The Purification of Aminopterin

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Aminopterin, or N-{4-[(2,4-diamino-6-pteridinylmethyl)amino]benzoyl}glutamic acid, is a 4-amino analog of folic acid that enjoys notable clinical application in the treatment of acute leukemias.[1] Commercially available aminopterin is grossly impure and frequently the contamination amounts to more than 10% based on chromatographic and spectrometric analyses.

Most multisubstituted pteridine compounds are extremely difficult to prepare in a state of high purity mainly because of their insolubility in most solvents. The criteria of purity are also hard to establish on account of the infusibility and lack of definite melting point of these pteridines. Good elementary analytical data, especially nitrogen determination, cannot be readily obtained[2] without meticulous care and ardous practice. In addition, they inevitably exhibit a strong tenacity for water owing, undoubtedly, to the addition of water across a double bond, a phenomenon quite commonly observed in this type of π-deficient N-heterocyclic compounds.[3] Fractions of water often show up in the analysis regardless of how thoroughly the sample is dried. As a consequence, a special technique must be designed for the purification of pteridines.

Small amounts of pure aminopterin can be prepared in milligram quantities by subjecting crude aminopterin to column chromatography on diethylaminoethyl (DEAE) cellulose followed by linear gradient elution with ammonium bicarbonate buffer of pH 8, increasing the molarity from 0.1 to 0.4.[4a-c] This method is tedious, time consuming, and not suitable for preparative purpose. A convenient technique for gram-scale preparation of chromatographically pure aminopterin in crystalline form is now described.

(1) S. Farber, L. K. Diamond, R. D. Mercer, R. F. Sylvester, Jr., and J. A. Wolff, *New Engl. J. Med.*, **238**, 787 (1948).

(2) L. M. Brancone and W. Fulmor, *Anal. Chem.*, **21**, 1147 (1949).

(3) A. Albert, "Heterocyclic Chemistry," The Athlone Press, University of London, London, 1959, p. 84.

Experimental

To a solution near boiling of 5 g. of aminopterin[5] in 400 ml. of water was slowly added magnesium oxide powder (calcined magnesia, light) in small portions with vigorous stirring, until only a slight amount of MgO remained undissolved. The magnesium oxide required was about 0.7 g., and it raised the pH of the solution from 3–4 to 7–8. To the hot solution was added 1 g. of activated charcoal (Darco G-60). The hot mixture was filtered at once through a large funnel with sealed-in fritted disk of medium porosity and lined with a wet pad of diatomaceous earth (Celite), 2–3 mm. thickness. The filtrate was cooled in ice, and the crystalline magnesium salt of aminopterin was collected by filtration, recrystallized from 200 ml. of boiling water, and washed with alcohol.

Anal. Calcd. for $C_{19}H_{18}MgN_8O_5 \cdot 2.5H_2O$: Mg, 4.79; N, 22.07. Found: Mg, 4.60; N, 21.75.

The magnesium salt was redissolved in 200 ml. of boiling water. The boiling solution was carefully acidified, accompanied by vigorous agitation, with 2 ml. of glacial acetic acid. The pure aminopterin that came down in fine crystalline form was easily filtered. It was again collected and washed with water and acetone. The yield was about 3 g. In comparison with the aminopterin before purification, this material showed only one peak (and a negligible trace of an unidentified simple pteridine amounting to much less than 0.1%) when chromatographed on DEAE cellulose column.[4a-c] Repetition of this procedure completely removed the last trace of the contaminant. In 0.1 *N* NaOH, aminopterin exhibited the following ultraviolet characteristics: λ_{max} 261 mμ (log ϵ 4.41), 282 (4.39), and 373 (3.91).

Anal. Calcd. for $C_{19}H_{20}N_8O_5 \cdot 0.75H_2O$: C, 50.27; H, 4.77; N, 24.69. Found: C, 50.35; N, 4.99; N, 24.65.

Pure aminopterin showed no difference from crude aminopterin in antileukemic activity when screened against advanced L1210 mouse leukemia. Clinical comparison of the purified *vs.* the crude material is in progress.

(4) (a) V. T. Oliverio and T. L. Loo, *Proc. Am. Assoc. Cancer Res.*, **3**, 140 (1960); (b) V. T. Oliverio, *Anal. Chem.*, **33**, 263 (1961); (c) R. L. Kisliuk and M. D. Levine, *J. Biol. Chem.*, **239**, 1900 (1964). Dr. Kisliuk kindly informed me that the aminopterin purified by the present procedure was six times more potent for inhibition of *Streptococcus faecalis* as the aminopterin purified by column chromatography.

(5) The aminopterin (grade C) was supplied by Calbiochem, Los Angeles, Calif. Column chromatography on DEAE cellulose resolved this material into 6 components as detected by absorption of ultraviolet light of 254 mμ. It was estimated that 80% of it was aminopterin, 15% folic acid, and the remaining 5% various pteridines.

New Compounds

Some 3,4,5-Trimethoxy-Substituted Benzamides[1]

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In continuation of our investigations of compounds having depressant effects on the central nervous system,[2] a number of trimethoxy benzene derivatives were prepared by a variety of methods. Most of these compounds were found to be CNS depressants by gross observation of intact mice and rats and by avoidance behavior studies. Some new trimethoxybenzene derivatives have been synthesized by us and these compounds were also found to potentiate pentobarbital hypnosis in albino mice and exert a moderate influence on the conditioned-avoidance response of trained rats.

(1) The authors gratefully acknowledge financial support from Council of Scientific and Industrial Research, New Delhi, India.

(2) (a) P. C. Dandiya and H. Cullumbine, *J. Pharmacol. Exptl. Therap.*, **125**, 353 (1959); (b) P. C. Dandiya and M. K. Menon, *Brit. J. Pharmacol.*, **20**, 434 (1963); (c) P. C. Dandiya, P. K. Sharma, and M. K. Menon, *Indian J. Med. Res.*, **50**, 750 (1962).

Experimental[3]

Except for compound VII, all of the amides in Table I were prepared by the following general method.

A calculated quantity of 3,4,5-trimethoxybenzoyl chloride (0.2 mole) in dry tetrahydrofuran was added slowly with stirring to a cooled (below 10°) solution of the calculated amount of the desired amine (0.4 mole) in the same solvent. The mixture was stirred at room temperature for about 1 hr. and then allowed to

(3) The melting points of the above compounds were determined in open capillaries using an electrically heated block and are uncorrected.