CHEMICAL
&
PHARMACEUTICAL BULLETIN

Vol. 8 No. 1 January 1960

UDC 547.812.5

1. Studies on Pyrone Derivatives. VI.[*3] On the Reaction between Dehydroacetic Acid and Several Ammonium Salts in Solution.

Sadao Iguchi, Kazuhito Hisatsune, Shigeru Goto, Eiko Futagami,[*1] Hiroshi Miyake, and Junzo Iida[*2]

*(Pharmaceutical Institute[*1] and First Department of Surgery,[*2] Faculty of Medicine, University of Kyushu)*

It was briefly reported in the preceding paper of this series[1] that dehydroacetic acid (DHA) or its sodium salt (DHA-Na) easily reacts not only with ammonia but also with various ammonium salts, either organic or inorganic, finally yielding 3-(1-iminoethyl)-4-hydroxy-6-methyl-2-pyrone (DHA-imide). In this paper the details of the experiment are reported.

The following ten ammonium salts were used in the experiment: Ammonium chloride, ammonium bromide, ammonium thiocyanate, ammonium nitrate, ammonium carbonate, ammonium sulfate, ammonium phosphate (dibasic), ammonium acetate, ammonium tartrate, ammonium citrate (dibasic). Test solutions were made by adding an equivalent of each ammonium salt into 5% DHA-Na solution and the mixture was kept in an incubator at 37°. Excepting ammonium citrate, all salts dissolved readily to give a clear solution and the pH values of these solutions lay between 7 and 8 (Table I). When ammonium citrate was added, the test solution promptly became cloudy by separation of DHA and $N/50$ sodium hydroxide was added to clarify the solution. In order to detect the formation of a condensation product, paper chromatography was applied periodically on all the test solutions which were kept in the incubator. As a developing solvent, the BuOH:AcOH:H_2O (4:1:5) system was mainly used and the ascending method adopted.

After 48 hours' storage, a new spot positive to Dragendorff's reagent appeared with all solutions at the position of Rf 0.82~0.83. Moreover, after 72~96 hours white crystalline substance began to precipitate out and the amount seemed to increase with passage of time. The precipitated substance was purified by recrystallization from hydrous alcohol. As a result of chemical and spectrophotometrical examination, it was proved that they were all the same and identical with DHA-imide, and that the aforementioned new spot on paper chromatogram was also due to this substance.

Such reactivity to ammonium salts was observed with DHA itself, but it was difficult to carry out the experiment on DHA under the same conditions as was applied on DHA-Na because of its poor solubility in water. Therefore, the reaction of DHA was examined in an alcoholic solution. For example, when an alcoholic solution con-

---

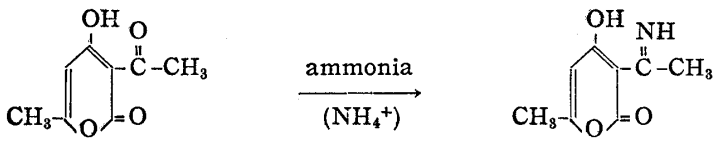
*1,2 Katakasu, Fukuoka (井口定男, 久恒和仁, 後藤 茂, 二神英子, 三宅 博, 飯田順造).

*3 Part V: Yakugaku Zasshi, **79**, 1235(1959).

1) S. Iguchi, *et al.*: This Bulletin, **7**, 323(1959).

NII-Electronic Library Service

taining DHA (5%) and NH_4NO_3 (2.5%) was refluxed for 1 hour, a new spot positive to Dragendorff's reagent was obtained on paper chromatogram and after cooling the solution DHA-imide also separated out.



DHA
m.p. 109~110°

DHA-imide
m.p. 206~207°

Table I. Amount of DHA-imide produced by the Reaction between DHA-Na and several Ammonium Salts
(Test solution was kept for 168 hr. in incubator at 37°)

NH_4 Salt	Amount added into 15 cc. of 5% DHA-Na soln.* (mg.)	pH of test soln.	Amount of DHA-imide formed (mg.)	yield (%)
NH_4Cl	192.8	7.4	126.9	21.1
NH_4SCN	274.4	7.3	109.8	18.2
NH_4NO_3	288.6	7.4	121.4	20.2
$(NH_4)_2SO_4$	238.2	7.5	108.8	18.1
$(NH_4)_2HPO_4$	237.9	8.0	136.2	22.6
$NH_4C_2H_3O_2$	277.8	7.3	132.6	22.0
$(NH_4)_2C_4H_4O_6$	331.6	7.5	100.7	16.7

(Theoretical amount of DHA-imide from 15 cc. of 5% DHA-Na Soln.* ⟶ 602.2 mg.)
* DHA-Na monohydrate ($C_8H_7O_4Na \cdot H_2O$ (208.1)) was used throughout this experiment.

On the other hand, for the object of examining reactivity, yield was calculated by weighing the product obtained when DHA-Na was used in combination with several ammonium salts. As shown in Table I, about 20% yield was generally obtained in each case when test solutions were stored for 168 hours at 37° and no remarkable difference could be seen between them. By raising the reaction temperature, yield of the product increased, especially when DHA-Na was used in quantities greater than the equivalent.

Examples of these experimental results are shown in Table II, which were obtained with NH_4Cl solution. Thus it was confirmed that DHA or DHA-Na could easily react with ammonium ion and form DHA-imide.

Table II. Amount of DHA-imide obtained by Heating the Test Solution

Heating condition / mol. ratio (NH_4Cl : DHA-Na)	100°, 30 min.	100°, 60 min.
1 : 1	0.6563 g. (26.3%)	2.344 g. (93.8%)
1 : 2	1.1929 g. (47.9%)	quantitative

40 cc. of test solution containing 0.8 g. of NH_4Cl was used. Amount of DHA-Na added was 3.112 g. and 6.23 g., respectively. (Theoretical amount of DHA-imide → 2.4992 g.)

This is an interesting new finding and with other carbonyl compounds, i.e. salicylaldehyde and acetylacetone which commonly have 1,3-diketone structure analogous to DHA, such a tendency was not so widely recognized as with DHA or its salt.[2] Besides this it is already well known that DHA and DHA-Na have strong antibacterial and antifungal activities and are widely used as preservatives especially because of their relatively low toxicities. Responses on the values of blood-ammonia were observed when these substances were administered into the animal body. For this purpose, dogs

2) Unpublished data.

NII-Electronic Library Service

with Eck's fistula were used as preliminary test animals, since it is known that dogs treated by "Eck's operation" often exhibit higher blood-ammonia level postoperatively. They are therefore frequently used in researches on hyper-ammonemia or hepatic coma.

Measurement of the content of ammonia in blood was carried out by Conway's micro-diffusion method. As a result, it was observed that the blood-ammonia values of Eck's dogs*[4] revealed downward curves after intramuscular injection of DHA-Na solution. It is quite interesting that this tendency was more marked when DHA-Na was applied together with a small amount of NH_4Cl solution.*[5]

In this case the main rôle of NH_4Cl given exogenously to dogs may presumably be in its effect on NH_3/NH_4^+ ratio in aminal body, but this requires further examination for clarification. Details of part of these animal experiments have already been reported[3] and typical examples are shown in Fig. 1.

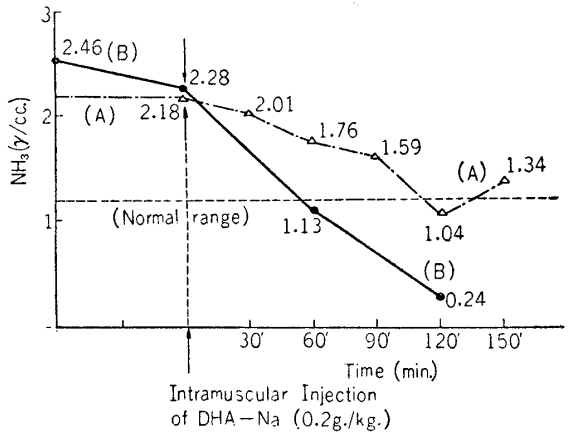


Fig. 1. Reduction of NH_3 Content in Blood of Eck's Dog by Injection of DHA-Na

(A) Eck's dog ♂ (body wt., 7.5 kg.)
(B) 〃 ♀ (〃 5.9 kg.)

In the case of (B), the intravenous droplet injection of 0.25% NH_4Cl was made at the rate of 1.5 cc./min. via the right femoral vein throughout the experiment. Blood sampling was made at regular intervals from the left femoral vein and ammonia value was measured by Conway's method. (Normal range in dogs, 0~1.2 γ/cc.).

These facts led to the supposition that DHA-Na reacted with ammonia or ammonium ion also *in vivo* and improvement of the syndrome might be brought about chiefly by this reaction. Concerning the problem of the reaction *in vivo*, two positive data were obtained as follows: (1) The concentrated chloroform extract from fresh urine of Eck's dogs after administration of DHA-Na gave a similar spot as DHA-imide on paper chromatogram, which had never been observed without application of this compound. (2) From the urine of rabbits injected with DHA-Na and NH_4Cl, occurence of a similar spot was also recognized by paper chromatography.

These facts seem to support the view that DHA-Na *in vivo* is condensed with ammonia or ammonium ion to some extent and excreted in the form of DHA-imide.

Although investigations are still in the preliminary state, it seems quite interesting that in the treatment of hyper-ammonemia the fairly positive response could also be obtained by the material which was considered to be quite different in its mode of action from those of medicines used at present, i.e. glutamic acid, arginine, or thioctic acid.

Experimental

General Procedure of Paper Chromatography—0.02 cc. of test solution was spotted on Toyo Roshi No. 50 and developed for 16 hr. at 15~18° by the ascending method. As developing solvent

*[4] Dogs with Eck's fistula.
*[5] Successive droplet intravenous injection throughout experiment or a single intramuscular injection after administration of DHA-Na.
3) J. Iida: Kyushu J. Med. Sci., **10**, 37(1959).

NII-Electronic Library Service

BuOH-AcOH-H_2O (4:1:5) system was used and Dragendorff's reagent was sprayed. Recognition limit of DHA-imide by this reagent was 10 γ.

Identification of DHA-imide from Urine—(1) The fresh urine of Eck's dog, which had been injected daily with DHA-Na (0.2 g./kg./day), was collected and immediately extracted with $CHCl_3$. Total volume of $CHCl_3$ used for extraction was about equal to that of urine. After centrifugation, $CHCl_3$ layer was separated and dehydrated over $CaCl_2$. Then the soln. was concentrated to 1/100 of the original volume under a reduced pressure at low temperature(below 40°), and paper chromatography was carried out on the solution thus obtained. A new spot positive to Dragendorff's reagent, which was not found before administration of the DHA, was clearly recognized.

(2) In the same manner as (1), examination was also made on fresh urine of normal rabbits which had received daily administration of both DHA-Na (1 g./head/day) orally and 10% NH_4Cl soln. (3 cc./head/day) intracutaneously. From the urine obtained on the 5th day after the beginning of daily administration, the same spot of DHA-imide was also found on the paper chromatogram.

Table III. Result of Paper Chromatography on Urine Extract

	Urine		Paper chromatogram	
	pH	Vol. (cc.)	Dragendorff reagent	Rf
Eck's dog	5.6	24	+	0.89~0.92
	6.2	15	+	
	5.8	145	+	
Control-E*	(5.8)		+	0.88~0.89
Rabbit	7.2	50	+	0.79~0.81
Control-R*	(7.6)		+	0.83~0.84

* Control-E and control-R were prepared by similar $CHCl_3$ extraction after adding a small amount of DHA-imide into the urine of Eck's dog or normal rabbit which had never been treated with DHA-Na.

The authors express their gratitude to Prof. H. Matsumura of this University for his encouragement. Thanks are due to Misses Hoshide, Ichihara, and Horikoshi for their technical help, and also to the Taito-Pfizer Co. for their kindness in supplying DHA.

Summary

It was found that dehydroacetic acid or its sodium salt reacted in the solution with not only ammonia but with various organic and inorganic ammonium salts under extremely mild condition and formed its imide, 3-(1-iminoethyl)-4-hydroxy-6-methyl-2-pyrone (DHA-imide). Dehydroacetic acid and its sodium salt are known to have low toxicity and examinations were made to see if a similar reaction would occur *in vivo* when dehydroacetic acid is administered. In a preliminary test with a dog with Eck's fistula, administration of sodium dehydroacetate in hyper-ammonemia was effective to a certain extent in reducing the value of blood ammonia.

(Received June 8, 1959)

NII-Electronic Library Service