Derivatives of the Nucleoside Antibiotics, Toyocamycin and Sangivamycin. Analogs of N^6-(Δ^2-Isopentenyl)adenosine.

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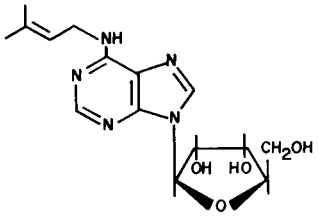
The isolation and characterization (1) of a minor nucleoside from yeast t-RNA as 6-(3-methyl-2-butenyl)amino-9-(β-D-ribofuranosyl)purine [I, N^6-(Δ^2-isopentenyl)adenosine] was followed by reports (2) on the occurrence of this minor nucleoside in several other t-RNA's. It is usually found adjacent to the anticodon which has generated considerable interest in studies involving the chemotherapeutic (antitumor) (3) and biological (cytokinin-like) (4) activity, as well as a study on the mode of action (5) and catabolic fate (6) of I. Our laboratory has



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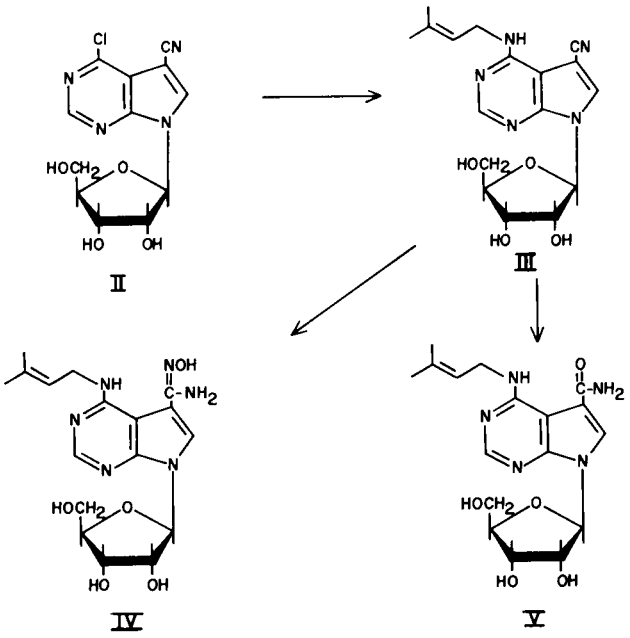
been involved in several studies designed to increase the biological and chemotherapeutic activity of the adenosine type nucleoside antibiotics toyocamycin (7) and sangivamycin (8). These nucleosides have demonstrated a unique ability to function as substrates for the anabolic enzymes (*e.g.* adenosine kinase) (9) and yet are resistant (9) towards catabolism by other enzymes (*e.g.* phosphorolysis and oxidation) which accept adenosine as a substrate. This prompted us to synthesize the N^6-(Δ^2-isopentenyl)-adenosine analogs of these nucleoside antibiotics.

Treatment of 4-chloro-5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (II) (10) with 3-methyl-2-butenylamine hydrochloride (11) in alcoholic solution in the presence of a proton acceptor furnished a good yield of 4-(3-methyl-2-butenyl)amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (III). The infrared spectrum displayed a peak at 2240 cm^{-1} and established that the exocyclic cyano group had not been changed under the reaction conditions. Treatment of III with peroxide in aqueous ammonia furnished a good yield of 4-(3-methyl-2-butenyl)amino-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine-5-carboxamide (V). That a chemical reaction had occurred only at the exocyclic cyano group



REACTION SCHEME

was established by infrared and pmr spectroscopy. The synthesis of V is of especial interest since sangivamycin, *per se*, has demonstrated sufficient anticancer activity to warrant clinical trials (12). The reported (13) antitumor activity of 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-5-carboxamidoxime prompted us to synthesize the corresponding derivative in this series. Treatment of III with ethanolic hydroxylamine furnished 4-(3-methyl-2-butenyl)amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime (IV).

EXPERIMENTAL

Ultraviolet spectra were obtained from a Beckman DK2-spectrophotometer and infrared spectra were obtained from a Beckman IR-8 as nujol mulls. PMR spectra were recorded on a Varian A-56/60 or XL 100 spectrometer in DMSO-d_6 and DMSO-d_6/deuterium oxide solution with TMS as an internal standard. Thin-layer chromatograms were run on glass plates

coated with a 0.25 mm layer of SilicAR-7GF (Malinckrodt Chem. Co.) and developed by the ascending technique in the following solvents: A, ethyl acetate; B, ethyl acetate-1-propanol-water-(4:1:2, v/v/v, upper phase); C, ethanol-water (7:3, v/v); D, ethanol-ethyl acetate (1:1, v/v); E, water; F, ethyl acetate-ethanol (9:1, v/v). All evaporations were conducted on a Buchler rotary evaporator under reduced pressure except where noted. Melting points were determined on a Thomas-Hoover Unimelt and are uncorrected. Elemental analysis were conducted by Heterocyclic Chem. Co., Harrisonville, Missouri, U.S.A.

4-(3-Methyl-2-butenyl)amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine. (III).

A mixture of 4-chloro-5-cyano-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (10) (II, 0.82 g., 2.64 mmoles), 3-methyl-2-butenylamine hydrochloride (11) (0.35 g., 2.87 mmoles), 1.5 ml. of triethylamine and 20 ml. of 1-butanol was heated at reflux temperature for 1.25 hours. The mixture was cooled to room temperature, 2.9 ml. of 1*N* sodium hydroxide diluted to 10 ml. with water was added and the mixture was evaporated to dryness. The residue was stirred with 20 ml. of ice-water to produce a white solid which after two recrystallizations from aqueous ethanol gave 500 mg. (52%, 1.39 mmoles) of III, dried *in vacuo* at 110°, m.p. 170-173°; ultraviolet λ max (*p*H 1) 278 nm (ε, 19,800) and 237.5 nm (ε, 18,500); λ max (ethanol) 296 nm (sh) (ε, 14,200), 285 nm (ε, 20,300), 277 nm (sh) (ε, 18,100) and 233 nm (ε, 10,000); λ max (*p*H 11) 284 nm (ε, 21,000), 277 nm (sh) (ε, 19,200), 234.5 nm (ε, 12,400); thin-layer chromatography solvents, A(R_f = 0.49), B(R_f = 0.91), C(R_f = 0.89), D(R_f = 0.94).

Anal. Calcd. for $C_{17}H_{21}N_5O_4$ (359.4): C, 56.86; H, 5.89; N, 19.49. Found: C, 56.79; H, 5.88; N, 19.43.

4-(3-Methyl-2-butenyl)amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-5-carboxamidoxime Hemihydrate (IV).

The nucleoside III (1 g., 2.79 mmoles) was dissolved in 100 ml. of absolute ethanol and hydroxylamine (14) (1.0 g.) was then added. The mixture was heated at reflux temperature for 2 hours, cooled to room temperature and evaporated to dryness. The residue was dissolved in 60 ml. of boiling water, treated with charcoal, filtered and the volume increased to 80 ml. with boiling water. The solution was allowed to cool slowly and when an oil separated, the warm aqueous supernatant was decanted from the oil and allowed to stand at 5° for 18 hours to produce a white solid. Two recrystallizations from water gave 440 mg. of pure IV, (39%, 1.09 mmoles), m.p. 120-124°, after drying *in vacuo* at 80°; ultraviolet λ max (*p*H 1) 278 nm (ε, 18,000); λ max (ethanol) 282 nm (ε, 18,300), λ max (*p*H 11) 283 nm (ε, 18,600); thin-layer chromatography solvents, A(R_f = 0.17, R_{III} = 0.35), B(R_f = 0.88), C(R_f = 0.94), D(R_f = 0.45).

Anal. Calcd. for $C_{17}H_{24}N_6O_5 \cdot 0.5H_2O$ (401.4): C, 50.87; H, 6.27; N, 20.94. Found: C, 50.80; H, 6.32; N, 20.87.

4-(3-Methyl-2-butenyl)amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide Hemihydrate (V).

The nucleoside III (350 mg., 0.973 mmoles) was suspended in 25 ml. of concentrated ammonium hydroxide and then 7 ml. of ethanol and 2.5 ml. of 30% hydrogen peroxide were added. The mixture was heated on a steam bath. Additional quantities of concentrated ammonium hydroxide (2.0 ml.) and 30% hydrogen peroxide (0.5 ml.) were added at two hour intervals until no unreacted III could be detected by thin-layer chromatography (Solvent B). The reaction mixture was evaporated to dryness under a stream of air, the residue dissolved in methanol (20 ml.) and again evaporated to dryness under a stream of air. This procedure was repeated twice and the solid residue was recrystallized from a methanol-toluene solution to give V (200 mg., 53%, 0.518 mmoles). The pure product was obtained by an additional recrystallization (78% recovery) from methanol-toluene, dried *in vacuo* at 80°, m.p. forms a glass at 110°; ultraviolet λ max (*p*H 1) 279 nm (ε, 19,500) and 253 nm (sh) (ε, 14,100); λ max (ethanol) 288.5 nm (ε, 19,300) and 255 nm (ε, 9,800); λ max (*p*H 11) 286 nm (ε, 19,700), 257 nm (sh) (ε, 11,800) and 237 nm (ε, 14,300); thin-layer chromatography solvents, A(R_f = 0.06 R_{III} = 0.12), E(R_f = 0.38), F(R_f = 0.52).

Anal. Calcd. for $C_{17}H_{23}N_5O_5 \cdot 0.5H_2O$ (386.4): C, 52.84; H, 6.26; N, 18.12. Found: C, 52.75; H, 6.51; N, 18.12.

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