Notes

Nucleosides. XXXV. 1-β-D-Arabinofuranosyl-5-methylcytosine[1]

Iris L. Doerr, John F. Codington, and Jack J. Fox

Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York 21, New York

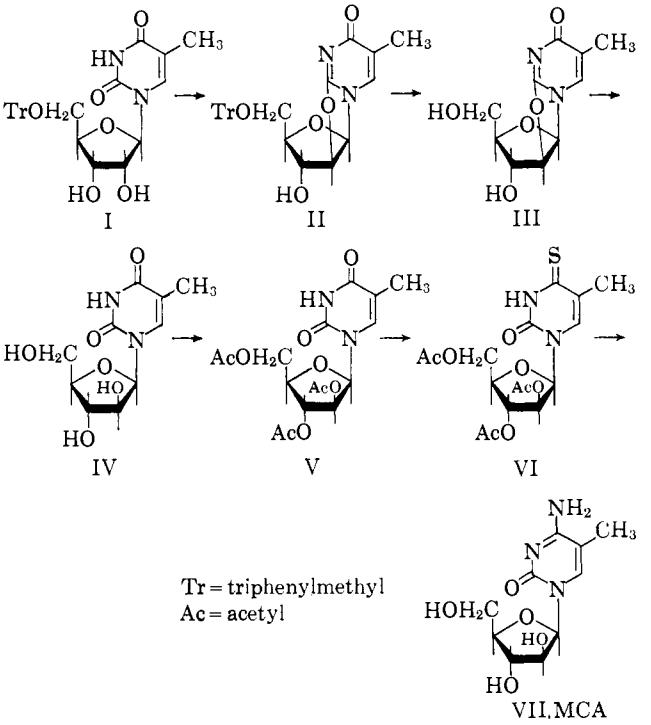
Received July 28, 1966

Pyrimidine nucleosides containing the 1-β-D-arabinofuranosyl moiety have exhibited interesting biological properties. Among these compounds are 1-(β-D-arabinofuranosyl)cytosine (CA)[2] which is active against several experimental tumors;[3] 1-β-D-arabinofuranosyl-5-iodouracil which is active against herpes and vaccinia virus in cell cultures;[5] 1-β-D-arabinofuranosyl-5-fluorouracil (FUA)[6] which is active against leukemia B82 and Sarcoma 180; and finally 1-β-D-arabinofuranosyl-5-fluorocytosine (FCA)[4] which is active against Sarcoma 180 and leukemias P388, P815, and L1210, as well as against the 5-fluorouracil-resistant strains of the latter two. Both CA and FCA are currently undergoing clinical trials.[7,8]

These biological data suggested the synthesis of 1-(β-D-arabinofuranosyl)-5-methylcytosine (VII, MCA, see Chart I) because of its structural similarity to CA and FCA. 1-(5-*O*-Trityl-β-D-ribofuranosyl)thymine (I)[9] was converted directly into the 2,2′-anhydroarabino derivative (II)[9b] (67%) by use of the thionocarbonate procedure[4,10] developed for nucleosides. Detritylation of II by anhydrous hydrogen chloride in ether gave the crystalline hydrochloride of III[9b] in quantitative yield which was subsequently converted to nucleoside III (92%). Refluxing the 2,2′-anhydronucleoside III in 1 *N* sulfuric acid for 1 hr yielded 1-β-D-arabinofuranosylthymine (IV)[9a] in 88% yield.[11]

The arabinosyl nucleoside (IV) was converted to MCA (VII) by modification of the thiation process previously reported for pyrimidine nucleosides.[12] Acetylation of IV gave a 94% yield of the tri-*O*-acetate (V) which was thiated with phosphorus pentasulfide in pyridine to the 4-thione (VI) in quantitative yield.

Chart I



Reaction of the thione (VI) with liquid ammonia at 55° overnight gave MCA (VII) in 80% yield.

Preliminary Screening Studies.—At doses of 100 mg/kg ip daily for ten doses, MCA was inactive in all experiments against mouse leukemia P815. MCA was administered intraperitoneally to Sarcoma 180 tumor-bearing mice twice daily starting with the day following tumor implantation. With MCA, tumor hosts showed no toxic signs after 13 injections of 125 mg/kg/day. The ratio of the average diameter of treated tumors to the average diameter of the control tumors (T/C) was 1.0 indicating no retardation of growth of tumors over the controls by MCA.

Experimental Section[13]

2,2′-Anhydro-1-(5-*O*-trityl-β-D-arabinofuranosyl)thymine (II).—This procedure is similar to that used to prepare 2,2′-anhydro-1-(5-*O*-trityl-β-D-arabinosyl)uracil from 5′-*O*-trityluridine.[4] A suspension of 5′-*O*-trityl nucleoside (I, 4.5 g, 9.0 mmoles) in anhydrous toluene (100 ml) was azeotroped to remove water. The suspension was cooled to about 80° and 1.8 g of thiocarbonyldiimidazole (preparation in ref 4) dissolved in toluene was added. Immediately a bright yellow solution formed which was boiled for 1 hr. After the pale yellow solution had cooled, crystalline plates of II precipitated and were collected, 3.1 g, mp 190–195°. Evaporation of the toluene filtrate to dryness and treatment of the residue with chloroform gave a second crop of 0.6 g, mp 225–230°. Recrystallization of both crops from ethanol (100 ml) gave micaceous plates, 2.9 g (67%), mp 225–230°, $[\alpha]^{25}D$ −29° (*c* 0.51, methanol). Compound II prepared from the 2′-*O*-tosyl derivative of I[9b] gave mp 230–233° and $[\alpha]^{25}D$ −28°.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 08748).

(2) E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 84 (1959).

(3) For pertinent references see ref 4.

(4) J. J. Fox, N. Miller, and I. Wempen, *J. Med. Chem.*, **9**, 101 (1966).

(5) M. Privat and J. de Rudder, *Compt. Rend.*, 259 (1964).

(6) N. C. Yung, J. H. Burchenal, R. Fecher, R. Duschinsky, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4060 (1961).

(7) J. H. Burchenal, H. H. Adams, N. S. Newell, and J. J. Fox, *Cancer Res.*, **26**, 370 (1966).

(8) Leukemia Task Force Report XV, Cancer Chemotherapy Program, National Cancer Institute, June 1966.

(9) (a) J. J. Fox, N. Yung, and A. Bendich, *J. Am. Chem. Soc.*, **79**, 2775 (1957); (b) J. F. Codington, I. L. Doerr, and J. J. Fox, *J. Org. Chem.*, **29**, 558 (1964).

(10) J. J. Fox and I. Wempen, *Tetrahedron Letters*, 643 (1965).

(11) The over-all yield of IV was 46% based on 1-β-D-ribosylthymine. This is an improvement over the 20% over-all yield previously reported.[9a]

(12) J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).

(13) Melting points are corrected. Elemental analyses were made by the Spang Microanalytical Laboratory, Ann Arbor, Mich. Ultraviolet absorption data were obtained using the Cary recording spectrophotometer, Model 15.

2,2'-Anhydro-1-β-D-arabinofuranosylthymine[9b] (III).—The hydrochloride of III was prepared by detritylation of II with ethereal HCl in the manner described.[9b] The free nucleoside III was obtained from its hydrochloride salt by a procedure which is simpler and affords higher yields than the Dowex 1 (acetate) method previously described.[9b]

A suspension of the hydrochloride of III (1.0 g) in 15 ml of ethanol was stirred and 1 equiv of triethylamine was added dropwise. The reaction mixture was allowed to stand at room temperature 1 hr. The solid (III) was filtered off and washed with ethanol followed by ether. The product had a mp 225–228° and was obtained in 92% yield.[14] Ultraviolet spectra and chromatographic data obtained for III were the same as for an authentic sample.[9b]

1-β-D-Arabinofuranosylthymine (IV).[9a]—Twenty milliliters of 1 *N* H_2SO_4 was added to the 2,2'-anhydro nucleoside (III, 1.0 g, 4.0 mmoles) and the reaction mixture was refluxed for 45 min. On cooling the reaction mixture, 0.84 g of 1-β-D-arabinosylthymine (IV), mp 245–248°, precipitated. The mother liquor was neutralized ($BaCO_3$) and the salts were removed by filtration. On evaporation of the filtrate, an additional 0.11 g (mp 248–250°, over-all yield 88%) was obtained. Crystallization of the combined solids from 25% ethanol gave a 93% recovery. Chromatographic data, infrared, and ultraviolet spectra of this sample were identical with those reported for authentic IV.[9a]

1-(Tri-*O*-acetyl-β-D-arabinofuranosyl)thymine (V).—To a stirred suspension of 1.3 g (5.2 mmoles) of 1-β-D-arabinofuranosylthymine in 20 ml of pyridine was added 1.6 ml (17 mmoles) of acetic anhydride. The suspension was heated at 45° for a few minutes until solution occurred. The reaction mixture was allowed to stand at room temperature for 18 hr. Ethanol (0.5 ml) was added to quench the reaction and the pyridine evaporated off. The syrup was evaporated twice with 50% ethanol and then azeotroped with absolute ethanol. The crystalline residue was triturated with ether and filtered, 1.9 g (94%), mp 138–140°. Crystallization from ethanol gave short colorless needles, mp mp 140–142°, $[\alpha]^{26}D$ +65° (*c* 0.3, ethanol).

Anal. Calcd for $C_{16}H_{20}N_2O_8$: C, 50.00; H, 5.25; N, 7.29. Found: C, 50.00; H, 5.22; N, 7.28.

1-(Tri-*O*-acetyl-β-D-arabinofuranosyl)-4-thiothymine (VI).—To a stirred pyridine (50 ml) solution containing 1.9 g (4.9 mmoles) of acetylated nucleoside V was added 2.4 g (10.9 mmoles) of P_2S_5. The mixture was refluxed and when solution was complete (within the first 30 min) 0.12 ml of water was added and the reaction mixture was refluxed for 3 hr. After the reaction mixture had cooled, the pyridine solution containing the product was decanted from a yellow hygroscopic precipitate. The solid was extracted twice with pyridine, and the combined pyridine solutions were evaporated *in vacuo* to an amber-colored syrup. The syrup was treated with 50% ethanol and the ethanol–water mixture was concentrated. This procedure was repeated. The yellow residue was taken up in chloroform and filtered from a small residue. The $CHCl_3$ solution was evaporated *in vacuo*, and ethanol was added to the yellow glass. Precipitation of a light yellow solid occurred, 2.0 g, mp 124–126°. Crystallization of the solid from ethanol gave light yellow needles, mp 126–127°, $[\alpha]^{26}D$ +148° (*c* 0.25, acetone). Spectral properties in 50% ethanol showed maxima at 332 and 243 mμ, minima at 275 and 222 mμ; spectral ratio in 50% ethanol 332/243 mμ = 5.3.

Anal. Calcd for $C_{16}H_{20}N_2O_8S$: C, 48.00; H, 5.04; N, 6.99; S, 7.99. Found: C, 47.87; H, 4.86; N, 7.08; S, 7.92.

1-β-D-Arabinofuranosyl-5-methylcytosine (VII).—The thionucleoside (VI, 1.3 g, 3.25 mmoles) within a glass container was treated with liquid NH_3 (20 ml) and heated in a steel bomb for 20 hr at 55–60°. The liquid ammonia was driven off by a stream of dry nitrogen. The resulting pale yellow syrup was dissolved in water (30 ml), neutralized with 2 *N* acetic acid, and applied to a Dowex 50 (H^+) 100–200 mesh column (1.4 × 14 cm). The column was washed with water until the effluent was free from ultraviolet-absorbing material and then eluted with 2 *N* NH_4OH. The ultraviolet absorbing fractions were evaporated to dryness. The white residue was dissolved in 50% ethanol, treated with charcoal, and filtered using a filter aid of diatomaceous earth. Colorless cubic crystals precipitated, 0.72 g (80%), which sintered at ~150°, and effervesced at 160–165°; $[\alpha]^{26}D$ +105° (*c* 0.39, water); ultraviolet absorption properties at pH 7.5 (neutral species), maximum at 277 mμ (ε 8600), minimum at 253 mμ (ε 4600); in 0.1 *N* HCl (cationic species), maximum at 287 mμ (ε 12,700), minimum at 244 mμ (ε 1400).

Anal. Calcd for $C_{10}H_{14}N_2O_5 \cdot H_2O$: C, 43.63; H, 6.22; N, 15.14. Found: C, 44.00; H, 6.26; N, 15.14.

Acknowledgments.—The authors are indebted to Dr. J. H. Burchenal for the screening data of MCA in leukemia P815 and to Drs. G. S. Tarnowski and F. Schmid for data on Sarcoma 180. The authors are grateful to Dr. G. B. Brown for his warm and continued interest.

(14) The hydrochloride of 2,2'-anhydro-1-β-D-arabinofuranosyluracil was also converted to its free base in 97% yield by this treatment.

Synthesis of Some Orotic Acid Analogs as Potential Antimetabolites[1]

SAUL BORODKIN, SIGFRIDUR JONSSON,[2] GEORGE H. COCOLAS, AND ROBERT L. MCKEE

School of Pharmacy and Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27515

Received June 17, 1966

The elucidation and biosynthesis of pyrimidine nucleotides has established orotic acid (2,6-dihydroxy-4-carboxypyrimidine) as an important metabolite. The significance of inhibition of this metabolic route is generally accepted as being reflected in antiviral, antineoplastic, and antibiotic effects on the organism.[3] Many isosteres and analogs of orotic acid as well as other pyrimidine derivatives have been shown to interfere with orotic acid metabolism and subsequently with nucleic acid formation.[4] We wish to report on the synthesis and biologic evaluation of a series of 5-substituted orotic acid analogs designed as potential antimetabolites of orotic acid. Inhibition by pyrimidines at this level of nucleic acid biosynthesis has the advantage of the use of molecules which can be more easily prepared than their corresponding nucleotides and also have the opportunity of being more specific in action since earlier metabolites (*e.g.*, aspartic acid, DPN, etc.) have more diverse metabolic applications.

In view of the antimetabolic activity of some 2,4-diaminopyrimidines[4f,5] on orotic acid a series of 2-amino-, 2-hydroxy-, and 2-thio-6-amino-4-carboxypyrimidines substituted at the 5 position was synthesized. Although the 5 position of orotic acid is not involved in the biosynthesis of the pyrimidine nucleotides, it has been mentioned by Stone and Potter[6] as the choice position for substitution in the preparation of orotic acid antagonists. A limited number of 5-substituted orotic acids have been synthesized and shown to possess anti-

(1) (a) Abstracted from a portion of the thesis submitted by S. Borodkin to the Graduate School of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1962. (b) Supported by a research grant from Smith Kline and French Laboratories, Philadelphia, Pa.

(2) Deceased.

(3) G. B. Elion and G. H. Hitchings, *Advan. Chemotherapy*, **2**, 91 (1965).

(4) (a) W. L. Holmes, *J. Am. Chem. Soc.*, **76**, 2899 (1954); (b) Z. B. Papanastassiou, A. McMillan, V. J. Czebotar, and T. J. Bardos, *ibid.*, **81**, 6056 (1959); (c) P. Pithova and F. Sorm, *Collection Czech. Chem. Commun.*, **28**, 2977 (1963); (d) S. Udaka and B. Vennesland, *J. Biol. Chem.*, **237**, 2018 (1962); (e) E. Bresnick, *Texas Rept. Biol. Med.*, **21**, 505 (1963); (f) J. E. Stone and V. R. Potter, *Cancer Res.*, **16**, 1033 (1956); (g) R. E. Hafschemacher, *ibid.*, **23**, 634 (1963).

(5) K. A. Shellenberg and G. B. Gourley, *Biochem. Pharmacol.*, **6**, 113 (1961).

(6) J. E. Stone and V. R. Potter, *Cancer Res.*, **17**, 801 (1957).