Volume 3 no.12 December 1976 Nucleic Acids Research

An acid-stable analogue of the 3-β-D-ribofuranoside of Y-base

Colin B. Reese and Neil Whittall

Department of Chemistry, King's College, Strand, London WC2R 2LS, UK

Received 10 November 1976

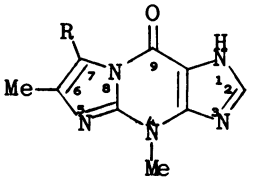
ABSTRACT

A cyclonucleoside analogue of Y_{TU} riboside has been prepared and shown to be relatively stable in M-hydrochloric acid solution at room temperature.

The Y-nucleosides are perhaps the most chemically interesting group of modified nucleosides which have so far been found to occur in tRNA[1-5]. Not only do their aglycones, the Y-bases (1), contain the most complex heterocyclic chromophore yet observed in nucleic acids but perhaps even more notably their glycosidic bonds are exceptionally labile to acidic hydrolysis. Thus Thiebe and Zachau found[2] that the half-time for the release of Y_{SC}-base (1a) from $tRNA^{Phe}$, isolated from baker's yeast (Saccharomyces cerviseae), was ca. 1-2 hr at pH 2.9 and 37°; these workers also found[2] that, under the same conditions, (1a) was released at a comparable rate from a hexanucleotide and from the putative Y-riboside, both obtained by the enzymatic digestion of yeast $tRNA^{Phe}$. In an elegant piece of work, Nakaniski and his co-workers[6] assigned the structure (1a) to Y_{SC}-base and later confirmed this assignment by synthesis[7]. Subsequently, Y_{TU}- and Y_L-bases (1b and 1c) have been obtained by the very mild acidic hydrolysis of $tRNA^{Phe}$, isolated from Torulopsis utilis[8] and mammalian liver[9], respectively.

Y_{SC}-Nucleoside has been shown[10,11] to be derived biogenetically from guanosine. Furthermore, chromatographic and chemical evidence consistent with its being a riboside has been obtained[2]. On the basis of this evidence[2] it has been suggested that Y_{SC}-nucleoside is the 3-β-D-ribofuranoside of (1a)[12]. In order to clarify further the structures of the Y-nucleosides, it would clearly be desirable to synthesize an authentic 3-β-D-ribofuranoside of a Y-base (1). However, it may not be possible to carry out such a synthesis unambiguously until 3-N-methylguanosine (2)

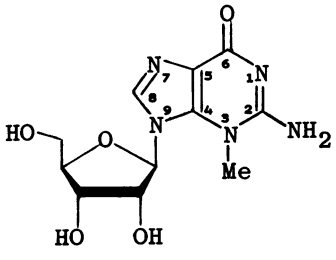
becomes available as a starting material. We now report the synthesis of 4,5'-cyclo-3-β-D-ribofuranosyl-4,9-dihydro-6-methyl-9-oxoimidazo[1,2-a]-purine (4b), a close analogue of the 3-β-D-ribofuranoside of (1b), starting from 2',3'-O-isopropylidene-3,5'-cycloguanosine[13] (3a), the only fully characterized 3-N-alkylguanosine system of which we are aware. Kasai et al. have recently reported[14] that (4a) may also be obtained by the cyclization of (5).



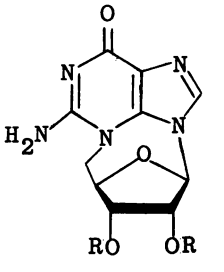
(1) a; R=$CH_2CH_2CH(NHCO_2Me)CO_2Me$

b; R=H

c; R=$CH_2CH(O_2H)CH(NHCO_2Me)CO_2Me$

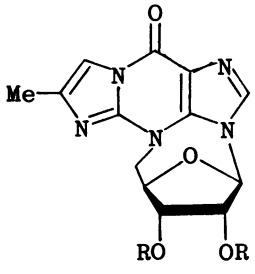


(2)



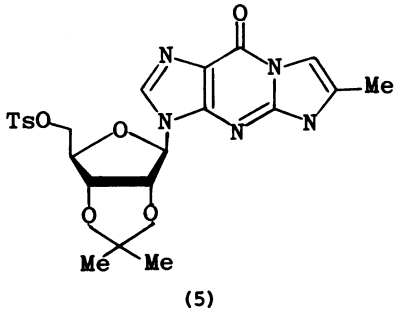
(3) a; R,R=$Me_2C<$

b; R=H

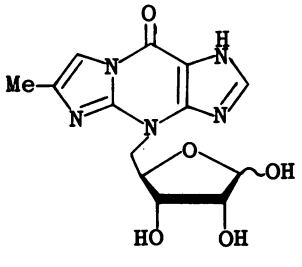


(4) a; R,R=$Me_2C<$

b; R=H



(5)



(6)

Treatment of (3a) with an excess (ca. 1.5 molecular equivalents) of sodium hydride, followed by an excess (ca. 2 molecular equivalents) of freshly-distilled bromoacetone in anhydrous dimethylformamide solution at room temperature gave, after 24 hr, (4a) as colourless fibrous needles in 52% isolated yield. Removal of the isopropylidene group (M-hydrochloric acid, 20°, 60 hr) gave (4b) as colourless needles in 84% isolated yield. The comparative stability of (4b) to acidic hydrolysis was confirmed by the observations that it remained virtually unchanged after it had been allowed to stand in 0.09 M-hydrochloric acid at 37° for 68 hr and that it underwent only ca. 30% hydrolysis in 27 hr at 37° in 0.9 M-hydrochloric acid. Under the latter conditions, guanosine and 3-N,5'-cycloguanosine (3b) underwent ca. 80% and virtually quantitative hydrolysis, respectively. Thus the glycosidic linkage of the Y-riboside analogue (4b) is more stable to acidic hydrolysis than that of guanosine or 3-N,5'-cycloguanosine (3b). When (4b) was treated with M-hydrochloric acid at 45° for 24 hr, a major product with an ultraviolet spectrum closely similar to that reported for Y_{TU}-base was obtained. This product, which is believed to be (6),[14] is unstable under more stringent acidic conditions; thus when (4b) was heated in M-hydrochloric acid solution at 100° for 30 min, a higher R_F major product and virtually no putative (6) was obtained.

The half-time of hydrolysis of 2'-deoxyguanosine[15] is 3.4 hr at pH 2.8 and 48.4°. Therefore Y_{SC} (and presumably Y_{TU})-nucleoside[2] is more labile to acidic hydrolysis than 2'-deoxyguanosine by a factor of ca. one order of magnitude. As the glycosidic bond of 2'-deoxyguanosine is more labile to acidic hydrolysis than that of guanosine by a factor of more than two orders of magnitude[15], it follows that Y_{SC} (or Y_{TU})-nucleoside undergoes acidic hydrolysis at a rate more than three orders of magnitude greater than that of guanosine and at a rate approaching 10^4 times that of the Y-riboside analogue (4b). This is a very unexpected result inasmuch as it is unclear why Y_{SC} (or Y_{TU})-3-riboside should be appreciably more susceptible to acidic hydrolysis than (4b). The rates of acidic hydrolysis of (4b) and 3-N,5'-cycloguanosine (3b) are of the same order of magnitude and it therefore seems probable that the rates of acidic hydrolysis of Y_{SC} (or Y_{TU})-riboside and 3-N-methylguanosine (2) will be similar. It also seems unlikely that the glycosidic bond of the latter compound (2) will prove to be significantly more labile to acidic hydrolysis than that of 3-N,5'-cycloguanosine (3b) which is also a 3-N-alkyl derivative of guanosine. However, the effect of the extra ring on the stability of the glycosidic linkage of the latter

compound (3b) will remain uncertain until 3-N-methylguanosine (2) becomes available. Support for the hypothesis that the glycosidic bonds of Y_{SC} (or Y_{TU})-riboside and the Y-riboside analogue (4b) are likely to have similar stabilities to acid is provided by the observation that the synthetic putative Y_{TU}-1-D-ribofuranoside[12] is comparatively stable to acidic hydrolysis, even in 2M-hydrochloric acid at 37°. Usually, purine 7-β-D-ribofuranosides are somewhat less stable to acidic hydrolysis than their 9-isomers[16] but 7-(β-D-ribofuranosyl)-guanine is an exception in that it is five times more stable to acidic hydrolysis than guanosine.

Despite previous studies[2] on the sugar component of Y_{SC}-nucleoside, a virtually inescapable conclusion of the present study is that Y_{SC}- and Y_{TU}-nucleosides are unlikely to be ribonucleosides. Nevertheless, it would seem from ultraviolet spectroscopic data that Y_{TU}-nucleoside[14] is very probably a 3-derivative of Y_{TU}-base (1b). All the nucleosides which have been isolated from tRNA and fully characterized have so far proved to be β-D-ribofuranosides or 2'-O-methyl-β-D-ribofuranosides. It should be noted in this context that 2'-O-methyladenosine has been reported[17] to be ca. 2.5 times more stable to acidic hydrolysis than adenosine. The full characterization of the sugar moiety of the Y-nucleosides must await further investigations. One interesting possibility is that the Y-nucleosides are 2'-deoxyribonucleosides. However, it is by no means clear that this possibility is consistent with their extreme sensitivity to acidic hydrolysis.

EXPERIMENTAL

U.v. absorption spectra were measured with a Unicam SP 800 spectrophotometer. ^{1}H N.m.r. spectra were measured at 90 MHz with a Bruker HFX 90 spectrometer. Merck silica gel 60 F_{254} was used for t.l.c. in solvent system A [$CHCl_3$-MeOH (82:18 v/v)].

4,5'-Cyclo-3-(2',3'-O-isopropylidene-β-D-ribofuranosyl)-4,9-dihydro-6-methyl-9-oxoimidazo[1,2-a]purine (4a). - A dispersion of sodium hydride (60%, 0.098 g, ca. 2.5 mmole) in oil was washed with petroleum ether (b.p. 40-60°, 3 x 20 ml) under N_2. Dry dimethylformamide (10 ml) was added to the residue and the cooled (ice-water bath) mixture treated with 2',3'-O-isopropylidene - 3-N,5'-cycloguanosine[13] (0.50 g, 1.64 mmole). The reactants were allowed to warm up to room temperature, then stirred for a further period of 1 hr, cooled again and treated with freshly-distilled bromoacetone (0.3 ml, 3.6 mmole). The reaction mixture was then stirred at room temperature. After 24 hr, the products were partitioned between dichloro-

methane (30 ml) and M-potassium phosphate buffer (pH 6.0, 50 ml). The organic layer was separated, washed with more phosphate buffer (3 x 20 ml), dried ($MgSO_4$) and concentrated under reduced pressure to give a glassy solid. Crystallization of this material from ethanol-water (1:2 v/v) gave 4,5'-cyclo-3-(2',3'-O-isopropylidene-β-D-ribofuranosyl)-4,9-dihydro-6-methyl-9-oxoimidazo[1,2-a]purine. [Found: C, 54.7; H, 4.7; N, 20.0. $C_{16}H_{17}N_5O_4.$ $\frac{1}{2}H_2O$ requires: C, 54.5; H, 5.15; N, 19.9%] as colourless fibrous needles, m.p. 295-296° (lit.[14] 292.5°); yield 0.295 g (52%); τ($CDCl_3$): 2.47 (1H, br.s), 2.74 (1H, t, J ∿ 1.2 Hz), 3.87 (1H, s), 4.81 (1H, dd, J = 2.5 and 14 Hz), 4.91 (1H, d, J = 6 Hz), 5.04 (1H, t, J = 2.5 Hz), 5.28 (1H, d, J = 6 Hz), 6.00 (dd, J = 2.5 and 14 Hz), 7.70 (3H, d, J ∿ 1.2 Hz), 8.41 (3H, s), 8.63 (3H, s); ultraviolet absorption: (a) in water, λ_{max} 293, 236 (ε 3,900, 14,200) λ_{min} 259 nm (ε 1,300); (b) in 0.1 M-sodium hydroxide, λ_{max} 291, 235 (ε 3,400, 12,300), λ_{min} 258 nm (ε 1,200); (c) in 0.1 M-hydrochloric acid, λ_{max} 276, 231 (ε 5,300, 14,300), λ_{min} 250 nm (ε 1,200); M^+ at m/e = 343.1278 (100%), calc. for $C_{16}H_{17}N_5O_4$: 343.1280; R_F 0.67 (system A).

4,5'-Cyclo-3-β-D-ribofuranosyl-4,9-dihydro-6-methyl-9-oxoimidazo-[1,2-a]purine (4b). - A suspension of the above isopropylidene derivative (0.048 g, 0.14 mmole) in M-hydrochloric acid (5 ml) was stirred at room temperature. After 60 hr, the products were neutralized with aqueous ammonia (d 0.88) and the resulting suspension concentrated under reduced pressure. Recrystallization of the residue from water gave 4,5'-cyclo-3-β-D-ribofuranosyl-4,9-dihydro-6-methyl-9-oxoimidazo[1,2-a]purine [Found, in material dried in vacuo over P_2O_5 at 100°: C, 50.7; H, 4.5; N, 23.0. $C_{13}H_{13}N_5O_4.0.25H_2O$ requires: C, 50.7; H, 4.4; N, 22.8%] as fine colourless needles, m.p. 266°; yield 0.036 g (84%); τ[$(CD_3)_2SO$-CD_3OD-$(CD_3)_2CO$-(9:1:10 v/v)]: 2.06 (1H, s, H-2), 2.65 (1H, m, H-7), 3.71 (1H, s, H-1'), 4.92 (1H, dd, J = 2 and 16 Hz, H-5'), 5.22 (1H, m, H-4'), 5.58 (1H, dd, J = 3.5 and 6.5 Hz, H-3'), 5.85 (1H, dd, J = 3 and 16 Hz, H-5'), 5.95 (1H, d, J = 6.5 Hz, H-2'), 7.74 (3H, d, J = 1.3 Hz, 6-CH_3); ultraviolet absorption: (a) in water, λ_{max} 293, 235 (ε 7,300, 28,600), λ_{min} 257 nm (ε 4,200); (b) in 0.1 M-sodium hydroxide, λ_{max} 293, 235 (ε 8,200, 30,300), λ_{min} 258 nm (ε 5,500); (c) in 0.1 M-hydrochloric acid, λ_{max} 275, 231 (ε 13,000, 34,000), λ_{min} 248 nm (ε 6,100); M^+ at m/e = 303.0958 (100%), calc. for $C_{13}H_{13}N_5O_4$: 303.0967; R_F 0.36 (system A).

Acidic hydrolysis of 4,5'-cyclo-3-β-D-ribofuranosyl-4,9-dihydro-6-methyl-9-oxoimidazo[1,2-a]purine (4b) - (a) A solution of (4b) in 0.09 M-hydrochloric acid was maintained at 37° for 68 hr. Ultraviolet spectros-

copic and t.l.c. (system A) evidence revealed that no hydrolysis had occurred. Under the same conditions, guanosine appeared to undergo ca. 5% and 3-N,5'-cycloguanosine ca. 10% hydrolysis.

(b) A solution of (4b) in 0.9 M-hydrochloric acid was maintained at 37° for 27 hr. It appeared from ultraviolet spectroscopic and t.l.c. (system A) evidence that ca. 30% hydrolysis had occurred. Under the same conditions, guanosine appeared to undergo ca. 80% and 3-N,5'-cycloguanosine ca. 100% hydrolysis.

(c) A solution of (4b) (0.015 g) in M-hydrochloric acid (100 ml) was maintained at 45° for 24 hr, then neutralized with aqueous ammonia and concentrated under reduced pressure. T.l.c. (system A) revealed one main product (R_F 0.27) and virtually no starting material (R_F 0.36). Ultraviolet absorption of major product: (a) in water, λ_{max} 295, 232, λ_{min} 252 nm; (b) in 0.1 M-sodium hydroxide, λ_{max} 295, 232, λ_{min} 252 nm; (c) in 0.1 M-hydrochloric acid, λ_{max} 277, 228, λ_{min} 245 nm.

(d) When the hydrolysis of (4b) was carried out in M-hydrochloric acid at 100° for 30 min, a major fluorescent product with R_F (system A) 0.65 and several minor lower R_F products were obtained.

ACKNOWLEDGEMENTS

We thank the Science Research Council and Imperial Chemical Industries Ltd., Pharmaceuticals Division for support (CASE Studentship awarded to N.W.).

REFERENCES

1. RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M., and Khorana, H. G. (1967) Proc. Natl. Acad. Sci. U.S.A., 57, 751.

2. Thiebe, R., and Zachau, H. G. (1968) Eur. J. Biochem. 5, 546.

3. Dudock, B. S., Katz, G., Taylor, E. K., and Holley, R. W. (1969) Proc. Natl. Acad. Sci. U.S.A. 62, 941.

4. Fink, L. M., Goto, T., Frankel, F., and Weinstein, I. B. (1968) Biochem. Biophys. Res. Commun. 32, 963.

5. Keith, G., Picaud, F., Weissenbach, J., Ebel, J.P., Petrissant, G., and Dirheimer, G. (1973) FEBS Lett. 31, 345.

6. Nakanishi, K., Furutachi, N., Funamizu, M., Grunberger, D., and Weinstein, I. B. (1970) J. Amer. Chem. Soc. 92, 7617.

7. Funamizu, M., Terahara, A., Feinberg, A. M., and Nakanishi, K. (1971) J. Amer. Chem. Soc. 93, 6706.

8. Kasai, H., Goto, M., Takamura, S., Goto, T., and Matsuura, S. (1971) Tetrahedron Lett. 2725.

9. Nakanishi, K., Blobstein, S., Funamizu, M., Van Lear, G., Grunberger, D., Lanks, K., and Weinstein, I. B. (1971) Nature New Biol. 234, 107.

10. Thiebe, R. and Poralla, K. (1973) FEBS Lett. 38, 27.

11. Li, H. J., Nakanishi, K., Grunberger, D., and Weinstein, I. B. (1973) Biochem. Biophys. Res. Commun. 55, 818.

12. Blobstein, S. V., Gebart, R., Grunberger, D., Nakanishi, K., and Weinstein, I. B. (1975) Arch. Biochem. Biophys. 167, 668.

13. Holmes, R. E. and Robins, R. K. (1963) J. Org. Chem. 28, 3483.

14. Kasai, H., Goto, M., Ikeda, K., Zama, M., Mizuno, Y., Takemura, S., Matsuura, S., Sugimoto, T., and Goto, T. (1976) Biochemistry 15, 898.

15. Hevesi, L., Wolfson-Davidson, E., Nagy, J. B., Nagy, O. B., and Bruylants, A. (1972) J. Amer. Chem. Soc. 94, 4715.

16. Panzica, R. P., Rousseau, R. J., Robins, R. K., and Townsend, L. B. (1972) J. Amer. Chem. Soc. 94, 4708.

17. Martin, D. M. G., Reese, C. B., and Stephenson, G. F. (1968) Biochemistry 7, 1406.