glucose units.[19,20] If the adsorption of phenylhydrazine is due to entrapment in the center of these helices, it might be expected that no noticeable amount of adsorption[21] would occur until the average chain length of the dextrin was approximately six to eight glucose units, as is observed. It is conceivable that by varying the solvents used and by controlling the conditions of precipitation it might be possible to eliminate adsorption and to obtain the derivatives in the more stable hydrazone form.

Summary

1. A method for isolating the dextrins from corn sirup in large quantities is given.

2. Repeated alcohol fractionation gives fractions ranging in mean molecular weight from less than two glucose units to 26 units, and the higher fractions are concluded to be relatively free of maltose and glucose.

3. Oxidation of the dextrins to the potassium salts of the dextrinic acids can be carried out in good yields and the potassium content of the products checks with the reducing value of the original dextrin, indicating the iodine reaction to be quantitative and a true measure of molecular size.

4. The specific rotations of the dextrins agree with the values calculated from iodine molecular weights, lending further evidence to the reliability of iodine values.

5. The Freudenberg and Boppel method of methylation is found to be very satisfactory for both the dextrins and dextrinic acids.

6. Tetramethyl glucose assay of the methylated dextrins indicates the chains to be essentially unbranched, and non-reducing fractions to be absent. The former conclusion is substantiated by the almost complete absence of dimethyl glucose, the latter, by the agreement of the rotations with the calculated values.

7. The reaction of phenylhydrazine with the smaller starch dextrins is found to be quantitative, but the derivatives are unstable and postulated to be largely of the phenylhydrazide type.

8. The dextrin fractions averaging greater than about six glucose units in length show a strong tendency to adsorb phenylhydrazine and a possible explanation is given.

(19) Hanes, *New Phytologist*, **36**, 101, 189 (1937).

(20) Bear, THIS JOURNAL, **64**, 1388 (1942).

(21) The word is here used with the realization that neither the term adsorption nor absorption as used in the usual sense fits this phenomenon.

AMES, IOWA RECEIVED MAY 8, 1942

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, STATE UNIVERSITY OF IOWA]

Antioxidants and Autoxidation of Fats. XIV. The Isolation of New Antioxidants from Vegetable Fats[1]

BY CALVIN GOLUMBIC

The tocopherols have been found in a wide variety of vegetable fats but are generally absent from animal fats.[2,3] The observations of Olcott and Emerson[4] and later those of Golumbic[5] have shown that they and related compounds function as fat antioxidants and they are responsible in part for the greater stability of vegetable fats toward oxidative deterioration.

When added to animal fats that are exposed to air or oxygen, tocopherols are rapidly oxidized during the period in which they exert their antioxygenic action and their complete disappearance practically coincides with the end of the induction period of the fat.[6] At this point there is a readily detectable increase in the rate of oxygen uptake and of peroxide formation[6] (Table I). In an autoxidizing hydrogenated vegetable fat, on the other hand, the pronounced acceleration of peroxide formation does not occur until a considerable time after the total disppearance of tocopherol (Table I). This observation suggested the presence of hitherto unrecognized antioxidants which were less susceptible to oxidation than the tocopherols. They were obviously not phenolic in nature because the oxidized fat gave no test with the ferric chloride–dipyridyl reagent of Emmerie and Engel.[7]

In some respects, the oxidized vegetable fat behaved as though it contained quinoid substances.

(1) Presented before the Division of Food and Agricultural Chemistry, American Chemical Society meeting, Memphis, Tenn., 1942.

(2) Olcott and Mattill, THIS JOURNAL, **58**, 1627 (1936).

(3) Karrer and Keller, *Helv. Chim. Acta*, **21**, 1161 (1938).

(4) Olcott and Emerson, THIS JOURNAL, **59**, 1008 (1937).

(5) Golumbic, *ibid.*, **63**, 1142 (1941).

(6) Golumbic and Mattill, *ibid.*, **63**, 1279 (1941).

(7) Emmerie and Engel, *Rec. trav. chim.*, **57**, 1357 (1938).

TABLE I

THE OXIDATION OF TOCOPHEROLS IN AN ANIMAL AND IN A VEGETABLE FAT AT 60°

Lard			Hydrogenated vegetable fat		
Time, days	Tocopherol,[a] per cent.	Peroxide value[b]	Time, days	Tocopherol,[a] per cent.	Peroxide value[b]
0	0.05[c]	1.3	0	0.105[d]	0.9
3	.01	3.2	14	.008	17.0
7	.004	10.3	35	.000	30.0
14	.003	19.0	49	.000	42.7
21	.001	27.7	58	.000	74.0
28	.000	61.5	63	.000	150.0

[a] Determined by the Emmerie–Engel method as modified by Parker and McFarlane, *Can. J. Res.*, **18**, 403 (1940). [b] Millimoles of peroxide oxygen per kg. of fat. [c] This amount of synthetic α-tocopherol was added to the fresh lard. [d] Naturally occurring tocopherol.

Thus, the light yellow color of the fresh fat gradually deepened to an orange-yellow during the course of the autoxidation. The oxidized fat was decolorized by treatment with reducing agents but its color soon reappeared after removal of the reducing agent and re-exposure to air. Upon continued exposure to air, no further change was noted until the period of accelerated peroxide formation was reached, whereupon the color of the fat faded out rapidly. Vegetable fats in general exhibit this decolorization at the point of extreme rancidity.[8] It seemed pertinent, therefore, to separate the substances responsible for the color production and to study their antioxidant and chemical properties.

For this purpose, hydrogenated vegetable fats were allowed to undergo sufficient atmospheric oxidation to destroy all the tocopherols and petroleum–ether solutions of these partially oxidized oils were twice chromatographed on permutit. This operation gave a red zone whose eluate yielded a red oil from which most of the sterols and fat were removed by cooling in alcoholic solution. Upon repeated adsorption of the residual oil on silicic acid, a homogeneous red zone was obtained which yielded a red oil possessing definite antioxygenic and quinoid properties (Table II).

The red concentrates prepared in this manner were subjected to the action of reducing, acetylating and cyclizing agents in order to gain further information as to the chemical nature of their antioxygenic constituents. In addition, their absorption spectrum was measured and their biological (vitamin E) activity was determined. In all these properties and reactions, these red anti-

(8) Joyner and McIntyre, *Oil and Soap*, **15**, 184 (1938).

TABLE II

ANTIOXYGENIC ACTION OF THE QUINONE CONCENTRATES

Substrate	% of inhibitor added	Anti-oxygenic index[a]
Lard	0.05 Quinone concentrate[b]	3
	.02 Red oxidation product of α-tocopherol	2–4
Purified ethyl esters of hydrogenated cottonseed oil	.80 Quinone concentrate	3

[a] Ratio of the induction period of stabilized fat to that of control. [b] Analytically equivalent to 0.025–0.035% red oxidation product of α-tocopherol.

oxygenic concentrates showed a marked similarity to the chroman-5,6-quinones resulting from the treatment of tocopherols with nitric acid.[9] Previous attempts[9] and our own efforts to purify these latter compounds have been unsuccessful, hence no direct qualitative comparison can be made between them and the antioxygenic quinoid concentrates just described. Until this is accomplished, the definitive chemical structure of the active constituents of the quinoid concentrates will remain open to question.

The red quinoid substances were found in oxidized cottonseed and soybean oils as well as in mixed hydrogenated vegetable fats. They do not occur as such in the fresh fats but are gradually formed in increasing amounts from colorless precursors as the fats undergo autoxidation. When the oxidized fats were dissolved in a butanol chloroform mixture[10] and compared colorimetrically with similar solutions of the chroman-5,6-quinone derived from α-tocopherol, the maximum amounts appearing in oxidized hydrogenated vegetable fats were of the order of 0.02 to 0.03%. Even at these low concentrations, chroman-5,6-quinones exhibit marked antioxygenic properties.[11]

The colorless precursors of these quinoid compounds are not the tocopherols. The quinoid compounds were never detected in autoxidizing animal fats or in purified fat substrates containing only added tocopherol. Furthermore, the addition of α-tocopherol or of α-tocoquinone to a fresh hydrogenated vegetable fat did not increase the amount appearing during the induction period.

Both the red quinoid substances and their precursors are destroyed by alkaline saponification; hence, methods analogous to those usually employed for concentrating vitamin E had to be

(9) Smith, Irwin and Ungnade, THIS JOURNAL, **61**, 2424 (1939).

(10) Quackenbush, Gottlieb and Steenbock, *Ind. Eng. Chem.*, **33**, 1276 (1941).

(11) Golumbic, THIS JOURNAL, **63**, 1163 (1941).

abandoned. The quinoid precursors could be separated with little apparent destruction by chromatographic adsorption on activated alumina according to the method introduced by Moss and Drummond for the isolation of tocopherols from wheat germ oil.[12] To secure selective adsorption of all the antioxygenic constituents of hydrogenated vegetable fats, it was first necessary to convert them to their crude ethyl esters by acid alcoholysis.[2] Petroleum ether solutions of these esters were adsorbed on activated alumina and afforded a yellow zone containing the quinoid precursors in association with the tocopherols. The antioxygenic activity of this adsorbed fraction was lost after acetylation but not after quantitative oxidation with gold chloride.

Tocoquinones, the expected oxidation products if tocopherols alone were present, are devoid of stabilizing action[5]; hence, the adsorbed zone contained phenolic inhibitors other than tocopherols. By repeated chromatographic adsorption on silicic acid, of the fraction oxidized by gold chloride, a homogeneous red zone was obtained which yielded a red antioxygenic oil exhibiting the chemical behavior of the chroman-5,6-quinones. The most likely source of these quinoid substances is thus their corresponding hydroquinones, possibly 5-hydroxy tocols.

Quackenbush, Gottlieb and Steenbock[10] found that the application of the Furter–Meyer method[13] to the determination of tocopherols in vegetable oils sometimes gave high results because of the presence of a chromogen other than tocopherol, which produced a color not unlike that obtained by vigorous oxidation of tocopherol. The fact that this unidentified chromogen was mainly lost upon saponification of the vegetable oils strongly suggests that it is the alkali-labile precursor of the red quinoid substances whose isolation is here reported.

These antioxygenic compounds are also responsible for certain characteristics of the autoxidative behavior of vegetable fats; a discussion of these relations will be presented elsewhere.

Experimental

Preparation of the Antioxygenic Quinoid Concentrates.—Hydrogenated vegetable fats were the most satisfactory starting materials; crude vegetable oils introduced complications because of the difficulty of removing the carotenoid pigments

(12) Moss and Drummond, *Biochem. J.*, **32**, 1953 (1938).

(13) Furter and Meyer, *Helv. Chim. Acta*, **22**, 240 (1939).

The hydrogenated vegetable fat (500 g.) was subjected to acid alcoholysis by refluxing with absolute alcohol containing 2–3% hydrogen chloride.[2] The crude ethyl esters obtained by this process were dissolved in sufficient petroleum ether (b. p. 60–70°) to make a 20% solution and chromatographed on 500 g. of activated alumina. The column exhibited one or two yellow zones depending on the kind of alumina used. When two bands were obtained, as with Brockmann's alumina, all the antioxygenic substances were confined to the second (lower) of the two bands whereas with other commercial aluminas, all the antioxygenic compounds were adsorbed in the single zone. The elutions were made with chloroform and evaporation of this solvent left a semi-solid residue. This was taken up in ethyl alcohol, cooled at −5° and the fat and sterols which crystallized were filtered off. Repetition of this process yielded a concentrate containing about 10% tocopherol (Emmerie–Engel analysis). It was quantitatively oxidized in alcoholic solution with gold chloride.[3] The precipitated gold was filtered off and the clear orange-red solution concentrated *in vacuo* to a small volume. This was taken up in ether, washed with water and dried. The ether residue was dissolved in petroleum ether (b. p. 60–70°) and chromatographed on 60 g. of silicic acid–hyflosupercel mixture (2:1). Two highly colored zones were obtained which were eluted with chloroform. The lower yellow band afforded an oil which gave a positive Furter–Meyer test but a negative Emmerie–Engel test and thus contained tocoquinones. The upper red zone yielded a red oil which on a colorimetric basis was equivalent to about 50 mg. of the chroman-5,6-quinone prepared from α-tocopherol. This fraction was cooled in alcoholic solution to remove further amounts of fats and sterols and the recovered oil was again adsorbed on the silicic acid–hyflosupercel mixture. Repetition of these last two steps yielded a concentrate which exhibited a chromatographically homogeneous zone upon adsorption and which contained 5–10% of the quinone, based on colorimetric comparison with the chroman-5,6-quinone from α-tocopherol.

To secure the red antioxygenic substance from incipiently rancid hydrogenated vegetable fats, these were allowed to undergo atmospheric oxidation, usually at 60°, until all the tocopherol had disappeared as determined by Emmerie–Engel analysis. Petroleum ether solutions of the partially oxidized oils were twice chromatographed on permutit, a process which afforded one red zone. Further purification of this adsorbed fraction was secured in essentially the same manner as with the gold chloride oxidized fractions, namely, by crystallization of fats and sterols from alcoholic solution and chromatographic adsorption on silicic acid. The antioxygenic red oil finally obtained was indistinguishable in properties from the concentrates secured from the fresh fat.

Another though less successful means of separating the antioxygenic quinoid substances from tocopherols was to extract petroleum ether solutions of their concentrates with Claisen alkali. It has previously been established that the reduced forms of the red oxidation products of the tocopherols are somewhat soluble in Claisen's alkali whereas according to Scudi and Buhs,[14] tocopherols themselves are not extracted by this reagent. Although this

(14) Scudi and Buhs, *J. Biol. Chem.*, **141**, 451 (1941).

method permits only partial recovery of the red antioxygenic substances, it was of some value in detecting their presence in highly pigmented vegetable oils.

Chemical and Biological Properties.—The absorption spectrum of the concentrates in the visible region, as measured in alcoholic solution by a Bausch and Lomb spectrophotometer, is identical with that of authentic chroman-5,6-quinones[9] and shows maximal absorption in the range 560–570 mμ.

The quinone concentrates are efficient stabilizers for lard and other fat substrates (Table II). They are instantly decolorized by reducing agents but quickly regain their initial color after separation from the reducing agent and re-exposure to air. Reductive acetylation, however, yields stable colorless oils which possess no antioxygenic properties. When the quinone concentrates react with *o*-phenylenediamine, they form products whose ether solutions exhibit the greenish fluorescence in daylight and in ultraviolet light, characteristic of the phenazines of authentic chroman-5,6-quinones.[9] The antioxygenic quinoid substances are destroyed when the concentrates are saponified. Their lability to alkali, first noted by John and Emte[15] with the authentic compounds, is markedly diminished when sodium hydrosulfite is present. However, when the quinone concentrates were saponified in the presence of this reducing agent, only a small proportion of the total quinone was isolated from the unsaponifiable matter. The greater proportion of it appeared to remain in the saponified fraction. Likewise, only partial recovery of the chroman-5,6-quinone oxidation product of α-tocopherol was secured when it was subjected to the same treatment.

(15) John and Emte, *Z. physiol. Chem.*, **261**, 24 (1939).

Conflicting statements have appeared regarding the vitamin E activity of the chroman-5,6-quinone derived from α-tocopherol. Evans, as reported in a paper of Smith and co-workers,[9] found that it was inactive in doses up to 6 mg. whereas Ridgway, Drummond and Wright[16] reported that it showed some activity in amounts of 5 mg. In our hands, the red oxidation product of α-tocopherol as well as its hydroquinone diacetate and the red quinoid substances obtained from vegetable oils were devoid of biological activity in doses of 10–15 mg. (eight animals).

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Summary

Cottonseed and soybean oils and mixed hydrogenated vegetable fats contain alkali-labile antioxygenic substances other than the tocopherols. The chemical behavior of these fat antioxidants showed that they are similar to, if not identical with, the chroman-5,6-quinones and occur in fresh vegetable fats in a colorless, possibly quinol form. Their isolation and concentration were accomplished by chromatographic adsorption and the use of selective solvents. These antioxygenic quinoid substances, like the chroman-5,6-quinone product of α-tocopherol, were devoid of vitamin E activity.

(16) Ridgway, Drummond and Wright, *Biochem. J.*, **34**, 1569 (1940).

IOWA CITY, IOWA RECEIVED JUNE 15, 1942

[CONTRIBUTION FROM THE DERMATOLOGICAL RESEARCH LABORATORIES, DIVISION OF ABBOTT LABORATORIES]

N^1-Sulfanilylamino-alkyl-pyrimidines

BY GEORGE W. RAIZISS AND MORRIS FREIFELDER

The substitution of an amide hydrogen in sulfanilamide by some heterocyclic nuclei has resulted in compounds with increased therapeutic activity. In continuing our work[1] in this field, we have synthesized a series of sulfanilylamino alkylpyrimidines. Some of these were prepared concurrently by other investigators and have been described[2]; in addition, we have mentioned herewith several which were previously unpublished.

The 4-alkyl and 4,5-dialkyl substituted 2-aminopyrimidines reacted readily with *p*-acetsulfanilyl chloride, forming the acetsulfanilyl derivatives which were subsequently hydrolyzed to the sulfanilylaminoalkylpyrimidines. Our attempts to combine the acid chloride with amino hydroxypyrimidines, such as isocytosine (2-amino-4-hydroxypyrimidine) or divicine (2,5-diamino-4,6-dihydroxypyrimidine), and purines such as adenine or guanine, failed.

Most of the aminopyrimidines used were prepared according to Benary's method[3] by treating guanidine carbonate with sodium oxymethylene ketones; these were obtained by condensation of methyl alkyl ketone and ethyl formate in presence of sodium methylate. 2-Amino-4-ethyl-5-methylpyrimidine was prepared from sodium oxymethylene-α-methyl-methyl ethyl ketone (derived from diethyl ketone and ethyl formate); 2-amino-4-isobutylpyrimidine from sodium oxy-

(1) (a) Raiziss, Clemence and Freifelder, THIS JOURNAL, **63**, 2739 (1941); (b) Raiziss and Clemence, *ibid.*, **63**, 3124 (1941).

(2) (a) Roblin, Williams, Winnek and English, *ibid.*, **62**, 2003 (1940); (b) Caldwell, Kornfeld and Donnell, *ibid.*, **63**, 2189 (1941); (c) Sprague, Kissinger and Lincoln, *ibid.*, **63**, 3028 (1941).

(3) Benary, *Ber.*, **63**, 2601 (1930).