CHROM. 4202

THIN-LAYER CHROMATOGRAPHIC AND ELECTROPHORETIC BEHAVIOUR OF PRIMARY AROMATIC AMINES ON WEAK ION EXCHANGERS

D. COZZI, P. G. DESIDERI, L. LEPRI AND V. COAS
Institute of Analytical Chemistry, University of Florence, Florence (Italy)
(First received May 14th, 1969; revised manuscript received June 5th, 1969)

SUMMARY

The authors have studied extensively the thin-layer chromatographic and electrophoretic behaviour of primary aromatic amines on weak ion exchangers such as alginic acid and carboxymethylcellulose. The eluents were solutions of organic and inorganic acids, water, buffer solutions and aqueous organic solvents. The R_F values were correlated with the acidity and basicity of many amines.

INTRODUCTION

Many authors[1–4] have investigated the separation of primary aromatic amines by partition chromatography on thin layers. However, little work has been published on the use of ion-exchange chromatography for this purpose, although it scores over partition chromatography by being simpler and by giving more reproducible results. Furthermore, the thin-layer chromatographic separation by this method can be repeated on a larger scale on a column of the same adsorbent.

The present work is part of a large scale investigation in our Institute on the chromatographic behaviour of organic[5] and inorganic[6,7] compounds on weak ion exchangers. The adsorbents were carboxymethylcellulose (H^+ or Na^+ form) and alginic acid, whose use as a thin-layer chromatographic stationary phase was first proposed by us[6]. The samples were also subjected to electrophoretic separation on these ion exchangers.

EXPERIMENTAL

Chromatography

The chromatographic use of these two stationary phases has been described earlier[7]. A small amount of the amine was dissolved in a 1:1 (v/v) mixture of 0.1 *N* HCl and isopropanol. Fresh solutions were used in cases when the amines tend to be photolysed (*e.g.* phenylenediamines). The amount of each amine (*cf.* Tables I–III) was fixed on the basis of the spot size and the sensitivity of the visualizing agent, found in preliminary tests.

Electrophoresis

Thin layers (20 × 20 cm) were used throughout in a Camag high potential electrophoresis apparatus with water cooling at 18°. The electrolytes contained acetic acid with or without sodium acetate in the same concentration.

Visualization

The visualizing agent was a saturated solution of *p*-dimethylaminobenzaldehyde (DMAB) in benzene, containing 5% of acetic acid.

With sodium carboxymethylcellulose plates, it is best to use a saturated solution of DMAB in glacial acetic acid. *o*-Anisidine and some isomers, such as those of toluidine, bromoaniline, and chloroaniline, could not be detected on such plates when the eluent is an acetate buffer. However, brown spots could later be found at the start by spraying the plates with a 5% solution of sodium nitrite in 0.2 *N* HCl, drying, and spraying with a methanolic 5% solution of α-naphthol.

With the amines visualized by DMAB, the diazotisation with nitrous acid followed by coupling with α-naphthol, reveals two spots on the sodium carboxymethylcellulose plates: one is at the start, and the other is in the same position where it was found by DMAB . Exceptions are *o*-, and *p*-aminophenol and *o*-, and *p*-nitroaniline for which the spots at the start are not observed.

Using neutral and weakly acidic eluents, BECKETT AND CHOULIS[8] found multiple spots for some aliphatic amines on paper and cellulose layers.

RESULTS AND DISCUSSION

Alginic acid plates with acidic eluents

Solutions of organic and inorganic acids were used at various concentrations as eluents. A change in the concentration of the eluent did not produce such an appreciable difference as was brought about by a change in the nature of the eluent. Table I shows the R_F values of thirty-three amines, obtained with acetic, formic, chloroacetic, and hydrochloric acid. Comparison shows that the R_F values increase with the strength of the acids. In most cases, the isomers had the same R_F value under the same conditions. Others, however, were separated; their R_F values increasing from the *meta*- to the *para*- and then to the *ortho*-isomer.

Hydrochloric acid and chloroacetic acid gave the same elution pattern, except in the case of the nitroanilines, where they gave opposite ones. Acetic acid was advantageous in the separation of the isomers (*cf.* Fig. 1 for nitroanilines), and in differentiating between variously substituted amines (*cf.* Fig. 2). In this second case, the amines can be separated not only because they differ in the nature of the substituents, but also because they have different acidities and basicities. Thus, the markedly acidic sulphanilic acid migrated with the solvent front, while the markedly basic *p*-phenylenediamine remained at the start.

Alginic acid plates with mixed eluents

The aqueous organic solvents, used previously with amino acids[5], generally gave less satisfactory results than the aqueous solvents. The following systems were used: HCl–acetone, HCl–dioxan, chloroacetic acid–dioxan, and chloroacetic acid–isopropanol; the latter gave some interesting results. The addition of alcohol to the acidic

TABLE I

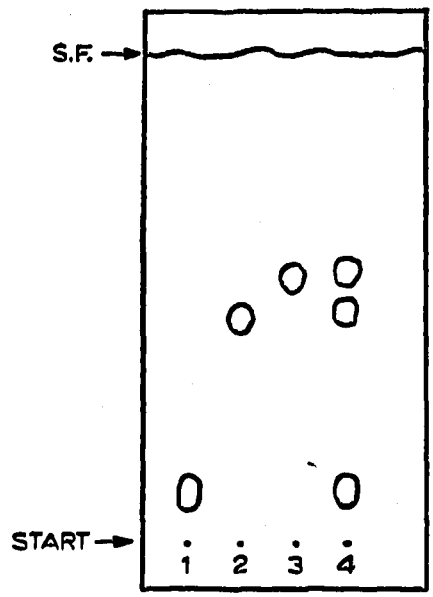
R_F VALUES OF SOME AROMATIC AMINES ON ALGINIC ACID

Eluents: (1) 1 *N* acetic acid; (2) 1 *N* formic acid; (3) 1 *N* chloroacetic acid; (4) 0.1 *N* hydrochloric acid.

Amine	*Eluent*				*Amount* (*μg*)
	1	*2*	*3*	*4*	
m-Aminobenzoic acid	0.10	0.21	0.48	0.62	0.6
o-Aminobenzoic acid	0.23	0.30	0.55	0.67	0.6
p-Aminobenzoic acid	0.16	0.24	0.51	0.61	0.4
Sulfanilic acid	0.95	0.94	0.94	0.96	2.0
o-Arsanilic acid	0.82	0.78	0.86	0.81	2.0
p-Arsanilic acid	0.41	0.42	0.64	0.72	1.5
m-Aminophenol	0.08	0.18	0.49	0.60	0.6
o-Aminophenol	0.09	0.18	0.51	0.60	1.2
p-Aminophenol	0.08	0.18	0.51	0.60	1.0
α-Naphthylamine	0.06	0.12	0.34	0.40	1.2
β-Naphthylamine	0.06	0.12	0.34	0.40	1.2
m-Anisidine	0.08	0.18	0.53	0.62	1.3
o-Anisidine	0.10	0.21	0.56	0.67	1.3
p-Anisidine	0.08	0.18	0.52	0.62	0.6
Benzidine	0.00	0.00	0.04	0.13	0.3
m-Bromoaniline	0.08	0.16	0.44	0.56	10.0
o-Bromoaniline	0.09	0.19	0.51	0.63	15.0
p-Bromoaniline	0.06	0.16	0.44	0.58	1.2
m-Chloroaniline	0.08	0.18	0.45	0.58	10.0
o-Chloroaniline	0.13	0.25	0.48	0.64	15.0
p-Chloroaniline	0.08	0.18	0.43	0.60	0.8
m-Phenylenediamine	0.01	0.02	0.08	0.28	1.0
o-Phenylenediamine	0.06	0.09	0.20	0.63	1.2
p-Phenylenediamine	0.00	0.02	0.08	0.27	0.5
m-Nitroaniline	0.11	0.21	0.40	0.59	0.6
o-Nitroaniline	0.55	0.52	0.62	0.51	2.0
p-Nitroaniline	0.47	0.47	0.56	0.52	0.8
m-Toluidine	0.08	0.25	0.50	0.66	8.0
o-Toluidine	0.08	0.26	0.50	0.66	8.0
p-Toluidine	0.07	0.25	0.50	0.66	1.2
p-Aminodimethylaniline	0.00	0.04	0.14	0.36	2.0
p-Aminosalicylic acid	0.26	0.27	0.46	0.53	1.2
p-Aminoacetophenone	0.20	0.29	0.51	0.67	0.6

eluent brought about different changes in the R_F values of the isomers, as can be seen in Table II. In the case of aminobenzoic acids, for example, it lowered the R_F value of the *meta*-isomer and raised that of the other two, particularly that of the *ortho*-isomer. Therefore, 1 *N* chloroacetic acid in 50% isopropanol gave a separation which could not be achieved with aqueous eluents.

Like *o*-arsanilic acid, *o*- and *p*-nitroaniline migrated with the solvent front. The use of aqueous organic solvents permitted the use of smaller samples.



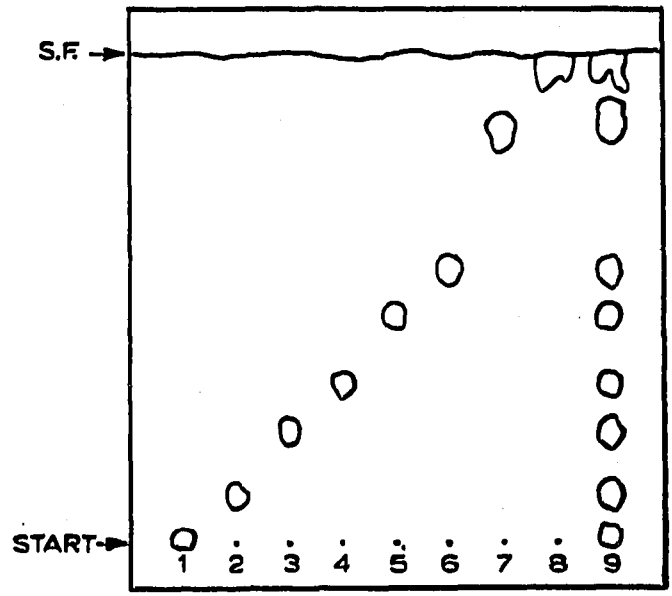


Fig. 1. Thin-layer chromatogram of nitroanilines. 1 = *m*-nitroaniline; 2 = *p*-nitroaniline; 3 = *o*-nitroaniline; 4 = mixture.

Fig. 2. Thin-layer chromatogram of aromatic amines. 1 = *p*-phenylenediamine; 2 = *p*-anisidine; 3 = *o*-aminobenzoic acid; 4 = *p*-arsanilic acid; 5 = *p*-nitroaniline; 6 = *o*-nitroaniline; 7 = *o*-arsanilic acid; 8 = sulphanilic acid; 9 = mixture.

Carboxymethylcellulose (CMC) plates (H^+ form)

As has been found earlier and confirmed now, the retention is weaker here than on alginic acid plates, and so only the results for water and acetic acid are reported. The aqueous organic solvents gave no useful results.

The R_F values in Table III show that water gives a very good separation of aminobenzoic acids, and between *p*-aminosalicylic acid and *m*-aminophenol. This second separation is of particular interest, because *m*-aminophenol is a harmful impurity formed in the preparation of *p*-aminosalicylic acid. The possibility of separating them may be of practical importance, if thin plates are replaced by columns.

The more water-soluble amines with a very low K_b value ($< 10^{-10}$) and/or a marked acidity (*e.g.* sulphanilic acid) exhibited a higher mobility in water. On the other hand, there was an equalization of the mobilities in 1 *M* acetic acid, as indicated by the uniformity of the R_F values (about 0.4), exceptions being strong bases (phenylenediamines, benzidine, and *p*-aminodimethylaniline) and pronounced acids (arsanilic acids and sulphanilic acid).

Relationship between the pK_b and the R_F values

In the case of some aromatic amines, the R_F values obtained on both types of plate can be correlated with the acid–base character of the isomers. Thus, among the isomeric aminobenzoic acids and nitroanilines, the affinity for the stationary phase decreases (and so the R_F value increases) as the pK_b value increases (isomers whose R_F values differ by 0.02 or less are considered to have the same affinity). Thus, the pK_b values for *m*-, *p*-, and *o*-aminobenzoic acid are 11.44, 11.80, and 12.20, respectively[9], and the corresponding R_F values on both stationary phases increase as $m <$

TABLE II

R_F VALUES OF SOME AROMATIC AMINES ON ALGINIC ACID

Eluents: (1) 1 *N* chloroacetic acid in 25% isopropyl alcohol; (2) 1 *N* chloroacetic acid in 50% isopropyl alcohol.

Amine	*Eluent*		*Amount* (μg)
	1	*2*	
m-Aminobenzoic acid	0.45	0.43	0.3
o-Aminobenzoic acid	0.58	0.72	0.3
p-Aminobenzoic acid	0.57	0.61	0.2
Sulfanilic acid	0.76	0.51	0.8
o-Arsanilic acid	0.86	0.96	0.8
p-Arsanilic acid	0.55	0.50	0.6
m-Aminophenol	0.45	0.31	0.5
o-Aminophenol	0.46	0.34	0.6
p-Aminophenol	0.45	0.29	0.5
α-Naphthylamine	0.44	0.43	0.6
β-Naphthylamine	0.48	0.47	0.6
m-Anisidine	0.51	0.37	0.6
o-Anisidine	0.54	0.37	0.6
p-Anisidine	0.48	0.35	0.3
Benzidine	0.00	0.00	0.3
m-Bromoaniline	0.44	0.48	3.5
o-Bromoaniline	0.50	e.s.*	5.0
p-Bromoaniline	0.46	0.46	0.6
m-Chloroaniline	0.46	0.50	3.5
o-Chloroaniline	0.53	0.70	5.0
p-Chloroaniline	0.46	0.49	0.8
m-Phenylenediamine	0.02	0.00	1.0
o-Phenylenediamine	0.17	0.11	1.2
p-Phenylenediamine	0.02	0.00	0.5
m-Nitroaniline	0.44	0.62	0.6
o-Nitroaniline	0.90	0.97	2.0
p-Nitroaniline	0.83	0.97	0.8
m-Toluidine	0.48	0.47	4.0
o-Toluidine	0.45	0.44	3.0
p-Toluidine	0.49	0.48	0.8
p-Aminodimethylaniline	0.09	0.00	2.0
p-Aminosalicylic acid	e.s.*	0.28	0.6
p-Aminoacetophenone	0.61	0.73	0.5

* e.s. = elongated spot.

$p < o$. Similarly, the pK_b values of *m*-, *p*-, and *o*-nitroaniline are 11.54, 13.00 and 14.26, respectively[10], and the R_F values again increase in the order $m < p < o$.

This sequence of R_F values was obtained on alginic acid with 1 *N* acetic acid, formic acid, and chloroacetic acid (*cf.* Table I), and on CMC with water as the eluent (*cf.* Table III).

Among other isomers, whose pK_b values do not decisively affect their chromatographic behaviour, one must draw a distinction between the aminophenols and naphthylamines (whose isomers have the same R_F value, but a different pK_b value[11,12])

TABLE III

R_F VALUES OF SOME AROMATIC AMINES ON CARBOXYMETHYLCELLULOSE

Eluents: (1) water; (2) 1 *N* acetic acid.

Amine	*Eluent*		*Amount* (μg)
	1	*2*	
m-Aminobenzoic acid	0.14	0.44	0.3
o-Aminobenzoic acid	0.44	0.57	0.3
p-Aminobenzoic acid	0.28	0.45	0.2
Sulfanilic acid	0.96	0.96	0.4
o-Arsanilic acid	0.92	0.91	0.6
p-Arsanilic acid	0.85	0.81	0.5
m-Aminophenol	0.01	0.38	0.5
o-Aminophenol	0.01	0.40	0.5
p-Aminophenol	0.00	0.40	0.5
α-Naphthylamine	0.00	0.28	0.6
β-Naphthylamine	0.00	0.26	0.6
m-Anisidine	0.02	0.38	0.6
o-Anisidine	0.02	0.40	0.6
p-Anisidine	0.01	0.38	0.3
Benzidine	0.00	0.08	0.3
m-Bromoaniline	0.03	0.40	2.0
o-Bromoaniline	0.03	0.52	3.0
p-Bromoaniline	0.02	0.38	0.5
m-Chloroaniline	0.04	0.40	2.0
o-Chloroaniline	0.04	e.s.*	3.0
p-Chloroaniline	0.03	0.38	0.5
m-Phenylenediamine	0.01	0.18	0.5
o-Phenylenediamine	0.05	0.35	1.0
p-Phenylenediamine	0.00	0.16	0.3
m-Nitroaniline	0.25	0.49	0.6
o-Nitroaniline	0.52	0.52	2.0
p-Nitroaniline	0.47	0.55	0.8
m-Toluidine	0.02	0.40	1.5
o-Toluidine	0.03	0.44	1.5
p-Toluidine	0.01	0.41	0.3
p-Aminodimethylaniline	0.01	0.24	1.0
p-Aminosalicylic acid	0.50	0.42	0.5
p-Aminoacetophenone	0.35	0.58	0.5

* e.s. = elongated spot.

and the chloroanilines, bromoanilines and phenylenediamines (where the *meta*- and *para*-isomers only have very close R_F values). Thus the pK_b values of *p*-, *m*-, and *o*-isomers of chloroaniline[9], bromoaniline[9], and phenylenediamine[11], are 9.25, 10.60, 11.64; 10.18, 10.68, 11.68; 7.92, 9.12, and 9.54, respectively; the corresponding R_F values showing the pattern: $p = m < o$ on both stationary phases.

These sequences were obtained on CMC with 1 *N* acetic acid since the R_F values, which are very low for these compounds, do not differ sufficiently after elution with water.

TABLE IV

MIGRATION DISTANCE (mm)* OF AROMATIC AMINES ON ALGINIC ACID (AA) AND CARBOXYMETHYLCELLULOSE (CMC) THIN LAYERS WITH 1 *N* ACETIC ACID AS ELECTROLYTE

Electric potential: 1200 V.

Amine	*Migration time (min)*		
	AA	*CMC*	
	60	*10*	*20*
m-Aminobenzoic acid	45	38	57
o-Aminobenzoic acid	51	27	35
p-Aminobenzoic acid	43	28	44
Sulfanilic acid	42	14	11
o-Arsanilic acid	47	16	16
p-Arsanilic acid	43	19	24
m-Aminophenol	51	40	68
o-Aminophenol	55	38	68
p-Aminophenol	51	36	68
α-Naphthylamine	31	27	44
β-Naphthylamine	31	28	48
m-Anisidine	49	35	70
o-Anisidine	58	34	72
p-Anisidine	55	34	77
Benzidine	6	21	45
m-Bromoaniline	54	37	60
o-Bromoaniline	56	45	e.s.**
p-Bromoaniline	54	38	60
m-Chloroaniline	56	38	68
o-Chloroaniline	65	41	e.s.**
p-Chloroaniline	57	40	70
m-Phenylenediamine	20	39	86
o-Phenylenediamine	35	25	76
p-Phenylenediamine	17	43	96
m-Nitroaniline	56	19	42
o-Nitroaniline	38	6	8
p-Nitroaniline	39	6	10
m-Toluidine	67	37	79
o-Toluidine	63	36	80
p-Toluidine	65	34	78
p-Aminodimethylaniline	24	38	90
p-Aminosalicylic acid	41	5	12
p-Aminoacetophenone	56	13	26

* The reported data are the means of several determinations.

** e.s. = elongated spot.

The smaller affinity of the *o*-isomer with respect to the *para*- and *meta*-isomers for many types of amine (aminobenzoic acids, nitroanilines, chloroanilines, bromoanilines, phenylenediamines on both stationary phases; toluidines on CMC and anisidines on alginic acid) suggests that steric hindrance influences the reaction between the cationic groups of the amine and the adsorbent.

Electrophoretic results

The electrophoretic behaviour of amines on alginic acid and CMC depends mainly on the retentive power of the stationary phase, the ionic mobility, and the migration of the zones. The first of these is the controlling factor in the case of alginic acid, because migration on it is at least three times as slow as on CMC (*cf.* Table IV). With CMC, which is known to have a weaker retentive power than alginic acid, the electrophoretic behaviour is governed mainly by the other two factors mentioned above.

Carboxymethylcellulose permits the correlation of the electrophoretic behaviour of the amines with their K_b values, since the zone mobility U is also a function of the ionized fraction of the amine[13];

$$U = u \frac{[ArNH_3^+]}{[ArNH_3^+] + [ArNH_2]} = u \frac{K_b}{[OH^-] + K_b} \quad (1)$$

where u is the ionic mobility. This equation clearly shows the effect of the pH of the medium on the migration of the zone. These relationships are illustrated by the data in Tables IV and V; these were obtained on CMC with 1 *M* acetic acid and with an

TABLE V

MIGRATION DISTANCE (mm) AND R_F VALUES OF AROMATIC AMINES ON SODIUM CARBOXYMETHYLCELLULOSE

Electrophoresis: electrolyte, 0.1 *N* acetic acid–0.1 *N* sodium acetate; electric potential, 800 V; time 25 min. Chromatography: eluent 0.1 *N* acetic acid–0.1 *N* sodium acetate.

Amine	*Migration distance (mm)*	R_F *value*
m-Aminobenzoic acid	−14	0.96
o-Aminobenzoic acid	−14	0.96
p-Aminobenzoic acid	−15	0.96
Sulfanilic acid	−31	0.96
o-Arsanilic acid	−18	0.96
p-Arsanilic acid	−13	0.96
m-Aminophenol	11	e.s.*
o-Aminophenol	16	0.38
p-Aminophenol	37	0.29
α-Naphthylamine	7	0.37
β-Naphthylamine	7	0.32
m-Anisidine	8	e.s.*
p-Anisidine	38	0.35
Benzidine	3	0.06
m-Phenylenediamine	25	0.33
o-Phenylenediamine	12	0.36
p-Phenylenediamine	48	0.25
m-Nitroaniline	8	0.80
o-Nitroaniline	5	0.75
p-Nitroaniline	6	0.68
p-Aminodimethylaniline	60	0.38
p-Aminoacetophenone	4	0.81

* e.s. = elongated spot.

equimolecular mixture of acetic acid and sodium acetate. It should be noted that at the pH of the acetate buffer the aminobenzoic and arsanilic acids and the sulphanilic acid are mainly present as anions, which is indicated by the electrophoretic data and the R_F values.

The mobility of the zones is important from the analytical point of view in that one can find the pH at which the separation is optimal, provided that the dissociation constants of the amines are known. For example, the isomeric aminophenols cannot be separated at pH 2.37 (in 1 *M* acetic acid), but can at pH 4.74 (in acetate buffer), as shown in Tables IV and V.

Table VI gives those fractions of the isomeric aminophenols which exist in the ionic form at the above-mentioned pH values, as found by the use of eqn. (1). Separation is thus possible at pH 4.74, since the difference between the α_b values of the isomers can be enhanced at this pH value. At pH 2.37, on the other hand the values for α are too close to each other.

TABLE VI

FRACTION OF THE TOTAL CONCENTRATION OF AMINOPHENOLS AND PHENYLENEDIAMINES IN THE IONIC FORM (α)

Aminophenol	pK_b (*ref. 11*) (21°)	α	
		pH = 2.37	*pH = 4.74*
meta-	9.83	0.986	0.210
ortho-	9.28	0.996	0.490
para-	8.51	0.999	0.850

Phenylene-diamine	$pK_{b(1)}$ (20°)	$pK_{b(2)}$ (*ref. 11*) (20°)	*pH = 2.37*		*pH = 4.74*	
			$\alpha_{(1)}$	$\alpha_{(2)}$	$\alpha_{(1)}$	$\alpha_{(2)}$
ortho-	9.53	13.40	0.993	0.016	0.349	0.000
meta-	9.12	12.11	0.997	0.248	0.579	0.001
para-	7.92	10.86	0.999	0.858	0.951	0.024

Similar results were obtained for anisidines (whose K_b values[10] are of the same order as those of the aminophenols) and for the phenylenediamines. The data listed in Table VI reveal that the α values from the phenylenediamine isomers, calculated from $K_{b(2)}$ differ considerably at pH 2.37, whereas the α values corresponding to the $K_{b(1)}$ values are practically the same. At pH 4.74, the situation is the reverse.

CONCLUSIONS

The following conclusions can be drawn from the chromatographic and electrophoretic behaviour of thirty-three primary aromatic amines on thin-layers of alginic acid and carboxymethylcellulose: (1) Alginic acid has a stronger retentive power than carboxymethylcellulose. Both stationary phases exhibit a marked selectivity for the amines investigated. (2) High-voltage electrophoresis sometimes enables one to separate amines that differ only a very little in their acidity or basicity.

REFERENCES

1 S. HERMANEK, V. SCHWARZ AND Z. CEKAN, *Pharmazie*, 16 (1961) 566.
2 M. GILLIO-TOS, S. A. PREVITERA AND A. VIMERCATI, *J. Chromatog.*, 13 (1964) 571.
3 A. CEE AND J. GASPARIC, *Mikrochim. Acta*, (1966) 295.
4 I. GEMZOVA AND J. GASPARIC, *Mikrochim. Acta*, (1966) 310.
5 D. COZZI, P. G. DESIDERI, L. LEPRI AND V. COAS, *J. Chromatog.*, 40 (1969) 138.
6 D. COZZI, P. G. DESIDERI, L. LEPRI AND G. CIANTELLI, *J. Chromatog.*, 35 (1968) 396.
7 D. COZZI, P. G. DESIDERI, L. LEPRI AND G. CIANTELLI, *J. Chromatog.*, 35 (1968) 405.
8 A. H. BECKETT AND N. H. CHOULIS, *J. Pharm. Pharmacol.*, 15 (1963) 236 T.
9 MYRBÄCK, *Z. Physiol. Chem.*, 158, 261; (BEILSTEIN, *Organische Chemie*, E II 12, (1950) 315, 321, 323, 341, 342 and 344; E II 14, (1951) 206, 238 and 246).
10 F. D. SNELL AND C. L. HILTON (Editors), *Encyclopedia of Industrial Chemical Analysis*, Vol. 5, Interscience, New York, 1967, p. 422.
11 R. KUHN AND A. WASSERMANN, *Helv. Chim. Acta*, 11 (1928) 3
12 R. C. FARMER AND F. J. WARTH, *J. Chem. Soc.*, 85 (1904) 1726.
13 R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *J. Biochem.*, 40 (1946) 33.