[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF STANFORD UNIVERSITY]

METALLIC SALTS OF LOPHINE, 1,2,4-TRIAZOLE AND TETRAZOLE

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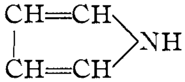
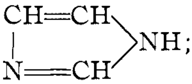
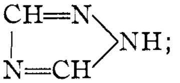
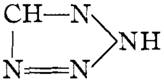
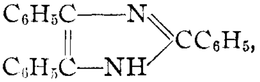
Introduction

When negative groups are substituted for one or two hydrogen atoms of a molecule of ammonia, a compound is obtained which is related to ammonia as the familiar oxygen acids are related to water. Such ammonia derivatives must therefore be regarded as acids of an ammonia system of compounds,[1] and in order to distinguish them from the oxygen or aquo acids, Franklin has called them ammono acids. A number of these ammono acids are known; for example, hydrocyanic acid or ammono carbonous acid, cyanamide or ammono carbonic acid, benzamidine or ammono benzoic acid, etc.

In general, the ammono acids are much weaker than the corresponding aquo acids, as shown by the inability of many of them to form metallic salts in the presence of water. However, by carrying out reactions in liquid ammonia, a poorer solvolytic solvent than water, Franklin[2] has shown that it is possible to prepare metallic salts of the very weak ammono acids pyrrole, indole and carbazole.

The ammono acids just mentioned owe their acid properties to the negative influence of the doubly bonded carbon atoms. Now, since nitrogen is a more electronegative element than carbon, it seems reasonable to expect that the acid properties of a compound such as pyrrole would be accentuated by substituting a nitrogen atom for a CH-member of the ring, while further substitution by nitrogen atoms would produce still stronger acids. Accordingly, pyrrole,  $\left.\begin{array}{l}\mathrm{CH{=}CH}\\ |\\ \mathrm{CH{=}CH}\end{array}\right\rangle\mathrm{NH}$; imidazole,  $\left.\begin{array}{l}\mathrm{CH{=}CH}\\ |\\ \mathrm{N{=}CH}\end{array}\right\rangle\mathrm{NH}$; 1,2,4 triazole,  $\left.\begin{array}{l}\mathrm{CH{=}N}\\ |\\ \mathrm{N{=}CH}\end{array}\right\rangle\mathrm{NH}$; and tetrazole,  $\left.\begin{array}{l}\mathrm{CH{-}N}\\ |\\ \mathrm{N{=}N}\end{array}\right\rangle\mathrm{NH}$ should be ammono acids, their strength increasing in the order given. Furthermore, alkyl and aryl derivatives of the above compounds should be acids, providing one hydrogen atom remains attached to a nitrogen atom, the strength of the resulting acid being dependent upon the negative character of the substituted group. Thus, lophine, triphenylimidazole,  $\left.\begin{array}{l}\mathrm{C_6H_5C{-}N}\\ \|\\ \mathrm{C_6H_5C{-}NH}\end{array}\right\rangle\mathrm{CC_6H_5}$, should be a stronger acid than imidazole.

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[1] Franklin and Stafford (a) *Am. Chem. J.*, **28**, 83 (1902); (b) Franklin, *Eighth Int. Cong. App. Chem.*, **6**, 119 (1912); (c) THIS JOURNAL, **37**, 2279 (1915); (d) **46**, 2137 (1924).

[2] Franklin, *J. Phys. Chem.*, **24**, 81 (1920).

Since, formally at least, the nitrogen compounds just mentioned are ammono acids, they should react with the more electropositive elements and their amides to form salts[1a,c] if the reactions be carried out in a poor solvolytic solvent such as ammonia. These compounds should also show conductivities characteristic of weak acids when dissolved in liquid ammonia.

The following experiments show that imidazole, lophine, triazole and tetrazole react with sodium, calcium and magnesium and with the amides of lithium, sodium, potassium, copper and silver, forming salts, while conductivity measurements indicate that the strength of the acid is dependent upon the negative character of the components of the molecule.

Unless otherwise stated, all salts were prepared in liquid ammonia solution. The methods of manipulation, such as handling the liquid ammonia, drying the salts, determining the ammonia of crystallization and preparing the salts for analysis, were essentially the same as those previously described by Franklin.[3] All the salts thus prepared were unstable in the presence of water, being hydrolyzed to the acid and to the metallic hydroxide.

Metallic Salts of Imidazole

Imidazole dissolves abundantly in liquid ammonia, forming a clear solution from which the imidazole may be obtained by evaporation of the solvent, there being no formation of an ammonium salt. Wenzel,[4] working in this Laboratory, found that imidazole in ammonia solution attacks magnesium slowly, setting hydrogen free and forming a slightly soluble magnesium salt. Wenzel has also prepared the potassium, sodium, calcium and silver salts of imidazole. However, Wyss[5] had previously prepared the silver salt.

Metallic Salts of Lophine

Lophine, prepared by heating hydrobenzamide,[6] is only slightly soluble in liquid ammonia at room temperature, while at —35° it is practically insoluble. Lophine does not form an ammonium salt.

Metallic Salts of 1,2,4-Triazole

Preparation of Triazole.—According to Pellizzari,[7] two molecules of formamide distilled with one molecule of hydrazine hydrochloride form 1,2,4-triazole in poor yields. Substitution of hydrazine sulfate for hydrazine hydrochloride did not increase the yields. It was found, however, that a yield amounting to one-sixth of the theoretical could be

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[3] Franklin, *J. Phys. Chem.*, **15**, 513 (1911).

[4] Wenzel, unpublished work.

[5] Wyss, *Ber.*, **10**, 1373 (1877).

[6] Radziszewski, *Ber.*, **10**, 70 (1877).

[7] Pellizzari, *Gazz. chim. ital.*, **4**, II, 222 (1894), *Ber.*, **27**(R), 801 (1894).

TABLE I

SALTS OF LOPHINE[a]

Metal	Formula	Cryst. form	Color	Solubility	M. calcd.	M. found	N calcd.	N found	Moles of NH_3 of cryst.
Mg[b]	$Mg(C_{21}H_{15}N_2)_2$	...	colorless	sl. sol.	3.96	3.4 3.6	9.3	... 14.3	... ...
Ca[c]	$Ca(C_{21}H_{15}N_2)_2\cdot 6NH_3$ and	Long prisms with pointed ends	colorless	m. sol.	6.4	6.5 6.2	8.9	10.7 9.4	6.0 6.7
	$Ca(C_{21}H_{15}N_2)_2$	white powder				6.4		10.4	5.9
K[d]	$KC_{21}H_{15}N_2\cdot 3NH_3$	...	colorless	v. sol.	11.7	11.6	8.4	...	...
	$KC_{21}H_{15}N_2$					11.3 11.2		8.8 ...	3.5 3.0
Ag[e]	$AgC_{21}H_{15}N_2\cdot 2NH_3$	small prisms	colorless	sl. sol.	26.8	27.4	7.0	7.0	...
	$AgC_{15}H_{15}N_2$	powder	gray			27.3		6.6	2.0
Cu^{++}[f]	$Cu(C_{21}H_{15}N_2)_2\cdot XNH_3$	large cubes or plates	greenish-blue	sl. sol.	9.7	9.5	8.6	8.5	6.2
	$Cu(C_{21}H_{15}N_2)_2$	powder				9.6		8.2	7.5
Cu^{+}[g]	$CuC_{21}H_{15}N_2$	small prisms	light orange	sl. sol.	17.7	17.1	7.8	7.8	None
Li[h]	$LiC_{21}H_{15}N_2\cdot XNH_3$	large prisms	colorless	v. sol.	2.3	2.0	...	...	not determined
	$LiC_{21}H_{15}N_2$								
Na[i]	$NaC_{21}H_{15}N_2$	Not crystallized	...	v. sol.	7.2	8.7	...	...	...

[a] The abbreviations used in this table are: M., metal; N, nitrogen; sol., soluble; sl., slightly; m., moderately; v., very. The percentage compositions of these compounds are all based on the anammonous (dry) salts.

[b] Magnesium and calcium lophine react with liquid ammonia, forming ammono basic salts as indicated by the high nitrogen analyses. If a reducing agent is present, these salts form highly red-colored solutions.

[c] The calcium salt was prepared by the action of lophine on a solution of metallic calcium in liquid ammonia and by the action of lophine on calcium amide.

[d] Prepared by the action of lophine on potassium amide.

[e] Prepared by the action of lophine on silver amide and by the action of potassium lophine on silver nitrate.

[f] Prepared by treating a solution of cupric nitrate with potassium lophine. The vapor pressure of the ammonated salt did not reach a constant value, hence the ammonia of crystallization could not be accurately determined.

[g] Prepared by treating cuprous amide with lophine.

[h] Prepared by the action of lophine on lithium amide.

[i] Prepared by the action of lophine on sodium amide.

obtained by adding two molecules of finely divided potassium hydroxide to the above mixture and allowing it to dissolve completely before distilling.

The distillation was made in a Pyrex flask over a luminous flame, while the distillate was collected in a balloon flask cooled in an ice-and-salt mixture. The semi-solid condensate was extracted with boiling ethyl acetate from which the triazole separated upon cooling. The crystals, after recrystallization from alcohol, melted at 120–121°.

Triazole is very soluble in liquid ammonia, forming a clear solution. Upon evaporation of the solvent, crystals of triazole are obtained, thus showing that triazole does not form an ammonium salt.

Until the following preparations were made, cupric triazole was the only metallic salt of triazole known. It was prepared by Pellizzari and Cuneo[8] as a blue precipitate when triazole was added to a neutral or slightly alkaline aqueous solution of a copper salt.

TABLE II

SALTS OF 1,2,4-TRIAZOLE[a]

Metal	Formula	Cryst. form	Color	Solubility	M. calcd.	M. found	Moles of NH_3 of crystallization
Na[b]	$NaC_2H_2N_3$	...	colorless	v. sol.	25.3	26.4	Not determined
Ag[c]	$AgC_2H_2N_3\cdot NH_3$	tetrahedra	colorless	m. sol.	61.4	60.8	1.0
	$AgC_2H_2N_3$	powder	gray			61.8	...
Mg[d]	$Mg(C_2H_2N_3)_2\cdot 4NH_3$	small prisms	white	sl. sol.	15.1	13.5	4.3
	$Mg(C_2H_2N_3)_2$					12.8	3.7
						15.0	4.1
Ca[e]	$Ca(C_2H_2N_3)_2\cdot XNH_3$	small prisms	white	sl. sol.	22.8	22.6	Not determined
	$Ca(C_2H_2N_3)_2$						
Cu^{+}[f]	$CuC_2H_2N_3\cdot XNH_3$	finely cryst.	white	sl. sol.	48.3	50.4	Not determined
	$CuC_2H_2N_3$						

[a] Since the salts were removed from the reaction tubes in aqueous solution, the Kjeldahl method for the determination of nitrogen was the only one which was applicable. However, it was found that unchanged triazole escapes with the vapors of the acid; hence, no nitrogen determinations were made. The analytical results are based on the anammonous salts.

[b] Prepared by the action of triazole on sodium amide.

[c] Prepared by the action of triazole on silver amide.

[d] Prepared by the action of triazole on magnesium.

[e] Prepared by treating a solution of calcium in liquid ammonia with triazole.

[f] Prepared by the action of triazole on cuprous amide.

Metallic Salts of Tetrazole

The tetrazole used in the following preparations was made from its carboxylic acid which was prepared according to the method of Oliveri-Mandalá and Passalacqua.[9] After the tetrazole had been recrystallized thrice it was deemed sufficiently pure.

Tetrazole is sufficiently acidic so that many of its salts have been pre-

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[8] Pellizzari and Cuneo, *Ber.*, **27**(**R**), 407 (1894).

[9] Oliveri-Mandalá and Passalacqua, *Gazz. chim. ital.*, 41 (II), 430 (1911).

pared in aqueous solution. Thiele and Ingle[10] have reported the sodium and barium salts, Bladin[11] has prepared the silver and copper salts, while Bauer[12] has measured the electrical conductivity of aqueous solutions of free tetrazole.

Ammonium Tetrazole, NH_4CHN_4.—Tetrazole reacts with gaseous ammonia, forming the ammonium salt which is fairly soluble in liquid ammonia. This salt was analyzed by distilling the ammonia from a dilute aqueous solution made alkaline with sodium hydroxide.

Anal. Calcd. for NH_4CHN_4: NH_3, 19.8. Found: 19.5.

Calcium Tetrazole, $Ca(CHN_4)_2$.—Tetrazole was allowed to react with metallic calcium in liquid ammonia solution. The blue color due to the calcium was rapidly dispelled, a quantity of hydrogen almost equivalent to the amount of metal used was rapidly evolved, while long, needle-like crystals were obtained by concentrating the solution.

A second preparation of this salt was made by treating calcium amide with tetrazole, the calcium being determined as oxalate.

Anal. Calcd. for $Ca(CHN_4)_2$: Ca, 22.2. Found: Ca, 21.8.

Conductivity Measurements

The electrical conductances of ammonia solutions of pyrrole, imidazole, triazole and tetrazole[13] were measured in an apparatus similar to that described by Elsey.[14] Lophine is so insoluble in ammonia that a sufficiently concentrated solution could not be obtained for accurate measurements.

In Table III the dilution is given in liters per mole. The values of the equivalent conductivity in Kohlrausch units were obtained by interpolation of the curves plotted from the conductivity measurements.

TABLE III

EQUIVALENT CONDUCTIVITIES OF SOLUTIONS OF PYRROLE, IMIDAZOLE, TRIAZOLE AND TETRAZOLE IN LIQUID AMMONIA

Dilution $\Phi \times 10^{-3}$	8	16	32	64	128	256	512	1024	2048	4096	8192
Pyrrole Λ	0.034	0.044	0.070	...	...	...	...	...	...	...	...
Imidazole Λ	...	0.59	0.70	0.90	1.10	1.40	...	...	...	...	...
Triazole Λ	...	...	60	75	91	115	142	169	197	225	245
Tetrazole Λ	...	...	...	128	151	174	197	217	236	249	259

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[10] Thiele and Ingle, *Ann.*, **287**, 247 (1895).

[11] Bladin, *Ber.*, **25**, 1413 (1892).

[12] Bauer, *Ann.*, **287**, 249 (1895).

[13] The pyrrole used in measuring the conductivity was purified according to the method of Ciamician and Dennstedt [*Ber.*, 19, 173 (1886)]. The imidazole, which was contributed by Wenzel and which he had purified by distillation, was recrystallized from toluene. The triazole and tetrazole were obtained sufficiently pure by recrystallizing portions of the specimens used in preparing the metallic salts.

[14] Elsey, THIS JOURNAL, **42**, 2454 (1920).

The author wishes to take this opportunity to express his sincere thanks to Dr. E. C. Franklin, at whose suggestion and under whose careful and kindly direction this investigation was carried out.

Summary

Any compound which is derived from ammonia by substituting negative groups for one or two of the three hydrogen atoms is an ammono acid.

The strength of the ammono acids is dependent upon the negative character of the components of the molecule; thus, the acidity of pyrrole, imidazole, triazole and tetrazole increases in the order given as shown by the conductivity measurements.

A number of the metallic salts of the ammono acids lophine, triazole and tetrazole have been prepared.

STANFORD UNIVERSITY, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, No. 550]

ESTABLISHMENT OF THE OPTIMAL HYDROGEN-ION ACTIVITIES FOR THE ENZYMIC HYDROLYSIS OF STARCH BY PANCREATIC AND MALT AMYLASES UNDER VARIED CONDITIONS OF TIME AND TEMPERATURE[1]

BY H. C. SHERMAN, M. L. CALDWELL AND MILDRED ADAMS

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The development of our investigation of the amylases and related enzymes makes it important now to know, and to be able experimentally to establish and maintain, the reaction most favorable to the activity of each enzyme under investigation throughout a wider range of time and temperature than is involved in the determination of the so-called diastatic powers, for which conditions only had optimal hydrogen-ion activities been established in the course of the earlier work of this Laboratory.

Experiments were therefore undertaken to establish the optimal hydrogen-ion activities for malt and pancreatic amylases when the temperature and time of enzymic hydrolysis were varied. The data obtained are discussed briefly below.

Experiments and Discussion

The experimental methods of measuring amylase activity have been described in detail in previous papers from this Laboratory.[2]

The slight reducing action of the substrate without enzyme was measured for each of the times, temperatures and hydrogen-ion activities

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[1] We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

[2] Sherman and co-workers, THIS JOURNAL, **41**, 231 (1919); **32**, 1073 (1910); **43**, 2461 (1921); **37**, 623 (1915).