A STUDY ON THE DARK COLOURED DURAMEN OF EBONY

by

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A. Introduction.

Many trees form duramen after a certain period in their life. They begin to eliminate the central part of the xylem as a transport tissue by closing the elements with various products. Many times the process is accompanied by a coloration of the substances. The formation of duramen is supposed to serve as a defence against infection and to strengthen the mechanical system.

Gürnik (15) has thoroughly investigated the formation of duramen. In his thesis he gives also a discussion of literature on this subject and cites the important work of Tschirch.

Tschirch states that by the formation of duramen a metamorphosis of the membrane itself never took place, but the coloured contents of the cells originated from between the layers of the wall. Gürnik has found that locally in the secundary membrane a mucilaginous layer is formed. This layer forms the duramen, which yields various substances such as bassorins, resins and oils. If the wall is coloured, this is caused by matters in the wall itself; perhaps originated by an enzymatic action on the lignin complex.

Prael (28) has investigated ebony-wood. In the young alburnum the vessels and other elements always are without contents, while in the duramen the lumina of the cells become filled with gummy- or resinous substances.

The colouring matters of the cell contents and those of the coloured cell walls are not of the same composition.

Already in 1879 Molisch (24) has tried to elucidate the nature of the colouring matter of ebony-wood. He stated a gummification process (chemical alterations of the walls of the elements) followed by a humification. At first humic acids should proceed (which may be extracted with Na_2CO_3 and precipitated with HCl) and later humic coals (may be isolated with NaOH and HCl).

Afterwards all kinds of coaly substances should be formed. According to Molisch ebony contains 4.63 % humic acids and 1.3 % humic coals.

Belohonbek (2) agreed with Molisch on the principal points. He considered the process to be of the nature of a reduction, leading to carbonization, whereby the soluble parts should be humic acids and the insoluble parts coals. He stated that the colouring matter was bound to calcium.

Another field of black plant matters is that of the so-called "phytomelans" found by Hanausek (16) in the pericarp of many fruits of Compositae. Afterwards these matters were investigated by Dafert and Miklauz (6) in a more chemical way. Dafert concluded that the colouring matters of ebony cannot be classed with these substances, but he did not speak about the possible engagement between those products.

According to the above-mentioned authors the middle lamella is the "melanogenous" layer; which is an important fact in connection with our problem.

B. Properties of the ebony-wood and the isolated products.

§ 1. *The material.*

For chemical investigation I used a piece of ebony from the collection of the laboratory, catalogized under the name of *Gjaja merah* = *Diospyros spec.* Duramen as well as alburnum were ground in a "disintegrator" and afterwards sieved by a fine sieve. For the analyses I made use of this "wood-flour".

§ 2. *Composition of the wood.*

a. Moisture.

The moisture content is determined by drying in a drying-oven to constant weight (t. = 105°). The air-dry duramen contains 7.75 % moisture and the alburnum 7.10 %.

b. Ash.

The quantity of ash is determined by incinerating the wood in a china crucible; a white voluminous ash remains, containing much Ca-carbonate (originated from the Ca-oxalate in the wood). The ash content of the duramen is 0.61 %, while the alburnum contains 0.54 % ash. The ash of the latter is more compact, greyish and contains relatively more phosphorus.

c. Cellulose content.

To obtain the cellulose quantitatively I made use of the method of Kürschner and Hoffner, (Chem. Zeit. 55, 161 (1931)). This method is based on the fact that the lignin and other products are changed into so-called „nitro"-compounds soluble in alcohol, and the cellulose remains.

The cellulose obtained in this way still contained brown substances (see p. 792). The cellulose content of the duramen is 43.2 % and of the alburnum 41.0 %.

d. Lignin content.

The quantity of lignin is determined according to the method of Geo A. Richter (Journ. Ind. Eng. Chem. 23^2, 131 (1931)).

The cellulose is dissolved by treatment with 72 % H_2SO_4. The residue does not consist only of lignin, but also humic matters, tannins, phlobaphenes etc. The amount of lignin in the duramen is 39.6 % and in the alburnum 38.5 %.

e. Pentosan determination.

The so-called furfural method of B. Tollens was used, which method is based upon the hydrolysis of pentosan into pentose and the transformation of this compound into furfural

by distillation with 12 % HCl. The furfural is quantitatively precipitated with phloroglucinol.

The method as described in Abderhalden "Handbuch d. biol. Arbeitsmethoden", Abt. 1, Teil 5, 195—206 (1922), where the conversion tables are mentioned too, has to be followed in detail.

The duramen appears to contain 12.9 % pentosan and the alburnum 16.5 %.

All the data are calculated on the absolute dry basis. Parallel determinations with fir-wood are included for comparison.

TABLE 1.

Composition of the wood samples.

	Ebony		Fir-wood
	Duramen percent	Alburnum percent	percent
Moisture	7.7	7.1	7.3
Ash	0.57	0.51	
Cellulose	39.9	38.1	47.9
Lignin	36.6	35.8	25.9
Pentosan	11.8	15.3	12.3

§ 3. *Isolation and properties of the humic acids.*

The investigations of Molisch warranted the assumption that the brown substances in the duramen had to be considered wholly or in part as humic matters. These substances had to be isolated, their properties should be investigated and compared with well-known products.

If these matters are really humic substances (and for a great part humic acids) alkalies should be the indicated extraction agents.

I used sodium hydroxide solution (from preliminary experiments concentrated ammonia appeared to be also very useful). The application of Na-fluoride as a neutral

extraction agent, as recommended by K. Simon (32) did not prove succesful on the duramen, otherwise this method should be preferred to the use of alkalies, because the latter are able to dissolve still other substances from the wood and also because solutions of alkali humates begin to change very soon. (U. Springer (34)).

Before treating the wood with Na-hydroxide, it must be freed from other substances present such as resins, fats, phytosterols and other organic products. Therefore the wood-flour was extracted with a mixture of equal parts of benzene and ethanol during twelve hours. The brown extract contained only a very small quantity of solid matter, which was not further analyzed. Humic acids soluble in alcohol (hymetomelanic acids) could scarcely be extracted from the wood, while the products isolated with NaOH afterwards contained again a part soluble in ethanol. In the wood it is probably present partly in an insoluble form.

Extractions with water were not carried out, because it appeared from preliminary experiments that the extraction did not lead to a good result; not only a small quantity of water-soluble products, but also important quantities of the brown acid formed a colloidal solution, which yielded coloured filtrates even after repeated extraction.

The wood-flour thus cleaned and dried was shaken with an eightfold quantity of a 2/3 n. NaOH solution during 2—2½ hours. The Buchnerfiltrate contained the sodium salt of humic acid. From the Na-humate solution obtained in this way the humic acid was liberated by carefully acidifying with hydrochloric acid to acid reaction. The humic acid is insoluble in water and HCl and therefore precipitated from the solution. It is necessary that the solution is acid, otherwise the humic acid does not precipitate easily.

In order to prevent all kinds of difficulties during the

filtration of the humic acid, the following method is recommended. The acidified fluid is quietly left in a high cylinder glass, the precipitate settles and after two days the clear supernatant fluid may be siphoned off. The cylinder is then filled with distilled water, well mixed, left again for settling (½—1 day) and the supernatant fluid is siphoned off. This process is repeated for about five times. The supernatant fluid will now be free from salts, but must still react slightly acid, otherwise the particles dispersed in the water, do not precipitate anymore, and, moreover acid-free water yields a colloidal solution with humic acid.

The clear fluid is now siphoned off, the remainder is carefully filtered through a Buchner funnel with hard filter and the residue is washed once or twice with hot water. If we dry this mass in the air, glittering products of a hard, coaly consistence appear. These products cannot very easily be treated further. Therefore it is a better method to grind the half-humid mass in small portions with ether in a mortar. The operation must be repeated, since the humic acid does not soon yield the strongly adsorbed water. The preparation obtained in this way is a fine dark brown powder. This powder is dried further in vacuo above P_2O_5 at 65°. The entire procedure takes a few weeks.

Besides this preparation, marked A, I still isolated a humic acid by extracting dry wood-flour with an eightfold quantity of 10 % ammonia, precipitating this solution with HCl, cleaning and drying it in the above-mentioned way (Preparation B).

For comparison of the obtained material I isolated a humic acid from brown coal. For this purpose a ground brown coal briquet was used at first carefully treated with hot ethanol during some days (large quantities of bitumen were extracted by the alcohol). After this the humic acid was isolated (Preparation C).

At last Cassel Brown served as material for comparison (Prep. D). Cassel Brown is a species of brown coal which is very rich in humic acids. Afterwards it appeared from unpublished data of this laboratory that the used material of Cassel Brown contained about 50 % of these acids!

The properties of the four products (A—D) are given in table 2. The table shows that the properties of the four products agree in the main points. A small part of the products is soluble in alcohol, this part is the hymetomelanic acid. Little differences in solubilities are the result of variations in methods of preparation.

TABLE 2.

Solubility of the humic acids.

	Prep. A	Prep. B	Prep. C	Prep. D
cetone	Almost non soluble	Almost non soluble	Almost non soluble	Non soluble
thanol.....	Partly soluble	Little soluble	Little soluble	Almost non soluble
a-hydroxide ..	Completely „	Completely „	Completely „	Completely soluble
mmonia	Slowly „	Slowly „	Soluble	Soluble
a-fluoride	Very little „	Very little „	Very little „	Completely „
Vater.....	Non soluble	Non soluble	Non soluble	Non soluble
ydrochloric acid	Non „	Non „	Non „	Non „

The reason for complete solubility of Cassel Brown in Na-fluoride is still obscure. It is possible that here the humic acid is polymerisated less far and is therefore present in a form soluble in Na-fluoride (with this solvent I could easily extract humic acid from peat, this in agreement with the results of Simon).

The properties of the products from the wood suggest indeed humic acids.

§ 4. *The "nitro"-compounds of the products obtained.*

In the preparation of the "nitro"-compounds the method

of Kürschner was followed (a mixture of ethanol and nitric acid) as well as the method of Fuchs (5 n. nitric acid).

Preliminary experiments showed the superiority of the first method. The humic acid isolated from ebony-wood is boiled with the alcoholic nitric mixture (4 : 1) in a bottle with reflux condensor on a water-bath during an hour. The hot fluid is filtered and poured out by drops in hot water; an ochre-coloured precipitate settles very quickly. This precipitate is boiled with the alcoholic nitric mixture for a moment and after filtration precipitated again with water.

The "nitro"-compound is filtered through a Buchner funnel, washed with water which is acidified with nitric acid (water free of acid yields a colloidal solution, causing much loss of material). Afterwards the product is treated with absolute ether and an orange to yellowish brown powder is obtained. Because a rather large part of the material is lost during the operation, a quantitative determination of the "nitro"-product is omitted.

TABLE 3.

Solubility of the "nitro"-compounds.

	"Nitro"-humic acids from		
	Ebony (method Kurschner)	Brown coal (idem)	Brown coal (method Fuchs)
Colour	Yellowish brown	Orange to brown	Brown
Acetone	Soluble	Soluble	Soluble
Ethanol	"	"	"
Ether	Very little soluble	Very little soluble	Very little soluble
Benzene.....	Non soluble	Non soluble	Non soluble
Glacial acetic acid	Soluble	Soluble	Soluble
Na-hydroxide 2n..	"	"	"
Na-fluoride	"	"	"

For comparison a humic acid from brown coal was treated with alcohol and nitric acid and a brown "nitro"-humic acid was obtained. The method of Fuchs was also used to prepare a "nitro"-product from brown coal.

Also from this table there appears to be a complete agreement between the properties of the products from ebony-wood and of those from brown coal.

§ 5. *Elementary analysis of the products and "nitro"-bodies isolated.*

The *analyses are determined on my request by Mr. L. M. v. d. Valk according to the method of ter Meulen en Heslinga (Rec. trav. chim., (1924) p. 551). Data of other investigators are included for comparison.

TABLE 4.
Composition of the isolated products.

Preparations	C percent	H_2 perc.	N_2 perc.
*Humic acid from ebony (NH_4OH isolation)	60.4	4.1	
* idem (NaOH „)	59.7	4.9	0.8
Natural humic acid (after Eller).....	59.6–60.2	3.4	?
idem cleaned with NaOH.....	60.7	3.5	1.5
Humic acids (after Sven Odén)	58.6	4.3	
*"Nitro"-humic acid from ebony	50.9	3.9	5.3
* idem from brown coal.....	52.5	4.0	3.2
"Nitro"-humic acid from Cassel Brown (after Fuchs)	53.8	3.6	2.2
"Nitro"-humic acid from Cassel Brown (after Kürschner)	53.6	4.6	5.1

§ 6. *The fusion with potassium hydroxide.*

This fusion is an important method for a closer investigation of the composition of certain plant materials. Hoppe-Seyler (19) used this method already in 1889

on humic substances. His results agree with those found by later investigators. Organic acids such as protocatechuic acid and phenoles such as pyrocatechol and phloroglucinol are formed. However the latter products have not been found by Tropsch and Schellenberg (35), who isolated isophtalic acid.

Also Eller (8) has occupied with the fusion with KOH of humic acids. That fusion yielded brown masses as accessory products. According to Eller the fusion with KOH of humic acids yielded humins with a high C-content.

The method of the fusion and the treatment of the isolated products are carefully worked out by Van Scherpenberg (30) for other products.

My own investigations on this point have been very superficial, but the results obtained warrant a report.

For this work lignin of fir-wood, of ebony and humic acid of ebony were used. From each sample 0.5 gramme was fused with 3 gramme KOH in a silver tube in an oil-bath at 250° during half an hour, the mass being stirred continually. After cooling the melt was taken up in HCl and water and a dark brown precipitate of humins settled. The filtrate was shaken with ether. The further operation took place according to Van Scherpenberg. The results are given in table 5.

TABLE 5.

Products present in the extract from the fusion with KOH.

	Protocatechuic acid	Phloroglucinol	Pyrocatechol
From fir-wood...	Present	Present	Present
From ebony	„	„	„
From humic acid.	„	„	—

§ 7. *Attempts to isolate the total humic matters.*

In the preceding paragraphs the alkali-soluble fraction of the humic acids was investigated; the reddish brown

product of the wood parenchyma and of the medullary ray cells.

In order to isolate also the dark brown and black products cellulose and lignin must be removed in some way. To dissolve lignin and cellulose from humus containing matter Karrer and Bodding-Wiger (21, 22) recommended a prolonged treatment of the material with a sixfold quantity of acetyl bromide (addition of glacial acetic acid promotes the reaction). This method was applied also in this case, although a preliminary study on the action of acetyl bromide on different lignins and humic bodies seems desirable, before a conclusion may be drawn to the absolute usefulness of the method.

To 1 gramme of the duramen-flour of ebony 12.5 gramme acetyl bromide was added and the whole was warmed in a flask with reflux condensor on a water-bath at 40—45° during five days. After filtering, washing and drying the residue amounted to 15—16 % of the wood. A longer treatment with the solvent should certainly yield still more. By a treatment with a tenfold quantity of phenol crystals and a few drops of HCl so much dissolved that the residue amounted only to about 5 % of the original quantity of wood. This residue was a brownish black powder.

It was still considered to isolate humic matters by treatment of the wood with phenol and HCl with which lignin is converted in a product soluble in ethanol and to liberate afterwards the residue from cellulose. Finally a residue of about 3 % was obtained. However this method with phenol and HCl is insufficiently investigated and many difficulties adhere to it, for phenol seems to dissolve centain fractions of humic acids (Kreulen (23)).

It seems to me of importance to control the influence of the reaction time in this case, for I believe that by very long exposure to the phenol also the cellulose may be

attacked. Macro- and microchemical reactions seem to point in this direction.

As is formerly stated, the cellulose isolated in the quantitative determinations of ebony contains brown pieces, which cannot be removed after repeated boiling in alcoholic nitric acid. If we dissolve the cellulose in 72 % sulfuric acid it appeared that this brown part remains and amounted to about 3 % of the weight of the wood. All these data do not tell us very much.

The quantitative determination of the humic acids soluble in alkali is also very difficult, for after a 3 week treatment with NaOH the filtrates are still brown. Moreover, we have to keep in mind that, under the influence of alkali, autoxidation of the lignin proceeds continually. The alkali extracts of the wood, assembled after 3 weeks, appeared to contain, after precipitation with HCl, about 5.5 % humic acid, calculated on the dry wood basis.

Roughly estimated there should be present in the duramen 5—6 % humic acid and 3—5 % humic bodies insoluble in alkali.

§ 8. *The "Russell Effect" of the wood.*

The Russell effect is the action of the wood on a photographic plate. The method employed was to place a piece of wood with its smooth surface on a photographic plate in the dark and to keep it about a week in an incubator at 40°. (29, 37).

It was found that the duramen had a rather marked action, while the alburnum was without influence (fig. 1). Therefore the duramen should be the seat of oxidations. In this connection I may refer to the study of Haslam (18), who has found that substances like coal have an action on a photographic plate. According to him the main cause of this process must be ascribed to oxidation, while moisture plays an important part.

It is not possible, however, to conclude much from this experiment, as the basis of the Russell effect still seems obscure.

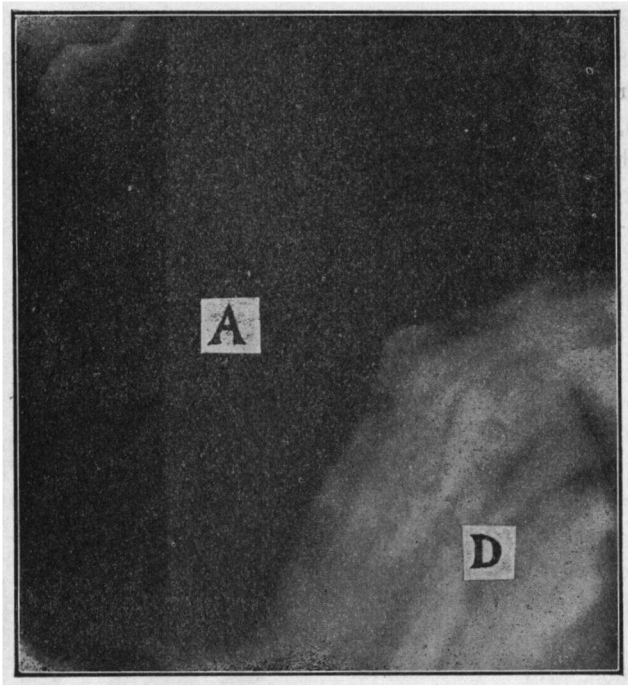

Fig. 1. "Russell Effect" of Diospyros ferrea after a week at 40°. A = alburnum, negative effect. D = duramen, positive effect.

C. Microscopy of the ebony-wood.

§ 1. *Introduction.*

For the microscopic investigation was chiefly used *Gjaja merah.* This wood was also used for chemical investigation. Also observations were made on sections of *Diospyros ferrea, D. celebica* and *D. discolor.* The material of these species was kindly sent to me by the "Forestry

Experiment Station" at Buitenzorg, Java. The sections were cut with a wood microtome from blockletts, which had undergone no other operation than boiling in water during some hours.

§ 2. *Anatomy.*

The anatomic characterisation of the wood may be brief as on this subject good descriptions are given by Molisch (24), Parmentier (27), Wright (38) and Janssonius (20).

Ebenaceae are marked by a characteristic position of the wood parenchyma namely:

1° paratracheal: in one row around the vessels,
2° metatracheal: in narrow, tangential rows, one cell broad, interrupted here and there, and
3° sometimes some scattered parenchyma cells.

Vessels occur either isolated, or in radial rows; tracheids are only very sparsely distributed. The medullary rays are generally in one row, but here and there also cells occur in two or three rows next to each other, many containing crystals of Ca-oxalate. (fig. 2).

The species investigated showed these structures. The duramen is very dark in these samples, especially in D. ferrea, where it is nearly black. The points of difference and agreement in the anatomy of the various species are stated in table 6.

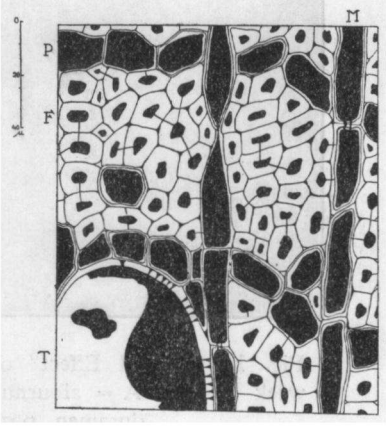

Fig. 2. Transverse section through the duramen of Gjaja merah = Diospyros spec. P = wood parenchyma; M = medullary rays; F = wood fibres; T = wood vessel. The lumina of P and M are filled with a reddish brown mass, those of F and T with a dark brown to black substance.

TABLE 6.

Colour of the cell contents of the three Ebony species.

		Gjaja merah	D. ferrea	D. celebica
Alburnum	Medullary ray cells	Reddish brown	Empty, a few sometimes yellowish brown	Empty, sometimes yellow
	Wood parenchyma cells	Reddish brown	Empty	Empty
	Wood fibres	Without coloured contents	Without coloured contents	Without coloured contents
	Wood vessels	Some with yellow contents, further empty	Some with yellow contents, further empty	Some with yellow contents, further empty
		Gjaja merah	D. ferrea	D. celebica
Duramen	Medullary ray cells	Reddish brown	Yellowish brown, sometimes black	Dark brown to black
	Wood parenchyma cells	Reddish brown	Brown	Dark brown to black
	Wood fibres	Dark brown to black	Black	Light coloured, brownish?
	Wood vessels	Black	Black	Black

The walls of the elements are not coloured, except in the wood of D. ferrea, where they have a yellowish tinge. The structure of the duramen of D. celebica and D. ferrea showed at places a "coaly" aspect in consequence of the very large quantities of black products present in the cell lumina. The limit between duramen and alburnum is rather sharp, only a very narrow transition zone is present.

§ 3. *Microchemical reactions.*

The results of the microchemical reactions performed by me are collected in the following table.

If we look at this table, we can percieve great differences between alburnum and duramen. The middle lamella plus primary lamella of the two parts are strongly lignified.

TABLE 7. MICROCHEMICAL PROPERTIES OF

Reagents	DURAMEN			
		Secundary layers		
	Middle lamella + prim. lamella	Vessels	Libriform	Wood parenchyma medullary rays
Phloroglucinol and HCl	Dark red	Red	Red, with here and there almost uncoloured inner lamella	
Aniline sulphate	Yellow	Yellow more feeble		
Fuchsin + HCl	Red to a little brown	Yellowish red		
Mäule react.	Brownish red	Yellowish red		
Ruthenium red	Red	Almost uncoloured		
Oxamin blue 4 Rx	Violet to light-brown	Yellowish brown, here and there lighter		
Methyl red		Yellow to orange, sometimes more pink		
Diethyl red	Yellow	Yellow, here and there more pink		
Iodine in ZnCl in KI + H_2SO_4		Yellow, on the damaged spots blue		
Sulphuric acid 72 %	Does not dissolve, swells up and becomes brown. (humification)	Dissolved for a great part; party brown	Dissolved	
Phenol + HCl after prolonged treatment		Walls dissolved; contents not dissolved become lighter		Walls dissolved Contents dissolved
Trichlor acetic acid after prolonged treatment		Walls dissolved; contents not dissolved become lighter		Walls dissolved contents not dissolved
Sodium hydroxide		Unchanged		Contents dissolved
Alcoholic nitric acid		Contents almost not dissolved, become light yellow		Contents dissolved
Acetyl bromide		Contents not dissolved		

ECTIONS OF EBONY-WOOD.

ALBURNUM				NOTES
Middle lamella prim. lamella	Secundary layers			
	Vessels	Libriform	Wood parenchyma; medullary rays	
Dark red	Red, no colourless zones			Colour alburnum darker than duramen
Deep yellow	Yellow			
Red	Red			
Red	Red with something orange			
Red	Colourless			Consequently stains red strongly lignified lamella
Blueish violet	Colourless, here and there on the damaged spots blue			
	Red			pH duramen. about 5.8—6.0?
	Red			pH alburnum about 4.4
	Yellow; here and there fibres with a lighter zone in the wall			Walls of the vessels more brown
Not dissolved brown (humification)	A little dissolved brown	Dissolved		Cellulose dissolved lignin not dissolved, but not visible in the sections; contents unchanged
	Walls dissolved; no contents		Walls dissolved contents dissolved	After boiling for a short time only lignin is dissolved. (maceration mixture)
	Walls dissolved; no contents		Contents not dissolved	After short treatment the tissues are only broken, not dissolved. (maceration agent)
	Unchanged		Contents dissolved	After prolonged treatment
			Contents dissolved	Lignin is dissolved cellulose reaction positive
			Contents partly not dissolved	Lignin and cellulose dissolved

(It is not certain that the red colouring with ruthenium red of the lamella points to pectin). It is remarkable that the cellulose reactions (with iodine in zinc chloride and with iodine in potassium iodide plus sulfuric acid) are not positive, only a yellow colouring of the undamaged walls takes place. With 72 % sulfuric acid we see the walls swell very clearly. Afterwards they dissolve, except the primary

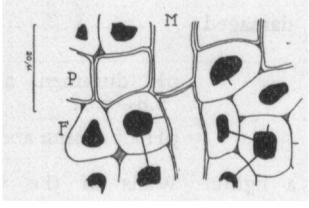

Fig. 3. Transverse section through the duramen of Diospyros spec. after treatment with sodium hydroxide. Lettering as in fig. 2. From P and M the substance has disappeared and from F it has not.

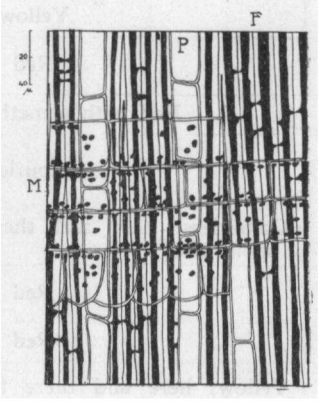

Fig. 4. Radial section through the duramen of Diospyros spec. after treatment with sodium hydroxide. Lettering as in fig. 2. From P and M the substance has disappeared except from the pits in the walls (the black spots in the figure.)

lamella that swells, but does not dissolve and becomes brown. (A humification, perhaps because this lamella is very rich in lignin). Sulfuric acid does not attack the cell contents.

These contents, according to their behaviour towards different reagents, may be divided in at least two groups, namely the reddish brown mass from the parenchymatic

tissues and the dark brown to black products from the vessels and the fibres. Treatment with sodium hydroxide solution causes the reddish brown contents to be totally dissolved, while the black masses remain intact (fig. 3 and 4). The wall pits of the medullary ray cells are filled with a black mass insoluble in alkali. The behaviour towards alkalies is characteristic of humic matters. The reddish brown matter dissolves also in phenol and hydrochloric acid (after prolonged treatment) and in an alcoholic nitric mixture (4 : 1), but is insoluble in trichlor acetic acid and in acetyl bromide. With these reagents the colour of the substance becomes light-yellow, but does not dissolve.

The black contents of vessels and fibres appear to be insoluble in sodium hydroxide, acetyl bromide, alcoholic nitric mixture, trichlor acetic acid and phenol plus hydrochloric acid.

As in some cases the colour changed to lightbrown, some action of these reagents cannot be denied.

The weaker lignin reactions of the duramen make us justify the supposition that by the "duramification" the lignin has been changed into humic acids, deposited into the cell lumina. G. van Iterson Jr. believes that perhaps this process finds an explanation in the fact that Ca-humate is very insoluble, so that the formed humic acid could be accumulated in the Ca-containing cell contents.

The presence of the black matter, insoluble in alkalies, in the vessels and the fibres leads moreover to the supposition that these humic acids have undergone a decarboxilation under the formation of humins.

This opinion, already mentioned in my preliminary communication (13) finds a rather strong support in the coloration of the sections with metachromatical dyes. On the behaviour of these dyes regarding cell walls in general Czaja published interesting results (5). After colouring with two of such dyes; oxamin blue 4 Rx and brillant

congo blue, the alburnum appeared to be colourless and the duramen yellow to brown. Consequently the wall substance (cellulose and lignin) in the alburnum is so dense that even the smallest particles of the dye cannot penetrate. Apparently in the duramen the lignin has partly disappeared, the wall structure has become less dense and the finest particles of the dye, which stain yellow, are able to penetrate. The damaged parts of the preparation are coloured blue with the dyes; the cellulose which has the largest interstices adsorbs the largest particles that stain blue. (That the strongly lignified primary lamella is stained violet is not to be explained only by different staining by particles of different size).

A second argument that is to be quoted for our supposition, but in my opinion cannot be fully accepted without criticism, is the fact that the walls of the duramen react less acid than those of the alburnum. This could be explained by accepting that the lignin complex which has acid character has partly disappeared from the duramen. The Range Indicator Method (R.I.M.) from Small (33) is applied to this pH determination. This method may be used too for suberin, cutin and lignin walls, according to an investigation of Armstrong on higher fatty acids (1). Just now the analogy between higher fatty acids and lignin is less plain and therefore the data obtained cannot be trusted implicitly. I used as indicators methyl red and diethyl red, recommended by Armstrong. They have their range from pH 4.4—6.0 (resp. 6.5) and change from red to yellow. The walls of the duramen are coloured yellow, with here and there a feebly pink tinge, those of the alburnum are red.

From these facts it may be derived that the lignin from the walls (and chiefly from the secundary layers) undergoes a humification.

According to the latest publication of Harlow in 1933 (17) now the presence of lignin in these layers seems

to be demonstrated, a fact, formerly disputed by some investigators.

In general the results of the microchemical reactions agree with those of Ohara (26), who has investigated the wood of a coffin, more than 1800 years old, found in Korea. While the inner parts of the wood were kept unchanged, the outer layers had undergone a humification and carbonization process. The structure was lost for a great part, but remains of vessels and medullary rays pressed together were still to be seen lying in a reddish brown ground mass. The humification began in the secundary layers of the walls. In these layers Ohara could show the presence of small globules of humic matter.

D. A note on the concept and the formation of "Humus".

While an exhaustive discussion of this matter lies outside the scope of this paper, a few investigations will be mentioned that are pertinent to our problem.

We are indebted to Sven Odén (25) for an extensive study about humic acids with a large literature index up to 1919. In this study he classified the humic products in this way: humus coals or humins (the part insoluble in alkalies) and the soluble parts, which are "humic acid", "hymetomelanic acid" and "fulvic acid". Especially Waksman, the prominent American soil investigator, has shown us the great confusion which is caused by the highly complex nomenclature of the humus. "Words have been used for preparations, giving the impression that they are chemical entities", (36). The "humic acids" for which Odén gives an empirical formula $C_2H_{1\cdot75}O_{0\cdot95}$ should be tetravalent; they have colloidal properties, adsorb water and other matters very strongly and may change into humins under evolution of CO_2.

Eller (7) has also worked on humic acids. He compared

the natural acids with artificiel acids obtained from carbo hydrates and phenoles. He thinks that the properties of the natural humic acids agree more closely with the artificiel acids from phenolic bodies than with those derived from carbohydrates.

From an investigation of the products yielded by treatment of lignin and of natural humic acids with nitric acid Fuchs (11) concluded that lignin and humic acid must be built up from very closely related molecules. He even gives a structural formula for humic acid and he believes that the molecule should be built up from "den Benzolcarbonsäuren lieferenden Grundkomplex des Lignins, doppelt oder mehrfach durch Ringschliessung verknüpft und vermindert um die hydroxylreichen und Protocatechusäure lieferenden Bauelemente des Lignins" and he adds that the processes which give rise to such molecules, finally should lead to the formation of graphite (12).

The formation of humus in nature has occupied many investigators. Hoppe Seyler (19) already stated that the humic substances are formed by dying plant material. Do they originate from cellulose or from lignin? Chardet, Marcusson, Burian (4) and others plead for the formation from cellulose, because of the presence of a furan ring in the molecule. On the contrary Eller, Fischer and Schrader, Grosskopf etc. are supporters of the lignin theory which accepts a benzene nucleus in the molecule.

Schrader (31) is able to prove from his experiments that lignin yields humic acid by autoxidation in the presence of KOH. According to this investigator this process also occurs in nature in the presence of alkaline substances!

Fischer and Schrader (10), building on Schrader's study, gave their important theory on the formation of coal. In the course of time the lignin is transformed to humic acids and afterwards this procedure goes on until the formation of coal; the cellulose is decomposed by the

activities of microorganisms. According to Fischer (9) at first lignin would yield acetic acid and this should lead to the formation of humic acids.

The investigations of Grosskopf (14) are of great importance to our subject. He used natural materials, which were in successive stages of humification; the youngest parts consisted of almost green needles and remains of fir-wood; the eldest parts consisted of brown coal. He now compared the chemical composition of these materials and stated that the lignin content decreases markedly concomittant with a proportional increase of humic products. He believes the course of the process to be as follows: lignin → intermediary substances → humic acids → humins. All these products which should contain aromatic complexes are hardly attacked by microorganisms. During the humification the cellulose disappeared for the greater part, because this substance is attacked by bacteria.

Waksman (36), in one of his latest publications, is convinced that the questions of humification are not so simple as Grosskopf and others seem to imply. Waksman shows that it is very reasonable to accept the presence of ligno-protein complexes with variable chemical composition. These complexes are rendered resistant to further rapid attack of microorganisms. These complexes are the "humic acids", but he wishes to avoid the latter term, because of the confusing status of the entire terminalogy.

It should be mentioned in this connection that Bergius (3) has still an other conception of coal formation. He states that cellulose as well as lignin give rise to coal, but the way should be different for both matters. From cellulose humic acids may be formed as intermediary products and from lignin such products do not originate.

E. Discussion.

In the course of this investigation it appears that the

lignin from the cell walls of the duramen has partly disappeared and that in the cell lumina products have been deposited with characteristic properties of humic acids. From the literature a transition of lignin to humic acids seems to be possible, but it remains obscure in what way this transition takes place in the ebony-wood. It is possible that still other products participate in this formation. For instance it has appeared that in the duramen phlobaphenes are present and these could perhaps be transformed into humic substances (Hoppe-Seyler). Moreover, the constitution of lignin in general is still relatively uncertain and it is very well possible that the lignin of ebony-wood has again a somewhat other composition than the lignin of other plants. We must conceive of the formation of humic acids from lignin as an oxidation process. The disappearance of the lignin during the formation of duramen may be explained with the current ideas on the presence of complicated oxidation and reduction systems in the living cells.

In the wood parenchyma and medullary ray cells, which elements remain alive during the longest time, we meet substances which are still soluble in alkali. These matters are to be regarded as some of the first oxidation products. The lignin oxidises out of the walls and the oxidised products probably precipitate, perhaps as Ca-compounds in the cell lumina, because in the cell contents calcium is present (crystals of Ca-oxalate). In the wood non-"duramified" — the alburnum — we see already the appearance of this humification, which therefore may be regarded as the initial stage of "duramification".

While during this "duramification" the cells die, the oxidation goes on with full strength and more brown coloured substances are found, which become increasingly less-soluble in alkalies; but in the case investigated in this paper, the products from wood parenchyma and

medullary rays have not been transformed for the largest part so far that they are non-soluble in alkalies.

What is condition of the wood fibres and the vessels? In these elements we meet nearly exclusively with the substance insoluble in NaOH, which is dark brown to black, consequently a product already oxidised further and probably decarboxilated. How is it possible that this substance is present in the lumina of these elements? They do not contain calcium, so that the explanation which may be accepted for medullary rays and wood parenchyma does not apply. Still the lumina of vessels and fibres are filled with the dark mass while the walls have been left nearly uncoloured! It is not very clear, moreover, why in these elements the soluble reddish brown mass is not present anywhere. It might be possible that with the oxidation the first stages have been passed very quickly, or that a different process has taken place. The more the "duramification" proceeds, the darker the products become. Probably further decarboxilation and incomplete carbonization take place (to be seen very plainly in the black duramen of Diospyros ferrea).

Whatever, the intermediary and final products of this humification may be, these matters seem to be peculiar to the duramen of the Ebenaceae.

It appears acceptable to the author that the above-mentioned processes of "duramification" would be able to proceed so far that they give rise to products with a very high C-content, products which are identical with or in any case very closely related to the phytomelans of the Compositae which are also formed in the primary lamella of the cell wall. This idea has perhaps a support in the opinion of Fuchs that finally graphite should originate from humic acids and related compounds.

F. Summary.

1. The composition of the duramen and of the alburnum of ebony-wood have been compared with that of firwood.
2. In the duramen substances occur in the cell contents which are partly soluble and partly insoluble in alkali.
3. The substance soluble in alkali appeared to have the properties and the composition of a humic acid.
4. The "nitro"-products agreed in qualities with "nitro"-humic acid.
5. From a microscopic examination it could be demonstrated that the duramen behaved microchemically in another way than the alburnum. The lignin of the duramen has partly disappeared.
6. The humic substances in the cell contents might find their origin in the lignin that disappeared from the walls.
7. Due to the lack of good methods the determination of total humic matter did not succeed.

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