The Serial Solubility of Some Rare Earth Bromates.—Bromates of dysprosium with holmium, containing some yttrium, erbium and terbium, were crystallized with bromates of lanthanum, praseodymium and neodymium, and fractionated for about three months. Such fractions as had the same appearance were united. Each fraction was then separated into the elements of the yttrium group and into those of the cerium group by the sodium sulfate method. In the least soluble fraction the separation was carried out by crystallizing the double magnesium nitrates together with bismuth magnesium nitrate. The absorption spectra of the fractions, 26 in all, were examined; the elements were arranged in the following order: (most soluble) erbium, lanthanum, yttrium, holmium, praseodymium, dysprosium, neodymium, terbium, (gadolinium), (least sol.). In crystallizing the bromates, the fractions were cooled to room temperature (20–25°); it is probable that by allowing them to crystallize at a lower temperature, the elements might show another order. Especially neodymium, which was found only slightly more soluble than terbium, might then come between terbium and gadolinium.

These results are confirmed by direct determinations of solubilities carried out in this Laboratory; this work will be published in the near future.

CONTRIBUTION FROM THE
CHEMICAL LABORATORIES OF THE
UNIVERSITY OF NEW HAMPSHIRE
DURHAM, NEW HAMPSHIRE

J. ZERNIKE AND C. JAMES

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A NEW COLOR REACTION FOR PROCAINE AND SOME OTHER LOCAL ANESTHETICS, AND ITS APPLICATION TO THE DETERMINATION OF PROCAINE

BY E. RAYMOND RIEGEL AND JOHN F. WILLIAMS

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Introduction

When to a solution containing approximately 1 mg. of procaine hydrochloride per cubic centimeter, a few drops of hydrochloric acid, of a solution of sodium nitrite, and of concd. aqueous ammonia are added, in the order named, an intense yellow color develops. Several other local anesthetics respond to this test, while others do not, as recorded in Part I of this paper, which part also includes the results of the test on a number of additional substances.

On suitable dilution, the yellow solution obtained with procaine may be made the basis of a colorimetric method for its determination. The procedure and necessary cautions are detailed in Part II.

Part I

The local anesthetics that respond to the test are procaine, tutocaine, butyn, butesin, propaesin, benzocaine and orthoform, all closely related in chemical structure; these will be designated as Group 1a.[1]

Saligenin gives a yellow color with hydrochloric acid and sodium nitrite before the addition of aqueous ammonia; hence saligenin, forming Group 1b, interferes with the test for Group 1a, the members of which give no color until after the addition of aqueous ammonia, but its presence would be detected.

None of the remaining local anesthetics gives a color, either before or after the addition of aqueous ammonia; they are cocaine, tropacocaine, alypine, β-eucaine, apothesine, benzyl alcohol, stovaine, quinine and phenacaine, which comprise Group 1c.

Among the additional substances tested, epinephrine, forming Group 2a, responds to the test, but the color forms very slowly.

Morphine and apomorphine give a red color on the addition of acid and nitrite, before aqueous ammonia; with the last-mentioned reagent the color changes to a mahogany brown; these two substances form Group 2b, and correspond in action to the members of Group 1b.

The following substances form Group 2c: codeine, heroine, dionine, thebaine, meconin, cinchonidine, quinidine, piperine, strychnine, theobromine, caffeine, veratrine, atropine, hyoscyamine, pilocarpine, creatine, picrotoxin, acetanilide, milk sugar and corn sugar. They do not form a color, either before or after the ammonia addition, and therefore do not interfere with the qualitative test; but they may interfere with the quantitative estimation and in their presence the absolute delicacy of the method is not so great as in their absence (see Part II).

The members of Group 1a do not react with the same intensity; with this reservation, the test may perhaps be considered a class test for this group. An opportunity to narrow the possible substances present is afforded by their solubilities; procaine[2] and tutocaine[3] hydrochlorides and butyn sulfate,[2] are water-soluble, while butesin, propaesin,[4] benzocaine[2] and orthoform[2] (the free bases) are insoluble. Further distinction between the first three and last four must be made by auxiliary tests.

Part II

Within the limitations set forth in the preceding part, the new reaction may be made the basis of a colorimetric method for the determination

[1] The first six are esters of *p*-aminobenzoic acid, and orthoform is an ester of aminosalicylic acid.

[2] "New and Non-official Remedies," **1925**, the American Medical Association, Chicago, Ill.

[3] Manufactured by the Winthrop Chemical Co., New York.

[4] Thorpe's "Dictionary of Applied Chemistry," Longmans, Green and Co., New York. Procaine and benzocaine are also U. S. P. chemicals.

of procaine. A number of qualitative and two quantitative methods are in use at the present time.[5] In addition to these, Cheramy[6] proposes modifying the qualitative β-naphthol test and extending it to a colorimetric quantitative one, by substituting potassium guaiacol sulfonate for the β-naphthol. Denigès[7] describes a microchemical method for the identification of procaine which involves the formation of perchlorate crystals.

The present method would seem to possess the advantage of simplicity and rapidity. The reagents are such as may be found in any laboratory; it is not even necessary to have procaine crystals for the preparation of standards, for it has been found that solutions of potassium dichromate may be substituted to advantage. The test is rapid; once the proper concentration, about 1 mg. per cubic centimeter, has been obtained, the addition of the reagents, the dilution to a suitable depth of color, and the comparison with the standard may be done in ten minutes.[8]

Procedure

An amount of the sample representing 10 to 15 mg. of procaine is weighed and dissolved in 10 cc. of water placed in a 100cc. graduate; this solution will be called the test solution. There are added 0.5 cc. of 10% hydrochloric acid, 1 cc. of a 2% solution of sodium nitrite, and after gentle mixing, 1 cc. of concd. ammonium hydroxide solution distributed evenly by one or two shakings. The color is allowed to develop until the turbidity, which appears simultaneously, has apparently reached a maximum, which will require about 30 seconds; enough water is now poured in to make the volume 100 cc. This solution will be called the stock solution; it is still intensely colored, a deep orange, and will usually be clear. The following portions are pipetted into 50cc. Nessler tubes by means of a 5- or 10cc. pipet graduated in 0.1 cm.: 1, 1.25, 1.5, 2 and 2.5 cc.; each tube is then diluted to the mark.

The five tubes are compared with a potassium dichromate standard tube prepared by diluting 8.5 cc. of a 0.1% potassium dichromate solution to the 50cc. mark in a Nessler tube. This tube constitutes the main standard, and represents a total content of 0.25 mg. of procaine. From the portion of the stock solution which matches the standard, the procaine content is computed. The accuracy of the comparison is increased markedly if about 10 cc. of the solution in the matched Nessler is placed in a slender

[5] "Methods of Analysis," **1925**, Association of Official Agricultural Chemists, Washington, D. C., pp. 402–403.

[6] Cheramy, *C. A.*, **19**, 1325 (1925).

[7] Denigès, *ibid.*, **18**, 3677 (1924).

[8] Tutocaine may be estimated by the same procedure, with potassium dichromate standards based on known weights of tutocaine powder, subject to the limitations indicated in Part I.

test-tube of the ordinary kind, 12 by 150 mm., and compared with a similar tube containing an equal column of the standard.

All solutions and the diluting water should be at 20°; this is particularly important for the test solution and the three reagents that are added to it.

The dichromate standard replaces a standard made with procaine crystals; if these are available, the standard may be prepared by dissolving 10 mg. in 10 cc. of water, adding the reagents as directed above, and pipetting 2.5 cc. of the resulting stock solution into the 50cc. Nessler tube. The dichromate standard is preferable because after an hour or two, the procaine color begins to fade. On diluting the equivalent colored solutions from procaine and from dichromate, at the strength of the standard, to one-half, and one-quarter, they are still equivalent; to the eye, at least, the persistence of the two colors is the same. Other standards containing fewer cubic centimeters of the 0.1% dichromate solution may be prepared, and will represent correctly the proportionate amount of procaine.

The Nessler tubes should be selected of equal height to the 50cc. mark and the bottoms of the tubes should be polished. The graduate for the stock solution should be checked at the 10cc. and at the 100cc. marks. The pipets should be similarly tested for the weight of water they deliver, to within 0.05 g. All glass surfaces should be freed from grease, which may best be done by placing them in a warm caustic solution for five minutes. Large test-tubes may be substituted for the Nessler tubes without loss of accuracy; in some respects they are preferable.

If no color forms in the test solution after addition of the reagents, there is either no procaine present or the amount present in the 10 cc. is less than 0.05 mg.

Discussion

There is a concentration of procaine which gives maximum color; lower as well as higher concentrations give less color, so that in such cases, without this caution, the amount of procaine reported would be low. The concentration giving maximum depth lies between 10 mg. and 15 mg. in 10 cc. of solution. It so happens that with concentrations below 10 mg., the test solution remains clear after the addition of the reagents and the development of the color, and before the dilution to 100 cc.; the turbidity forms only in solutions having a concentration of or very near 10 mg., and higher. The absence of turbidity is, therefore, an indication that the concentration is too low. The effect of concentration above and below the range of maximum color is shown in Table I; the solution indicated in Rows 2 and 5 forms 50 cc. and is contained in a Nessler tube; the solutions indicated in Rows 3 and 6 also measure 50 cc. in similar tubes and are made up by diluting the indicated number of cubic centimeters of a 0.1% potassium dichromate solution. The content of procaine in each tube is 0.25 mg.,

and the standard each should have matched is the main standard, 8.5 cc. of 0.1% dichromate in 50 cc. of water.

TABLE I

EFFECT OF CONCENTRATION ON DEPTH OF COLOR

Stock soln., mg. of procaine per 100 cc.....	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
Stock soln. taken to give 0.25 mg. of procaine, cc.	25	12.5	8.33	6.25	5	4.17	3.6	3.12	2.77
Standards matched in cc. of dichromate soln.....	2	4	5	5.5	6.5	7.	7.5	8	8.5
Stock soln., mg., of procaine per 100 cc., cont'd.	0.1	0.11	0.12	0.13	0.14	0.15	0.2	0.25	0.5
Stock soln. taken to give 0.25 mg. of procaine, cc.	2.5	2.27	2.08	1.92	1.80	1.67	1.25	1.0	0.5
Standards matched in cc. of dichromate soln.....	8.5	8.5	8.5	8.5	8.5	8.5	8.	7	5.5

Diluting the yellow solution obtained at the concentration of maximum color formation permits a detection of color when the amount of procaine is 0.00045 mg. per cc. When the concentration is not the maximum color-producing one, but lower, the limit of color formation is reached when the amount of procaine is 0.005 mg. per cc. The color obtained at the favored concentration is about ten times stronger than at the minimum concentration that still gives the reaction.

The effect of temperatures near 10° and lower is to retard the formation of color; even after a time interval of several minutes the color is weaker than at 20°; at the latter temperature the color in the test solution develops within one-half minute, and is a maximum. Above 20°, the color is again less, as indicated by the following data: at 15°, 50 cc. containing 0.25 mg. matched standard 8.5; at 20°, 8.5; at 25°, 7.

The rate at which the procaine color fades was determined by allowing a 50cc. tube containing 0.25 mg. of procaine yellow, to stand, and matching it from time to time with different standards in terms of cubic centimeters of 0.1% dichromate solution. Table II contains the results.

TABLE II

RATE OF FADING

Elapsed time, hrs.....	2	18	24	42	120
Standard matched.....	8.5	6	5	2	0

The stock solution fades at a somewhat slower rate.

The use of a colorimeter in the general procedure was found to offer no advantage.

Effect of Admixture of Cocaine

In order to determine the effect of an admixture which does not produce color on the quantitative estimation of procaine, mixtures of the latter

with cocaine were studied.[9] Ten mg. of procaine was dissolved in the customary 10 cc. of water which contained also 10 mg. of cocaine in one case, 25 mg. in another and 50 mg. in a third; the reagents were added, and it was noted that the turbidity was greater than for pure procaine solutions. The test solution was diluted to the stock solution volume (100 cc.) and 2.5 cc. was placed in a Nessler tube, diluted to 50 cc., and compared with dichromate standards; all three should have matched the standard 8.5. With 10 mg. of cocaine mixed with 10 mg. of procaine, the standard matched was 7; with 25 mg., it was 5.5; and with 50 mg., it was 4.2. The reason for the low results is probably that the cocaine base, precipitated by the ammonia, encloses some of the yellow substance, a part of which is also solid in the test solution, and prevents its subsequent intended solution. The procedure must be modified in such cases by first preparing a table for mixtures of cocaine and procaine, and referring the color obtained to such a table. If no cocaine is available, the reaction should be performed in alcohol solution, but the color values for the procaine must be established for the solvent chosen. The cocaine does not interfere in alcohol. We have found that for 95% alcohol the color is less, in 20% alcohol, the color is deeper than for water solutions. The turbidity in the test solution when water is the solvent will not appear when alcohol is used.

A similar reduction of color must be expected when any other admixture, soluble in water but insoluble in aqueous ammonia, is present. This precipitated material will also reduce the delicacy of the qualitative test in a degree that will vary with the amount of the admixture.

The greatest use of the method will probably be in the analysis of solutions, powders or tablets containing pure procaine with perhaps a slight admixture of a water- and ammonia-soluble, non-reacting filler. Epinephrine, which is sometimes mixed with procaine, gives the yellow color so slowly (see Part I) that the test solution is diluted before the epinephrine color has developed, and in the stock solution the concentration of the reagents is too low to allow an appreciable reaction for the exceedingly small amounts of epinephrine usually present. Other substances that might interfere are indicated in Part I.

Unknowns

In order to test the method for its quantitative value, the following samples containing amounts of procaine unknown to the analyst were prepared, and the results shown in Table III were obtained.

In Analysis 6, duplicate samples were handed to the analyst.

The details of the trial tests for one of these unknowns will render the procedure clear.

[9] By cocaine is meant the water-soluble hydrochloride.

TABLE III

APPLICATIONS OF THE METHOD TO SAMPLES OF UNKNOWN CONTENT

	Material	Found, mg.	True content, mg.	Found, %	Analyst
1.	Solution, 14 cc.	23	25	92	R
2.	Solution, 25 cc.	25	25.9	97	W
3.	Powder, mixed with lactose.	75	73	103	W
4.	Solution, about 12 cc.	49	50	98	R
5.	Powder mixed with lactose.	37	40	92	W
6.	Powder mixed with lactose.	5	5	100	W

In No. 4, the volume of the solution was indefinite; it was made up exactly to 17 cc. by adding water in a 25cc. graduate. One cc. diluted to 10 cc. gave the color but no turbidity, indicating that the concentration was too low. In a second trial, 4 cc. was used to make up the test solution; a turbidity developed with the color. To be certain that the concentration was not too high, a third trial with 3.5 cc. was made; after dilution to 10 cc. and adding the reagents, a fair turbidity developed with the color. Of the resulting stock solution, 2.5 cc. diluted to 50 cc. matched Standard 8.5 and represented, therefore, 0.25 mg. of procaine. The total apparent content was thus $0.25 \times (100/2.5) \times (17/3.5)$, or 49 mg., while the true content was 50 mg.

The method is reliable to within 10%.

Materials

All the substances used in this work were the commercial materials. The procaine crystals were tested for the melting point, a titration with standard sodium nitrite, and a chlorine analysis; these proved that the substance was the material listed in the "New and Nonofficial Remedies"[10] as procaine (or novocaine). Specimens were obtained from four different sources and were found to be identical.

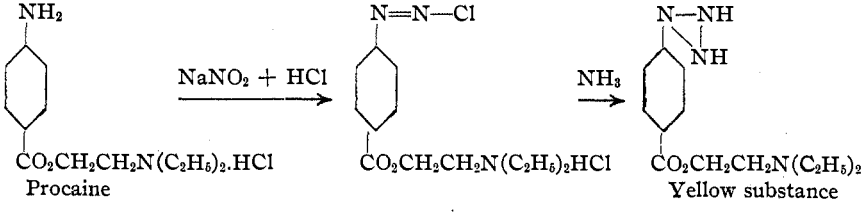
For the dichromate standards, potassium dichromate crystals marked C. P. were used.

The Nature of the Color

On diazotizing with sodium nitrite and hydrochloric acid the diazonium chloride forms; after the addition of aqueous ammonia and reacidification, the coupling power is not restored. This means either that the diazo grouping is destroyed by the ammonium hydroxide, or that the reformed diazonium chloride is the stereo-isomer, which does not couple. When sodium hydroxide is used instead of ammonia, a faint yellow color appears which after 30 minutes is fairly intense, but not so strong as the one due to ammonium hydroxide. Nitrogen gas is not developed if the procedure is followed, but during the fading, the gas forms. Of several solvents employed for the extraction of the color and of the insoluble product

[10] Ref. 2, p. 34.

which on the addition of more water produces color, amyl alcohol is the most promising. Work in that direction will be continued. For the present, the yellow substance may be assigned the following formula.



Procaine Yellow substance

Summary

1. A new color reaction is described for the local anesthetics procaine, tutocaine, butyn, butesin, propaesin, benzocaine and orthoform; no special reagents are necessary.

2. The reaction is extended to a quantitative method for procaine. The conditions which must be observed in order to obtain reliable results are detailed; they include temperature (20°), amounts, concentration, and order of addition of the reagents. The concentration of the procaine must lie between 10 mg. and 15 mg. in 10 cc. of solution.

3. The preparation of dichromate color standards is described.

4. The possible interference of admixed materials has been investigated.

5. The accuracy of the quantitative method is within 10%.

BUFFALO, NEW YORK

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BIPHENYL DERIVATIVES OF AMMONIA, PARA-PHENYLENEDIAMINE AND BENZIDINE. MERIQUINONIC SALTS

BY JEAN PICCARD

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The first meriquinonic salt was described by C. Wurster,[1] and its constitution was established by Willstätter and Piccard.[2] Later, the present author described the preparation of the simplest of all the meriquinonic salts, that obtained by oxidation of *p*-phenylenediamine itself.[3] By adding two atoms of bromine for two molecules of the base we get the meriquinone di-imonium bromide.[4]

[1] Wurster, *Ber.*, **12**, 1803 (1879).

[2] Willstätter and Piccard, *Ber.*, **41**, 1458 (1908).

[3] Piccard, *Ann.*, **381**, 351 (1911).

[4] Coördination formula instead of quinquivalent nitrogen [see THIS JOURNAL, **48**, 2354 (1926)]. In dye chemistry we need the coördination formulas even if we keep the quinquivalent nitrogen, because most dyes are intramolecular or extramolecular addition compounds.