[Contribution from the Socony-Vacuum Laboratories (A Division of Socony-Vacuum Oil Co.)]

Aminomethylation of Thiophene. V. The 2-Thenylaminomethylsulfonic Acids. The Mechanism of the Aminomethylation Reaction

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Introduction

Prior articles in this series[1–4] have dealt with reaction factors and intermediates in the aminomethylation of thiophene, I, and its derivatives with formaldehyde and ammonium chloride.

The addition of sodium bisulfite to the carbonyl group in aldehydes is a well established reaction but its use in typical Mannich reactions is not reported. Eibner[5] has prepared salts of sulfurous acid and the complex reaction products of formaldehyde and aniline and has studied the action of sulfurous acid on Schiff bases. The use of catalysts or inhibitors for the Mannich reaction has not been investigated prior to this communication, although acetic acid was shown previously to have a direct influence on the formation of di-(2-thenyl)-amine.[3]

This communication deals with the addition of sulfurous acid to Schiff bases of the type $-N{=}CH_2$, derived from thenylamines. In addition, evidence for catalysis (by sulfurous acid) and inhibition (by alcohols) of the Mannich reaction is reported. The mechanisms involved and some general observations on the Mannich reaction are discussed.

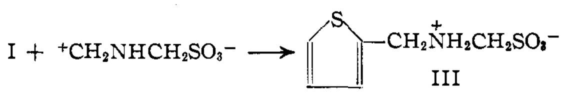
Discussion of New Results

Sulfurous acid catalyzes the aminomethylation reaction of thiophene with formaldehyde and ammonium chloride. Evidence of catalysis was obtained by causing the reaction of thiophene, formaldehyde and ammonium chloride to proceed rapidly at ambient temperatures with evolution of heat and high conversions of thiophene to resins. Previously, it was reported that reaction temperatures of 70–75° with high ratios of formaldehyde to thiophene were necessary to produce resins from this reaction.[1] It was noted in prior work that acetic acid, when used as a solvent or as a catalyst, materially increased the reaction rate for the aminomethylation with thiophene and changed the product distribution.[1,3] However, it does not appear to have entered into the reaction in a manner comparable to sulfurous acid. Other acids such as orthophosphoric acid catalyze this reaction to a considerable extent but they have not been as fully investigated as sulfurous acid.

A stepwise investigation of this reaction revealed that aminomethylsulfonic acid,[6] II, is formed when sulfur dioxide is bubbled into an aqueous formaldehyde solution containing ammonia or ammonium chloride. II reacts further with thiophene and formaldehyde to give 2-thenylaminomethylsulfonic acid, III. A possible course of the reaction involves the addition to the hypothetical Schiff base, $CH_2{=}NH$, subsequent formation of the hypothetical $CH_2{=}NCH_2SO_3H$, and addition to thiophene as represented below.

$$CH_2{=}NH + H_2SO_3 \longrightarrow \underset{\text{II}}{{}^+NH_3CH_2SO_3{}^-}$$

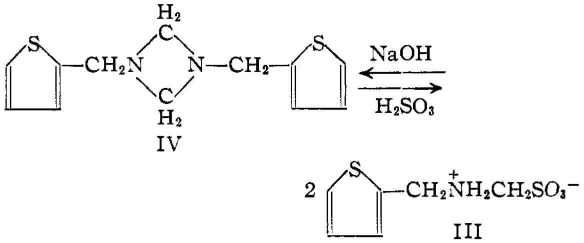
$$\text{II} + CH_2O \rightleftarrows CH_2{=}N{-}CH_2SO_3H \longrightarrow {}^+CH_2NHCH_2SO_3{}^-$$



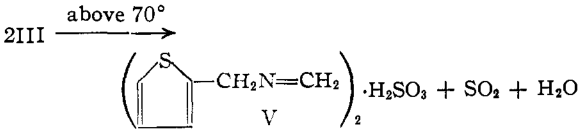
2-Thenylaminomethylsulfonic acid, III, is isolated as a crystalline water-insoluble product by controlling the reaction temperature below 60°.

This reaction appears to be general to alkylthiophenes. In addition, a 5-chloro-2-thenylaminomethylsulfonic acid has been prepared (see Table I). III or its analogs are isolated when sulfur dioxide or sodium bisulfite is introduced into the reaction mixture of thiophene, aqueous formaldehyde and ammonium chloride.

III, when treated with sodium hydroxide, gives N,N′-di-(2-thenyl)-tetrahydro-1,3-diazacyclobutane, IV. This reaction is reversible and IV with sulfurous acid yields III.



In alcoholic or aqueous solutions, III is unstable to heat and loses water and sulfur dioxide in a manner similar to ammonium bisulfite.



2-Thenylformaldine sulfite

Continued heating of V in water or alcohols causes polymerization of the basic unit 2-thenylformaldi-

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(1) Hartough, Lukasiewicz and Murray, This Journal, **70**, 1146 (1948).

(2) Hartough, Meisel, Koft and Schick, *ibid.*, **70**, 4013 (1948).

(3) Hartough and Meisel, *ibid.*, **70**, 4018 (1948).

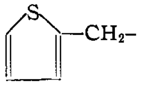
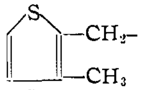
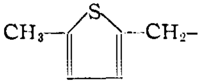
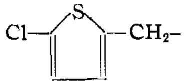
(4) Hartough and Dickert, *ibid.*, **71**, 3922 (1949).

(5) Eibner, *Ann.*, **316**, 89 (1901).

(6) Aminomethylsulfonic acid is an internal salt difficultly soluble in water. It has been prepared previously by the action of ammonia on $HOCH_2SO_3Na$ followed by acidification; see Backer and Mulder, *Rec. trav. chim.*, **52**, 454 (1933); **53**, 1120 (1934); Reinking, Dehnel and Labhardt, *Ber.*, **38**, 1069 (1905).

TABLE I

AMINOMETHYLSULFONIC ACIDS, $R—\overset{+}{N}H_2—CH_2SO_3^-$

R	Yield, %	Decomposition point, °C.	Formula	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
H-	83	183–184	CH_5NO_3S[a]	12.61	12.23	28.8	28.3
CH_3-	[b]	165.5–167.5	$C_2H_7NO_3S$	11.20	11.13	25.6	25.7
	95	135–136	$C_6H_9NO_3S_2$[c]	6.71	6.65	30.9	31.0
	90	140–141[d]	$C_7H_{11}NO_3S_2$	6.34	6.49	29.0	28.0
	85	138–140	$C_7H_{11}NO_3S_2$	6.34	6.65	29.0	29.2
		141–142	$C_6H_8ClNO_3S_2$	5.79	6.06	26.5	26.3
tt-Octyl-2-thenyl-	64	140–142	$C_{14}H_{25}NO_3S_2$	4.39	4.58	20.1	20.2

[a] Calcd.: C, 10.72; H, 4.50. Found: C, 11.20; H, 4.61. [b] Not determined. [c] Calcd.: neut. equiv., 216. Found: neut. equiv., 206. [d] Turns brown at 137–139°.

mine. Pure white resins, VI, are then obtained by treating the aqueous solution with sodium hydroxide.

The effect of temperature on the end-products of the aminomethylation reaction has led to some rather interesting results when sulfur dioxide is introduced as a catalyst in the reaction of I with formaldehyde and ammonium chloride. At 25–30°, both the dimer, IV, and III were formed (III being formed in amounts corresponding to the sulfurous acid used). At 45° only the dimer, IV, and higher polymers were obtained. At 55° only di-(2-thenyl)-amine, VII, could be isolated along with higher polymers. At 70°, 2-thenylamine, VIII, and VII are obtained along with higher polymers. When *t*-butylthiophene was substituted for thiophene, it was noted that a trimer of N-(*t*-butyl-2-thenyl)-formaldimine formed at 70° to the exclusion of the tri-(*t*-butyl-2-thenyl)-amine reported in an earlier work.[1]

The nitrogen–sulfur ratios of the resins, VI, can be varied by adjusting the molecular ratios of the reactants. Analyses of some of these resins are listed in Table II. Sodium bisulfite was used as a source of sulfurous acid in these reactions. The physical characteristics of VI vary from white, putty-like thermoplastic masses to white, infusible powders. The infusible powders are not listed in Table II due to attendant difficulties in removing inorganic salts to obtain accurate analyses. They are best obtained from high ratios of formaldehyde to thiophene, usually greater than 2:1, and long heating periods above 70°.

The ease of formation of resins, VI, when sulfurous acid is employed in the aminoalkylation reaction would appear to involve a direct polymerization of the $-N{=}CH_2$ moiety or a reaction of this material with the unsubstituted 5-position of thiophene. Resin formation occurs when this position is blocked by a methyl group but more slowly than in the case of thiophene itself. III does not hydrolyze to 2-thiophenealdehyde at a *p*H of 3 to 6 in a manner similar to IV by the method previously described.[4] This suggests that the tendency of the $-N{=}CH_2$ unit to polymerize is augmented by the sulfite ion in the reaction mixture even at *p*H values as high as 4 and the prototropic shift required to produce 2-thiophenealdehyde by a simple Sommelet mechanism is inhibited. VI redissolves in excess hydrochloric acid with the evolution of formaldehyde. Steam distillation of this mixture yields 1–2% of 2-thiophenealdehyde. These properties are characteristic of methylene linkages between amines. The following structures, IX and X, have been considered and our observations would indicate that both are present.

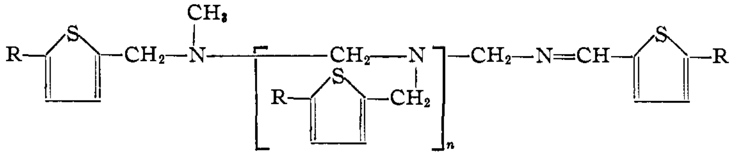
TABLE II

RESINOUS AMINOALKYLATION PRODUCTS USING SODIUM BISULFITE CATALYST WITH AQUEOUS FORMALDEHYDE

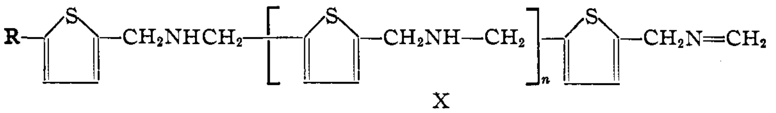
Moles of reactants C_4H_4S	CH_2O	NH_4Cl	$NaHSO_3$	Product, g. Soluble	Product, g. Insoluble	Analyses of benzene insolubles, % Nitrogen	Analyses of benzene insolubles, % Sulfur
5[a]	5	5	1	226[b]	80	...	...
9[c]	15	15	6	60	900	13.55	20.9
9[d]	15	15	3	55[e]	800	8.6	20.9
2	4	2	2	5[f]	24[f]	9.81	20.1
6	3	6	2	9[f]	24[f]	12.7	18.9
5	10	5	[g]	14[f]	18[f]	7.24	25.0
5	20	5	4	9[f]	18[f]	7.11	21.1

[a] 365 g. of thiophene recovered. [b] Contained 23 g. of 2-thenylamine and di-(2-thenyl)-amine. [c] 300 g. of thiophene recovered. [d] 370 g. of thiophene recovered. [e] Obtained by steam distillation. [f] From 50-g. aliquot. [g] Sulfur dioxide gas bubbled in at a rate of 400 cc. per minute throughout reaction period.

Structure IX appears to be correct where R is other than a hydrogen unless the 3,4-positions enter into the reaction. In the case where R is a hydrogen, polymerization may take place in the same manner or through the active 5-position and the linear type resin, X, may form. X could then react with excess formaldehyde in dilute acid or



IX



X

caustic to produce cross-linkages which would evolve formaldehyde upon treatment with strong acid. It has been noted that sub-resinous products of this type can be further resinified with formaldehyde in alkaline solutions and can be depolymerized again with acid solutions. Due to insolubility of these resins in all common solvents and their tendency to evolve formaldehyde in aqueous acid solutions, no molecular weight studies have been made.

Sulfurous acid (from sodium bisulfite) does not appear to exert a catalytic effect upon the aminomethylation reaction when anhydrous polymeric formaldehyde is substituted for the usual commercial aqueous formaldehyde containing from 1 to 6% methanol. Instead of resins, VI, sub-resinous products soluble in benzene and similar to those described in a prior work[7] were obtained. Table III lists the analyses and molecular weights of several of these sub-resinous amines.

The small amounts of methanol (1–6%) contained in commercial formaldehyde appear to exert a desirable inhibitory effect upon the aminomethylation reaction. This tendency of methanol to inhibit the aminomethylation of thiophene has only been studied in a superficial manner. Aqueous 30% methanol-free formaldehyde reacts much faster than commercial 36% formaldehyde, the yield of IV is substantially lower (about 35% as compared to 56%) and the amount of polymer of VI is increased. If methanol is introduced into an aminomethylation reaction while it is proceeding at 68°, the reaction is immediately stopped. Attempts to use ethyl alcohol and butyl alcohol as solvents with polymeric formaldehyde have led to almost quantitative yields of the corresponding formals to the total exclusion of the aminomethylation reaction.

Generalizations Concerning the Aminomethylation Reaction

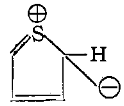
Inasmuch as our work in this field is now complete, it seems pertinent to set down several generalizations concerning the aminomethylation reaction that have become apparent during the course of several hundred experiments devoted to this subject. Other portions of this work have been summarized in prior publications.[1–4,8]

There can be little question that the aminomethylation reaction is a type of Mannich reaction. According to Blicke, the mechanism of the Mannich reaction has not been established.[9]

It is the purpose of this section to discuss the probable course of this reaction.[10]

It was previously reported[1] that the only ammonium salts to undergo the aminomethylation reaction with thiophene were the ammonium halides. Primary amines react only to a very limited extent and secondary amines do not react at all. These facts are specific to thiophene. It has been observed in these laboratories that phenol (or acetone) reacts with formaldehyde and secondary amines or with ammonium sulfate or bisulfate. The products from the latter ammonium salts are similar to those obtained from ammonium chloride. Further, it has been found that thiophene could not be aminomethylated at a *p*H above 3 but the reaction proceeded rapidly at *p*H of 1 to 2. Since ketones and other reactive compounds such as phenol are known to undergo this reaction at a *p*H above 7, it would appear that two fundamental mechanisms are involved; the first an acid-catalyzed reaction, involving hydrohalide salts of ammonia and primary amines; the second, a reaction involving secondary amine salts or reactions of ammonia, primary or secondary amines as the free bases.

In the first reaction, *i. e.*, the acid-catalyzed reaction, let us consider the case of thiophene in its most commonly represented resonating form as



XI

and the reaction of formaldehyde and ammonium chloride to take place in the following manner

$$CH_2O + NH_4Cl \rightleftharpoons CH_2{=}NH + HCl + H_2O$$

$$CH_2{=}NH \overset{H^+}{\rightleftharpoons} {}^+CH_2NH_2$$

XII

The carbonium ion, XII, would then attach itself to XI and expel the proton which would then combine with the nitrogen to form 2-thenylamine hydrochloride. This reaction was more graphically demonstrated with hydroxylamine hydrochloride

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(7) Reference 1 describes formation of sub-resinous amines quite similar to these materials in appearance, analysis and molecular weight, but obtained from aqueous formaldehyde instead of polymeric formaldehyde. Molecular weight determinations were carried out in benzene solution.

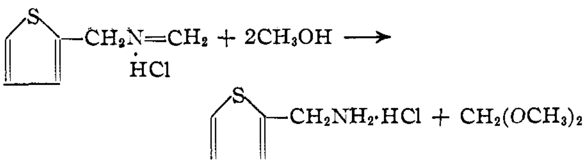
(8) Hartough, THIS JOURNAL, **69**, 1355 (1947).

(9) Blicke, "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 306, summarizes the prior attempts to establish this mechanism.

(10) An acid-base catalysis mechanism for the Mannich reaction was disclosed by Lieberman and Wagner, Abstracts of the Philadelphia Meeting-in-Miniature, Jan. 20, 1949, p. 49, prior to submission of this paper.

and formaldehyde with thiophene[8] wherein it was found that if the reaction was carried out stepwise with formation of the formaldoxime in acid solution using an excess of hydroxylamine hydrochloride much higher yields of 2-thenylhydroxylamine hydrochloride were obtained. Since formaldehyde and hydroxylamine are known to form formaldoxime ($CH_2{=}NOH$), this fact suggests that the oxime is an actual intermediate in the formation of 2-thenylhydroxylamine. In the aminomethylation reaction, formaldimine is an analogous species.

It was shown in a prior paper[3] that the $=CH_2$ group of the N-(2-thenyl)-formaldimine monomer could be cleaved easily with methanol in acid solution to yield the 2-thenylamine.



It has also been shown in these laboratories that butanol when used as a solvent for the aminomethylation reaction gives a high yield of di-(*n*-butyl)-formal. Reasoning on analogy with the reaction above it would appear that formation of XII takes place and interaction with the butanol yields the formal and regenerates the ammonium chloride.

$$\text{XII} + 2CH_3CH_2CH_2CH_2OH \xrightarrow{HCl} (CH_3CH_2CH_2CH_2O)_2CH_2 + NH_4Cl$$

Our superficial study of the inhibition of the aminomethylation reaction of thiophene with methanol indicated a very rapid reaction of methanol with XII which immediately stopped a reaction that was proceeding between thiophene, ammonium chloride and formaldehyde.

It was further noted that ammonium bisulfite and formaldehyde reacted when placed together in solution in a manner quite similar to ammonium chloride and formaldehyde, *i. e.*, the *p*H immediately falls to about 1 upon mixing the two solutions. By analogy, with ammonium chloride one would expect to obtain aminomethylsulfonic acid, II, from a recombination of the $CH_2{=}NH$ and sulfurous acid, but this does not take place. Addition of hydrochloric acid to this solution gave only ammonium chloride rather than the expected II. This suggests that the reaction of ammonium bisulfite and formaldehyde gives a relatively stable methylol derivative, $HOCH_2{-}NH_3{-}SO_3H$, which does not decompose to $CH_2{=}NH$, the intermediate necessary to undergo the addition of sulfurous acid. Additional evidence of the formation of a stable methylol form lies in the fact that ammonium bisulfite fails to undergo the aminomethylation reaction with formaldehyde and thiophene while the aminomethylsulfonic acid reacts at a rate much faster than ammonium chloride. Considering some of the known facts about the ionic nature of inorganic ammonium salts two generalizations may be pointed out. First, ammonium halides are the most highly ionized and consequently more dissociated than ammonium bisulfite thus allowing the ammonium ion to act more or less independently. Second, the inductive effect of the bisulfite ion would also tend to prevent the dehydration of the methylol group. Since the inductive effect of the sulfur exerts itself on the methylene group in aminomethylsulfonic acid (a Zwitterion type compound), dehydration or detachment of the hydroxyl ion would be expected to take place more readily and leave the carbonium ion (as in XII) free to attack the carbanion in the thiophene.

There appears to be little doubt then of the existence of formaldimine, $CH_2{=}NH$, in aqueous acidic solutions. Its existence was predicted in 1917 by Werner[11] but this has caused considerable controversy and the current views as emphasized by Walker[12] tend to favor the methylol type mechanisms in illustrating the formation of methylamines from formaldehyde and ammonium chloride.

There appears to be little question that a great majority of base-catalyzed Mannich reactions with secondary amines proceed through the normal methylol type intermediates. Therefore the authors consider the mechanism postulated by Blicke[9] to be correct when applied to systems having a *p*H of 7 or above or when the salts of dialkylamines are employed.

Experimental[12a]

Preparation of Aminomethylsulfonic Acid. Method A.—Sulfur dioxide was bubbled into an equimolar mixture of formaldehyde (36% aqueous) and ammonium chloride and the temperature controlled at 75–80° by means of an ice-bath. When the amount of sulfur dioxide reached the saturation point the mixture suddenly crystallized. The sulfur dioxide stream was continued until the temperature fell to 50°. After cooling, the crystalline product was removed by filtration, washed with cold water and dried. Yields varied over a series of experiments from 60–70%. Recrystallization of this material from water was necessary in order to obtain an analytical sample free from ammonium chloride, but for further reactions this final purity is not necessary. Aminomethylsulfonic acid is insoluble in cold water, soluble to the extent of about 8 g. in 100 g. of boiling water, and extremely insoluble in alcohol or alcohol–water mixtures.

Method B.—Sulfur dioxide was bubbled into a cooled equimolar mixture of 36% aqueous formaldehyde and commercial 28–30% ammonium hydroxide. The rate of sulfur dioxide was adjusted to maintain the reaction temperature below 50° while the reaction vessel was surrounded by an ice-bath. After saturation of the solution with sulfur dioxide, the pure white crystals were filtered off, washed with cold water and dried. Yields vary from 75–83% at 50°. At higher temperatures the yields fell to about 60%.

Method C.—Hexamethylenetetramine as a 25% aqueous solution was employed instead of ammonia and form-

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(11) Werner, *J. Chem. Soc.*, **111**, 844 (1917).

(12) Walker, "Formaldehyde," A. C. S. Monograph 98, Reinhold Publishing Corp., New York, N. Y., 1944, p. 122.

(12a) All melting points are corrected; boiling points are uncorrected.

aldehyde. Yields ranging from 60–70% were obtained using the techniques of Method B. Two moles of ammonium chloride may also be added to one mole of hexamethylenetetramine to obtain equimolar ratios of ammonia to formaldehyde in the subsequent acid hydrolysis with sulfur dioxide.

Preparation of N-Methylaminomethylsulfonic Acid.—Somewhat lower yields (about 30–40%) were obtained from methylamine by Method B above. The product was recrystallized from water to obtain an analytical sample.

General Methods for the Preparation of 2-Thenylaminomethylsulfonic Acids. Method A.—Into a well-stirred mixture of one mole of thiophene, two moles of 36% formaldehyde and one mole of ammonium chloride was passed a stream of sulfur dioxide gas at a rate of 400 cc. per minute. The temperature was maintained at 25–35° by means of an ice-bath. After one hour, the flow of sulfur dioxide was discontinued and the crystalline product was filtered off and recrystallized from 50% aqueous alcohol three times to obtain an analytical sample. The yields were nearly quantitative.

Method B.—An alternate procedure can be followed if the dimeric or trimeric N-(2-thenyl)-formaldimines are available. This method consists of bubbling sulfur dioxide gas into an aqueous slurry of the formaldimines in water until crystallization is complete. The yields by this method approach the theoretical.

Method C.—2-Thenylamine (55.5 g.) was dissolved in 100 ml. of water and 42.5 g. of 36% formaldehyde was added. A considerable amount of heat was evolved and a white viscous oil separated from the solution. After cooling to room temperature, the mixture was saturated with sulfur dioxide and the crystalline mass allowed to stand twenty-four hours. Filtration yielded 71 g. (69%) of pure white N-(2-thenyl)-aminomethylsulfonic acid. An analytical sample was obtained by a single recrystallization from 50% alcohol.

Method D.—One mole of thiophene (2-, or 3-methyl, 2-chloro- or *tt*-octylthiophene) was mixed with one mole of aqueous formaldehyde, 50–100 ml. of water and one mole of aminomethylsulfonic acid. The well-stirred mixture was heated to 50–60° whereupon a noticeable reaction began and crystallization of the thenylaminomethylsulfonic acid started. In the case of thiophene the entire reaction mixture became solid in about five minutes and addition of more water was necessary to continue stirring. The source of heat was removed and the mixture was allowed to cool to room temperature before filtering. The crystalline material was dried and analytical samples were prepared by crystallization in the following manner. Fifty grams of absolute alcohol was brought to a vigorous boil and 5 g. of the crude sulfonic acid was added. The mixture was vigorously stirred for a few seconds with a glass spatula and quickly filtered into an ice-cooled flask. The crystalline material that separated was analytically pure. The unreacted aminomethylsulfonic acid was totally insoluble in boiling alcohol. 2-Thenylaminomethylsulfonic acid cannot be purified in this manner due to its very low solubility in boiling alcohol. Instead, the product that separated from alcohol in trace amounts is the di-(N-(2-thenyl)-formaldimine) sulfite, m. p. 135–136° with decomposition.

Anal. Calcd. for $C_{12}H_{16}N_2O_3S_3$: N, 8.43; S, 28.92. Found: N, 8.68; S, 29.05.

Continued warming of alcoholic or aqueous solutions of these thenylaminomethylsulfonic acids completely converted them to water-soluble sulfite salts, which when treated with sodium hydroxide yielded the corresponding (2-thenyl)-formaldimines. For example, treatment of the 5-methyl-2-thenylaminomethylsulfonic acid with excess caustic gave a yellow oil that dissolved in ether and crystallized to the trimeric formaldimine, tris-N,N′,N″-(5-methyl-2-thenyl)-hexahydro-1,3,5-triazine. After two recrystallizations from absolute alcohol the product melted at 87–88°. A mixed melting point with an authentic sample[2] gave no depression. This same crystalline trimeric formaldimine was isolated in low yields (15%) by neutralization of the filtrate from the reaction mixture. Dimeric formaldimines from thiophene, 3-methylthiophene and 2-chlorothiophene are also present in small amounts in the filtrates of the corresponding reaction systems as the sulfite salts.

Preparation of N,N′-Di-(2-thenyl)-tetrahydro-1,3-diazacyclobutane, IV.—To ten moles each of thiophene and ammonium chloride was added twenty moles of 36% formaldehyde. Into this well-stirred mixture, maintained at 35° by means of an ice-bath, was introduced 200 cc. of sulfur dioxide per minute for a period of two and one-half hours. The crystalline III (135 g.) was filtered off and the aqueous reaction mixture was neutralized with 40% sodium hydroxide. The oil was taken up in benzene. The benzene and any dissolved water was removed by atmospheric distillation and the residue was distilled *in vacuo* in the apparatus previously described[2] to give 462 g. (36%) of VI, b. p. 119–146° at 2 mm.[13] The refractive index varied from 1.6144 to 1.6162 throughout that range. A polymeric residue of 245 g. remained in the still.

If the reaction mixture is maintained at 70° during the addition of the sulfur dioxide and the time is shortened to one hour, the distillate consists of 2-thenylamine (20%) and di-(2-thenyl)-amine (15%). The former was identified as the phenylthiourea, m. p. 123.5–124°,[14] and the latter as the hydrochloride, m. p. 252–253°.[15]

Tris-N,N′,N″-(*t*-butyl-2-thenyl)-hexahydro-1,3,5-triazine.—In a manner similar to that described above, sulfur dioxide was passed into a mixture of 0.5 mole each of *t*-butylthiophene and ammonium chloride and one mole of 36% formaldehyde at 70°. After cooling the reaction mixture, 68 g. of crude *t*-butyl-2-thenylaminomethylsulfonic acid was filtered off; 31 g. of unreacted *t*-butylthiophene was recovered from the filtrate. Neutralization of the filtrate with caustic gave only 1 g. of an oily amine. The crude crystalline sulfonic acid was digested with excess sodium hydroxide and the resultant insoluble oil was extracted with benzene. Removal of the benzene by distillation gave 28 g. of white crystalline material. After three recrystallizations from alcohol, the product melted at 106–106.5°.[16]

Anal. Calcd. for $C_{30}H_{45}N_3S_3$: C, 66.30; H, 8.28; N, 7.74; S, 17.5; mol. wt., 543. Found: C, 65.99; H, 8.28; N, 7.92; S, 17.5; mol. wt.,[17] 512.

General Procedure for Preparation of Resinous V. A. With Aqueous Formaldehyde.—A well-stirred mixture of thiophene, 36% formaldehyde, ammonium chloride and sodium bisulfite (for mole ratios see Table II) was refluxed for one to three hours at 70°. After neutralization with sodium hydroxide the resinous products were either steam distilled to remove small amounts of 2-thenylamine and di-(2-thenyl)-amine or digested with benzene. In most cases, the products were totally insoluble in cold benzene and only partially soluble in hot benzene. Some were totally insoluble in boiling benzene. Table II indicates the molar ratios used and the distribution of products. In most cases the yield of light yellow to pure white resin was quantitative.

B. With Paraformaldehyde.—The procedure in this instance was essentially the same as in A above with the exception that 20 ml. of acetic acid was added to initiate the depolymerization of the paraformaldehyde. The mole ratios and the products obtained are listed in Table III.

Inhibitory Effect of Methanol on the Aminomethylation Reaction.[18]—Ten moles of thiophene, 20 moles of paraformaldehyde and ten moles of ammonium chloride were

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[13] Ref. 2. gives b. p. of 115–125° at 3 mm.

[14] Ref. 1 lists m. p. of 123.5–124°.

[15] Ref. 1 lists m. p. of 252–253°.

[16] Although the *t*-butylthiophene used in this work was later found to be a mixture of 2- and 3-*t*-butylthiophene, the sharp melting point of the derivative indicates a single compound rather than a mixture of isomers.

[17] Determined by freezing point methods in cyclohexane.

[18] The following study was carried out by S. L. Meisel in connection with the work in references 2 and 3.

TABLE III

SUB-RESINOUS AMINOALKYLATION PRODUCTS USING SODIUM BISULFITE CATALYST WITH PARAFORMALDEHYDE

Molecular ratio of reactants				Analyses, %		Mol. wt.[b]
C_4H_4S	CH_2O	NH_4Cl	$NaHSO_3$	N	S	
2	6	1.5	0.5	6.64	22.0	706
2	6	2.0	.5	6.91	22.0	521
2	4	2.0	.5	8.00	26.5	416
2	6	2.0	1.0	8.67[a]	24.1[a]	...

[a] Resinous product, only partially soluble in hot benzene, obtained by adding the sodium bisulfite to the reaction mixture as a 40% solution while the reaction was proceeding at 75°. [b] Determined by boiling point method in benzene.

reacted in the presence of 25 ml. of acetic acid in the manner described in a prior reference.[3] When the temperature rose to 68° the addition of one liter of methanol was begun. The temperature immediately rose to 72° and external cooling was necessary to continue the addition. Rectification of the products yielded only 150 g. of 2-thenylamine and 90 g. of di-(2-thenyl)-amine. From a similar run in which methanol was excluded, 220 g. of 2-thenylamine and 390 g. of di-(2-thenyl)-amine were obtained.

The paraformaldehyde in the above experiment was replaced with 2000 g. of 30% formaldehyde (methanol-free) and the reaction was conducted to obtain a maximum yield of N-(2-thenyl)-formaldimine as described in reference 2. The reaction proceeded very rapidly and it was difficult to maintain the correct temperature of 65–68° by means of an ice-bath. Rectification of the products gave only 250 g. (35% based on thiophene reacted) of N-(2-thenyl)-formaldimine as the dimer, b. p. 110–145° (at 3 mm.), and larger amounts of resinous products. In a controlled experiment the 36% commercial formaldehyde containing 6% methanol reacted slower, permitting better temperature control. A 56% yield of the N-(2-thenyl)-formaldimine dimer was obtained. Thus, a small amount of alcohol is deemed necessary to slow down the reaction so that resinification and other side reaction are held at a minimum to assure formation of primary products.

Use of *n*-Butyl Alcohol as a Solvent in the Aminomethylation Reaction.—One mole each of thiophene, paraformaldehyde and ammonium chloride was mixed with 150 g. of *n*-butyl alcohol, vigorously stirred and warmed to 70–75° for three hours. The ammonium chloride, 54 g., was recovered by filtration. Distillation of the filtrate through a 6-plate column gave unreacted thiophene, water, unreacted butyl alcohol and 110 g. (92%) of di-(*n*-butyl)-formal, b. p. 180–181°.

Acknowledgment.—The authors are grateful to Dr. D. E. Badertscher for his advice and interest in this problem and to Emily Burns, Loretta Conley and Paul Gee for laboratory assistance.

Summary

1. A study of the action of sulfurous acid as a catalyst for the aminoalkylation reaction has led to the isolation of 2-thenylaminomethylsulfonic acid. Mechanism studies have been carried out which give further indication for the existence of $CH_2{=}NH$, formaldimine. This compound appears to be the reactive intermediate in the aminoalkylation reaction.
2. Alcohols have been found to have definite inhibitory action in the aminomethylation reaction due to formal formation.
3. The structures of resins formed from this reaction are discussed.
4. Generalizations concerning the mechanism for aminomethylation reactions with formaldehyde and ammonium halides, hydroxylamine salts and monoalkylamine hydrohalides are presented.

PAULSBORO, N. J. RECEIVED JANUARY 26, 1949

[CONTRIBUTION FROM THE MEDICAL NOBEL INSTITUTE, BIOCHEMICAL DEPARTMENT, STOCKHOLM]

The Properties of the Enzyme-Substrate Compounds of Lactoperoxidase[1]

BY BRITTON CHANCE[2]

Theorell[3] in addition to his work in crystallizing lactoperoxidase has obtained many important data upon which an explanation of the mode of action of this enzyme might be based. Among other things, two types of lactoperoxidase–hydrogen peroxide complexes have been found, one red and the other brown, probably corresponding to the horse-radish peroxidase-peroxide complexes II and III. In addition, a heme-linked hydroxyl group has been found as in the case of the horse-radish peroxidase.[4] However, the activity of lactoperoxidase and hydrogen peroxide toward pyrogallol was found[3] to be only P.Z. = 71.5 compared with P.Z. = 900 for the horse-radish peroxidase.[5] Such a great difference of activity could be attributed to the difference in the hemin or the protein components of these two peroxidases,[6] or to the fact that no primary lactoperoxidase–hydrogen peroxide complex was formed, in contrast with horse-radish peroxidase.

These experiments, however, show that lactoperoxidase not only forms a primary peroxide complex in the same way as horse-radish peroxidase but also oxidizes pyrogallol and ascorbic acid considerably faster than the horse-radish enzyme. Lactoperoxidase also forms highly active complexes with the alkyl hydrogen peroxides. There is a great similarity between the reaction

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(1) This is paper 10 of a series on catalases and peroxidases.

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(3) H. Theorell and Å. Åkeson, *Ark. Kemi Mineral. Geol.*, **B17**, No. 7 (1943).

(4) H. Theorell and K. G. Paul, *Ark. Kemi. Mineral. Geol.*, **A18**, No. 12 (1944).

(5) H. Theorell, *Ark. Kemi Mineral. Geol.*, **A16**, No. 2 (1942).

(6) H. Theorell and K. O. Pedersen, "The Svedberg," Almquist & Wiksell, Uppsala, 1944.