

ON THE MECHANISM OF FLORENCE'S CRYSTAL FORMATION

I. PREPARATION OF NEURINE CHLORIDE

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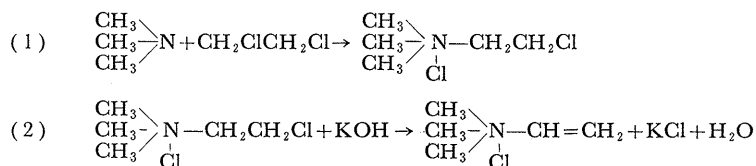
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INTRODUCTION

Presence or absence of only one molecular water makes the difference between choline and neurine. In the past, it was comparatively difficult to distinguish neurine from choline, since, for example, *Baeyer* believed that neurine reported by *Liebreig* might have been mixture of neurine and choline. The preparation of neurine has usually been accomplished by the removal of hydrogen halide from a β -halo-ethyltrimethylammonium salt except in the case of a few methods. It is supposed that the difficulties of preparation and purification of neurine chloride have been greater than those which have attended the synthesis of other neurine halides, since fewer papers have been published concerning the preparation of neurine chloride than with regard to other neurine halides. Of the investigators who have employed bromocholine bromide as intermediate for the preparation of neurine, *Baeyer*, *Hofmann*, *Rensaw* (1) and *Meyer* et al. (2) have made use of moist silver oxide for dehydrobromination of the bromide; *Lucius* (3) and *Rensaw* et al. (4), alcoholic potash; *Schmidt*, barium hydroxide; and *Riley* (5), trisilver phosphate or disilver monophenyl phosphate, respectively. *Baeyer*, *Schmidt* et al., and *Rensaw* (1) have stated that the reaction of silver oxide or alcoholic potash with iodocholine iodide results in the production of neurine. On the contrary, no one seems to have been successful in converting chlorocholine chloride into neurine. Such methods as those involving the removal of hydrogen halide from a β -halo-ethyltrimethylammonium halide were quite suitable and generally used for the preparation of neurine, but there have also been the attempts to synthesize neurine directly without dehydrohalogenation. *Schmidt's* attempts to convert choline into neurine by dehydrating agents such as H_2SO_4 and P_2O_5 were unsuccessful. In their recent paper on acetylen reactions, however, *Gardner* et al. (6) stated that the interaction of aqueous trimethylamine and acetylene under pressure yields neurine only if the reaction is carried out at or below $50^\circ C$. On the other hand, *Schmidt* et al. has found that the reaction of trimethylamine with ethylene bromide yields neurine with some other products when the reaction is carried out in alcohol and at $100^\circ C$. Besides the works mentioned above, biological or biochemical works have been performed by some of the other investi-

gators, but these were never available for the preparation of neurine. For instance, *Schmidt* et al. (7) have reported that the addition of putrescent blood to choline causes to give rise to neurine, *Brieger* (8-11) has observed that neurine results from the putrefaction of the flesh, and *Kutscher* has obtained neurine from *Liebig's* meat extract.

The study of the so-called *Florence's* crystal which is produced in the rectangular rhombic form by the addition of *Florence's* reagent to human semen required that neurine chloride be purely prepared, if possible, without a trace of choline chloride as impurity, since it also reacts with the same reagent to form the similar crystal. The present author attempted to prepare pure neurine chloride, consulting the method of *Krüger* et al. (12) and the method of *Rensaw* et al. (4). In the author's procedure trimethylamine was allowed to react with ethylene chloride to yield chlorocholine chloride, from which hydrogen chloride was later removed by the action of alcoholic potash. The reaction proceeds as follows:



EXPERIMENTAL

Synthesis of Chlorocholine Chloride

In contrast to a larger number of procedures published for the synthesis of bromocholine bromide, only a few contributions have been made to that of chlorocholine chloride. In 1904, *Schmidt* obtained chlorocholine chloride by adding phosphorus oxychloride to choline. *Kaufmann* et al. (13) observed that the reaction of benzenesulfonyl chloride with choline chloride in a common salt bath yielded this compound. In their paper concerning new basic choline-derivatives, *Fränkel* et al. (14) reported a method for the synthesis of chlorocholine chloride, in which the interaction of ethylene chloride with alcoholic solution of trimethylamine at room temperature was employed. In 1931, *Harada* (15) prepared chlorocholine chloride by using trimethylamine hydrogen chloride and ethylene chloride as starting materials. In the case of the present author's work, the following procedure was used.

Vapor phase trimethylamine generated by heating 50 cc. of 39.9% aqueous trimethylamine, dried through a calcium oxide tube, was little by little conducted into 100 cc. of ethylene chloride, which was distributed into three large test tubes connected one another, and which was continually heated at 80°C. in an oil bath. The procedure was carefully continued for three hours under dry conditions

until vapor phase trimethylamine generator was heated to 60°C. to prevent a back-flowing of ethylene chloride into the generator. With such treatment, an increasing turbidity and crystallization of the contents of the test tubes were observed. The contents, including chlorocholine chloride, were cooled by applying ice water until a large amount of snow-white crystals formed. The crystals were separated from mother liquor by suction through a glass filter, washed swiftly with dry ether, recrystallized repeatedly from solvent consisting of absolute alcohol and dry ether, and finally dried over P₂O₅ under reduced pressure. Chlorocholine chloride thus obtained was a needle-like plate, which was very hygroscopic, giving no definite m.p. It finally melted at 243°C. and changed in color into a stained brown. *Harada* (15) gave m.p. 242°C. for chlorocholine chloride. Chlorocholine chloride was freely soluble in water, soluble in methyl alcohol, ethyl alcohol, formic acid and in acetic acid, very slightly soluble in n-amyl alcohol, n-butyl alcohol and in acetone, and essentially insoluble in ether, petroleum ether, benzene, ethyl acetate, chloroform and in carbon tetrachloride. It should be described here that chlorocholine chloride, as well as bromocholine bromide synthesized by the method of *Krüger et al.* (12), reacted with *Florence's* reagent, giving rise to microscopic blade-like crystals, which were to be distinguished from *Florence's* crystal. Chlorocholine chloride also reacted with phosphomolybdic acid, yielding yellow precipitates.

As for the identification of chlorocholine chloride, platinum double salt was prepared by the addition of platinum chloride to chlorocholine chloride. The double salt recrystallized from hot water containing hydrochloric acid, melted and decomposed at the temperature between 250 and 260°C. This is in agreement with the result obtained by *Flänkel et al.* (14). The recrystallized crystals were analysed with the following result:

Subst.: 0.2322g., Pt: 0.0697g.
Found: Pt, 30.02%
Calc. for C₁₀H₂₆N₂Cl₈Pt: Pt, 29.89%

Preparation of Neurine Chloride

The conversion of chlorocholine chloride into neurine chloride was carried out according to the method of *Rensaw et al.* (4) devised for the preparation of neurine bromide. Six g. of pure chlorocholine chloride, covered by 6 cc. of absolute alcohol, were treated with 43.2 cc. of absolute alcohol containing 4.00 g. of commercial potassium hydroxide. The flask was vigorously shaken during the addition of alcoholic potash, and allowed to stand for an hour, by which time dehydrohalogenation was completely carried out, under conditions of excluding moisture from the air. The separation and purification of neurine chloride were far more difficult than those of neurine bromide, but satisfactorily carried out by the following procedure. After the potassium chloride was filtered off by suc-

tion, the filtrate was evaporated under reduced pressure at room temperature to get whitish-yellow mass, and a small amount of water was added to dissolve it. The aqueous solution was neutralised with 4N-hydrochloric acid to pH 6.8, concentrated under reduced pressure at room temperature, and then dried over P_2O_5 in a high vacuum. The dry residue was treated with about four-fold absolute alcohol, and filtered by suction. To the filtrate an equal volume of dry ether* was added. The resulting solution was allowed to stand for a week over P_2O_5 in a dessicator at $0^\circ C$. With such treatment, neurine chloride separated in the large forms of tetragonal prism of first or second order and tetragonal pyramid of first order. Neurine chloride separated from the mother liquor by suction through a glass filter was rapidly washed with dry ether, and dried over P_2O_5 in a high vacuum. In accordance with the conditions pointed out above, neurine chloride was recrystallized. Neurine chloride obtained finally was very hygroscopic, white, tetragonal, and melted at $193-194^\circ C$., changing in color into a stained brown. Gardner et al. (6) gave m.p. $193-194^\circ C$. for neurine chloride. Aqueous solution of neurine chloride showed the properties of neurine, reducing potassium permanganate, reacting with phosphomolybdic acid. The synthetic neurine chloride was freely soluble in water, soluble in methyl alcohol, ethyl alcohol, formic acid and in acetic acid, very slightly soluble in acetone, n-butyl alcohol, chloroform and in n-amyl alcohol, and essentially insoluble in benzene, ether, petroleum ether and in carbon tetrachloride, when extra pure preparations of relevant solvents were used. It should be pointed out that neurine chloride, as well as neurine bromide prepared by the method of Rensaw et al. (4), reacted with Florence's reagent, giving rise to the microscopic rectangular crystals which were quite similar to Florence's crystal.

Any substance is characterized by its infra-red spectra. For this reason, identification of the specimen obtained was performed by infra-red spectroscopy. The spectrogram was obtained on Hilger Recording Infra-red Spectrophotometer H 800 with a NaCl prism. The infra-red sample was prepared as $\frac{1}{2}$ inch KBr pellet weighing approximately 0.15 g. and containing approximately 1 mg. of the specimen obtained. The infra-red spectrum of the specimen obtained is shown in Fig. 1.

The positions of the absorption peaks related to the C=C stretching vibrations, 1643 cm.^{-1} and 1653 cm.^{-1} , are ascribed to the terminal double-bond types $CHR = CH_2$ and $CR^1R^2 = CH_2$, respectively (16). In general, the C=C stretching vibration of the terminal double-bond gives rise to a somewhat intense band, which is lower in frequency than a weaker band pertaining to the C=C stretching vibration of a double bond situated in the middle part of the chain (for instance,

* The solution is liable to separation into two layers in the presence of a minute amount of water contained in ether, or in the presence of ether larger in amount than was specified in the author's procedure.

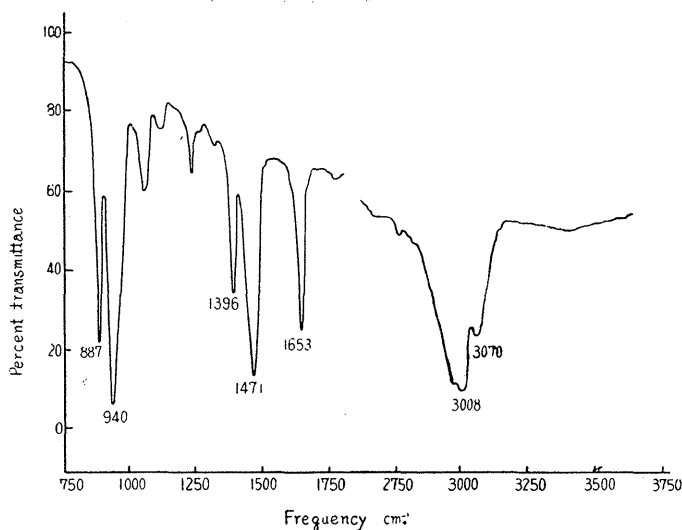


Fig. 1. Infra-red absorption spectrum of neurine chloride.

- 887 cm.^{-1} : δ CH (out-of-plane) of $=\text{CH}_2$
- 940 cm.^{-1} : δ CH (out-of-plane) of $-\text{CH}=\text{}$
- 1396 cm.^{-1} : δ CH (in-plane) of $=\text{CH}_2$
- 1471 cm.^{-1} : δ CH of $-\text{CH}_3$
- 1653 cm.^{-1} : ν C=C of $-\text{CH}=\text{CH}_2$
- 3008 cm.^{-1} : ν CH of $=\text{CH}-$
- 3070 cm.^{-1} : ν CH of $=\text{CH}_2$

$\text{CHR}^1 = \text{CHR}^2$ or $\text{CR}^1\text{R}^2 = \text{CHR}^3$). It was, therefore, apparent that the strong absorption peak at 1653 cm.^{-1} shown in Fig. 1 was due to the C=C stretching vibration of terminal double-bond.

As a rule, the CH out-of-plane deformation vibration of $=\text{CH}_2$ in $\text{RCH}=\text{CH}_2$ type gives rise to band at 910 cm.^{-1} , but the specimen obtained had an absorption at lower frequency, 887 cm.^{-1} . Since the positive inductive effect (electron attraction) of $-\text{N}^+\text{R}_3$ radical is stronger than that of $-\text{R}$ radical (electron release in fact), it would be appropriate to presume the absorption which was expected to appear at 910 cm.^{-1} shifted to 887 cm.^{-1} , which frequency was to be typical for $=\text{CH}_2$ radical. For the same reason, an absorption at 940 cm.^{-1} may correspond to the expected absorption at 990 cm.^{-1} where absorption pertaining to the CH out-of-plane deformation vibration of $-\text{CH}=\text{}$ in $\text{RCH}=\text{CH}_2$ type occurs.

It was probable that the very weak absorption somewhere near 3400 cm.^{-1} was attributed to a trace of moisture from the air into the specimen obtained, since the sample was very hygroscopic.

Thus the infra-red spectrum of the specimen shown in Fig. 1 was consistent with what was expected from the structural formula of neurine chloride.

As for further identification of the synthetic neurine chloride, the platinum

double salt was prepared. The compound gave m.p. (decomp.) 213–214°C. *Lucius* (3) gave 213°C. for m.p. of neurine chloride-platinum double salt. The double salt dried at 100°C. in an oven was analysed with the following result:

Subst.: 0.3975 g., Pt: 0.1336 g.

Found: Pt, 33.61%

Calc. for $C_{10}H_{24}N_2Cl_6$ Pt: Pt, 33.64%

SUMMARY

Neurine chloride could be prepared by dehydrochlorination of chlorocholine chloride synthesized by the interaction of ethylene chloride and trimethylaminé. It was a tetragonal prism or pyramid, reacting with *Florence's* reagent to give microscopic rectangular crystals which could not be distinguished from typical *Florence's* crystal. The infra-red spectrum of neurine chloride was briefly discussed.

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