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

Antioxidants and the Autoxidation of Fats. II

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Certain compounds possess the property, when present in minute amounts, of inhibiting for long periods of time the autoxidation of natural fats and oils. Notable among these are several of the polyphenols. Mattill[1] studied the behavior of a number of phenolic compounds in this connection and arrived at several tentative conclusions concerning the necessary chemical structure of an antioxidant. He pointed out that the activity is associated in some manner with the ortho or para configuration and is not possessed by compounds with the meta configuration or its equivalent, that the presence of free hydroxyl groups is apparently necessary, and that these must be bound directly to an aromatic nucleus. This study was continued for the purpose of throwing more light upon the mechanisms of autoxidation and inhibition, and, by analogy, upon the structure of the antioxidants, the presence of which has been demonstrated in the unsaponifiable lipid fractions of plants.[2,3,4] Since concentrates of these plant antioxidants contain no nitrogen, sulfur or halogens, the investigation has been limited to compounds containing only C, H and O.

Experimental

The oxygen absorption method, as described by Mattill,[1] was used for measuring the length of the induction period. Lard or mixtures of lard and cod-liver oil were the substrate fats. Results were interpreted in terms of the antioxidant index, the ratio of the induction period with inhibitor to that of the unprotected fat. The indices given in Table I with the exceptions noted, were determined in lard at 75°, at which temperature the induction period of the samples used varied from six to fifteen hours. A reasonable degree of correlation was obtained between the indices with lard and those with lard–cod-liver oil mixtures.

The compounds used, when not available, were synthesized by standard methods. Triquinoyl and hexahydroxybenzene were obtained from chloranil through sodium nitranilate.[5,6]

TABLE I

INACTIVE COMPOUNDS

Hydroquinone diacetate[a]	Anthraquinol
Hydroquinone monobenzoate	1,5-Dihydroxyanthraquinol
Hydroquinone dibenzoate	2,4,6-Tribromoresorcinol
Hydroquinone di-(*p*-nitrobenzoate)	Saligenin
	1,4-Cyclohexanediol
p-Dimethoxybenzene	Thymoquinone
Hydroxyhydroquinone triacetate	Triquinoyl
	Cyclohexanol
Apionol tetracetate	Tannic acid
Hexahydroxybenzene	Tartaric acid
Dipyrogallol tricarbonate	Citric acid
1,4-Naphthalenediol	Maleic acid

ANTIOXIDANTS AND INDICES[b]

Catechol[a]	41	Pyrogallol carbonate	2
Hydroquinone[a]	38	Apionol	20
Hydroquinone monomethyl ether	6	Naphthoresorcin	5
		1,8-Naphthalenediol[c]	20
Toluhydroquinone	7	α-Naphthol[a]	22
Thymohydroquinone	2	Quinone[a]	4
Hydroxyhydroquinone	60	Toluquinone	2
Pyrogallol[a]	60+	β-Naphthoquinone[a]	8

PRO-OXIDANTS AND INDICES

Carotene[d]	0.5	Xanthophyll[d]	0.7
Lycopene[e]	.5	Perbenzoic acid[f]	.5

[a] Repeated for purposes of orientation.[1] [b] The indices represent the ratio of the induction period of lard or lard–cod-liver oil plus the antioxidant to that of the lard alone. The results in this section were determined by adding 0.5 mg. of the compound in question to 5 g. of lard. The determinations were run at 75°. [c] 0.02% 1,8-naphthalenediol in lard–cod-liver oil mixture. [d] 0.10% in lard–cod-liver oil mixture. [e] 0.04% in lard–cod-liver oil mixture.[4] [f] Trace.

Only two compounds showed activity comparable in degree with that of the simple polyphenols, pyrogallol, hydroquinone and catechol, previously examined. These were the closely related hydroxyhydroquinone and apionol (1,2,3,4-tetrahydroxybenzene). Hexahydroxybenzene had no antioxygenic action. Since this compound is very easily oxidized, it may again be emphasized that there exists no simple relationship between reducing action and antioxidant activity.[7] The antioxygenic effect seems to de-

(1) H. A. Mattill, *J. Biol. Chem.*, **90**, 141 (1931).
(2) H. A. Mattill and B. Crawford, *Ind. Eng. Chem.*, **22**, 341 (1930).
(3) H. S. Olcott and H. A. Mattill, *J. Biol. Chem.*, **93**, 59, 65 (1931).
(4) E. M. Bradway and H. A. Mattill, THIS JOURNAL, **56**, 2405 (1934).
(5) J. U. Nef, *Am. Chem. J.*, **11**, 17 (1889).
(6) R. Nietzki and T. Benckiser, *Ber.*, **18**, 499 (1885).
(7) N. A. Milas, THIS JOURNAL, **52**, 739 (1930).

pend in some way on an imbalance of the benzene molecule. Thus, phloroglucinol is a very poor antioxidant; orcinol (5-methylresorcinol), though also weak, is definitely more active.[1]

Esterification of one or both hydroxyl groups in hydroquinone destroyed its action. The only ester investigated which was not completely inactive was pyrogallol monocarbonate. Alkylation was not quite as destructive. *p*-Dimethoxybenzene was inactive, but the monomethyl ether of hydroquinone possessed some antioxygenic activity. Guaiacol, the monomethyl ether of catechol, was much less active.[1] The simplest homolog of hydroquinone, toluhydroquinone, was only one-fifth as active as an antioxidant; thymohydroquinone possessed even less activity.

Mattill's conclusion[1] that both hydroxyl groups must be bound to the ring is borne out by the inactivity of saligenin (*o*-hydroxybenzyl alcohol). The inactivity of *cis*-1,4-cyclohexanediol[8] emphasizes the important part played by the aromatic ring.

α-Naphthol is a strong antioxidant, β-naphthol is weak.[1] Of the three naphthalenediols investigated, the 1,8 compound was most active, the 1,3, less so and the 1,4, had no activity. The naphthalene analog of hydroquinone was thus less active than the analog of resorcinol. Anthraquinol and 1,5-dihydroxyanthraquinol were without activity.

The quinones were much less active than the corresponding quinols. Triquinoyl ($C_6O_6 \cdot 8H_2O$) and thymoquinone had no activity. Toluquinone was a less active antioxidant than benzoquinone. 2,4,6-Tribromoresorcinol, which possesses pseudo-quinoid properties,[9] was inactive.

Under the conditions used, maleic[10] and some aliphatic hydroxy acids had no antioxygenic action.

Of the many organic compounds whose effects on the induction period of fats have been observed in this Laboratory, only perbenzoic acid and three carotenoid pigments, carotene,[11] xanthophyll[12] and lycopene[4] have exerted a definite pro-oxygenic effect by shortening the induction period. Presumably the series of aliphatic conjugated double bonds form the metastable peroxide intermediates of autoxidation reactions more easily than do the unsaturated fat molecules, and thus serve to initiate the reaction chains of the autocatalytic oxidation. The pigments were bleached as the fat began to oxidize.

The data at hand are not sufficient to warrant any theoretical treatment. They serve the purpose of suggesting further compounds for investigation, and of outlining in some degree the configurations which compounds must possess in order to act as antioxidants. It should be emphasized that the data hold strictly only for the experimental method which has been described. The effectiveness of these compounds on other and more complex fats and oils requires further study. Greenbank and Holm[10] have confirmed some of the previous results[1] with the polyphenols using cottonseed oil instead of lard.

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Summary

A number of compounds have been tested for their antioxygenic activity toward lard. Besides pyrogallol, hydroquinone and pyrocatechol, hydroxyhydroquinone and apionol are excellent antioxidants. The 1,3 and 1,8 naphthalenediols are effective while the 1,4 derivative is inactive. Esterification and alkylation of one or more of the hydroxyl groups destroys or greatly reduces antioxygenic activity. Side chains on the benzene nucleus reduce the activity of hydroquinone. 1,4-Cyclohexanediol and saligenin are inactive, the quinones possess slight activity. Maleic, tartaric and citric acids are inactive.

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(8) We are indebted to Professor Homer Adkins of the University of Wisconsin for the sample tested.

(9) T. L. Davis and V. F. Harrington, THIS JOURNAL, **56**, 129 (1934).

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

(11) H. S. Olcovich and H. A. Mattill, *J. Biol. Chem.*, **91**, 105 (1931).

(12) We are indebted to Dr. F. M. Schertz of the Bureau of Chemistry and Soils for the xanthophyll.