1011. *Substituted Benzidines and Related Compounds as Reagents in Analytical Chemistry. Part XVII.* The* NNN'N'*-Tetracarboxymethyl Derivatives of Some* 3,3'*-Disubstituted Benzidines.*

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NNN'N'-Tetracarboxymethyl derivatives of some 3,3'-disubstituted benzidines have been prepared and their properties as analytical reagents have been examined. The dimethoxy- and diethoxy-derivatives are particularly useful as metallofluorescent indicators in the titration of copper(II) and mercury(II) with ethylenediaminetetra-acetic acid (EDTA). A similar but less sensitive reaction is shown by 3,3'-dicarboxybenzidine-*NNN'N'*-tetra-acetic acid in the titration of calcium with EDTA.

Many compounds possessing the characteristic group $-N(CH_2 \cdot CO_2H)_2$ have found extensive application as analytical reagents because of their ability to form complex compounds with many cations. The introduction of this group into the molecule of an acid–base indicator can result in the formation of an indicator suitable for complexometric titrations with EDTA.[1] Its effect on redox indicators and, in particular, the 3,3'-disubstituted derivatives of benzidine, which have recently been shown to have interesting redox

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[1] Schwarzenbach, Kampitsch, and Steiner, *Helv. Chim. Acta*, 1945, **28**, 828; Körbl and Pribil, *Chem. and Ind.*, 1957, 233; Belcher, Leonard, and West, *J.*, 1958, 2390.

properties,[2] was thought to be of interest. Accordingly, the *NNN'N'*-tetracarboxymethyl derivatives of benzidine, *o*-dianisidine, *o*-diphenetidine, *o*-tolidine, and benzidine-3,3'-disulphonic, -3,3-dicarboxylic, and -3,3'-di(oxyacetic acid) have been prepared. The preparation of the tetracarboxymethyl derivatives of 3,3'-dichloro-, 3,3'-dihydroxy-, and 3,3'-diamino-benzidine by a similar procedure was not at all satisfactory and these substances have not been included in the present study.

Condensation of sodium chloroacetate with organic amines has been extensively used by Schwarzenbach and others for the preparation of many aminocarboxylic acids, although this reaction has not been used with diamines of the benzidine type. Benzidine-*NN*-diacetic acid and -*NNN'N'*-tetra-acetic acid had been prepared by Reissert [3] in 1914. When these two polyacids were prepared according to his directions, they were obtained in a very impure state and were easily oxidised by air. Similarly, when *o*-dianisidine-*NNN'N'*-tetra-acetic acid was prepared by the original method, considerable difficulty was experienced in isolating the free acid in pure form because of its hygroscopic nature and lack of crystallinity. The isolation of these polyacids as sodium salts proved more satisfactory and it was possible to obtain tetrasodium *o*-dianisidinetetra-acetate as a stable, water-soluble, white powder. A general procedure was developed for the preparation of the tetrasodium salts of the various complexans based on these observations. This involved very slow addition of a solution of sodium chloroacetate in slight excess to an aqueous suspension of the appropriate benzidine at 100°, whilst the reaction mixture was stirred and maintained at pH 8 by addition of a solution of sodium carbonate. The product is then isolated as barium salt; this is converted into the sodium salt which is precipitated from water by a large excess of ethanol. A disadvantage of this procedure is that the product contains a small amount of sodium carbonate which cannot successfully be removed without appreciable loss. This residual inorganic matter has no effect on the analytical uses of the material.

An alternative to repeated crystallisation was developed for purification of tetrasodium *o*-dianisidine-*NNN'N'*-tetra-acetate. A slow stream of hydrogen chloride was passed through a suspension of the slightly impure sodium salt in cold ethanol; sodium chloride was filtered off, the excess of hydrogen chloride removed by concentration *in vacuo*, a solution of sodium ethoxide was added, and the precipitate of tetrasodium salt collected. This procedure could doubtless be used for other complexans of this type.

3,3'-Disulpho-*o*-dianisidine-, *o*-diphenetidine-, and -benzidine-*NNN'N'*-tetra-acetic acid and 3,3'-di(carboxymethoxy)benzidine-*NNN'N'*-tetra-acetic acid show intense fluorescences at pH 4—10 when their aqueous solutions are exposed to ultraviolet light. Although benzidine- and *o*-tolidine-*NNN'N'*-tetra-acetic acid also fluoresce over a wide pH range, the fluorescence is much weaker than that given by the above-mentioned compounds. The fluorescence of 3,3'-dicarboxybenzidine-*NNN'N'*-tetra-acetic acid is also fairly weak and only occurs above pH 7.

Indicator Properties.—The new derivatives reacted in a similar manner to the parent benzidines when treated with strong oxidising agents. Similarly coloured oxidation products to those given by the parent materials were obtained. The end-points obtained when the complexans were used as redox indicators in conventional titrations were very sluggish and their use in this connection was not further studied. Preliminary experiments indicated that the addition of various cations, especially Cu^{2+}, to aqueous solutions of the complexans had a pronounced effect on the fluorescence; with the *o*-dianisidine, *o*-diphenetidine, 3,3'-di(carboxymethyl)- and 3,3'-dicarboxy-benzidine derivatives the fluorescence was quenched completely. The derivatives of benzidine and tolidine are too weakly fluorescent for objective observation, whilst the fluorescence of 3,3'-disulphobenzidine-*NNN'N'*-tetra-acetic acid was only partially quenched by the addition of

[2] (*a*) Belcher, Nutten, and Stephen, *J.*, 1958, 2336; Rees and Stephen, *Talanta*, 1959, **2**, 361; (*b*) Rees and Stephen, *J.*, 1960, 4225.

[3] Reissert, *Ber.*, 1914, **47**, 679, 680.

metallic ions. These observations indicated that certain of these complexans might be applied as metallofluorescent indicators.

The substances were used initially as aqueous solutions of their sodium salts. However, the sharpness of the end-points deteriorated when these solutions were kept and it was necessary to prepare fresh solutions at least once a week. The solutions were more stable in dark bottles but it was more convenient to use the indicators as 1% solid mixtures with an inert salt such as sodium chloride or potassium nitrate.

A distinct advantage of the metallofluorescent indicator system is that the detection of the end-point is not affected by the colour of the solution, as happens with most of the metallochromic indicators.

Titration of Copper.—Solutions of copper(II) were titrated satisfactorily with EDTA when *o*-dianisidine-, *o*-diphenetidine-, and 3,3′-di(carboxymethoxy)benzidine-*NNN′N′*-tetra-acetic acid were used as indicators; the very sharp end-point was denoted by the appearance of fluorescence in solutions buffered over the pH range of 4—10. Slight overtitration occurred in the presence of the dioxyacetic acid derivative. 3,3′-Dicarboxybenzidine-*NNN′N′*-tetra-acetic acid gave an end-point only in solutions buffered to pH 10. An equally sharp end-point denoted by the disappearance of fluorescence was also obtained in the reverse titration. Bi^{3+}, Cd^{2+}, Ce^{3+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, In^{3+}, La^{3+}, Ni^{2+}, Pb^{2+}, Th^{4+}, Tl^{3+}, Sn^{2+}, and Zr^{4+} interfere with the titration because they consume titrant. However, they do not affect the nature of the end-point, and so these ions can be titrated with an excess of EDTA, the excess being determined by titration with copper(II) in the presence of the metallofluorescent indicator. Since the indicators are themselves capable of binding metal ions, the indicator blank could be appreciable if the amount of indicator used were excessive; however, the fluorescence of these compounds is sufficiently intense to enable an amount of indicator equivalent only to a fraction of a drop of titrant to be used and this does not impair the quality of the end-point.

Titration of Mercury.—Stoicheiometric results were obtained in the titration of mercury(II) with EDTA at pH 4 when *o*-dianisidine-, and *o*-diphenetidine-*NNN′N′*-tetra-acetic acid were used as indicators. Although the end-points were not quite so sharp as those obtained in the titration of copper they were, nevertheless, quite distinct. The reverse titration was not so satisfactory because of the slight fluorescence that remained when a slight excess of mercury was present in the solution.

Titration of Calcium.—The fluorescence of 3,3′-dicarboxybenzidine-*NNN′N′*-tetra-acetic acid in alkaline solution was quenched by Ca^{2+}. Variable results were obtained initially in the titration of calcium with EDTA over the range pH 10—13 when an aqueous solution of the compound was used as indicator. Although the fluorescence of this solution was still intense a few days after its preparation, its indicator properties had disappeared, probably because of decarboxylation to the fluorescent *NNN′N′*-tetramethylbenzidine-3,3′-dicarboxylic acid. When the compound was used as a 1% solid mixture with sodium chloride this difficulty was overcome. However, there was still slight residual fluorescence in the presence of a small excess of calcium which is thought to be due to the presence of unchanged benzidine-3,3′-dicarboxylic acid. In spite of this, quite a sharp end-point was obtained with this indicator in the direct titration of calcium with EDTA in alkaline solution. Barium and strontium interfered, but one part of magnesium in ten parts of calcium could be tolerated.

Electrophoretic Studies.—With the exception of benzidine-*NNN′N′*-tetra-acetic acid and its 3,3′-dicarboxy-derivative the compounds gave a single intense blue fluorescent spot on paper electrophoresis at pH 10 (borate buffer). Benzidine-*NNN′N′*-tetra-acetic acid gave six fluorescent spots, the leading one being the most intense. Likewise, a complex pattern was obtained with 3,3′-dicarboxybenzidine-*NNN′N′*-tetra-acetic acid, but here no one spot was outstanding. The various blue-fluorescent bands which were obtained when a large amount of the latter material was subjected to electrophoresis were cut out and dissolved in water. The band immediately behind the leading one was

completely quenched by calcium. This indicated that the highly purified compound would give a very sharp end-point in the titration of calcium with EDTA.

Fluorimetric Studies.—*o*-Dianisidine-, *o*-diphenetidine-, and 3,3′-di(carboxymethoxy)-benzidine-*NNN′N′*-tetra-acetic acid showed maximum fluorescence at pH 5·5—6·0. The intensity of the fluorescence given by the other complexans was not sufficient for measurements to be undertaken with the available equipment except in the case of 3,3′-dicarboxybenzidine-*NNN′N′*-tetra-acetic acid. This substance showed slightly more fluorescence in sodium hydroxide and diethylamine solutions than in a buffer solution of pH 10.

Experimental

Preparation of o-*Dianisidine*-NNN′N′-*tetra-acetic Acid* (*Tetrasodium Salt*).—*o*-Dianisidine (24·4 g.) was suspended in water (*ca.* 100 ml.) containing a small amount of phenolphthalein, in a 700-ml. round-bottomed flask fitted with a stirrer and two separatory funnels. The whole was placed on a steam-bath. A solution of sodium chloroacetate (49 g.) in water (100 ml.) was added dropwise from one funnel and 2N-sodium carbonate from the other in order to maintain the mixture at pH 8·0 corresponding to the colour of phenolphthalein. When all the sodium chloroacetate solution had been added, the reaction was allowed to proceed until further additions of sodium carbonate solution were unnecessary. The mixture was filtered to remove any unchanged diamine, and a concentrated solution of barium chloride was added until precipitation was complete. The mixture was warmed for about 30 min. on a steam-bath and barium *o*-dianisidine-*NNN′N′*-tetra-acetate was filtered off, washed with water, and dried under a vacuum (yield 80 g.). The barium content was determined by sulphate ashing (0·3293 g. gave 0·1969 g. of barium sulphate. Theor., 0·2058 g. for a dibarium salt). The barium salt was suspended in water and the required amount of sodium sulphate in solution was added. The mixture was stirred and warmed on a steam-bath for about 1 hr. and the precipitate of barium sulphate was filtered off. Ethanol was slowly added to the filtrate until precipitation was complete. The white precipitate was filtered off, washed with a little ethanol, and dried under a vacuum. The yield of white powder was 46·3 g. (32%).

The crude sodium salt (30 g.) was dissolved in sufficient water and boiled for a short time with charcoal. The filtrate from this treatment was diluted slowly with ethanol until precipitation just occurred. This precipitate was filtered off and rejected, and a large excess of ethanol was added to the filtrate. The white precipitate which was formed on stirring was filtered off, washed with a small amount of ethanol, and vacuum-dried (yield, 12 g.) (Found: Equiv., by non-aqueous titrimetry, 145; Na, from sulphated ash, 15·8%).

This product was considered sufficiently pure for use in the analytical studies of its properties as an indicator. Ultimate analysis gave results about 2% low for carbon, indicating the probable presence of inorganic salts as impurities. A purer product was obtained by suspending the sodium salt (10 g.) in ethanol (50 ml.) and cooling the suspension in salt–ice. A slow stream of hydrogen chloride was passed into the suspension until conversion of the sodium salt into the free acid was judged to be complete. The precipitated sodium chloride was filtered off and the filtrate was concentrated to half its bulk under reduced pressure. This solution was again filtered to remove a further small quantity of sodium chloride, and then poured into a fresh solution of sodium ethoxide (2 g. of sodium in 100 ml. of ethanol). The white, highly hygroscopic precipitate was filtered off, washed with ethanol, and vacuum-dried. On exposure to air, it rapidly absorbed moisture and slowly changed to a stable, dry white solid (Found: C, 44·0; H, 4·3; N, 4·8. Calc. for $C_{22}H_{20}Na_4N_2O_{10}$: C, 47·1; H, 3·6; N, 5·0. Calc. for $C_{22}H_{20}Na_4N_2O_{10},2H_2O$: C, 44·0; H, 4·1; N, 4·7%). An equivalent-weight determination by non-aqueous titrimetry gave a value of 148 (Calc. for the dihydrate, 150). The sodium content determined from the sulphated ash was 15·5% (Calc. for the dihydrate, 15·3%). These results indicate that the dihydrate is the normal product obtained when the tetrasodium salt is precipitated from aqueous solution by ethanol.

The other complexan salts were prepared similarly, the following yields being obtained, before final purification by fractional precipitation, from the corresponding benzidine derivatives: benzidine, 47%; *o*-diphenetidine, 29%; *o*-tolidine, 59%; 3,3′-di(carboxy-methoxy)benzidine, 59%; 3,3′-dicarboxybenzidine, 17%; 3,3′-disulphobenzidine, 25%

Only *o*-diphenetidine complexan was further purified *via* the free acid, the anhydrous tetrasodium salt forming a *dihydrate* on exposure to moist air (Found: C, 46·0; H, 3·7; N, 4·7. $C_{24}H_{24}Na_4N_2O_{10},2H_2O$ requires C, 45·9; H, 3·9; N, 4·5%). The sodium salt of 3,3′-dicarboxybenzidine-*NNN′N′*-tetra-acetic acid could not be purified in this way because of the rapid decomposition of the free acid in the presence of hydrogen chloride. It and the remaining complexan salts were assayed by non-aqueous titrimetry and by determination of sulphated ash.

Titration of Metal Ions with EDTA.—The apparatus used is described by Belcher and Nutten.[4] The ultraviolet source was a Mazda mercury vapour electric discharge lamp of type MBW/U, 230—240 v, 125 w.

An intimate mixture of the sodium salt of the complexan and sodium chloride in the ratio of 1 : 100 was used as indicator.

Procedure. The 0·02M-cation solution (10 ml.) in a 250 ml. conical flask was diluted with about 50 ml. of water. Sufficient of an appropriate buffer solution and of the solid indicator mixture (5—10 mg.) was added. The titration was carried out in a darkened room with the solution in the titration vessel exposed to the source of ultraviolet light. The solution was titrated with EDTA (0·02M) to the appearance of fluorescence.

Electrophoresis. The apparatus and procedure previously described [2b] were used.

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Edgbaston, Birmingham 15. [*Received, December 28th,* 1960.]

[4] Belcher and Nutten, "Quantitative Inorganic Analysis," 2nd edn., Butterworths Scientific Publ., London, p. 174.