

FURTHER STUDIES ON THE STRUCTURE AND COMPOSITION OF VARIABLY DEGRADED ANCIENT WOODY TISSUES

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ABSTRACT

Ancient wood of *Heritiera fomes*, *Bischofia javanica* and *Picea excelsa* has been studied with reference to: chemistry of these woods and of their normal representatives in relation to anatomical degradation; structure and texture of the walls of tracheids and fibres and their chemical degradation; microscopic architecture of the cell-wall in terms of its sub-microscopic organization, and of its composition.

The cellulose in ancient wood is degraded and disappears relatively quickly, whereas lignin is largely retained. When cellulose is destroyed, its original trend of orientation is left in the form of texture in lignin. In some cases the original submicroscopic structure of cellulose is, however, well preserved. Probably the mineralization of the decaying cell-wall is mainly controlled by the surviving cellulose or the pattern it leaves in the texture of lignin.

INTRODUCTION

THE present investigation is one of a series concerning the organization of the cell-wall of fibres and tracheids, with general reference to their composition, in ancient wood. The state of preservation of different cell-wall constituents and their role in maintaining the physical structure or texture¹, or both, in ancient fibres and tracheids have been studied, and an attempt made to correlate the nature of the physical structure of the cell-wall with the various stages of its chemical degradation. The microscopic organization of the cell-wall has also been considered in relation to its submicroscopic structure or texture, or both, and to its chemical composition. The problem has also been considered from the point of view of fossilization.

The details of structure in normal wood fibres and tracheids have been admirably reviewed by Frey-Wyssling (1953, 1957), Preston (1952) and Northcote (1958), and the degraded fibres and tracheids have been considered by, among others, Bailey and Vestal (1937b), Barghoorn (1949a-1952), Müller-Stoll (1951), Schulze *et al.* (1938—

see HÄGGLUND, 1951), Sen (1948), and Sen and Basak (1955). Recently the physical (SEN, 1956) and chemical (SEN & BASAK, 1957) nature of ancient wood has also been reviewed.

According to Bailey and Berkley (1942) the use of the polarizing microscope and of X-ray diffraction patterns alone may lead to serious misconceptions regarding the orientation of cellulose in cell-wall layers, unless other morphological, histological, and chemical and other variables in plant materials are accurately examined and accounted for. That is why the present author has made use of all available methods for studying the structure of the ancient fibres and tracheids. Unfortunately the usually low and at the same time degraded cellulose content in some of the ancient fibres and tracheids does not always retain sufficiently the original structure for detailed investigations.

MATERIALS

The materials consist of (1) ancient wood samples of *Heritiera fomes* and *Bischofia javanica* collected from different localities by the present author from the alluvial clay lying immediately below the Calcutta peat layer, which is about 25 ft. below the surface soil, (2) variously degraded buried wood of *Picea excelsa* (about 450 years old) occurring below the ground water level, which formed timber pilings of buildings in Rotterdam (obtained through the courtesy of Dr. W. W. Varossieau, Delft, who made some microscopic examinations of this wood in 1949, and later along with Breger, he chemically investigated this and other materials from Rotterdam — 1952), and their normal representatives (used as control).

Additional materials of ancient wood collected from different sources are now being studied in detail. Most of the observations on such samples, some of which are recorded in this paper, confirm the findings presented in this paper.

1. 'Structure' refers to the crystalline fine cellulose structure of the plant cell-wall, whereas 'texture' is only the arrangement and distribution of some structural units.

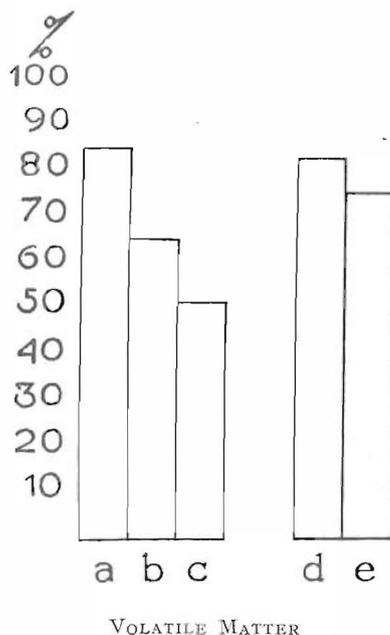
The materials are free from fungi or from visible effects of other micro-organisms. Such materials may show the nature of non-biologically or anaerobically degraded ancient fibres and tracheids.

MICROSCOPIC ORGANIZATION

The microscopic nature of ancient wood showing various stages of degradation has been studied by Bailey and Barghoorn (1942), Barghoorn (1949a-1952), Barghoorn and Bailey (1938), Barghoorn and Spackman (1950), Chowdhury (1953), Jahn and Harlow (1942), Sen and Basak (1955) and Varossieau (1949). Most of these authors are inclined to correlate the microscopical structural changes to the series of chemical as well as physical changes in the modification of the cell-wall. The present author follows this up with an attempted demonstration of the details of fine morphology, etc., in the surviving remnants of the ancient wood of *Heritiera fomes*, *Bischofia javanica*, *Shorea robusta*², and *Picea excelsa* as discussed in the subsequent sections of this paper. The following brief account, however, deals with the ordinary microscopic morphology of the degraded remains of these plants as occurring in Nature, so that such observations may help in their detailed studies.

The details of microscopic disorganization in the buried wood samples of *Picea excelsa* (FIG. 1) have been admirably described by Varossieau (1949) from whom the present author has got the material for further studies. In the ancient wood of *Picea excelsa* the central and the inner secondary wall layers are usually removed or disorganized, the persisting compound middle lamella (according to Kerr's & Bailey's, 1934, usage) are highly resistant. The residual thin wall or 'membrane' in such samples actually constitutes a five-layered structure comprising of the primary walls of contiguous cells, the outermost layer of their secondary walls, and the middle lamella in between the two such adjacent cells, as has already been observed by Barghoorn (1949a, b). Sometimes in a few cells of the late wood, all the wall layers are excellently preserved (FIG. 1). The inner portions of this buried wood, however, remain relatively well preserved so far as

2. The details about this material have appeared in another paper of this series (SEN & BASAK, 1955).



TEXT-FIG. 1 [a, *Heritiera fomes* (recent wood). b, *Heritiera fomes* (ancient wood from Calcutta peat — Madhyamgram site). c, *Heritiera fomes* (ancient wood from Calcutta peat — Jadavpur site). d, *Picea excelsa* (recent wood). e, *Picea excelsa* (ancient wood from Rotterdam)].

the lamellar organization of the cell-wall is concerned.

In the samples of the ancient wood of *Heritiera fomes*, the secondary wall layers of the libriform fibres are usually found displaced and occasionally totally disorganized (FIG. 2). It has not been possible to trace the order of decay of the various secondary wall layers. In *Bischofia javanica* masses of cell-wall residues, besides the infiltrating gummy substances, often accumulate within the cell cavities. However, this ancient wood, like that of *Shorea robusta*, has been found to be generally excellently preserved (FIG. 3).

It appears that the process of degradation "involves certain basic structural changes which appear to be similar in all plant tissues; these changes are directly related to basic physical and chemical features in the organization of the cell wall" (BARGHOORN, 1949a). The degradation is due to the loss of cellulose in sequence from the different cell-wall layers in the following order — (1) central and inner layers of the secondary

wall, (2) outer layer of the secondary wall, and (3) primary wall. From comparative studies on the decomposition of the plant cell-wall and certain tracheary features of the *Lepidodendrales*, it has now been established by Barghoorn and Scott (1958) that the primary walls, associated at times with the outermost portion of the secondary wall, are most resistant to decay. It is rather difficult to assign any definite reason to the selective decomposition of cellulose in the different cell-wall layers, but perhaps the protective nature of lignin and/or the intrinsic chemical nature of the cell-wall layers is chiefly responsible for selective degradation.

Possibly the remnant layers of the cell-wall subsequently undergoes mineralization for preservation as fossils. The present author is in agreement with Barghoorn (1949a, b), that mineralization may occur at a stage when cellulosic residues are still retained. This is apparent from the chemical and other physical data of some ancient wood (*vide infra*).

COMPOSITION OF ANCIENT WOOD

Wood and Peat Analysis — The literature on the chemical analysis of ancient wood has recently been reviewed (SEN & BASAK, 1957). It has generally been found that increasing degradation is usually accompanied by increase in ash, organo-soluble extractives and lignin, with the simultaneous decrease of cellulose and hemicellulose. Jahn and Harlow (1942), Varossieau and Breger (1952), and many others analysed ancient wood from different places, and arrived almost at the

same conclusion that their wood samples yield high percentage of apparent lignin chiefly due to the loss of cellulose. However, in a Miocene coniferous wood the hemicellulose content has been found not to differ greatly from that found in living sapwoods (JONES & MERLER, 1956).

Representative samples of normal and degraded wood have been selected for chemical analyses, and the materials have been prepared following the usual procedure. The moisture content of the ancient wood of *Heritiera fomes* and *Bischofia javanica* immediately after unearthing has been found to be about 80 and 78 per cent respectively. Both the wood samples lose moisture very fast as a result of the loss of water-holding capacity, which is possibly due to molecular simplicity. In *Heritiera fomes* the moisture content of thoroughly air-dried material is usually 10 per cent, that of normal *Heritiera fomes* being 12.3 per cent. This higher value in normal material is perhaps due to greater moisture-holding capacity. The moisture contents in relatively well-preserved ancient wood of *Bischofia javanica* (18.71 per cent) and *Picea excelsa* (10.06 per cent) are, however, greater than those of their control (16.9 and 8.13 per cent respectively).

It appears from the analytical data that cellulose and pentosans in the ancient wood of *Heritiera fomes* and *Bischofia javanica* (see TABLE 1), buried under compact sediments and apparently free from fungi or other micro-organisms, have possibly suffered from non-biological decomposition. The lignin in the ancient wood of *Bischofia javanica* has been left almost intact and in all cases it is

TABLE 1 — ANALYSIS OF ANCIENT AND NORMAL WOOD OF *HERITIERA FOMES* AND *BISCHOFIA JAVANICA**

ANALYSIS	ANCIENT <i>Heritiera fomes</i> (basis oven dry material)	NORMAL <i>Heritiera fomes</i> (basis oven dry material)	ANCIENT <i>Bischofia javanica</i> (basis oven dry material)	NORMAL <i>Bischofia javanica</i> (basis oven dry material)
Density (sp. gr.)	0.29	0.98	0.39	0.74
Volatile matter (%)	51.20	86.01	80.09	85.91
Ash (%)	4.62	2.31	3.02	2.10
Pentosans (%)	4.19	22.00	8.75	16.26
Lignin (%)	22.00	16.80	33.00	20.01
Cellulose (C & B) (%)	5.95	60.50	45.40	61.48

*Carried out at the Department of Applied Chemistry, University College of Science, Calcutta.

far more resistant to decay than the polysaccharides. Both lignin and cellulose of the two equally ancient samples, occurring in the same peat bed, are greatly different in their resistance to decay. This is significant.

These data are consistent with the findings of previous workers on ancient wood chemistry in that the lignin apparently increases due to the loss of the cellulose content. The pentosans are also considerably destroyed. Possibly the conditions in the Calcutta peat swamp were not generally favourable for the preservation of the polysaccharides specially in materials like wood of *Heritiera fomes*.

Some amount of lignin in the ancient sample of *Heritiera fomes* must have been converted into other substances; possibly the degradation products have accumulated as humic materials. The methoxyl content of the lignin of this ancient wood and peat is now being determined to find out the validity of this assumption following the generalizations of Varossieau and Breger (1952).

In *Picea excelsa* the amount of cellulose progressively decreases from normal to ancient wood (see TABLE 2) as in the case of hard wood species. The visibly decomposed peripheral tissues of the ancient material of the conifer show further loss of cellulose, but the

lignin content slightly rises due to decrease of the original cellulose (see TABLE 2). Similar tendencies in buried spruce wood have also been noted earlier (GORTNER, 1938; GROSSKOPF, 1929 — see VAROSSIEAU & BREGER, 1952). The pentosan content, however, gives almost constant values. Possibly it is decomposed very slowly (VAROSSIEAU & BREGER, 1952). Recalculation of analytical figures are not likely to give any result of further significance.

The density of all the wood samples has been determined following the procedure adopted by Jahn and Harlow (1942). The density value of the ancient materials is expressed as grams of dry wood substance per cubic centimetre of space occupied by the wet tissue. This value needs comparison with the density of normal wood, which is the weight in grams of dry wood substance per cubic centimetre of the fresh tissue. The density values of normal wood are always considerably higher than those of the ancient ones (see TABLES 1 and 2). This is quite expected.

A number of samples of ancient and normal wood of *Heritiera fomes*, *Bischofia javanica* and *Picea excelsa* have been made free of moisture, and their volatile content determined (see TABLES 1 and 2).

The usual higher values of volatile matter in normal wood and their decline in ancient representatives may be directly correlated with the relative abundance of cellulose in such materials, the normal wood being always richer in cellulose than the ancient ones (TEXT-FIG. 1). The extent of general anatomical preservation is, as expected, inversely related to reduction in volatiles and, therefore, of cellulose. Similar correlations have also earlier been noted by Barghoorn and Spackman (1950).

An increase in the ash contents in all the samples of ancient wood (see TABLES 1 and 2) is the result of the accumulation of mineral and other extraneous substances due to infiltration or like processes. This tendency of increase in ash in ancient wood has been previously noted by many workers. The accumulation of mineral matters, etc., appears to be natural in course of fossilization, and perhaps the ancient woods under investigation are at some early stages of fossilization. This possibility has also been indicated, with evidence, in the subsequent chapters.

The chemical investigation of the Calcutta peat matrix, in terms of coal petrology, is

TABLE 2 — ANALYSIS OF ANCIENT AND NORMAL WOOD OF *PICEA EXCELSA*

ANALYSIS	ANCIENT <i>Picea excelsa</i> (basis oven dry material)	APPARENTLY SOUND CORE OF ANCIENT <i>Picea excelsa</i> (basis oven dry material)	NORMAL <i>Picea excelsa</i> (basis oven dry material)
Density (sp. gr.)	0.29	0.40	0.43†
Volatile Matter (%)	75.01	82.51	84.92
Ash (%)	3.52	1.57	0.77†
Pentosans (%)	{ 11 ca*	11.01	{ 11.30
Lignin (%)	{ 31 ca	28.07	{ 28.29
Cellulose (C & B) (%)	{ 45 ca	50.02	{ 63.95

*From Varossieau and Breger (1952). The data of these authors are quite consistent with the similar estimations made in the present author's laboratory, and, therefore, the already published data have been shown.

†From "Chemistry of Wood" by Hägglund (1951).

TABLE 3—COAL ANALYSIS: CALCUTTA PEAT*

		AIR DRIED	DRY	REMARKS
Proximate analysis	Moisture	13.2	Nil	Colour of ash: brick red
	Ash	33.4	38.5	
	Volatile matter	35.5	40.9	
	Fixed carbon	17.9	20.6	
	<i>Total</i>	<u>100.0</u>	<u>100.0</u>	
	Calorific value in B.t.u./lb.	4918	5666	
Ultimate analysis	Carbon	30.15	34.74	Hydrogen in water of hydration is not subtracted
	Hydrogen	2.55	2.94	
	Sulphur	3.43	3.95	
	Nitrogen	1.45	1.67	
	Phosphorus in coal	0.0316	0.364	

*Carried out at the Fuel Research Institute, Jealgora, Dhanbad.

primarily interesting as fuel analysis (see TABLE 3). It is difficult to correlate these analytical results with the source materials, since only a few of them (e.g. *Heritiera fomes*, *Bischofia javanica*, *Pandanus* sp., etc., *Oryza quartata* and some species of grasses) have been known. The application of the broad generalization on the Brandon lignite made in this direction by Barghoorn and Spackman (1950) does not appear to be of much value in this study. But the high sulphur content of the peat indirectly shows the possibility of direct chemical degradation of cellulose in *Heritiera fomes*, where the cellulose content is low and degraded (see TABLES 1 and 4).

Nature of Cellulose — The loss of cellulose in ancient wood samples appears to be a universal feature, and the residual cellulose that persists in such materials has usually been found to be degraded (BARGHOORN & SPACKMAN, 1950; JAHN & HARLOW, 1942). To demonstrate the degradation of cellulose in the ancient wood samples under investigation, the copper number of these samples and their normal (and recent) representatives (as control) has been determined.

The copper number data (see TABLE 4) are very consistent with reasonable expectations. The higher values of copper number in the ancient materials, as compared to their recent representatives, indicate their gradually increasing reducing power resulting from the formation of numerous shorter chains of cellulose, each possessing a reducing end group. It is, therefore, evident that the cellulose in the ancient samples suffers both

TABLE 4—COPPER NUMBER OF CELLULOSE IN REPRESENTATIVE SAMPLES OF ANCIENT AND NORMAL WOOD OF *HERITIERA FOMES*, *BISCHOFIA JAVANICA* AND *PICEA EXCELSA*

MATERIAL, CELLULOSE (C & B) OF:	COPPER NUMBER
Ancient <i>Heritiera fomes</i>	10.60
Recent <i>Heritiera fomes</i>	4.05
Ancient <i>Bischofia javanica</i>	7.92
Recent <i>Bischofia javanica</i>	5.35
Ancient <i>Picea excelsa</i>	6.51
Ancient <i>Picea excelsa</i> (apparently sound core)	4.04
Recent <i>Picea excelsa</i>	3.02

loss and degradation, and the extent of such damages has been considerable in the ancient wood of *Heritiera fomes*.

It is perhaps necessary to point out that any comparison of the results of chemical analyses and copper number determination of ancient wood samples with their normal representatives, as the foregoing, collected from widely different sources, should always be made largely in empirical terms. Therefore, such analytical data may only be used for broad comparison with great caution.

Some Other Aspects of the Chemical Nature of Ancient Wood — Only a few aspects giving unambiguous results have been considered. Water at ordinary temperature does not appear to chemically react with sound wood.

The degraded wood which has possibly already suffered from hydrolytic action of water, however, loses very negligible weight in cold water after prolonged treatment. In hot water the hydrolytic actions are readily accelerated specially in degraded wood. These are all indications of decay specially in the ancient wood of *Heritiera fomes*. This is associated with lower resistance and molecular simplicity (FREEMAN, 1946).

The wood decay is accompanied by an increase in solubility in 1 per cent NaOH. This is probably due to easy delignification of partially degraded lignin-rich cell-wall, which has been chemically simplified. Conversely, ancient wood samples are less soluble in cellulose solvents, e.g. sulphuric acid, monoethanolamine and Schweitzer's reagent, than their normal representatives. This is due to lower cellulose content in ancient wood. Many previous authors obtained similar nature of results as recorded by the present author.

Mäule test gives a positive reaction, which is rather easily visible in patches, where the wood of ancient *Heritiera fomes* has retained the colour of freshly exposed surface. This appearance of positive reaction in patches may be due to either of the following alternatives: (1) some sort of selective degradation of lignin in portions which cannot produce positive reaction, (2) portions of the wood not showing apparently visible positive reaction are already black (without any trace of freshness) and as such possibly mask the positive reaction even if it is effected. The test is strongly positive in case of the ancient wood of *Bischofia javanica*. But the ancient wood of *Picea excelsa*, as expected, does not react positively to Mäule test. Whatever may be the cause of Mäule reaction, it is apparent that angiosperm lignin reacts to it even when the hardwoods are in advanced stages of decomposition.

It has been found that newly unearthed ancient wood of *Heritiera fomes* looking like freshly exploited timber often turns black on drying during exposure to air. Erdtman (1943) has ascribed similar dark colouration as due to the oxidation of phenols, and the colouring matter appears to be similar to the artificial phenol-humic acids. The phenols are oxidized and produce phenol-humic acids, to which the characteristic black colour of the exposed ancient wood of *Heritiera fomes* is possibly due. These substances are now being identified.

STAINING AND MICROCHEMICAL TESTS

Thin transverse and longitudinal sections were prepared from a number of pieces of ancient wood of *Heritiera fomes*, *Bischofia javanica* and *Picea excelsa*, and from the corresponding normal and sound wood of these species when necessary. Then they were variously studied using some common microchemical reagents and stains. The limitations of these tests are obvious, because "cell-wall chemistry can hardly progress beyond the point where the effect of the reagents used is known qualitatively at least by workers in wood chemistry" (HARLOW, 1946).

The Possibility of the Presence of Pectin in the Middle Lamellar Region — According to the widespread practice of staining pectin in the middle lamella with aqueous Ruthenium red (1:10,000), for which phenomenon the uronic carboxyl groups must be held responsible, the freshly cut sections of the ancient wood of *Picea excelsa*, *Bischofia javanica* and *Heritiera fomes* have been treated in this reagent. The staining of the previously untreated sections of *Picea excelsa* does not show any specific selection of the dye by a particular layer, because only the delignified cell-walls reveal the presence of pectin (ONSLow, 1931). All the wall layers, including the middle lamella, are coloured. This may possibly also be due to the presence of some fractions of oxycellulose produced during the decomposition of the original cellulose in different cell-wall layers. It has been said that oxycellulose has a strong affinity for Ruthenium red (KERR & BAILEY, 1934), and perhaps that is why all the wall layers are generally stained. In any case the Ruthenium red test should not be considered as a reliable evidence for the presence of pectin.

The pretreatment of a few sections of the ancient wood of *Picea excelsa* with dilute acid and alkali produces the desired effect, possibly with the removal of the suspected oxycellulose (?), and partial delignification. The stain is mostly restricted to the so-called compound middle lamella of Kerr and Bailey (1934) when the secondary cell-wall layers are preserved intact (FIG. 4). Sometimes the stained compound middle lamella tends to blend with the outer secondary layer. The compound middle lamella in *Bischofia javanica* behaves almost similarly. It appears that a pectin-like substance may

possibly be present in the middle lamella of the ancient wood of *Picea excelsa*. The presence of pectin in ancient wood has also been previously demonstrated by Yasui (1925).

In the ancient wood of *Heritiera fomes*, however, such demonstration of pectin or like substance is more difficult due to the usually displaced and partially disorganized wall layers; but when the net of the middle lamella is recognizable, it always exhibits a very strong affinity for Ruthenium red usually after very slow and careful pre-treatment. Unfortunately it has not been possible to produce any desirable contrast between the intercellular substance and other cell-wall layers in the photograph. This is possibly due to the natural dark brownish colour of the cell-wall layers (FIG. 5).

Nature of the Secondary Wall — The secondary walls of the normal wood of *Heritiera fomes*, *Bischofia javanica* and *Picea excelsa* swell violently in 72 per cent sulphuric acid, and gradually tend to disintegrate into thread-like fragments of varying sizes. The ancient woods of *Picea excelsa* and *Bischofia javanica* also show swelling in sulphuric acid, because of the presence of sufficient amount of cellulose.

When the sections of the ancient wood of *Picea excelsa* are carefully delignified in 5 per cent chromic acid and subsequently stained in iodine, followed by treatment in 72 per cent sulphuric acid, cellulose walls of intact cells are greatly swollen and stained violet. The cellulose reaction is also faintly visible irregularly in some disorganized cell-walls (FIG. 6). The reaction is visibly absent in similarly treated sections of the ancient wood of *Heritiera fomes*. The reactions are indicative of the relative abundance of cellulose in the cell-wall layers of different ancient wood under investigation.

The lignin patterns, which may be produced by careful treatment with 72 per cent sulphuric acid (following DADSWELL, 1931 — see WARDROP & DADSWELL, 1950), are remarkably similar in the normal and ancient wood of *Picea excelsa*. In both, the patterns are concentric (FIGS. 7, 8) as in the ancient wood of *Pinus sylvestris* (SEN & BASAK, 1955). The persistence of original lignin pattern in ancient wood is probably due to the very good state of preservation of lignin. By careful chlorination of transverse sections of *Bischofia javanica* similar concentric pattern (FIG. 9) may be obtained due to the removal

of lignin. Unfortunately, the fibres of *Heritiera fomes* fail to produce any clear pattern even after repeated trials.

Jahn and Harlow (1942) employed bromination-alkali method and found that the secondary cell-walls in ancient beech stakes are chiefly composed of lignin. But the variably degraded samples of the three species of ancient wood investigated by the present author, however, behave differently to the reagents used by Jahn and Harlow (1942), obviously because of their varying lignin : cellulose ratio. Usual bromination of sections of *Heritiera fomes* followed by washing and subsequent treatment with 10 per cent ammonia results in the complete dissolution of the secondary wall (FIG. 10). Chlorination appears to be more effective in that relatively brief treatment alone clearly removes the secondary wall layers, etc., of *Heritiera fomes*, usually leaving a network of middle lamella. It is, therefore, very apparent that the secondary cell-walls of *Heritiera fomes* are highly rich in lignin. The secondary cell-walls of the ancient wood of *Bischofia javanica* are partially removed by rather prolonged chlorination (FIG. 9) indicating thereby that possibly the cellulosic framework of this well-preserved wood is relatively less resistant compared to ancient *Shorea robusta*, *Picea excelsa* and all control samples. This shows that at least some species of ancient wood have retained relatively more resistant cellulose in the secondary wall (as is also evident from other methods), and that the control samples behave according to reasonable expectation.

The staining method evolved by Coppick and Fowler (1939) (a modified Tollens reaction) very nicely helps to locate the relative abundance of lignin in cell-wall layers. "Structures high in lignin are stained dark brown to black, whereas lightly lignified areas are pale yellow to amber coloured." The method consists of chlorination (when reducing substances are formed from the lignified tissues) followed by treatment in 5 per cent aqueous silver nitrate (when silver is deposited in the lignified areas).

A few transverse sections of ancient *Heritiera fomes* are chlorinated and subsequently treated in silver nitrate solution, as a result of which the compound, middle lamella and the remnants of secondary wall turn dark brownish and light brownish respectively. Hence the cell-wall layers are all expected to be more or less heavily lignified. The

technique has been more successfully employed in studying the lignin distribution in the cell-wall layers of the ancient wood of *Picea excelsa*. The cell-wall layers, on treatment, turns into brownish to black specially in the disorganized layers of the late wood and the compound middle lamellar zone. When the secondary cell-wall layers are left intact, as in some portions of the late wood, the central layer looks light brownish often with shades of yellow; hence intact central secondary wall layers and similarly lightly coloured portions are relatively less rich in lignin possibly due to the retention of cellulosic and other substances. In ancient *Bischofia javanica* the secondary layers are also similarly rich in cellulose and relatively poor in lignin. This fact has since been substantiated by other chemical and physical methods.

The effect of phloroglucinol in hydrochloric acid on the sections of ancient wood of *Picea excelsa* is rather interesting, because on treatment with this reagent all wall layers including the middle lamella turn a deep pink. But when the secondary wall layers of late wood are left intact, the central and inner layers are recognizably less purplish, which indicates proportionately lesser amount of lignin in these wall layers. The sections of naturally brownish (often with reddish shades) ancient wood of *Heritiera fomes* and *Bischofia javanica* do not, however, show marked visible reaction on treatment with phloroglucinol in hydrochloric acid. Probably the reaction has been mostly masked by the natural colouration of the wood.

The excellent colour reaction (yellow) produced by Aniline sulphate with the lignified cell-wall of ancient wood of *Picea excelsa*, *Bischofia javanica* and *Heritiera fomes* is highly convincing. The intensity of the reaction is throughout uniform in the sections of ancient wood of *Bischofia javanica* and *Heritiera fomes*, and as such the relative abundance of lignin in the different cell-wall layers of fibres cannot be located. In a transverse section of the ancient wood of *Picea excelsa* the lignin reaction is relatively weak in the secondary layers of the late wood (FIG. 11). This is possibly due to the presence of more of cellulosic and other non-lignin substances in the secondary walls of the late wood.

Coppick's and Fowler's (1939) comparative study of the progressively delignified white spruce tissues prove the importance of a number of reagents as useful indicators

of the extent of lignification and/or the presence of cellulosic substances. This procedure could not, however, be followed by the present author, as the pre-chlorination of all the preparations would inevitably destroy the almost exclusively lignified cell-walls of ancient *Heritiera fomes*.

The present author's observations of the comparative effects of phloroglucinol, the Mäule test, and 72 per cent sulphuric acid may be summarized in the words of Bailey and Kerr (1937) as quoted by Harlow (1946) — "In the wood of both gymnosperms and angiosperms, walls or layers which persist as coherent structural residues upon treatment with strong mineral acids usually give an intense coloration with phloroglucin-HCl; whereas those which disintegrate commonly do not, although they may give a strongly positive coloration with either the Mäule test or the chlorine-sodium sulfite reaction." The last reagent has not been used by the present author. Coppick's and Fowler's (1939) chlorination-silver nitrate technique has been found remarkably useful in demonstrating and locating lignin in the cell-wall layers of ancient wood.

STRUCTURE OF ANCIENT TRACHEIDS AND FIBRES

Fibrillar Organization — Since the ancient wood fibres of *Heritiera fomes* and the ancient tracheids of *Picea excelsa* are not sufficiently suitable for employing Wardrop's and Dadswell's (1948) modification of Herzog's technique for revealing fibre structure, the ordinary drying technique has been adopted by the present author for studying the apparent residual fibrillar texture (principally of lignin in *Heritiera fomes*) of these ancient wood elements. The orientation of the microscopic structural units producing the apparent fibrillar texture, which often appears even after the cellulose degradation, is likely to reveal the original patterns of cellulose chain directions. Usually the patterns in the central secondary layers can be shown effectively by this method.

Unfortunately it appears that the residual substances have suffered variable degradation in the same sections, and often in the same fibres or tracheids. This has made it difficult to assign any type of specific orientation to the fibrillar texture or to measure the angle of these orientation with confidence. It is clear that the fibrillar texture of the adjacent

cells in *Heritiera fomes* shows different degrees of orientation; the narrower cell showing highly steep and more or less regular spiral orientation, whereas the fibrillar texture in the broader cells is more nearly parallel to the fibre axis (FIG. 12). A more difficult situation has been created by the texture of the ancient tracheids in *Picea excelsa*. Some of these tracheids show texture of highly steep spiral fibrillar orientation (FIG. 16) of varying degrees, but there are others which exhibit highly degraded and disoriented stages.

Wardrop's and Dadswell's (1948) technique for crushing of fibre and tracheid sections has been employed for studying the fibrillar orientation in well-preserved wood samples of *Bischofia javanica*, *Shorea robusta* and apparently sound core of *Picea excelsa*. In *Bischofia javanica* and *Shorea robusta* excellent crossed spiral has been found, the fibrillar orientation being at 19°-20° and 25°-26° respectively to the longitudinal cell axes (FIGS. 13, 15). In *Bischofia javanica* the orifices of pits and the spiralling of a wall layer lying in another plane are also photographed together, which are at right angles to each other (FIG. 14). This is due to the considerable focal depth of the objective used. The angle of the spiral slopes is 22° in the apparently sound core of the ancient wood of *Picea excelsa*.

The crushing and swelling of the wood elements, as above, do not appear to appreciably alter the orientation of the cellulose within the cell-wall excepting in *Shorea robusta*, because it is held that the major extinction position usually does not change ordinarily by more than 5° during crushing and swelling treatment (WARDROP & DADSWELL, 1948).

Optical Properties — It is rather difficult to interpret the optical nature of the ancient tracheids and fibres in view of the unsatisfactory birefringent measurements of the optically irregular cell-wall layers. The difficulties in getting correct measurements of cell-wall birefringence and m.e.p. determinations are primarily due to (a) variably degraded cell-wall layers giving erratic results in case of visibly decomposed ancient *Picea excelsa* (FIG. 18), (b) form birefringence in ancient *Heritiera fomes* which is due to the presence of imbibition liquid (FIG. 17) and (c) that the degraded walls and wall layers are not always clearly distinguishable. The ancient materials of *Picea excelsa* may be studied rather qualitatively, i.e. the visible differences in the optical behaviour of the

various cell-wall layers in the transverse sections may simply be interpreted in terms of general differences in micellar arrangement within them. But the apparently sound core of the ancient sample of *Picea excelsa* (FIG. 18) and the samples of ancient *Bischofia javanica* and *Shorea robusta* exhibit almost characteristic optical heterogeneity when viewed between the crossed nicols.

It is now known that the secondary cell-wall of fibres and tracheids is organized in a series of three coaxial micellar spirals such that the outer and inner spirals are flatter than the central micellar spiral separating them (BAILEY & VESTAL, 1937a; WARDROP & PRESTON, 1947; WARDROP & DADSWELL, 1948). The anisotropy of the outer and inner secondary wall layers in transverse sections of wood ordinarily indicates that the cellulose micellar chain directions therein are flatter with reference to the long axis of the cell than the chain directions in the isotropic central layer; the optical properties in the cell-wall layers in the samples of ancient wood may be interpreted accordingly.

Jahn and Harlow (1942) have observed that the transverse sections of the ancient beech wood for the most parts fail to transmit polarized light, but in scattered patches the outer secondary layer showed birefringence. In the ancient fibre of *Heritiera fomes* also the composite wall layers (the individual layers appearing indistinguishable), as already described, are weakly birefringent due to form birefringence of the outer secondary layers. The birefringence is so weak that it takes long exposure for successful photographic reproduction. The so-called birefringence of the indistinguishable wall layers gradually disappears after about two months.

It is, therefore, clear that the apparent fibrillar texture in ancient fibres of *Heritiera fomes*, and their temporary form birefringence are produced mostly by non-cellulosic substances. In ancient *Heritiera fomes* the pictures thus obtained may only refer to micellar texture which gives information about the arrangement of the residual elements in the material, in contrast to the micellar structure which characterizes the fine structure of gels in general. The original structures of the wall layers in *Heritiera fomes* do not persist, but they have left their texture in the highly resistant lignin. This conclusion finds support in some previous observations made by the present author (SEN, 1955a).

But the original wall structure of at least the outer layer of the secondary cell-wall has been found preserved in the ancient wood samples of *Bischofia javanica* and *Shorea robusta*, which are truly anisotropic in transverse sections. This is consistent with other available data on these materials. In transverse sections the birefringence of the outer layer of the secondary wall excepting at the position of extinction, which merges with the primary wall, indicates the presence of flatter micellar spirals with reference to the long axis of the cell in this layer. The isotropic central layer shows the presence of steep orientation of cellulose micelles. The presence of an inner layer has been rendered generally obscure by degradation, and consequent loss of birefringence.

The tracheids from 450 years old buried wood samples of *Picea excelsa* appear to have retained some of their original cellulosic structure as evident from most of the chemical and physical methods of study. It is usually difficult to optically distinguish the five-layered wall of the early wood (consisting of two birefringent outer secondary layers, and primary walls of each of the two adjacent cells, and the compound middle lamella — isotropic — in between). In most of the cases the other secondary layers are found in collapsed conditions, but when preserved in the late wood, sometimes they exhibit optical heterogeneity in transverse sections, which is characteristic in their recent and normal representatives and the apparently sound core of the ancient wood of the same species (FIG. 18). The outer (indistinguishable from the primary wall) and inner secondary wall layers are characteristically birefringent, excepting at the position of extinction, indicating the presence of flatter micellar spirals, and the isotropic central layer denotes the presence of relatively steep spirals. The birefringence of the inner layer of the secondary wall, when present, is weaker than the outer layer of the same cell. The outer layer is more prominently birefringent possibly because it forms, together with others, an indistinguishable five layered structure. The relatively weak and rather irregular birefringence of the inner secondary wall layer is possibly due to variable degradation which often starts from this layer. As has been stated earlier in this chapter, only broad generalization as to the cell-wall structure in ancient wood is possible.

Texture Pattern in Dark-field with Spierer Lens — It has earlier been demonstrated by Thiessen (1932) that the microscopic organization in cellulose fibres, wood fibres, etc., may be brought out with contrast in dark-field by the Spierer lens. The structural and/or textural units of the organic fibres, thus revealed, have since been designated as super-micelles (SEIFRIZ, 1936). According to Thiessen (1932), these super-micelles are characteristically arranged in cotton, ramie and wood fibres, and verify the structure postulated by X-ray method. In some cases, however, the structural units involved in the two methods of study are different (SEN, 1956b).

The Spierer pictures of the longitudinal sections of the ancient wood of *Heritiera fomes*, already subjected to natural decay, show microscopic super-micelles, which are regularly arranged parallel to the long axes of the cells as in the corresponding sound materials (FIG. 19). In the ancient tracheids of *Picea excelsa* the helical pitch of the super-micelles is generally very steep, sometimes appearing almost parallel to the long axes of the cells (FIG. 20). The orientation of these microscopic units like those in the normal materials can, therefore, survive the general degradation of wood. The contrast pictures of *Heritiera fomes* also show regular line-up in the secondary walls (SEN, 1955a). The visibly irregular patches of super-micelles are due to strain.

The loss of cellulose and enrichment of lignin in wood produces better super-micellar pictures (SEN, 1955a; THIESSEN, 1932). So it is chiefly lignin that preserves the original architectural plan of wood when the cellulosic structural framework is gradually destroyed. That is why the cellulose rich materials like *Bischofia javanica* and *Shorea robusta* do not produce clear Spierer pictures.

X-ray Analysis — Small pieces of variously degraded untreated sapwood samples of ancient wood of *Heritiera fomes* (from different localities), *Bischofia javanica*, *Shorea robusta* and *Picea excelsa* (and its apparently sound core) (about 0.5 mm. thick) have been thoroughly washed in distilled water and subsequently air-dried, and exposed to the X-rays. The sample to film distance is 3 cm., using Cu target. The photographs are taken in a cylindrical camera with the X-ray beam normal to the fibre and tracheid axes of the tangential sections. The general background scattering is due to extraneous

matter accumulated during long burial and the lignin.

The X-ray examination of ancient fibres has been previously done by Jahn and Harlow (1942) and Sen and Basak (1955) almost according to the same method as adopted by the present author. Jahn's and Harlow's (1942) X-ray photograph of ancient beech stake from the Boylston Street Fishweir, U.S.A., contains cellulosic substance in a non-oriented arrangement with reference to the fibre axis. The 002 interference in the ancient beech is a weak, but sharp, continuous ring. Similarly, in the X-ray diffraction photographs of visibly decomposed wood of ancient *Picea excelsa* and *Heritiera fomes* (from Madhyamgram site), there are slightly blurred appearance of the (002) reflections of the lattice of native cellulose. The marked loss of orientation and also broadening of the lines are clear (FIGS. 22, 24). It is certain that the degradation process has decreased the size of the crystalline domains and at the same time destroyed their orientation.

But the X-ray diffraction photographs of apparently sound core of the ancient wood of *Picea excelsa* (FIG. 25) and *Shorea robusta* (previously studied by SEN & BASAK, 1955) show very clear preferred orientation of the cellulose micelles. In the latter the spreading of the equatorial arcs indicates a micellar spiral angle of about 16° with reference to the long axes of the cells. The micellar angles are slightly greater in *Picea excelsa*. Both these samples of ancient wood are anatomically nicely preserved showing thereby that the cellulosic framework is primarily related to tissue preservation. Similarly the ancient wood of *Bischofia javanica*, showing good preservation of tissues, produces only slightly blurred preferred X-ray diffraction pattern with greater spreading of the equatorial arcs (FIG. 23).

It is rather interesting to note that there is a great difference between the X-ray diffraction patterns of the apparently sound core of the ancient log of *Picea excelsa* and its considerably degraded peripheral portions (FIGS. 24, 25). The amount of cellulose contents in them differs by about 5 per cent. The cellulosic structural framework has been mostly broken down in the visibly decaying wood, possibly both by mechanical action and, oxidation, whereas the structure has been excellently preserved in the apparently sound core of the same wood of *Picea excelsa*. The difference in the reducing properties of

cellulose in the two samples of variously preserved ancient wood of *Picea excelsa* bears additional testimony to the assumption that the cellulose in the visibly decaying wood has been more degraded (see TABLE 4). It is the state of preservation of cellulose rather than its amount, which is responsible for maintaining the structural framework of the cell-wall.

Unfortunately in the X-ray diffraction photograph of ancient wood of *Heritiera fomes* from Jadavpur site (FIG. 21) there is almost no trace of cellulose left in it. The chemical analysis of the Jadavpur material reveals the presence of a small fraction (see TABLE 1) of highly degraded cellulose (see TABLE 4), which is evidently not clearly reproducible in the X-ray diffraction photograph. Besides there is a rather distinct ring of some inorganic matter which is not oriented. It would, therefore, appear that the original submicroscopic structure of the wood has been very strongly destroyed in these samples.

It has already been pointed out that the cellulose is mostly destroyed in the ancient wood of *Heritiera fomes* from Jadavpur site (FIG. 21) unlike that in similar materials collected from Madhyamgram site (FIG. 22). The cellulose is abundantly preserved and oriented in the equally ancient wood of *Bischofia javanica* (FIG. 23), unearthed from the same clay bed below the Calcutta peat layer wherefrom variably degraded samples of *Heritiera fomes* have been collected. In still more ancient wood of *Shorea robusta* collected from an archaeological site near Patna in India, the cellulose has been found to be very well oriented. Therefore, it is apparent that local factors of preservation, intrinsic nature of wood, etc., are responsible for this variable preservation of equally ancient wood. As has been indicated, even older wood samples may be better preserved than younger ones.

Possibly the inorganic ring in the X-ray diffraction photograph of *Heritiera fomes* (from Jadavpur site), and scattered spots in that of *Shorea robusta*, indicate the accumulation of extraneous matter, which possibly had permeated the tissues in course of fossilization and effected 'fixation' of structure.

DISCUSSION

By employing different techniques in the present investigation it has been possible to correlate the results obtained by diverse

methods of study, and to interpret the microscopic feature of the cell-walls in ancient wood in terms of their submicroscopic structure or texture, or both, and of their chemical composition. The degradation and the loss of cellulose in greatly affected materials have resulted in non-oriented and blurred cellulose X-ray diagrams (FIGS. 21, 22, 24) and gradual loss of characteristic birefringence. As such the persistent fine morphology in highly degraded ancient wood elements, which is probably mostly retained by lignin, can mainly be inferred from the microscopic examination. However, when the ancient materials are well-preserved and retained a good amount of original native cellulose, they produce well-oriented X-ray diffraction diagrams (FIGS. 23, 25). The wonders of their submicroscopic morphology are thus revealed with accuracy.

The results of chemical analyses of the normal and ancient wood of *Heritiera fomes*, *Bischofia javanica* and *Picea excelsa*, and the microchemical reactions, etc., of these species of ancient wood are generally consistent with the X-ray and other optical evidence. All this evidence clearly shows that very little cellulose has been preserved in the ancient wood of *Heritiera fomes*, whereas structurally preserved cellulose is present in the ancient wood of *Bischofia javanica* and apparently sound core of *Picea excelsa*. The X-ray diffraction photographs of the two variously preserved samples of *Picea excelsa* are widely different (FIGS. 24, 25), but surprisingly the difference in the quantity of cellulose contained in them is about 5 per cent (see TABLE 2). The cellulose of the visibly decaying wood samples has been strongly destroyed. This is also evident from the copper number data (see TABLE 4). It is apparent that the preservation of the structural framework of cellulose does not depend upon the amount of cellulose present in the material.

The possibility of the persistence of the original micellar architecture may be questioned in ancient materials where the cellulosic framework is destroyed. It has been demonstrated by the present author in this and in some other papers (SEN, 1955a, 1956a), and earlier by Thiessen (1932), that in all probability the persistent lignin records the original cellulose distribution pattern. Thus a micellar texture is retained in which the general arrangement of the original structural units is preserved mainly by the persistent

lignin. Obviously the original micellar structure has been mostly destroyed in seriously degraded materials. The apparent demonstration of fibrillar orientation in the ancient fibres and tracheids of *Heritiera fomes* and *Picea excelsa* (FIGS. 12, 16), is, therefore, mainly produced by the micellar texture preserved by the persistent lignin. The ancient tracheids of *Picea excelsa* may, however, retain the original structure to some extent as it appears from the accumulated data. The original fine structure may also be excellently preserved, e.g. in the apparently sound core of ancient *Picea excelsa*, *Bischofia javanica* (FIGS. 23, 25) and *Shorea robusta*, where the original fibrils are also naturally present. A sort of fibrillar texture was previously noted even in petrified wood (ZIMMERMANN, 1953).

The transverse section of an ancient late wood tracheid of *Picea excelsa* between the crossed nicols, when preserved with all the layers, indicates the presence of a steep spiral in the central isotropic secondary layer (as also exhibited by the fibrillar texture) and relatively flatter spirals in the birefringent layers. But unfortunately it is difficult to accept such a clear picture for the structure of the ancient tracheid of *Picea excelsa* in view of the weak and sometimes irregular birefringence and the degraded nature of the cellulose. In fact the X-ray diffraction photograph of the ancient wood of *Picea excelsa* exhibits the presence of only strongly destroyed native cellulose consisting of highly non-oriented short micellar units. But the texture appears to be preserved. The ancient fibres of *Heritiera fomes* (from Jadavpur site) do not exhibit truly birefringent cell-wall layers in sections (FIG. 17), and consequently hardly produce any trace of cellulose in the X-ray diffraction pattern. In similar wood from Madhyamgram site, the presence of cellulose is, however, detectable, but it does not transmit polarized light. The cellulose has been found strongly destroyed. Therefore, these fibres do not retain any original structure. Only the texture is preserved in lignin. But the cell-wall layers in the other samples of ancient wood are often birefringent, which under certain conditions may be an indication of the preservation of cellulosic framework.

It is, difficult to account for the high longitudinal shrinkage in the ancient wood of *Picea excelsa*. There is no trace of fungus in this material, which might have caused the

shrinkage cracks. According to Varossieau (1949) these cracks are believed to be due to the lower cellulose content of the outer layers and the irregular distribution of cellulose in these and successive layers brought about by the removal of this component.

Greater orientation of the micelles in the tension wood of *Eucalyptus regnans* than that in the normal wood, which is perhaps due to the observed longitudinal shrinkage of the tension wood has been demonstrated by Wardrop and Dadswell (1948). It appears, therefore, that the longitudinal shrinkage of certain fibres and tracheids, at least up to a stage, may result in still greater orientation. The shrinkage properties of tension and ancient woods are as such directly related to the fibre and tracheid structures.

In general there is a high longitudinal shrinkage and low lateral shrinkage in specimens in which the micellar angle is large and the reverse of this when the micellar angle is small. The ancient, tension and similar type of woods in question appear to be exceptions to this, or, rather, they are cases in which a different set of factors predominate. Thus, in flax and ramie in which the micellar angle is very small, and relatively little lignin is present in the cell-wall, the longitudinal shrinkage is about one per cent. The wood in question may be similar, i.e. the composition (if this is the cause) appears to override the influence of micellar orientation, but there is no proof of this in any case. Unfortunately it is difficult to correlate a micellar texture to the shrinkage properties of the ancient wood of *Picea excelsa*.

The lignin patterns of ancient wood are often excellently preserved. In *Picea excelsa* the patterns are strikingly concentric (FIG. 8) as in their corresponding normal representatives (FIG. 7). When lignin is apparently removed by chlorination, the pattern is also concentric in the ancient wood of *Bischofia javanica* (FIG. 9). This observation is not necessarily in conflict with the opinion of Bailey and Kerr (1935) that the lignin is a continuous matrix. It has been suggested by Traynard *et al.* (1954) that the residual cellulosic material in the delignified sections may still contain lignin so that the spaces between these residues represent regions of more easily removable lignin, relatively free from polysaccharides.

The compound middle lamella in ancient wood is always resistant to the reagents that usually solubilize the other wall layers.

This region is perhaps richest in lignin (ASUNMAA, 1955; BAILEY & KERR, 1935; HARLOW, 1932; MEIER, 1955). Whether a pectin-like substance is also present in this layer is not yet definitely established.

The nature of degraded cell-wall, as discussed in the foregoing paragraphs, has been assumed by Barghoorn (1949a, b) and Varossieau (1949) to be the first of a series of changes which wood undergoes during conversion to coal or during petrification. This idea has also been supported by the data presented in this article. The inorganic infiltrating substances in the ancient wood of *Heritiera fomes* (from Jadavpur site) and *Shorea robusta* are disoriented. At the 'incipient stage of fossilization' the disorientation may be due to a small amount of mineral precipitates within the empty cell cavities. But in advanced stages, when the cell cavities are being gradually filled up, the minerals infiltrating the tissues may be oriented in certain directions due to consolidation and newly acquired structural internal equilibrium of the developing petrification. (For mechanism of petrification of plant tissues, see ARNOLD, 1947; WALTON, 1953). Such orientation is possibly mainly controlled by the orientation of the surviving cellulosic structural pattern (as in some samples), or by the texture left by the cellulosic structural framework in the persisting lignin. This possibility is to some extent apparent from the petro-fabric patterns of some petrified wood samples (SEN, 1955b.) It is no wonder, therefore, that the submicroscopic organization is sometimes nicely preserved in petrified wood, as it is apparent from the electron microscopic investigations by Eicke (1952-1957).

The presence of humic materials in the ancient wood of *Picea excelsa*, as reported by Varossieau and Breger (1952), and in *Heritiera fomes*, is also significant. The possibility of the origin of humus from structurally altered lignin molecule is not yet definitely known, but it is worth considering. It has been found that the methoxyl content in wood decreases with the degradation of lignin, which is 1 per cent in peat. Moreover, it has been always observed that the "cellulose disappears with a corresponding decrease in the thickness of the cell-wall". The cellulose breaks down into substances which do not remain in the wall (VAROSSIEAU & BREGER, 1952). All these facts, according to these authors, support the lignin theory of coal

genesis. Recently Kinney and Doucette (1958) have shown that a comparison of the infrared spectra of the coalification series from cellulose and lignin to anthracite may give an insight into the chemical changes that occur during coalification. The possible use of lignin as an indicator of the local thermal history in regions containing fossilized vascular plants has been investigated by Siegel *et al.* (1958). They have found that the survival of lignin in identifiable soluble form was favoured by lower temperature, the absence of oxygen, and association with normal cell-wall constituents of woody tissues. The possible use of the methods, as above, in future studies of the lignin in ancient buried wood in relation to fossilization is now being explored.

Such is the history of the persistence of the structural features in the ancient fibres and tracheids. At least in some materials the original cellulosic structure is preserved almost intact. In the materials where the cellulose is destroyed, the original structure is left in the form of textural patterns. The lignin has faithfully preserved the characteristic pattern after the breakdown of the cellulose, and retains its original line up, and thus reveals the texture of some of the entrancing beauty of the ancient plant life, going down to the smallest limits of visibility. The same lignin often persists in the petrifications when the original structural units are considerably modified, and thus perhaps greatly help in the preservation of the general anatomical plan of the original plants. The cellulose usually controls the texture in the lignin, which carries its patterns through the ages. But when the cellulose itself is preserved on occasions, its wonderful architecture is more easily and truly revealed. Such cases have been recorded above in this paper.

SUMMARY

1. The chemical and physical (including optical) analyses of variably degraded ancient wood of *Heritiera fomes*, *Bischofia javanica* and *Picea excelsa* have been attempted with a view to find out the nature and structure of non-biologically and anaerobically decomposed materials generally with reference to the organization in their normal representatives. Another object in view has been to bring out facts of interest in the stages of

degradation of the plant materials, and with reference to fossilization. Some of the data on the cell-wall structure of another previously investigated ancient wood (*Shorea robusta*) have also been incorporated in this paper as a basis of comparison. The examination of other ancient wood samples, now being done, generally support the conclusions arrived at in this paper.

2. The relatively important constituents of the ancient wood and corresponding normal wood samples, and the reducing properties of cellulose in such materials have been determined. The general chemical and microchemical behaviour, and the effect of some common staining reagents in the wood samples have also been recorded. For studying the nature of the cell-wall structure of the ancient tracheids and fibres, polarized light, Spierer lens, and X-ray diffraction method have been employed, followed by an examination of the fibrillar texture of the wood elements. An attempt has also been made to correlate the results obtained from all these methods of study. The microscopic evaluation of the ancient cell-wall architecture has been specially brought out to interpret in terms of its submicroscopic structure and/or texture, and of its chemical composition.

3. All the methods of study point to the same conclusion that the cellulose in ancient wood is variably degraded, and disappears relatively quickly. The lignin may also be partially destroyed, but it is obviously more resistant to decay than the cellulose. Generally the lignin content increases apparently due to the loss of cellulose. The rate of decomposition of pentosan is variable; possibly it is related to the extent of cellulose degradation.

4. Possibly there is a pectin-like substance located still in the compound middle lamella. This has not yet been definitely proved. The relative abundance of the cellulosic fraction (which is possibly an oxycellulose in some cases) and the lignin in the different cell-wall layers of the ancient wood have also been shown.

5. The extent of degradation of the anatomical organization is accompanied by gradual changes in the cellulosic wall structure and composition, and consequently the loss of volatile matters. In *Picea excelsa* there is a general uniformity of plan of degradation of the lamellar organization of the cell-wall, as observed by the previous authors. The resistant wall and wall layers primarily

preserve the general anatomical organization before and during mineralization.

6. From all the evidence at hand, and that furnished by Varossieau and Breger, it appears that lignin is the principal source material for coal formation. The possibility of the occurrence of humic materials in the ancient wood of *Heritiera fomes* is now being systematically worked out.

7. An attempt has been made to study the structure of the changing cell-wall during its degradation. It has been found that the original micellar structure of cellulose is highly degraded in the visibly affected ancient fibres and tracheids, but the general nature (texture) of the original structural patterns of some wall layers are often largely retained by the persisting lignin. The original submicroscopic structure is wonderfully retained in about two thousand years old *Shorea robusta*, the oldest of the ancient wood, *Bischofia javanica*, and in the apparently sound core of the ancient *Picea excelsa*.

It has been found in the case of *Picea excelsa* that the quantity of cellulose present in the ancient wood may not necessarily be an index to the extent of preservation of its structural framework.

8. The mineralization or 'biological fixation' of decaying plant tissues appears to be mainly controlled by the surviving cellulose

or the pattern it leaves in the texture of the lignin. The disorientation of inorganic matters in the X-ray diffraction patterns of some ancient wood samples is possibly due to the small amount of early precipitates. However, these precipitates may not develop into a disoriented fabric pattern in the mineralized wood at its final stage of consolidation. This is also apparent from one of the earlier papers of this series.

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EXPLANATION OF PLATES

PLATE 1

1. Transverse section of the ancient wood of *Picea excelsa* showing a few intact cells of the late wood. $\times 500$.
2. Transverse section of the ancient wood of *Heritiera fomes* showing collapsed walls of libriform fibres. $\times 310$.
3. Transverse section of the ancient wood of *Bischofia javanica* showing general nature of good preservation of the fibre walls. $\times 250$.
4. Transverse section of the ancient wood of *Picea excelsa* after acid-alkali treatment followed by staining with Ruthenium red. The heavily stained structural residue consists of the compound middle lamella tending to blend with the outer secondary layer. The collapsed secondary walls of the late wood are also stained, but when preserved intact, they do not show any affinity for Ruthenium red. $\times 260$.
5. Transverse section of the ancient wood of *Heritiera fomes* after acid-alkali treatment followed by staining with Ruthenium red. It has not been possible to produce desirable contrast between heavily stained intercellular substances and other wall layers due to the natural dark brownish colour of the latter. $\times 260$.

PLATE 2

6. Transverse section of the ancient wood of *Picea excelsa* showing iodine-sulphuric acid reaction of cellulose. Note the greatly swollen and coloured walls of the intact cells, and some irregular swelling, indicative of cellulose reaction. $\times 310$.
7. Transvers section of the normal late wood of *Picea excelsa* after treatment with 72 per cent sulphuric acid, showing concentric distribution of lignin in the secondary cell-wall. $\times 350$.
8. Transverse section of the ancient late wood of *Picea excelsa* after treatment with 72 per cent sulphuric acid, showing concentric distribution of lignin in the cell-wall. $\times 360$. Compare Fig. 7.
9. Transverse section of the ancient wood of *Bischofia javanica* after chlorination. Note the partially removed secondary wall, and the concentric distribution of cellulose. $\times 260$.
10. Transverse section of the ancient wood of *Heritiera fomes* after bromination. Note that the secondary wall layers are removed. $\times 360$.

PLATE 3

11. Transverse section of the ancient wood of *Picea excelsa* after usual treatment in aniline sulphate. Note that the lignin reaction is relatively less perceptible in the secondary walls of intact cells. $\times 260$.
12. Tangential longisection of the ancient wood of *Heritiera fomes* showing apparent texture (of lignin) of the wood elements after drying. $\times 190$.
13. A fibre of the ancient wood of *Bischofia javanica* that has been cut longitudinally and crushed after staining with congo red. Note the angle of spiral in cell-wall. $\times 250$.
14. Longitudinal section of the ancient wood of *Bischofia javanica* showing a fibre with the orifices of pits and the spiralling of a wall layer lying in another plane photographed together. Such images

are possible due to the considerable focal depth of the objective used. $\times 345$.

15. A fibre isolated from the ancient wood of *Shorea robusta* that has been cut longitudinally and crushed after staining with congo red. Note the angle of spiral in cell-wall. $\times 530$.

16. Tangential longisection of the ancient wood of *Picea excelsa* showing apparent fibrillar texture of the tracheids after drying. $\times 180$.

PLATE 4

17. Transverse section of the ancient wood of *Heritiera fomes* viewed between crossed nicols. Note the form birefringence mainly produced by the outer secondary wall layers. About 30 seconds exposure against super high speed panchromatic photographic plate. $\times 120$.

18. Transverse section of the late wood of the ancient *Picea excelsa* viewed between crossed nicols. Note the characteristic birefringence of the outer and inner secondary layers, and lack of birefringence of the central secondary layers in some intact cells. $\times 375$.

19. Spierer picture of tangential longisection of the lignin rich ancient wood of *Heritiera fomes* showing highly preferred texture (i.e. the supermicelles are arranged parallel to the long axes of the cells).

20. Spierer picture of tangential longisection of the ancient wood of *Picea excelsa* showing that the supermicelles are arranged in steep spirals with reference to the long axes of the cells. $\times 1120$.

21. X-ray diffraction photograph of the ancient wood of *Heritiera fomes* (from Jadavpur site), taken with the X-ray beam normal to the fibre axis of the tangential section, using Cu target and a specimen-film distance of 3 cm. Note the lack of intensity spots, and the appearance of a distinct ring of inorganic matter which is non-oriented. Compare other patterns.

22. X-ray diffraction photograph of the ancient wood of *Heritiera fomes* (from Madhyamgram site), taken with the X-ray beam normal to the fibre axis of the tangential section, using Cu target and a specimen-film distance of 3 cm. Note the slightly blurred continuous ring of 002 interference. Compare other patterns.

23. X-ray diffraction photograph of the well preserved ancient wood of *Bischofia javanica* (as old as *Heritiera fomes*), taken with the X-ray beam normal to the fibre axis of the tangential section, using Cu target and a specimen-film distance of 3 cm. Note the large spreading of the equatorial arcs. Compare other patterns.

24. X-ray diffraction photograph of the degraded ancient wood of *Picea excelsa*, taken with the X-ray beam normal to the tracheid axis of the tangential section, using Cu target and a specimen-film distance of 3 cm. Note the slightly blurred continuous ring of 002 interference. Compare other patterns.

25. X-ray diffraction photograph of the apparently sound core of the ancient wood of *Picea excelsa*, taken with the X-ray beam normal to tracheid axis of the tangential section, using Cu target and a specimen-film distance of 3 cm. Note the small spreading of the equatorial arcs indicating a steep spiral micellar orientation. Compare other patterns.

