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117. Shozo Kamiya and Akitada Nakamura[*1]: Ring-Substituted α-Amino Acids. I. The Michael-type Addition of Maleic Hydrazide and Related Compounds leading to the α-Amino Acids.

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The Michael-type addition of maleic and phthalic hydrazide derivatives with an α,β-unsaturated carbonyl compound such as methyl vinyl ketone or acrolein was examined. All of the products were comfirmed to be the N-addition products by comparing their infrared spectra with those of the corresponding O- and N-deuterated compounds.

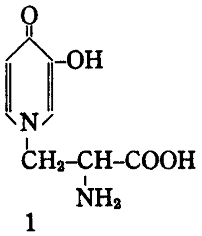
By the Bucherer synthesis these addition products were converted to the corresponding hydantoins from which new, heterocyclic ring-substituted α-amino acids were obtained by hydrolyzing with barium hydroxide. Some of the hydantoins were independently synthesized from 2-amino-2-methyl-4-hydroxybutyric acid *via* 4-(2-iodoethyl)-4-methylimidazolidine-2,5-dione.

(Received July 29, 1966)

Recently, natural amino acids bearing a pyridone or pyrimidone molecule at the β-position of alanine moiety have attracted much attention. Mimosine1) isolated from *Mimosa pudica* Benth and leucenol from *Leucaena glauca* Benth are both 3-hydroxy-4-(1*H*)-pyridone derivative (1), and willardin (2) from *Acacia willardina* has a uracil molecule. The former, structurally similar to tyrosine, was found to inhibit tyrosine decarboxylase, and competitively inhibits tyrosinase.2) Therefore, it inhibits the growth of hair in amagen phase in animals.

The biological activities of mimosine prompted us to synthesize various kinds of unnatural, ring-substituted α-amino acids.

The present paper deals with the Michael-type addition of maleic hydrazide[*2] and related compounds with an α,β-unsaturated carbonyl compound such as methyl vinyl ketone or acrolein leading to the corresponding hydantoins and α-amino acids. These amino acids which have a pyridazinone or phthalazinone molecule in place of the amino group in α,γ-diaminobutyric acid[*3] moiety are expected to have selective toxicity for microorganisms by exhibiting specific metabolic reactions, and therefore may be candidates for the chemotherapy of cancer and virus.



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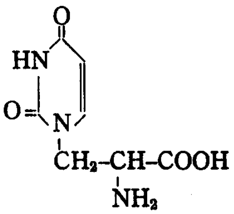

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Maleic hydrazide and phthalic hydrazide are able to tautomerize by prototropy to the dihydroxy form (A), to the monohydroxy-monoketo form (B), and to the diketo

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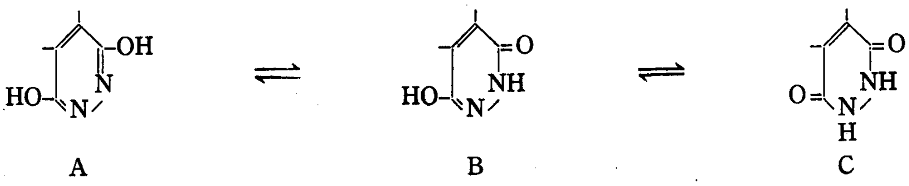
[*2] Maleic hydrazide has an antiauxin effect which counteracts the growth stimulation caused by applied 3-indoleacetic acid in the slit pea curvature test. It is also an interesting growth retardant, and therefore, known as an inhibitor of cell division in plant field. W. A. Andreae: "Metabolic Inhibitors—A Comprehensive Treaties," Ed. by R. M. Hochster, J. H. Quastel, Vol. II, 247 (1963), Academic Press.

[*3] α,γ-Diaminobutyric acid was recently isolated from the hydrolysate of Polymixin, a polypeptide antibiotic produced by the growth of *Bacillus polymyxa*. K. Vogler, *et al.*: Helv. Chim. Acta, **43**, 1751 (1960); Experientia, **7**, 223 (1961).

1) "The Chemistry of Heterocyclic, Compounds, Pyridine and its Derivatives," Part 3. Ed. by E. Klingsberg, 689 (1962), Interscience Pub.; W. J. Haggerty, Jr., R. H. Springer, C. C. Cheng: J. Heterocyclic Chem., **2**, 1 (1965).

2) R. G. Crounse, J. D. Maxwell, H. Blank: Nature, **194**, 694 (1962); D. Kostermans: Rec. trav. chim., **65**, 319 (1946) (C. A., **40**, 7193 (1946)).

form (C). Their structures have been extensively studied by the aids of ultraviolet, infrared spectroscopy, and also of alkylation with diazomethane or dimethyl sulfate. From these results, they exist predominantly as the monohydroxy-monoketo form (B) both in solid and solution.[3]



In the preceding paper[4] it has been reported that the Mannich reaction of maleic hydrazide (I) with bis(2-chloroethyl)amine gave neither the O- nor C-, but the N-mustard derivative, 2[bis(2-chloroethyl)amino]methyl-6-hydroxy-3(2*H*)-pyridazinone.

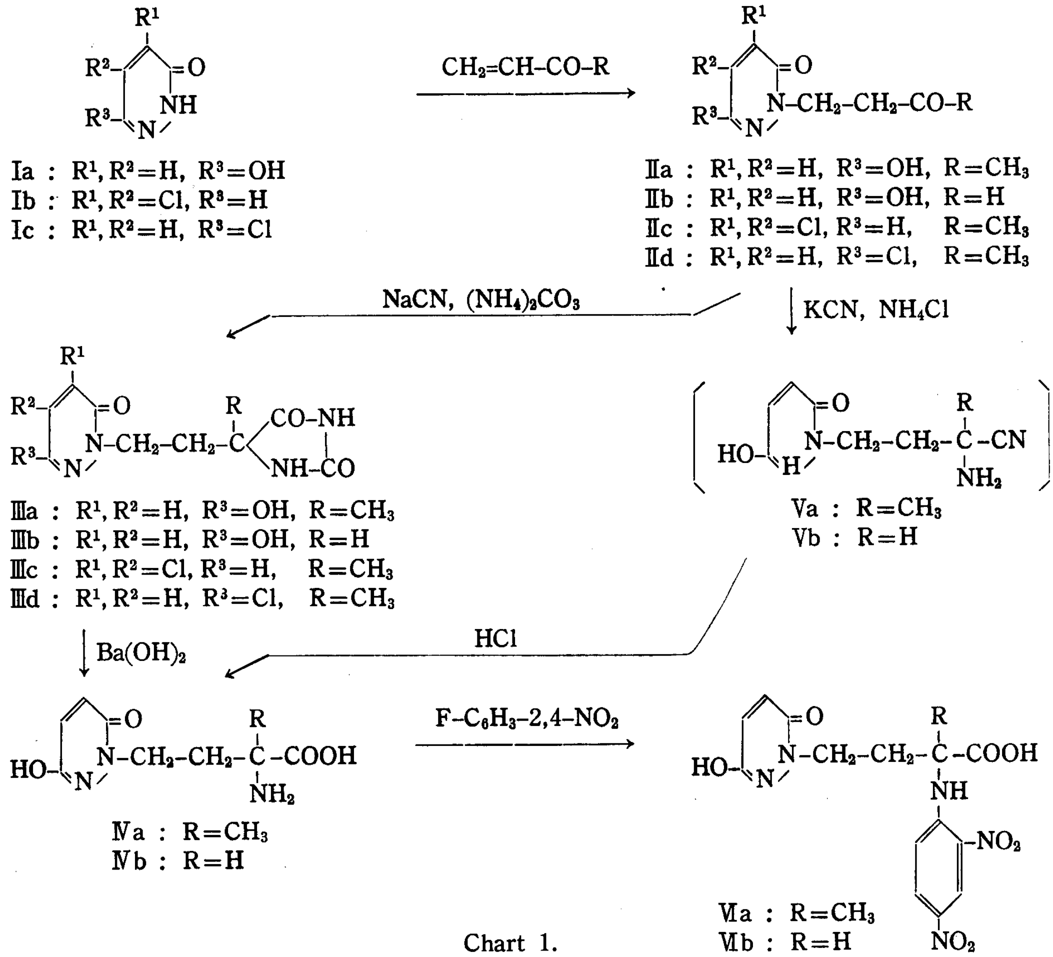


Chart 1.

The Michael-type addition of maleic hydrazide (I) with methyl vinyl ketone in the presence of catalytic amount of sodium hydroxide gave a monoaddition product in 67% yield. Similarly, reaction of I with acrolein afforded a monoaddition product in 49% yield. These addition products formed the corresponding 2,4-dinitrophenylhydrazones.

Considering from the Mannich reaction[4] of I, this reaction seems to take place at the nitrogen of 2-position. Structures of these addition products were confirmed by infrared and ultraviolet spectroscopy as follows.

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3) A.R. Katritzky, J.M. Lagowski: "Advances in Heterocyclic Chemistry," Ed. by A.R. Katritzky, Vol. 1, 367 (1963), Academic Press.
4) S. Kamiya, *et al.*: Yakugaku Zasshi, **86**, 1099 (1966).

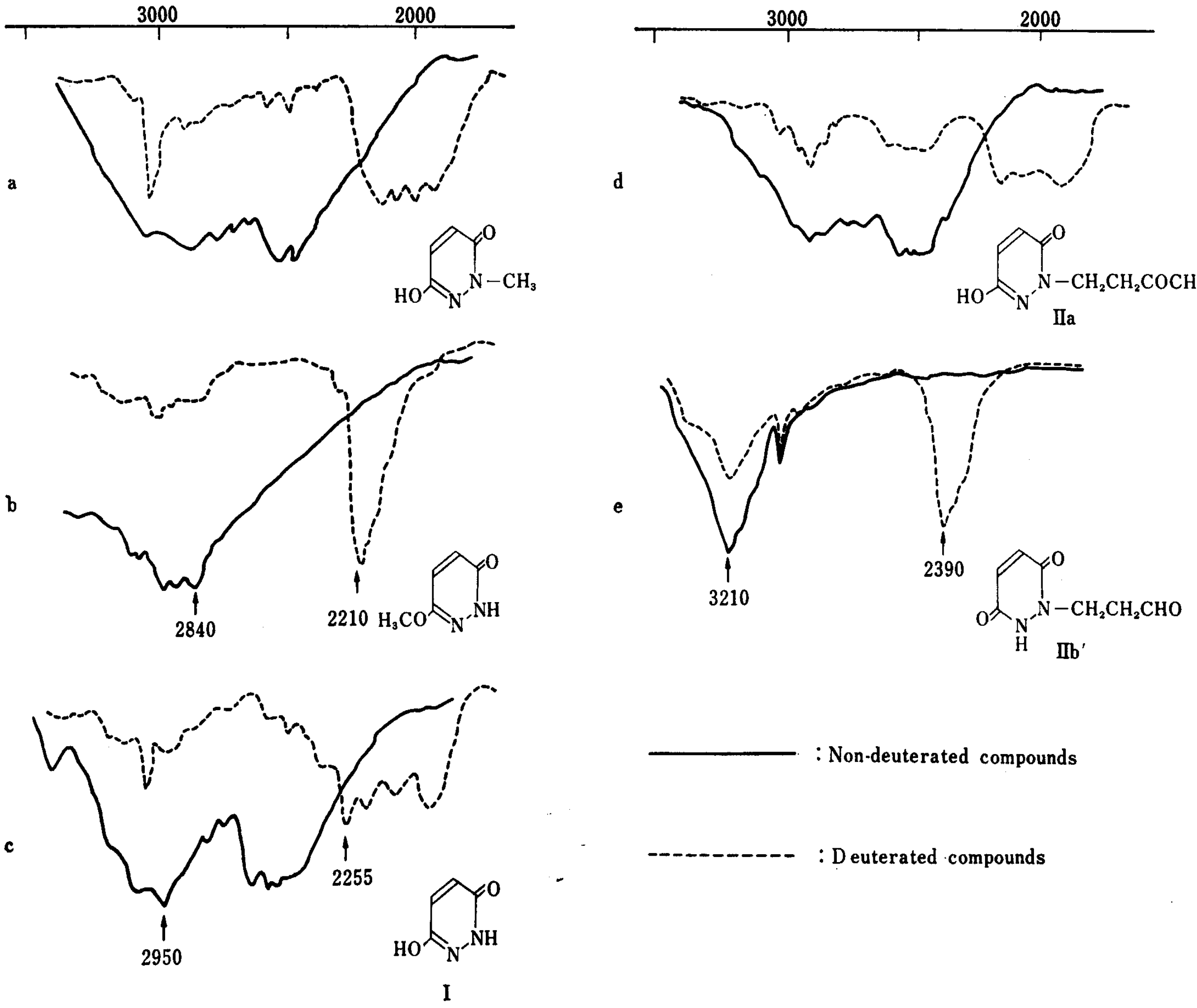

Fig. 1. Infrared Spectra of Maleic Hydrazide Derivatives and Their Deuterated Compounds at 3200~1800 cm^{-1} Region (in KBr)

As shown in Fig. 1, it is difficult to distinguish the absorptions of NH and OH in the infrared spectra of maleic hydrazide derivatives because of their complication in the region of 3200~1800 cm^{-1}.

Shindo⁵⁾ studied the tautomerism of 2-pyridone series by comparing their spectra with those of the corresponding N-deuterated compounds. Nitta and Yoneda⁶⁾ confirmed that the products arising from reaction of maleic hydrazide with substituted benzyl chlorides are the O-benzyl compounds by the same method.

Spectral informations for structures of the addition products were obtained by comparing their infrared spectra with those of the corresponding O- and N-deuterated compounds, and also from their ultraviolet spectra.

Maleic hydrazide derivatives show the strong, very broad absorption with several maxima extending from 3200 cm^{-1} down to 2400 cm^{-1} as shown in Fig. 1a~e. However, these bands disappeared on deuteration, and the OD stretching absorption appeares as a broad band in the region of 2400~1800 cm^{-1} as a, c, and d. On the other hand, the ND stretching band is observed as a sharp band at 2210 cm^{-1} (b), 2255 cm^{-1} (c) and 2390 cm^{-1} (e), respectively. As shown in Fig. 2a~e, the OH in-plane deformation frequencies (δ OH) are identified as the strong band of the hydroxy form at 1270 cm^{-1}

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5) H. Shindo: This Bulletin, **7**, 407 (1959).

6) Y. Nitta, F. Yoneda: *Ibid.*, **11**, 737 (1963).

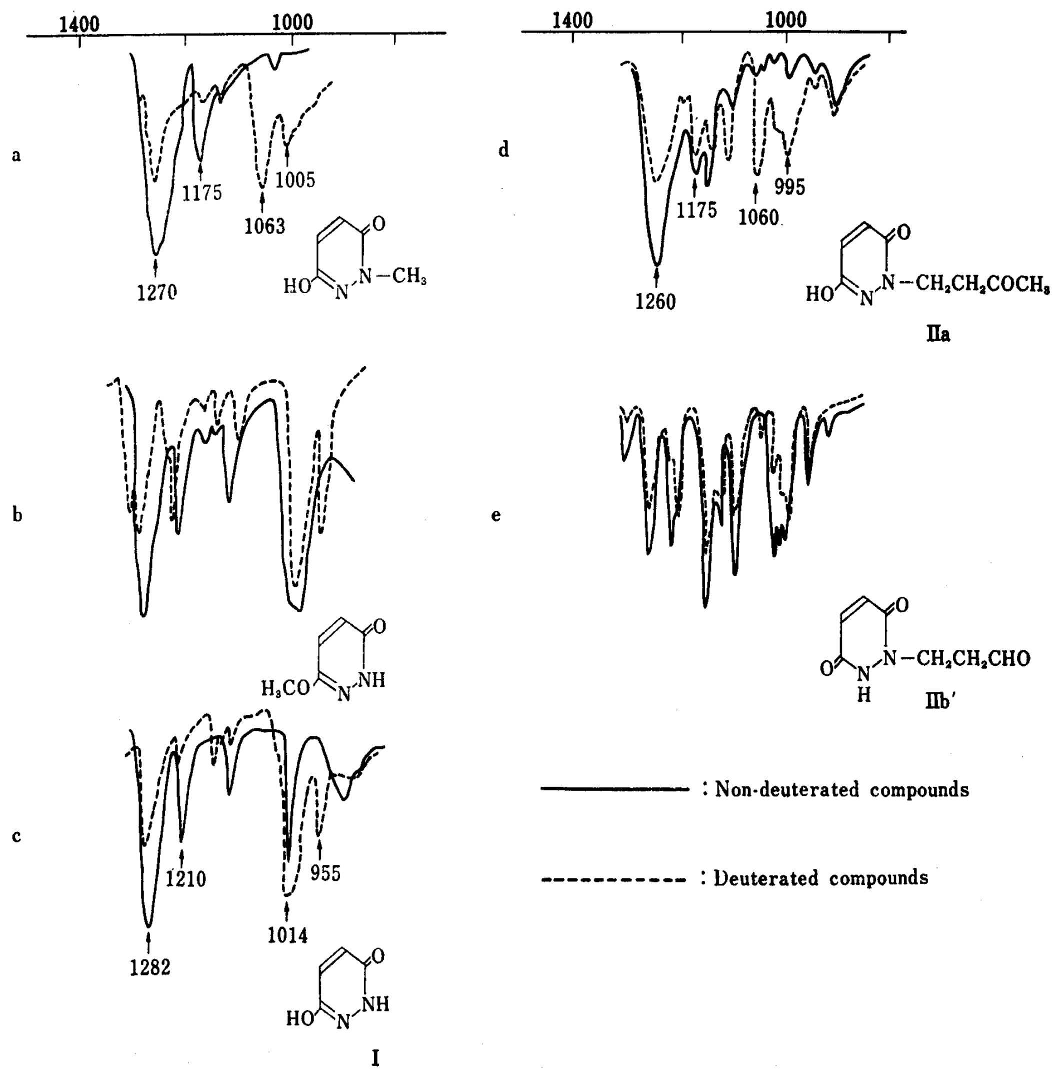


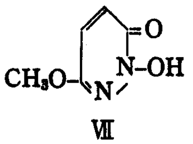
Fig. 2. Infrared Spectra of Maleic Hydrazide Derivatives and Their Deuterated Compounds at 1000~1300 cm^{-1} Region (in KBr)

(a), 1282 cm^{-1} (c) and 1260 cm^{-1} (d), being displaced on deuteration to give δ OD at 1063 cm^{-1} (a), 1014 cm^{-1} (c) and 1060 cm^{-1} (d).

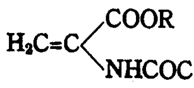
Consequently, the monoaddition product arising from reaction of I with methyl vinyl ketone should present predominantly as the monoketo-monohydroxy form, 2-(3-oxobutyl)-6-hydroxy-3(2*H*)-pyridazinone (IIa). This structure is also supported from its ultraviolet spectrum ($\lambda_{\max}^{\text{EtOH}}$ 320 mμ) which agrees with that of various N-substituted pyridazinones as reported in our preceding paper.$^{4)}$

On the other hand, the monoaddition product (IIb) arising from I with acrolein is considered to be either N-substituted derivative in the diketo-form or O-substituted derivative in the monoketo-monohydroxy form. However, its ultraviolet spectrum ($\lambda_{\max}^{\text{EtOH}}$ 328 mμ) indicates to be predominantly of the diketo-form$^{4)}$ rather than the monoketo-monohydroxy form. In addition, its infrared spectrum shows the CO stretching band at 1680 cm^{-1} which is unusually low comparing with ordinary aldehydes. This result could be explained by considering an intramolecular hydrogen bond between the imido group and aldehyde one. This interpretation is supported by the fact that the position and intensity of the CO stretching band are insensitive to concentration in chloroform solution. It is consequently concluded that IIb presents predominantly as the diketo form IIb' both in solid and solution.

Analogously, the Michael-type addition of 4,5-dichloro-3(2*H*)-pyridazinone (Ib) and 6-chloro-3(2*H*)-pyridazinone (Ic) gave the monoaddition products in 52% and 84% yield, respectively. The disappearance of the absorptions at 3100~3200 cm^{-1} due to NH observed in Ib and Ic shows that the reaction also took place at the nitrogen of 2-position producing 2-(3-oxobutyl)-4,5-dichloro-3(2*H*)-pyridazinone (IIc) and 2-(3-oxobutyl)-6-chloro-3(2*H*)-pyridazinone (IId).



VII



$H_2C{=}C\begin{smallmatrix}COOR\\NHCOCH_3\end{smallmatrix}$

VIII

The Michael-type addition of a cyclic hydroxamic acid, 2-hydroxy-6-methoxy-3(2*H*)-pyridazinone (VII) with methyl vinyl ketone did not give the expected O-addition product, recovering most of the starting material. It is considered that the proton of N-hydroxy group in 2-position is too acidic to react with such an α,β-unsaturated compound. The same reaction of maleic hydrazide and related compounds with 2-acetoaminoacrylic acid derivatives (VIII) is currently in progress.

The Michael-type addition of phthalic hydrazide (IX) with methyl vinyl ketone afforded a monoaddition product in 52% yield. This addition product has an ultraviolet spectrum with λ_{max}^{EtOH} mμ (log ε) : 255(3.43), 265(3.47) and 307(3.75) which agrees with the similar datum[7] for the N-methylphthalic hydrazide, λ_{max}^{EtOH} mμ (log ε) : 255(3.51), 264(3.54) and 304(3.78). In addition, it has been reported that the Mannich reaction of IX[8] gave only the N-Mannich base. These results suggest that this compound is the N-addition product, 2-(3-oxobutyl)-4-hydroxy-1(2*H*)-phthalazinone (X).

Treatment of IIa~d and X with sodium cyanide and ammonium carbonate, followed by acidification gave the corresponding hydantoins (IIIa~d) and XI in 45~65% yields as shown in Table I. All hydantoins exhibit the strong infrared absorption due to the hydantoin carbonyl at 1705 cm^{-1} with a shoulder at 1760 cm^{-1}.

In order to establish the structures definitely as the N-addition products, IIIa and IIIc were independently synthesized from DL-2-amino-2-methyl-4-hydroxybutyric acid (XII) as shown in Chart 2. Treatment of XII with potassium cyanate followed by heating

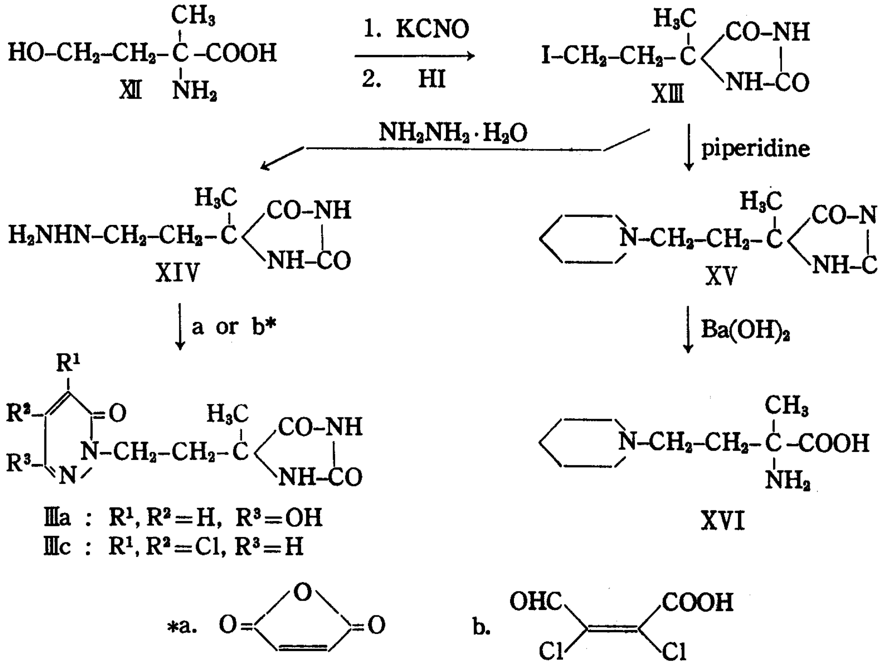


Chart 2.

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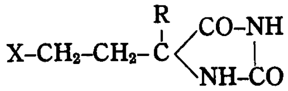
[7] J. A. Elvidge, A. P. Redman : J. Chem. Soc., **1960**, 1710.

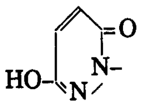
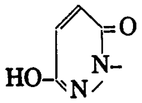
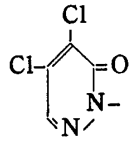
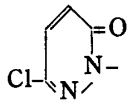
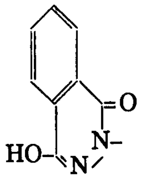
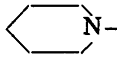
[8] H. Hellmann, I. Löschmann : Chem. Ber., **89**, 594 (1956).

with concentrated hydrogen iodide afforded 4-(2-iodoethyl)-4-methylimidazolidine-2,5-dione (XIII). Then, XIII was heated with 80% hydrazine hydrate, and the produced 4-(2-hydrazinoethyl)-4-methylimidazolidine-2,5-dione (XIV) cyclized by heating with maleic anhydride to give IIIa in a 20% overall yield. Reaction of XIV with mucochloric acid gave IIIc, though in poor yield.

Then, IIIa and IIIb were hydrolyzed by heating with barium hydroxide to give DL-2-(3-amino-3-carboxybutyl)-6-hydroxy-3(2*H*)-pyridazinone (IVa) in 32% yield, and DL-2-(3-amino-3-carboxypropyl)-6-hydroxy-3(2*H*)-pyridazinone (IVb) in 50% yield. DL-2-(3-Amino-3-carboxybutyl)-4-hydroxy-1(2*H*)-phthalazinone (XVII) was similarly prepared

Table I. The Hydantoins derived from various 2-(3-Oxobutyl)- and 2-(3-Oxopropyl)-3(2*H*)-pyridazinones

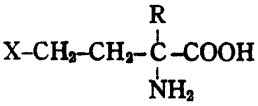


No.	X	R	Yield (%)	Cryst. Solv.	Cryst. Form
IIIa		CH_3	65	EtOH·H_2O	powder
IIIb		H	78	EtOH·H_2O	leaflets
IIIc		CH_3	45	EtOH	leaflets
IIId		CH_3	78	EtOH	pillars
XI		CH_3	82	EtOH·H_2O	powder
XV		CH_3	34	EtOH	pillars

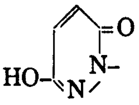
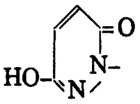
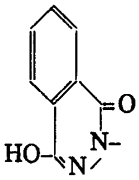
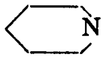
No.	m.p. (°C)	Formula	Analysis Calcd. C	H	N	Found C	H	N
IIIa	192~195 decomp.	$C_{10}H_{12}O_4N_4$	47.62	4.80	22.22	47.18	4.94	22.44
IIIb	161	$C_9H_{10}O_4N_4\cdot\frac{1}{2}H_2O$	43.72	4.48	22.66	43.59	4.24	22.26
IIIc	223~225[a]	$C_{10}H_{10}O_3N_4Cl_2$	39.36	3.30	18.36	39.77	3.40	18.43
IIId	154~156	$C_{10}H_{11}O_3N_4Cl$	44.37	4.10	20.70	44.96	4.02	20.88
XI	167~168 decomp.	$C_{14}H_{14}O_4N_4\cdot H_2O$[b]	52.49	5.04	17.49	52.47	5.17	17.72
XV	205~207	$C_{11}H_{19}O_2N_3$	58.64	8.50	18.65	58.85	8.65	18.52

a) in a sealed tube.
b) Prolonged heating in vacuum did not remove the water of crystallization.

Table Ⅱ. Various 4-Ring-substituted 2-Aminobutyric Acid Derivatives



$$X\text{-}CH_2\text{-}CH_2\text{-}\overset{R}{\underset{NH_2}{C}}\text{-}COOH$$

No.	X	R	Yield (%) a)	Yield (%) b)	Cryst. Solv.	Cryst. Form	d.p. (°C)
Ⅳa		CH_3	32	34	$EtOH \cdot H_2O$	needles	272~273
Ⅳb		H	50	63	H_2O	needles	272
XVII		CH_3	21		H_2O	needles	263
XVI		CH_3	67		EtOH	pillars	267

No.	Rf Value c) p.p.c.	Formula	Analysis Calcd. C	H	N	Found C	H	N
Ⅳa	0.38	$C_7H_9O_3N_3$	47.57	5.77	18.49	47.21	6.02	18.02
Ⅳb	0.30	$C_8H_{11}O_4N_3$	45.07	5.20	19.71	44.72	5.55	20.19
XVII	0.58	$C_{13}H_{15}O_4N_3$	56.31	5.45	15.16	56.03	5.61	14.86
XVI	0.29	$C_{10}H_{20}O_2N_2 \cdot \frac{1}{2}H_2O$	57.86	10.11	13.39	57.86	10.29	13.79

a) By the Bucherer synthesis.
b) By the Strecker synthesis.
c) Ascending method. Solvent: *n*-BuOH-AcOH-H_2O (4:1:5). Paper: Toyo Roshi No. 50. Color reagent: 0.2% ninhydrin acetone solution.

in 21% yield. But, hydrolysis of Ⅲc with barium hydroxide or sulfuric acid gave a ninhydrin positive product of which analysis was incorrect.

Reaction of XIII with piperidine gave N-[2-(2,5-dioxo-4-methyl-4-imidazolidinyl)]-ethylpiperidine (XV) from which DL-2-methyl-2-amino-4-piperidinobutyric acid (XVI) was obtained in 67% yield.

Although much has been written concerning the syntheses of usual α-amino acids by the Strecker synthesis,[9] those did not give satisfactory results for Ⅳa and Ⅳb. Isolation of these heterocyclic α-amino acids from the reaction mixture was difficult by usual procedures. By the Strecker synthesis modified by the present authors Ⅳa and Ⅳb were directly obtained from Ⅱa in 34% yield, and Ⅳb in 63% from Ⅱb. Namely, Ⅱa and Ⅱb were treated with ammonium chloride and potassium cyanide in the presence of ammonium hydroxide, and without isolation the produced aminonitriles (Va) and (Vb) were hydrolyzed with concentrated hydrochloric acid to give the hydrochlorides of Ⅳa and Ⅳb. From the acidic solution, Ⅳa and Ⅳb were absorbed onto

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[9] "Chemistry of Protein," Ed. by S. Akahori, S. Mizushima, Vol. 1, 330 (1954), Kyoritsu Shuppan.

the Amberlite IR-120 (H-form) from which free amino acids were eluted with diluted ammonia solution after washing out impure substances. However, the Strecker synthesis of IIc to obtain the corresponding α-amino acid was failed.

These heterocyclic α-amino acids show intense blue color on heating with ninhydrin, and the corresponding N-2,4-dinitrophenyl derivatives (VIa, VIb) were prepared in good yields.

Paper chromatography of these amino acids using *n*-butanol-acetic acid-water (4:1:5) was carried out, and in each case only a single spot was observable.

The derivatives prepared in the present work have been submitted to biological test, the result of which will be reported separately.

Experimental*4

2-(3-Oxobutyl)-6-hydroxy-3(2*H*)-pyridazinone (IIa)——A mixture of 22.4 g. (0.20 mole) of maleic hydrazide, 16.8 g. (0.24 mole) of methyl vinyl ketone, 150 ml. of ethanol, and 5 drops of 20% sodium hydroxide solution was refluxed for 3 hr. After filtration of unchanged maleic hydrazide, the filtrate was allowed to stand in a refrigerator overnight, the separated crystals were filtered, washed with water, and dried. Recrystallization from acetone afforded colorless needles, m.p. 144~146°(reported,[10] 148~149°). UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε): 320 (3.48). IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 1706 (CO) and 1650 (ring CO). Yield, 24.5 g. (67%).

2,4-Dinitrophenylhydrazone: Orange needles, m.p. 223~224°(from ethanol). *Anal.* Calcd. for $C_{10}H_{12}O_4N_4$: C, 46.41; H, 3.90. Found: C, 46.77; H, 4.08.

2-(3-Oxopropyl)-6-hydroxy-3(2*H*)-pyridazinone (IIb)——A mixture of 5.60 g. (0.05 mole) of maleic hydrazide, 3.92 g. (0.07 mole) of acrolein, 50 ml. of ethanol, and 3 drops of 20% sodium hydroxide solution was heated at reflux temperature for strictly 10 min. While being hot, unchanged maleic hydrazide was filtered, and the filtrate was allowed to stand at room temperature. The deposited crystals were filtered, washed with ethanol, and dried. Recrystallization from ethanol gave colorless leaflets, m.p. 178~180°. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε): 328 (3.43). IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 1680 (CHO) and 1625 (ring CO). Yield, 4.12 g. (49%). *Anal.* Calcd. for $C_7H_8O_3N_2$: C, 50.00; H, 4.80; N, 16.66. Found: C, 49.64; H, 4.89; N, 16.36.

2,4-Dinitrophenylhydrazone: Yellow needles (from methanol), m.p. 225~226°(decomp.). *Anal.* Calcd. for $C_{13}H_{12}O_6N_6$: C, 44.83; H, 3.47; N, 24.13. Found: C, 45.13; H, 3.60; N, 24.08.

2-(3-Oxobutyl)-4,5-dichloro-3(2*H*)-pyridazinone (IIc)——A mixture of 16.50 g. (0.10 mole) of 4,5-dichloro-3(2*H*)-pyridazinone,[11] 8.40 g. (0.12 mole) of methyl vinyl ketone, 75 ml. of ethanol, and 3 drops of 20% sodium hydroxide solution was refluxed for 5 hr. The reaction mixture was concentrated on a rotary evaporator, and the resulting brownish solution was treated with water. The separated crystals were filtered, washed with water, and dried. Recrystallization from a mixture of ethanol and water gave 20.10 g. (85%) of IIc. Yellowish needles, m.p. 85~87°. IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 1696 (CO) and 1634 (ring CO). *Anal.* Calcd. for $C_8H_8O_2N_2Cl_2$: C, 40.87; H, 3.42; N, 11.92. Found: C, 41.43; H, 3.43; N, 12.26.

2,4-Dinitrophenylhydrazone: Orange yellow needles, m.p. 191~192°(from methanol). *Anal.* Calcd. for $C_{14}H_{12}O_5N_6Cl_2$: C, 40.50; H, 2.88. Found: C, 41.04; H, 3.02.

2-(3-Oxobutyl)-6-chloro-3(2*H*)-pyridazinone (IId)——This compound was prepared from 6-chloro-3(2*H*)-pyridazinone (Ic) and methyl vinyl ketone in 52% yield by the method similar to that used for the preparation of IIa. Straw yellow prisms, m.p. 42~43°(from a mixture of ethanol and *i*-propylether). IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 1700 (CO) and 1650 (ring CO). *Anal.* Calcd. for $C_8H_9O_2N_2Cl$: C, 47.89; H, 4.40; N, 13.96. Found: C, 47.83; H, 4.73; N, 13.78.

2,4-Dinitrophenylhydrazone: A yellow, crystalline powder, m.p. 155~156°(from ethanol). *Anal.* Calcd. for $C_{14}H_{13}O_5N_6Cl$: C, 44.16; H, 3.45. Found: C, 43.96; H, 3.61.

2-(3-Oxobutyl)-4-hydroxy-1(2*H*)-phthalazinone (X)——This compound was prepared from phthalic hydrazide and methyl vinyl ketone in 52% yield by the method similar to that used for the preparation of IIa. Slightly yellow needles, m.p. 155~156°(from ethanol). IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 1708 (CO) and 1625 (ring CO). *Anal.* Calcd. for $C_{12}H_{12}O_3N_2$: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.27; H, 5.41; N, 12.41.

2,4-Dinitrophenylhydrazone: Orange granules (from ethanol), m.p. 237°(decomp.). *Anal.* Calcd. for $C_{18}H_{16}O_6N_6$: C, 52.43; H, 3.91. Found: C, 52.26; H, 4.19.

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*4 All melting points are uncorrected. Infrared and ultraviolet spectra were measured on a JASCO Model-IR infrared spectrophotometer, and on a Hitachi Model EPS-2 ultraviolet spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian HR-100 spectrophotometer.

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2-(2,5-Dioxo-4-methyl-4-imidazolidinyl)ethyl-6-hydroxy-3(2*H*)-pyridazinone (IIIa)——A typical experiment for these hydantoins, IIIa~d and XI, is described with IIIa. Their physical properties and analytical data are shown in Table I.

To a solution of 6.00 g. (0.033 mole) of 2-(3-oxobutyl)-6-hydroxy-3(2*H*)-pyridazinone and 10.0 g. of ammonium carbonate in a mixture of 80 ml. of ethanol and 60 ml. of water was added dropwise a solution of 1.72 g. (0.035 mole) of sodium cyanide in 10 ml. of water, and the mixture was heated at 75~80° for 1.5 hr. Additional 2.0 g. of ammonium carbonate was added, and the mixture was heated again at 75~80° for 1 hr. Meanwhile, the excess of ammonium carbonate was evaporated, and the reaction mixture was concentrated in a rotary evaporator. The resulting solution was carefully acidified with 10% hydrochloric acid in a hood, the separated crystals were filtered after 3 hr., washed with ice-water, and dried. Recrystallization from ethanol gave a colorless, crystalline powder, m.p. 192~195°(decomp.). IR ν_{max}^{Nujol} cm^{-1}: 1705 (hydantoin CO) and 1658 (ring CO).

DL-2-(3-Amino-3-carboxybutyl)-6-hydroxy-3(2*H*)-pyridazinone (IVa)——1) From the hydantoin (IIIa). A mixture of 2.52 g. (0.01 mole) of IIIa, 6.94 g. (0.022 mole) of barium hydroxide octahydrate, and 30 ml. of water was refluxed for 4 hr. After the reaction mixture was diluted with 100 ml. of water, nitrogen was passed through to exclude ammonia which was produced during hydrolysis, and the reaction mixture was filtered. The filtrate was acidified with 20% sulfuric acid, the precipitated barium sulfate was filtered, and washed with water. The combined filtrate was passed through an Amberlite IR-120 (H-form) column,[*5] and the column was washed with water till no more sulfate ion was detected with barium nitrate solution. The absorbed IVa was eluted by passing a diluted ammonia solution (twenty ml. of conc. ammonia solution was diluted to 500 ml. with water). The elution of IVa was checked by heating with 0.2% ninhydrin ethanol solution. The eluant was concentrated on a ratory evaporator until white flakes began to form. The residue was treated with ethanol, and the mixture was allowed to stand in a refrigerator overnight. The deposited crystals were filtered, washed with ethanol, and dried. Colorless fine needles, m.p. 272~276°(decomp.). Yield, 0.73 g. (32%). UV $\lambda_{max}^{H_2O}$ mμ (log ε): 314 (3.45). NMR spectrum of IVa in deuterium oxide shows the signals (τ) at 1.54 (singlet, 3-CH_3), 2.29 (multiplet, 2-CH_2), 4.15 (triplet, 1-CH_2), and at 7.10 (ring 4-H) and 7.32 (ring 5-H) as a pair of doublets.

N-2,4-Dinitrophenyl derivative (VIa): A yellow powder (from ethanol·water), m.p. 186~187°. IR ν_{max}^{Nujol} cm^{-1}: 1702 (COOH), 1655 (ring CO) and 1322 (NO_2). *Anal.* Calcd. for $C_{15}H_{15}O_8N_5$: C, 45.80; H, 3.84. Found: C, 45.58; H, 3.54.

2) By the Strecker synthesis from 2-(3-oxobutyl)-6-hydroxy-3(2*H*)-pyridazinone (IIa). To a solution of 5.20 g. (0.08 mole) of potassium cyanide and 4.55 g. (0.085 mole) of ammonium chloride in 80 ml. of water, was added 9.6 ml. of conc. ammonium hydroxide solution. To this solution was added dropwise a solution of 14.56 g. (0.08 mole) of IIa in a mixture of 140 ml. of methanol and 50 ml. of water under ice-cooling. After stirring at 5~10° for 2 hr., the mixture was heated on a water bath at 70~75° for 10 hr. Then, 140 ml. of conc. hydrochloric acid was added dropwise with stirring under ice-cooling, hydrogen chloride was passed through the reaction mixture for 20 min., and the reaction mixture was allowed to stand overnight. To the reaction mixture was added the same volume of water, the mixture was heated in a boiling water bath for 2 hr., and evaporated to dryness under reduced pressure. On concentration the deposited inorganic crystals had to be filtered off several times. The residue was extracted with ethanol, the extract was evaporated to dryness under reduced pressure, and the syrupy residue was dissolved in 250 ml. of water. The solution was passed through an Amberlite IR-120 (H-form) column, and IVa was eluted as described in 1).

DL-2-(3-Amino-3-carboxypropyl)-6-hydroxy-3(2*H*)-pyridazinone (IVb)——This compound was prepared from the hydantoin (IIIb) derived from 2-(3-oxopropyl)-6-hydroxy-3(2*H*)-pyridazinone (IIb), and also by the Strecker synthesis from IIb. These procedures employed were similar to those used for the preparation of IVa. UV $\nu_{max}^{H_2O}$ mμ (log ε): 314 (3.42).

N-2,4-Dinitrophenyl Derivative (VIb): Yellow granules (from ethanol), m.p. 233°(decomp.). IR ν_{max}^{Nujol} cm^{-1}: 1703 (COOH), 1650 (ring CO) and 1336 (NO_2). *Anal.* Calcd. for $C_{14}H_{13}O_8N_5$: N, 18.47. Found: N, 18.33.

DL-2-(3-Amino-3-carboxybutyl)-4-hydroxy-1(2*H*)-phthalazinone (XVII)——A mixture of 7.7 g. (0.026 mole) of the hydantoin (XI), 18.0 g. (0.057 mole) of barium hydroxide octahydrate and 50 ml. of water was heated at reflux temperature for 4 hr. After cooling, nitrogen was passed through the reaction mixture to remove ammonia produced during hydrolysis. The reaction mixture was filtered, the filtrate was acidified with 20% sulfuric acid, and the precipitated barium sulfate was filtered off. The filtrate was allowed to stand in a refrigerator overnight, the separated granules were filtered, and recrystallized from water to give colorless needles, m.p. 261°(decomp.). UV $\lambda_{max}^{H_2O}$ mμ (log ε): 263 (3.31), 272 (3.33), 316 (3.75). Yield, 1.53 g.

4-(2-Iodoethyl)-4-methylimidazolidine-2,5-dione (XIII)——To a solution of 4.52 g. (0.034 mole) of 2-methyl-2-amino-4-hydroxybutyric acid[12] (XII) in 15 ml. of water was added dropwise a solution of 2.97 g. (0.037 mole) of potassium cyanate in 5 ml. of water with stirring at room temperature, and the mixture was

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[*5] Before preparing a column, Amberlite IR-120 was washed with 10% hydrochloric acid several times, then with water till almost neutral to litmus paper.

heated at 70～75° for 4 hr. Twenty-five ml. of conc. hydroiodic acid was added dropwise into the reaction mixture with stirring under ice-cooling, and the mixture was heated at 90～95° for 3 hr. The reaction mixture was evaporated to almost dryness under reduced pressure, the residue was extracted with hot acetone, and acetone was evaporated to dryness. To the brown residue was added 25 ml. of conc. hybroiodic acid again, the mixture was heated at 90～95° for 5 hr., and allowed to stand overnight at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, the residue was dissolved in hot water, and the resulting solution was kept in a refrigerator overnight. The needles separated were collected, washed with ice-water, and dried. Recrystallization from hot water gave yellow needles, m.p. 177～178°. Yield, 2.62 g. (27%). IR $\nu_{\max}^{\text{Nujol}}$ cm^{-1}: 1770 and 1714 (hydantoin CO). *Anal.* Calcd. for $C_6H_9O_2N_2I$: C, 26.87; H, 3.62; N, 10.45. Found: C, 27.62; H, 3.34; N, 10.62.

2-(2,5-Dioxo-4-methyl-4-imidazolidinyl)ethyl-6-hydroxy-3(2*H*)-pyridazinone (IIIa) from 4-(2-Iodoethyl)-4-methylimidazolidine-2,5-dione (XIII)——A mixture of 2.68 g. (0.01 mole) of XIII, 20 ml. of ethanol, and 2.0 g. of 80% hydrazine hydrate was refluxed for 2 hr. The reaction mixture was evaporated to dryness under reduced pressure, 5 ml. of 10% hydrochloric acid was added to the residue, and the mixture was evaporated again to dryness under reduced pressure. This syrupy residue was heated with 1.96 g. of maleic anhydride and 20 ml. of water at reflux temperature for 3 hr. After cooling, the separated leaflets were filtered, and dried. IR spectrum of this product, m.p. 288～291° (decomp.) was identical with that of maleic hydrazide (0.37 g.). The filtrate was concentrated under reduced pressure, and the resulting solution was allowed to stand in a refrigerator overnight. The separated leaflets were filtered, washed with ethanol, and dried. Recrystallization from ethanol gave colorless leaflets, m.p. 188～192°. The infrared spectrum of this product was identical with that of IIIa. Yield, 0.51 g. (20%, calculated from XIII).

Similarly, IIIc was synthesized by heating XIV with mucochloric acid in an overall yield of 6% from XIII.

Preparation of Deuterated Compounds——Replacement of the hydrogens of NH and OH in these compounds was accomplished as follows. A sample was dissolved in an excess amount of deuterium oxide, the solution was allowed to stand overnight, and evaporated under reduced pressure. This procedure was repeated two or three times. The residue was dried over potassium hydroxide in vaccum, and its infrared spectrum was measured in a potassium bromide disk.

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