Azo Dyes as Indicators for Calcium and Magnesium

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▶ From a study of 26 selected monoazo compounds it is concluded that the minimum structural features necessary for union with magnesium and calcium are two hydroxyl groups, o- and o'- respectively, to the azo group, or alternatively, one o-hydroxyl group and one o'-carboxyl group. The combining ratio of such compounds with magnesium and with calcium is invariably one to one in water solution. The formation constant for the magnesium compound is always greater than that for the corresponding calcium compound.

THE GREAT utility of Eriochrome Black T in the (ethylenedinitrilo)-tetraacetate titration of calcium plus magnesium leads one naturally to wonder just what elements in the molecule are essential to the indicator function. Biedermann and Schwarzenbach give no hint of this in their first paper (*3*), merely naming four dyes which will work by their ingenious scheme of employing the color change which accompanies the extraction of the metal from the slightly dissociated magnesium-dye compound. In a later paper (*28, 29*) they report the combining ratio and the formation constants toward magnesium and calcium of one of the four dyes, Eriochrome Black T. This is the dye which was recommended and which has found wide acceptance although it is less than satisfactory because of its instability in water solution. All four of the dyes named in the first paper—Eriochrome Blue Black B (F 239; C.I. 201), Eriochrome Blue Black R (F 240; C.I. 202), Eriochrome Black T (F 241; C.I. 203), and Eriochrome Black A (F 242; C.I. 204)—are *o,o'*-dihydroxyazo dyes, all are acid-base indicators, all four bear sulfonic acid groups, and two have nitro groups. Obviously, the chromophore and an acid group so situated to produce chelation with the alkaline earth metal are essential, but what of the second hydroxyl group? What of the nitro and sulfonic groups, distant in the molecule from the azo group? We can draw on the related work of Drew and Landquist (*11*), of Haendler and Smith (*16*), of Drew and Fairbairn (*10*), and of Beech and Drew (*2*) on the manganese, iron, cobalt, nickel, copper, and zinc derivatives of azo mono-*o*- and di-*o,o'*- substituted azo compounds, but it would probably be unsafe to conclude that the alkaline earths will behave toward these reagents in a fashion identical to the transition elements.

In the present work we describe some 30 hydroxyazo dyes, selected so as to throw light on the structural question, and their behavior toward calcium and magnesium.

EXPERIMENTAL WORK

Apparatus and Materials. The absorption spectra of the dyes mentioned in this paper have been reproduced in full (*13*) and were obtained using Beckman DK2 and DU spectrophotometers as were those of the calcium and magnesium compounds. All spectrophotometric data used in obtaining combining ratios and formation constants were obtained using a Beckman DU spectrophotometer.

All pH measurements were made with a Beckman Model G pH meter with a Type E glass electrode. The meter was standardized against an appropriate National Bureau of Standards reference buffer (*1*).

Standard calcium solutions were prepared by weight from Mallinckrodt analytical reagent primary standard calcium carbonate. Standard magnesium solutions were prepared using magnesium chloride hexahydrate. The strength of these solutions was determined by titration with (ethylenedinitrilo)tetraacetate (EDTA) previously standardized against Mallinckrodt analytical reagent primary standard calcium carbonate.

All inorganic chemicals used were of reagent grade quality. The organic chemicals were purified as needed by recrystallization or redistillation.

All spectrophotometric data were obtained on buffered solutions at a constant ionic strength (μ = 0.100). The pH 10 buffer was prepared by mixing 67.5 grams of ammonium chloride with 570 ml. of ammonium hydroxide and diluting to 1000 ml. with distilled water. Potassium chloride was used to bring all solutions to a constant ionic strength.

The preparation of each of the azo dyes is given with each dye. The dyes were all carefully purified and the majority of them were obtained in crystalline form.

Acid Dissociation Constants. The acid dissociation constants of the various dyes studied were determined spectrophotometrically.

Combining Ratio and Formation Constants. Two methods were used to determine the combining ratio of the alkaline earth metals to dye: the method of continuous variations (*19, 30*) and the log-ratio method (*7, 8, 13, 18*).

Monazo Dyes Which Do Not Combine with Calcium or Magnesium. The following dyes, A-1 through A-17,

Table I. Monoazo Dyes Which Do Not Combine with Calcium or Magnesium

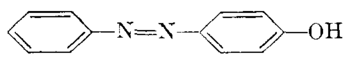
Dye	Absorption Max. at pH 10, Mμ	Molar Extinction Coefficient	Melting Point, ° C. Found	Melting Point, ° C. Reported	Reference
A-1	405	16,200	154.5–55	152	(*22*)
A-2	450	9,200	218	216–16.5	(*5*)
A-3	428	17,000	166–66.5	169–70	(*21*)
A-4	455	16,900	228	226	(*4*)
A-5	415	[a]	139	146–47	(*24*)
A-6	445	18,600	181	180	(*12*)
A-7	325	14,200	235–37	238–39	(*15*)
A-8	485	15,800	133.5	133–34	(*14*)
A-9	435	19,050	136	136	(*25*)
A-10	348	20,500	174–74.5	[b]	(*32*)
A-11	495	[a]	198[c]	206[c]	(*33*)
A-12	415	12,500	253	251–52	(*14*)
A-13	410	19,600	210	209–10	(*23*)
A-14	495	[a]	186–88	194	(*20*)
A-15	475	14,600	194.5–95	192–93	(*27*)
A-16	505	[a]	169–70	178–79	(*26*)
A-17	530	22,050	227–28	229–30	(*6*)

[a] Compound not sufficiently pure to calculate molar extinction coefficient.
[b] No melting point listed.
[c] Decomposition temperature.

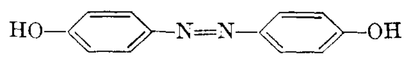
[1] Present address, The Ohio Oil Co., Littleton, Colo.

do not combine with calcium nor with magnesium as evidenced by the lack of any change in the absorption spectrum of the dye on the addition of calcium or magnesium salts. The structure and preparation of each dye are given below; melting point, literature reference to preparation and melting point if known, and absorption data are given in Table I.

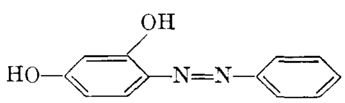
A-1. 1-(4-Hydroxy-1-benzeneazo)-benzene. Purchased from Eastman Kodak Co., recrystallized from hot ethyl alcohol with Norite A.



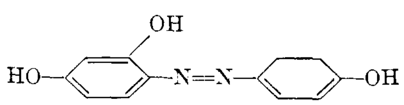
A-2. 1-(4′-Hydroxy-1′-benzeneazo)-4-hydroxybenzene. Prepared by the diazonium reaction from p-aminophenol and phenol; crystals from benzene.



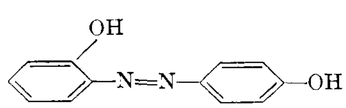
A-3. 1-(2′,4′-Dihydroxy-1′-benzene-azo)benzene. Purchased from the Eastman Kodak Co. Crystals from hot ethyl alcohol with Norite A.



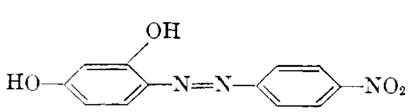
A-4. 1-(2′,4′-Dihydroxy-1′-benzene-azo)-4-hydroxybenzene. Prepared by the diazonium reaction from p-aminophenol and resorcinol; bright red crystals from ethyl alcohol with Norite A.



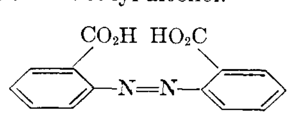
A-5. 1-(4′-Hydroxy-1′-benzene)-2-hydroxybenzene. Prepared by the diazonium reaction from o-aminophenol and phenol. Attempts at recrystallization were unsuccessful.



A-6. 1-(2′,4′-Dihydroxy-1′-benzene-azo)-4-nitrobenzene. Prepared by the diazonium reaction from p-nitroaniline and resorcinol; crystals from hot ethyl alcohol and Norite A.



A-7. 1-(2′-Carboxy-1′-benzeneazo)-2-benzoic acid. Prepared by heating o-nitrobenzoic acid, water, and sodium hydroxide at 200° C.; bright orange crystals from ethyl alcohol.



A-8. 1-(2′-Hydroxy-1′-naphthylazo)-benzene. Prepared by the diazonium reaction from aniline and 2-naphthol; orange crystals from ethyl alcohol.

Table II. Monoazo Dyes Which Combine with Calcium and Magnesium

(Absorption maxima and molar extinction coefficients at pH 10)

Dye	Absorption Maximum, Mμ			Molar Extinction Coefficient		
	Dye	Calcium compound	Magnesium compound	Dye	Calcium compound	Magnesium compound
B-1	570	510	510	24,300	19,400	22,000
B-2	480	[a]	480	12,000	[a]	17,000
B-3	475	475	475	24,300	24,400	30,100
B-4	485	[a]	485	21,200	[a]	16,800
B-5	325	325	325	6,700	6,600	6,600
B-6	500	490	490	10,200	8,700	13,300
B-7	560	515	510	12,200	16,000	19,100
B-8	570	545	550	33,500	31,100	41,900
B-9	548	523	528	31,100	29,500	37,600

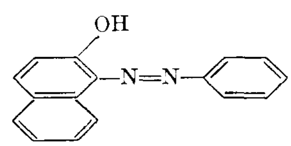
[a] Does not form calcium compound.

Table III. Monoazo Dyes Which Combine with Calcium and Magnesium

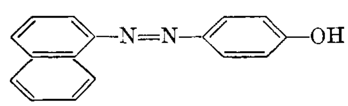
(Combining ratios and formation constants at pH 10)

Dye	Combining Ratio and Method Used				Formation Constant	
	Calcium		Magnesium		Calcium	Magnesium
B-1	1.08	Log-ratio	1.00	Cont. var.	2.26	4.59
B-2	[a]	[a]	1.00	Log-ratio	[a]	4.85
B-3	1.06	Log-ratio	1.03	Log-ratio	1.23	3.50
B-4	[a]	[a]	1.34	Log-ratio	[a]	2.10
B-5	1.19	Log-ratio	1.15	Log-ratio	1.39	2.39
B-6	1.13	Log-ratio	1.03	Log-ratio	1.68	3.68
B-7	1.39	Log-ratio	1.00	Cont. var.	1.75	4.29
B-8	1.01	Log-ratio	0.95	Log-ratio	2.50	3.81
B-9	0.96	Log-ratio	1.16	Log-ratio	3.08	4.50

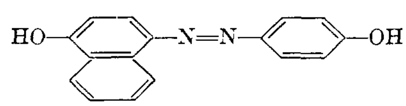
[a] Does not form a calcium compound.



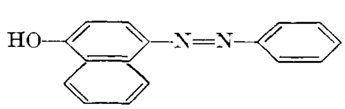
A-9. 1-(4′-Hydroxy-1′-benzeneazo)-naphthalene. Prepared by the diazonium reaction from 1-naphthylamine and phenol; long red needles from methanol.



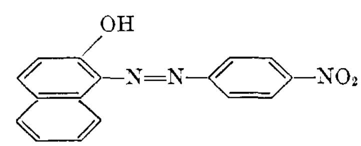
A-10. 1-(4′-Hydroxy-1′-naphthylazo)-4-hydroxybenzene. Prepared by the diazonium reaction from p-aminophenol and 1-naphthol; crystals from ethyl alcohol with Norite A.



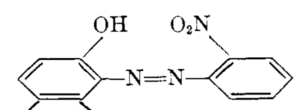
A-11. 1-(4′-Hydroxy-1′-naphthyl-azo)benzene. Prepared by the diazonium reaction from aniline and 1-naphthol. Attempts to obtain a crystalline material were unsuccessful.



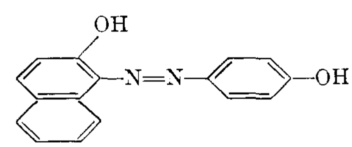
A-12. 1-(2′-Hydroxy-1′-naphthyl-azo)-4-nitrobenzene. Prepared by the diazonium reaction from p-nitroaniline and 2-naphthol; bright red crystals from chloroform.



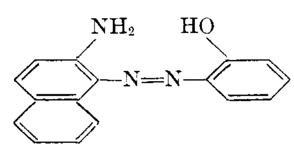
A-13. 1-(2′-Hydroxy-1′-naphthyl-azo)-2-nitrobenzene. Prepared by the diazonium reaction from o-nitroaniline and 2-naphthol; crystals from ethyl alcohol with Norite A.



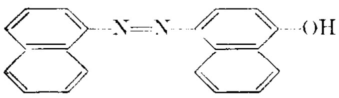
A-14. 1-(2′-Hydroxy-1′-naphthyl-azo)-4-hydroxybenzene. Prepared by the diazonium reaction from p-aminophenol and 2-naphthol; crystals from benzene.



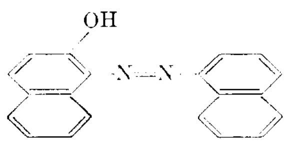
A-15. 1-(2′-Amino-1′-naphthylazo)-2-hydroxybenzene. Prepared by the diazonium reaction in glacial acetic acid from o-aminophenol and 2-naphthylamine; crystals from ethyl alcohol.



A-16. 1-(4′-Hydroxy-1′-naphthylazo)naphthalene. Prepared by the diazonium reaction from 1-naphthylamine and 1-naphthol. Repeated attempts at purification failed to yield a crystalline material.

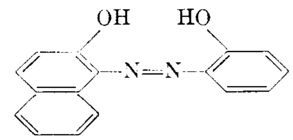


A-17. 1-(2′-Hydroxy-1′-naphthylazo)naphthalene. Prepared by the diazonium reaction from 1-naphthylamine and 2-naphthol; crystals from ethyl alcohol.

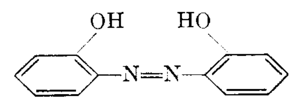


Monoazo Dyes Which Combine with Calcium and Magnesium. The following dyes unite with calcium and with magnesium at pH 10 as evidenced by a change in absorption spectrum on the addition of calcium or magnesium salts. The structure, preparation, and melting points are given immediately below; absorption maxima and molar extinction coefficients of the dyes and the calcium and magnesium compounds are given in Table II; combining ratios and formation constants at pH 10 are given in Table III; ionization constants and the various color changes are given in Table IV. Combining ratios for some of the dyes with calcium and magnesium at pH values other than 10 are given in Table V. Combining ratios and formation constants were determined at more than one wave length for all compounds formed and irrespective of which method was used.

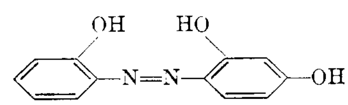
B-1. 1-(2′-Hydroxy-1′-benzeneazo)-2-hydroxynaphthalene. Prepared by the diazonium reaction from *o*-aminophenol and 2-naphthol; crystals from ethyl alcohol with Norite A; melting point 192–3° C., reported 192° C. (*11*).



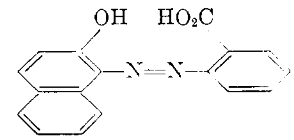
B-2. 1-(2′-Hydroxy-1′-benzeneazo)-2-hydroxybenzene. Prepared by fusing *o*-nitrophenol with sodium hydroxide and water at 180° C.; crystals from ethyl alcohol with Norite A; melting point, 171.5° C., reported 172° C. (*31*).



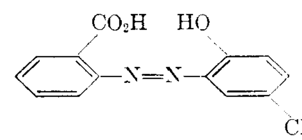
B-3. 1-(2′-Hydroxy-1′-benzeneazo)-2,4-dihydroxybenzene. Prepared by the diazonium reaction from *o*-aminophenol and resorcinol; bright yellow crystals from ethyl alcohol and Norite A; decomposition at 183–4° C., reported 184° C. (*9*).



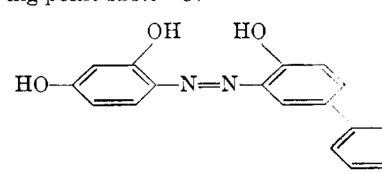
B-4. 1-(2′-Carboxy-1′-benzeneazo)-2-hydroxynaphthalene. Prepared by the diazonium reaction from anthranilic acid and 2-naphthol; bright red needles from butyl alcohol; melting point 273.5–74.5° C., reported 268° C. (*11*). Only one ionization constant could be determined. The first ionization probably occurs at a very low pH, neutralization did not produce a color change, and potentiometric titration failed owing to the extreme insolubility of the dye.



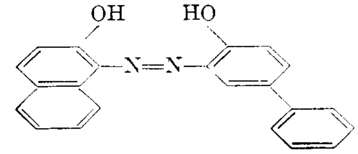
B-5. 1-(2′-Carboxy-1′-benzeneazo)-2-hydroxy-5-methylbenzene. Prepared by the diazonium reaction from anthranilic acid and *p*-cresol; bright red crystals from ethyl alcohol; melting point 193° C., reported 186° C. (*11*). The absorption maxima for both the calcium and the magnesium derivative are at longer wave lengths than that of the dye, contrary to all of the other dyes investigated. The color changes on forming the calcium and magnesium derivatives are almost imperceptible visually and the dye would not be satisfactory as an indicator.



B-6. 1-(2′,4′-Dihydroxy-1′-benzeneazo)-2-hydroxy-5-phenylbenzene. Prepared by the diazonium reaction from 2-amino-4-phenylphenol and resorcinol; orange-red crystals from benzene; melting point 123.5° C.



B-7. 1-(2′-Hydroxy-1′-naphthylazo)-2-hydroxy-5-phenylbenzene. Prepared by the diazonium reaction from 2-amino-4-phenylphenol and 2-naphthol; red crystals from benzene; decomposition about 155° C., melted at 191–3° C. The combining ratio of this dye with magnesium in water is 1 to 1, but is 2 dye to 1 magnesium in 20% acetone and 80% water. The dye functions well as an indicator in water-acetone mixtures.



B-8. 2-(2′-Hydroxy-1′-benzeneazo)-8-amino-1-naphthol-3,6-disulfonic acid. Prepared by the diazonium reaction from *o*-aminophenol and 8-amino-1-naphthol-3,6-disulfonic acid (*17*). All attempts to obtain crystals failed. No melting

Table IV. Monoazo Dyes Which Combine with Calcium and Magnesium

(Ionization constants and color change at pH 10 on union with calcium or magnesium)

	Ionization Constants				Color Change[a]	
Dye	pK_1	pK_2	pK_3	Wave Length, Mμ	Dye	Calcium or magnesium compound
B-1	7.7	12.4		560	Purple	Orange
B-2	7.8	11.5		475	[b]	[b]
B-3	6.6	8.7	12.2	450	Red	Yellow
B-4	...	12.0		350–485	[b]	[b]
B-5	...	11.4		370–450	[b]	[b]
B-6	6.7	8.1	11.4	500	Red	Yellow
B-7	8.0	11.8		600	Purple	Orange
B-8	8.4	11.9		575	Blue	Red
B-9	7.4	11.6		500	Purple	Red-orange

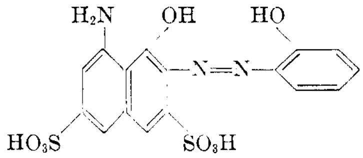
[a] Dyes suitable as indicator for calcium and magnesium.
[b] Color change difficult to detect by eye.

Table V. Monoazo Dyes Which Combine with Calcium and Magnesium

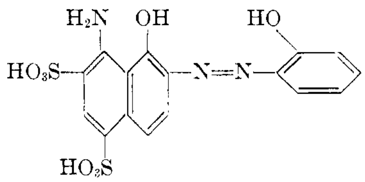
(Combining ratios at pH values other than 10)

Dye	Metal	Combining Ratio Metal to Dye	pH	Method
B-1	Mg	1	9.5	Continuous variations
B-1	Mg	1	11.2	Continuous variations
B-1	Ca	1.04	11.4	Log-ratio
B-2	Mg	0.98	9.0	Log-ratio
B-3	Mg	1.04	9.1	Log-ratio
B-3	Ca	0.97	11.4	Log-ratio

point could be obtained on the blue-black solid isolated.



B-9. 2-(2′-Hydroxy-1′-benzeneazo)-8-amino-1-naphthol-5,7-disulfonic acid. Prepared by the diazonium reaction from *o*-aminophenol and 8-amino-1-naphthol-5,7-disulfonic acid (*17*). Attempts to obtain a crystalline material were unsuccessful. A well purified blue-black solid having no melting point was used. The dye functions well as an indicator but is difficult to purify and unstable in solution.



CONCLUSIONS

From the work just described on 26 selected monoazo dyes, it is concluded that magnesium and calcium do not form compounds with dyes bearing only one ortho-hydroxyl group. Two hydroxyl groups, *o*- and *o*′-, respectively, or, alternatively, one *o*-hydroxyl and one *o*′-carboxyl, are necessary and sufficient.

Magnesium and calcium unite with *o,o*′-dihydroxyazo and with *o*-hydroxy-*o*′-azo dyes to form compounds only in the ratio of one molecule of dye to one atom of metal. The situation may be different in nonaqueous solvents because the one dye so investigated, 1-(2′-hydroxy-1′-naphthaleneazo)-2-hydroxy-5-phenylbenzene, showed in 80% water–20% acetone mixture a two to one compound in this solvent in addition to the one to one compound in water.

The formation constants of magnesium with *o,o*′-dihydroxyazo dyes are always greater than that of calcium toward the dye, although the difference between the two is variable.

The formation constants of magnesium and calcium with *o*-hydroxy-*o*′-carboxyazo dyes are of the order of a hundred times smaller than with *o,o*′-dihydroxyazo dyes.

LITERATURE CITED

(1) Bates, R. G., "Electrometric pH Determination," p. 117, Wiley, New York, 1954.
(2) Beech, W. F., Drew, H. D. K., *J. Chem. Soc.* **1940**, 608.
(3) Biedermann, W., Schwarzenbach, G., *Chimia (Switz.)* **2**, 56 (1948).
(4) Borsche, W., Frank, R., *Ann.* **450**, 75 (1926).
(5) Cook, A. H., Jones, D. G., *J. Chem. Soc.* **1939**, 1309.
(6) Cumming, W. M., Howie, G., *Ibid.*, **1931**, 3181.
(7) Diehl, Harvey, Lindstrom, F., Anal. Chem. **31**, 414 (1959).
(8) Diehl, Harvey, Sealock, R. F., *Record Chem. Progr. (Kresge-Hooker Sci. Lib.)* **13**, 10 (1952).
(9) Drew, H. D. K., Dunton, F. G., *J. Chem. Soc.* **1940**, 1064.
(10) Drew, H. D. K., Fairbairn, R. E., *Ibid.*, **1939**, 823.
(11) Drew, H. D. K., Landquist, J. K., *Ibid.*, **1938**, 292.
(12) Elbs, K., *J. prakt. Chem.* **108**, 209 (1924).
(13) Ellingboe, J. L., Ph.D. thesis, Iowa State University, Ames, Iowa, 1956.
(14) Ernsberger, M. L., Brode, W. R., *J. Org. Chem.* **6**, 331 (1941).
(15) Greiss, P., *Ber.* **10**, 1868 (1877).
(16) Haendler, H. H., Smith, G. M., *J. Am. Chem. Soc.* **62**, 1669 (1940).
(17) Hartwell, J. L., Fieser, L. F., "Organic Syntheses," J. R. Johnson, ed., Vol. 16, p. 12, Wiley, New York, 1936.
(18) Job, Paul, *Ann. chim. (Paris)* [10] **9**, 113 (1928).
(19) *Ibid.*, [11] **6**, 97 (1936).
(20) Johnson, T. B., *J. Am. Chem. Soc.* **25**, 483 (1903).
(21) Kostanecki, St. v., *Ber.* **21**, 3114 (1888).
(22) Kostanecki, St. v., Schreiber, B., *Ibid.*, **38**, 2748 (1905).
(23) Meldola, R., Hughes, F., *J. Chem. Soc.* **59**, 372 (1891).
(24) Meyer, F., *Ann.* **287**, 212 (1895).
(25) Niementowski, S., *Ber.* **26**, 49 (1893).
(26) Nietzki, R., Goll, O., *Ibid.*, **19**, 1281 (1886).
(27) Sachs, O., *Ibid.*, **18**, 3125 (1885).
(28) Schwarzenbach, Gerold, "Die komplexometrische Titration," 2nd ed., pp. 28–30, Ferdinand Enke Verlag, Stuttgart, 1956.
(29) Schwarzenbach, G., Biedermann, W., *Helv. Chim. Acta* **31**, 678 (1948).
(30) Vosburg, W. C., Cooper, G. R., *J. Am. Chem. Soc.* **63**, 437 (1941).
(31) Willstätter, R., Benz, M., *Ber.* **39**, 3492 (1906).
(32) Witt, O. N., Buntrock, A., *Ibid.*, **27**, 2358 (1894).
(33) Witt, O. N., Dedichen, J., *Ibid.*, **30**, 2655 (1897).

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Indicator for the Titration of Calcium Plus Magnesium with (Ethylenedinitrilo)tetraacetate

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▶A new *o,o*′-dihydroxyazo indicator, 1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid, is recommended for the titration of calcium plus magnesium with EDTA. The new indicator has the same color change as Eriochrome Black T but the color change is clearer and sharper, and aqueous solutions of the indicator are stable indefinitely. It may be substituted for the older indicator without change in the procedure. It has been given the common name Calmagite.

Since its introduction by Schwarzenbach and Biedermann in 1948 (*7*), Eriochrome Black T has been used almost exclusively as the indicator in the direct complexometric titration of calcium plus magnesium with EDTA. Unfortunately, solutions of Eriochrome Black T are not stable and a recent review (*1*) lists seven publications describing efforts to stabilize solutions of the dye. The most successful of these schemes claims a solution stable for about 7 months (*4*). The instability of Eriochrome Black T quite likely arises from the simultaneous presence in the molecule of groups having oxidizing and reducing powers. The minimum requirement for an azo dye molecule to react with an alkaline earth ion is the presence of hydroxyl groups in both positions *o*- and *o*′- to the azo group (*3*). Azo dyes are easy to synthesize in number, albeit difficult to purify, and it appeared likely that one could be found having the indicator properties of Eriochrome Black T but lacking the nitro group and its oxidizing power. This paper describes one result of such