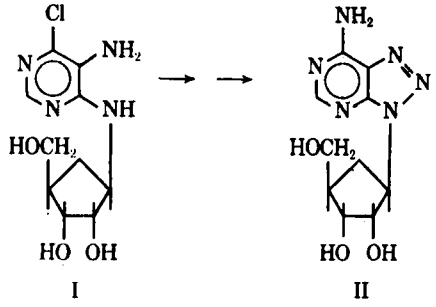
Carbocyclic Analog of Purine Ribonucleosides with Antileukemic Activity

Keyphrases ☐ Carbocyclic analogs of purine nucleosides (8-azaadenosine analog)—synthesis, antileukemic activity ☐ *v*-Triazolo[4,5-*d*]pyrimidines—synthesis of 8-azaadenosine analog of purine nucleoside, antileukemic activity ☐ Antileukemic activity—synthesis of a *v*-triazolo[4,5-*d*]pyrimidine (carbocyclic analog of purine nucleoside) ☐ Purine ribonucleosides—synthesis of carbocyclic analog with antileukemic activity

Sir:

Carbocyclic analogs of several naturally occurring and biologically active purine nucleosides have been synthesized (1–4). In these analogs a methylene group occupies the position of the ring oxygen atom of the ribo- or deoxyribofuranosides. Because of the presence of a stable carbon–nitrogen bond at position 9 of the purine moiety, these analogs are not subject to cleavage by enzymes that remove or transfer the ribofuranosyl moiety of the nucleosides or nucleotides. Biochemical studies (5–7) have shown that the racemic analog (III) of adenosine can serve either as a substrate for or as an inhibitor of some of the enzymes involved in purine nucleotide metabolism, one of the more interesting findings being the inhibition of guanylic acid kinase by the phosphate (7). The antibiotic aristeromycin (8), the optically active form (9, 10) of the adenosine analog, inhibits certain plant pathogens (8, 11). Several racemic carbocyclic analogs of purine nucleosides are cytotoxic to neoplastic cells growing in culture (4). In this communication, antileukemic activity *in vivo* by a carbocyclic analog, the 8-azaadenosine analog (II), is reported.

The 8-azaadenosine analog II, (±)-*trans*-3-(7-amino-3*H*-*v*-triazolo[4,5-*d*]pyrimidin-3-yl)-*trans*-5-(hydroxymethyl)-*cis*-1,2-cyclopentanediol, was synthesized from the diaminopyrimidine I (Scheme I) (1, 2). The pyrimidine was diazotized with sodium nitrite in hydrochloric acid. Since 6-chloro-8-azapurines are known to be highly reactive, the 6-chloro-8-azapurine formed by this reaction was not isolated. The reaction mixture was neutralized and then lyophilized, and the residue was treated with anhydrous ammonia. The yield of II after recrystallization from water was 76%, m.p. 276–278° dec.; UV_{max}: 264 (ε 12,400) and 275 (sh) in 0.1 *N* HCl, 279 (ε 12,400) in phosphate buffer (pH 7), and 279 nm. (ε 12,300) in 0.1 *N* NaOH.



Scheme I

Anal.—Calc. for $C_{10}H_{14}N_6O_3$: C, 45.11; H, 5.30; N, 31.57. Found: C, 44.90; H, 5.46; N, 31.39.

In tests of II against leukemia P-388[1] in mice, the median survival time of treated animals was prolonged by 40–70%. Thus, at doses of 100, 75, 67, and 37 mg./kg./day given intraperitoneally, q.d. 1–9, the observed median increases in lifespan (ILS) were 68, 53, 63, and 42%, respectively. Higher doses produced evidence of toxicity; in two tests at 150 mg./kg./day and in one at 225 mg./kg./day, ILS were 73, 18, and 36%, respectively, but the average differences in weight change between treated and untreated animals were −2.5, −3.4, and −5.1 g., respectively. In comparison, the carbocyclic analog (III) of adenosine was not active against P-388 leukemia in a dose–response test at 50 (toxic), 25 (toxic), 12.5, and 6.25 mg./kg./day performed simultaneously with the dose–response test of II (at 225, 150, 100, and 67 mg./kg./day).

In tests *versus* leukemia L-1210, Compound II displayed, at best, borderline activity. Average ILS were 18–22% after doses of 150, 100, 67, or 33 mg./kg./day given intraperitoneally, q.d. 1–9. Average ILS were 23 and 26% after doses of 200 and 100 mg./kg., respectively, given on Days 1, 5, and 9; but in a second test at 150 and 100 mg./kg., ILS were 11 and 14%, respectively.

Both II and the carbocyclic analog of adenosine (III) are highly cytotoxic to human epidermoid carcinoma cells, No. 2, in culture. The concentration of II producing 50% inhibition of growth (ED_{50}), determined by protein measurements, was about 0.3 mcg./ml. The cytotoxicity of III is essentially the same; values of ED_{50} determined by this method and by the clone-colony method were about 0.7 (4) and 0.2 mcg./ml. (6), respectively. The fact that II is tolerated *in vivo* at higher doses than III suggests that the observed activity *in vivo* may be due to greater selectivity in the action of II.

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Y. FULMER SHEALY▲
JOE D. CLAYTON
Kettering-Meyer Laboratories
Southern Research Institute
Birmingham, AL 35205

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▲ To whom inquiries should be directed.

Failure of USP Tablet Disintegration Test to Predict Performance in Man

Keyphrases ☐ Aminosalicylic acid enteric-coated tablets—results of compendial disintegration testing compared to plasma levels in man ☐ Plasma levels, aminosalicylic acid and metabolites—after administration of enteric-coated tablets, compared to dissolution data ☐ Tablet disintegration, compendial method—results for enteric-coated aminosalicylic acid tablets compared to plasma levels in man ☐ Excretion, fecal—enteric-coated aminosalicylic acid tablets, man

Sir:

An enteric-coated tablet of aminosalicylic acid[1], purchased on the open market in the United States by the Food and Drug Administration (FDA), was shown to pass all USP specifications in the FDA laboratories. In a four-way crossover study in eight normal adult volunteers, single oral doses of either 1 g. of aminosalicylic acid or the equivalent of 1 g. of the acid were administered as the enteric-coated tablet[1], a compressed tablet of aminosalicylic acid, a suspension of aminosalicylic acid in water, and a solution of the sodium aminosalicylate in water.

Blood samples were taken at 0, 0.33, 0.67, 1, 2, 3, 4, 6, 8, 12, and 24 hr. postadministration of each dose. The plasma samples, derived from the blood, were assayed for both aminosalicylic acid and its metabolite, *N*-acetyl-*p*-aminosalicylic acid, by a new specific analytical method (1). Plasma samples of all eight subjects at each sampling time assayed "zero" for both drug and metabolite following oral administration of the enteric-coated tablet. The assay sensitivity level was 0.5 mcg./ml. The average peak plasma levels of unchanged drug were 43.5, 16.7, and 8.86 mcg./ml. following oral administration of the solution, the suspension, and the compressed tablet, respectively. Drug levels were measurable over 6–8 hr. following the latter three dosage forms. The average peak plasma levels of the metabolite were 11.6, 12.3, and 10.9 mcg./ml. following the solution, the suspension, and the compressed tablet, respectively. Metabolite levels were measurable over 6–8 hr. following these dosage forms. Detailed results of this study will be published.

Since we did not know about the zero plasma levels of unchanged drug and metabolite in every subject following the enteric-coated tablet until all assays were completed, we never thought to ask the subjects to check their stools for intact tablets or large fragments of tablets. After the results were known, however, one of the eight subjects volunteered to take two more of the enteric-coated tablets. One tablet was excreted in his feces essentially intact (but with a small "hole" in one face) about 30 hr. postingestion. The other tablet did break up, but large pieces were excreted in the same feces. Figure 1 is a photograph showing the original intact tablet as it appeared before ingestion and the essentially intact tablet and the large fragments of the other tablet that were isolated from the feces. After photographing, the drug content of the material isolated from the feces was determined. The essentially intact tablet isolated from the feces assayed 489 mg. (98% of labeled dose), and the fragments[2] of the other tablet assayed 240 mg. (48% of labeled dose).

It is obvious that an enteric-coated tablet that gave zero plasma levels of bioactive aminosalicylate and that was excreted in the feces would be clinically ineffective. It would be unethical to perform a clinical study in patients with tuberculosis to prove such a point. The results obtained are attributable to both poor disintegration of the tablets and slow dissolution of drug from the fragments of the tablets once they did disintegrate or the coating ruptured. This was readily

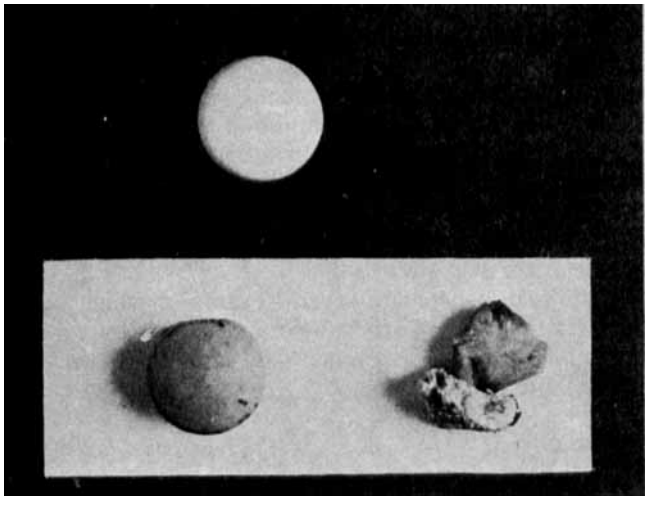

Figure 1—*(Top) Enteric-coated tablet before ingestion. (Bottom) Enteric-coated tablets excreted in the feces.*

[1] 4-Aminosalicylic acid; Parasal enteric-coated 0.5-g. tablet (Lot No. 722165B), containing 0.5 g. aminosalicylic acid USP (Panray Division of Ormont Drug and Chemical). The other dosage forms had the same trademark and were made by the same manufacturer.

[2] Only some fragments were recovered. Since some remained in the feces, the assay is lower than it should be.