## *Structure of Compound A, a Hydrolysis Product of Roseothricin A*

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Roseothricin is a streptothricin-like complex antibiotic, produced by *Streptomyces Roseochromogenus*[1] and has been separated into three components, roseothricin A, B and C, by means of ion exchange chromatography[2]. Chemical investigations have been undertaken on roseothricin A.

Acid hydrolysis of roseothricin A afforded two β-amino acids, β-lysine (I) and roseonine (II), as reported previously,[3] and a glucosamine-like sudstance (III) with properties as follows: crystalline hydrochloride, dec. p. 145–155°C, paper chromatography BuOH : AcOH : $H_2O$ (4 : 1 : 1) Rf. 0.33 (glucosamine 0.33, chondrosamine 0.25), collidine 0.44 (G. 0.43, C. 0.35), phenol : $NH_3$ 0.74 (G. 0.70, C. 0.64), AcOEt : pyridine : $H_2O$ (2 : 1 : 2) 0.86 (G. 0.87), Me COEt 0.03 (G. 0.03), Fehling, triphenyltetrazolium, ninhydrin, Elson-Morgan and Tollens reactions are positive like glucosamine; $\nu_{max.}^{KBr}$ (III. HCl) 1605 m, 1498m, 1404m, 1332 w, 1319 w, 1253 w, 1230 w, 1149 m, 1121s, 1100 s, 1070 s, 1040 s $cm^{-1}$ (s: strong, m: medium, w: weak). The infrared spectrum is slightly different from that of glucosamine, but bands at 1605 and 1498[4] due to a primary amino group are present and the shape of the absorption at 1100-1000 (alcoholic hydroxyl) is very similar to that of glucosamine. It is almost indistinguishable from glucosamine by means of paper chromatography, and these facts together with the analytical data of compound A strongly suggest that substance III is a stereoisomer of glucosamine.

Compound A, colorless needles, dec. p. 215–220°C, which consists of II and III, was obtained as one of the acid hydrolysis products of roseothricin A. Counter current distribution (CCD) (BuOH : MeOH: AcOH : $H_2O$, 18 : 2 : 1 : 19) of the partial DNP (2,4-dinitrophenyl) product of compound A afforded two peaks at K=0.16 and 9.0, as measured by the absorption at 350 mμ. From the number of the peaks it may be deduced that two amino groups capable of being DNP-ated exist in one molecule of compound A. The product of complete DNP-ation, DNP-compound A, m. p. 192–196°C (dec.), had an $E_{1\,cm.}^{1\%}$ value of 306 at 350 mμ[5] and the molecular wight calculated on the assumption of the presence of twe amino groups is ca. 682.[6] Analytical, pKa′, I. R., and other data point to 1 : 1 ratio of II and III in the compound A. Found: C, 32.65; H, 6.25; N, 16.12. Calcd. for $C_{24}H_{44}O_{13}N_{10}$. 6HCl (2 moles each of II and III minus $3H_2O$; mol. wt., 680.67[6]) : C, 32.05; H. 5.60; N, 15.57%. Compound A has eight pKa′s; to at ca. 2.4 (II-$CO_2H$), to at 6.5 (III-$NH_3^+$),[7] two at 8.9 (II β-$NH_3^+$) and two at 10.4 (II guanidinium). The mol. wt. obtained from this potentiometric curve is ca.645.[6] $\nu_{max.}^{KBr}$ (compound A. HCl) 1732 m (-$CO_2H$[8]), 1658 s (sym. trisubstituted guanidine band[9]), 1609 m, and 1490 m, br (-$NH_3^+$ of both II and III), 1405 m, 1388 m, 1220 m, br, 1075 s, and 1054 s, $cm^{-1}$ (br: broad). Since 5.96 moles of periodate are consumed by 1 mole of compound A, it is suggested that $C_2$-$NH_2$, $C_3$-and $C_4$-OH of III, and the β-$NH_2$ and OH of II in compound A may all be free.

1) S. Hosoya, M. Soeda, N. Komatsu, S. Imamura, M. Iwasaki, Y. Sonoda and K. Okada, *Jap. J. Exptl. Med.*, **20**, 121 (1949); *J. Antibiotics*, **3**, (4), 217 (1950).

2) Y. Saburi, *J. Antibiotics*, **6**, (8), 402 (1953).

3) K. Nakanishi, T. Ito, M. Ohashi, I. Morimoto and Y. Hirata. *J. Am. Chem. Soc.* **76**, 2843 (1954); This Bulletin, **27**, 539 (1954); ibid., submitted for publication.

4) K. Nakanishi, T. Goto, M. Ohashi, this Bulletin, submitted for publication.

5) Calculation was made as ε=15500 (at 350 mμ) per one mole of DNP group.

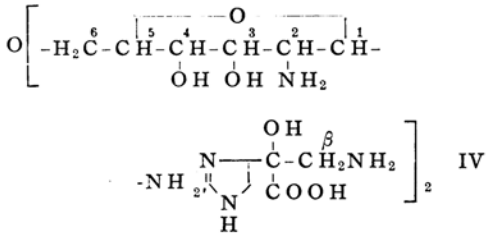
6) Molecular weight as free base.

7) The reason that these amino groups are not DNP-ated, is that rate of DNP-ation of III-$NH_2$ is much slower than that of II β-$NH_2$, and DNP-ation of III-$NH_2$is prevented by precipitation of DNP-compound A.

8) The fact that it is not an ester may be deduced from the following. When trimethylamine is added to compound A and the mixture is immediately evaporated to dryness, this band is absent and a band at ca. 1600 $cm^{-1}$ due to a carboxylate group is seen. Furthermore, this band is absent in DNP-compound A; similar to the case of DNP-roseonine, this is due to the fact that the guanidine portion cannot be DNP-ated and forms an intramolecular salt with this carboxyl group. This corresponds to the two pka′ values at 2.4.

9) N N′-Disubstituted guanidine hydrochlorides possess, two strong absorptions at 1680 and 1600 $cm^{-1}$ (roseonine-2HCl, 1682, 1581), whereas N. N′, N′′-trisubstituted guanidine hydrochlorides possess only one strong absorption at ca. 1650 $cm^{-1}$. On the other hand, N, N, N′-trisubstituted guanidine hydrochlorides show three bands at 1650s, 1620s, and 1570m $cm^{-1}$. Hence the guanidine group in compound A is symmetrically trisubstituted, i. e., the 2′-amino group is linked to some other group (T. Goto, K. Nakanishi, this Bulletin, submitted for publication).

Compound A, therefore, contains a N-glucoside linkage; the Elson-Morgan reaction is positive, but the Fehling reaction is negative, and the tetrazolium reaction becomes positive after longer heating period than that of III. The strong electron attractive effect of the guanidinium ion probably accounts for the lower pKa' of compound A (6.5) than that of III (7.8). The fact that the linkage between II and III



IV

is more stable under acidic conditions than alkaline is also explicable in terms of this effect. Compound A is extremely resistant towards acid hydrolysis; i. e., no appreciable hydrolysis occurred upon heating for 22 days with concentrated hydrochloric acid at 34°, and ca. 15% was hydrolysed upon heating for 11 days with 20% hydrochloric acid at 50°. This difficulty in hydrolysis points to the absence of an acetal linkage. Ester or amide bands were not observable in the region 1750–1500 $cm^{-1}$. These together with the amount of periodic acid consumption suggested that a primary hydroxyl group participates in an ether linkage. We wish to present IV for compound A.

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