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# The Dipole Moments of Esters of Amino Acids and Peptides

BY JEFFRIES WYMAN, JR., AND T. L. MCMEEKIN

## I. Introduction

In the preceding paper in THIS JOURNAL it was pointed out that qualitative estimates of the electric moments of amino acids and peptides in solution in water and alcohol–water mixtures afford strong evidence that these substances exist in the form of zwitter ions: (a) the moments are all abnormally large; (b) they are the same for all amino acids of a given type; (c) they show a linear increase with the length of chain separating the acid and basic groups in the molecule.

The argument just outlined, however, convincing as it is, leaves out of consideration the possibility of these effects being explained in terms of electric moments inherent in the un-ionized molecules. For example, in regard to the first point, it is not certain that the undissociated amino acids and peptides are not themselves sufficiently polar to account directly for the high values of the electric moment observed. In regard to the last point, it is not wholly clear that the differences of electric moment between the various types of amino acids may not be due to the different orientation of certain groups, or that the greater moments of the larger peptides may not be due to the greater size of the molecules. It was in order to investigate these possibilities and to place the argument on a more secure basis that the present investigation was undertaken. This comprises an exact determination of the electric moments, in the un-ionized condition, of as many of the compounds previously studied in water and alcohol–water mixtures as was possible. The results however have a certain independent interest in themselves.

## IIa. Choice of Materials

The ideal procedure would have been to dissolve each of the substances in some non-polar solvent, such as benzene, in which the formation of zwitter ions would undoubtedly be suppressed and in which alone it is possible to make quantitative measurements of moments in accordance with the theory developed by Debye.[1] Unfortunately, however, none of the ampholytes in question are appreciably soluble in any of the non-polar solvents, no doubt for the very reason that ionization cannot take place. In view of this difficulty, it was decided to study the esters, many of which are readily soluble in benzene. In particular, the ethyl esters were selected in preference to the methyl ones, as being more stable.

Ample justification for applying the values obtained for the moments

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(1) Debye, "Polar Molecules," Chemical Catalog Company, Inc., New York, 1929.

of the esters to the acids themselves is to be found in the well-known generalization that the substitution of a hydrogen atom by a methyl or ethyl group has very little effect on the moment of the molecule involved.[2]

Unfortunately, even by using this procedure, it was not possible to study all the materials in question. Although the esters of the amino acids themselves are readily soluble in benzene, it was found that the esters of the peptides were very insoluble, to an extent increasing with the number of glycine units in the molecule. For this reason, it was only possible to study one of the peptides, glycylglycine, and even this was only sufficiently soluble for measurement at 50°. Another difficulty was encountered in the case of the ester of $\gamma$-aminovaleric acid, which was found to pass over rapidly into the anhydride.[3] The anhydride thus unintentionally obtained was found to be soluble in benzene and was included among the compounds measured. In view of the instability of the ester of $\gamma$-aminovaleric acid no attempt was made to study the esters of any of the $\delta$ or $\epsilon$ acids.

A list of the substances investigated, with references to the method of preparation, is given in the following section. In addition to observations on the boiling points, or melting points, and, in the case of the anhydride, analytical data on the nitrogen content, determined by the Kjeldahl method, this list also gives values of the density $\rho$ and index of refraction $n$, both at 25°. The density was measured with a pycnometer. In the case of the esters of glycine, $\alpha$-alanine and $\beta$-alanine, the index of refraction was determined for red light with an Abbé refractometer made by Bausch and Lomb; in all other cases, for sodium light with a Pulfrich–Zeiss instrument. In the case of the ester of glycylglycine, which melts at 86°, these data on the density and index of refraction are lacking.

## IIb. Preparation of Materials

All $\alpha$-amino acids used as starting materials in the preparation of the esters were obtained from the Eastman Kodak Company. They were recrystallized from alcohol–water mixtures before using.

**Glycine Ethyl Ester.**—Glycine ethyl ester was made by the well-known Fischer[4] method of preparing esters of amino acids; boiling point 43–44° at 12 mm. pressure; $\rho = 1.0169$; $n = 1.4213$.

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(2) This is illustrated by the following data taken from the appendices to Smyth, "Dielectric Constant and Molecular Structure," Chemical Catalog Co., Inc., New York, 1931.

| Substance | Moment (e. s. u. $10^{-18}$) |
|---|---|
| Acetic acid | 1.73 |
| Methyl acetate | 1.75 |
| Ethyl acetate | 1.81 |
| Propionic acid | 1.74 |
| Ethyl propionate | 1.79 |

(3) This is not surprising since the usual method of preparation of the anhydride is through the ester as an intermediate stage.

(4) Fischer, *Ber.*, **34**, 433 (1901); **34**, 2868 (1901).

**α-Alanine Ethyl Ester.**—α-Alanine ethyl ester was prepared by the method of Fischer; boiling point 48–52° at 12 mm. pressure; $\rho = 0.9722$; $n = 1.4155$.

**α-Aminobutyric Acid Ethyl Ester.**—This ester was also prepared by the method of Fischer;[4] boiling point 60–62° at 12 mm. pressure; $\rho = 0.9533$; $n = 1.42070$.

**α-Aminovaleric Acid Ester.**—This compound was prepared by the method of Slimmer;[5] boiling point 73–75° at 12 mm. pressure; $\rho = 0.9377$; $n = 1.4241$.

**α-Valine Ethyl Ester.**—The method of Slimmer[5] was used to prepare this compound; boiling point 66–70° at 12 mm. pressure; $\rho = 0.9390$; $n = 1.4267$.

**α-Aminocaproic Acid Ethyl Ester.**—The method of Fischer[4] was used to prepare this compound; boiling point 91–92° at 12 mm. pressure; $\rho = 0.9246$; $n = 1.42829$.

**l-Leucine Ethyl Ester.**—Fischer's[4] method was used to prepare this compound; boiling point 82–84° at 12 mm. pressure; $\rho = 0.9215$; $n = 1.42655$.

**β-Alanine Ethyl Ester.**—The method of S. Hoogewerff and van Dorp[6] was used to prepare this substance; boiling point 56–58° at 12 mm. pressure; $\rho = 0.9892$; $n = 1.4250$.

**β-Aminobutyric Acid Ethyl Ester.**—The method of Fischer and Groh[7] was followed in the preparation of this compound; boiling point 60–62° at 12 mm. pressure; $\rho = 0.9409$; $n = 1.4221$.

**Glutamic Acid Diethyl Ester.**—This compound was prepared by the method of Fischer;[4] boiling point 140–142° at 12 mm. pressure; $\rho = 1.0620$; $n = 1.44045$.

**Glycylglycine Ethyl Ester.**—Fischer's[4] method was used to prepare this substance; melting point 86° (uncorrected).

**Anhydride of γ-Aminovaleric Acid.**—The method of Tafel[8] was used to prepare this substance; boiling point 132–135° at 12 mm. pressure; nitrogen, found 14.20%; calcd. 14.14%; $\rho = 1.0285$; $n = 1.4695$.

## IIc. Determination of Electric Moments

The values of the electric moments were calculated from data at a single temperature in dilute solutions in benzene in the usual way. This procedure involves a knowledge of the total and optical polarizations, $P_1$ and $P_{10}$, of the solute at a variety of concentrations. From the value of $P_1 - P_{10}$ extrapolated to zero concentration the electric moment is calculated from the expression

$$\mu = 0.0127\,(P_1 - P_{10})T \times 10^{-18} \text{ e. s. u.}$$

where $T$ is the absolute temperature.[1]

The determination of $P_1$ requires the direct measurement of the density and dielectric constant of the solution, together with a knowledge of its composition. For this purpose the values of the density were measured with a chainomatic immersion densitometer, which greatly facilitated the measurements, particularly at high and low temperatures. The accuracy of the determinations was between 0.02 and 0.03%, and, although this is much less than that attainable with a pycnometer, the errors were well below those involved in measurements of the dielectric constant and were not significant.

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(5) Slimmer, *Ber.*, **35**, 400 (1902).

(6) Hoogewerff and van Dorp, *Rec. trav. chim.*, **10**, 4 (1891).

(7) Fischer and Groh, *Ann. Chem. Pharm.*, **383**, 363 (1911).

(8) Tafel, *Ber.*, **19**, 2414 (1886).

The method employed for the determination of the dielectric constant was the same as in the study of solutions of the free acids and peptides described in the previous paper.[9] Owing, however, to the much lower values of the dielectric constant of the benzene solutions, it was necessary to use a far larger resonator in order to keep the resonance points within the frequency range of the oscillator (lower wave length limit about 2.2 meters). This introduced a problem in regard to the amount of solution required, for it is essential to the method that the resonator be immersed in a volume of material sufficient to include sensibly its entire electric field. In order to reduce this volume as far as possible a special type of resonator was constructed having a minimum stray field. This is shown, in vertical section, in the accompanying figure, together with the glass container for the solution and a glass jacket to allow the circulation of water to control the temperature. This circuit consisted of three concentric brass cylinders, the inner and outer ones being connected and forming one plate of a condenser. The opposite plate, formed by the middle cylinder and separated from the two others by a clearance of 0.8 mm., was supported rigidly by the single brass loop forming the inductance. Small holes were appropriately drilled to allow the entrance of liquid and the escape of air when the resonator was immersed, and the whole structure was heavily gold-plated to provide a clean surface. This circuit had a resonant wave length of 4.193 meters in air and of about 6.3 meters in the benzene solutions (depending on the exact value of the dielectric constant). With this arrangement it was possible to make absolute measurements of the dielectric constant on only 60 cc. of solution.

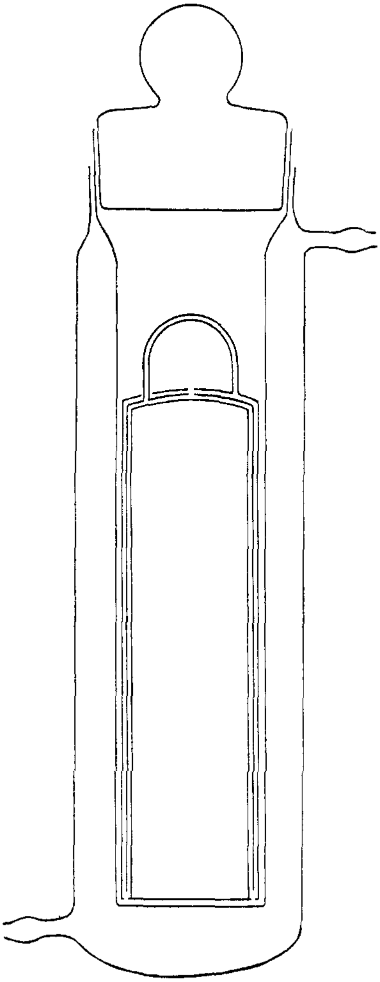

Fig. 1.

Before accepting the results obtained with this resonator, it was necessary to investigate the extent of errors due to residual stray lines of force extend-

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[9] Wyman, *Phys. Rev.*, **35**, 623 (1930).

ing out into the glass and surrounding water. Owing to the shielding effect of the outer cylinder, these were bound to be small, but at the high frequencies involved there was the possibility of capacity effects along the surface of the outer cylinder and across the loop forming the inductance, which might be expected to spread out into the surrounding media to an appreciable extent. To ascertain the magnitude of these, determinations were made of the resonant wave length of the circuit under the following conditions: (a) suspended freely in air; (b) in the bottle filled with air and standing on the table; (c) in the bottle filled with air and jacketed with water. The values obtained in the first two cases were indistinguishable. The value obtained in the last case was 0.17% higher. When benzene was substituted for air the resonant wave length determined according to conditions (c) was 0.13% too high. Since the dielectric constant is given by the square of the ratio of the resonant wave length in the solution divided by the resonant wave length in air, it is evident that these small errors nearly cancel one another and involve a net error of less than 0.08% in the dielectric constant. Since this is scarcely more than the random errors involved in the measurements, no attempt has been made to correct for these effects in the following results.

The values of the optical polarization $P_{10}$ were for the most part calculated from the data on the density and index of refraction of the pure compounds given above. In the case of glycylglycine, however, owing to the high melting point, no attempt was made to measure directly the density and refractive index of the pure material. Instead, the optical polarization was determined indirectly from the density and index of refraction of the benzene solutions, together with the optical polarization of benzene itself, in exactly the same way that the total polarization $P_1$ is calculated from corresponding data on the density and dielectric constant.

## III. Results

In the case of glycine and β-alanine the measurements were extended over a range of temperature from 5 to 75° in order to test the constancy of $(P_1 - P_{10})T$, which is to be expected on theoretical grounds (see Debye (1)). The results are given in Table I.

TABLE I

| Temp., °C. | $(P_1 - P_{10})T$ | Temp., °C. | $(P_1 - P_{10})T$ |
|---|---|---|---|
| Glycine Ethyl Ester | | Alanine Ethyl Ester | |
| 5 | 28,200 | 5 | 29,000 |
| 20 | 28,000 | 20 | 29,500 |
| 30 | 26,800 | 30 | 29,000 |
| 50 | 27,600 | 45 | 28,300 |
| 70 | 28,100 | 60 | 28,000 |
| | | 75 | 27,500 |

The values of $(P_1 - P_{10})T$ listed in the table were derived by extrapolation to infinite dilution from results obtained on dilute solutions. Despite a considerable fluctuation, attributable mainly to difficulties of temperature control at the upper and lower limits, it is clear that the values of $(P_1 - P_{10})T$ show no systematic variation with temperature. In this respect these esters are like the majority of other substances which have been studied and behave in accordance with the standard theory.

The results of the entire investigation are summarized in Table II. This contains the following data for each of the esters: the optical polarization $P_{10}$; values of $(P_1 - P_{10})T$ tabulated against mole fraction of the ester; the limiting value of $(P_1 - P_{10})T$, extrapolated to infinite dilution, given in parentheses; the corresponding dipole moment $\mu$; the temperature at which the measurements were made.

TABLE II

| Ester | Temp., °C. | $P_{10}$ | Mole fraction | $(P_1 - P_{10})T$ | Mole fraction | $(P_1 - P_{10})T$ | Dipole moment, $\mu$ |
|---|---|---|---|---|---|---|---|
| Glycine ethyl ester | 5–75 | 25.73 | 0.0175 | 27,600 | | | $2.11 \times 10^{-18}$ |
| | | | .00882 | 27,700 | | | |
| | | | ( .00000) | (27,700) | | | |
| α-Alanine ethyl ester | 25 | 30.20 | .0530 | 29,000 | 0.0175 | 28,700 | $2.09 \times 10^{-18}$ |
| | | | .0422 | 29,000 | .00875 | 27,100 | |
| | | | .0294 | 29,100 | ( .00000) | (27,000) | |
| α-Aminobutyric acid ethyl ester | 25 | 34.90 | .0778 | 24.000 | .0113 | 29,100 | $2.13 \times 10^{-18}$ |
| | | | .0530 | 26,600 | .00679 | 28,600 | |
| | | | .0350 | 28,300 | ( .00000) | (28,000) | |
| | | | .0208 | 29,600 | | | |
| α-Aminovaleric acid ethyl ester | 25 | 39.50 | .03577 | 27,600 | .01106 | 28,100 | $2.13 \times 10^{-18}$ |
| | | | .02665 | 28,100 | .005505 | 28,300 | |
| | | | .01853 | 28,300 | ( .000000) | (28,000) | |
| Ethyl ester of α-aminovaline | 25 | 39.66 | .02968 | 28,400 | .01646 | 27,800 | $2.11 \times 10^{-18}$ |
| | | | .02065 | 28,200 | ( .00000) | (27,700) | |
| α-Aminocaproic acid ethyl ester | 25 | 44.31 | .03223 | 27,000 | .00807 | 27,700 | $2.13 \times 10^{-18}$ |
| | | | .02563 | 27,100 | .00645 | 29,300 | |
| | | | .02041 | 27,500 | ( .00000) | (28,000) | |
| | | | .01627 | 28,000 | | | |
| β-Alanine ethyl ester | 5–75 | 30.27 | .0264 | 29,100 | .00296 | 27,500 | $2.14 \times 10^{-18}$ |
| | | | .00757 | 28,700 | ( .00000) | (28,500) | |
| β-Aminobutyric acid ethyl ester | 25 | 35.42 | .0397 | 25,900 | .0099 | 27,400 | $2.11 \times 10^{-18}$ |
| | | | .027 | 26,300 | ( .0000) | (27,700) | |
| | | | .01644 | 27,200 | | | |
| Leucine ethyl ester | 25 | 44.37 | .0377 | 25,100 | .0116 | 24,800 | $2.03 \times 10^{-18}$ |
| | | | .0280 | 25,500 | .00693 | 25,520 | |
| | | | .0195 | 25,800 | ( .00000) | (25,500) | |
| Diethyl ester of glutamic acid in $C_6H_6$ | 25 | 50.54 | .03924 | 38,900 | .008193 | 41,500 | $2.56 \times 10^{-18}$ |
| | | | .03113 | 39,600 | .006130 | 40,400 | |
| | | | .02314 | 39,800 | ( .000000) | (40,600) | |
| | | | .01374 | 39,000 | | | |
| Ethyl ester of glycylglycine | 50 | 42.6 | .01763 | 65,600 | .006689 | 66,000 | $3.20 \times 10^{-18}$ |
| | | | .01405 | 66,800 | ( .000000) | (63,500) | |
| | | | .01120 | 67,100 | | | |
| Anhydride of γ-aminovaleric acid | 25 | 26.87 | .05542 | 28,600 | .01024 | 38,300 | $2.62 \times 10^{-18}$ |
| | | | .04189 | 30,000 | .00464 | | |
| | | | .02930 | 31,810 | ( .00000) | (42,500) | |
| | | | .01464 | 38,100 | | | |

## IV. Discussion

So far as the authors are aware, there have been no previous determinations of the moments given in this table. It is of interest however to compare them with Estermann's[10] values of $1.4 \times 10^{-18}$ and $2.7 \times 10^{-18}$ for the methyl esters of $\alpha$- and $\delta$-aminovaleric acids, respectively. The discrepancy between the former of these and the authors' value of 2.13 for the ethyl compound is far too great to attribute to the difference between the ethyl and methyl groups, and no other explanation suggests itself. In regard to the latter result, it appears possible that in reality the ester had passed over spontaneously into the anhydride before the measurement was made, as in the case of the $\gamma$-acid studied by the authors. In fact their value of $2.62 \times 10^{-18}$ for the moment of the anhydride of $\gamma$-valeric acid is close to Estermann's figure of $2.7 \times 10^{-18}$ for what he supposed to be free $\delta$-amino form.

None of the moments listed in Table II are large, and it appears reasonably certain that the high values of the dielectric constant of the aqueous solutions of the free acids must depend on the effect of the zwitter ions. Further support in favor of this view may be drawn from the moments of the methyl esters of *m* and *p* aminobenzoic acids, reported by Estermann[10] as 2.4 and $3.3 \times 10^{-18}$, respectively. Although both of these are greater than the moments of glycine and most other amino acids listed above, the free acids form aqueous solutions of dielectric constant less than that of water. This is, of course, readily intelligible in terms of the zwitter ion theory when it is recalled that calculations based on dissociation constants indicate that the aminobenzoic acids dissolved in water do not exist mainly in the ionized form.[11]

Another strong argument in favor of the zwitter ion hypothesis, which provides an answer to one of the objections raised at the outset of this paper, is the close agreement between the moments of the esters of the $\alpha$ and $\beta$ acids, in marked contrast to the behavior of the free acids in aqueous solution. The slightly lower moment of the leucine ester was at first attributed to the branched structure of the molecule but this appears unlikely in view of the results on valine which also has a branched chain. It is in any case relatively unimportant. The large moment of the ester of glutamic acid is undoubtedly due to the presence of two carboxyl groups.

Another objection referred to in the introduction, namely, that the progressive increase in the electric moments of the peptides of glycine in aqueous solution with the size of the molecule might be due to the greater inherent moments of the larger molecules, associated with the possession of

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(10) Estermann, *Z. physik. Chem.*, **B1**, 134 (1928).

(11) Bjerrum, *ibid.*, **104**, 147 (1923). Similar considerations might be advanced in regard to glycylglycine and glycylglycine anhydride. Although data on the moment of the anhydride are lacking, it may be assumed by analogy with the anhydride of $\gamma$-aminovaleric acid that this is greater than in the case of the peptide itself. Nevertheless, solutions of glycylglycine have dielectric constants greater than water, those of the anhydride. less [see Devoto, *Gazz. chim. ital.*, **61**, 897 (1932)].

a greater number of polar groups, rather than to the formation of zwitter ions, is not conclusively answered by the present results. Nevertheless, in a general way they are against it. The moment obtained for the ester of glycylglycine is only half again as great as that of the ester of glycine itself. On the other hand, in contrast to this, the data on the dielectric constant indicated that the electric moments of the peptides in aqueous solution were directly proportional to the number of glycine units in the molecule, as if due to the formation of zwitter ions. It is unfortunate that their great insolubility in non-polar solvents makes it impossible to measure the moments of the esters of the higher peptides.

## V. Summary

Highly approximate values of the electric moments of amino acids and peptides have been obtained from measurements on solutions of the esters in benzene. An analysis of these results furnishes additional support for the zwitter ion hypothesis.

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[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

# Kinetics of the Unimolecular Dissociation of Gaseous Ethyl Bromide[1]

BY ELVIN L. VERNON AND FARRINGTON DANIELS

A search for unimolecular reactions has been in progress in this Laboratory for several years. Another communication[2] describes the thermal decomposition of several organic compounds and, of these, the dissociation of ethyl bromide was selected as the reaction most promising from a theoretical standpoint. It has been subjected to further intensive study as described in this article.

In the first investigation[2] it was established, experimentally, that the reaction is

$$C_2H_5Br \rightleftharpoons C_2H_4 + HBr$$

and various ways of calculating the rate of dissociation were discussed. The course of the reaction was followed in both researches by measuring the pressure as a function of time, using glass diaphragms[3] and all-glass or all-quartz vessels, totally immersed in a lead bath. In the present investigation the measurements were carried to lower pressures for the purpose of testing recent theories of unimolecular reactions and an improved method

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[1] Further details of this investigation may be obtained from the Ph.D. thesis of Elvin L. Vernon filed in the Library of the University of Wisconsin in June, 1932.

[2] Lessig, *J. Phys. Chem.*, **36**, 2325 (1932).

[3] Daniels, THIS JOURNAL, **50**, 1115 (1928).