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Synthesis of Actinomycin Related Compounds. I[1]

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Amino acid derivatives bearing an actinomycin chromophore was synthesized in a hope of finding one or another with more carcinolytic activity and less toxicity than the actinomycin. That is, DL-valine ethylester, DL-serine ethylester, DL-phenylalanine ethylester, α-aminocaprolactam, 3-aminopiperidone (2), and 3-aminopyrrolidone (2) were acylated with 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride. These compounds were catalytically hydrogenated to 3-hydroxy-4-methylanthraniloyl derivatives and then oxidized to actinocyl derivatives.

In the course of these investigation, it was observed that dehydric cyclization occurs between carboxyl and amino group when 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-proline is hydrogenated. Similar dehydric cyclization were observed in hydrogenation process of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-hydroxyproline and *o*-nitrobenzoyl-L-proline.

Actinomycin is an antibiotic which was first isolated in 1940 by Waksman and Woodruff.[3] It was soon discovered that there are several types of actinomycin.[4–7] Though it is highly active against gram positive bacteria, because of its high toxicity, it has not been applied clinically as a chemotherapeutic agent. Since the discovery of the carcinolytic activity of actinomycin by Hackmann[8] several investigations have been made on its antitumor effect.[9–15] The chemical structure of one component, actinomycin C_3 (I), of the actinomycin C mixture has been determined by Brockmann and his co-workers.[16] The chromophoric part of the molecule, responsible for the yellow-red color of the actinomycin, is the 2-amino-4,6-dimethyl-3-oxophenoxazine-1,9-dicarboxylic acid, which is bonded with its two carboxyl groups to two peptide lactone groups of equal structure. The chemical differences in the various actinomycins involve the nature and order of the amino acids in the peptide chains, but the phenoxazin-3-one nucleus seems to be common to all the actinomycins so far examined.[17] The total synthesis of actinomycin C_3 was performed by Brockmann, *et al.*[18] It has been

1) This work was presented at the 19th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo (April 5, 1964).
2) Location: *Takara-machi, Kanazawa.*
3) S.A. Waksman and H.B. Woodruff, *Proc. Soc. Exp. Biol. Med.*, **45**, 609 (1940).
4) C.E. Dalgliesh and A.R. Tood, *Nature*, **164**, 830 (1949).
5) H. Brockmann and N. Grubhofer, *Naturwiss.*, **36**, 376 (1949).
6) R. A. Manaker and G.G. Gregory, L.C. Vining, and S.A. Waksman, *Antibiotics Annual*, 853 (1954).
7) A. Nishibori, *J. Antibiotics* (Tokyo), *Ser. A*, **9**, 31 (1956).
8) C. Hackmann, *Z. Krebsforsch.*, **58**, 607 (1952).
9) G. Schulte, *Z. Krebsforsch.*, **58**, 500 (1952).
10) G. Schulte and Lings, *Strahlentherapie*, **90**, 301 (1953).
11) H.C. Reily, C.C. Stock, S.M. Buckley, and R.A. Clarke, *Cancer Research*, 13, 684 (1953).
12) C. Hackmann, *Z. Krebsforsch.*, **60**, 250 (1954).
13) H. Umezawa, *Chemotherapy*, **3**, 117 (1955).
14) S. Shiba, *Gann*, **47**, 440 (1956).
15) C. Hackmann and G. Schmidt-Kastner, *Z. Krebsforsch.*, **61**, 607 (1957).
16) H. Brockmann, G. Bohnsack, B. Franck, H. Grone, H. Muxfeldt, and C. Suling, *Angew. Chem.*, **68**, 70 (1956).
17) H. Brockmann, *Ann. New York Acad. Sci.*, **89**, 323 (1960).
18) H. Brockmann and H. Lackner, *Naturwiss.*, **47**, 230 (1960).

Table I. 3-Benzyloxy-4-methyl-2-nitrobenzoyl Derivatives (III)

Amino Acid Derivatives	Formula	Recrystallization Solvent	Yield (%)	mp (°C)	Analysis (%) Calcd. C	H	N	Found C	H	N
Glycine Ethylester	$C_{19}H_{20}O_6N_2$	Isopropylether	50	97— 98	61.28	5.41	7.52	61.41	5.72	7.37
DL-Alanine Ethylester	$C_{20}H_{22}O_6N_2$	Isopropylether	65	96— 97	62.16	5.74	7.25	62.32	5.78	7.31
DL-Valine Ethylester	$C_{22}H_{26}O_6N_2$	Isopropylether	40	113—114	63.75	6.32	6.76	63.63	6.34	7.22
DL-Phenylalanine Ethylester	$C_{26}H_{26}O_6N_2$	Isopropylether	52	118—119	67.52	5.67	6.06	67.28	5.70	6.44
DL-Serine Ethylester	$C_{20}H_{22}O_7N_2$	Isopropylether	60	103—105	59.69	5.51	6.96	59.92	5.28	6.54
L-Proline Ethylester	$C_{22}H_{24}O_6N_2$	Isopropylether	73	110—112	64.06	5.87	6.79	63.72	5.74	7.11
Glycine	$C_{17}H_{16}O_6N_2$	50% EtOH	86	192—193	59.30	4.68	8.14	59.51	4.61	8.51
DL-Alanine	$C_{18}H_{18}O_6N_2$	Me_2CO Benzene	87	150—152	60.33	5.06	7.82	59.95	5.30	8.10
α-Amino Caprolactam	$C_{21}H_{23}O_5N_3$	EtOH	50	203—205	63.46	5.83	10.58	63.80	6.10	10.61
3-Amino Piperidone (2)	$C_{20}H_{21}O_5N_3$	EtOH	78	201—202	62.65	5.52	10.96	63.01	5.90	11.21
3-Amino Pyrrolidone (2)	$C_{19}H_{19}O_5N_3$	EtOH	86	195—196	61.78	5.19	11.38	61.61	5.37	11.38

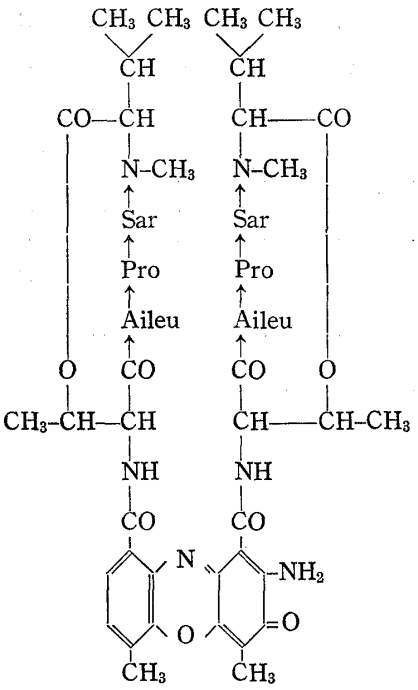
Table II. 3-Hydroxy-4-methylanthraniloyl Derivatives (IV)

Amino Acid Derivatives	Formula	Recrystallization Solvent	Yield (%)	mp (°C)	Analysis (%) Calcd. C	H	N	Found C	H	N
DL-Valine Ethylester	$C_{15}H_{22}O_4N_2$	Isopropylether	63	90— 92	61.20	7.53	9.52	61.23	7.88	9.51
DL-Phenylalanine Ethylester	$C_{19}H_{22}O_4N_2$	Isopropylether	87	123—124	66.65	6.48	8.18	66.47	6.62	7.99
DL-Serine Ethylester	$C_{13}H_{18}O_5N_2$	Isopropylether	89	119—120	55.31	6.43	9.92	55.18	6.54	9.64
α-Amino Caprolactam	$C_{14}H_{19}O_3N_3$	EtOH	82	232—233	60.63	6.91	15.15	60.30	6.94	15.09
3-Amino Piperidone (2)	$C_{13}H_{17}O_3N_3$	EtOH	73	240—241	59.30	6.51	15.96	59.38	6.60	16.28
3-Amino Pyrrolidone (2)	$C_{12}H_{15}O_3N_3$	EtOH	75	230—232	57.82	6.07	16.86	58.09	6.21	16.42

Table III. Actinocyl Derivatives (V)

Amino Acid Derivatives	Formula	Recrystallization Solvent	Yield (%)	mp (°C)	Analysis (%) Calcd. C	H	N	Found C	H	N
DL-Valine · Ethylester	$C_{30}H_{38}O_8N_4$	$CHCl_3$·Petr. Ether	50	255—256	61.84	6.57	9.62	61.63	6.87	9.59
DL-Phenylalanine Ethylester	$C_{38}H_{38}O_8N_4$	$CHCl_3$·Petr. Ether	60	218—220	67.24	5.64	8.26	66.85	5.85	7.95
DL-Serine Ethylester	$C_{26}H_{30}O_{10}N_4$	$CHCl_3$·Petr. Ether	60	239—241	55.91	5.41	10.03	55.76	5.57	9.66
α-Amino Caprolactam	$C_{28}H_{32}O_6N_6$	$CHCl_3$·Petr. Ether	35	300	61.30	5.88	15.32	61.02	6.26	14.95
3-Amino Piperidone (2)	$C_{26}H_{28}O_6N_6$	$CHCl_3$·Petr. Ether	33	289—290	59.99	5.42	16.15	59.69	5.51	16.35
3-Amino Pyrrolidone (2)	$C_{24}H_{24}O_6N_6$	$CHCl_3$·Petr. Ether	50	300	58.53	4.91	17.07	58.40	5.01	16.63

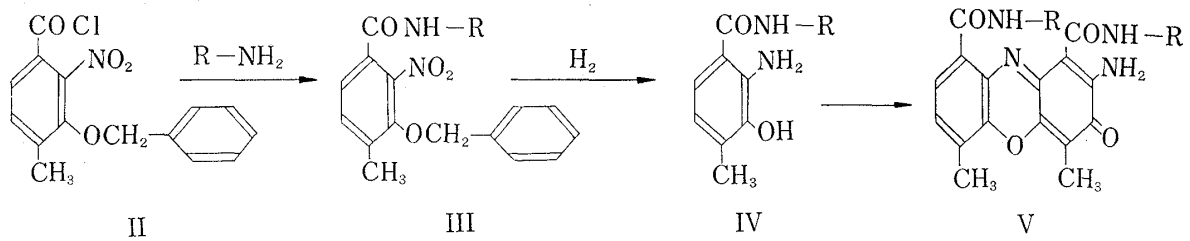
of great interest whether or not there are differences among the actinomycins in toxicity and in carcinolytic activity. From animal studies it was learned that there are indeed differences in the carcinolytic activity. It was reported by Kawamata, *et al.*[19] that actinomycinic acid S, which was obtained by treatment of actinomycin S with methanolic sodium hyrdoxide, has less acute toxicity, but it has an anticancer effect, though the amount of the acid was much greater than actinomycin S. From these observation, it is suggested that there are difference in biological activity for variety of peptide moiety of actinomycins.



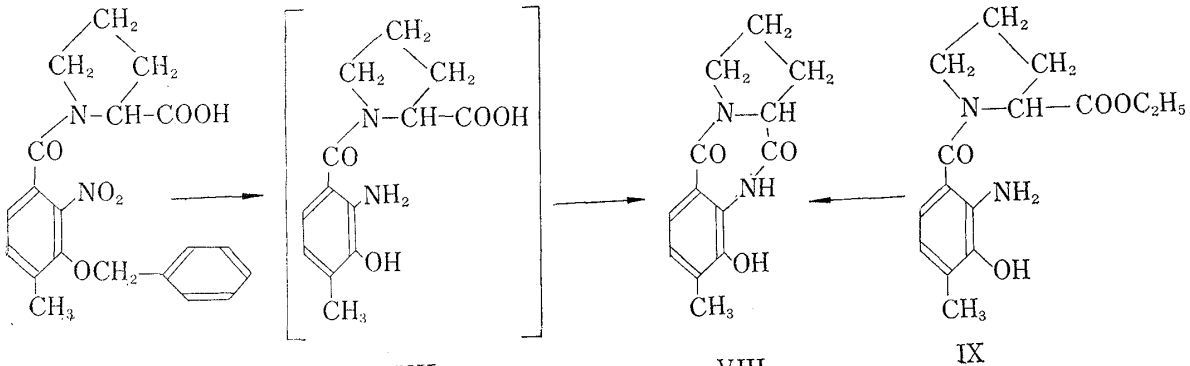
I

The authors have attempted to obtain derivatives of actinomycin in the hope of finding one or another with more carcinolytic activity and less toxicity than the actinomycin. This paper describes the synthesis of the compounds consisting of amino aicd derivatives bearing an actinomycin chromophore.

3-Benzyloxy-4-methyl-2-nitrobenzoyl chloride (II) was prepared by chlorination of the carboxylic acid compound[10] with thionyl chloride. Amino acid ethyl esters or their lactams were treated with the above chloride in ether at 5—10°. By these processes, glycine ethyl ester, DL-alanine ethyl ester, DL-valine ethyl ester, DL-serine ethyl ester, DL-phenylalanine ethyl ester, L-proline ethyl ester, α-aminocaprolactam, 3-aminopiperidone (2), and 3-aminopyrrolidone (2) were derived to N-(3-benzyloxy-4-methyl-2-nitrobenzoyl) compounds (III) in 40—86% yield (Table I). These compounds were then hydrogenated in the presence of palladium charcoal catalyst, in order to reduce the nitro group and split off the benzyl group (Table II). N-(3-Hydroxy-4-methyl-



II III IV V



VI VII VIII IX

Chart 1

19) J. Kawamata, H. Fujita, and R. Ikegami, *J. Antibiotics* (Tokyo), *Ser. A*, **13**, 415 (1960).
20) W.G. Hanger, W.C. Howell, and A.W. Johnson, *J. Chem. Soc.*, **1958**, 496.

anthraniloyl) compounds (IV) thus obtained were then oxidized by means of potassium ferricyanide in phosphate buffer at pH 7.2 to actinocyl derivatives (V) (Table III).

Next, we have examined a similar reaction on the compound bearing L-proline. However, when 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-proline was hydrogenated, unexpected pale yellow crystal (VIII), mp 278—280°, was obtained instead of 3-hydroxy-4-methylanthraniloyl-L-proline (VII). This product was not dissolved in dil. $NaHCO_3$ or dil. HCl and give a violet color reaction with $CHCl_3$ solution of anhydrous $FeCl_3$, which was suggested that the absence of free carboxyl group or amino group and the presence of phenolic hydroxyl group. Also, VIII was not oxidized by potassium ferricyanide. The presence of amide carbonyl groups in VIII was shown by IR band at 1690 cm^{-1} and 1630 cm^{-1} (in $CHCl_3$) (Fig. 1).

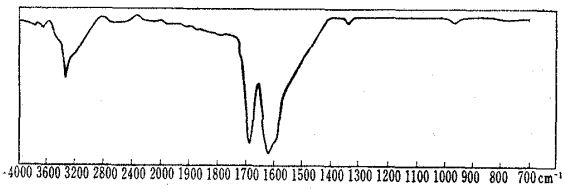


Fig. 1. Infrared Absorption Spectra of 3-Hydroxy-4-methylanthraniloyl-L-proline Lactam (in $CHCl_3$)

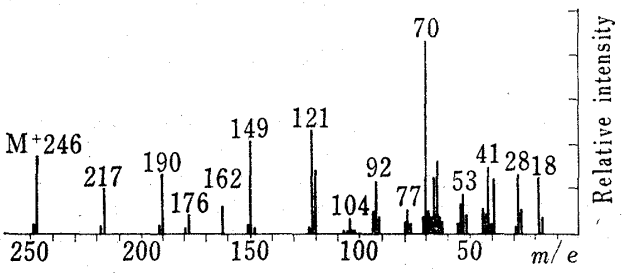


Fig. 2. Mass Spectrum of 3-Hydroxy-4-methylanthraniloyl-L-proline Lactam

The elemental analysis of VIII suggested a molecular formula, $C_{13}H_{14}O_3N_2$ (molecular weight: 246.26). The mass spectrum analysis, giving parent peak at *m/e* 246 (Fig. 2), also supported this molecular formula. Furthermore, VIII was obtained from 3-hydroxy-4-methylanthraniloyl-L-proline ethyl ester (IX) by heating at 140° under reduced pressure. These results suggest that dehydric cyclization occurred between 2-amino group and carboxyl group in VII. Similar dehydric cyclization occurred in the case of catalytic hydrogenation of *o*-nitrobenzoyl-L-proline or 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-hydroxyproline. On the other hand, 3-benzyloxy-4-methyl-2-nitrobenzoylglycine and 3-benzyloxy-4-methyl-2-nitrobenzoyl-DL-alanine were hydrogenated to 3-hydroxy-4-methylanthraniloyl glycine and DL-alanine respectively. These compounds were cyclized with loss of one molecule of water to 3-hydroxy-4-methylanthraniloylglycine lactam or DL-alanine lactam by heating at 200° under reduecd pressure, respectively.

Experimental

3-Benzyloxy-4-methyl-2-nitrobenzoyl Amino Acid Ethyl Esters (Table I)——3-Benzyloxy-4-methyl-2-nitrobenzoic acid[20] (0.02 moles) was suspended in $CHCl_3$ (50 ml), added a few drops of pyridine, and heated under reflux with $SOCl_2$ (3 ml) for 10 min. The resulting solution was evaporated *in vacuo* to remove the excess of $SOCl_2$, the residue was dissolved in benzene, filtered off the pyridinum chloride, the filtrate was evaporated *in vacuo* to dryness and the residual acid chloride (II), which solidified on cooling, was redissolved in dry ether. This solution was added in a small portions to a solution of amino acid ethyl ester (0.02 moles), triethylamine (3 g) and ether with stirring and ice cooling. After 1 hr, ether solution was washed with dil. HCl and dil. $NaHCO_3$ solution and dried over Na_2SO_4. The ether solution was evaporated *in vacuo* to dryness and the crystalline residue was recrystallized from isopropyl ether to give the ester (III) as colorless crystals.

3-Benzyloxy-4-methyl-2-nitrobenzoyl-α-aminolactam (Table I)——A solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride (II) (prepared from 0.02 moles of the acid with 3 ml of $SOCl_2$ as above) in ether was added in a small portion to a solution of α-amino lactam (0.02 moles) and triethylamine (3 g) dissolved in acetone under stirring and ice cooling. After 1 hr, reaction mixture was evaporated *in vacuo* to dryness and the residue was washed with H_2O and ether. The crystalline residue was recrystallized from EtOH to give (III) as colorless crystals.

3-Benzyloxy-4-methyl-2-nitrobenzoyl Amino Acid (Table I)——To 30 ml of MeOH solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl amino acid ethyl ester (0.01 mole), N NaOH solution (15 ml) was

added and allowed to stand 2 hr at room temperature. The reaction mixture was concentrated *in vacuo* to remove the MeOH, the residue was dissolved in H_2O, and extracted with AcOEt. The aqueous layer was acidified with HCl, extracted with AcOEt, the AcOEt extract was evaporated *in vacuo* to dryness and the residue was recrystallized from 50% EtOH to give 3-benzyloxy-4-methyl-2-nitrobenzoyl amino acid as colorless needles.

3-Hydroxy-4-methylanthraniloyl Amino Acid Ethyl Ester or α-Amino Lactam (IV), (Table II)——3-Benzyloxy-4-methyl-2-nitrobenzoyl derivative (III) (0.01 mole) from the preceeding experiment was dissolved in EtOH and hydrogenated over 5% Pd-C (0.5 g) at atmospheric pressure and room temperature. After the absorption of hydrogen had ceased, the catalyst was separated and the filtrate was concentrated to dryness *in vacuo*. The residue, which solidified by treating with petr. ether, was purified by recrystallization from isopropyl ether, EtOH or H_2O.

Actinocyl Di-amino Acid Ethylester or Di-α-amino Lactam (V), (Table III)——Finely powdered 3-hydroxy-4-methylanthraniloyl amino acid ethyl ester or amino lactam (IV) (0.001 mole) was suspended in phosphate buffer (600 ml, pH 7.2), kept at 40° while a solution of potassium ferricyanide (0.9 g) in water (40 ml) was added, dropwise under stirring, and allowed to stand overnight at 37°. After cooling, the product which had separated as bright orange flocculent solid was collected, washed, and dried. It recrystallized from $CHCl_3$·petr. ether.

3-Benzyloxy-4-methyl-2-nitrobenzol-L-proline (VI)——To 10 ml of MeOH solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-proline ethyl ester (2 g), N NaOH solution (5 ml)was added and allowed to stand 1 hr at room temperature. The reaction mixture was concentrated *in vacuo* to remove MeOH, the residue was dissolved in H_2O and extracted with AcOEt. The aqueous layer was acidified with HCl, extracted with AcOEt, the AcOEt extract was dried over Na_2SO_4, and evaporated *in vacuo* to dryness. The oily residue (1.3 g) could not be solidified and was used for next reaction without further purification.

Catalytic Reduction of 3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-proline (VI)——3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-proline (VI) (1.3 g) was dissolved in EtOH and hydrogenated over 5% Pd-C (0.5 g) at atmospheric pressure. After the absorption of hydrogen had ceased, the catalyst was removed by filtration and the filtrate was concentrated to dryness *in vacuo*. The residue was recrystallized from EtOH to give a plae yellow needles (0.5 g), mp 278—280°. It was not dissolved in dil. $NaHCO_3$ and dil. HCl, and give a violet color reaction with $CHCl_3$ solution of anhydrous $FeCl_3$. *Anal.* Calcd. for $C_{13}H_{14}O_3N_2$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.20; H, 5.90; N, 10.98.

3-Acetyloxy-4-methylanthraniloyl-L-proline Lactam——A mixture of 0.2 g of 3-hydroxy-4-methylanthraniloyl-L-proline lactam (VIII) and 10 ml of Ac_2O was heated at 100° for 1 hr. The reaction mixture was evaporated *in vacuo* to dryness. The residue was crystallized by adding of H_2O, the solids obtained were recrystallized from H_2O to give 3-acetyloxy-4-methylanthraniloyl-L-proline lactam (0.1 g) as colorless needles, mp 258°. *Anal.* Calcd. for $C_{15}H_{16}O_4N_2$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.05; H, 5.88; N, 10.02.

3-Hydroxy-4-methylanthraniloyl-L-proline Ethyl Ester (IX)——3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-proline ethyl ester (1.5 g) was dissolved in EtOH (50 ml) and hydrogenated over 5% Pd-C (0.5 g) at atmospheric pressure and room temperature. After the absorption of hydrogen had ceased, the catalyst was removed and the filtrate was evaporated *in vacuo* to dryness. The residue was recrystallized from isopropyl ether to give 3-hydroxy-4-methylanthraniloyl-L-proline ethyl ester (IX) (0.7 g), as pale yellow prisms, mp 110—112°. *Anal.* Calcd. for $C_{15}H_{20}O_4N_2$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.89; H, 7.20; N, 9.45.

Cyclization of 3-Hydroxy-4-methylanthraniloyl-L-proline Ethyl Ester (IX) by Heating——3-Hydroxy-4-methylanthraniloyl-L-proline ethyl ester (IX) (0.2 g) was heated at 140° in oil bath under reduced pressure for 30 min. The reaction products was recrystallized from EtOH to give (VIII) (0.1 g) as pale yellow needles, mp 278—280°. It was identical with the catalytic reduction product of 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-proline by the comparison of the IR spectrum.

3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-hydroxyproline——A solution of 3-benzyloxy-4-methyl-2-nitrobenzoyl chloride (II) (prepared from 4 g of the acid with $SOCl_2$ as above) in ether was added in a small portion to a solution of L-hydroxyproline (2 g) dissolved in N NaOH (20 ml) under stirring and ice cooling. During the reaction, the solution was kept alkaline to thymolblue with N NaOH solution. The stirring was continued for 20 min after addition of the acid chloride. The reaction mixture was extracted with ether, and ether layer was discarded. The aqueous layer was acidified with HCl, and extracted with AcOEt. The AcOEt extract was dried with Na_2SO_4 and evaporated *in vacuo* to dryness. The residue was washed several times with ether to remove 3-benzyloxy-4-methyl-2-nitrobenzoic acid and recrystallized from 50% EtOH to give 3-benzyloxy-4-methyl-2-nitrobenzoyl-L-hydroxyproline (4 g) as colorless scales, mp 175—176°. *Anal.* Calcd. for $C_{20}H_{20}O_7N_2$: C, 59.99; H, 5.04; N, 7.00. Found: C, 59.93; H, 5.09; N, 7.38.

Catalytic Reduction of 3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-hydroxyproline——3-Benzyloxy-4-methyl-2-nitrobenzoyl-L-hydroxyproline (0.7 g) was dissolved in EtOH and hydrogenated as above described (IV). The product was recrystallized from H_2O to give 3-hydroxy-4-methylanthraniloyl-L-hydroxyproline lactam (0.2 g) as pale yellow needles, mp 264—266°. *Anal.* Calcd. for $C_{13}H_{14}O_4N_2$: C, 59.53; H, 5.38; N, 10.68. Found: C, 59.09; H, 5.63; N, 10.68.

***o*-Nitrobenzoyl-L-proline**——*o*-Nitrobenzoyl chloride (1.8 g) was added in a small protion to a solution of L-proline (1.1 g) dissolved in N NaOH (10 ml) under stirring and ice cooling. The recation continued for 30 min and treated the same method as above described. The AcOEt extract wsa chromatographed in benzene on silica gel (20 g). The fractions (1000 ml) eluted with benzene–ether (4:1) affortded *o*-nitrobenzoyl-L-proline (1 g), which was recrystallized from H_2O as colorless prisms, mp 123—125°. *Anal.* Calcd. for $C_{12}H_{12}O_5N_2$: C, 54.54; H, 4.58; N, 10.60. Found: C, 54.93; H, 4.24; N, 10.45.

Catalytic Reduction of *o*-Nitrobenzoyl-L-proline——*o*-Nitrobenzoyl-L-proline (0.4 g) was dissolved in EtOH and hydrogenated as above described (IV). The product was recrystallized from H_2O to give anthraniloyl-L-proline lactam (0.25 g) as colorless needles, mp 205—207°. *Anal.* Calcd. for $C_{12}H_{12}O_2N_2$: C, 66.65; H, 5.59; N, 12.96. Found: C, 66.73; H, 5.34; N, 12.62.

3-Hydroxy-4-methylanthraniloylglycine——3-Benzyloxy-4-methyl-2-nitrobenzoylglycine (1.9 g) was dissolved in EtOH (50 ml) and hydrogenated as above described (IV). The product was recrystallized from EtOH to give 3-hydroxy-3-methylanthraniloylglycine (1.2 g) as yellow needles, mp 215—216°. *Anal.* Calcd. for $C_{10}H_{12}O_4N_2$: C, 53.57; H, 5.39; N, 12.50. Found: C, 53.70; H, 5.38; N, 12.32.

Dehydric Cyclization of 3-Hydroxy-4-methylanthraniloylglycine by Heating——3-Hydroxy-4-methylanthraniloylglycine (0.2 g) was heated at 200° in oil bath under reduced pressure for 1 hr. The reaction product was recrystalilzed from EtOH to give a lactam (0.1 g) as colorless prisms, mp 280—283°. *Anal.* Calcd. for $C_{10}H_{10}O_3N_2$: C, 58.25; H, 4.89; N, 13.58. Found: C, 58.30; H, 5.23; N, 13.23.

3-Hydroxy-4-methylanthraniloyl-DL-alanine——3-Benzyloxy-4-methyl-2-nitrobenzoyl-DL-alanine (2.3 g) was dissolved in EtOH (50 ml) and hydrogenated as above described (IV). The product was recrystallized from H_2O to give 3-hydroxy-4-methylanthraniloyl-DL-alanine (1.5 g) as yellow prisms, mp 170—180° (decomp.) 300°. *Anal.* Calcd. for $C_{11}H_{14}O_4N_2$: C, 55.45; H, 5.92; N, 11.76. Found: C, 55.16; H, 6.21; N, 11.65.

Dehydric Cyclization of 3-Hydroxy-4-methylanthraniloyl-DL-alanine by Heating——3-Hydroxy-4-methylanthraniloyl-DL-alanine (0.2 g) was heated at 200° in oil bath under reduced pressure for 1 hr. The reaction product was recrystallized from H_2O to give lactam (0.1 g) as pale yellow needles, mp 300°. *Anal.* Calcd. for $C_{11}H_{12}O_3N_2$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.19; H, 5.80; N, 12.67.

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