THE PAPER CHROMATOGRAPHY OF SOME COMPLEXONES AND THEIR IRON CHELATES

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INTRODUCTION

The complexones may be defined as the series of polyaminopolyacetic acids related to ethylenediaminetetra-acetic acid, EDTA. The ferric iron chelates formed by some of these compounds are of importance in both practical and experimental horticulture as sources of iron for plant growth. Investigations here into their behaviour in soils and plants have included experiments on the identification by paper chromatography of these chelates or their possible decomposition products. The paper chromatography of EDTA and a few other complexones has been reported by HAMAKER[1], HEMWALL[2] and HILL-COTTINGHAM AND LLOYD-JONES[3], but the number and nature of the compounds examined has now been extended greatly.

EXPERIMENTAL

Paper

Metallic impurities in normal paper interfere with the separations of the complexone acids. Hence before use the paper, Whatman No. 1, is leached with *N* HCl, then water and finally dried in air.

Solvents

The solvents found most satisfactory are those commonly used for amino acid separations, namely:

(*a*) *Phenol–water*, 4:1 w/v, with solid potassium cyanide, approx. 1 g, and ammonia, 2 ml of 0.880, present in the tanks. (Tank volume approximately 35 l.)

(*b*) *n-Butanol–acetic acid–water*, 4:1:5 by volume, using the organic phase after equilibration at room temperature.

Methods of detection on paper

(*a*) Some acids can be detected by a modification of the DARBEY[4] reaction for EDTA as proposed by HAMAKER[1]. The procedure is: (*i*) dip paper through solution of $NiSO_4$ (0.01 % in methanol); (*ii*) expose to ammonia vapour and then dry in air; (*iii*) dip paper through solution of dimethylglyoxime, DMG, (0.1 % in ethanol) and dry again.

The positions of those acids capable of chelating the nickel sufficiently strongly to prevent the formation of Ni-DMG are revealed on the paper as white or yellow spots on a deep pink background.

(*b*) Ninhydrin (0.2 % in acetone) forms blue or purple spots with amino and imino-compounds if, after dipping the paper and allowing to dry at room temperature, the paper is heated at 100° for 5 min.

(*c*) The complexone acids that contain phenolic groups form characteristically-coloured red or purple iron chelates. These compounds may be formed on the paper by dipping through a solution of ferric citrate (0.02 % in 80 % acetone).

(*d*) Alternatively the positions of the phenolic compounds may be revealed by dipping the paper first through a solution of *p*-nitrobenzene diazonium fluoroborate, 0.1 % in acetone, then through potassium hydroxide solution, 0.1 % in ethanol.

(*e*) The ferric iron chelates can be seen as coloured spots in daylight, or for lower concentrations, as dark spots under U.V. light.

TABLE I

	Chelating agents	*Abbreviations*	*Colour formed with detection reagents*	
			Nickel-DMG	*Ninhydrin*
1	Triethylenetetraminehexa-acetic acid	TTHA	White	—
2	Diethylenetriaminepenta-acetic acid	DTPA	Pale yellow	—
3	Ethylenediaminetetra-acetic acid	EDTA	White	—
4	Propylene-1,2-diaminetetra-acetic acid	Me-EDTA	White	—
5	1,2-Diaminocyclohexanetetra-acetic acid	CDTA	White	—
6	1,3-Diaminopropan-2-ol-tetra-acetic acid	DPTA	White	—
7	Ethyleneglycol-bis-(2-aminoethyl ether)-tetra-acetic acid	EGTA	Pale yellow*	—
8	N-2-Hydroxyethyl-ethylenediamine-N,N',N'-triacetic acid	HEEDTA	White	—
9	N-2-Hydroxycyclohexyl-ethylenediamine-N,N',N'-triacetic acid	OETA, RA 155	White	—
10	N,N'-Di-2-hydroxyethyl-ethylenediamine-N,N'-diacetic acid	HEEDDA	White	—
11	Ethylenediamine-N,N'-diacetic acid	EDDA	White	Purple
12	2-Ketopiperazin-1-yl-acetic acid	cyclic EDDA	—	Yellow
13	Ethylenediamine-N,N'-bis-(2-hydroxyphenylacetic acid)	EDHPA, Chel 138	White	—
14	Ethylenediamine-N,N'-bis-(2-hydroxy-5-methylphenylacetic acid)	RA 157	White	—
15	N,N'-Bis-(2-hydroxy-5-methylbenzyl)-ethylenediamine-N,N'-diacetic acid	RA 156	White	—
16	N,N'-Bis-(2-hydroxy-5-sulphobenzyl)-ethylenediamine-N,N'-diacetic acid	RA 159	White	—
17	Nitrilotriacetic acid	NTA	White	—
18	N-2-Hydroxyethyl-iminodiacetic acid	HEIDA	White	—
19	N,N-Di-2-hydroxyethyl-glycine	DHEG	White*	Purple
20	N-Methyliminodiacetic acid	Me-IDA	White*	—
21	Iminodiacetic acid	IDA	White*	Purple
22	Glycine	Glyc	—	Purple
23	Ethylenediamine	ED	—	Blue-grey

* Spots of these acids slowly revert to the pink colour of the background.

During preparation of the chromatograms it must be remembered that iron chelate solutions are photosensitive. Exposure of the solutions to U.V. light, in particular direct sunlight, results in reduction of the ferric iron and partial decomposition of the organic ligand.

RESULTS

The full chemical names of the compounds investigated together with their abbreviations are given in Table I. This Table also gives their responses to the Ni-DMG and the ninhydrin detection reagents.

The mean R_F values found for the complexone acids and their iron chelates in the two solvents are given in Table II.

All the results were obtained by descending chromatography.

TABLE II

MEAN R_F VALUES × 100 OF THE CHELATING AGENTS AND OF THEIR FERRIC-IRON CHELATES

	Chelating agent	*Phenol–water (+ NH_3, KCN)*		*Butanol–acetic acid–water (4:1:5)*	
		Free acid	*Iron chelate*	*Free acid*	*Iron chelate*
1	TTHA	22	75	07	03
2	DTPA	26	50	08	08
3	EDTA	25	63	14	06
4	Me-EDTA	42	77	17	08
5	CDTA	55	77	25	14
6	DPTA	20	82	04	09
7	EGTA	66	81	08	07
8	HEEDTA	57	80	16	13
9	RA 155	69	88	31	30
10	HEEDDA	83	87	15	19
11	EDDA	53	—	07	—
12	Cyclic EDDA	80	—	18	—
13	EDHPA	71	88	0	54 Red-brown 66 Crimson
14	RA 157	82	91	0	73
15	RA 156	64	84	75	84
16	RA 159	03	12	0	05
17	NTA	22	76	10	09
18	HEIDA	54	87	16	11
19	DHEG	83	92	21	45
20	Me-IDA	65	74	21	—
21	IDA	24	—	12	—
22	Glyc	45	—	17	—
23	ED	88	26	11	11

DISCUSSION

The R_F values found with the phenol solvent are lower than those obtained by HAMAKER[1] or HEMWALL[2] who, however, did not run the chromatograms in the presence of ammonia. It has been advantageous here to use the ammoniacal solvent as it precipitates un-chelated iron, and some other metals, on the origin.

As reported by LEBLANC[5], EDDA (No. 11) and its cyclic form (No. 12 in Table I)

are readily interconvertible, the di-acetic acid being transformed into the cyclic imide in hot acid solution and the reverse action taking place in hot basic solutions. It has been found here that, on standing, even neutral solutions of EDDA contain considerable amounts of the cyclic form.

It is of interest to note that in the butanol solvent the ferric iron chelate of EDHPA separates into two components of approximately equal concentrations but different colours. These two iron chelates have been shown to have different stabilities, the more stable component being of red-brown colour with the lower R_F. It is suggested that Fe-EDHPA exists in two isomeric forms but their structures are unknown as yet.

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SUMMARY

R_F values for complexone acids and their ferric iron chelates in phenol–water and in butanol–acetic acid–water solvents are given, together with methods of detecting these substances on paper.

REFERENCES

1 J. W. HAMAKER, personal communication.
2 J. B. HEMWALL, *Soil Sci.*, 86 (1958) 126.
3 D. G. HILL-COTTINGHAM AND C. P. LLOYD-JONES, *J. Sci. Food Agr.*, 12 (1961) 69.
4 A. DARBEY, *Anal. Chem.*, 24 (1952) 373.
5 R. B. LEBLANC, *Anal. Chem.*, 31 (1959) 1840.

NOTE ADDED IN PROOF

It has now been stated by D. P. RYSKIEWICH AND G. BOKA, *Nature*, 193 (1962) 472, that the two components of Fe-EDHPA separated by the butanol solvent are, in fact, the *meso* form and the *dl*-racemic mixture.