hydrogen chloride through a benzene solution of the quinaldine. The hydrochloride separated from the benzene solution as a white solid. The melting point of the purified compound was 200.5° (cor.).

Anal. Calcd.: neut. equiv., 287.6. Found: neut. equiv., 288.

Preparation of 6-Methyl-3-phenylmercaptoquinaldine.—Three grams of 6-methyl-3-phenylmercapto-4-quinaldinecarboxylic acid was placed in a 25-cc. distilling flask attached to an air condenser, and the mass heated on an oil-bath to 290°. At this temperature the acid melted and carbon dioxide was evolved. After the effervescence had ceased the liquid was distilled under diminished pressure, and the fraction boiling at 280° (1–2 mm.) was collected as a heavy oil which solidified on cooling. After two recrystallizations from alcohol the white 6-methyl-3-phenylmercaptoquinaldine melted at 79.8° (cor.).

Anal. Calcd. for $C_{17}H_{15}NS$: N, 5.28. Found: N, 5.90.

Preparation of the Picrate of 6-Methyl-3-phenylmercaptoquinaldine.—The picrate was prepared by dissolving the substituted quinaldine in hot alcohol and pouring into a saturated aqueous solution of picric acid. The picrate separated immediately and after recrystallization melted at 217.5° (cor.).

Summary

1. Pfitzinger's method has been extended to include the utilization of thiophenoxyacetone in the synthesis of 3-phenylmercapto-4-quinaldinecarboxylic acid and 6-methyl-3-phenylmercapto-4-quinaldinecarboxylic acid from isatin and 5-methylisatin, respectively.

2. Phenoxyacetone has been condensed with 5-methylisatin to form 6-methyl-3-phenoxy-4-quinaldinecarboxylic acid.

3. The thio acids have been decarboxylated, and some derivatives of their quinaldines prepared.

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[CONTRIBUTION FROM THE NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Oxidation of Thio-ethers by Unsaturated Fatty Acids

BY WILLIAM V. B. ROBERTSON, JONATHAN L. HARTWELL AND SYLVIA KORNBERG

In the course of a toxicological investigation of β,β'-dichloroethyl sulfide it was observed that 1% and 5% olive oil solutions of the sulfide gradually decreased in toxicity and after standing several weeks at room temperature deposited varying amounts of crystals. These were identified as β,β'-dichloroethyl sulfoxide by mixed melting point determination with an authentic specimen. Fresh olive oil solutions of the sulfide when kept under nitrogen did not deposit crystals, nor did the pure sulfide when exposed to oxygen for over one year. The possibility that bacterial or other enzymes caused the reaction was excluded since olive oil which had been heated at 150° for two hours under nitrogen was still able to effect the oxidation of the sulfide.

A review of the literature revealed only one brief note about such an oxidation. In 1925 Walker[1] reported the oxidation of β,β'-dichloroethyl sulfide, diphenylchloroarsine and diphenyl arsenious oxide by aged turpentine or rancid olive oil. Investigation of this reaction seems to have been dropped, since no other reports appeared. Destruction of carotene,[2] vitamin A,[3,4] biotin,[5] vitamin D,[6] vitamin E,[3] and of the carcinogenic azo dye *p*-dimethylaminoazobenzene[7] by rancid fats has been noted. Recently Burr and Barnes[8] have discussed the deterioration of dietary essentials caused by the presence of unsaturated fats in the diet. In view of these physiological implications a more detailed study of the oxidation of thio-ethers was undertaken. This paper presents the results of experiments dealing with the mechanism and specificity of the reaction.

Highly unsaturated linseed oil, when substituted for olive oil, was found to react even more rapidly, whereas mineral oil was completely inactive. Various other oils were then tested for the ability to mediate the oxidation of the β,β'-dichloroethyl sulfide. The oils were selected either because of general interest or for their content of specific fatty acids. The results, which are shown in Table I, indicated that all unsaturated oils tested, with the exception of those consisting mainly of glycerides of fatty acids with conjugated unsaturation (tung oil and oiticica oil), were able to oxidize the sulfide to sulfoxide. Neither the yield nor the time until appearance of sulfoxide crystals was related to the degree of unsaturation. The addition of driers was necessary in some cases in order to overcome the effect of naturally-occurring oxidation inhibitors; that the drier did not effect the oxidation in the absence of unsaturated oils is seen from the table in the results with liquid petrolatum and tricaprylin. The deposition of crystals was always preceded by oxygen consumption, as indicated by a strong vacuum within the reaction flask. Although both tung and oiticica oil absorbed oxygen, subsequent oxidation of the sulfide did not

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(1) Walker, *J. Chem. Soc.*, **127**, 1491 (1925).

(2) Olcovich and Mattill, *J. Biol. Chem.*, **91**, 105 (1931).

(3) Mattill, *J. Am. Med. Assoc.*, **89**, 1505 (1927).

(4) Sumner, *J. Biol. Chem.*, **146**, 215 (1942).

(5) Pavcek and Shull, *ibid.*, **146**, 351 (1942).

(6) Fritz, Halpin, Hooper and Kramke, *Ind. Eng. Chem.*, **34**, 979 (1942).

(7) György, Tomarelli, Ostergard and Brown, *J. Exptl. Med.*, **76**, 413 (1942).

(8) Burr and Barnes, *Physiol. Rev.*, **23**, 256 (1943).

TABLE I

OXIDATION OF β,β′-DICHLOROETHYL SULFIDE WITH OILS AND CRUDE FATTY ACIDS

Oil	Iodine number	Oxygen consumption[i]	Time to appearance of crystals, days[j]	Yield, % of calcd.
Liquid petrolatum U. S. P.	0	− (D)	No crystals	0
Tricaprylin[a]	0	− (D)	No crystals	0
Coconut	8.9	− (D)	No crystals	0
Olive	81.0	+	8	34
Castor	85.6	+ (D)	22	6
Chaulmoogra	96.2	+ (D)	7	29
Mustard seed	101.4	+	9	42
Rapeseed	102.8	+ (D)	3	24
Parsley seed[b]	105.6	+ (DD)	>60	..
Henbane	107.7	+ (D)	3	26
Wheat germ	122.8	+ (DD)	6	46
Poppy seed	128.6	+	11	65
Oiticica	143.9	+ (D)	No crystals	0
Cod liver	149.6	+ (D)	5	29
Walnut	150.0	+ (D)	2	33
Tung	158.6	+ (D)	No crystals	0
Linseed, raw	172.5	+	10	34
Linseed, boiled	161.8	+	4	50
Linseed, boiled, and drier	...	+	3	59
Linseed boiled, and anti-oxidant[c]	...	−	No crystals	0
Neo-fat No. 190[d,e]	205[h]	+	3	52
Animal fatty acids[d]	60[h]	+ (D)	180	9
Linseed acids dist.	164.7	+	2	60
Neo-fat No. 17[d,f]	190[h]	+	>60	..
Neo-fat No. 19[d,g]	235[h]	+	6	..

[a] Hartwell, *Am. J. Path.*, **16**, 313 (1940), m. p. 8.2–9.2°. [b] Obtained from the petroleum ether extract of ground parsley seeds after removal of the essential oil by vacuum distillation. [c] 0.5% of hydroquinone as anti-oxidant. [d] Obtained from Armour and Co., Chicago, Illinois. [e] Stated by manufacturer to be produced by esterification of Neo-Fat No. 19 with glycerol. [f] Stated by manufacturer to consist of a mixture of polymerized glycerides and fatty acids of the C_{20} and C_{22} unsaturated series (marine origin). [g] Stated by manufacturer to consist mainly of unsaturated C_{20} acids of marine origin. [h] Data supplied by manufacturer. [i] D signifies addition of drier (0.5% of cobalt linoleate): DD signifies two additions of drier. [j] The identity of the crystalline sulfoxide was checked by melting point determinations.

occur. In the case of most of the oils the identity of the crystalline sulfoxide was checked by melting point determinations.

The occurrence of at least small amounts of linoleic glycerides in most of the above oils precluded drawing conclusions about the specificity of the reaction. Purified fatty acids or their simple esters were then tested, and the results are given in Table II. All of the olefinic acids except undecylenic, ricinoleic, and α-eleostearic, were able to mediate the oxidation of the sulfide. Whereas polyethenoid acids absorbed oxygen spontaneously, the mono-ethenoid acids required the addition of driers. The acid with conjugated double linkages, α-eleostearic, absorbed oxygen rapidly but did not oxidize the sulfide. The acetylenic acids, tariric and stearolic, neither absorbed oxygen nor oxidized the sulfide.

TABLE II

OXIDATION OF β,β′-DICHLOROETHYL SULFIDE WITH PURIFIED FATTY ACIDS AND ESTERS

Fatty acid or ester	Iodine number Calcd.	Iodine number Found	Time to appearance of crystals, days[s,t]	Yield, % of calcd.
Mono-ethenoid:				
Ethyl undecylenate[a]	119.5	116.4	No crystals	0
Methyl petroselinate[b]	85.6	81.2	1 (D)	52
Oleic acid, A[c]	89.9	93.5	5 (D)	47
Oleic acid, B[d]	89.9	89.7	2 (D)	13
Oleic acid, C[e]	89.9	87.9	13 (D)	11
Methyl oleate, A[f]	85.6	85.1	6 (D)	63
Methyl oleate, B[g]	85.6	72.0	8 (D)	66
Methyl erucate[h]	72.0	67.3	6 (D)	17
Ricinoleic acid[i]	85.0	84.8	No crystals	0
Methyl chaulmoograte[j]	86.2	81.9	3 (D)	29
Elaidic acid[k]	89.9	89.3	3 (D)	49
Di-ethenoid:				
Methyl linoleate[l]	172.4	157.8	5	87
Ethyl linoleate[m]	164.5	141.7	2, 3	63, 52
Methyl gorlate[n]	173.5	145.4	2	76
Tri-ethenoid:				
Ethyl linolenate[o]	248.3	244.5	2	58
α-Eleostearic acid[p]	...	...	No crystals	0
Mono-acetylenic:				
Stearolic acid[q]	90.5	92.0	No crystals	0
Methyl tarirate[r]	...	...	No crystals	0

[a] Eastman Kodak No. P5052. [b] Prepared by esterification of the free acid; b. p. 185–186° (4 mm.). Free acid prepared according to Hilditch and Jones, *J. Soc. Chem. Ind.*, **46**, 174 T (1927). No trace of turbidity was obtained in the bromination test. [c] Commercial product, labeled "C. P. free from linolic acid" but giving a voluminous precipitate on bromination in petroleum ether. [d] Furnished by Mr. Louis L. Shapiro; water-white. Stated by source to have thiocyanate number of 86.3, which indicates (with the iodine number given in the table) the presence of 3.8% linoleic acid. Bromination in petroleum ether gave a slight turbidity. [e] Furnished by Dr. J. P. Kass; water-white. The bromination test gave a slight turbidity. [f] Furnished by Dr. J. B. Brown; water-white. The bromination test gave no evidence of poly-ethenoid esters. [g] Prepared by esterification of the free acid obtained by alkaline hydrolysis of oleamide. Oleamide was a commercial product purified by repeated crystallization. Methyl oleate gave a very slight turbidity in the bromination test. [h] Prepared by esterification of the free acid. Erucic acid (Eastman Kodak No. 2233) was purified by a lead salt separation (Twitchell, *Ind. Eng. Chem.*, **13**, 806 (1921)) followed by crystallization. Methyl ester distilled at 202–203° (2 mm.) and yielded no turbidity in the bromination test. [i] Commercial product. Bromination test was inapplicable. [j] Prepared by esterification of the free acid; b. p. 173–174° (2 mm.). Bromination test gave a slight turbidity. Free acid (Power and Gornall, *J. Chem. Soc.*, **85**, 838 (1904)) formed glistening white prisms, m. p. 68.5–70.2°. [k] Lyutenberg, *Fettchem. Umschau*, **42**, 89 (1935); m. p. 44.5–45.0°. [l] Furnished by Mr. Louis L. Shapiro, prepared from linseed oil foots by fractional distillation. Bromination in ether of the free

acid gave a precipitate indicative of linolenic acid. [m] Prepared from poppyseed oil by method of McCutcheon, *Org. Synth.*, **21**, 75 (1942); b. p. 178° (2 mm.). Original poppyseed oil gave no evidence for linolenic ester. [n] Cole and Cardoso, THIS JOURNAL, **60**, 612 (1938); b. p. 167–168.5° (1.5 mm.). Bromination in petroleum ether caused separation of large quantity of yellow oil which obscured the test for linoleic ester. [o] McCutcheon, *Org. Synth.*, **21**, 82 (1942); b. p. 166–168° (1 mm.). [p] Majima, *Ber.*, **42**, 674 (1909). After several crystallizations from alcohol the free acid melted at 48–49.5°. [q] Prepared by method based on that of Overbeck, *Ann.*, **140**, 49, 61 (1866) but the debromination of oleic acid dibromide was conducted in one step. [r] Prepared by esterification of the free acid. Tariric acid was prepared from petroselinic acid through the dibromide by a procedure similar to that of Vongerichten and Köhler, *Ber.*, **42**, 1638 (1909), for stearolic acid. No derivation of tariric acid from petroselinic acid appears in the literature. The tariric acid formed glistening white platelets from alcohol, m. p. 50.4–51.0° (50–51°, Steger and van Loon, *Rec. trav. chim.*. **52**, 593 (1933)); iodine no. 89.9 (calcd. 90.5). Petroselinic acid dibromide (6,7-dibromostearic acid) was prepared pure by crystallization from hexane; white platelets, m. p. 36.6–36.9° (30–36° in Eibner, Widenmayer and Schild, *Chem. Umschau*, **34**, 312 (1927)). [s] (D) signifies addition of drier (0.5% of cobalt linoleate). [t] The identity of the crystalline sulfoxide was checked by melting point determinations.

As has been noted, oxygen consumption always preceded deposition of sulfoxide crystals; moreover, it was found that previously oxygenated oils oxidized added sulfide even after replacement of the oxygen atmosphere by pure nitrogen. During exposure to oxygen the peroxide number of the fatty acid increased to the same extent as the iodine number decreased; after addition of sulfide the peroxide number decreased without further change in iodine number. It thus appeared that the reaction proceeded in two distinct steps: (1) The oxidation of the unsaturated fatty acid to a peroxide, (2) the oxidation of the thio-ether to sulfoxide by the fatty acid peroxide. Of these steps the second is by far the more rapid. Conducted at room temperature as all the experiments reported here were, the first step required from a few days to several weeks and frequently required the use of an accelerating agent, while the second step was substantially completed in less than an hour.

TABLE III

REACTION OF PEROXIDIZED ETHYL LINOLEATE AND ETHYL SULFIDE

Experiment		Peroxidized ethyl linoleate, millimoles of peroxide O	Ethyl sulfide, millimoles
1	Initial amount	16.3	9.7
	Amount consumed	8.6	8.6
2	Initial amount	16.2	12.4
	Amount consumed	9.2	10.8
3	Initial amount	11.8	30.6
	Amount consumed	8.9	25.2
4	Initial amount	1.29	8.2
	Amount consumed	1.29	4.0

The oxidation of fatty acids has been studied by numerous workers in great detail; we have, therefore, restricted this study to the oxidation of thio-ethers by fatty acid peroxides. It was possible to follow this reaction by determining the amounts of reacted sulfide and peroxide after addition of ethyl sulfide to peroxidized linoleic acid ester in an atmosphere of nitrogen. These results are given in Table III. In the presence of an excess of peroxidized ethyl linoleate, the oxidation of the sulfide proceeded approximately stoichiometrically, one mol of peroxide oxygen being utilized to oxidize one mol of ethyl sulfide. When, however, the sulfide was in excess considerably more sulfide disappeared than could be accounted for by the peroxide used. In these latter experiments about three times as much sulfide disappeared as peroxide. Since the formation of the corresponding amount of sulfoxide would require more oxygen than was available in the original peroxidized fatty acid ester, and as no method for determining directly the amount of ethyl sulfoxide formed was available, it would appear that either gaseous oxygen was not wholly excluded from the reaction or some less oxidized product than the sulfoxide was formed. It was not possible to study this point further at this time.

Other thio-ethers were also found to react with peroxidized oils at room temperature. In the case of β,β'-dihydroxyethyl sulfide, *n*-heptyl sulfide, and benzyl sulfide, crystalline sulfoxides whose melting points agreed with those recorded in the literature were obtained; the identity of β,β'-dihydroxyethyl sulfoxide was proved by a mixed melting point determination with an authentic specimen. Furthermore, ethyl sulfide and *p*-cresyl methyl sulfide were found to react. Although the sulfoxides were not isolated, the reaction was indicated by evolution of heat and by an accompanying reduction in peroxide content when the sulfide was added to peroxidized ethyl linoleate. Phenyl sulfide and thiophene gave no evidence of reaction. Thus, those sulfides with at least one alkyl residue were attacked while the aromatic sulfides were not. Although methionine also failed to react, its behavior is not regarded as exceptional since, unlike the other thio-ethers, it was completely insoluble in peroxidized ethyl linoleate. Biotin, in which the sulfur atom appears to be in a saturated ring and thus of aliphatic nature, rapidly lost most of its potency upon addition of peroxidized linseed oil, as determined by assay with yeast.[9] With regard to compounds investigated other than thio-ethers, *p*-dimethylaminoazobenzene and carotene were both decolorized by peroxidized ethyl linoleate.

Materials and Methods[10]

Chemicals used in this study were either the best grade commercially available and generally used without further purification, or were prepared by methods readily accessible in the literature.

In order to ascertain whether a given oil or derivative

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(9) We are indebted to Dr. Dean Burk of this Institute for these assays.

(10) All melting points reported in this paper are corrected unless otherwise specified.

could cause the oxidation of β,β'-dichloroethyl sulfide, the following procedure was adopted: One cc. of the sulfide was added to 9 cc. of the oil contained in a 125-cc. Erlenmeyer flask fitted with a well-lubricated glass stopper. The flask was flushed with oxygen and set aside at room temperature (21–32°) in a darkened room. Oxygen consumption was tested by manipulation of the stopper. If no vacuum was apparent after about one week, drier was added in the form of 0.9 cc. of a 5% hexane solution of cobalt linoleate. After oxygen consumption was apparent, the mixture was seeded with a few crystals of β,β'-dichloroethyl sulfoxide and the flask again flushed with oxygen. When it seemed that no further deposition of crystals occurred, the mixture was diluted with hexane; the crystals were then filtered, washed with hexane and dried. The procedure in the case of solid acids was essentially the same except that the minimum amount of hexane necessary to effect solution was added to the mixture.

The reaction between peroxidized ethyl linoleate and thio-ethers was carried out as follows: Dry oxygen was bubbled through ethyl linoleate at room temperature until the peroxide oxygen content was approximately 1.5 millimoles per gram (calcd. for two double bonds, 5.37). Oxygen absorption was very slow unless catalyzed by the addition of 0.15% cobalt linoleate (dry weight basis) or by irradiation with a 50-watt tungsten lamp. Under either of the latter conditions, the desired degree of peroxidation was attained in one to three days. Dissolved oxygen was then removed by a stream of dry nitrogen. A weighed amount of the sulfide was added to a definite quantity (about 8 to 10 g.) of peroxidized ethyl linoleate. The major part of the reaction took place in a short time, as evidenced by the evolution of heat. However, aliquots were taken for peroxide and sulfide analysis over a period of several hours until constant values were obtained.

Iodine number was determined by the method of Rosenmund and Kuhnhenn.[11] The method described by French, *et al.*,[12] was used for the determination of peroxide. Reliable results were obtained in the presence of unsaturated fatty acids and sulfoxides. Slightly low values were found when sulfides were present, so that in the experiments with ethyl sulfide the unreacted sulfide was removed by vacuum before analysis.

The determination of ethyl sulfide was based on its reaction with mercurous nitrate which results in the formation of metallic mercury.[13] Since this reaction has not been reportedly used in this way as the basis for an analytical method, a brief description of the procedure developed and observations on possibly interfering substances are given. An aliquot containing about 100 mg. of ethyl sulfide was shaken a few minutes with a six-fold excess of a 10% aqueous mercurous nitrate solution containing 0.5% nitric acid. The mixture was centrifuged and the precipitated mercury washed several times with alcohol, water and chloroform until clean. The bright droplet of mercury was dried at 100° and then weighed. Ninety-six per cent. of the theoretical yield of mercury was regularly obtained. Ethyl sulfoxide, unsaturated fatty acids and their peroxides did not affect the reaction.

We are greatly indebted to Mr. Louis L. Shapiro of Wilson and Co., Inc., Philadelphia, for samples of oleic acid and methyl linoleate; to Dr. J. P. Kass of the Interchemical Corp., New York, for oleic acid; to Professor J. B. Brown, The Ohio State University, Columbus, Ohio, for methyl oleate; to Mr. Dale V. Stingley of Armour and Co., Chicago, for several technical fatty acid mixtures, and to Brazil Oiticica, Inc., New York, for samples of oiticica oil.

Summary

1. It was observed that β,β'-dichloroethyl sulfide was oxidized at room temperature to the sulfoxide by unsaturated oils in the presence of oxygen.
2. All the unsaturated acids and their esters which were examined were found to mediate the oxidation, with the following exceptions: α-eleostearic (in which the double bonds are conjugated), Δ^{10}-undecylenic, ricinoleic, and the acetylenic acids tariric and stearolic.
3. The reaction with thio-ethers was found to be limited to those in which at least one of the groups attached to sulfur is aliphatic.
4. The agents found to be responsible for the oxidation of the thio-ethers were the peroxides of the unsaturated fatty acids or esters. One mol of peroxide oxygen was consumed for each mol of thio-ether oxidized.
5. In addition, carotene and *p*-dimethylaminoazobenzene were decolorized, and biotin was found to be inactivated by peroxidized oils.
6. Tariric acid was synthesized from petroselinic acid.
7. The crystalline dibromide of petroselinic acid (6,7-dibromostearic acid) was prepared and characterized by melting point.

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[11] Rosenmund and Kuhnhenn, *Z. Untersuch. Nahr.- u. Genussm.*, **46**, 154 (1923).

[12] French, Olcott and Mattill, *Ind. Eng. Chem.*, **27**, 724 (1935).

[13] Faragher, Morrell and Comay, THIS JOURNAL, **51**, 2774 (1929).