For the case where the rate of exchange is measured by the growth of activity, y, in B

$$y(t) = y(\infty)\ [1 - \exp(-s)] \quad (4)$$

$$\mathrm{d} \ln s / \mathrm{d} \ln (y/y(\infty)) = (\exp(s) - 1)/s = F(s) \quad (5)$$

The figure contains a plot of this function, too. In this case, of course, the fractional error in y cannot possibly be constant as y approaches zero; an estimate of σ_y as a function of y is needed for selecting an optimum reaction time or calculating the error of a particular determination.

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A Convenient Synthesis of N,N-Dimethyl-*p*-nitroaniline and N,N-Dimethyl-*o*-nitroaniline

BY TOD W. CAMPBELL

There are a number of methods in the literature for the preparation of N,N-dimethylnitroaniline[1]; but most of them are troublesome and result in low yields of a product which is usually of not too high purity. The methods in the literature[1g,i] which give acceptable or good yields involve a reaction in which a nitrohalobenzene is allowed to react with dimethylamine.

The author has found that this reaction is most conveniently brought about by refluxing a pyridine solution of nitrohalobenzene with a mixture of dimethylamine hydrochloride and sodium bicarbonate. The desired product is obtained in virtually quantitative yield; in the case of the para isomer, the product can be crystallized directly from the reaction solvent in a high state of purity. This procedure is therefore recommended for the preparation of these two substances.

Experimental Part

p-Nitrodimethylaniline.—A mixture of 42 g. of *p*-bromonitrobenzene, 300 cc. of pyridine, and 50 g. of sodium bicarbonate was placed in a 500-cc. round-bottom flask. To this mixture was added 30 g. of dimethylamine hydrochloride dissolved in about 10 cc. of warm water. The mixture was refluxed for ten hours. Mechanical stirring was not employed, since serious bumping did not occur. At the end of the reflux period, the hot solution was filtered free of inorganic salts, and the latter was extracted with 200 cc. of acetone, which was added to the pyridine solution. The mixed extracts were boiled, and water added to near the cloud point. On cooling, bright yellow needles of *p*-nitrodimethylaniline, 1–3 cm. in length, crystallized out. The melting point was observed to be 163.7–164.1° (lit. 163–166°) on a calibrated Anschütz thermometer in a Hershberg apparatus. The mother liquor on concentration to one third of its original volume gave an additional small yield of fine yellow needles, which had a melting point anywhere from 1–10 degrees low, for various experiments. One recrystallization from methanol raised the melting point to 163.5–164°. The over-all yield of pure product was 32.4–33.6 g. (94–97%).

o-Nitrodimethylaniline.—The above procedure was employed to prepare the ortho substituted derivative. Ten grams of *o*-nitrochlorobenzene gave 8.9 g. (85%) of product; b. p. 149 at 20 mm.; $n^{25}D$ 1.6080.

Anal. Calcd. for $C_8H_{10}N_2O_2$; C, 57.81; H, 6.06. Found: C, 57.53; H, 6.21.

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(1) (a) Beilstein "Handbuch," Vol. XII, 690, 714, and first Supplement; (b) Le Fevre, *J. Chem. Soc.*, 147 (1930); (c) Davies, *Bull. soc. chim.*, [5] **2**, 295 (1935); (d) Donald and Reade, *J. Chem. Soc.*, 53 (1935); (e) Marsden and Sutton, *ibid.*, 599 (1936); (f) Shorygin, Topchier and Anan'ina, *J. Gen. Chem.* (*U. S. S. R.*), **8**, 981 (1938); (g) Hodgson and Kershaw, *J. Chem. Soc.*, 280 (1930); (h) Evans and Williams, *ibid.*, 1199 (1939); (i) Senear, Rapport, Mead, Maynard and Koepfli, *J. Org. Chem.*, **11**, 378 (1946).

Vapor Density of Diborane[1]

BY E. M. CARR, J. T. CLARKE AND H. L. JOHNSTON

In connection with the process of adding diborane to a calorimeter it was necessary to determine its density at 275.16°K. The diborane was obtained from the Naval Research Laboratory, subjected to a two-plate distillation and shown by the cryoscopic method to have a purity of 99.95 mole per cent. The diborane was introduced into an evacuated, weighed and calibrated 1-liter Pyrex bulb immersed in a constant temperature water-bath at 275.16°K. and constant to 0.01° C. during the measurement. The pressure was read on a 15 mm. i. d. manometer using a Gaertner cathetometer and a standard meter bar in an insulated case; the readings were converted to standard conditions and meniscus corrections made according to Cawood and Patterson.[2] The bulb was then reweighed (using a similar bulb as tare) on a Troemner 4-kg. balance. One bulb had 1/8 inch Pyrex helices with a surface area 16.0 times that of the interior part of the bulb added to it. Since all density measurements were made at approximately atmospheric pressure the amount of adsorption was assumed to be constant.

The results were used to calculate the density and the second virial coefficient B in the equation

$$n - A = \frac{PV}{RT + PV}$$

where A equals moles of diborane adsorbed on the surface of a 1-liter bulb and was found to have a value of 2.4×10^{-5} mole. A summary of the data is

Pressure, atm., P	Temperature, °K., T	Moles in gas phase, $n - A$	Volume of bulb, ml., V	Second virial coef. B (ml.) 275.16° K.	Density g./l. 1 atm. 275.16°K.
0.98832	275.20	0.048232	1090.2	−247	1.2398
.98201	275.14	.047296	1076.4	−233	1.2393
.99445	275.16	.048488	1090.2	−223	1.2386
.94553	275.16	.046104	1090.2	−234	1.2393
.96219	275.17	.046314	1076.4	−227	1.2388
.97098	275.15	.046690	1076.4	−199	1.2374
		Average		−227	1.2389
		Av. deviation		±11	±0.0006

(1) This work was carried out under contract between the Office of Naval Research and The Ohio State University Research Foundation.

(2) Cawood and Patterson, *Trans. Faraday Soc.*, **29**, 514–523 (1933).

The value B equals -227, determined experimentally agrees very well with the value B equals -240 calculated from Berthelot's equation and the critical constants of A. E. Newkirk.[3]

(3) A. E. Newkirk, THIS JOURNAL, **70**, 1978 (1948).

THE CRYOGENIC LABORATORY
DEPARTMENT OF CHEMISTRY
THE OHIO STATE UNIVERSITY

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The Preparation of Xanthopterin

BY GERTRUDE B. ELION, AMOS E. LIGHT AND GEORGE H. HITCHINGS

The method of Totter[1] for the preparation of xanthopterin (2-amino-4,6-dihydroxypteridine) has several advantages with respect to the time involved, convenience and availability of starting materials, over those of Purrmann[2,3] and Koschara.[4] As will be shown, however, the product of this procedure is impure, as determined spectrographically. Moreover, such impure xanthopterin is shown to have a microbiological activity quite different from that of pure xanthopterin. The possibility exists that some of the reported biological activities of xanthopterin may be attributable to such impurities. This note describes modifications of the Totter procedure which result in their elimination.

Experimental: Preparation of Xanthopterin

Leucopterin.—The leucopterin was prepared by the method of Purrmann.[5] On standing, after neutralization, the acid filtrate from the crystallization of leucopterin deposited a red precipitate which had microbiological activity (Precipitate A, Expt. 9, Table I).

Dihydroxanthopterin.—Leucopterin (6 g., 0.03 mole) was suspended in 40 ml. of water and 4% sodium amalgam (88 g., 0.153 mole) was added in small portions with stirring, the temperature being maintained below about 50°. On completion of the reduction the mixture was decanted from the mercury and chilled in an ice-bath. The sodium salt of dihydroxanthopterin precipitated in shiny crystals which were filtered off, washed with 5 ml. of ice water and dried *in vacuo* (3.73 g., 60%). A small additional quantity of dihydroxanthopterin (0.46 g.) was obtained by acidification of the mother liquors. The filtrate from this fraction, on standing several days, deposited a red precipitate which had purine-like activity (Precipitate B, Expt. 10, Table I).

The sodium dihydroxanthopterin (3.73 g.) was dissolved in 300 ml. of hot water, with the aid of a small quantity of sodium hydroxide solution, filtered and acidified with acetic acid. Dihydroxanthopterin monohydrate precipitated as pale yellow microcrystals (3.4 g.); *cf.* Hitchings and Elion.[6]

Xanthopterin.—Dihydroxanthopterin monohydrate (2.5 g., 0.0125 mole) was dissolved at room temperature in 200 ml. of water containing 1.4 g. of potassium hydroxide. Potassium permanganate solution (84 ml. of 0.01 M) was added dropwise over the course of ten minutes. After coagulation of the manganese dioxide, the solution was separated by centrifugation. The manganese dioxide was extracted with 100 ml. of water; the combined supernatant fluids were filtered and acidified with acetic acid. The yellow-orange xanthopterin precipitate was collected by centrifugation, washed seven times with water, then with alcohol, finally with ether and dried *in vacuo*. The yield was 1.95 g. of xanthopterin monohydrate (79%). At *p*H 11.0 the monohydrate has an $E^{1\%}_{1\,cm.}$ of 0.92 at 255 mμ and 0.355 at 390 mμ.

(1) Totter, *J. Biol. Chem.*, **154**, 105 (1944).
(2) Purrmann, *Ann.*, **546**, 98 (1940).
(3) Purrmann, *ibid.*, **548**, 284 (1941).
(4) Koschara, *Z. physiol. Chem.*, **277**, 159 (1943).
(5) Purrmann, *Ann.*, **544**, 182 (1940).
(6) Hitchings and Elion, THIS JOURNAL, **71**, 467 (1949).

Microbiological

Each compound was tested for its ability to serve as a substitute for adenine in the growth of *Lactobacillus casei* with thymine as nutrilite at a concentration of 10 γ per ml.[7] It will be seen (Table I) that whereas pure xanthopterin (Expt. 1) and dihydroxanthopterin (Expt. 5) have only inhibitory effects, the product of the complete Totter procedure (Expt. 2) the product of further purification[8] of this material (Expt. 3) and that obtained by the oxidation of pure dihydroxanthopterin by silver oxide (Expt. 4) all possess purine-like activity. This activity is not due to the starting material (Expt. 6), the intermediate oxalyl derivative (Expt. 7) or leucopterin (Expt. 8). The activities appear to be properties of by-products which are formed in the various steps and in some instances deposit slowly on standing of the solutions (Expt. 9, Expt. 10). This finding demonstrates the necessity for the isolation and purification of the intermediates as a requisite for the preparation of pure xanthopterin.

TABLE I

PURINE-LIKE ACTIVITY OF XANTHOPTERIN AND INTERMEDIATES

Expt.	Compound	Titer: With compound 1 mg. per 10 ml.	Titer: Control
1	Xanthopterin I[a]	0.4	1.1
2	Xanthopterin II[b]	2.5	1.1
3	Xanthopterin III[c]	2.25	1.0
4	Xanthopterin IV[d]	1.9	1.3
5	Dihydroxanthopterin	0.3	0.6
6	2,4,5-Triamino-6-hydroxypyrimidine	0.5	0.5
7	2,4-Diamino-6-hydroxy-5-oxalamidopyrimidine	0.8	1.1
8	Leucopterin	0.4	0.6
9	Precipitate A	3.7	1.2
10	Precipitate B	5.0	1.2
11	Adenine sulfate (0.1 mg.)	7.1	1.2

[a] Permanganate oxidation of purified dihydroxanthopterin. [b] Silver oxide oxidation of crude dihydroxanthopterin. [c] Purified sample of II.[8] [d] Silver oxide oxidation of purified dihydroxanthopterin.

THE WELLCOME RESEARCH LABORATORIES
TUCKAHOE 7, NEW YORK

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(7) Hitchings, Falco and Sherwood, *Science*, **102**, 251 (1945).

(8) Crude xanthopterin prepared by Totter's method was dissolved in N sulfuric acid, treated with norite and filtered. The product was precipitated with ammonium hydroxide, washed, dried, rewashed and redried. This treatment increased the $E^{1\%}_{1\,cm.}$ at 390 mμ in glycine buffer of *p*H 11.0 from a value of 0.31 to 0.35, the latter indicating approximate purity. The greater part of the microbiological activity remained, however.