containing low concentrations of residual "free" gossypol. This procedure promises to provide another analytical tool for the study of residual material in processed cottonseed meal that causes egg discoloration when fed to laying hens.

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A Comparison of Alkylated Phenols as Antioxidants for Lard[1]

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ALTHOUGH CONSIDERABLE ATTENTION has been focused recently on the alkyl substituted phenols (hindered phenols), no systematic study relating their structure to antioxidant activity in fats has been published. Rosenwald *et al.* (1) has compared a number of alkyl phenols as antioxidants for gasoline in oxygen bomb tests. Wasson and Smith (2) recently reported a study of copper-catalyzed oxidation of lubricating oils to which various trialkyl phenols were added at 0.1% concentration (weight basis). The present paper reports the effects of some of the same and other compounds when equimolar quantities of them were added to lard.

Materials and Methods

Synthetic phenols were obtained from commercial sources whenever possible. All were recrystallized or redistilled before use. Four of the desired compounds which were not available commercially were synthesized in this laboratory. Sources and references are given in Table I for all except 2,4,6-tri-methylphenol, which was prepared as follows: 9.6 ml. conc. sulfuric acid were added to 20.0 g. mesitylene in a 125-ml. Erlenmeyer flask, and the mixture was held at 60°C. for 4 hrs. with frequent shaking. The supernatant liquid was decanted, the solid 2,4,6-trimethylbenzylsulfonic acid was dissolved in water, and 10% aqueous sodium chloride solution was added until the sodium salt of the sulfonic acid precipitated. This was filtered in a Buchner funnel, washed thoroughly with ether, and dried under reduced pressure; then 30 g. were added slowly to 84 g. of molten potassium hydroxide in a 500-ml. nickel crucible. The temperature was raised to 330°C. and held there for 10 min. The partly-cooled melt was poured into ice, the solution was acidified with hydrochloric acid and filtered. The crystalline product (2–3 g.) was recrystallized repeatedly from a methanol-ether solution until a constant melting point (68–69°C.) was obtained. Bruson and MacMullen reported a melting point of 69° for 2,4,6-tri-methylphenol (3).

The antioxidant activities of the various compounds were compared by addition of equivalent molar quantities to a lard of low antioxidant content, one micromol of antioxidant per gram of lard. The detailed procedure has been described elsewhere (4). All determinations were replicated: the active compounds five times, the inactive three times. In most cases replicates were started on different days.

Results and Discussion

Of the compounds tested, only those having an alkyl group in the *ortho*-position showed any antioxidant activity in lard (Table I). In all cases those having two alkyl group in the *ortho*-positions were more active than those having one, and an alkyl group in the *para*-position further increased the activity. Alkyl groups in the *meta*-position showed little or no influence on activity.

The nature of the alkyl group was less important than its position in most cases. However in the *ortho*-position *tertiary* butyl groups seemed to be most effective, and in the *para*-position methyl groups seemed most effective in enhancing antioxidant activity. Accordingly 2,6-di-*tert*-butyl-4-methyl phenol was the most active compound tested. This compound was more than twice as active as an equivalent molar quantity of catechol.

Nitro or halogen groups in *ortho* and *para* positions were not effective substitutes for alkyl groups since tribromophenol, triiodophenol, and picric acid showed no antioxidant effect. Evidently the function of the alkyl group is more fundamental than merely to provide a "steric hindrance" to the reactivity of the phenolic group. The antioxidant activity does not seem to bear an inverse relation to the acidity of the phenolic hydrogen or the inductive effects of substituent groups. Trinitro- and trihalogen phenols react readily with alkalies as do also the simple phenols. In contrast 2,4,6-trimethylphenol forms a sodium salt only on several hours of refluxing with alkali (3); 2,4,6-tri-*tert*-butylphenol does not form a sodium salt with alkali, and its solubility is diminished by the presence of alkali in alcohol (5).

While the inductive effect of the alkyl substituents

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TABLE I
Comparison of Antioxidant Activities of the Phenols

Phenol	Commercial Name and Source or Reference	Activity [a] (Catechol Index)
4-octylphenol [b]	octylphenol [c]	0.00
4-nonylphenol [b]	nonylphenol [c]	0.00
3-methylphenol	*m*-cresol [d]	0.00
3-isopropylphenol	*m*-isopropylphenol [d]	0.00
4-isopropylphenol	*p*-isopropylphenol [d]	0.00
4-*tert*-butylphenol	*p*-*tert*-butylphenol [e]	0.00
2-methyl-4-*tert*-butylphenol	*p*-*tert*-butyl-*o*-cresol [d]	0.14
2-*tert*-butylphenol	*o*-*tert*-butylphenol [f]	0.26
3-methyl-6-*tert*-butylphenol [b]	mono-*tert*-butyl-*m*-cresol [d]	0.35
3-methyl-6-amylphenol [b]	mono-amyl-*m*-cresol [d]	0.37
2,4-di-*tert*-butylphenol	2,4-di-*tert*-butylphenol [f]	0.66
3-methyl-4,6-di-*tert*-butylphenol	4,6-di-*tert*-butyl-*m*-cresol [e]	0.69
2-*tert*-butyl-4-methylphenol	*o*-*tert*-butyl-*p*-cresol [d]	0.84
2,4-di-*tert*-butyl-6-methylphenol [b]	di-*tert*-butyl-*o*-cresol [d]	0.90
2-methyl-6-*tert*-butylphenol	*o*-*tert*-butyl-*o*-cresol [d]	1.00
2,4-di-*tert*-butyl-6-isopropylphenol [b]	di-*tert*-butyl-*o*-isopropylphenol [d]	1.16
2,4,6-tri-methylphenol [g]		1.19
2,4,6-tri-isopropylphenol [g]	(8)	1.19
2-isopropyl-6-*tert*-butylphenol	*o*-isopropyl-*o*-*tert*-butylphenol [d]	1.30
2,4,6-tri-*tert*-butylphenol [g]	(5)	1.75
2,6-di-*tert*-butyl-4-methylphenol	2,6-di-*tert*-butyl-*p*-cresol [d]	2.35
2,4,6-tri-bromophenol [g]	(9)	0.00
2,4,6-tri-iodophenol	2,4,6-tri-iodophenol [e]	0.00
2,4,6-tri-nitrophenol	picric acid [h]	0.00

[a] Five replicates on active phenols, three on inactive. Variance of individual analyses: 0.00198 units; standard error of replicates (active): 0.0154 units.
[b] Including other isomers.
[c] Rohm and Haas Company.
[d] Koppers Company Inc.
[e] Eastman Organic Chemicals.
[f] The Dow Chemical Company.
[g] Synthesized in this laboratory.
[h] Bakers Chemical Company.

prevents ion-formation by the phenolic hydrogen, it also promotes the formation of hydrogen free-radicals. Taylor (6) has stated that the production of radicals stabilized by resonance always means that there is a low activation energy for withdrawal of a hydrogen atom and the *ortho*-alkyl phenoxy radicals have a capacity for a number of resonance forms. In addition, any aromatic compound exhibiting resonance has greater thermochemical stability than non-resonating compounds (7). This ease of radical formation is undoubtedly related to the effectiveness of these compounds in breaking the reaction chains of fat autoxidation.

Our results differ substantially from those of Wasson and Smith (2), especially in the activity of trimethyl phenol, which under their conditions was inactive and under ours was more active than catechol. Substantial quantitative differences also exist between our results and those of Rosenwald *et al.* (1). Whether these differences reflect a fundamental difference in antioxidant structural requirements for fats as compared to petroleum products remains for further work under common testing procedures.

Summary

Tests with 24 substituted phenols showed that position was more important than the nature of the alkyl group in influencing antioxidant potency for lard. The most active compound tested was 2,6-di-*tert*-butyl-4-methylphenol.

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Letter to the Editor

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IN A RECENT ARTICLE Reiser (1) has restated his original contention (2) that no significant amount of fatty acid exchange occurs in the intestinal lumen of the rat during fat digestion. The validity of this reasoning has been questioned repeatedly by the present author (3–6), who has shown that such exchanges take place in the rat and in the human being and that the reaction is of considerable magnitude. This letter is written in order to correct any misunderstandings which may have arisen out of our data.

In Reiser's original experiments (2) rats were fed a synthetic unsaturated triglyceride labelled both in the glycerol and acid part. This was mixed with an unlabelled saturated triglyceride. From the relative amounts of labelled glycerol in the saturated and unsaturated triglycerides of the thoracic duct lymph, calculations were made as to the species of glyceride absorbed. These calculations were based on the assumption that no recombination of glyceride ester