Activated Hydrogens in Compounds Related to Thiamine*

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The exchange rate of hydrogens with solvent of a series of model compounds related to thiamine has been measured. The results show that ylid formation in thiamine is primarily due to the carbon-nitrogen double bond in a cyclic system and not to sulfur-shell expansion. Resonance forms utilizing the sulfur increase the stability of the ring to hydrolytic cleavage and aid in carbanion formation in later reactions of thiamine.

All known mechanisms of reactions catalyzed by thiamine require prior ionization of the proton at the 2-position of the thiazolium ring. (Breslow, 1958; White and Ingraham, 1962) The carbanion or ylid thus formed then condenses with a carbonyl group of the substrate. The rate of formation of ylids in thiazolium rings as measured by exchange rates in D_2O (Breslow, 1956, 1957; Fry *et al.*, 1957) is remarkable compared with exchange via other known nitrogen ylids. For example, Breslow (1957) found that 3,4-dimethylthiazolium iodide exchanges hydrogen at the 2-position with a half-life of minutes in neutral solution at room temperature, whereas tetramethylammonium iodide requires over 10 days in refluxing 0.27 N base to exchange only 0.1% of each hydrogen (Doering and Hoffman, 1955).

Clearly, ylid formation in thiazolium compounds is much faster than in simple model quaternary ammonium salts. Ideas have already been given to explain this anomaly. The purpose of this paper is to present the results of some exchange experiments that help to provide further explanation.

EXPERIMENTAL

Preparations

4-Methyloxazole.—The preparation was made according to Cornforth and Cornforth (1953) by refluxing ethyl chloroacetoacetate with ammonium formate in formic acid. The resulting ester (bp 98–106° 20 mm, 29% yield) was saponified with dilute NaOH. 4-Methyloxazolcarboxylic acid (mp 238°) was obtained in 55% yield. The decarboxylation was performed in boiling quinoline. The product (bp 89°, 73% yield) gave a single peak on gas chromatographic analysis.

3,4-Dimethyloxazolium Iodide.—One g of 4-methyloxazole was refluxed with 3.4 g methyliodide for 24 hours. After removal of the surplus reagent in vacuum, 2.7 g (92% of the theory) of a resinous product remained, easily soluble in water, ethanol, acetone, acetonitrile; soluble in butanol; insoluble in chloroform ethylacetoacetate, ether, dioxane, benzene. Crystallization from butanol was possible, but better results were obtained by dissolving the compound in acetonitrile and precipitating it slowly with ether. Very hygroscopic colorless crystals were obtained (mp 109–110°).

Anal. Calcd. for C_5H_8NOI: C, 26.68; H, 3.58; N, 6.22; I, 56.40. Found: C, 26.76; H, 3.84; N, 5.74.

N-Methylthiazolium Iodide.—One g of thiazole and 3.7 g of freshly distilled methyl iodide were allowed to stand for several hours at room temperature. The crystals (65% yield) that formed were recrystallized from ethanol in long white needles, which were not hygroscopic, were soluble in high polar solvents, and insoluble in low polar solvents (mp 150–150.5°).

Anal. Calcd. for C_4H_6NSI: C, 21.15; H, 2.66; N, 6.16; S, 14.11; I, 55.89. Found: C, 21.40; H, 2.76; N, 6.16; S, 13.91; I, 55.70.

3,4-Dimethylthiazolium Iodide.—Material prepared by F. White (White and Ingraham, 1962) was used.

Dianilinoethane.—This was prepared by the method of Wanzlick and Loechel (1953) by heating aniline with ethylenedibromide. The resulting anilide was obtained in 82% yield and had a melting point of 65° (monohydrate, recrystallized from methanol).

Monoformyldianilinoethane.—Monoformyldianilinoethane was obtained in good yield by heating dianilinoethane (Zienty, 1946) with 97% formic acid. Dilution of the formic acid with water gave a product which was recrystallized from methanol (mp 57–59°).

1,3-Diphenylimidazolinium Chloride.—The preparation was made by dissolving monoformyldianilinoethane in 2 N hydrochloric acid (Zienty, 1946). In contradiction to previous reports (May *et al.*, 1951) the product can be obtained in crystalline form by crystallization from 0.001 N hydrochloric acid (mp 239–241° decomp.).

Benzal-p-toluidine.—This was prepared (Hantzsch and Schwab, 1901) by heating 3-g portions of *p*-toluidine and benzaldehyde. The product was purified by distillation (bp 90°, 0.08 mm). A yellow oil, which solidified, was obtained in 74% yield (mp 27.5–28.5°).

N-Methylbenzal-p-toluidinium Iodide.—Benzaltoluidine was refluxed with a surplus of freshly distilled methyliodide. Atmospheric moisture was excluded by using a phosphorous pentoxide drying tube. Residual reactants were removed in vacuum (0.03 mm) at 100°. The product can be crystallized by stirring with a small amount of cold acetonitrile. It has the same melting point as described by Hantzsch and Schwab (1901). A further purification is possible by dissolving the compound in dry acetonitrile and precipitating with absolute ether (mp 149–151°). In the mother liquor was found more material (mp 138–140°) which analyzed for *N*-methyl-*p*-toluidinium iodide.

Anal. Calcd. for $C_{18}H_{12}NI$: C, 38.57; H, 4.86; N, 5.62; I, 50.95. Found: C, 38.52; H, 4.91; N, 5.12; I, 50.50.

N-Methylbenzal-*p*-toluidinium iodide is very sensitive to water and had to be stored carefully in a vacuum dessicator. It reacts at room temperature with all solvents that have only slightly nucleophilic properties and is insoluble in nonpolar solvents. The compound

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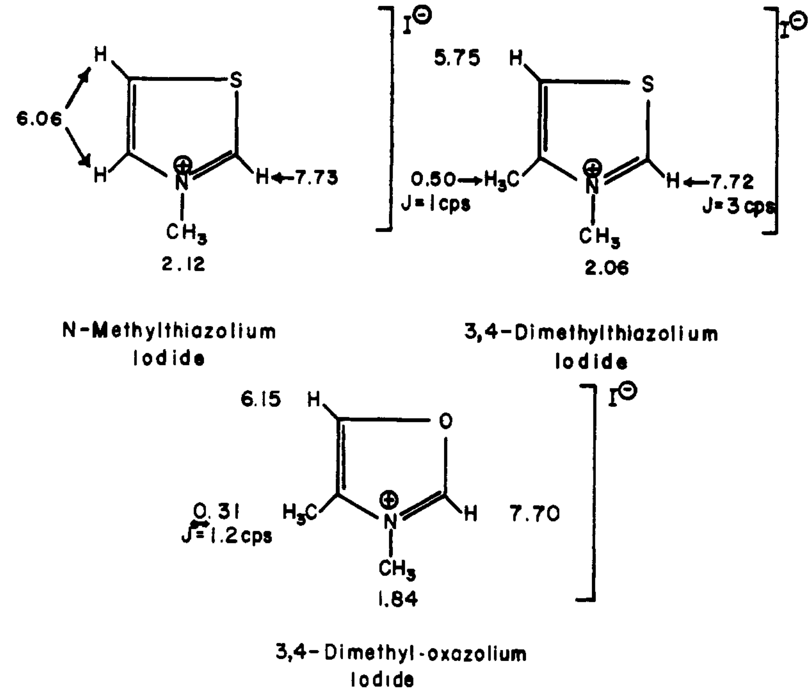

FIG. 1.—Nuclear magnetic resonance data for various heterocyclic salts. The shielding values were measured in 0.1 N *d*-acetate buffer at *p*D 3.6 and are given in the ppm downfield shift from the methyl group of internal acetic acid. The J-values refer to splittings by the proton in the 5-position.

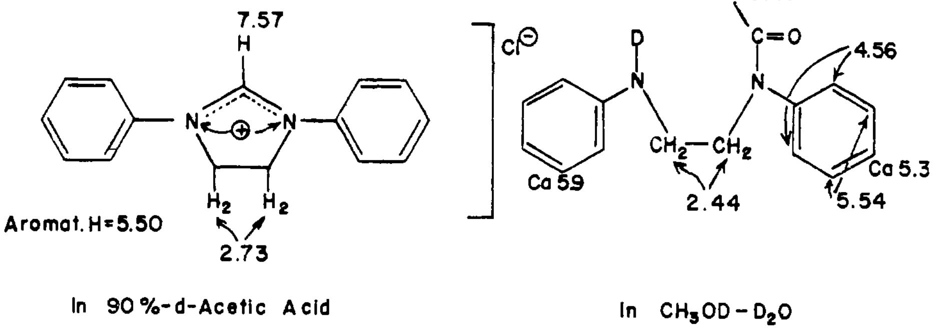

FIG. 2.—Nuclear magnetic resonance data for 1,3-diphenylimidazolinium chloride and monoformyldianilinoethane. The shielding values are given in the ppm downfield shift from the internal acetic acid. The 1,3-diphenylimidazolinium chloride was measured in 90% *d*-acetic acid and the monoformyldianilinoethane in CH_3OD-D_2O solution.

described by Decker and Becker (1913), which has a melting point of 114–117° when recrystallized from chloroform-ligroin, was not formed. Methylation in a sealed tube at 100° also gave the compound with the 151° melting point.

Exchange Studies by Spectral Methods

Nuclear Magnetic Resonance Studies of Hydrogen Exchange in 3,4-Dimethyloxazolium Iodide, 3,4-Dimethylthiazolium Iodide, and 3-Methylthiazolium Iodide.—Proton magnetic resonance spectra of these compounds, obtained with a Varian DP-60 spectrometer system,[1] were used to follow the exchange and (sometimes) decomposition as 5% solutions in a suitable deuterated buffer. As soon as possible after mixing, the spectra were observed either on the oscilloscope or recorder of the spectrometer. When possible the amplitudes of changing peaks were determined as a function of time (integrals were obtained when feasible). The shielding values (measured from approximate centers-of-gravity of complex bands) in ppm from the methyl protons of internal acetic acid are reported in Figures 1 and 2 and the half-times in Table I.

Acetic acid is far from an ideal internal reference, but was the only compound common to the various studies which were made. In one case a change from a

TABLE I

EXCHANGE RATES IN 0.1 N D_2O ACETATE BUFFER AT 28°

Compound	*p*D	Half-Life
3-Methylthiazolium iodide	5.55	2.5 min[a]
	5.02	8 min
	4.60	17 min
	4.11	200 min
	3.71	350 min
3,4-Dimethylthiazolium iodide	5.60	13 min
3,4-Dimethyloxazolium iodide	4.65	*ca.* 5 sec
	3.80	6 min

[a] The error limit of the half-life times can be estimated to be in the order of ±10–20%.

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[1] Reference to a company and/or product name by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

TABLE II
INFRARED SPECTRA OF PROTONATED AND DEUTERATED 3,5-DIMETHYLOXAZOLIUM IODIDE

Protonated		Deuterated in 2-Position	
3100 cm^{-1}	very st[a]	3100 cm^{-1}	st
—		2290	st
1650	st	1650	st
1550	st	1545	st
1440	m	1440	m
1380	w	1380	m
—		1330	m
1235	m	1280	w
1185	st	1200	m
1090	w	1100	st
1040	st	1070	w
—		1030	w
1010	w	1005	w
900	st	—	
860	w	850	st
810	w	805	m
790	w	—	
—		725	m
750	m	705	m

[a] Key: st = strong, m = medium, w = weak.

*p*D of 3.6 to 4.6 caused an apparent upfield shift of all peaks by 0.08 ppm. Almost certainly this shift resulted from a change in the environment of the reference hydrogens. The amplitude of the peak corresponding to the proton in the 2-position was followed with time.

Further evidence that the deuterated 3,4-dimethyloxazolium iodide contained deuterium at the 2-position in the intact ring was obtained by dissolving the salt in D_2O, lyophilizing, and observing the infrared spectra of the residue (Table II).

1,3-Diphenyl-2-imidazolinium Chloride.—It was necessary to exchange this in a mixture of *d*-methanol (350 μl) and 0.1 N *d*-acetate buffer (150 μl). The mixture had a *p*D of 5.6. The half-life of the decrease of the peak from the proton in the 2-position was about 10 minutes. Downfield at 6.41 the peak assigned to the formyl proton of the hydrolysis product appeared. A strong singlet peak assigned to the four methylene protons (equivalent by hydridization) of the diphenylimidazolinium system decreases with a half-life of about 30 minutes (Fig. 1, see spectra Figs. 3 and 4).

N-Methylbenzaltoluidinium Iodide.—Study of this material was made in a nonaqueous buffer which contains deuterium ions and has no nucleophilic properties. A mixture of 4 ml acetonitrile, 4 ml d_1-acetic acid, and 140 mg sodium acetate showed a *p*D of 2.51 (measured with a glass electrode) and had the ability to keep this value constant if smaller amounts of acid or base were added. About 40 mg of *n*-methylbenzal-*p*-toluidinium iodide was dissolved in 600 ml of this mixture. The NMR[2] spectrum showed a slow decrease of the azomethine-proton peak accompanied by the formation of new peaks. This indicates that there was only a reaction, no hydrogen exchange.

Exchange Experiments in Tritiated Water

All counting was performed with a Packard Tri-Carb Liquid Scintillation Spectrometer[1] in toluene containing 5 g of 2,5-diphenyloxazole and 0.3 g of 2,2-*p*-phenylene-bis-(5-phenyloxazole) per liter.

1,3-Diphenylimidazolinium Chloride.—A solution of 63.7 mg of the chloride in 0.5 ml of methanol and 0.5 ml of 2 N acetate buffer (*p*H 5.5) containing 50 μc/ml T was allowed to stand overnight at 24°. It was then extracted twice with 5 ml ether and the ether was removed on a water bath. The residue was lyophilized with a large surplus of water to remove mobile T, four times recrystallized from methanol, and dried in vacuum. N-Monoformyldianilinoethane (mp 61–62°) was obtained in a yield of 13.5 mg. The radioactivity of this compound counted in toluene scintillation solution on a Packard Tri-Carb Liquid Scintillation Spectrometer[1] was found to be 509.6 disintegrations/mg/minute.

In order to prevent errors due to quenching, exchange at the amino group of the same compound was used as a standard. Nontritiated *N*-monoformyldianilinoethane (16.4 mg) was dissolved in 0.5 ml of methanol and 0.5 ml of tritiated water (50 μc/ml T). The emulsion was heated on a water bath and then was frozen and lyophilized, and radiation was counted in the same manner. The activity was 665.0 disintegrations/mg/minute.

These results gave a composite rate for hydrolysis and exchange. In order to separate these rate constants, it was necessary to measure the rate of hydrolysis by ultraviolet spectroscopy. The ultraviolet absorption of the compound (12.5 μg/ml) was measured in methanol-acetate buffer, 1:1 (*p*H of the mixture, 5.6) as a function of time. It showed, in agreement with May *et al.* (1951), maxima at 235 mμ and 312 mμ. The disappearance of the latter (312 mμ) as a function of time was studied. A logarithmic plot of the optical density versus time showed that the reaction was first order over two half-lives.

N-Methylbenzaltoluidinium Iodide.—A solution of 8.8 g of K_2HPO_4 in 22 ml tritiated water (4.0 μc/ml) was brought to *p*H 9.0 by adding dilute sulfuric acid. To this solution was added 2.97 g of *N*-methylbenzaltoluidinium iodide with cooling and stirring at 8°. A brown oil appeared as the *p*H of the solvent dropped to 7.6 (after 5 minutes). The reaction mixture was extracted several times with ether and the ether was shaken four times with twice its volume of water (to remove exchangeable T) and dried with anhydrous Na_2SO_4. Removal of the ether *in vacuo* left 0.82 g of a residual oil, which yielded 0.2 g benzaldehyde.

The aldehyde was dissolved in 10 ml diethyleneglycol, and 0.5 ml hydrazine hydrate was added. Water and the surplus of hydrazine hydrate were removed by a slow distillation. The hydrazone was decomposed by adding 1 g KOH and heating the solution to 200°. A distillate, which had two phases, was obtained. The organic layer (91 mg) dried over sodium and distilled, had an infrared spectrum identical to that of toluene. A further purification was obtained by adding 10 ml "cold" toluene and distilling through a column. The radioactivity of the 5-ml middle fraction was counted by liquid scintillation.

A solution of 23.9 mg of toluene, obtained by the reduction, in toluene counting solution was found to have 271 ± 10 (standard deviation) disintegrations per minute. The background was 275 ± 11.

Rate of Exchange of 2-Tritio-N-methylthiazolium Iodide.—In order to obtain an isotopic rate effect for the exchange, the loss of tritium from 2-tritio-*N*-methylthiazolium iodide was measured in deuterated solvent. This result could then be compared with the loss of hydrogen from the protonated salt in deuterated solvent as measured by NMR.

N-Methylthiazolium iodide tritium labeled in the 2-position was obtained by adding 0.5 ml tritiated water (50 mc) to 200 mg of the protonated compound. The solution was allowed to stand overnight at room temperature, lyophilized, and dried in high vacuum over P_2O_5 for several hours. The 2-tritiothiazolium salt was dissolved in 2 ml deuterated acetate buffer (*p*H

[2] Abbreviation used in this paper: NMR, nuclear magnetic resonance.

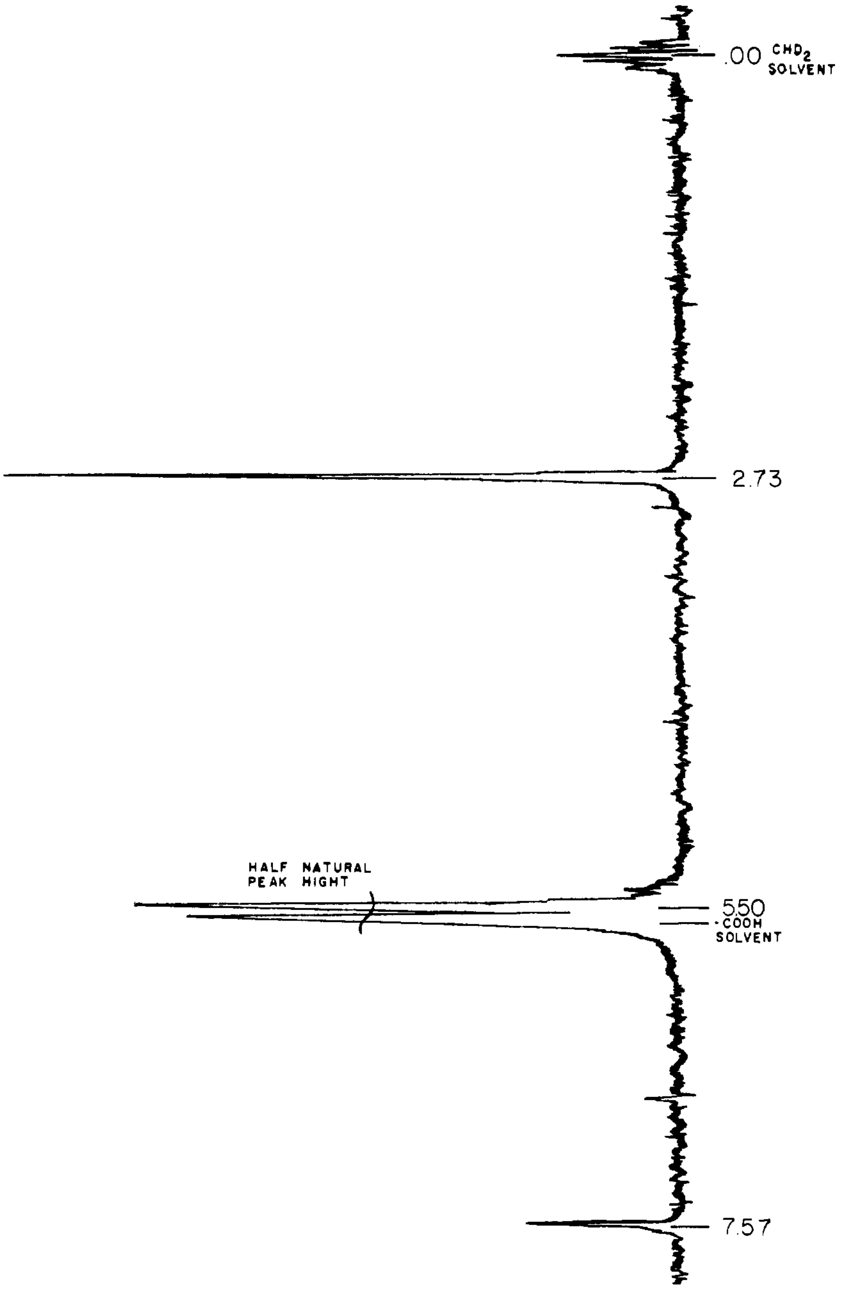

FIG. 3.—Nuclear magnetic resonance spectrum of 1,3-diphenylimidazolinium chloride.

5.55) and kept at 26.5°. At certain times samples of 100 μl were removed and promptly injected into counting vials which contained 100 μl glacial acetic acid. The samples were frozen, lyophilized, and dried over P_2O_5 *in vacuo*. The residue was dissolved in 1 ml methanol containing 0.1 ml glacial acetic acid and 10 ml toluene scintillation solution. (The solution became slightly yellowish, but decolorized upon shaking with air). Counts between 12,736 (after 34 seconds) and 2,157 (after 36.5 minutes) were obtained. The half-life was determined by a least squares treatment of sixteen points.

Attempted Reaction of Benzaldehyde with 3,4-Dimethyloxazolium Iodide.—To a solution of 25 mg 3,4-dimethyloxazolium iodide in 1 ml of 1.0 M potassium phosphate buffer, *p*H 8, was added 12 mg of benzaldehyde in 1.2 ml cyclohexane. The mixture was stirred overnight at room temperature (23°), the layers were separated, and the organic phase was dried with Na_2SO_4. A gas-liquid chromatogram of the organic phase showed only starting material present. The gas-liquid chromatography was performed on a 2-ft silicone rubber column with temperature programing (70–250°, 13°/min).[3] A test experiment gave a clear separation of benzaldehyde, benzophenone, and benzoin. A fluorescence test (White and Hoffman, 1957) showed the absence of benzoin in the aqueous phase. The tested sample showed fluorescence after addition of a trace of benzoin. In other experiments reaction products were searched for by means of the ultraviolet spectra. The spectrum of an equimolar solution of benzaldehyde (absorption max, 247 mμ) and 3,4-dimethyloxazolium iodide (absorption max, 217–220 mμ) in phosphate buffer (*p*H 5.0 and 7.0) and 1-propanol (containing 1.3×10^{-3} mole Tris) was recorded as a function of time. The absorption of the mixture was very similar to the sum of the single spectra and did not change with time.

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[3] We wish to express our thanks to Dr. P. Bernhard, University of California, Davis, Department of Food Technology, who performed this GLC experiment.

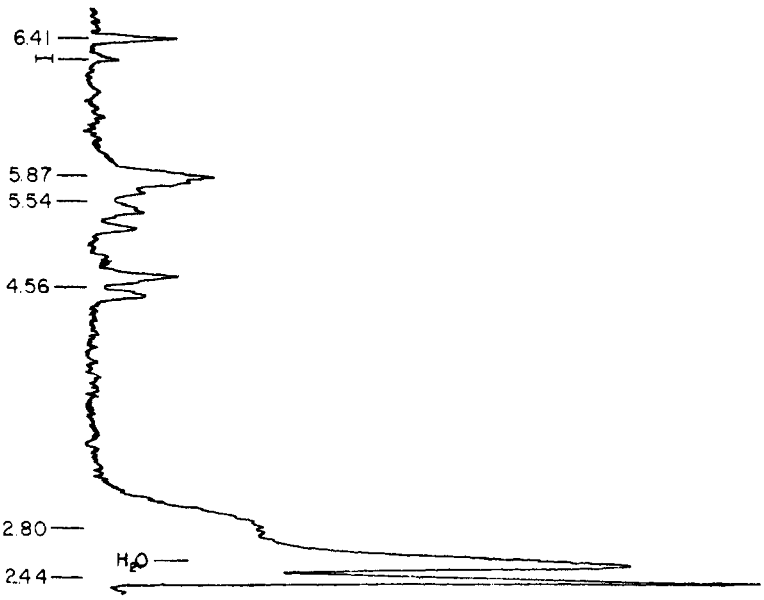

FIG. 4.—Nuclear magnetic resonance spectrum of monoformyldianilinoethane.

When the solution in *p*H 5.3 buffer was heated to 80° for 3.5 hours, no change of the absorption occurred. After extraction with ether the spectrum of the aqueous phase was identical to the pure 3,4-dimethyloxazolium iodide; a large amount (about 83%) of the starting material was still present.

RESULTS

The highest rate of hydrogen exchange was found in 3,4-dimethyloxazolium iodide. The half-life for the exchange of the hydrogen at the 2-position of the oxazolium ring was found to be of the order of 5 seconds at *p*D 4.7 and 6 minutes at *p*D 3.8 (0.1 M sodium acetate buffer in D_2O) at 28°. This exchange is too rapid to be observed in neutral solution. The NMR spectrum in neutral D_2O lacks the peak corresponding to the hydrogen at the 2-position but otherwise is identical to the spectra in weakly acidic buffers. This indicates that neither ring opening nor exchanges, other than the 2-hydrogen, occur at neutral *p*H.

The strong infrared band at 900 cm^{-1}, normally present in the 3,4-dimethyloxazolium iodide, disappeared in the product deuterated in neutral solution This result parallels the investigation of thiazolium salts by Breslow (1957) who correlated the peak at 900 cm^{-1} in 3,4-dimethylthiazolium iodide with the out-of-plane bending in the 2-position.

3,4-Dimethyloxazolium iodide was found to have no catalytic activity in the benzoin condensation, in agreement with the results found for *N*-methyl-4,5-benzo-oxazolium iodide. In fact the oxazolium salt was found by ultraviolet spectroscopy to give no reaction with benzaldehyde.

The half-life of *N*-methylthiazolium iodide in D_2O 0.1 N acetate buffer, *p*D 4.6, is 17 minutes. Thiazole is available commercially, the methylated product is easy to purify and not hygroscopic, so it is useful as a standard. An isotopic rate effect was measured for this compound by measuring both the exchange of the 2-tritio and 2-proto derivatives in D_2O. The decrease of counts in the 2-tritio derivative in D_2O was found by the least squares method to have a half-life of 11.2 ± 2.3 minutes. The relatively large error was due to the unpredictable yellowing of the solutions. The half-life of the protonated species under identical conditions

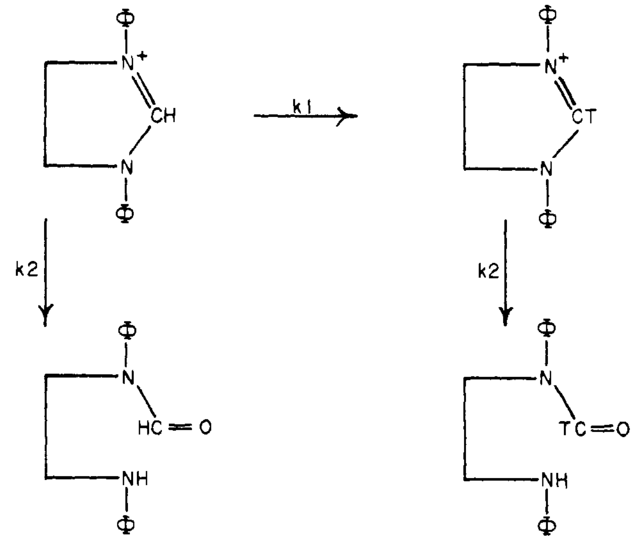

FIG. 5.—The reactions occurring when 1,3-diphenylimidazolinium chloride is placed in tritium-labeled water.

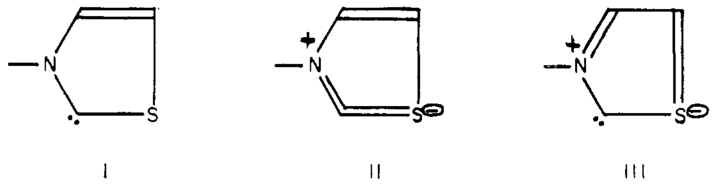

FIG. 6.—Resonance forms of the thiazolium carbanion.

was found by NMR measurements to be 2.2 minutes, giving an isotopic rate effect of $kH/kT = 5.2 \pm 1.0$. The pH dependence for the exchange of 3-methyl thiazolium iodide is reported in Table II.

The exchange rate of 3,4-dimethylthiazolium iodide is still less, with a half-life of 13 minutes at pD 5.6. The exchange of both thiazolium compounds in neutral D_2O, in phosphate buffers (pD 7) and in CH_3OD was too fast to observe. Breslow (1957) measured the half-life of this compound in unbuffered neutral D_2O and found a half-life of 20 minutes at 27°.

Observation of the NMR spectra of 1,3-diphenyl-2-imidazolinium chloride showed that the peak corresponding to the proton at the 2-position disappeared with a half-life of 10 minutes, whereas the CH_2 peak adjacent to the positive nitrogens disappeared with a half-life of 30 minutes. These results give the half-life of k_1, $T_2 = 7.5$ minutes ($1/T_2 = {}^1/_{10} + {}^1/_{30}$) and the half-life for k_2, $T_2 = 30$ minutes, where k_1 and k_2 refer to the reactions in Figure 5.

In a parallel experiment the exchange was measured by dissolving the 1,3-diphenyl-2-imidazolinium chloride in tritiated water. The product was *N*-formyl-*N,N'*-diphenylethylene diamine labeled 77% of the maximum for one hydrogen with tritium. The rate constant k_2 was found from the decrease of the ultraviolet spectra to correspond to a half-life of 18 minutes. Assuming steady-state kinetics (exact kinetics give the same results) for the concentration of 2-tritio-1,3-diphenyl-2-imidazolium ion and using both the values for the per cent labeling of the product and the rate constant k_2 it is possible to calculate a half-life of 5.5 minutes for the exchange reaction. The observed rates show fair agreement with those obtained by NMR, allowing for a solvent isotope effect.

The reaction of *N*-methylbenzaltoluidinium iodide in tritiated water showed no detectable exchange. An estimation of the minimum exchange which could be detected by the experiment may be based on the error limits of 0 ± 15 (obtained by the square root of the sum of the squares of the error of product and blank) disintegrations per minutes per 23.9 mg. A total exchange of 1 H of *N*-methylbenzaltoluidinium iodide with the tritiated water used and hydrolysis afterward would yield toluene with 9×10^8 disintegrations/g/minute. So the relation of exchange to hydrolysis under the described conditions was equal to or larger than 1 to 7×10^6.

Experiments with *N*-methyl-pyridinium iodide did not show any anion formation in 2-position, in agreement with Kursanov and Setkina (1954). In this compound carbene character of the carbanion is at the expense of the aromatic resonance. Likewise, no hydrogen exchange could be detected at the 2-position of imidazole hydrochloride.

DISCUSSION

There are several possible explanations for faster exchange of the hydrogen at the 2-position of the thiazolium ring over exchange through the usual nitrogen ylids. Important resonance structures (Fig. 6) include the carbene (I), sulfur expansion (II), and both carbene and sulfur expansion (III). Breslow considered these structures and ruled out (II) on the basis of steric strain.

Another factor is the hybridization of the bond at the 2-position. Bonds rich in S-character are known to be more acidic. However, since no exchange can be detected from the hydrogen at the 4-position of 3-methylthiazolium iodide whereas the hydrogen at the two position exchanges quite rapidly, the SP^2 hybridization must not be of great importance.

The observation that oxazolium salts exchange faster than thiazolium salts at the 2-position is in disagreement with theoretical linear combinations of atomic orbitals–molecular orbital (LCAO–MO) calculations which predicted that oxazolium salts are not catalytic because they form pseudo bases instead of a carbanion formation (Pullman and Spanjaard, 1961). This result is an indication that the sulfur is unimportant in the ylid formation because the electronegativity of the oxygen overbalances expansion of the sulfur shell. This is not true in reactions where expansion of the sulfur shell is important. For example, butyl lithium will add to phenylvinylthioether but there is no reaction with the corresponding oxygen analog (Parham and Motter, 1959).

The fast exchange rate with the 3,4-dimethyloxazolium iodide indicates it might be a good catalyst. However, no benzoin was formed from benzaldehyde and, in fact, no reaction could be detected between 3,4-dimethyloxazolium iodide and benzaldehyde. Presumably the anion is so stable that it will not even condense with benzaldehyde.

The observation that *N,N*^{1}-diphenylimidazolinium ion exchanges at approximately the same rate as thiazolium ion indicates again that structure (I), carbene character, is important but that sulfur is unimportant. Carbene character is known to be important in this carbanion because it will add to tetracyanoethylene to form a cyclopropane derivative characteristic of carbene-olefin reactions (Wanzlick, 1962). The comparison of the rapid exchange of thiazole and oxazole with this known carbene leads to the conclusion that the thiazolium and oxazolium anions are also strongly stabilized by carbene character.

Both these comparisons indicate the unimportance of sulfur in ylid formation and the importance of structure (I). In order to stress the importance of the double bond so that structure (I) is possible, the term "ylenid" should be substituted for "ylid."

The function of sulfur is partially answered by the great instability of *N*-methylbenzaltoluidinium ion. Quaternized Schiff bases are extremely unstable but can be stabilized by the resonance of the thiazolium ring. Sulfur tends to act partially like a double bond (Lonquet-Higgins, 1948) and thus adds stability to the thiazolium ring. The lack of exchange in *N*-methylbenzaltoluidinium ion is tentatively ascribed to a lack of stability of singlet carbene structures in acyclic systems. Evidence for this proposal will be given in a future publication.

Although this investigation found that *N,N*-diphenyl-

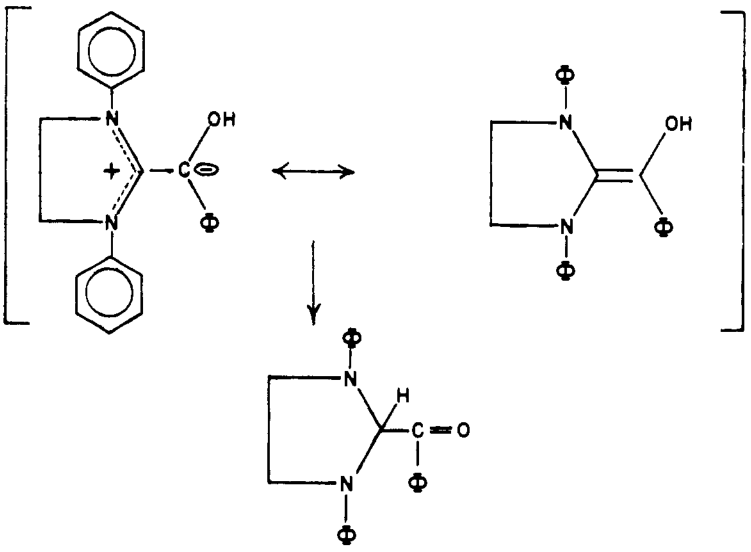

FIG. 7.—Product of the reaction of 1,3-diphenylimidazolinium chloride with benzaldehyde.

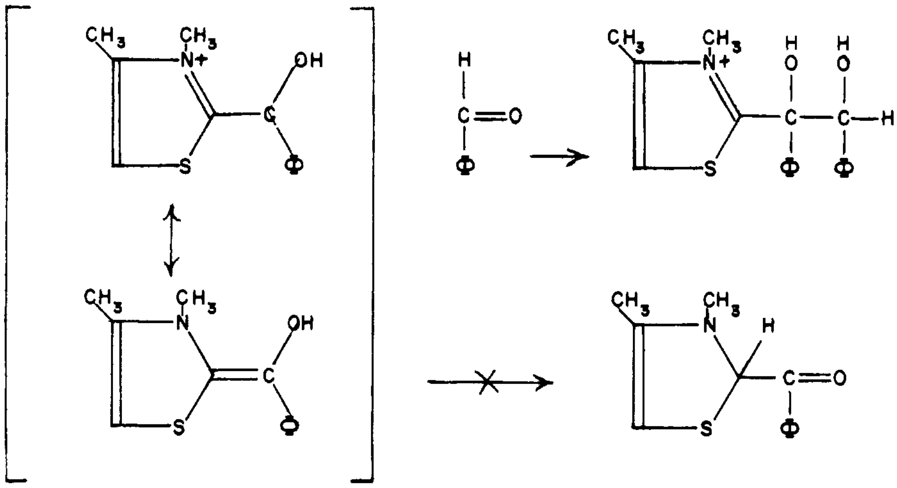

FIG. 8.—Product of the reaction of 3,4-dimethylthiazolium iodide with benzaldehyde.

imidazolinium iodide exchanges the proton at the 2-position quite readily, Wanzlick (1962) found that the corresponding carbene was a poor catalyst. The main product with benzaldehyde was the product VI in Figure 7. This product must have arisen because carbanion IV has a large amount of enol character V. The carbanion therefore acts as an enol and rearranges to VI instead of condensing with another molecule of benzaldehyde.

The corresponding reaction does not occur in the thiazolium salts (see Fig. 8) because there is less enol character in the carbanion. The carbon-nitrogen double bond tends to be held in the ring by the partial aromatic character of the thiazolium ring in which sulfur again acts as a partial double bond.

Thus the partial double bond character is the property of sulfur responsible for its role in stabilizing both the ring and the second carbanion. If a double bond is necessary it should be possible to insert an actual double bond instead of sulfur and use a pyridinium ring instead of thiazolium ring as a benzoin condensation catalyst. However, in pyridinium salts the resonance is too great. The ring is quite stable but no measurable exchange occurs at the α-position because carbene formation is at the expense of the aromatic resonance. A good catalyst requires some resonance for stability and second carbanion formation but not enough to prevent carbene character, and sulfur appears to fill this role.

It is clear that nature has done a good job of designing the thiazolium portion of the thiamine molecule.

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The Participation of the Amide Group in the Solvolysis of Phosphoric Acid Esters.

II. Phosphotriesters in Neutral Ethanolic and Aqueous Media*

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The syntheses of phosphotriesters of *N*-acylethanolamine, and *N*-acetylserinamide are recorded. Treatment of these triesters in ethanolic potassium acetate at 78° results in the quantitative formation of a Δ^2-oxazoline or dihydrooxazine and a phosphodiester ion. The solvolysis of these triesters proceeds equally well in aqueous media (*p*H 4–7). The finding that, under both sets of conditions, the rate of expulsion of the phosphodiester fragment is independent of potassium acetate concentration or *p*H supports the hypothesis that the facile solvolysis is due to the effective anchimeric assistance of the *un-ionized amide (peptide) function.* In ethanol, formation of diphenylphosphate ion from the serine derivative IVb occurs 1200 times faster than from the simple phosphotriester ethyl diphenylphosphate.

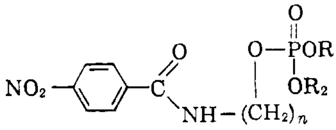
While many of the functional groups located in the amino acid side chains of proteins have been implicated in the catalytic action of enzymes, little direct evidence has been adduced so far to support the possible role of the peptide bond in these processes.

The amide group has long been known to be able to participate in displacement reactions, by nucleophilic attack of either the carbonyl oxygen or the amide nitrogen atom. Much of the early literature has been reviewed by Winstein and Boschan (1950) and more recently by Cohen and Witkop (1961) and Bruice (1962). As a result of attempts to devise model systems for the catalytic center of esteratic and proteolytic enzymes, particular attention has been focused upon intramolecular reactions of amide groups and carboxylic acid derivatives. Bernhard *et al.* (1962) have investigated the unusually rapid alkaline hydrolysis of the β-benzyl esters of carbobenzoxyaspartylserinamide and related compounds. The mechanism of these reactions appears to involve intramolecular participation of the amide anion, leading to the transient formation of succinimide derivatives. Similar cyclic intermediates had earlier been reported to be formed during the alkaline solvolysis of carbobenzoxy-L-aspartamine methyl ester and carbobenzoxy-L-glutamine methyl ester (Sondheimer and Holley, 1954, 1957). In an extension of these studies, Adler *et al.* (1963) have described the conversion of poly-β-benzyl-L-aspartate to polysuccinimide by catalytic amounts of sodium methoxide. On the other hand, poly-γ-benzyl-L-glutamate gave rise to the sodium salt of D,L-2-pyrrolidone-5-carboxylic acid in the presence of stoichiometric sodium methoxide. The solvolysis of the amide bond may itself be accelerated by a neighboring amide function, both under acidic (Cohen and Lipowitz, 1961) and alkaline (Morawetz and Otaki, 1962) conditions.

The study of the influence of the amide (peptide) function in the solvolysis of esters of phosphoric acid is of interest in connection with catalytic mechanisms. Furthermore, information pertinent to the chemistry of phosphoproteins may also be derived. In a previous communication (Zioudrou and Schmir, 1963), the facile solvolysis of certain phosphotriesters in alkaline media was shown to occur via intramolecular participation of a neighboring amide group (in its anionic form). The present report describes the results of a study of the solvolysis in neutral ethanolic and aqueous media of phosphotriesters derived from serine and ethanolamine. Efficient assistance to the solvolytic process has been found to be provided by the *un-ionized amide group.*

RESULTS

Earlier studies (Zioudrou and Schmir, 1963) have demonstrated the ready transformation of phosphotriesters of type I to heterocyclic compounds II and phosphodiesters, in the presence of alkoxide ion at room temperature. It has now been observed that



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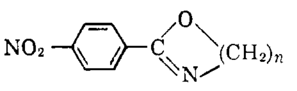

II

Ia $R_1 = R_2 = C_6H_5$ $n = 2$ IIa $n = 2$
Ib $R_1 = R_2 = C_6H_5CH_2$ $n = 2$ IIb $n = 3$
Ic $R_1 = R_2 = C_6H_5$ $n = 3$
Id $R_1 = C_6H_5$ $n = 2$
R_2 = 2-*p*-nitrobenzamidoethyl

treatment of such phosphotriesters with ethanolic potassium acetate at 78° results in the formation of the same products. High yields (80–95%) of Δ^2-oxazoline IIa and of the dihydroöxazine IIb have been isolated from triesters Ia and Ic, respectively; in both cases, diphenyl hydrogen phosphate was also obtained in

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