The Conversion of the 2',3'-*O*-Isopropylidene Derivative of 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA Riboside) into 2',3'-*O*-Isopropylidene-isoguanosine

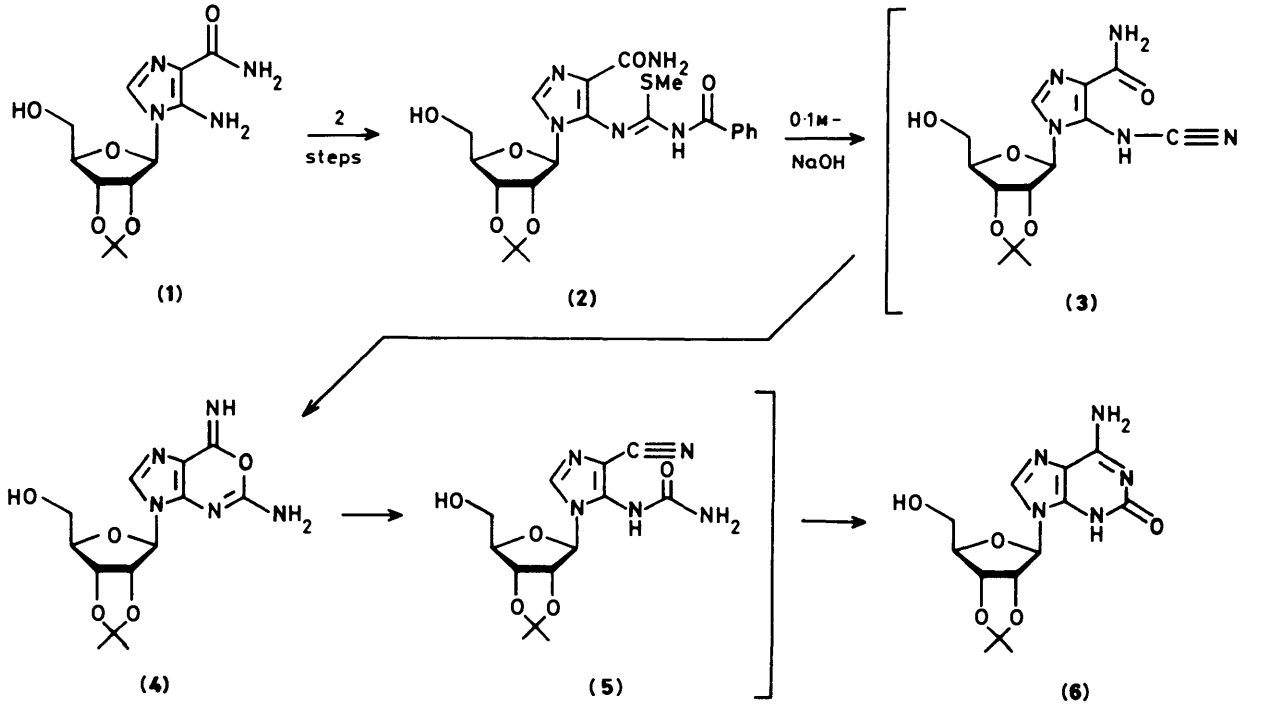
Colin B. Reese * and Yogesh S. Sanghvi
Department of Chemistry, King's College London, Strand, London WC2R 2LS
Reiko Kuroda
Department of Biophysics, King's College London, 26—29 Drury Lane, London WC2B 5RL

Treatment of the putative methoxyacetyl thioureido derivative (**14**), which was prepared in two steps from 5-amino-1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**1**), with mercury(II) perchlorate in the presence of pyridine in tetrahydrofuran solution at room temperature and then with methanolic ammonia gives the ureido nitrile (**5**); treatment of (**15**) with mercury(II) ions under the same conditions gives (**17**). When (**5**) is allowed to react with N^1,N^1,N^3,N^3-tetramethylguanidine and water in tetrahydrofuran at room temperature, 2',3'-*O*-isopropylideneisoguanosine (**6**) is obtained in high yield; however, when (**5**) is heated, under reflux, with triethylamine in dioxane–water (9:1, v/v), the amino nitrile (**23**) is obtained in good yield.

A number of years ago, Yamazaki and his co-workers showed [1] that when compound (**2**), which they had prepared in two steps (*i.e.* reaction with benzoyl isothiocyanate, followed by treatment with methyl iodide and alkali) from 5-amino-1-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**1**), was heated in 0.1M-aqueous sodium hydroxide, under reflux, 2',3'-*O*-isopropylideneisoguanosine [2] (**6**) was obtained in rather modest yield. The mechanism proposed [1] by these workers (Scheme) which, in essence, involves three non-isolated intermediates [(**3**), (**4**), and (**5**)] would appear to be plausible. The alkali-promoted conversion of (**2**) into (**6**) is of particular interest in that it was found that when (**2**) was heated, under reflux, in 6M-aqueous sodium hydroxide, 2',3'-*O*-isopropylideneguanosine (**7**) was obtained,[1] in relatively good yield, instead of the corresponding isoguanosine derivative (**6**).

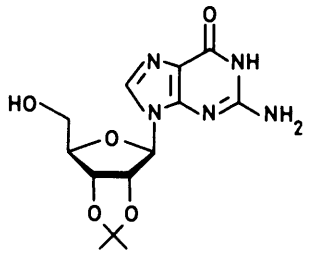
Somewhat in contrast to these results, Yamazaki *et al.* reported [1] that when the *S*-methylisothiourea derivative (**8**) was heated, under reflux, in 0.1M-aqueous sodium hydroxide for 2—3 min, compound (**9**) could be isolated from the products. However, the latter compound (**9**) was found to be unstable, and readily to undergo conversion into isoguanine (**10**) under the reaction conditions. In an accompanying paper,[3] Okutsu and Yamazaki reported that when (**11**) was treated with 0.5M-aqueous sodium hydroxide at room temperature, a crystalline product, to which they assigned the anhydronucleoside structure (**12**), was obtained rather than isoguanosine.

Our interest in the above studies originated in an attempt to prepare oxanosine,[4] *via* its 2',3'-*O*-isopropylidene derivative [5] (**13**), from the 2',3'-*O*-isopropylidene derivative (**1**) of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICA riboside).

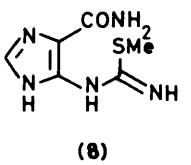


(1) (2) (3) (4) (5) (6)

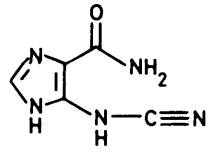
Scheme.



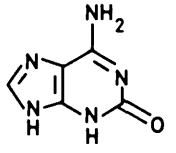
(7)



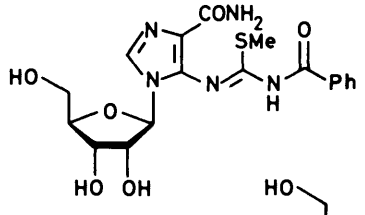
(8)



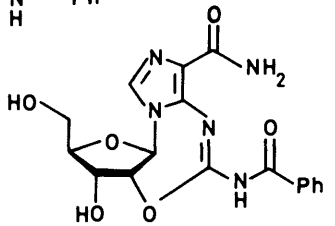
(9)



(10)



(11)



(12)

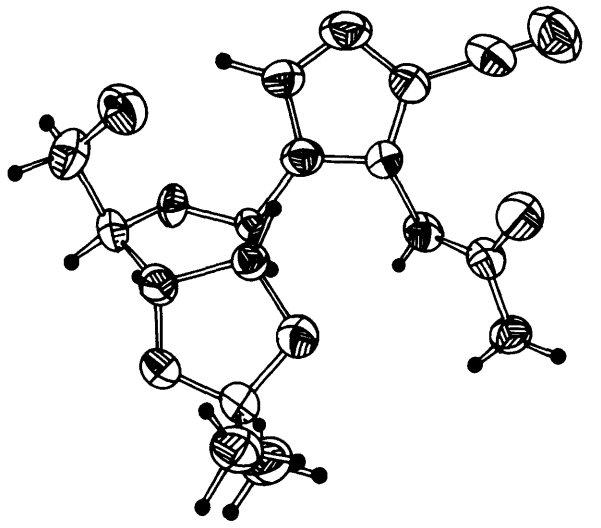
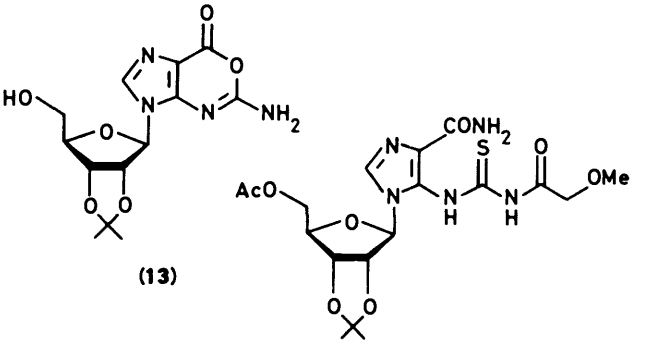
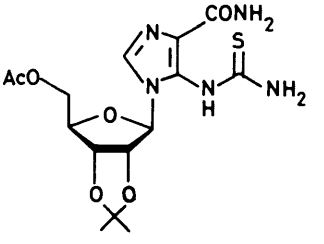


Figure. Computer-drawn plot of molecular structure of 1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-5-ureidoimidazole-4-carbonitrile (**5**)

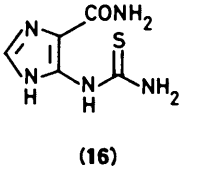


(13)

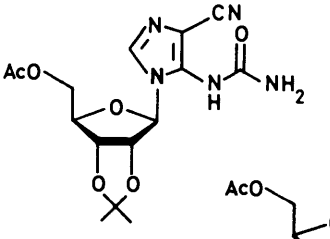
(14)



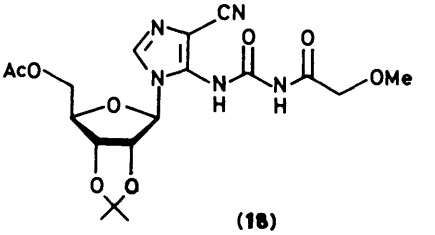
(15)



(16)



(17)



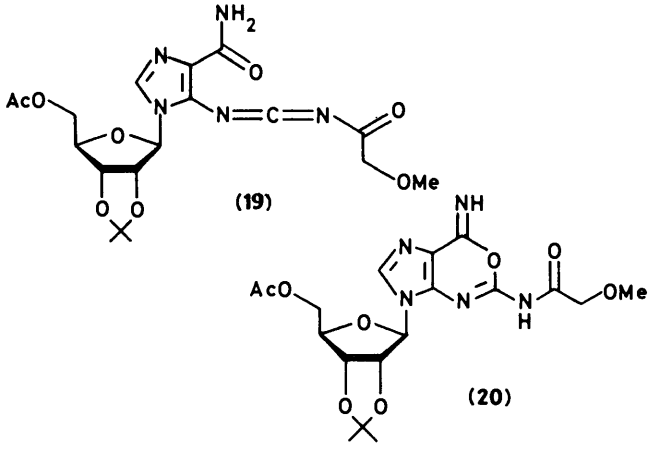
(18)

The latter compound (**1**) was treated with acetic anhydride in pyridine solution, and the resulting 5′-*O*-acetyl derivative was allowed to react with a very slight excess of methoxyacetyl isothiocyanate [prepared from methoxyacetyl chloride and lead(II) thiocyanate] in acetonitrile solution at room temperature. Treatment of the resulting putative methoxyacetyl thioureido derivative (**14**) with *ca.* 1M-methanolic ammonia led to the selective removal of the methoxyacetyl group, and gave (**15**) as a crystalline solid in 49% overall yield for the three steps. Although 5-thioureidoimidazole-4-carboxamide (**16**), the precursor of (**8**), was reported [1] not to react with mercury(II) oxide, when the thioureido derivative (**15**) was treated with a slight excess of mercury(II) perchlorate in the presence of pyridine in tetrahydrofuran solution at room temperature, it was rapidly converted into the ureido nitrile (**17**). The latter compound (**17**) was isolated as a crystalline solid in satisfactory yield, and was characterized on the basis of microanalytical and spectroscopic data and an *X*-ray crystal structure determination.[6] When the putative methoxyacetyl thioureido derivative (**14**) was treated in the same way with mercury(II) perchlorate in the presence of pyridine in tetrahydrofuran at room temperature, it was rapidly converted into what was assumed to be the methoxyacetylureido nitrile (**18**). Treatment of the latter compound with *ca.* 3M-methanolic ammonia at room temperature for 2 h led to the removal both of the methoxyacetyl and acetyl groups, and gave (**5**). This compound, which had previously been proposed as an intermediate in the conversion of (**2**) into (**6**) (Scheme), was isolated as a crystalline solid and was again characterized on the basis of microanalytical and spectroscopic data, and an *X*-ray crystal structure determination [6] (Figure).

It is reasonable to assume that the conversion of (**15**) into (**17**) follows the pathway suggested in the Scheme. Mercury(II) ion-promoted removal of the elements of hydrogen sulphide from (**15**) would lead to the corresponding 5-cyanamido derivative

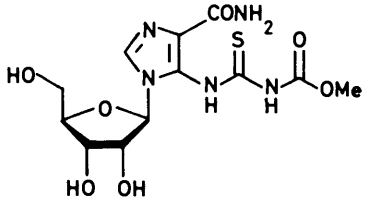
[the 5′-acetate of (3)] which presumably spontaneously cyclizes [to give the 5′-acetate of (4)] and then ring opens to give (17) [the 5′-acetate of (5)]. Similarly, the mercury(II) ion-promoted removal of the elements of hydrogen sulphide from (14) would lead to the corresponding carbodi-imide derivative (19) which again would presumably spontaneously cyclize to give (20), and then ring open to give (18). Very recently, Townsend and his co-workers have reported[7] that when (21) is treated with *N*,*N*′-dicyclohexylcarbodi-imide in dimethylformamide solution at room temperature, it undergoes conversion into (22). These workers[7] have suggested that this transformation involves intermediates corresponding to (19) and (20), and have carried out an ^{18}O-labelling study that supports such a pathway.

In further confirmation of the original proposal[1] of Yamazaki and his co-workers (Scheme), when (5) was treated with an excess of N^1,N^1,N^3,N^3-tetramethylguanidine in slightly wet tetrahydrofuran at room temperature, it was smoothly converted into 2′,3′-*O*-isopropylideneisoguanosine[2] (6) in high yield. However, when (5) was heated, under reflux, with a relatively large excess of triethylamine in dioxane–water (9:1, v/v), the 5-*N*-carbamoyl group was removed and 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (23) was obtained in good yield. The latter compound (23) was also readily obtained[8] as a colourless crystalline solid in satisfactory yield by allowing (1) to react with a slight excess of toluene-*p*-sulphonyl chloride in pyridine solution at room temperature. In contrast to a recent observation in the literature,[9] the dehydration of this 4-carboxamide function is facile and proceeds cleanly.

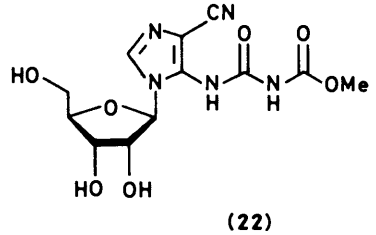


(19)

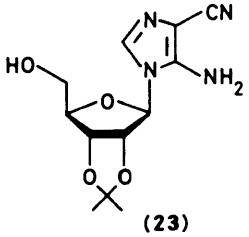
(20)



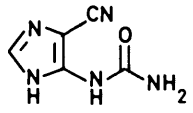
(21)



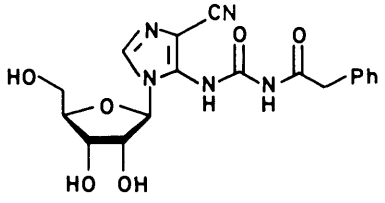
(22)



(23)



(24)



(25)

It may be concluded from the present studies that, although treatment of (14) and (15) with mercury(II) ions very probably leads to the formation of intermediate 7-iminoimidazo[4,5-*d*]-[1,3]oxazine nucleosides [(20), and the 5′-acetate of (4), respectively], spontaneous ring-opening occurs. It would therefore seem that, if the synthesis of oxanosine [*via* its 2′,3′-*O*-isopropylidene derivative (13)] from (1) is to be accomplished successfully, the 4-carboxamide function of, say, (14) would need to be deaminated before the step involving treatment with mercury(II) ions is carried out. Finally, the conversions of (8) into (9) and (11) into (12), reported[1,3] by Yamazaki and his co-workers, call for some comment. First, while the base-catalyzed elimination of methanethiol from (8) would be expected to lead to (9), it seems very likely that (9) would spontaneously cyclize and then ring-open to give (24). This is, of course, analogous to the above mercury(II) ion-promoted conversion of (15) into (17). The putative product (9) has a band at 2 190 cm^{-1} in its i.r. spectrum[1] that can be assigned to the stretching frequency of a cyano group. The corresponding band in the i.r. spectrum of (5) is at somewhat higher frequency (2 234 cm^{-1}). Secondly, treatment of (11) with 0.5M-sodium hydroxide at room temperature would be expected to lead to the 4-carbonitrile (25) rather than to the anhydronucleoside[3] (12). However, the reported chemical reactions[3] of the actual product obtained cannot all be accounted for in terms of structure (25).

Experimental

^1H and ^{13}C N.m.r. spectra were measured with a Bruker WM 250 spectrometer; tetramethylsilane was used as an internal standard. U.v. absorption spectra were measured with a Kontron Uvikon 820 recording spectrophotometer. Merck silica gel H was used for short column chromatography; Merck silica gel 60 F_{254} plates were used for t.l.c. Pyridine and tetrahydrofuran (THF) were dried by heating, under reflux, with calcium hydride, and were then distilled.

Methoxyacetyl Isothiocyanate.—Freshly distilled methoxyacetyl chloride (43.4 g, 0.40 mol) was added in one portion to a stirred suspension of lead(II) thiocyanate (167 g, 0.52 mol) in dry benzene (500 ml), and the reactants were heated, under gentle reflux, for 12 h. The cooled products were filtered through Celite, and the residue was washed with benzene (2 × 100 ml). The combined filtrate and washings were concentrated under reduced pressure, and the dark-red coloured residue was distilled (N_2 bleed) to give *methoxyacetyl isothiocyanate* as a colourless liquid (35.3 g, 67%), b.p. 78 °C/39 mmHg; $\nu_{max.}$(film) 1 718 and 1 966 cm^{-1}.

1-(5′-O-*Acetyl*-2′,3′-O-*isopropylidene*-β-D-*ribofuranosyl*)-5-*thioureidoimidazole*-4-*carboxamide* (**15**).—5-Amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide (**1**; 5.0 g, 16.8 mmol) and acetic anhydride (1.83 ml, 19.4 mmol) were stirred together in anhydrous pyridine (25 ml) solution at room temperature. After 6 h, methanol (5 ml) was added and, after a further period of 30 min, the products were concentrated under reduced pressure and chromatographed on silica gel. Fractions [eluted with $CHCl_3$–EtOH (95:5, v/v)] that contained the main product were combined and evaporated under reduced pressure to give a glass (5.0 g). A solution of the latter material (3.4 g) and methoxyacetyl isothiocyanate (1.36 g, 10.4 mmol) in dry acetonitrile (35 ml) was stirred at room temperature. After 3 h, the products were concentrated under reduced pressure and chromatographed on silica gel. The fractions [eluted with $CHCl_3$–EtOH (96:4, v/v)] were combined and evaporated under reduced pressure to give a glass (3.67 g). 8M-Methanolic ammonia (0.3 ml) was added to a stirred solution of this material (**14**; 1.0 g) in methanol (2 ml) at room temperature. After 5 min, the products were concentrated under reduced pressure and then fractionated by chromatography on silica gel to give recovered starting material (**14**; 0.25 g) and a more polar product. Crystallization of the latter from ethanol gave the *title compound* (**15**) (0.45 g, 49% overall yield for the three steps) (Found: C, 45.3; H, 5.3; N, 17.55. $C_{15}H_{21}N_5O_6S$ requires C, 45.1; H, 5.3; N, 17.5%), m.p. 188 °C; $\lambda_{max.}$ (95% EtOH) 245 (ε 11 600), $\lambda_{min.}$ 227 (ε 9 500) nm; δ_H[$(CD_3)_2SO$] 1.32 (3 H, s), 1.50 (3 H, s), 2.00 (3 H, s), 4.10 (2 H, m), 4.26 (1 H, m), 4.91 (1 H, m), 5.20 (1 H, m), 5.91 (1 H, br s), 7.13 (1 H, br s), 7.26 (1 H, br s), 7.84 (1 H, s), and 9.24 (1 H, br s), R_F 0.13 [$CHCl_3$–MeOH (9:1, v/v)].

1-(5′-O-*Acetyl*-2′,3′-O-*isopropylidene*-β-D-*ribofuranosyl*)-5-*ureidoimidazole*-4-*carbonitrile* (**17**).—A solution of mercury(II) perchlorate trihydrate (0.90 g, 2.0 mmol) in THF (10 ml) was added to a stirred solution of 1-(5′-*O*-acetyl-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-5-thioureidoimidazole-4-carboxamide (0.66 g, 1.65 mmol) and pyridine (0.4 ml, 4.9 mmol) in THF (20 ml) at room temperature. After 15 min, hydrogen sulphide was gently bubbled through the suspension of products for a period of *ca.* 5 min after which no more black precipitate was obtained. The products were then flushed with nitrogen, filtered through Celite, and the residue was washed several times with small quantities of chloroform. The combined filtrate and washings were concentrated under reduced pressure and the residue was chromatographed on silica gel. Evaporation of the appropriate fractions [eluted with $CHCl_3$–EtOH (97:3, v/v)] and crystallization of the residue from ethanol gave the *title compound* (**17**) (0.37 g, 61%) (Found: C, 49.5; H, 5.3; N, 19.6. $C_{15}H_{19}N_5O_6$ requires C, 49.3; H, 5.2; N, 19.2%), m.p. 173 °C; δ_H[$(CD_3)_2SO$] 1.32 (3 H, s), 1.50 (3 H, s), 1.97 (3 H, s), 4.09 (1 H, m), 4.35 (1 H, m), 4.91 (1 H, dd, *J* 3.3, 6.0 Hz), 5.19 (1 H, dd, *J* 2.1, 6.0 Hz), 5.78 (1 H, d, *J* 2.1 Hz), 6.36 (2 H, br s), 7.96 (1 H, s), and 8.60 (1 H, br s); R_F 0.30 [$CHCl_3$–MeOH (9:1, v/v)].

1-(2′,3′-O-*Isopropylidene*-β-D-*ribofuranosyl*)-5-*ureidoimidazole*-4-*carbonitrile* (**5**).—A solution of mercury(II) perchlorate, trihydrate (4.08 g, 9.0 mmol) in THF (30 ml) was added to a stirred solution of putative 1-(5′-*O*-acetyl-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-5-[3-(methoxyacetyl)thioureido]-imidazole-4-carboxamide (**14**; 2.82 g, 5.98 mmol) obtained as above by the action of acetic anhydride, followed by methoxyacetyl isothiocyanate on 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide and pyridine (2.4 ml, 29.7 mmol) in THF (50 ml) at room temperature. After 15 min, the products were treated with hydrogen sulphide, worked-up as in the experiment above, and then purified by chromatography on silica gel. The appropriate fractions [eluted with $CHCl_3$–EtOH (97:3, v/v)] were combined and evaporated to give a colourless glass (2.25 g). Two-thirds of this material (1.5 g) was dissolved in methanol (15 ml) and the solution was treated with 8M-methanolic ammonia (10 ml) at room temperature. After 2 h, the products were concentrated under reduced pressure and the residue was crystallized from ethanol to give the *title compound* (**5**) [0.66 g, 51% overall yield for the two steps; 35% overall yield for the four steps starting from (**1**)] (Found: C, 48.5; H, 5.4; N, 21.9. $C_{13}H_{17}N_5O_5$ requires C, 48.3; H, 5.3; N, 21.7%), m.p. 181 °C; $\nu_{max.}$(KBr) 1 703 and 2 234 cm^{-1}; $\lambda_{max.}$ (95% EtOH) 230 (ε 8 600), $\lambda_{min.}$ 211 (ε 6 200) nm; δ_H[$(CD_3)_2SO$] 1.31 (3 H, s), 1.50 (3 H, s), 3.48 (2 H, m), 4.17 (1 H, m), 4.87 (1 H, dd, *J* 2.7, 6.0 Hz), 5.07 (1 H, dd, *J* 2.4, 6.0 Hz), 5.17 (1 H, br s), 5.75 (1 H, d, *J* 2.4 Hz), 6.33 (2 H, br s), 8.00 (1 H, s), 8.58 (1 H, br s); R_F 0.16 [$CHCl_3$–MeOH (9:1, v/v)].

5-*Amino*-1-(2′,3′-O-*isopropylidene*-β-D-*ribofuranosyl*)*imidazole*-4-*carbonitrile* (**23**).—(a) A solution of 1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-5-thioureidoimidazole-4-carbonitrile (0.20 g, 0.62 mmol) and triethylamine (1.0 ml, 7.17 mmol) in dioxane (9 ml) and water (1 ml) was heated, under reflux, for 1 h. The cooled products were then evaporated under reduced pressure, and the residue was fractionated by chromatography on silica gel. Fractions eluted with $CHCl_3$–EtOH (95:5, v/v) were combined and evaporated under reduced pressure. Crystallization of the residue from ethyl acetate gave the *title compound* (**23**) as colourless needles (0.12 g, 70%) (Found: C, 51.4; H, 5.8; N, 20.3. $C_{12}H_{16}N_4O_4$ requires C, 51.4; H, 5.75; N, 20.0%), m.p. 182 °C; $\nu_{max.}$(KBr) 2 216 cm^{-1}; $\lambda_{max.}$ (95% EtOH) 246 (ε 13 500), $\lambda_{min.}$ 207 (ε 1 800) nm; δ_H[$(CD_3)_2SO$] 1.31 (3 H, s), 1.53 (3 H, s), 3.52 (2 H, m), 4.13 (1 H, m), 4.86 (1 H, dd, *J* 2.7, 6.2 Hz), 5.04 (1 H, dd, *J* 3.7, 6.3 Hz), 5.32 (1 H, m), 5.77 (1 H, d, *J* 3.7 Hz), 6.38 (2 H, br s), 7.45 (1 H, s); R_F 0.35 [$CHCl_3$–MeOH (9:1, v/v)].

(b) 5-Amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxamide (0.50 g, 1.68 mmol) and toluene-*p*-sulphonyl chloride (0.35 g, 1.84 mmol) were dissolved in pyridine (5 ml) and the solution was stirred at room temperature. After 2 h, methanol (1 ml) was added and the products were concentrated under reduced pressure. The residue was partitioned between chloroform (100 ml) and saturated aqueous sodium hydrogen carbonate (50 ml). Evaporation of the dried ($MgSO_4$) organic layer and crystallization of the residue from ethyl acetate gave 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (0.30 g, 64%), m.p. 180 °C. This material was identical [t.l.c., ^1H n.m.r.] with the product obtained in (a) above.

2′,3′-O-*Isopropylideneisoguanosine* (**6**).—N^1,N^1,N^3,N^3-Tetramethylguanidine (0.2 ml, 1.6 mmol) and water (0.2 ml, 11.1 mmol) were added to a solution of 1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-5-ureidoimidazole-4-carbonitrile (0.20 g, 0.62 mmol) in THF (10 ml) at room temperature. After 16 h, the products were evaporated under reduced pressure. The residue was co-evaporated with ethanol (2 × 10 ml), dissolved in

methanol (15 ml), and acetic acid (0.2 ml) and then ether (250 ml) were added. The resulting precipitate was collected by centrifugation and recrystallized from ethanol to give the *title compound* (**6**) (0.16 g, 80%) as colourless crystals (Found: C, 47.0; H, 5.7; N, 21.3. $C_{13}H_{17}N_5O_5 \cdot 0.5H_2O$ requires C, 47.0; H, 5.5; N, 21.1%), m.p. 280 °C; $\lambda_{max.}$ 299, 249 (ε 10 700, 9 100), $\lambda_{min.}$ 268, 230 (ε 2 300, 3 900) nm; $\delta_H[(CD_3)_2SO]$ 1.29 (3 H, s), 1.54 (3 H, s), 3.53 (1 H, dd, *J* 3.8, 12.0 Hz), 3.62 (1 H, dd, *J* 3.5, 12.1 Hz), 4.21 (1 H, m), 4.92 (1 H, dd, *J* 1.9, 5.9 Hz), 5.21 (1 H, dd, *J* 3.9, 5.8 Hz), 5.84 (1 H, d, *J* 3.9 Hz), 7.80 (1 H, s); R_F 0.07 [$CHCl_3$–MeOH (9:1, v/v)], 0.30 [$CHCl_3$–MeOH (4:1, v/v)].

Acknowledgements

We thank the Cancer Research Campaign for financial support, and the Ajinomoto Company for a gift of 5-amino-1-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide; we also thank Dr. Halina Serafinowska for her help.

References

1 A. Yamazaki, M. Okutsu, and Y. Yamada, *Nucleic Acids Res.*, 1976, **3**, 251.
2 A. Yamazaki, I. Kumashiro, T. Takenishi, and M. Ikehara, *Chem. Pharm. Bull.*, 1968, **16**, 2172.
3 M. Okutsu and A. Yamazaki, *Nucleic Acids Res.*, 1976, **3**, 237.
4 N. Shimada, N. Yagisawa, H. Naganawa, T. Takita, M. Hamada, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1981, **34**, 1216.
5 N. Yagisawa, T. Takita, H. Umezawa, K. Kato, and N. Shimada, *Tetrahedron Lett.*, 1983, **24**, 931.
6 R. Kuroda, C. B. Reese, and Y. S. Sanghvi, unpublished work.
7 J.-W. Chern, M. P. Groziak, and L. B. Townsend, *J. Heterocyclic Chem.*, 1986, **23**, 153; M. P. Groziak, J.-W. Chern, and L. B. Townsend, *J. Org. Chem.*, 1986, **51**, 1065.
8 T. Meguro, T. Saito, I. Kumashiro, and T. Takenishi, J.P. 1534, 1966 (*Chem. Abstr.*, 1966, **64**, 12781e).
9 J.-W. Chern and L. B. Townsend, *Tetrahedron Lett.*, 1985, **26**, 6419.

Received 21*st July* 1986; *Paper* 6/1467