[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

1,2,4-Triazine Analogs of the Natural Pyrimidines

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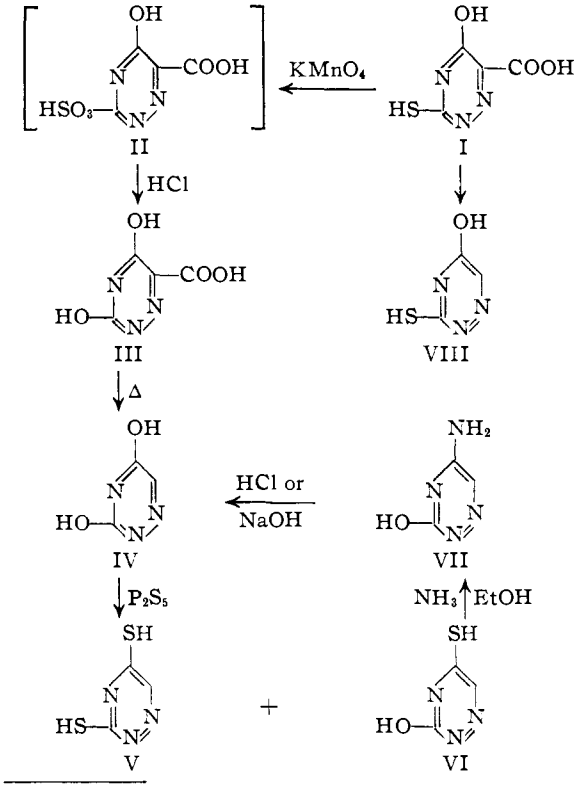
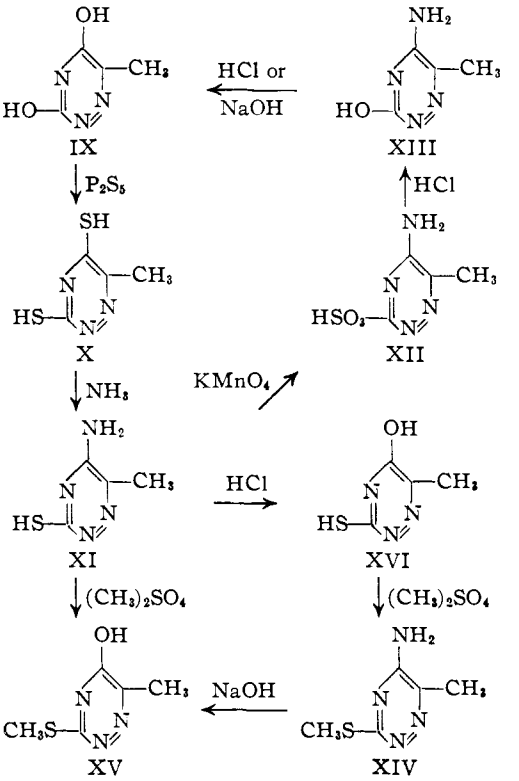
RECEIVED SEPTEMBER 23, 1955

A number of 1,2,4-triazines, including those structurally related to thymine, cytosine, 5-methylcytosine and uracil, have been synthesized. Both 3,5-dihydroxy- and 3,5-dihydroxy-6-methyl-1,2,4-triazine (6-azauracil and 6-azathymine, respectively) served as intermediates for transformation reactions leading *via* the 3,5-dimercapto derivatives to mercaptoamino, hydroxyamino and diamino derivatives. The amino groups of the triazine analogs of cytosine and 5-methylcytosine are extremely labile.

The present paper deals with two series of 1,2,4-triazines which were prepared in connection with a broad program dealing with the study of antagonists of nucleic acid derivatives.[1-3] A number of useful antagonists had been found among 5-substituted uracils, several years ago.[4] Thus, 5-bromouracil was found to be an antagonist of thymine[4] and 5-hydroxyuracil a competitor of uracil.[3,4] The possibility that the 1,2,4-triazine analogs of the pyrimidines might be suitable antagonists of the natural pyrimidines was first investigated in connection with studies on 2,4-diaminopyrimidines.[5] The analogs of thymine and 5-methylcytosine and more recently those of uracil, cytosine and a considerable variety of related and intermediate substances have been prepared in the further extensions of these studies. Meanwhile, 3,5-dihydroxy-6-methyl-1,2,4-triazine ("6-azathymine") has been rather intensively studied by Welch and his colleagues.[6-8]

REACTION SCHEME





Neither 6-azathymine (IX) nor 6-azauracil (IV) is a new substance. The former was first prepared by a rather round-about method by Thiele.[9] Later it was prepared by desulfurization of the 3-mercapto-5-hydroxy derivative XVI which is formed by the cyclization of the thiosemicarbazone of pyruvic acid.[10] Azauracil was synthesized first from the semicarbazone of glyoxylic ester.[11] It has now been prepared by a rather more fruitful and accessible route. Oxomalonic ester was condensed with thiosemicarbazide, and the resulting thiosemicarbazone was cyclized to 3-mercapto-5-hydroxy-1,2,4-triazine-6-carboxylic acid (I). De-

(1) G. B. Elion and G. H. Hitchings, *J. Biol. Chem.*, **185**, 651 (1950).

(2) G. H. Hitchings, G. B. Elion and E. A. Falco, *ibid.*, **185**, 643 (1950).

(3) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *ibid.*, **183**, 1 (1950).

(4) G. H. Hitchings, E. A. Falco and M. B. Sherwood, *Science*, **102**, 251 (1945).

(5) G. H. Hitchings, A. Maggiolo, P. B. Russell, H. VanderWerff and I. M. Rollo, THIS JOURNAL, **74**, 3200 (1952).

(6) W. H. Prusoff and W. L. Holmes, *Federation Proc.*, **11**, 271 (1952).

(7) W. H. Prusoff, *ibid.*, **12**, 358 (1953).

(8) M. T. Hakalu, W. H. Prusoff and A. D. Welch, *ibid.*, **13**, 223 (1954).

(9) J. Thiele and J. Bailey, *Ann.*, **303**, 82 (1898).

(10) J. Bougault and L. Daniel, *Compt. rend.*, **186**, 1216 (1928).

(11) W. Seibert, *Ber.*, **80**, 498 (1947).

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF 1,2,4-TRIAZINES

1,2,4-Triazine	pH 1 Max., mμ	pH 1 E_m	pH 1 Min., mμ	pH 1 E_m	pH 11 Max., mμ	pH 11 E_m	pH 11 Min., mμ	pH 11 E_m
3-OH-5-SH-6-COOH	270	18,300	300	4,500	265	20,600		
	330	6,400						
3,5-Di-OH-6-COOH	273	8,900			260	5,490		
3-OH-5-SH	243	4,640	262	2,258	245	7,050	280	3090
	325	11,400			330	9,410		
3-SH-5-OH	265	12,000			260	12,680		
3,5-Di-SH	275	18,100	305	9,700	270	14,500		
	320	10,100			310–320	6,900		
3,5-Di-OH	258	5,200			250	5,140		
5-NH_2-3-OH	275	10,520			267	9,120		
3,5-Di-SH-6-CH_3	275	22,050	300	10,310	265	16,700	300	7550
	315	11,500			318	8,250		
5-NH_2-3-OH-6-CH_3	278	4,790			252	5,850		
5-NH_2-6-CH_3-3-CH_3S	250	35,000			240	11,500[e]	280	4300
					295	4,670		
5-NH_2-3-SH-6-CH_3	270–275	24,200			265	18,750	300	2480
					325	2,960		
5-OH-6-CH_3-3-CH_3S	235	20,400			287	3,290	270	1800
5-OH-6-CH_3-3-SO_3H	240	[a]	250		240	[b]	260	
	275				290			
5-NH_2-6-CH_3-3-SO_3H	250	[c]			240	[d]	260	
					290			
3,5-Di-OH-6-CH_3	263	7,000			252	7,300		
5-OH-3-SH-6-CH_3	268	17,000			262	36,000		

[a] Concentration unknown; O.D. at 240 mμ/O.D. at 275 mμ = 0.65. [b] Concentration unknown; O.D. at 240 mμ/O.D. at 290 mμ = 1.56. [c] Concentration unknown. [d] Concentration unknown; O.D. at 240 mμ/O.D. at 290 mμ = 1.69. [e] Inflection.

carboxylation of this and of the dihydroxy derivative led to the 6-unsubstituted derivative (IV, VIII). The usual hydrolytic methods for replacement of mercapto by hydroxyl were unsuccessful with the 6-carboxylic acid I and recourse was had to oxidative procedures.

The transformation reactions and the interrelations of the derivatives are summarized in the reaction scheme.

Both dihydroxy-1,2,4-triazines (IV, IX) served as intermediates for the preparation of mercapto and amino derivatives. In both instances treatment with phosphorus pentasulfide to produce mercaptan intermediates was found to be preferable to chlorination as a route to the amino derivatives. The major product with azauracil was a hydroxymercapto derivative VI different from that previously obtained by decarboxylation of 3-mercapto-5-hydroxy-1,2,4-triazine-6-carboxylic acid (I), and therefore presumably the 3-hydroxy-5-mercapto derivative (VI).

Azathymine gave a dimercapto derivative X which reacted with ammonia to form an aminomercapto derivative XI. The latter was readily hydrolyzed to the same hydroxymercapto derivative XVI which is formed by the cyclization of pyruvic acid thiosemicarbazone. The lability of the amino group in this derivative prevented the replacement of the mercapto group by hydroxyl through the usual hydrolytic procedures. However, this end was accomplished through oxidation *via* the 3-sulfonic acid which could be isolated but was too unstable to permit purification (XII).

In general the properties, both physical and chemical, of the 6-azapyrimidines resemble those of the pyrimidines closely. This is particularly marked with respect to the ultraviolet absorption spectra (Table I). The most notable differences between triazines and pyrimidines are to be found in the instability of the 5-amino group in the hydroxy and mercaptoamino derivatives. Whereas both cytosine and 5-methylcytosine are stable to boiling in 6 *N* hydrochloric acid,[12,13] the corresponding azapyrimidines are exceedingly unstable. Relevant kinetic data for the hydrolysis of the two azacytosines are supplied in Table II.

TABLE II

DEAMINATION[a] OF 5-AMINO-1,2,4-TRIAZINES BY ACID AND BASE

Compound	0.01 *N* soln.	Time, min.	Deamination, %
6-Azacytosine	NaOH	5	39
	NaOH	10	58
	HCl	10	43
	HCl	30	75
	HCl[b]	3 days	54
6-Aza-5-methylcytosine	NaOH	5	40
	NaOH	10	71
	NaOH	30	92
	HCl	10	87
	HCl	30	100

[a] Samples were diluted to a concentration of 100 mg./l. and aliquots were heated in a boiling water-bath. The deamination was followed by changes in the ultraviolet absorption spectra at 275 and 258 mμ for azacytosine and 280 and 265 mμ for the methyl derivative; the hydrolysis of the amino group was accompanied by a shift to the lower wave length. [b] The mixture was kept at room temperature.

(12) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **29**, 492 (1903).
(13) H. L. Wheeler and T. B. Johnson, *ibid.*, **31**, 591 (1904).

It is seen that the 6-methyl derivative is somewhat more stable than the unsubstituted.

Acknowledgments.—The authors are indebted to Samuel W. Blackman, Mrs. Veronica Purdy and P. R. W. Baker for microanalyses.

Experimental

5-Hydroxy-3-mercapto-1,2,4-triazine-6-carboxylic Acid (I).—To 80 g. (0.485 mole) of diethyl oxomalonate was added 55 g. (0.6 mole) of thiosemicarbazide and 2.5 liters of 95% ethanol, and the mixture was heated for 24 hours at reflux temperature. The ethanol was removed *in vacuo* and the residual solid extracted with one liter of ether. The ether was dried over sodium sulfate and evaporated to dryness. The yield was 75 g. of the yellow semi-crystalline thiosemicarbazone of diethyl oxomalonate. A portion recrystallized from ether gave shiny yellow plates, m.p. 108–109°.

Anal. Calcd. for $C_8H_{13}N_3O_4S$: C, 38.9; H, 5.26. Found: C, 38.5; H, 5.25.

The remaining 70 g. of crude thiosemicarbazone was heated with two equivalents of 1 *N* sodium hydroxide on the steam-bath for 6 hours. The reaction mixture was adjusted to *p*H 1 with 6 *N* hydrochloric acid and evaporated to dryness *in vacuo*. The residue was taken up in about 200 ml. of cold water and filtered. The yield was 36 g. of a pale yellow compound which, after recrystallization from water, melted at 222–224°.

Anal. Calcd. for $C_4H_3N_3O_3S \cdot H_2O$: C, 25.13; H, 2.62. Found: C, 25.17; H, 2.26.

5-Hydroxy-3-mercapto-1,2,4-triazine (VIII).—To 1 g. of 5-hydroxy-3-mercapto-1,2,4-triazine-6-carboxylic acid was added 5 ml. of diphenyl ether and the mixture was heated in a bath for 10 minutes at 180–185°. The reaction mixture was cooled, filtered, and the precipitate washed with diethyl ether. Recrystallization from ethyl acetate afforded a pale yellow powder, m.p. 227–228°.

Anal. Calcd. for $C_3H_3N_3OS$: C, 27.91; H, 2.33; N, 32.6; S, 24.81. Found: C, 28.03; H, 2.69; N, 33.03; S, 25.34.

3,5-Dihydroxy-1,2,4-triazine-6-carboxylic Acid (III).—This compound may be prepared by either of two methods. The first method, oxidation with nitric acid, tends to give variable yield. The permanganate method is better suited to large scale preparation.

A. Oxidation with Nitric Acid.—To 7.6 g. of the 3-mercapto compound was added 45 ml. of 8 *N* nitric acid and the mixture was allowed to react spontaneously. After the reaction ceased the mixture was cooled and the precipitated 3,5-dihydroxy-1,2,4-triazine-6-carboxylic acid was removed and washed with water (50 ml.). After one recrystallization from water the colorless powder melted at 238–239°. The yield was 5 grams.

Anal. Calcd. for $C_4H_3N_3O_4$: C, 30.6; H, 1.91. Found: C, 30.4; H, 2.00.

B. Oxidation with Alkaline Permanganate.—To 4.0 g. of 5-hydroxy-3-mercapto-1,2,4-triazine-6-carboxylic acid in 20 ml. of 1 *N* sodium hydroxide was added 7.3 g. of potassium permanganate previously dissolved in 1 liter of water. The mixture was allowed to stand 10 minutes and about 10 ml. of methanol was added to remove the excess permanganate. The manganese dioxide was removed, washed with water and the filtrates adjusted to a *p*H value of 1 with 6 *N* hydrochloric acid. The filtrate was then concentrated to a volume of 100 ml. and cooled. The compound (2.9 g.) obtained is identical with the acid described above.

3,5-Dihydroxy-1,2,4-triazine (6-Azauracil) (IV).—The carboxylic acid (2.9 g.) described above was heated in 29 ml. of diphenyl ether for 30 minutes at 190–210°. The mixture was cooled, the precipitate removed, and washed several times with diethyl ether. It was purified by sublimation *in vacuo* (200° (1 mm.)) to give a white compound (1.2 g.) melting at 277–279°.

Anal. Calcd. for $C_3H_3N_3O_2$: C, 31.9; H, 2.65. Found: C, 31.9; H, 2.58.

Thiation of 3,5-Dihydroxy-1,2,4-triazine.—To 5 g. of azauracil (IV) was added 15 g. of freshly ground phosphorus pentasulfide and 50 ml. of dry pyridine. After heating this mixture at reflux temperature for 3 hours, the pyridine was removed *in vacuo* and the residue taken up in 100 ml. of cold water. The aqueous material was adjusted to a *p*H value of 9 by the addition of 2 *N* sodium hydroxide, and shaken with ether (3 × 100 ml.) to remove the pyridine. The aqueous layer was then adjusted to a *p*H value of 3 with 2 *N* hydrochloric acid and then shaken several times with 100-ml. portions of ether. The ether extract was dried over sodium sulfate and allowed to evaporate to dryness on the steam-bath to give 4 g. of a bright orange powder. The 4 g. of crude material was dissolved in 230 ml. of 0.02 *N* sodium hydroxide and put on a Dowex 1 (formate) column, 4 cm. × 16 cm. After washing with water (50 ml.) the column was eluted with 0.025 *N* formic acid (3–4 liters). The eluate was neutralized by the addition of 2 *N* sodium hydroxide and concentrated *in vacuo* to 200 ml. The concentrated solution was then adjusted to *p*H 1 and shaken three times with 100 ml. of ether. The ether was dried over sodium sulfate and allowed to evaporate to dryness. The residue (2.8 g.) a bright orange powder, melted at 213–214° after recrystallization from ethyl acetate. Comparison of its ultraviolet absorption spectrum with that of the isomeric compound VIII (see Table I) led to the identification of the compound as **3-hydroxy-5-mercapto-1,2,4-triazine (VI).**

Anal. Calcd. for $C_3H_3N_3OS$: C, 27.9; H, 2.33; S, 24.8. Found: C, 28.2; H, 2.1; S, 23.9.

Further elution of the column with 0.1 *N* formic acid (3–4 liters) followed by treatment similar to that for the first fraction afforded 400 mg. of a brilliant orange compound which melted at 209–210° after recrystallization from 50% ethanol.

Anal. Calcd. for $C_3H_3N_3S_2$: C, 24.8; H, 2.07; S, 44.1. Found: C, 24.5; H, 2.1; S, 43.9.

This compound was identified as **3,5-dimercapto-1,2,4-triazine (V).**

5-Amino-3-hydroxy-1,2,4-triazine (Azacytosine) (VII).—To 200 mg. of 3-hydroxy-5-mercapto-1,2,4-triazine was added 50 ml. of alcoholic ammonia (saturated at 0°). This mixture was heated in a sealed tube at 143° for 16 hours and then the tube contents were allowed to evaporate to dryness on the steam-bath. The residue was taken up in boiling methanol and deposited beige needles on cooling. The compound does not melt at 320°.

Anal. Calcd. for $C_3H_4N_4O$: C, 32.1; H, 3.57; N, 50.0. Found: C, 32.31; H, 3.77; N, 50.3.

Stability of Azacytosine and 5-Methylazacytosine to Acid and Base. Stability to Acid.—Ten milligrams of azacytosine was dissolved in 100 ml. of cold 0.01 *N* hydrochloric acid. Aliquots (10 ml.) were heated in lightly stoppered tubes in a boiling water-bath for 10 and 30 minutes, cooled and the ultraviolet absorption spectra were recorded; a third aliquot was held at room temperature (25–28°) for three days. Similar studies were made with 5-methylazacytosine.

Stability to Base.—Ten milligrams of compound was dissolved in 100 ml. of cold 0.01 *N* sodium hydroxide and 10-ml. portions were heated 5 and 10 minutes, respectively, and a sample was held at room temperature for three days. The data of the above experiments are recorded in Table II.

3,5-Dimercapto-6-methyl-1,2,4-triazine (X).—To 10 g. of 3,5-dihydroxy-6-methyl-1,2,4-triazine (azathymine) were added 30 g. of freshly ground phosphorus pentasulfide and 100 ml. of tetrahydronaphthalene. This mixture was stirred at 180–190° for three hours and then cooled. The precipitate was filtered and washed with petroleum ether. The precipitate was decomposed by the addition of 250 ml. of water and then the aqueous solution shaken three times with 200 ml. of ether. The ether was dried over sodium sulfate and then allowed to evaporate to dryness on the steam-bath. The orange residue (7.5 g.) was crystallized from boiling water to form orange plates melting at 217–218°.

Anal. Calcd. for $C_4H_5N_3S_2$: C, 30.2; H, 3.14. Found: C, 30.37; H, 3.13.

The ultraviolet absorption data are given in Table II.

5-Amino-6-methyl-3-mercapto-1,2,4-triazine (XI).—Treatment of the above dimercapto compound X with alcoholic ammonia (saturated at 0°) at 122° for 16 hours gave a quantitative yield of a compound which may be dissolved in cold, very dilute acid and precipitated with cold, very dilute alkali at *p*H 7. The compound forms beige needles from water

which darken at 270° and do not melt at 310°. Heating this compound, 10 mg. in 2 ml. of 2 *N* sodium hydroxide, gives a product with an ultraviolet absorption spectrum identical with that previously found for 3-mercapto-5-hydroxy-6-methyl-1,2,4-triazine.

Anal. Calcd. for $C_4H_6N_4S$: C, 33.8; H, 4.22; N, 39.4. Found: C, 33.94; H, 4.44; N, 39.13.

5-Amino-3-hydroxy-6-methyl-1,2,4-triazine (6-Aza-5-methylcytosine) (XIII).—Ten grams of the above compound was dissolved in 500 ml. of 0.02 *N* sodium hydroxide. To this was added slowly 12.5 g. of potassium permanganate dissolved in 250 ml. of water. The manganese dioxide was removed, washed well with water and neutralized by the addition of glacial acetic acid. The neutral solution was taken to dryness *in vacuo*. The residue was recrystallized from about 50 ml. of water, to give 5.7 g. of a colorless material XII. To 500 mg. of this white crystalline material was added 11.5 ml. of 0.4 *N* hydrochloric acid and the mixture was allowed to stand at room temperature for two days. An odor of SO_2 was detected and white crystals slowly deposited. Those (200 mg.) were collected and washed with a few ml. of water. The compound was recrystallized from 5 ml. of water and melted with decomposition at 327°.

Anal. Calcd. for $C_4H_6N_4O$: C, 38.1; H, 4.76; N, 44.4. Found: C, 38.6; H, 4.76; N, 44.87.

5-Amino-6-methyl-3-methylmercapto-1,2,4-triazine (XIV).—To 5 g. of 5-amino-3-mercapto-6-methyl-1,2,4-triazine in 100 ml. of 0.35 *N* alkali there was added with shaking 3.4 ml. of dimethyl sulfate over a period of 30 minutes. The precipitate which formed was removed and crystallized from methanol–ether–petroleum ether to form colorless plates melting at 164–165°.

Anal. Calcd. for $C_5H_8N_4S$: C, 38.5; H, 5.1. Found: C, 38.1; H, 4.92.

Treatment of 5-Amino-6-methyl-3-methylmercapto-1,2,4-triazine with 0.1 *N* Sodium Hydroxide.—To 500 mg. of the above compound there was added 20 ml. of 0.1 *N* sodium hydroxide and this mixture was boiled for 90 minutes at reflux temperature (until the evolution of ammonia ceased). A pale yellow crystalline compound was obtained which melted at 224–225°. The ultraviolet absorption spectrum is identical with that of the compound given below.

5-Hydroxy-6-methyl-3-methylmercapto-1,2,4-triazine (XV).—The 5-hydroxy-3-mercapto-6-methyl-1,2,4-triazine (20 g.) was added to 261.5 ml. of 0.114 *N* NaOH and methyl iodide (19.9 g.) was added portionwise with shaking, over one hour. After standing an additional hour the reaction mixture was adjusted to *p*H 5 by the addition of acetic acid. The precipitate which formed on cooling (13.3 g.) was recrystallized from boiling water; m.p. 222–223° dec.

Anal. Calcd. for $C_5H_7N_3OS$: C, 38.2; H, 4.45. Found: C, 37.9; H, 4.8.

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[CONTRIBUTION FROM LOS ALAMOS SCIENTIFIC LABORATORY, UNIVERSITY OF CALIFORNIA]

Oxazole Quaternary Salts[1]

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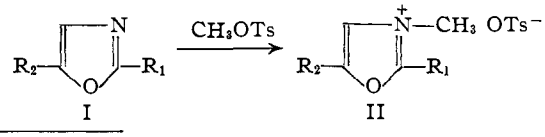
RECEIVED SEPTEMBER 29, 1955

2,5-Disubstituted-3-methyloxazolium salts have been prepared for pharmacological investigations of their notable inhibition of thermoregulation in animals. General procedures are described for the anions tosylate, perchlorate, iodide and chloride. Hydrolytic studies on the oxazolium ring ion have been carried out. Ultraviolet absorption maxima and representative infrared absorption data are given.

The original interest in 3-methyloxazolium salts in the Biomedical Research Group of this Laboratory arose in connection with an extensive study of liquid solution scintillators. Observations[2] that certain of these compounds seemed to produce specific inhibition of thermoregulation in animals led to the synthesis of a number of additional salts. It appears that a new, previously overlooked series of compounds has been opened to pharmacological research.

In Table I are listed the new oxazolium salts, as well as 2,5-diphenyl-3-methyloxazolium iodide[3] and those given in an earlier communication.[4] The methyl *p*-toluenesulfonate salts (tosylates) are readily formed by heating the oxazole with an excess of methyl tosylate. Salts containing other anions were prepared by taking advantage of solubility characteristics. Oxazolium perchlorates precipitate when an aqueous solution of the tosylate is treated with perchloric acid. The perchlorate salts are soluble in acetone, and therefore the metathetical reaction with sodium iodide may be used to produce the acetone-insoluble iodides. Chloride salts may be prepared from the iodides through consideration of the difference in solubility of silver iodide ($K_{sp} = 1.5 \times 10^{-16}$) and silver chloride ($K_{sp} = 1.0 \times 10^{-10}$); an aqueous solution of the iodide salt is stirred with a slurry of silver chloride which precipitates silver iodide. The chloride salt is isolated by evaporation of the filtered reaction mixture. Another method which provides methyl chloride salts of amines without the necessity of using sealed reaction vessels and methyl chloride employs an ion exchange resin. An aqueous solution of the tosylate salt is passed through a column of anion exchange resin in the chloride form; the chloride salt is isolated by evaporation of the eluate. Presumably, this procedure is applicable to amines in general.



I II

In acidic solution the oxazolium salts are quite stable, being only slightly decomposed by refluxing for 8 hours with 48% hydrobromic acid. In dilute base, however, they are rapidly and quantitatively converted at room temperature to the N-methyl-α-acylamido ketone by hydrolytic ring

$$\text{II} \xrightarrow[(HO^-)]{H_2O} R_2COCH_2N(CH_3)COR_1$$

IV

(1) Work performed under the auspices of the U. S. Atomic Energy Commission.

(2) C. C. Lushbaugh, F. N. Hayes, W. H. Langham, D. G. Ott and P. C. Sanders, *J. Pharm. Exptl. Therap.*, **116**, February, (1956).

(3) E. Fischer, *Ber.*, **29**, 208 (1896).

(4) F. N. Hayes, B. S. Rogers and D. G. Ott, THIS JOURNAL, **77**, 1850 (1955).