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STRUCTURE OF AMIPURIMYCIN, A NUCLEOSIDE ANTIBIOTIC HAVING A NOVEL BRANCHED SUGAR MOIETY[1]

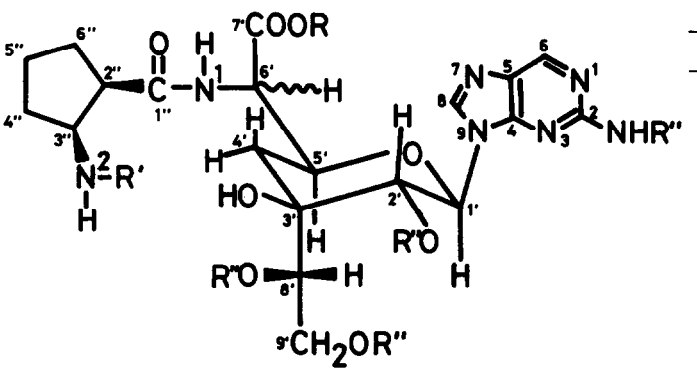
Toshio Goto,* Yoshiaki Toya, Tadaaki Ohgi and Tadao Kondo†

Laboratory of Organic Chemistry, Faculty of Agriculture, and
†Chemical Instruments Center; Nagoya University,
Chikusa, Nagoya 464, Japan

Summary: Structure of amipurimycin was determined chemically to be 1.

Harada and Kishi[2] isolated from Streptomyces novoguineensis a new antibiotic amipurimycin, which was active against Pyricularia oryzae, had a molecular formula $C_{20}H_{27-31}N_7O_8 \cdot H_2O$ and showed pKa' 3.7 and 9.1 and the signals in the pmr and cmr spectra as well as the maxima of its uv spectra characteristic to those of 2-aminopurine moiety. We now report here structure determination of amipurimycin (1) [$C_{20}H_{29}N_7O_8$ from fd mass m/z 496 (M+1)].

Hydrolysis of amipurimycin (APM)[3] (1) with 6N HCl at 80 °C gave a ninhydrin positive compound [fd mass m/z 130 (M+1)] whose spectral data as well as those of its N-acetyl methyl ester [m/z 185 (ei mass)] suggested its structure to be 2-aminocyclopentanecarboxylic acid. Its stereochemistry was determined to be cis (3) by comparison with synthetic cis-[4] and trans-2-aminocyclopentanecarboxylic acid.[5]



(1) R = R' =R" = H
(4) R = CH_3, R' = R" = H (HCl salt)
(5) R = CH_3, R' = R" = Ac
(6) R = CH_3, R' = Ac, R" = H

Pmr Coupling Patterns (Hz)

	(1)	(5)	(7)
1'	9	9	9
2'			
4eq'	4		13.5 2
4ax'	12		11.5
5'	4	3	4
6'		8	8
N^1H			
8'		3	7 7
9a'		12	7
9b'			

Although presence of the 2-aminopurine moiety in 1 was suggested from the spectral data[1], hydrolysis of 1 with hydrochloric acid did not afford 2-aminopurine base (2). We found that on treatment with trifluoroacetic acid at 130 °C in a sealed tube 1 did afford 2 in 41% yield.

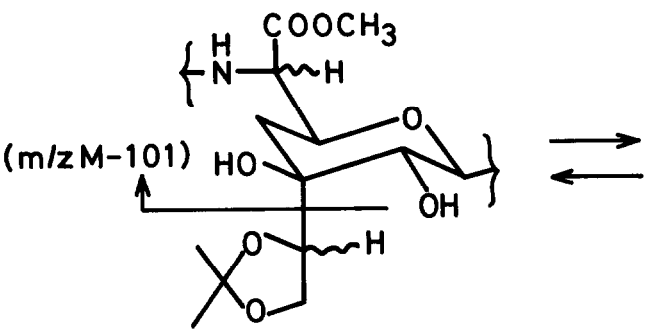
Treatment of APM (1) with methanolic 1N HCl at 5 °C afforded APM methyl ester (4) [fd mass m/z 510 (M+1)], which was acetylated with N-acetylimidazole in dichloroethane at 100 °C to pentaacetyl APM methyl ester (5)[6] [m/z 719 (M)]. Extensive analysis of pmr and cmr spectra of APM[3] and 5[6] suggested the formula 1 for APM except the stereochemistry at 3' and 8' positions. For confirmation of this formula the presence of the tertiary hydroxy group at 3' position had to be established. It was carried out as follows.

APM methyl ester (4) was treated with p-nitrophenyl acetate in aq pyridine to give N-acetyl APM methyl ester (6) [40% from 1; fd mass m/z 552 (M+1); ninhydrin test negative; δ 2.15 (s, Ac)], which was then treated in acetone with 2,2-dimethoxypropane and camphorsulfonic acid giving two isomeric acetonides; N^2-acetyl APM methyl ester acetonide I (7)[7] [yield 81%; crystals; mp 248-9 °C; exact mass 591.2681 (M)] and II (8)[8] [yield 11%; fd mass 592 (M+1)]. Position of the acetonide ring in 7 was assumed from the analysis of its pmr spectrum and from the fragment peak at exact m/z 490.2040 (M-101 in formula 7) in its mass spectrum, and further confirmed by deriving the acetonide I (7) with acetylimidazole to its monoacetate [2' position; H-2' δ 4.74 in 7 → 5.83 in 7 acetate] and N-acetyl monoacetate [arom-amino and 2']; no acetyl group was introduced to 8' or 9' position. Similarly the position of acetonide ring in the acetonide II (8) was assumed and confirmed by deriving it to its diacetate [2' and 9'; no peak at m/z 574 (M-101); H-2' δ 4.74 in 8 → 5.79 in 8 diacetate; H-9' δ 4.20 or 4.06 in 8 → 4.70 in 8 diacetate].

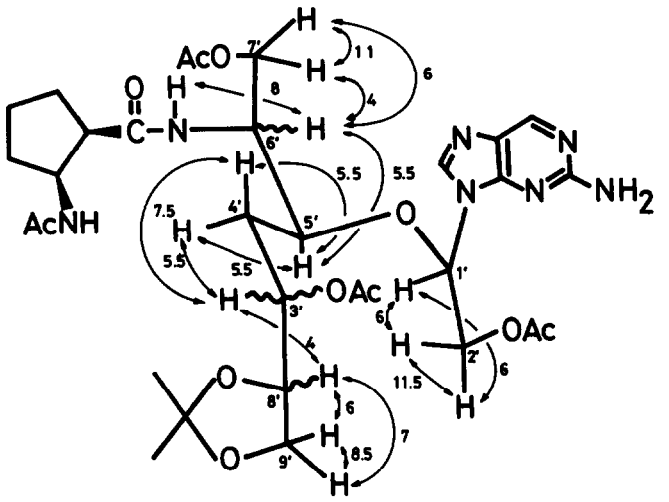
The acetonide I (7) was treated with aq $NaIO_4$ at 5 °C followed by reduction with $NaBH_4$ to give two products (2:1 ratio), seco-alcohol A (9) and B (10) [both fd mass m/z 566 (M+1)], which were separated by silica gel tlc. These seco-alcohols were isomeric at the newly produced sec-hydroxy group at 3' position. Seco-alcohol A (9) was acetylated with acetylimidazole to the seco-alcohol A triacetate (11) [m/z 691 (M)], whose structure was proven by extensive analysis of its pmr spectrum (formula 11); these results indicated correctness of the proposed structure 1 (except stereochemistry at 3' and 8' positions) having the tert-hydroxy group at 3' position.

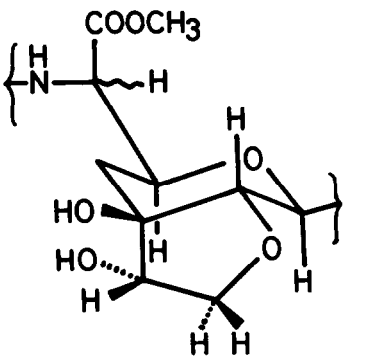
The acetonide I (7) and II (8) were interconvertible under the acetonidation condition, but no diacetonide has been detected in the reaction mixture. Both of the monoacetonides could be hydrolyzed with 20% acetic acid to the starting N-acetyl APM methyl ester (6) in a nearly quantitative yield, and hence no rearrangement occurred during the acetonidation. The formation of no diacetonide suggested that the 2',3'-diol should be trans each other, and this was established by conversion of the N-acetyl APM methyl ester (6) by treatment with mesyl chloride in dry pyridine at 4 °C to the tetrahydrofuran derivative 13[9] [yield 45 %; oil; fd mass 534 (M+1); acetylation gave 13 O-acetate; H-8' δ 3.93 in 13 → 5.49 in 13 acetate], which was accompanied with the epoxide 14 [yield 25 %; H-2' δ 4.39 in 14 → 5.83 in 14 2'-mesylate]. Under the same condition, the acetonide II (8) could not form its tetrahydrofuran derivative, but its 9'-O-mesyl and 2',9'-di-O-mesyl derivatives, indicating that the 3' and 8'-hydroxy

groups in the tetrahydrofuran 13 should be trans each other. Absolute configuration of APM as well as the stereochemistry at C-6' position has not yet been determined.



Acetonide I (7) Acetonide II (8)





Seco-alcohol triacetate (11) (13)

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REFERENCES AND FOOTNOTES

1. This paper was presented at the 8th Symposium on the Nucleic Acids Chemistry held in Sapporo, Japan, on August 22, 1980.
2. S. Harada and T. Kishi, J. Antibiotics, 30, 11 (1977); see also T. Iwasa, T. Kishi, K. Matsuura and O. Wakae, J. Antibiotics, 30, 1 (1977).
3. APM (1): uv (H_2O) nm (ε) 303 (6500), 243 (5900), 216 (21,000); pmr (D_2O, t-BuOH =1.23

as standard) δppm (J in Hz) 8.53 (s, H-6), 8.20 (s, H-8), 5.80 (d, 9, H-1'), 4.48 (m, 12, 4 and 4, H-5'), 4.38 (d, 4, H-6'), 4.32 (d, 9, H-2'), 3.72 (bq, 8, 7 and 7, H-3"), 3.96-3.75 (H-8', 9a', and 9b'), 2.94 (bq, 8, 7 and 7, H-2"); cmr (D_2O, MeOH = 49.3 as standard) δppm 175.8 and 175.4 (s, C-7' and 1"), 159.5 (s, C-2), 152.7 (s, C-4), 149.2 (d, C-6), 142.3 (d, C-8), 125.7 (d, C-5), 80.5 (d, C-1'), 75.6, 73.5 and 70.3 (d, C-5', 2' and 8'), 74.8 (s, C-3'), 61.9 (t, C-9'), 58.5 (d, C-6'), 53.8 (d, C-3"), 45.1 (d, C-2"), 33.1 (t, C-4'), 30.1, 28.6 and 22.0 (t, C-4", 6" and 5").

4. E. Nativ and P. Rona, Isr. J. Chem., 10, 55 (1972).
5. H. Plieninger and K. Schneider, Chem. Ber., 92, 1594 (1959).
6. Pentaacetyl APM methyl ester (5): pmr ($CDCl_3$, 70 °C) 10.58 (s, arom-NHAc), 8.97 (s, H-6), 8.09 (s, H-8), 7.04 (d, 8, N^1-H), 6.32 (d, 8, N^2-H), 6.07 (d, 9, H-1'), 5.36 (d, 9, H-2'), 5.18 (dd, 7 and 3, H-8'), 4.66 (dd, 8 and 3, H-6'), 4.46 (dd, 12 and 3, H-9a'), 4.35 (m, H-5'), 4.28 (m, H-3"), 4.02 (dd, 12 and 7, H-9b'), 3.76 (s, OMe), 2.88 (bq, 7, H-2"), 2.46, 2.12, 1.99, 1.93 and 1.68 (s, 5 x Ac).
7. N^2-Acetyl APM methyl ester acetonide I (7): pmr (Py-d_5, 70 °C, 270 MHz) δppm (J in Hz) 9.13 (bd, 8, N^1-H), 8.69 (s, H-6), 8.37 (s, H-8), 7.72 (bd, 8, N^2-H), 6.24 (d, 9, H-1'), 5.15 (bdd, 8 and 4, H-6'), 4.87 (m, H-5'), 4.74 (d, 9, H-2'), 4.74 (m, H-3"), 4.55, 4.37 and 4.13 (each t, 7, H-8', 9a' and 9b'), 3.56 (s, OMe), 3.41 (q, 7, H-2"), 2.46 (bt, 11.5 and 13.5, H-4ax'), 2.26 (dd, 13.5 and 2, H-4eq'), 2.11 (s, Ac), 1.9-1.6 (4H, m, H-4" and 6"), 1.43 and 1.35 (s, CMe_2); cmr (CD_3OD; CD_3OD = 49.0 as standard) δppm 175.7 and 171.0 (s, C-7' and 1"), 172.9 (s, COMe), 161.9 (s, C-2), 154.7 (s, C-4), 149.7 (d, C-6), 143.0 (d, C-8), 127.6 (d, C-5), 110.8 (s, CMe_2), 82.4, 81.8, 74.9 and 71.5 (each d, C-1', 5', 2' and 8'), 73.9 (s, C-3'), 66.0 (t, C-9'), 57.1 (d, C-6'), 54.3 (d, C-3'), 52.9 (q, OMe), 48.6 (d, C-2"), 34.6 (t, C-4'), 33.0, 28.7 and 23.8 (each t, C-4", 6" and 5"), 26.5 and 25.4 (q, CMe_2), 23.0 (q, Ac).
8. N^2-Acetyl APM methyl ester acetonide II (8): pmr (Py-d_5, 70 °C, 270 MHz) δppm (J in Hz) 9.19 (bd, 8, N^1-H), 8.68 (s, H-6), 8.24 (s, H-8), 7.78 (bd, 8, N^2-H), 6.20 (d, 9, H-1'), 5.19 (bdd, 8 and 4, H-6'), 5.06 (t, 6, H-8'), 4.80 (m, H-5'), 4.74 (d, 9, H-2'), 4.20 (dd, 11 and 6, H-9a'), 4.06 (dd, 11 and 6, H-9b'), 3.56 (s, OMe), 3.24 (q, 7, H-2"), 2.54 (bt, 13 and 11, H-4ax'), 2.35 (dd, 13 and 1.5, H-4eq'), 2.15 (s, Ac), 1.9-1.6 (4H, m, H-4" and 6"), 1.54 (6H, s, CMe_2), 1.5-1.0 (2H, m, H-5"); cmr (CD_3OD) δppm 175.7 and 171.0 (s, C-7'and 1"), 172.7 (s, COMe), 161.8 (s, C-2), 154.5 (s, C-4), 149.5 (d, C-6), 142.5 (d, C-8), 127.4 (s, C-5), 110.1 (s, C-1"), 83.6 (s, C-3'), 83.4 (d, C-1'), 78.0, 75.3 and 69.0 (d, C-5', 2' and 8'), 61.1 (t, C-9'), 57.0 (d, C-6'), 54.2 (d, C-3"), 52.8 (q, OMe), 48.4 (d, C-2"), 34.8 (t, C-4'), 33.0, 28.7 and 23.9 (t, C-4", 6" and 5"), 29.3 and 26.8 (q, CMe_2), 23.0 (q, Ac).
9. 2',9'-Dehydro-N^2-acetyl APM methyl ester (13): pmr (Py-d_5,110 °C) δppm (J in Hz) 8.71 (s, H-6), 8.61 (d, 8, N^1-H), 8.23 (s, H-8), 7.29 (d, 8, N^2-H), 6.56 (d, 9.5, H-1'), 6.29 (bs, arom-NH_2), 5.24-4.88 (m, H-5' and 6'), ca 4.80 (m, H-3"), 4.66 (d, 9.5, H-2'), 4.60-4.40 (m, H-9a' and 9b'), 3.93 (m, H-8'), 3.55 (s, OMe), 3.16 (bq, 8, 7 and 7, H-2"), 2.89 (dd, 13 and 11.5, H-4ax'), 2.39 (dd, 13 and 3, H-4eq'), 2.03 (s, Ac), 2.3-1.2 (6H, m).

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