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Synthesis of Orotidine from Uridine

Orotidine, uridine 6-carboxylic acid, is the riboside moiety of orotidylate which is the key intermediate of the biosynthetic pathways of pyrimidine nucleotides. The nucleoside has been isolated from mutants of *Neurospora crassa* in 1951[1] and the chemical synthesis has been achieved by Curran and Angier in 1966[2] from butyl orotate *via* mercuri-procedure.[3]

We wish to report a facile synthetic procedure of orotidine which involves the chemical transformation of uridine.[4]

Treatment of 2',3'-O-isopropylidene-5-bromouridine (Ia)[5] with 4 molar equivalents of potassium cyanide in dimethylformamide at 80° for one hour afforded three products. One of them was identified as 5-cyanouridine derivative (IIa), mp 221.5—222.5°, *Anal.* Calcd. for $C_{13}H_{15}O_6N_3$: C, 50.48; H, 4.89; N, 13.59. Found: C, 50.54; H, 4.90; N, 13.61. UV $\lambda_{max}^{H_2O}$ (mμ): 276, 216; $\lambda_{min}^{H_2O}$ (mμ): 239, which are similar to those of 5-cyanouridine reported.[6,7] IR: 2260 cm^{-1} (ν C≡N). NMR: 8.68 ppm in d_6-DMSO (H-6). The second product, 6-cyanouridine derivative (IIIa), was obtained as a crude form together with the known O^6,5'-cyclonucleoside (IV).[8] In order to avoid the formation of (IV), the 5'-acetyl derivative (Ib) was prepared (mp 166—167°, *Anal.* Calcd. for $C_{14}H_{17}O_7N_2Br$: C, 41.50; H, 4.23; N, 6.92; Br, 19.72. Found: C, 41.46; H, 4.24; N, 7.06; Br. 19.61). Compound Ib was likewise treated with sodium cyanide in dimethylformamide at 80° for 70 min and the resulting solution was concentrated and the residue was extracted with ethyl acetate (dilute hydrochloric acid was added to neutralize the medium). The ester soluble material was applied to preparative thin-layer chromatography (silica gel, developed with $CHCl_3$–EtOH, 7:1) from which (IIIb) was obtained as a solid mass. *Anal.* Calcd. for $C_{13}H_{17}O_7N_3$: C, 51.28; H, 4.88; N, 11.96. Found. C, 51.08; H, 4.95; N, 12.16. UV λ_{max}^{EtOH} (mμ, ε): 281, 8600; λ_{min}^{EtOH}: 229, 2000. IR: 2280 cm^{-1} (ν C≡N). NMR: 6.29 ppm in d_6-DMSO (H-5). Mass Spectrum *m/e*: 336 $(M-CH_3)^+$. Compound IIIb was treated with 0.5N NaOH at room temperature for 110 min and the solution was neutralized by Dowex 50 (H^+) resin. The filtrate was concentrated and the residue was applied to silica gel column chromatography. From the fraction eluted with AcOEt–EtOH the carboxamide (V) was obtained in 68% overall yield as a solid mass. *Anal.* Calcd. for $C_{13}H_{17}O_7N_3$: C, 47.71; H, 5.23; N, 12.84. Found: C, 47.72; H, 5.29; N, 12.71. UV $\lambda_{max}^{pH.6.4}$ (mμ, ε): 267, 8800; $\lambda_{min}^{pH.6.4}$: 235, 3200. NMR: 5.67 ppm (H-5), 8.06 and 8.40 ppm (6-$CONH_2$) in d_6-DMSO. Mass Spectrum *m/e*: 312 $(M-CH_3)^+$.

When the reaction of Ib with cyanide was carried out in pyridine IIIb was the sole product from which V was derived in 91% yield. Compound V (640 mg) was next treated with 0.5N NaOH at 90—100° for 3.5 hours and the mixture was acidified by the addition of Dowex 50 (H^+) resin to pH 2—3 and kept for 22 hours at 36°. The hydrolyzate was applied to DEAE-cellulose column chromatography with the linear concentration gradient elution of triethylammonium bicarbonate (0—0.1M, pH 7.8). The fraction containing orotidine (VI)

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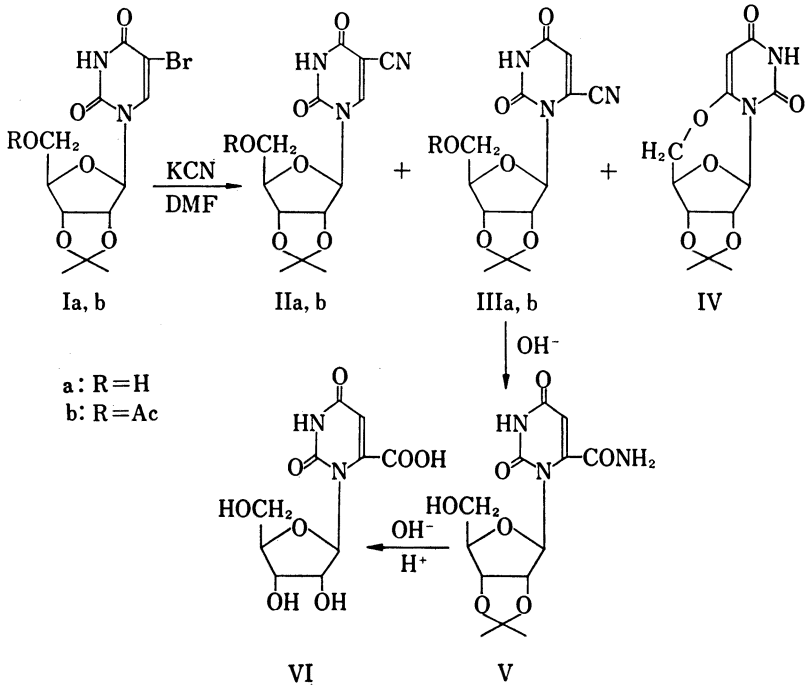
(11000 OD unit at 267 mμ) was pooled and concentrated to dryness, and the residue was taken up in water and acidified through Dowex 50 (H^+) resin column from which VI was obtained as an amorphous powder (300 mg, 50% yield). The spectroscopic and chromatographic nature of VI was identical with those of an authentic material.[9]

5-Bromouridine affords 6-cyanouridine with the concomitant formation of 5-cyanouridine by the treatment with cyanide. The mixture was treated with 1.0N NaOH and after separation by DEAE-cellulose column chromatography, VI was obtained in 20% yield. Some decomposition was observed during the alkaline hydrolysis of the amide group in all cases.

The formation of 6-cyanouridines (III) from 5-bromouridines (I) involves the initial nucleophilic addition of cyanide to 5,6-double bond of I and successive elimination of hydrogen bromide. Similar mechanism has been advanced in the formation of IV[8] from Ia by ethoxide treatment and of O^6,5′-cyclonucleosides by *tert*-butoxide treatment of 5-iodopyrimidine nucleosides.[10] The introduction of a carbon unit into C-6 position by nucleophilic addition-elimination mechanism here described is, to our knowledge, the first example.



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