SYNTHESIS OF POTENTIAL ANTICANCER AGENTS. II.[1] 6-MERCAPTO-9-β-D-RIBOFURANOSYLPURINE

Sir:

Although the antibacterial and antileukemic activities of 6-mercaptopurine are well known,[2,3] the mechanism of action of this drug is still not well understood. Since there is a distinct possibility that 6-mercaptopurine must be converted to its riboside or ribotide[4] before it becomes an effective drug, a number of workers have attempted the synthesis of 6-mercaptopurine riboside.[5,6]

For the synthesis of 6-mercaptopurine riboside, 6-chloropurine riboside[7] appeared to be a promising starting material. The reactivity of the chloro group of this compound was demonstrated by Brown and Weliky,[8] who treated 6-chloropurine riboside with ammonia at 100° to get a smooth conversion to adenosine in 86% yield. Since 6-chloropurine has been converted to 6-mercaptopurine by reaction with thiourea in ethanol,[9] the same reaction with 6-chloropurine riboside was the first one studied. In boiling ethanol, the reaction was quite rapid, and in spite of considerable cleavage to 6-mercaptopurine by the acid formed in the reaction, pure 6-mercapto-9-β-D-ribofuranosylpurine, micro m.p. 198–200° dec., could be isolated in low yield. The addition of calcium carbonate to the reaction mixture as an acid acceptor failed to improve the yields.

6-Mercaptopurine has also been prepared by reaction of 6-chloropurine with aqueous potassium hydrogen sulfide at 100° for seven hours.[10] The use of this reagent with 6-chloropurine riboside has the disadvantage that the imidazole moiety of nucleosides of this type is rapidly cleaved in the presence of aqueous alkali to give a 4-glucosylamino-5-formamido-6-chloropyrimidine.[11] However, under anhydrous conditions the 6-chloro group of a 6-chloropurine nucleoside can be replaced with methoxide without ring cleavage.[12] These observations suggested the use of methanolic sodium hydrogen sulfide as a reagent with which 6-chloropurine riboside might be treated without ring cleavage.

Anhydrous methanolic sodium hydrogen sulfide, prepared by saturating 10.5 ml of 1*N* methanolic sodium methoxide with hydrogen sulfide, was added to a refluxing suspension of 2.49 g. 6-chloro-9-β-D-ribofuranosylpurine in 40 ml. of methanol. The mixture was refluxed for 10 minutes, solution being complete in 7 minutes.[13] The solution was evaporated to dryness and the residue redissolved in 15 ml. of hot water and then acidified with acetic acid. On cooling, the solution deposited 2.00 g. (81%) of nearly pure 6-mercapto-9-β-D-ribofuranosylpurine, micro m.p. 196–201° dec. Recrystallization from 20 ml. of water afforded 1.70 g.(69%) of pure riboside as cream-colored needles; micro m.p. 198–200° dec., capillary m.p. 207–210° dec.; $[\alpha]^{23}D$ −73° (2.0% in 0.1 *N* NaOH); $\lambda_{max}^{pH\ 1}$ 322 mμ (a_M 22,500), $\lambda_{max}^{pH\ 6.7}$ 320 mμ (a_M 21,500), $\lambda_{max}^{pH\ 13}$ 310 mμ (a_M 22,130).[14] *Anal.* Calcd. for $C_{10}H_{12}N_4O_4S$: C, 42.2; H, 4.26; N, 19.7. Found: C, 42.2; H, 4.49; N, 19.8.

When tested against Adenocarcinoma 755 in the CBF_1 mouse, 6-mercaptopurine riboside profoundly inhibited tumor growth. The riboside administered at several dose levels was found to be equally as carcinostatic and equally as toxic as similar doses of 6-mercaptopurine on a molar basis.[15] Whether this compound is effective due to phosphorylation to the ribotide or due to enzymatic hydrolysis to 6-mercaptopurine and whether or not the riboside is effective against 6-mercaptopurine-resistant bacteria and leukemias is now under study in these laboratories.[15]

(1) For Paper I of this series cf. J. A. Montgomery, THIS JOURNAL, **78**, 1928 (1956). This work was supported by funds from the Kettering Foundation, the Sloan Foundation and the Black–Stevenson Foundation.

(2) G. B. Elion, G. H. Hitchings and H. VanderWerff, *J. Biol. Chem.*, **192**, 505 (1951).

(3) J. H. Burchenal, R. R. Ellison, M. L. Murphy, D. A. Karnofsky, M. P. Sykes, T. C. Tan, A. C. Mermann, M. Yuceoglu, W. P. L. Myers, I. Krakoff, and N. Alberstadt, *Ann. N. Y. Acad. Sci.*, **60**, 359 (1954).

(4) Preliminary experiments in this laboratory have shown that 6-mercaptopurine-8-C^{14}, when added to a growing culture of *Streptococcus faecalis* (ATCC 8043), is rapidly converted to a number of other compounds, as yet unidentified. On two dimensional chromatography several of these materials move to the same region of the chromatogram as do known nucleotides.

(5) The deoxy compound, 6-mercapto-9-(2′-deoxy-β-D-ribofuranosyl)-purine, has been synthesized enzymatically by M. Friedkin, *Biochim., Biophys. Acta*, **18**, 447 (1955).

(6) I. Goodman, G. B. Elion, and G. H. Hitchings, *Fed. Proc.*, **14**, 219 (1955), have synthesized 6-mercapto-9-β-D-glucopyranosylpurine in low yield, starting with 6-benzylmercaptopurine.

(7) Pure material was prepared in 28–30% yield by suitable modifications of the procedure of Brown and Weliky,[8] to be published.

(8) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(9) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954).

(10) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **76**, 4027 (1954).

(11) Private communication from Drs. G. B. Brown and M. P. Gordon, Sloan-Kettering Institute, New York, New York. A similar observation has been made in this laboratory on treatment of 6-chloro-9-α-L-rhamnopyranosylpurine with aqueous sodium hydroxide (B. R. Baker and K. Hewson, unpublished results).

(12) B. R. Baker and K. Hewson, unpublished results.

(13) Inspection of the ultraviolet spectra of aliquots from a pilot run showed that the reaction was complete three minutes after all solid had dissolved. With longer reflux time, some decomposition of the mercapto compound takes place, as indicated by further changes in the ultraviolet spectrum.

(14) The 6-mercaptopurine riboside showed the following R_f values from ascending paper chromatography on 1-inch strips of Whatman No. 1 filter paper: R_f 0.64 in 0.1 *M* phosphate buffer, *p*H 6.9 R_f 0.62 in 5% ammonium sulfate–5% isopropyl alcohol in water.[5]

(15) F. M. Schabel, Jr., and H. E. Skipper, to be published.

(16) Affiliated with Sloan–Kettering Institute.

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ON THE MECHANISM OF ACTION OF PARATHORMONE[1]

Sir:

In the years since Dickens first reported a high bone content of citrate,[2] there has been a growing interest in the possibility of a relationship between calcium metabolism and serum citrate. A recent literature review[3] emphasizes the strong correlation between serum levels of the two ions and the re-

(1) This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

(2) F. Dickens, *Biochem. J.*, **35**, 1010 (1941).

(3) T. F. Dixon and H. R. Perkins, Chapter 11 in "The Biochemistry and Physiology of Bone," ed. by G. H. Bourne, Academic Press, New York, N. Y., 1956.