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CHEMICAL STUDIES ON KASUGAMYCIN. V.
THE STRUCTURE OF KASUGAMYCIN

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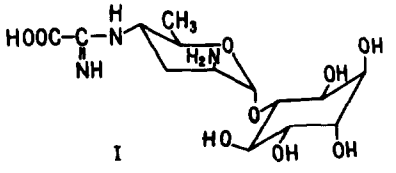
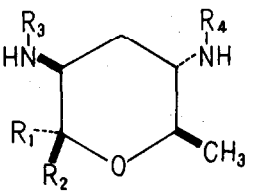
Kasugamycin (I) is an antibiotic produced by Streptomyces kasugaensis and exhibits inhibition against various kinds of bacteria including Pseudomonas and a strong preventive effect against rice blast.[1] The structural elucidation of the three fragments, i.e., d-inositol, methylkasugaminide (II), and kasuganobiosamine (III), obtained from the degradation of kasugamycin, have been described in the previous communications[2] in this series. The present studies deal with the structure of the amino derivative at C_4 of the amino sugar moiety. The following results, considered with previous findings,[2-3] permit the assignment of the gross structure I to kasugamycin, which has an unique amidine group.



I

The degradation products of kasugamycin were investigated toichiometrically. Reaction of the hydrochloride of I with barium hydroxide at 100° for 10 hours gave III, oxalic acid, and ammonia in 97%, quantitative, and 91% yields, respectively. The same reaction at room temperature for 36 hours gave III, kasugamycinic acid (IV), oxalic acid, and ammonia in 56, 35, 56 and 21% yields, respectively.



II, $R_1=R_3=R_4=H$; $R_2=OCH_3$

III, R_1=d-inositol; $R_2=R_3=R_4=H$

IV, R_1=d-inositol; $R_2=R_3=H$; $R_4=C(=O)-C(=O)-OH$

V, $R_1=R_3=H$; $R_2=OCH_3$; $R_4=CN$

VI, $R_1=R_3=H$; $R_2=OCH_3$; $R_4=C(=NH)-COOH$

VII, R_1=d-inositol; $R_2=R_3=H$; $R_4=C(=O)-C(=O)-NH_2$

These evidences show that the amino derivative at C_4 is derived from equivalent amounts of III, oxalic acid, and ammonia and suggest either an amide structure ($R_4=COCONH_2$) or an amidine structure ($R_4=C(=NH)-COOH$). To clarify such point, the following reactions have been carried out. Methanolysis of the hydrochloride of I in saturated methanolic hydrogen chloride gave an amine and d-inositol. The amine hydrochloride shows m.p. 210-213° (dec.), pK'a 10.8, 7.2, and below 2 and has a molecular formula,* $C_9H_{17}O_4N_3 \cdot HCl \cdot 1/2H_2O$.

Calcd: C 39.06, H 6.92, N 15.19, Cl 12.81; Found: C 38.86, H 7.09, N 14.99, Cl 12.98. Treatment of the amine with barium hydroxide gave II and oxalic acid, and oxidation with lead tetraacetate or

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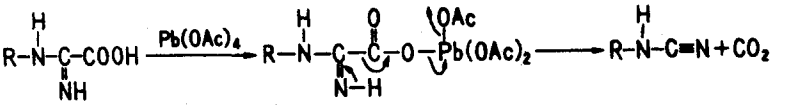
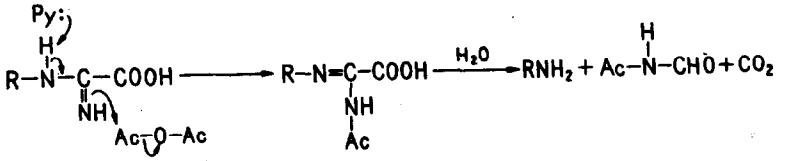
* Although the molecular formula was previously assigned to $C_{10}H_{19}O_4N_3 \cdot HCl$, [2a] it is now revised to $C_9H_{17}O_4N_3 \cdot HCl \cdot 1/2H_2O$.

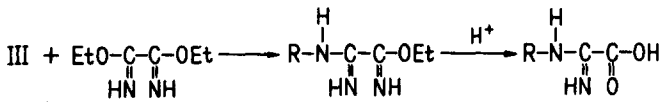
sodium periodate afforded a nitrile amine (V), with evolution of carbon dioxide, showing m.p. 123-126°, ν_{max}^{KBr} 2200 cm^{-1}, and a positive test for nitroprusside reagents.[4] Calcd. for $C_8H_{15}O_2N_3$: C 51.87, H 8.16, N 22.69. Found: C 51.63, H 8.03, N 22.37. Therefore, the structure of the amine is assigned to (VI). The results show that the $-\overset{\frown}{N}-C\equiv N$ group of V is formed by oxidative decarboxylation and can be easily rationalized by the present understanding of such reagents[5-6] as shown in the following sheme.



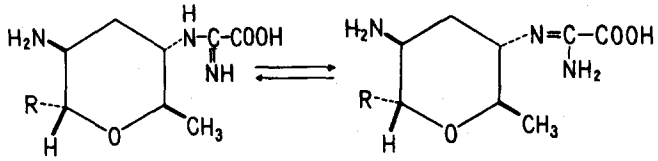
On the other hand, the treatment of I with acetic anhydride-pyridine afforded heptaacetylated III, carbon dioxide, and acetylformamide, m.p. 64-66°, ν_{max}^{KBr} (cm^{-1}) 3250-3150, 1740, 1670, 1600, 1215, and 1180, n.m.r. (δ) 2.21 (singlet, 3H, $-\overset{O}{\overset{\|}{C}}-CH_3$), 9.10 (doublet, 1H, -CHO), and 9.67 (broad, 1H, $-\overset{H}{\overset{|}{N}}-$), which had been previously prepared by the reaction of ethyl formimidate and acetic anhydride by Pinner.[7] The formation of acetylformamide can be explained in the following way.



Kasugamycin forms a chelate compound with basic cupric carbonate[8], stable to acid[9] and unstable to heat and base. These evidences together with the results obtained above strongly support an amidine structure for the amino derivative at C_4. Unumbiguous proof of the amidine structure has been successfully achieved by a partial synthetic approach to I. The amide (VII) prepared by the reaction of III and methyl oxalate followed by ammonolysis was found to be different from I spectroscopically and showed a different biological activity.[2d] The amidine compound has been prepared by the reaction of III with diethyl ester of oxalimidic acid[10] and subsequent mild acid hydrolysis with hydrochloric acid. The synthetic material in state of hydrochloride has been found to be completely identical with the natural kasugamycin hydrochloride (mixed m.p., I.R., n.m.r., and bio-assay).



Since the position of attachment of d-inositol to amino-sugar moiety has been decided by X-ray crystallographic analysis on the hydrobromide of I,[3] the whole structure of kasugamycin has been assigned to I. A large amount of experimental evidence[9] indicates that monosubstituted amidines having different groups on the nitrogen atoms exhibit tautomerism. Supporting evidence for the existence of tautomerism in case of kasugamycin lies in the fact that the hydrolysis with barium hydroxide produces a mixture of III and IV. Therefore, the intrinsic structure of kasugamycin is considered as follows:



R = d-inositol

It should be mentioned also that the pK'a of kasugamycin shows 7.1, 10.6, and below 2 which can be assigned to the amino group at C_2, amidine, and carboxylic acid, respectively, and the infrared spectrum displays an absorption at 1670cm^{-1} for the carboxylic acid attached to the amidine.

Acknowledgement

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