background effects 1601 reflections were judged observed, $|F_o| > 3\sigma(F_o)$. Three standard reflections were measured every hour and these showed no appreciable decline. The quantity $\sigma(F_o)$ was computed from $\{[l + \sigma(l)]/L_p\}^{1/2} - F_o$[18] and $\sigma(1)$ was computed from [total count + background count + 0.05(total count)^2 + 0.05(background)^2]$^{1/2}$.

Determination and Refinement of Structure. The observed structure factor amplitudes ($|F_o|^2$) were converted to normalized structure factors by removing the angular dependence of the reflections.[19] The solution of this 36 atom problem in the space group P_1 presented a severe challenge to direct methods. The largest 150 E's ($E \geq 1.6$) were assigned phases using the multisolution tangent formula approach.[19] Of the resultant 32 solutions the one with the lowest ψ_o residual was used to generate a Fourier map. The map was discouraging in that it resembled a continuous net of hexagons resembling chicken wire. The only encouraging aspect was the absence of outstandingly large peaks which meant that not much information had been destroyed by the squaring effect.[20] Nevertheless attempts to expand the model from various plausible fragments failed both through the use of the tangent formula[21] and through difference Fourier calculations using only those structure factors for which $F_c \geq 0.5F_o$. In both methods and for every fragment the model could not be forced to give additional atomic positions. Finally least-squares refinements with unit weights[22] did expand a 13-atom fragment into all 36 nonhydrogen atoms. Many cycles of least-squares refinements with anisotropic temperature factors for the nonhydrogen atoms and fixed-hydrogen atoms lowered the conventional discrepancy index to 0.048 for the 1601 observed reflections. Figure 2 is a computer generated perspective drawing of the final X-ray mode.[23] The absolute configuration is based on the negative Cotton effect CD measurement as only the relative configuration was determined by the diffraction experiment. (The estimated standard deviation in the bond lengths given in Table IV is 0.01 Å.) Table III is a listing of the fractional coordinates.

Acknowledgment. Financial assistance from the National Institutes of Health (Grants No. GM-06840 and RR-00612) is gratefully acknowledged.

Registry No.—**4a,** 53534-44-4; **4b,** 53534-45-5; **5a,** 53586-51-9; **5b,** 53534-46-6.

References and Notes

(1) For part LXIX, see M. Kaisin, Y. M. Sheikh, L. J. Durham, C. Djerassi, B. Tursch, D. Daloze, J. C. Braekman, D. Losman, and R. Karlsson, *Tetrahedron Lett.*, 2239 (1974).
(2) From part of the Ph.D. Thesis of W. L. Tan.
(3) Camille and Henry Dreyfus Foundation Teacher-Scholar Grand Awardee 1972–1977 and Fellow of the Alfred P. Sloan Foundation 1973–1975 at Iowa State University.
(4) B. W. Halstead, "Poisonous and Venomous Marine Animals of the World," Vol. 1, U. S. Government Printing Office, Washington, D.C., 1965, p 567.
(5) (a) S. L. Friess, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **31,** 1146 (1972). (b) M. M. Anisimov, E. B. Fronert, T. A. Kuznetsova and G. B. Elyakov, *Toxicon*, **11,** 109 (1973).
(6) J. S. Grossert, *Chem. Soc. Rev.*, **1,** 1 (1972).
(7) P. J. Scheuer, "Chemistry of Marine Natural Products," Academic Press, New York, N. Y., 1973, p 23–35.
(8) I. Rothberg, B. Tursch, and C. Djerassi, *J. Org. Chem.*, **38,** 209 (1973).
(9) S. Shimada, *Science*, **163,** 1462 (1969).
(10) (a) G. B. Elyakov, T. A. Kuznetsova, and V. E. Vaskovshy, *Khim. Prir. Soedin.*, **4,** 253 (1968), *Chem. Abstr.*, **70,** 45173v (1969); (b) G. B. Elyakov, T. A. Kuznetsova, A. K. Dzizenko, and Yu. N. Elkin, *Tetrahedron Lett.*, 1151 (1969).
(11) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill, New York, N. Y., 1960, p 73–114.
(12) P. Roller, C. Djerassi, R. Cloetens, and B. Tursch, *J. Amer. Chem. Soc.*, **91,** 4918 (1969).
(13) B. Tursch, R. Cloetens, and C. Djerassi, *Tetrahedron Lett.*, 467 (1970).
(14) (a) N. Entwistle and A. D. Pratt, *Tetrahedron*, **24,** 3949 (1968); (b) for a comparison of some 3α- and 3β-lanostane derivatives, see H. K. Adam, T. A. Bryce, I. M. Campbell, and N. J. McCorkindale, *Tetrahedron Lett.*, 1461 (1967).
(15) D. G. Buckley, G. H. Green, E. Ritchie, and W. C. Taylor, *Chem. Ind. (London)*, 298 (1971).
(16) We are indebted to Professor G. B. Elyakov, Pacific Institute of Bio-organic Chemistry, USSR Academy of Sciences, Vladivostok, for samples.
(17) Kindly provided by Dr. R. I. Dorfman of Syntex Research, Palo Alto, Calif.
(18) D. E. Williams and R. E. Rundle, *J. Amer. Chem. Soc.*, **86,** 1660 (1964).
(19) P. Main, M. Woolfsor, and G. Germain, MULTAN, Department of Physics, University of York, York, England, 1971.
(20) J. Gassman and K. Zechmeister, *Acta Crystallogr., Sect. A*, **28,** 270 (1972).
(21) J. Karle, *Acta Crystallogr., Sect. B*, **24,** 182 (1968).
(22) W. R. Busing, K. O. Martin, and H. A. Levy, "A Fortran Crystallographic Least Squares Program," USAEC Report ORNL-TM-305, Oak Ridge National Laboratory, 1965.
(23) C. K. Johnson, "ORTEP, A Fortran Therman Ellipsoid Plot Program for Crystal Structure Illustrations," USAEC Report ORNL-3794, Oak Ridge National Laboratory, 1965.

Synthesis of Dipeptides of Aminophosphonic Acids[1]

M. Hariharan, R. J. Motekaitis, and A. E. Martell*

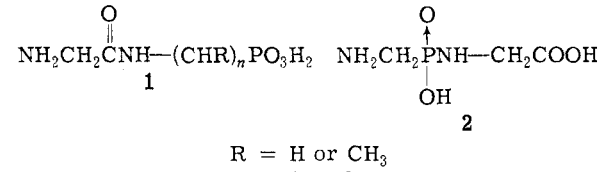
Department of Chemistry, Texas A&M University, College Station, Texas 77843

Received June 26, 1974

Of the two classes of phosphono dipeptide derivatives, three new parent dipeptides were synthesized using a new method followed by the removal of the protective groups and were characterized by elemental and detailed nmr analyses. In addition, for two dipeptides containing amide linkages and terminal phosphonic acid groups the pK_a's and metal binding constants were determined. The peptides containing phosphonamide linkages could not be obtained in the free state because of their sensitivity toward acids and bases.

The recent isolation of 2-aminoethylphosphonic acid (2AEP) from several organisms[2a–e] and human beings[2f,g] has clearly shown that aminophosphonic acids are biologically an important class of compounds. Early publications of the natural occurrence of 2-aminoethylphosphonic acid suggested participation of the compound in lipid structures,[2a–e,3] but Quin[4] showed that occurrence in protein structures was also possible. Quin suggested[5] that the aminophosphonic acids could form part of polypeptide chains by amide formation through either one or both of their amino and phosphonic acid groups.

In a preliminary communication[6] we reported the preparation of the derivatives of several members of two classes (1 and 2) of phosphonic acid dipeptides. This note provides



1 2

R = H or CH_3
n = 1 or 2

complete details for the removal of the protective groups; describes the isolation of three new dipeptides, glycyl-1- and 2-aminoethylphosphonic acid and glycylaminomethylphosphonic acid; provides nmr characterization; and reports the proto- and metallophilicity of the dipeptides acting as ligands. Furthermore, a much simpler route was

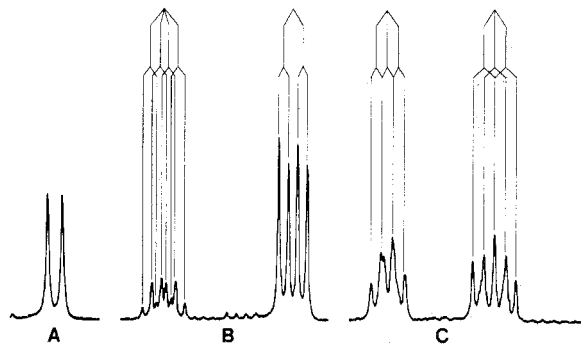


Figure 1. Nmr spectra of aminophosphonic acids in D_2O–NaOD solution; A, aminomethylphosphonic acid; B, 1-aminoethylphosphonic acid; C, 2-aminoethylphosphonic acid (assignments in text).

found for the synthesis of P-terminal phosphono dipeptides in the mixed carboxyliccarbonic anhydride method and was used to replace the acylated amino acid chloride method.

Results and Discussion

CON Peptides. The previously reported condensation of the acylated amino acid chloride with the aminophosphonic acid was abandoned as it proved to be rather tedious, resulted in low yields, and presented purification problems. A new, simpler mixed carboxylic–carbonic anhydride method was substituted for the preparation of class 1 peptides as shown in eq 1 and 2.

$$\underset{3}{\text{RNCHR'COOH}} + \underset{4}{\text{R''OCOCl}} \xrightarrow{Et_3N} \underset{5}{\text{RNCHR'CO—O—COR''}} \quad (1)$$

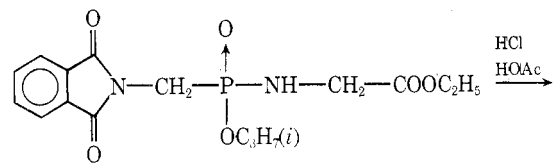
$$5 + \underset{6}{NH_2CH_2\overset{\overset{O}{\uparrow}}{P}(ONa)_2} \longrightarrow \underset{7}{\text{RNCHR'CONHCH}_2\overset{\overset{O}{\uparrow}}{P}(ONa)_2} \quad (2)$$

The method is similar to the preparation of normal peptides by the mixed anhydride method,[7] and essentially consists in treating a cold anhydrous dioxane solution of the acyl (R = phthaloyl or carbobenzoxy) aminocarboxylic acid with an equimolar quantity of ethyl chlorocarbonate (**4**, R'' = C_2H_5) in the presence of triethylamine and then adding an alkaline solution of the appropriate aminophosphonic acid (**6**). Since alcohol and carbon dioxide are the only by-products of the reaction, the dipeptides were obtained in fairly pure form by this method. An added advantage of the method is that when the phthalyl or carbobenzoxy[8] derivative of an optically active amino acid is used as one of the reactants, very little optical deactivation occurs in the formation of the mixed anhydride and hence the dipeptide. The removal of the protective phthalyl groups from the peptide derivatives was achieved using hydrazine under mild conditions.

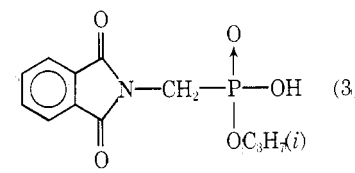
PON Peptides. In contrast to the two methods for the synthesis of peptides with free phosphonic acid groups as described above, the condensation of aminomethylphosphonic acid with *N*-acylated aminocarboxylic acids in the presence of dicyclohexylcarbodiimide (DCC) failed. Perhaps the aminophosphonic acids are too acidic for this reaction to occur. Evidence for this interpretation comes from the observation that aminophosphonic acid esters do condense with *N*-acylated aminocarboxylic acids in the presence of DCC.[9]

The peptide of the type represented by **2** was found to be more difficult to synthesize than are the peptides represented by **1**. *N*-Acylated aminophosphonic acids were found not to condense with ethylglycinate in the presence of dicyclohexylcarbodiimide. Hence an alternate route for the synthesis of P–N peptides was developed as outlined earlier[6] by activating the phosphorus ester group with phosphorus pentachloride to form the phosphonochloridate intermediate, which in turn was reacted with the glycine ester.

The removal of the phthalyl group from the peptide **8** was found to be difficult because of the sensitivity of the P–N bond even to mild acids. Thus when **8** was allowed to stand in hydrochloric acid–acetic acid mixture for a short time (~30 min), the half-ester **9** separated out. The hydrolysis reaction is represented by eq 3.



8



9 (3)

Nmr Spectra. The nmr spectra of the two classes of peptides and their derivatives provided clear proof of their structures. A comparison of the spectra of the dipeptides with those of the corresponding amino and aminophosphonic acids is very useful in identifying the resonances and understanding the complex spin–spin interactions between the protons and the ^{31}P nucleus. All the nmr spectra were obtained in solvents consisting of D_2O with added sodium deuteroxide in order to avoid complications due to pH dependence. In the spectrum of aminoethylphosphonic acid the $-CH_2$ protons are split into a doublet, τ 7.33 and J_{P-H} = 11 Hz, by the ^{31}P nucleus. The spectrum of 1-aminoethylphosphonic acid is more complex. The –CH proton signals are split into an octet (τ 7.08 and J_{P-H} = 10.4 Hz); the quartet expected from the J_{H-H} spin–spin interaction with the $-CH_3$ protons is further split by the spin of one-half of the ^{31}P nucleus as shown in Figure 1. Similarly the $-CH_3$ proton signal is split into a quartet (τ 8.74 and J_{P-H} = 7 Hz) by long-range coupling with the ^{31}P nucleus. In the spectrum of 2-aminoethylphosphonic acid a quartet (τ 6.98 and J_{P-H} = 8 Hz) and quintet (τ 8.27 and J_{P-H} = 16 Hz) are observed for the N–CH_2 and the $-CH_2$–P protons, respectively. These multiplets for the methylene protons are separately a composite of six lines (as shown in Figure 1) with some of them overlapping one another. Here again these multiplets arise from the secondary splitting of the spin–spin interactions of the $-CH_2$ protons with the ^{31}P nucleus.

The spectra of the dipeptides contain the same features as those of the corresponding phosphonic acids in addition to the singlet due to the $-CH_2$ group of the amino acid moiety. In the nmr spectra of all the free dipeptides containing terminal phosphonic acid groups the signal for the $-CH_2$ group of the aminocarboxylic acid moiety overlaps the signals due the $-CH_n$–P (n = 1 or 2) protons. Thus the spectrum of glycylaminomethylphosphonic acid consists of only two lines (Figure 2; J_{P-CH} = 11 Hz). Similar features are seen in the spectra of the other two peptides. Thus in the case of the Gly-1-Aep peptide, J_{P-CH} = 10.2 Hz and

Table I[a]
Log Equilibrium Constants for Gly-Amp (H_2L), Gly-2-Aep (H_2L), Gly-Gly (HL), and Gly-β-Ala (HL) at 25.0 ± 0.05° and μ = 0.100 M KNO_3

Equilibrium quotient	Gly-Amp[b]			Gly-2-Aep[b]			Gly-Gly[c]			Gly-β-Ala[c]
$K_1^H = [HL]/[H][L]$	8.34 (2)			8.32 (1)			8.07			8.09
$K_2^H = [H_2L]/[H][HL]$	6.19 (1)			6.84 (1)			3.13			3.91
	Cu^{2+}	Ni^{2+}	Co^{2+}	Cu^{2+}	Ni^{2+}	Co^{2+}	Cu^{2+}	Ni^{2+}	Co^{2+}	Cu^{2+}
$K_{ML} = [ML]/[M][L]$	6.86 (1)	4.75 (1)	3.68 (1)	7.55 (1)	4.44 (1)	3.53 (1)	5.50	4.05	3.01	5.70
$K_{MHL} = [MHL]/[ML][H]$	5.19 (1)	5.79 (1)	6.29 (1)	5.21 (1)	6.64 (1)	6.87 (1)		6.29		
$K_A = [ML]/[MH_{-1}L][H]$	5.17 (1)			5.26 (1)			4.07			4.57

[a] Gly-Amp is glycylaminomethylphosphonic acid; Gly-2-Aep is glycyl-2-aminoethylphosphonic acid; Gly-Gly is glycylglycine; Gly-β-Ala is glycyl-β-alanine. [b] This work. [c] Reference 15.

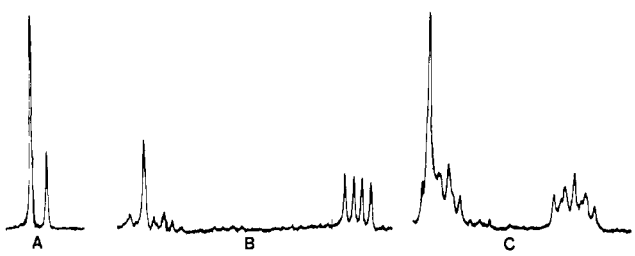


Figure 2. Nmr spectra of free phosphono dipeptides with terminal phosphonic acid group in NaOD solution; A, glycylaminomethylphosphonic acid; B, glycyl-1-aminoethylphosphonic acid; C, glycyl-2-aminoethylphosphonic acid (assignments in text).

J_{P-CH_3} = 7 Hz, and the Gly-2-Aep peptide, $J_{CH_2-CH_2}$ = 10 Hz and J_{P-CH_2} = 16 Hz. (More detailed nmr data on the compounds are given in the Experimental Section.)

pK_a's and Stability Constants. Due to the importance of the dipeptides synthesized in this work both the pK_a's and log stability constants with three representative metal ions were determined by potentiometric measurements at 25° and μ = 0.1. The results are indicated in Table I together with literature values of analogous compounds alongside for comparison purposes.

A comparison of each log K_1^H value for the phosphono dipeptides with the values of the ordinary dipeptides of Table I indicates its assignment to the protonation of the terminal amino group. Similarly, log K_2^H can be concluded to represent the protonation of the terminal P group.

The further comparison of the log K_{ML} data with the available data indicates that copper affinity increases one order of magnitude when a terminal phosphonate group is substituted for a carboxylate in a peptide. For Ni(II) and Co(II) the increase is less dramatic.

A comparison of log K_{MHL} values with the corresponding log K_2^H values strongly suggests that in the two CuHL chelates, Cu^{2+} is coordinated to the terminal amino group as well as to the peptide carbonyl. Likewise, in the NiHL and CoHL chelates the initial bonding must be to the PO_3^{2-} terminal and amido-O groupings explaining the approximately 2 log unit drop in the terminal N acidity constants. The case of glycylglycinenickel(II) corroborates this finding.[15]

Only copper(II) ion assists in the dissociation of the amidic proton present in each peptide (K_A), presumably because higher pH's result in M hydrolysis before this phenomenon could be observed.

Experimental Section

The reagents benzene, ether, tetrahydrofuran (THF), chloroform, and triethylamine used in the P–N peptide synthesis were completely dried before use. Triisopropyl phosphite was donated by Mobil Chemical, Richmond, Virginia, and was redistilled at 88° (33 mm) before use. The melting points given are uncorrected. The nmr spectra were recorded using a Varian T-60 nmr spectrometer. Elemental analyses were provided by Galbraith Laboratories, Knoxville, Tenn.

Phthalylglycine (Phtgly). Finely powdered phthalic anhydride (74 g, 0.5 M) and glycine (37.5 g, 0.5 M) were thoroughly mixed together and heated in an erlenmeyer flask to 145–150° for 0.5 hr over an oil bath. The fused mass was crystallized from methanol–water: mp 194 (lit.[10] 192–194); yield 90 g (90%).

Phthalylglycylaminomethylphosphonic Acid (Phtglyamp). A solution of 4.10 g (0.020 M) of phthalylglycine and 2.04 g (0.020 M) of anhydrous triethylamine in 40 ml of dry p-dioxane was cooled to −5° and treated with 2.17 g (0.020 M) of ethyl chloroformate. After 25 min of mixing a cold solution of 2.22 g (0.020 M) of aminomethylphosphonic acid and 1.06 g (0.020 M) of anhydrous sodium carbonate in 20 ml of water was added and the mixture was stirred for 3–4 hr allowing it to warm to room temperature. The mixture was acidified with concentrated hydrochloric acid and cooled, and the product that separated out as white crystalline material was filtered and dried. Any unreacted phthalylglycine was removed with hot ethylacetate and the product was recrystallized from alcohol–water: yield 4.5 g (75%); mp 195°; nmr (NaOD) 2.43 (s, 4, C_6H_4), 5.90 (s, 2, N–CH_2–CO), 7.70 (d, 2, –CH_2–P).

Phthalylglycyl-1-aminoethylphosphonic Acid (Phtgly-1-Aep). The procedure employed was similar to that described for phtglyamp. The carboxylic–carbonic anhydride formed by the reaction of 4.10 g (0.020 M) of phthalylglycine, 2.04 g (0.020 M) of triethylamine, and 2.17 g (0.020 M) of ethylchloroformate was treated with a neutral solution of 2.50 g (0.020 M) of 1-aminoethylphosphonic acid for 3 hr. The product was acidified and cooled, and the peptide was filtered off and was recrystallized: yield 4.1 g (66%); mp 225°; nmr (NaOD) 2.40 (s, 4, C_6H_4), 5.92 (s, 2, N–CH_2–CO), 6.01 (m, 1, CH–P), 8.75 (q, 3, –CH_3).

Phthalylglycyl-2-aminoethylphosphonic Acid (Phtgly-2-Aep). The procedure employed is similar to that employed for the preparation of Phtglyamp. The mixed anhydride formed by the reaction between 4.10 g (0.020 M) of phthalylglycine, 2.04 g (0.020 M) of triethylamine, and 2.17 g (0.020 M) of ethyl chloroformate was allowed to react with a neutral solution of 2-aminoethylphosphonic acid for 3 hr. The product was acidified and cooled, and the phthalyldipeptide obtained was filtered and recrystallized from alcohol–water: yield 4.2 g (67%); mp 236–238°; nmr (NaOD) 2.30 (s, 4, C_6H_4), 5.58 (s, 2, N–CH_2–CO), 6.33 → 6.73 (q, 2, CH_2–P), 7.82 → 8.40 (m, 2. NH–CH_2).

Dephthalylatin. The following procedure is representative of those employed for the dephthalylation of the phthalyated dipeptides.

Glycylaminomethylphosphonic Acid (Glyamp). Phthalylglycylaminomethylphosphonic acid (4.8 g, 0.016 mol), 1.0 g of sodium carbonate, and 1.0 ml of hydrazine were mixed with about 30 ml of distilled water and the clear mixture was stirred for 40 hr at room temperature. The precipitated phthalyl hydrazide was removed after acidifying with 15 ml of concentrated hydrochloric acid. On concentrating the filtrate, sodium chloride and hydrazine hydrochloride separated out. The addition of alcohol to the filtrate resulted in the separation of the free dipeptide. It was recrystallized from a water–alcohol mixture: yield 1.5 g (30%).

(1) **Glyamp:** mp 205° dec; nmr* (NaOD) 6.67 (s, 2, NH_2–CH_2), 6.71 (d, 2, CH_2–P). *Anal.* Calcd for $C_3H_9N_2O_4PH_2O$; C, 19.46; H, 6.00; N, 15.14; P, 16.73. Found: C, 19.28, H, 5.95; N, 14.94; P, 16.78.

(2) **Gly-1-Aep:** mp 235° dec; nmr (NaOD) 6.42 (s, 2, NH_2CH_2),

6.22 → 6.68 (m, 1, NH-CH-P), 8.30 → 8.80 (q, 3, $-CH_3$). *Anal.* Calcd for $C_4H_{11}N_2O_4P$: C, 26.37; H, 6.08; N, 15.38; P, 17.00. Found: C, 26.19; H, 5.92; N, 15.20; P, 17.10.

(3) **Gly-2-Aep:** mp 250° dec; nmr (NaOD) 6.37 (s, 2, NH_2–CH_2), 8.03 → 8.62 (m, 2, NH–CH_2), 6.30 → 6.82 (m, 2, CH_2–P). *Anal.* Calcd for $C_4H_{11}N_2O_4P$: C, 26.37; H, 6.08; N, 15.38; P, 17.00. Found: C, 26.46; H, 6.11; N, 15.33; P, 16.89.

Acknowledgment. The authors express their thanks to Dr. A. F. Isbell, Chemistry Department, Texas A&M University, for providing the aminophosphonic acids and for helpful discussions.

Registry No.—Glyamp, 30211-73-5; Gly-1-Aep, 53626-51-0; Gly-2-Aep, 53626-52-1; Phtglyamp, 38416-67-0; Phtgly, 4702-13-0; aminomethylphosphonic acid, 1066-51-9; Phtgly-1-Aep, 51814-60-9; 1-aminoethylphosphonic acid, 6323-97-3; Phtgly-2-Aep, 51814-61-0; 2-aminoethylphosphonic acid, 2041-14-7.

References and Notes

(1) This work was supported by a research grant, GM 20511, from The National Institute of General Medical Sciences.
(2) (a) M. Horiguchi nd M. Kandatsu, *Nature (London)*, **184**, 901 (1959); (b) J. S. Kittredge, E. Roberts, and D. G. Simonsen, *Biochemistry*, **1**, 624 (1962); (c) L. D. Quin, *ibid.*, **4**, 324 (1965); (d) A. J. Koning, *Nature (London)*, **210**, 113 (1966); (e) T. Hori, O. Itasaka, H. Inoue, and K. Yamada, *J. Biochem. (Tokyo)*, **56**, 447 (1964); (f) J. A. Alhadeff and G. D. Davies, Jr., *Biochemistry*, **9**, 4866 (1970); (g) *Biochim. Biophys. Acta*, **244**, 211 (1971).
(3) (a) G. Rouser, G. Kritchevsky, D. Heller, and E. Lieber, *J. Amer. Oil Chem. Soc.*, **40**, 425 (1963); (b) E. Baer and N. Z. Stanacev, *J. Biol. Chem.*, **239**, 3209 (1964).
(4) L. D. Quin, *Science*, **144**, 1133 (1964).
(5) L. D. Quin, "Topics in Phosphorus Chemistry," Vol. 4, M. Grayson and E. J. Griffith, Ed., Interscience, New York, N.Y., 1966.
(6) M. Hariharan and A. E. Martell, *Syn. Commun.*, **3**, 375 (1973).
(7) J. P. Greenstein and M. Winetz, "Chemistry of the Amino Acids," Wiley, New York, N.Y., 1961.
(8) M. Hariharan and A. E. Martell, unpublished results.
(9) K. Yamauchi, M. Kinoshita, and M. Imoto, *Bull. Chem. Soc. Jap.*, **45**, 2528 (1972).
(10) J. H. Billman and W. E. Harting, *J. Amer. Chem. Soc.*, **70**, 1473 (1948).
(11) F. E. King, J. W. Clark-Lewis, R. Wade, and W. A. Swindin, *J. Chem. Soc.*, 873 (1957).
(12) J. C. Sheehan and V. S. Frank, *J. Amer. Chem. Soc.*, **71**, 1856 (1949).
(13) S. R. Buck, *J. Amer. Chem. Soc.*, **69**, 254 (1947).
(14) S. Gabriel, *Ber.*, **41**, 242 (1908).
(15) A. E. Martell and R. M. Smith, "Critical Stability Constants," Vol. I, Plenum Press, New York, N.Y., 1974.

Mechanism and Stereochemistry of Oxetane Reactions. I. Stereospecific Synthesis of the Diastereoisomeric 2-Phenyl-3-methyloxetanes and Study of Their Configuration and Conformation by Nuclear Magnetic Resonance Spectroscopy

Aldo Balsamo, Giulio Ceccarelli, Paolo Crotti, and Franco Macchia*

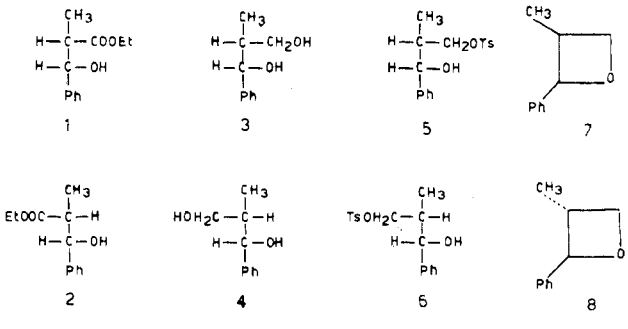
Istituti di Chimica Organica, Chimica Fisica e Chimica Farmaceutica, Università di Pisa, 56100 Pisa, Italy

Received March 5, 1974

The stereospecific synthesis of the diastereoisomeric 2-phenyl-3-methyloxetanes **7** and **8** has been achieved from the corresponding 1,3-diols through their monotosylates. The relative configurations of **7** and **8** have been unequivocally established by an extensive study of their nmr spectra. The assignment of the proton resonance signals has been effected on the basis of the shielding effects and of the coupling constants, and confirmed through additive shielding parameters. By using the J_{trans}/J_{cis} ratio it has been possible to obtain some informations on the conformational preference of the oxetane ring in the examined compounds.

The stereochemistry of the ring opening of small ring heterocycles such as oxiranes[1-3] and aziridine[4,5] in acid media is well known and documented. However, practically no information is available on the steric course of the analogous reactions of oxetanes.[6] Furthermore no "stereospecific" synthesis of diastereoisomeric couples of oxetane has been reported and only a few pairs of diastereoisomeric oxetanes have been prepared.[7] Since we are strongly interested in the study of mechanism and stereochemistry of the ring opening of small ring systems,[3,5] it was thought desirable to prepare and study diastereoisomerically pure oxetanes of unquestionable configuration. Oxetanes **7** and **8** appeared as promising substrates for this purpose and their synthesis and stereochemical characterization was the aim of this work.

The Reformatsky reaction of benzaldehyde and ethyl 2-bromopropionate gave a mixture of the diastereoisomeric esters *erythro*-**1** and *threo*-**2** whose relative configurations were deduced from their nmr spectra on the basis of the higher coupling constant of the benzylic proton for the threo isomer **2**, as found for the corresponding methyl esters.[8] On the other hand reduction of **1** and **2** with $LiAlH_4$ afforded the known diols *erythro*-**3** and *threo*-**4**.[8c,9] Reaction of **3** and **4** with *p*-toluenesulfonyl chloride in pyridine gave the respective monotosylates **5** and **6**. The constitution of **5** and **6** could be inferred by the known fact that tosyl chloride should react preferentially with the primary rather than with the secondary hydroxyl group,[10] and confirmed from their nmr spectra. In fact whereas the chemical shift of the signals of the benzylic proton is almost the same for the diols **3** and **4** and the corresponding tosylates, the signals of the protons of the methylene group are shifted toward low field in the cases of tosylates. Treatment of **5** and **6** with potassium *tert*-butoxide in *tert*-butyl alcohol at room temperature led to the diastereoisomerically pure oxetanes **7** and **8**. The configurations of oxetanes **7** and **8** were



deduced from their method of synthesis. The complete stereospecificity of the formations of oxetanes **7** and **8** is in accordance with the mechanism of their formation from **5**