*An Attempt at the Synthesis of Roseonine**

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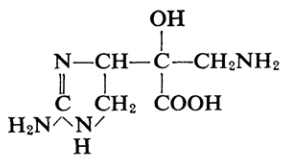
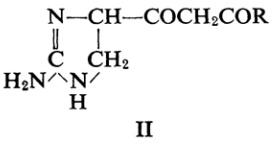
In 1954, Nakanishi et al. isolated a new amino acid, roseonine, as one of the hydrolysis products of a peptide antibiotics roseothricin; they proposed that the structure was 2-amino-4-(1-carboxy-1-hydroxy-2-amino)-ethyl-2-imidazoline (I)[1]. Since the compounds firmly



I



II

identified as the degradation products of roseonine were very few in number, information about its structure was obtained mainly from a comparison of its physicochemical properties with those of many model compounds. Later, some similar amino acids were isolated from other sources and were named differently (e. g., geamine isolated from geomycin[2]), but they were proved to be identical with roseonine.

In order to confirm the structure of this amino acid, the synthesis of roseonine was attempted. As the most facile path to roseonine appeared to be through a ketonic compound II, the studies were directed to the preparation of this ketonic compound. When 2,3-diaminopropionic acid monohydrobromide (III) was treated with bromocyan in the presence of two moles of sodium hydroxide, 2-amino-2-imidazoline-4-carboxylic acid (IV) was obtained. The acetylation of IV with a mixture of acetic acid, acetic anhydride and concentrated sulfuric acid afforded a monoacetyl derivative. The acetyl derivative was formulated as V in view of the following reaction sequence. Ethanolic hydrogen chloride converted V into its ester, VI. Numerous attempts to effect the condensation of VI with ethyl acetate or acetone in order to afford II were unsuccessful. However, ethyl acetoacetate condensed with it in the presence of sodium ethylate, and the product was isolated as its hydrochloride. Based on the following physicochemical properties and reactions of this condensation product, the bicyclic structure VIIa was assigned to it. IV and V may have a dipolar structure, judging from their pK_a values (Table II). The infrared spectra of IV, V, VI and VIIa in the 1800~

* After the manuscript of this article was submitted for publication, a revised structure was proposed for roseonine: H. E. Carter et al., *J. Am. Chem. Soc.*, 83, 4297 (1961).

1) K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto and Y. Hirata, This Bulletin, 27, 539 (1954); K. Nakanishi and M. Ohashi, ibid., 30, 725 (1957).

2) H. Brockmann and H. Musso, *Chem. Ber.*, 88, 648 (1955).

Chart 1

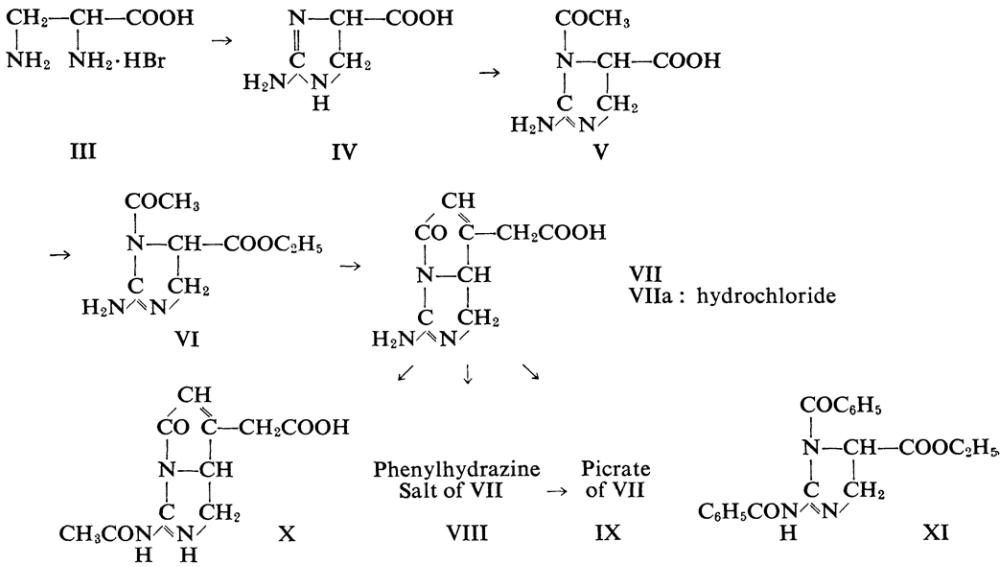
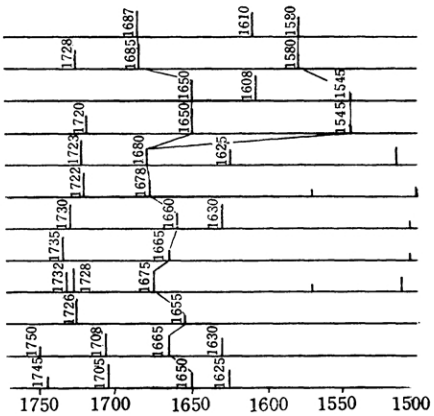


TABLE I. INFRARED SPECTRA

A	IV (KBr)
B[a]	IV hydrochloride (KBr)
C[b]	IV (D_2O solution)
D[b]	IV (D_2O-HCl solution)
E	V (KBr)
F[a]	V hydrochloride (KBr)
G[b]	V (D_2O solution)
H[b]	V (D_2O-HCl solution)
I[a]	VI hydrochloride (KBr)
J[b]	VI (D_2O-HCl solution)
K	VIIa (KBr)
L[b]	VIIa (D_2O solution)



a. Determined for the sample obtained by dissolving IV, V or VI in a slight excess of 5% hydrochloric acid and keeping the solution over solid sodium hydroxide and then over phosphorus pentoxide at room temperature (1 mmHg.).

b. Determined in a KRS cell.

1500 cm^{-1} region were measured in different states and were compared with each other (Table I). The carboxylate band (1630~1608 cm^{-1} in A, C, E, G) of IV and V shifted to the carboxyl band (1735~1720 cm^{-1} in B, D, F, H) in the acidic state. Goto et al.[3] reported that the disubstituted guanidium group generally showed in the 1700~1580 cm^{-1} region, two bands separated from each other by 90 cm^{-1}; they were named guanidium I and II. The bands at 1687 and 1580 cm^{-1} of IV were, therefore, thought to be guanidium I and II; in accordance with this expectation, they shifted to a lower wave number in the deuteriated state (C, D). However, V and VI have only one band that shifted when deuteriated. It was assumed from this result that the two guanidium bands of IV were reduced to one band by acetylation. The bands at 1735~1720 cm^{-1} of V and VI, which were unaffected by the change in state, were assigned to amide carbonyl[4] overlapped by carboxyl in

3) T. Goto, K. Nakanishi and M. Ohashi, This Bulletin, 30, 723 (1957).

4) It has been reported that the amide carbonyl band shifts toward a higher wave number when the amide nitrogen atom constitutes a five-membered ring system (*N*-acetylimidazole, *N*-acetylindol, etc.): W. Otting, *Chem. Ber.*, 89, 1940 (1956); H. A. Staab, ibid., 90, 1320 (1957).

Table II. Values of pK_a's

Compound	pK_{a1}	pK_{a2}
IV	2.5	12.1
V	1.6	8.7
VI	—	7.7
VIIa	2.0	3.8
X	3.1	—

the case of F and H in Table I or by ester in the case of I and J.

Bearing these facts in mind, the band assignments of VIIa were made as follows: 1750 cm^{-1} to amide carbonyl (shifted to a higher wave number by five-membered cyclization), 1708 cm^{-1} to carboxyl, 1665 cm^{-1} to guanidium (shifted to a lower wave number by deuterium substitution in the case of L), and 1630 cm^{-1} to a C=C double bond.

Further evidence supporting this structure (VIIa) was also obtained. Phenylhydrazine reacted with VIIa, but the product was not the phenylhydrazone, but the phenylhydrazine salt of VII (VIII), because phenylhydrazine was easily removed from VIII by the action of picric acid to afford the picrate of VII (IX). The hydrochloride VIIa was not susceptible to the acid or to alkali hydrolysis, and the titration with alkali revealed the presence of two titratable groups, which were attributable to the guanidine and carboxyl groups (see below). The acetylation of VIIa afforded the acetyl derivative X, which had only one titratable group. Although the observations described above were consistent with the bicyclic structure VIIa, the hydrochloride exhibited unexpectedly low pK_a values, 2.0 and 3.8. Even when the higher observed value of pK_a was attributed to the ionization of the guanidine group, it appeared to be too low to be regarded as the pK_a value of normal guanidine derivatives. A specific steric environment caused by the fusion of two rings is probably responsible for this unusually low pK_a value.

In order to prevent the cyclization of the acetyl derivative VI to VIIa, the benzoylation of IV was carried out. However, the dibenzoyl ester (XI or its isomer, according to the position of the benzoyl groups), which was directly obtained from IV, resisted condensation with ethyl acetate or acetone to prepare II.

As α,β-diamino compounds were found to be a convenient starting material for the synthesis of 2-amino-2-imidazoline derivatives, an artifice of synthesizing from the side chain was next adopted. As the immediate synthetic objective, an olefinic compound XII was chosen in order to prepare for the desired diamino compound XIII.

Chart 2

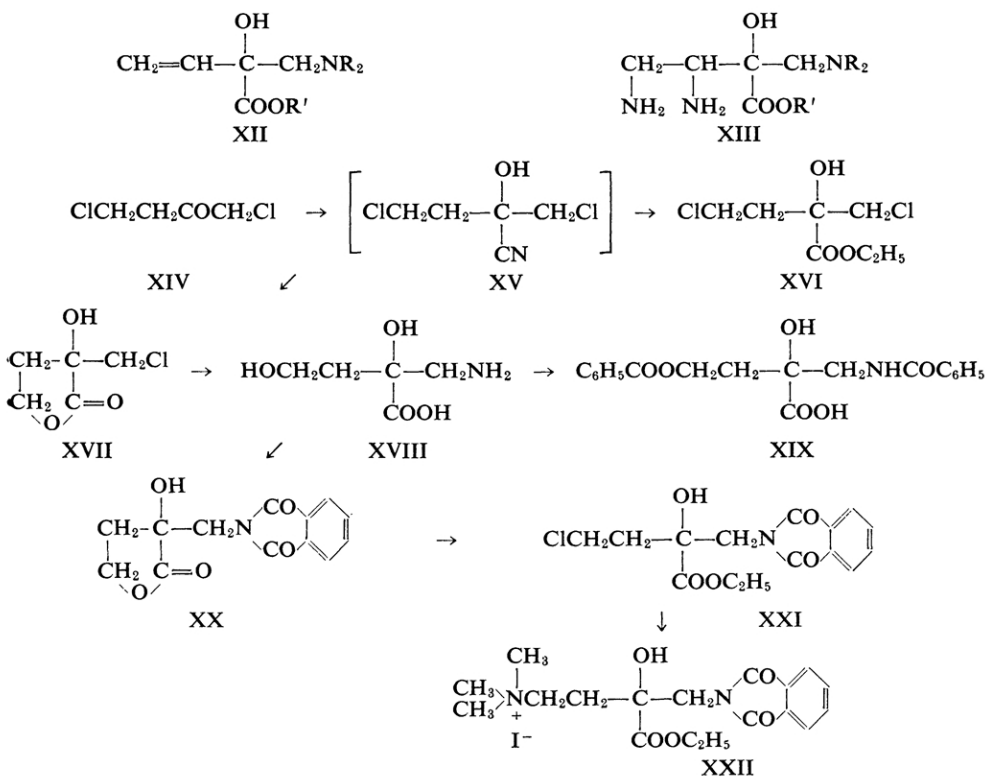
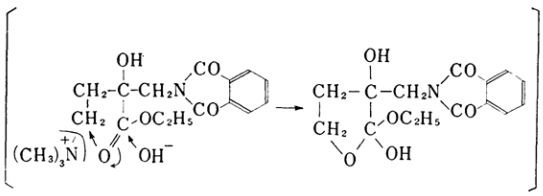


Chart 3



XXIII XXIV

When 1,4-dichloro-2-butanone (XIV) was treated with sodium bisulfite and then with potassium cyanide, an oil was obtained. This oil, despite its fairly constant boiling point, contained the starting ketone, which was detected by infrared analysis. The oil exhibited bands of hydroxyl and nitrile in its infrared spectrum and afforded a considerable amount of the starting ketone on being left standing. It may be inferred from these results that the oil is an equilibrium mixture of the cyanhydrin XV and the starting ketone XIV. Further support for the cyanhydrin structure was obtained by converting it into its chloroester XVI by ethanol and concentrated sulfuric acid, and into a chlorolactone XVII by hydrochloric acid in acetic acid. The amino acid XVIII was obtained from the lactone XVII by aqueous ammonia at 100°C. Chemical evidence for the β-amino-γ-hydroxy acid structure of XVIII was obtained by Schotten-Baumann benzoylation, which afforded the dibenzoylcarboxylic acid XIX, and by reaction with phthalic anhydride in pyridine, which afforded the phthaliminolactone XX. The amino acid XVIII has a side chain of roseonine, and its behavior in reaction against ninhydrin was similar to that of roseonine dihydrochloride. Ethanolic hydrogen chloride converted the phthaliminolactone XX into the ester XXI. As the direct dehydrochlorination of XXI was unsuccessful, it was converted into tertiary amine methiodide XXII. The Hofmann degradation of XXII afforded a glassy substance. The structure of this substance may be assumed to be the hemiacetal XXIV derived from the hydroxide XXIII by the scheme shown in Chart 3, since it afforded the starting lactone XX on treatment with dilute hydrochloric acid.

Experimental

The infrared spectra were determined on a Koken infrared recording spectrophotometer, model DS 301.

The acid constants refer to apparent values derived with a glass electrode system in water.

2-Amino-2-imidazoline-4-carboxylic Acid (IV).—Eleven grams of 2,3-diaminopropionic acid monohydrobromide were dissolved in 48 ml. of 10% sodium hydroxide and 50 ml. of an aqueous solution containing 6.3 g. of bromocyan were added. After being left standing overnight, it was evaporated to dryness in vacuo, and the residue was recrystallized from water. Yield, 3.5 g.; m. p., 303°C (decomp.).

Found: C, 36.90; H, 5.41; N, 32.80. Calcd. for $C_4H_7N_3O_2$: C, 37.21; H, 5.43; N, 32.55%.

The picrate was prepared and recrystallized from water, m. p., 208°C.

Found: N, 23.73. Calcd. for $C_{10}H_{10}N_6O_9$: N, 23.46%.

1-Acetyl-2-amino-2-imidazoline-5-carboxylic Acid (V).—Seventy milliliters of acetic anhydride and one drop of concentrated sulfuric acid were added to a solution of 8 g. of IV in 70 ml. of glacial acetic acid, and the solution was kept at 40~45°C for 45 min. After concentration in vacuo on a water bath, 200 ml. of methanol were added. The resulting crude product (m. p. 225°C (decomp.)) was recrystallized from glacial acetic acid-methanol; m. p., 232°C (decomp.).

Found: N, 24.73. Calcd. for $C_6H_9N_3O_3$: N, 24.55%.

Ethyl 1-Acetyl-2-amino-2-imidazoline-5-carboxylate (VI).—A suspension of 5 g. of the acid V in 100 ml. of ethanol was saturated with dry hydrogen chloride without cooling and then left to stand for 2 hr. After concentration in vacuo, the residue was neutralized with aqueous sodium carbonate and taken up in chloroform. The extract was dried over sodium sulfate, and the solvent was distilled. The residual ester was recrystallized from chloroform-petroleum ether; m. p., 152~154°C.

Found: C, 48.14; H, 6.72; N, 21.42. Calcd. for $C_8H_{13}N_3O_3$: C, 48.23; H, 6.58; N, 21.10%.

The picrate was prepared in water and recrystallized from ethanol; m. p., 163~165°C.

Found: N, 19.74. Calcd. for $C_{14}H_{16}N_6O_{10}$: N, 19.62%.

Bicyclic Compound VII.—A mixture of 0.3 g. of the ester VI, 0.3 g. of ethyl acetoacetate, and 1.1 ml. of a sodium ethylate solution (prepared by dissolving 1 g. of sodium in 20 ml. of ethanol) was heated at reflux for 1.5 hr. The solid was then filtered out and dissolved in a small amount of water. The solution was acidified with hydrochloric acid (1 : 1) and concentrated in vacuo. The resulting solid was recrystallized from ethanol; m. p., 242°C.

Found: C, 41.37; H, 4.56; N, 18.00. Calcd. for $C_8H_{10}ClN_3O_3$: C, 41.54; H, 4.31; N, 18.11%.

The phenylhydrazine salt VIII was obtained by heating a mixture of the hydrochloride and phenylhydrazine in ethanol for 10 min. Recrystallization from ethanol afforded white needles; m. p., 162~163°C.

Found: C, 55.65; H, 5.72; N, 23.07. Calcd. for $C_{14}H_{17}N_5O_3$: C, 55.53; H, 5.62; N, 23.11%.

The picrate IX was prepared from the hydrochloride VIIa or the phenylhydrazine salt VIII by treatment with picric acid in water; m. p., 125°C.

Found: N, 19.27. Calcd. for $C_{14}H_{12}N_6O_{10}$: N, 19.85%.

The acetyl derivative X was prepared by heating a solution of the hydrochloride in a mixture of glacial acetic acid and acetic anhydride (1 : 1) in the presence of a small amount of pyridine and then recrystallizing it from ethanol; m. p., 224°C (decomp.).

Found: C, 50.90; H, 4.70; N, 17.59. Calcd. for $C_{10}H_{11}N_3O_4$: C, 50.75; H, 4.64; N, 17.72%.

Ethyl 1-Benzoyl-2-benzoylamino-2-imidazoline-4-carboxylate (XI).—A suspension of 1 g. of IV in 20 ml. of ethanol was saturated with dry hydrogen chloride without cooling. After 2 hr., the ethanol was removed in vacuo. The resulting oil was dissolved in a small amount of ethanol, and the ethanol was again removed in vacuo. The same procedure was once repeated with ethanol and then twice with pyridine, and 3.6 g. of benzoic anhydride was added. The solution was heated at reflux for 4 hr., and the pyridine was removed in vacuo. The resulting oil, when triturated with a small amount of ethanol, crystallized; this was then recrystallized from ethanol in the form of white needles; m. p., 189~190°C.

Found: C, 65.85; H, 5.60; N, 11.80. Calcd. for $C_{20}H_{19}N_3O_4$: C, 65.74; H, 5.24; N, 11.50%.

2-Chloromethyl-2-hydroxy-4-chlorobutyronitrile (XV).—A solution of 89 g. of sodium bisulfite in 222 ml. of water was added to 62 g. of 1,4-dichloro-2-butanone (XIV), and the mixture was shaken vigorously for 20 min. To the resulting homogeneous solution, some crushed ice, 150 ml. of ether, and then a solution of 56 g. of potassium cyanide in 168 ml. of water were added. The ether layer was separated, and the aqueous layer was shaken with ether several times. The combined ether solution was dried over sodium sulfate and distilled; b. p., 102~103°C/1 mmHg.

The infrared spectrum showed bands of hydroxyl (3340 cm^{-1}) and nitrile (2219 cm^{-1}). The carbonyl band (1724 cm^{-1}) of the starting ketone was also observed.

Ethyl 2-Chloromethyl-2-hydroxy-4-chlorobutyrate (XVI).—A mixture of 5 g. of the cyanhydrin XV, 25 ml. of ethanol and 7.5 ml. of concentrated sulfuric acid was heated at reflux for 5 hr. and then poured onto crushed ice. This was shaken with ether, and the ether extract was dried over sodium sulfate. After the solvent had been removed, the residue was distilled; b. p. 111~115°C/4 mmHg.

Found: C, 38.43; H, 5.32. Calcd. for $C_7H_{12}Cl_2O_3$: C, 39.23; H, 5.58%.

The infrared spectrum showed an ester band at 1733 cm^{-1}.

α-Chloromethyl-α-hydroxy-γ-butyrolactone (XVII).—A mixture of 61 g. of the cyanhydrin (XV), 92 ml. of glacial acetic acid and 183 ml. of concentrated hydrochloric acid was heated on a water bath for 6 hr. After the ammonium chloride had been filtered off, the filtrate was concentrated in vacuo and extracted with ether. The ether extract was dried over sodium sulfate, and the solvent was removed. Vacuum distillation afforded a white viscous oil. Yield, 39 g.; b. p., 128~136°C/5 mmHg.

Found: C, 38.46; H, 4.69. Calcd. for $C_5H_7ClO_3$: C, 39.84; H, 4.65%.

The infrared spectrum showed a lactone band at 1760 cm^{-1}.

2-Aminomethyl-2,4-dihydroxybutyric Acid (XVIII).—A mixture of 6 g. of the lactone (XVII) and 120 ml. of concentrated aqueous ammonia was heated at 100°C in an autoclave for 5 hr. The solution was evaporated to dryness in vacuo, and the resulting solid was recystallized from water. Yield, 3.5 g.; m, p. 237°C (decomp.).

Found: C, 40.39; H, 7.55; N, 9.31. Calcd. for $C_5H_{11}NO_4$: C, 40.26; H, 7.43; N, 9.39%.

The amino acid showed no color development on treatment with ninhydrin at 100°C. However, a purple-brown precipitate was obtained when the reaction was carried out at 150°C in a sealed tube.

2-Benzaminomethyl-2-hydroxy-4-benzoxybutyric Acid (XIX).—A small amount of the amino acid XVIII was dissolved in 10% aqueous sodium hydroxide and shaken with benzoyl chloride, with the solution being kept alkaline. Acidification with hydrochloric acid afforded white crystals, which were washed with ether to remove any benzoic acid. The ether-insoluble product was recystallized from 50% ethanol; m. p., 173°C.

Found: N, 3.86. Calcd. for $C_{19}H_{19}NO_6$: N, 3.93%.

α-Hydroxy-α-phthaliminomethyl-γ-butyrolactone (XX).—A mixture of 3.5 g. of the amino acid XVIII, 5.5 g. of phthalic anhydride, 50 ml. of pyridine was heated at reflux for 6 hr. The solution was concentrated in vacuo, and water was added. The resulting solid (5.5 g., m. p. 147~149°C) was recrystallized from ethanol; m. p., 155°C.

Found: N, 5.26. Calcd. for $C_{13}H_{11}NO_5$: N, 5.37%.

Ethyl 2-Hydroxy-2-phthaliminomethyl-4-chlorobutyrate (XXI).—A suspension of 15 g. of the lactone XX in 350 ml. of ethanol was saturated with dry hydrogen chloride without cooling. After standing for 2hr. at room temperature, the solution was concentrated in vacuo. The addition of water afforded an oil which soon solidified. Recrystallization from 50% ethanol afforded white needles of XXI; m. p., 99~100°C.

Found: C, 55.47; H, 5.16; N, 4.32. Calcd. for $C_{15}H_{16}ClNO_5$: C, 55.22; H, 4.90; N, 4.28%

Ethyl 2-Hydroxy-2-phthaliminomethyl-4-dimethylaminobutyrate Methiodide (XXII).—Four grams of the chloroester XXI were dissolved in 20 ml. of ethanol containing 2.8 g. of dimethyl amine and then heated at 100°C for 4 hr. in a sealed tube. The ethanol was removed in vacuo, and the residue was extracted with ether (Soxhlet). Evaporation of the ether afforded a pale yellow viscous oil. The oil was dissolved in 50 ml. of dry acetone, and 15 g. of methyl iodide was added. A yellow solid was recrystallized from water; m. p., 219°C.

Found : C, 44.44 ; H, 5.56 ; N, 5.91. Calcd. for $C_{18}H_{25}IN_2O_5$: C, 45.31 ; H, 5.25 ; N, 5.87%.

Thermal Decomposition of the Hydroxide Obtained from XXII.—Wet silver oxide which had freshly been prepared from 1.5 g. of silver nitrate was added to a solution of 1.5 g. of the methiodide XXII in 30 ml. of water, stirred for 30 min., and filtered. The filtrate was evaporated to dryness under reduced pressure and kept at 150°C for 2 hr. (2 mmHg.). The residue was taken up in chloroform and treated with charcoal. Evaporation of the chloroform afforded a white glassy substance. Treatment of this substance with dilute hydrochloric acid afforded the lactone XX ; m. p. and mixed m. p., 155°C.

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