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Conformational Aspects of Polypeptides.[1] X. Helical and Associated Forms of Oligomeric Peptides and Polymers Derived from β-Methyl L-Aspartate

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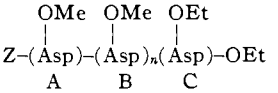
This report presents the results of studies on the conformational characteristics in solution of oligomeric and polymeric peptides derived from β-methyl L-aspartate. Model compounds (containing L-aspartic acid and glycine) were prepared, from which predictions of the molar rotations of the oligomers in the random coil conformation were made. There was excellent agreement between the predicted and experimentally found values for the dimer through hexamer in dimethylformamide solution indicating that all of these L-aspartate oligomers exist in the random coil conformation in this solvent. In dichloroacetic acid, a plot of molar rotation *vs.* the number of internal residues gives a straight line indicating that aspartate oligomers exist as random coils in this solvent also. Large deviations from model compound predictions were noted for the molar rotations of the oligomers in chloroform. Infrared spectra in chloroform show that the octamer possesses chiefly β-structure while the two higher oligomers prepared (undecamer and tetradecamer) possess helical, random and β-conformations. Optical rotatory dispersion measurements in chloroform of the undeca- and tetradecapeptides, as well as the high polymer, show that the helix for this series is left-handed. A modified approach to the interpretation of b_0 data for the oligomers is presented which eliminates non-helical contributions to this constant. In the mixed solvent chloroform–dichloroacetic acid (97%:3%), the predicted values of the molar rotations agree well with the values of the oligomers through the octamer indicating that all of these oligomers exist as random coils in this solvent system. Deviations were observed for the undeca- and tetradecapeptides indicating the existence of stable helices. The conclusions reached from a study of model compounds are consistent with those reached on the basis of optical rotatory dispersion and infrared spectroscopy. In weakly hydrogen bonding solvents such as chloroform and chloroform containing 3% dichloroacetic acid, helical forms become stable at a chain length of about eleven.

Introduction

The polymers of most amino acids in the L-configuration form right-handed helices.[3] Recently, Blout, Karlson, Norland and Fasman,[4] and Elliott, Downie, Bradbury and Hanby[5] reported that poly-β-benzyl L-aspartate forms a left-handed helix. In order to investigate the conformation of L-aspartate peptides in the critical size range of helix formation, we synthesized a series of oligomers derived from β-methyl L-aspartate having 2 through 14 residues.[1] Polymers of β-methyl L-aspartate were also prepared for comparison with these oligomers. We investigated the conformations of the oligomers in three solvents: chloroform, a solvent supporting the formation of secondary structure of the peptide chain; dimethylformamide, a solvent permitting the existence of only very stable helices; and dichloroacetic acid, a solvent favoring the highly solvated random coil conformation for most polypeptides. Since measurements of optical rotation[3] and infrared absorption[6] have been of great value in the past for the determination of polypeptide conformations, we have employed these techniques in our studies of the oligomers.

Results and Discussion

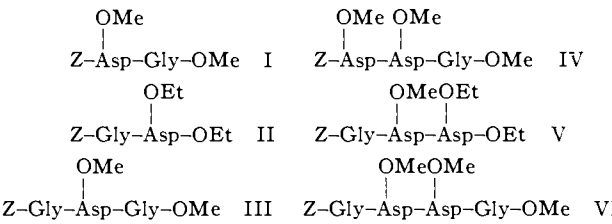
Optical Activity. Model Compounds.—Brand[7] and Doty[8] suggested that the optical rotation of a peptide depends on the sum of the contributions from the internal and terminal residues of a peptide. This approach has been successfully used by Goodman, Listowsky and Schmitt[9] for the prediction of the optical rotations of oligomers derived from γ-methyl L-glutamate. We have applied this method to the oligomers of the L-aspartate series



(1)

where n varies from 0 to 12.

The following model compounds were prepared in order to predict the random coil value of the molar rotation for each of the positions A, B and C, in the peptide chain



The molar rotation, ϕ, for any aspartyl oligomer in a random coil conformation should equal the sum of the molar rotations of compounds I and II plus the molar rotation of compound III multiplied by the number of internal residues (n).

$$\phi = \frac{[\alpha]\text{mol. wt.}}{10{,}000} \frac{\text{deg.}}{\text{cm. moles}}$$

$$\phi_{\text{peptide}}^{\text{random coil}} = \phi_A + \phi_C + n\phi_B = \phi_I + \phi_{II} + n\phi_{III}$$

where ϕ_A, ϕ_B and ϕ_C denote the molar rotation contributions of residues noted in the polymer formula (1) and ϕ_I, ϕ_{II} and ϕ_{III} represent the molar rotations of compounds I, II and III, respectively.

One cause for failure of agreement of predicted values of the molar rotations with those actually obtained is the presence of interaction between neighboring aspartyl residues. The more complex model compounds (IV, V and VI) take such interactions into account. The sum of the rotations of compounds IV and V predicts the rotation of a tetrapeptide. For each internal residue beyond the tetrapeptide stage, one-half of the rotation of compound VI must be added to the tetrapeptide rotation. Values for the

(1) This investigation was generously supported by a grant from the National Science Foundation (G8614) and Grant GM08974 from the National Institutes of Health. Previous paper in this series, M. Goodman and F. Boardman, *J. Am. Chem. Soc.*, **85**, 2483 (1963).

(2) Submitted to the faculty of the Polytechnic Institute of Brooklyn, 1962, in partial fulfillment of the requirements for the Ph.D. degree.

(3) E. R. Blout, in "Optical Rotatory Dispersion," by C. Djerassi, McGraw-Hill Book Co., Inc., New York, N. Y., 1961, Chapter 17.

(4) R. H. Karlson, K. S. Norland, G. D. Fasman and E. R. Blout, *J. Am. Chem. Soc.*, **82**, 2268 (1960).

(5) A. Elliott, E. M. Bradbury, A. R. Downie and W. E. Hanby, *Proc. Roy. Soc.* (London), **A259**, 110 (1960).

(6) C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956.

(7) E. Brand, B. F. Erlanger and H. Sachs, *J. Am. Chem. Soc.*, **73**, 3508 (1951).

(8) P. Doty and E. P. Geiduschek, in "Optical Properties of Proteins," H. Neurath and K. Bailey, Ed., Academic Press, Inc., New York, N. Y., 1953, p. 393.

(9) M. Goodman, I. Listowsky and E. E. Schmitt, *J. Am. Chem. Soc.*, **84**, 1296 (1962).

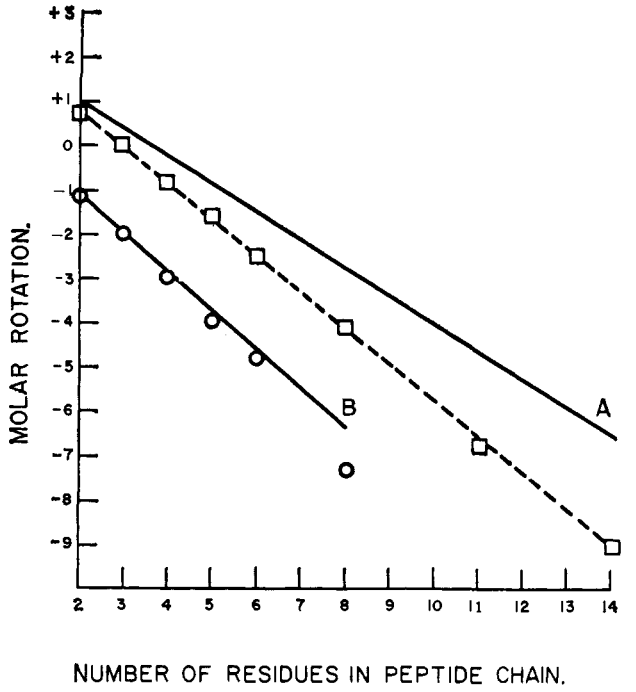


Fig. 1.—Molar rotations at 589 mμ of the oligomeric peptides derived from β-methyl L-aspartate and calculated molar rotation values based on model compounds. Line A, dichloroacetic acid solutions: solid line, calculated values from model compounds; squares, experimental values for oligomeric peptides. Line B, dimethylformamide solutions: solid line, calculated values from model compounds; circles, experimental values for oligomeric peptides.

di- and tripeptides may be calculated by extrapolating the plot of predicted molar rotation *vs.* number of residues (n'). Table I lists the molar rotations of the model compounds in various solvents.

$$\phi_{\text{peptide}} = \phi_{IV} + \phi_V + n'\phi_{VI}$$

(random coil with neighboring residue effects)

where ϕ_{IV}, ϕ_V and ϕ_{VI} denote the molar rotations of compounds IV, V and VI.

TABLE I

SPECIFIC AND MOLAR ROTATIONS OF MODEL COMPOUNDS FOR OLIGOMERS DERIVED FROM β-METHYL L-ASPARTATE AT 589 mμ AND 25°

	Solvent							
	Dichloroacetic acid		Dimethyl-formamide		Chloroform		Chloroform–dichloroacetic acid (97:3)	
Compound	$[\alpha]$	ϕ	$[\alpha]$	ϕ	$[\alpha]$	ϕ	$[\alpha]$	ϕ
I								
I	+ 1.6	+0.056	−20.5	−0.722	+16.9	+0.59	...	...
II	+25.1	+ .954	−15.7	− .597	+36.1	+1.37	...	...
III	−15.4	− .630	−22.0	− .900	− 8.0	−3.3	...	...
IV	−14.8	− .712	−20.6	−1.28	+ 4.1	+0.20	− 7.6	−0.365
V	+ 4.1	+ .21	−35.7	−1.81	+20.4	+1.04	+12.1	+0.616
VI	−23.2	−1.25	−36.3	−1.95	− 3.2	−0.17	−20.0	−1.076

Figure 1 (line B) shows a comparison of predicted and actual molar rotations of the aspartyl oligomers in dimethylformamide. The line represents the identical values predicted by both sets of model compounds, indicating that interaction between neighboring aspartyl residues does not occur. The experimental points (circles) of dimer through hexamer fall on this line indicating that these oligomers are in the random coil conformation. The small deviation of the octamer point from this line may indicate the presence of some secondary structure. Unfortunately, the higher oligomers and polymers are insoluble in dimethylformamide

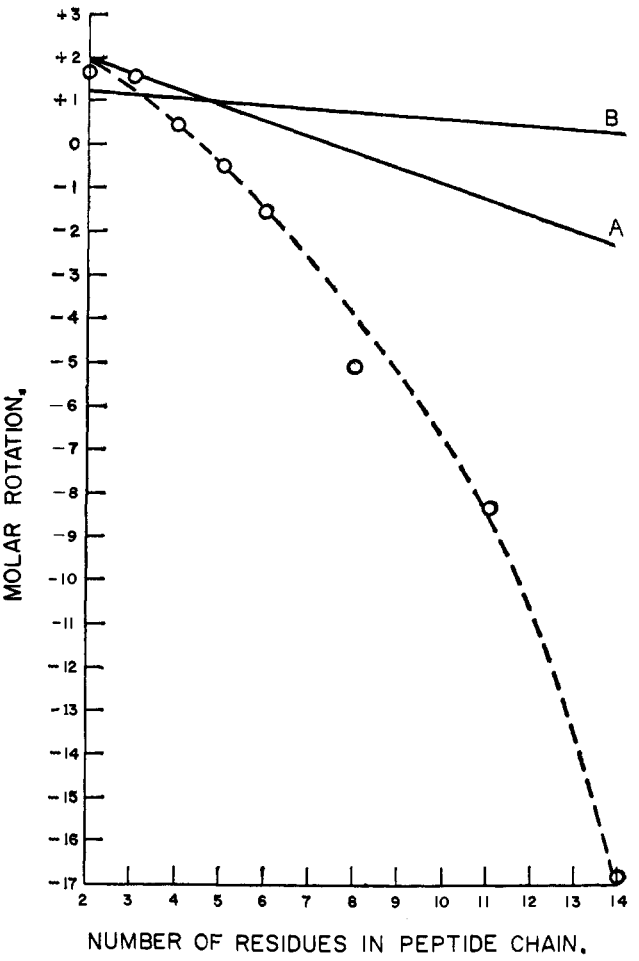


Fig. 2.—Molar rotations at 589 mμ of the oligomeric peptides derived from β-methyl L-aspartate and calculated values based on both sets of model compounds (see text) in chloroform solutions: line A, calculated values from simple model compounds; line B, calculated values from complex model compounds; circles, experimental values for oligomeric peptides.

and therefore the significance of this deviation remains obscure. However, through the hexamer, rotation data for the model compound predict the optical activity of the oligomers very well.

Figure 1 (line A) shows a similar comparison for the oligomers in dichloroacetic acid (DCA). Since a straight line can be drawn through all of the experimental points connecting dimer through tetradecamer, all of these peptides must be in the random coil conformation. However, the slope of this line differs slightly from that of the line derived from the model compounds. Thus in DCA the model compounds can only yield approximate molar rotations for random coil oligomers. We are at present investigating the causes for the difference in slope encountered in DCA. As in dimethylformamide, the data in DCA from both sets of model compounds lead to essentially the same predicted values of molar rotations for the oligomers.

A more complex pattern is seen when the graph for the aspartyl oligomers in chloroform is examined (Fig. 2). Infrared studies of the larger oligomers (see later section of this paper) show that random, associated and helical forms can co-exist in chloroform solution. Since we do not know the contribution to the structure of associated and helical forms, neither the simple nor complex model compounds can be used to predict the optical rotations for the oligomers.

In order to eliminate association of the aspartate oligomers, we utilized the mixed solvent, chloroform–

TABLE II

OPTICAL ROTATORY DISPERSION DATA FOR POLY-β-METHYL L-ASPARTATE AND POLY-β-BENZYL L-ASPARTATE AT 25°

			Chloroform			Dichloroacetic acid		
Ester	Initiator	A/I	$[\alpha]_D$	b_0	λ_c, mμ	$[\alpha]_D$	b_0	λ_c, mμ
Methyl	$NaOCH_3$	970	−193[a]	+410[a]	162[a]	−63.5	−290	257
	Et_2NH	48	−126	+395	213	−60.0	−200	241
	Et_2NH	5.30	− 77.4	− 76	227	−52.2	−195	258
	Et_2NH	1.32	− 41.3	− 94	250	−24.9		
Benzyl[b]	$NaOCH_3$	200	−172[c]	+630	141[d]	−18	−250	NL[e]

[a] Measurements made in 97% chloroform–3% dichloroacetic acid. [b] Data of Blout, *et al.*[4] [c] Measurement made at 546 mμ. [d] Calculated by Schellman.[11] [e] A non-linear Drude plot was obtained.

TABLE III

OPTICAL ROTATORY DISPERSION DATA FOR OLIGOMERIC PEPTIDES DERIVED FROM β-METHYL L-ASPARTATE AT 25°

	Dichloroacetic acid				Dimethylformamide			
Oligomer	Concn., %	$[\alpha]_D$	b_0[a]	λ_c, mμ	Concn., %	$[\alpha]_D$	b_0[a]	λ_c, mμ
Di-	3.06	+17.9	−101	151[b]	2.80	−25.7	− 46	232
Tri-	3.11	− 1.01	−142	NL[c]	1.25	−34.5	− 85	239
Tetra-	1.10	−13.1	−152	325	0.76	−44.4	−111	237
Penta-	0.138	−19.1	−188	273	.18	−48.1	−150	242
Hexa-	.740	−26.6	−155	269	.709	−48.0	−141	240
Octa-	.514	−35.1	...[d]	..[d]	.200	−60.8	− 83	227
Undeca-	.453	−42.9	−223	..[d]	Insoluble			
Tetradeca-	.321	−46.0	−221	258	Insoluble			
	Chloroform				97% chloroform–3% dichloroacetic acid			
Di-	0.645	+38.2	−282	12[b]	0.130	−27.2[e]	+ 75	236
Tri-	0.664	+26.2	−294	NL[c]	.130	0		
Tetra-	2.00	+ 8.8	−262	NL[c]	.121	−10.9[e]	−306	296
Penta-	0.651	− 8.5	−272	333	.208	−15.8	−303	319
Hexa-	.680	−16.1	−308	309				
Octa-[e,f]	.250	−41.7	−134	248	0.860	−12.6	−288	329
Undeca-[e,f]	.224	−50.7	− 44	219	.0826	−37.5	−181	245
Tetradeca[e,f]	.168	−83.8	+278	131	.100	−42.4	− 26	217

[a] In order to obtain molar b_0, the listed b_0 values must be multiplied by the appropriate number of residues. [b] These values of λ_c were calculated by using only the portion of the plot that was linear. [c] A non-linear (NL) Drude dependence was obtained. [d] It was impossible to get reproducible results. [e] The value of $[\alpha]_D$ is an extrapolated point from the Moffitt–Yang plot. [f] Readings were taken after solution was stored 24 hr. at 25° since the rotations changed with time after the first 10 hr.

dichloroacetic acid (97:3 volume–volume). Data for the compounds in this solvent system (Fig. 3) show excellent agreement between model compound predictions and actual molar rotations for the oligomers from dimer through octamer. This agreement indicates that these oligomers are in the random coil conformation. The molar rotations for the undecamer and tetradecamer deviate from the predicted values. In the study of γ-methyl-L-glutamate oligomers in dimethylformamide we attributed such deviations from predicted optical activity values to the onset of helicity.[9] Therefore, in the aspartate series, we suggest that the deviations from predicted optical activity values for the undeca- and tetradecamer noted above are indicative of the presence of helical structures.

Optical Rotatory Dispersion Studies of the Aspartate Polymers.—Optical rotatory dispersion constants for the various samples of poly-β-methyl L-aspartate are shown in Table II. For the high molecular weight polymers the b_0 constants in chloroform were positive, denoting left-handed helices.[4,5] Because of solubility problems it was necessary to study the material of highest molecular weight in a mixed solvent, chloroform–dichloroacetic acid (97:3), which may account for the relatively low b_0 value, +410. Fasman[10] states that as little as 5% dichloroacetic acid in chloroform can cause the helix to coil transition. From the Drude equation the λ_c for this polymer in the mixed solvent system is 162 mμ, which is similar to the λ_c value for poly-β-benzyl L-aspartate in chloroform calculated by Schellman.[11]

The lower molecular weight polymers from β-methyl L-aspartate have b_0 values of −76 and −94 in chloroform. These values are comparable to those obtained by Elliott, *et al.*,[5] for low molecular weight poly-β-benzyl L-aspartate in *m*-cresol. In dichloroacetic acid the polymers show b_0 constants between −195 and −290. It will be shown in the discussion of rotatory dispersion data for the oligomers that the b_0 values in chloroform (above) are indicative of helical conformational contributions while the results obtained in dichloroacetic acid denote non-helical structures. It should be noted that as the size of the oligopeptides is increased the optical rotation values approach those of the polymers (see Tables II and III).

Rotatory Dispersion Studies on Oligomers (Modified Moffitt–Yang Treatment).—In a previous report[12] we discussed an empirical approach to the Moffitt–Yang constant[13] b_0 for oligomeric peptides based on an additivity principle. Our treatment, shown in the equation below, attempts to separate terminal residue effects, non-helical and helical contributions to this constant. For all b_0 values the λ_0 has been set equal to 212 mμ.

$$\text{molar } b_0^t = \text{molar } b_0^E + (n-2)b_0^i + (n-8)f_H b_0^H$$

where

(1) molar b_0^t is the total b_0, obtained by utilizing the entire molecular weight instead of mean residue weight, in the Moffitt–Yang equation
(2) molar b_0^E represents the end-group effects and is obtained from the molar b_0^t of the dipeptide
(3) n is the number of residues in the peptide
(4) b_0^i is the configurational b_0 contribution per internal residue

(10) G. D. Fasman, in "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p. 222.
(11) J. Schellman and C. G. Schellman, *J. Polymer Sci.*, **49**, 129 (1961).
(12) M. Goodman, I. Listowsky, Y. Masuda and F. Boardman, *Biopolymers*, **1**, 33 (1963).
(13) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, **42**, 596 (1956).

and is obtained from the slope of the non-helical peptides in a plot of molar b_0^t *vs.* number of residues

(5) f_H is the fraction of residues in the helical form; this factor is zero for the octamer and smaller peptides

(6) $(n - 8)$ is the maximum number of residues which can be aligned in a helical structure

(7) b_0^H is the helical b_0 contribution per residue; helical residues are those which are doubly intramolecularly hydrogen bonded

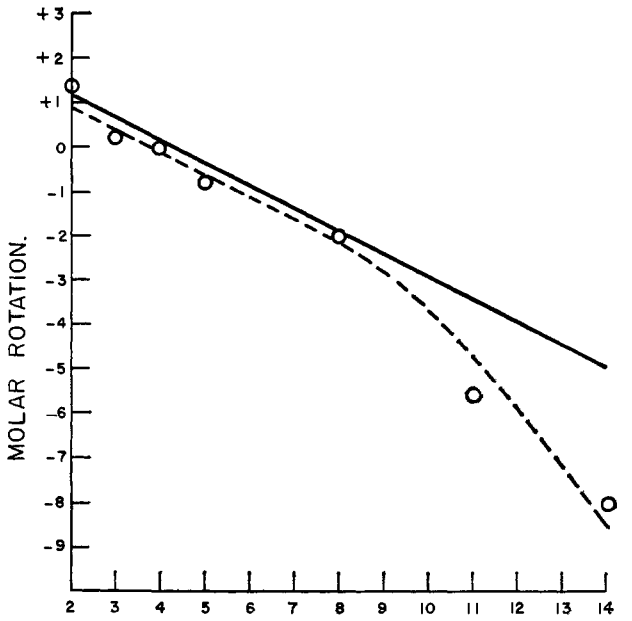


Fig. 3.—Molar rotations at 589 mμ of the oligomeric peptides derived from β-methyl L-aspartate and the calculated values from complex model compounds (see text) in 97% chloroform–3% dichloroacetic acid: solid line, calculated values from model compounds; circles, experimental values for oligomeric peptides.

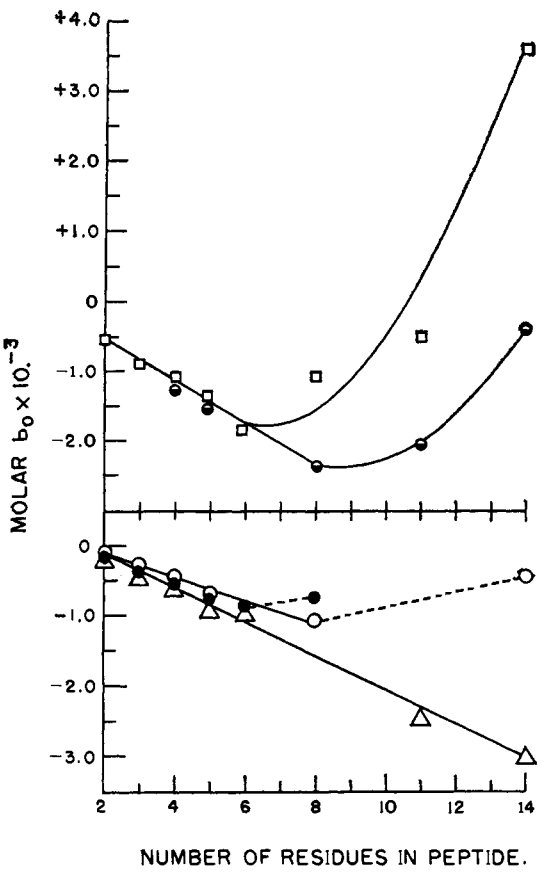


Fig. 4.—Molar b_0 values for oligomeric peptides derived from β-methyl L-aspartate: □, in chloroform; ◒, in 97% chloroform–3% dichloroacetic acid; ○, in trifluoroethanol; ●, in dimethylformamide; △, in dichloroacetic acid.

It is readily seen from Fig. 4 that the b_0^i values are very nearly the same (about −250) in each of the solvents studied. The terminal effects b_0^E do vary somewhat in the different solvents. These values of b_0^i and b_0^E are not zero as predicted for random structures. The reason for this may reside in the fact that a completely random structure does not exist in solution for these compounds, since the polar side chain is so close to the main chain.[14,15] This causes the peptide to assume specific conformations.

The contribution of the helical structures for the oligomeric peptides is negligible in dimethylformamide and in dichloroacetic acid. In chloroform the b_0 values are difficult to interpret on the basis of the Moffitt–Yang equation, because of complications arising from β-associated structures.[16] The contributions of β-structures commence at about the octapeptide for these aspartate oligomers in this solvent. For γ-methyl L-glutamate peptides in solvents such as dioxane and chloroform, the β-structure exists in smaller peptides.[17]

In 97% chloroform–3% dichloroacetic acid and in trifluoroethanol solutions, there appears to be no association and helical structures commence at about the undecamer (Fig. 4). Utilizing the high polymer b_0 value of +600 (from the Moffitt–Yang equation) in chloroform–dichloroacetic acid, and +860 in trifluoroethanol, the helical fractions (f_H) of a given oligopeptide in each solvent may be determined.

(14) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, W. E. Hanby and T. R. R. McDonald, *Nature*, **183**, 1736 (1959).

(15) G. D. Fasman, ref. 10, p. 271.

(16) A. Wada, M. Tsuboi and E. Konishi, *J. Phys. Chem.*, **65**, 1119 (1961).

(17) M. Goodman, *J. Polymer Sci.*, **49**, 150 (1961).

In this treatment, it must be recalled that we consider helical residues *only* those that form intramolecular hydrogen bonds both through their carbonyls and N–H groups. Since the four terminal residues at each end cannot be doubly intramolecularly hydrogen bonded, a maximum of three residues in the undecamer and six residues in the tetradecamer can be aligned in a rigid helical array similar to a residue of the high polymer in a helical form. The number of actual residues in the helical form is given by N_H

$$N_H = f_H(n - 8)$$

The f_H value for the undecamer is about 0.60 in chloroform–dichloroacetic acid (97:3) which gives an N_H of about two residues (Table IV). The tetradecamer has an f_H value of about 0.92 in chloroform–DCA (97:3) (N_H of about 6 residues) and 0.60 in trifluoroethanol (N_H of about 4 residues). This is in contrast to the γ-methyl L-glutamate oligomers in dimethylformamide and trifluoroethanol where the f_H values are close to 1, indicating that the maximum number of residues which *can be* helical are in the helical conformation. The nonamer, therefore, has one, undecamer three, and tridecamer five residues that are doubly intramolecularly hydrogen bonded. In this analysis of rotatory dispersion data, the assumption is made that the b_0^i values are essentially the same in the helical as in the random coil form. We also assume that the b_0 value for the completely helical high polymer can be used as the b_0^H for the oligomers. It is possible that b_0^H for oligomers is less than the value of b_0 obtained from high polymer data. If the above assumptions are not valid, our estimate of the f_H values obtained for all helical oligomers would be low.[18,19]

TABLE IV
HELICITY OF OLIGOPEPTIDES DERIVED FROM β-METHYL L-ASPARTATE

	Chloroform–DCA (97:3)			Trifluoroethanol	
Oligomer	f_H	N_H	N_H^{max}	f_H	N_H
Octamer	0	0	0	0	0
Undecamer	0.6	2	3	...	..
Tetradecamer	0.92	6	6	0.6	4

peptides. Our results are most easily interpreted if we assume that the helical amide I peak occurs at 1675 cm.$^{-1}$ and the β-form 1642 cm.$^{-1}$ and the random form at 1685 cm.$^{-1}$. An intense peak at 1675 cm.$^{-1}$ has been reported by Bradbury and Elliott for the heated film of poly-*n*-propyl aspartate which characterizes the ω-helix.[21,22] They suggested that this helical conformation is stable only in the solid state. However, since we find bands close to 1675 cm.$^{-1}$ in films and solutions, β-methyl L-aspartate high oligomers and polymers may exist in a conformation similar to the ω-helix.

TABLE V
INFRARED SPECTRA OF OLIGOMERIC AND POLYMERIC PEPTIDES DERIVED FROM β-METHYL L-ASPARTATE IN CHLOROFORM[a]

			Frequency, cm.$^{-1}$						
			Ester carbonyl			Amide			
Oligomer	Concn., %	Time,[b] hr.	1745	1735	1715	Random 1685	Helical 1675	Associated 1642	Temp., °C.
Tetra-	0.3	0.5	...	1	...	0.56	..	..	25
Penta-	.3	.5	...	1	...	.57	..	..	25
Hexa-	.3	.5	...	1	...	.61	..	..	25
Octa-	.25	.5	1	...	...	.44	..	0.79	25
	.06	.5	1	...	...	.67	..	.42	25
	.01	24	...		(←1→)[c]	.68	..	.18	25
Undeca-	.10	0.5	...	1	...	(←0.66→)		.25	25
	.10	24	...		(←1→)	(←0.79→)		.50	25
	.03	24	...	1	...	(←0.49→)		.24	25
	.01	24	...	1	...	..	0.76	.25	25
Tetradeca-	.10	0.5	...	1	...	(←0.71→)		.27	25
	.10	24	...	1	...	(←0.41→)		.41	25
	.03	24	...	1	...	(←0.41→)		.41	25
	.01	24	...		(←1→)[c]	(←0.23→)		..	25
Polymers									
$(A/I\ 5.3)$[d]	0.3	0.5	1	...	...	..	0.67	0.42	25
$(A/I\ 46)$[d]	0.1	0.5	(←1→)		...	..	0.67	0.14	25

[a] The intensities of all peaks are related to the ester carbonyl stretching bond which is arbitrarily set at a value of one in each spectrum. [b] The measurements were carried out after the solution was stored for the indicated time. [c] (←→) indicates the breadth of the band. [d] (A/I) indicates the anhydride to initiator ratio.

Infrared Spectra.—The infrared spectra of the oligomers and polymers in chloroform solution and as solid films were measured. Because of the absorption characteristics of chloroform, only the region of 2000 to 1620 cm.$^{-1}$ was investigated, covering the ester carbonyl frequencies and the amide I frequencies. The films were studied over the range 5000 to 600 cm.$^{-1}$.

Table V lists the absorption frequencies of the peptides in chloroform in the range 1745 to 1620 cm.$^{-1}$. In order to compare the absorption intensities on a semiquantitative basis the intensity of the ester carbonyl peak in each spectrum was arbitrarily set at a value of "1." Amide I intensities were then related to the ester carbonyl absorption intensities. The marking "(↔)" indicates the breadth of the given bands covering the frequencies under which the marking occurs.

Miyazawa's compilation[20] of amide I peaks for the various conformations of poly-γ-benzyl L-glutamate assigns amide I absorptions for the random coil at 1658 cm.$^{-1}$, for the helix at 1650 cm.$^{-1}$, and for β-structure at 1630 cm.$^{-1}$.

None of these assignments appears to be applicable to the aspartate oligomers and polymers which we studied. Elliott, Bradbury, *et al.*,[21] showed that for films of poly-β-*n*-propyl L-aspartate, the helical amide I peak occurs at 1662 cm.$^{-1}$ while the β-amide I peak occurs at 1637 cm.$^{-1}$. These values are much closer to those which we obtained for the β-methyl L-aspartate

TABLE VI
INFRARED SPECTRA OF OLIGOMERIC PEPTIDES DERIVED FROM β-METHYL L-ASPARTATE AT 25° AS FILMS CAST FROM CHLOROFORM[a]

	Frequency, cm.$^{-1}$				
	Ester carbonyl		Amide		
Oligomer	1745	1735	Random 1685	Helical 1670	Associated 1642
Octa-	1	....	....	..	1.3
Undeca-	1	....	....	..	1.2
Tetradeca-	1	....	....	..	2.3
Polymer $(A/I\ 46)$	(←1→)		..	0.74	0.23

[a] Solutions of these materials were cast as films 30 min. after the peptides dissolved.

An unexpected feature of poly-β-methyl L-aspartate spectra in chloroform is the variable range of the ester carbonyl band (from 1745 to 1715 cm.$^{-1}$). Sometimes this band actually splits into two bands. The tetramer, pentamer and hexamer show a sharp peak at 1735 cm.$^{-1}$ which is, we believe, correlatable with the random coil conformation. In the octamer (0.25% and 0.06%) the peak shifts to 1745 cm.$^{-1}$ with a simultaneous appearance of a new intense peak at 1642 cm.$^{-1}$ Thus the ester carbonyl absorption at 1745 cm.$^{-1}$ is relatable to the β-associated form. Since only one methylene separates the ester group from the peptide main chain, it is not surprising that hydrogen bonding of the amide groups affects the ester absorption frequency. The highly dilute solution of octamer (0.01%) exhibits a broad ester absorption band at about 1730 cm.$^{-1}$, with a simultaneous decrease in the 1642 cm.$^{-1}$

(18) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 464 (1961).

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(21) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, R. D. B. Fraser, W. E. Hanby, and T. R. R. McDonald, *J. Mol. Biol.*, **2**, 276 (1960).

(22) E. M. Bradbury, L. Brown, A. R. Downie, A. Elliott, R. D. B. Fraser, and W. E. Hanby, *ibid.*, **5**, 230 (1962).

band and an increase in the 1685 cm.$^{-1}$ band. These shifts suggest that most of the β-structure has been replaced by random forms.

In the solid state, association is at a maximum and polypeptides which form only weak helices in solution probably exist solely in the β-form. The films of octamer and undecamer show very narrow peaks at 1745 cm.$^{-1}$ while the tetradecamer and low polymer have these peaks broadened as far as 1735 cm.$^{-1}$

Changes in the ester absorption frequency of the polypeptides have not been studied in great detail, although they have been reported by Elliott and his co-workers.[5] The polypeptide most studied, poly-γ-benzyl L-glutamate, does not have side chains which interact with the main chain amide groups. The frequency of the ester absorption, therefore, is always at 1735 cm.$^{-1}$.[5] Films of polymers of benzyl aspartate and propyl aspartate show ester absorption peaks from 1730 to 1737 cm.$^{-1}$ and 1738 to 1740 cm.$^{-1}$, respectively.[5]

As stated above, solutions of poly-β-methyl L-aspartate show the amide I band as a sharp peak at 1675 cm.$^{-1}$, which we believe is characteristic of a helical form. Solutions of tetramer, pentamer and hexamer show a band at 1685 cm.$^{-1}$ (as stated above) characteristic of the random coil.[23] The octamer solutions show a peak at 1685 cm.$^{-1}$ as well as a new peak at 1642 cm.$^{-1}$; the latter we identify with β-structure. In addition, the 1642 cm.$^{-1}$ band is the only amide I peak in the spectrum of the octamer film. The octamer, in chloroform, therefore exists partially as a random coil and partially in a β-associated form but does not contain helical conformers.

The spectra of the undecamer and tetradecamer present a more complicated picture. The amide I peak for the 0.10% solutions appears from 1675 to 1685 cm.$^{-1}$ and at 1642 cm.$^{-1}$, indicating the presence of helical structures, β-forms and random structures. After storage for 24 hr., the solutions show an increase in both 1675 and 1642 cm.$^{-1}$ peaks. This is probably caused by the conversion of some of the random coil material in solution to helical and β-structures.

Conclusions.—With the aid of infrared spectroscopy, it is possible to detect the presence of β-structures and separate their contribution from those of the helical and random coil conformations. This is not possible from optical rotatory measurements. However, the separation of helical from random coil contributions is more accurately made by optical rotatory measurements than by infrared spectroscopy. Thus both techniques are necessary in the determination of oligomer conformations.

Infrared spectral studies agree with the conclusions from optical rotatory measurements in chloroform. The dimer through hexamer exist in the random coil conformation; the octamer possesses β-structure in concentrated solution which upon dilution reverts to the random conformation. The undecamer and tetradecamer possess varying amounts of random and β-associated forms and also significant amounts of helical structure. Helical forms in chloroform probably commence when there are approximately 11 residues present.

The optical rotatory data show the great dependence of conformation on the nature of the solvent. Dichloroacetic acid forms strong hydrogen bonds with the oligomers and their conformation (which is random) is independent of the number of residues in the peptide chains. Dimethylformamide is a solvent of limited use for aspartate oligomers since peptides containing more than 8 residues are insoluble.

Chloroform is sufficiently non-polar to permit most types of hydrogen bonding in the oligomers. As mentioned previously, chloroform is unique in its solvent power for the aspartate oligomers. Measurements in chloroform show that β-structure is not observed until there are about 8 residues in the chain and helical forms are not stable until there are about 11 residues in the chain. Chloroform–dichloroacetic acid (97:3) and trifluoroethanol seem to be solvents which support some helical structure for β-methyl L-aspartate oligomers with 11 or more residues.

These studies attempt to clarify the interpretation of optical rotatory data and infrared spectra for aspartate oligomers and polymers. The influence of a polar side chain near the main chain of a polypeptide has been examined and explained in part. We have presented consistent data relating our findings from molar rotations, rotatory dispersion and infrared spectroscopy.

Experimental

Optical Rotations.—All rotations were measured using a Rudolph model 70 polarimeter with two modifications. The readings were determined by a photoelectric cell (Rudolph model 200).

The voltage applied to the photoelectric cell was varied by means of a Keithley voltage supply model 240. All measurements were recorded between 500 and 600 v. Increasing the voltage increased the sensitivity of the photoelectric cell galvanometer.[24]

Two light sources were employed. The polarimeter was first equipped with a sodium lamp (and filter for 589 mμ), a mercury lamp (and filters for 546, 435, 405, and 365 mμ), a cadmium lamp (and filters for 509 and 480 mμ), and a thallium lamp (with a filter for 535 mμ). We also employed a Bausch and Lomb grating monochromator in conjunction with a xenon–mercury lamp (Hanovia 901B). For these measurements we covered the spectral range 600 to 360 mμ. The polarimeter tubes used were 2 dm. in length with a bore not less than 3 mm. in diameter. The temperature of the tube was kept constant by a circulating pump connected to a constant temperature bath.

Infrared Spectra.—All infrared spectra were recorded on a Perkin–Elmer model 21 spectrophotometer using sodium chloride optics. The solution spectra were measured in matched cells ranging in thickness from 0.01 to 0.10 mm. All sample solutions were measured against the pure solvent. The chloroform used was reagent grade manufactured by the Brothers Chemical Corp., Orange, N. J.

Preparation of Compounds.[25]—Preparations of the oligomers derived from β-methyl L-aspartate were reported in the preceding paper in this series.[1]

Benzyloxycarbonylglycyl–Diethyl L-Aspartate.—Benzyloxycarbonylglycine (1.05 g., 0.005 mole) was dissolved in a 50–50 mixture of ethyl acetate and dimethylformamide and cooled to 0°. Isobutyl chloroformate (0.7 ml., 0.005 mole) was added, followed by triethylamine (0.7 ml., 0.005 mole). The reaction proceeded for 20 min. Diethyl L-aspartate·hydrochloride (1.13 g., 0.005 mole) was then added, followed by the slow addition of triethylamine (0.7 ml., 0.005 mole). The reaction was allowed to proceed for 4 hr. after which time the reaction mixture was diluted with 100 ml. of ethyl acetate. The solution was extracted with hydrochloric acid (2 *N*), and aqueous potassium chloride and aqueous sodium bicarbonate solutions. The organic solution was dried over magnesium sulfate and the solvent distilled under reduced pressure. The resulting oil was crystallized and recrystallized from ethyl acetate–ether to give needles, 1.5 g. (79%), m.p. 48–49°.

Anal. Calcd. for $C_{18}H_{24}N_2O_7$: C, 56.84; H, 6.32; N, 7.37. Found: C, 56.99; H, 6.48; N, 7.45.

Benzyloxycarbonyl-β-methyl-L-aspartylglycine Methyl Ester.—Benzyloxycarbonyl-β-methyl L-aspartate (0.66 g., 0.0025 mole) was dissolved in a 50–50 mixture of ethyl acetate–dimethylformamide and cooled to 0°. Isobutyl chloroformate (0.35 ml., 0.0025 mole) and triethylamine (0.35 ml., 0.0025 mole) were added. The reaction proceeded for 20 min. Glycine methyl ester hydrochloride (0.32 g., 0.0025 mole) was then added followed by slow addition of triethylamine (0.35 ml., 0.0025 mole). The reaction proceeded for 4 hr. and the mixture was then diluted

(23) These values are higher than those encountered with poly-γ-benzyl L-glutamate (see, for example, E. R. Blout and A. Assadourian, *J. Am. Chem. Soc.*, **78**, 955 (1956)).

(24) We wish to thank Dr. J. Rank and Prof. W. Kauzmann for the suggestion to improve sensitivity by increasing the voltage across the photocells.

(25) All melting points are corrected. All analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, Long Island, N. Y.

with 75 ml. of ethyl acetate. The reaction mixture was extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and sodium bicarbonate solutions. The organic solution was dried over magnesium sulfate and the solvent removed under reduced pressure. The crystals obtained were recrystallized several times from ethyl acetate–ether solution yielding 0.66 g. (75%), m.p. 127–128°.

Anal. Calcd. for $C_{16}H_{20}N_2O_7$: C, 54.55; H, 5.68; N, 7.96. Found: C, 54.52; H, 5.66; N, 7.80.

Benzyloxycarbonylglycyl-β-methyl-L-aspartyl-glycine Methyl Ester.—Benzyloxycarbonyl glycine (1.4 g., 0.0066 mole) was dissolved in dimethylformamide and the solution cooled to 0°. Triethylamine (1 ml., 0.0066 mole) and isobutyl chloroformate (1 ml., 0.0066 mole) were added, and the reaction was allowed to proceed for 20 min. β-Methyl α-*p*-nitrophenyl-L-aspartate (2.3 g., 0.0066 mole) in dimethylformamide was then added followed by slow addition of triethylamine (1 ml., 0.0066 mole). After 4 hr., glycine methyl ester hydrochloride (0.82 g., 0.0066 mole) was added followed by slow addition of triethylamine (1 ml., 0.0066 mole). After 14 hr., the solution was diluted with ethyl acetate (125 ml.) and extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and saturated sodium carbonate solutions until the yellow *p*-nitrophenoxide color disappeared. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The resulting solid was recrystallized a few times from a minimum of hot ethyl acetate yielding 1.55 g. (57%), m.p. 141°.

Anal. Calcd. for $C_{18}H_{23}N_3O_8$: C, 52.81; H, 5.63; N, 10.25. Found: C, 53.04; H, 5.83; N, 10.21.

Benzyloxycarbonyl-di-(β-methyl-L-aspartyl)-glycine Methyl Ester.—Benzyloxycarbonyl-β-methyl-L-aspartyl-β-methyl-α-*p*-nitrophenyl L-aspartate (0.56 g., 0.001 mole) was dissolved in dimethylformamide and cooled to 15°. Glycine methyl ester hydrochloride (0.25 g., 0.002 mole) was added followed by slow addition of triethylamine (0.14 ml., 0.001 mole). The reaction proceeded for 12 hr. after which time the volume was reduced under vacuum and ethyl acetate (150 ml.) was added. The solution was extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and saturated sodium carbonate solutions until colorless. The organic layer was dried over magnesium sulfate and the solvent removed under reduced pressure. The resulting solid was recrystallized three times from hot ethyl acetate, yielding 0.3 g. (63%), m.p. 115°.

Anal. Calcd. for $C_{21}H_{27}N_3O_{10}$: C, 52.39; H, 5.61; N, 8.73. Found: C, 51.97; H, 5.74; N, 8.53.

Benzyloxycarbonyl-glycyl-β-methyl-L-aspartyl–Diethyl L-Aspartate.—Benzyloxycarbonylglycine (0.29 g., 0.001 mole) was dissolved in dimethylformamide and cooled to 0°. Isobutyl chloroformate (0.14 ml., 0.001 mole) and triethylamine (0.14 ml., 0.001 mole) were added and the reaction allowed to proceed for 20 min. A solution of β-methyl α-*p*-nitrophenyl-L-aspartate hydrobromide was added (0.35 g., 0.001 mole) followed by addition of triethylamine (0.14 ml., 0.001 mole) and the reaction was allowed to proceed for 4 hr. Diethyl L-aspartate hydrochloride (0.23 g., 0.001 mole) was then added followed by slow addition of triethylamine (0.14 ml., 0.001 mole). After 12 hr., the solution was diluted with ethyl acetate and extracted with 2 *N* hydrochloric acid, aqueous potassium chloride and sodium carbonate solutions until colorless. The organic layer was dried over magnesium sulfate and the solvent distilled under reduced pressure. The resulting compound was recrystallized twice from ethyl acetate–ether–petroleum ether to give 0.4 g. (79%) of the desired product, m.p. 115°.

Anal. Calcd. for $C_{23}H_{31}N_3O_{10}$: C, 54.22; H, 6.09; N, 8.25. Found: C, 53.88; H, 6.24; N, 8.10.

Benzyloxycarbonylglycyl-di-(β-methyl-L-aspartyl)-glycine Methyl Ester.—Benzyloxycarbonyl-di-(β-methyl-L-aspartyl)-glycine methyl ester (0.5 g., 0.0012 mole) was treated with 1.5 ml. of dry hydrogen bromide (33%) in glacial acetic acid. After 30 min. the compound dissolved with evolution of carbon dioxide. An oil separated on addition of ether. The ethereal solution was decanted, and the oil triturated several times with ether. The oil was dissolved in methanol, precipitated twice with ether, and dried under vacuum to yield 0.43 g. of product (0.001 mole) which was used immediately in the following reaction.

Benzyloxycarbonylglycine (0.2 g., 0.001 mole) was dissolved in dimethylformamide and cooled to 0°. Isobutyl chloroformate was added (0.14 ml., 0.001 mole) followed by addition of triethylamine (0.14 ml., 0.001 mole). The reaction was allowed to proceed for 20 min. The hydrobromide oil (0.43 g., 0.001 mole) prepared above was added and then triethylamine (0.14 ml., 0.001 mole) was added slowly.

After 4 hr. the solution was diluted with ethyl acetate (200 ml.) and washed with 2 *N* hydrochloric acid, aqueous potassium chloride, and sodium bicarbonate solutions. The ethyl acetate layer was dried over magnesium sulfate and the solvent removed under reduced pressure. The resulting solid was recrystallized from warm ethyl acetate several times to give 0.28 g. (51%) of product, m.p. 181°.

Anal. Calcd. for $C_{23}H_{30}N_4O_{11}$: C, 51.30; H, 5.58; N, 10.41. Found: C, 50.94; H, 5.66; N, 10.19.

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND NUTRITION, GRADUATE SCHOOL OF PUBLIC HEALTH AND BIOCHEMISTRY SCHOOL OF MEDICINE, UNIVERSITY OF PITTSBURGH, PITTSBURGH, PENNA.)

Gas–Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances[1]

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The separation and estimation of carbohydrates and related polyhydroxy compounds by gas–liquid chromatography of trimethylsilyl (TMS) derivatives is described. The formation of the TMS derivative, in pyridine containing hexamethyldisilazane and trimethylchlorosilane, occurs very rapidly at room temperature so that analyses can be made within a few minutes. Comparative studies of the reaction product of methyl α-glucopyranoside and authentic methyl (tetra-*O*-trimethylsilyl)-α-glucopyranoside indicate that silylation of all free hydroxyl groups occurs and that the yield of TMS derivative is virtually quantitative. Conditions are described for chromatography of a wide variety of carbohydrates from C_2 (glycolaldehyde) to C_{24} (stachyose) and related substances such as glycosides, deoxysugars, inositols, hexosamines, and N-acetylneuraminic acid. Most of the studies have been made with a silicone column (SE-52) and a polyester column (polyethylene glycol-succinate) but separations of the TMS derivatives are possible on other polar and non-polar columns. Isothermal conditions are usually employed for separations within a narrow range of molecular weight; separations of more complex mixtures, with components of widely differing molecular weights, may be made by linear temperature-programmed analysis. Excellent separations are generally observed with anomeric pairs as well as configurational isomers within a given class such as pentoses, hexoses, disaccharides, etc. The identity of an unknown sugar may be determined by multiple analyses on a number of liquid phases or, alternatively, by analyses of the parent sugar and various derivatives such as methyl glycoside, alcohol, lactone, oxime, and acetal. In all such cases TMS derivatives are prepared prior to gas chromatography. Comparisons are reported for the compositions of aqueous equilibrium solutions of aldoses, by gas chromatographic analysis, with those reported by measurements of optical rotation and bromine oxidation. In several cases unexpected retention times are interpreted in terms of conformational differences of the sugars.

The application of gas chromatography to the separation of carbohydrates and related polyhydroxy compounds has tended to lag behind the development of this technique with other classes of compounds. A major difficulty has been the preparation of volatile derivatives of the polyhydroxy compounds by rapid and general techniques. Investigations so far described have generally made use of either *O*-methyl ethers or acetyl derivatives; a comprehensive review of separa-

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