Although the analytical procedure reported here was designed primarily for the analysis of cottonseed products, it has been found to be applicable to the analysis of peanut products and a variety of other agricultural materials. These applications will be outlined in a subsequent communication.

ACKNOWLEDGMENT

G. A. Harper, National Cottonseed Products Association, supplied some of the experimental cottonseed samples; G. C. Cavanagh, Ranchers Cotton Seed Oil Co., Stanley Nesheim of FDA, and J. A. Robertson, Jr., of this Laboratory gave suggestions, analytical technique, and Mrs. Louise S. Lee and Mrs. Alva F. Cucullu of this Laboratory performed some of the analyses.

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[Received January 8, 1965—Accepted March 8, 1965]

Identification of Elementary Sulfur and Sulfur Compounds in Lipid Extracts by Thin-Layer Chromatography

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Abstract

Elementary sulfur, long chain thiols and sulfides in lipid mixtures can be separated and identified by thin layer chromatography (TLC), preparation of derivatives, development of typical flourescent colors with Rhodamine 6G under ultraviolet light, and colors with other spray reagents. Silica gel mixed with magnesium silicate and the same adsorbent plus silver nitrate are used for polar stationary phase and silver nitrate complexing chromatography, respectively.

Elementary sulfur yields a purple fluorescent spot with Rhodamine 6G in contrast to the yellow fluorescent of most lipids. Compounds isolated by means of TLC were further identified by spectroscopic methods. The sulfur bacterium (*Chromatium sp.*), and the Orgueil carbonaceous meteorite were analyzed by the new technique. Elementary sulfur was identified in both samples, but the lipid compositions of the bacteria and meteorite were found to be entirely different. The meteorite lipids and hydrocarbons were also different from the abiological hydrocarbons synthesized in a Miller high frequency spark discharge experiment.

The new analytical technique is suitable for the analysis of recent biological matter, petroleum, bitumens and organic matter from marine sediments.

Introduction

A STUDY OF LIPIDS in sulfur bacteria, petroleum, and carbonaceous stony meteorites resulted in the development of a chromatographic technique for the detection of trace quantities of elementary sulfur and organic sulfur compounds. The desirability of a technique to identify sulfur and sulfur compounds in lipid extracts was emphasized by the appearance of a number of well defined but unknown spots on the thin-layer chromatograms of lipid mixtures isolated from substances known to contain sulfur in some form. Standard methods of sulfur anaylsis did not appear to be applicable to sulfur determination in lipids.

Several of the standard analytical methods for determining trace quantities of sulfur and sulfur compounds are based on reaction with metal ions (1,2), oxidation (3,4,5,) and colorimetric determinations (6,7). Chromatographic methods have been used repeatedly in recent years. Sulfur compounds from large samples of petroleum have been identified by Smith et al. (8,9) by column chromatography. Ertel and Horner (10) separated a few sulfur compounds from microgram quantities of samples by TLC. Mangold (11) has reviewed some of the applications of TLC for lipids and quantitative TLC of lipids has been discussed in detail by Privett et al. (12,13,14).

Two approaches were used in the course of developing the present analytical procedure. First, experiments were performed with pure standards. Secondly, unidentified components of natural samples were separated by TLC, eluted from the adsorbent, and analyzed by suitable chemical means as well as IR and UV spectroscopy. TLC included 1) polar stationary phase and 2) polar stationary phase impregnated with silver nitrate. The overall technique permits identification of sulfides, thiols and elementary sulfur in the presence of hydrocarbons, fatty acids, fatty alcohols, esters and amines.

Materials and Methods

The following materials were used:

Methyl octadecanoate, Nutritional Biochemicals Corp, 99% purity.

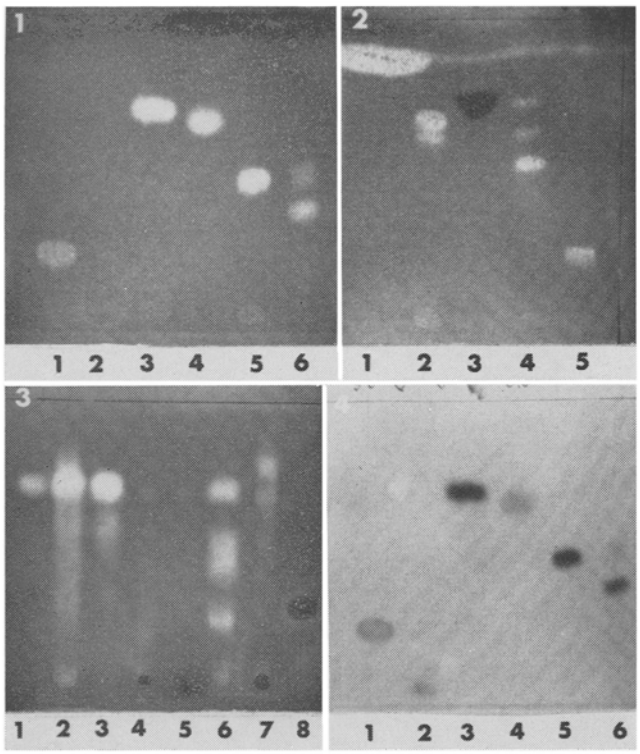


FIG. 1. TLC to show migration of standards. Ascending chromatography with silicic acid-magnesium silicate adsorbent, n-hexane as solvent, development at room temperature, and Rhodamine 6G spray. Impurities in adsorbent pushed to end of plate by ascending development wash with chloroform prior to application of samples. Applications: 1) 20 μg squalene; 2) 10 μg β-carotene (no spot seen); 3) 20 μg dotriacontane; 4) 20 μg octadecane; 5) 20 μg di-n-octadecyl trisulfide, and 6) 20 μg octadecylthiol (upper two spots from sulfides, lower spot from thiol). Note the absence of line across top of chromatogram produced from impurities (see Fig. 2 without chloroform prewash). Note wide separation of unsaturated hydrocarbon (squalene) from saturated hydrocarbons demonstrating the pronounced effect of double bonds and the very small difference in migration with large change in chain length. Sulfide and thiol are separated from each other and from saturated hydrocarbons (compare Fig. 4 where the same chromatogram was sprayed with the charring reagent).

FIG. 2. TLC to show improved separation of standards at 4C. Silicic acid-magnesium silicate adsorbent, neohexane as solvent, ascending development at 4C temperature, and Rhodamine 6G spray. Applications: 1) 40 μg octadecane; 2) 20 μg of di-n-octadecyl trisulfide; 3) 20 μg elementary sulfur; 4) 20 μg octadecylthiol; and 5) 20 μg squalene. Note the improved resolution at 4C compared to 25C (Fig. 1) and the presence of an impurity line at the solvent front (no $CHCl_3$ prewash) in contrast to its absence in Fig. 1.

FIG. 3. TLC to illustrate components in various samples. Silicic acid-magnesium silicate adsorbent, n-hexane as solvent, ascending development at 25C, and Rhodamine 6G spray. Applications: 1) 20 μg of octadecane; 2) 100 μg commercial motor oil; 3) 100 μg of laboratory pump oil; 4) 100 μg of Rozel point crude petroleum (seep); 5) 100 μg of a naphthenic acid fraction isolated from combined petroleum stocks; 6) 100 μg of extract from banana palm leaves; 7) 100 μg of material scraped from the electrode of a Miller high frequency spark discharge apparatus in which CH_4, NH_3 and H_2O were subjected to sparking; and 8) 20 μg of phenanthrene. The motor oil sample contained polar components not seen in pump oil. Crude petroleum and the naphthenic acid fraction of petroleum contained smaller amounts of the saturated and unsaturated hydrocarbons characteristic of pump and motor oils. The banana palm extract is seen to contain a variety of hydrocarbons. The presence of hydrocarbons in the Miller experiment is seen (application 7). Note the unidentified component in application 7 which has moved ahead of octadecane.

FIG. 4. TLC to demonstrate result of spraying with the sulfuric acid-dichromate spray after Rhodamine 6G. Same chromatogram shown in Fig. 1. The relatively light spot of octadecane (application 4) indicates loss of the more volatile hydrocarbons by heating at 180C.

Di-n-octadecyl sulfide, di-n-octadecyl disulfide, di-n-octadecyl trisulfide, and di-n-octadecyl tetrasulfide, obtained through the courtesy of N. Nicolaides of the University of Oregon Medical School.

Aromatic hydrocarbons: p,p'-bitolyl, retene, perylene and p-quinquephenyl, Metro Scientific, Inc., Long Island, N.Y. Hydrocarbon Kits 1 and 2 supplied by K & K Labs, Inc., Plainview, N.Y.

Phenanthrene, practical grade, Eastman Chemicals Company.

Sulfur flowers, Baker and Adamson. Dissolved in hexane to give a 1 mg/ml solution.

N-Ethylmaleimide, Delta Chemical Works, Inc., practical grade, recrystallized from hexane before use.

Hydrogen peroxide (Superoxol, 30%).

Rhodamine 6G, Allied Chemical Corp., 1 ml. aqueous stock solution (1 mg/ml) diluted with 100 ml water to give a 0.001% solution.

Hexane, chloroform, benzene, methanol (spectroquality; Matheson, Coleman, and Bell) were freshly distilled in 300 mm columns packed with Raschig rings before use.

Ether, absolute anhydrous, Mallinckrodt Chemical Co.

Silica Gel, Plain, Research Specialties Co., Richmond, Calif.

Silica Gel G, Brinkmann Instruments Co., Great Neck, Long Island, N.Y.

Magnesium silicate, synthetic; ratio of magnesium oxide to silica 2:5, moisture 12%; Allegheny Industrial Chemical Corp., Butler, N.J.

Preparation of Thin Layers

A mixture of Silica Gel Plain containing 10% (by wt) magnesium silicate was heat activated (120–150C for 3–6 hr) and then ground in a ball mill with lightweight ceramic balls for ½ to 1 hr. The activated adsorbent was cooled in a closed container to prevent uptake of moisture and extraneous substances from air and stored in a tightly capped container until use. Other adsorbents were not heat activated before spreading over plates.

A thin slurry of the Silica Gel-magnesium silicate adsorbent consisting of 25 g of adsorbent and 70 ml of water provided a very uniform layer when spread from a Desaga applicator (fixed distance, set at 0.25 mm) on glass plates 20×20 cm in size. Similarly, Silica Gel G layers were prepared from a slurry of 25 gm of adsorbent and 63 ml of water. Thin layers were heat activated just before use (120C for 20 min.), cooled for 30 min, and prewashed with chloroform before sample application (15).

The silver nitrate impregnated layers were prepared by spraying a normal Silica Gel-magnesium silicate layer with a saturated aqueous solution of silver nitrate. Each layer was dried for ½ hr at 120C, and after removal from the oven was protected from direct light before spotting.

Prewashing the Thin Layers

A preliminary wash with chloroform is desirable with both Silica Gel-magnesium silicate and Silica Gel G layers for removal of various impurities in the adsorbents, particularly when lipids are to be isolated from the adsorbents. Washing was performed by the ascending technique in chromatography chambers lined on all sides with solvent saturated Whatman No. 1 or No. 3 filter paper.

Sample Application

Samples were dissolved in chloroform, carbon tetrachloride, or hexane (usually at a concentration of 2.5 mg/ml) and were stored, before application, in graduated glass stoppered centrifuge tubes to prevent changes in concentration due to solvent evaporation. Samples were applied to the adsorbent from a 50μl syringe by spotting several small drops of the sample in a horizontal row 1 cm long. This spotting technique resulted in better resolution of components of the chromatograms by giving oval-shaped spots (16).

Development of Chromatograms

Chromatograms were developed in glass chambers (11–¾ × 11 × 4 in.) lined on all sides with Whatman No. 1 or No. 3 filter paper. The solvent (200 ml) was added and the liners wet by tilting chambers first to one side and then to the other a few minutes before insertion of plates. It is important to avoid interference from silicone grease and other common laboratory contaminants during development. Suitable tight-fitting, ground-glass chamber tops can be held in place satisfactorily with a weight and without grease.

Spraying of Chromatograms

Chromatograms were sprayed within a few minutes after removal from the developing chamber. Lipids were detected with one of several reagents. Rhodamine 6G either as a 0.001% solution in water or a 0.002% solution in 2 N KOH are useful general spray reagents (16,17). Chromatograms were viewed wet under short wave UV light and then allowed to dry since some lipids show characteristic color changes on drying and some spots become more intense. Spots may be more intense with the alkaline reagent, but the dye in water (no KOH) must be used when lipids are to be isolated from chromatograms. All lipids, hydrocarbons, and elementary sulfur give spots with these dye solutions. After drying, chromatograms sprayed with the aqueous reagent can be sprayed with the sulfuric acid-dichromate (charring) or phosphomolybdate reagents. Plates sprayed with 0.6% solution of potassium dichromate in 55% (by wt) sulfuric acid (15) were heated at 180C until fuming ceased, producing brown or black charred spots from lipids. Elementary sulfur does not react and is lost from the plate during heating. Phosphomolybdic acid as a 10% solution in absolute ethanol produces blue spots on a white or pale yellow background after heating for about 10 min at 120C. Elementary sulfur and lipids such as bitolyl that are lost from the plate prior to reaction at 120C can give good spots with this reagent if color development is begun in an oven at about 40C and the temperature increased to 120C over a 3–5 min (Fig. 6). A saturated aqueous solution of silver nitrate applied as a very fine mist provides a useful and sensitive spray for elementary sulfur (producing an orange colored spot) and to a lesser extent thiols and sulfides. Reaction is instantaneous at room temp.

N-Ethylmaleimide Derivatives

The reaction of thiols with N-ethylmaleimide was carried out either in solution or directly on the thin layer plates (see Fig. 5). The reagent appears to be specific for thiol groups in a manner analogous

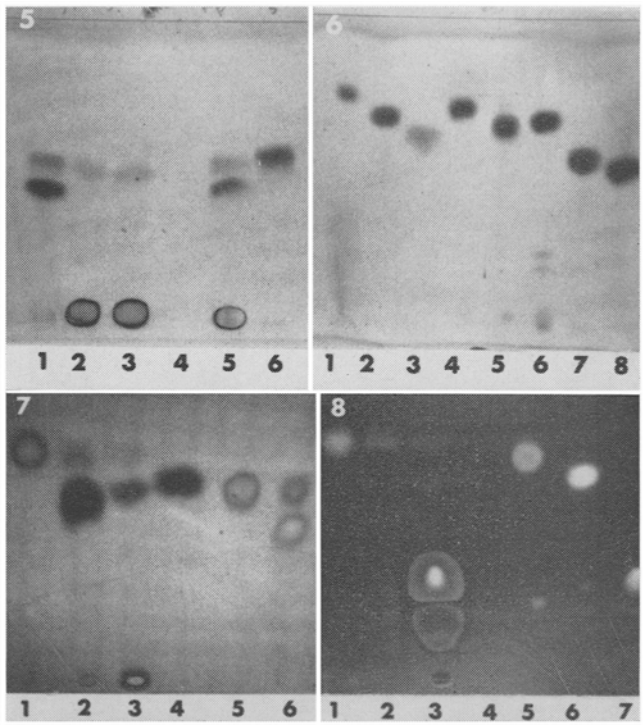


FIG. 5. TLC to show reaction of thiol with N-ethylmaleimide and elementary sulfur. Silicic acid-magnesium silicate adsorbent, ascending development with n-hexane at 25C, sprayed with sulfuric acid-dichromate reagent and heated for spot development. Applications: 1) 50 μg of octadecylthiol (upper spot from corresponding sulfide); 2) and 3) 40 μg of thiol reacted in n-hexane with 3– and 5–fold excess of NEM, respectively; 4) 50 μg of NEM reagent only (no spot); 5) 50 μg of octadecylthiol spotted then spotted over with 50 μg NEM in n-hexane; 6) mixture of 25 μg of octadecylthiol and 25 μg of elementary sulfur.

FIG. 6. TLC to show reversal of the order of migration of elementary sulfur with respect to thiol and sulfide. Silicic acid-magnesium silicate adsorbent, ascending development at 25C with n-hexane/diethyl ether 96/4, sprayed with phosphomolybdic acid reagent and heated to develop spots. Applications: 1) 10 μg of dotriacontane; 2) 25 μg of octadecane; 3) 25 μg of elementary sulfur; 4) 25 μg of di-n-octadeyl trisulfide; 5) 25 μg of octadecylthiol; 6) 25 μg of squalene; 7) 25 μg of p, p'-bitolyl; and 8) 25 μg of retene. Note that elementary sulfur (3) migrates behind sulfide (4) and thiol (5) in contrast to the reverse order with n-hexane as solvent. Both elementary sulfur (3) and bitolyl (7) gave good spots with the phosphomoybdate reagent since color was developed by placing the chromatogram in an oven at 40C and allowing the temp to rise to 120C over a period of 4 min to avoid heat loss of volatile substances prior to reaction.

FIG. 7. TLC to show elementary sulfur and lipids in the Orgueil carbonaceous stony meteorite and the sulfur bacterium (*Chromatoum sp.*). Commercial Silica Gel G adsorbent ($CaSO_4$ as the binder), ascending development with n-hexane at 25C and spots developed with Rhodamine 6G spray. Applications: 1) 25 μg of octadecane; 2) 100 μg Orgueil meteorite extract; 3) 100 μg of sulfur bacterium extract; 4) 50 μg of elementary sulfur; 5) 25 μg of di-n-octadecyl trisulfide; and 6) 25 μg of octadecylthiol. Note the presence of hydrocarbon (upper spot) and sufur (larger, dark spot) in the meteorite (2) and bacterial (3) extracts. The bacterial extract (3) contains more polar components that do not migrate from the origin (compare Fig. 9).

FIG. 8. TLC to show the more polar components of the Orgueil carbonaceous meteorite and the sulfur bacterium (*Chromatium sp.*). Commercial Silica Gel G adsorbent ($CaSO_4$ as binder), ascending development at 25C with n-hexane/diethyl ether/glacial acetic acid (70/30/1, v/v/v), and alkaline Rhodamine 6G spray. Applications: 1) 25 μg octadecane; 2) 100 μg of Orgueil meteorite extract; 3) 100 μg of sulfur bacteria extract; 4) 20 μg of elementary sulfur (the purple spot not seen against the dark purple background produced by the reagent); 5) 25 μg of octodecylthiol; 6) 25 μg of methyl octadecanoate; and 7) 25 μg of octadecanoic acid. Note the light spots in the region for fatty acids in the meteorite extract (2) that are much more prominent in bacterial extract (3) and trace of ester in bacterial extract only.

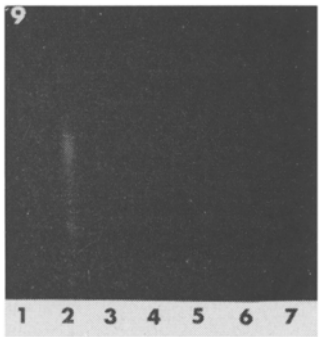


FIG. 9. TLC potograph to show the presence of natural fluorescent material in the Orgueil meteorite. The same chromatogram as shown in Fig. 8 but photographed under UV light prior to staining with Rhodamine 6G. Fig. 9 shows the presence of fluorescent material in (2) from the Orgueil meteorite and the lack of the streak of fluorescent substances in the bacterial extract (3).

to reactions with amino acids and proteins (18,19). In a small glass-stoppered centrifuge tube 0.5 ml of a lipid solution was added to a threefold excess (three times the milliequivalent weight of the lipid standards) of N-ethylmaleimide reagent in carbon tetrachloride solution. Reaction was almost instantaneous. To form the derivative directly on the adsorbent, the thiols were spotted in the usual way and then at least a threefold excess of the N-ethylmaleimide reagent was added directly over the sample area at the origin on the plate. Since the N-ethylmaleimide reagent itself gives no color with Rhodamine 6G and the other reagents, it does not interfere in the visualization of lipids (Fig. 5).

Oxidation with Hydrogen Peroxide

The oxidation of thiols with hydrogen peroxide (20) was carried out in a manner similar to that described for the formation of the N-ethylmaleimide derivatives of the sulfhydryl compounds. The samples were dissolved in carbon tetrachloride or chloroform (0.5 ml) and they were shaken vigorously for 1 hr with 0.5 ml of 30% hydrogen peroxide. After removal of the water layer from the sample, traces of peroxide were removed by evaporation of the solvents under nitrogen (excess peroxide may cause streaking on chromatograms). Attempts to form derivatives with hydrogen peroxide directly on the plates were successful, but the excess peroxide caused some streaking.

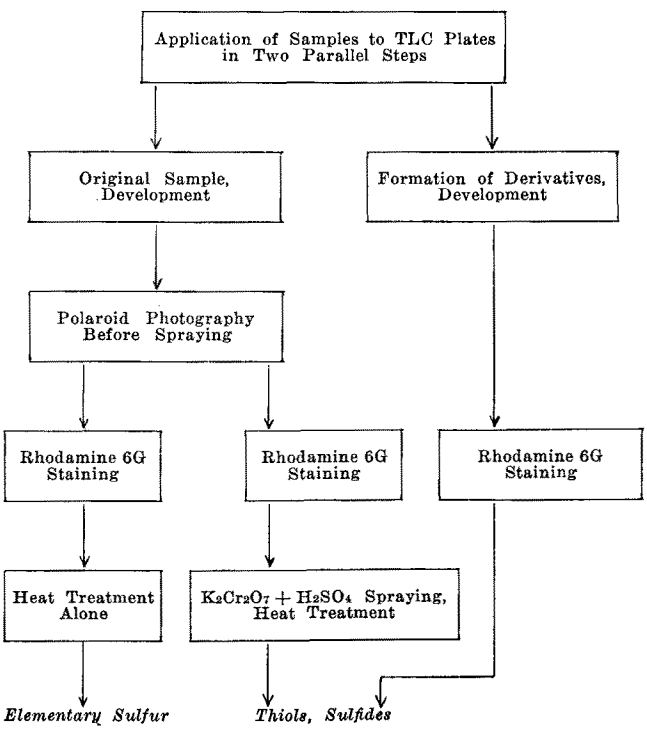


FIG. 10. Flow sheet illustrating the basic steps in the thin-layer chromatographic analysis. Additional procedures, such as phosphomolybdic acid and silver nitrate staining, may be added to this scheme for purposes of confirmation.

Recovery of Sulfur and Sulfur Compounds After TLC

Individual components of the lipid material separated by TLC were located with 0.001% Rhodamine 6G, scraped off with a razor blade, and eluted from the adsorbent with hexane or chloroform. When Rhodamine 6G was used as a visualizer, elution of substances from the adsorbent was accomplished by placing the adsorbent over a short column (2–3 in) of silica gel (Mallinckrodt) packed in chloroform and eluting with chloroform. Silica gel retained the dye and released the desired components for further analysis.

Results

The overall technique is summarized in Figure 10.

Migration Characteristics and Color Reactions

Sulfur and the sulfur compounds examined in this study did not migrate as far as saturated hydrocarbons but migrated farther than fatty acids, esters, alcohols and amines on Silica Gel-magnesium silicate layers (Figs. 1,3,4,8). The following migration sequence was observed in the various solvent systems: saturated hydrocarbons > elementary sulfur > sulfur compounds > unsaturated hydrocarbons > esters > acids > alcohols > amines. Hexane and hexane-ether mixtures are suitable solvents for separating the lipids of low polarity.

Depending on the information desired, the chromatograms were sprayed with Rhodamine 6G, phosphomolybdic acid, the sulfuric acid charring reagent, or silver nitrate solution. The sulfur compounds examined in this study gave yellow flourescent spots on a pink or light purple background with Rhodamine 6G under UV light. Elementary sulfur gave an intense dark purple fluorescent spot with the same indicator on a pink or light purple background. Spraying with the other reagents produces colored spots on a white or yellow background under visible light. Phosphomolybdic acid gives an immediate blue color reaction at room temp with the thiol group only; other components are detectable after heating at 120C for 10–20 min. Elemental sulfur gives a spot that can be observed without staining by photography with the Polaroid camera on Pola Pan 200/Type 42 film with short wave UV illumination. The spot is not detectable by eye.

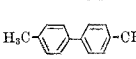
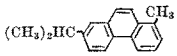
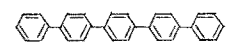
On thin layers containing Silica Gel impregnated with silver nitrate, sulfur compounds reacted immediately with the silver ions prior to development and gave orange colored spots. These spots remain at the origin with hexane, hexane-ether (94/6) and hexane-ether (80/20) as solvents.

Developing Solvents

The R_f values of some of the compounds in different solvent systems are listed in Table I. The resolutions of the nonpolar components of the samples obtained

TABLE I

R_f Values for Hydrocarbons and Sulfur and Sulfur Compounds on Silica Gel Plain with 10% Magnesium Silicate (The Arithmetic Mean of These R_f Values is ±0.06, Based on 26 Determinations)

Substance	Structure or Structural Formula	Solvent			
		Hexane at room temp.	Hexane at 4C	Hexane-ether 96/4	Hexane-ether 94/6 (on Ag^+
Saturated hydrocarbons					
Dotriacontane	$C_{32}H_{66}$	0.81	0.92	0.74	0.97
Octadecane	$C_{18}H_{38}$	0.74	0.90	0.67	0.95
Sulfur and sulfur compounds					
Sulfur	S_x	0.61	0.80	0.61	origin
Di-n-octadecyl trisulfide	$(C_{18}H_{37})$-S_3-$(C_{18}H_{37}$	0.59	0.78	0.70	origin
Octadecanethiol	$C_{18}H_{37}SH$	0.47	0.61	0.65	origin
Unsaturated hydrocarbon					
Squalene	$[(CH_3)_2C{=}CHCH_2CH_2C(CH_3){=}CHCH_2CH_2$-$C(CH_3){=}CHCH_2$-$)]_2$	0.37	0.41	0.66	0.06
Aromatic hydrocarbons					
p,p'-Bitolyl		0.33	0.41	0.54	not detected
Retene		0.31	0.39	0.50	0.78
Perylene		0.22	.....	0.36	0.14
p-Quinquephenyl		origin	.....	not detected	origin

with the branched chain solvents, neohexane and neopentane, and with cyclohexane, were approximately the same as the resolution obtained with n-hexane. A significant increase in the resolution of hydrocarbons and sulfur compounds did occur, however, when the chromatograms were developed with hexane at 4C or 10C rather than room temperature (compare Figs. 1 and 2). Two impurities in the octadecane thiol sample became apparent on a chromatogram developed in the cold (Fig. 2). Improvement in resolution with the other solvents at the low temps was not significant. The migration sequence was reversed in the hexane-ether (96/4) system (Fig. 6) with sulfide and thiol migrating ahead of elementary sulfur.

Affect of Plate Lengths

Increased resolution was obtained by doubling the length of the adsorbent layer from the usual 15 cm to 30 cm. Hydrocarbons and sulfur compounds were well separated on a double length layer. Dotriacontane migrated in front of octadecane when developed with hexane. One of the impurities in the octadecane thiol sample, which was resolved in cold development, did not appear on the double length layer, even though the separation of the two other components of this sample was increased over that found at room temperature or at 4C.

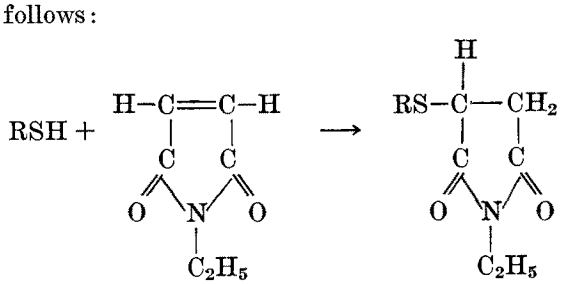
Interactions Between Thiols and Elementary Sulfur

Mixtures of elementary sulfur and thiols did not show the typical migrations of either substance alone (Fig. 5). Reaction evidently takes place. With a molar excess of elementary sulfur, the thiol spot is missing and a new spot, showing yellow fluorescence with Rhodamine 6G, appears in the sulfide region of the chromatogram. With a molar excess of thiol, the sulfur spot disappears, some thiol can be seen, and what appears to be a sulfide spot is observed. When a stochiometric mixture of sulfur and thiol is made in carbon tetrachloride and chromatographed on a thin layer plate, a new product is obtained which appears to be a sulfide. Similar reactions between these two substances have been observed by other investigators (2,21,22,23). The exact reaction mechanism is not known, although it appears to be analogous to the usual disulfide exchange reaction of RSH and R-S-S-R. This reaction occurs when samples contain both thiols and elementary sulfur and may account for the absence of thiols in sulfur bacteria (*Chromatium sp.*) and carbonaceous meteorite organic matter, both of which contain an excess of elementary sulfur (Fig. 7).

Derivatives

The presence of sulfides or thiols in synthetic lipid mixtures was confirmed by derivative formation followed by TLC. The plates were compared with the original chromatograms containing no derivatives. The N-ethylmaleimide (NEM) addition products of thiols and the hydrogen peroxide oxidation products of thiols and sulfides remained at the origin when hexane was used to develop the chromatograms (Fig. 5). Both of these reagents were insensitive to the spray reagents used for visualization of lipid compounds and gave no color reactions by themselves. The identifications of questionable components on the original chromatogram became possible in this mannner because of the absence of the spots from their original position. When small samples were used, the derivatives were prepared directly on the adsorbent layer. After a sample was applied to the adsorbent in the usual manner, excess reagent was added over the sample area.

The reaction of NEM with thiols can be visualized as follows:



Note the disappearance of the thiol spot, (applications 2 and 3) on Figure 5, when reaction with NEM is carried out with excess reagent in the test tube and the incomplete reaction when the reagent

is spotted over the thiol spot on the plate. Reaction with NEM produces a derivative that does not migrate from the origin, (applications 2,3,5). No thiol or sulfur spots are seen when equal wts of sulfur and thiol are mixed and a spot in the sulfide region appears.

Hydrogen peroxide oxidizes thiols to disulfides according to the following equation:

$$2RSH + H_2O_2 \longrightarrow RSSR + 2H_2O$$

The derivatives are useful to obtain confirmatory evidence for the presence of thiols and sulfides in samples.

Detection of Hydrocarbons in Oils and Other Samples

Figure 3 shows the application of the chromatographic procedure to several types of samples. Pump and motor oils contain hydrocarbons (Fig. 3) that can be introduced into the analytical samples unless precautions are taken to vent vacuum pump exhaust completely and remove traces of oil from laboratory glassware and equipment. TLC is helpful in disclosing such contaminations. Plants such as the banana palm yield a variety of hydrocarbons (Fig. 3, application 6) as native components. The petroleum, (application 4) naphthenic acid (application 5) and the hydrocarbons (application 7) abiologically synthesized by Miller high frequency spark discharge experiment from methane, ammonia and water (simulating conditions on the primitive earth) are of interest for comparison with the Orgueil meteorite lipids (24). The Orgueil lipids seem to lack some of the components present in each of these samples. The Orgueil sample seems to represent a unique composition (25).

Analysis of Two Natural Samples Containing Sulfur

The new chromatographic methods of analysis were used with saponified benzene-methanol (60/40) Soxhlet extracts and the substances soluble in chloroform from the sulfur bacterium (*Chromatium sp.*), and from organic matter in the Orgueil carbonaceous meteorite (Fig. 7). After the Rhodamine 6G spray, purple fluorescent spots appeared on the plates in addition to the customary yellow spots. The purple spots had the same R_f value as elementary sulfur (Fig. 7). Both the purple and some of the yellow staining spots were scraped off the plates with a razor blade, eluted from the adsorbent with chloroform or hexane/ether mixtures and subjected to further identification by spectroscopy. IR spectra were run with a Beckman Model IR-4 infrared spectrophotometer (NaCl optics) and the KBr pelleting technique. The major nonpolar, yellow staining fractions were identified as hydrocarbons. The purple staining material moving just below the hydrocarbon spot in the position of elementary sulfur crystallized from solution as a yellowish-white and finely divided crystalline substance similar to a sample of elementary sulfur isolated in the same manner. This crystalline fraction darkened silver upon heating and gave an IR spectrum identical with that of elementary sulfur. UV spectra obtained with a Beckman DK2A spectrometer and hexane as solvent, showed absorption bands at 260 mμ and 280 mμ wavelengths as observed for elementary sulfur. It was therefore concluded that the purple fluorescent material on the plates was indeed elementary sulfur.

The results obtained with natural mixtures make it apparent that the technique is applicable for the analysis of natural lipid mixtures containing sulfur. If the technique is followed carefully, differences in sample compositions can be defined. This is illustrated by the similarities and differences found between the sulfur bacteria (*Chromatium sp.*) and the Orgueil carbonaceous meteorite organic matter. Both of these samples contained hydrocarbons. Thiols were not detected, but elementary sulfur was present. They differed by the fact that the meteorite lacked some components, such as the ester fraction present in the bacteria and that the fatty acid content of the carbonaceous meteorite was by an order of magnitude less than that of *Chromatium sp.* (Fig. 8). The bacteria, on the other hand, did not contain a number of the naturally fluorescening, polar components of the meteorite (Fig. 8). The two samples, shown in Figures 7–9, were prepared by different methods; the bacterial sample was a saponified benzene-methanol Soxhlet extract and the meteorite sample was a cold chloroform extract. It may be of interest to note that these differences between these two substances were also clearly noticeable when both samples were prepared by the identical saponification process. This points out that the two samples are basically different in character. The results (see Figs. 3,7,8,9) suggest that the technique is applicable to the analysis of recent biological matter, and may be useful for analysis of petroleums, bitumens and organic matter in marine sediments.

ACKNOWLEDGMENTS

Prof. Harold C. Urey of the University of California provided valuable suggestions. Mrs. Mary Alice Lumpkins and Mr. Vincent Modzeleski of the University of California, San Diego assisted in the experiments. Samples of the Orgueil meteorite were provided by Prof. Jean Orcel of the Musée National d'Histoire Naturelle, Paris, France, and D. A. Cavaille of the Musée d' Histoire Naturelle de Montauban, Tarn-et-Garonne, France. The bacteria sample was supplied by Prof. Andrew Benson of the University of California, San Diego. Prof. Stanley Miller of the University of California, San Diego provided a sample from his high frequency spark discharge experiments and Dr. Sol Silverman of the California Research Corporation in La Habra, California provided the petroleum and naphthenic acid samples. The investigation was supported by the National Aeronautics and Space Administration, NsG–541 and in part by U.S. Public Health Service Grants NB–01847–06 from the National Institute of Neurological Diseases and Blindness, CA–03134–08 from the National Cancer Institute, and Research Grant DA–AMC–18–035–71(A) from the U.S. Army Chemical Research and Development Laboratories, Army Chemical Center, Maryland.

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[Received November 30, 1964—Accepted January 29, 1965]