CXL. CONSTITUTION OF CERTAIN NUTSHELLS.

I. THE SEED-COAT OF *BERTOLLETIA EXCELSA* (BRAZIL-NUT).

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(Received April 6th, 1936.)

SURVEY of the literature reveals that little or no work has been done on the constitution of nutshells. In fact a quantitative determination of the main constituents of peanut hull (*Arachis hypogea*) is the only investigation recorded for this type of tissue [Emley, 1928]. The following table gives the results of Emley's analysis:

				%
Water-soluble, cold	...	...	...	8.5
Water-soluble, hot	...	...	...	9.8
1% alkali-soluble, cold	...	...	...	21.5
Ether-soluble	...	...	...	0.5
Alcohol-soluble	...	...	...	3.2
Pentosans	...	...	...	18.5
Lignin	...	...	...	33.4
Cellulose	...	...	...	44.9
Pentosans in pulp	...	...	...	13.5

One point stands out clearly, that the pentosan content of the shell is high and in particular that part of the pentosan associated with the cellulose is large compared with that from the celluloses of other lignified tissues.

These figures suggested that other "nut" shells might repay investigation by bringing to light general characteristics of this group of tissues. The results obtained from the first of these investigations, on the Brazil-nutshell, are recorded in this paper.

Preliminary analysis.

The shells carefully freed from all traces of the oily endosperm were ground to a fine powder. Very fine grinding was essential in order that the cellulose might be obtained from the lignocellulose complex without considerable oxidation taking place. No attempt was made to separate the layers which made up the shell. The following table gives an average of the analyses of the samples taken from the bulk:

				%
Benzene-soluble	...	...	...	4.69
Alcohol-soluble	...	...	...	1.06
Water-soluble	...	...	...	1.64
5% NaOH (cold)-soluble	...	...	...	16.00
Lignin	...	...	...	53.25
Cellulose	...	...	...	25.47
				102.11

Preparation of fractions.

The mass of the powdered shell was treated by the methods used in the quantitative analysis. The benzene, alcohol and water extracts were prepared with an extractor working on the Soxhlet principle and holding about 1000 g.

at each filling. These three fractions were obtained by evaporation of the solvents under reduced pressure. The NaOH-soluble fraction was obtained by treating the residue from the water extraction with 5% NaOH in the proportion of 100 g. of the residue to 1 l. of solution for 24 hours at room temperature with constant stirring. The residue was filtered off and the filtrate acidified with a slight excess of acetic acid. The bulky, fawn-coloured precipitate was centrifuged. This precipitate designated hemicellulose A was washed on the centrifuge with increasing concentrations of alcohol, finally with absolute alcohol and ether and dried in a vacuum desiccator. When the filtrate was neutralised with solid sodium carbonate and gently warmed a second precipitate came down. This precipitate, hemicellulose A 1, was separated, washed on the centrifuge and dried as before. The filtrate was dialysed for 8 days; it was then concentrated *in vacuo* to low bulk, filtered to remove a small amount of precipitate and treated with 3 vols. of 95% alcohol. The resulting precipitate was collected, washed and dried as before—hemicellulose B. The lignin was prepared by treating part of the NaOH-insoluble residue with fuming HCl overnight, diluting to 5% and boiling for 6 hours. The residue, washed free from acid and dried by treatment with alcohols of increasing strength and with ether, was regarded as lignin. Cellulose was prepared by chlorination of part of the NaOH-insoluble residue and solution of the chlorolignin in either 3% sodium sulphite or 95% alcohol. Both methods gave similar results.

Benzene-soluble fraction.

After removal of the solvent the residue was obtained as a dark brown viscous mass which on standing deposited a white solid "stearin".

The crude product had the following characteristics:

Sap. equiv.	199.6
I.V.	110.9
Sp. gr.	0.933 at 15°
Reichert-Meisel value	2.11
Solidified at	-5°

These values are closely similar to those given by Schuette and Enz [1931] and Schuette *et al.* [1930] for the expressed and residual oils from the endosperm of the Brazil-nut, except that the shell-oil has a somewhat higher Reichert-Meisel value suggesting that it had become slightly rancid during storage of the shell before use.

The crude extract was saponified and the fatty acids separated by the lead salt process. The unsaponifiable residue was negligible in quantity. After repeated crystallisation the "solid" lead salts retained an appreciable quantity of the "liquid" fraction.

The "solid" acids had equiv. by titration 236 and I.V. 12 and the "liquid" acids equiv. by titration 187 and I.V. 131.5, figures which again indicate the essential identity of the shell-oil with the endosperm oil examined by Schuette *et al.*

It is probable therefore that the oil in the shell is a casual intrusion by diffusion from the endosperm and has no part in the structure of the lignified tissue which forms the shell.

Alcohol-soluble fraction.

The dark brown amorphous mass was readily soluble in pyridine and partially soluble in water leaving a brown, gummy residue. The water-soluble part gave a green colour with methylene blue, indicating the presence of tannins. Further,

the green colour with ferric chloride and the yellowish brown precipitate with bromine confirmed the first test and indicated that the tannins were of the catechol type. Further investigation of this fraction yielded no other conclusive results.

Water-soluble fraction.

Part was dissolved to a strong solution, but fractional precipitation was of no value in the isolation of any of the constituents. An aqueous solution of the fraction, decolorised with charcoal, was acid in reaction and failed to reduce Fehling's solution. The solution was reduced to a small volume *in vacuo* and on standing deposited crystals which, on dissolving in water, gave a precipitate with lead acetate solution, and on testing with resorcinol and sulphuric acid gave a reddish tint and on heating a deep violet colour. They were therefore considered to be tartaric acid.

5% NaOH-soluble fraction.

The three fractions hemicelluloses A, A 1, B, were brown or fawn-coloured amorphous bodies. A and A 1 made up the major part of the alkali-soluble fraction, B being present only to the extent of 1%. A was partially soluble in warm water and was divided into water-soluble and insoluble fractions, the former being separated by acidification; the acid-insoluble precipitate was collected and dried by a sequence of alcohols of increasing strengths.

The quantitative determinations were as follows.

(1) *Lignin content.* The hemicellulose was treated with fuming HCl for 18 hours, the acid diluted to 5% and boiled for 6 hours. The residue washed free from acid was regarded as lignin.

(2) *Furfuraldehyde yield.* The phloroglucide precipitation method was used. The precipitate was washed with hot absolute alcohol to remove methylfurfuraldehyde and ω -hydroxyfurfuraldehyde. In view of the confused state of the literature as to the method to be used to obtain accurate results, this procedure is followed without any claim being made concerning the accuracy of the results obtained, but at least minimum furfuraldehyde yields can be calculated.

(3) *Uronic anhydride content* by the established carbon dioxide yield method.

The identification of the acids and sugars contained in these fractions was based on their acid hydrolysis products. The hemicelluloses were hydrolysed with 3% H_2SO_4 for 6–8 hours. The residue was filtered off and the filtrate half-neutralised with barium hydroxide and completely with barium carbonate. The precipitate which came down was filtered off and will be referred to as precipitate 1. The filtrate was reduced *in vacuo* to a small bulk and treated with three volumes of alcohol. Any precipitate which came down was filtered off and will be referred to as precipitate 2. The filtrate was evaporated to dryness *in vacuo* and extracted twice with hot absolute alcohol, then with 80% alcohol and finally with water. These solutions were evaporated to dryness, taken up in water and decolorised with charcoal.

In no case was the attempt to isolate the uronic acid successful; the small quantity present was probably decomposed by the hot acid during the hydrolysis. In two cases a trace of pentose was found among the sugars and it is possible that this represents the remains of a decarboxylated uronic acid.

	Hemicelluloses			
	A		A 1	B
	Water-soluble %	Water-insoluble %	%	%
Lignin content	70.8	41.0	67.5	34.5
Uronic anhydride	8.16	9.2	11.24	18.4
Furfuraldehyde	1.8	3.1	4.6	6.2

Hemicellulose A.

Water-soluble. Precipitate 1 on decomposition with dilute H_2SO_4 yielded no identifiable products. Precipitate 2 was very small in quantity. In aqueous solution it gave barium reactions with H_2SO_4 and with an aqueous solution of rhodizonic acid. (The latter is a micro-test using a freshly-prepared 1% aqueous solution of the acid which gives a brown stain when in contact with barium salts on filter-paper.) The Ba content of this salt, after being twice precipitated with alcohol from solution in water, was 39.6%. The amount of the salt was too small to permit of further examination.

The absolute alcohol extract gave a faint pentose reaction with orcinol and HCl but the quantity was too small to permit of identification of the sugar. The aqueous extract, treated in the usual way, yielded mannosephenylhydrazone, m.p. 190°.

Water-insoluble. Precipitate 1 contained no organic matter. Precipitate 2 was a barium salt (identified as before) having Ba 30.7%, but obtained in too small quantity to permit of any examination of the acid radical.

The absolute alcohol extract gave a strong pentose reaction with the reagents previously used and on treatment with bromine and cadmium carbonate the characteristic crystals of cadmium xylobromide were obtained. The water-soluble fraction reduced Fehling's solution and yielded glucosazone, m.p. 203°.

Hemicellulose A 1.

Precipitate 1 contained no organic matter. Precipitate 2 was decomposed with dilute H_2SO_4 in the calculated quantity. The precipitate was removed and the filtrate concentrated *in vacuo*. Part was neutralised with barium hydroxide, yielding a crystalline barium salt with Ba 25.5%. Another part was warmed and neutralised with cinchonine. The resulting cinchonine salt crystallised from 90% alcohol melted at 186°. A third portion was evaporated to a gummy mass on a boiling water-bath; when this syrup was kept in a vacuum desiccator over H_2SO_4 for a long time, crystals separated which, after freeing from the residual gum, melted at 130°. Gluconic acid forms a crystalline salt containing 26.0% Ba, forms a cinchonine salt, m.p. 187°, and on keeping in a desiccator for a long time forms a lactone, m.p. 134–136°.

The absolute alcohol extract gave a pentose reaction and on treatment with the usual reagents formed crystals of cadmium xylobromide. The 80% alcohol extract gave a pentose reaction and on treatment with *p*-bromophenylhydrazine hydrochloride and sodium acetate in the cold gave a crystalline mass which when recrystallised from 60% alcohol melted at 160°. The corresponding derivative of arabinose melts at 162°.

Hemicellulose B.

This was a dark brown amorphous solid with a lignin content of 34.5%, a uronic anhydride content of 18.4% and a furfuraldehyde yield of 4.2%. The amount available was too small to permit of further analysis.

Cellulose fraction.

This was prepared from the lignocellulose residue of the 5% NaOH extraction. This residue was washed free from alkali, dried and treated for periods of 5 min. with gaseous chlorine. After each chlorination the excess chlorine was removed by washing with brine and the chloro-derivative removed by boiling either with sodium sulphite solution or 95% alcohol. The process was repeated until cellulose

was obtained free from lignin. The resulting cellulose was a hard, greyish mass with a uronic anhydride content of 4.68 % and a furfuraldehyde yield of 17.4 %. Allowing for the furfuraldehyde from the uronic acid, the pentosan content was high and, using Kröber's tables, was calculated as 29.3 %. The pentosan was extracted from the cellulose by boiling it with 12 % NaOH for 5 hours. The residue was filtered off, the filtrate cooled and treated with an equal bulk of 95 % alcohol at 35°. The precipitate accumulated overnight and was filtered off as a granular mass. The pentosan was hydrolysed by the method of Heuser and Jayme [1923] by boiling it with 3 % HNO₃ for 1 hour, the solution neutralised with barium hydroxide and evaporated to dryness *in vacuo*. The dry mass was extracted with boiling absolute alcohol three times. The alcohol was removed, the residue taken up in water, decolorised with charcoal, the colourless solution was evaporated to dryness *in vacuo* and the resulting mass crystallised from absolute alcohol. The crystals after twice recrystallising from absolute alcohol melted at 142°. With bromine and cadmium carbonate crystals of cadmium xylobromide were obtained. The cellulose fraction appears to be associated with a large amount of xylan.

Lignin fraction.

The lignocellulose residue from the 5 % NaOH extraction was used in this preparation. An attempt was made to isolate the lignin as a chloro-derivative by treatment with gaseous chlorine, removal of the excess by washing with brine and extraction of the derivative with 95 % alcohol. The chloro-derivative was obtained from the alcoholic solution by precipitation with water. This method was unsuccessful, the lignin having undergone oxidation and having a variable chlorine content. Acid hydrolysis however yielded a uniform product. The dried lignocellulose residue was treated with fuming HCl for 18 hours, the solution diluted to 5 % and boiled for 6 hours. The residue was washed free from acid and on combustion gave the following figures, C, 58.62; H, 6.63 %. C₉H₁₂O₄ requires C, 58.70; H, 6.63 %. Treatment with acetic acid and acetic anhydride containing a few drops of sulphuric acid for 1 hour in a boiling water-bath yielded no acetyl derivative, the lignin being recovered unchanged. Benzoylation either in alkali or pyridine resulted in a complex change which obscured any addition of benzoyl groups. This change has been observed in the attempted benzoylation of lignin from other sources. By the semi-micro method of Clarke [1932] the product gave 3.2 % methoxyl.

DISCUSSION.

As already suggested the fat content of the shell is probably a casual intrusion from the endosperm where it is the main reserve product of the seed. The hemi-cellulose fractions are similar in constitution to those found in other plant tissues, with the exception that acids of the gluconic type have not previously been isolated, uronic acids being the usual form. The furfuraldehyde-yielding bodies with the exception of uronic acids are present only in small quantities in this fraction and in no wise compare in quantity with the 5 % of pentosan not in pulp which Emley [1928] found in the peanut hull. The cellulose however with its large xylan content is similar to that from the peanut hull. The uronic acid content is much higher than that found in other lignified tissues. The greatest precautions were taken to avoid over-chlorination and consequent cellulose oxidation and exclude any possibility that the high uronic acid value was a result of the method of cellulose preparation. In view of the hardness of

the shell it is interesting to note that the cellulose was obtained as a hard mass whatever method of drying was used. This can be compared with the cellulose obtained from timber after the attack of the dry-rot fungus, *Merulius lachrymans*. In this case the cellulose was isolated as a hard grey mass with a high uronic acid content [Barton-Wright and Boswell, 1931]. There appears to be a close relationship between the uronic acid content and the texture of the cellulose obtained. The lignin basic formula of $C_9H_{12}O_4$ fits into the C_{36} formula which appears to be the basis of lignins isolated from many sources. Owing to the unreactive and insoluble nature of the substance it is extremely difficult to obtain even an approximate value for its mol. wt.

This investigation has shown that these two nutshells, peanut and Brazil, have certain characteristics in common (pentosan content) and other shells are being examined to determine to what extent the common ground can be extended.

SUMMARY.

Brazil-nutshells reduced to a fine powder were extracted with benzene, alcohol, water and 5% NaOH successively, lignin and cellulose being prepared from the residue. These fractions were examined and their constituents identified as far as possible. The hemicellulose and lignin fractions were similar to those isolated from other lignified tissues. On the other hand the cellulose fraction with its high furfuraldehyde yield was similar to that of peanut hull. The fat isolated from the benzene extract was identical with that isolated by other workers from the endosperm.

My thanks are due to Messrs Rowntree of York for their kindness in supplying the nutshells, to the Trustees of the Dixon Fund of the University of London for the use of the micro-balance required for the combustions, to Prof. J. W. Edington for the use of the centrifuge which so greatly reduced the time and labour required for the preparation of the slimy hemicellulose fractions and finally to Prof. B. H. Bentley for his interest in this work and the many ways in which he has facilitated it.

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