

INVESTIGATION OF THE MEMBRANES OF SPORES AND POLLEN.

3 Fossil Sporopollenins from Tasmanite and the Moscow brown coal.

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TRANSPARATION OF SEPARATUM

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To the products of nature, which meet us in an apparently well preserved condition still to-day, a long time after the death of the single individuals which have built them up, belong the membranes of the spores and Pollen. In almost all coals and many oil shales of quite different geological age the spore membranes of the plants existing at that time can be established. These delicate structures have been converted completely into a fossil mineral. However, widely distributed are the rocks containing fossil spores, as is shown from petrographic investigations carried out on the thin slice, thin section and relief methods, still there are only a few suitable for the isolation of the spore membranes. Since it was established in the previous investigation that the sporopollenins of recent plants differ little from each other, so was this same conclusion also to be drawn for the extinct plant world of the past. It was desirable, therefore, that the spore material should contain only one kind of spore. Further it was desired that the breakable stone should contain as high percentage as possible of sporecases, in order to overcome the treatment of not too great quantities, probably with difficulty too, of inorganic or organic accompanying material. Besides it was desired for an easier isolation to work with the most friable rock obtainable. Rocks of this nature were found in an oil shale in Tasmania (Tasmanite) and in a Russian brown coal in the Moskauer district. Although both Kaustobioliths possessed suitable loose structure yet there were still some difficulties in getting from it a material, that was free for analysis, and uniform. The greatest difficulty was caused by those spore cases that in consequence of the pressure of overlying rocks were bound together formerly with principally clay and humic material which in Tasmanite amounted to over half, in the brown coal to 90% of the total quantity of spores. Just as in the case of the recent Spores and Pollen the alternate application of mechanical and chemical methods of cleaning lead to the goal. Especially favoured was the separation of the spore cases in that in both cases it is concerned with macrospores by, that are understood spores over 0.1 mm. diameter diverging from botanical use into coal. The material was coarsely ground in order to avoid as much as possible the disintegration of these, and then divided by sifting into a finer and a coarser part. The coarser part contains the undamaged spores. Both parts are further treated separately. In Tasmanite the coarser part contains large quantities of sand and the smaller proportion of clay whilst the finer part contains the main part of the clay, fine sand and pounded spore material. The best way to get rid of the sand is to wash with carbon tetrachloride, and through frequent washing of the clay with soapy water it is possible to cause the clay to go into suspension. The spore material obtained in this way out of both parts is still high in ash which is caused principally through the constituent parts clinging firmly to each other in consequence of the earth pressure as mentioned above. The only way to separate it is to make a chemical treatment of the spores, in which they are submitted to the influence of hydrofluoric acid. The grains of the associated inorganic material are so greatly freed through this, that they can be reduced through renewed washing with carbon tetrachloride and soap solution to a proportion of ash of under 1%. Then the spore membranes are extracted with copper oxide ammonia solution for the separation of the cellulose introduced through the numerous filtering operations,

and finally for the separation of soluble bitumen with organic solvents. In this way you get about 17% of Sporopollenin which represents a minimum figure since in the working of the greater proportion of rock material, losses are inevitable, especially in the pouring out.

The difficulties of getting pure Macrospores from the Moscow brown coal are considerably greater than with Tasmanite. Indeed the main part of the spores can easily be obtained here also. Through grinding and sieving, yet the separation from the inorganic parts, which consist almost exclusively of sand does not cause half as much trouble as the separation from the humic coal substance. Instead of carbon tetrachlorite a liquid of lower specific gravity must be chosen for this purpose which is obtained through the addition of carbon disulphide to carbon tetrachlorite. However, the separation is much more imperfect and must be repeated more often. The spores obtained in this way appear under the magnifying glass still considerably contaminated with the firmly clinging humic material. Frequent intermittent boiling with alkali and acid removes them followed by a subsequent treatment with hydrofluoric acid since the spores obtained in this way contain at the most 0.4% Ash. But here a solid extraction with organic solvents, preferably pyridine, is still more important than with the spores of the Tasmanite. The yield amounts to 8.5%, yet it is hard to give the figure since we have worked with only suitable pieces rich in spores. Previously it was mentioned that fossil sporopollenin is very far spread, as shown by coal-petrographic investigation. From the great power of resistance of the modern Cuticular substances Sporopollenin and Cutin, to the most numerous chemical influences, it is not surprising that these substances have offered considerable resistance to the biochemical and geochemical sections which are connected with the formation of sapropelic material and coal. Their recognition depends certainly less on chemical reactions than on the shape of the Cuticles and spore cases preserved in spite of all the influences so that the latter are called simply fossil spores and Pollen, although not more than on constituent part whose form can be definitely recognised has remained preserved.

By virtue of microchemical reactions one previously took for granted that the membrane of the spores is still completely unchanged, since it gives the reactions of the modern cuticular substances. The reaction with concentrated chromic acid, concentrated sulphuric acid, zinc-chloride-iodine, Potassium Tri-iodide solutions and the colour reactions with gentian, violet, Sudan 3 - saffron, Erythrosin and Chlorophyll solution, and concentrated potash lye almost corresponds to that of the modern Sporopollenin. The investigations on the modern Sporopollenins have already shown that the maintenance of the shape alone gives no proof for the immutability of the fossil spores. So we have taken for example a series of brominations and subsequent debrominations through potash lye and after being treated three times during which treatment the greater part of the Lycopodium Sporopollenins was dissolved, the outer shape was still completely preserved, only the pale yellow colour was changed towards brown. Likewise were sulphonated Sporopollenins of Lycopodium and Pinus prepared, which remained insoluble and kept their shape in spite of the drastic treatment and opportunity for salt formation. In spite of the resistant nature power of the Sporopollenins it was scarcely probable that the countless chemical influences during the formation of coal and sapropelic matter have been without any influence on the Sporopollenin. To discover the intensity and ultimate effect of these influences, it could be attempted only through comparison of the fossil Sporopollenin with recent Sporopollenin. Since the spores of the Tasmanite cannot be correlated by the palaeobotanists with certainty to any fossil plant we name the fossil Sporopollenin after the mineral Tasmanin, the spores from the Moscow district are assigned to the species Bothrodendrin and we, therefore, call the second fossil Sporopollenin. (Bothrodendrin). Tasmanin is obtained after our method of separation in the shape of bright redbrownish, transparent spore cases of about 0,5 - 1 mm. diameter. Under the microscope the undamaged cases show the shape of flat, pressed together discs of a smooth surface

which is mostly more or less creased owing to the consolidating pressure of the rock. But a great part appears in the shape of large fragments. The substance is free of sulphur and nitrogen. Bothrodendrin when dry is of bright brown colour, unpolished and opaque, but when moist is dark brown. The undamaged spore cases show low magnification the shape of a flat, pressed together semi-spherical form, whose base-forms a tetrahedron. They have, on an average a diameter of 2 mm. Bothrodendrin is free of nitrogen but contains a varying quantity of sulphur. From numerous pieces of coal we have obtained fossil Sporopollenin with 0.1 - 0.2% sulphur, from others Sporopollenin with 2 - 3% sulphur. Since the Moscow brown coal is very rich in sulphur, the sulphur might be considered with safety to have been absorbed during the coal formation; the variable quantity of sulphur supports this view. Therefore, we may neglect this quantity. It shows further that even this membrane substance which is so resistant is chemically changeable. This vulcanized Sporopollenin is also much more brittle than the Tasmania (which has no sulphur). The reaction to heat is, on the other hand, in the case of both fossil Sporopollenins like that of the recent Lycopodium-Sporonins, whilst at 200 degrees an evolution of water is noticeable, which in Tasmania seems to be lesser than in Bothrodendrin, and over 300 degrees with the appearance of a brown distillate and a smell characteristic for each fossil Sporopollenin decomposition occurs to a dark bitumen. Those colour reactions of the recent Sporopollenins obtained with concentrated sulphuric acid are not given since the yellow colouring materials giving these reactions are altered very quickly from the material of the Sporopollenine during the carbonisation. Even though of recent origin Sporemembranes separated from peat give very seldom one of these colour reactions. How quickly this colouring material part is changed is already shown by the reaction of the recent Sporopollenins in boiling in glacial acetic acid whereby those which gave colour reactions with concentrated sulphuric acid were coloured dark brown. But analytically a change was not established. With longer standing or with heating in concentrated sulphuric acid or phosphoric acid Tasmanin and Bothrodendrin are likewise coloured dark brown like the recent Sporopollenine. By virtue of analysing we can by accepting the empirical formula established for the recent Sporopollenins come to the formula for Tasmanin: $C_{90}H_{136}O_{17}$ and for Bothrodendrin, whereby we neglect for the present the trivial quantity of sulphur, to the formula $C_{90}H_{120}O_{21}$. Even in this fossil Sporopollenin a part of the oxygen was left through acetylation still as OH species, and even in Tasmanin 2 Hydroxyl groups and in Bothrodendrin 9 Hydroxyl groups; so that the above-mentioned formulae can be resolved into $C_{90}H_{134}O_{15}(OH)_2$ for Tasmanin and $C_{90}H_{111}O_{12}(OH)_9$ for Bothrodendrin. If one puts these formulae opposite to that of the Lycopodium Sporopollenin: $C_{90}H_{142}O_{27}$ or $C_{90}H_{127}O_{12}(OH)_{15}$ so there is shown a surprisingly close relationship in quite characteristic changes. The oxygen (perhaps ether-oxygen) not more closely specified by us previously has apparently remained unchanged through bio and geo-chemical influences. The changes extend to the hydrogen and the Hydroxyl groups. Both fossil Sporopollenins show a lessened Hydroxyl and hydrogen content. Yet the relation of Tasmanin to Bothrodendrin is completely reverse. Tasmanin has **lost** more (Hydroxyl) and less hydrogen, Bothrodendrin less Hydroxyl and very much more hydrogen. The cause of these phenomena will have to be sought in the history of the development of both Kaustobidiths Tasmanite and brown coal. Tasmanite is a Sapropelith which owes its existence to the intensive reducing processes of the decomposing mud. And so essentially the Hydroxyl groups have been reduced to hydrogen atoms. Quite differently formed are the relations perhaps reactions during the carbonisation. Carbonisation stands out also in the example of the Bothrodendrin as a particularly dehydrogenating process. The loss of the Hydroxyl groups is to be traced back to the dehydrating process. Bothrodendrin must have an unsaturated character, Tasmanin a saturated one. The reaction with oxidising agents among which we favour fuming nitric acid can serve as proof. If in a glass-stoppered bottle samples of the three Sporopollenins are set aside with an excess of fuming nitric acid Lycopodium-Sporopollenin is dissolved at room temperature

after 12 hours, Bothrodendrin 24-30 hours, but Tasmanin is dissolved only after 3 weeks. It remains like paraffin. The point of attack of the oxidising process seems to lie in the free Hydroxyl-groups. It should be expected that Bothrodendrin would be dissolved, more quickly than Lycopodium-Sporonin yet the previously mentioned primary processes of reduction, dehydrogenation and dehydration may give rise to secondary changes as for example cyclisation, which as a final result cause a greater resistance to oxidation in the case of the Bothrodendrin than in the case of Lycopodium-Sporonin. We are occupied with the explanation of these important processes, and we hope to get further insight into the fossilisation process of organic matter in the above described way i.e. through the separation of the clean fossil Sporopollenine from the numerous Kaustobioliths and their comparison with the recent Sporopollenins. The still surprisingly high Hydroxyl content of the Bothrodendrin may also be explained by the fact that the Moscow coal has not been buried at a great depth in spite of its great age and, therefore, the dehydration like carbonisation in general has not progressed very much since the influence of a higher temperature, caused through a high pressure of rock masses was lacking. For the donation of the valuable brown coal we heartily thank the Prussian Geological Institution in Berlin and especially the Principal W. Bennhold.

THE EXTRACTION OF TASMANIN FROM TASMANITE.

770g. Tasmanite were pulverised with a hammer and ground in a coffee mill. The bright brown powder was boiled with 5l distilled water for several hours and filtered. The weak yellow filtrate was evaporated. There were obtained 12g. = 1.6% of a residue consisting mainly of colourless crystals, which on being completely dried turned brown and gave out a caramel smell. The residue gave positive K and negative PO_4 . - ions reactions. The rock powder of the stone was dried and passed through a hair sieve. The finer part I in the weight of 422g. went through, whilst the coarser Part II with a weight of 324g. remained behind. Now both parts were separated and put into carbon tetrachloride. After longer standing the part floating on top was skimmed. After drying 88 grams of spores from Part I were obtained and 116 grams from Part II (Part IIA). The residue from Part I consisted almost exclusively of sand and appeared so poor in spores under the microscope that a further treatment was not worth while. The residue from Part II of the weight of 210g. (Part IIB). was very rich in clay and still contained plentiful spores. Now each part was put into hydrofluoric acid for the removal of the silicic acid and for the freeing of the clay. Since strong heating followed in consequence of the high quantity of silicic acid, the material was slowly added in portion. The lead vessels were cooled during this operation by placing in ice. There was so much hydrofluoric acid used that a thin pulp resulted. Then it was left standing well shut up for 2 days, soaked off through filter linen, washed at first with hydrofluoric acid then with concentrated hydrochloric acid and finally washed several times with boiling water. Now the spores were boiled in a porcelain basin for half an hour several times with 10% potash lye and filtered until the previously yellow filtrate was colourless. Then the soap washing followed, whilst the spores were shaken vigorously for a longer time with the 30 fold quantity of a warm 15% soft soap solution in a large Erlenmeyer flask. After a short standing of the obliquely placed flask the soapy solution, muddy through the suspended clay, was decanted from the settled spores by a gradual tilting of the vessel, and this treatment with soap was repeated until the soapy water no longer appeared muddy. This operation can be greatly simplified for the coarser Part II which contains the undamaged Macrospores, whilst the spores are freed with the help of a sieve from the impurities instead of being left to settle and poured off after the shaking with soapy water. Then the spores were repeatedly washed in this way with the hot water which was weakly acidulated with hydrochloric acid in the last washing, and drained. Whilst the quantity of ash in the sporemembranes from Part I after this method of treatment amounted to 0.1 - 0.2%, in the Parts IIA and B. it amounted to 2.5%. In order to reduce this to under 1%, the whole of the operation from the treatment with hydrofluoric acid onwards must be repeated. For the complete removal of the oil bitumen, so far as it had not already dissolved in the carbon tetrachloride, the alkali lye or the soapy water, the spores were submitted to a treatment with organic solvents. To this end they were extracted for a long time in turns with alcohol, ether bisulphide of carbon and Lyndine with the aid of heat on the waterbath until the filtrates remained clear. Then for the removal of the Pyndine they were washed free of acid with glacial acetic acid to which some hydrochloric acid was added and then with hot water, whereby one always let this take effect for a longer time before each filtration. Since, in consequence of the numerous filtrations, the Sporopollenin obtained in this way appeared greatly polluted through the filter paper fluff, so it was put into a 20 fold quantity of copper tetrammin hydroxyd solution and left to stand for 2 days amid frequent shaking. In order to avoid renewed pollution with cellulose the spore cases were now drained off through a glass filter crucible, washed several times with 24% ammonia and left to stand for 2 days with diluted hydrochloric acid. Now they were washed free of acid and boiled first with alcohol then with ether, and dried in the air, then in the vacuum apparatus at 100 degress. From Part I. 65 grams of Sporopollenin were obtained, from Part IIA 35g. and Part IIB. 38g., in all 138g. = 17% of the original material. This yield represents only a fraction since there were unavoidable losses due to pouring off of the soapy extract. (Caution). You should know that the

by repeated grinding in the mill. By this mechanical treatment the earthy substance is detached from the membranes. On now repeating the separation with the mixture of carbon tetrachloride and carbon disulphide, the dark impurities were for the most part removed. The remaining impurities were eliminated by the final washing with soap. In conclusion the bothrodendrin was treated with ammoniacal cupric oxide solution &c., as described for Tasmanin. From Portion I 7 grams were obtained and from Portion II 10 grams, in all 17 grams = 8.5%

Appearance and general properties of bothrodendrin¹ see above. Acetylation and acetyl estimation follows according to foot note p.76.

(1). Structure of Bothrodendrin and Tasmanin we will give loc. cit.

0.1508 grms. Sub (0.1503 ashfree) gave 0.3859 grms. CO₂ & 0.1070 grms. H₂O

0.1562 grms. Sub (0.1557 ashfree) gave 0.3982 grms. CO₂ & 0.1081 grms. H₂O.

0.5044 grms. ash & 3. free subst. required 23.7cc. $\frac{N}{10}$ KOH.

0.4910 grms. ash & 3 free subst. required 23.6 cc. $\frac{N}{10}$ KOH.

CgO	H111	O12	(OH) ₉	calculated	C	H.	CH ₃	CO
					70.26	7.87	20.20	
				Found	(70.04	7.97	20.29	
					(69.77	7.77	20.66	

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