$[Ni(NH_3)_5NO]NO_3$. Briner and co-workers[9] have found that nitrogen peroxide is formed slowly in pure nitric oxide under pressure at ordinary temperature, and also in liquid nitric oxide under atmospheric pressure below its boiling point. The presence in the reacting gases of nitrogen peroxide so formed may explain the oxidation of the complex compound first formed, and may also explain the failure to obtain a pure solid product from the reaction of nitric oxide with nickel carbonyl.

In view of the unexplained erratic behavior of oxygen and nickel carbonyl,[10] the observations concerning mixtures of nitrous oxide and nickel carbonyl cannot be taken as final. Nitrous oxide may react equally unpredictably with nickel carbonyl.

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Summary

Some observations have been reported concerning the reactions of nickel carbonyl with nitrogen peroxide, nitric oxide, nitrous oxide, ammonia and a mixture of ammonia and nitric oxide.

The suggested relationship of the results of Job and Reich[7] to those of Mond and Wallis[5] was also investigated.

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(9) Briner, *et al.*, *Compt. rend.*, **149**, 1372 (1909); **156**, 228 (1931); *J. chim. phys.*, **23**, 157 (1926).

(10) Hieber and Kaufmann, *Z. anorg. allgem. Chem.*, **204**, 174 (1932); Berthelot, *Bull. soc. chim.*, **7**, 434 (1892); *Compt. rend.*, **113**, 679 (1892); Lenher and Loos, THIS JOURNAL, **22**, 114 (1900); Blanchard and Gilliland, *ibid.*, **48**, 872 (1926).

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

Antioxidants and the Autoxidation of Fats. VII. Preliminary Classification of Inhibitors[1]

BY H. S. OLCOTT AND H. A. MATTILL

It was recently proposed[2] to give the name "inhibitols" to those as yet unidentified constituents of the unsaponifiable matter of various vegetables and vegetable oils which possess the capacity to delay oxidative rancidity in certain fats. This term indicates their activity as inhibitors and also the invariable occurrence of hydroxyl groups upon which their inhibiting action depends. Methods of preparation of inhibitol concentrates and some of their physical and chemical properties have been described in some detail.[2]

Inhibitol concentrates are effective antioxidants for animal fats and for highly purified unsaturated fatty acids and esters, but they have consistently failed to show any antioxygenic activity when added, even in relatively large amounts, to the vegetable oils from which they were obtained (Table I). The inactivity of inhibitols in vegetable oils is also implied in the experiments of Royce,[3] who added a crude sterol fraction of cottonseed oil to hydrogenated cottonseed oil without observing any prolongation of the induction period, in those of Greenbank and Holm[4] who reported an unsuccessful attempt to isolate an antioxidant from cottonseed oil, using vegetable oils as the assay substrate, and in those of Bau-

TABLE I

EFFECT OF INHIBITOLS ON DIFFERENT FATS

Substrate	Per cent. inhibitol concentrate added[a]	Induction period, days: With inhibitor	Control
Organoleptic method, 63°			
Cottonseed oil	0.10 C58	3.5, 4	4, 4.5
	.05 W5-10	3	3.5
	.05 W5-10	6½	8
	.05 C44	8½	8
Hydrogenated cottonseed oil	.02 W48	27, 31	28, 32
	.03 W5-10	42, 49	41, 49
Lard	.02 W5-10	9, 9	3, 3
	.01 W5-10	10.5, 11	4, 4
Oxygen absorption method, 75°		Hrs.	Hrs.
Lard[b]	0.02 W5-10	50	10
Oleic acid	.03 W5-10	10	4
Methyl oleate	.02 W5-10	30	4

[a] W indicates wheat germ oil inhibitol, C that from cottonseed oil. [b] See also reference 2, Tables V and VI.

(1) Presented before the Division of Agricultural and Food Chemistry at the 92nd meeting of the American Chemical Society, Pittsburgh, Pa., September 7 to 11, 1936.

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

(3) H. D. Royce, *Oil and Soap*, **9**, 25 (1931).

(4) G. R. Greenbank and G. D. Holm, *Ind. Eng. Chem.*, **26**, 243 (1934).

mann and Steenbock[5] who found that, in cottonseed oil, carotene losses were not reduced by an inhibitol-containing material like wheat germ oil.

These facts prompted an investigation of the factor or factors in the vegetable oils responsible for their refractoriness to protection by inhibitols and conversely, a study of the types of inhibitors which could protect these oils. The experiments to be described deal with the second of these two questions. Refined and hydrogenated cottonseed oils were used in most of the experiments, but the same phenomena were also demonstrable with palm, sesame, soy bean and other vegetable oils.

The oxygen absorption method which we have used[6] in the study of lard and its protection by inhibitors could not be applied satisfactorily to the study of vegetable oils. Whereas lard demonstrates a definite induction period after which the absorption of oxygen is rapid, vegetable oils absorb oxygen slowly and the end of the induction period is not sharply defined. Furthermore, vegetable oils are more stable than lard so that the oxygen absorption method is time consuming unless high temperatures are used. Since, in our hands, a temperature of 70–80° has proved to be a convenient one at which to measure oxygen absorption, we investigated several methods of reducing the induction period of these oils to convenient lengths at 75°, attempting at the same time to retain the properties of the original oils with regard to inhibitors, especially their absolute refractoriness to protection by inhibitols.

The most satisfactory method proved to be the use of the ethyl esters of the fatty acids, prepared as follows. Two parts of absolute alcohol containing 2–3% hydrogen chloride were added to one part of the fat and refluxed on a steam-bath for eighteen to twenty-four hours. The mixture was cooled and diluted with water. The ester layer was washed repeatedly with water to remove hydrochloric acid and ethyl alcohol, then centrifuged free from occluded water, and finally heated on a steam-bath in a vacuum to remove traces of solvents. Theoretically this light brown, mobile liquid, hereafter referred to as crude esters, contained the ethyl esters of the fatty acids, the unsaponifiable constituents of the original fat and possibly traces of unhydrolyzed glycerides; the glycerol and any water-soluble constituents should have been removed.

The product so obtained from a hydrogenated cottonseed oil could be used to assay inhibitors by the oxygen absorption method, since it had a convenient induction period at 75° (four to sixteen hours), and a rapid rate of oxygen absorption at the end. Furthermore, the protection afforded the crude esters by various inhibitors was qualitatively parallel to that conferred on the original fat; the crude esters were not protected by relatively large amounts of inhibitol concentrates but were protected to a remarkable degree by several substances which have been suggested for use as antioxidants in vegetable fats, namely, oxalic acid,[7] maleic acid,[4] sulfuric and phosphoric acids and their acid salts,[8] and lecithin.[9,10]

The effectiveness of so many substances that are alike only in the possession of an ionizable hydrogen atom prompted an investigation of the antioxygenic activity of other acids, both organic and inorganic. The results, summarized in Table II, permit of no generalizations, but suggest certain interesting relationships. In the aliphatic series, pyruvic acid is the only active compound not containing two free carboxyl groups; the adjacent carbonyl apparently has an activating effect. The two carboxyl radicals must not be separated by more than one CH_2 group unless an active group or groups, such as hydroxyl, or an unsaturated bond is also present. The inactivity of the salts and esters of the dicarboxylic acids indicates that the carboxyl groups must be free. Among the inorganic acids tested, sulfuric and phosphoric acids were the only ones to show marked antioxygenic action. Calcium acid phosphate was effective in contrast to the inactivity of the sodium and potassium salts, presumably due to its greater acidity. Any explanation of the action of these acids must take account of the fact that the medium in which they act is practically anhydrous. The solubility of cephalin doubtless accounts in part for its efficiency.

With the exception of hydroquinone, the phe-

(5) C. H. Baumann and H. Steenbock, *J. Biol. Chem.*, **101**, 561 1933).

(6) R. B. French, H. S. Olcott and H. A. Mattill, *Ind. Eng. Chem.*, **27**, 724 (1935).

(7) T. H. Rogers, U. S. Patent 1,826,258; *C. A.*, **26**, 613 (1932).

(8) E. W. Eckey, U. S. Patent 1,982,907; 1,993,152; A. S. Richardson, F. C. Vibrans and J. T. R. Andrews, U. S. Patent 1,993,181; *C. A.*, **29**, 518, 2770 (1935).

(9) H. Bollman, U. S. Patent 1,464,557; *C. A.*, **17**, 3234 (1923).

(10) The antioxygenic action of commercial lecithin has been shown to be due to its contained cephalin, and the activity of cephalin in turn, appears to depend on the monobasic phosphoric acid radical [H. S. Olcott and H. A. Mattill, *Oil and Soap*, **13**, 98 (1936)].

TABLE II

THE ANTIOXYGENIC EFFECT OF ACIDIC AND PHENOLIC COMPOUNDS ON THE CRUDE ESTERS OF HYDROGENATED COTTONSEED OIL

Inactive Compounds[a]			
Hydrochloric acid		Azelaic acid	
Hydrobromic acid		Dihydroxystearic acid	
Hydriodic acid		Aspartic acid	
Iodic acid		Benzoic acid	
Nitric acid		Phthalic acid	
Boric acid		Isophthalic acid	
Arsenious oxide		α-Naphthoic acid	
Tungstic oxide		β-Naphthoic acid	
Sodium dihydrogen phosphate		*d*-Camphoric acid	
Potassium dihydrogen phosphate		Tannic acid	
		Ethyl oxalate	
Formic acid		Ethyl malonate	
Acetic acid		Ethyl tartrate	
Lactic acid		Sodium bitartrate	
Succinic acid		Sodium oxalate	
Adipic acid		Oxamide	
Mucic acid		Inhibitol concentrates	
Active Compounds and Indices[b]			
Sulfuric acid (95%)[c]	15–20	Maleic acid	4–6
Phosphoric acid (85%)[c]	15–20	Citric acid	10–15
		Malic acid	8–12
Calcium dihydrogen phosphate	4–6	Pyruvic acid	10–15
		Hydroquinone	1.2–1.6
Cephalin	4–6	Catechol	12
Perchloric acid	3	Pyrogallol	26
Arsenic acid	3	α-Naphthol	9
Oxalic acid	15–20	1,5-Naphthalenediol	5
Malonic acid	10–15		
Tartaric acid	10–15		

[a] For the most part these compounds were assayed by adding 1 mg. to 5 g. of the crude esters (0.02%). The induction period of the "protected" sample was never more than twice that of the control sample; in most cases, no antioxygenic action could be observed. When 5 mg. was used (0.1%), some of the compounds exhibited slight antioxygenic activity. Among these were lactic and phthalic acids, sodium and potassium dihydrogen phosphates, and ethyl tartrate.

[b] The index represents the ratio of the induction period with inhibitor to that of the control. For example, the data for several runs of tartaric acid: with inhibitor, 130, 100, 170 hours; without, 6, 9, 10. Most of the inhibitors were assayed at a 0.02% level. 1,5-Naphthalenediol was tested at 0.01%. All results were obtained at 75°.

[c] Dilution of the sulfuric and phosphoric acids with an equal amount of water did not decrease their activity.

nolic inhibitors were relatively as active on the ester preparations as on lard.[11,12]

The reactions of the vegetable oil crude esters to inhibitors thus provided an entirely different picture from that presented by lard and purified fatty acids, in which the acid inhibitors mentioned above are only very slightly antioxygenic if at all, while the phenolic inhibitors and the inhibitols are very efficient.[2] Crude methyl and ethyl esters prepared from lard responded to inhibitors in the same manner as did the original lard (Table III), indicating that the process of esterification was not responsible for the reaction toward inhibitors of the vegetable oil esters. Crude methyl esters of the vegetable oils reacted exactly like those prepared with ethyl alcohol.

TABLE III

THE EFFECT OF INHIBITORS ON LARD AND ON THE CRUDE ESTERS OF LARD

Substrate	% inhibitor added	Induction period, hours: With inhibitor	Control
Lard (75°)	0.20 cephalin	8	5
	.20 tartaric acid	15	9
	.20 malonic acid	13	9
	.20 citric	16	11
	.20 phosphoric acid	19	10
	.20 oxalic acid	38	13
	.20 W5–10	62	15
	.01 hydroquinone	410	14
Crude methyl esters of lard (60°)	.20 cephalin	10	9
	.20 tartaric acid	14	9
	.10 W5–10	45	9
Crude ethyl esters of lard (65°)	.10 tartaric acid	4	2
	.10 malonic acid	4	1.5
	.10 citric acid	4	1.5
	.10 phosphoric acid	5	1.5
	.10 oxalic acid	25	2
	.10 W5–10	12	1.5
	.02 hydroquinone	60	2

When the crude esters were distilled *in vacuo*, 95% distilled below 160° at 0.1 mm. The properties of the water-white distillate, with regard to its amenability to protection by various inhibitors, were strikingly different from those of the crude esters. The inhibitols were effective, although not as active as in lard. Hydroquinone was an efficient inhibitor, and the dibasic and inorganic acids were relatively ineffective (Table IV). This table also demonstrates the same significant difference in the reactions of palm oil esters to different inhibitors before and after distillation.

From these results it is apparent that, unless some destruction or alteration occurred during the distillation, the residue should contain compounds which could (a) inhibit the antioxygenic activity of inhibitols and hydroquinone and (b) activate the acid type inhibitors.

It was possible to demonstrate (b) but not (a) by adding small amounts of the residue to the distilled esters. The acid type inhibitors were effec-

(11) H. A. Mattill, *J. Biol. Chem.*, **90**, 141 (1931).
(12) H. S. Olcott, THIS JOURNAL, **56**, 2492 (1934).

TABLE IV

THE EFFECT OF INHIBITORS ON THE DISTILLED ESTERS OF HYDROGENATED COTTONSEED OIL, AND ON THE CRUDE AND DISTILLED ESTERS OF PALM OIL

Substrate	% inhibitor added	Induction period, hrs. With inhibitor	Control
Distilled ethyl esters of hydrogenated cottonseed oil	0.02 citric acid	3	3
	.20 tartaric acid	6	6
	.02 maleic acid	4	3
	.20 cephalin	5.5	6
	.10 malonic acid	6	6
	.10 W5–10	52	6
	.02 hydroquinone	77	2
Palm oil crude ethyl esters	.02 tartaric acid	94	11
	.10 W5–10	11	11
	.02 hydroquinone	16	11
Distilled ethyl esters of palm oil	.02 tartaric acid	18	6
	.10 W5–10	24	6
	.02 hydroquinone	280	6

TABLE V

EFFECT OF MIXTURES OF INHIBITORS ON VARIOUS SUBSTRATES

% inhibitor added	Induction period, hrs. With inhibitor	Control
Distilled ethyl esters of hydrogenated cottonseed oil		
0.04 W5–10	9.5	5
.10 tartaric acid	8	5
.04 W5–10 + 0.10% tartaric acid	148	5
.02 W5–10	7.5	5
.10 cephalin	4	5
.02 W5–10 + 0.10% cephalin	78	5
.02 citric acid	3.5	3
.02 W5–10 + 0.02% citric acid	98	3
.02 H_3PO_4	4.5	4
.02 W5–10 + 0.02% H_3PO_4	73	4
Lard		
0.02 W5–10	30	7
.10 tartaric acid	17	7
.02 W5–10 + 0.10% tartaric acid	84	7
.10 cephalin	5	7
.02 W5–10 + 0.10% cephalin	69	7
.10 malonic acid	5	4
.02 W5–10 + 0.10% malonic	89	4
.02 $Ca(H_2PO_4)_2 \cdot H_2O$	11	10
.02 W5–10 + 0.02% $Ca(H_2PO_4)_2 \cdot H_2O$	93	10
Palm oil fatty acids		
0.10 W5–10	8	3
.10 tartaric acid	7	3
.10 W5–10 + 0.10% tartaric acid	88	3
Methyl oleate		
0.02 W5–10	22	10
.02 W5–10 + 0.1% tartaric acid	136	10
Octadecene		
0.10 W5–10	86	13
.10 tartaric acid	13	13
.10 W5–10 + 0.10% tartaric acid	304	13

tive for this mixed substrate but the antioxygenic action of inhibitols was not suppressed. A series of investigations designed to determine the nature of the compound present in the residue and responsible for the activation of the acid type inhibitors, disclosed that it occurred in the nonsterol unsaponifiable fraction and that it was destroyed by acetylation but not by hydrogenation. These were also properties of the inhibitols, and it was possible to demonstrate that a highly concentrated inhibitol fraction exhibited this remarkable synergism with acid type inhibitors when the distilled esters of hydrogenated cottonseed oil were used for the substrate fat (Table V). Apparently the inhibitol originally present in the vegetable oil and remaining in the residue when the crude esters were distilled, is responsible for the activation of the acid type inhibitors in the crude esters.

This synergistic effect of inhibitol and some one of the acid inhibitors could also be demonstrated in lard, purified fatty acids and esters and octadecene (Table V), but not in vegetable oils.

The close relationship between hydroquinone and the inhibitols led to an investigation of the effect of mixtures of phenolic and acid inhibitors. The synergistic effect could easily be demonstrated, for example, with orcinol and phosphoric acid (Table VI). Other combinations of phenolic and acidic inhibitors varied in their effectiveness. Citric and tartaric acids did not show the synergistic effect with orcinol when assayed on lard fatty acids.

It is thus apparent that the observation of Holmes, Corbet and Ragatz[13] on the superior stabilizing effect of combinations of lecithin and hydroquinone on vitamin A over that of either alone, is only an individual case of a more or less general phenomenon.

Based upon our experiments we have tentatively classified the inhibitors studied into three groups: (1) the acid type inhibitors, (2) inhibitols and hydroquinone, and (3) the phenolic type including α-naphthol, pyrogallol, catechol and others. Table VII outlines the qualitative differences in behavior of these kinds of inhibitors on various substrates. The boundaries between the three types are not especially well defined. For example, oxalic acid has a definite antioxygenic action on lard, greater than that of the other acid type inhibitors. The anomalous behavior of hy-

(13) H. N. Holmes, R. E. Corbet and R. A. Ragatz, *Ind. Eng. Chem.*, **28**, 133 (1936).

TABLE VI

SYNERGISTIC EFFECT OF ORCINOL AND PHOSPHORIC ACID

Substrate	% inhibitor added	Induction period, hrs. With inhibitor	Control
Lard (75°)	0.02 orcinol	75	12
	.10 phosphoric acid	21	12
	.02 orcinol + 0.10% phosphoric acid	314[a]	12
Lard fatty acids (60°)	.02 orcinol	29	2.5
	.10 phosphoric acid	5	2.5
	.02 orcinol + 0.10% phosphoric acid	300[a]	2.5
	.02 orcinol + 0.10% citric acid	30	2.5
	.02 orcinol + 0.10% tartaric acid	41	2.5

[a] When discontinued, these samples were still fresh.

droquinone in contrast to the other phenolic inhibitors is in accord with the observations of others concerning hydroquinone in gasoline.[14]

TABLE VII

QUALITATIVE DIFFERENTIATION OF THREE TYPES OF INHIBITORS

	Acid type[a]	Inhibitols and hydroquinone[b]	Phenolic type[c]
Vegetable oils	+	−	+
Crude vegetable oil esters	+	−	+
Distilled vegetable oil esters	−	+	+
Lard and lard esters	−	+	+
Purified fatty acids and esters	−	+	+

[a] Oxalic acid protects lard to an appreciable extent (Table III).

[b] Hydroquinone is effective in vegetable oils, but is included in this grouping because it is relatively ineffective on crude vegetable oil esters.

[c] Includes some natural products, such as gossypol, gallic acid, as well as catechol, pyrogallol, α-naphthol, etc.

In general, any type 1 inhibitor when used with any type 2 or 3 compound, prolongs the induction period of certain fats and other unsaturated compounds to a much greater extent than would be expected from a summation of the effects of each used alone.

There is, of course, an almost infinite number of possible combinations of antioxidants, some of which should be utilizable not only in the edible fat industry, but wherever compounds or mixtures are subject to oxidative deterioration.

In conclusion, it may again be emphasized that the complex reactions which result in rancidity are subject to many factors, some unknown, which operate to make duplication of results uncertain at times. The data recorded in this paper are merely representative of those obtained from a large number of experiments and assays.

Further observations on the causes of the fundamental differences between vegetable and animal fats, with respect to their reactions toward different inhibitors, will appear in subsequent publications.

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Summary

The crude esters of hydrogenated cottonseed and other vegetable oils, prepared by refluxing the oil with absolute methyl or ethyl alcohol containing dry hydrogen chloride, are protected to a remarkable degree by oxalic, malonic, maleic, citric and other aliphatic dibasic acids, by phosphoric and sulfuric acids and cephalin, and by some phenolic inhibitors. Hydroquinone is only slightly effective and inhibitol concentrates prepared from the unsaponifiable fraction of vegetable oils are inactive.

When the esters are partially purified by fractional distillation *in vacuo*, they are only slightly protected by the acids or cephalin, but are protected by hydroquinone and the inhibitols.

The acid inhibitors and inhibitol concentrates have a pronounced synergistic effect when used together in the distilled ester preparation; the protection afforded by the mixture is much greater than by either alone. This phenomenon can also be demonstrated in lard, and in purified fatty acids and esters, and also with certain mixtures of phenolic and acid type inhibitors.

Based upon these observations, a tentative classification of inhibitors into three groups is proposed: Group 1, acid type inhibitors; Group 2, inhibitols and hydroquinone; Group 3, other phenolic inhibitors.

(14) C. D. Lowry, Jr., G. Egloff, J. C. Morrell and C. G. Dryer, *Ind. Eng. Chem.*, **25**, 804 (1933).

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