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THE STRUCTURE OF ARUGOMYCIN, A NEW ANTHRACYCLINE ANTIBIOTIC PART I. STRUCTURAL ELUCIDATION OF DEGRADATION PRODUCTS, AG1, AG2 AND AG3.

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Summary: Based on ^{1}H- and ^{13}C-NMR and mass spectral analysis and chemical degradation, the structures of degradation products of arugomycin (arugorol, AG1, AG2 and AG3) have been determined as shown in Fig. 1.

In the previous paper[1] we reported the isolation of a new anthracycline antibiotic arugomycin produced by Streptomyces violochromogenes 1098-AV2. Arugomycin showed antibacterial activity against Gram-positive bacteria and marked inhibitory effect on Ehrlich ascite carcinoma. This paper describes structural analysis of several degradation products of arugomycin.

The physicochemical properties of arugomycin (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} 235 nm (ε 61500), 258(28300), 292 (10300) and 476 (17600).

Acid hydrolysis of AGM (40% HCOOH at 85° C, 40 min) gave a mixture of the aglycone and sugar moieties which were separated by Diaion HP-20 column chromatography. Development of the column with MeOH gave a red fraction, from which, after an appropriate work up, the chromophore, arugorol(AGR) was obtained. The physicochemical properties of AGR are as follows; mp. 210-212° C, $[\alpha]_D^{21}$= +483°, SIMS m/z 586 (MH^+), $C_{29}H_{31}O_{12}N$, UV λ_{max}^{MeOH} 236nm (ε 43000), 258 (20000), 292(7500), and 475(12800). As shown in table 1, the ^{13}C-NMR spectrum of AGR is very close to that of nogalarol(NOG)[2] except for the amino sugar moiety [C-2' to C-6' and $N(CH_3)_2$]. Taking into account of the large upfield shift of C-2' (73.5 in NOG to 67.4 in AGR), these chemical shift differences may be ascribed to the stereochemical change of C-4' (γ-effect). The ^{1}H-NMR spectrum of AGR displayed the following resonances:(400MHz, in CD_3OD) 1.50(s, 3H, H-6'), 1.68(s, 3H, H-13), 1.96(dd, J=4.0, 14.0Hz, 1H, H-8a), 2.55(dd, J=5.1, 14.0Hz, 1H, H-8b), 2.58(s, 6H, $-N(CH_3)_2$), 2.95(dd, J=2.6, 11.0Hz, 1H, H-3'), 3.68(s, 3H, $-COOCH_3$), 3.80(s, 1H, H-10), 4.07(d, J=2.6Hz, 1H, H-4'), 4.42(dd, J=3.0, 11.0Hz, 1H, H-2'), 5.21(dd, J=4.0, 5.1Hz, 1H, H-7), 5.88(d, J=3.0Hz, 1H, H-1'), 6.72(s, 1H, H-11), and 7.25(s, 1H, H-3). These signals indicate that the amino sugar moiety is 3,6-dideoxy-3-dimethylamino-galactopyranose with axial configurations for H-2', H-3' and an equatorial configuration for H-4' as shown in Fig. 1 with the uncertainty about the absolute configuration. Thus AGR is the 4'-epimer of nogalarol, the chromophore of nogalamycin[2].

The carbohydrate fraction contained in the HP-20 effluent was separated by

silica gel column chromatography ($CHCl_3$:MeOH = 9:1) to give three sugars which were identified as L-2-deoxyfucose[3,4] (deFUC), L-diginose[5] (DIG) and L-decilonitrose[6] (DEC) by ^{1}H-NMR spectral analysis, their optical rotation and comparison with authentic samples.

On mild acid hydrolysis with 50% CH_3COOH at 85° C for 20 min, AGM gave a mixture of red pigments named AG1[7], AG2[8], AG3[9] and AG4 which were separated by preparative silica gel TLC ($CHCl_3$:MeOH = 10:1) followed by column chromatography (Toyopearl HW-40, developed with MeOH). The ^{13}C-NMR spectral data of these compounds are summarized in Table 1, and that of AG4 will be reported in next paper.

Table1. Assignments of ^{13}C-NMR signals of AGR (CD_3OD), AG1, AG2 and AG3 ($CDCl_3$)

c	NOG[2]	AGR	AG1	AG3	AG2	(T_1)#
1	148.0	147.5	147.8	147.6	147.5	(1.46)
2	138.1	138.6	139.6	139.4	139.4	(0.89)
3	125.2	123.8	122.1	121.7	121.8	(0.18)
4	155.7	157.0	155.8	155.5	156.2	(1.75)
4a	117.2	117.8	116.3	116.4	117.0	(3.09)
5	192.3	191.1	190.5	190.1	190.9	-
5a	114.6	114.5	113.8	113.8	114.2	(1.24)
6	161.6	161.4	159.7	161.2	161.8	(2.51)
6a	131.3	133.4	132.6	131.3	131.5	(1.67)
7	63.0	63.8	63.9	71.4	71.4	(0.29)
8	40.9	40.9	41.6	41.5	41.2	(0.14)
9	69.7	70.5	70.5	70.4	70.5	-
10	56.9	59.0	56.3	56.7	56.5	(0.28)
10a	144.0	143.8	141.7	142.5	143.0	(1.36)
11	119.8	120.3	119.0	118.3	119.0	(0.25)
11a	134.1	133.9	133.4	132.8	133.4	(2.57)
12	179.8	180.1	179.1	178.7	179.8	(0.73)
12a	115.4	115.7	114.3	114.1	114.7	(1.41)
13	29.7	30.1	29.1	29.1	28.9	(0.27)
COO-	171.9	172.3	172.5	171.9	172.4	(3.84)
$-OCH_3$	52.4	52.9	52.9	52.8	52.7	(0.87)
1'	97.8	97.5	97.4	97.1	97.0	(0.23)
2'	73.5	67.4	67.0	68.2	68.5	(0.24)
3'	66.8	64.7	61.5	61.4	62.1	(0.25)
4'	70.8	73.2	81.4	81.3	81.0	(0.29)
5'	76.0	77.5	77.3	77.8	77.7	-
6'	24.2	22.6	24.0	24.2	23.8	(0.13)
$N-CH_3$	41.4	43.1	44.5	44.4	44.1	-
S-1		deFUC[4]				
C-1		99.1	99.1	99.2	99.5	(0.32)
C-2		33.0	33.7	33.6	33.7	(0.20)
C-3		65.9	64.6	64.5	64.6	(0.23)
C-4		71.6	82.6	82.6	82.7	(0.26)
C-5		68.4	68.8	66.9	67.1	-
C-6		17.9	17.1	17.1	16.6	(0.61)
S-2		DEC[6]				
C-1		98.6	99.3	99.2	99.6	(0.36)
C-2		41.7	41.6	41.5	41.8	(0.24)
C-3		88.6	89.4	89.2	88.7	(2.62)
C-4		77.0	76.1	76.7	83.8	(0.45)
C-5		71.1	71.4	71.4	70.3	-
C-6		18.3	18.6	18.5	18.6	(0.59)
$-CH_3$		25.2	25.2	25.2	25.1	(0.53)

c	DIG[6]	AG3	AG2	(T_1)#
S-3				
C-1	98.7		101.7	(0.43)
C-2	29.4		30.0	(0.22)
C-3	74.8		74.1	(0.41)
C-4	67.8		67.3	(0.43)
C-5	65.5		67.1	-
C-6	16.8		16.8	-
$-OCH_3$	54.8		55.6	-
S-4				
C-1	98.7	100.9	101.0	(0.33)
C-2	29.4	29.7	29.6	(0.13)
C-3	74.8	74.9	74.9	(0.27)
C-4	67.8	68.2	68.1	(0.34)
C-5	65.5	66.9	66.6	(0.37)
C-6	16.8	17.1	16.8	-
$-OCH_3$	54.8	55.7	55.6	-

#T_1 was obtained by inversion recovery method

Hydrolysis of AG1 ($C_{42}H_{52}O_{19}N_2$, Fig. 1) under a stronger condition (40% HCOOH, 85° C, 40 min) gave AGR, deFUC and DEC. The ^{1}H- and ^{13}C-NMR spectra of AG1 demonstrated that it consists of one mole each of these components [anomeric protons, δ_H H-1(S-1)* 5.36 and H-1 (S-2) 4.68, anomeric carbons δ_C C-1(S-1) 99.1 and C-1(S-2) 99.3]. Comparison of the ^{13}C-NMR data of AG1 and NOG revealed the glycosidation shift of C-4' (δ_C 73.2 in AGR vs. 81.4 in AG1) but

*For numbering of sugar units, see Fig.1.

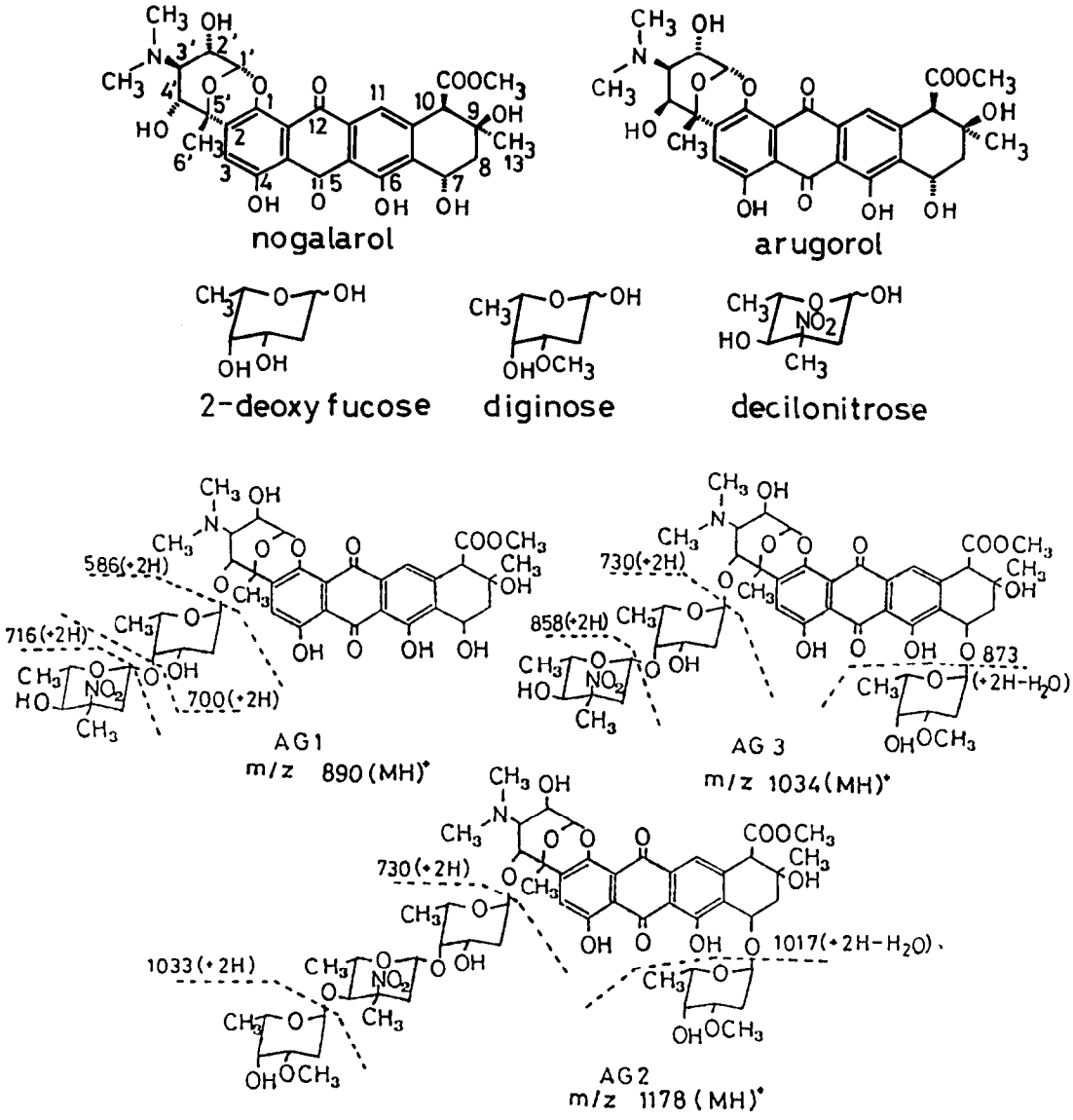

Fig. 1. The structures and SIMS-diagnostic ions of AG1, AG2 and AG3

not of C-7 (δ_C63.8 vs. 63.9) and C-2'(67.4 vs. 67.0) in the aglycone part. This evidence suggested the attachment of the carbohydrate moiety only to C-4' of the chromophore. The similar glycosidation shift[10] was observed with C-4 of deFUC(S-1) from δ_C 71.6 to 82.6 suggesting the linkage of DEC to this carbon. The SI-mass spectrum of AG1 showed the protonated molecule (MH^+) at m/z 890 and fragmentation peaks at m/z 586, 700 and 716 due to aglycone - deFuc(S-1) and deFuc(S-1) - DEC(S-2) bond cleavages (see Fig. 1). The anomeric configurations of deFUC(S-1) and DEC(S-2) were determined to be α and β, respectively, based on the coupling constants of the anomeric protons [H-1 of deFuc(S-1); $J_{1,2ax}$=2.0, $J_{1,2eq}$=<1 Hz, H-1 of DEC(S-2); $J_{1,2ax}$=11.4, $J_{1,2eq}$=<1 Hz]. Thus, the structure of AG1 has been determined to be as shown in Fig. 1.

Hydrolysis of AG3[9] ($C_{49}H_{64}O_{22}N_2$, Fig. 1) gave DIG in addition to the components of AG1, i.e. AGR, deFUC and DEC. The ^{1}H- and ^{13}C-NMR spectra of AG3

showed that it consists of one mole each of these components (anomeric signals, δ_H 4.74, 5.32 and 5.46, δ_C 100.5, 100.9 and 102.6). Consequently AG3 is a mono diginosyl derivative of AG1. Comparison of the ^{13}C-NMR data of AG3 and AG1 revealed the glycosidation shift of C-7 in the aglycone part of AG3 from δ_C 63.9 to 71.4. This downfield shift is reasonably explained by positioning DIG at C-7 of AG1. The SI-mass spectrum of AG3 showed the protonated molecule ion peak at m/z 1034 (MH^+) and fragment peaks at m/z 730, 858 and 873 originating from the cleavages of the linkages between the aglycone - deFUC(S-1), deFUC(S-1) - DEC(S-2) and the aglycone - DIG(S-4) as shown in Fig 1. The glycosidic linkage between aglycone-DIG(S-4) is shown to be α[H-1 of DIG(S-4); $J_{1,2ax}$=2.0, $J_{1,2eq}$=<1 Hz].

The SI-mass spectrum of AG2[9] ($C_{56}H_{76}O_{25}N_2$, Fig. 1) gave the protonated molecule ion peak at m/z 1178 (MH^+) and fragment peaks at m/z 1033, 730 common to AG3. However, the fragment peak at m/z 873 in AG3 shifted to 1017 in AG2. The ^{1}H- and ^{13}C-NMR spectra revealed AG2 to consist of one mole each of AGR, deFUC and DEC, and two moles of DIG (δ_C 99.5, 99.6, 101.0 and 101.7, δ_H 4.98, 5.27, 5.33 and 5.48). Therefore AG2 contains one additional DIG moiety as compared with AG3. Comparison of the ^{13}C-NMR spectra of AG2 and AG3 proved the glycosidation shift of C-4 of DEC(S-2) from δ_C 76.7 to 83.8. The fragment ion peaks at m/z 730 and 1017 in the SI-mass spectrum of AG2 suggested that the deFUC(S-1) - DEC(S-2) - DIG(S-3) and DIG(S-4) moieties attached to C-4' and C-7 of the aglycone, respectively. The glycosidic linkage of DIG(S-3) moiety was shown to be α[H-1 of DIG(S-3); $J_{1,2ax}$=2.0 Hz, $J_{1,2eq}$=<1 Hz]. Thus, the structure of AG2 has been determined as shown in Fig. 1. The biological activities of AG1, AG2 and AG3 will be reported elsewhere.

The assignments of the ^{13}C-NMR spectra of AG1 and AG3 could be easily accomplished based on selective proton decoupling experiments and comparison with the literature values of the individual component. Distinction of the two DIG units in AG2 was made by T_1 values assuming that the longitudinal relaxation time (T_1) of S-4 would be shorter than S-3 which is located at the terminal of the longer sugar chain.

The structures of the other degradation products and total structure of arugomycin will be reported in the following paper.

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7) AG1: $[\alpha]_D^{22}$= +610°(c 0.1, MeOH), m.p. 235-236°C, M.W. 889, UV λ_{max}^{MeOH} 237 nm (ε63100), 255(34700), 290(11600), 478(15200).
8) AG3: $[\alpha]_D^{22}$= +430°(c 0.1, MeOH), m.p. 208-209°C, M.W. 1033, UV λ_{max}^{MeOH} 235 nm (ε47800), 257(22400), 290(8300), 478(14800).
9) AG2: $[\alpha]_D^{22}$= +369° (c 0.1, MeOH), m.p. 201-203°C, M.W. 1177, UV λ_{max}^{MeOH} 235 nm (ε47800), 259(22600), 293(7400), 478(13800), 546(13100).
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