Structural Identity of Anthelmycin with Hikizimycin

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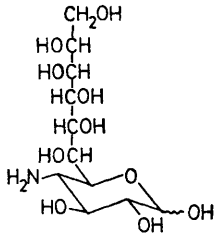
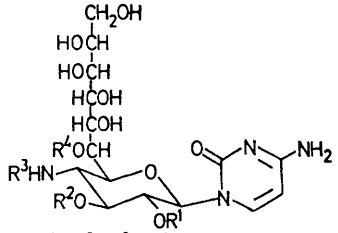
Summary The antibiotics, hikizimycin and anthelmycin, are the same compound, and the correct structure is (**17**).

HIKIZIMYCIN[1] is a nucleoside antibiotic having the empirical formula $C_{21}H_{37}N_5O_{14}$ and has been shown to consist of a 3-amino-3-deoxy-D-glucopyranosyl unit[2] β-glycosidically linked to a C_{11} amino sugar, named hikosamine[3] (**1**) which in turn is attached to a cytosine residue[2,3] forming a β-*N*-glycosidic bond.



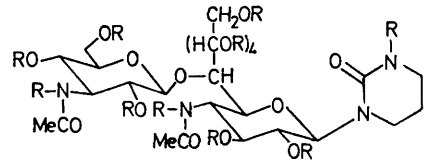
(1)



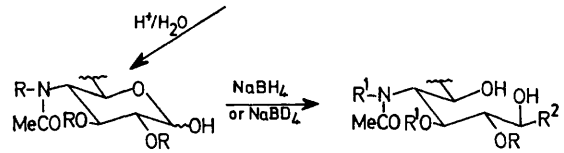
(2) $R^1 = R^2 = R^3 = R^4 = H$
(3) R^1 = 3-amino-3-deoxy-β-D-glucopyranosyl; $R^2 = R^3 = R^4 = H$
(4) R^2 = 3-amino-3-deoxy-β-D-glucopyranosyl; $R^1 = R^3 = R^4 = H$
(5) R^4 = 3-acetamido-3-deoxy-β-D-glucopyranosyl; $R^1 = R^2 = H$; R^3 = COMe

The configuration of the polyol side chain of hikosamine (**1**) followed from the isolation and characterization of D-glycero-D-galactoheptose, obtained by periodate cleavage between C-4 and C-5 of 2,3,6,7,8,9,10,11-*O*-octa-acetyl-hikosamine.[4] Evaluation of the periodate oxidation studies on hikizimycin, its *NN'*-diacetate, and some of its degradation products, led to structure (**3**) for hikizimycin with a 1 → 2 glycosidic linkage.[4,5] The ^{13}C n.m.r. spectra of hikizimycin and hikosaminylcytosine (**2**) were claimed as lending support to structure (**3**) for hikizimycin.[6]

Anthelmycin[7] was also shown to have a 3-amino-3-deoxy-D-glucopyranose residue β-glycosidically attached to a C_{11} amino sugar which has a β-nucleosidic linkage with cytosine. Decoupling experiments in the ^1H n.m.r. spectrum of anthelmycin trihydrobromide ($C_{21}H_{37}N_5O_{14}\cdot 3HBr$) indicate

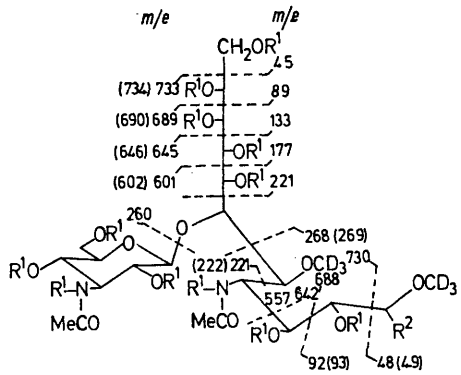


(6) R = H
(7) R = Me



(8)

(9) R^1 = Me; R^2 = H
(10) R^1 = Me; R^2 = D



(11) R^1 = Me; R^2 = H: $M^{+\bullet}$ 778
(12) R^1 = Me; R^2 = D: $M^{+\bullet}$ 779

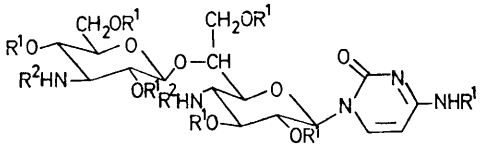
[Numbers in parentheses represent the corresponding fragment ion masses in the mass spectrum of (12)]

that the *N*-anomeric proton at δ 5·70 is coupled to the adjacent 2'-proton at δ 3·88 in the C_{11} sugar. The corres-

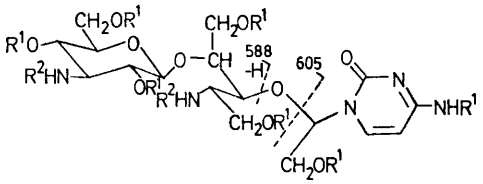
ponding *N*-anomeric and 2′-protons in peracetyl anthelmycin are centred at δ 6·02 and 5·15, respectively. This downfield shift of 1·27 p.p.m. for the 2′-proton in going from anthelmycin to its peracetyl derivative can only be rationalized as a consequence of acetylation of a hydroxy-group at the 2′-position. Consequently, structure (**4**) with a $1 \rightarrow 3$ intersugar linkage was suggested for anthelmycin.[8] Furthermore, comparison of *X*-ray powder patterns and bioautograms of anthelmycin and hikizimycin suggested that the two antibiotics may be the same compound.[8] Comparison of the ^{13}C n.m.r. spectra of hikizimycin and anthelmycin as well as those of *NN*′-diacetylhikizimycin and *NN*′-diacetylanthelmycin established that the two antibiotics are identical.

Analysis of the ^{13}C n.m.r. spectra of anthelmycin and its peracetyl derivative ruled out[9] the possibility of a $1 \rightarrow 2$ attachment between the two sugar units in this antibiotic. The work described below establishes structure (**17**) for anthelmycin, and consequently for hikizimycin. *NN*′-Diacetylanthelmycin (**5**) was hydrogenated to (**6**)† and subsequently methylated to (**7**). Acid hydrolysis of (**7**) gave 3,4,5,6-tetrahydropyrimidin-2-one and the product (**8**). The anomeric mixture (**8**) on reduction with $NaBH_4$ as well as $NaBD_4$ afforded the diols (**9**) and (**10**), respectively. The alcoholic groups at C-1 and C-5 in (**9**) and (**10**) were trideuteriomethylated with CD_3I, and after t.l.c. purification gave (**11**) and (**12**), respectively. The mass spectra of (**11**) and (**12**) exhibit the correct molecular ion peaks at *m/e* 778 and 779, respectively. The presence of peaks at *m/e* 730, 686, and 642 in both mass spectra clearly eliminates substitution of the 3-amino-3-deoxy-D-glucopyranosyl unit at the 2′ or 3′-positions in the C_{11} sugar of anthelmycin. Peaks at *m/e* 733, 689, 645, and 601, as well as the peaks at *m/e* 45, 89, 133, 177, and 221 in the mass spectrum of (**11**) rule out substitution of the hexosamine between C-7′ and C-11′ of the C_{11} sugar. Finally, the crucial peaks at *m/e* 268 in (**11**) and 269 in (**12**), along with the peak at *m/e* 557 [not shifted in the mass spectrum of (**12**)], establish the attachment of the 3-amino-3-deoxy-D-glucopyranosyl residue at position 6′ of the C_{11} sugar.

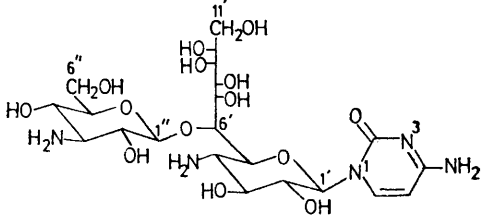
Oxidation of *NN*′-diacetylanthelmycin (**5**) with excess of sodium metaperiodate, followed by reduction of the products with $NaBH_4$ and subsequent acetylation, afforded a mixture of products. Separation by column chromatography gave the peracetates (**13**) and (**14**) in 3% and 34% yields, respectively. These peracetates have the empirical formulae $C_{35}H_{47}N_5O_{19}$ and $C_{35}H_{49}N_5O_{19}$, and exhibit $M+1$ peaks at *m/e* 842 and 844, respectively, in their mass spectra. The peaks at *m/e* 605 ($C_{25}H_{37}N_2O_{15}$) and 588 ($C_{25}H_{36}N_2O_{14}$) in (**14**) are consistent with the *seco* structure for the major product (**14**). In the ^1H n.m.r. spectrum of (**13**), the β-*N*-glycosidic proton occurs at δ 6·08 as a doublet with *J* 9·0 Hz, while in (**14**) the corresponding proton is a triplet at δ 6·28 with *J* 5·0 Hz. Deacetylation of (**13**) and (**14**) with $Ba(OH)_2$ afforded (**15**) and (**16**), respectively. The ^1H and ^{13}C n.m.r. and field desorption mass spectra are fully consistent with the assigned structures. The above data unequivocally establish the structure (**17**) for anthelmycin (hikizimycin).



(13) $R^1 = R^2 = COMe$
(15) $R^1 = H, R^2 = COMe$



(14) $R^1 = R^2 = COMe$
(16) $R^1 = H, R^2 = COMe$



(17)

In view of the above results, the ^{13}C n.m.r. assignments of hikizimycin by Uchida *et al.*[6] need re-evaluation. Our own studies of the ^{13}C n.m.r. spectra of anthelmycin (hikizimycin) and model hexopyranosyl nucleosides are compatible with structure (**17**).

We thank Dr. R. L. Hamill for a large supply of anthelmycin.

(*Received*, 25*th October* 1976; *Com.* 1197.)

† All new compounds gave correct composition by elemental analysis and/or mass spectrometry, and the spectral data were consistent with assigned structures.

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