ary reactions which occur with tyrosine may have taken place, under the conditions chosen, to the extent of very roughly 10% with Ea during the deamination. The indications are, therefore, that in the deamination of Ea under controlled conditions the main reaction is a removal of a portion of the free $-NH_2$ groups and that the extent of the secondary reactions is minimized, though not eliminated.

(4) **Immunological Specificity.**—The classical studies of Obermayer and Pick[13] and of Landsteiner[14] demonstrate that the specificity of proteins is strongly modified by the introduction of substituents into the tyrosine residues, although exceptions are known.[15] The virtual identity of the specificity of fraction B with that of unmodified crystalline Ea may therefore, be interpreted as supplementing the other evidence that in this undenaturated fraction, at least, the effects of substitution are minimal. Whether or not substitution plays any part in the immunological differences observed between Fraction A, insoluble at its isoelectric point, and acid-denatured Ea is less certain.

Moreover acid denatured egg albumin (DnEa) which had been deaminated 58% by means of free nitrous acid was deep orange in color, yet its reactivity with anti-serum to DnEa was only slightly less than that of DnEa itself (Paper III, Fig. 3 ⬖). Similarly the reactivities of the orange Ea FNA (Fig. 3 ◐) and of 8A FNA (◪) were only slightly less than that of the corresponding Fraction 6A (□). These data indicate that the specificity of DnEa is not greatly influenced either by the removal of roughly one-half of its free $-NH_2$ groups or by the substitution primarily of aromatic amino acid residues by nitrous acid.

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The Self-Interaction of Mandelic Acid as Determined from Solubilities in Salt Solutions

BY W. F. MCDEVIT[1] AND F. A. LONG

One of the most common ways to determine the effects of salts on the activity coefficients of non-electrolytes is to compare solubilities in salt solutions with those in pure water. The data usually obey the Setschenow equation, $\log S_0/S = KC_s$, where S_0 and S are molar solubilities in pure water and salt solution, respectively, C_s is molar salt concentration and K is a constant, and it is common to identify $\log S_0/S$ with $\log f_i$ (where f_i is the molar activity coefficient of the non-electrolyte) and call K the salting out parameter. However, in general two types of interaction enter, ion–non-electrolyte interaction and self-interaction, *i.e.*, interaction of the non-electrolyte with itself. If one wishes to compare salt effects with theory or salt effects for two non-electrolytes of different solubility with each other, it is necessary to make a distinction between these two. In particular most theories of salt effect[2] deal only with the ion–non-electrolyte interaction. The contribution from the two types of interaction is shown by the following.

The activity coefficient of a species in solution, referred to the infinitely dilute solution, can usually be well represented by a power series in the concentrations[3] which for dilute solutions of a neutral molecule reduces to

$$\log f_i = k_sC_s + k_iC_i \qquad (1)$$

where the subscripts s and i refer to salt and non-electrolyte, respectively, and k_s and k_i are the parameters for salting out and for self-interaction. Combining this with the usual equation for equilibrium between saturated solutions

$$f_iS = f_i^0S_0 \qquad (2)$$

and utilizing the fact that

$$\log f_i^0 = k_iS_0$$

one obtains

$$\log \frac{f_i}{f_i^0} = \log \frac{S_0}{S} = k_sC_s + k_i(S - S_0) \qquad (3)$$

When the solubility of the non-electrolyte is quite low, f_i^0 is unity and the term $k_i(S - S_0)$ is negligible. Here the salting out parameter k_s can indeed be obtained from a plot of $\log S_0/S$ against C_s. However, when the solubility is high the last term of Eq. (3) can be large and only with a knowledge of k_i can the value of k_s be determined.

A very interesting study by Ross, Morrison and Johnstone[4] on the influence of salts on the solubilities of racemic and optically active mandelic acids can be used to evaluate both k_s and k_i for this particular non-electrolyte and hence indicate the order of magnitude of the latter. The solubilities of these two forms of mandelic acid in water are high and quite different; at 25° the solubility of the racemic acid is 1.335 molal whereas that of the active form is only 0.738 molal. The striking features of the salt effects reported by the above authors for the two forms are: the observed salt effects are quite different for the active and racemic forms being considerably larger for the latter; the experimental values of the Setschenow parameters are very large when compared with similar molecules of low solubility (*e.g.*, benzoic and phthalic acids).

Table I gives for several salts the observed values of the Setschenow parameters, K^r and K^a for the racemic and active forms respectively. The other columns of the table give values of k_s and k_i calculated on the assumptions that the observed results are due to a combination of ion–non-electrolyte interaction and self-interaction and that the

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[2] (a) P. P. Debye, *Z. physik. Chem.*, **130**, 56 (1927); (b) J. G. Kirkwood in Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, Chap. 12.

[3] Ref. 2b, Chap. 3.

[4] J. D. M. Ross, T. J. Morrison and C. Johnstone, *J. Chem. Soc.*, 264 (1938).

values of k_s and k_i are the same for the two forms. Using S_0^r, S^r, S_0^a and S^a for the solubilities of the racemic and active forms in water and salt solutions, Eq. (3) can be applied to the results for each. Then, for solubility experiments in solutions of a given salt, k_sC_s can be eliminated giving

$$k_i = \frac{\log S_0^r/S^r - \log S_0^a/S^a}{(S^a - S_0^a) - (S^r - S_0^r)} \qquad (4)$$

In practice when the data obey the Setschenow equation it is more convenient to use

$$k_i = \frac{K^r - K^a}{\dfrac{S^a - S_0^a}{C_s} - \dfrac{S^r - S_0^r}{C_s}} \qquad (5)$$

Once k_i is obtained, the value of k_s can be calculated from Eq. (3), written for either form. Table I gives the results of such calculations for several salts. In making the calculations the recorded molal concentrations have been converted to molar using an apparent molar volume of 125 ml./mole for mandelic acid. Actually the results are very similar if molal concentrations and activity coefficients are used throughout. Where necessary, notably for experiments with the mandelate salts, corrections for the dissociation of mandelic acid have been made.

TABLE I

SALTING OUT AND SELF-INTERACTION PARAMETERS FOR MANDELIC ACID AT 25°

Salt	K^r	K^a	k_i	k_s
LiCl	+0.40	+0.20	−0.30	0.125
NaCl	+ .30	.185	− .25	.12
KCl	+ .155	+ .10	− .22	.07
RbCl	+ .135	+ .072	− .28	.04
CsCl	− .002	− .001	...	− .002
NH_4Cl	+ .21	+ .11	− .26	.07
$LiNO_3$	+ .21	+ .115	− .27	.07
$NaNO_3$	.095	+ .07	...	.04
KNO_3	− .04	− .00	...	− .02
$CsNO_3$	− .165	− .097	− .22	− .06
Na Mandelate	− .35	− .205	− .23	− .12
Cs Mandelate	− .89	− .31	− .27	− .18
		Av.	− .255	

The most significant point from Table I is that the values of k_i calculated from the data for the various salts are constant to within the variation of the experimental data. Furthermore the resulting values of k_s are similar to those for the comparable non-electrolytes, benzoic acid[5] and phthalic acid.[6] Finally the negative value of k_i shows, as expected, that mandelic acid is "salted in" by itself. The average value of k_i, −0.25, is quite large but it seems reasonable when compared with the value for phenol for which, from the freezing point data of Jones and Bury,[7] the k_i value is about −0.16.

These results for mandelic acid suggest that a comparison of salt effects from solubility measurements for two non-electrolytes of different solubility can be quite misleading unless the self-interaction is corrected for. Similarly for a non-electrolyte of high solubility, salt effects obtained from solubility studies can be quite different from those obtained by other procedures, for example vapor pressure or distribution.

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Coördination of Silver Ion with Methyl Esters of Oleic and Elaidic Acids

BY PETER L. NICHOLS, JR.[1]

The coördination of silver ion with unsaturated compounds has been studied extensively by Lucas and co-workers.[2,3] Their information was obtained largely by partition studies using carbon tetrachloride and 1 *N* aqueous silver nitrate as the immiscible solvent pair. An attempt to determine the argentation constants of methyl oleate and methyl elaidate with the above solvent pair was quickly found to be impractical since the amount of material transferred to the aqueous silver nitrate phase was negligible. Preliminary experiments showed that satisfactory results could be obtained with isoöctane as one phase and aqueous methanol as the other phase. The *cis*-isomer was found to have a greater argentation constant than the *trans*-isomer, but the magnitudes of the constants for the most part were considerably greater than those found for *cis*- and *trans*-olefins distributed between carbon tetrachloride and 1 *N* aqueous silver nitrate. Accordingly, it is recognized that a distribution of this type might be used as a basis for the separation of *cis*- and *trans*-isomers as well as for the separation of saturated and unsaturated fatty acid esters or olefinic compounds in general.

Experimental

Preparation of Materials.—We are indebted to H. B. Knight for generous samples of methyl oleate and methyl elaidate. The iodine values of these preparations were about one unit lower than theoretical. However, the only possible contamination (as indicated by spectrophotometric analysis) was that of saturated esters and these would have a negligible effect on the distribution experiments.

Reagent grade chemicals were used throughout. Isoöctane (A.S.T.M. grade) had an iodine value of 0.07, a satisfactorily low value. Solutions of isoöctane and methanol containing various amounts of water were prepared as follows: upper layer, a large amount of isoöctane was shaken successively with several small portions of methanol containing a given amount of water; lower layer, methanol containing a given amount of water was shaken successively with several small portions of isoöctane.

The solutions of isoöctane saturated with a particular methanol solution were added to weighed amounts of the methyl esters in a volumetric flask and made to the mark at 25 ± 0.01°. All solutions were stored at this temperature until used in the distribution experiments.

Solutions of aqueous methanol containing silver nitrate were prepared by dissolving silver nitrate in the appropriate amount of water and then diluting to the mark and saturating the solution as described above. Flasks containing the silver nitrate solutions were wrapped at all times with a

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[2] S. Winstein and H. J. Lucas, THIS JOURNAL, **60**, 836 (1938).

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