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Structure of Angelmicin B, A Novel *src* Signal Transduction Inhibitor

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Abstract: The structure of angelmicin B, which has been found to be a specific inhibitor of tyrosine kinase and *src*-dependent cell transformation, was determined. Angelmicin B consists of six deoxyhexoses and eight fused six-membered rings containing highly oxidized naphthylnaphthoquinone as a chromophore. Copyright © 1996 Elsevier Science Ltd

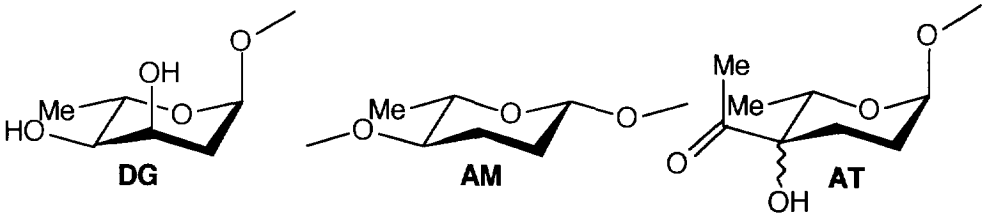
Angelmicin B (AGB), an inhibitor of specific oncogenic signal transduction, was isolated along with angelmicin A_1 and A_2 from a culture broth of a strain of *Microbispora* sp. in 1993[1,2]. Angelmicins suppress *src*-dependent cell transformation and inhibit tyrosine kinases which have been known as key enzymes for tumor formation. Recently, it has been reported that angelmicins induce differentiation of the human myeroid leukemia cell line HL-60[3]. Although such interesting biochemical properties had been reported, the structures were unknown. We wish to report the chemical structure of angelmicin B in this paper.

FAB-MS analysis of AGB gave $[M]^+$, $[M+H]^+$, $[M+Na]^+$ and $[M]^-$ at *m/z* 1724.7, 1725.7, 1747.8 and 1724.7, respectively. Elemental analysis showed that AGB did not contain nitrogen. The molecular formula of AGB was determined to be $C_{85}H_{112}O_{37}$ based on these data and the ^{13}C- and ^1H-NMR. The UV and visible spectra of AGB in MeOH showed absorbance bands at 240, 278, 432 and 511 nm. In alkaline MeOH solution, the band at 511 nm disappeared and two bands appeared at 614 and 647 nm. The IR spectrum showed absorbance bands at 3450, 1705 and 1620 cm^{-1}. This spectroscopic behavior indicated that AGB was a phenolic quinone.

The ^{13}C-NMR spectrum of AGB showed 85 signals of carbons containing 6 carbonyl, 17 aryl quaternary carbons and an aryl methine. In addition, six anomeric methine carbons and protons were observed in the ^{13}C- and ^1H-NMR spectra indicating that AGB contained six sugars.

2D-NMR spectra such as PFG-DQF-COSY, TOCSY, CH-COSY and PFG-HMBC suggested that AGB contained β-amicetopyranosyl (AM), α-digitoxopyranosyl (DG)

and α-4-*C*-acetyl-2,3,6-trideoxyhexopyranosyl (AT) residues in pairs. The anomeric configurations of these sugars were determined by the coupling constants ($J_{1,2}$). Although the relative configuration at C-4 of AT residues has not been determined, chemical shifts of H-5, 6 and 8 in the residues were close to those of synthetic methyl α-4-*C*-acetyl-2,3,6-trideoxy-*threo*-hexopyranoside[4] which had an axial hydroxy group and an equatorial acetyl group at C-4. Absolute configurations of the sugars have not yet been determined.



The aglycon of AGB was divided into two similar units, which we call the east and west units for convenience. Two *n*-propyl groups (C-19,20,21 and C-19',20',21') and two C_5 units (C-8,9,10,11,12 and C-8',9',10',11',12') were assigned by COSY, TOCSY and decoupling experiments. The HMBC spectrum indicated that these two C_5 units, hydroxyl quaternary carbons C-13,13',14 and 14'(79.44, 82.69, 77.19, 85.73 ppm), and carbonyl carbons C-15 and 15' (203.44 and 195.54 ppm) composed ring A-B and G-H. The HMBC spectrum also showed correlation peaks between H-19'b (1.09 ppm) and C-13', indicating that a *n*-propyl group was attached to C-13' (east unit). NOE of H-12 and H-AM1 was observed when H-21 was saturated, indicating that the other propyl group bonded to C-13 (west unit). In the east unit, there was an ether bridge between C-13' and 8', while a corresponding oxygen atom in the west unit formed a hydroxyl group at C-13 instead of the ether.

NOESY and HMBC experiments indicated that the sugars were linked to east and west units symmetrically; two α-digitoxoses were attached to C-10 and 10', respectively, and α-4-*C*-acetylhexoses were attached to C-4 of β-amicetoses which were linked to C-12' and 12.

The HMBC spectrum indicated that C-8 and 8' connected to aromatic rings F and C, respectively. It was assumed that carbonyl carbons C-15 and 15' in ring G and B also bonded to the aromatic ring on the basis of their chemical shifts. The structures of rings E and F were determined by the HMBC experiment except for a fragment C-3-C-4-C-5 in ring E. The structure of the fragment was elucidated by NOE between methoxyl protons at C-3 and 4, and by double LSPD experiments to detect $^2J_{H\text{-}6,\,C\text{-}5}$, $^4J_{CH3O\text{-}4,C\text{-}5}$ and $^4J_{OH\text{-}1,\,C\text{-}3}$ which could not be detected by HMBC experiment. Thus, the structure of the west unit was determined.

The structure of ring C was also elucidated by the HMBC experiment. Consideration of the chemical shift of carbons in ring C indicated that carbonyl carbons (184.42 and 187.82 ppm) bonded to C-18' and C-5', respectively. Hetero nuclear NOEs were observed at C-5', 6' and the carbonyl carbon at 184.82 ppm when HO-6' was irradiated, indicating that the carbonyl carbon (184.82 ppm) was C-4' and the residual carbonyl carbon at 187.82 ppm, therefore, should be C-1'. It was elucidated that the residual three carbons, a methoxylaryl group (60.79 and 158.40

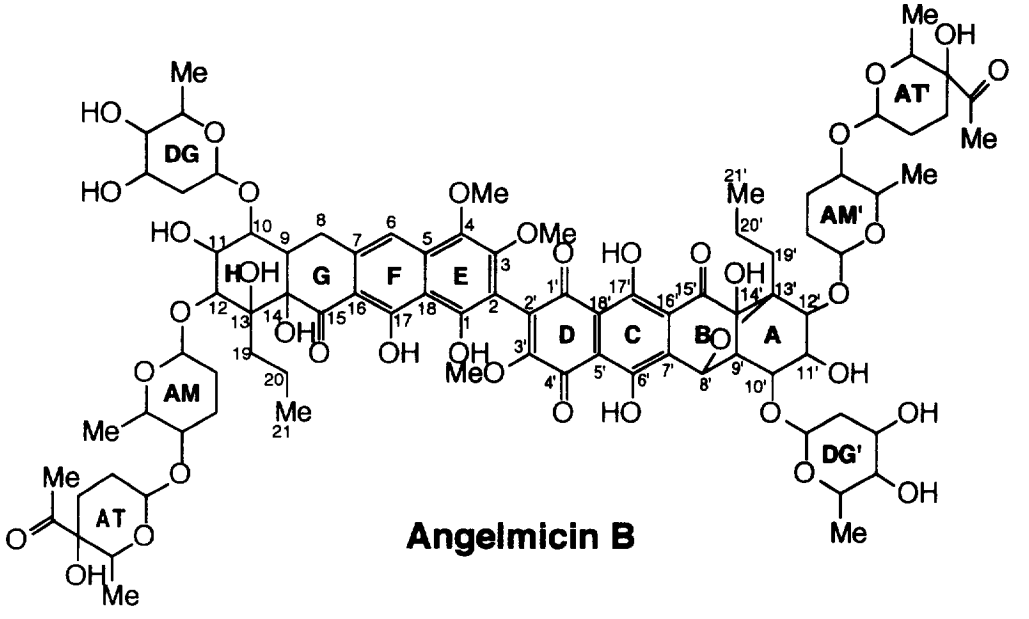
Table ^{1}H and ^{13}C chemical shifts (ppm) of angelmicin B in $CDCl_3$

West	^{13}C	^{1}H	East	^{13}C	^{1}H	OH	^{1}H
1	152.05		1'	187.82		1-OH	9.700
2	107.93		2'	125.62		11-OH	4.680
3	153.32		3'	158.40		13-OH	1.974
4	138.56		4'	184.82		14-OH	3.934
5	135.44		5'	116.28		17-OH	14.980
6	111.97	7.379	6'	150.90		6'-OH	12.215
7	139.34		7'	147.98		11'-OH	4.822
8	27.79	3.768,3.038	8'	67.85	5.836	14'-OH	4.375
9	44.51	2.693	9'	55.79	2.645	17'-OH	13.945
10	76.18	4.223	10'	77.03	4.160		
11	70.75	4.151	11'	75.34	4.098		
12	86.60	3.755	12'	85.44	3.954		
13	79.44		13'	82.69			
14	77.19		14'	85.73			
15	203.44		15'	195.54			
16	110.54		16'	124.84			
17	164.34		17'	157.23			
18	108.37		18'	112.98			
19	37.11	1.52,1.68	19'	34.25	1.09,1.84		
20	18.00	1.15,1.37	20'	16.58	0.99,1.33		
21	15.12	0.872	21'	14.88	0.789		
3-OMe	60.87	3.973	3'-OMe	60.79	3.950		
4-OMe	61.12	3.873					
DG1	98.63	5.428	DG1'	98.95	5.350	DG3-OH	3.727
DG2	34.81	1.956,2.431	DG2'	35.29	1.95,2.405	DG4-OH	2.497
DG3	67.27	4.03	DG3'	67.12	3.995	AT4-OH	d 3.527
DG4	72.83	3.167	DG4'	72.59	3.125	DG3'-OH	3.382
DG5	65.15	3.820	DG5'	65.00	3.685	DG4'-OH	2.500
DG6	17.85	1.294	DG6'	17.66	1.150	AT4'-OH	d 3.541
AM1	103.24	4.524	AM1'	103.24	4.460		
AM2	a 30.38	1.92,1.68	AM2'	a 30.53	1.88,1.76		
AM3	29.43	1.63,2.20	AM3'	29.49	1.63,2.20		
AM4	78.75	3.260	AM4'	78.88	3.260		
AM5	75.42	3.535	AM5'	75.21	3.527		
AM6	18.12	1.331	AM6'	18.09	1.331		
AT1	98.91	4.940	AT1'	98.91	4.940		
AT2	24.81	1.70,2.03	AT2'	24.81	1.70,2.03		
AT3	27.79	1.50,2.22	AT3'	27.79	1.50,2.22		
AT4	78.62		AT4'	78.62			
AT5	66.75	4.275	AT5'	66.68	4.275		
AT6	14.64	b 0.946	AT6'	14.64	b 0.952		
AT7	210.59		AT7'	210.59			
AT8	e 25.04	c 2.245	AT8'	e 25.01	c 2.252		

a-e; interchangeable. The NMR spectra were measured in $CDCl_3$ or ^{12}C enriched $CDCl_3$ at room temperature at 270 and 400MHz for ^{1}H, and 67.8 and 100 MHz for ^{13}C except for the PFG-HMBC experiment at 600MHz.

ppm) and an aryl carbon (125.62 ppm) comprised ring D because there was no other possibility. The position of the methoxyl group on ring D was determined by comparison of the chemical shift of the carbonyl carbons in ring D with those of an analogous compound such as *O*-methyllapachol; HMBC spectrum of *O*-methyllapachol indicated that the chemical shift of α-methoxylated quinone carbonyl was smaller than that of α-alkylated quinone carbonyl. Thus, the structure of the east unit was determined. The east and west units were joined together by a bond between C-2(ring E) and C-2' (ring D).

In conclusion, we determined the structure of angelmicin B as shown below. The structure of other angelmicins and the stereochemistry of angelmicin B are now under investigation.



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