CCCXXIII.—*Plant Cuticles. Part II. Fossil Plant Cuticles.*

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FOR the final stage of this research, the chemical examination of cuticle from bituminous coals, the collection of sufficient material presented difficulties. Fortunately, however, during the lengthy and laborious process of accumulating supplies of bituminous coal cuticles, we have been able to study similar material obtained from the so-called *Papierkohle* of Russia, which consists largely of readily separable cuticles. Belonging as it does to the Carboniferous period, this " paper-coal " could reasonably be assumed to contain cuticles closely allied chemically to those in bituminous coal, so that their examination, coupled with the knowledge obtained from the study of modern plant cuticles, helped considerably towards elucidating the nature of the cuticular tissues that form such a conspicuous feature of many bituminous coals.

(A) *Cuticles from Papierkohle.*

For our original supplies of Papierkohle we are indebted to Dr. Marie Stopes, whose scheme of coal research (see *Brit. Assoc. Rep.*, Newcastle, 1916), as exemplified by this work, we have followed. Later, Dr. Zalessky, on whose studies our knowledge of the character of the Papierkohle deposits mainly rests, kindly sent us a quantity of material from Tovarkovo, in Toula.

The deposits of Papierkohle, so named by Auerbach and Trautschold (*Nouv. Mem. Soc. Imp. Natural. Moscou*, 1860, **13**, 1) because of their superficial resemblance to accumulations of sheets of brown paper, cover an area of many square miles in the Moscow basin and vary in thickness from less than one cm. to more than one m. The deposits are near the surface, being covered by a thin layer of either sand or clay. They occur principally in the three adjoining provinces of Kalouga, Toula, and Raizan, lying west to east across the Moscow basin about 100 miles south of Moscow. Geologists are agreed that the deposits belong to the lower Culm of Russia, corresponding with the lower carboniferous period of Western Europe, but there is little agreement as to their mode of formation.

Zalessky (*Mem. du Com. Géolog.*, 1915, 125) at one time believed that the deposits resulted from the accumulation of remains of a single genus of plants, carried by chance to the shallower portions of the basin where, in running water, decomposition rapidly occurred with the formation of ulmins by all save the cuticles. Each successive layer of decomposed débris was retained in position by a covering of undecomposed material and, finally, by a layer of clay or sand. He now considers it more probable (*loc. cit.*) that the accumulation of stems that yielded the cuticles occurred in any of the sunken places surrounding the Moscow basin near the trunks of the corresponding trees, their interiors being destroyed as much by aerial decay as by maceration in swampy ground. Later, these sunken places became inundated with water, causing accumulated plant material, which now consisted mainly of the cuticularised sheaths of twigs and separated fragments of cuticles, to be carried away and deposited within the basin. The water, having percolated through marsh land rich in decaying vegetation, would be impregnated with ulmins, which would be deposited with the cuticles. A similar theory had been put forward by Renault (*Bull. Soc. Hist. Nat. Autun*, 1895, **3**, 136), who suggested also, as another possibility, that the hollow places where trunks and branches of the trees had accumulated and undergone bacterial decay might at a later date have been filled with ulmin-bearing water. Zalessky does not support this alternative suggestion, because the deposits of paper-coal contain little, if any, woody material.

When freed from ulmins, which may amount to over 80% of the mass, the fossil cuticles appear as thin, pliable fragments of dark brown colour. Earlier investigators recognised these as belonging to the stems and twigs of a single species of plants, and Zalessky has proved that species to be *Lepidodendron Olivieri*.

The only previous chemical examination of the cuticles seems to be an analysis recorded by Bertrand and Renault (*Bull. Soc. Ind. Min.*, 1899, **13**, 865) : C, 74·69; H, 9·75; N, 0·97; and O, 14·59%, and an observation by Zeiller (*Ann. Sci. Nat. Bot.*, 1882, **13**, 217), which we confirm, that the cuticles are not attacked by boiling alkalis.

Examination of Papierkohle from Toula.—The crude material, which was contaminated with sand and miscellaneous plant débris, was resolved into (1) ulmins soluble in ammonium hydroxide (35%), (2) ulmins insoluble in ammonium hydroxide (45%), and (3) fossil cuticles (20%).

Photomicrographs showing the appearance of the cuticles are reproduced on Plate 1, in surface view in Figs. 1—3 and in section in Fig. 4. The perforations of the surfaces of the cuticles were produced by the falling away of the leaf traces from the stems, and the narrow " tongues " of cellular material extending within the perforations (Figs. 2 and 3) are fragments of cuticle detached from the dorsal sides of the leaves. In the section, Fig. 4, which is highly magnified, cutin " teeth " projecting from the inner surface of the cuticle are faintly visible (compare Fig. 4 of J., 1925, **127**, 1412).

As might be expected from their history, the fossil cuticles, unlike those from modern plants, contained neither water-soluble material nor cellulose. On extraction with organic solvents, however, as much as 4% of wax was found to be associated with the cutin.

Examination of the Fossil Cutin.—Treatment with oxidising agents yielded compounds of similar character to those obtained from modern cutin but of lower carbon-content, no doubt because the fossil cutin (C, 73%) was more resistant than the modern (C, 68%) and required longer treatment. With dilute nitric acid, a 50% yield of sparingly soluble and insoluble acid products was obtained. From the sparingly soluble fraction sebacic acid was isolated and from the insoluble fraction an acid to which the formula $C_{15}H_{28}O_3$ is assigned. This acid was also obtained on oxidising the cutin by hydrogen peroxide and by alkaline potassium permanganate.

On distillation in a vacuum, the fossil cutin gave a high yield of condensable products (40%) of similar character to those from modern cutin. Details regarding these products, and of the gases accompanying them during the distillation, are given under " Experimental " (see also Fig. 1).

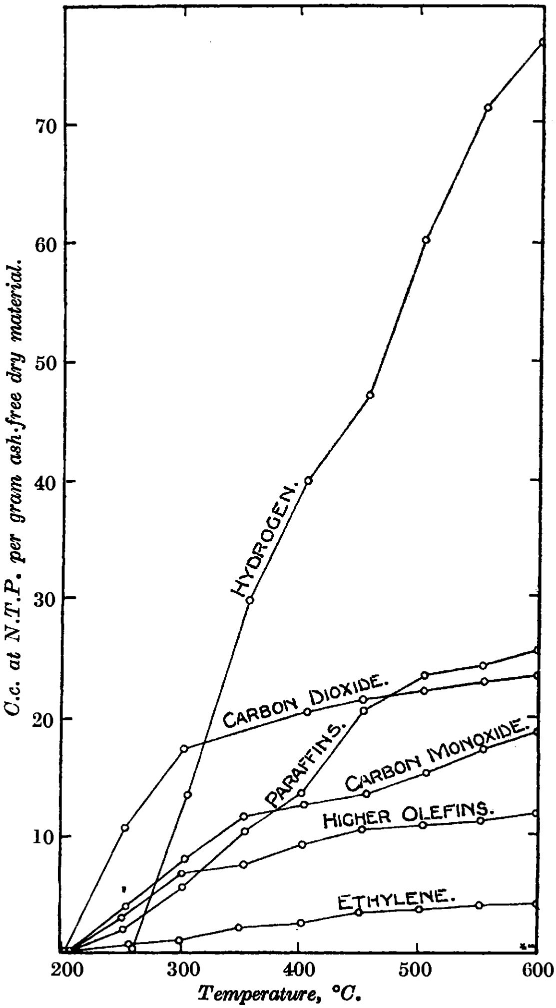
Examination of the Fossil Wax.—In marked contrast with the wax associated with modern cutin, that from fossil cutin consisted mainly of acids. From an examination of the limited quantity available it appeared that oleic acid and saturated oxy-aliphatic acids predominated.

(B) *Cuticles from Bituminous Coals.*

Although cuticles are an easily recognisable and prominent constituent of most bituminous coals (clarains and durains), their mass contribution is usually small and the isolation of any considerable quantity is, in consequence, a lengthy process. We have succeeded in isolating, by a process of mild oxidation of the main

FIG. 1.

Distillation of fossil cutin in a vacuum. Gases evolved in c.c. per gram ash-free dry material.



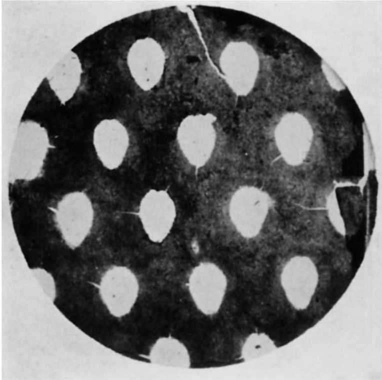


Fig. 1.—*Surface view of fossil cutin, showing perforations produced by the falling away of leaf traces.* (×4.)

Fig. 2.—*Surface view of fossil cutin, showing "tongues" of cuticular substance originally attached to the dorsal sides of the leaves.* (×5.)

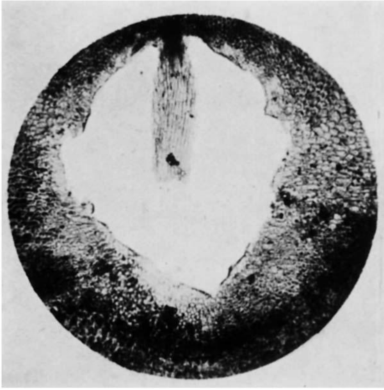


Fig. 3.—*Part of Fig. 2 more highly magnified.* (×20.)

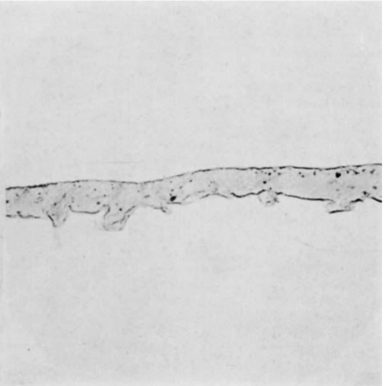


Fig. 4.—*Section of fossil cutin in which the remains of the "teeth" on the inner surface of the cuticle are still visible.* (×200.)

Plate 1.

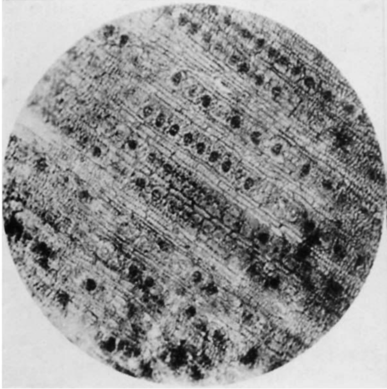


FIG. 1.—*Surface view of cuticle isolated from Top Hard durain, showing impress of cell structure and stomata.* (× 60.)

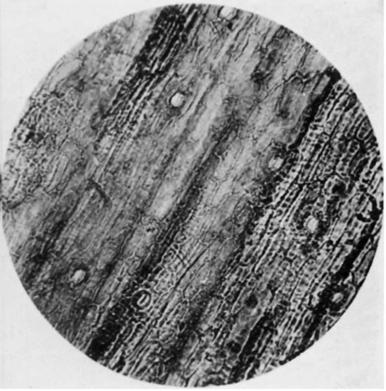


FIG. 2.—*Surface view of another cuticle from Top Hard durain.* (× 60.)

FIG. 3.—*As Fig.* 1, *more highly magnified.* (× 170.)

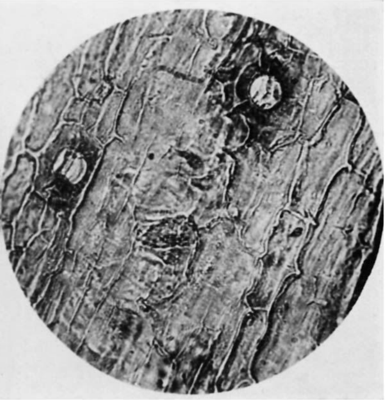


FIG. 4.—*As Fig.* 2, *more highly magnified.* (× 170.)

PLATE 2.

mass of the coal, sufficient cuticles with which to carry out oxidation and distillation tests similar to those performed on cuticles from modern plants and from Papierkohle.

The coal chosen was the durain from the Top Hard seam, Nottingham (Found on pure coal : C, 83·0 ; H, 5·5%). About 50 kg. were partly extracted with pyridine in an autoclave and the residue was treated alternately with hydrogen peroxide and dilute ammonium hydroxide until all soluble ulmins had been removed. There remained the plant entities resistant to oxidation, namely, spore exines, cuticular tissues and miscellaneous plant structures. From these the cuticles were removed either by hand-picking or by air-elutriation. The yield, 2·5 g. (0·005%), did not represent the cuticle-content of the coal, since there was considerable loss during oxidation and by fragmentation. Photomicrographs of typical cuticular tissues thus isolated are reproduced on Plate 2. The impress of the cell structure and the openings of the stomata remain undamaged. Referring to the Papierkohle, D. H. Scott (" Studies in Fossil Botany," p. 184. London, 1900) has observed : " That a vegetable tissue should have come down to us, fresh and unaltered, from a period more ancient than that of the Coal Measures, is certainly one of the most remarkable among the curiosities of palæontology." The same remark applies with equal force to the preservation, apparently unaltered, of cuticles in bituminous coals.

On analysis, the cuticles, which had undergone some oxidation during their isolation, were found to contain : C, 65·2 ; H, 7·4 ; ash, 4·9%. Traces of nitrogen and sulphur were also present.

The results of oxidation and distillation tests, recorded under " Experimental," showed the cuticles to resemble closely those from Papierkohle and to be, therefore, not far removed in character from the cutin of modern plants.

EXPERIMENTAL.

(A) *Cuticles from Papierkohle.*

The crude Papierkohle (6 kg.) was treated with successive quantities of dilute ammonium hydroxide solution until the filtrate was no longer coloured brown. The residue was then repeatedly agitated with water and the suspended cuticles were decanted from the heavier particles of insoluble ulmins, from which they could be readily separated. The final residue of cuticles, when dried, formed a mass of dark brown fragments (Found : C, 73·9 ; H, 11·4 ; N, 1·1 ; S, 2·1 ; ash, 13·4%). Yield, 1·2 kg. or 20%. It was exhaustively extracted under reflux with (1) chloroform and (2) alcohol. The chloroform extract was a dark brown soft wax (yield, 1·5% of the cuticles). The alcoholic extract was a pale brown hard wax (yield, 2·5%).

Resolution of Fossil Cutin by Oxidation.—(1) *With dilute nitric acid.* In a typical experiment, 100 g. of the fossil cutin were boiled under reflux with 4 l. of 30% nitric acid during 6 hours. An oxidation product separated as a dark brown oily layer on the surface of the hot acid solution. When cold, the acid solution, from which a quantity of crystalline material had separated, was decanted and evaporated to dryness (yield, 5%); the oil had hardened to a dark brown crust. This was washed free from acid and dried (yield, 45%).

Acids insoluble in cold water. The brown crust was powdered and repeatedly extracted under reflux with light petroleum (b. p. 60—80°). From the hot filtered solutions, a pale yellow powder separated on cooling. This, after several recrystallisations from light petroleum, had m. p. 81—82°. It contained neither nitrogen nor sulphur and appeared to be saturated (Found: C, 70·4; H, 11·0. $C_{15}H_{28}O_3$ requires C, 70·3; H, 10·9%). Yield, 10%. The *silver* salt was obtained from the neutral ammonium salt (Found: Ag, 30·3. $C_{15}H_{27}O_3Ag$ requires Ag, 29·7%).

The residue insoluble in light petroleum was a hard black mass, soluble in alkalis, alcohol and acetone and forming dark brown solutions. From alkaline solutions, the material was recovered unchanged on acidification. Attempts to resolve the lead, calcium, barium, and copper salts by solvents were unsuccessful (Found: C, 65·8; H, 8·9; ash, 2·2%). Yield, 35%.

Acids sparingly soluble in cold water. The residue from the nitric acid solution consisted of a reddish oil containing a crystalline solid in suspension. Separation by organic solvents was only partly successful, but several recrystallisations from boiling water enabled the solid to be recovered, m. p. 128—130° (Found: C, 59·7; H, 9·4. Calc. for $C_{10}H_{18}O_4$, sebacic acid: C, 59·4; H, 8·9%).

(2) *With hydrogen peroxide.* 100 G. of fossil cutin were added to a mixture of equal volumes of (100 vol.) hydrogen peroxide and glacial acetic acid and maintained at room temperature during 15 days. The acid solution was then decanted, and the cutin, which had become pale brown in colour, was dried and extracted with acetone under reflux. From the acetone solution a dark green solid was obtained. A second treatment of the residual cutin with hydrogen peroxide enabled further acetone-soluble material to be obtained (total yield, 40%). The whole of this material was extracted in a Soxhlet apparatus with light petroleum, in which it was only partly soluble. From the hot filtered solution a yellow flocculent precipitate separated on cooling and yielded after several recrystallisations

a pale yellow powder, m. p. 80—81° (Found : C, 70·6; H, 11·5. $C_{15}H_{28}O_3$ requires C, 70·3; H, 10·9%). Yield, 22% of acetone extract.

(3) *With alkaline potassium permanganate.* 50 G. of fossil cutin were boiled during 2 hours with 2½ l. of 5% potassium hydroxide solution containing an excess of powdered potassium permanganate. On filtration a red liquid was obtained which on cooling and acidification yielded a yellow flocculent precipitate. This was washed free from acid, dried, ground with sand, and extracted in a Soxhlet apparatus with light petroleum. A white powder separated from the solution on cooling, m. p. after recrystallisation 82—83° (Found : C, 71·0; H, 10·9. $C_{15}H_{28}O_3$ requires C, 70·3; H, 10·9%). The *silver* salt was prepared from the neutral ammonium salt (Found : Ag, 30·5. $C_{15}H_{27}O_3Ag$ requires Ag, 29·7%). The *ethyl* ester, prepared by interaction of the silver salt with ethyl iodide, was obtained as a white powder, m. p. 57—59°, after crystallisation from light petroleum (Found: C, 73·7; H, 11·4. $C_{19}H_{36}O_3$ requires C, 73·1; H, 11·5%).

Destructive Distillation of Fossil Cutin.—(1) *Condensable products.* 35 G. of the dried cutin were distilled under high vacuum in the same manner as described for modern plant cutin (*q.v.*). Liquids first made their appearance at 265° and for the most part distilled at 300—350°. They were dissolved in chloroform, filtered, and recovered as a dark brown mobile oil (yield, 40% of the ash-free, dry cutin). After removal of acids, phenols and bases by the usual means, part of the oil was treated with small quantities of cold acetone. The insoluble brown residue was recrystallised from boiling alcohol and yielded a pale yellow solid hydrocarbon, m. p. 68—71° (Found : C, 85·3; H, 14·1%). Yield, 6% of the total oil. A portion of the oil was fractionated by means of solvents and reagents; there were then obtained : saturated hydrocarbons, 25; unsaturated hydrocarbons, 33; aromatic hydrocarbons, 9; phenolic and acidic oils, 10; bases, 1; oxygenated compounds, 16; ether-soluble resin, 3; and chloroform-soluble pitch, 3%.

(2) *Gaseous products.* 1 G. of the dried cutin was distilled in the manner described for modern plant cutin, and the gases evolved over each temperature range were analysed. The results are recorded graphically in Fig. 1 (compare figure on p. 2448).

Examination of the Fossil Wax.—(1) *Chloroform-soluble portion.* 18 G. of the chloroform extract, a dark brown soft wax, were treated successively with small quantities of cold ethyl ether until the filtrate was no longer coloured.

The ether-soluble material (10 g.) was a dark red plastic mass. It was redissolved in ether, and the solution extracted successively with 4% sodium bicarbonate, 4% sodium hydroxide, and 4% hydrochloric acid solutions to remove acids and bases. Acids soluble in sodium bicarbonate solution were recovered as a viscous red oil (0·5 g.). Neither the acids themselves nor their lead, calcium, barium, and copper salts could be resolved by recrystallisation from single or mixed solvents (Found: C, 70·1; H, 10·1%). The acids soluble in sodium hydroxide solution (2·0 g.) were similar (Found: C, 69·1; H, 10·1%). Bases were not present in any quantity. The neutral compounds (7·5 g.) were saponified during 2 hours with 10% alcoholic potassium hydroxide. The unsaponified material, dissolved in light petroleum, was resolved into hydrocarbons (0·15 g.) and oxygenated compounds (0·82 g.) by repeated shaking with a concentrated solution of hydrochloric acid saturated with ferric chloride. The acids were recovered as a viscous red oil (6·5 g.) from the saponified material and separated into saturated and unsaturated acids by means of their lead salts. The unsaturated acids, which predominated, were partly soluble in cold acetone. The soluble portion was a transparent yellow oil which partly solidified on cooling (Found: C, 76·5; H, 11·9. Calc. for $C_{18}H_{34}O_2$, oleic acid: C, 76·6; H, 12·1%). Yield, 4·5 g. From the acetone-insoluble portion, a white solid, m. p. 56—60°, was obtained by crystallisation from alcohol (Found: C, 75·8; H, 12·4%). The saturated acids, which were crystallised from a mixture of alcohol and water, had an indefinite melting point, 51—57° (Found: C, 75·5; H, 12·5. Calc. for $C_{16}H_{32}O_2$, palmitic acid: C, 75·0; H, 12·5%. Calc. for $C_{18}H_{36}O_2$, stearic acid: C, 76·0; H, 12·7%). Yield, 0·8 g.

The ether-insoluble material (8 g.) was a pale brown powder, part of which (1·2 g.) was insoluble in boiling light petroleum. The soluble portion was boiled during 2 hours with excess of 10% alcoholic potassium hydroxide. The unsaponifiable material was resolved into hydrocarbons (0·3 g.) and oxygenated compounds (0·5 g.). The saponified acids were recovered in the usual way and a small quantity of unsaturated acids was removed from them by extraction of the lead salts with ethyl ether. The saturated acids were recrystallised several times from light petroleum and separated as a pale yellow powder, m. p. 69—71° (Found: C, 75·0; H, 12·1. $C_{24}H_{48}O_3$ requires C, 75·5; H, 12·5%. Found for the *silver* salt: Ag, 21·3. $C_{24}H_{47}O_3Ag$ requires Ag, 22·0%).

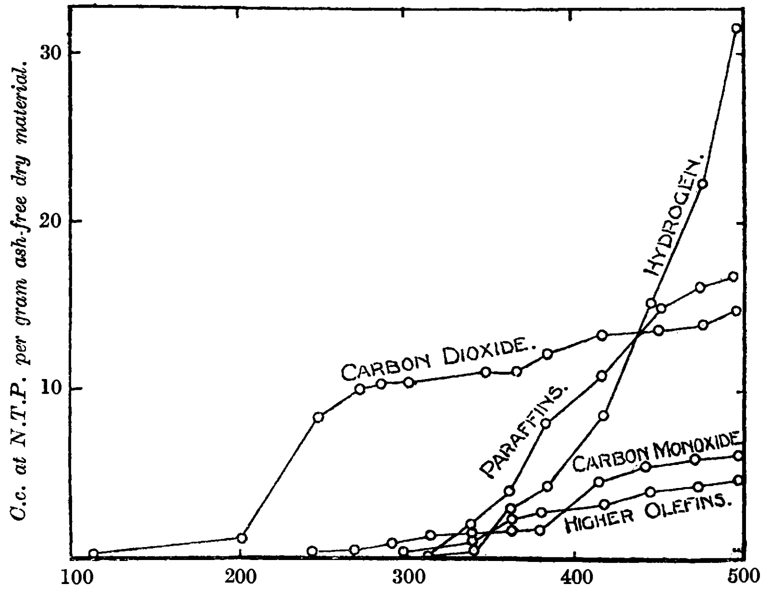
(2) *Alcohol-soluble portion.* 30 G. of the alcoholic extract, a pale brown hard wax, were extracted in a Soxhlet apparatus successively with (1) light petroleum (b. p. 40—60°), (2) light petroleum (b. p. 60—80°), (3) ethyl ether, and (4) alcohol, each extraction being carried to completion.

Extract (1). A yellow soft wax (13 g.). When this was resolved in the manner described for the chloroform-soluble wax, there were obtained: hydrocarbons (Found: C, 84·9; H, 13·7%. Yield, 1·0 g.) and acids, pale yellow, m. p. 88—89° (Found: C, 72·1; H, 12·0. $C_{18}H_{36}O_3$ requires C, 72·0; H, 12·0%. Found for the *silver* salt: Ag, 25·7. $C_{18}H_{35}O_3Ag$ requires Ag, 26·5%).

Extract (2). A pale yellow solid (7 g.), containing a little unsaponifiable material. Acids were recovered and recrystallised from light petroleum (b. p. 60—80°), separating as a white powder, m. p. 89° (Found: C, 71·9; H, 11·4. $C_{18}H_{36}O_3$ requires C, 72·0; H, 12·0%. Found for the *silver* salt: Ag, 26·2. $C_{18}H_{35}O_3Ag$ requires Ag, 26·5%).

FIG. 2.

Distillation of cuticles from bituminous coal in a vacuum. Gases evolved in c.c. per gram ash-free dry material.



Extracts (3) *and* (4). The ethyl ether extract (4 g.) and the alcoholic extract (5 g.) were separately saponified, unsaponifiable material (traces only) removed, and the acids (dark brown) recovered but not further examined.

(B) *Cuticles from Bituminous Coal.*

Oxidation with Dilute Nitric Acid.—2 G. of the coal cuticles were boiled under reflux with 30% nitric acid during 6 hours, and were but little affected. On increasing the strength of the acid to 50% and boiling during 2 hours, the cuticles were attacked and the acid became red. The residue was washed with water, dried, and extracted with light petroleum; white acids similar in character to those from Papierkohle cutin were then obtained. Evaporation of the nitric acid solution to dryness yielded resinous material too small in quantity to be identified.

Destructive Distillation.—0·5 G. of the coal cuticles was distilled in the manner previously described. Oils made their appearance at 350—370°, the yield being 0·1 g., or 20% on the ash-free, dry cuticle. Resolution of this oil by means of solvents showed: saturated hydrocarbons, 11; unsaturated hydrocarbons, 22; aromatic hydrocarbons, 22; phenolic and acidic oils, 1; bases, trace; oxygenated compounds, 22; ether-soluble resin, 11; and chloroform-soluble pitch, 11%.

The gases evolved over successive temperature ranges were collected separately and analysed. The results are recorded graphically in Fig. 2 (compare Fig. 1, and Fig. 1 of previous paper).

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