TABLE VII

Twitchell Isooleic Determinations on Known Isooleate Mixtures

Fatty Acid Mixture	Lead Salt Solubility	Twitchell Recovery	
		Actual	Calculated (SFA wt. 1.2g. IV 30)
	g./100 ml. 95% EtOH	%	%
Elaidic	.030	95	87
Elaidic/Petroselaidic (1:1)	.047	93	81
Elaidic/vaccenic[a]/ Petroselaidic (1:1:1)	.109	85	65
Palmitic .5g Stearic .2g 9-trans- .5g 11-trans- .1g 12-trans- .2g Oleic 1.0g Linoleic 0.5g	.069 (SFA IV=44)	83	74
Isooleic Acids from Hydrogenated CSO[b]	.085	67	70
Isooleic Acids from Hydrogenated SBO[b]	.13	70	60

The lead salt-solubility data for CSO and SBO are low inasmuch as they were determined by a third lead salt crystallization of a regular Twitchell run. The more soluble lead isooleates were lost in the first two crystallizations.

[a] Vaccenic acid from partially hydrogenated tung oil.

[b] Hydrogenated CSO and SBO stocks are known to contain at least 6 trans-isooleic acids of different double bond position, based on unreported x-ray spectrophotometer studies of dihydroxy derivatives.

a) The solubility of lead isooleate salts increases in a more or less regular manner as the number of component isooleic acids increases.

b) The presence of "tung oil vaccenic acid," which is a mixture of mostly trans-11 and -12 acids, causes anticipated low recoveries.

c) The Twitchell recoveries with three and more individual isooleic acids approach the same order as those indicated by comparison of Twitchell and infrared isooleic analyses on hydrogenated stocks.

d) The broken lines indicate the anticipated Twitchell recoveries of six component mixtures.

Conclusion

The Twitchell and infrared isooleic methods have been compared. Assuming that all trans-isooleic acids are of equal significance as regards influence on shortening plasticity as "solid unsaturated acids," the infrared method is superior. This method has all the advantages of speed and accuracy of spectrophotometric methods. It handles simple and complex isooleate mixtures with equal ease in view of identical infrared absorption for trans-isomers of different double bond position.

The Twitchell method was shown, on close analysis, to have fair accuracy for simple trans-acid mixtures but give very low values (errors of 35 to 45%) with ordinary hydrogenated oils. The reason for the failure of the Twitchell method apparently lies in the increasing lead isooleate solubility with increase in the number of trans-isooleic acids.

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Phenolic Antioxidants for Carotene

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THE stability of carotene has been the subject of numerous investigations, most of which have been concerned with the rate of loss of carotene naturally present or added to food or feed ingredients. When antioxidants are incorporated into such systems, the interpretation of the results is often complicated by the possibility of synergistic action of the added antioxidants with those naturally present in the various materials comprising the provitamin A carrier (or by pro-oxidant materials which nullify the effect of the antioxidant). The results of studies, comparing antioxidant efficiency in natural and purified systems, indicate that the effectiveness of added antioxidants may be very different under various conditions of storage (1,2,5,9).

A wide variety of antioxidants is available for study, two of the most important groups being phenolic compounds and aromatic amines. The present investigation was primarily concerned with phenolic compounds and included aminophenols. A study of the effect of various substituting groups on the antioxidant efficiency of these compounds was undertaken in order to obtain a better understanding of the relationship of antioxidant activity to structure. The results obtained were expected to be applicable to the stabilization of carotene in natural materials. For this work a simplified system comprising a solution of pure crystalline carotene in relatively inert solvent was employed. More than 100 phenolic compounds were studied.

Experimental

Commercial crystalline beta-carotene, repurified by precipitation from chloroform with methyl alcohol, was employed in this study. The substrate used for testing the antioxidants was a solution of this carotene in a highly refined medicinal mineral oil. The antioxidants in a suitable solvent, usually peroxide-free ether, were added to this solution and the solvent removed by bubbling a stream of nitrogen through the mixture. To facilitate the comparative evaluation of the antioxidants, the compounds were incorporated on an equivalent molecular basis rather than on a weight basis. Thus, in all cases, the test solution contained the added antioxidant in the proportion of one molecule of antioxidant to two molecules of carotene.

Stability Test. The details of the stability test have been published previously (11). Briefly, it consists

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

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of a determination of the time required for breakdown of 20% of the carotene in the oil solution, stored as a thin layer at 75°C., under specified conditions, as measured by a chromatographic-colorimetric method (stability value$_{20}$). Chromatographic adsorption on magnesia was employed to remove the colored oxidation products of carotene which developed during the storage period of the test (10).

The use of accelerated tests is necessary if rapid progress is to be made in the evaluation of antioxidants. It is important however that the test used be a reliable index of the results to be expected from storage at normal temperature over a longer period of time. In an earlier study it was found that, in general, the results obtained at 75°C. were in agreement with those found at 40° and 25° storage (3). This conclusion has been more recently confirmed in storage tests in which antioxidants were added to alfalfa meal (9).

Results

Monohydroxybenzene Derivatives. Since all the experiments reported here followed the same pattern, the quantitative data can be concisely reported in several tables. Table I shows the comparative stabilizing effect of monohydroxybenzene derivatives. The stability value$_{20}$ of the control (i.e., the time in hours for 20% loss of carotene in the absence of added antioxidants) was about two hours. The unsubstituted phenol was ineffective as an antioxidant. Introduction of nitro, carboxyl, primary alcohol, or ketonic groups onto the ring led to little if any enhancement of antioxidant potency. Alkylation of the hydroxyl group (as in phenetole) produced a very slight enhancement of the activity. The potency of phenol was increased by insertion of alkyl groups into the ring. The enhancement was most marked when substitution occurred in both the ortho- and para-positions as in 2,6-di-t-butyl,-4-methylphenol. Alkyl substitution also enhanced the activity of the so-called bisphenols, such as 2,2-bis(4-hydroxy-3-isopropylphenyl) propane. Phenyl substitution on the ring as in *o*-phenylphenol or *p*-hydroxydiphenyl led to a small enhancement of the activity. However the substitution of a phenolic radical as in 4,4′-dihydroxy-3,3′-dimethyl diphenyl was very effective. Presence of the two methyl groups may also have contributed to the antioxidant efficiency.

TABLE I

Antioxidant Efficiency of Monohydroxybenzene Derivatives for Carotene in Mineral Oil Solution at 75°C.

Compound	Stability Value$_{20}$
	hours
Control	2
Phenol	1
p-Nitrophenol	1
Salicylic Acid (*o*-Hydroxybenzoic Acid)	1
m-Hydroxybenzoic Acid	2
p-Hydroxybenzoic Acid	2
o-Hydroxyacetophenone	2
p-Hydroxyacetophenone	2
Phenyl Salicylate	2
Phenetole (Ethyl Phenyl Ether)	3
m-Ethylphenol	3
Anisic Acid (*p*-Methoxybenzoic Acid)	3
o-Hydroxybenzyl Alcohol	3
Anisaldehyde (*p*-Methoxybenzaldehyde)	3
p-t-Butylphenol	3
4-Methoxy-t-Amylbenzene	3
4-t-Butyl-2-Chlorophenol	4
p-Cresyl Methyl Ether	4
o-Hydroxydiphenyl	4
Bisphenol A [2,2-Bis (4-Hydroxyphenyl) Propane]	5
5-Hydroxy-1,3-dimethylbenzene	5
p-Hydroxydiphenyl	5
3,5-Methylethylphenol	6
p-Cumylphenol	8
2,2′-Dihydroxy-4,4′-dichlorodiphenylmethane	10
p-Octylphenol	12
Bisphenol G [2,2-Bis (4-Hydroxy-3-isopropyl phenyl) Propane]	52
Bisphenol C [2,2-Bis (4-Hydroxy-3-methyl phenyl) Propane]	68
Octylcresol	99
4,4′-Dihydroxy-3,3′-dimethyldiphenyl	106
2,6-Di-t-butyl-4-methylphenol	142
2,4-Dimethyl-6-t-butylphenol	188

Dihydroxybenzene Derivatives. The addition to phenol of a second hydroxyl group in the ortho position to form catechol greatly enhanced the antioxidant properties (Table II). The data indicated that substitution of an alkyl group into the catechol nucleus enhanced antioxidant effectiveness in the same manner as noted above for phenol. Furthermore, substitution in the 3 position was more effective than substitution into the 4 position although both the 3- and 4-alkyl-substituted catechols were very effective antioxidants. Substitution of a phenyl group into the catechol ring as in 3,4-dihydroxydiphenyl led to a slight enhancement in effectiveness. Nordihydroguaiaretic acid, which may be considered to be composed of two molecules of catechol linked together through a four-carbon-atom chain, was almost as effective as 3-lauryl catechol, which was the most effective catechol derivative tested. Ethyl hydrocaffeate was also an effective antioxidant. Methylation of one of the hydroxyl groups of catechol as occurs in guaiacol or eugenol greatly reduced antioxidant activity. This loss of activity due to methylation could be partly counteracted by ring substitution of an alcohol or ketone group as in vanillyl alcohol or vanillal acetone. However substitution of an aldehyde group as in vanillin or veratraldehyde almost completely destroyed the antioxidant efficiency.

TABLE II

Antioxidant Efficiency of Catechol Derivatives for Carotene in Mineral Oil Solution at 75°C.

Compound	Stability Value$_{20}$
	hours
Catechol (1,2-Dihydroxybenzene)	84
Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	3
Veratraldehyde (3,4-Dimethoxybenzaldehyde)	3
3-Ethoxy-4-hydroxybenzaldehyde	4
Guaiacol (*o*-Methoxyphenol)	6
Eugenol (2-Methoxy-4-allylphenol)	7
2-Hydroxy-3-methoxybenzaldehyde	8
Propenylguaethol	12
Vanillyl Alcohol (4-Hydroxy-3-methoxybenzyl alcohol)	20
2-Methoxy-4-methylphenol	30
Vanillalacetone	35
3,4-Dihydroxydiphenyl	96
4-t-Butylcatechol	124
Dibenzylcatechol	165
4-Octylcatechol	166
4-Laurylcatechol	166
Ethyl Hydrocaffeate	174
4-Hexylcatechol	182
Nordihydroguaiaretic Acid	208
3-Laurylcatechol	226

Resorcinol (1,3-dihydroxybenzene) was a much less effective antioxidant than catechol. Substitution of chlorine, acyl, aldehydic, or ketonic groups into the nucleus of resorcinol further reduced the activity. The hydrogenated compound, dimethyldihydroresorcinol, was completely inactive.

Hydroquinone (Table III) was only slightly more effective than resorcinol.

Alkyl substitution into the ring as in toluhydroquinone reduced the antioxidant efficiency by half and further substitution as in 2,5-di-t-butylhydro-

TABLE III

Antioxidant Efficiency of Hydroquinone Derivatives for Carotene in Mineral Oil Solution at 75°C.

Compound	Stability Value$_{20}$
	hours
Hydroquinone(1,4-Dihydroxybenzene)	8
2,5-Di-t-butylhydroquinone	1
2,5-Di-t-amylhydroquinone	1
2,5-Di(2′,4′-dihydroxyphenyl)hydroquinone	2
2,5-Dibenzylhydroquinone	2
Hydroquinone Dibenzyl Ether	2
Hydroquinone Diacetate	2
Trichloroethylbenzenehydroquinone	3
Toluhydroquinone	4
Tetrachlorohydroquinone	14
Chlorohydroquinone	23
2-Phenylhydroquinone	31
Hydroquinone Monobenzyl Ether	45
Hydroquinone Monomethyl Ether	46
2,5-Di-t-butylhydroquinone Monomethyl Ether	76
2-t-Butyl-4-methoxyphenol (butylated hydroxyanisole)	130
2,5-Dihydroxydiphenyl	146
Alpha Tocopherol	160

quinone produced a compound which had pro-oxidant properties. Contrary to the results with catechol, methylation of one of the hydroxyl groups of hydroquinone as in hydroquinone monomethyl ether greatly enhanced the antioxidant effectiveness. Furthermore, alkyl substitution of the ring of the monomethyl ether as with 2-t-butyl-4-methoxy phenol resulted in a three-fold increase in antioxidant efficiency. Methylation of both hydroxyl groups of hydroquinone destroyed antioxidant activity completely. Acylation also destroyed activity. Both quinhydrone and quinone were about equal in effectiveness to hydroquinone as antioxidants. Substitution of one chlorine atom in the ring enhanced the antioxidant effectiveness about three-fold. Tetrachlorohydroquinone was less effective. Phenyl substitution as in 2,5-dihydroxydiphenyl proved effective. Since alpha-tocopherol can be prepared by the condensation of trimethyl hydroquinone with phytol, it may be thought of as a derivative of hydroquinone. In accordance with the results of many reports in the literature, alpha-tocopherol exhibited very marked antioxidant activity.

Trihydroxybenzene Derivatives. In comparing the trihydroxybenzene derivatives, position of the hydroxyl groups is of prime importance. Thus phloroglucinol (1,3,5-trihydroxybenzene) with a stability value$_{20}$ of 4 was almost completely ineffective as an antioxidant, whereas pyrogallol (1,2,3-trihydroxybenzene) was a potent antioxidant (Table IV). Methylation of two of the hydroxyl groups reduced the activity to some extent whereas methylation of all three hydroxyl groups completely destroyed the effectiveness of the compound. Alkyl substitution in the ring of the completely methylated compound as in 4,6-di-t-butylpyrogallol trimethyl ether increased the antioxidant activity to a small extent. Introduction of a carboxyl group into the ring as in gallic acid caused the loss of most of the antioxidant activity. However the gallic acid esters, such as lauryl or ethyl gallate, were as effective as pyrogallol.

TABLE IV

Antioxidant Efficiency of Pyrogallol Derivatives for Carotene in Mineral Oil Solution at 75°C.

Compound	Stability Value$_{20}$
	hours
Pyrogallol(1,2,3-Trihydroxybenzene)	155
Pyrogallol Tribenzyl Ether	2
Pyrogallol Triacetate	15
Pyrogallol Trimethyl Ether	2
Tannic Acid [Penta-(m)Digalloyl]-Glucose	5
Gallic Acid(3,4,5-Trihydroxybenzoic Acid)	7
4,6-Di-t-butylpyrogallol Trimethyl Ether	17
Di-t-butylpyrogallol	82
Pyrogallol-1,3-Dimethyl Ether	89
Lauryl Gallate	138
Ethyl Gallate	165

TABLE V

Antioxidant Efficiency of Naphthol Derivatives for Carotene in Mineral Oil Solution at 75°C.

Compound	Stability Value$_{20}$
	hours
Alpha-Naphthyl Ethyl Ether	1
2-Aceto-1-Naphthol	2
Benzyl-Alpha-Naphthyl Ether	2
Di-Beta-Naphthol	6
Beta-Naphthol	18
2,7-Dihydroxynaphthalene	51
Alpha-Naphthol Benzene	145
Alpha-Naphthol	154
1,5-Dihydroxynaphthalene	204

Naphthol Derivatives. Table V presents the relative antioxidant efficiencies of the naphthol derivatives studied. Alpha naphthol was considerably more effective than beta-naphthol, comparing in efficiency with the alkyl substituted catechols. The introduction of an aceto-group, as in 2-aceto-1-naphthol, as well as alkylation of the hydroxyl group destroyed the antioxidant activity completely. The linkage of two beta-naphthol molecules at the alpha position to form di-beta-naphthol reduced the activity to one-third that of beta-naphthol. Of the dihydroxy compounds tested, 1,5-dihydroxynaphthalene proved to be four times as effective as 2,7-dihydroxynaphthalene, which is consistent with the results for alpha- and beta-naphthol.

Amino-phenols. Table VI contains the comparative stability data for the amino-phenols. *o*-Aminophenol was about as effective as the corresponding dihydroxy derivative, catechol, whereas *p*-aminophenol was much more effective than hydroquinone. Acetyl-*o*-aminophenol is only a third as active as *o*-aminophenol. *m*-Amino phenol was a weak antioxidant. Alkyl substitution on the nitrogen atom of *p*-aminophenol more than doubled the effectiveness of the compound, the N-sec.-butyl derivative being more effective than the *n*-butyl compound. Interposition of a methylene group between the nitrogen atom and the benzene ring almost completely destroyed the activity, which was however slightly enhanced by alkyl substitution

TABLE VI

Antioxidant Efficiency of Amino-Phenol Derivatives for Carotene in Mineral Oil Solution at 75°C.

Compound	Stability Value$_{20}$
	hours
Dimethylaminomethylphenol	2
o-Dimethylaminomethyl-*p*-Butyl Phenol	3
o-Phenetidine(*o*-Aminophenol Ethyl Ether)	3
Acetyl-*p*-phenetidine	3
m-Phenetidine	5
Aminohydroquinone Dimethyl Ether	6
o-Dimethylaminomethyl-*p*-octylphenol	6
p-Anisidine(*p*-Aminophenol Methyl Ether)	10
p-Phenetidine	11
m-Aminophenol	15
Acetyl-*o*-Aminophenol	27
Aminohydroquinone Diethyl Ether	44
o-Aminophenol	90
p-Aminophenol	92
2-Amino-4-phenylphenol	175
n-Butyl-*p*-aminophenol	183
Isobutyl-*p*-aminophenol	190
N-sec.-Butyl-*p*-aminophenol	230

on the benzene nucleus as in *o*-dimethylaminoethyl-*p*-octylphenol. In most cases methylation of the phenolic group in an aminophenol caused a tremendous loss of activity.

Discussion

In general, it was found, in accordance with stabilization studies on gasoline (8) and paraffin wax (6) that alkyl substitution enhanced the activity of phenol. Furthermore this effect was more than additive. Substitution in the meta position was less effective than in either the ortho or para positions. In agreement with work on other substrates (6,8) maximum potency was obtained with the trisubstituted phenols when the substitution occurred in the highly reactive 2, 4, and 6 positions.

Although alkyl substitution was effective in enhancing the activity of phenol, catechol, and pyrogallol, it was not effective for hydroquinone. Thus addition of two butyl groups to hydroquinone reduced the activity to zero under our experimental conditions. This is contrary to results obtained in other substrates. For example, 2,5-ditertiary butyl hydroquinone was found to be an extremely effective antioxidant for carotene when added to alfalfa meal (9). The reason for the ineffectiveness of this compound in mineral oil solution is not immediately apparent. Golumbic (4) has also shown that the stabilizing action of hydroquinone was finally lost with progressive nuclear methylation.

Conversely, 2-t-butyl-4-methoxyphenol (butylated hydroxyanisole), which is a very effective antioxidant for carotene in mineral oil solution, is only slightly effective in alfalfa meal (9). In the same way ethyl gallate, which is one of the best antioxidants tested for carotene in mineral oil solution, is completely ineffective for carotene in alfalfa meal under our experimental conditions (9).

Work with 2,5-di(2′,4′-dihydroxyphenyl) hydroquinone affords a further illustration of the difficulties involved in attempting to correlate the results obtained with antioxidants when employed in different substrates. Thus Lovern (5) has shown this compound, which he called diresorcinylquinol, to be an active inhibitor for carotene in ethyl acetate solution whereas the present studies show it to be completely ineffective under our experimental conditions (Table III). It should therefore be emphasized that generalizations which may be made regarding nuclear substitution of phenolic inhibitors and their stabilizing effect apply only under the conditions studied. That the generalizations do not always hold is shown by comparison with other work. They may or may not apply to other systems.

Summary

Relative values for the carotene-stabilizing effects in mineral oil solutions of a number of phenolic-type antioxidants were determined. Certain relationships between molecular structure and antioxidant activity were observed. Alkylation of the phenolic compounds in general enhanced activity. Hydroquinone was shown to be an exception in the system studied.

Several antioxidants which have been accepted for use in lard have been found to be effective antioxidants for carotene in mineral oil solution. These include nordihydroguaiaretic acid, 2-t-butyl-4-methoxyphenol, alpha tocopherol, and esters of gallic acid. In addition, certain bisphenols used as anthelmintics were effective antioxidants for carotene.

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The Flavor Problem of Soybean Oil. VII. Effect of Trace Metals[1]

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IN any consideration of flavor stability of fats the importance of trace metals is fully recognized because of the pro-oxidant effects of such impurities. Enhanced oxidation soon lowers the flavor quality and reduces the stability of the product. Although the problem of metal contamination is quite generally appreciated, its rather insidious and stealthy nature has kept it from being regarded at its full importance. Work on fat stability and the greatly increased activity in the entire field of antioxidants has developed a greater interest in metallic contamination. The lack of adequate methods for determining metal concentrations of less than 1 p.p.m. has been, no doubt, one of the most serious handicaps workers have faced in making a complete evaluation of the metal problem.

The detrimental influence of copper in developing fishiness and other oxidized flavors in butter is well-

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