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Structure of Carzinophilin. II.[1] A New Amino Acid and Its Derivative from Carzinophilin

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There have been obtained several compounds from the alkaline hydrolysate of carzinophilin. Three of them are 3-methoxy-5-methylnaphthalene-1-carboxylic acid (XIII), its amide (XIV), and 3-hydroxy-5-methylnaphthalene-1-carboxylic acid (XV). The remaining compounds are a new amino acid (XII) and its derivative (XVI). XII is identified with synthetic *dl-erythro*-4-amino-2,3-dihydroxy-3-methylbutanoic acid and, finally, the absoulte configuration of XII is determined to be the (*S*)-configuration both at C-2 and C-3 by circular dichroism. Hydrolysis of XVI with 20% hydrochloric acid gives XII and XV. From mass and nuclear magnetic resonance spectra, the structure of XVI is assigned as shown in Chart 4.

Carzinophilin is an anti-tumor antibiotic isolated from *Streptomyces sahachiroi* by Hata[3] and his collaborators in 1954. Its molecular formula was first given as $C_{60}H_{60}O_{21}N_6$ but Tanaka[4] and others corrected it to $C_{50}H_{58}O_{18}N_5$ in 1959 and further reported that alkaline hydrolysis of carzinophilin gave 3-methoxy-5-methylnaphthalene-2-carboxylic acid. We recently re-examined this alkaline hydrolysis and described in our communication[1] that this carboxylic acid was 3-methoxy-5-methylnaphthalene-1-carboxylic acid. In the present series of work, a new amino acid and its derivative were isolated from another fraction obtained from the alkaline hydrolysis of carzinophilin and their structures were determined, which are reported herein.

As shown in Chart 1, treatment of carzinophilin with dilute ammonia resulted in separation of a solid substance (fract.-1) which was found to be rather labile and difficult to purify.

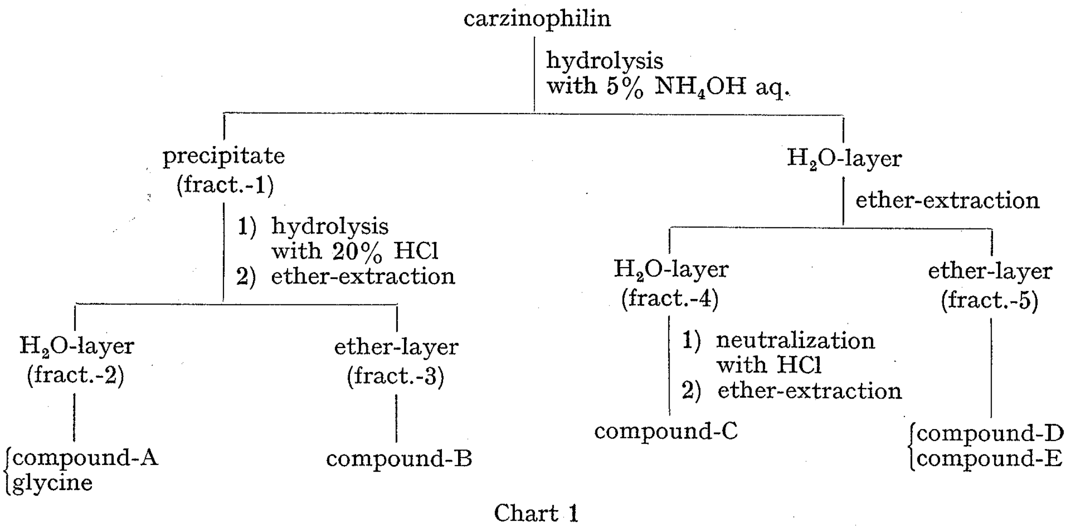

Chart 1

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[1] Part I: M. Onda, Y. Konda, A. Noguchi, S. Ōmura, and T. Hata, *J. Antibiotics*, Ser. A, 22, 42 (1969).
[2] Location: *5-9-1 Shirokane, Minato-ku, Tokyo.*
[3] T. Hata, F. Koga, T. Sano, K. Kanamori, A. Matsumae, R. Sugawara, T. Shima, S. Ito, and S. Tomizawa, *J. Antibiotics*, Ser. B, 7, 107 (1954).
[4] M. Tanaka, T. Kishi, and Y. Maruta, *J. Antibiotics*, Ser. B, 12, 361 (1959).

After hydrolysis of fract.-1 with 20% hydrochloric acid, the aqueous layer (fract.-2) and the ethereal layer (fract.-3) were separated by extraction with ether. The thin-layer chromatography (TLC) of fract.-2 revealed three spots which colored with ninhydrin. The results of amino acid analysis and paper electrophoresis confirmed the presence of glycine and an unknown substance in the neutral amino acid region and another substance in the basic amino acid region. This hydrolysate was purified by column chromatography and afforded colorless plates (compound-A), mp 208—210.5°. Fract.-3 gave colorless needles (compound-B), mp 246°. The aqueous layer obtained after separation of fract.-1 by decantation was extracted with ether and separated into the aqueous layer (fract.-4) and the ethereal layer (fract.-5). Neutralization of fract.-4 with hydrolchloric acid and extraction with ether gave colorless needles (compound-C), mp 180°. Fract.-5 was a mixture of two neutral substances and separated by alumina chromatography into colorless needles (compound-D), mp 178°, and colorless plates (compound-E), mp 180°.

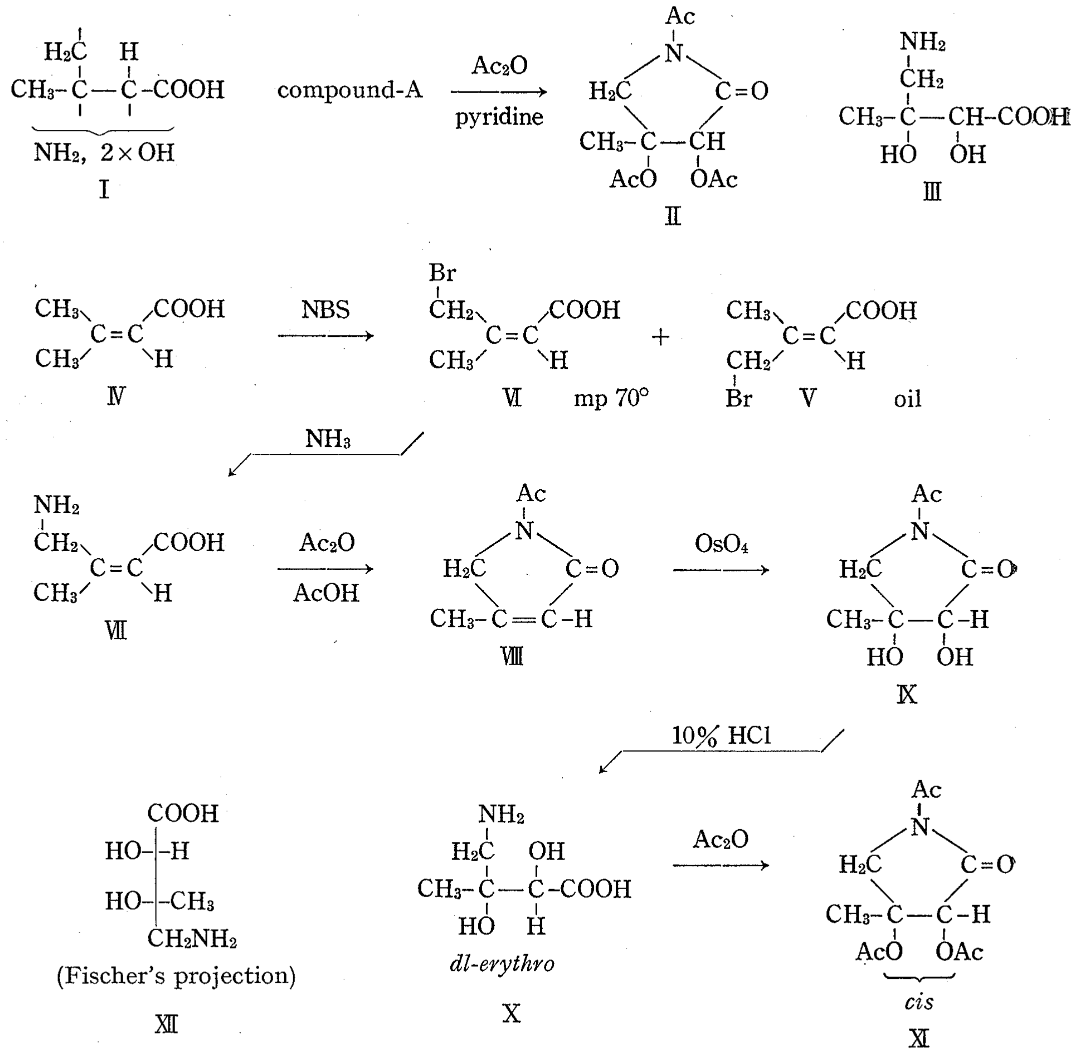

Chart 2

Structure of Compound-A: Compound-A has a composition corresponding to $C_5H_{11}O_4N$, colors violet to ninhydrin, and was assumed to be a neutral amino acid from the results of amino acid analysis[5] and paper electrophoresis.[6] Its infrared (IR) spectrum (KBr) showed

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5) Beckman Model 120C Amino Acid Analyser.

6) Toyo Roshi (No. 51), 10×65 cm; solvent, pyridine:AcOH:H_2O=10:1:89 (pH 6); 3000 V, 10°, 30 min.

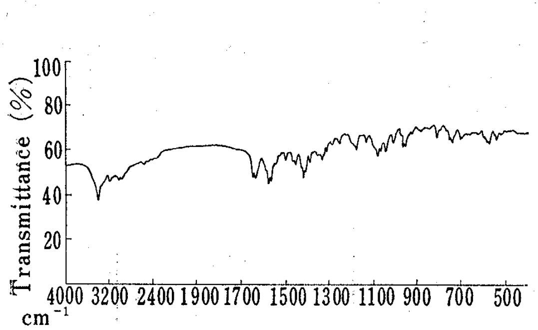
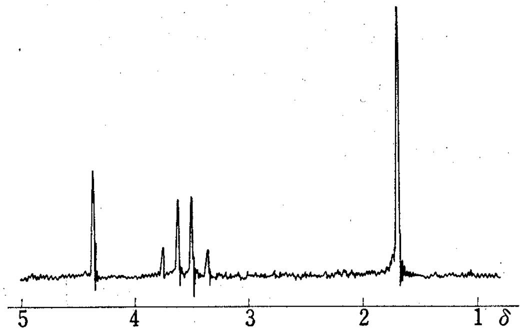


Fig. 1. IR Spectrum of Compound-A(XII) (KBr)



Fug. 2. NMR Spectrum of Compound-A (XII) (100 Mc, D_2O)

the absorptions at 3600—2400 cm^{-1} corresponding to -OH and -NH, and at 1640 cm^{-1} due to -COO$^-$ (Fig. 1). Its nuclear magnetic resonance (NMR) spectrum (D_2O) exhibited the signals at 1.75 (3H, s), 3.56 (1H, d, $J=14$), 3.68 (1H, d, $J=14$), and 4.4 (1H, s) (Fig. 2). These data suggest a partial structure (I) for compound-A (Chart 2). Acetylation of compound-A with acetic anhydride gave an oily substance (II), $C_{11}H_{15}O_6N$, whose IR spectrum ($CHCl_3$) exhibited the absorptions at 1750 and 1707 cm^{-1} due to the carbonyl group of ester and amide, respectively, and its NMR spectrum showed signals at 1.71 (3H, s), 2.05 (3H, s), 2.22 (3H, s), 2.52 (3H, s), 3.55 (1H, d, $J=14$), 4.49 (1H, d, $J=14$), and 5.45 (1H, s). These spectral data indicate that II should be 1-acetyl-3,4-diacetoxy-4-methyl-2-pyrrolidone and, consequently, compound-A would be 4-amino-2,3-dihydroxy-3-methylbutanoic acid (III). In order to prove this structure, an authentic sample was synthesized for the sake of comparison.

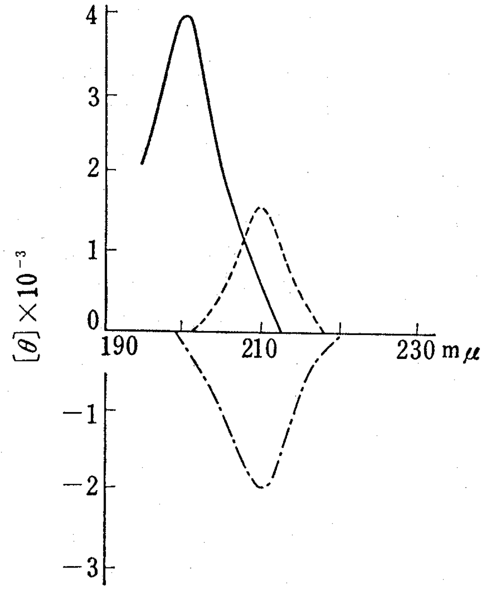


Fig. 3. CD Curves of Compound-A (XII), L-(−)-Malic Acid and D-(−)-Lactic Acid

——: compound-A ($c=6.5\times10^{-3}$ in H_2O)
--------: L-(−)-malic acid ($c=1.4\times10^{-2}$ in H_2O)
------: D-(−)-lactic acid ($c=2.8\times10^{-2}$ in H_2O)

Bromination of 3-methylcrotonic acid (IV) with N-bromosuccinimide in benzene and purification of its product through silica gel chromatography afforded two monobromo compounds. Amination of the *cis* compound (VI)$^{7)}$ with conc. ammonia gave *cis*-4-amino-3-methylcrotonic acid (VII), 185—187°. Acetylation of VII with acetic anhydride in acetic acid gave colorless scaly crystals (VIII), mp 85°, $C_7H_9O_2N$, whose IR spectrum ($CHCl_3$) did not show the absorptions corresponding to -OH and -NH but did show the absorptions at 1725 and 1690 cm^{-1}. Its NMR spectrum exhibited the signals at 2.1 (3H, m), 2.5 (3H, s), 4.27 (2H, br. s), and 5.85 (1H, q, $J=1.5$). These data indicate that lactam formation took place during this acetylation and also indicate that VI was actually a *cis* compound. Hydroxylation of VIII with osmium tetroxide in benzene gave an oily diol (IX), $C_7H_{11}O_4N$, whose IR spectrum in a dilute carbon tetrachloride solution showed the presence of intramolecular hydrogen bonding by the absorptions at 3565 and 3547 cm^{-1}. Hydrolysis of IX with 10% hydrochloric acid afforded colorless plates (X), mp 208—211°, $C_5H_{11}O_4N$, whose behavior in

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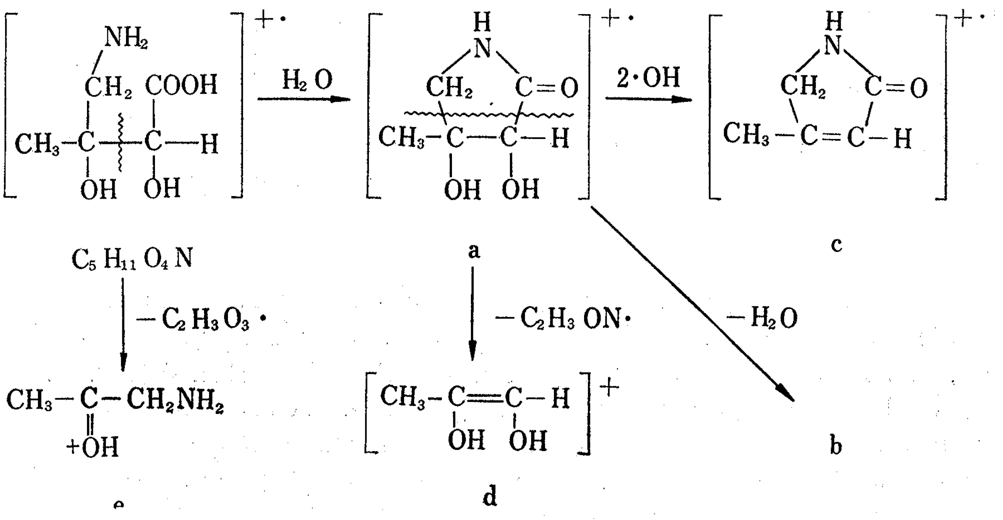
7) *cis* is referred to COOH.

the TLC[8] and paper chromatography (PPC),[9] and NMR spectrum were entirely identical with those of compound-A. Acetylation of X afforded an oily substance (XI), $C_{11}H_{15}O_6N$, whose IR and NMR spectra were entirely identical with those of II. Since X was derived from *cis*-glycol (IX), X would be in *erythro* form and compound-A would consequently be the optically active *erythro* compound.



	m/e	%		Calcd.	Found
a	131	(29)	$C_5H_9O_3N$	0.058	0.059
b	113	(48)	$C_5H_7O_2N$	0.051	0.047
c	97	(100)	C_5H_7ON	0.054	0.052
d	74	(93)	$C_3H_6O_2$	0.036	0.037
e			C_3H_8ON	0.060	0.060

Chart 3

In order to know the absolute configuration of C_2 in compound-A, circular dichroism absorption due to *n*-*π** transition of the carbonyl group in compound-A was compared with that in the model compounds, ᴅ-(—)-lactic acid ((*R*)-configuration) and ʟ-(—)-malic acid ((*S*)-configuration) (Fig. 3). Since compound-A showed a positive Cotton effect as did ʟ-(—)-malic acid, C_2 in compound-A is known to have the (*S*)-configuration. As stated above, compound-A is an *erythro* compound and it may be concluded that C_3 also has the (*S*)-configuration, *i.e.*, (2(*S*): 3(*S*))-4-amino-2,3-dihydroxy-3-methylbutanoic acid (XII). The high-resolution mass spectrum of compound-A did not show a molecular ion and only four important fragment ions were found. From the composition of these ions calculated from this mass spectrum, the fragmentation course shown in Chart 3 may be considered.

Structures of Compound-B, -C, and -D: Compound-C has a molecular composition corresponding to $C_{13}H_{12}O_3$ and its decarboxylation in quinoline afforded 7-methoxy-1-methylnaphthalene. From the absence of intramolecular hydrogen bonding in its IR spectrum and from the data of NMR and mass spectra, compound-C was determined as 3-methoxy-5-methylnaphthalene-1-carboxylic acid (XIII).[1]

Compound-D corresponds to the molecular formula of $C_{13}H_{13}O_2N$ and, from its conversion to compound-C by hydrolysis and the presence of an amide absorption in its IR spectrum, compound-D was determined as 3-methoxy-5-methylnaphthalene-1-carboxamide (XIV).

Compound-B has the molecular formula of $C_{12}H_{10}O_3$ and was indentified with 3-hydroxy-5-methylnaphthalene-1-carboxylic acid (XV) derived from acidic hydrolysis of compound-C (XIII) by IR spectrum and mixed fusion.

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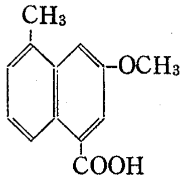
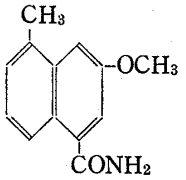
8) A microcrystalline cellulose (Avicel SF); solvent, BuOH: AcOH: $H_2O=4:1:1$.
9) Toyo Roshi (No. 51); solvent, BuOH: pyridine: $H_2O=1:1:1$.

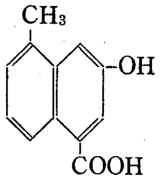
Structure of Compound-E: Compound-E corresponds to the molecular formula of $C_{18}H_{22}O_5N_2$. Its hydrolysis with 20% hydrochloric acid afforded an aromatic acid and an amino acid. The aromatic acid was identified with XV by mixed fusion. The amino acid was determined as XII from the results of TLC, PPC, amino acid analysis, and paper electrophoresis. Since compound-E is neutral, it is known that compound-E was formed by the ester or amide bonding of the carboxyl group in XIII with XII whose carboxyl group has been converted into an amide. The IR spectrum (KBr) of compound-E showed the absorptions at 3400—3280 (-OH and -NH) and 1670 cm^{-1} (-CO-N<), but no absorption in the region corresponding to the ester-carbonyl. This indicates that XVI is the most probable of the three possible structures for compound-E (Chart 4). The signals at 2.85 (2H, s), 5.32 (1H, d, J=2.5), 5.75 (1H, d, J=5), and 7.0 (1H, br. s) in the NMR spectrum (acetone-d_6) of compound-E disappear by deuteriation and, at the same time, the quartet (J=14, and 5) at 3.2 (1H) changes into a doublet (J=14), the octet (J=14, 8, and 2.5) at 3.92 (1H) into a doublet (J=14), and the doublet (J=5) at 4.05 (1H) into a singlet. The signals that disappear by deuteriation correspond to -OH and -NH, and the signals that change into a simpler splitting are known to be coupled with -OH and/or -NH. Irradiation of the signal at 5.75 results in conversion of the doublet at 4.05 into a singlet and irradiation of the signal at 3.92 results in change of the doublet at 5.32 into a singlet. From these data, the protons in compound-E (XVI) can be assigned as shown in Chart 4. The important fragment ions indicated by the high-resolution mass spectrometry may be considered to correspond to the ions derived from the cleavage of the bond shown by wave-like lines (Chart 4), and it seems appropriate to give the structure (XVI) for compound-E.



XIII

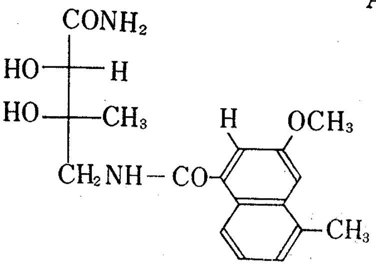


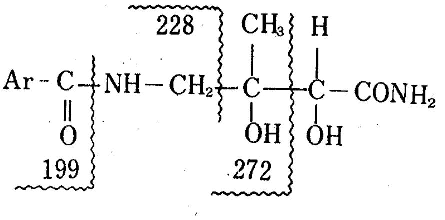
XIV



XV

$$\text{compound-E} \xrightarrow{20\%\ \text{HCl}} \text{XII} + \text{XV}$$





m/e	%		Cacld.	Found.
272	(9)	$C_{16}H_{18}O_3N$	.128	.130
228	(24)	$C_{14}H_{14}O_2N$	.102	.102
199	(100)	$C_{13}H_{11}O_2$	.076	.074

XVI

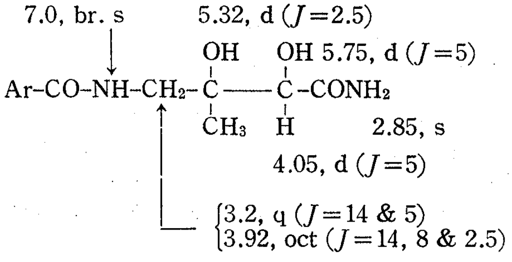


Chart 4

Experimental

Melting points were determined on a micro hot-stage and were uncorrected. Infrared spectra were measured with a Shimadzu IR-27G and intramolecular hydrogen bonding was measured with a JASCO Model DS-403G. Nuclear magnetic resonance spectra were taken in $CDCl_3$ unless otherwise noted on a JEOL's JNM-4H-100 spectrometer (100 Mc). Chemical shifts were given in δ (ppm) downfield from internal Me_4Si, and coupling constants (J) in cps. Abbreviation used were as follows: s, singlet; d, doublet; q, quartet; m, multiplet; br, broad. Circular dichroism was taken with a JASCO ORD/UV-5 spectropolarimeter. Mass spectra were measured with a JEOL's JMS-OIS.

Hydrolysis of Carzinophilin——A solution of carzinophilin (3.0 g) in 5% NH_4OH (73 ml) was heated at 80° for 30 min. After cooling, the precipitate (900 mg) (fract.-1) was separated from H_2O by decantation. The H_2O-layer was extracted with ether to separate into H_2O-layer (fract.-4) and ethereal layer (fract.-5).

Isolation of Compound-A (XII) and -B (XV)——A solution of fract.-1 (400 mg) in 20% HCl (20 ml) was heated at 110° in a sealed tube for 24 hr. After cooling, the reaction mixture was extracted with ether to separate into H_2O-layer (fract.-2) and ethereal layer (fract.-3). Fract.-2 was evaporated *in vacuo* to dryness and washed with $CHCl_3$. The residue (120 mg) was chromatographed over a microcrystalline cellulose (Avicel SF) (600 g) by using BuOH–pyridine–H_2O (4 : 1 : 1 v/v) as eluent. The first fraction included two substances (50 mg) which were not able to be identified. The second afforded colorless plates (XII) (33 mg), which showed mp 108—110.5° on recrystallization from 90% EtOH. TLC: *Rf* 0.23. PPC: *Rf* 0.36. Paper electrophoresis: distance from center line, 1 cm. *Anal.* Calcd. for $C_5H_{11}O_4N$: C, 40.26; H, 7.55; N, 9.26. Found: C, 40.27; H, 7.43; N, 9.39. Fract.-3 was evaporated and then recrystallized from benzene–AcOEt to afford colorless needles (XV) (100 mg), mp 246°. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3300—2800 (OH), 1690 (COOH). Mass Spectrum Calcd. for $C_{12}H_{10}O_3$: MW,10) 202.063. Found: M^+, 202.064.

Isolation of Compound-C (XIII)——Fract.-4 was neutralized with conc. HCl and extracted with ether. The residue (150 mg) was recrystallized to afford colorless needles (XIII) (120 mg), mp 180°. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3420—3200 ($\mathrm{CO\underline{OH}}$), 1690 ($\mathrm{\underline{C}OOH}$). NMR (acetone-$d_6$): 2.65 (3H, s), 3.95 (3H, s), 7.35 (aromatic 2H, m), 7.55 (aromatic 1H, d, $J=3$), 7.85 (aromatic 1H, d, $J=3$), 8.67 (aromatic 1H, q, $J=7$ and 3). Mass Spectrum Calcd. for $C_{13}H_{12}O_3$: MW, 216.079. Found: M^+, 216.079.

Isolation of Compound-D (XIV) and -E (XVI)——Fract.-5 was evaporated *in vacuo* to dryness. The residue (100 mg) was chromatographed over Al_2O_3 (11 g). The first fraction of benzene afforded colorless needles (XIV) (26 mg), mp 178° (from benzene). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 3400—3200 (-NH-), 1640 (-CON<). NMR (acetone-d_6): 2.66 (3H, s), 3.96 (3H, s), 7.2—7.4 (aromatic 4H, m), 8.15 (aromatic 1H, q, $J=7$ and 3). Mass Spectrum Calcd. for $C_{13}H_{13}O_2N_2$: MW, 215.095. Found: M^+, 215.096. The second of benzene–AcOEt (3:7 v/v) afforded XVI (50 mg). Recrystallization of XVI from benzene gave colorless plates (31 mg), mp 180°. Mass Spectrum Calcd. for $C_{18}H_{22}O_5N_2$: MW, 346.153. Found: M^+, 346.151.

Hydrolysis of Compound-E (XVI)——A solution of XVI (8 mg) in 20% HCl (4 ml) was heated at 120° in a sealed tube for 24 hr, and the reaction mixture was extracted with ether. The ethereal layer afforded colorless needles (2 mg), mp *ca.* 245°, which was identified with compound-B (XV) by IR spectrum and mixed fusion. The H_2O-layer mainly showed one spot in TLC, which was identified with compound-A (XII) by TLC, PPC, and paper electrophoresis.

(3(*S*):4(*S*))-1-Acetyl-3,4-diacetoxy-4-methyl-2-pyrrolidone (II)——A solution of XII (10 mg) in AcOH (1.5 ml) and dry pyridine (0.5 ml) was allowed to stand at room temperature for 72 hr. After evaporation *in vacuo*, the residue was extracted with benzene to give oily compound (II) (13 mg). Mass Spectrum Calcd. for $C_{11}H_{15}O_6N$: MW, 257.089. Found: M^+, 257.085.

***cis*-4-Amino-3-methylcrotonic Acid (VII)**——A solution of 3-methylcrotonic acid (IV) (2 g) and NBS (3.45 g) in benzene (100 ml) was refluxed for 3 hr and then allowed to stand at room temperature overnight. The reaction mixture was filtered to remove off succinimide and the filtrate was evaporated *in vacuo* to dryness. The residue was chromatographed over silica gel (150 g). The first fraction of *n*-hexane–benzene (3:1 v/v) afforded oily *trans*-compound (V) (1.5 g) and the second of *n*-hexane–benzene (2:1 v/v) afforded *cis*-compound (VI) (900 mg), colorless needles, mp 70° (from petr. ether). A mixture of VI (200 mg) and $(NH_4)_2CO_3$ (860 mg) in conc. NH_4OH was heated at 60° for 6 hr and then evaporated *in vacuo* to dryness. The residue was extracted with EtOH to remove NH_4Br and recrystallized from dil. EtOH to give colorless pillars (VII) (30 mg), mp 185—187°. Mass Spectrum Calcd. for $C_5H_9O_2N$: MW, 115.063. Found: M^+, 115.066.

1-Acetyl-4-methyl-2-keto-3-pyrroline (VIII)——A solution of VII (60 mg) in Ac_2O (0.2 ml) and AcOH (0.2 ml) was heated at 120° for 30 min with stirring. After evaporation *in vacuo*, the residue was extracted with benzene. The benzene residue was recrystallized from *n*-hexane to give colorless plates (VIII) (40 mg), mp 85—87°. Mass Spectrum Calcd. for $C_7H_9O_2N$: MW, 139.063. Found: M^+, 139.065.

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10) MW: molecular weight.

***dl-cis*-1-Acetyl-3,4-dihydroxy-4-methyl-2-pyrrolidone (IX)**——A mixture of VIII (36 mg), OsO_4 (76.2 mg), and pyridine (0.05 ml) in benzene (5 ml) was allowed to stand at room temperature overnight. The reaction mixture was filtered to collect black precipitate. The black precipitate was dissolved in dioxane (2 ml) and treated with H_2S gas. After filtration, the filtrate was evaporated *in vacuo*. The residue was chromatographed over silica gel (2 g) by using $CHCl_3$–MeOH (200:1 v/v) as eluent, affording oily compound (IX) (20 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (-OH), 1750 and 1700 (2 -CON<). NMR: 1.48 (3H, s), 2.5 (3H, s), 3.27 (2 × OH, br. s), 3.36 (1H, d, $J=12$), 4.04 (1H, d, $J=12$), 4.18 (1H, s). Mass Spectrum Calcd. for $C_7H_{11}O_4N$: MW, 173.068. Found: M^+, 173.067.

***dl-erythro*-4-Amino-2,3-dihydroxy-3-methylbutanoic Acid (X)**——A solution of IX (70 mg) in 10% HCl (15 ml) was heated at 90° for 1 hr. The reaction mixture was evaporated *in vacuo* to dryness with repeated addition of H_2O. The residue was chromatographed over a microcrystalline cellulose (35 g) by using BuOH–pyridine–H_2O (4:1:1 v/v) as eluent, affording X (35 mg). Recrystallization of X from 90% EtOH gave colorless plates (30 mg), mp 208—211°. TLC: *Rf* 0.23. PPC: *Rf* 0.36. NMR (D_2O): 1.75 (3H, s), 3.56 (1H, d, $J=14$), 3.68 (1H, d, $J=14$), 4.4 (1H, s). Mass Spectrum Calcd. for $C_5H_{12}O_4N$: MW, 150.076. Found: M^++1, 150.074.

***dl-cis*-1-Acetyl-3,4-diacetoxy-4-methyl-2-pyrrolidone (XI)**——A solution of X (5 mg) in EtOH (0.5 ml) and Ac_2O (0.2 ml) was allowed to stand at room temperature for 17 hr. After addition of H_2O, the reaction mixture was evaporated *in vacuo*, affording oily compound (XI) (7 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (-COO-), 1707 (-CON<). NMR: 1.71 (3H, s), 2.05 (3H, s), 2.22 (3H, s), 2.52 (3H, s), 3.55 (1H, d, $J=14$), 4.49 (1H, d, $J=14$), 5.45 (1H, s). Mass Spectrum Calcd. for $C_{11}H_{15}O_6N$: MW, 257.089. Found: M^+, 257.092.

Acknowledgement The authors are indebted to Kyowa Hakko Kogyo Co., Ltd. for the supply of carzinophilin.