galactoarabotriose.

Concerning the reaction of acetolysis of polysaccharide like galactoaraban, it did not proceed uniformly, and an intermediate product of homogeneous constitution could not be obtained.

The lower polymerids were extracted with ether from the crude product and the residue was acetolysed again.

Thus the twice acetolysed product was extracted perfectly with

ether again and the residue was acetolysed for the third time. This product was extracted almost completely with ether.

The substance extracted from ether assumed the form of a white crystal of needle shape by purification.

Molecular weight, quantities of acetyl radicals, and quantity of arabinose radicals were determined. Galactose was detected, and from all these results, it might be explained that the crystal was nonacetyl-galactoarabotriose which had been assumed.

	Melt. point	Acetic acid%	$[\alpha]_D$	Mol. weight	C %	H %	Arabinose %
Nonacetyl-galacto-arabotriose obtained.	115°~118°C	65.99	+95.44	820.9–835.9	49.56	5.58	35.29
Calculated value.	—	65.70	—	822.36	49.61	5.64	36.50

It was expressed as follows from the calculation:

	Acetic acid %	Arabinose radical free from hydroxyl radical %	Two oxygen bridge %	Galactose radical free from hydroxyl radical %
Nonacetyl-galactoarabotriose obtained.	64.60	19.95	3.89	11.56
Calculated value.	64.88	19.20	3.89	10.92

The polymerisation theory combined with the primary valency in the polysaccharide prevailed, but the association theory associated with the auxiliary valency was seldom considered.

Though in few cases the polysaccharide might be considered to be associated, HAWORTH[37, 38] concluded that it must have been the polymerid from the products of acetolysis and assumed it to be a long chain of sugar radicals combined with the primary valency.

The author then presumed that the galactoaraban was made up of a long chain of sugar radicals, and on this assumption, he tried to make further research on the construction.

The residue which was obtained by the ether extraction of the acetolysis product was divided into three fractions according to the solubility in alcohol and every fraction was purified except the insoluble substance.

	Melt. point	Acetic acid %	C %	H %
Cold alcohol soluble substance	128°~135°C	60.09	49.79	5.84
Hot alcohol soluble substance	140°~146°C	59.17	50.55	5.82
Calculated heptacetyl-galactoaraban		58.92	50.47	4.66

The properties of the two fractions were determined and from these results it may be thought that the fraction soluble in hot alcohol is heptacetyl-galactoaraban and the fraction soluble in cold alcohol is of more decomposed acetyl substances.

The yield of the product of the acetolysis has been frequently discussed in the case of cellulose. The yield of cellobiose acetate was reported to be 37.2 % by OST[39], 43 % by MADSON[40] and reached up to 51 % by HESS[36], but this highest yield is doubted not only in the yield by SPENCER[41], but also in the purity by HAWORTH[38].

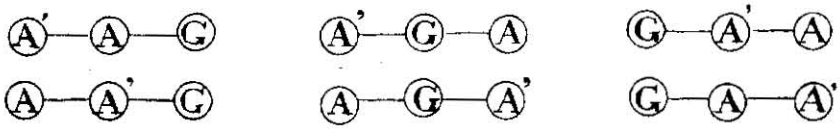
Concerning the yield of acetolysis, it is appreciably smaller than the theoretical value, but it was assumed that this smaller value was caused only by one fraction in the intermediate products of decomposition, and then FRËUDENBERG[42] proposed a formula to calculate the velocity of reaction of acetolysis, after determining the velocity of decomposition of the products.

The author decomposed the galactoaraban by acetolysis and determined the quantity of acetyl radical of the product and found that the yield of crude acetate reached to 63 % of the theoretical yield.

From all these findings and hypotheses the following conclusions might be derived.

1) The galactoaraban from peanuts can be decomposed into the trisaccharide constructed in the ratio of two molecules of arabinose to one molecule of galactose, and therefore the galactoaraban is assumed to be galactoarabotriose combined uniformly.

2) Six arrangements of the components of the trisaccharide, which is constructed of two molecules of arabinose and one molecule of galactose, by definite bond, can be made.

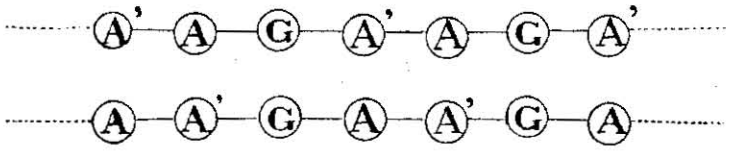


Ⓐ Ⓐ' represent the each arabinose radical.

Ⓖ represents the galactose radical.

3) Galactoaraban may be considered to be formed with galactoarabotriose which are combined with each other to make a chain with definite bond.

4) Then the arrangements of the trisaccharide in the galactoaraban come under the following two cases:



EXPERIMENTS

(1) Acetolysis and its reaction temperature.

The method of reaction at high temperature used by SKRAUP and KÖNIG was applied to the galactoaraban, but the product was too much decomposed to be made acetyl trisaccharide.

When the treatment of WISE and RUSSEL was applied to this galactoaraban, a few products of acetolysis was obtained but the quantity was too little to purify as crystal.

The treatment at low temperature as applied by HAWORTH to methyl xylan, could not decompose the galactoaraban for this purpose.

(2) Acetolysis with several kinds of acid mixture.

Acetolysis were carried out with the solutions that were used by HESS, OST and KNOTH, NISHIDA, and a solution containing 40 g.

of acetic anhydride, 40 g. of glacial acetic acid and 4 g. of sulphuric acid respectively, at room temperature in September.

At the end of the reactions, every solution was cooled and sodium acetate was added to remove the free sulphuric acid and every solution was dropped into ice cooled water to precipitate the acetates.

After filtering, the precipitates were washed, dried and then weighed as crude acetate.

Reagent of the acetolysis

No. of group	Sample	Glacial acetic acid	Acetic anhydride	Sulphuric acid
	g	g	g	g
1	1	3.6	3.6	0.4
2	1	3.5	5.0	0.4
3	1	5.0	4.5	0.4
4	1	4.0	4.0	0.4

Change of the yield caused by time of reaction

No. of group	6 days g	9 days g	12 days g	15 days g	18 days g	20 days g
1	0.86	0.90	1.10	1.02	1.10	0.90
2	0.90	0.91	1.00	1.09	1.10	1.01
3	0.88	0.92	0.98	0.99	1.07	1.10
4	0.89	0.90	1.03	1.07	1.11	1.10

In the above mentioned table, the solutions of No. 1, No. 2, No. 3 corresponded to the treatments of HESS, OST and KNOTH and NISHIDA respectively.

(3) Influence of concentration of sulphuric acid on acetolysis.

By using the different concentrations of sulphuric acid on acetolysis, the yields were determined after 15 days.

The acetolysed reagent contained 4 g. of glacial acetic acid, 4 g. of acetic anhydride and variable quantity of sulphuric acid for 1 g. of sample.

	No. 1	No. 2	No. 3	No. 4
	g	g	g	g
Sulphuric acid used	0.1	0.2	0.4	0.8
Yield	—	—	0.99	0.87

(4) The influence of the time of reaction.

If the free galactoaraban was reacted on with acid mixture in such a way as to take several days to dissolve, it was thought that, on the one hand, the first acetylated surface of the grain dissolved to acid solution and was acetolysed progressively, and, on the other hand, the solid neucleous remained unchanged.

Thus the reaction did not proceed uniformly and therefore the yield of intermediate substance might have been reduced.

To avoid this defect, the free galactoaraban was acetylated preliminarily, and then acetolysed so as to react smoothly.

Galactoaraban was acetylated with acetic anhydride by using methyl sulphate as catalizer, and after it was acetylated, the other acid solution was added to it to make the solution contain 40 g. of acetic anhydride, 40 g. of glacial acetic acid, 2 g. of methyl sulphate, and 3 g. of sulphuric acid for 10 g. of sample. Thus it was acetolysed as follows:

		No. 1	No. 2	No. 3	No. 4	No. 5
Reaction time	days	12	15	15	18	18
Crude acetate obtained	g	9.12	11.23	10.17	11.96	12.13
Yield as acetate	%	91.2	112.3	101.7	119.6	121.3

		No. 6	No. 7	No. 8	No. 9	No. 10
Reaction time	days	18	20	20	23	26
Crude acetate obtained	g	12.07	11.53	10.67	9.87	8.87
Yield as acetate	%	120.7	115.3	106.7	98.7	88.7

The crude acetate which was obtained by the acetolysis of No. 6 contained 58.41 % acetic acid, and the product came to be 63.12 % of the theoretical value.

(5) Acetolysis of the substances insoluble in ether.

The residue which was obtained from the acetolysed product by extraction with ether was acetolysed again.

The results of the second acetolysation are indicated as Nos. 11, 12, 13, 14, and the residue of these product was acetolysed for the third time, and result being shown as No. 15.

		No. 11	No. 12	No. 13	No. 14	No. 15
Residue used	g	10	10	10	10	5
Reaction time	days	10	10	12	15	12
Crude acetate obtained	g	6.91	7.12	7.00	5.78	2.68
Yield	%	69.1	71.2	70.0	57.8	53.6

(6) Treatment of the acetolysis product and its properties.

The crude acetate of the acetolysis was separated into two fractions by extraction with ether.

a) The substance soluble in ether.

The acetate which was obtained by evaporating ether from the ether solution was washed with hot water and purified with bone charcoal and recrystallised with methanol.

	Crude acetate used	Ether ext. subst. obtained	Purified ether extract subst. Acetic acid determined	Melt. point
	g	g	%	
No. 2	11.23	2.3027	66.82	104°~106°C
No. 5	11.96	3.4972	66.48	102°~105°C
No. 11	6.97	3.1018	67.31	103°~108°C
No. 15	2.63	2.6652	67.12	104°~108°C

	Anal. subst.	CO_2 determined	H_2O determined	C found	H found
	g	g	g	%	%
No. 2	0.0911	0.1650	0.0451	49.39	5.54
No. 5	0.0539	0.0983	0.0288	49.73	5.98
No. 11	0.0517	0.0932	0.0279	49.15	6.03
No. 15	0.0821	0.1448	0.0419	49.42	5.71
Calculated value		$[C_6H_{10}O_5(CH_3CO_2)_2]_x$		49.61	5.64

Mol. weight, measured by the freezing point depression, benzol being used as solvent.

$$\text{No. 2} \quad M = \frac{5120 \times 0.4016}{0.185 \times 15 \times 0.879} = 843$$

$$\text{No. 5} \quad M = \frac{5120 \times 0.3507}{0.160 \times 15 \times 0.879} = 851$$

$$\text{No. 11} \quad M = \frac{5120 \times 0.3387}{0.175 \times 15 \times 0.879} = 797$$

$$\text{No. 15} \quad M = \frac{5120 \times 0.3652}{0.175 \times 15 \times 0.879} = 810$$

Specific rotary power was determined, benzol being used as solvent.

$$\text{No. 2} \quad [\alpha]_D^{20^\circ} = \frac{(4.60-0.64) \times 100}{2 \times (0.3054 \times \frac{100}{15})} = 97.25^\circ$$

$$\text{No. 5} \quad [\alpha]_D^{20^\circ} = \frac{(3.62-0.64) \times 100}{2 \times (0.3812 \times \frac{100}{15})} = 97.72^\circ$$

$$\text{No. 11} \quad [\alpha]_\alpha^{20^\circ} = \frac{(4.62-0.64) \times 100}{2 \times (0.5127 \times \frac{100}{15})} = 97.04^\circ$$

$$\text{No. 15} \quad [\alpha]_D^{20^\circ} = \frac{(5.07-0.64) \times 100}{2 \times (0.5762 \times \frac{100}{15})} = 96.10^\circ$$

b) The substance insoluble in ether.

The residue of the ether extraction was divided into three fractions by alcohol extraction; first with cold alcohol, second with hot alcohol, and third, the residue. Each fraction other than the residue was purified.

The substance soluble in cold alcohol.

It melted at 128°~135°C, acetic acid being measured after FRÉUDENBERG 60.09 %.

Anal. subst. 0.0677g. CO_2=0.1236g. H_2O=0.0354g. found C=49.79% H=5.84%

The substance soluble in hot alcohol.

It melted at 140°~146°C, acetic acid being measured after FRÉUDENBERG 59.17 %.

Anal. subst. 0.0812g. CO_2=0.1507g. H_2O=0.0432g. found C=50.62% H=5.71%
Calc. for $\{C_{15}H_{18}O_6(CH_3CO_2)_7\}_X$ C=50.47% H=4.66%

c) Fractional precipitation of the substance extracted with ether.

The purified substance which was extracted with ether was further purified after being dissolved with hot 50 % methanol and separated by fractional precipitation.

First fraction.

This fraction was precipitated at the temperature between 7° and 10°C, and it melted at 108°~113°C.

Mol. weight, measured after freezing point depression, benzol being used as solvent.

$$M=\frac{5120\times0.3389}{0.102\times15\times0.879}=842$$

Second fraction

This fraction was precipitated under 7°C and it melted at 106°~113°C.

Mol. weight measured after depression of freezing point, benzol being used as solvent.

$$M=\frac{5120\times0.3319}{0.153\times15\times0.879}=838$$

Third fraction

This fraction was obtained from the filtrate of the above mentioned fraction.

It melted at 88°~72°C, mol. weight measured after freezing point depression, benzol being used as solvent.

$$M=\frac{5120\times0.3747}{0.235\times15\times0.879}=619$$

d) Nonacetyl-galactoarabotriose.

The difference between the properties of the first fraction and the second fraction were thought to be an error of measurement.

The second fraction was recrystallised with methanol containing a small quantity of gracial acetic acid, and then a white crystal was separated.

Mol. weight measured after freezing point depression, benzol being used as solvent.

$$M = \frac{5120 \times 0.4164}{0.197 \times 15 \times 0.819} = 821$$

$$M = \frac{5120 \times 0.3767}{0.157 \times 15 \times 0.879} = 836$$

Anal. subst.	0.0801g.	CO_2=0.1456g.	H_2O=0.0408g.	founnd	C=49.67% H=5.70%
	0.1117g.	0.2030g.	0.0557g.		C=49.56% H=5.58%
Calc. for		$C_{16}H_{18}O_5(CH_3CO_2)_2$			C=49.61% H=5.64%

Acetic acid was measured after FREUDENBERG			65.99 %
Calc. for	$C_{16}H_{18}O_5(CH_3CO_2)_2$		65.70 %
Arabinose was measured as furfurol		35.12	34.45 %

Galactose was detected as mucic acid, it melted at 212°C and it was transformed into calcium salt.

Calcium oxide was measured		CaO=19.99 %
Calc. for	$CaC_6H_4O_8 + 1\frac{1}{2}H_2O$	CaO=20.93 %

CHAPTER III

Methylation of Galactoaraban and its Decomposition Products.

As already described, galactoaraban could be thought to be a long chain made of galactoarabotrioses, which was the trisaccharide constructed of one molecule of galactose and two molecules of arabinose.

But it was not known by which bonds of the sugar radicals they combine with each other and whether every sugar belongs to pyranose or furanose.

Then the author tried to methylate the galactoaraban and to decompose the fully methylated derivative into each component, and also tried to investigate the connected point of every sugar radical after the decomposed product was distillated to several fractions.

Concerning the methylation of polysaccharide, cellulose was first methylated with methyl iodide and silver oxide by DEHNHAM and WOODHAUSE, then methyl-sulphate and alkali were used to methylate the polysaccharides such as cellulose[44], starch[45], and inulin[46].

Though these investigators endeavoured to get fully methylated products, the methyl derivatives still contained poor methoxyl, moreover the purifications of the methyl derivatives were imperfect, but finally URBAN[47] purified the methylated polysaccharide as chloroform solution.

It was hard to replace the total hydroxyl radicals of polysaccharide with methoxyl radicals, and the methyl products always contained less methoxyl than the calculated value.

Afterward HAWORTH[48] methylated the acetyl cellulose with one trial of methylation into trimethyl cellulose containing the theoretical methoxyl value.

Attempts were made to methylate many polysaccharides by this treatment and the products were reported to have much methoxyl content, but BELL[49] doubted if the methyl cellulose might be decomposed because the decomposition usually accompanied the acetylation.

It is hard to think that the polysaccharide can be obtained unchanged from the natural state, and it is not clear if all of the polysaccharides decomposed with one trial of acetylation.

If a polysaccharide could not be methylated completely on account of the combination of basic metals or phosphoric acid of ashy substances, it would have been possible to purify by acetylation and the purified acetate could then be methylated easily.

The galactoaraban prepared by the author might be changed more or less from that of the natural state by the treatments of alkali extraction and acid purification.

On one hand the galactoaraban was methylated 12 times repeatedly by common treatment, and on the other hand the acetyl-galactoaraban also was methylated three times according to HAWORTH's method, and both of the methyl derivatives almost corresponded to the heptamethyl-galactoaraban in so far as the methoxyl content was concerned.

The mother substance might be concluded to be polymerid for the methoxyl content of the methyl galactoaraban.

Concerning the yield of the methyl product, it could not reach the theoretical value, and further, the substances which were investigated were restricted to the substances which had been prepared.

On one hand 30 g. of galactoaraban was methylated into 6 g.

of heptamethyl galactoaraban and on the other hand the acetyl product obtained from the acetylation of 30 g. of galactoaraban was also methylated and 11 g. of heptamethyl-galactoaraban was prepared.

Though it was reported that the methyl product of arabinose was hydrolised with the solution containing 5 % or 8 % of hydrochloric acid, recently decomposition has been noticed when treated with dilute hydrochloric acid[50].

BOT and HIRST[51] found that the methyl derivatives of xylose and arabinose were decomposed almost to furfurol when they were heated with 12 % hydrochloric acid, and the pentose derivatives were also decomposed to furfurol with 3 % dilute hydrochloric acid, so the author gave consideration to this point and selected the following treatment.

Heptamethyl-galactoaraban was heated with absolute methanol containing 1 % HCl in the sealed tube to make a cleavage product, and the reduction of the quantity was found to be small, and the cleavage products were separated by vacuum distillation.

After distillating the methyl derivative to four fractions, each fraction was investigated.

If the treatments proceeded to completion on these conditions, the produced methyl monosaccharides would come to contain one free hydroxyl radical when they were connected with the primary carbon atom in the polysaccharide.

If the primary carbon atom was occupied with methoxyl radical completely by this treatment, it could be detected that it was the derivative of arabinose or galactose by the determination of methoxyl content only.

Though the methoxyl content of heptamethyl-galactoaraban approached the theoretical value, it was always smaller than that of the theoretical value, and it was doubtful whether the simultaneous decomposition and methylation was carried out completely and also whether the arabinose derivatives were not decomposed further by the treatment of hydrochloric acid, and it was hard to think that they were separated completely with one distillation only.

Considering the above mentioned matters, the author deter-

mined the substances after investigating the properties of every fraction.

	Yield g	$[\alpha]_D$	N_D	Methoxyl %
1st fraction	0.7825	98.72	1.4450	45.08
2nd fraction	3.8912	67.51	1.4493	44.75
3rd fraction	4.3107	63.04	1.4508	44.90
4th fraction	4.2105	100.10	1.4781	41.06

After methylating each of the fractions in order to replace the free hydroxyl radical with methoxyl one perfectly, they were tested by methoxyl content to judge whether they were tetramethyl-methylgalactosid or trimethyl-methylarabinosid, and the results are as follows:

	2nd fraction	3rd fraction	4th fraction
Methoxyl determined %	60.00	60.61	61.08
$[\alpha]_D^{19°}$ (alcohol solution)	+187.15	+185.63	+151.44
Calc. for	$C_5H_8O(OCH_3)_4$	60.20 %	
Calc. for	$C_6H_7O(OCH_3)_5$	61.70 %	

The problems to be investigated are thought to be two, one of which might be to determine the positions of carbon atoms which are connected to other sugar radicals, and the other to determine to whether the sugar in each fraction belonged to furanose or to pyranose.

Then the author tried to examine the properties of each carbon atom in the sugar radicals as follows:

1) The methoxyl radical at the first carbon atom.

The methoxyl radical at the first carbon atom may be hydrolised off by heating with 5 % hydrochloric acid, but arabinose derivative may be decomposed to furfurol on this condition, and then every fraction was hydrolised with 2 % hydrochloric acid on the water bath and clarified.

They were discovered to be as follows:

	1st fraction	2nd fraction	3rd fraction	4th fraction
Yield %	36.64	23.33	23.81	74.20
Methoxyl measured %	41.71	33.70	33.25	39.61

All these findings might be explained by assuming that the first carbon atom was to be combined with methoxyl radical in each fraction.

2) Methoxyl radical at the second carbon atom.

Though the above hydrolised syrups of every fraction reduced FEHLING's solution, they did not build the osazon at all. These phenomena might be explained by assuming that the hydroxyl radicals of the second carbon atoms in all the fractions are replaced with methoxyl radical.

3) CH_2OH radical.

The author tried in vain to get the trityl compounds with the syrups of all the fractions, after treating with triphenyl chlormethan respectively.

Therefore every syrup of all the fractions may be ascertained to contain no CH_2OH radical.

4) The fifth carbon atom in the arabinose.

In order to show that the derivatives of arabinose to be pyranoses, the fully methylated arabinoses of the second and the third fractions, after being oxidised, were determined to have been transformed into trimethyl glutaric acid, and the trimethyl glutaric acid were also ascertained with the crystals of diamid.

Then the arabinoses of the second and the third fractions were found to be pyranoses and the author was convinced that all the judgements hitherto were true for the crystal, for arabinose derivatives were not isolated as crystals except diphenyl hydrazon.

5) The fifth carbon atom in the galactose.

For the purpose of obtaining tetramethyl-galactopyranose-anilid, tetramethyl-methylgalactosid was saponified to tetramethyl-galactose and afterwards it was treated with anilin, anilid was separated as crystal and then it was found that the properties of the crystal corresponded to that of the 2:3:6-tetramethyl-d-galatopyranose-anilid.

This finding might be explained by assuming that the hydroxyl radical of the fifth carbon atom was used to make oxide ring in the fourth fraction.

6) The sixth carbon atom in the galactose.

Though it was suggested by 3) that the hydroxyl radical at the sixth carbon atom in the galactose might be methylated, it was demonstrated with dimethyl mucic acid obtained by the oxidation.

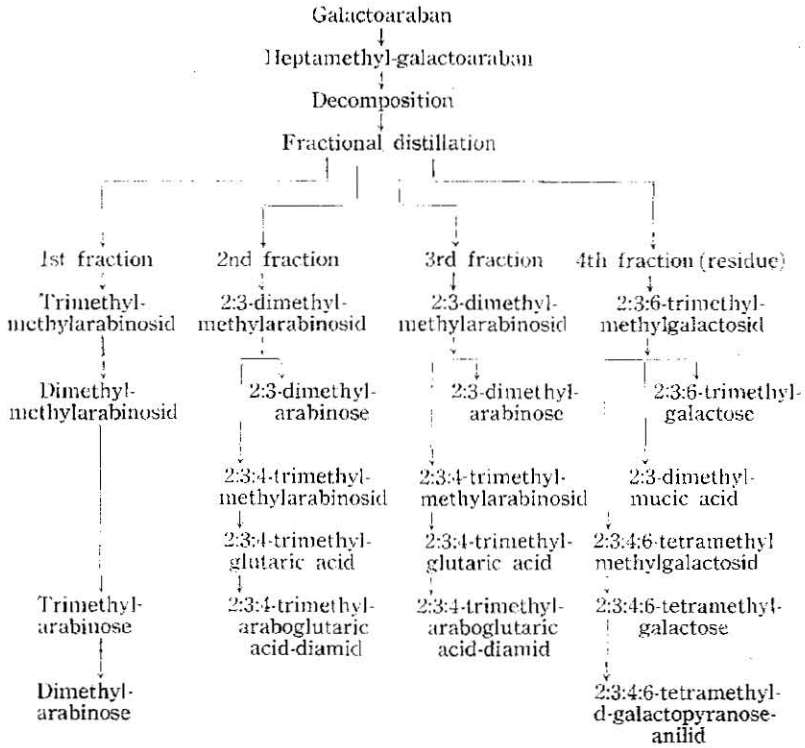
This phenomenon might be explained as that the hydroxyl radicals at the first and the sixth carbon atom are replaced by methoxyl radicals.

7) The methoxyl radical in the third and the fourth carbon atom.

It was impossible to ascertain the position of methoxyl radical combined with either the 3rd carbon atom or the 4th carbon atom in the methyl monosaccharide.

Then they were assumed to be combined with the 4th carbon atom as usual.

All the courses of the investigation are graphed as follows:



The first fraction was assumed to be the mixture of trimethyl-methylarabinosid and dimethyl-methylarabinosid, and it seemed that this fraction played no important part in this investigation on account of its small quantity.

The second fraction and the third fraction were detected to be 2:3-dimethyl-methylarabinosid and the residual fraction was considered to be constructed mainly of 2:3:6-trimethyl-methyl-galactose.

If we considered the sugar radicals in the heptamethylgalacto-araban by the scission products which were decomposed under the above mentioned condition, the connecting aldehyde radicals would be not only hydrolised from other sugars, but also methylated simultenously, and the sugar radicals must have been 2:3-dimethyl-arabinose and 2:3:6-trimethylgalactose and they became to combine with the first carbon atom and the fourth carbon atom of other sugar with oxygen bridge in the mother substance.

In the galactoaraban, the molecular ratio of arabinose and galactose was 2:1, but, in the methylated scission product, the arabinose could not be detected to be two kinds of the derivative.

Therefore they must be connected as same radical.

The large quantity of methoxyl content in the first fraction is supposed to mean that the galactoaraban is the chain formulae, so the last sugar radical has much methoxyl content.

EXPERIMENTS

(1) Methylation.

a) After dissolving 30 g. of galactoaraban in 400 c.c. of 30 % caustic soda solution, methylsulphate was added drop by drop to the solution which was always mixing, to methylate smoothly, and the temperature of the solution was guarded that it should not rise.

This treatment was continued until a neutral reaction was obtained with Lacmus paper, and after being left standing for several hours and then 200 c.c. of 40 % caustic soda solution being added to the solution, the solution was methylated again.

After this methylation was finished, the solution was heated and filtered while hot and the precipitate was washed with hot water repeatedly.

The methoxyl content of the precipitate was determined after it was dried and powdered.

Anal. subst.	5.51 mg.	AgJ=14.20. mg.	Found OCH_3=33.8 %
	4.15 mg.	AgJ=11.00 mg.	OCH_3=33.2 %

After repeating the methylation twice;

Anal. subst.	5.66 mg.	AgJ=15.75 mg.	Found OCH_3=36.8 %
	5.93 mg.	AgJ=16.47 mg.	OCH_3=36.7 %

Again repeated the methylation 4 times;

Anal. subst.	6.480 mg.	AgJ=19.025 mg.	Found OCH_3=38.8 %
	6.075 mg.	AgJ=18.225 mg.	OCH_3=39.2 %

The methylation was repeated twice, and the methyl galactoaraban was purified as chloroform solution;

Anal. subst.	3.725 mg.	AgJ=11.405 mg.	Found OCH_3=40.45 %
	3.123 mg.	AgJ= 9.580 mg.	OCH_3=40.52 %
Calc. for	$[C_5O_5H_{10}(OCH_3)_3OH]_n$		OCH_3=36.6 %
Calc. for	$[C_5O_5H_{10}(OCH_3)_4]_n$		OCH_3=41.5 %

b) 30 g. of galactoaraban was dissolved in 400 c.c. of 0.5 % caustic soda solution and neutralised with acetic acid, 1.5 *l.* of alcohol was added to precipitate the galactoaraban and the precipitate was filtered, washed with absolute alcohol and ether, the syrup was evaporated to a semidried condition, and then it was acetylated with the mixed solution which contained 200 c.c. of acetic anhydride and 12 c.c. of methylsulphate as catalizer.

The solution was poured into water drop by drop to precipitate the acetate and the precipitate was separated from the solution.

The acetate was methylated in aceton solution with 300 c.c. of methylsulphate and a corresponding quantity of caustic soda solution, according to HAWORTH's method.

The aceton was evaporated from the reaction mixture, the syrup was made as water solution, and methyl-galactoaraban was obtained from it by the usual treatment and purified as chloroform solution.

Anal. subst.	4.48 mg.	AgJ=14.875 mg.	OCH_3=40.53 %
	3.58 mg.	AgJ=11.010 mg.	OCH_3=40.63 %

Again repeated the same methylation.

Anal. subst.	5.342 mg.	AgJ=16.689 mg.	OCH_3=41.41 %
	4.390 mg.	AgJ=13.725 mg.	OCH_3=41.30 %
Calc. for	$[C_6O_5H_{10}(OCH_3)_5OH]_n$		OCH_3=36.6 %
	$[C_6O_5H_{10}(OCH_3)_7]_n$		OCH_3=41.5 %

Specific rotation was measured as chloroform solution.

$$[\alpha]_D^{23^\circ} = \frac{82.78 \times 100}{1 \times 0.1426} = +579.94^\circ$$

Anal. subst.	3.885 mg.	CO_2=7.3910 mg.	H_2O=2.719 mg.	C=52.25 % H=7.89 %
Anal. subst.	2.992 mg.	CO_2=5.685 mg.	H_2O=2.096 mg.	C=51.82 % H=6.66 %
Calc. for	$[C_6O_5H_{10}(OCH_3)_7]_n$			C=52.63 % H=7.71 %

(2) Decomposition of heptametyl-galactoaraban.

16.5 g. of heptamethyl-galactoaraban was dissolved in 250 c.c. of absolute methanol containing 1 % hydrochloric acid and was decomposed in a sealed tube at 100°C for 50 hours and then at 130°C for 50 hours.

The decomposed solution was neutralised with silver carbonate and was bleached with bone charcoal, evaporated to syrup and then the syrup was extracted with ether, and after the ether was evporated at 40°C under reduced pressure the syrup was dried at 100°C in vacuum on phosphor pentaoxide for 24 hours, and was separated into four fractions by vacuum distillation.

	Pressure	Boiling point	Yield g	Colour
1st fraction	4 mm.	124°~128°C	0.7825	Colourless
2nd fraction	6 mm.	132°~138°C	3.8912	Pale yellow
3rd fraction	6 mm.	133°~141°C	4.3107	Yellow
4th fraction (residue)			4.2105	Yellowish

(3) Decomposition product.

a) Properties of the fractions.

First fraction;

Anal. subst.	5.446 mg.	AgJ=18.615 mg.	OCH_3=45.15 %
	3.775 mg.	AgJ=12.860 mg.	OCH_3=45.00 %
Calc. for	$C_5H_8O(OCH_3)_4$		OCH_3=60.20 %
	$C_5H_7O_2(OCH_3)_3$		OCH_3=48.44 %

$$N_D^{20^\circ} = 1.4450$$

Specific rotation was determined with alcoholic solution.

$$[\alpha]_D^{20^\circ} = \frac{1.39 \times 100}{1 \times 1.408} = +98.72^\circ$$

Second fraction;

Anal. subst.	3.340 mg.	AgJ=11.295 mg.	Found OCH_3=44.67 %
	3.655 mg.	AgJ=12.405 mg.	OCH_3=44.83 %
Calc. for	$C_6H_7O_2(OCH_3)_3$		OCH_3=48.44 %

$$N_D^{20^\circ} = 1.4493$$

Specific rotation was determined with alcoholic solution.

$$[\alpha]_D^{20^\circ} = \frac{1.44 \times 100}{1 \times 2.133} = +67.51^\circ$$

Third fraction;

Anal. subst.	4.655 mg.	AgJ=15.842 mg.	Found OCH_3=44.96 %
	5.566 mg.	AgJ=18.890 mg.	OCH_3=44.83 %
Calc. for	$C_6H_7O_2(OCH_3)_3$		OCH_3=48.44 %

$$N_D^{20^\circ} = 1.4508$$

Specific rotation was determined with alcoholic solution.

$$[\alpha]_D^{20^\circ} = \frac{1.75 \times 100}{1 \times 2.776} = +63.04^\circ$$

Fourth fraction (residue);

Anal. subst.	4.765 mg.	AgJ=14.860 mg.	Found OCH_3=41.20 %
	4.420 mg.	AgJ=13.690 mg.	OCH_3=40.92 %
Calc. for	$C_6H_9O_2(OCH_3)_1$		OCH_3=52.54 %

$$N_D^{20^\circ} = 1.4781$$

Specific rotation was determined with alcoholic solution.

$$[\alpha]_D^{20^\circ} = \frac{2.04 \times 100}{1 \times 2.038} = 100.10^\circ$$

b) Saponification of the fractions.

All the fractions were saponified with 2% hydrochloric acid on the water bath and neutralised with barium carbonate and then dried in vacuum.

After they had been purified as ether solution, each of the solutions was evaporated to syrup.

	Sample g	Time of hydrolysis hrs.	Yield g	Saponified soluton
1st fraction	0.5485	7	0.2012	Yellowish solution.
2nd fraction	1.6823	5	0.3925	Dark solution with black ppt.
3rd fraction	1.8147	5	0.4321	Dark solution with black ppt.
4th fraction	1.6523	8	1.2260	Faintly coloured solution.

The second and the third fractions seemed to be decomposed on saponification by the facts that the black ppt. occurred and the yield was reduced remarkably.

In the case of the first fraction, though it seemed to be pentose, it was not decomposed much. This phenomenon corresponded to the report according to which it was difficult to decompose to furfurol when the hydroxyls were replaced entirely with methoxyl radical.

These syrups were dried and methoxyl content were determined as follows:

First fraction;

Anal. subst.	2.970 mg.	AgJ = 9.430 mg.	Found $OCH_3 = 41.94$ %
	4.505 mg.	AgJ = 14.180 mg.	$OCH_3 = 41.58$ %
Calc. for	$C_5H_7O_2(OCH_3)_3$		$OCH_3 = 45.59$ %
	$C_5H_8O_3(OCH_3)_2$		$OCH_3 = 32.98$ %

Second fraction;

Anal. subst.	3.475 mg.	AgJ = 9.120 mg.	Found $OCH_3 = 33.93$ %
	3.555 mg.	AgJ = 9.005 mg.	$OCH_3 = 33.46$ %
Calc. for	$C_5H_8O_3(OCH_3)_2$		$OCH_3 = 32.98$ %

Third fraction;

Anal. subst.	4.125 mg.	AgJ = 10.365 mg.	Found OCH_3 = 33.19 %
	3.540 mg.	AgJ = 8.925 mg.	OCH_3 = 33.30 %
Calc. for	$C_5H_5O_3(OCH_3)_2$		OCH_3 = 32.98 %

Fourth fraction (residue);

Anal. subst.	3.575 mg.	AgJ = 10.825 mg.	Found OCH_3 = 40.00 %
	4.495 mg.	AgJ = 10.420 mg.	OCH_3 = 39.38 %
Calc. for	$C_5H_5O_3(OCH_3)_3$		OCH_3 = 41.90 %

The first carbon atom of every fraction might be explained to have been in free condition by the reactions that they reduced FEHLING's solution and the reduction of methoxyl quantity after saponifications.

Then it may be concluded that the first saponified fraction is a mixture of trimethyl-arabinose and dimethyl-arabinose, the second and the third saponified fractions are dimethyl-arabinose and the saponified residue is constructed of trimethyl-galactose mainly.

c) Osazon.

The syntheses of osazons were tried but in vain. These phenomena may be explained by supposing that the hydroxyl radicals of the second carbon atoms in the all fractions were replaced with methoxyl radicals.

d) Methylation of every fraction.

Methylations of the second fraction, the third fraction and the residue were attempted with methyl iodide and silver oxide respectively.

Second fraction;

1.2165 g. of the second fraction was methylated and the yield as a fully methylated derivative was 1.0073 g.

Anal. subst.	4.080 mg.	AgJ = 18.570 mg.	Found OCH_3 = 60.12 %
	3.390 mg.	AgJ = 15.355 mg.	OCH_3 = 59.88 %
Calc. for	$C_5H_6O(OCH_3)_4$		OCH_3 = 60.20 %

Specific rotation was measured with alcoholic solution.

$$[\alpha]_D^{19^\circ} = \frac{2.68 \times 100}{1 \times 1.432} = +187.15^\circ$$

Third fraction;

1.7217 g. of the third fraction was methylated and the yield as a fully methylated derivative was 1.2152 g.

Anal. subst.	4.460 mg.	AgJ=20.510 mg.	Found OCH_3=60.75 %
	4.125 mg.	AgJ=18.880 mg.	OCH_3=60.46 %
Calc. for	$C_5H_6O(OCH_3)_4$		OCH_3=60.20 %

Specific rotation was measured with alcoholic solution.

$$[\alpha]_D^{19^\circ} = \frac{1.99 \times 100}{1 \times 1.072} = +185.62^\circ$$

Fourth fraction (residue);

1.8994 g. of the fourth fraction was methylated and the yield as fully methylated derivative was 1.3725 g.

Anal. subst.	4.420 mg.	AgJ=20.480 mg.	Found OCH_3=61.21 %
	3.515 mg.	AgJ=16.215 mg.	OCH_3=60.94 %
Calc. for	$C_6H_7O(OCH_3)_5$		OCH_3=61.76 %

Specific rotation was measured with alcoholic solution.

$$[\alpha]_D^{19^\circ} = \frac{1.84 \times 100}{1 \times 1.215} = +151.44^\circ$$

From these results, it was recognized that the fractional distillation was not carried out completely, but it can be concluded that the second fraction and the third fraction may be 2:3:4-trimethyl-methylarabinosid and the residue may be 2:3:4-trimethyl-methyl-galactosid.

e) The reaction with triphenyl chlormethan.

After evaporating each syrup, 0.3 g. of the second fraction, 0.3 g. of the third fraction and 0.35 g. of the residue were dissolved in 10 c.c. of pyridin respectively, and 1.5 g. of triphenyl chlormethan were added to each of them, and they were left standing at room temperature, then diluted with water, cooled and filtered. The solution were decanted respectively into cold water containing ice, and every precipitate was filtered and purified with aceton.

The melting point of these precipitates, which were recrystallised with aceton, corresponded to that of triphenylchlormethan and showed no depression of melting point on admixture with triphenyl-chlormethan which was used.

The syrups of all the fractions were not combined with triphenylchlormethan into trityl compound, and it may be concluded that there were no free CH_2OH radicals in these galactose and arabinose derivatives.

f) Oxidation of trimethy-l-methylarabinosid and dimethylmethylgalactosid.

The trimethyl-methylarabinosid obtained from the second fraction and the third fraction, and trimethyl-methylgalactosid of the fourth fraction, were oxidised respectively with nitric acid of S. G. 1.20 on the water bath heating gradually to reach 80°C for the first three hours, then at 90°C for one hour, and at last at 60°C for three hours, so as not to be oxidised intensively.

For the purpose of evaporating off the nitric acid from these oxidised solutions, the oxidised solutions were evaporated at 40°C under reduced pressure repeatedly after adding water until the syrup gave no nitric acid reaction, and at last alcohol was added to them and again evaporated. After these treatments, yellowish syrups were obtained from all the fractions.

Second fraction;

0.8971 g. of yellowish syrup was obtained from the 0.9561 g. of the second methylated fraction which was oxidised with 15 c.c. of nitric acid and was found to be as follows:

Anal. subst.	5.650 mg.	AgJ=17.810 mg.	Found OCH_3=41.64 %
	3.410 mg.	AgJ=10.875 mg.	OCH_3=42.13 %
Calc. for	$C_3H_2(OCH_3)_3(COOH)_2$		OCH_3=41.90 %

The acidity of the syrup was determined as alcoholic solution, calculated by the data which was obtained by back titration with N/10 sulphuric acid solution after adding N/10 caustic soda solution.

Anal. subst.	0.1126 g.	Consumed 9.4 c.c.	N/10 caustic soda solution.
Calc. for	$C_3H_5(OCH_3)_3(COOH)_2$	10.14 c.c.	N/10 caustic soda solution.

Third fraction;

Trimethyl-methylarabinosid 1.1261 g. of the third fraction was oxidised and 0.9611 g. of oxide was obtained.

Anal. subst.	3.885 mg.	AgJ=12.080 mg.	Found OCH_3=40.87 %
	3.630 mg.	AgJ=11.195 mg.	OCH_3=40.74 %
Calc. for	$C_3H_3(OCH_3)_3(COOH)_2$		OCH_3=41.90 %

The acidity of the syrup was determined with alcoholic solution, and calculated by the data which was obtained by back titration with N/10 sulphuric acid solution after adding N/10 caustic soda solution.

Anal. subst.	0.1228 g.	Consumed	10.7 c.c. N/10 caustic soda solution.
Calc. for	$C_3H_5(OCH_3)_3(COOH)_2$		11.05 c.c. N/10 caustic soda solution.

Fourth fraction (residue);

0.8011 g. of trimethyl-methylgalactosid was oxidised and 0.6012 g. of oxide was obtained.

Anal. subst.	4.025 mg.	AgJ=8.560 mg.	OCH_3=28.09 %
	4.400 mg.	AgJ=9.435 mg.	OCH_3=28.33 %
Calc. for	$C_4H_6O_2(OCH_3)_2(COOH)_2$		OCH_3=29.82 %

The acidity of the syrup was determined with alcoholic solution, and calculated by the data which was obtained by back titration with N/10 sulphuric acid solution after adding N/10 caustic soda solution.

Anal. subst.	0.1768 g.	Consumed	16.00 c.c. N/10 caustic soda solution.
Calc. for	$C_4H_6O_2(OCH_3)_2(COOH)_2$		16.99 c.c. N/10 caustic soda solution.

None of them seemed to be composed of a single substance, but it may be concluded that the main substances of the oxidised products obtained from the second fraction and the third fraction were the lactons of trimethoxyl glutaric acid, and that of the fourth fraction were the lacton of dimethyl mucic acid.

g) Tetramethyl-galactose anilid.[53]

Tetramethyl-methylgalactosid was saponified with 5 % hydrochloric acid on the water bath for five hours and was neutralised with barium carbonate, and after evaporating the solution to syrup, it was extracted with absolute alcohol to purify it.

Anilin was added to the alcoholic extract and was heated at 96°C for three hours, and its volume was reduced by vacuum evaporation.

The anilid was crystallised in the shape of a white needle, and after being recrystallised with hot alcohol, it melted at 93°C, and showed no depression of melting point on admixture with an authentic specimen of tetramethyl-d-galactopyranose-anilid prepared from lactose

Anal. subst.	5.080 mg.	AgJ=15.210 mg.	Found	OCH_3=39.54 %
	3.165 mg.	AgJ= 9.270 mg.		OCH_3=38.75 %
Calc. for	$C_6H_7O(OCH_3)_4NHC_6H_5$			OCH_3=39.88 %

Anal. subst.	3.56 mg.	N_2=0.150 c.c. (20°C 772 mm.)	N=4.89 %
Calc. for	$C_6H_7O(OCH_3)_4NHC_6H_5$		N=5.50 %

Anal. subst.	3.445mg. H_2O=2.578mg.	CO_2=7.620mg.	C=60.32% H=8.36%
Calc. for	$C_6H_7O(OCH_3)_4NHC_6H_5$		C=60.70% H=8.10%

h) 2 : 3 : 4-trimethyl-glutaric-acid-diamid.[52]

The glutaric acids obtained from the second and the third fractions were treated respectively to obtain the crystals of diamid in order to ascertain whether they were araboglutaric acids. Then each of the oxidation products was methylated with absolute methanol containing 3 % hydrochloric acid in a hot water bath for six hours, neutralised with silver carbonate, and purified with chloroform to get trimethyl-araboglutaric-acid-dimethyl ester.

These syrups were each dissolved in absolute methanol saturated with dry ammonia gas and were left standing in the ice box for four days and then diamids were crystallised out from both of the solutions several days later.

They were melted at 230°C after being recrystallised with methanol twice, and the properties corresponded to that of the 2 : 3 : 4-trimethyl-araboglutaric-acid-diamid.

Anal. subst.	3.540 mg.	AgJ=13.08 mg.	Found	OCH_3=48.81 %
	5.250 mg.	AgJ=19.63 mg.		OCH_3=49.11 %
Calc. for	$C_6H_3(OCH_3)_3(NH_2)_2$			OCH_3=49.47 %

Anal. subst.	2.35 mg.	N_2=0.307 c.c. (21°C 772 mm.)	N=15.41 %
Calc. for	$C_6H_3(OCH_3)_3(NH_2)_2$		N=14.89 %

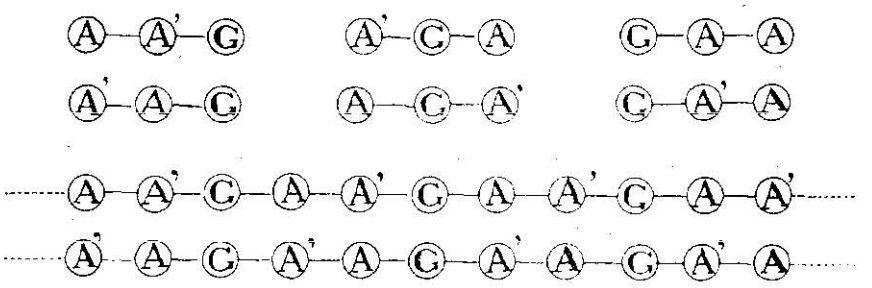
CONCLUSION

The author ascertained that the galactoaraban which was prepared from the seeds of the peanut is constructed in the ratio of two molecules of arabinose to one molecule of galactose though a small part of it is oxidised (in chapter I).

The galactoaraban was decomposed by acetolysis into the derivatives of galactoarabotriose which is of the same componental

ratio as the mother substances except for the acetyl radical, which is assumed to be polymerised galactoarabotriose, with the result that the acetyl higher polymerid obtained, contained less acetyl radical corresponding to the heptacetyl-galactoaraban (in chapter II).

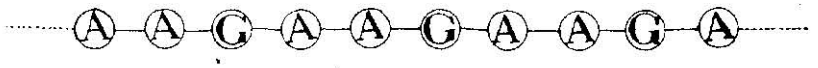
As already described, there are six cases of association in the arrangement of the sugar radicals in the trisaccharide, but they come under two cases when connected as shown below.



In chapter III, it is described that the galactoaraban was methylated to heptamethyl-galactoaraban, and reconsidered to be the polymerised substance by the methoxyl content.

After it was decomposed to componental sugars the scission product of the methyl derivative was investigated and the heptamethyl-galactoaraban was found to be constructed of 2:3:4-trimethyl-d-galactopyranose and 2:3-dimethyl-l-arabopyranose, and then all the components came to be combined with the first and the fourth carbon atoms.

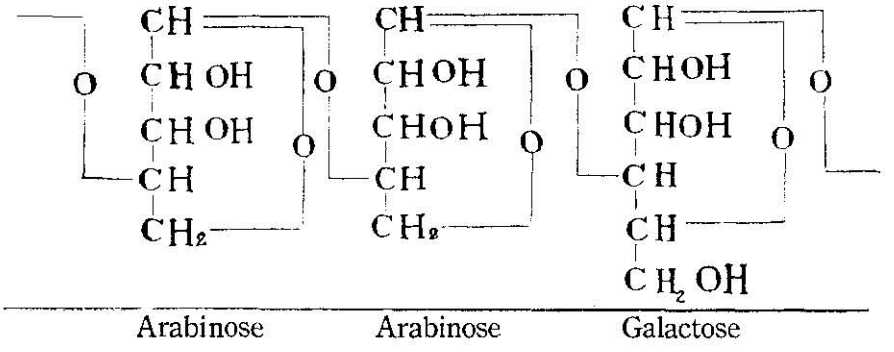
Moreover the two kinds of arabinose radicals supposed were proved to be the same substance, so the above two arrangements of the galactoaraban became the same arrangement as follows:

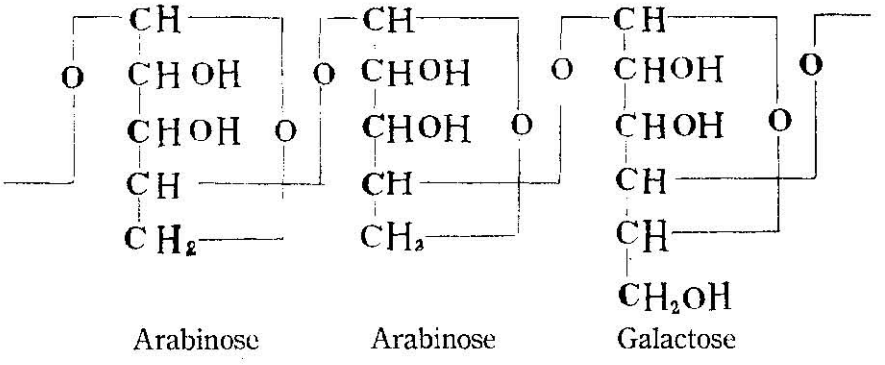


2:3:6-trimethyl-d-galactopyranose and 2:3-dimethyl-l-arabopyranose were inserted to this arrangement so as to combine with the first carbon atom of the sugar to the fourth carbon atom of other sugar with oxygen bridge as connected usually in the polysaccharide.

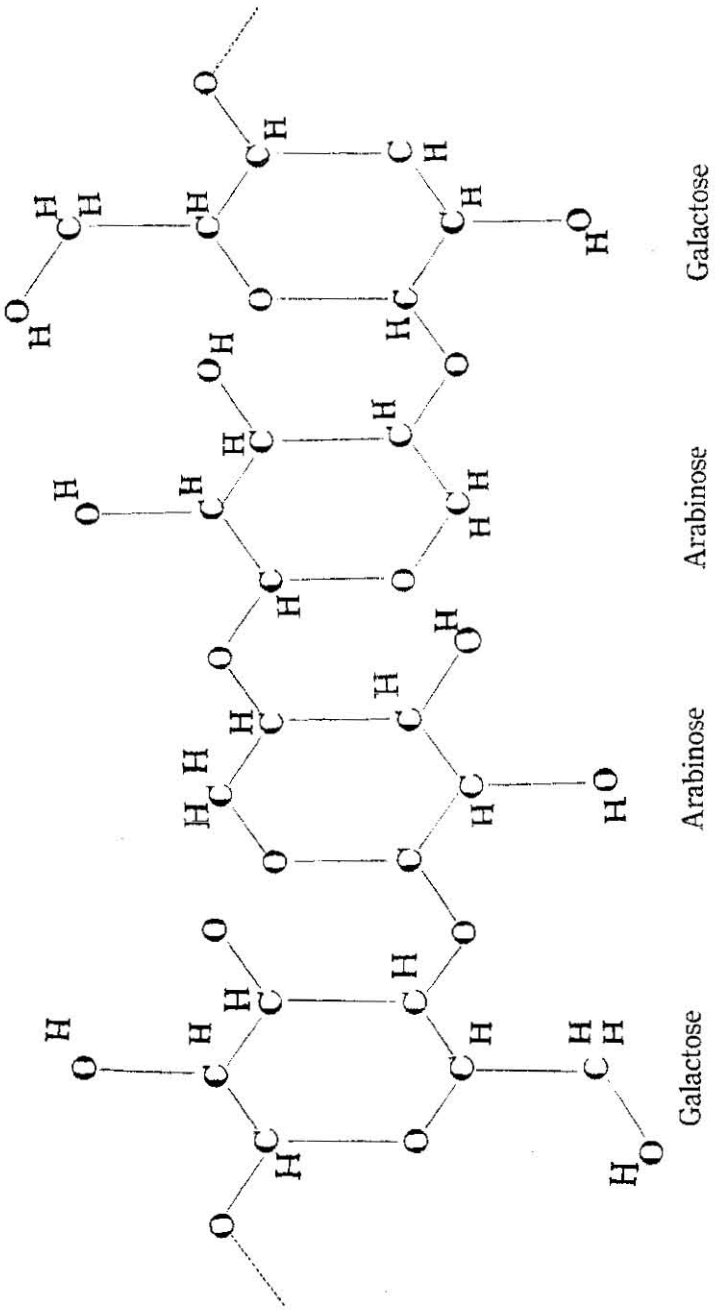
Of these connections there are two cases to make the formulae

which were connected as 1-4, 1-4, 1-4, and 4-1, 4-1, 4-1, in the plain formulae as follows:





But these two formulae become the same formula when arranged on the stereochemical standpoint as follows:


Galactose
Arabinose
Arabinose
Galactose

The author recommends the constitutional formula of galactoaraban above given which has been formed from his experiments as well as the suppositions made by other authors who partook to the composition of the common formulae of polysaccharide.

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