isomeric *o*-aminobenzoic acid proved to be much less inhibiting. The replacement of the amino group by the hydroxyl, the carboxylic acid by the sulfonic acid group, and the introduction of a third substituent into *p*-aminobenzoic acid resulted in a substantial destruction of the inhibitory effect. Nicotinic acid, cevitamic acid and even to a lesser degree thiamin chloride have only a slight inhibitory effect on sulfapyridine.

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A Note on the Determination of Arsenic in Organic Arsenical Compounds*

By F. B. Rodman† and Harold N. Wright‡

Numerous methods of analysis have been advocated for the determination of the arsenic content of organic arsenical compounds. Foremost among these are the gravimetric method of Treadwell and Hall (1) and the volumetric method of Lehmann (2). The Treadwell–Hall gravimetric method has been recognized as having a comparatively high degree of accuracy, but is laborious and time consuming. The Lehmann volumetric method was adopted as the official method of assay for arsphenamine and neoarsphenamine in U. S. P. X (1926) and has remained the official method of assay since that time. While the Lehmann method has been considered satisfactory for the assay of arsphenamine and neoarsphenamine it has not been considered satisfactory for the analysis of some of the more refractory arsenicals such as arsanilic acid and sodium cacodylate.

Myers and DuMez (3) made a comparative study of several methods of analysis for the assay of arsphenamine and neoarsphenamine. They assayed a number of specimens of these drugs by four methods, namely (*a*) the Treadwell–Hall gravimetric method, (*b*) Lehmann's, (*c*) Ewins' (4), and (*d*) Gaebel's (5) titration methods. These workers concluded that the Treadwell–Hall and Lehmann methods gave nearly identical results, while the results obtained by Ewins' or Gaebel's methods were invariably low.

Wright, *et al.* (6), in a study of the crystalloid and colloid fractions of arsphenamine and neoarsphenamine, found it impossible to employ the official Lehmann volumetric method in this work since "the oxidation with potassium permanganate and sulfuric acid employed in this method is apparently inadequate to cause the complete breakdown of 'aged' solutions of the arsphenamines, which become decidely refractory to oxidation and almost invariably give low results with the Lehmann method." This was found to be particularly true with the colloidal fractions. Robertson (7) also has concluded that the Lehmann method, while useful in routine assays, is not satisfactory for research purposes.

EXPERIMENTAL

In connection with an investigation of the comparative distribution and retention of the crystalloid and colloid fractions of the arsphenamines to be reported elsewhere (8) a series of comparative analyses of the arsenic content of the sample of neoarsphenamine employed in this study was made by both the Treadwell–Hall and Lehmann methods.

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TABLE I.—RESULTS OF COMPARATIVE ANALYSIS BY THE TREADWELL–HALL AND LEHMANN METHODS OF THE PER CENT OF ARSENIC IN A SINGLE SAMPLE OF NEOARSPHENAMINE

Analysis No.	Treadwell–Hall Method, Per Cent	Lehmann Method, Per Cent
1	20.17	19.59
2	20.34	19.20
3	20.19	19.27
4	19.92	19.72
5	20.44	19.54
6	20.14	19.55
7	20.24	19.32
8	20.23	19.87
9	20.60	19.82
10	19.81	19.21
11	20.17	19.55
12	...	19.61
13	...	19.79
14	...	19.91
Mean	20.20 ± 0.04	19.57 ± 0.04

This furnished an opportunity to present a statistical analysis of the results of the assay by the two methods. Each analysis represents a different ampul from a batch of drug of a single lot number. All analyses were made in duplicate and the results reported are the means of those duplicate analyses. The results obtained are shown in Table I.

We find therefore that the mean per cent of arsenic found for 11 duplicate analyses from 11 different ampuls of a single lot of neoarsphenamine by the Treadwell–Hall gravimetric method was 20.20% with a probable error of the mean of ±0.04%, while 14 duplicate analyses made similarly by the Lehmann volumetric method gave an arsenic content of 19.57 ± 0.04%.

Applying the familiar formula for the test of a significant difference between means, namely,

$$\text{Sig. Diff.} > 3\sqrt{\text{P.E.M.}_x^2 + \text{P.E.M.}_y^2}$$

we find that a difference between the means in excess of

$$3\sqrt{0.04^2 + 0.04^2} = 0.17$$

would be significant. Since the actual difference between the means is 0.63, this difference is unquestionably significant.

Further evidence of the significance of this difference in the results by the two methods is obtained by a statistical analysis of the results of analyses by Myers and DuMez (3) on 21 samples of arsphenamine of different lot numbers and from several manufacturers. These results are given in Table II.

It may be noted that of these 21 analyses, 18 gave lower results by the Lehmann than by the Treadwell–Hall method, the mean difference being 0.146%, with a probable error of the mean difference of ±0.036. Since the mean difference is well in excess of three times the probable error we may conclude that the Lehmann method also gives significantly lower results than does the Treadwell–Hall method in the assay of arsphenamine.

TABLE II.—ARSENIC CONTENT OF COMMERCIAL SAMPLES OF ARSPHENAMINE (MYERS AND DUMEZ)

Lot No.	Treadwell–Hall Method, Per Cent	Lehmann Method, Per Cent
740	31.58	31.32
750	31.16	31.34
555	31.13	30.94
799	31.52	31.40
809	30.87	30.46
841	31.54	31.38
873	31.38	31.22
886	31.46	31.18
890	31.35	31.22
900	31.07	30.94
928	31.17	31.03
952	31.07	31.03
DBB	31.16	31.22
DFB	31.47	31.32
FBB	31.85	31.50
HBB	31.65	31.28
LBB	31.24	31.20
175	31.15	30.68
176	31.15	31.55
177	31.80	31.61
179	31.56	31.47

The experience of Wright, *et al.* (6), in attempts to employ the Lehmann method in the analysis of dialyzed samples of arsphenamine and neoarsphenamine, would suggest that the low results by the Lehmann method are due to incomplete oxidation of the organic arsenical, particularly of the colloidal component. The probability that the colloid fraction of neoarsphenamine and arsphenamine is resistant to oxidation is also shown by the fact that Wright and Rodman (8) experienced difficulty in the analysis of the colloidal portions of both of these compounds obtained by dialysis when analyzed by the regular Treadwell–Hall method. The use of fuming nitric acid in amounts considerably larger than those recommended by Robertson (7) was required to complete the breakdown of this fraction of the two arsenicals. Since the amount of the colloidal component in these compounds differs not only from one brand to another, but even from one lot to another within the same brand, the degree to which the Lehmann method will give lower results might be expected to vary in the same manner.

SUMMARY

1. The Lehmann volumetric method of analysis for arsenic in organic arsenicals, the official method of assay of the arsenic content of arsphenamine and neoarsphenamine, has been shown to give significantly lower values with both of these drugs than does the Treadwell–Hall gravimetric method.

2. It is suggested that the lower results by the Lehmann method may be due to incomplete oxidation of the organic arsenical, particularly of the colloidal fraction.

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The Synthesis of 3-Hydroxyphthalic Acid

*By Ole Gisvold**

Oxidative studies carried out upon the pigment celastrol, a naturally occurring β-naphthoquinone, yielded a small quantity of material that melted at 244° C. and gave a positive phthalein test. Because the hydroxyl group that is present in this pigment was suspected of being adjacent to the carbonyl groups, 3-hydroxyphthalic acid was an anticipated oxidation fragment. A search of the literature revealed an apparent discrepancy in the melting point of 3-hydroxyphthalic acid. The "Handbook of Chemistry and Physics" gives the melting point of 3-hydroxyphthalic acid as 244° C., and solubilities which corresponded quite closely with those of the above-mentioned oxidation fragment. Heilbron's "Dictionary of Organic Compounds" reports the melting point of 3-hydroxyphthalic acid as 151° C. (with anhydride formation at 161–163° C.) and the melting point of the corresponding anhydride as 198–199° C., with solubilities which differed from those given in the "Handbook." In addition it reports a red color with ferric chloride. No red color with ferric chloride is given by the unknown.

It was therefore necessary to synthesize 3-hydroxyphthalic acid in order to make comparisons with the unknown. No attempt was made to develop good yields of any of the products obtained in this synthesis. 3-Aminophthalic acid was prepared in a pure state in order to report for the first time its melting point. It was not, however, used pure as an intermediate. The reduction of 3-nitrophthalic acid was accomplished catalytically with either platinum or Raney nickel; whereas in the previous syntheses reported in the literature this was accomplished by tin or iron and hydrochloric acid, and no 3-aminophthalic acid was isolated as the intermediate. The 3-hydroxyphthalic anhydride prepared corresponded with that recorded in Heilbron's "Dictionary of Organic Compounds" with respect to solubilities and color reaction. The melting point was somewhat lower; however, no correction was made for stem emergence.

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EXPERIMENTAL

3-Nitrophthalic Acid.—This compound was prepared according to the method given in "Organic Syntheses," Vol. I.

3-Aminophthalic Acid.—The 3-nitrophthalic acid was reduced in alcohol by means of platinum black or Raney nickel at 40 to 50 lbs. pressure and at room temperature. The reduction was accompanied by the formation of large quantities of an unidentified material that appeared to be a polymer. This side reaction was greatest with platinum. The catalyst was removed by filtration, the filtrate concentrated by spontaneous evaporation and the residue digested with water. The aqueous solution was separated from an oily water-insoluble residue. Concentration by spontaneous evaporation yielded crude 3-aminophthalic acid which after several crystallizations from water yielded orange rosettes that melted at 231–232° C. (uncorr.).

3-Hydroxyphthalic Anhydride.—3-Aminophthalic acid was diazotized by the usual method and the crude diazotized product was collected and washed several times with cold water. The diazonium product thus prepared was decomposed according to the directions given in "Organic Syntheses" for the preparation of *m*-nitrophenol. The reaction mixture was then extracted with ether in order to collect the crude 3-hydroxyphthalic acid. Attempts to purify the product by recrystallization from suitable solvents were unsuccessful. The crude material was