A FURTHER STUDY OF Cu** CHELATE OF KANAMYCIN

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In the previous paper (1), it was reported that kanamycin (KM), an antibiotic found by Umezawa et al. (2), formed a chelate with Cu** at a molar ratio of 2:1. The structure shown in Fig. 1-a was proposed for this chelate where the propylene diamine moiety of KM took part in the formation of the chelation compound. To confirm this structure, deoxystreptamine (DOSA) was prepared by hydrolysis of KM and a comparative study of Cu** chelates of DOSA and ethylene diamine (EDA) with that of KM was made by infrared spectroscopy and polarography.

![Fig. 1. Structures of the Cu** chelates of KM and DOSA.](image)

EXPERIMENTAL METHODS

The CuCl₂·2H₂O and EDA·2HCl employed were reagent grade compounds from Wako Pure Chemicals. KM·H₂SO₄, was a product of Takeda Chemical Industries. DOSA was prepared from KM according to the procedures described by Daly et al. (3). KM of 1.4 g was hydrolyzed in 30 ml of 48% HBrO₃ for 18 hours. The resulting brownish syrup was treated with 10 ml of hot methanol and then filtered yielding a crude preparation of DOSA. This was converted in its picrate which was then hydrolyzed by the ion-exchange column method and 120 mg of crystalline powder of DOSA (m.p. 228°C) were obtained. Chemical analysis of this material gave the following values in %:

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Found: C 44.28, H 8.60,
Calculated for C_{14}H_{12}N_{2}O_{3}: C 44.43, H 8.70.

This DOSA was converted in its monohydratesulphate.

The Cu**^{2+}** chelate of KM was prepared as follows: Two mM of KM and 1.0 mM of CuCl_{2}\ were dissolved in 10 ml of distilled water and 5.0 mM of NaOH were gradually added with stirring. When chelate formation was complete, 10 ml of acetone were added. The resulting purple precipitate was obtained by centrifugation and after recrystallization was dried *in vacuo*. The Cu**^{2+}** chelate of DOSA was prepared in the same way.

The Cu**^{2+}** chelate of EDA was prepared as follows: One mM of Cu(OH)_{2}\ prepared from a solution of CuCl_{2}\ was dissolved in 5.0 ml of a hot solution containing 2.0 mM of EDA. On concentration and cooling, a purple precipitate of the chelate deposited. This was recrystallized and dried *in vacuo*.

IR spectra were obtained with a Perkin-Elmer, model 21, spectrophotometer using the KBr disk method. Alternating-current polarograms were obtained with a Shimadzu Recording Polarograph with an H-type cell and a saturated calomel electrode, in a thermostat at 25.0°C. Gelatin at 0.00025% was used as a maximum suppressor. The test solutions contained 0.025 M of KM, DOSA or EDA, 5×10^{-4} M of Cu(NO_{3})_{2}, 0.01 M of KH_{2}PO_{4}, and NaOH to give various pH values.

**RESULTS AND DISCUSSION**

The IR spectra of KM and its Cu**^{2+}** chelate are shown in Fig. 2. As reported previously, the bands at 6.25 μ and at 6.72 μ seen in the spectrum of KM were assigned to the degenerated bending vibration and the symmetric bending vibration of the NH_{3}^{+} group, respectively. On the other hand, the band at 6.33 μ seen in the spectrum of the

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![Fig. 2. IR spectra of KM and its Cu**^{2+}** chelate.](image)
chelate was assigned to the bending vibration of the NH₂ group, since no band was observed at 6.7 μ. This fact indicates that the NH₂+ groups in the KM molecule participated in the chelate formation by liberating H⁺.

The IR spectra of DOSA and its Cu⁺⁺ chelate are shown in Fig. 3. A similar change in the spectra occurred in the 6-7 μ region and the disappearance of the bands at longer wave length on chelation corresponds to the change in the structure from Fig. 1-b to 1-c. From these facts it seems likely that the amino groups taking part in formation of a KM chelate were those of the DOSA moiety. This was further con-
firmed by a comparison of the IR spectra of EDA and its Cu" chelate, as shown in Fig. 4. It is clear from the figure that a similar situation occurred in the formation of an EDA chelation compound and therefore it is concluded that the propylene diamine moiety of KM is essential for the formation of a chelation compound.

Fig. 5 shows alternating-current polarograms of the Cu" chelates of KM, DOSA and EDA at pH 7.5. The peak of the reduction wave corresponds to the half-wave potential, $E_{1/2}$. Only single waves are observed in the polarograms of chelates of DOSA and EDA, while double waves are obtained in the case of KM. Accordingly, it is apparent that in the KM-Cu system the cupric complex was reduced first to the cuprous state before being further reduced to the amalgam. These facts show that there is a certain discrepancy in the nature of their chelates among KM and DOSA. The plot of $E_{1/2}$ against pH is given in Fig. 6. It is

![Graph showing pH-$E_{1/2}$ relations of Cu" chelates of KM, DOSA and EDA.](image)

(a): KM-Cu (the first wave); (b): DOSA-Cu; (c): EDA-Cu.

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apparent that for all the systems \( E_{24} \) is a linear function of pH, as is the case with many other Cu** chelates (4).

From these results it may be concluded that although KM forms a Cu** chelate with its DOSA moiety, the nature of this chelate is somewhat different from that of DOSA. It is an interesting problem whether or not the above conclusion is correlated with the fact that KM has a high antituberculous activity, whereas DOSA is less active (5).

SUMMARY

IR spectra and alternating-current polarograms of the Cu** chelates of KM, DOSA and EDA were compared. From this comparison it was concluded that KM forms its Cu** chelate through the propylene diamine moiety but the nature of the chelate is somewhat different from that of DOSA.

REFERENCES

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4) Li, N.C. AND DOODY, E. : Ibid. 72, 1891 (1950)
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