[Contribution from the Department of Chemistry of Clark University]

The Preparation and Properties of Ethylenebisoxypropylamine-N,N,N′,N′-tetraacetic Acid[1]

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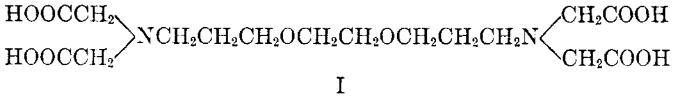
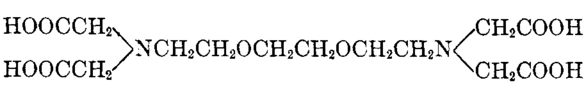
The synthesis of a new chelating agent, ethylenebisoxy-*n*-propylamine-N,N,N′,N′-tetraacetic acid, is described, and its acid-base properties and affinity for calcium(II) and copper(II) ions are discussed. Through the semi-quantitative use of potentiometric titration data, the probable structure of the calcium and copper chelates are deduced.

Since ethylenebisoxy-*n*-propylamine has recently become available, it was considered of interest to prepare and study the corresponding tetraacetic acid derivative (Formula I) as a possible metal chelating agent. This substance is of particular interest in view of the fact that a closely-related compound, ethylenebisoxyethylamine-N,N,N′,N′-tetraacetic acid II has been found by Schwarzenbach[2] to have considerable affinity for calcium ions. In order to explain this property, he suggested that the ether linkages are involved in coordination with the metal ion, as indicated by Formula IV. The chelating agent reported in this paper would presumably form a similar metal derivative III, which differs from IV in that two five-membered rings are replaced by 6-membered rings. It would be of considerable interest to determine whether the weaker 6-rings of III would prevent coordination of the ether linkages with metal ions.



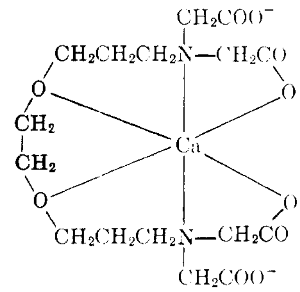
$$(HOOCCH_2)_2NCH_2CH_2CH_2OCH_2CH_2OCH_2CH_2CH_2N(CH_2COOH)_2$$

I

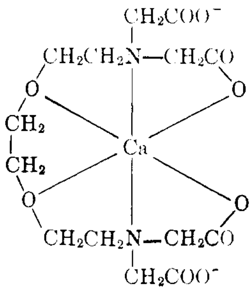


$$(HOOCCH_2)_2NCH_2CH_2OCH_2CH_2OCH_2CH_2N(CH_2COOH)_2$$

II



III



IV

The carboxymethylation of ethylenebisoxy-*n*-propylamine was carried out by a modification of the general procedure described by Smith, *et al.*[3] It was converted to the acid and separated from inorganic salts by treatment with the acid form of Dowex-50 cation exchange resin. The purified product I was found to be strongly acidic, very soluble in hot water, and moderately soluble in cold water. Through the use of potentiometric titrations in the presence and absence of calcium(II) and copper(II) ions it was possible to determine the molecular weight, approximate basicities of the functional groups, and probable structures of the calcium and copper chelates. The potentiometric titrations are given in Figure 1.

Acid dissociation. The potentiometric titration curve obtained in the absence of divalent metal ions indicates that the amino acid I contains two strongly acid groups and two strongly basic groups. Addition of base to the acid results in the formation of a flat low-*p*H buffer region which is maintained until two moles of base have been added per mole of amino acid. Addition of a further small amount of base results in a very sharp inflection whereby the solution becomes strongly alkaline. The addition of the third and fourth moles of base results in a flat, high-*p*H buffer region corresponding to the overlapping neutralizations of two strongly basic groups. Through a comparison of this titration curve with those of analogous compounds, such as methylaminediacetic acid, it becomes apparent that the two strongly acidic groups are acetic acid residues attached to the posi-

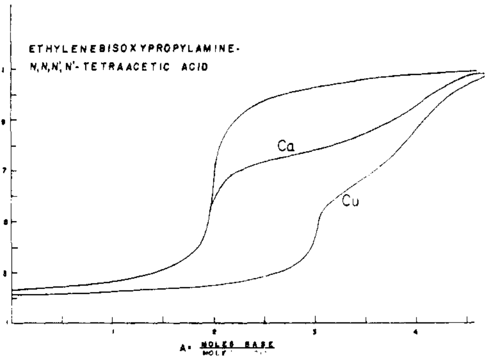


Fig. 1. Potentiometric Titrations of Ethylenebisoxy-*n*-propylamine-N,N,N′,N′-tetraacetic Acid in the Presence of and in the Absence of Equimolar Amounts of Ca(II) and Cu(II) Ions. A = Moles of KOH Added per Mole of Amino Acid.

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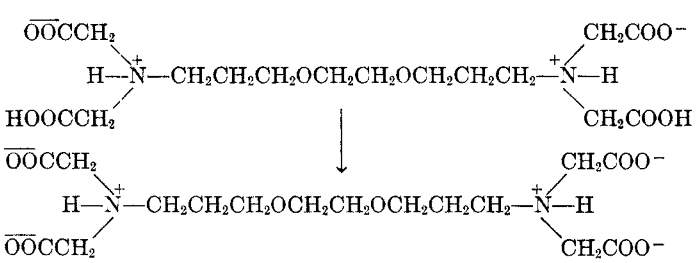
[1] Taken from a Thesis submitted by Harold C. Jigarjian to the Faculty of Clark University in partial fulfillment of the requirements for the degree of Master of Arts.

[2] Schwarzenbach, G., private communication.

[3] R. Smith, J. L. Bullock, F. C. Bersworth, and A. E. Martell, *J. Org. Chem.*, 14, 355 (1949).

tive nitrogen of a dipolar ion. Hence the sharp break in the titration curve of I, given in Figure 1, corresponds to the end point of the reaction in which the dipolar ion V is converted to the doubly negative anion VI:



$$^{-}OOCCH_2(HOOCCH_2)\overset{+}{N}H-CH_2CH_2CH_2OCH_2CH_2OCH_2CH_2CH_2-\overset{+}{N}H(CH_2COO^-)(CH_2COOH) \quad V$$

$$\downarrow$$

$$(^{-}OOCCH_2)_2\overset{+}{N}H-CH_2CH_2CH_2OCH_2CH_2OCH_2CH_2CH_2-\overset{+}{N}H(CH_2COO^-)_2 \quad VI$$

From the nature of the charge distributions in Formula V, it appears that the strong acidity of the carboxyl groups is a result of the strong inductive effect of the positive nitrogen in the *alpha* position with respect to the carboxyl group.

The last two steps in the neutralization of the amino acid correspond to the removal of the two hydrogen ions attached to the highly basic tertiary amino groups. In other diaminotetraacetic acids, such as ethylenediaminetetraacetic acid, the dissociation of one of the nitrogens takes place at a much lower *p*H than does the other, and the two dissociation reactions are separated by three to four *p*H units. This has been described as due to the mutual inductive effects of the positive nitrogen atoms on each other.[4] In the present instance, however, with ten atoms between the two nitrogen atoms, there should be very little electrostatic repulsion between the nitrogens, and the overlapping of their neutralization reactions thus seems reasonable. In fact the flatness of both the low- and high-*p*H buffer regions indicate that both pairs of dissociation[1] steps may occur simultaneously, with only the usual statistical factor between the successive constants. A rough estimate of the dissociation constants from Figure 1 gives ~ 2 for pK_1 and pK_2, and ~10 (or slightly higher) for pK_3 and pK_4.

Metal chelate formation. The calcium titration curve of Figure 1 coincides with that of the free acid until two moles of base have been added per mole of amino acid. After an inflection region involving a relatively small *p*H rise, there is a broad buffer region from *p*H 7 to 9, in which two moles of base are neutralized per mole of chelate formed. Below *p*H 5 there is, therefore, no reaction between the calcium ion and the dinegative form of the ligand, or any of its more acid forms. Above *p*H 5, chelate formation takes place with the displacement of two hydrogen ions, according to the following reaction:

$$Ca^{-2} + H_2A^{-2} \rightleftharpoons CaA^{-2} + 2H^+$$

where H_4A represents the amino acid with its four dissociable protons. The *p*K value of this two-step dissociation, estimated from the *p*H of the midpoint of the corresponding buffer region (*i.e.*, at $A = 3$), is approximately 13. When this value is combined with the values of pK_3 and pK_4 given above, a stability constant for the calcium chelate of the order of 10^7 is obtained. This value is about the order of what we would expect for a hexadentate calcium chelate. It is considerably less stable, for example, than the chelate formed by ethylenediamine tetraacetic acid, which forms five five-membered rings with the calcium ion, while it has approximately the same stability as the calcium chelate of ethylenediamine-N,N′-diacetic-N,N′-dipropionic acid, which it resembles in forming two 6-rings and three 5-rings with the metal ion. The magnitude of the stability constant rules out all of the probable alternatives to formula III. It is 3 log units higher, for example, than that of the calcium chelate of methylaminediacetic acid.[5] Hence the coordination compound does not involve merely the iminodiacetate group at one end of the ligand molecule.

The copper(II) titration curve of Figure 1 is seen to lie considerably below the free amino acid curve even in the lowest *p*H regions. Hence the copper chelate is stable enough to form directly from the most acid form of the chelating agent. Since the first inflection (*i.e.*, *p*H rise) in the titration curve occurs at $A = 3$, formation of the 1:1 chelate involves the displacement of only one of the hydrogen ions bound to the amino group. This compound is therefore tridentate, and its structure is indicated by formula VII. It is not possible in this case to estimate closely, or to calculate, the stability constant from a simple titration curve, except to state that log of the copper(II) formation constant is greater than 10. This is roughly in agreement with the stabilities of copper chelates having analogous structures. The corresponding values for the copper derivatives of iminodiacetic acid and 3-hydroxypropyl iminodiacetic are above 10 in both cases.[6]

The second step of the copper chelate neutralization curve is evidenced by a buffer region at somewhat higher *p*H, in which the fourth hydrogen ion is neutralized by added base. Thus the whole titration curve for copper can be summarized by the following two equations.

$$Cu(H_2O)_4^{++} + H_4A + 3OH^- \longrightarrow CuHA(H_2O)^- + 6\,H_2O$$

$$CuHA(H_2O)^- + OH^- \longrightarrow CuA^{--} + 2\,H_2O$$

The nature of the final copper chelate formed, CuA^{--}, can be deduced by considering the various alternatives. The proton which is removed from VII may come from either the nitrogen or the

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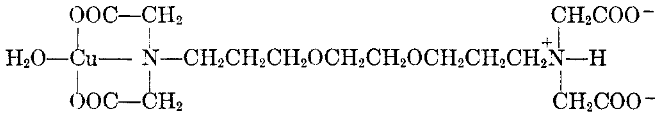
(4) G. Schwarzenbach and H. Ackermann, *Helv. Chim. Acta*, **31**, 1029 (1948).

(5) G. Schwarzenbach, E. Kampitsch, and R. Steiner, *Helv. Chim. Acta*, **28**, 1133 (1945).

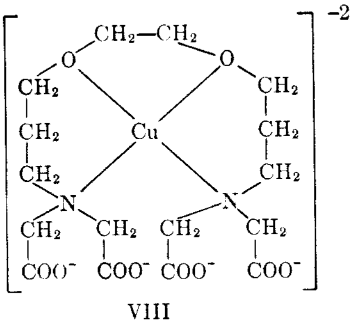
(6) S. Chaberek and A. E. Martell, *J. Am. Chem. Soc.*, **76**, 215 (1954).

solvated water molecule. If the former is the case, the reaction must be assisted by coordination of this nitrogen atom with the copper(II) ion, since its basicity (for hydrogen ions) would otherwise be too great. This would result in formation of a structure indicated by Formula VIII.

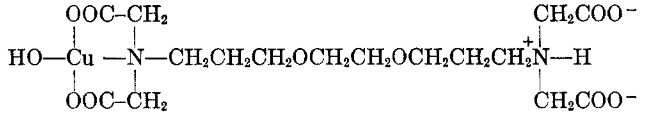


$$\begin{array}{lr}
OOC{-}CH_2 & CH_2COO^- \\
H_2O{-}Cu{-}N{-}CH_2CH_2CH_2OCH_2CH_2OCH_2CH_2CH_2\overset{+}{N}{-}H & \\
OOC{-}CH_2 & CH_2COO^-
\end{array}$$

VII



VIII



$$\begin{array}{lr}
OOC{-}CH_2 & CH_2COO^- \\
HO{-}Cu{-}N{-}CH_2CH_2CH_2OCH_2CH_2OCH_2CH_2CH_2\overset{+}{N}{-}H & \\
OOC{-}CH_2 & CH_2COO^-
\end{array}$$

IX

If the hydrogen ion comes from the coordinated water molecule, structure IX would be formed. It is not possible to decide between VIII and IX on the basis of titration data. However, it is now known that copper chelates of iminodiacetic acid and methylamine diacetic acid do not form hydroxy derivatives except possibly at much higher *p*H. For this reason Formula VIII is preferred as the structure of the copper chelate compound.[7]

EXPERIMENTAL

Ethylenebisoxy-n-propylamine-N,N,N′,N′-tetraacetic acid. To a solution of 32 g. of ethylenebisoxypropylamine and 25 ml. of water in a 1-liter reaction flask fitted with a stirrer, distillation condenser, and a dropping-funnel was added 30 ml. of an aqueous solution containing 13.5 g. of sodium cyanide. The mixture was stirred and heated to the point of slow distillation, while a stream of nitrogen was passed through the liquid. Then 14.6 g. of an 18.5% aqueous formaldehyde solution was added over the period of one hour, and three 23-g. portions of the same solution were subsequently added over a period of four hours. After each addition of formaldehyde except the last, three equal portions of an aqueous solution containing a total of 28 g. of sodium cyanide were added rapidly. The heating was stopped after 120 hours, when the rate of evolution of the ammonia had decreased to a relatively small value.

The aqueous sodium salt of the product then was passed through a column containing a liberal excess of Dowex-50 ion exchange resin. A portion of the product was obtained in a relatively pure form by elution of the resin with water and evaporation to a small volume. The resulting off-white crystalline material was purified by dissolving it in hot water, and addition of acetone to the cooled aqueous solution. The batch of colorless crystals thus obtained amounted to 30% of the theoretical amount. Elution of the column with ammonia resulted in the isolation of more of the product as the very water-soluble ammonium salt. The total yield of amino acid was estimated as between 60 and 70% of the theoretical amount. The compound is very soluble in hot water, sparingly soluble in cold water, insoluble in ethanol, acetone, and solvents of lower polarity, and decomposes above 200°.

Anal. Calc'd for $C_{16}H_{28}N_2O_{10}$: C, 47.06; H, 6.86; N, 6.86. Found: C, 47.22; H, 7.22; N, 6.83.

Determination of molecular weight. The neutral equivalent was found to be 205 based on the inflection in the titration curve (*p*H vs. ml. standard base) of the amino acid in the absence of divalent metal ions. From the relative positions of the inflections of this curve, and of the curves obtained in the presence of 1:1 ratios of calcium(II) and copper(II) ions, it is apparent that the molecular weight must be at least two times the neutral equivalent. Examination of all reasonable structures obtainable on the basis of the starting materials used in the synthesis indicates that the molecular weight is indeed two times the neutral equivalent.

Anal. Calc'd for $C_{16}H_{28}O_{10}N_2$: M.W., 408. Found: M.W., 410.

Potentiometric titration. The potentiometric titrations were carried out with a Beckman Model G *p*H meter used in conjunction with extension glass and calomel electrodes, calibrated with acid phthalate buffer. The temperature, though not precisely controlled remained in the range of 24 ± 1°, and carbon dioxide-free nitrogen was passed through the solution during the titrations. The concentrations of ligand and metal ions were approximately 10^{-3} *M*. Since ionic strength was not controlled, no calculations of the exact stability constants were made from the potentiometric data.

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(7) Another structure, in which copper is bound to two bidentate aminoacetate groups, is also a possibility. This would result in a titration curve resembling that obtained from a 2:1 ratio of iminodiacetic acid to Cu(II) ion, which is actually the case. However, this arrangement of donor groups would leave a 10-atom bridge between the nitrogens, with the ether oxygens uncoordinated. Hence the more stable ring structure of VIII is preferred.