TABLE IV

Composition of Mixed Fatty Acids

Acid	In Solid Fraction	In Liquid Fraction	Total
Myristic	0.4	0.5	0.9
Palmitic	9.3	1.2	10.5
Stearic	6.4	......	6.4
Tetradecenoic	......	0.4	0.4
Hexadecenoic	......	0.8	0.8
Oleic	3.1	16.0	19.1
Linoleic	......	61.9	61.9
Total	19.2	80.8	100.0

The distillation and analytical data of the solid and liquid fractions are respectively recorded in Tables II and III.

Discussion

The seed of *Citrullus vulgaris* (large green citron) yield a linoleic rich oil, which is light yellow in color and has an agreeable odor (3) and could be utilized as an edible oil. Curtis also points out that the yield of seed per acre is about four times the yield of seed from soybeans. The mixed fatty acids consist of myristic, palmitic, stearic, tetradecenoic, hexadecenoic, oleic, and linoleic acids. The seed contain about 19% of oil.

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[Received October 5, 1951]

Comparative Evaluation of Antioxidants for Carotene

E. M. BICKOFF, A. L. LIVINGSTON, JACK GUGGOLZ, and C. RAY THOMPSON,
Western Regional Research Laboratory,[1] Albany, California

CONSIDERABLE success has been achieved in stabilizing carotene by means of phenolic antioxidants (1, 2, 3), three of which have been used in food products. Although aromatic amines such as diphenylamine have long been known to be effective antioxidants for carotene (6, 8), they have not been considered seriously for use in edible products for reasons of possible toxicity. In 1949 the use of N,N′-diphenyl-p-phenylenediamine was patented (4) for preserving carotene in alfalfa. Feeding tests with chicks showed that 10 to 15 times the amount recommended (0.015% in the dried meal) gave no toxic symptoms. This led to an increased interest in related compounds for carotene stabilization. In a recent report from this laboratory (7) a number of aromatic amines were tested as antioxidants for carotene in dehydrated alfalfa meal. In the present report a simplified system was employed to compare the antioxidant efficiency of a number of aromatic nitrogenous compounds in an attempt to obtain a more absolute evaluation. As far as possible the compounds tested were chosen to permit observation of change in activity with systematic change in structure.

Experimental

The procedure employed for testing the antioxidants was identical to that reported previously (3). A solution of purified crystalline beta-carotene in medicinal-grade mineral oil was employed as the substrate. The antioxidant compounds were dissolved in the oil solution and the time required for breakdown of 20% of the carotene at 75°C. was determined.

Results

Under the conditions of the test the control sample containing no added antioxidant lost 20% of its original carotene in about two hours. Since all the experiments reported here were carried out in the same way, the quantitative data are reported in a single table.

Aniline Derivatives. Aniline and all derivatives tested were either very weak antioxidants or were without effect. Of those tested, 2-aminobenzenethiol was most effective.

Naphthylamine Derivatives. Monoamino substitution on naphthalene produced moderately effective antioxidants. The corresponding phenyl derivatives (phenyl-alpha- and phenyl-beta-naphthylamine) were very effective antioxidants. Diamino substituted naphthalene was one of the most potent antioxidants tried in this study.

Secondary Amine Derivatives. Diphenylamine was much more effective than either monophenylamine (aniline) or triphenylamine. The effectiveness of diphenylamine could be greatly enhanced by suitable ring substitution. Thus monoalkyl substitution (4-heptyldiphenylamine) caused only slight antioxidant enhancement whereas dialkyl substitution (4,4′-diheptyl or 4,4′-dioctyldiphenylamine) more than doubled the antioxidant effectiveness. Hydroxy or alkoxy substitution (4-hydroxydiphenylamine, 4-isopropoxydiphenylamine) more than quadrupled the antioxidant efficiency. Amino substitution (4-aminodiphenylamine) enhanced the antioxidant effectiveness of the parent compound five-fold. p-Nitroso substitution more than tripled the activity of diphenylamine whereas nitro substitution caused the loss of antioxidant effect. Acetylation of diphenylamine destroyed most of the antioxidant effectiveness of the compound. Interposition of methylene groups between the nitrogen atom and the benzene ring (benzylaniline or dibenzylamine) greatly reduced the activity.

Phenylenediamine Derivatives. Both ortho- and para-phenylenediamines were effective antioxidants, the para isomer being the more effective of the two. An alkyl substituted derivative of m-phenylenediamine (2,4-diaminotoluene) had about the same effectiveness as p-phenylenediamine. Acetylation of one of the amino groups (p-aminoacetanilide) caused the loss of all the antioxidant activity. Derivatives of p-phe-

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

TABLE I

Effect of Various Antioxidants on the Stability of Carotene in Mineral Oil

Compound Tested	Time for 20% Loss of Carotene at 75°C.
	Hours
Aniline Derivatives	
2-Aminobenzenethiol	32
Benzidine	6
p-Toluidine	6
o-Aminodiphenyl	5
4-Amino-1,2-dimethylbenzene	5
Aniline	3
p-Aminodiphenyl	2
Dimethylaniline	2
Dibenzylaniline	2
Benzalaniline	2
Benzylaniline	2
Naphthylamine Derivatives	
1,8-Diaminonaphthalene	232
Phenyl-alpha-naphthylamine	193
Phenyl-beta-naphthylamine	150
Beta-naphthylamine	15
Alpha-naphthylamine	14
Diphenylamine Derivatives	
4-Aminodiphenylamine	234
2,4-Diaminodiphenylamine	225
4-Isopropoxydiphenylamine	205
4-Hydroxydiphenylamine	196
4,4′-Methoxydiphenylamine	190
4-Nitrosodiphenylamine	155
4.4′-Diheptyldiphenylamine	102
4,4′-Dioctyldiphenylamine	95
4-Heptyldiphenylamine	60
Diphenylbenzidine	50
Diphenylamine	45
Benzeneazodiphenylamine	45
Carbazole	9
Acetyldiphenylamine	8
Acridone	5
Triphenylamine	4
2,4-Dinitrodiphenylamine	3
Phenylenediamine Derivatives	
N,N′-Diphenyl-p-phenylenediamine	245
N,N′-Di-sec-butyl-p-phenylenediamine	245
N,N′-Di-4(2,6-dimethylheptyl)-p-phenylenediamine	226
Di-beta-naphthyl-p-phenylenediamine	210
p-Phenylenediamine	106
2,4-Diaminotoluene	98
o-Phenylenediamine	78
p-Aminoacetanilide	3
Quinoline Derivatives	
Polymer of 2,2,4-trimethyl-1,2-dihydroquinoline	154
6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline	140
Acid rearranged-2,2,4-trimethyl-1,2-dihydroquinoline	92
6-Phenyl-2,2,4-trimethyl-1,2-dihydroquinoline	51
2,2,4-Trimethyl-1,2-dihydroquinoline	44
8-Hydroxyquinoline	6
2-Hydroxylquinoline	2
Quinoline	2
Isoquinoline	2
Urea and Related Compounds	
sym-Diphenylcarbazide	205
1-Phenylsemicarbazide	195
1-Phenyl-thiosemicarbazide	190
4,4-Diphenylsemicarbazide	78
Diphenylthiocarbazide	75
Di-o-tolylthiourea	60
Diphenylguanidine	9
Allylthiourea	8
N,N′-Diphenylurea	6
Diphenylthiocarbazone	4
N-Phenyl-beta-hydroxyethyl-thiourea	5
Urea	3
N,N′-Diphenylurea	2
Thiourea	2
Triphenylguanidine	2
Phenothiazine Derivatives	
Phenothiazine	155
Phenothiazone	210
Thionol	160
Azobenzene Derivatives	
p-Aminoazobenzene	8
p-Hydroxyazobenzene	5
Azobenzene	2
Diphenylmethane Derivatives	
sym-Diphenylethylenediamine	14
p,p′-Diaminodiphenylmethane	6
4,4′-Tetramethyl diaminotriphenylmethane	5
Tetramethyl diaminodiphenylmethane	2

nylenediamine having alkyl (N,N′-disec-butyl-p-phenylenediamine) or aryl (N,N′-diphenyl-p-phenylenediamine, N,N′-di-beta-naphthyl-p-phenylenediamine) substitution on the N atom were the most potent antioxidants found for carotene under these conditions.

Quinoline Derivatives. Neither quinoline nor its hydroxy derivatives (2-hydroxyquinoline, 8-hydroxyquinoline) were effective antioxidants. However several dihydroquinolines were powerful antioxidants (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline). The effectiveness of 2,2,4-trimethyl-1,2-dihydroxyquinoline was enhanced by acid rearrangement or polymerization.

Urea Derivatives. A number of carbazides, semicarbazides, and related thio derivatives were powerful antioxidants. In comparing the phenylcarbazides with their corresponding thio derivatives, it is interesting to note that although 1-phenylsemicarbazide and 1-phenylthiosemicarbazide had the same antioxidant effectiveness, N,N′-diphenylcarbazide was more than twice as effective as its corresponding thio analogue (N,N′-diphenylthiocarbazide). The carbazone tested (diphenylthiocarbazone) was inactive.

Phenothiazine Derivatives. Phenothiazine and its derivatives, phenothiazone and thionol, were also effective antioxidants.

Hydrazine Derivatives. Although phenylhydrazine was ineffective, tetraphenylhydrazine was a potent antioxidant.

Discussion

These studies indicate that certain classes of compounds, such as naphthylamines, diphenylamines, phenylenediamines, and dihydroquinolines, when properly substituted are effective for preserving carotene from oxidation in the presence of mineral oil. Many of these compounds have also been found to be effective for carotene in alfalfa (7), and it is suggested that they will probably act similarly in other carotene-containing systems.

A method of estimating the relative activities of antioxidants independent of animal or vegetable fats or oils should prove of value in obtaining a more absolute comparison of antioxidant potency. The employment of carotene as the substrate in such a study was recently suggested (5) since it is a highly unsaturated compound of constant identity found intimately associated with fats and oils, and its destruction parallels the formation of peroxides and the break-down of naturally occurring antioxidants in fats. Carotene dissolved in a relatively inert carrier, as employed in the present study, may serve as the basis for such a test.

Summary

A variety of compounds were evaluated as antioxidants for carotene in mineral oil solution by an accelerated stability test. These included derivatives of aromatic amines, substituted dihydroquinolines, ureas, phenothiazine, azobenzene, and diphenylmethane.

The most effective antioxidants were hydroxy- and amino-substituted diphenylamines, p-substituted phenylenediamines, and derivatives of 2,2,4-trimethyl-1,2-dihydroquinoline.

Acknowledgment

The authors are indebted to Monsanto Chemical Company and Universal Oil Products Company and David F. Houston for some of the compounds studied. The technical assistance of I. V. Ford is gratefully acknowledged.

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[Received March 19, 1952]