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STUDIES ON ARUGOMYCIN, A NEW ANTHRACYCLINE ANTIBIOTIC
PART II. STRUCTURAL ELUCIDATION OF ARUGOMYCIN

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Summary: The total structure of arugomycin has been determined as shown in Fig. 1 based on spectroscopic data and chemical degradation.

In the previous paper[1] we reported the structures of degradation products of arugomycin (AGM)[2], i.e. arugorol, AG1, AG2 and AG3 based on the ^{1}H- and ^{13}C-NMR and mass spectral analysis. This paper describes structural determination of an additional hydrolysis product, AG4, and hydrogenolysis products, compounds A and B; these compounds gave enough experimental evidence to establish the total structure of AGM (Fig. 1).

The physicochemical properties of AGM are as follows; mp. 208-212°C, $[\alpha]_D^{25}$ +112° (c 0.1, $CHCl_3$:MeOH = 9:1), $C_{80}H_{110}O_{37}N_2$, Anal. found: C 56.22, H 6.85, O 35.14, N 1.65 %, calcd.: C 56.71, H 6.67, O 34.97, N 1.65 %, SIMS m/z 1694 (MH^+), IR ν_{max}^{KBr} 3430, 2930, 1740, 1660, 1545, 1450, 1410, 1380, 1300, 1105 and 1000 cm^{-1}, UV λ_{max}^{MeOH} 235nm (ε 61500), 258(28300), 292 (10300) and 476 (17600).

In addition to the sp^2 carbons in the chromophore unit (Table 1, C-1 to C-13, $-COOCH_3$, C-1' to C-6' and $(CH_3)_2N$-), AGM contains two carbonyl (δ_C 166.0 and 168.9) and two olefinic methine carbons (δ_C 134.3 and 139.3) which were assigned to a fumaric acid residue based on comparison with the literature value[3]. The olefinic protons observed as an AB-type quartet at δ_H 6.81 and 6.86 (J=17 Hz) are in agreement with this partial structure.

Methanolysis of AGM gave inter alia a methyl ester of methyl 4-O-fumaryl-α-L-diginoside[4], $C_{13}H_{20}O_7$. Since a resonance corresponding to the methyl ester signal (δ_H 3.81) in this compound was not found in the ^{1}H-NMR spectrum of AGM, and since AGM was methylated by diazomethane to give a methyl ester of AGM Me-AGM[5], $C_{81}H_{114}O_{37}N_2$, one of the carboxyl functions of the fumaric acid residue must be free in the parent compound. Consequently the 4-O-fumaryl-α-L-diginose moiety must be glycosidically combined to the terminal of a sugar chain.

The SI-mass spectrum of AG4[6] ($C_{62}H_{86}O_{28}N_2$) obtained as a partial hydrolysis product of AGM[1] gave the protonated molecule at 1308 (MH^+) which is

Table 1. 100MHz ^{13}C-NMR data of compound A (in $CDCl_3$), AG4 (in $CDCl_3$), AGM (in d_5-pyridine)

		A	AG4	AGM
1		147.7	147.6	147.9
2		139.4	139.4	140.1
3		121.8	121.7	-**
4		155.9	155.5	156.6
4a		116.6	116.4	117.8
5		190.9	190.0	191.8
5a		112.6	113.2	114.8
6		159.8	161.4	162.2
6a		131.2	131.2	131.2
7		21.0	71.2	70.6
8		33.4	41.0	39.9
9		70.5	70.7	69.3
10		55.8	55.6	59.0
10a		141.8	142.5	144.4
11		119.2	119.0	119.8
11a		133.0	132.8	131.2
12		114.6	114.2	115.3
13		28.9	29.3	30.0
COO-		173.0	171.7	171.9
-OCH_3		52.9	52.7	52.0
1'		97.4	97.3	97.9
2'		68.8	68.5	67.4
3'		61.5	61.3	62.5
4'		81.6	81.3	81.6
5'		77.2	77.5	77.2
6'		24.0	24.2	23.6
NCH_3		44.7	44.3	44.3
C=				139.3
C=				134.3
COOH				168.9
OCO-				166.0
S-1	C-1	99.6	99.4	100.0
	C-2	33.9	33.7	34.6
	C-3	64.8	64.6	65.0
	C-4	82.9	82.5	82.3
	C-5	67.5	67.9*	67.9
	C-6	17.0	16.8	17.1
S-2	C-1	99.1	99.2	99.7
	C-2	42.1	41.8	41.9
	C-3	88.7	88.5	89.5
	C-4	83.9	83.6	83.6
	C-5	69.0	70.3	70.6
	C-6	19.0	18.8	18.6
	-CH_3	25.4	25.2	24.9
S-3	C-1	101.6	101.4	102.3
	C-2	30.3	30.9	30.9*
	C-3	74.3	74.4	75.4*
	C-4	67.2	67.3*	67.5
	C-5	66.9	66.1*	67.2
	C-6	17.3	17.0	17.3
	-OCH_3	55.8	56.3	56.1*
S-4	C-1		100.7	102.1
	C-2		30.1	31.9*
	C-3		74.1	74.9*
	C-4		75.3	75.9*
	C-5		67.9*	68.5*
	C-6		17.6*	17.6*
	-OCH_3		56.8	56.1
S-5	C-1		99.2	100.0
	C-2		32.4	34.4*
	C-3		65.8	65.8
	C-4		67.9*	81.1
	C-5		67.3*	67.4
	C-6		17.6*	17.2*
S-6	C-1			100.4*
	C-2			31.9*
	C-3			74.9*
	C-4			75.3*
	C-5			68.3*
	C-6			17.6*
	-OCH_3			56.1*
S-7	C-1			100.0*
	C-2			31.7*
	C-3			73.8
	C-4			72.3
	C-5			65.4
	C-6			17.6*
	-OCH_3			55.9*

*Tentative assignments

**Obscured by the solvent peak

larger than AG2[1] by 130u. Since hydrolysis of AG4 gave 2-deoxy-fucose(deFUC), diginose(DIG), and decilonitrose (DEC), this difference corresponds to the incarement of a deFUC unit. The ^{1}H- and ^{13}C-NMR spectra of AG4 revealed five anomeric signals (δ_C 99.2, 99.2, 99.4, 100.7 and 101.4, δ_H 4.98, 5.01, 5.25, 5.32 and 5.52), two methoxy signals due to DIG (δ_C 56.2 and 56.3) and one characteristic tertiary CH_3 signal due to DEC (δ_C 25.2, δ_H 1.75 singlet). These data indicate that AG4 is a mono deoxyfucosyl derivative of AG2 comprising arugorol (ARG), DEC, deFUC and DIG in the ratio of 1:1:2:2. Comparison of the ^{13}C-NMR spectra of AG2 and AG4 revealed the glycosidation shift[7] of C-4 of DIG from δ_C 68.2[1] to 75.3 in AG4. However, since there exist two terminal DIG units (S-3 and S-4) with almost identical ^{13}C chemical shifts in AG2, it was impossible to determine which of the terminal sugars in AG2 is glycosydated by deFUC(S-5) in AG4. Thus, AGM was subjected to catalytic hydrogenation (5 % $Pd/BaSO_4$) which resulted in the formation of hydrogenolysis products, compound A and compound B.

The ^{13}C-NMR spectrum of A[8] ($C_{49}H_{64}O_{21}N_2$) in $CDCl_3$ indicated the presence of a new methylene group at δ_C 20.7 due to C-7 of the chromophore and three anomeric carbons (δ_C 99.1, 99.6 and 101.6, δ_H 4.97, 5.25 and 5.33). The SI-mass spectrum of A showed the molecular ion peak at m/z 1018. These spectral data

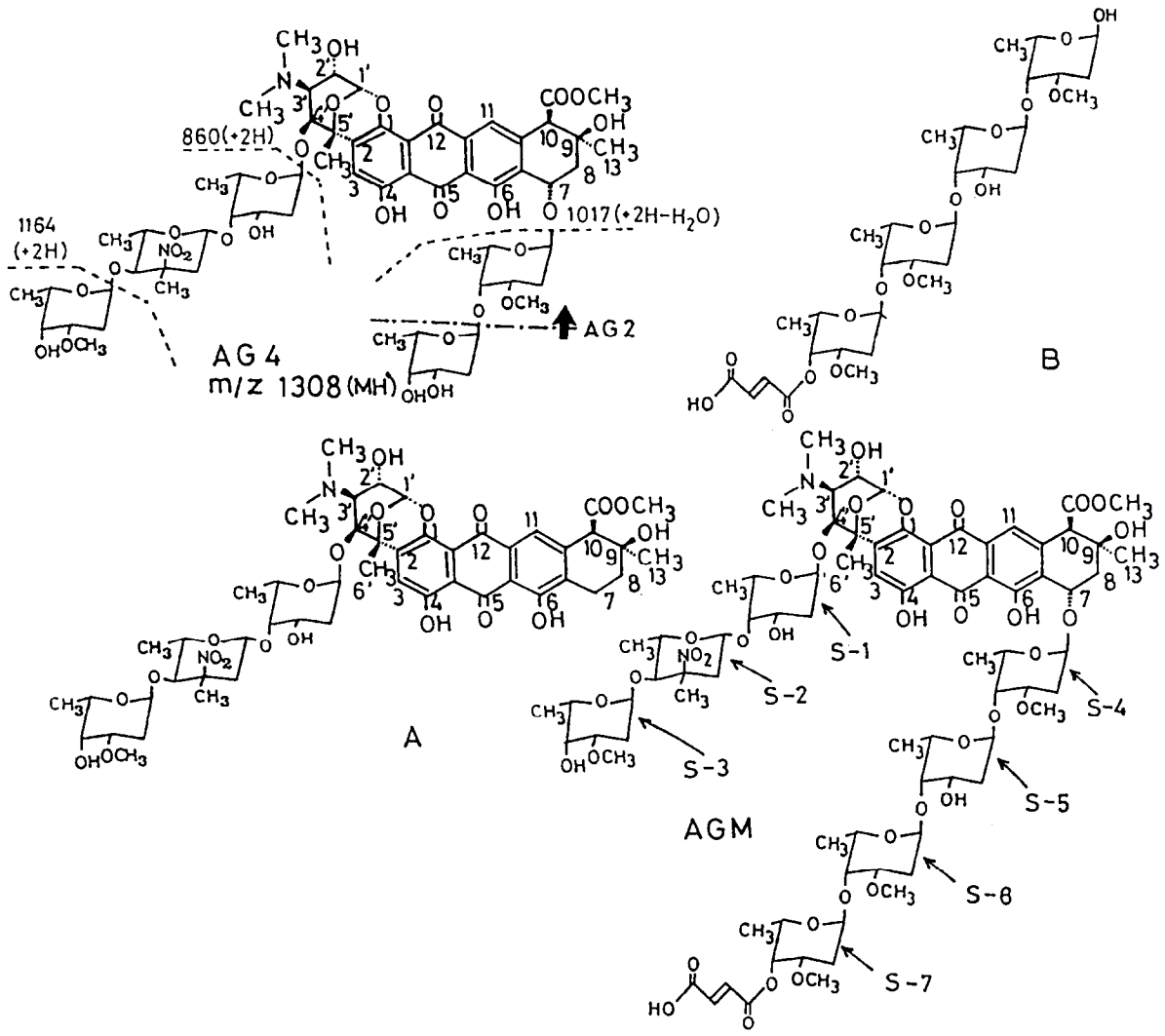

Fig. 1. SIMS-diagnostic ions of AG4 and the structures of AG4, A, B, and AGM.

proved the structure of A to be 4'-(L-diginosyl-L-decilonitrosyl-2-deoxyfucosyl)-7-deoxyarugorol (Fig. 1). Therefore, the additional deFUC(S-5) moiety is combined to C-4 of DIG(S-4) in AG2 and consequently, the structure of AG4 is illustrated as shown in Fig. 1. This conclusion was supported by detailed analysis of the SI-mass diagnostic ions at m/z 860, 1017, 1164 and 1308 (see Fig. 1). The anomeric configuration of deFuc(S-5) was determined to be α[H-1 of DIG(S-5); $J_{1,2ax}$=2.0, $J_{1,\ 2eq}$=< 1Hz].

Compound B[9] ($C_{31}H_{50}O_{16}$) which was isolated as an anomeric mixture, gave DIG and deFUC on acid hydrolysis (40% HCOOH, 85°C, 40 min). The ^{1}H-NMR spectrum of B proved the presence of four sugars [δ_H 4.76, dd, J=10.4, 2.0 Hz (β-form) δ_H 5.42, dd, J=2.0, <1 Hz(α-form), δ_H 4.97, dd, J=2.0, <1 Hz, δ_H 4.99, dd, J=2.0, <1 Hz, δ_H 5.05, dd, J=2.0, <1 Hz] and three methoxy groups (δ_H 3.36, 3.41, 3.42) due to DIG. The IR spectrum of B gave absorption at 2400 - 2800, and 1720 cm^{-1}. Furthermore, B showed a characteristic AB-type quartet at δ_H

6.80 and 6.90 (J=17 Hz) arising from a fumaric acid half ester residue. Thus B consists of one mole each of fumaric acid and deFUC and three moles of DIG. Since the hydroxy group at C-3 of deFUC(S-5) in B was revealed to be free by ^{1}H-NMR spectral analysis of its diacetyl derivative (vide infra) and, since the linkage of deFUC to DIG has been established in AG4, the 4-O-fumaryl-DIG-DIG unit must be attached to C-4 of deFUC(S-5).

Reaction of B with acetic anhydride in pyridine gave an anomeric mixture of diacetate. Since the separation of these anomers was very difficult, the diacetate was analyzed without further purification. In its ^{1}H-NMR spectrum, two acylated proton signals were observed at δ_H 6.24 and 5.67 (the total area of these anomeric proton signals being equal to 1H), and 5.21. Irradiation of the proton at 5.21 collapsed the methylene signal (ddd) at δ_H 1.95 and 2.10 to double doublets* and thus, the acylated proton was unambiguously assigned to H-3 of 2-deFuc. Therefore, the fumaryl-Dig(S-7)-DIG(S-6) unit must be attached to deFUC(S-5) through an α-1,4 linkage and the structure of B is represented as shown in Fig. 1.

Based on these experimental results, the structure of AGM has been established as shown in Fig. 1 with some ambiguity about the absolute configuration.

* Due to the overlapping of complicated signals in this region, the changes of signals were detected by difference spectral method.

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REFERENCES AND FOOTNOTES

1) H. Kawai et al., The preceding paper.
2) H. Kawai et al., J. Antibiotics, 36, 1569 (1983).
3) H. Seto et al., Tetrahedron Lett. 23, 2667 (1982).
4) Methyl 4-O-fumaryl-α-L-diginoside methyl ester : $[\alpha]_D^{21}$ = -149° (c 0.6, MeOH), M.W. (EI-mass) m/z 288 (M^+), oily substance.
5) Me-AGM : $[\alpha]_D^{22}$ = +219°, m.p. 214-215°C, M.W. 1708, UV λ_{max}^{MeOH} 234 nm(ε53500), 257(25100), 289(16600), and 478(14200).
6) AG4 : $[\alpha]_D^{22}$ = +330°, m.p. 209-210°C, M.W. 1307, UV λ_{max}^{MeOH} 235 nm (ε52700), 257(24300), 290(9500), and 478(16100).
7) S. Seo et al., J. Amer. Chem. Soc. 100, 3331 (1978).
8) Compound A : $[\alpha]_D^{21}$ = +393° (c 0.1, MeOH), m.p. 165-168°C, M.W. 1017, UV λ_{max}^{MeOH} 235 nm(ε54900), 248(28500), 292(11900), and 478(16000).
9) Compound B : $[\alpha]_D^{21}$ = -93.9° (c 0.1, MeOH), m.p. 115-118°C.

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