The Shedlovsky Extrapolation Function

BY HARRY M. DAGGETT, JR.[1]

In a recent note, Fuoss and Shedlovsky[2] have shown that in evaluating the limiting equivalent conductance, Λ_0, and the dissociation constant, K, from conductance data of electrolytes in non-aqueous solutions, it is preferable to use the Shedlovsky[3] extrapolation function rather than that[4] proposed by Fuoss. The values obtained for Λ_0 by these two procedures are identical, but those for K are sometimes significantly different. In the range $10^{-3} \leqslant K \leqslant 1$, the value of K obtained through the Shedlovsky function is preferable, while for $K \leqslant 10^{-3}$ either procedure is satisfactory.

The method of Shedlovsky is based on the solution of the equation[5]

$$\Lambda = \theta\Lambda_0 - \alpha(\Lambda/\Lambda_0)\sqrt{c\theta}$$

in terms of the function

$$S(z) = [z/2 + \sqrt{1 + (z/2)^2}]^2$$
$$= 1 + z + z^2/2 + z^3/8 - z^5/128 + z^7/1024 - \ldots$$

where $z = \alpha\sqrt{c\Lambda}/\Lambda_0^{3/2}$ (for details see ref. 2 or 3).

While it is a relatively easy matter to calculate the required values of $S(z)$ from the expanded form,[6] the procedure is rather time-consuming when a large number of calculations are to be made. It would, therefore, be convenient to have available a table of the function $S(z)$ similar to that given by Fuoss[4] for $F(z)$.

Such a table has been constructed in the course of another investigation. Values of the function $S(z)$ for the range $0.000 \leqslant z \leqslant 0.209$ are presented in Table I. Linear interpolation in this table is readily carried out to give $S(z)$ for any z in this range. The values of $S(z)$ so obtained should be significant to ± 1 in the fourth decimal.

TABLE I

VALUES OF THE SHEDLOVSKY EXTRAPOLATION FUNCTION, $S(z)$

z	0.000	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	Diff.
0.000	1.0000	1.0010	1.0020	1.0030	1.0040	1.0050	1.0060	1.0070	1.0080	1.0090	10
.010	1.0101	1.0111	1.0121	1.0131	1.0141	1.0151	1.0161	1.0171	1.0182	1.0192	10
.020	1.0202	1.0212	1.0222	1.0233	1.0243	1.0253	1.0263	1.0274	1.0284	1.0294	10
.030	1.0305	1.0315	1.0325	1.0335	1.0346	1.0356	1.0367	1.0377	1.0387	1.0398	10
.040	1.0408	1.0418	1.0429	1.0439	1.0450	1.0460	1.0471	1.0481	1.0492	1.0502	11
.050	1.0513	1.0523	1.0534	1.0544	1.0555	1.0565	1.0576	1.0586	1.0597	1.0608	11
.060	1.0618	1.0629	1.0640	1.0650	1.0661	1.0671	1.0682	1.0693	1.0704	1.0714	11
.070	1.0725	1.0736	1.0746	1.0757	1.0768	1.0779	1.0789	1.0800	1.0811	1.0822	11
.080	1.0833	1.0843	1.0854	1.0865	1.0876	1.0887	1.0898	1.0909	1.0920	1.0930	11
.090	1.0941	1.0952	1.0963	1.0974	1.0985	1.0996	1.1007	1.1018	1.1029	1.1040	11
.100	1.1051	1.1062	1.1073	1.1084	1.1095	1.1107	1.1118	1.1129	1.1140	1.1151	11
.110	1.1162	1.1173	1.1184	1.1196	1.1207	1.1218	1.1229	1.1240	1.1252	1.1263	11
.120	1.1274	1.1285	1.1297	1.1308	1.1319	1.1331	1.1342	1.1353	1.1365	1.1376	11
.130	1.1387	1.1399	1.1410	1.1421	1.1433	1.1444	1.1456	1.1467	1.1479	1.1490	11
.140	1.1501	1.1513	1.1524	1.1536	1.1547	1.1559	1.1570	1.1582	1.1594	1.1605	12
.150	1.1617	1.1628	1.1640	1.1652	1.1663	1.1675	1.1686	1.1698	1.1710	1.1721	12
.160	1.1733	1.1745	1.1757	1.1768	1.1780	1.1792	1.1803	1.1815	1.1827	1.1839	12
.170	1.1851	1.1862	1.1874	1.1886	1.1898	1.1910	1.1922	1.1934	1.1945	1.1957	12
.180	1.1969	1.1981	1.1993	1.2005	1.2017	1.2029	1.2041	1.2053	1.2065	1.2077	12
.190	1.2089	1.2101	1.2113	1.2125	1.2137	1.2149	1.2161	1.2174	1.2186	1.2198	12
.200	1.2210	1.2222	1.2234	1.2246	1.2259	1.2271	1.2283	1.2295	1.2308	1.2320	12

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(2) R. M. Fuoss and T. Shedlovsky, THIS JOURNAL, **71**, 1496 (1949).

(3) T. Shedlovsky, *J. Franklin Inst.*, **225**, 739 (1938).

(4) R. M. Fuoss, THIS JOURNAL, **57**, 488 (1935).

(5) The symbols used in this note are the same as those of Fuoss and Shedlovsky, ref. 2.

(6) Ordinarily, it is not necessary to employ terms higher than z^2 in evaluating $S(z)$ [H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd ed., Reinhold Publishing Corporation, New York, N. Y., 1950, p. 189; also *cf.* ref. 3, p. 742].

Identification of Histidine and Tyrosine by Partition Chromatography of Their Azo Dyes[1a]

BY JAMES E. DE VAY,[1b] WEN-HUA CHANG AND RALPH L. HOSSFELD

The identification of histidine and tyrosine in biological materials by paper partition chromatography has proven difficult because these amino-acids give weak color reactions with ninhydrin. A more sensitive spot-test method was described by Dent[2] who coupled histidine and tyrosine with diazobenzene-*p*-sulfonic acid to reveal their presence after separating them on a one-dimensional paper chromatogram.

One difficulty encountered in applying Dent's method[2] was that other amino-acids such as glycine and alanine gave yellow-orange spots when treated with diazotized sulfanilic acid and sodium carbonate and thus interfered with the identification of histidine which gave an orange-red spot. When irrigated with Dent's collidine–lutidine mixture on a one-dimensional chromatogram these

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(2) C. E. Dent, *Biochem. J.*, **41**, 240 (1947).

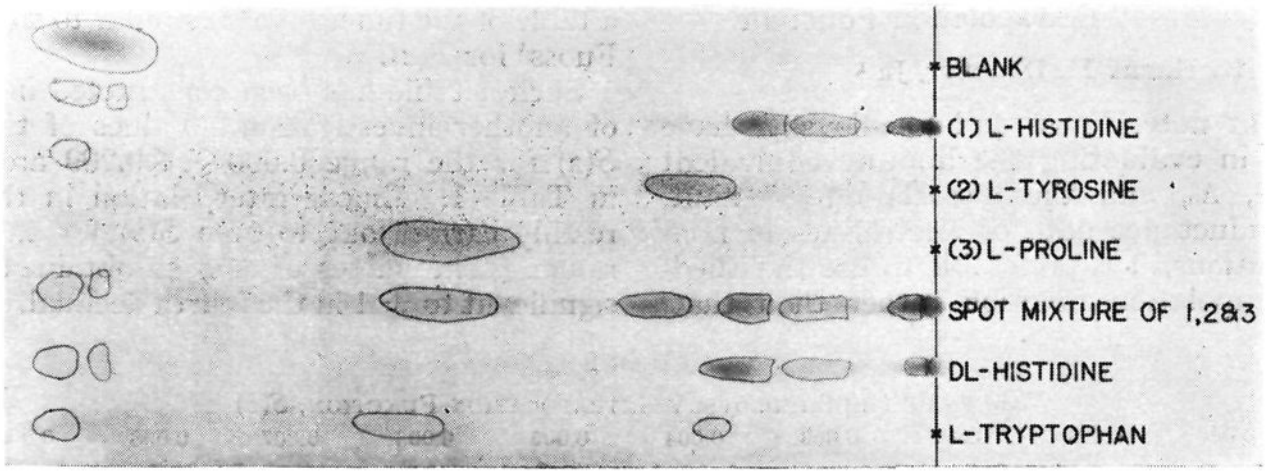

Fig. 1.—Chromatogram of the azo dyes of L-histidine, L-tyrosine, L-proline, DL-histidine and L-tryptophan; irrigated with *s*-butanol–H_2O for 40 hr. at 13°.

amino-acids overlap or lie sufficiently close to histidine that the identification of histidine is not always possible. The identification of tyrosine with this method was difficult because phenylalanine and tryptophan gave color reactions similar to tyrosine and lay near enough to tyrosine on a one-dimensional chromatogram that the identity of the spots was questionable.

The present method affords a more positive identification of histidine and tyrosine because the azo dyes from histidine could be resolved into a distinct pattern of three different colored spots while the azo dye from tyrosine gave a colored spot with an R_f value greater than any of the spots of histidine.

It has been found that a satisfactory chromatographic separation of histidine and tyrosine can be made by modifying the method described by Hossfeld for the partition chromatography of simple phenols.[3] Histidine and tyrosine were coupled with diazotized *p*-nitroaniline[4] in the presence of 4% sodium carbonate. The resulting azo dyes were spotted on Whatman No. 1 filter paper and developed by descending irrigation with secondary butanol (1 vol.)–water (1 vol.). After drying at room temperature, the chromatogram was sprayed with 4% sodium carbonate to intensify the color of the spots (Fig. 1). The azo dyes from L-histidine gave a pattern of three-colored spots: purple, yellow-orange and orange-red in the order of their increasing R_f values. A brown spot remained at the origin. L-Tyrosine gave a purple spot having an R_f value greater than that for the orange-red spot of L-histidine.

If an excess of diazotized *p*-nitroaniline was used in the coupling procedure, a red spot appeared near the solvent front, usually followed by a small yellow spot. The ultraviolet absorption curve of an absolute methanol extract of these colored areas was similar to the absorption curve obtained for the red spot from the blank (Fig. 1).

The possibility of separating the isomeric forms of a racemic mixture of histidine was investigated (Fig. 1); however, the pattern of the azo dyes from DL-histidine was similar to that from L-histidine.

As a control measure, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, proline, hydroxyproline, valine, leucine, phenylalanine, tryptophan, arginine, lysine, methionine, cystine, histamine and glucosamine were treated with diazotized *p*-nitroaniline under the above conditions and the resulting colored products chromatographed. Proline, hydroxyproline, tryptophan and arginine yielded derivatives which when chromatographed gave yellow spots. The spot due to the azo dye of hydroxyproline followed that of proline and the two dyes when spotted together on a filter paper strip were easily separated. The derivatives of histamine gave two spots: one was purple having approximately the same R_f value as the yellow-orange spot of histidine, the other was orange-red corresponding in position to an area between proline and tyrosine. The spots resulting from the arginine, tryptophan, proline, hydroxyproline and histamine derivatives did not interfere with the identification of histidine and tyrosine.

Resolution of the azo dyes of L-histidine, L-tyrosine and L-proline was effected from superimposed spots of a mixture of the dyes at one location on the paper (Fig. 1).

Experimental

Preparation of the Azo Dyes.—Diazotized *p*-nitroaniline was prepared according to the method of Smith and Irwin[4] and coupled to the amino-acids in a 4% sodium carbonate solution using 0.01 molar quantities of the reactants. The resulting reaction mixtures were spotted directly by means of a small nichrome wire loop at the appropriate position on the paper strip (22 × 56 cm. Whatman No. 1 filter paper).

Development of the chromatograms was accomplished using the method described by Hossfeld[3] except that the chromatograms were sprayed with 4% sodium carbonate after development instead of before. Trailing of the spots was decreased by developing the chromatograms at 13°, controlling the temperature (±0.5°).

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(3) R. L. Hossfeld, THIS JOURNAL, **73**, 852 (1951).
(4) L. I. Smith and W. B. Irwin, *ibid.*, **63**, 1036 (1941).

The Preparation of *trans*-4-Chlorocyclohexanol

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The action of concentrated hydrochloric acid on 1,4-cyclohexanediol is reported to yield an oil which has been characterized as a mixture of *cis*- and *trans*-4-chlorocyclohexanol.[1,2] The separation

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