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ISOLATION AND STRUCTURE OF REBECCAMYCIN - A NEW ANTITUMOR ANTIBIOTIC FROM NOCARDIA AEROCOLIGENES

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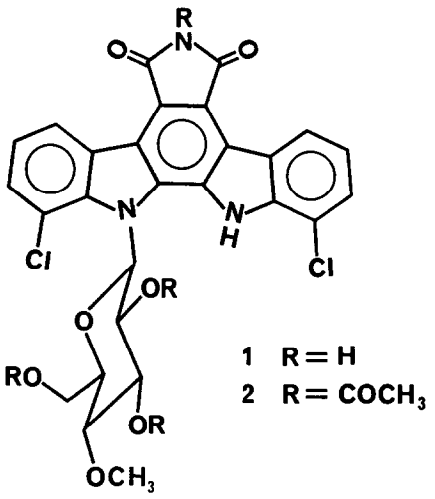
Summary The isolation and structure elucidation of rebeccamycin 1, a new antitumor agent from Nocardia aerocoligenes, is described. The NMR spectra of 1 and its peracetate 2 are discussed.

Recently we have isolated a novel antitumor antibiotic rebeccamycin 1 from fermentations of Nocardia aerocoligenes, strain C38383-RK-2 (ATCC 39243).[1] Rebeccamycin was isolated by extraction of the mycelial mat of the fermentation with THF. Evaporation of the extracts followed by trituration of the crude solids with ether yielded crude rebeccamycin which was recrystallized from THF-MeOH to yield pure rebeccamycin as a yellow solid, mp326-330°C (decomposition), α_D^{21} (THF) = + 131°. The IR spectrum of 1 had bands characteristic of hydroxyl groups and a cyclic imide (IR bands at 3418, 3355, 1752 med., 1704 strong, cm^{-1}). The UV spectrum of 1 in methanol showed two maxima at 238 (a=75.75) and 314 (a=90.51) with shoulders at 256, 293, 362 and 390 nm. Addition of dilute base gave bands at 314, 285, and 237 nm. No shifts were observed on addition of dilute acid. The elemental formula $C_{27}H_{21}Cl_2N_3O_7$ was determined by elemental analysis and mass spectroscopy.[2] The CI mass spectrum of 1 exhibited an ion at M/e 570(M+1) and a parent ion at M/e 394($M-C_7H_{14}O_5$). Minor ions at M/e 422 and M/e 436 were also observed. The ^1H NMR spectrum of 1 in DMSO-d_6 exhibited resonances at δ11.37(S, 1H, N6-H), 10.30(s, 1H, N13-H), 9.27(d, 1H, C8-H), 9.09(d, 1H, C4-H), 7.74(d, 1H, C10-H), 7.69(d, 1H, C2-H), 7.45(t, 2H, C3-H+C9-H), 6.97(d, 1H, C1'-H), 5.45(d, 1H, C3'-OH), 5.36(t, 1H, C6'-OH), 5.03(d, 1H, C2'-OH), 3.90(bt, 2H, C6'-CH_2), 3.66(quin, 1H, C5'-H), 3.56(dt, 1H, C2'-H) 3.53(t, 1H, C4'-H) 3.48(s, 3H, C4'-OCH_3) 3.45(obscured, 1H, C3'-H). The ^{13}C NMR spectrum of 1 is listed in Table 1. Treatment of 1 with acetic anhydride/pyridine gave the tetracetate 2 in good yield as a yellow solid, mp145-147°C. The ^1H NMR spectrum of 2 was very similar to that of 1 in the



Proton	Compound 1	2	Δδ
C1'-H	6.97	7.36	+ .39
C2'-H	3.44	5.14	+1.70
C3'-H	3.59	5.60	+2.01
C4'-H	3.67	3.98	+0.31
C5'-H	3.87	4.73	+0.86
C6'-H	3.99	4.68,4.82	+0.75
C4'-OCH_3	3.61	3.61	-
C2'-OAc	-	1.04	-
C3'-OAc	-	1.86	-
C6'-OAc	-	2.10	-

Fig. 1. Structures of 1 and 2. ^{1}H NMR assignments to sugar residue (recorded in DMSO-d6 at360 MHz. Chemical shifts in δ).

Table 1. ^{13}C NMR Spectrum of 1 and 2[a]

Carbon	δ 1	2	Carbon	δ 1	2	Carbon	δ 1	2
C1	116	116	C11	116.5	116.5	C1'	84.2	81.3
C2	129.7	130.4	C10	126.9	127.2	C2'	72.0	70.1
C3	121.9	122.5	C9	122.4	123.6	C3'	77.5	73.9
C4	123.1	123.9	C8	123.1	123.6	C4'	79.3	77.9
C4a	121.4	122.6	C7c	125.3	124.6	C5'	80.3	76.7
C4b	117.6	117.3	C7b	119.2	119.4	C6'	59.7	62.7
C4c	118.4	118.1	C7a	121.4	121.1	C7'	60.0	60.0
C5	170.1	165.3	C7	170.3	165.2			
C12b	129.5	129.9	C12a	129.5	130.1			
C13a	137.0	137.2	C11a	137.4				

a. Recorded at 90 MHz in DMSO-d6.

aromatic region of the spectrum. The glycoside protons became first order and readily led, together with the ^{1}H NMR spectrum of 1, to an assignment of 4-O-methyl glucose as the carbohydrate portion of the molecule linked by a β-glycoside linkage to the aglycone (see table Fig. 1). From the chemical shifts of C1'-H in both 1 and 2 and the position of the anomeric carbon in the ^{13}C NMR spectra of 1 and 2 (84.2 and 81.3 PPM respectively) it was evident that the sugar residue was either a C- or N- glycoside attached to an aromatic or heteroaromatic aglycone. The chemical shift of the C2' acetyl function supported this assignment. That the aglycone of 1 had a good deal of symmetry could also

be seen from the ^{1}H NMR of the aglycone protons as well as the ^{13}C NMR spectrum of 1 in which many of the signals appeared to be paired. At this junction crystals of 1 suitable for x-ray analysis become available.

Crystals were grown by slow evaporation of a dioxane solution and a roughly rectangular specimen with dimensions 0.5x0.3x0.2 mm was used. Preliminary x-ray photographs showed monoclinic symmetry, and accurate lattice constants of a=7.616(1), b=20.610(4), c=18.912(2) Å, and β=82.27(2)° were determined from a least-squares fit of fifteen 2θ-values. The systematic extinctions, crystal density, and presence of chirality were uniquely accomodated by space group $P2_1$ with two molecules of composition $C_{27}H_{21}Cl_2N_3O_7$ and some solvent forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected using variable speed, 1° ω-scans and graphite monochromated Cu Kα radiation (1.54178 Å). Of the 4468 reflections measured in this way, 3304 (74%) were judged observed ($|F_o| \geq 3\sigma(F_o)$).[3] A phasing model was achieved with some difficulty using a multisolution tangent formula approach and extensive tangent formula recycling of plausible molecular fragments.[4] The structure was finally completed with $2F_o-F_c$ syntheses. The final x-ray model consisted of the 78 anisotropic nonhydrogen atoms of rebeccamycin, 42 isotropic hydrogens, and ten solvent atoms. Block diagonal least-squares refinements with this model have converged to a standard crystallographic residual of 0.058 for the observed reflections.[5]

Figure 1 is a computer generated perspective drawing of the final x-ray model of rebeccamycin. Both molecules comprising the asymmetric unit have the same conformation, and only one is illustrated. Hydrogens are omitted for clarity, and the enantiomer illustrated was selected on the basis of the known absolute configuration of the D-4-methoxyglu-

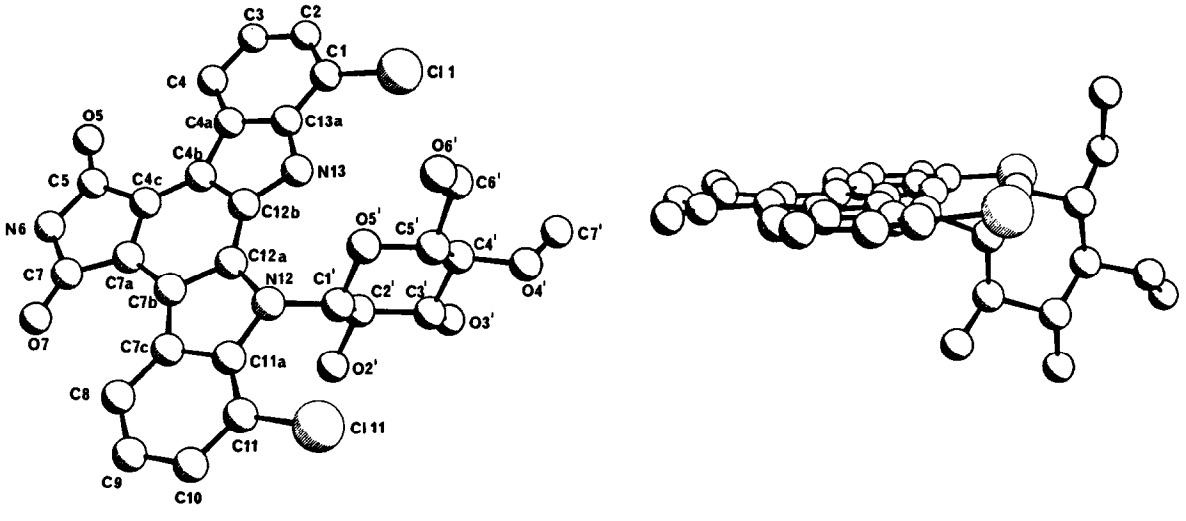


Fig. 2. Computer generated perspective drawings of rebeccamycin 1.

cose component. Bond distances and angles generally agree well with accepted values. The aromatic portion of the molecule is planar within experimental error. The plane of the sugar fragment is rotated 90° to the plane of the aromatic ring. There is some evidence of steric interactions between the sugar and the chlorine closest to the sugar. Atoms Cl 11 and Cl' are on opposite sides of the aromatic plane by 0.27 and -0.65 Å respectively. From the position of the C2'-OH function in 1 the shielding of the acetate methyl function in 2 may be readily understood since the acetyl group would lie under the aromatic chromophore. It is also notable that the aromeric nitrogen is pyramidal. This may explain some of the chemical shift differences noted in the ^{13}C NMR spectrum of 1 for C4a vs C7c, C4b vs C7b and C4c vs C7a.[6]

From the x-ray analysis it was not possible to assign the absolute configuration of 1. All attempts to hydrolyse the glycosidic linkage in 1 and recover the resultant glycoside failed. Consequently the absolute configuration was determined by total synthesis as reported in the accompanying communication.[7]

References and Notes

1. Details of the fermentation and biological evaluation of rebeccamycin will be published elsewhere. J.A. Bush, J.J. Catino, and W.T. Bradner, to be submitted to J. Antibiotics.
2. Compounds 1 and 2 exhibited satisfactory elemental analyses. While 1 exhibited a molecular ion in CIMS compound 2 only showed ions at M/e 394 and M/e 436 for the aglycone and acetylated aglycone respectively.
3. All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. For a listing of principal programs employed see: D.M. Balitz, F.A. O'Herron, J. Bush, D.M. Vyas, D.E. Nettleton, R.E. Grulich, W.T. Bradner, T.W. Doyle, E. Arnold and J. Clardy, J. Antibiotics 34, 1544-1555 (1981).
4. Karle, J. Acta Crystallogr. 1968, B24, 182.
5. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, ENGLAND CB2 1EW and are available from them.
6. The 360.13 MHz ^{1}H and 90.6 MHz ^{13}C NMR spectra were obtained using a Bruker WM360WB FTNMR spectrometer equipped with an ASPECT 2000A computer. The chemical shifts are relative to internal $(CH_3)_4$ Si. The proton assignments were confirmed by 2D homonuclear shift correlated spectroscopy and the carbon assignments were confirmed by 2D heteronuclear shift correlated spectroscopy.
7. Kaneko, T., H. Wong, K.T. Okamoto and J. Clardy, next paper.

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