organophosphorus compounds were synthesized in our laboratories.[18] Because of the possible toxic nature of the phosphorus compounds, they were handled with rubber gloves in a fume hood. Also, a small Plexiglas fume hood was placed over the sample space of the spectrophotometer to remove the strong odor characteristic of many of the samples. The spectra were recorded with a Perkin-Elmer Model 21 spectrophotometer equipped with interchangeable sodium chloride and potassium bromide optics.

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BREA, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF UTAH]

Porphyrins in Gilsonite

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A crystalline porphyrin, isolated from gilsonite, was identified to be deoxophyllerythroetioporphyrin or an isomer. The isolation creates the inference that it is the porphyrin originally present. It occurs in the form of the Ni(II) complex.

A knowledge of types of porphyrins in asphaltic materials can provide useful information in interpreting their origin and mode of formation. The presence of pyrroles[1] in the products of pyrolysis of gilsonite and the occurrence of nickel[2] in fractions of gilsonite soluble in organic solvents suggested the presence of porphyrin-nickel complexes in this asphaltite. Accordingly, a study was initiated to clarify this possibility.

A preliminary concentration procedure was found to be desirable to decrease the requirements for hydrogen bromide in acetic acid. An exhaustive extraction of gilsonite with ethyl acetate removed porphyrins into the soluble fraction, which constituted about one fourth of the total. A colorimetric method was used to follow the efficiency of the extraction procedure. A series of six extractions at room temperature removed the major portion of the desired compounds. The colorimetric analysis indicated the porphyrin content in gilsonite to be 0.03%.

The reaction of the porphyrin-metal complex in the concentrate with hydrogen bromide in acetic acid released the free porphyrins. The treatment with hydrogen bromide in acetic acid was assumed to have no effect on the porphyrin moiety of the metal complex. Using a sealed tube for this reaction, as described by Treibs,[3] was found to be unnecessary, when the reaction was repeated once. The modified approach permitted processing larger batches than would otherwise be possible. Basic porphyrin molecules were carried into the aqueous acid phase by this procedure. Accompanying neutral compounds were extracted partially with benzene. The porphyrin fraction was then placed in ether, and basic compounds removed by aqueous hydrochloric acid, leaving behind a further amount of contaminants.

Basic compounds, aside from porphyrins, in gilsonite[4] were contained in the main, in the crude fraction at this point. A procedure was devised to extract these compounds from the desired porphyrins by forming the porphyrin-nickel complex. The latter is not basic and is stable to mineral acids in general but cleaved by concentrated sulfuric acid.[5] Accordingly, the crude extract was treated with Ni(II) ion, and the resulting Ni(II) porphyrins in an ether solution were extracted with hydrochloric acid. The complex was cleaved with concentrated sulfuric acid, and the cycle was repeated to remove essentially all of the nonporphyrin bases.

Additional purification and separation of types of porphyrins were effected by applying a hydrochloric acid fractionation[6] and by chromatography. Using 2.5, 4, and 7% aqueous acid solutions, the porphyrins were separated into fractions, amounting approximately to 80%, 10%, and 10%, respectively. Final purification and separation were achieved by chromatography on calcium carbonate. Each of the fractions gave chromatograms with two red colored zones, moving away from small amounts of dark material adsorbed more tightly on the column. The major component from the 2.5% hydrochloric acid fraction amounted to about 73% of the total porphyrins.

Visible spectra were determined for the major and minor components in the 2.5% acid fraction

(1) J. M. Sugihara and D. P. Sorenson, *J. Am. Chem. Soc.*, 77, 963 (1955).

(2) P. L. Morse, American Gilsonite Co., private communication.

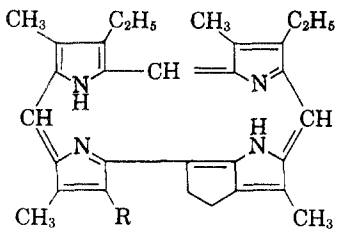
(3) A. Treibs, *Ann.*, 509, 103 (1934); 510, 42 (1934); 517 172 (1935).

(4) D. P. Sorensen, Ph.D. dissertation, University of Utah, 1955.

(5) W. S. Caughey and A. H. Corwin, *J. Am. Chem. Soc.*, 77, 1509 (1955).

(6) R. Willstätter and W. Mieg, *Ann.*, 350, 1 (1906).

and the major components of the other two fractions. Essentially identical spectra were found in all instances with peaks at 618 (I), 566 (II), 534 (III), and 499 (IV) mμ and with relative intensities in the order IV, II, III, and I. These characteristics are suggestive of the phyllo-type of porphyrins,[7] and more specifically of deoxophyllerythroetioporphyrin (I). The presence of the two components in each hydrochloric acid fraction may be interpreted as reflecting the presence of a given porphyrin together with its precursor, a monocarboxylic acid. The spectra of deoxophyllerythroetioporphyrin (I) and deoxophylloerythrin (II) are reported[3] to be identical.



I, R = C_2H_5
II, R = $CH_2CH_2CO_2H$

A sufficient amount of only the principal component of the 2.5% hydrochloric acid fraction was available for added characterization studies. The porphyrin was crystallized from chloroform and methanol in the form of reddish-brown needles. Additional recrystallizations from this solvent and from pyridine and methanol provided a sample, whose ultimate analyses for carbon and hydrogen agreed with the calculated values for deoxophyllerythroetioporphyrin. Its infrared spectrum contained a peak at 3.06 μ, charcteristic of an NH stretching frequency displaced to a higher wave length because of hydrogen bonding. Absorption characteristic of a carbonyl group was missing, indicating the absence of a carboxyl group.

As an added contribution to the identification of the major porphyrin, deoxophyllerythroetioporphyrin was synthesized for comparison purposes. This extended synthesis was carried out, as detailed in the experimental section, by procedures described in the literature with a few modifications. Separation of the porphyrins from unreacted pyrromethenes was readily accomplished by using the same procedure as was applied in separating nonporphyrin bases from the crude extract. Unfortunately the yield of the synthetic compound was so limited that a visible spectrum determination only was possible. When this spectrum was compared to that of the major gilsonite porphyrin, determined on the same instrument, by adjusting the peaks at 499 mμ to the same heights, the positions and the heights of the other three peaks were found to be identical. This and the additional data described strongly suggest that the major porphyrin isolated is deoxophyllerythroetioporphyrin. However, isomeric compounds with different sequences of side-chains cannot be discounted as possibilities, although, to our knowledge, the natural occurrence of such porphyrins has not been demonstrated.

Studies directed toward the identification of the cation associated with the gilsonite porphyrins have been made. The concentrate, obtained by ethyl acetate extraction of the bitumen, was chromatographed three times on alumina and twice on silica gel to obtain a chromatographically homogeneous fraction. A visible spectrum of this porphyrin-metal complex exhibited peaks at 552 and 514 mμ. This spectrum was found to be identical with that of the nickel complex formed by reaction of Ni(II) acetate with the porphyrin fraction prior to hydrochloric acid fractionation. Complexes formed from Cu(II) and Co(II) ions were found to possess distinctly different spectra. A flame spectrum of the isolated complex showed peaks at 349.2 and 353.5 mμ, in agreement with those for nickel.[8] An arc spectrum showed a dominant nickel line together with indications of traces of cations, which might be expected to be derived from the adsorbents used in the chromatographic purification. The absence of the vanadium line in the arc spectrum and the absence of any absorption at 570 mμ in the visible spectrum of the complex provide strong evidence for the absence of vanadium, commonly found as the cation in metal complexes of porphyrins of asphaltic materials.

The findings in this study permit several speculations. Gilsonite appears to have been derived largely, if not entirely, from plants. The predominance of decarboxylated porphyrins indicates that the temperature range involved in the formation of this bitumen was, at one time, not less than 250° to 350°, since such temperatures are normally required to decarboxylate porphyrins containing propionic acid side chains.[9] However, temperatures appreciably in excess of 400° could not have been involved, since porphyrins undergo degradation at such temperatures.

EXPERIMENTAL[10]

Isolation of total porphyrins. Bonanza gilsonite[11] (200 g.) was placed in a 1-quart Waring Blendor with 500 ml. of ethyl acetate and agitated for 1 min. The suspension was allowed to settle, and the liquid was decanted. A second 500-ml. portion of the solvent was added, and the agitation and decantation repeated. After eight extractions, removal of ethyl acetate-soluble compounds was essentially complete. Combination of the extracts and distillation of the solvent left 53.4 g. (26.7% of the gilsonite) of a black, tarry mass.

(7) R. Lemberg, *Fortschr. Chem. org. Naturstoffe*, 11, 299 (1954).

(8) G. R. Harrison, R. C. Lord, and J. R. Loofbourow, *Practical Spectroscopy*, Prentice-Hall, New York, 1948, p. 578.

(9) A. Treibs, *Angew. Chem.*, 49, 682 (1936).

(10) All melting points are uncorrected. Analyses were made by G. Weiler and F. B. Strauss, Oxford, England.

(11) Gilsonite mined from the Bonanza vein.

The latter (269 g.) was dissolved in a minimum volume (1 l.) of benzene, an equal volume of 15% hydrogen bromide in acetic acid was added, and the resulting solution was allowed to stand for 1 hr. at room temperature. The reaction mixture was then poured over cracked ice, and the resulting red acid layer was removed. Concentrated hydrochloric acid, used instead of hydrogen bromide in acetic acid, failed to extract any red color, indicating absence of free porphyrins and failure to cleave porphyrin-metal complexes. The benzene solution was retreated with hydrogen bromide in acetic acid in the same manner. The organic layer was finally extracted with one half its volume of 7% hydrochloric acid and discarded. The combined acid solutions (about 2 l.) were washed three times with 300-ml. portions of benzene to remove accompanying neutral compounds. The porphyrin fraction was extracted by ether (1 l.) by reducing the acidity of the aqueous solution with sodium acetate. The red color was transferred from the aqueous to the organic phase. The ether solution was extracted with 200 ml. of 7% hydrochloric acid, and the resulting red acid solution was extracted three times with 50-ml. portions of chloroform. The chloroform solution was washed with aqueous sodium bicarbonate and with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue (about 1 g.) was dissolved in 100 ml. of acetic acid, and the resulting solution was treated with 1 g. of nickel(II) acetate and refluxed for 15 min. The reaction mixture was poured onto ice and water, and a sufficient volume of ether (500 ml.) was added to dissolve the nickel(II) porphyrins. When the ether solution was repeatedly washed with water, a solid (B) (120 mg.) separated at the ether-water interface. The ether solution was washed three times with 50-ml. portions of concentrated hydrochloric acid to remove accompanying basic contaminants. The ether solution was then washed with aqueous sodium bicarbonate and with water, dried over anhydrous sodium sulfate, and evaporated to leave a solid (A) (140 mg.). The latter was dissolved in 50 ml. of concentrated sulfuric acid to cleave the complex, and the resulting solution was poured on ice and water. Any unchanged complex was removed by extraction of the aqueous solution with ether.

The solid B was dissolved in 500 ml. of chloroform. This solution was treated as described in the above to remove accompanying basic contaminants to yield 120 mg. of the nickel complex, which was cleaved in concentrated sulfuric acid.

Each of the sulfuric acid solutions resulting from A and B was extracted with 100-ml. portions of chloroform (total volume, 500 ml.) until all red color was removed from the acid phase. The chloroform solution was washed with aqueous sodium bicarbonate and with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in acetic acid and treated with nickel(II) acetate as previously described, and the cycle was repeated. Fractions derived from both A and B yielded a red solid at the ether-water interface during the washing operation, but not at the chloroform-water interface. The combined free porphyrins from A and B yielded 220 mg. of red solid.

An ether solution of the porphyrin fraction was extracted with 10% sodium hydroxide. A separation of insoluble porphyrin salts was not observed.

A solution of 70 mg. of porphyrins in 500 ml. of ether was fractionated with 100-ml. portions of 2.5, 4, and 7% hydrochloric acid. The completeness of removal of porphyrins was followed by use of ultraviolet light. When no fluorescence was evident in a given acid extract, the next higher concentration of acid was applied. Each acid fraction was extracted with 50-ml. portions of chloroform until removal of red color from the aqueous acid solutions was complete. Three extractions were usually required. The chloroform solutions were washed with aqueous sodium bicarbonate and with water, dried over anhydrous sodium sulfate, and evaporated to dryness, leaving 55 mg., 7 mg., and 7 mg., respectively, from the 2.5, 4, and 7% hydrochloric acid fractions.

Chromatography of porphyrins. The porphyrins (50 mg.) from the 2.5% hydrochloric acid fraction were dissolved in 100 ml. of chloroform and placed on a chromatographic column, packed with calcium carbonate (180 × 53 mm. diam.).[12] Development was effected with a mixture of benzene–petroleum ether (60–70°) (4 : 1, by vol.). The major portion of the sample moved rapidly down the column as a discrete band. A sufficient volume of the developer was introduced to move the leading zone near the bottom of the column. A second, red zone appeared near the top of the chromatogram. The adsorbent bed was extruded, and the two red-colored portions sectioned. Each of these was eluted with acetone, and solvent was evaporated from the resulting solutions. Each of the two fractions was dissolved in 100 ml. of chloroform and rechromatographed on calcium carbonate in the same fashion. The chromatogram containing the more tightly adsorbed fraction was developed by the same solvent as used initially, but with 1% (by vol.) of *t*-butyl alcohol added. The rapidly moving fraction contained 35 mg. of deoxyphyllerythroetioporphyrin, and the second fraction about 3 mg. of material. Crystallization of deoxophyllerythroetioporphyrin from chloroform and methanol provided reddish-brown needles, which did not melt when heated to 350°, although decomposition began at about 300°. The analytical sample was recrystallized twice from chloroform and methanol, once from pyridine and methanol, and once again from chloroform and methanol.

Anal. Calcd. for $C_{32}H_{36}N_4$: C, 80.63; H, 7.61. Found: C, 80.49; H, 7.64.

Chromatography of the porphyrins from the 4 and 7% hydrochloric acid fractions showed essentially the same chromatograms, with rapidly moving zones containing the major portions of the samples. Total weight of porphyrins isolated from 70 mg. of the porphyrin fraction was about 48 mg.

Spectra. Visible spectra determinations were made on a Beckman DK-2 spectrometer. Deoxophyllerythroetioporphyrin from gilsonite, dissolved in chloroform, gave a spectrum[13] with maxima at 618 (I), 566 (II), 534 (III), and 499 (IV) mμ and with relative intensities in the order IV, II, III, and I. The second portion from the 2.5% hydrochloric acid fraction and the principal portions from the 4 and 7% hydrochloric acid fractions gave essentially the same spectra with maxima appearing at the same wave lengths. Relative intensities varied very slightly, possibly because the small amounts of materials did not permit exhaustive recrystallizations.

Extinction coefficients of deoxophyllerythroetioporphyrin from gilsonite were determined with a Beckman DU spectrophotometer at a concentration of 0.0165 mg. per milliliter of chloroform. Molar extinction coefficients at 618, 566, 534, and 499 mμ were found to be 3530, 5220, 5100, and 11200, respectively.

The infrared spectrum of deoxophyllerythroetioporphyrin from gilsonite was run on a Perkin-Elmer, Model 21, spectrophotometer with a sodium chloride prism at a concentration of 6 mg. per ml. of carbon disulfide. A small NH peak at 3.06 μ and the absence of a carbonyl absorption at 5.8 μ were noted.

Deoxophyllerythroetioporphyrin. 2,4-Dimethyl-3-ethylpyrrole was prepared using the procedure of Treibs and Schmidt.[14] 2,3-Dimethyl-4-ethylpyrrole was synthesized as described by Fischer and Klarer,[15] except that a Huang-Minlon modification was used in place of the standard Wolff-Kishner reduction. 4-(β-Bromovinyl)-5-formyl-3-methyl-2-pyrrolecarboxylic acid was prepared by a slight modifica-

(12) Dimensions of the adsorbent.

(13) This and other spectral curves are given in the Ph.D. dissertation of L. R. McGee, University of Utah, 1956.

(14) A. Treibs and R. Schmidt, *Ann.*, **577**, 105 (1952).

(15) H. Fischer and J. Klarer, *Ann.*, **450**, 181 (1926).

tion of the method of Fischer and Süs,[16] since their procedure in our hands yielded only polymeric products. 5-Carbethoxy-2,4-dimethyl-3-pyrrole-α,β-dibromopropionic acid[16] (16 g.) was dissolved in 50 ml. of ethanol and 150 ml. of water added. The resulting solution was heated over a boiling water bath until carbon dioxide ceased to be evolved. The product, which separated, was filtered and dissolved in 200 ml. of ether. The ether solution was washed with 2% aqueous sodium carbonate and with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was crystallized from ethanol to yield 5.5 g. (39%) of light brown crystals of ethyl 4-(α,β-dibromoethyl)-3,5-dimethyl-2-pyrrolecarboxylate, m.p. 132–133,° reported[17] for compound prepared by bromination of ethyl 4-vinyl-3,5-dimethyl-2-pyrrolecarboxylate, 133°. A solution of 5.5 g. of ethyl 4-(α,β-dibromoethyl)-3,5-dimethyl-2-pyrrolecarboxylate in 150 ml. of absolute ether was cooled in an ice bath, and while stirring, a solution of 5.5 g. of sulfuryl chloride in 20 ml. of absolute ether was added slowly. After addition was complete, stirring was continued for 1 hr., and then the reaction mixture was allowed to stand overnight. The ether was removed at room temperature under reduced pressure. The residue was dissolved in 10 ml. of ethanol, 50 ml. of water was added, and the mixture was heated on a boiling water bath for 30 min. Ethyl 4-(β-bromovinyl)-5-formyl-3-methyl-2-pyrrolecarboxylate separated first as an oil, which crystallized upon cooling. Recrystallization from ethanol gave 2.3 g. (52%) of light brown crystals, m.p. 138–140°; reported,[16] 140°. The latter compound was then processed as described[17] to form 4-(β-bromovinyl)-5-formyl-3-methyl-2-pyrrolecarboxylic acid.

5-Bromo-5′-bromomethyl-3,4′-dimethyl-3′,4-diethylpyrromethene hydrobromide[15] and 3-(β-bromovinyl)-5-carboxy-3′-ethyl-4,4′,5′-trimethylpyrromethene hydrobromide[18] were prepared by the methods described. Condensation of the two (500 mg. each) was effected in 10 ml. of 90% formic acid (instead of succinic acid[18]) by refluxing at 110° for 8 hrs. Isolation of the desired porphyrin was effected by a modified procedure. Chloroform (200 ml.) was added to the reaction mixture. The resulting chloroform solution was washed thoroughly with water, dried over anhydrous sodium sulfate, and evaporated. The residue was dissolved in 100 ml. of glacial acetic acid containing 1 g. of nickel(II) acetate, and the mixture was refluxed for 15 min. The solution was then poured onto ice, and sufficient ether (500 ml.) was added to dissolve all the precipitated solid material. The ether solution was extracted four times with 100-ml. portions of concentrated hydrochloric acid to remove unreacted pyrromethenes, washed well with aqueous sodium bicarbonate and with water, dried over anhydrous sodium sulfate, and evaporated. The residue was dissolved in 50 ml. of concentrated sulfuric acid to cleave the nickel complex. The acid solution was poured onto ice and water. Ether (200 ml.) was added and sufficient sodium acetate was introduced to reduce acidity and permit extraction of the porphyrins into the organic phase. The ether solution was extracted four times with 50-ml. portions of 2% hydrochloric acid, the red color going to the acid phase. The latter was extracted four times with 50-ml. portions of chloroform. The chloroform solution was washed with sodium bicarbonate and with water, dried over anhydrous sodium sulfate, and concentrated to a volume of about 25 ml. The latter was placed on a chromatographic column, packed with talc (180 × 53 mm., diam.).[12] Development of the chromatogram was effected with benzene: *t*-butyl alcohol (1000:1, by vol.). The major product, etioporphyrin I, moved more rapidly down the column. The column was extruded and sectioned. The colored segments were eluted with acetone. The solvent was evaporated from the eluates, the solids obtained were redissolved in chloroform, and each of the resulting solutions was rechromatographed on talc. Deoxophyllerythroetioporphyrin was obtained in an amount of about 0.3 mg. as determined spectrophotometrically.

The visible spectrum of deoxophyllerythroetioporphyrin showed maxima at the same wave lengths as the spectrum of the major gilsonite porphyrin. When the absorbancies of the maxima at 499 mμ of the two curves were adjusted to coincide, the heights of the other three maxima were found to be identical. The major synthetic product, etioporphyrin I, possessed a distinctly different visible spectrum with maxima at 619, 566, 533, and 497 mμ with relative intensities in the order IV, III, II, and I.

Isolation and identification of the metal-porphyrin complex. A solution containing 4 g. of the ethyl acetate extract in 10 ml. of benzene was placed on a chromatographic column packed with alumina (150 × 35 mm., diam.)[12]. The chromatogram was developed with 200 ml. of benzene: *t*-butyl alcohol (1000:1, by vol.). The column was extruded and divided into two parts. The lower half of the column, orange-red in color, containing the porphyrin-metal complex in almost its entirety, was eluted with acetone. Two additional chromatograms on alumina and two on a mixture of silica gel-Celite[19] (3:1, by wt.) provided chromatographically-homogeneous material, in an amount of about 0.8 mg.

An arc spectrum of the sample showed a dominant line for nickel together with indications of trace amounts of cations contained in the adsorbents used in the chromatographic purification. A flame spectrum of the sample determined on a Beckman DK-2 spectrophotometer with a flame attachment showed peaks at 349.2 and 353.5 mμ, in agreement with those for nickel.[8]

Portions of the porphyrin fraction purified by converting into the nickel complex two times, were dissolved in acetic acid and reacted with nickel(II), copper(II), and cobalt(II) acetates. The complexes were purified by chromatography on silica gel. Visible spectra were determined, and the maxima for the nickel, copper, and cobalt complexes were found to be 552 and 514 mμ, 562 and 528 mμ, 558 and 517 mμ, respectively. The spectrum of the porphyrin-metal complex isolated possessed maxima at 552 and 514 mμ.

Colorimetric determination of porphyrins as the nickel complex. Chromatographically-homogeneous porphyrin-nickel complex, prepared as described in the previous section, was dissolved in benzene to form solutions of varying concentrations. Using a Klett-Summerson photoelectric colorimeter with a green filter, 480–550 mμ, readings were made on solutions containing from 1 to 25 parts per million of the nickel porphyrins. A plot demonstrated that Beer's Law applied over this concentration range.

The porphyrin content in any given crude fraction was approximated by this colorimetric method. In each instance samples were processed through the same purification procedure as described for the reference sample. The efficiency of the ethyl acetate extraction procedure was followed by this technique. After the sixth extraction, percentage of porphyrins in the residue dropped markedly. Based upon this colorimetric method, gilsonite was found to contain 0.03% porphyrins.

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(17) H. Fischer and K. Zeile, *Ann.*, **462**, 210 (1928).

(18) H. Fischer and H. J. Hofmann, *Ann.*, 517, 274 (1935).

(19) A diatomaceous earth, Johns-Manville Co., New York, N. Y.