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Studies on Stable Free Radicals. III.*1 Reactions of Stable Nitroxide Radicals with *S*-Radicals Derived from Benzenethiols and Thiamine

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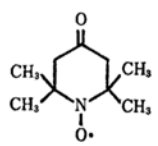
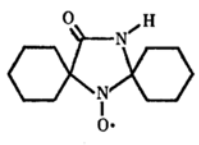
The reaction of the extremely stable free radical, 2,2,6,6-tetramethyl-4-oxopiperidine-1-oxyl (I), was carried out with benzenethiols (IV). The formation of diphenyl disulfides (VI), benzenesulfinic acids (VII), and 1-(*p*-substituted benzenesulfinyl)-2,2,6,6-tetramethyl-4-oxopiperidines (X) from the above reaction shows that the radical (I) can act as a hydrogen-abstracting agent, an oxidizing agent, and a radical scavenger. Possible mechanisms for the coupling reaction of the radical (I) with the *S*-radicals (V) to give the sulfinamides (X) were discussed. A substituent effect was observed in the formation of the disulfides (VI). The *N*-oxyl radical (I) and another stable free radical, such as 2,6-di-*t*-butyl-4-methylphenoxyl radical (XXV), were shown to react with the *S*-radical (XVII) derived from thiamine (XIV) to give new types of thiamine derivatives, *S*-(2,2,6,6-tetramethyl-4-oxopiperidino)thiamine (XX) and *S*-(2,6-di-*t*-butyl-4-methylphenoxy)thiamine (XXVI) respectively.

Many studies of the synthetic methods and physical properties of extremely stable *N*-oxyl radicals have recently been reported.[2,3] However, few papers have reported the roles of such radicals as oxidizing agents, hydrogen-abstracting agents, or radical scavengers. Especially, there have been few studies of the reactivities of radicals by means of isolating the coupled products of the radicals with the other free radicals.[1,4] In the preceding paper[1] we studied the reactive sites in such radicals as 2,2,6,6-tetramethyl-4-oxopiperidine-1-oxyl (I) and cyclohexane-1-spiro-2′-(4′-oxoimidazolidine-1′-oxyl)-5′-spiro-1″-cyclohexane (III) from the structure of the coupled products of I and III with a *C*-radical derived from α,α′-azo-bis(isobutyronitrile) (ABIN).



(I)



(III)

Chart 1

In this paper the authors wish to report that the radical (I) can act as a hydrogen-abstracting agent, an oxidizing agent, and a radical scavenger, by using the fact that the radical (I) reacts with benzenethiols (IV) to give diphenyl disulfides (VI), benzenesulfinic acids (VII), and 1-benzenesulfinyl-2,2,6,6-tetramethyl-4-oxopiperidine (X). Most importantly, the fact that the radical (I) acts as a radical scavenger is very interesting because this result can be applied to other experiments to determine whether a reaction proceeds *via* a free radical intermediate or not.

Todd and Sykes,[5] and Yurugi[6] have studied the pyrolysis of thiamine disulfide (XVIII) and the reaction of the *S*-anion (XV) of thiamine (XIV) with potassium ferricyanide to give thiochrome (XIX). Although they have postulated a reaction mechanism involving an *S*-radical intermediate in the above reaction, no coupled products of the *S*-radical (XVII) of thiamine with other free radicals have been obtained. We applied the reaction of the free radical (I) with the *S*-radical derived from benzenethiol to the case of another *S*-radical (XVII) derived from thiamine (XIV).

Results and Discussion

The reaction of the *N*-oxyl radical (I) with various benzenethiols (IV) was found to proceed

*1 Part II: Ref. 1.

1) K. Murayama, S. Morimura and T. Yoshioka, This Bulletin, **42**, 1640 (1969).

2) M. B. Neiman, Yu. G. Madedova and E. G. Rozantzev, *Azerb. Khim. Zh.*, **1962**, 37.

3) E. G. Rozantzev and M. B. Neiman, *Tetrahedron*, **20**, 131 (1964).

4) E. G. Rozantzev and V. A. Golubev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1966**, 891.

5) a) P. Sykes and A. R. Todd, *J. Chem. Soc.*, **1951**, 534. b) P. Nesbitt and P. Sykes, *ibid.*, **1954**, 4584.

6) S. Yurugi, *Yakugaku Zasshi (J. Pharm. Soc. Japan)*, **77**, 259, 264 (1957).

vigorously in the absence of a solvent at room temperature. This reaction took place even at −55°C in an ethereal or a chloroform solution to give the disulfides (VI), the substituted benzenesulfinic acids (VII), and 1-(*p*-substituted benzenesulfinyl)-2,2,6,6-tetramethyl-4-oxopiperidines (X), as Chart 2 shows. Two reduction products, 2,2,6,6-tetramethyl-4-oxopiperidine (XII) (*ca.* 35% yield) and 1-hydroxy-2,2,6,6-tetramethyl-4-oxopiperidine (XI) (*ca.* 6% yield), were also isolated.

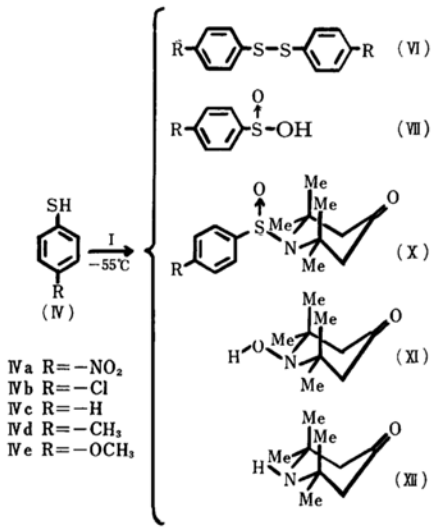


Chart 2[7-9]

The structural assignments of these products were based on the results of the elemental analyses and spectral data. The melting points of the products were identical with those of normal products produced by the independent syntheses of X from the corresponding sulfinyl chlorides and XII, or that of authentic samples.

Our preliminary communication showed that, in our NMR studies of the amides X, four methyl protons of the amides X gave rise to two broad absorptions indicative of magnetic non-equivalence.[10]

The reaction mechanism can be formulated as is shown in Chart 3. The sulfhydryl hydrogen of thiols (IV) was abstracted by the radical (I). The *S*-radicals (V) thus obtained then dimerized to give the dimer (VI). The *N*-oxyl radical (I) or (II) can further react with *S*-radicals (V) to give intermediate, (VIII) or (IX), which, on isomerization, furnish amides (X).

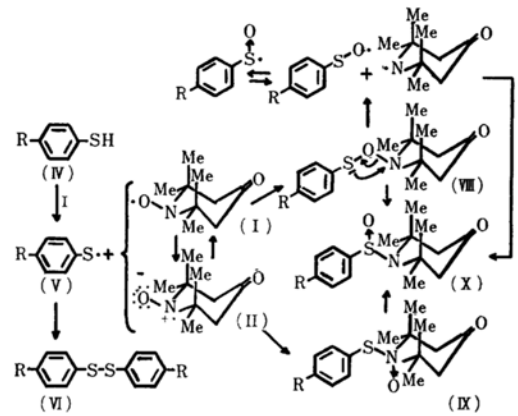
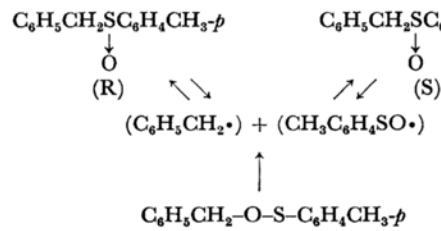


Chart 3[7-9]

The formally analogous thermal rearrangement of sulfenates to sulfoxides has been reported in racemization studies of optically-active sulfoxides by Mislow *et al.*,[11] who have proposed the following homolytic dissociation mechanism:



Similar mechanisms involving an –O–N< bond cleavage in VIII may also be possible.

Sulfinic acid formation, *i. e.*, VII, can hardly be explained by the hydrolysis of the sulfinamides (X), since the reaction of the *N*-oxyl radical (I) with the thiols proceeds in anhydrous media. The details of this reaction mechanism will be presented elsewhere.

Thus, the *N*-oxyl radical (I) appears to have the properties of a hydrogen-abstracting agent, an oxidizing agent, and a radical scavenger. The change of the *p*-substituent on the phenyl group from hydrogen to $-NO_2$, $-Cl$, $-CH_3$, or $-OCH_3$ had only a small effect. Table 1 shows that such an electron-donating group as *p*-OCH_3 tended to decrease the yield of the disulfides (VI). The yield was determined by UV-intensity measurements (λ_{max}) after separation using silica gel-column chromatography. This fact can be explained in terms of the small effect of the substituents on the stability

7) In this chart a piperidine ring was conveniently shown in a chair form as represented for the amine XII by Aroney *et al.*[8] However, Rozantzev and Cur'yanova[9] proposed a twist and a boat form for the *N*-oxyl radical (I) and 1,4-dihydroxy-2,2,6,6-tetramethylpiperidine respectively.

8) M. J. Aroney, C.-Y. Chen, R. J. W. Le Fèvre and A. N. Singh, *J. Chem. Soc. B*, **1966**,98.

9) E. G. Rozantzev and E. N. Cur'yanova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1966**, 979.

10) K. Murayama and T. Yoshioka, *Tetrahedron Letters*, **1968**, 1363.

11) E. G. Miller, D. R. Rayner and K. Mislow, *J. Am. Chem. Soc.*, **88**, 3138, 3139 (1966).

TABLE 1. *p*-SUBSTITUENT EFFECT ON THE YIELD OF DIPHENYL DISULFIDE

Substituent (*p*)	$-NO_2$	$-Cl$	$-H$	$-CH_3$	$-OCH_3$
Yield (%)	55.0	51.5	51.5	52.0	30.5

of the *S*-radical, or on the rate of the hydrogen-abstraction from benzenethiols.

These results appeared to be inconsistent with those proposed by Kooyman *et al.*,[12] who have studied the hydrogen-abstraction reaction by the 1-methyl-1-cyanoethyl radical from substituted benzenethiols (ρ-value: -0.4). However, a direct comparison is impossible because of the uncertainty of the reaction mechanism; in the system studied, there was no evidence that the disulfides were kinetically-controlled products.

Since the free radical (I) was found to exhibit the property of a radical scavenger, we considered using it in the reaction of the radical (I) with the *S*-radical of thiamine (XIV) in a thiol form in order to investigate whether the reaction proceeds *via* a free radical intermediate (XVII) or not. As is

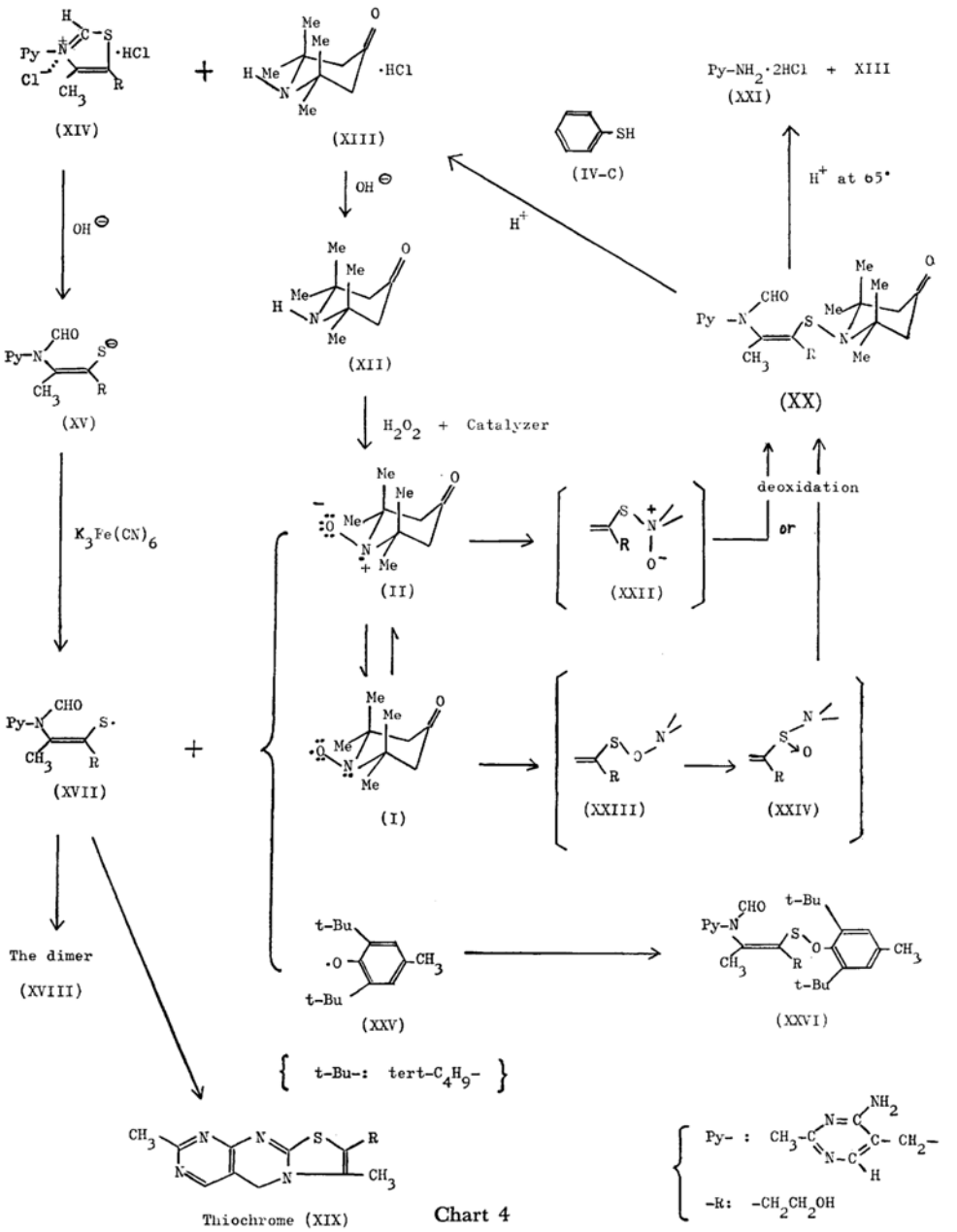


Chart 4

12) Y. Schaafsma, A. F. Bickel and E. C. Kooyman, *Rec. Trav. Chim.*, **76**, 180 (1957).

summarized in Chart 4, thiamine (XIV) was transformed into the *S*-anion (XV) by normal procedures.[13] This *S*-anion (XV) was oxidized with a 1-equivalent electron-abstracting agent, potassium ferricyanide, at 5°C to give the corresponding *S*-radical (XVII).[14] The reaction of the *N*-oxyl radical (I) with the *S*-radical (XVII) was expected to give a coupled product, a new thiamine derivative.[15] From the results of elemental analyses, the UV, IR, NMR, and mass spectra, and the R_f-value in paper chromatography and a thiochrome test,[16] (to be shown in the Experimental section), the structure of the isolated product, $C_{21}H_{33}N_5O_3S$, was deduced to be *N*-[(4-amino-2-methyl-5-pyrimidyl)methyl]-*N*-{4-hydroxy-1-methyl-2-[(2,2,6,6-tetramethyl-4-oxopiperidino)thio]-1-butenyl}-formamide (XX). Further, the hydrolysis of the new derivative, XX, with hydrochloric acid gave 2-methyl-4-amino-5-aminomethylpyrimidine dihydrochloride (XXI) and 2,2,6,6-tetramethyl-4-oxopiperidine hydrochloride (XIII). The reduction of XX with benzenethiol and hydrochloric acid gave thiamine hydrochloride (XIV) and 2,2,6,6-tetramethyl-4-oxopiperidine hydrochloride (XIII) quantitatively. These results are consistent with the structure XX. However, the above observations could not ascertain whether or not a sulfenamide group, –S–N<, was present in this molecule XX. Strong support for the existence of the sulfenamide group was given by the mass spectrometric fragmentation of XX using a high-resolution double-focussing mass spectra; the large fragment peaks at *m/e* 122.073, 186.098, and 249.136 corresponded with $C_6H_8N_3^+$, $C_9H_{16}NOS^+$, and $C_{12}H_{17}N_4O_2^+$ respectively, as is shown in Chart 5. Therefore, the isolated product, XX, was deduced to be a deoxy-form of the expected normal coupled product. Further, the structure of XX was established by X-ray analysis.[17]

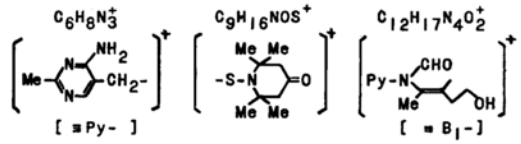


Chart 5

In the reaction of benzenethiols with the *N*-oxyl radical, the coupled products were isolated as sulfinamides, –S(=O)–N<, as has been described above. On the other hand, the coupled product of the *N*-oxyl radical (I) with the *S*-radical (XVII) of thiamine was found to be a sulfenamide, –S–N<, (XX), which was considered to have been produced *via* a normal coupling intermediate, XXII, XXIII, or XXIV. The difference between the two cases will be reported on elsewhere.

Sulfenamides have been produced by Dunbar and Rogers by the following procedure.[18] Although the thiamine derivative in the RSO_2–S–R′ form could not be detected in our laboratory, the thiol-sulfonate, RSO_2–S–R, can be produced by the reaction of the *N*-oxyl radical (I) with the disulfide (XVIII). Therefore, it is possible to consider that

$$RSO_2\text{–}S\text{–}R' + 2\ HN\begin{matrix}R''\\R'''\end{matrix} \rightleftharpoons R'\text{–}S\text{–}N\begin{matrix}R''\\R'''\end{matrix} + RSO_2^{\ominus}\cdot{}^{\oplus}H_2N\begin{matrix}R''\\R'''\end{matrix}$$

the sulfenamide (XX) of thiamine was produced by such a process.

In order to exclude the possibility of the formation of the sulfenamide (XX) by such a course, and in order to prove the existence of the *S*-radical (XVII) of thiamine, it was felt necessary to carry out the reaction of XVII with a free radical other than the *N*-oxyl radical (I). The phenoxyl radical, the 2,6-di-*t*-butyl-4-methylphenoxyl radical (XXV), was chosen as the other free radical. As expected, the *S*-radical (XVII) of thiamine reacted with the phenoxyl radical (XXV) to give a coupled product XXVI, mp 197°C (from MeOH) and the disulfide XVIII under almost the same conditions as those used for the reaction of the *N*-oxyl radical (I) with the *S*-radical (XVII) of thiamine. The molecular formula of $C_{27}H_{40}N_4O_3S$, (XXVI) was assigned to the coupled product of the *S*-radical (XVII) with the phenoxyl radical (XXV) on the basis of the elemental analysis. The structures XXVI-a, XXVI-b, and XXVI-c were reasonable for the coupled products (XXVI), as Chart 6 shows.

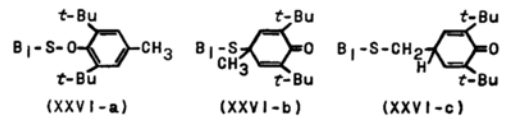


Chart 6

The UV spectrum of the product (XXVI) showed λ_{max}^{EtOH} 235 mμ (log ε 4.22) and 276 mμ (log ε 4.03) (shoulder) in an ethanol solution. The thiochrome test for the product (XXVI) was negaitve. After the reduction of the product (XXVI) with cysteine, the test was positive. Since a thiamine alkylsulfide with the partial structure of =C–S–C could not be

13) R. R. Williams, *Ergev. Vitamin u. Hormon Forsch.*, **1**, 256 (1938).

14) R. Stewart, "Oxidation Mechanisms," W. A. Benjamin, New York, N. Y. (1964), p. 85.

15) K. Murayama and T. Yoshioka, *Chem. Pharm. Bull. (Tokyo)*, **15**, 723 (1967).

16) W. Karrer, *Helv. Chim. Acta*, **20**, 369 (1937).

17) T. Tamura, S. Sato and T. Yoshioka, *Tetrahedron Letters*, **1969**, 547.

18) J. E. Dunbar and J. H. Rogers, *J. Org. Chem.*, **31**, 2842 (1966).

generally reduced with cysteine, the structures XXVI-b and XXVI-c may be excluded.[19]

Thus, the reaction of thiamine in the thiol form with potassium ferricyanide was understood to proceed *via* a free radical intermediate. In these results the existence of new types of thiamine derivatives was established.

Experimental

All the melting points were uncorrected.

The NMR spectra were obtained by means of a Varian A-60 NMR spectrometer, using tetramethylsilane as the internal standard at 32°C.

The mass spectra were obtained using a JEOL-JMS-OlS mass spectrometer with an ionizing voltage of 70 eV.

The paper chromatograms of thiamine derivatives were run with an *n*-butanol - acetic acid - water mixture (4 : 1 : 5) on Toyofilter paper No. 51, using the ascending technique, while spots were detected by spraying with a dilute Dragendorff reagent.

Materials. The benzenethiol (IV-c), *p*-nitro- (IV-a), *p*-chloro- (IV-b), and *p*-methylbenzenethiol (IV-d) used in this work were obtained from the Tokyo Kasei Kogyo Co., Ltd. Compound IV-a was twice recrystallized from dichloroethane after the solution of the corresponding potassium salt of IV-a had been treated with active charcoal at 5°C under a nitrogen atmosphere and then neutralized with a 10% hydrochloric acid solution to furnish an almost pure compound (IV-a). The other thiols, (IV-b), (IV-c), and (IV-d), were purified by distillation under diminished pressure. The *p*-methoxy derivative (IV-e) was prepared by the general method described in the literature.[20]

2,2,6,6-Tetramethyl-4-oxopiperidine-1-oxyl (I) was prepared by the oxidation of the corresponding amine (XII) by hydrogen peroxide in the presence of sodium tungstate and ethylenediaminetetraacetic acid (EDTA).[3]

General Procedure for the Reaction of *p*-Substituted Benzenethiols (IV) with 2,2,6,6-Tetramethyl-4-oxopiperidine-1-oxyl (I). Into a solution of 0.01 mol of IV in 15 m*l* of anhydrous chloroform, maintained at an internal temperature of −55—−60°C with stirring under a dry nitrogen atmosphere, there was slowly added, drop by drop, a solution of 0.01 mol of I in 5 m*l* of anhydrous chloroform. After the addition was completed, the stirring was continued for 1 hr at −55°C; then the internal temperature was gradually raised to room temperature. The reaction mixture was allowed to stand at room temperature for 10 hr, and then the solvent was evaporated under diminished pressure at 30°C. The oily residues or crude crystals were chromatographed on 100 g of silica gel activated at 150°C for 2 hr. By using anhydrous *n*-hexane as the eluting solvent, *p*-substituted diphenyl disulfides (VI) were isolated. Then 1-(*p*-substituted benzenesulfinyl)-2,2,6,6-tetramethyl-4-oxopiperidines (X) (*ca.* 10%), 2,2,6,6-tetramethyl-4-oxopiperidine (XII) (*ca.* 35%), and 1-hydroxy-2,2,6,6-tetramethyl-4-oxopiperidine (XI) (*ca.* 6%) were eluted with benzene. Lastly, the salts of 2,2,6,6-tetramethyl-4-oxopiperidine with *p*-substituted benzenesulfinic acids (VII) was eluted with methanol. The disulfides (VI) were recrystallized from ethanol to give analytically-pure samples which were identical with authentic samples. The sulfinamides (X) were recrystallized from ethanol or ether to give analytically-pure samples which were identical with the normal samples produced by the reaction of *p*-substituted benzenesulfinyl chloride with 2,2,6,6-tetramethyl-4-oxopiperidine (XII) in dry triethylamine at 50°C. The sulfinamides (X) are listed in Table 2.

The benzenesulfinates of VII (*ca.* 20%) obtained from the last fraction were recrystallized from ethanol to give analytically-pure samples which were identical with authentic samples.

***N*-[(4-Amino-2-methyl-5-pyrimidyl)methyl]-*N*-{4-hydroxy-1-methyl-2-[(2,2,6,6-tetramethyl-4-oxopiperidino)thio]-1-butenyl}formamide (XX). [Abbr., *S*-(2,2,6,6-Tetramethyl-4-oxopiperidino)thiamine].** Into a mixture of 33.4 g (0.1 mol) of thiamine hydrochloride (XIV) and 17.0 g (0.1 mol) of the *N*-oxyl radical (I) in 100 m*l* of water, there was slowly added a solution of 12 g of sodium hydroxide in 50 m*l* of water at 5°C with vigorous stirring. After the reaction mixture was allowed to stand at 20°C for 30 min, a solution of 32.9 g (0.1 mol) of potassium ferricyanide in 100 m*l* of water was added at 5°C with vigorous stirring. The crude crystals which were separated after several hours were then filtered and washed with cold water three times. The crude product was recrystallized from wet

TABLE 2. *p*-SUBSTITUTED DERIVATIVES OF 1-BENZENESULFINYL-2,2,6,6-TETRAMETHYL-4-OXOPIPERIDINE

Compound	R	Mp (°C) (decomp.)		Analysis (%) Calcd C	H	N	S	Mw	Found C	H	N	S	Mw
X-a	$-NO_2$	179—180	$C_{15}H_{20}N_2O_4S$	55.55	6.17	8.64	9.88	324	55.35	6.20	8.60	9.92	328[c]
X-b	$-Cl$	163—164	$C_{15}H_{20}ClNO_2S$	57.42	6.34	4.47	10.21	313	57.38	6.51	4.44	10.09	345[a]
X-c	$-H$	116—117	$C_{15}H_{21}NO_2S$	64.51	7.53	5.02	11.47	279	64.30	7.58	5.01	11.41	286[a]
X-d	$-CH_3$	160—161	$C_{16}H_{23}NO_2S$	65.53	7.92	4.78	10.92	293	65.57	7.99	4.70	10.91	300[c]
X-e	$-OCH_3$	131—132	$C_{16}H_{23}NO_3S$	62.13	7.44	4.53	10.36	309	61.87	7.40	4.66	10.25	325[a]

Mw: VPO-method a: in an acetone solution, c: in a chloroform solution.

19) For the sake of less solubility, NMR spectrum of XXVI was not measured.

20) D. S. Tarbell and D. K. Fukushima, "Organic Syntheses," Coll. Vol. 3, p. 809 (1955).

methanol to afford colorless prisms with a mp of 179°C (monohydrate). The monohydrate was dried at 105—110°C for 10 hr under diminished pressure to give an analytically-pure sample (mp 179°C). Found: C, 57.78; H, 7.76; N, 16.29; S, 7.39%. Calcd for $C_{21}H_{33}N_5O_3S$: C, 57.91; H, 7.64; N, 16.08; S, 7.36%. UV λ_{max}^{EtOH} mμ (logε): 235 (4.17); 276 (3.77) (shoulder). IR cm^{-1} ($CHCl_3$ solution, 1.0 m/m NaCl, Grating):[21] ν_{O-H} 3620; $\nu_{as\ N-H}$ 3480; $\nu_{s\ N-H}$ 3335; $\nu_{C=O}$ 1715. NMR (τ) (in d-6 DMSO):[22] 2.08 (1H, singlet); 2.13 (1H, singlet). R_f (paper chromatography):[23] 0.70. The thiochrome test was negative, but became positive after reduction with cysteine.[24] High-resolution double-focussing mass spectra: molecular ion peak, *m/e* 435.230=$C_{21}H_{33}N_5O_3S$; large fragment peaks, *m/e* 122.073=$C_6H_8N_3^+$; *m/e* 186.098=$C_9H_{16}NOS^+$; *m/e* 249.136=$C_{12}H_{17}N_4O_2^+$.

The Hydrolysis of *S*-(2,2,6,6-Tetramethyl-4-oxopiperidino)thiamine (XX). A solution of 0.87 g (0.002 mol) of XX in 2 m*l* of methanol containing 0.5 g (0.005 mol) of concentrated hydrochloric acid (35% aq) was refluxed for 2 hr, and then the solvent was evaporated under diminished pressure. Scratching induced the oily residue to solidify upon the addition of absolute ethanol. The mixture thus obtained was filtered, and the solid was recrystallized from wet ethanol; mp 265—270°C (bubbling). Found: C, 34.25; H, 5.73; N, 26.48; Cl, 33.40%. Calcd for $C_6H_{12}N_4Cl_2$: C, 34.12; H, 5.69; N, 26.54; Cl, 33.65%. This compound was identical with authentic 4-amino-2-methyl-5-aminomethylpyrimidine·2HCl (XXI).[25] The filtrate was evaporated under diminished pressure. The oily residue, when scratched in ethyl acetate, gave a yellow semi-solid. After this semi-solid was filtered off, the filtrate was treated with fresh ethyl acetate to furnish a white precipitate which was then separated by filtration. To a suspension of this solid in ethyl acetate on a water bath, a small amount of ethanol was added until the solid dissolved. This solution was cooled at −30°C to give white crystals. This crystalline product was neutralized with potassium hydroxide in a methanol solution to give 2,2,6,6-tetramethyl-4-oxopiperidine (XII), which was identical with an authentic sample.

The Reduction of *S*-(2,2,6,6-Tetramethyl-4-oxopiperidino)thiamine (XX) with Benzenethiol. Into a mixture of 4.4 g (0.0101 mol) of XX and 4.4 g (0.0400 mol) of benzenethiol in 30 m*l* of methanol, 2.1 g of a 35% aqueous solution of hydrochloric acid (0.0101 mol) were slowly added, drop by drop, with stirring at 10°C. After the mixture had stood for 10 hr at 20°C, the solvent was evaporated under diminished pressure. The residue solidified upon being scratched in a small amount of ethanol (about 10 m*l*). As the solid was a mixture of thiamine hydrochloride (XIV) and diphenyl disulfide (VI), the solid was purified by fractional crystallization from ethanol. Thiamine hydrochloride monohydrate, mp 235°C was crystallized out first, and then the diphenyl disulfide, mp 61°C was obtained. These substances were identical with authentic samples. After the above filtrate was evaporated under diminished pressure, the residue, upon the addition of a small amount of ethyl acetate with scratching, furnished a small amount of a solid. The solids (crude VI and XIV) were removed, and then to the filtrate there was added a further amount of ethyl acetate. This solution deposited white crystals after standing for several hours. These crystals were treated with potassium hydroxide in methanol to afford 2,2,6,6-tetramethyl-4-oxopiperidine (XII), which was identical with an authentic sample.

***N*-[(4-Amino-2-methyl-5-pyrimidyl)methyl]-*N*-{4-hydroxy-2-[(2,6-di-*t*-butyl-4-methylphenoxy)thio]}-formamide (XXVI). [*S*-(2,6-Di-*t*-butyl-4-methylphenoxy)thiamine].** To a solution of 6.7 g (0.0199 mol) of thiamine hydrochloride (XIV) in 50 m*l* of water, with the internal temperature maintained at 5°C by effective stirring under a nitrogen atmosphere, there was slowly added, drop by drop, a solution of 2.4 g (0.06 mol) of sodium hydroxide in 10 m*l* of water. After the addition was completed, stirring was continued for 1 hr at 20°C to afford the *S*-anion (XV) of thiamine (XIV), which was, for convenience, named the "-$S^{\ominus}$-solution." Separately, to a mixture of 4.4 g (0.020 mol) of 2,6-di-*t*-butyl-4-methylphenol in 20 m*l* of benzene and 4.4 g (0.110 mol) of sodium hydroxide in 20 m*l* of water, while controlling the internal temperature at 5°C by effective stirring (nearly an emulsion) under a nitrogen atmosphere, there was slowly added, drop by drop, a solution of 6.5 g (0.0198 mol) of potassium ferricyanide in 20 m*l* of water. After the addition was completed, stirring was stopped. The benzene-layer was carefully separated under a nitrogen atmosphere with a large pipette. Immediately, a portion of the benzene solution in the large pipette was slowly added, drop by drop, to the "-$S^{\ominus}$-solution" with effective stirring at 5°C (nearly an emulsion) under a nitrogen atmosphere; meanwhile, a solution of 6.6 g (0.0205 mol) of potassium ferricyanide in 20 m*l* of water was alternately added in an approximately equivalent amount to the above benzene solution. After this alternating addition was completed, the benzene layer was separated by means of a large pipette as soon as possible. Subsequently the benzene solution which separated was allowed to stand for several hours; the crude crystalline product was isolated and then recrystallized from methanol, mp 197°C. Found: C, 64.52; H, 8.04; N, 10.98; S, 6.26%. Calcd for $C_{27}H_{40}N_4O_3S$: C, 64.80; H, 8.00; N, 11.20; S, 6.40%. UV λ_{max}^{EtOH} mμ (logε): 234.5 (4.22); 276.5 (4.03). Thiochrome test: negative, but positive after reduction with cysteine.

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21) These values were disagreement with the standard value in nonpolar dilute solution becuase of the measurement in concentrated chloroform solution.

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