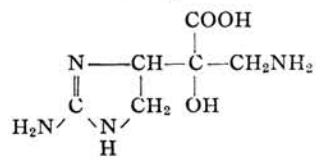
*Confirmation of Structure of Roseonine**

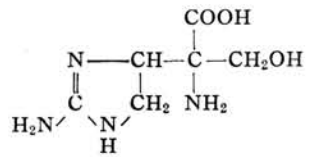
By Koji NAKANISHI and Mamoru OHASHI

(Received April 25, 1957)

Roseonine[1] is one of the two β-amino acids obtained as the hydrolysis products of roseothricin[2,3], and is identical with streptolidine from streptolin[4] and geamine from geomycin[5]; it has also recently been obtained from racemomycin B[6]. On account of the poor crystallizing properties of the reaction products from roseonine, and especially because the starting material was so scarce, the reasoning that led to the previously proposed structure I[1,7] was somewhat indirect. The validity of structure I and the possibilities of three



(I)



(II)

* Reported at 9th Annual Meeting, *Chem. Soc. Japan*, *Kyoto*, April, 1956.

1) K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto and Y. Hirata, This Bulletin, **27**, 539 (1954).
K. Nakanishi, T. Ito and Y. Hirata, *J. Am. Chem. Soc.*, **76**, 2845 (1954).
K. Nakanishi, *J. of Antibiotics*, Ser. B., VII-7, 228 (1954).

2) S. Hosoya, M. Soeda, N. Komatsu, S. Imamura, M. Iwasaki, Y. Sonoda and K. Okada, *Jap. J. Exp. Med.*, **20**, 121 (1949); *J. of Antibiotics*, Ser. B, III-4, 217 (1950).

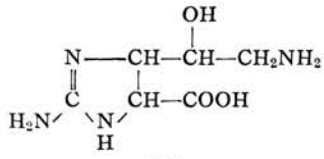
3) T. Goto, Y. Hirata, S. Hosoya and N. Komatsu, following paper.

4) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers", 120th Meeting, Am. Chem. Soc., New York, N. Y., September, 1951, p. 31.
E. E. Smissmann, R. W. Sharpe and E. E. van Tamelen, "Abstracts of Papers", 121st Meeting, Am. Chem. Soc., Milwaukee, Wisc., April 1952, p. 80.
Identity of roseonine with streptolidine confirmed by direct comparison of infrared spectra (personal communication from Dr. van Tamelen).

5) H. Brockamnn and H. Musso, *Ber.*, **88** 648 (1955).

6) H. Taniyama and S. Takemura, "Abstracts of Papers", 10th Annual Meeting, Pharm. Soc. Japan, Tokyo, April, 1957, p. 88.

7) For simplicity the structures I-IV are represented in the classical fashion and not in the zwitterionic form in which distribution of the positive charge within the guanidinium moiety should have to be considered. However, see structure I of preceding paper.



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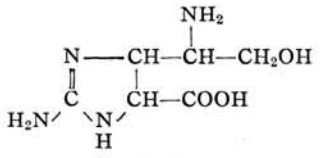
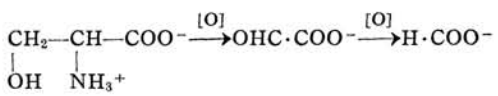


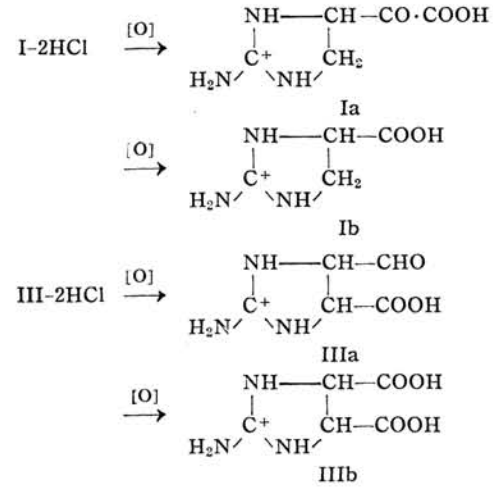
(IV)

other structures, II–IV, have accordingly been re-investigated. In the present paper we wish to furnish additional evidence pointing to structure I, and thus to clarify a few ambiguities[8]. Firstly, potentiometric titration and consideration of the hydrogen ion-binding curve[9] excluded structures III and IV, and secondly, evidence from infrared spectra pointed to the correctness of structure I.

Free D,L-serine was chosen as the model for the first method, and this was subjected to a two-mole periodate oxidation. Potentiometric titration of the aqueous reaction solution gave a pK'_a value of 3.8, and the hydrogen ion-binding curve occurred *below* the zero reference line (i.e., the inflection corresponded to a hydrogen ion-binding group). The dissociation constant corresponded to that of formic acid, and thus the two oxidative steps may be correctly formulated by the following scheme:



Oxidation of roseonine dihydrochloride with one mole periodate yielded one mole each of formaldehyde and ammonia[1] and an unisolated product with a pK'_a of 2.1[10]. Oxidation with a second mole of periodate afforded a product with a pK'_a value of 3.3. Since neither formaldehyde nor 2-amino-2-imidazoline hydrochloride was attacked by periodate to any measurable extent under similar conditions, it is apparent that the inflection at 3.3 is neither due to the transformation of formaldehyde to formic acid, nor to an oxidative decomposition of the 2-imidazoline nucleus. Hence the oxidative steps may be represented as follows for structures I (or II)[11] and III (or IV)[11], respectively:



Ia

Ib

IIIa

IIIb

The oxidation product could not be isolated from the reaction solution owing to poor crystallizing properties and the minute amount of starting material available. Otherwise a simple analysis would have sufficed to differentiate between structures Ib and IIIb. Though no clear differentiation between structures Ia and IIIa, or between Ib and IIIb is possible from the two

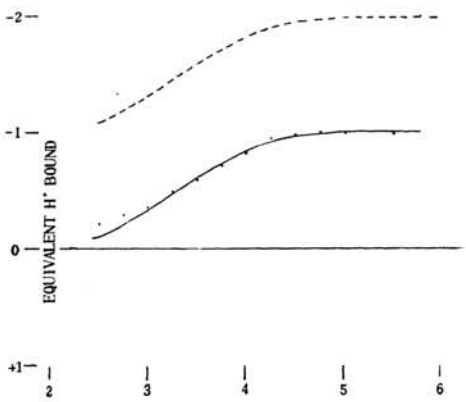


Fig. 1. Hydrogen ion-binding curve for 2 mole periodate oxidation product.
·····: Experimental
——: Theoretical curve for pK'_a 3.3
– – –: Hypothetical curve for structure IIIb.

8) Ref. 5 and H. Musso, *Angew. Chem.*, **68**, 313 (1956).
9) T. V. Parke and W. W. Davis, *Anal. Chem.*, **26**, 642 (1954).
10) The titration was carried out only in the region of pH 2.3 to 6, since the end point was obscured in the other pH regions by the effect of the slightly excess periodic acid (pK_{a_1} at 1.64, pK_{a_2} 6.92 : N. V. Sidgwick, "The Chemical Elements and their Compounds", Vol. II, p. 1237 (1950), Oxford) and the buffering action of the formed ammonium chloride. The inflection at 2.1 was obtained by extrapolation.
11) Inasmuch as the side chain aminoethanol structure is destroyed by the first mole of periodate, it makes no difference whether the structure is I or II. The same applies to III and IV.

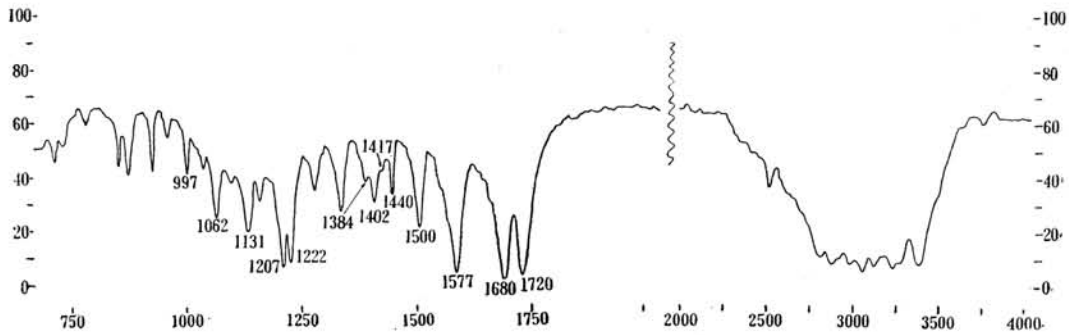


Fig. 2. Roseonine-2HCl (1.1 mg./300 mg. KBr): 1720 (COOH), 1680 (G—I)*, 1577 (G—II, NH_3^+)*,**, 1500(NH_3^+)**

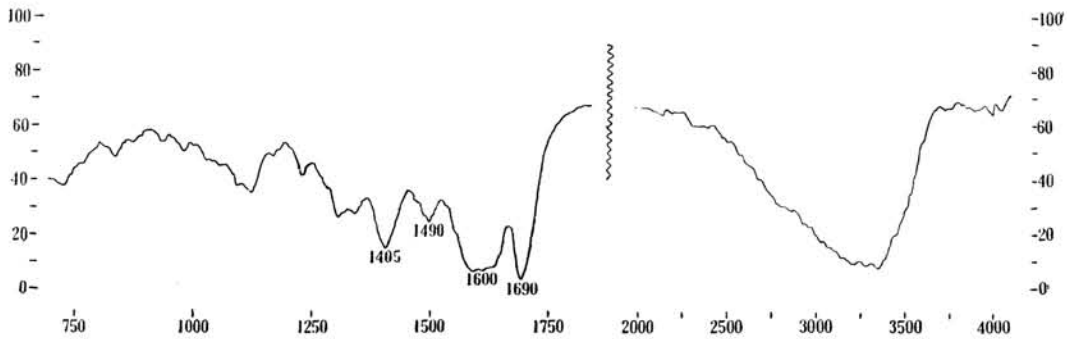


Fig. 3. Free roseonine (crude) (KBr): 1690 (G—I)*, 1600 (NH_3^+, G—II, COO^-)*,**, 1490(NH_3^+)**

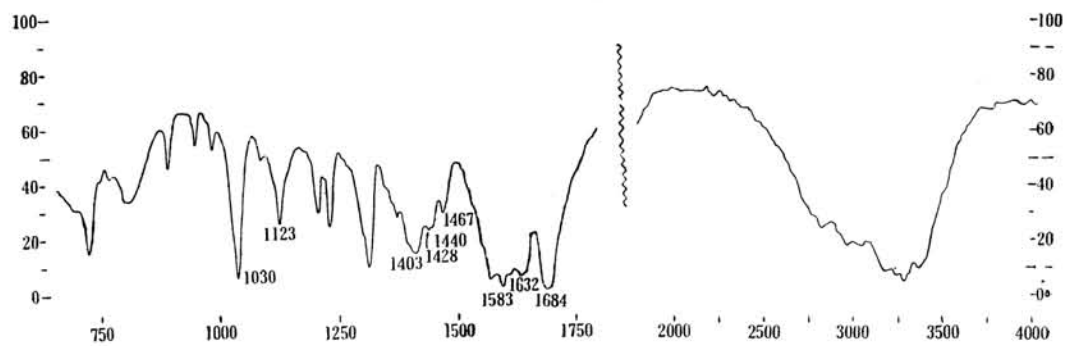


Fig. 4. Roseonine Diol (1.0 mg./300 mg. KBr): 1684 (G—I)*, 1632 (COO^-), 1583 (G—II)*

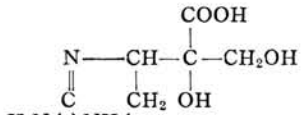
* cf. Guanidinum I and II band, Goto, Nakanishi, Ohashi, preceding paper.
** cf. Nakanishi, Goto, Ohashi, This Bulletin, **30**, 403 (1957).

pK'_a values of 2.1 and 3.3, differentiation may be made by taking into account the hydrogen ionbinding curve for the two-mole oxidation product (Fig. 1). It is to be noted that since the dihydrochloride was used as the starting material, structure Ib possesses one hydrogen ion-releasing carboxyl group, and structure IIIb possesses two such groups; the entire titration curve, in either case, should thus lie *above* the zero line. The incompatibility of structure IIIb with Fig. 1 may be inferred as follows. First, assuming 3.3 to be the first pK'_a value, the second dissociation constant arising from the second carboxyl group must then lie somewhere above 7 since the upper end of the experimental curve ending at about pH 5 as yet shows no sign of a second inflection. The pK'_a2 values for 1,2-*cis*- and *trans*-cyclopentanedicarboxylic acid are at 6.51 and 5.91, respectively[12], and

12) H. C. Brown, D. H. McDaniel and O. Häfliger in "Determination of Organic Structures by Physical Methods", Academic Press (1955), p. 625.

if the additional acid strengthening effect of the guanidinium group is considered, a pK'_{a2} value above 7 would be unexplicable. Second, assuming 3.3 to be the second pK'_a value, the first pK'_a should then be below 2 and the experimental curve for the second constant should be that represented by the dotted lines in Fig. 1. Thus it becomes apparent that the observed inflection at 3.3 is due to the carboxylic group of Ib strengthened by the guanidinium group[13], and that roseonine possesses either structure I or II.

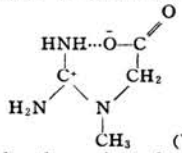
The decision between these two structures, i.e., whether the side chain is an isoserine residue or a serine residue, was further made possible from the infrared spectra. Namely, conversion of roseonine dihydrochloride into the diol V[14] resulted in the appearance of a band at 1467 cm^{-1}



(V)

(Fig. 4) which is absent in the spectra of roseonine dihydrochloride (Fig. 2) or free roseonine (Fig. 3, see experimental for preparation of this compound). This new band can only be assigned to a normal methylene bending vibration, and a new methylene group in the diol could, in turn, only be derived from formula I and not from II. The appearance of a conspicuous absorption at 1030 cm^{-1} in the diol (*prim*-hydroxyl; corresponding to a similar strong band in the spectra of L- and D,L-serine at 1017 and 1036 cm^{-1}, respectively—D-serine was not examined) is also to be noted.

13) The pK'_a of creatine, which like Ib also has a guanidinium group alpha to the carboxyl group is 3.0 at 20°C (Eadie and Hunter, *J. Biol. Chem.*, **67**, 237, 243 (1926)). This slightly enhanced acidity might be explained by structure VI for the zwitterion.



(VI)

14) Obtained by the action of silver nitrite on the dihydrochloride this was the only relatively easily accessible derivative.

Experimental

Potentiometric Titration of Periodate Oxidation Products.—Roseonine dihydrochloride (9.7 mg., 0.037 m.mol.) was dissolved in 3 ml. of water, and to this there was added 3 ml. of an aqueous 0.03 m.mol./ml. solution of sodium metaperiodate. After being kept in an incubator at 35°C, 2 ml. of this mixed solution was pippetted out after 30 minutes and after 20 hours. These solutions were acidified by addition of 1 ml. of 0.0480 N-hydrochloric acid and were titrated with 0.0480 N-sodium hydroxide using a Beckman type-G pH-meter. The pK'_a values were determined by the method of Parke and Davis[9]. The blank curve was obtained by carrying out an identical procedure on a periodate solution to which 3 ml. of water had been added instead of the roseonine-dihydrochloride solution. A parallel reaction mixture was employed for following the periodate consumption; this was measured by conventional techniques. The periodate consumption after 30 minutes was 1.0 mol., and that after 20 hours was 2.1 mol. The titrations were carried out at 11±2°C and not under thermostatted conditions.

Free Roseonine.—This was prepared by dissolving 10 mg. of sodium (0.44 m.mol.) in 1 ml. of absolute ethanol, adding 52 mg. of roseonine-dihydrochloride (0.21 m.mol.), stirring, and leaving overnight. The precipitates of sodium chloride and free roseonine were collected, dried, and pressed into disks. Though the infrared bands were diffuse owing to the presence of sodium chloride, it was sufficient for the purpose of showing the absence of strong absorptions around 1000 cm^{-1} and 1470 cm^{-1} (Fig. 3).

Infrared Absorption Measurements.—The spectra were recorded on a Hilger H 800 double beam instrument equipped with a sodium chloride prism; when necessary the region 1300–1750 cm^{-1} was scanned with a calcium fluoride prism. Potassium bromide disks were used, and the die and the handpress were those supplied by Hilger and Watts, Co. Analytical grade potassium bromide was ground to pass a 200 mesh sieve and dried at 150°C for 24 hours; the samples (ca. 1 mg.) were ground evenly with 300 mg. of this potassium bromide for 5 minutes.

The authors wish to express their gratitude to Professor Yoshimasa Hirata for his guidance and interest in this research and to Mr. Toshio Goto for valuable discussion. We are also grateful for a grant from the Ministry of Education.

Department of Chemistry, Faculty of Science, Nagoya University
Chikusa, Nagoya