[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE, MENLO PARK, CALIFORNIA]

Potential Anticancer Agents.[1] XLV. Alkylating Agents Related to Phenylalanine Mustard. III.[2] Synthesis of Phenylpyruvate Mustard

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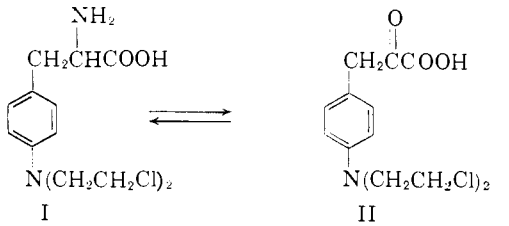
Phenylpyruvate mustard (XII), a nitrogen mustard with a new carrier, has been synthesized in seven steps from *p*-nitrobenzaldehyde *via* the key intermediate, methyl α-benzamido-*p*-[bis-(2-chloroethyl)-amino]-cinnamate (XIV). The benzamido blocking group of XIV was removed by acid-catalyzed methanolysis, then the ester group by short aqueous acid hydrolysis, resulting in a 50% yield of pure phenylpyruvate mustard (XII). Because of the acid instability of XII, the direct conversion of XIV to XII by acid hydrolysis was poor.

The discovery of the interesting anticancer properties of phenylalanine mustard (I)[3,4] has led to continued effort towards synthesis of related compounds[3,5-10] that may have a different tumor spectrum[5,7,10] or may be more efficacious in man. The mechanism whereby phenylalanine mustard (I)



I II

shows a selective action against some tumors compared to their hosts remains a problem. The first step in the catabolism of aromatic amino acids is usually transamination to the corresponding α-keto acid. Jacquez, *et al.*,[11] have observed that phenylalanine transaminase is essentially absent (deleted[12]) in nine out of fifteen rodent tumors but present in all the normal rodent tissues that were examined. Since rats bearing Sarcoma 45 can be cured with phenylalanine mustard (I),[5,7] the observations of Tustanovskiĭ and Ivanova[13] are also pertinent. They noted that the D-amino oxidase in rat tissues could deaminate D-phenylalanine mustard (but not the L-form) and that kidney homogenate had the highest deamination activity of all the tissues measured, in contrast to the Sarcoma 45 homogenate, which showed the lowest.

A possible mechanism for the selective action of phenylalanine mustard (I) against susceptible tumors may be that normal cells are able to convert I to phenylpyruvate mustard (II), an enzymic reaction deleted in many types of tumors.[11] If phenylpyruvate mustard (II) is less toxic than phenylalanine mustard (I), then a selective toxicity to the tumor would result.[14] In order to test this hypothesis, it would be of interest to synthesize phenylpyruvate mustard (II) and determine its toxicity as well as other biological properties.

Phenylpyruvate mustard (II) is also an example of a new type of metabolic carrier[8,15] for the mustard group which may have antitumor activity; II is also a possible exo-alkylating irreversible inhibitor[16] of lactic dehydrogenase, since phenylpyruvate is a good reversible inhibitor[17] of this enzyme. The synthesis of phenylpyruvate mustard (II) is the subject of this paper.

The availability of methyl *p*-nitrophenylpyruvate (III), readily obtained by condensation of *p*-nitrotoluene with methyl oxalate,[18] would appear to make III an attractive starting material for the synthesis of phenylpyruvic acid mustard (XII). However, it would be necessary to find a suitable blocking group for the ketone function in order to carry out the remaining rigors of a nitrogen mustard synthesis. Preliminary attempts to block the ketone

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, *cf.* L. O. Ross, L. Goodman and B. R. Baker, *J. Org. Chem.*, **25**, in press (1960).

(2) For the second paper on phenylalanine mustard, see W. A. Skinner, K. A. Hyde, H. F. Gram and B. R. Baker, *ibid.*, **25**, in press (1960), paper XXXVIII of this series.

(3) F. Bergel, V. C. E. Burnop and J. A. Stock, *J. Chem. Soc.*, 1223 (1955); F. Bergel and J. A. Stock, *ibid.*, 2409 (1954). These authors use the synonym of Merphalan for DL-phenylalanine mustard and Melphalan for L-phenylalanine mustard.

(4) L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaia, O. S. Vasina, V. I. Trusheikina and A. M. Novikova, *Lancet*, **269**, 169 (1955). These authors use the synonym of sarcolysin for DL-phenylalanine mustard.

(5) An excellent, comprehensive review of screening data—some previously unpublished—of compounds in this area can be found in *Cancer Chemotherapy Reports*, No. 6, 61 (1960), published by the Cancer Chemotherapy National Service Center, National Institutes of Health.

(6) (a) T. A. Conners and W. C. J. Ross, *Chem. and Ind.*, **13**, 492 (1960); (b) F. Bergel, J. M. Johnson and J. A. Stock, *ibid.*, **12**, 1487 (1959); (c) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 90 (1959), and previous papers. For a review of the earlier British work, see J. A. Stock in G. E. W. Wolstenholme and C. M. O'Connor, "Amino Acids and Peptides with Antimetabolic Activity," Little, Brown and Co., Boston, Mass., 1958, pp. 89–103.

(7) For a review of recent accomplishments in this area in the U.S.S.R., including screening data on new compounds not previously reported, see L. F. Larionov, *Akad. Med. Nauk Vestnik*, **14**, No. 6, 25 (1959).

(8) H. F. Gram, C. W. Mosher and B. R. Baker, THIS JOURNAL, **81**, 3103 (1959), paper XVII of this series.

(9) T. S. Osdene, D. N. Ward, W. H. Chapman and H. Rakoff, *ibid.*, **81**, 3100 (1959).

(10) J. Scholler, E. Tholen and L. H. Schmidt, *Proc. Am. Assoc. Cancer Research*, **3**, 60 (1959).

(11) J. A. Jacquez, R. K. Barclay and C. C. Stock, *J. Exper. Med.*, **96**, 499 (1952).

(12) V. R. Potter, *Univ. Michigan Med. Bull.*, **23**, 401 (1957).

(13) A. A. Tustanovskiĭ and T. I. Ivanova, *Doklady Akad. Nauk S.S.S.R.*, **122**, 665 (1958); *Chem. Abstr.*, **53**, 2470g (1959); see also reference 5.

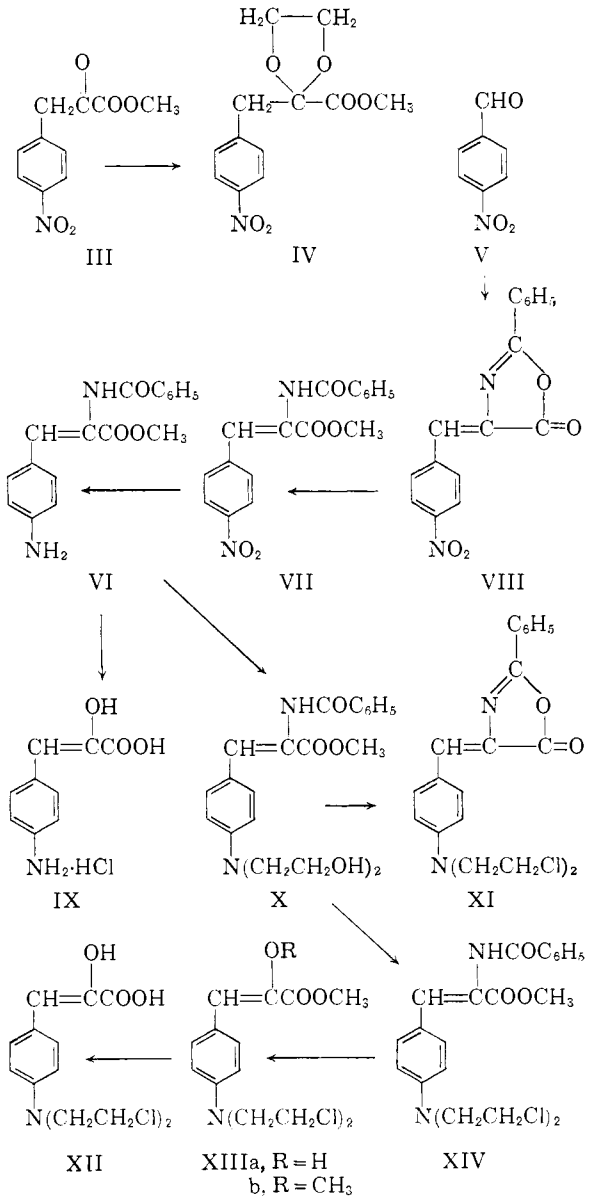
(14) J. A. Jacquez, C. C. Stock and R. K. Barclay, *Cancer*, **6**, 828 (1953), have proposed this principle for taking advantage of an enzyme system deleted in a tumor.

(15) F. Bergel, *N. Y. Acad. Sci.*, **68**, 1238 (1958).

(16) B. R. Baker, *Cancer Chemotherapy Reports*, No. 4, 1 (1959), published by the Cancer Chemotherapy National Service Center, National Institutes of Health.

(17) Unpublished observation made in this Laboratory

(18) W. Wislicenus and E. Thoma, *Ann.*, **436**, 61 (1924).



function of III by conversion to the dioxalane IV were not promising, since at least three components could be detected by paper chromatography. The major crystalline product was not the expected dioxalane IV and remains unidentified; part of the difficulty might be attributed to the completely enolic nature of III, as shown by its chelated carbonyl infrared absorption near 5.9 μ.

Little additional effort was devoted to the dioxalane IV approach, since α-benzamidocinnamic acids such as VII are good precursors of phenylpyruvic acids,[19-22] are easily prepared by the azlactone synthesis[23,24] (such as V to VIII) and are properly blocked for a nitrogen mustard synthesis. The major difficulty that could be anticipated would be the hydrolysis[19-22] of the benzamido blocking group to convert XIV to XII. Basic hydrolysis would not be compatible with the chloroethyl groups and acid hydrolysis might be expected to increase the reported[22] instability of a *p*-dialkylaminophenylpyruvic acid. This step did prove to be the major stumbling block in the synthesis, but was solved by a two-step methanolysis-hydrolysis procedure, as described later.

Condensation of *p*-nitrobenzaldehyde V with hippuric acid afforded the azlactone VIII in near quantitative yield.[25,26] Treatment of VIII with methanol containing a catalytic quantity of sodium methoxide[27] proceeded smoothly to methyl α-benzamido-*p*-nitrocinnamate (VII) in 85–93% yield, m.p. 187–188°.[28] Selective reduction of the nitro group of VII, without concurrent reduction of the side-chain double bond, proceeded smoothly with zinc and ammonium chloride in 90% methanol to give a quantitative yield of crystalline methyl *p*-amino-α-benzamidocinnamate (VI)[29]; this compound gave one spot (R_f 0.60) when paper chromatographed in system A.[30]

In order to establish early in the sequence to XII whether or not conditions for hydrolysis of the benzamido group would be compatible with a phenylpyruvic acid bearing an amine group in the *p*-position, VI was subjected to hydrolysis with hot concentrated hydrochloric acid. The recovery of benzoic acid was quantitative only after a 2.5 hr. hydrolysis period, shorter reaction times leading to incomplete hydrolysis of the benzamido group. After the proper hydrolysis time, *p*-aminophenylpyruvic acid hydrochloride could be isolated pure in 86% yield as yellow crystals. The infrared absorption spectrum clearly indicated that IX was enolic, since a hydroxyl band was present at 2.95 μ. On paper, this compound traveled as a single spot with R_f 0.72 in system A and R_f 0.67 in system B.

Hydroxyethylation of methyl *p*-amino-α-benzamidocinnamate (VI) with ethylene oxide in aqueous acetic acid in the usual manner[3,8] proceeded slower than usual due to the insolubility of VI. However, at the end of 36 hr. at room temperature with stirring, the reaction was complete. Methyl α-benzamido-*p*-[bis-(2-hydroxyethyl)-amino]-cinnamate (X) was obtained in 90% yield as an amorphous solid that could not be crystallized; nevertheless, the compound was analytically pure

(19) E. Erlenmeyer, *ibid.*, **271**, 137 (1892).

(20) Plöchel, *Ber.*, **16**, 2815 (1883).

(21) (a) H. R. Snyder, J. S. Buck and W. S. Ide, "Organic Syntheses," Coll. Vol. II, 333 (1943); (b) R. M. Herbst and D. Shemin, *ibid.*, II, 519 (1943).

(22) L. Hellerman, C. C. Porter, H. J. Lowe and H. F. Koster, THIS JOURNAL, **68**, 1890 (1946).

(23) E. Erlenmeyer, *Ann.*, **275**, 1 (1893).

(24) J. S. Buck and W. S. Ide, "Organic Syntheses," Coll. Vol. II, 55 (1943).

(25) R. L. Douglas and J. M. Guilland, *J. Chem. Soc.*, 2893 (1931).

(26) A. Pedrazzolli, *Helv. Chim. Acta*, **40**, 80 (1957).

(27) G. E. McCasland, R. Horvat, J. Korntvedt and A. Furst, *J. Org. Chem.*, **23**, 1568 (1959), have used this procedure in ethanol to convert VIII to the ethyl ester related to VII.

(28) M. Vanghelovici and A. Stefanescu, *Soc. Chim. România Sect. Sec. române Stiinte*, Bul. Chim. pura apl. [2] **3A**, 159 (1942); *Chem. Abstr.*, **38**, 5501 (1944), used the less satisfactory methanolic sodium hydroxide and recorded a m.p. of 190°.

(29) Pedrazzoli (reference 26) prepared the corresponding ethyl ester by selective hydrogenation of the nitro group with a palladium-on-alumina catalyst at room temperature.

(30) Paper chromatograms were run by the descending technique with benzene–methanol–water (2:6:1) on Schleicher and Schuell No. 2495 acetylated paper (system A)[31] or on Whatman No. 1 paper (system B). The compounds were detected by their ultraviolet absorption.

(31) T. Wieland and W. Kracht, *Angew. Chem.*, **69**, 172 (1957).

and traveled as a single spot (R_f 0.79) when paper chromatographed with system A.

Treatment of X with phosphorus oxychloride at the boiling point not only converted the hydroxyethyl group to chloroethyl but yielded XI by elimination of methanol with regeneration of the azlactone function. This red, crystalline compound was difficult to obtain pure without considerable loss; however, it could be obtained pure as a toluene solvate and was homogeneous (R_f 0.24) on paper with system A.[30] After considerable investigation of reaction times and temperatures, solvents and chlorinating agents, suitable conditions were established for avoiding appreciable azlactone and tar formation and for obtaining the optimum yield of the methyl ester XIV. When X was heated in phosphorus oxychloride at 70–75° for 30 minutes, a 45% yield of pure, crystalline XIV was obtained which was homogeneous (R_f 0.63) on paper with system A[30] and free of XI (R_f 0.24).

Even though the hydrolysis of methyl *p*-amino-α-benzamidocinnamate (VI) to *p*-aminophenylpyruvic acid hydrochloride (IX) proceeded in excellent yield, considerable difficulty with the hydrolysis of XIV to phenylpyruvate mustard was experienced, as anticipated earlier. A check of the time in boiling 12 *N* hydrochloric acid necessary for removal of the benzamido group of XIV showed that 2 hr. were required—similar to the case with VI→IX. Unfortunately, a great deal of decarboxylation took place in this reaction time, resulting primarily in condensation products of *p*-[bis-(2-chloroethyl)-amino]-phenylacetaldehyde.

This difference in stability of the phenylpyruvic acid substituted in the *p*-position with a primary amino group (IX) compared to a *t*-amino group (XII) is remarkable. In one run, an 18% yield of the desired phenylpyruvate mustard XII could be isolated in nearly pure form, m.p. 137–140°, but the procedure could not be duplicated. With shorter hydrolysis times, the intermediate α-benzamido-*p*-[bis-(2-chloroethyl)-amino]-cinnamic acid could be isolated. Since the ester group of XIV could be removed under milder conditions than the benzamido, as would be expected, and since decarboxylation should not take place if the ester group is intact, conditions were sought to remove the benzamido group selectively with retention of the ester function to form XIII; the sequence could then be completed by a short aqueous acid hydrolysis in order to hold decarboxylation to a minimum. Methanolysis of the benzamido group proceeded slowly—but effectively—in boiling methanolic hydrogen chloride, reaction being complete in 18 hr. The resultant product was free of the benzamido group, as shown by its infrared absorption spectrum. Although the compound was not characterized, its spectrum indicated that it was probably the enol ether XIII rather than the enol, since a normal ester carbonyl was present at 5.72 μ; a chelated enol ester (XIIIa) should have a carbonyl band near 5.9 μ. The lack of a color with ferric chloride also indicated the ether structure (XIIIb). The crude enol ether XIIIb, without further purification, was hydrolyzed further by 12 *N* hydrochloric acid to give a 70% yield of XII, m.p. 137–139°, comparable in purity to the sample obtained by direct hydrolysis of XIV. The compound (XII) was readily purified to yellow crystals, m.p. 154–155°, in 50% over-all yield. In common with *p*-aminophenylpyruvic acid hydrochloride (IX), XII was enolic, as shown by its infrared spectrum.

Experimental[32]

Methyl *p*-Amino-α-benzamidocinnamate (VI).—To a mixture of 35.7 g. (0.112 mole) of methyl α-benzamido-*p*-nitrocinnamate (VII),[27,28] 12.6 g. of ammonium chloride, 100 ml. of water and 1 l. of methanol was added 72 g. of zinc dust. The reaction commenced at room temperature and VII dissolved in 15 minutes. The mixture was then refluxed for 1 hr., filtered hot through a Celite[33] pad and the filtrate evaporated to dryness *in vacuo*. Trituration of the residue with water gave 34.0 g. (96%) of product as a hydrate melting at 90–98°, resolidifying and remelting at 165–170°. This material was suitable for the next step. Recrystallization of a sample from absolute ethanol–petroleum ether (b.p. 30–60°) gave orange-yellow crystals of a monoethanolate, m.p. 90–100°, resolidifying and remelting at 173–175°; $\lambda_{max(\mu)}^{Nujol}$ 2.92, 2.99, 9.50 (COH of ethanol); 3.04, 3.09 (NH); 5.91, 8.46 (ester); 6.05 (amide C=O); 12.17 (*p*-disubstituted benzene); 14.12 (benzoyl). The compound traveled on paper as a single spot (R_f 0.60) in system A.[30]

Anal. Calcd. for $C_{17}H_{16}N_2O_3 \cdot C_2H_5OH$: C, 66.7; H, 6.48; N, 8.18. Found: C, 67.0; H, 6.80; N, 8.40.

***p*-Aminophenylpyruvic Acid Hydrochloride (IX).**—A solution of 2.0 g. (7.0 mmoles) of methyl *p*-amino-α-benzamidocinnamate (VI) monohydrate in 70 ml. of 12 *N* hydrochloric acid was refluxed for 2.5 hr. The solution was extracted with ether (2 × 100 ml.), giving an 86% yield of benzoic acid. The aqueous solution was cooled to about −10° for 2 hr. The product was collected on a glass filter and washed with 12 *N* hydrochloric acid; yield, 1.4 g. (93%) of reddish-tan crystals. The product was dissolved in ethanol, treated with Norit and the filtrate evaporated to dryness *in vacuo*, giving 1.3 g. (86%) of light yellow crystals, m.p. >300°; $\lambda_{max(\mu)}^{Nujol}$ 2.95 (OH); 3.5–4.0, 5.87, 7.00, 8.20 (COOH); 3.90 (NH_3^+); 12.14 (*p*-disubstituted benzene); The compound traveled on paper at R_f 0.72 in system A[30] with a trace component at R_f 0.87 and in system B[30] at R_f 0.67 with the trace component at R_f 0.76.

Anal. Calcd. for $C_9H_9NO_3 \cdot HCl$: C, 50.2; H, 4.67; Cl, 16.5; N, 6.48. Found: C, 50.1; H, 4.84; Cl, 16.5; N, 6.20.

Methyl α-Benzamido-*p*-[bis-(2-hydroxyethyl)-amino]-cinnamate (X).—To a stirred mixture of 6.0 g. (0.019 mole) of methyl *p*-amino-α-benzamidocinnamate (VI) monohydrate, 56 ml. of water and 30 ml. of acetic acid cooled in an ice-bath was added 16 ml. of ethylene oxide. The air was displaced with nitrogen and the mixture was magnetically stirred at room temperature in the loosely stoppered flask for 36 hr. VI dissolved after about 20 hr. The solution was neutralized with sodium bicarbonate, then extracted with methylene chloride (2 × 125 ml.). The combined extracts were washed with water (2 × 200 ml.), dried with magnesium sulfate, then evaporated to dryness *in vacuo;* yield, 6.6 g. (90%) of an amorphous, yellow solid, m.p. 68–72°, which could not be crystallized and had $\lambda_{max(\mu)}^{Nujol}$ 2.80–3.20 (OH, NH); 5.82, 8.42 (ester); 6.02 (amide C=O); 12.25 (*p*-disubstituted phenyl); 14.0 (benzoyl). The compound traveled on paper as a single spot (R_f 0.79) in system A.[30]

Anal. Calcd. for $C_{21}H_{24}N_2O_6$: C, 65.6; H, 6.29; N, 7.29. Found: C, 65.3; H, 6.37; N, 7.27.

The 4-{*p*-[Bis-(2-chloroethyl)-amino]-benzylidene}-2-phenyl-2-oxazolin-5-one (XI).—A solution of 1.0 (2.6 mmoles) of methyl α-benzamido-*p*-[bis-(2-hydroxyethyl)-amino]-cinnamate (X) in 7.5 ml. of phosphorus oxychloride was refluxed for 10 minutes, then the deep red solution was poured on ice and stirred well for 30 minutes. The aqueous mixture was extracted with chloroform. The extracts were

(32) Melting points were taken on a Fisher–Johns block and are uncorrected.

(33) Johns-Manville Co. diatomaceous earth.

washed with 100 ml. of water, dried over magnesium sulfate and evaporated to dryness *in vacuo*. The dark metallic-colored hydrochloride (0.7 g.) was dissolved in methylene chloride and stirred with 2 g. of anhydrous sodium acetate until the dark metallic color of the solution changed to red (about 1 hr). The filtered solution was evaporated to dryness *in vacuo*, leaving 0.60 g. (59%) of red crystals, m.p. 115–120°. Recrystallization from toluene–petroleum ether (b.p. 30–60°) gave red crystals (about 50% recovery) as a toluene solvate, m.p. 60–70°, resolidifying and remelting at 139–141°; $\lambda_{max(\mu)}^{Nujol}$ 5.60 (azlactone C=O); 6.20, 6.29 (C=C, C=N, aryl); 12.2 (*p*-disubstituted benzene); 13.2 (monosubstituted benzene of toluene). The compound traveled as a single spot (R_f 0.24) in system A,[30] giving an intense yellow fluorescence under ultraviolet light.

Anal. Calcd. for $C_{30}H_{18}Cl_2N_2O_2 \cdot 1/2\ C_7H_8$: C, 64.9; H, 5.06; Cl, 16.3. Found: C, 65.1; H, 5.38; Cl, 16.2.

A sample dried at 100° (0.5 mm.) then melted at 139–141° and had the analysis:

Anal. Calcd. for $C_{20}H_{18}Cl_2N_2O_2$: C, 61.7; H, 4.67; Cl, 18.3; N, 7.20. Found: C, 61.6; H, 4.82; Cl, 18.3; N, 7.12.

Methyl α-Benzamido-*p*-[bis-(2-chloroethyl)-amino]-cinnamate (XIV).—A solution of 10.0 g. (0.026 mole) of methyl α-benzamido-*p*-[bis-(2-hydroxyethyl)-amino]-cinnamate (X) in 60 ml. of freshly distilled phosphorus oxychloride was heated in an oil-bath at 70–75° for 30 minutes. The red solution was poured into excess crushed ice and stirred for 15 minutes. The mixture was extracted with methylene chloride (2 × 150 ml.). The combined extracts were thoroughly shaken with 50 ml. of saturated aqueous sodium acetate, then dried with magnesium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in 100 ml. of methylene chloride, then clarified with Norit. Addition of 100 ml. of ether and 25 ml. of petroleum ether (b.p. 30–60°) followed by cooling in an acetone-Dry Ice-bath gave 4.9 g. (45%) of light yellow needles, m.p. 138–139°; $\lambda_{max(\mu)}^{Nujol}$ 3.10 (NH); 5.82, 8.32, 8.45 (ester); 6.04, 6.58 (amide); 12.2 (*p*-disubstituted benzene); 13.8 (C–Cl); 14.1 (benzoyl); no COH near 2.9 and 9.5; no azlactone C=O near 5.6. The compound traveled on paper as a single spot (R_f 0.63) in system A.[30]

Anal. Calcd. for $C_{21}H_{22}Cl_2N_2O_3$: C, 59.9; H, 5.28; Cl, 16.9; N, 6.66. Found: C, 60.0; H, 5.35; Cl, 16.8; N, 6.66.

***p*-[Bis-(2-chloroethyl)-amino]-phenylpyruvic Acid (XII).**—To 30 ml. of reagent methanol, saturated with hydrogen chloride at 5°, was added 1.0 g. (2.4 mmoles) of methyl α-benzamido-*p*-[bis-(2-chloroethyl)-amino]-cinnamate (XIV). The solution was refluxed for 18 hr. protected from moisture, then evaporated to dryness *in vacuo*. The crude residue (XIIIb) gave no color with ferric chloride and showed a normal ester carbonyl at 5.73 μ in the infrared.

A solution of the intermediate XIIIb in 25 ml. of 12 *N* hydrochloric acid was heated on the steam-bath for 20 minutes, then quickly chilled to about −10°. The cold mixture was washed with ether (3 × 20 ml.) to remove benzoic acid. The aqueous solution was neutralized with solid sodium acetate, diluted with about two volumes of water and extracted with ether. Dried with magnesium sulfate, the ether solution was diluted with 50 ml. of toluene, then evaporated to dryness *in vacuo*, leaving 0.7 g. (97%) of a green oil that solidified on cooling. A solution of the crude product in 15 ml. of ethyl acetate was diluted with 75 ml. of petroleum ether (b.p. 30–60°). The clear solution was decanted from the dark green gum that separated, then evaporated *in vacuo*, leaving a light green solid. Recrystallization from methylene chloride–petroleum ether (b.p. 30–60°) gave 0.50 g. (70%) of light green crystals, m.p. 137–139°. Further recrystallization from methylene chloride afforded 0.40 g. (55%) of yellow crystals, m.p. 154–155°; $\lambda_{max(\mu)}^{Nujol}$ 2.93 (enolic OH); 3.5–4.5 (carboxyl OH); 6.07 (chelated C=O and COOH); 6.22, 6.55 (aryl); 8.15, 8.42 (COOH); 12.32 (*p*-disubstituted phenyl); free of alcohol C-OH near 9.5.

Anal. Calcd. for $C_{13}H_{15}Cl_2NO_3$: C, 51.3; H, 4.97; Cl, 23.4; N, 4.61. Found: C, 51.4; H, 5.26; Cl, 23.8; N, 4.79.

No suitable solvent for paper chromatography could be found that gave consistent results, although the best results were obtained with system A; in most runs the compound streaked. Occasionally a paper chromatogram was obtained without streaking, in which case the compound moved as a single spot with R_f 0.81.

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[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA, CHARLOTTESVILLE, VA.]

The Association of Divalent Cations with Anserine[1]

BY R. BRUCE MARTIN

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The substitution of a methyl group in the 1-position of the imidazole ring of carnosine to yield anserine does not alter the chelating properties with cupric ion. However, the tendency to chelate with nickel ion is reduced by methylation. For both dipeptides the ionization of the amide hydrogen is induced by either metal ion at about neutral *p*H values. Plausible structures of the chelate compounds are discussed.

A recent study[2] reported the association of divalent copper, nickel and zinc ions with L-carnosine (β-alanyl-L-histidine). The first two ions promoted the ionization of the amide hydrogen at physiological *p*H values or less. The recent availability of L-anserine (β-alanyl-L-1-methylhistidine) makes possible a test of the proposals which have been made[2,3] for the association and subsequent ionization of the chelate complexes formed. This paper describes the results of titrating mixtures of divalent cations and anserine with standard base. A comparison is made with the results obtained from similar titrations with carnosine.

Experimental

The techniques have previously been described.[3] Carnosine and anserine nitrate were products of the California Corporation for Biochemical Research.

Results and Discussion

The results of the titrations of mixtures of copper(II) or nickel(II) ions with anserine and, for comparison, with carnosine are shown in Fig. 1. The curves for copper(II) ion are nearly identical for both peptides. In the case of nickel(II) ion the curve for anserine is displaced to higher *p*H values indicating a weaker interaction.

(1) This research was supported by a grant from the National Science Foundation (G9796).

(2) R. B. Martin and J. T. Edsall, THIS JOURNAL, **82**, 1107 (1960).

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