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).

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The Preparation of Xanthopterin

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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).

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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.

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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.