Development of Color in Fats Stabilized with Amino-Hexose-Reductones[1]

PATRICIA M. COONEY, J. E. HODGE, and C. D. EVANS, Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois

ONE REQUIREMENT for an acceptable antioxidant is the absence of color development in the fatty substrate. It was known at the time this investigation was undertaken that reductones would impart some added browning to stabilized fats. However, because amino reductones are among the most potent substances to prevent oxidation of fats and are among the very few substances that show an appreciable antioxidant activity in soybean oil (5), this investigation on color development in reductone-treated oils was undertaken. Hodge (7, 8, 9) has discussed the browning reactions of amino and nonamino reductones in nonfat systems. The development of color by nonamino reductones (ascorbic acid and its esters, catechol, and hydroquinone) when used as antioxidants in fats has apparently not been a problem. These reductones however are well known for their browning reactions in foods and aqueous systems. Regier and Tappel (12) report that 1% concentrations of ascorbic acid, morpholino-, piperidino-, and dimethylamino-hexose-reductones induced rapid visual browning in freeze-dried beef.

Aqueous solutions of amino-hexose-reductones darken upon standing in air. The dehydro form of reductone further oxidizes and polymerizes, changing from yellow (in dilute solutions) or reddish (in concentrated solutions) to dark brown melanoid-like substances (3).

Experimental

Various reductones in antioxidant concentration ranges of 0.005 to 0.02% were evaluated for color development in soybean oil, cottonseed oil, lard, and shortening. The reductones were added as a 1% solution in 90% ethyl alcohol to fats on the cooling side of deodorization. With this technique, peroxide-free systems were attained; also a minimum of air was introduced into the sample. All samples of fats and oils were of commercial origin and, as judged by physical constants and stability tests, were typical and representative materials.

Color was measured by the A.O.C.S. tentative spectrophotometric method (11), and a few investigations were made in the visible and ultraviolet spectra with a Beckman Model DU spectrophotometer. Color development was measured on fats stored in loosely stoppered bottles held at 60°C. (Schaal oven conditions) for periods up to three months and on samples stored at 100°C. Tests for shorter periods of time were conducted on fat samples under A.O.M. oxidizing conditions. Deep-fat frying tests were conducted at 196°C. to determine the browning developed under these conditions (1). For uniformity the samples for evaluation were removed periodically from a single, large lot of material that was undergoing test.

The lack of a good method for determining low concentrations of peroxides and reductones in the presence of each other, and the fact that previous tests had shown that oils stored under Schaal oven conditions in the presence of 0.01% reductone did not reach the autoxidation stage until storage had exceeded 100 days, led to the decision not to determine peroxide values. There is a distinct need in all antioxidant work for effective methods of removing or separating peroxides before proceeding with the analysis for antioxidants in fat systems.

Lacking adequate methods, we proceeded on the assumption that if reductones react with peroxides, the error in the peroxide determination would be no greater than the known amount of the added reductone. Conversely in analyzing for reductones, any reducing value would indicate the presence of some reductone. However a zero value would also mean that peroxides or oxidizing substances were present in excess and the reductone content was not necessarily zero. In nonoxidized fat systems any of the standard methods for determining ascorbic acid or the Emmerie-Engel method for tocopherol are quite satisfactory for the determination of reductones. The Emmerie-Engel method is satisfactory for small samples and is more sensitive than titrimetric procedures. Reductones were determined directly in the fat system, using the iron-bipyridyl reaction. A standard curve prepared with pure reductones points to a direct linearity of concentration with color.

Table I and Figures 1 and 2 present the data obtained on color development for several reductones in three different fat systems stored at 60°C. Figure 1 indicates that pronounced differences are observed among the three different types of piperidino reductones. Anhydropiperidino reductone, $C_5H_{10}N \cdot C_6H_5O_2$, shows a very high color development and piperidino reductone, $C_5H_{10}N \cdot C_6H_7O_3$, considerably less, however piperidino reductone continues to develop some color at a rather constant rate over several weeks. Dihydroanhydropiperidino reductone, $C_5H_{10}N \cdot C_6H_7O_2$, developed the least color and was the best reductone evaluated insofar as browning is concerned. Very slight browning developed in the first 8–10 days, which gradually faded during the next sev-

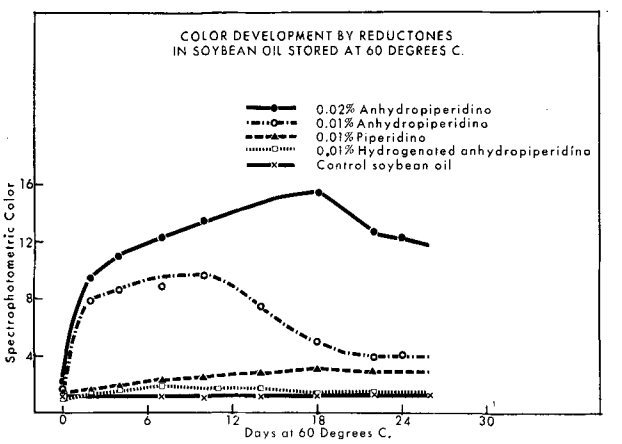


FIG. 1. Color development during storage at 60°C. of soybean oil treated with various reductones.

[1] Presented at the spring meeting, American Oil Chemists' Society, New Orleans, La., April 28–May 1, 1957.

TABLE I
Color Development in Reductone-Treated Fats

Treatment	Conc. %	Days of storage 0	4	7	11	14	18	22	25	35	56
						Soybean oil at 60°C.					
Morpholino	0.005	0.8	1.3	1.3	1.2	1.2	1.1	1.1	—	0.9	0.6
	0.01	1.0	1.6	1.6	1.5	1.3	1.3	1.2	—	1.0	0.5
	0.02	1.1	1.7	1.7	2.0	1.9	1.8	1.5	—	1.4	0.9
Control	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—	0.9	0.3
						Soybean oil at 100°C.					
Morpholino	0.01	0.7	1.5	1.6	1.6	1.5	—	1.3	—	—	—
	0.02	0.8	2.4	2.8	2.5	2.1	—	2.1	—	—	—
Control	—	0.8	1.1	1.1	1.1	1.3	—	1.4	—	—	0.3
						Cottonseed oil at 60°C.					(77 da.)
Anhydropiperidino	0.005	4.9	10.1	8.5	8.1	7.9	8.1	—	6.2	—	3.3
	0.01	5.4	11.7	10.1	9.9	9.9	10.0	—	8.9	—	2.2
Piperidino	0.005	4.8	7.0	7.0	5.4	5.6	5.4	—	4.4	—	1.1
	0.01	4.9	8.3	6.3	6.3	6.2	5.9	—	5.7	—	1.2
Control	—	4.6	5.2	4.8	4.9	4.7	4.6	—	4.6	—	1.1
						Lard at 60°C.					
Morpholino	0.005	2.5	2.6	2.7	2.8	2.7	2.7	—	—	2.2	—
	0.01	2.4	2.8	2.8	2.9	2.8	2.7	2.5	—	2.4	—
	0.02	2.4	3.3	3.3	3.4	3.3	3.3	3.2	—	3.3	—
Control	—	2.3	2.4	2.0	1.7	1.3	1.2	1.0	—	0.8	—

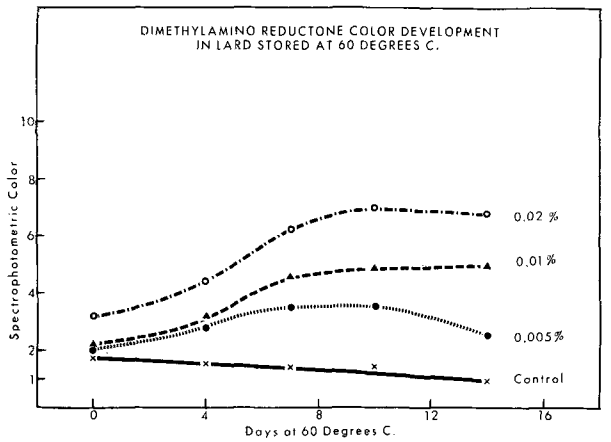


FIG. 2. The effect of concentration of dimethylamino-hexose-reductone upon the color development in lard stored at 60°C.

eral weeks; the value after 56 days in storage was about the same as after 25 days. The break point in the development of color, as will be shown later, is not associated with the break point in the autoxidation curve. Normally this reductone is one of the most active antioxidants, and other experiments with the same oil gave peroxide levels of less than 1 after 64 days and below 2 after 100 days of storage at 60°C. (5).

Dimethylamino reductone develops more color in lard than morpholino reductone, and the effect of concentration on color development is seen in Figure 2 and Table I. Lard stored under these conditions showed a bleaching action within a few days while all samples containing reductones showed an increase in color for about 10 days. After 30 days the stored sample with 0.005% concentration of reductone had bleached to a value equal to the control, but lard samples containing higher concentrations maintained the maximum color which they had developed and no oxidative deterioration was detected. The initial differences in color (Figure 2), we feel, are a result of air oxidation of the reductone in aqueous alcoholic solution which occurred before it was added to the fat. The reductones are much more sensitive to air oxidation in aqueous systems than in fat systems. The long induction period needed in oxygen absorption tests (2) is indicative of their stability to oxygen in fat systems.

Table I presents the effects of concentration of moropholino reductone on color development in soybean oil and lard. Morpholino- and dihydro-anhydropiperidino are the two reductones having the least browning in fat systems. Only slight differences in color are observed between controls and samples treated with 0.005 or 0.01% morpholino reductone. At 0.02% only small increases are seen in soybean oil stored at 60° and 100°C. The increase in color at 100° over 60° is negligible in the soybean oil. except at the 0.02% level. More rapid bleaching occurred in lard at 100°, but the total browning color which developed appears to be the same under both conditions of storage. Because of the rapid peroxide development in lard at 100° the difference between the color of the reductone-stabilized sample and the bleached control is large. However, if the initial level of color of the samples is used for comparison, only small increases in absorption are seen at 100°, even at the 0.02% level.

The increased color developed in shortenings under deep-fat frying conditions is illustrated in Figure 3 for the different reductones. Similar results but slightly less browning were observed for lard. Lard also exhibited high initial values of 4.8 for piperidino and 3.3 for morpholino, which dropped to 3.8 and

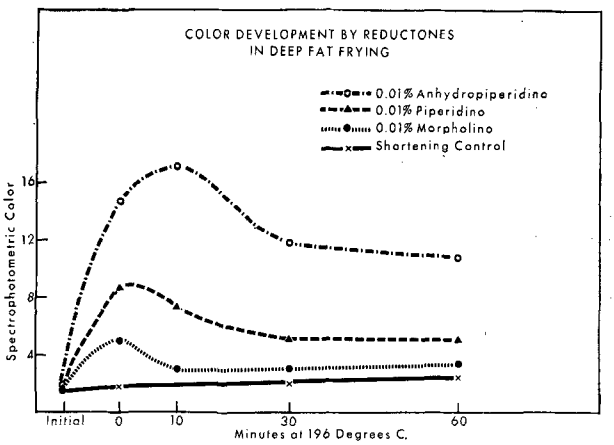


FIG. 3. Color development during deep-fat frying of shortening treated with various amino-hexose-reductones.

3.2, respectively, within 10 min. and remained constant thereafter. Color values for the control lard remained constant over the 1-hr. test period at 2.4–2.5. Color development during deep-fat frying can be attributed to both thermal destruction and to oxidation of the reductone. A 0.01% piperidino reductone sample heated under vacuum at 196°C. showed the initial rise in color from 2.9 to 4.0, but the maximum of 4.6 was not attained until after an hour's heating. About 20% of the reducing power of the reductone was destroyed under these conditions in 1 hr.

In the evaluation of antioxidants, color development is most generally observed under A.O.M. conditions. Figures 4, 5, and 6 show such results. The loss of anhydropiperidino reductone under A.O.M. conditions in lard and the simultaneous development of color and peroxides are given in Figure 4. Maximum rate of color development occurs during the first stages of aeration. The maximum color is attained after 70 hrs. of aeration and when approximately 65% of the reductone has been destroyed. These results would indicate that the color begins to bleach when a relatively low level of peroxide is attained, perhaps even at levels of less than 1 milliequivalent.

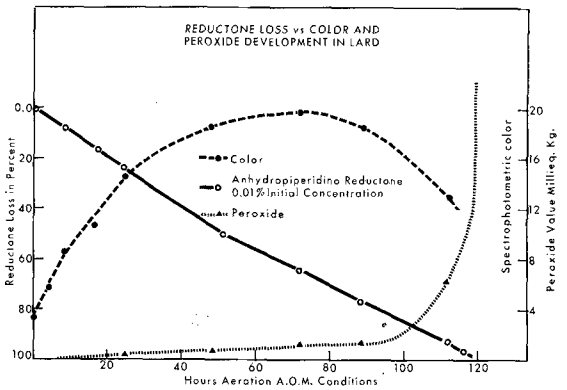


FIG. 4. Destruction of 0.01% anhydropiperidino reductone in soybean oil oxidized under A.O.M. conditions and the development of color and hydroperoxides in the system.

Subject to the limits of the analytical methods, it appears that bleaching occurs with the simultaneous rise in peroxides. The initial rise in color is linear up to approximately 20 hrs., and the deviation from linearity at this point could result from a constantly increasing bleaching rate while other color bodies are still being formed. In this particular test the accumulation of an appreciable peroxide concentration corresponds to the time when bleaching becomes predominant over color-body formation, or roughly after about 70 hrs. of aeration.

The time to obtain the stage of rapid autoxidation, normally considered to be the break point, also corresponds to the complete destruction of the antioxidant at approximately 120 hrs. The bleaching indicated by the decrease in color, if attributed only to hydroperoxides (none to free air), might indicate a higher peroxide level in the pre-autoxidation stage than can be determined by titrimetric methods. The rate of destruction of reductones (Figure 4) is linear at the outset although it is plotted as two linear segments with a slight change in slope at about 50% destruction. The relationship and significance of these curves are amplified in the following section. Under A.O.M. conditions it was found that 0.01% morpholino reductone developed color at a slower rate than the oxidizing soybean-oil control. The maximum color obtained before the break point was however about the same in each case.

Figure 5 is the same system as in Figure 4 except that the samples were held under high vacuum (continuous pumping) at 100°C. The slight increase in color ran be attributed to air dissolved in the system, which could not be removed by the initial vacuum-pumping at room temperature. After deaeration, continuous heating under vacuum gave no further increase or decrease in color of the lard containing 0.01% reductone. Analysis for the reductone by its reducing power showed a thermal destruction of 30% in 100 hrs. at 100°C. The loss is indicated as linear rate with time.

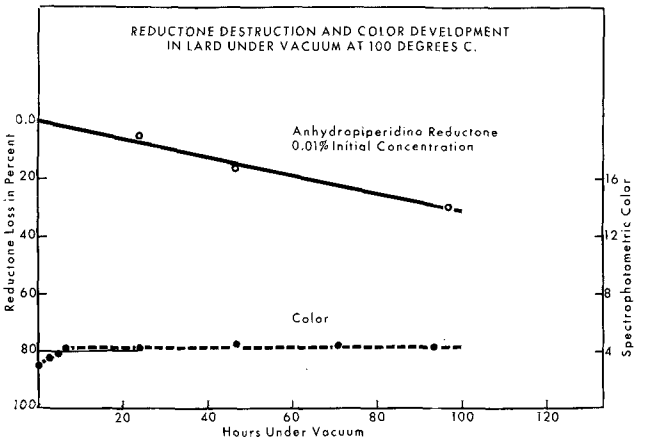


FIG. 5. Destruction of 0.01% anhydropiperidino reductone in soybean oil held at 100°C. under vacuum and the development of color.

Practically all commercial antioxidant mixtures contain free polyfunctional acids, such as citric acid; it is well known that mild acid conditions inhibit the browning of foods. Citric acid has a pronounced effect in retarding the development of browning in reductone-treated soybean oil. Figure 6 presents re-

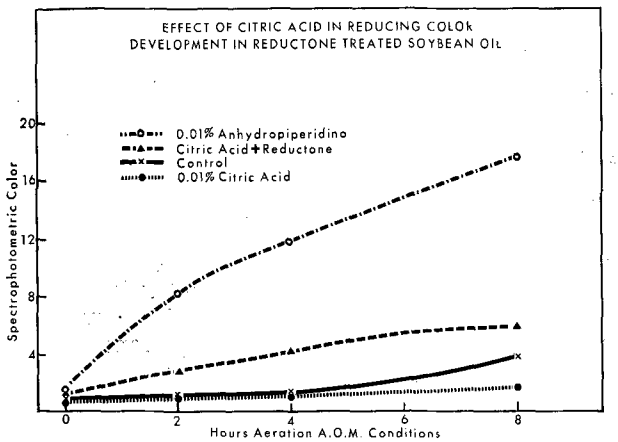


FIG. 6. The effect of citric acid upon the development of color in a reductone-treated soybean oil oxidized under A.O.M. conditions.

sults obtained under A.O.M. conditions with 0.01% each of anhydropiperidino reductone and citric acid. Although color does develop in this system, the use of citric acid reduced the browning by at least 50%. In tests with the morpholino reductone-citric acid combinations the browning and color development are virtually the same as that of the control oil.

Discussion

Absorption methods are the most widely used techniques to measure the extent of browning in spite of the fact that a large amount of visual browning is derived from reflected and fluorescent light. Absorption curves in the visible range exhibit no characteristic structure for a reductone-oil system. The presence of 0.02% anhydropiperidino reductone sharpens the absorption and gives exceedingly high absorption at 4200 Å. Oils containing reductones that are submitted to oxidation show only a more intense general absorption. Pure crystalline piperidino reductone has a high absorption at 315 mμ (E = 1510 in MeOH), and the oxidized form of the reductone absorbs at 228 mμ (7, 9). Virgin soybean oil also exhibits absorption at these wavelengths, where a slight increase is seen at 315 mμ and a stronger absorption at 228 mμ. The absorption data obtained on oil systems at these concentrations were not suitable for the analytical or reaction type of studies.

Color values on undiluted fats by the A.O.C.S. photometric method indicated that about 90% of the color was produced by the absorption at 550 mμ. Thus the color readings are closely related to the absorption at this wavelength and compare favorably with the visual amount of red color in the samples. The absorption at 460 mμ contributes only a small amount to the color values because of the low conversion factor. This absorption increases at a similar rate in both the control oil and reductone-treated oil upon storage. The magnitude of the difference between the treated and untreated oil is small at 460 mμ, compared to the difference observed in absorptions at 520 mμ. Absorptions at the two higher wavelengths were negligible.

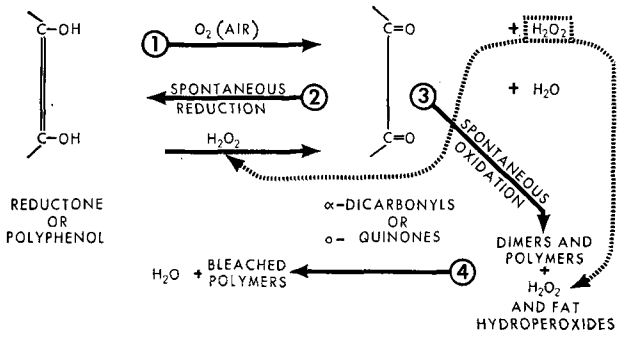
In some respects reductones behave in anhydrous fat systems as they do in aqueous systems. Figure 5 shows that no color develops when air is excluded from a reductone-containing soybean oil held at 100° C. although the reductone concentration slowly diminishes. The necessity for dehydrogenation of reductones to α-dicarbonyl compounds and of polyphenols to *o*-quinones before typical browning can occur (in aqueous systems) was emphasized by Hodge in 1953 (8). Further evidence has been provided in support of that contention (2, 3, 4, 6, 10). The present work indicates that the dehydrogenation hypothesis should be extended to include browning in oils and in fatty systems.

Color is assumed to be formed from the reductones by dehydrogenation of the enediols to α-dicarbonyl compounds, which are colored and readily generate deeper-colored bodies of higher molecular weight. Recently it has been shown that α-dicarbonyl compounds (dehydro reductones) and quinones disproportionate in the absence of air to regenerate the enediol and at the same time undergo oxidative dimerization and further polymerization to deeply colored substances (2, 3, 4, 6, 10). If such a spontaneous oxidation-reduction occurs in oils, the enediol form of the antioxidant would be continuously regenerated while color bodies are being formed. Constant regeneration of reductone could explain the zero-order reaction observed in this work, that is, the rate of reductone loss was found to be independent of reductone concentration (Figure 4).

Citric acid inhibited development of color in reductone-soybean oil systems. Whether the general inhibiting effect of acids on browning reactions or the sequestering effect of citric acid for heavy metals (that would otherwise catalyze oxidation of the reductone) is responsible for the slower development of color cannot now be decided. Because citric acid does not exhibit a synergistic antioxidant effect with amino reductones in soybean oil (5), the metallic-ion sequestering action may not be the reason for the inhibition of browning.

The amino reductones studied behave as true antioxidants in a variety of oils. In anhydrous oils, dissociation of protons from the enediol group would not be expected; consequently hydrogen transfer should be limited in comparison with aqueous and other polar systems. Yet the reductones do react with oxygen slowly in anhydrous fatty media. Oxidation could proceed by free radical mechanism, as with catechol in nonpolar solvents, and hydrogen peroxide would be generated (6). The colored substances are bleached by the peroxides; hence the colored substances or their intermediates could exert direct antioxidant action on the peroxides. More than one type of antioxidant mechanism is probably involved in our experiments.

Our study indicates that at least four reaction processes should be considered for the mechanism of antioxidation in oils by enediols (catechols, other polyphenols, and reductones): a) Air-oxidation of the enediols to α-dicarbonyl compounds, b) spontaneous reduction of the α-dicarbonyl compounds to enediols, accompanied by c) spontaneous oxidation, independent of oxygen, of the α-dicarbonyl compounds to deeper-colored bodies, and d) oxidation-reduction between the colored bodies and the fat peroxides. The possible reactions are outlined below. The manner in which the enediols *per se* interfere with peroxide formation remain to be determined.



Summary

Amino reductones derived from hexoses were evaluated for color development in heated, oxidizing fat systems. Browning was observed to some extent with all the amino-hexose-reductones. The brown color frequently faded upon long heating of the oils. The density of color increased with reductone concentration and varied markedly among the different amino

reductones. Morpholino-hexose-reductone could be used in lard and vegetable oils at concentrations up to 0.01% without introducing visually detectable amounts of color. Heating soybean oil solutions of the amino reductones at 100°C. under vacuum slowly destroyed the reductone but did not cause development of color. Air or oxygen was required for color production. Addition of citric acid along with the reductone reduced the amount of color developed. Reductones in fat systems show similarities in browning to reductones in aqueous systems. New considerations for the mechanism of antioxidation by polyphenols and reductones in oils are presented.

Acknowledgment

The help of E. H. Melvin and Miss Jean Mallan in making and interpreting absorption spectra of the reductones is gratefully acknowledged.

REFERENCES

1. Bennion, Marian, and Henning, Flora, Food Tech., *6*, 290–292 (1956).
2. Doskocil, J., Coll. Czechoslav. Chem. Communications, *15*, 780–796 (1950).
3. Dulkin, S. I., and Freidemann, T. E., Food Res., *21*, 519–527 (1956).
4. Erdtman, H., and Granath, M., Acta Chem. Scand., *8*, 811–816 (1954).
5. Evans, C. D., Moser, Helen A., Cooney, Patricia M., and Hodge, J. E., J. Am. Oil Chemists' Soc., in press.
6. Hathaway, D. E., and Seakins, J. W. T., Nature, *176*, 218 (1955).
7. Hodge, John E., and Rist, Carl E., J. Am. Chem. Soc., *75*, 322–324 (1953).
8. Hodge, John E., Agr. and Food Chem., *1*, 928–943 (1953).
9. Hodge, John E., and Fisher, B. E., Abstracts, Am. Chem. Soc., 130th meeting, Atlantic City, September 16, 1956.
10. Mason, H. S., Nature *175*, 771–2 (1955).
11. "Official and Tentative Method of the American Oil Chemists' Society," edited by V. C. Mehlenbacker and T. H. Hopper, 2nd ed., rev. to 1956, Chicago, 1946–56.
12. Regier, L. W., and Tappel, A. L., Food Res., *21*, 640–649 (1956).

[Received August 7, 1957]

The Preparation and Properties of Surface-Active N-Acylamino-Methanesulfonates

ROBERT A. FALK,[1] J. FRED GERECHT, and IRVING J. KREMS,[2] Research and Development Department, Colgate-Palmolive Company, Jersey City, New Jersey

THE IGEPON-T surface-active agents have proved to be one of the most popular types in current use (13). These compounds may be considered to be derivatives of 2-aminoethanesulfonic acid, taurine. It therefore seemed desirable to investigate derivatives of the analogous aminomethanesulfonic acid since they might prove functionally and economically advantageous. Such surface-active compounds have been mentioned in the patent literature (2, 11, 18). Yoshizaki (19) discusses the preparation *via* the high-temperature reaction between fatty amides and sodium hydroxymethanesulfonate and describes some surface-active properties. The present investigation was designed to explore the chemistry of the aminomethanesulfonic acids and their acylation. This route to the desired surface-active compounds was judged preferable because of its greater simplicity and unambiguity.

While it is only fairly recently that the structure of aminomethanesulfonic acids has been firmly established (1, 2, 16), there is little doubt that these substances have long been known. Thus Petersen (7) reported the compound corresponding to 1-aminoethanesulfonic acid in 1852; and Reinking *et al.* described aminomethanesulfonic acid in 1905 (9). More recent interest in these compounds has been generated as a result of their discovery as antimicrobial agents (4, 5, 6, 15, 17).

Aminomethanesulfonic acids may be generally prepared, as described by Raschig and Prahl (8), by condensing a primary amine or ammonia with sodium 1-hydroxyalkanesulfonates, which are obtained by the addition of sodium bisulfite to aldehydes.

$$RCH(OH)SO_3Na + R'NH_2 \rightleftarrows RCH(NHR')SO_3Na + H_2O$$

The sodium aminomethanesulfonates are easily hydrolyzable, but they may be stabilized by acidification to form a zwitterion, or better, by acylation of the amino group. It is possible to vary the molecule over a wide range by changing one or more of the starting materials: aldehyde, amine, or acylating agent. In this work, surface-active agents were made by introducing the hydrophobic group through the latter two means.

The aminomethanesulfonic acids prepared in our investigation fall into two categories and are presented in Table I. In the first are those derived from low-molecular-weight amines or ammonia, and low-molecular-weight aldehydes. The acids prepared from higher amines and low-molecular-weight aldehydes fall into the second category.

TABLE I
Aminomethanesulfonic Acids

Amine	Aldehyde	Product	Yield	Analysis Calc.	Found
			%	%	%
NH_3	CH_2O	$NH_2CH_2SO_3H$[a]	87	N	12.60 12.49
				S	28.80 28.89
NH_3	CH_3CHO	$CH_3CH(NH_2)SO_3H$[c]	30	N	11.10 10.99
				S	25.39 25.26
CH_3NH_2	CH_2O	$CH_3NHCH_2SO_3H$[c]	60	N	11.17 11.03
				S	25.57 25.57
$(CH_3)_2CHNH_2$	CH_2O	$(CH_3)_2CHNHCH_2SO_3H$[c]	36	N	9.12 9.03
				S	20.90 20.47
$C_{12}H_{25}NH_2$	CH_2O	$C_{12}H_{25}NHCH_2SO_3H$[b]	93	N	5.01 4.90
				S	11.45 11.78
$C_{16}H_{33}NH_2$	CH_2O	$C_{16}H_{33}NHCH_2SO_3H$[b]	84	N	4.18 4.42
				S	9.55 9.07
$C_6H_{11}NH_2$[d]	CH_2O	$C_6H_{11}NHCH_2SO_3H$[a]	70	N	7.24 7.13
				S	16.58 16.34

[a] Analytical sample obtained by recrystallization from water.
[b] Analytical sample obtained by recrystallization from alcohol.
[c] Analysis on dried crude sample.
[d] Cyclohexylamine.

Our attempts to apply the experimental procedure of Raschig and Prahl (8) gave fair yields of 1-aminoethanesulfonic acid and N-isopropylaminomethanesulfonic acid. However highly variable results for aminomethanesulfonic acid and practically negligible

[1] Present address: Sperry Gyroscope Corporation, Great Neck, N. Y.
[2] Present address: Medical Faculty, University of Vienna, Vienna, Austria.