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.

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[Received August 7, 1957]

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

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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

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yields of N-methylaminomethanesulfonic acid led to a detailed study of the reaction.

When ammonia and sodium hydroxymethanesulfonate interact, it has been shown (8) that the following equilibria occur:

$$HOCH_2SO_3Na + NH_3 \rightleftarrows H_2O + NH_2CH_2SO_3Na \quad (I)$$

$$NH_2CH_2SO_3Na + HOCH_2SO_3Na \rightleftarrows NH(CH_2SO_3Na)_2 + H_2O \quad (II)$$

It is apparent from these equations that in order to obtain the maximum yield of aminomethanesulfonic acid it is necessary to minimize forward reaction (II). This may be accomplished first by adding an excess of ammonia to drive reaction (I) towards completion to the right, thereby reducing the concentration of sodium hydroxymethanesulfonate, and secondly by rapidly quenching the alkaline reaction mixture. This is achieved by pouring the reaction mixture into an excess of mineral acid in order to freeze the equilibrium and precipitate the aminomethanesulfonate as the zwitterion. It was observed that slow acidification of the ammoniacal reaction mixture substantially reduces the yield of the desired product since ammonia is gradually removed, thereby reversing equilibrium I and favoring the forward reaction of equilibrium II. By following these precautions, 85% yields of aminomethanesulfonic acid were obtained consistently.

When methylamine was substituted for ammonia, only low yields of the condensation product, N-methylaminomethanesulfonic acid, could be obtained by either the method of Backer and Mulder (1) or our modification of the Raschig and Prahl technique. An alternative preparation of aminomethanesulfonic acids was recently reported by Hartough *et al.* (3), who prepared them from formaldehyde, sulfur dioxide, and ammonia or an amine. It is interesting to note that they also obtained poorer results for N-methylaminomethanesulfonic acid. However by a variation of their method we were able to increase the yield to 60%.

When the low-molecular-weight amine or ammonia is substituted by long-chain amines, such as dodecyl- or hexadecylamine, the preparation is best conducted in alcohol because of solubility considerations. The resulting aminomethanesulfonic acids are relatively insoluble in water even as the sodium salts. Their chemical properties however are similar to the lower homologues, as indicated by the hydrolytic instability of the sodium salts and the formation of stable zwitterions.

Aminomethanesulfonic acid may be titrated with standard alkali (12). The stability of aminomethanesulfonic acid as a function of pH at 25°C. was studied in order to determine the importance of this factor on its titrimetry as well as in acylation. From the curves (Fig. 1) it can be seen that if the titration is conducted in a reasonable time (less than 6 minutes), a maximum decomposition of only 2% is to be expected.

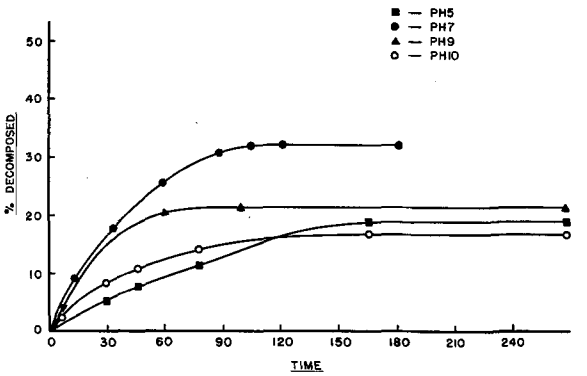

FIG. 1. Stability of aminomethanesulfonic acid as a function of pH at 25°C.

The other aminomethanesulfonic acids were found to decompose during titration, as evidenced by low results. For example, a slow titration of N-methylaminomethanesulfonic acid at 30°C. gave an inflection equivalent to a purity of 80%, but at 5°C. a fast titration indicated a purity of 98%. An extreme example is N-cyclohexylaminomethanesulfonic acid, which, though analytically pure, assayed 40% by titration. The marked instability of the alkylaminomethanesulfonic acids, in contrast to aminomethanesulfonic acid, made it possible to analyze them iodimetrically, after decomposition in borax-carbonate buffer as described by Shupe for the hydroxymethanesulfonates (14).

Although the aminomethanesulfonates derived from dodecyl and hexadecylamines show surface-active properties as exhibited by the foaming power of their aqueous solutions, they are handicapped by poor hydrolytic stability. They were converted into the practically useful acetyl derivatives (Table II) by treatment with acetic anhydride in pyridine.

The reaction could also be conducted in the absence of pyridine by using a five-fold excess of acetic anhydride as solvent. Attempts to use water as a solvent were not satisfactory. The acetylated products are soluble in water and give clear, foaming solutions that are quite stable to hot and cold acid or alkali.

In order to stabilize the low-molecular-weight aminomethanesulfonic acids and at the same time provide the hydrophobic chain necessary for surface-active properties, they were acylated with long chain acid chlorides, such as lauroyl and palmitoyl chlorides (Table II).

Although reasonable yields could be obtained in the acylation of aminomethanesulfonic acid by a

TABLE II
Acylated Aminomethanesulfonic Acids

Aminomethanesulfonic acid	Acylating agent	Product	Yield		Analysis Calc.	Found
			%		%	%
$NH_2CH_2SO_3H$	$C_{11}H_{23}COCl$	$C_{11}H_{23}CONHCH_2SO_3Na$	65[a]	N	4.44	4.42
$NH_2CH_2SO_3H$	$C_{15}H_{31}COCl$	$C_{15}H_{31}CONHCH_2SO_3Na$	40[a]	N	3.78	3.90
$CH_3NHCH_2SO_3H$	$C_{11}H_{23}COCl$	$C_{11}H_{23}CON(CH_3)CH_2SO_3Na$	30	N	4.26	4.17
$C_{12}H_{25}NHCH_2SO_3H$	$(CH_3CO)_2O$	$CH_3CON(C_{12}H_{25})CH_2SO_3Na$	50	N	4.08	3.96
$C_{16}H_{33}NHCH_2SO_3H$	$(CH_3CO)_2O$	$CH_3CON(C_{16}H_{33})CH_2SO_3Na$	70	N	3.51	3.47

[a] Using method I (experimental section); with coco fatty acid chlorides and method II, 90% yield of the acylated product was obtained.

Schotten-Bauman procedure in the presence of sodium carbonate, diffculties were encountered in the acylation of N-methylaminomethanesulfonic acid. Because of this and the potential commercial importance of the acylated aminomethanesulfonic acid a more detailed study of the acylation conditions was undertaken.

For investigation of the effect of pH, benzoyl chloride was chosen instead of a fatty acid chloride since it simplified the analytical problem. Figure 2 presents the data of the acylation conducted in the pH range 4–8.

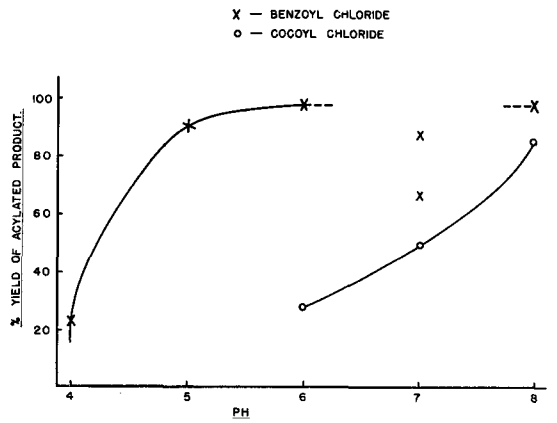

FIG. 2. Acylation of aminomethanesulfonic acid at different acidities.

Since it would be practically desirable to conduct the acylation of aminomethanesulfonic acid without the necessity of its isolation, the benzoylation of ammonium sulfate, which would be present, was also studied throughout this region. These data are presented in Figure 3.

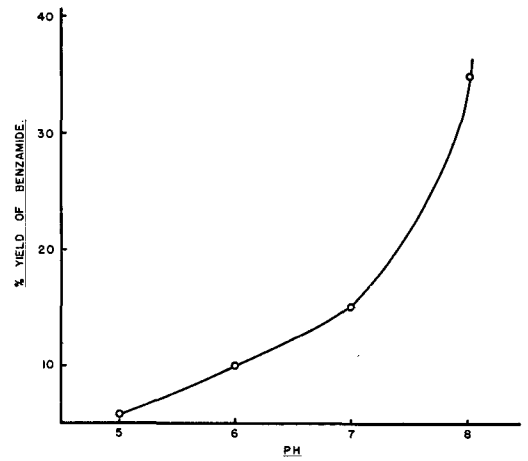

FIG. 3. Benzoylation of ammonium sulfate at different acidities.

Comparing both curves, it is seen that pH 6 is the optimum. A possible explanation of the peculiar behavior in the benzoylation of aminomethanesulfonic acid at pH 7 may be that not only is the decomposition of the sulfonic acid greatest here (Fig. 1) but that also the rate of its acylation relative to that of ammonia is very low at this pH.

When cocoyl chloride was substituted for benzoyl chloride, optimum yield was obtained in the vicinity of pH 8. In contrast to benzoyl chloride this eliminates consideration of acylating in the presence of ammonia for, as indicated by Figure 3, excessive amide would result.

In view of the instability of aminomethanesulfonic acid, it would be presumed that temperature would be important. It was found necessary to operate between 0° and 5°C. to obtain the best yield of acylated product.

Several typical acyl aminomethanesulfonates were compared with Igepon T in pour foam and detergency tests in soft and hard water to obtain some idea of their merit as surface-active compounds. Since Igepon T was a commercial product containing 65% of salt, an equal quantity of salt was added to the pure compounds in these tests. Sodium N-palmitoylaminomethanesulfonate could not be examined because of its insolubility in water.

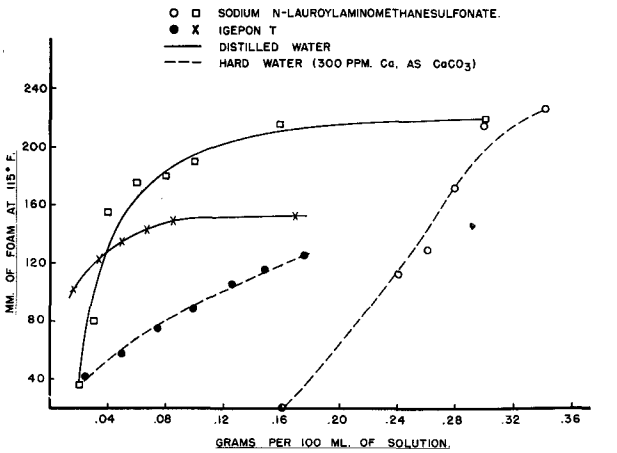

FIG. 4. Pour foam comparison of sodium N-lauroylaminomethanesulfonate with Igepon T at 46°C.

Figure 4 demonstrates that although the pour foam property of sodium N-lauroylaminomethanesulfonate is generally superior to that of Igepon T in soft water, the reverse is true in hard water at reasonably low concentrations. Sodium N-acetyllaurylaminomethanesulfonate is generally better in pour foam than the cetyl homolog, and both are decidedly better than Igepon T or sodium N-lauroylaminomethanesulfonate in both hard and soft water (Figure 5).

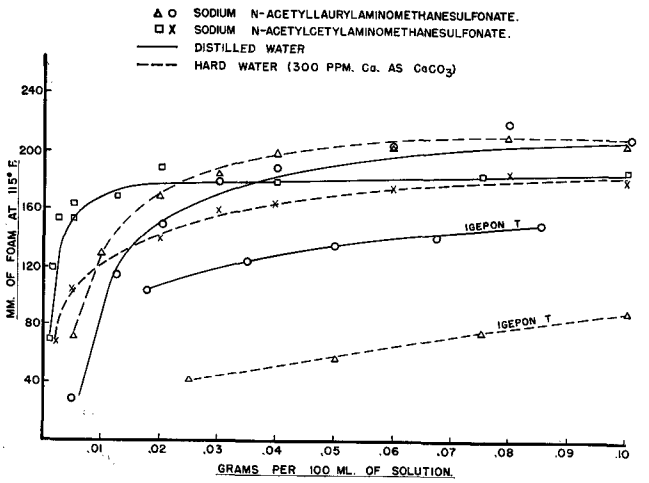

FIG. 5. Pour foam comparison of sodium N-acetyllauryl- and sodium N-acetylcetylaminomethanesulfonates with Igepon T at 46°C.

The soil-removal data in soft water of Figure 6 indicate that only sodium N-acetylcetylaminomethanesulfonate is on a par with Igepon T. In hard water however (Figure 7) it is less efficient than Igepon T although still superior to the other two aminomethanesulfonates.

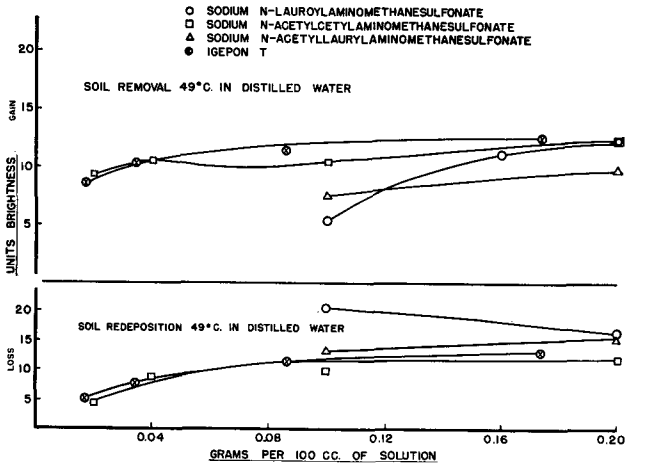

FIG. 6. Soil removal and redeposition in distilled water at 49°C.

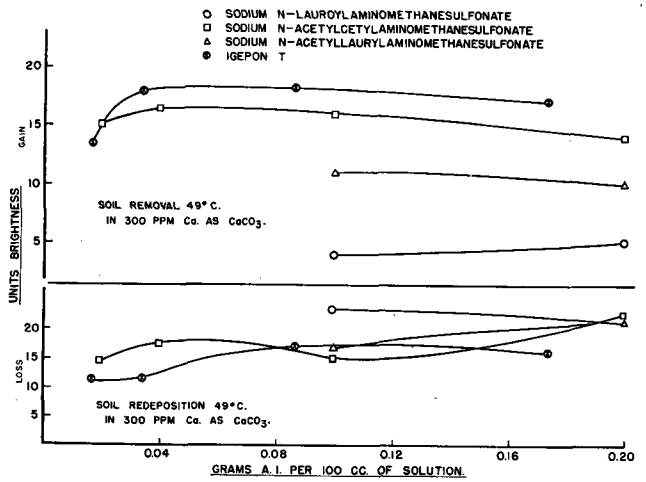

FIG. 7. Soil removal and redeposition in 300 p.p.m. hard water at 49°C.

In soil redeposition the general superiority of the aminomethanesulfonates to Igepon T in both hard and soft water is self-evident (Figures 6 and 7).

It may thus be concluded that sodium N-acetylcetylaminomethanesulfonate is the best of the acyl aminomethanesulfonates examined. With respect to Igepon T it has better foaming properties although its over-all detergency is not as good.

Experimental

Preparation of Aminomethanesulfonic Acids

1-Aminoethanesulfonic and N-isopropylaminomethanesulfonic acids were prepared according to the method of Raschig and Prahl (8).

Aminomethanesulfonic acid was prepared according to the improved method. Ten hundred forty grams of sodium bisulfite (10.0 moles) were dissolved in 2,000 ml. of water, and sulfur dioxide was bubbled through the solution until the yellow-green color, characteristic of the bisulfite ion, appeared. Then 752 ml. of 37% formaldehyde solution (10.0 moles) were added with stirring and cooling. When the reaction had gone to completion, as was evidenced by no further temperature rise, the solution was cooled to 0°C., and 1,360 ml. of 30% ammonium hydroxide solution (10.5 moles) were added rapidly. The solution was stirred two hours at 0°C. and was then added to 1,800 ml. of previously cooled 1:1 sulfuric acid solution at a rate such that the temperature of the reaction mixture did not exceed 5°C. The mixture was then allowed to rise gradually to room temperature in one hour in order to dissolve any salts that had precipitated with aminomethanesulfonic acid. The product was collected by filtration, washed with 3A ethanol, and vacuum dried. One thousand grams of product were obtained, which contained 4.0% water. It assayed 93.2% as determined by titration with sodium hydroxide.

N-methylaminomethanesulfonic acid was prepared by a modification of the Hartough procedure (3). Five hundred seventeen grams of 37% aqueous formaldehyde solution (6.37 moles) and 495 grams of 40% aqueous methylamine solution (6.37 moles) were combined, and sulfur dioxide was passed through the mixture until no more precipitation occurred and the temperature showed no tendency to rise. The rate of addition of sulfur dioxide was adjusted to maintain the reaction temperature at 25°C. while the reaction vessel was immersed in an ice bath. The crystals of N-methylaminomethanesulfonic acid were separated by filtration, washed with 3A ethanol, and dried under vacuum.

N-cyclohexylaminomethanesulfonic acid was made by another modification of the Hartough method. Formaldehyde (0.2 mole, 15 ml. of 37% solution) was dissolved in 100 ml. of dioxane. The solution was cooled below 15°C., and cyclohexylamine (0.2 mole, 19.8 g.) was slowly added with stirring at a temperature of 0–15°C. Sulfur dioxide was then slowly bubbled into the solution below 20°C. as N-cyclohexylaminomethanesulfonic acid precipitated out of solution. An additional volume of solvent, 150 ml. of dioxane — H_2O (2:1), was added, and the solution was again saturated with sulfur dioxide. After standing one-half hour, the mixture was filtered through sintered glass, and the crystalline product was air-dried on the filter.

Higher-Molecular-Weight N-Alkylaminomethanesulfonic Acids

Sodium Hydroxymethanesulfonate. Two hundred and five grams (2 moles) of sodium bisulfite were dissolved in 300 ml. of water, and 160 ml. of 37% formaldehyde (2 moles) were added. The mixture became warm, and, after standing one-half hour, the solution was poured into 1.5 liters of 3A alcohol. The produce separated as a second phase, which gradually crystallized. These crystals were collected on a suction funnel, washed with ethanol, and dried over calcium chloride in vacuum; yield was 259 g. The product was analyzed iodimetrically (14) and was found to contain 86.5% sodium hydroxymethanesulfonate monohydrate, corresponding to a yield of 73.5%.

N-Alkylaminomethanesulfonic Acids. To 120 ml. of 40% 3A ethanol were added 45 g. (0.25 mole) of 86.5% sodium hydroxymethanesulfonate monohydrate with the aid of some heat to obtain a saturated solu-

tion. After cooling to room temperature, 0.25 mole of fatty amine was added with stirring. A fairly homogeneous solution resulted, with heat evolution, which, after a short time, set into a plastic mass. After several hours it was separated on a Buchner funnel washed with 3A alcohol and dried in vacuum over calcium chloride. If desired, the product could be converted to the zwitterion by acidifying with mineral acid to pH 4. In this form it could be recrystallized from 3A ethanol.

Acylation of Aminomethanesulfonic Acids

Sodium N-Lauroylaminomethanesulfonate and Sodium N-Palmitoylaminomethanesulfonate, Method I. One-half mole of aminomethanesulfonic acid was dissolved in 800 ml. of water. Fifty-three grams (0.5 mole) of sodium carbonate were added, and this was followed by the addition of 0.5 mole of fatty acid chloride with vigorous agitation. The reaction proceeded with an evolution of carbon dioxide, and any foaming difficulties were countered by the addition of a little ether. The reaction mixture was stirred for three to four hrs. at room temperature, acidified to Congo red, and extracted with petroleum ether to remove fatty acids and amides. The inorganic salts were then separated by crystallizing the crude product from 80% 3A ethanol.

Sodium N-Lauroyl-N-Methylaminomethanesulfonate, Method II. N-methylaminomethanesulfonic acid (0.56 mole) was dissolved in 600 ml. of water in a vessel equipped with pH electrodes. After conversion to the sodium salt with 10% aqueous sodium hydroxide, 0.53 mole of lauroyl chloride was added simultaneously with 10% aqueous sodium hydroxide to maintain the pH at 7.3–7.8, employing vigorous agitation and keeping the temperature at 0°C. Upon completion of the reaction as evidenced by constancy of pH, the solution was acidified to pH 3 and dried (steam rolls were used). The dried product was extracted in the Soxhlet with petroleum ether to remove lauric acid and any N-methyllauramide and was finally recrystallized from 80% ethanol.

Acetylated Higher Alkylaminomethanesulfonates. To a solution of 30 ml. (0.3 mole) of acetic anhydride in 150 ml. pyridine there was added, with stirring, 0.2 mole of the fatty aminomethanesulfonate. After stirring for 2 hrs. at room temperature, the reaction mixture was heated for one hour on the steam bath. An almost homogeneous solution was obtained, which was filtered. The desired crude product was precipitated by the addition of anhydrous acetone (nitromethane was also found effective) and separated on the Buchner funnel. It was recrystallized from 99% isopropyl alcohol.

Acylation of Aminomethanesulfonic Acid or Ammonium Sulfate at Varying Conditions of Temperature and pH

A three hundred-ml., three-neck, round-bottom flask was fitted with electrodes and a mechanical stirrer. Provision was made for temperature control of the reaction by means of a water bath and for simultaneous addition of the acyl chloride and alkali. To a well agitated, aqueous solution of 0.05 mole of ammonium sulfate or aminomethanesulfonic acid, which had been adjusted to the desired temperature, sufficient 20% alkali was added to attain the desired pH. The rate of addition of acid chloride and alkali was adjusted so that the same pH and temperature were maintained during the reaction.

When all of the acid chloride had been added and the pH was constant, the reaction was complete. The mixture was then worked up by either of the following methods to determine the extent of reaction.

a) *Benzoyl Chloride-Ammonium Sulfate Reaction.* The reaction mixture was acidified, and the solid product was collected on a suction filter. After the solid had been dried, it was titrated with standard alkali. The amount of alkali consumed was a measure of the amount of benzoic acid and, by difference, the extent of reaction of benzoyl chloride with ammonia to form benzamide.

b) *Benzoyl Chloride-Aminomethanesulfonic Acid Reaction.* The reaction mixture was acidified and the insoluble material was collected, dried, and weighed. This was a measure of the extent of undesirable side-reactions; the the benzoylated aminomethanesulfonic acid is soluble in the aqueous medium.

c) *Fatty Acid Chloride-Aminomethanesulfonic Acid Reaction.* After the reaction mixture was acidified to pH 3, it was extracted several times with petroleum ether; alcohol was added as necessary to break any emulsions. The combined ether extracts were back-washed with 50% ethanol, and, after drying, the solvent was evaporated. The weight of the petroleum ether solubles is a measure of the acid chloride which did not react with the aminomethanesulfonic acid.

Stability of Aminomethanesulfonic Acid or a Function of pH at 25°C. Twenty ml. of 2% solutions of aminomethanesulfonic acid were made up in the following buffers: citric acid + disodium acid phosphate, pH 5–8; borax, pH 9; borax-carbonate, pH 10. Two-ml. aliquots of test solution were withdrawn with time, and, after acidifying in 15 ml. of 5% sulfuric acid, the solutions were titrated with 0.1 N iodine.

Surface-Active Tests

Pour Foam. The Ross and Miles method (10) at 46°C. was used. Hard water was made up with the necessary quantity of calcium chloride in distilled water; concentrations are expressed in terms of calcium carbonate.

Igepon T, $RCON(CH_3)C_2H_4SO_3Na$, RCO is oleoyl. This was a commercial sample from the General Aniline and Film Corporation, assaying 34% active. Appropriate concentrations for the pour foam test were made by diluting with the requisite amount of water at 46°C.

The acylaminomethanesulfonates were adjusted to 40% active ingredient with sodium sulfate. These samples were then diluted with water at 46°C. to achieve the desired concentration.

The hard-water solutions were aged at 46°C. for 10 min. before testing to ensure equilibrium.

The foaming properties in all cases were found relatively insensitive to pH.

Detergency—Soil Removal and Redeposition. The detergency values, unit brightness gain, were obtained at various concentrations. A Terg-O-Tometer type instrument was used under the following conditions: speed, 130 oscillations per minute; time, 20 min.; temperature, 49°C.; solution volume, 1 liter; cotton swatches, 30 1¾-in. squares, oildag soiled and six unsoiled 1¾-in. squares. On completion of washing, the test pieces were mildly rinsed by hand in lukewarm water and air dried. The test cloths were measured for reflectance on a Hunter Multi-Purpose Reflectometer with a green filter.

Summary

The preparation and properties of aminomethanesulfonic acid and several of its lower and higher N-alkyl derivatives have been studied. The acylation

of these compounds was investigated, and the optimum conditions were determined. Measurements of the surface-active properties, including pour foam, soil removal, and redeposition, of several of the acylated derivatives are described, with reference to Igepon T.

Acknowledgment

The authors are grateful for the analyses performed by the Analytical Division. Special thanks are due to Conrad Jakob of the Physical Chemistry Division for the surface-active measurements.

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[Received August 12, 1957]

Rancidity as a Factor in the Loss of Viability of Pine and Other Seeds

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THREE YEARS AGO the Southern Forest Experiment Station of the United States Forest Service and our laboratory started experiments on the preservation of pine seeds. Pine reforestation has become an important project of the U.S.D.A. Pine trees under Louisiana's conditions produce a normal crop of seeds at irregular intervals; the necessity therefore of storing the seeds of high yield years in order to meet the needs for seeds during the light crop years is apparent. Our experiments in this field deal not only with the preservation of seeds at various temperatures, dehydrofreezing techniques, and storage under an inert gas but also extend to a more fundamental study of the viability process in general, the development of rapid color methods for measuring viability using triphenyl tetrazolium chloride, and the development of treatments which might improve germinability. We thought also that since pine seeds are relatively rich in oil, the rancidity of the fat of the seeds might have something to do with the loss of viability.

The effect of rancidity of the fat on the vitamins and on other oxidizable compounds of foods is a well established fact (17). That vitamins together with other substances are important factors in conditioning germination of pollen (3), rice (16), and other seeds has also been shown by various experiments. To suppose therefore that a connection might exist in seeds rich in fat between its rancidity and the germinability of the seeds is quite a logical hypothesis. To ascertain however such a relationship on a strict cause-and-effect basis, if the hypothesis is correct, is not quite such a simple matter, considering our present limited knowledge concerning the various phases of rancidity development and the uncertainty of methods for measuring it (10). Our experiments on the study of germination of various seeds, *viz.*, rancidity development in the fat, indicate that a connection between the two does exist; but because of the complexity of factors determining viability and the variability of the individual seeds it is rather difficult to establish a strict correlation between the two in a preliminary experiment with a limited number of tests.

The data of Tables I and II show that seeds of high germinability have no actual rancidity in their fat and as the viability decreases, so the rancidity (peroxide value) increases more or less steadily. This correlation between viability as determined by sand flat germination tests and color tests does not hold well, especially in the low range of viability values. It seems rather certain that rancidity development is one among several other factors contributing to the loss of viability, such as protein coagulation, degeneration of oxidizing and digestive enzymes, exhaustion of stored foods, cell poisoning, etc. (2), which under certain conditions of storage undergo changes independently of rancidity development. Such might

TABLE 1
Rancidity and Other Fat Changes During the Gradual Loss of Germinability in Longleaf Pine Seeds

Storage conditions	Moisture	Fat (dry basis)	Germination (sand flat germ. test)	Free fatty acids	Peroxide value	Iodine number (Hanus)
	%	%	%	%	*mM/Kg.*[a]	
Fresh seeds (partially dried in air)	14.49	25.09	80	0.28	0.0	—
Fresh seeds (partially dried in air)	10.90	25.43	75	—	0.0	147.0
Seeds stored at 25°F. in burlap bag for 2 years	12.93	23.43	70	2.31	9.5	146.6
Seeds stored at 45°F. for 1 year in jars	13.96	23.08	48	4.51	25.0	—
Seeds stored in jars at room temp. for 1 year	6.53	23.72	18	2.82	70.0	—
Seeds stored in jars at room temp. for 2 years	7.96	24.50	0.0	2.54	130.0	141.7
Seeds stored in jars for 2 yrs. at 45°F	14.60	23.96	0.0	2.61	—	143.8
Seeds stored at 45°F. in CO_2 + ethylene oxide for 1 year	13.86	24.90	0.0	2.68	105.0	—
Seeds stored in CO_2 + ethylene oxide at room temp. for 1 year	11.29	24.60	5.0	1.97	55.0	—

[a] mM = millimoles.