Pyrimidine Derivatives and Related Compounds. Part 36.[1] Nucleophilic Addition Reaction of a Cyanide Ion to 6-Substituted 1,3-Dimethyl-5-nitrouracils. Synthesis of 5,6-Dihydrouracil and 5,6-Dihydrocyclothymine Derivatives[2]

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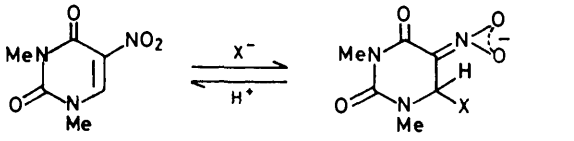
6-Substituted 1,3-dimethyl-5-nitrouracils (3a—c) react with potassium cyanide to give stereospecifically the 6-cyano-5-nitro-5,6-dihydrouracils (4a—c). Reaction of 6-bromomethyl-1,3-dimethyl-5-nitrouracil (7) with potassium cyanide gives 6-cyano-1,3-dimethyl-5-nitro-5,6-dihydrocyclothymine (8). The structures of the 5,6-dihydrouracils (4a—c) and the cyclothymine (8) were clarified using ^1H and ^{13}C n.m.r. spectroscopy.

NUCLEOPHILIC addition readily occurs across the 5,6-double bond of uracils and 5-halogenouracils. This fact has recently received much attention in connection with the biosynthesis[3] and chemical modification[4] of nucleic acids. For example, we reported[5] that 5-bromouracils react with sodium cyanide to afford 6-cyanouracils *via* the 5,6-dihydro-intermediates. These intermediates, formed by covalent addition of cyanide ion, were, however, not isolated and were only detected by deuterium-labelling experiments. 5-Nitrouracils are regarded as one of the most reactive uracils with respect to nucleophilic addition at the 6-position, because of the presence of the strongly electron-withdrawing nitro-groups. Pfleiderer *et al.* treated 1,3-dimethyl-5-nitro-uracil (1) with sodium ethoxide and obtained the sodium salt of the 5,6-dihydrouracil (2; X = OEt).[6] Fox[7] and Pitman[8] independently reported that the reaction of (1) with nucleophiles such as DO^-, EtO^-, and SO_3^{2-} gave the 5,6-dihydrouracil anions (2; X = OD, OEt, and SO_3^-) in solution, which have only been detected by ^1H n.m.r. and u.v. spectroscopy and have not hitherto been isolated. Compounds (2) reverted to the starting material (1) on neutralization.

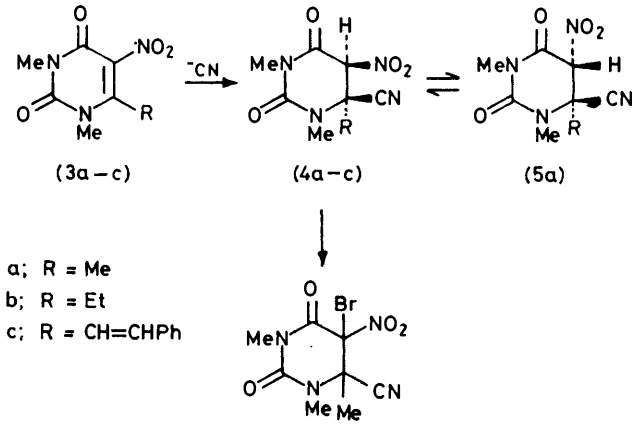


SCHEME 1

In the present study, 6-substituted 1,3-dimethyl-5-nitrouracils (3) † and potassium cyanide were used as a 5-nitrouracil compound and a nucleophile, respectively, and we have found that the reaction of (3) and (7) with cyanide ion gives the stable 6-cyano-5-nitro-5,6-dihydro-uracils (4) and (8), respectively.

† The reaction of the 6-unsubstituted 5-nitrouracil (1) with potassium cyanide did not cause the adduct formation described later.

A solution of 1,3,6-trimethyl-5-nitrouracil (3a) in dimethylformamide (DMF) was treated with potassium cyanide at room temperature for 1 h. The reaction mixture was diluted with water and acidified with hydrochloric acid to give stereospecifically a single adduct in 95% yield. The structural assignment of the adduct (4a) was made mainly on the basis of the elemental analysis and on spectral evidence. The u.v. spectrum



SCHEME 2

showed only end absorption, which was attributed to a 5,6-dihydrouracil ring system. The 6-cyano-6-methyl-5-nitro-5,6-dihydrouracil has two possible diastereoisomeric configurations, (4a) (*threo*) and (5a) (*erythro*), and our assumption that the adduct obtained was a *threo* isomer could be expected with the bulky methyl and nitro-groups preferring the *trans* equatorial position. The ^1H n.m.r. spectrum of the adduct (4a) in deuteriochloroform ($CDCl_3$) showed that it was a single compound. Addition of D_2O, however, caused not only hydrogen–deuterium exchange at the 5-position but also the appearance of signals for another diastereoisomer. The ^1H n.m.r. spectra of (4a) in protic solvents such as deuteriomethanol, trifluoroacetic acid, and $CDCl_3$ containing a catalytic amount of H_2O revealed a mixture of two compounds, presumed to be the *threo* (4a) and *erythro* (5a) diastereoisomers in the ratios 9 : 1, 8 : 2, and

7 : 3, respectively (Table 1). Thus, the adduct (4a) seems to come easily to equilibrium with (5a) in protic solvents, because of the lability of the 5-H. The major compound was identical with (4a). Attempts to isolate the minor diastereoisomer (5a) by chromatography on silica or alumina were unsuccessful and only the starting material (3a), formed by elimination of hydrogen cyanide, was obtained. Reaction of (4a) with bromine in chloroform containing a catalytic amount of H_2O gave the corresponding 5-bromo-5,6-dihydrouracil (6), whose configuration could not be determined.

Similar treatment of the uracils (3b) and (3c) with potassium cyanide furnished the 5,6-dihydrouracils (4b) and (4c), respectively, in good yields. These products, substituted with much more bulky groups at the 6-position, did not epimerize to the other diastereoisomers in protic solvents. This fact supports the conclusion that the adducts (4a—c) have the *threo* configuration.

Compounds (4a—c) were relatively stable under neutral and acidic conditions, but were less stable in alkaline media or in refluxing alcohol when they reverted to the starting materials. The i.r. spectra of (4a—c) did not show any absorption due to a cyano-group. However, in the ^{13}C n.m.r. spectra the characteristic band of a cyano-group appeared at δ 114.1. Peaks for C-5 and C-6 appeared at δ 87.2 and 54.6 respectively. Triplett *et al.* recently reported the ^{13}C n.m.r. spectra of hydrogensulphite–uracil adducts.[9] The ^{13}C n.m.r. spectra for the 5,6-dihydro-series (Table 2) are in close agreement with Triplett's data if the 5,6-substituent effect is considered. The ^1H n.m.r. data also support the structure as being the 5,6-dihydro-adduct.

It is noteworthy that the nitrouracil (1) forms no stable adduct with HCN. The stability of the adducts (4a—c), in which C-5 and C-6 are tetrahedral, probably is due to a partial relief of a strong *ortho*-interaction between the R group and the nitro-group of (3a—c). The corresponding interaction in (1) is between a hydrogen atom and a nitro-group.

When 6-bromomethyl-1,3-dimethyl-5-nitrouracil (7) was treated with potassium cyanide in DMF at room temperature, the corresponding adduct was not obtained, 6-cyano-1,3-dimethyl-5-nitro-5,6-dihydrocyclothymine (8) * being formed instead in 40% yield. The structure of (8) was established by the spectral data as follows.

TABLE 1

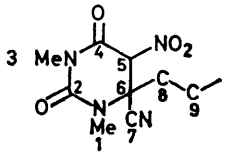
^1H N.m.r. data of 6-cyano-1,3,6-trimethyl-5-nitro-5,6-dihydrouracil and the ratio of its diastereoisomers (4a) and (5a) in various solvents [a]

Solvent	Chemical shifts (δ) 5-H	6-CH_3	NCH_3	Ratio (4a) (5a)
$CDCl_3$	5.38	1.88	3.22, 3.32	10 : 0
$CDCl_3$ (+catalytic H_2O)	5.42 (5.53)	1.88 (1.93)	3.22, 3.32 (3.16, 3.33)	7 : 3
$CDCl_3$ (+catalytic CF_3CO_2H)	5.48 (5.57)	1.90 (1.94)	3.25, 3.35 (3.19, 3.38)	7 : 3
CF_3CO_2H	5.78 (5.90)	2.02 (2.07)	3.37, 3.42 (3.32, 3.44)	8 : 2
CD_3OD	*b*	1.89 (1.93)	3.17, 3.25 (3.28, 3.31)	9 : 1

[a] δ Values for the minor isomer (5a) are given in parentheses. [b] Peaks not observed, removed by H–D exchange.

TABLE 2

^{13}C N.m.r. chemical shift assignments for the 5-nitrouracil (3a) and the 5,6-dihydrouracil derivatives



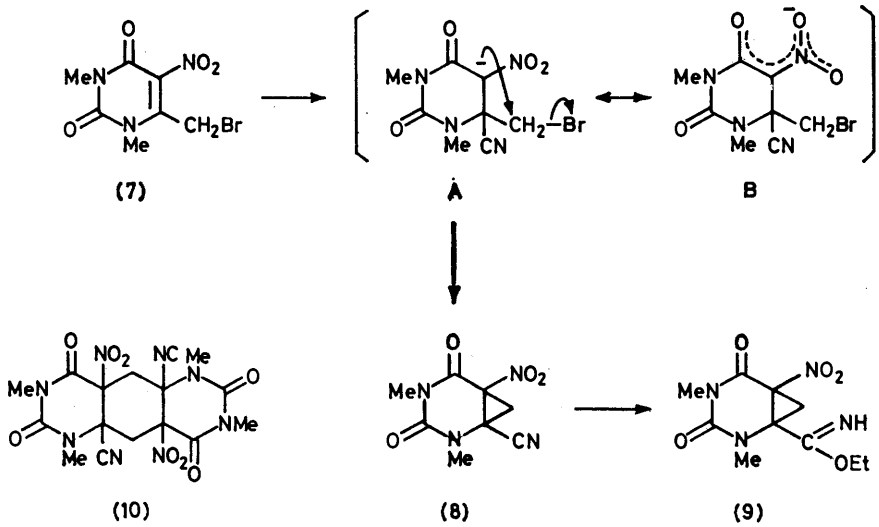
Compound	Solvent	Chemical shifts (δ) C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
(3a)	$[^2H_6]DMSO$	28.4	151.2	32.4	154.7	128.9	149.9		16.1	
(4a)	$CDCl_3$	31.1	150.9	29.2	157.3	87.2	54.6	114.1	22.7	
(4b) [a]	$CDCl_3$	32.6 (q)	150.5 (s)	28.9 (q)	157.1 (s)	84.3 (d)	59.5 (s)	113.3 (s)	29.3 (t)	7.8 (q)
(4c) [b]	$CDCl_3$	32.0	151.4	29.2	157.6	86.9	61.2	112.6	116.8	140.2
(5a)	$CDCl_3$	30.7	*c*	29.2	*c*	86.2	53.8	114.1	21.2	
(8)	$[^2H_6]DMSO$	34.1	148.4	28.5	158.2	68.2	37.4	112.5	24.7	

[a] Letters in parentheses denote multiplicity observed in off-resonance decoupled spectra; s = singlet; d = doublet; t = triplet; q = quartet. [b] Aromatic peaks were observed at δ 132.7, 127.4, 128.9, 130.2. [c] Signal was too weak for its chemical shift to be determined.

When 6-bromomethyl-1,3-dimethyl-5-nitrouracil (7) was treated with potassium cyanide in DMF at room temperature, the corresponding adduct was not obtained, 6-cyano-1,3-dimethyl-5-nitro-5,6-dihydrocyclothymine (8) * being formed instead in 40% yield. The structure of (8) was established by the spectral data as follows.

* The name of ' cyclothymine ' was introduced by Witkop.[10] The systematic name for compound (8) is 1-cyano-2,4-dimethyl-6-nitro-2,4-diazabicyclo[4.1.0]heptane-3,5-dione.

The u.v. spectrum of compound (8) showed only end absorption with no band at 291 nm corresponding to that of the nitrouracil (7). The ^{1}H n.m.r. spectrum in $CDCl_3$ showed the characteristic signals of *endo* and *exo* protons of a cyclopropane ring at respectively δ 1.98 and 3.11 with a coupling constant of 8.2 Hz. The i.r. and ^{13}C n.m.r. spectra showed bands due to a cyano-group at 2 150 cm^{-1} and δ 112.5, respectively. The signals of the three-membered-ring carbons displayed peaks at δ 68.2 (C-5), 37.4 (C-6), and 24.7 (C-8). A possible dimer



SCHEME 3

structure (10) for the product was eliminated since the molecular-ion peak ($M^+ + 1$, *m/e* 225) of (8) was observed in the mass spectrum. Furthermore, when compound (8) was allowed to react with triethylamine in absolute ethanol, the imidate (9) was obtained.

A reasonable mechanism for the formation of (8) would involve initial attack of cyanide ion at the 6-position to form an addition intermediate (B) which then would undergo an intramolecular cyclisation of the carbanion (A) with elimination of a bromo-anion to yield (8). General methods for the cyclothymine synthesis are primarily based on the intermolecular cyclopropane cyclization of uracils with dimethyloxosulphonium methylide [10] or metal carbenes.[11] To the best of our knowledge, the present finding is the first example affording a cyclothymine by an intramolecular cyclization.

EXPERIMENTAL

M.p.s were determined on a Yanagimoto hot-stage apparatus. ^{1}H N.m.r. spectra were recorded on a Hitachi Perkin-Elmer R-20B 60 MHz spectrometer with tetramethylsilane as an internal standard. I.r. spectra were obtained for KBr pellets with a Hitachi 215 instrument. U.v. spectra were measured on a Hitachi 323 spectrophotometer. ^{13}C N.m.r. spectra were recorded on a Varian XL-100A spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured on a JEOL JMS-D300 spectrometer.

6-Substituted 6-Cyano-1,3-dimethyl-5-nitro-5,6-dihydrouracils (4a—c).—*General procedure.* To a solution of the 5-nitrouracils (3a—c) (10 mmol) in dimethylformamide (DMF) (10 ml) was added a solution of KCN (0.65 g, 10 mmol) in H_2O (1.3 ml) with stirring. The mixture was stirred at room temperature for 1 h, diluted with ice–water (100 ml), and acidified with HCl. The resulting precipitate was collected by filtration to give the 5,6-dihydro-adducts (4a—c). Recrystallization from ether afforded prisms. *6-Cyano-1,3,6-trimethyl-5-nitro-5,6-dihydrouracil* (4a) (95%) had m.p. 106—110 °C (Found: C, 42.7; H, 4.35; N, 24.65. $C_8H_{10}N_4O_4$ requires C, 42.48; H, 4.46; N, 24.77%); $\nu_{max.}$ 1 730 and 1 690 cm^{-1} (C=O); *6-cyano-6-ethyl-1,3-dimethyl-5-nitro-5,6-dihydrouracil* (4b) (63%) had m.p. 93—95 °C (Found: C, 44.75; H, 4.85; N, 23.05. $C_9H_{12}N_4O_4$ requires C, 45.00; H, 5.04; N, 23.33%); $\nu_{max.}$ 1 740 and 1 710 cm^{-1} (C=O); δ($CDCl_3$) 1.20 (3 H, t, *J* 7.5 Hz, CMe), 2.07 (2 H, q, *J* 7.5 Hz, CH_2C), 3.26 (3 H, s, NMe), 3.31 (3 H, s, NMe), and 5.43 (1 H, s, 5-H); *6-cyano-1,3-dimethyl-5-nitro-6-styryl-5,6-dihydrouracil* (4c) (92%) had m.p. 118—123 °C (Found: C, 57.5; H, 4.4; N, 17.85. $C_{15}H_{14}N_4O_5$ requires C, 57.32; H, 4.49; N, 17.83%); $\nu_{max.}$ 1 745 and 1 680 cm^{-1} (C=O); δ($CDCl_3$) 3.12 (3 H, s, NMe), 3.33 (3 H, s, NMe), 5.56 (1 H, s, 5-H), 6.00 (1 H, d, *J* 16 Hz, C*H*=CHPh), 7.20 (1 H, d, *J* 16 Hz, CH=C*H*Ph), and 7.40 (5 H, s, aromatic).

5-Bromo-6-cyano-1,3,6-trimethyl-5-nitro-5,6-dihydrouracil (6).—A mixture of the adduct (4a) (0.45 g, 2 mmol) and bromine (0.32 g, 2 mmol) in $CHCl_3$ (15 ml) containing a catalytic amount of H_2O was stirred at room temperature for 24 h. The solvent was evaporated *in vacuo*. Water was added to the residue and the precipitate was collected and dried to give the *5-bromo-5,6-dihydrouracil* (6) (0.46 g, 75%). Recrystallization from ether afforded needles, m.p. 114—115 °C (Found: C, 31.7; H, 2.7; H, 18.3. $C_8H_9BrN_4O_4$ requires C, 31.49; H, 2.97; N, 18.37%); $\nu_{max.}$ 1 730 and 1 690 cm^{-1} (C=O); δ($CDCl_3$) 1.92 (3 H, s, NMe), 3.25 (3 H, s, NMe), and 3.34 (3 H, s, NMe).

6-Bromomethyl-1,3-dimethyl-5-nitrouracil (7).—A mixture

of 1,3,6-trimethyl-5-nitrouracil (3a) (10 g, 50 mmol) and bromine (12.1 g, 75 mmol) in acetic acid (80 ml) was refluxed for 0.5 h. The acetic acid was evaporated *in vacuo*. To the residue were added ethyl acetate (34 ml) and ether (134 ml) and the mixture was allowed to stand overnight at room temperature, after which time yellow prisms had separated. Filtration and washing of the crystals with ether gave the pure *bromouracil* (7) (12.1 g, 87%), m.p. 143—144 °C (Found: C, 30.3; H, 2,9; N, 15.35. $C_7H_8BrN_3O_4$ requires C, 30.23; H, 2.90; N, 15.12%); $\nu_{max.}$ 1 710 and 1 660 cm^{-1} (C=O); $\lambda_{max.}$ 291 nm (ε 7 413); $\delta(CDCl_3)$ 3.32 (3 H, s, NMe), 3.64 (3 H, s, NMe), and 4.32 (2 H, s, CH_2Br).

6-*Cyano*-1,3-*dimethyl*-5-*nitro*-5,6-*dihydrocyclothymine* (8).*—To a solution of the bromouracil (7) (2.8 g, 10 mmol) in DMF (10 ml) was added dropwise a solution of KCN (0.65 g, 10 mmol) in H_2O (1.3 ml) with stirring. The reaction mixture was maintained at room temperature for 1 h and poured into ice–water (100 ml). The precipitate was filtered, washed with cold water, and dried to give the product (0.88 g, 40%), m.p. 145—148 °C. Recrystallization of a sample from ligroin afforded pale yellow *needles*, m.p. 149—150 °C (Found: C, 42.95; H, 3.65; N, 24.75. $C_8H_8N_4O_4$ requires C, 42.86; H, 3.60; N, 24.99%); $\nu_{max.}$ 2 150 (CN), and 1 720 and 1 680 cm^{-1} (C=O); $\delta(CDCl_3)$ 1.98 (1 H, d, *J* 8.2 Hz, *endo*-7-H), 3.11 (1 H, d, *J* 8.2 Hz, *exo*-7-H), 3.32 (3 H, s, NMe), and 3.36 (3 H, s, NMe); *m/e* 225 ($M^+ + 1$) and 178 ($M^+ - NO_2$).

Ethyl 1,3-*Dimethyl*-5-*nitro*-5,6-*dihydrocyclothymine*-6-*carboximidate* (9).—A mixture of the cyanodihydrocyclothymine (8) (0.45 g, 2 mmol) and triethylamine (0.5 ml) suspended in absolute EtOH (10 ml) was stirred at room temperature for 36 h. The resulting precipitate was collected by filtration to give the *imidate* (9) (0.345 g). Concentration of the mother-liquor gave more product (0.042 g) (total yield 72%). Recrystallization from EtOH afforded *prisms*, m.p. 166 °C (Found: C, 44.6; H, 5.2; N, 20.95. $C_{10}H_{14}N_4O_5$ requires C, 44.44; H, 5.22; N, 20.73%); $\nu_{max.}$ 3 300 (NH), and 1 760 and 1 735 cm^{-1} (C=O); $\delta(CDCl_3)$ 1.34 (3 H, t, *J* 7 Hz, CMe), 3.00 (3 H, s, NMe), 3.08 (3 H, s, NMe), 3.48 (1 H, d, *J* 19 Hz, *endo*-7-H), 3.95 (1 H, d, *J* 19 Hz, *exo*-7-H), 4.35 (2 H, q, *J* 7 Hz, CH_2C), and 7.53br (1 H, s, NH); *m/e* 271 ($M^+ + 1$) and 224 ($M^+ - NO_2$).

* See footnote on p. 1897 regarding the nomenclature of this compound.

We thank Dr. M. Nishikawa, Central Research Division, Takeda Chemical Industries, Ltd., for the ^{13}C n.m.r. spectra measurements and analyses.

[0/1826 *Received*, 26*th November*, 1980]

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