30 cm.$^{-1}$) and might cancel each other, so that the observed isotope effect must arise elsewhere. The stretching frequencies of carbon–hydrogen bonds in methylene groups are the same in cyclopentanone and cyclopentanol, but the bending frequencies are about 1410 cm.$^{-1}$ in the ketone and 1438 cm.$^{-1}$ in the alcohol. The net frequency shift of $4 \times 28 =$ 112 cm.$^{-1}$ is enough to account for 70% of the observed isotope effect.

The comparison of relative frequencies of normal and deuterated compounds is possible in a few cases, among them acetone.[23] One of the striking observations is that the skeletal vibrations in acetone change markedly on deuterium substitution. Below 1000 cm.$^{-1}$, for example, frequencies at 906, 788, 530, 488 and 391 cm.$^{-1}$, ascribed to CH_3 rocking, C–C stretching, C—C=O bending (2) and C–C–C bending[24] are replaced by frequencies at 894, 700, 483, 413 and 335 cm.$^{-1}$. The skeletal vibrations in the hemiketal would be expected to change even more, from the arguments given above, but in the absence of definite information on the vibrational assignments for normal and deuterated alcohol, it is not possible to make quantitative calculations relating frequencies and the isotope effect.

In conclusion it may be said that it has been possible to make semi-quantitative correlations of the isotope effect in reactions of carbonyl compounds with the frequency changes in reactants and products, caused by isotopic substitution. A quantitative treatment of these frequency shifts is not possible at the present time, but qualitatively they seem to be directly attributable to the effect of changes in mass. Consequently, it appears that the observed secondary deuterium isotope effects in carbonyl compounds may be considered solely in terms of mass effects on vibrational frequencies.

(24) R. E. Pennington and K. A. Kobe, THIS JOURNAL, **79**, 300 (1957).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE UNIVERSITY, AMES, IOWA]

Alkylation of Carbon–Metallic Compounds with Trialkyl Phosphates

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The reactions of several sterically hindered Grignard reagents and organolithium compounds with trialkyl phosphate esters were studied. These reactions were found to proceed *via* nucleophilic displacements from carbon (alkylation), in contrast to earlier work with less hindered organometallic compounds which attack the ester at phosphorus. Some of the factors which may determine the position of attack in these reactions are discussed.

Recently we reported the reactions of trialkyl[1,2] and triaryl[3] phosphate esters with triphenylsilyllithium in tetrahydrofuran. The alkyl esters were found to react in a 1:1 mole ratio with this silylmetallic compound *via* nucleophilic displacements of oxygen from carbon, giving alkyltriphenylsilanes in high yields.[2] The use of three equivalents of triphenylsilyllithium and forced conditions in reactions with tri-*n*-butyl phosphate, however, gave only *ca.* 50% of the alkylation product, *n*-butyltriphenylsilane.[2] These reactions also afforded varying amounts of hexaphenyldisilane, hexaphenyldisiloxane, 4-triphenylsilylbutanol and triphenylsilanol. A mechanism involving a nucleophilic displacement of oxygen from phosphorus followed by a cleavage of the silicon–phosphorus bond was proposed to account for the formation of the disilane and the triphenylsilanol isolated.[2]

The reactions of triaryl phosphates with triphenylsilyllithium, likewise, were found to proceed *via* displacements from phosphorus. Here also, hexaphenyldisilane and triphenylsilanol were the major silicon-containing products of reaction. The phenol derived from the ester used was also found to be among the products of reaction.

Previously, esters of this type having the P–O–R linkage had only been observed to undergo attack at the central phosphorus atom when treated with either Grignard reagents or organolithium compounds.[4] Esters of other inorganic acids, except sulfates and sulfonates, also react with organometallic compounds *via* attack at the central element.[4b,c,5] The use of alkyl sulfates and sulfonates as alkylating agents for R–M compounds, on the other hand, is well known, and extensive use has been made of this reaction to prepare alkyl derivatives from Grignard reagents.[5]

Other nucleophiles besides Grignard reagents have been observed to show specificity as to the position of attack on phosphorus esters. For example, lithium chloride in ethoxyethanol solution reacts with tribenzyl phosphate to give benzyl chloride, nucleophilic attack by chloride ion having occurred on carbon.[6] Hydroxide ion, on the other hand, has been shown to react with alkyl phosphates and phosphonates *via* displacements from

(1) M. V. George, B. J. Gaj and H. Gilman, *J. Org. Chem.*, **24**, 624 (1959).

(2) H. Gilman and B. J. Gaj, *ibid.*, in press.

(3) H. Gilman and B. J. Gaj, *ibid.*, in press.

(4) See, for example (a) A. Michaelis and F. Wegner, *Ber.*, **48**, 316 (1915); (b) H. Gilman and C. C. Vernon, THIS JOURNAL, **48**, 1063 (1926); (c) H. Gilman and J. Robinson, *Rec. trav. chim.*, **48**, 328 (1929); (d) G. M. Kosolapoff, THIS JOURNAL, **72**, 5508 (1950); (e) A. Burger and N. D. Dawson, *J. Org. Chem.*, **16**, 1250 (1951); (f) G. M. Kosolapoff and R. M. Watson, THIS JOURNAL, **73**, 4101 (1951); (g) P. W. Morgan and B. C. Herr, *ibid.*, **74**, 4526 (1952); (h) R. H. Williams and L. A. Hamilton, *ibid.*, **74**, 5418 (1952); (i) N. D. Dawson and A. Burger, *J. Org. Chem.*, **18**, 207 (1953); (j) M. H. Maguire and G. Shaw, *J. Chem. Soc.*, 2039 (1955); (k) R. H. Williams and L. A. Hamilton, THIS JOURNAL, **77**, 3411 (1955); (l) R. C. Miller, J. S. Bradley and L. A. Hamilton, *ibid.*, **78**, 5299 (1956); (m) R. C. Miller, C. D. Miller, Wm. Rogers, Jr., and L. A. Hamilton, *ibid.*, **79**, 424 (1957); (n) B. B. Hunt and B. C. Saunders, *J. Chem. Soc.*, 2413 (1957); (o) J. L. Willans, *Chemistry & Industry*, 235 (1957); (p) M. Janczewski, *Roczniki Chem.*, **33**, 185 (1959).

(5) See M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Nonmetallic Substances," Prentice-Hall, Inc., New York, N. Y., 1954.

(6) V. M. Clark and A. R. Todd, *J. Chem. Soc.*, 2030 (1950).

phosphorus.[7] Hudson and Harper[7] used kinetic measurements to correlate the basicity of the nucleophile with the position of anionic attack. They suggested that only the more basic anions can remove alkoxyl groups from phosphorus esters *via* nucleophilic displacements from the phosphorus atom, whereas anions of strong acids preferentially attack carbon. Since organometallic compounds are decidedly more basic than either hydroxyl or chloride ions, attack by the former on compounds of this type should always occur at the phosphorus atom, providing the basicity of the nucleophile were the only factor involved.

In our earlier investigation,[2] we proposed the idea that steric requirements of the triphenylsilyl group were largely responsible for the occurrence of attack on carbon in reactions of triphenylsilyllithium with trialkyl phosphates. It was not unreasonable to assume that the large size of the triphenylsilyl group prevented its attack at the central phosphorus atom; accordingly the less favored displacement from carbon took place. The present investigation lends some support to the importance of size in the organometallic compound; however, the steric requirements and reactivity of the ester also appear to be important in determining the position of nucleophilic attack in these esters. Otherwise, a given organometallic compound, if alkylated by one ester, should be alkylated by all other trialkyl esters.

This was found not to be the case in the reactions employing mesitylmagnesium bromide. Although alkylation occurred when this Grignard reagent was treated with trimethyl phosphate, three attempts to accomplish this with tri-*n*-butyl phosphate in diethyl ether or tetrahydrofuran failed, and high recoveries of the ester were realized. The even more reactive mesityllithium also failed to undergo alkylation with this ester in a mixture of diethyl ether and tetrahydrofuran under stringent conditions. Apparently the steric requirements of both the organometallic compound and ester, and/or their reactivities were responsible for the failure to observe attack at either carbon or phosphorus. However, the limited experimental data available do not permit generalizations as to the relative importance of these factors.

In this study, several relatively hindered Grignard reagents and organolithium compounds were treated with trimethyl, tri-*n*-butyl or triisobutyl phosphate in diethyl ether or tetrahydrofuran. In all cases tested, except for the reaction of mesitylmagnesium bromide or mesityllithium with the *n*-butyl ester, attack on carbon occurred to give the alkylation products. The yields in most of these reactions were quite satisfactory

The size of the organometallic group was reduced from that of the triphenylmethyl group to that of the mesityl group, with the desire to establish the relative amount of hindrance necessary in the organometallic compound to obtain alkylation products. Since Willans[4o] had previously observed attack on phosphorus in the reaction of *o*-anisyllithium with diethyl phosphite, the mechanistic change, if due to steric requirements of the organometallic compound alone, ought to occur with an organometallic reagent more hindered than *o*-anisyllithium, but less hindered than triphenylsilyllithium.

To eliminate the possibility of the solvent effect due to the use of tetrahydrofuran, several of these reactions were carried out in diethyl ether. Both triphenylmethyllithium and triphenylmethylmagnesium chloride were alkylated with trimethyl phosphate in yields of 86.5 and 77%, respectively. The product, 1,1,1-triphenylethane, was identified by mixed melting point and infrared spectrum. Similarly, this ester, when treated in a 1:1 mole ratio with 9-phenyl-9-fluorenyllithium (in tetrahydrofuran), diphenylmethyllithium and mesityllithium, gave the corresponding alkylation products in yields of 88, 80.5 and 39.1%.

The use of tri-*n*-butyl phosphate gave similar results with triphenylmethyllithium, 9-phenyl-9-fluorenyllithium and diphenylmethyllithium. 1,1,-1-Triphenylpentane (77.5%), 9-*n*-butyl-9-phenylfluorene (94%) and 1,1-diphenylpentane (74.5%) were the products isolated. Triisobutyl phosphate was also used to alkylate 9-phenyl-9-fluorenyllithium in tetrahydrofuran. From two reactions 9-isobutyl-9-phenylfluorene was isolated in low yields. These reactions also gave a hydrocarbon whose infrared spectrum and analysis indicate it to be composed of two 9-phenylfluorenyl groups and one isobutyl group. The infrared spectrum was nearly identical with those of the 9-methyl and 9-butyl derivatives except for the intensity of absorption due to aliphatic C–H. Its structure, however, is not known.

All of the reactions employing mesitylmagnesium bromide or the corresponding organolithium compound gave varying amounts of mesitylene, even when the reactions were terminated by carbonation. Its mode of formation is not known, since the organometallic compounds appeared to be formed in high yields; freshly distilled esters and anhydrous solvents were employed, and care was taken to exclude moisture from the reaction mixtures. In this connection, it is surprising that the attempted reaction of tri-*n*-butyl phosphate with mesitylmagnesium bromide in tetrahydrofuran gave a considerably higher yield of β-isodurylic acid than did the corresponding reactions in diethyl ether, particularly since organolithium compounds are generally more stable in diethyl ether than in tetrahydrofuran.[8] A 65.5% yield of this acid was realized after refluxing a mixture of mesitylmagnesium bromide and tri-*n*-butyl phosphate in tetrahydrofuran for three days, whereas the corresponding reactions in diethyl ether gave this acid in yields of *ca.* 40%.

The results of these experiments indicate that trialkyl phosphate esters can be used to alkylate organometallic compounds, providing the latter are sufficiently hindered and reactive. The reaction seems to be best suited for the alkylation of more reactive organometallic compounds such as organosilyllithium reagents,[2] since yields are

(7) R. F. Hudson and D. C. Harper, *J. Chem. Soc.*, 1356 (1958).

(8) H. Gilman, A. H. Haubein and H. Hartzfeld, *J. Org. Chem.*, **19**, 1034 (1954); H. Gilman and B. J. Gaj, *ibid.*, **22**, 1165 (1957).

TABLE I

ALKYLATION OF ORGANOMETALLIC COMPOUNDS WITH TRIALKYL PHOSPHATES[a]

Compound alkylated (mole)	R of R_3PO_4	Solvent	Yield of alkylation product, %	M.p. or b.p., °C. (mm.)	$n^{20}D$
Triphenylmethylmagnesium chloride[b] (0.03)	CH_3	Et_2O	77	93.5–95	.....
Triphenylmethyllithium (0.02)	n-C_4H_9	Et_2O	77.5	60–61[c]	.....
9-Phenyl-9-fluorenyllithium (0.02)	CH_3	THF[d]	88	84–85[e]	.....
9-Phenyl-9-fluorenyllithium (0.02)	n-C_4H_9	THF[d]	94	98–99[f]	.....
Diphenylmethyllithium[g] (0.07)	CH_3	Et_2O	80.5	136–137 (11)[h]	1.5730[h]
Diphenylmethyllithium[g] (0.07)	n-C_4H_9	Et_2O	74.5	80–81 (0.005)[i]	1.5510[i]
Mesitylmagnesium bromide (0.03)	CH_3	Et_2O	39.1[j]	193 (atm.)	1.5125

[a] Reactions described in the Experimental section have been omitted from the table. [b] Prepared according to the directions of H. Gilman and E. A. Zoellner, THIS JOURNAL, **51**, 3493 (1929). [c] K. Ziegler and L. Jacob, *Ann.*, **511**, 45 (1934), report a m.p. of 60–61°. [d] Abbreviation for tetrahydrofuran. [e] E. Bergmann and A. Bond, *Ber.*, **64B**, 1455 (1931), report a m.p. of 84–85°. [f] *Anal.* Calcd. for $C_{23}H_{22}$: C, 92.58; H, 7.42. Found: C, 92.45, 92.54; H, 7.53, 7.41. [g] Prepared by metalation of diphenylmethane with n-propyllithium. [h] P. Sabatier and M. Murat, *Compt. rend.*, **154**, 1772 (1912), report: b.p. 136° (12.5 mm.), $n^{21}D$ 1.573. [i] K. T. Serijan and P. H. Wise, THIS JOURNAL, **73**, 5191 (1951), report: b.p. 307.8°, $n^{20}D$ 1.5510. [j] Mesitylene (30%), b.p. 165°, $n^{20}D$ 1.4992, was also isolated after hydrolysis.

TABLE II

ATTEMPTED ALKYLATIONS WITH TRI-n-BUTYL PHOSPHATE (1:1)[a]

Organometallic compound (mole)	Solvent	Hr. at reflux	Products (%)
Mesitylmagnesium bromide[b] (0.1)	Et_2O	24	β-Isodurylic acid[c] (44.2), mesitylene (9.6), recovered ester (86)
Mesitylmagnesium bromide[b] (0.1)	Et_2O	72	β-Isodurylic acid[c] (43.3), mesitylene (19.3), recovered ester (86)
Mesityllithium[d] (0.1)	Et_2O–THF	18	Mesitylene (73), recovered ester (42), polymeric distillation residue

[a] Reactions described in the Experimental section are omitted from the table. [b] Reaction terminated by carbonation. [c] M.p. 152–154°. [d] Prepared directly from 2-bromomesitylene and lithium in tetrahydrofuran; reaction terminated by hydrolysis.

generally better with these compounds than with carbon–metallic types.[9]

Experimental[10]

The reactions described below illustrate the procedure used. Other reactions are listed in Table I.

Reaction of Triphenylmethyllithium with Trimethyl Phosphate.—The triphenylmethyllithium was prepared by a modification of a reported[11] procedure. A mixture of 8.36 g. (0.03 mole) of triphenylmethyl chloride, excess lithium and 60 ml. of diethyl ether was refluxed with stirring for 2 days. The suspension was filtered through a stopcock (to remove the excess metal) into a solution of 4.2 g. (0.03 mole) of trimethyl phosphate in 50 ml. of diethyl ether. Reaction was instantaneous as evidenced by the rapid loss of color of the organolithium compound.

Work-up by hydrolysis, separation of the layers, drying of the organic layer over sodium sulfate and removal of the solvent left an oily solid. Two crystallizations of this residue from ethanol gave 6.7 g. (86.5%) of 1,1,1-triphenylethane, m.p. 93–95°. The compound was identified by a mixed melting point determination and by a comparison of its infrared spectrum, as a carbon disulfide solution, with that of an authentic sample.

Reaction of 9-Phenyl-9-fluorenyllithium with Tri-n-butyl Phosphate.—The 9-phenyl-9-fluorenyllithium was prepared from 9-phenylfluorene by a modification of a reported[12] procedure. A mixture of 4.84 g. (0.02 mole) of 9-phenylfluorene, 0.7 g. (0.1 g.-atom) of lithium wire, cut into five pieces, and 50 ml. of tetrahydrofuran was stirred without cooling for 24 hr. The darkly colored solution was filtered through a stopcock into an addition funnel, and then added to a stirred solution of 5.32 g. (0.02 mole) of tri-n-butyl phosphate in 30 ml. of the same solvent. No appreciable heat of reaction was observed, nor was the color of the organolithium reagent discharged; hence the solution was refluxed for 2 hr. (no color change), then hydrolyzed with water.

Separation of the layers after adding 50 ml. of diethyl ether, and removal of the solvent from the dried organic layer left a brown viscous oil, which was poured on a column of dry alumina. The solid eluted with petroleum ether (b.p. 60–70°) was recrystallized from ethanol to give 5.62 g. (94%) of 9-n-butyl-9-phenylfluorene, m.p. 98–99°.

Anal. Calcd. for $C_{23}H_{22}$: C, 92.58; H, 7.42. Found: C, 92.45, 92.54; H, 7.53, 7.41.

Reaction of 9-Phenyl-9-fluorenyllithium with Triisobutyl Phosphate.—A solution of 0.02 mole of 9-phenyl-9-fluorenyllithium in 50 ml. of tetrahydrofuran was added to a stirred solution of 5.32 g. (0.02 mole) of triisobutyl phosphate[13] in 25 ml. of the same solvent. The mixture was stirred at room temperature for 24 hr., carbonated and hydrolyzed. The organic layer was extracted with 10% sodium hydroxide and the basic extract was boiled to convert the salt of the acid to 9-phenylfluorene.[14]

The solid was filtered, washed with water and crystallized from ethanol to give 0.7 g. (14.5%) of 9-phenylfluorene, m.p. and mixed m.p. 145–146°.

The solvents were removed from the dried, darkly colored organic layer and the residue was passed through a column of alumina. The material eluted with petroleum ether (b.p. 60–70°) was boiled with 50 ml. of ethanol and filtered hot to remove 0.25 g. of a hydrocarbon, m.p. 208–209° (after recrystallization from a benzene–ethanol pair). The structure of this product is unknown; however, the analysis and infrared spectrum indicate it to be composed of two 9-phenylfluorenyl groups and one isobutyl group.

Anal. Calcd. for $C_{42}H_{34}$: C, 93.62; H, 6.38. Found: C, 93.53, 93.50; H, 6.43, 6.53.

The ethanolic filtrate gave, after two additional crystallizations from the same solvent, 1.7 g. (28.5%) of 9-isobutyl-9-phenylfluorene, m.p. 94–95°.

Anal. Calcd. for $C_{23}H_{22}$: C, 92.58; H, 7.42. Found: C, 92.59, 92.65; H, 7.64, 7.48.

No other pure compounds could be identified from the benzene, ethyl acetate and methanol eluates, which are colored, viscous oils.

(9) For a discussion concerning the relative reactivities of silyl-metallic compounds as compared to carbon–metallic types, see D. Wittenberg and H. Gilman, *Quart. Revs.*, **113**, 116 (1959); D. Wittenberg and H. Gilman, *J. Org. Chem.*, in press.

(10) Melting and boiling points are uncorrected. All reactions were carried out in oven-dried glassware under an atmosphere of dry, oxygen-free nitrogen. The diethyl ether used as solvent for the preparation and reactions of organometallic compounds was sodium-dried. The tetrahydrofuran was freshly distilled from a suspension of lithium aluminum hydride under dry nitrogen. The phosphate esters were freshly distilled before use.

(11) P. Tomboulain, *J. Org. Chem.*, **24**, 229 (1959).

(12) H. Gilman and R. D. Gorsich, *ibid.*, **23**, 550 (1958).

(13) Kindly supplied by Dr. J. B. Dickey of the Tennessee Eastman Co., Kingsport, Tenn.

(14) W. Schlenk and E. Bergmann, *Ann.*, **463**, 203 (1928).

A repeat reaction using the same quantities of reactants and work-up gave 0.6 g. of the unidentified hydrocarbon, m.p. 209–210°, and 1.6 g. of the 9-isobutyl derivative, m.p. 94–95°.

Reaction of Mesitylmagnesium Bromide with Tri-*n*-butyl Phosphate (Attempted).—A mixture of 0.1 mole of mesitylmagnesium bromide (prepared in 95% yield, as determined by acid titration[15] from 19.9 g. (0.1 mole) of 2-bromomesitylene and excess magnesium in 100 ml. of tetrahydrofuran) and 26.63 g. (0.1 mole) of tri-*n*-butyl phosphate in 50 ml. of the same solvent was refluxed gently for 3 days and then carbonated. Work-up in the usual manner for carbonation reactions gave 10.75 g. (65.5%) of β-isodurylic acid, m.p. and mixed m.p. 152–154°, after crystallization from petroleum ether (b.p. 60–70°).

(15) H. Gilman, P. D. Wilkinson, W. P. Fishel and C. H. Meyers, THIS JOURNAL, **45**, 150 (1923).

The neutral layer furnished 1.05 g. (9.2%) of slightly impure mesitylene, b.p. 65–70° (25 mm.), $n^{20}D$ 1.4980, identified by infrared spectra. In addition, 86% of recovered tri-*n*-butyl phosphate, b.p. 165–167° (15 mm.), was obtained, and identified by infrared spectra.

The results of other unsuccessful reactions of tri-*n*-butyl phosphate with mesitylmetallic compounds are reported in Table II.

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[CONTRIBUTION FROM THE VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA]

Studies on the Amino Acid Sequence of Tobacco Mosaic Virus Protein. III. The Amino Acid Sequence of a Pentadecapeptide from a Tryptic Digest[1]

BY DUANE T. GISH

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The amino acid sequence of a pentadecapeptide isolated from a tryptic digest of tobacco mosaic virus protein has been determined. This sequence was found to be pyroglu·phe·ser·gluNH$_2$·val·try·lys·pro·ser·pro·gluNH$_2$·val·thr·val·arg.

In earlier communications the countercurrent distribution of a tryptic digest of tobacco mosaic virus (TMV) protein[2] and the elucidation of the structures of six of these components[3] were reported. The isolation of the C-terminal peptide from the tryptic digest, establishing the presence of three tryptophan residues in TMV protein, has also been reported.[4] In this paper is presented[5] the amino acid sequence of a peptide, designated K-0.66-A, found in the material with distribution coefficient of 0.66[2] (see Fig. 1). Thus, from the tryptic digest of TMV protein, containing 12 peptides, the sequences of 7 of these peptides, comprising 48 of the approximately 160 residues, have now been established. These 48 residues include 7 of the 11 arginine residues and both of the lysine residues present in TMV protein.

The peptide K-0.66–A was separated from the countercurrent distribution fraction K-0.66 by electrophoresis on cellulose (see Fig. 2) and was found to have the amino acid composition, ser$_2$, glu$_3$, val$_3$, pro$_2$, phe, try, thr, lys, arg.[6] It gave a positive ninhydrin test (due to the ε-amino group of lysine) but failed to yield a free N-terminal amino group by either the dinitrophenyl (DNP) or the phenylthiohydantoin (PTH) method. In the discussion below, as partial sequences are disclosed, these are indicated in brackets. In accordance with common usage, in known sequences the amino acid designations are separated by dots and in unknown sequences the amino acid designations are enclosed in parentheses and are separated by commas.

The presence of a tryptophanyl–lysine bond was established by treating K-0.66-A with N-bromoacetamide. It had been shown by Patchornik, Lawson and Witkop[7] that N-bromosuccinimide splits tryptophanyl peptide bonds. From K-0.66-A both split products were isolated. One peptide had the composition glu$_2$, ser, val, phe (no free α-amino group) and the other had the composition lys (pro$_2$, val$_2$, ser, glu, thr, arg). Tryptophan is destroyed during the reaction. [(glu$_2$, ser, val, phe) try·lys (pro$_2$, val$_2$, ser, glu, thr, arg)].

Treatment of K-0.66-A with carboxypeptidase-B[8] confirmed that arginine was the C-terminal amino acid. A partial sequence at the C-terminal end of .....val·thr·val·arg was established by treating the peptide with a mixture of carboxypeptidases A and B (see Fig. 3). [(glu$_2$, ser, val, phe) try·lys (pro$_2$, ser, glu) val·thr·val·arg].

When K-0.66-A was hydrolyzed with chymotrypsin the peptides C-1 through C-7, shown in Table I, were obtained. The sequence of C-4 could be deduced both from the results with carboxypeptidases A and B just described and from the N-terminal amino acids and compositions of C-4 and C-3. The sequence val·thr·val·arg was confirmed by hydrolyzing the peptide with leucine aminopeptidase. The results shown in Fig. 4 were obtained.

Peptide C-1 was resistant to both aminopeptidase and carboxypeptidase. In each case the resistant bond involved proline. The sequence lys··

(1) This paper has been aided by a U. S. Public Health Service Grant.

(2) D. T. Gish, L. K. Ramachandran and W. M. Stanley, *Arch. Biochem. Biophys.*, **78**, 433 (1958).

(3) L. K. Ramachandran and D. T. Gish, THIS JOURNAL, **81**, 884 (1959).

(4) D. T. Gish, *Biochem. Biophys. Res. Communs.*, **1**, 67 (1959).

(5) A preliminary report of this work has been published elsewhere (*Biochim. Biophys. Acta*, **35**, 557 (1959)).

(6) Abbreviations for the amino acid residues are those suggested by E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947).

(7) A. Patchornik, W. B. Lawson and B. Witkop, THIS JOURNAL, **80**, 4747 (1958).

(8) J. E. Folk and J. A. Gladner, *J. Biol. Chem.*, **231**, 379 (1958).