CXXXIII. THE CONSTITUTION OF CERTAIN NUTSHELLS

II. THE PERICARP OF *CORYLUS AVELLANA* (FILBERT, COB, BARCELONA OR HAZEL NUT)

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(Received 30 April 1938)

THE postfertilization development of the ovary of the hazel flower results in the formation from the outer integument of the ovule of a thin fibrous seed coat closely adherent to the embryo and from the ovary wall of a hard, lignified pericarp which is quite free from the seed coat at the time when the fruit is ripe. The work recorded in this paper was done on the pericarp of the hazel fruit. The results obtained are therefore more comparable with those of Emley [1928] for the hull of the peanut, *Arachis hypogea*, than with those for the shell of the Brazil nut, *Bertholletia excelsa* [Boswell, 1936], as the hull of the former is the pericarp of the fruit, the seed coat being a thin fibrous membrane closely adherent to the kernel, while the shell of the latter is the seed coat.

EXPERIMENTAL

Preparation of the fractions. The shells, freed from all traces of kernel, were ground to a very fine powder in order to facilitate the separation of the cellulose from the ligno-cellulose complex. The benzene-, alcohol- and water-soluble extracts and the hemicelluloses A, A 1 and B were prepared by methods described previously [Boswell, 1936]. The lignin was prepared by treating the residue of ligno-cellulose insoluble in cold 4 % NaOH with 5 % H_2SO_4 at 100° for 3 hr. and afterwards with 72 % H_2SO_4 in the cold for 18 hr., followed by dilution to c. 3 % H_2SO_4 and heating for 5 hr. at 100° . The residue, washed free from acid and dried by increasing concentrations of alcohol and finally by ether, was regarded as lignin. The cellulose was isolated from another portion of the ligno-cellulose residue by the following method. The residue was treated with neutral solutions of NaOCl and Na_2SO_3 [Norman & Jenkins, 1933]. Three treatments removed all the easily soluble lignin leaving a light brown powder. Substitution of acid hypochlorite (5 ml. NaOCl with about 3 % available Cl and 2 ml. 20 % H_2SO_4) for the neutral solution removed the residual lignin only very slowly, 21.0 % of lignin still remaining insoluble after several treatments. When however gaseous Cl was used, each chlorination of 5 min. being followed by boiling with 3 % Na_2SO_3 [Sieber & Walter, 1913], three treatments sufficed to prepare the cellulose as a grey powder giving no red coloration with Na_2SO_3 after exposure to Cl. This difference in the response to Cl is of interest in view of the effects obtained by Norman & Shrikhande [1935] and their statement that "the results obtained after chlorination in solution were almost identical with those for gaseous chlorination". It may however be noted from the figures given by these workers that treatment of straw by gaseous Cl removed the lignin more rapidly than did treatment with Cl in solution although the differences between the lignin contents at corresponding stages were not large.

This problem is being investigated with other lignified tissues to determine the generality and probable cause of the phenomenon.

The methods outlined above were used not only for the preparation in quantity of the fractions for detailed examination but also for preliminary analysis of the powdered shell. The following table gives an average of the analyses of the samples taken from the bulk:

	%
Benzene-soluble	0.52
Alcohol-soluble	1.50
Water-soluble	1.92
4% NaOH (cold)-soluble	22.30
Cellulose	48.04
Lignin	29.40
	<hr/> 103.68

Benzene-soluble fraction. The benzene extract was dark green in colour and showed the absorption bands characteristic of chlorophyll. The residue after the removal of benzene was a brown, stiff gum which failed to solidify even after standing for several months, and was fractionated by dissolving it in boiling 95 % alcohol. On cooling, a first, small fraction (A) came down as a hard amorphous mass, the second fraction (B) (about 40 mg.) crystallized after concentration of the solution. On further evaporation a very large part of the chlorophyll was deposited, while the residue (C) was the major part of the original extract and was a brown-coloured, non-drying oil. The following table gives the values obtained:

	A	B	C
Saponification value	62.0	—	188.0
Iodine value	15.1	4.1	73.0

The s.v. of "A" suggests that this fraction is a wax and not a fat. The values for fraction "C" are comparable with those (187–197 for the s.v. and 83.2–90.2 for the i.v.) obtained for the crude oil from the embryo of the hazel seed by various workers.

The benzene-soluble material appears to consist in the main of chlorophyll and a non-drying oil with characteristics similar to those of the oil present in the kernel of the nut.

Alcohol-soluble fraction. A brown, gummy mass which would not crystallize remained after the removal of the solvent. It was insoluble in water but partially soluble in hot dilute acetic acid, by which, after three treatments, the mass appeared to be completely extracted. The filtrate became opaque on cooling and deposited a precipitate overnight. Both solution and precipitate gave a dark green colour with FeCl_3 , a brown colour and subsequently a precipitate with potassium dichromate and a green colour with methylene blue, indicating the presence of a tannin of the catechol type. The residue after extraction with hot dilute acetic acid was soluble in dilute NaOH and NH_4OH , and reprecipitated on acidification. This fraction was a hard amorphous mass, which melted below 100° and gave no reaction with FeCl_3 . It appears to be a tannin of the phlobaphene type. The crude alcohol-soluble fraction contained no organic acids, gave negative tests for alkaloids but contained a small quantity of a substance associated with the phlobaphene tannin forming an amorphous 2:4-dinitrophenylhydrazone and an amorphous semicarbazone.

Water-soluble fraction. On removal of the solvent a brown residue was obtained. The solution gave negative tests for reducing substances, organic acids, tannins and alkaloids. No recognizable organic material was isolated from it.

4% NaOH-soluble fraction. The three fractions A, A 1 and B, of which the first two made up the major part of the alkali-soluble material, were brown or fawn-coloured amorphous bodies. The pentose and uronic anhydride contents were determined by boiling with 12% HCl, the methoxyl by Clarke's semi-micro method and the lignin by the method described earlier in this paper. The following table gives the values obtained:

	A	A 1	B
	%	%	%
Lignin	26.7	58.4	—
Uronic anhydride	7.6	7.2	—
Pentose	51.4	12.0	70.2
Methoxyl	2.6	3.7	—

The quantity of "B" was too small to permit further examination. 5 g. of hemicelluloses A and A 1 were separately hydrolysed with 16 ml. conc. HCl in 500 ml. water at 100°, the residues filtered off and the filtrates neutralized with BaCO₃. Small precipitates separated and were filtered off but yielded no identifiable acids. The filtrate was evaporated to dryness *in vacuo* and the BaCl₂ separated from the reducing substances associated with it by the method described previously [Boswell, 1938]. The reducing substances were identified as follows:

Hemicellulose A	Hemicellulose A 1
Xylose	Xylose
Crystals m.p. 142°	Osazone m.p. 155°
Cadmium xylobromide crystals	Cadmium xylobromide crystals
	Galactose
	Mucic acid

The residues after dilute acid hydrolysis gave the following values:

	A	A 1
	%	%
Fraction of the original	31.0	71.2
Uronic anhydride	7.8 (2.4)	3.1 (2.20)
Pentose	12.8 (4.07)	0.0
Methoxyl	9.12 (2.81)	4.7 (3.34)

The values in the brackets are figures calculated as % of the original material. The methoxyl radical appears to be associated solely with the lignin fractions of the hemicelluloses; this is in accord with the examination of the sugars separated by hydrolysis. Colour tests and absorption spectra indicated the presence of pentoses (HCl and orcinol) and the absence of methyl pentoses (HCl and acetone) and xylose was identified as the only pentose present.

The dilute acid hydrolyses were repeated on the hemicelluloses A and A 1 using 1% H₂SO₄ in place of the HCl in order to test for the presence of organic acids whose Ba salts were soluble in water and insoluble in alcohol, but none was found.

Cellulose. The isolated product was a grey powder which in spite of the fact that it gave no red coloration with sodium sulphite solution after exposure to chlorine contained 3.3% of lignin: pentosan content 23.2%, uronic anhydride 4.12% and α -cellulose 48.2%. The pentosan was isolated by heating the cellulose for 3 hr. with 12% NaOH at 100°, filtering off the residual cellulose and precipitating by cooling the filtrate to 50° and adding an equal volume of 95% alcohol. The pentosan accumulated as a granular mass on standing overnight and was separated on a centrifuge. It was hydrolysed by boiling for 1 hr. with 3% HNO₃ [Heuser & Jayme, 1923], the solution neutralized with BaCO₃ and evaporated to dryness *in vacuo*. The pentose, after separation from the Ba(NO₃)₂

by extracting with absolute alcohol, was identified as xylose by the formation of the characteristic crystals of cadmium xylobromide.

Lignin. This fraction was dark brown in colour. Found: C, 60.91; H, 5.36; OMe, 13.01. Calc. for $C_{35}H_{31}O_{13}(CH_3O)_3$: C, 60.64; H, 5.32; OMe, 12.4.

DISCUSSION

The morphological relationships of the nutshells of *Bertholletia excelsa*, *Arachis hypogea* and *Corylus avellana* have been described previously and it is of interest to note how those of the last two are paralleled by similarities in their chemical constitution and how both differ from the nutshell of *Bertholletia excelsa*. The lignin/cellulose ratio for the hull of the peanut is 0.74 being of the same order as that for the hazel nutshell (0.61) but differing widely from that for the shell of the brazil nut (2.09). The values for the shells of the first two nuts are similar to those for the same ratio for the xylem of the stem of any tree, whether hard or soft wood, e.g. *Fraxinus americana*, 0.54; *Quercus alba*, 0.62; *Pinus ponderosa*, 0.64; and *Sequoia sempervirens*, 0.70. Consideration of the pentosan contents of the hemicelluloses further illustrates this parallelism (*Arachis hypogea*, 12.0%; *Corylus avellana*, about 7.0%; shell of *Bertholletia excelsa*, 0.60%); the corresponding value for the xylem in the stem of any hardwood tree is about 9.0%. Further, the absolute values for the lignin and cellulose without the associated pentosan for *Arachis hypogea* and *Corylus avellana* are in close agreement with those recorded for the xylem in the stem of hardwood trees while those for *Bertholletia excelsa* differ widely from such values.

It would appear therefore that the processes of lignification in the development of secondary xylem in hardwood trees and in the development of the woody pericarps of the fruits of certain of these trees proceed by similar stages resulting in lignified tissues which are alike not only in their lignin/cellulose ratios but also in their lignin and cellulose contents. Further, the hemicellulose fraction of *Corylus avellana*, mainly xylan with a small amount of galactan, is of the same type as that isolated from the secondary xylem of hardwood trees. As both the quantitative and qualitative constitutions of the lignified seed coat of the fruit of *Bertholletia excelsa* differ so widely from those of the tissues just considered it would suggest either that its lignification proceeds by a chain of reactions quite different from that in the lignification of the pericarp and the secondary xylem of hardwood trees, or that a different set of factors determines the constitution of the mature tissue.

These differences do not however extend to the celluloses isolated from the nutshells. The two examined in detail, those from *Corylus avellana* and *Bertholletia excelsa*, are of the same type, both being associated with large quantities of xylan and with relatively large amounts of uronic acid. The cellulose of *Arachis hypogea* is similarly associated with a large amount of pentosan. These celluloses with their large pentosan content and low α -cellulose value are very similar to those isolated from the secondary xylem of the stem of hardwood trees.

The cellulose associated with the lignin in the lignified tissues of hardwood trees would appear therefore to be of the same general form, independent of the morphological identity of the lignified organ, or of differences of constitution in the fractions associated with it, and unaffected by variations in its quantitative relationship to the whole tissue.

In view of our very scanty knowledge of the nature of lignin it is impossible to draw any useful conclusions from the values obtained by elementary analysis of the fractions isolated.

SUMMARY

The pericarp of the fruit of *Corylus avellana* reduced to a fine powder was fractionated with benzene, alcohol, water and 4% NaOH; lignin and cellulose being prepared from the residue. The results obtained from an examination of these fractions show that the morphological identity of the shells of *Arachis hypogea* and *Corylus avellana*, both being the pericarps of their fruits, is paralleled by a similarity in chemical constitution. Both show considerable differences from the shell of *Bertholletia excelsa*, which is the seed coat. It is noted that the lignified pericarps are similar in constitution to the secondary xylem in the stem of hardwood trees and it is suggested that lignification in both tissues proceeds by similar stages but that a different process or set of factors is involved in the development of a lignified seed coat.

My thanks are due to Messrs Gill and Duffus for their kindness in supplying me with the nutshells, to the Trustees of the Dixon Fund of the University of London for the loan of a microchemical balance and to Prof. B. H. Bentley for his interest in the work and for the many ways in which he has facilitated it.

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