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## The Interaction of Polyvinylpyrrolidone with Aromatic Compounds in Aqueous Solution. Part II.<sup>1</sup> The Effect of the Interaction on the Molecular Size of the Polymer<sup>2</sup>

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The systems studied in Part I have been further investigated by light scattering and viscometry to determine the effect of the interaction on the molecular size of the polymer. With anionic cosolutes the viscometric results indicate that, in general, the polymer expands; this is attributed to the coulombic repulsions between the bound anions on the polymer chain. At the higher concentrations of these cosolutes the increases in size reach essentially constant values, or actually fall with increasing concentration (with sodium phenanthroate); a lessened increase is observed, with sodium 2-naphthoate as cosolute, on the addition of a non-bound salt (sodium chloride); these effects are attributed to "screening," by the free counterions within the polymer molecule, of the coulombic repulsions between the bound anions. With non-ionic cosolutes the viscometric results show that the polymer molecule contracts; at sufficiently high concentrations of phenolic cosolutes polymer precipitation occurs. These effects seem to indicate the formation of labile "cross-links" due to hydrogen bonding between suitable groups on the bound cosolute molecules and on the polymer molecule. The light scattering data parallel those from viscometry for both types of cosolutes; they also indicate that the random-flight configuration is, in general, retained upon expansion or contraction; however, the most strongly bound non-ionic cosolutes produce deviations from this configuration, indicating some folding-up of the polymer coil due to extensive "cross-linking." No appreciable effects are produced by cationic cosolutes such as aniline hydrochloride or naphthylamine hydrochloride.

### Introduction

It has been shown<sup>4-6</sup> that bound cosolutes affect the size of the polyvinylpyrrolidone (PVP) molecule in aqueous solution; in the studies described in the present paper essentially the same types of aromatic cosolute species as those dealt with in Part I<sup>1</sup> have been used to investigate the effect of cosolute structure on these phenomena. The investigations have involved both viscometry and light scattering; the viscometric studies were extended over a wide range of cosolutes, whose molecular size and substituent groups were varied systematically; the light scattering studies involved fewer cosolutes but gave more direct data on the size and configuration of the polymer molecule in solution.

### Experimental

**Materials.**—The polymer and the cosolutes were from the same sources as those used in Part I<sup>1</sup>; the polymer was not, however, treated by ion-exchange or dialysis for either the viscometric or light scattering measurements.

**Viscometry.**—The measurements were carried out at  $30.00 \pm 0.05^\circ$ ; the instrument used was a suspended-level dilution viscometer, with a flow time for water of 273 sec. and a mean shear gradient of about  $1500 \text{ sec.}^{-1}$ ; kinetic energy corrections, and corrections for the change in the density of the solution with dilution, were considered sufficiently small to be neglected. All solutions were filtered through medium grade sinters before use to remove dust particles, etc. The concentrations of the polymer solutions were determined by evaporation of 5-ml. samples in an oven at  $125^\circ$ , followed by heating *in vacuo* at  $85^\circ$  to constant weight (6 hr.) to remove the residual moisture (1-6%). The viscosity runs in the presence of a cosolute covered by dilution the polymer concentration range  $c = 0.5$  to  $0.1 \text{ g./100 ml.}$ ; for each such run, flow times were also taken for water, for the cosolute solution in the absence of polymer

and for the polymer solution (at  $c = ca. 1 \text{ g./100 ml.}$ ) in the absence of cosolute. The flow times noted were in general the mean of two consecutive flow times differing by 0.2 sec. or less. The viscosity measurements in methanol were carried out in essentially the same fashion as those in aqueous solution.

**Light Scattering.**—Solutions, of known concentration, of the aromatic cosolutes in pure water and in solutions of PVP, also of known concentration (*ca.* 1 g./100 ml.), were prepared. The polymer-free solutions were used as "solvent" and were clarified by pressure filtration through ultrafine fritted discs; the PVP-cosolute solutions were clarified by high speed centrifugation (30 minutes at 15000 g) and weighed increments of these solutions were mixed with weighed amounts of the filtered "solvent." The light-scattering measurements were carried out on a B.-S. Phoenix light-scattering photometer, using unpolarized radiation of 4360 Å. wave length; readings were taken over a sufficient range of angles and polymer concentrations to give satisfactory Zimm plots. Depolarization and back-reflection corrections were applied. A refractive index increment of 0.185 ml./g.<sup>7</sup> was used. All measurements were taken at room temperature (*ca.*  $25^\circ$ ).

### Results

**Viscometry. Cosolute Solutions.**—It has been shown<sup>8</sup> that for dilute aqueous solutions the specific viscosity  $\eta_{sp}$  depends upon the molar concentration  $[A]$  of the solute according to a relation of the type  $\eta_{sp} = \alpha[A]^{1/2} + \beta[A]$ ; for non-electrolytes  $\alpha = 0$ . A plot of  $\eta_{sp}/[A]^{1/2}$  versus  $[A]^{1/2}$  should therefore give a straight line with slope  $\beta$  and intercept  $\alpha$ . From the flow times for water and for cosolute solutions in the absence of polymer, determined in the course of this work, values for  $\eta_{sp}/[A]^{1/2}$  were calculated and those for the most extensively studied cosolutes are plotted against  $[A]^{1/2}$  in Figure 1; as will be seen, the plots for the non-ionic cosolutes are straight lines going through the origin, as expected; the anionic cosolutes also give straight lines, but the intercepts ( $\alpha$  values) appear in some cases to be negative; in previous work,<sup>8</sup> where small inorganic ions were involved, the  $\alpha$  values always have been positive. The  $\beta$  values obtained from these plots show correlations with the size of, and substituents on, the cosolute molecules.

**Polymer Solutions in the Absence of Cosolute.**—Numerous runs were carried out on the polymer in

(1) Part I: P. Molyneux and H. P. Frank, *J. Am. Chem. Soc.*, **85**, 3169 (1961).

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(4) S. Barkin, H. P. Frank and F. R. Eirich, *Ric. Scient.*, "Simposio Internazionale di Chimica," **25A**, 844 (1955).

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(6) S. Barkin, Ph.D. thesis, Polytechnic Institute of Brooklyn, 1957.

(7) G. B. Levy and H. P. Frank, *J. Polymer Sci.*, **17**, 247 (1955).

(8) R. W. Gurney, "Ionic Processes in Solution," McGraw-Hill Book Co., New York, N. Y., 1953, Chapter 9.

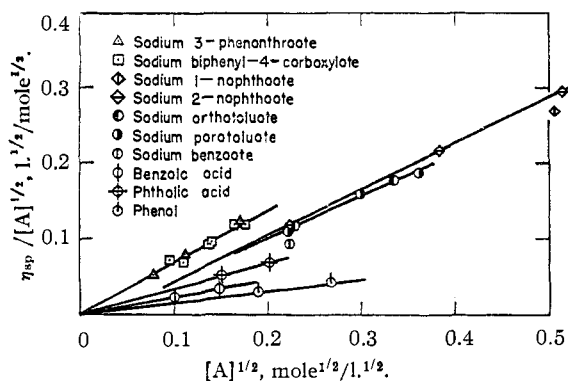


Fig. 1.—Viscometry on the cosolute solutions:  $\eta_{sp}/[A]^{1/2}$ ,  $l.^{1/2} \text{ mole}^{-1/2}$ , versus  $[A]^{1/2}$ ,  $\text{mole}^{1/2} l.^{-1/2}$ .

the absence of a cosolute and showed the intrinsic viscosity  $[\eta]$  to vary within the range  $1.38 \pm 0.04 \times 10^2$  g./ml. and the Huggins parameter  $k'^9$  to have the value  $0.354 \pm 0.008$  (one s.d.); the variations in intrinsic viscosity could be ascribed to the variations between different samples of the polymer and also to a time-dependent change (a fall, amounting maximally to about 4% per week) in the intrinsic viscosity for polymer solutions from individual polymer samples; from the weight-average molecular weight  $\bar{M}_w$  of  $1.01 \times 10^6$  determined in this work by light scattering and from the published relationship<sup>7</sup> between  $\bar{M}_w$  and  $[\eta]$  for the unfractionated polymer, one would expect an intrinsic viscosity value of  $1.13 \times 10^2$  g./ml. at  $25^\circ$ .

#### Polymer Solutions in the Presence of Cosolute.

The measurements on polymer solutions in the presence of cosolutes gave in general linear  $\eta_{sp}/c$  versus  $c$  plots from which the  $[\eta]$  and  $k'$  values were readily obtained; the most active cosolutes gave slightly curved plots which were made linear by adding suitable polymer-concentration-dependent correcting terms to the  $\eta_{sp}/c$  values. (All of the  $\eta_{sp}$  values were calculated assuming the liquid to have its analytical cosolute concentration  $[A]$ , i.e., neglecting any reduction in this concentration due to the binding of the cosolute by the polymer; calculations show that such binding has a negligible effect on the  $\eta_{sp}$  values, except for the most strongly bound cosolutes, where it may be responsible for the curvature in the  $\eta_{sp}/c$  versus  $c$  plots. The  $[\eta]$  values, of course, since they correspond to infinite dilution of the polymer, do refer in the strict sense to  $[A]$ .) For each run carried out the intrinsic viscosity of the polymer in the absence of cosolute,  $[\eta]_0$ , was calculated from the flow time for the polymer solution (at  $c = ca. 1$  g./100 ml.) and the known Huggins parameter (0.354); the ratio,  $[\eta]/[\eta]_0$ , is termed here the "intrinsic viscosity ratio,"  $V$ , and deviations of the ratio from unity indicate directly the effect of the cosolute on the size of the polymer molecule in solution; the use of this ratio also removes the uncertainties caused by variations of  $[\eta]_0$  with polymer sample and with time.

(a) **Anionic Cosolutes.**—Table I shows the results obtained with anionic cosolutes, the accompanying cation being in all cases the sodium ion; the results are set out essentially in the order of

decreasing  $V$  values. The numbers in the first column are run numbers. (In the cases of sodium biphenyl-4-carboxylate and sodium 3-phenanthroate the  $\eta_{sp}/c$  versus  $c$  plots were curved; the curvatures were removed by the addition of  $\alpha c^2$  terms (with the parameter  $\alpha$  constant for a given run) to the  $\eta_{sp}/c$  values; on analogy with the derivation of the dimensionless Huggins slope parameter  $k'$ , we may calculate a dimensionless curvature parameter,  $l'$ , given by  $l' = \alpha/[\eta]^3$ . The values for this parameter lay within the ranges 0.05–0.07 and 0.10–0.16 for the two cosolutes in question).

TABLE I

#### VISCOMETRY RESULTS WITH ANIONIC COSOLUTES

Run	Cosolute, A	$[A]$ , mole/l., $\times 10^3$	$V$	$k'$	$f_a$
57	Sodium 3-phenanthroate	6.2	1.765	0.40	0.65
56	Sodium 3-phenanthroate	12.6	1.73	.50	.785
59	Sodium 3-phenanthroate	29.1	1.455	.48	.90
67	Sodium biphenyl-4-carboxylate	8.95	1.41	.325	.40
68	Sodium biphenyl-4-carboxylate	12.1	1.42	.35	.475
18	Sodium biphenyl-4-carboxylate	18.4	1.455	.33	.58
45	Sodium biphenyl-4-carboxylate	27.1	1.46	.355	.67
16	Sodium biphenyl-4-carboxylate	31.0	1.46	.36	.70
43	Sodium 2-naphthoate	50	1.31	.295	.65
44	Sodium 2-naphthoate <sup>b</sup>	49	1.065	.30	.645
14	Sodium 2-naphthoate	116	1.405	.29	.81
49	Sodium 2-naphthoate	147	1.405	.28	.845
52	Sodium 2-naphthoate	264	1.425	.31	.905
6	Sodium 1-naphthoate	13.0	1.09	.27	..
8	Sodium 1-naphthoate	29.6	1.16	.31	..
7	Sodium 1-naphthoate	58.2	1.235	.30	..
5	Sodium 1-naphthoate	125	1.235	.32	..
66	Sodium 1-naphthoate	256	1.295	.33	..
22	Sodium <i>p</i> -toluate	49	1.11	.335	..
47	Sodium <i>p</i> -toluate	90	1.11	.36	..
19	Sodium <i>p</i> -toluate	131	1.19	.33	..
15	Sodium benzenesulfonate	91	1.135	.32	..
17	Sodium benzenesulfonate	91	1.13	.325	..
46	Sodium benzoate	49.8	1.095	.315	.62
3	Sodium benzoate	86.8	1.135	.30	.74
38	Disodium terephthalate	48.4	1.08	.355	..
21	Sodium <i>o</i> -toluate	52.1	1.05	.355	..
20	Sodium <i>o</i> -toluate	111.5	1.055	.38	..
51	Sodium thiocyanate	99	1.05	.36	..
42	Sodium chloride	100	1.015	.35	..
..	None	..	1.00	.355	.00
11	Sodium acetate	50	1.00	.375	..
40	Sodium phenolate	100	0.98	.42	..
35	Disodium phthalate	49.2	.98	.375	.33
13	Sodium <i>p</i> -hydroxybenzoate	122	.945	.45	..
33	Sodium hydrogen phthalate	23.5	.98	.35	..
31	Sodium hydrogen phthalate	49	.955	.355	..

<sup>a</sup> See text. <sup>b</sup> In the presence of 0.10 *M* sodium chloride.

The final column of Table I shows values of  $f$ , the fractional degree of coverage of the binding sites at that value of  $[A]$ , as calculated from  $f = k[A]/1 + k[A]$ , where  $k$  is the binding constant in liter/mole<sup>1</sup>; this derivation assumes that the concentration of free anions within the polymer coil is equal to  $[A]$ , whereas at finite degrees of binding it will be less; for the present systems, however, with only a small concentration of bound anions within the polymer coil even at saturation, the difference should be quite small.

Figure 2 shows plots of  $V$  versus  $[A]$  for the anionic cosolutes studied at two or more concentrations.

(b) **Non-ionic Cosolutes.**—Table II shows the results obtained with non-ionic cosolutes. With many of the cosolutes precipitation of the polymer

occurred above a certain cosolute concentration, and this limited the maximal concentrations at which, in particular, phenol, resorcinol and phloroglucinol could be studied; with the other cosolutes (and this applies also to sodium biphenyl-4-carboxylate in the anionic cosolutes) the solubility of the cosolute itself was the limiting factor. With the

TABLE II  
VISCOMETRY RESULTS WITH NON-IONIC COSOLUTES

Run	Cosolute, A	$[A]$ , mole/l., $\times 10^2$	$V$	$k'$	$f^a$	$D_{1/3}^a$
60	Nitrobenzene	7.7	0.985	0.365	0.38	0.01
41	Phenol	36	.88	.405	.345	.11
39	Phenol	72	.67	.585	.51	
28	Benzoic acid	10	.945	.385	.23	.09
24	Benzoic acid	16	.915	.415	.325	
29	Benzoic acid	22	.865	.40	.405	
26	Phthalic acid	23	.84	.40	.255	.25
30	Phthalic acid	41	.655	.53	.38	
48	Resorcinol	25	.515	.93	.395	.35
53	Phloroglucinol	10.4	.20	10.8	.50	.42
55	Phloroglucinol	8.8	.29	5.0	.46	
64	2-Naphthol	2.6	.71	0.885	.24	.42

<sup>a</sup> See text.

exception of phloroglucinol and 2-naphthol, all the runs gave linear  $\eta_{sp}/c$  plots from which the  $V$  and  $k'$  values given in Table II were obtained; in the cases of the two cosolutes cited the non-linearity was removed by the addition of terms of the form  $\beta/(c - 0.1)$  to the  $\eta_{sp}/c$  values,  $\beta$  being constant for a given run and ranging from 2 to  $4 \times 10^{-3}$  g./ml. for the three runs in question. In contrast to the behavior with most ionic cosolutes, the results in Table II for those cosolutes studied at more than one concentration show that here  $V$  falls almost linearly with increasing  $[A]$ . The penultimate column of Table II gives values of  $f$ , the fractional degree of saturation of the polymer sites at that particular cosolute concentration. The final column of the table indicates the values of  $D_{1/3}$ , the fractional degree of shrinkage at one-third saturation of the polymer sites, for each cosolute; these values were calculated by setting  $f = 1/3$  and hence obtaining  $[A]_{1/3}$  as  $1/2K$ , and then interpolating or estimating from a plot of  $V$  versus  $[A]$  the corresponding values of  $V_{1/3}$ ; subtraction of these from unity gave  $D_{1/3}$ . The values of  $D_{1/3}$  are measures of the "shrinking efficiencies" of the cosolutes (see Discussion).

(c) **Cationic Cosolutes.**—Table III shows the results obtained with three cationic cosolutes, the chloride ion being the counterion in each case.

TABLE III  
VISCOMETRY RESULTS WITH CATIONIC COSOLUTES

Run	Cosolute, A	$[A]$ , mole/l., $\times 10^2$	$V$	$k'$
10	Aniline hydrochloride	46	1.01	0.345
12	<i>p</i> -Hydroxyaniline hydrochloride	59	0.995	.38
70	2-Naphthylamine hydrochloride	91	0.995	.37

(d) **Viscometry in Methanol.**—Some measurements were carried out on representative cosolutes

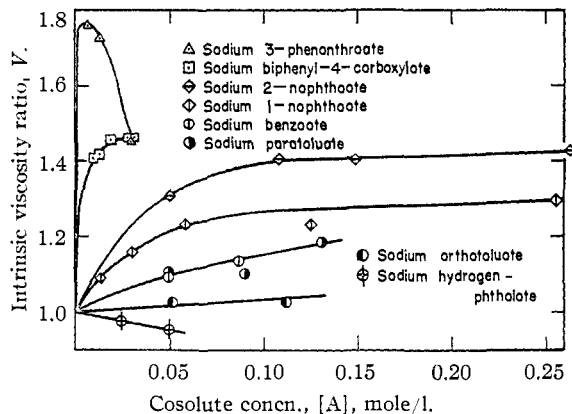


Fig. 2.—Intrinsic viscosity ratio,  $V$ , versus molar cosolute concentration,  $[A]$ , for anionic cosolutes.

using methanol as solvent; the intrinsic viscosity of the polymer in this solvent was  $1.82 \times 10^2$  g./ml. Table IV indicates the intrinsic viscosity ratio values that were obtained; in all three cases the  $\eta_{sp}/c$  versus  $c$  plots were linear.

TABLE IV  
VISCOMETRY RESULTS IN METHANOL

Cosolute, A	$[A]$ , mole/l., $\times 10^2$	$V$
Sodium 3-phenanthroate	25	0.935
2-Naphthol	3	.92
Phloroglucinol	10	.90

**Light Scattering.**—The light scattering data obtained in aqueous solution with various cosolutes were treated in the conventional manner on reciprocal intensity plots ("Zimm plots"). The results are summarized in Table V. The experimentally determined weight-average molecular weights  $\bar{M}_w$  are listed in the third column of this table; in all the PVP-cosolute systems the polymer will bind a certain amount of cosolute which will increase the apparent molecular weight, and from the equilibrium data presented in Part I,<sup>1</sup> it is possible to calculate this amount and hence the molecular weight that would be expected; the values of this calculated molecular weight are set out in the fourth column of Table V; if anything the corrections seem to overcompensate for the binding; the calculations do not take into account any possible changes in the refractive index increment due to the presence of the cosolute on the coil, but at low degrees of binding these changes should be quite small. The last three columns of Table V indicate the second virial coefficient,  $A_2$ , the  $z$ -average root mean square radius of gyration of the polymer chain,  $\langle \bar{R}^2 \rangle_z^{1/2}$ , and finally the intrinsic viscosity ratio,  $V$ , calculated from the viscometry data set out in the previous section.

## Discussion

**Viscometry.**—From the results obtained from viscometry, *i.e.*, from the values of  $V$ , the intrinsic viscosity ratio (which is a direct measure of the change in the volume of the polymer molecule), it is evident that in general anionic cosolutes produce increases in the volume of the polymer molecule in solution, non-ionic cosolutes produce de-

creases, and cationic cosolutes have no appreciable effect.

TABLE V  
LIGHT SCATTERING RESULTS FROM AQUEOUS PVP-COSOLUTE SYSTEMS

Cosolute, A	[A], mole/l., $\times 10^4$	$\bar{M}_w \times 10^{-5}$		$A_2$ , cm. <sup>2</sup> mole g. <sup>-2</sup> , $\times 10^4$	$\langle \bar{R}^2 \rangle^{1/2}$ , Å.	V
		Exp.	Calcd.			
Sodium 3-phenanthroate	8.1	1.11	1.16	7.1	710	1.76
Sodium biphenyl-4-carboxylate	7.65	1.02	1.08	6.5	687	1.40
Sodium biphenyl-4-carboxylate	10.8	1.05	1.10	5.6	675	1.42
Sodium biphenyl-4-carboxylate	25.5	1.065	1.14	5.9	660	1.46
Sodium benzoate	6.7	1.00	1.03	3.8	640	1.02
Sodium benzoate	50	1.02	1.06	3.6	630	1.09
None	...	1.01	1.01	3.3	590	1.00
Benzoic acid	13.1	0.99	1.04	2.7	590	0.93
Phenol	20	1.00	1.05	2.7	572	.90
Phenol	70	0.96	1.07	1.6	500	.68
2-Naphthol	2.2	1.00	1.04	3.0	520	.65
Phloroglucinol	8.2	1.075	1.07	1.2	327	.30

(a) **Anionic Cosolutes.**—With the four anionic cosolutes whose binding constants are known accurately,<sup>1</sup> the increases are directly in the order of these binding constants: benzoate,  $K = 33$  l./mole; 2-naphthoate,  $K = 37$  l./mole; biphenyl-4-carboxylate,  $K = 75$  l./mole; 3-phenanthroate,  $K = 300$  l./mole. It is apparent from this correlation that the observed expansions are due basically to the coulombic repulsions between the anions bound to the polymer coil; these repulsions are, however, reduced by the "screening" effect of the free counterions within the solution encompassed by the polymer molecule; this was clearly shown by the consequence of adding non-bound salt (sodium chloride) to a run with sodium 2-naphthoate (Table I, runs 43 and 44) when the tripling in the total sodium ion concentration reduced the expansion to about one-fifth of its former value. This counterion "screening" must also be responsible for the shape of the  $V$  versus  $[A]$  plots (Fig. 2), *i.e.*, for the flattening off with increasing concentration and, with the most active cosolute, phenanthroate, for the presence of the (unresolved) maximum in the plot.

From the correlations between the height and form of the  $V$  versus  $[A]$  plots and the known  $K$  values, it is evident that the  $V$  values can be used to obtain estimates of the binding constants where these were not determined by dialysis equilibria; these considerations indicate, for example, that the 1-naphthoate ion is bound a little less strongly than the 2-naphthoate ion, that the *p*-toluate and benzenesulfonate ions are bound about as strongly as the benzoate ion and that the *o*-toluate ion is bound much less strongly than these latter three. Further, there is a remarkable contrast between the results for disodium terephthalate (run 38,  $V = 1.08$ ) and disodium phthalate (run 35,  $V = 0.98$ ) (both at  $[A] = ca. 0.05 M$ ); the binding constant for the terephthalate ion is not known, that for the phthalate ion (see Part 1<sup>1</sup>) is very small (10 l./

mole) and at about the limit of detection by the dialysis method. It is evident that a group substituted *ortho* to the carboxyl group has a definite effect in reducing the binding strength (*i.e.*, compare 1- and 2-naphthoate, *o*- and *p*-toluate and phthalate and terephthalate). The low (*i.e.*, less than unity)  $V$  value for phthalate may indicate that, for the anions in general, in addition to the coulombic repulsions which cause the polymer coil to expand, there may also be a shrinking effect which is dominant in this particular case.

It will be also seen from Table I that a number of the ions—chloride, acetate and phenolate—are essentially inactive in the viscometry, indicating no appreciable binding of these; the small, but positive, effect with sodium thiocyanate (run 51,  $V = 1.05$ ) shows there to be a small degree of binding of this ion: the binding probably is a consequence of the polarizability of the ion and is in this respect reminiscent of the triiodide ion.<sup>4</sup>

The *reductions* in size caused by sodium *p*-hydroxybenzoate (run 13) and sodium hydrogen phthalate (runs 31 and 33) evidently are due to the non-ionic groups on the anions; apparently in these cases any expansive effect due to the coulombic repulsions between bound anions is masked by a shrinkage effect whose mechanism is probably the same as that (see below) which leads to the shrinkages caused by phenol, benzoic acid, etc.

The Huggins parameters,  $k'$ , for the polymer in the presence of anionic cosolutes (Table I, column five) show small but probably significant deviations from the value of 0.354 for the polymer in the absence of cosolute; the deviations are, however, in general too irregular for them to be correlated with any definite molecular effects.

(b) **Non-ionic Cosolutes.**—The results in Table II indicate that in all cases the presence of a non-ionic cosolute causes shrinking of the polymer coil.

It may be assumed, firstly, that at the low cosolute concentrations involved in these studies, the cosolute molecules in the liquid within the polymer molecule have no direct effect on the polymer-solvent interactions and that the changes in the coil size are solely and directly due to the bound cosolute molecules.

Now the values of  $D_{1/3}$  (the fractional degree of shrinkage for one-third coverage of the binding sites, last column of Table II), which are direct measures of the "shrinking efficiencies" of the cosolutes, show that these "efficiencies" vary greatly from one cosolute to another; from this it would seem that "iceberg" disordering is not likely to be a primary factor in the shrinking process, since it was shown (see Part 1<sup>1</sup>) that the  $\Delta S_u$  values for the four cosolutes: nitrobenzene, phenol, benzoic acid and 2-naphthol, do not differ very greatly (varying between 12 and 17 e.u.) thus indicating a similar degree of "iceberg" disordering, whereas their  $D_{1/3}$  values vary over a forty-fold range. Further, it is apparent from Table II that the  $D_{1/3}$  value for any cosolute is directly related to the number of hydroxyl or carboxyl groups that it bears, and it is therefore most plausible to attribute the shrinking to the production of labile "cross-links" by the bound cosolute molecules, through the formation of

hydrogen bonds between a substituent hydroxyl or carboxyl group (or groups) and another section of the same polymer molecule. (It is also evident, from the high  $D_{1/2}$  value for 2-naphthol (0.42) as compared with that for phenol (0.11), that the second ring of the naphthol molecule can also act as a "cross-link" former). Considerations from the theory<sup>10,11</sup> of tetrafunctional cross-links indicate that between 1 (for phenol) and 10 (for phloroglucinol) cross-links would be required to produce the observed degree of shrinking; since the polymer molecule has about 450 binding sites (as calculated from the weight-average molecular weight  $\bar{M}_w$  of  $1 \times 10^6$ , and hence a probable number-average molecular weight  $\bar{M}_n$  of  $5 \times 10^5$ , and with the monomer molecular weight of 111 and about 10 monomer units per site), then that at the degrees of saturation involved in the present studies only a few of the bound cosolute molecules would, at any instant, actually be involved in "cross-linking." Under the extreme conditions of very high degrees of "cross-linking" and of the formation of intermolecular "cross-links," polymer precipitation may result, and this is observed at higher concentrations of the phenolic cosolutes. Further, the Huggins parameters,  $k'$ , for the polymer in the presence of anionic cosolutes (Table II, column five) are all higher than the value (0.354) for the polymer in the absence of cosolute, the values becoming very high in the cases of the most active cosolutes; thus *inter-* as well as *intra-*molecular "cross-linking" is indeed favored by the presence of the bound cosolute molecules.

(c) **Cationic Cosolutes.**—The results in Table II show that cationic cosolutes have essentially no effect on the size of the polymer molecule; it is evident that aromatic molecules of this type, *i.e.*, the anilinium or naphthylammonium ions, are not bound by the polymer; a brief discussion of this already has been made in Part I, where it was suggested that either the tightness of binding of the water of hydration to the ionic center or the close proximity of the ionic charge to the aromatic system may be responsible; the same effects could explain the inactivity of the phenolate ion (Table I, run 40).

(d) **Viscometry in Methanol.**—The results in Table IV indicate there to be comparatively small reductions in the size of the polymer molecule for all three cosolutes; there is thus no coulombic effect for phenanthroate in this solvent but only a certain degree of shrinkage.

**Light Scattering.**—The light scattering results (Table V) essentially parallel the viscometric ones as far as change in molecular size is concerned, *i.e.* increases of the molecular dimensions were observed with the anionic cosolutes and decreases with the non-ionic ones.

As regards the actual values of the molecular parameters, from the application of statistical mechanics to polymer solutions, it has been shown<sup>12,13</sup> that under certain simplifying as-

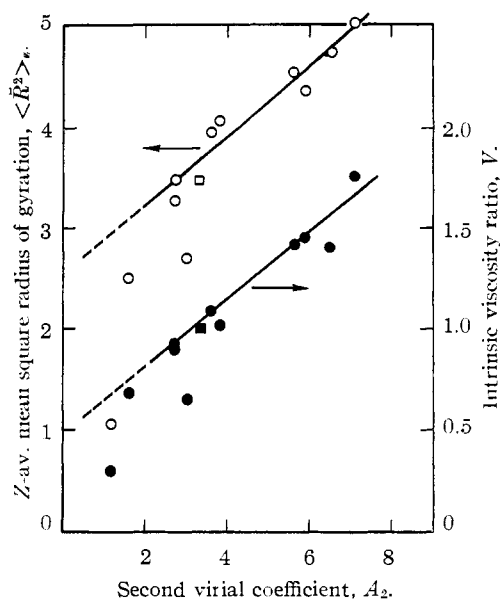


Fig. 3.— $Z$ -average mean square radius of gyration of the polymer molecule,  $\langle \bar{R}^2 \rangle_z$  (unfilled points and left-hand scale),  $\text{cm}^2 \times 10^{11}$  and intrinsic viscosity ratio,  $V$  (filled points and right-hand scale) versus second virial coefficient,  $A_2$ ,  $\text{cm}^3 \text{ mole g}^{-2} \times 10^4$ , for both anionic and non-ionic cosolutes. The squares represent polymer in the absence of cosolute.

sumptions, including an essentially random-flight configuration for the polymer molecule, the mean square radius of gyration of the polymer molecule,  $R^2$ , is proportional to the excluded volume, which is in turn linearly related to the second virial coefficient,  $A_2$ ; thus a relation of the following type results<sup>14</sup>

$$\langle R^2 \rangle_z = \langle R_0^2 \rangle_z (1 + \alpha A_2)$$

where  $R_0$  is the radius of gyration in the absence of long-range interactions, and the constant  $\alpha$  depends on a number of molecular parameters and also on the molecular heterogeneity; this relation is strictly valid only for a small range of  $A_2$ . It has been used and discussed in detail, for instance, for the system polystyrene-cyclohexane,<sup>14</sup> where variations in temperature were used to produce the changes in both virial coefficient and molecular dimensions. For the present results, Fig. 3 shows a plot of the  $z$ -average mean square radius (upper plot) and also the intrinsic viscosity ratio  $V$  (lower plot) against the second virial coefficient; for the upper plot the majority of the points lie close to the indicated straight line (the notable exceptions are phenol, 2-naphthol and, particularly, phloroglucinol, which all fall below, and it is evident that in these latter cases some "folding-up" of the polymer coil, due to extensive "cross-linking," had taken place). The value of  $\langle \bar{R}_0^2 \rangle_z$  derived from Fig. 3 is  $2.54 \times 10^{-11} \text{ cm}^2$ , leading to  $\langle \bar{R}_0^2 \rangle_z^{1/2} = 5.04 \times 10^{-6} \text{ cm}$ . (504 Å.); hence the  $z$ -average mean square unperturbed end-to-end distance,  $\langle \bar{L}_0^2 \rangle_z^{1/2}$ , has

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(11) C. D. Thurmond and B. H. Zimm, *J. Polymer Sci.*, **8**, 477 (1952).

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the value  $12.4 \times 10^{-6}$  cm., and also  $\langle \bar{L}_0^2 \rangle_z / \bar{M}_w = 1.52 \times 10^{-16}$  cm.<sup>2</sup> mole g.<sup>-1</sup>; this last value cannot be directly compared with analogous figures reported in the literature<sup>14</sup> without a heterogeneity correction. The slope,  $\alpha$ , of the upper line in the Fig. 4 is  $3.5 \times 10^{-8}$  g.<sup>2</sup> mole<sup>-1</sup> cm.<sup>-1</sup>.

The lower plot in Fig. 3, that of intrinsic viscosity ratio  $V$  versus  $A_2$ , is also essentially linear; an analogous linearity was observed by Krigbaum and Carpenter<sup>14</sup> on plotting  $[\eta]$  versus  $A_2$  for a poly-

styrene in cyclohexane over a range of temperatures.

Finally, it is possible to calculate from the data in Table V the values of the quantity  $\langle \bar{R}^2 \rangle_z^{1/2} / V^{1/3}$ , which should be constant if the intrinsic viscosity is to be proportional to the cube of a linear dimension of the polymer coil; the eleven values obtained from the data in Table V (omitting that for phloroglucinol) are indeed closely similar and give a mean value for  $\langle \bar{R}^2 \rangle_z^{1/2} / V^{1/3}$  of  $596 \pm 13$  Å. (one s.d.).

[CONTRIBUTION NO. 1653 FROM STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONN.]

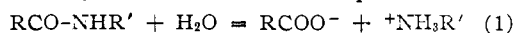
## Heats of Hydrolysis of Peptide Bonds<sup>1</sup>

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The heats of hydrolysis, to form fully charged products, of the tyrosine-glycine bond in benzoyl-L-tyrosylglycine and L-tyrosylglycinamide have been determined by direct calorimetry. The results obtained,  $\Delta H_{298} = -1330 \pm 90$  and  $-1300 \pm 150$  cal. per mole, respectively, are slightly less negative than the value previously reported for the related peptide benzoyl-L-tyrosylglycinamide, the small differences finding a reasonable interpretation in terms of electrostatic effects. The heat of hydrolysis of benzoyl-L-tyrosine,  $\Delta H_{298} = -1980 \pm 100$  cal. per mole, has also been determined. From this result and other data obtained in this Laboratory, the heat of hydrolysis of the benzoic acid-tyrosine bond in benzoyl-L-tyrosylglycinamide is calculated to be  $\Delta H_{298} = -2230 \pm 210$  cal. per mole. Accurate calorimetric values are now available for the heats of hydrolysis of eight different peptide bonds in synthetic peptides. These values range from  $-1240$  to  $-2250$  cal. per mole, with a mean value of approximately  $-1800$  cal. per mole.

It was reported in 1952<sup>3</sup> that the heat of hydrolysis of the tyrosine-glycine bond in benzoyl-L-tyrosylglycinamide (BTGA) to give fully charged products is  $\Delta H_{298} = -1550 \pm 100$  cal. per mole



Since BTGA carries no charge, it might be supposed that this small heat of hydrolysis would be more or less typical for interior peptide bonds in a protein. It was of interest to investigate the effect of having a charged or partially charged group close to the peptide bond under attack, as is the case in the hydrolysis of a terminal bond in a peptide. We have accordingly determined the heats of hydrolysis of the tyrosine-glycine bond in benzoyl-L-tyrosylglycine (BTG) and L-tyrosylglycinamide (TGA), using enzymic catalysis so that the reactions could be carried out at 25° at approximately neutral pH. Since the enzyme used with BTG, carboxypeptidase, also attacks the product, benzoyl-L-tyrosine (BT), it was necessary to determine the heat of hydrolysis of this compound.

### Experimental

The calorimetric equipment and method have been described in detail elsewhere.<sup>4</sup> In each experiment, 14.0 ml. of substrate solution was charged to the top compartment and 14.0 ml. of enzyme solution to the bottom compartment of one calorimeter and 28.0 ml. of water to the other calorimeter. Because of the necessity to remove solutions for analysis, it was impossible to make duplicate runs

on each filling of the calorimeters. All measurements were made at  $25.00 \pm 0.05^\circ$ . The enzymes employed, carboxypeptidase and leucineaminopeptidase, were purchased from Worthington Biochemical Company, Freehold, N. J., and were used without further purification. The enzymic hydrolyses were buffered by 0.03–0.05 M tris-(hydroxymethyl)-aminomethane (tris) plus enough HCl to give the desired pH.

**Preparation of Substrates.**—BT was prepared from benzoyl chloride and L-tyrosine; m.p.  $166\text{--}168^\circ$  after recrystallization several times from H<sub>2</sub>O. Titration equivalent weight 293; calcd. 285.3.

**BTG.**—Freshly distilled glycine ethyl ester was coupled to BT with N,N'-dicyclohexylcarbodiimide,<sup>5</sup> and the product was recrystallized from ethyl acetate and acetone; m.p.  $158\text{--}165^\circ$ ; yield 62%. The benzoyltyrosylglycine ethyl ester was dissolved in methanol and saponified at room temperature by addition of two equivalents of aqueous NaOH. After 20 min., the reaction mixture was brought to pH 5 by addition of HCl, and the methanol was removed by distillation at room temperature. The product was recrystallized from H<sub>2</sub>O; m.p.  $196\text{--}202^\circ$ , with softening at  $160^\circ$ ; yield 95%. Titration equivalent weight 341; calcd. 342.3.

*Anal.* Calcd. for C<sub>18</sub>H<sub>18</sub>O<sub>6</sub>N<sub>2</sub>: C, 63.15; H, 5.27; N, 8.18. Found: C, 63.09; H, 5.39; N, 8.01.

**TGA.**—Carbobenzoxytyrosine was prepared according to Martin, *et al.*<sup>6</sup>; m.p.  $98^\circ$  after recrystallization from H<sub>2</sub>O. It was treated with glycine ethyl ester and N,N'-dicyclohexylcarbodiimide,<sup>5</sup> and the product was recrystallized from ethyl acetate; m.p.  $163\text{--}170^\circ$ . Treatment of the ester with NH<sub>3</sub> in methanol<sup>7</sup> gave carbobenzoxytyrosylglycinamide; m.p. approximately  $113^\circ$  (melt not clear until  $135^\circ$ ). The carbobenzoxy group was removed by hydrogenation over palladinized charcoal in methanol containing an excess of acetic acid.<sup>8</sup> The resulting acetate of tyrosylglycinamide was recrystallized from methanol-ethanol (2:5). The product did not show a sharp melting point; there appeared to be a transition at  $163^\circ$ , followed

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