Extraction of the Wood Part of *Wistaria floribunda* DC.——The dried wooden part of *W. floribunda* (3.7 kg.) was extracted 3 times with MeOH. The methanolic extract was concentrated to 5 L. and treated with $(AcO)_2Pb$ and lead subacetate, successively. The filtrate was treated with H_2S to remove PbS, and extracted with Et_2O and AcOEt as described previously for the bark extract. Thus wistin was obtained in the yield of 0.003%.

From the precipitates formed by the addition of lead subacetate, a brownish syrup (19.5 g.) was isolated, which gave 3 fluorescent spots (Rf 0.95, 0.80, 0.45) under UV-illumination, on the paper chromatogram developed with BuOH-AcOH-H_2O (4:1:5). The upper two spots represented afromosin and wistin, respectively.

The authors wish to express their thanks to Prof. W. Cocker and Dr. T. B. H. McMurry, University of Dublin, for their kind gift of samples. The microanalyses, measurements of ultraviolet and infrared spectra were carried out by the members of Microanalytical Laboratories of this Faculty and the Institute for Applied Microbiology of this University. The authors are indebted to all of them.

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Summary

A new glucoside, $C_{23}H_{24}O_{10}\cdot H_2O$, m.p. 209~210°, $[\alpha]_D^{12}$ −67.15° (c=1.43, acetic acid) was isolated from *Wistaria floribunda* DC. and some allied plants, and named wistin. The aglycone of wistin was proved to be identical with afromosin (=7-hydroxy-6,4′-dimethoxyisoflavone (II)), which had been isolated by McMurry *et al.* from *Afromosia elata* HARMS. Wistin was formulated as in I.

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70. Sadao Iguchi, Atsuko Inoue, and Chieko Kurahashi: Studies on Pyrone Derivatives. (IX).*1 On the Reaction of Dehydroacetic Acid to the Primary Amines and Ammonia. (1).

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Dehydroacetic acid (DHA) is one of the officially recognized food-preservatives in Japan and also used frequently as an antiseptic in medicinal preparations. Therefore, we have been investigating its chemical behaviors with special interest. In previous papers,[1~4] it has been reported that DHA was reactive with ammonia, primary amines and some of the compounds possessing amino radical, such as amino acids and sulfanilamides, and Schiff's base type compounds were readily produced at first by the reaction between them in the solution. Such reaction of DHA was not always stopped at the step of the formation of Schiff's base, but it tended to proceed secondly to the transformation to pyridone derivatives even under mild conditions in some cases. The latter fact was

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*1 Part VIII, This Bulletin: **10**, 1070 (1962).
*2 Katakasu, Fukuoka (井口定男, 井上敦子, 倉橋千恵子).
1) S. Iguchi, *et al.*: Yakugaku Zasshi, **77**, 1258 (1957).
2) *Idem*: This Bulletin., **7**, 323 (1959).
3) *Idem*: *Ibid.*, **8**, 1 (1960).
4) *Idem*: *Ibid.*, **9**, 1016 (1961).

briefly reported in the communication.[4] The details of the detection of pyridone derivatives performed chiefly by paper chromatography are reported in this paper together with the results of antibacterial and antifungal tests on these reaction products.

As reactants to DHA in this experiment, we used ammonia, ammonium chloride, methylamine, ethanolamine and aniline. Test solutions were prepared by adding DHA at the rate of 2% (w/v) to each solution of various concentrations of ammonia or the amines, and then kept in an incubator (37°). The progress of the reaction was traced periodically by paper chromatography, chiefly using Dragendorff's reagent for spraying.

In the case of ammonia, formation of DHA-imide (3-iminoethyl-4-hydroxy-6-methyl-2-pyrone) was generally observed immediately after the addition of DHA, and this compound was apt to separate out as white needles.

However, when kept in the incubator, such crystals of DHA-imide once appeared in the solutions tended to decrease gradually in the course of time and finally disappear completely. Concerning this phenomenon, paper chromatographical investigation revealed the remarkable increase of the amount of two new compounds in proportion to the disappearance of unchanged DHA and DHA-imide, both of which were predominant in an earlier stage. But it was proved that these two compounds were lutidone and lutidonecarboxylic acid, because they showed a good agreement with authentic samples in their Rf values in paper chromatogram, infrared and ultraviolet spectra. These pyridone derivatives seem to be produced naturally by the transformation from DHA-imide (the primary reaction product) as shown in Chart 1. The result of the paper chromatography

Chart 1.

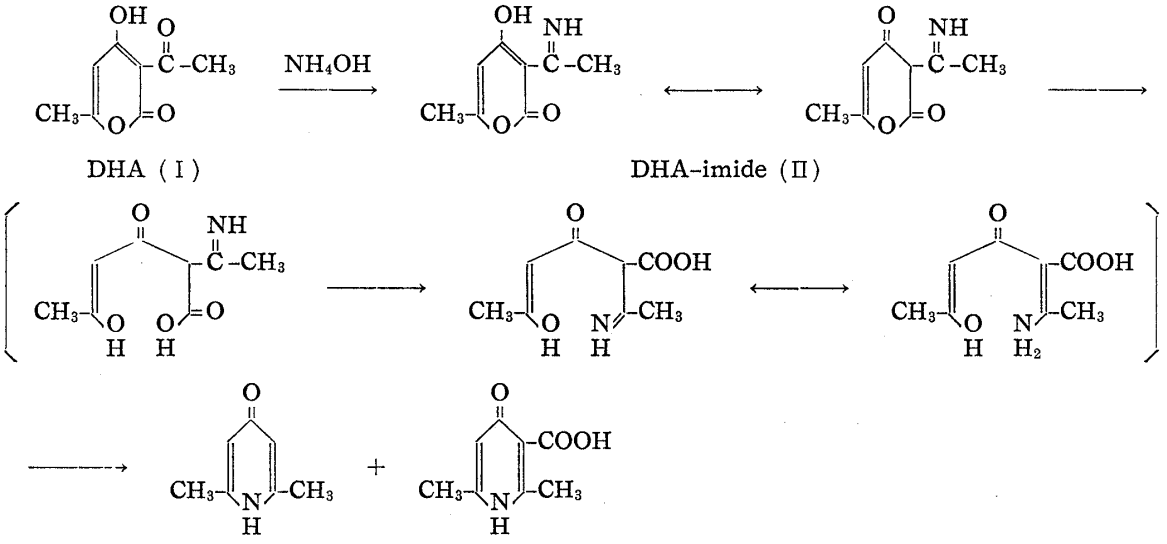


Chart 1.

is summarized in Table I, which shows clearly that not only DHA-imide but also lutidone and its carboxylic acid are rapidly formed even under a mild condition when DHA coexists with ammonia, and that this tendency is more remarkable in higher concentrations of ammonia. It was confirmed later that these pyridone derivatives were present stably and predominantly in the solutions even after 6 months. It is also noteworthy that the formation of lutidone was recognized in ammonium chloride solution containing DHA-Na, though a far longer time was necessary in this case.

We examined further this transformation reaction using DHA-imide as a starting material instead of DHA, as it seemed an interesting problem whether the presence of an excess of ammonium ion is essential or not in the transformation of DHA-imide. As shown in Table II, it was observed that DHA-imide also transformed into lutidone

Table I. Results of the Paper Chromatographical Investigation on the Reaction between DHA and Ammonia (at 37°)

Concn.	Product	Rf	S.R.	Time (hr.) 1	3	7	24	48	120	240
28% NH_4OH	I	0.91	F	+	+	+	+	−	−	−
	II	0.84	D	+	+	+	−	−	−	−
	III	0.68	D	−	−	+	+	+	+	+
	IV	0.73	D	−	−	±	+	+	+	+
10% NH_4OH	I			+	+	+	+	+	−	−
	II			+	+	+	+	−	−	−
	III			−	−	−	+	+	+	+
	IV			−	−	−	+	+	+	+
5% NH_4OH	I			+	+	+	+	+	+	−
	II			+	+	+	+	+	+	−
	III			−	−	−	+	+	+	+
	IV			−	−	−	±	+	+	+
10% NH_4Cl (pH 6.2)	I [a] (DHA-Na)			+	+		+	+		−
	II			−	+		+	+		+
	III			−	−		−	−		+

I : DHA, II : DHA-imide, III : lutidone, IV : lutidonecarboxylic acid
(Concn. of DHA→1 g. in 50 ml. soln.)
a) (DHA-Na→1.2 g. in 50 ml. of 10% NH_4Cl soln.)
paper : Toyo Roshi No. 50 Developing solvent : BuOH-AcOH-H_2O (4 : 1 : 5) S.R. : spraying reagent D : Dragendorff F : $FeCl_3$

in dilute NaOH solution, but the rate was very slow in comparison with the solution having an excess of ammonium ion. Moreover, the following fact seems very interesting that the recovery of DHA was seen prior to formation of lutidone in every solutions with or without an excess of ammonium ion. From this phenomenon, it may be assumed that ammonium ion generated from DHA-imide by hydrolysis takes an important part in this transformation, but the conclusive data have not yet been obtained.

Table II. Results of the Paper Chromatographical Investigation on the Reaction of DHA-imide in Alkaline Solutions (at 37°)

Concn.	Product	Rf	S.R.	Time (hr.) 1	2	5	12	24	48	72	96	168
5% NH_4OH (pH 12.2)	II	0.84	D	+	+	+	+	+	+	+	+	+
	I	0.91	F	±	+	+	+	+	+	+	+	+
	III	0.61	D	−	±	+	+	+	+	+	+	+
5% $(NH_4)_2HPO_4$ (pH 8.4)	II			+	+	+	+	+	+	+	+	+
	I			−	−	−	−	−	+	+	+	+
	III			−	−	−	−	−	±	+	+	+
dil. NaOH soln. (pH 8.4)	II			+	+	+	+	+	+	+	+	+
	I			−	−	−	−	±	+	+	+	+
	III			−	−	−	−	−	−	−	−	+

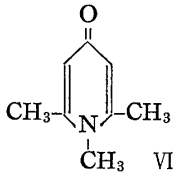
II : DHA-imide (starting material), I : DHA, III : lutidone
Concn. of DHA-imide→1 g. in 50 ml. of each soln.

In methylamine solutions the similar tendency was seen more markedly and rapidly than expected. For example, in 5% methylamine, formation of N-methyllutidone (1-methyl-2,6-dimethyl-4(1*H*)-pyridone) was clearly recognized already 1 hour after the addition of DHA, and after 24 hours, DHA (unchanged) and 3-methyliminoethyl-4-hydroxy-6-methyl-2-pyrone (the primary product) could no longer be detected by paper chromatography in contrast to the remarkable formation of N-methyllutidone (Table III). Both in ethanolamine solutions and in aniline solutions (50% methanol solution), the same phenomenon was also observed as shown in Table IV.

Table III. Results of the Paper Chromatographical Investigation on the Reaction between DHA and Methylamine (at 37°)

Concn.	Product	Rf	S.R.	Time (hr.) 1	3	5	24	48	96
30% CH_3NH_2	I	0.91	F	+	−	−	−	−	−
	V	0.83	D	+	±	±	−	−	−
	VI	0.60	D	+	+	+	+	+	+
5% CH_3NH_2	I			+	+	±	−	−	−
	V			+	+	+	±	−	−
	VI			+	+	+	+	+	+
1% CH_3NH_2	I			+	+	+	+	±	−
	V			+	+	+	+	+	+
	VI			−	±	+	+	+	+

I : DHA, V : 3-methyliminoethyl-4-hydroxy-6-methyl-2-pyrone,
VI : N-methyllutidone



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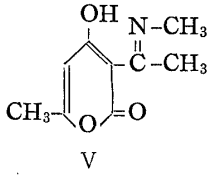

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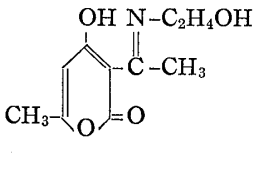
Table IV. Results of the Paper Chromatographical Investigation on the Reaction of DHA with Ethanolamine and Aniline (at 37°)

Concn.	Product	Rf	S.R.	Time (hr.) 1	3	5	7	24
10% Ethanolamine	I	0.91	F	−	−	−	−	−
	VII	0.76	D, N	+	+	+	+	+
	VIII	0.62	D	−	−	±	+	+
10% Aniline[a]	I	0.91	F	+	−	−	−	−
	IX	0.89	D	+	+	+	+	+
	X	0.79	D	−	±	±	+	+

I : DHA, VII : 3-[1-(2-hydroxyethyl)iminoethyl]-4-hydroxy-6-methyl-2-pyrone,
VIII : N-hydroxyethyllutidone, IX : 3-phenyliminoethyl-4-hydroxy-6-methyl-2-pyrone,
X : N-phenyllutidone (N : ninhydrin)



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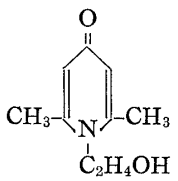

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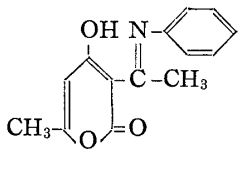
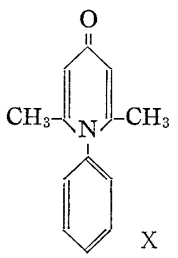


IX



X

a) In this case, 50% MeOH was used as the test soln. in order to dissolve aniline, and the following system was adopted for developing solvent in paper chromatography; BuOH-AcOH-*N* HCl (4 : 1 : 2).

On the other hand, concerning the transformation of DHA, only the following fact was reported in the past that DHA was converted to lutidone by treating with ammonia under drastic conditions, for example, by heating in a sealed tube. Contrary to this fact, we could confirm this time the rapid progress of the reaction under a mild condition in all the test solutions. Therefore, it may be said that the marked reactivity of DHA was clearly demonstrated by these experimental results, especially the ease with which DHA was converted into another heterocyclic compound.

Finally, the antibacterial and antifungal activities of lutidone, lutidonecarboxylic acid, N-methyllutidone and N-hexyllutidone were examined, but no activities could be found among them. Therefore, it seems also possible to conclude that DHA loses its activity when it reacts with ammonia or primary amines.

Experimental

Paper chromatography : An ascending method was adopted. Developing was carried out for 15 hr. at room temperature (18~25°) by using BuOH-AcOH-H_2O (4 : 1 : 5) system as developing solvent. Distance of development was generally about 30 cm. (Filter paper : Toyo Roshi No. 50). Dragendorff's reagent was chiefly used for spraying, because it was relatively sensitive both to Schiff's base type compounds of DHA and also to the pyridone derivatives derived from DHA.

Synthesis of Pyridone Derivatives

Lutidone (III) and Lutidonecarboxylic Acid (IV)[5,6]——(i) Two grams. of 2,6-dimethyl-4-pyrone was added to 100 ml. of 28% NH_3, and this mixed solution was refluxed for 10 hr. After the evaporation of the solution to dryness under reduced pressure, crude lutidone (2 g.) was obtained. Repeated recrystallization from the mixture of MeOH-ethylacetoacetate (1 : 1) gave the pure sample. Lutidone (Yield 1.5 g.) m.p. 224~225°.

(ii) To 500 ml. of 28% NH_3, was added 15 g. of DHA and refluxed for 15 hr. Yellowish white residue was obtained after this solution was evaporated under reduced pressure (13 g.). After the residue was washed with benzene (50 ml.) at room temperature, remaining crude products were immersed in 150 ml. of warm EtOH (about 70°). Lutidone was soluble into EtOH at this condition, whereas lutidonecarboxylic acid remained insoluble. Therefore, the separation of those reaction products could be well performed. Lutidone (Yield 7.5 g.). Lutidonecarboxylic acid monohydrate (Yield 5.2 g.). m.p. 257~258° (decomp.) (from EtOH).

N-Methyllutidone,[7] **(1-Methyl-2,6-dimethyl-4(1*H*)-pyridone) (VI)**——5.6 g. of DHA and 25 ml. of 25% methylamine solution were heated at 100~110° for 6 hr. in a sealed tube. After cooling, long needles were filtered off. Thus raw product obtained was recrystallized from hot water and then dried at 110°, m.p. 245°, white needles. (Yield 4.6 g.).

N-Hydroxyethyllutidone (1-Hydroxyethyl-2,6-dimethyl-4(1*H*)-pyridone) (VIII)——Three grams of DHA and 1.3 g. of ethanolamine were added to 50 ml. of water and warmed on a steam bath for 10 hr. After cooling, the solution was shaken with 50 ml. of Et_2O to remove unchanged material. Then the water layer was separated and evaporated under reduced pressure. The residue thus obtained was recrystallized from EtOH, m.p. 224~225°, white prism. (Yield 2 g.). *Anal.* Calcd. for $C_9H_{13}O_2N$: C, 64.7; H, 7.8; N, 8.4. Found : C, 64.9; H, 8.0; N, 8.1.

3-[1-(2-Hydroxyethyl)iminoethyl]-4-hydroxy-6-methyl-2-pyrone (VII)——Three grams of DHA and 1.1 g. of ethanolamine were added into 50 ml. of EtOH and refluxed for 30 min. After the solvent was evaporated, the residue was recrystallized from EtOH, m.p. 118~119°, white plates. (Yield 2.5 g.). *Anal.* Calcd. for $C_{10}H_{13}O_4N$: C, 56.9; H, 6.2; N, 6.6. Found : C, 56.7; H, 6.2; N, 6.6.

N-Phenyllutidone,[7] **(1-Phenyl-2,6-dimethyl-4(1*H*)-pyridone) (X)**——The mixture of 3.4 g. of DHA, 1.9 g. of aniline and 3.1 g. of conc. HCl was refluxed for 2 hr. After cooling, the mixture was neutralized with Na_2CO_3 and then extracted with $CHCl_3$. After the solvent was evaporated, the raw product was recrystallized from benzene, m.p. 196~197°, white prism. (Yield 5.4 g.).

Antibacterial and Antifungal Tests——Tested Bacteria and Fungi : *Staphylococcus aureus, Escherichia coli, Shigella flexneri, Penicillium notatum, Aspergillus niger.*

Culture condition

Bacteria : Bouillon broth (pH 7.1) at 37°

Fungi : Sabouroud broth (pH 7.0) at 30°

The examination was carried out by the multiplying dilution method, and the result was observed after 48 hr. (Bac.) and 14 days (Fung.) respectively. As standard active compounds, Streptomycin (Bac.) and DHA (Fung.) were used in this experiment.

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5) L. Haitinger : Ber., **18**, 452 (1885).
6) M. Conrad, M. Guthzeit : *Ibid.*, **20**, 159 (1887).
7) S. Hunig, G. Köbrich : Ann., **617**, 181 (1958).

Mrs. H. Matsuba and Mr. M. Shirōzu for the microanyalyses and to Messrs. H. Yano and H. Matsui for infrared and ultraviolet spectral measurements. This work was supported by the Grant-in-Aid for Scientific Reserch provided by the Ministry of Education, to which they are also grateful.

Summary

By means of paper chromatography, it was fonud that DHA was very reactive with ammonia, ammonium chloride, methylamine, ethanolamine or aniline in aqueous solutions even under a mild condition such as keeping in an incubator (37°), and pyridone derivatives were easily formed via Schiff's base type compounds (the first reaction product). It was also observed that DHA had lost its activity against some bacteria and fungi when it reacted with ammonia or primary amines.

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71. Sadao Iguchi and Atsuko Inoue: Studies on Pyrone Derivatives. X.*[1] On the Reaction of Dehydroacetic Acid to the Primary Amines and Ammonia. (2).

(*Institute of Pharmaceutical Sciences, Faculty of Medicine, Kyushu University*[*2])

As reported in previous paper,*[1] it seems noteworthy that dehydroacetic acid (DHA) is apt to transform readily into pyridone derivatives even under a mild condition when it coexists with ammonia or primary amines in aqueous solutions.

We continued to investigate the mechanism of the reaction in more detail, and by the success of capturing the intermediate of the reaction to pyridone which had not been able to detect by paper chromatography, it became possible to explain the reaction process of the pyridone transformation under a mild condition. The details of the experiment are described in this paper.

In this experiment, we used benzylamine as the representative of primary amines at first, because of its easiness of treatment.

Though Schiff's base I, m.p. 79~81°, was formed when DHA reacted with the equivalent mole of benzylamine at room temperature, a new compound II, $C_{21}H_{24}ON_2$ (m.p. 118~119°), was obtained in the presence of an excess of benzylamine. II was also obtained by treating I with an excess of benzylamine. The compound II was a relatively labile substance having fluorescent property. It gave blue color with ferric chloride solution, whereas the compound I did not show such an apparent color reaction. The compound II was apt to change into N-benzyllutidone monohydrate (III), m.p. 125~127°, at the presence of mineral acids or organic acids such as acetic acid. However, when DHA reacted with benzylamine in the absence of an acid in a sealed tube, only the compound III was isolated. Thus formed lutidone derivative was very stable and remained unchanged even in solutions.

The validity of the structures of I (the primary reaction product) and III (the final product) shown in Chart 1, was supported by infrared, ultraviolet spectra and an elemental analysis.

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*[1] Part IX. This Bulletin, **11**,385 (1963).
*[2] Katakasu, Fukuoka (井口定男, 井上敦子).