to obtain UDPGal essentially free of UDPG. The same method was used for preparation of labeled UDPGal from Gal(-1-C^{14})-1-P, but in this case the ratio of Gal-1-P to UDPG was reduced to nearly one, and residual UDPG was removed from the product. A typical reaction mixture consisted of the following ingredients, in a final volume of 40 ml. 0.1 *M* glycine, *p*H 8.9: 80 μmoles of UDPG, 200 μmoles of $MgCl_2$, 40 μmoles of cysteine, 100 μmoles of TPN, 5 mg. of lyophilized G-6-P dehydrogenase,[22] 150 mg. of lyophilized liver Gal-1-P uridyl transferase[26] and 12 ml. of the crude, heat-inactivated solution from reaction 5 containing 95 μmoles of Gal(-1-C^{14})-1-P. It was essential to inactivate the Gal-1-P incubation mixture before proceeding with the conversion to UDPGal, since the galactokinase preparation contained UDPGal-4-epimerase, which prevented the accumulation of UDPGal by conversion of most of this product to UDPG. It was not necessary to add phosphoglucomutase since the uridyl transferase preparation contained relatively large amounts of this enzyme. The course of the reaction could be followed by observing the increase in optical density at 340 mμ due to TPN reduction. Incubation of the reaction mixture was carried out at 37° for 1 hr. after which the mixture was assayed for UDPG and UDPGal with UDPG dehydrogenase and UDPGal-4-epimerase[42] and found to contain 10 μmoles of UDPG and 62 μmoles of UDPGal. After heating the reaction mixture at 100° for 2 min. 30 μmoles of DPN and a quantity of purified UDPG dehydrogenase[12] equivalent to 7 mg. of protein were added and the mixture was incubated for 30 min. at room temperature to convert UDPG to UDPGA. UDPGal was isolated as described for UDPG and assayed according to Maxwell.[41]

Alternative Procedure for the Preparation of UDPGal from Galactose.—This procedure used yeast as the source of Gal-1-P uridyl transferase and of phosphoglucomutase as well as of galactokinase. When the intermediate Gal-1-P is not desired, it has two advantages in that the necessary enzymes are easier to prepare and the reactions can be carried out in a single incubation. The procedure could be made practical for the preparation of UDPGal-C^{14} by decreasing the ratio of galactose to UDPG and removing residual UDPG from the product as described above.

The yeast fraction used was prepared from galactose-adapted *Saccharomyces fragilis* grown and harvested essentially as described above for the galactokinase preparation. In this case the medium consisted of 50 g. of galactose, 1.5 g. of yeast extract (Difco), 1.8 g. of $(NH_4)_2SO_4$ and 1.5 g. of KH_2PO_4 per liter of solution. The cells were grown at room temperature for 24 hr.; a yield of 250 g. of wet washed cells was obtained from 18 liters of medium. An extract was prepared by the procedure of Lamanna and Mallette.[43] The wet cell mass was placed in a Waring blendor containing 400 g. of Alconox-washed Superbrite glass beads (100–500 mμ diameter, #p–1091, from Minnesota Mining and Manufacturing Company) and 150 ml. of 0.1 *M* phosphate buffer, *p*H 7.0 and 0.005 *M* with respect to cysteine. Water (350 ml.) was added to fill the blendor, and homogenization was carried out at −10° for two successive 5 min. periods with intermediate cooling. The mixture was then centrifuged and 380 ml. of clear supernatant was obtained. Nucleic acid was removed by autolytic digestion[44]; 25 ml. of 1 *M* phosphate buffer, *p*H 7.0 was added to 320 ml. of the extract and the mixture was incubated at 37° for 2.5 hr. Ammonium sulfate fractionation was then carried out at 2–5° by the addition of solid ammonium sulfate; fractions precipitating between 0 and 40%, 40 and 50%, 50 and 60%, and 60 and 70% saturation (at 0°) were collected. The last fraction (60–70%) contained the bulk of Gal-1-P uridyl transferase as well as sufficient galactokinase and phosphoglucomutase for the preparation of UDPGal; UDPGal-4-epimerase was located in the 40–50% fraction. The precipitate from 60–70% saturation was dissolved in 10 ml. water and lyophilized. The dry powder stored at −20° was stable for at least three months.

For the preparation of UDPGal, a typical reaction mixture consisted of the following materials in a final volume of 80 ml. of 0.1 *M* glycine, *p*H 8.9: 400 μmoles of galactose, 80 μmoles of UDPG, 100 μmoles of TPN, 160 μmoles of ATP, 400 μmoles of $MgCl_2$, 80 μmoles of cysteine, 10 mg. of lyophilized G-6-P dehydrogenase[22] and 100 mg. of the lyophilized yeast fraction described above. Incubation of the reaction mixture was carried out at 37° for 30 min. UDPGal was then isolated as already described; about 60 μmoles of UDPGal (75% of theory from UDPG), containing less than 5% UDPG, was obtained.

Acknowledgment.—The authors would like to thank Dr. Herman M. Kalckar for helpful advice and stimulating discussion throughout the course of this work.

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(43) C. Lamanna and F. M. Mallette, *J. Bact.*, **67**, 503 (1954).

(44) P. Berg; W. E. Razzell, personal communications.

BETHESDA, MARYLAND

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, SYRACUSE UNIVERSITY]

Structural Influences on the Stability of Dipeptide–Metal Ion Complexes[1]

BY JOHN L. BIESTER[2] AND PAUL M. RUOFF

RECEIVED MAY 22, 1959

Dissociation constants of glycylglycine, glycyl-L-alanine, glycyl-L-phenylalanine, glycyl-D-phenylalanine and L-phenylalanylglycine and stability constants of the complexes of these peptides with Co(II) and Cu(II) ions have been determined by potentiometric titration. Variations in the stability constants are discussed in relation to the structural differences in the peptides. Substitution on the carbon adjacent to the peptide nitrogen produces small changes, whereas substitution on the carbon adjacent to the terminal amino group leads to more pronounced changes in stability constants. Both electronic and steric effects are produced by the structural changes studied. The sensitivity of stability constant values to the dissociation constants employed in their calculation is pointed out.

Introduction

Many proteolytic enzymes are activated by metal ions,[3–5] but the mechanism of this activation is still obscure. Smith's suggestion[6,7] that an enzyme–metal ion–substrate complex is formed has been criticized[8–10] as inconsistent with the complex

(1) (a) Abstracted from the dissertation submitted by John L. Biester to the Graduate School of Syracuse University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. (b) Presented at the 135th Meeting of the American Chemical Society, Boston, Mass., April, 1959 (Abstracts of that Meeting, p. 28C). (c) This work was supported in part by grants from the Bristol Laboratories, Inc., Syracuse, N. Y., and from the Research Corporation to J. L. B.

(2) Department of Chemistry, Beloit College, Beloit, Wis.

(3) M. J. Johnson and J. Berger, *Advances in Enzymol.*, **2**, 69 (1942).

(4) E. L. Smith in J. T. Edsall, Ed., "Enzymes and Enzyme Systems," Harvard University Press, Cambridge, Mass., 1951, pp. 47–76.

(5) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice–Hall, Inc., New York, N. Y., 1952, pp. 401–414.

(6) E. L. Smith, *J. Biol. Chem.*, **176**, 21 (1948).

(7) E. L. Smith, *Proc. Nat. Acad. Sci.*, **35**, 80 (1949).

(8) I. M. Klotz and W.-C. L. Ming, THIS JOURNAL, **76**, 805 (1954).

(9) J. B. Gilbert, M. C. Otey and J. Z. Hearon, *ibid.*, **77**, 2599 (1955).

(10) C. B. Murphy and A. E. Martell, *J. Biol. Chem.*, **226**, 37 (1957).

stability constants observed for the substrate and hydrolytic products with the metal ions involved. Detailed interpretation of the activation mechanism requires knowledge of the effect of several factors on complex stabilities.

One factor of particular interest is the effect of structural variations in the ligand molecule. Datta and Rabin[11,12] have employed a systematic sequence of ligands to determine the effects of structural differences on complex stabilities. They used a group of glycine peptides in which structural variations involved substitution on the amino or peptide nitrogen or esterification of the carboxyl group. The literature apparently does not contain any report of a similar study in which the structural differences involve systematic variations on the carbon skeletons of the peptides.

Many stability constants have been reported[13] involving a variety of structural variations in the ligand molecules. Although it might appear possible to select data from the literature that would reveal the effects of structure on complex stabilities, the varying experimental conditions employed make direct comparisons difficult if not impossible. The present work presents data on five structurally related peptides for which dissociation and stability constants have been experimentally determined under the same conditions. It is believed the results are internally consistent and subject to direct comparison. Any variations in stability constants may thus be ascribed to the structural differences in the peptides. Dissociation and stability constants have been determined for the Co(II) and Cu(II) complexes of glycylglycine, glycyl-L-alanine, glycyl-L-phenylalanine, glycyl-D-phenylalanine and L-phenylalanylglycine. Several of the values are believed to be previously unreported in the literature.

Experimental

Materials.—Glycylglycine, glycyl-L-phenylalanine and L-phenylalanylglycine were purchased from Mann Research Laboratories, New York. Glycyl-L-alanine was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Glycyl-D-phenylalanine was synthesized from D-phenylalanine (purchased from Nutritional Biochemicals Corp.) by the method of Birnbaum, *et al.*,[14,15] and recrystallized to constant rotation of $[\alpha]^{22}D$ −41.4° (*c* 2% in water). All peptides were dried *in vacuo* at 105° for 15 hours before use. Metal ion solutions were prepared from Fisher Certified Reagent Grade $CoCl_2 \cdot 6H_2O$ and $CuCl_2 \cdot 2H_2O$. The cobalt solution was standardized by complexometric titration with ethylenediaminetetraacetic acid (EDTA) by the method of Harris and Sweet.[16] The EDTA was prepared as a primary standard from Eastman Organic Chemical #6354 by the method of Blaedel and Knight.[17] The copper solution was standardized iodometrically. Reagent grade sodium chloride was used to maintain constant ionic strength. Carbon dioxide-free sodium hydroxide was prepared from Stansol concentrate and standardized potentiometrically against potassium acid phthalate. Hydrochloric acid was standardized potentiometrically against the sodium hydroxide.

Procedure.—Titrations were carried out using 20 ml. of solution contained in a 100-ml. beaker immersed in a constant temperature water-bath kept at 25 ± 0.05°. Acid or base was added from 10-ml. burets graduated in intervals of 0.02 ml. Extension tips were fitted to the burets so the titrant was added below the liquid surface. Electrical stirring was used. A stream of moist nitrogen was passed over the surface of the solution to prevent absorption of carbon dioxide and oxygen. Measurements of *p*H were made with a Beckman model G *p*H meter equipped with a general purpose glass electrode no. 1190-72 and a no. 1170 calomel electrode with extension leads. The electrodes were standardized against Beckman buffer of *p*H 7.00.

The peptide solutions were prepared to contain 0.0200 *M* peptide and 0.200 *M* sodium chloride. Metal ion solutions were 0.0100 *M* in metal ion. For determinations of ionization constants 10 ml. of peptide solution and 10 ml. of distilled water were used. For the stability constant determinations, 10 ml. of peptide and 10 ml. of metal ion solution were used.

Results

One source of confusion in stability constant studies is the multiplicity of symbols employed to identify the various equilibria involved. In general *K*, with appropriate subscripts, has been used to represent both acid dissociation and complex stability constants. Furthermore, most of the constants reported, including those in this paper, are based on concentrations or a combination of concentration and activity data and are not activity constants which the symbol *K* presumably represents. In the interest of clarity we have chosen to represent apparent acid dissociation constants by *K'* and stability constants or concentration quotients by *Q*. This distinction was recently used by McIntyre, *et al.*,[18] and earlier in a slightly different manner by Hearon and Gilbert.[19]

Apparent Dissociation Constants.—The apparent dissociation constants for the five peptides as determined in this work are given in Table I.

TABLE I

APPARENT DISSOCIATION CONSTANTS OF PEPTIDES

t = 25°; inert electrolyte (NaCl) = 0.100 *M*

Ligand	This work pK_1'	This work pK_2'	Literature pK_1'	Literature pK_2'
Glycylglycine	3.22 ± 0.02[a]	8.17 ± 0.02[a]	[b]	[c]
Glycyl-L-alanine	3.34 ± .02	8.19 ± .04	3.17[d]	8.23[d]
Glycyl-L-phenylalanine	3.12 ± .02	8.16 ± .02	[f]	[e]
Glycyl-D-phenylalanine	3.11 ± .02	8.18 ± .01	[f]	[e]
L-Phenylalanylglycine	3.13 ± .04	7.62 ± .02	[f]	[f]

[a] The ± values are estimates of the standard deviations computed from 25 or more calculations of each pK' value. [b] Eleven literature values range from 2.94 to 3.19. [c] Thirteen literature values range from 8.07 to 8.37. [d] E. Ellenbogen, THIS JOURNAL, **74**, 5198 (1952). [e] D. J. Perkins (*Biochem. J.*, **57**, 702 (1954)) reports 8.28 for glycyl-DL-phenylalanine. [f] Not recorded in the literature.

Representative literature values, where available, are also given. The values for this work were calculated by the method of Hitchcock[20] and are defined by the equations

$$K_1' = [H_3N^+RCOO^-][H^+]/[H_3N^+RCOOH] \quad (1)$$

$$K_2' = [H_2NRCOO^-][H^+]/[H_3N^+RCOO^-] \quad (2)$$

(11) S. P. Datta and B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1117 (1956).

(12) S. P. Datta and B. R. Rabin, *ibid.*, **52**, 1123 (1956).

(13) J. Bjerrum, G. Schwarzenbach and L. G. Sillen, "Stability Constants, Part I: Organic Ligands," Special Publication No. 6, The Chemical Society, Burlington House, London, 1957.

(14) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952).

(15) K. R. Rao, S. M. Birnbaum, R. B. Kingsley and J. P. Greenstein, *ibid.*, **198**, 507 (1952).

(16) W. F. Harris and T. R. Sweet, *Anal. Chem.*, **26**, 1649 (1954).

(17) W. J. Blaedel and H. T. Knight, *ibid.*, **26**, 741 (1954).

(18) G. H. McIntyre, Jr., B. P. Block and W. C. Fernelius, THIS JOURNAL, **81**, 529 (1959).

(19) J. Z. Hearon and J. B. Gilbert, *ibid.*, **77**, 2594 (1955).

(20) D. I. Hitchcock in C. L. A. Schmidt, Ed., "The Chemistry of the Amino Acids and Proteins," Charles C. Thomas, Springfield, Ill., 1945, pp. 596–609.

TABLE II

STABILITY CONSTANTS OF Co(II) AND Cu(II) COMPLEXES

$t = 25°$; inert electrolyte (NaCl) = 0.100 M

Ligand	Cobalt log Q_1	Cobalt log Q_1Q_2	Copper log Q_1	Copper log Q_1Q_2	Copper pQ_{1a}	Copper pQ_{2a}
Gly-Gly	2.94 ± 0.01[a]	5.42 ± 0.02[a]	5.43 ± 0.03[a]	8.64 ± 0.08[a]	4.17	9.67
Gly-L-Ala	3.10 ± .02	5.68 ± .03	5.81 ± .03	8.97 ± .14	4.35	9.28
Gly-L-Phe	2.96 ± .01	5.27 ± .02	5.45 ± .01	8.63 ± .06	3.86	9.78
Gly-D-Phe	2.91 ± .02	5.35 ± .02	5.26 ± .01	8.39 ± .08	3.70	9.77
L-Phe-Gly	2.12 ± .02	4.14 ± .01	4.66 ± .02	[b]	3.50	[b]

[a] The ± values are estimates of the standard deviations. [b] Not determined because of insufficient peptide.

Stability Constants of Co(II) and Cu(II) Complexes.—The calculated values for the first and over-all constants and the additional ionizations with copper are given in Table II.

The values given for the cobalt complexes were calculated by the method of Datta and Rabin[11] and are based on the equilibria

$$Q_1\text{: } Co^{++} + L^- \rightleftharpoons CoL^+ \quad (3)$$

$$Q_2\text{: } CoL^+ + L^- \rightleftharpoons CoL_2 \quad (4)$$

These results were also calculated by the method of Hearon and Gilbert[19] for a two-step complexing reaction employing the "indeterminate point" relationship. Values by this method were approximately 0.1–0.2 log unit higher. Because of the limited quantities of peptides available, it was impossible to obtain data for several titrations over a range of concentrations and some of the values needed for the Hearon and Gilbert calculations could not be determined with precision. Tests with glycine, however, gave identical values for Q_1 and Q_1Q_2 by both methods of calculation. The values for glycylglycine are the only ones previously reported in the literature with log Q_1 ranging from 3.04 to 3.5 and log Q_1Q_2 ranging from 5.30 to 5.88. Constants for glycyl-D-alanine have been reported[9] as log Q_1 = 3.23 and log Q_1Q_2 = 5.59.

In the titration of copper–dipeptide mixtures in 1:2 molar ratio it is found that four protons are liberated per copper atom during complex formation. Dobbie and Kermack[21] were the first to recognize this situation and to include the additional equilibria in their calculations. The equilibria involved can be represented by the equations

$$Cu^{++} + L^- \rightleftharpoons CuL^+ \quad (5)$$

$$CuL^+ \rightleftharpoons CuL + H^+ \quad (6)$$

$$CuL + L^- \rightleftharpoons CuL_2^- \quad (7)$$

$$CuL_2^- \rightleftharpoons CuL_2^{=} + H^+ \quad (8)$$

The corresponding equilibrium constants are defined by the equations

$$Q_1 = [CuL^+]/[Cu^{++}][L^-] \quad (9)$$

$$Q_{1a} = [CuL][H^+]/[CuL^+] \quad (10)$$

$$Q_2 = [CuL_2^-]/[CuL][L^-] \quad (11)$$

$$Q_{2a} = [CuL_2^{=}][H^+]/[CuL_2^-] \quad (12)$$

The procedures of Dobbie and Kermack[21] were used in calculating Q_1 and Q_2. The values of pQ_{1a} and pQ_{2a} were determined by the method of Datta and Rabin.[12] Constants for the copper–glycylglycine system are the only ones previously reported in the literature; log Q_1 ranges from 5.82 to 6.04 and log Q_1Q_2 is reported as 9.14[21] or 11.66.[22,23]

The computational method of Hearon and Gilbert[19] was applied to the copper–glycylglycine data and gave log Q_1Q_2 = 11.77 in good agreement with Monk[22] and Li and Doody.[23] However, values for Q_1 were negative by this procedure. A fundamental assumption of the Hearon–Gilbert treatment is the absence of proton-bearing complexes, an assumption which is not valid if the above sequence of reactions is correct. Fried and Gurd[24] and Datta and Rabin[12] have independently confirmed the suggestion of Dobbie and Kermack[21] that the proton released in reaction 6 comes from the peptide nitrogen. Further confirmation of this reaction sequence is found in the failure of the Hearon–Gilbert calculations which assume that reaction 6 does not intervene between 5 and 7. These results also support the suggestion of Datta and Rabin[12] that both Monk and Li and Doody did not take these additional proton-releasing reactions into consideration in determining the constants they reported.

Discussion

The formation of metal ion–dipeptide complexes is essentially a competition between the metal ion and hydrogen ion for the ligand anion. If all other factors are constant, the relative stabilities of complexes formed by a series of structurally related ligands and a given metal ion should be determined by the basic strength of the ligand anion.

Table III shows the general formula representing the five peptides included in this study and the structural variations they involve. Glycylglycine, as the simplest structure, is used as the reference compound. If R_1 is some group other than H, the electron density on the terminal amino nitrogen should be altered and hence the basic character of this group would also change. The first four pep-

TABLE III

GENERAL FORMULA AND STRUCTURAL FEATURES OF PEPTIDES

$$H_3N^+CH(R_1)CONHCH(R_2)COO^-$$

Peptide	R_1	R_2
Glycylglycine	H	H
Glycyl-L-alanine	H	L-CH_3–
Glycyl-L-phenylalanine	H	L-$C_6H_5CH_2$–
Glycyl-D-phenylalanine	H	D-$C_6H_5CH_2$–
L-Phenylalanylglycine	L-$C_6H_5CH_2$–	H

(21) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246 (1955).

(22) C. B. Monk, *Trans. Faraday Soc.*, **47**, 297 (1951).

(23) N. C. Li and E. Doody, THIS JOURNAL, **76**, 221 (1954).

(24) M. Fried and F. R. N. Gurd, p. 29C of the Abstracts of the New York Meeting of the American Chemical Society, Sept., 1957.

tides have R_1 = H and as expected these compounds showed essentially the same pK_2' value. In L-phenylalanylglycine R_1 = $-CH_2C_6H_5$ and the lowered pK_2' indicates a weaker base resulting from the withdrawal of electrons by the benzyl group. Based on the observed pK_2' values, equal complex stabilities would be expected for the first four peptides and a lower stability for the fifth. The data of Table II and the curves of Fig. 1 indicate that these predictions are only approximately realized and suggest that factors other than pK_2' are significant in these dipeptide–metal ion interactions.

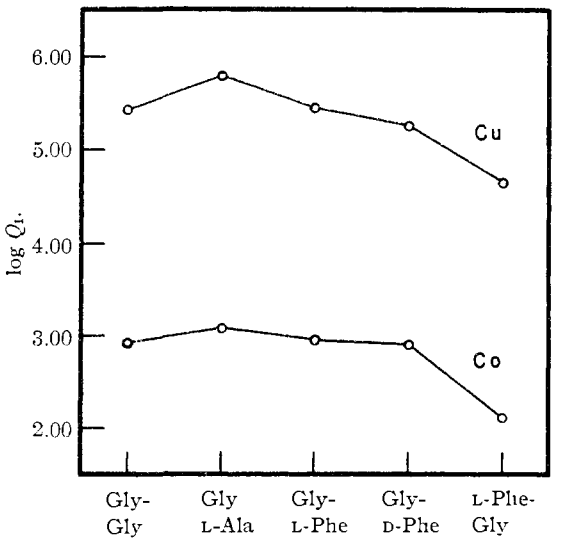


Fig. 1.—Graphical summary of log Q_1 values *vs.* structure.

Although the initial step in dipeptide–metal ion complexing is probably interaction between the terminal amino group and the metal ion, it is a well-established fact that chelation follows by interaction at the peptide bond. Structural influences in this region of the ligand molecule would presumably be less pronounced, because of the strong resonance character of the peptide bond which would tend to stabilize electronic character in this region. Coördination at the peptide bond can theoretically occur either through nitrogen or oxygen, but Rabin[25] has presented evidence that oxygen is the most likely site of initial bonding. With copper the peptide hydrogen is subsequently lost permitting a rearrangement of the chelate bond to the nitrogen atom without destruction of the resonance normally associated with the peptide bond. For the present discussion the precise location of the chelate bond is not critical since any change in electron density at the peptide bond would be distributed generally through the resonance structure.

The log Q_1 values for both cobalt and copper complexes indicate that the R_2 group exerts only a small influence on the stability constant. The group at R_1, however, produces a significant variation in stability, presumably because of its effect on the basic strength of the peptide. The results with L-phenylalanylglycine indicate that the benzyl group in position R_1 is electron withdrawing thereby reducing the electron density on the terminal amino group.

(25) B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1130 (1956).

The glycylphenylalanines show results that appear to be inconsistent with structural theory. These two peptides and their complexes are enantiomorphs and should show the same constants within the limits of experimental error. It has been determined that the differences reported in Table II for these peptides are the result of computational errors rather than reflecting real variations in stability constants. Because the computational procedures are complicated by several algebraic substitutions, it is practically impossible to appraise rigorously the error in the calculated stability constants. The most probable sources of error are the *p*H measurements and the value of pK'_2 used in the computations. Gilbert, *et al.*,[9] have discussed this problem. Since the glycylphenylalanines gave nearly identical titration curves, the *p*H measurements did not appear to be the source of the differences observed.

Although enantiomorphs should give identical values, the small difference of 0.02 log unit in the pK'_2 values for the glycylphenylalanines seemed to reflect the errors inherent in the titration techniques employed. The experimental values of 8.16 and 8.18 for pK'_2 were used in calculating the data of Table II. However, by assuming the value of 8.16 for both peptides, the calculated value of log Q_1 for glycyl-D-phenylalanine becomes 2.94 with cobalt and 5.40 with copper. These results indicate that an insignificant difference in the pK'_2 values used in the calculations can produce an apparently significant difference in the computed stability constant values. This observation indicates the necessity of treating stability constants with extreme caution if one is attempting to compare two or more values and ascribe significance to the differences observed.

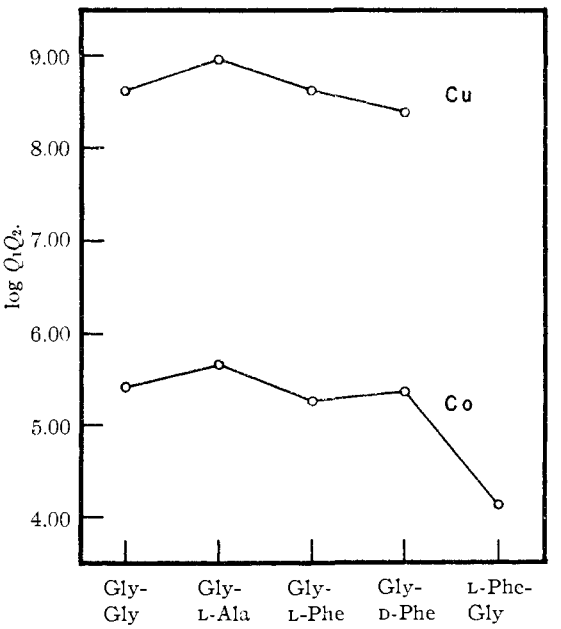


Fig. 2.—Graphical summary of log Q_1Q_2 values *vs.* structure.

Glycyl-L-alanine should show the same pK'_2 value as the glycylphenylalanines since all three peptides have the same structure at the amino end of the molecule. Using pK'_2 = 8.16 for glycyl-L-alanine

gives even higher log Q_1 values than reported in Table II. This suggests that this peptide probably shows some increased complex stability arising from electron release by the methyl group, enhanced in this instance by the positive character of the adjacent peptide nitrogen. The small size of the methyl group does not produce any steric interference in the complex formation.

A similar electronic effect might be expected with the benzyl group particularly under the influence of the positive peptide nitrogen, but the glycylphenylalanines show about the same stability as glycylglycine. A study of Fisher–Hirschfelder scale models shows that the phenyl ring does not directly interfere with chelation at the peptide bond, but when chelation occurs, the bulky phenyl ring is forced into close proximity to other atoms in the molecule. Such steric interaction may offset any electronic influence present.

The over-all stability constants (log Q_1Q_2) given in Table II and summarized in Fig. 2 show the same general trends revealed by the constants involving the uptake of the first peptide molecule, although the calculated differences are greater. This is to be expected if steric factors are important, for the presence of two phenyl groups in these complex structures should increase the steric interferences compared to structures containing only one ligand molecule. Calculations using $pK'_2 = 8.16$ for both glycylphenylalanines give stability constants for the D-isomer of 5.30 with cobalt and 8.58 with copper. The differences recorded in Table II for these peptides thus appear to be the result of reasonable experimental error in the determination of pK'_2 rather than a real variation in complex stabilities.

It is apparent that variations in stability constants of dipeptide–metal ion complexes can be correlated with peptide structures. The variations can to some extent be ascribed to the electron-releasing or withdrawing character of the group attached to the α-carbon, but steric interferences may be more significant when large side chains are present, particularly adjacent to the peptide bond. The results show that the largest changes occur when structural changes significantly alter the basic strength of the terminal amino group. Structural changes adjacent to the peptide bond are much less effective in altering complex stability.

It has been observed that small differences in the amino dissociation constants employed can lead to relatively large and apparently significant variations in computed stability constants. Direct comparison of stability constants must, therefore, be made with extreme caution and with a knowledge of the correctness of the dissociation constants used in the calculations.

SYRACUSE 10, N. Y.

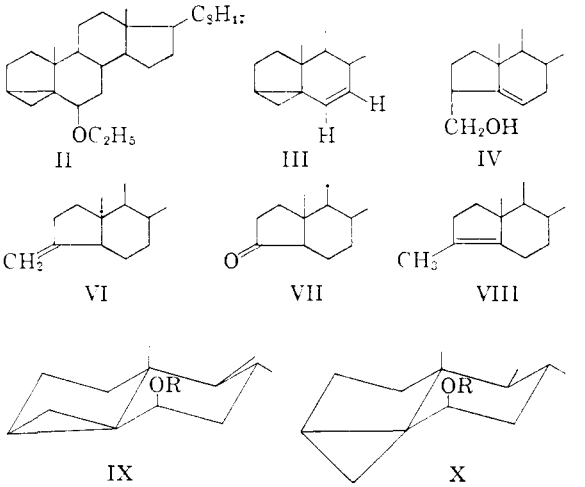
COMMUNICATIONS TO THE EDITOR

PHOTOCHEMICAL TRANSFORMATIONS. V. THE REACTION OF 3,5-CHOLESTADIENE[1,2]

Sir:

The photochemical sensitivity of homoannular dienes is well established,[3-5] and we now wish to report a photochemical induced transformation of a heteroannular diene.

The tetracyclic 3,5-cholestadiene, I ($C_{27}H_{44}$), in ethanol, upon irradiation with a mercury arc yielded 50% of an ethyl ether of a pentacyclic sterol,[6] II, ($C_{29}H_{50}O$, m.p. 104–105°, $[\alpha]^{25}D$ + 18.3°, ϵ_{200} 100, ν_{max} 3030 cm.$^{-1}$). II upon treatment with alumina at room temperature[7] lost one mole of ethanol and gave rise to an olefin, III ($C_{27}H_{44}$, m.p. 109.5–110.2°, $[\alpha]^{25}D$ + 41°, λ_{max} 207 mμ (ϵ 12,000), n.m.r., two vinyl hydrogens) which, in turn, upon reaction with osmium tetroxide yielded a saturated diol (m.p. 132–134°, $[\alpha]^{25}D$ + 52°, ϵ_{200} 100). These data establish in II a cyclopropylcarbinyl ethyl ether grouping such as is found in a 3,5-cyclo-6-ol steroid.



II upon reaction with aqueous acid in acetone yielded a β,γ-unsaturated primary alcohol, IV (m.p. 102–103°, $[\alpha]^{25}D$ −45°, no reaction with MnO_2 in acetone), and Oppenauer oxidation of IV gave rise to an oily α,β-unsaturated aldehyde (ν_{max} 2700, 1668, 1630 cm.$^{-1}$; 2,4-dinitrophenyl-

(1) For paper IV, see W. G. Dauben, K. Koch and W. E. Thiessen, THIS JOURNAL, in press.

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