STRUCTURE OF POLYETHER ANTIBIOTIC A28695B

Sir:

Polyether antibiotic A28695B (**1**) is the less abundant component of A28695 produced by *Streptomyces albus* NRRL 3883[1]. Extraction of the fermentation broth with organic solvents, followed by carbon-column chromatography, yielded a mixture, which was separated by silica-gel chromatography into **1** and A28695A (**2**, identical with septamycin);[2] **1** exhibits antibacterial, antifungal, antiviral, and insecticidal activity[1,3], and controls coccidial infections in poultry and rabbits[4]. The antibiotic is effective in improving feed efficiency in ruminants[5] and as an ionophore[1].

The field desorption (FD) mass spectrum of **1**, together with microanalysis, established a molecular formula of $C_{48}H_{82}O_{17}$ (Found: $(M+Na)^+$, 953.544. Calcd. for $C_{48}H_{82}O_{17}Na$, 953.545. Found: C, 60.49; H, 9.15; O, 31.32. Calcd. for $C_{48}H_{82}O_{17}\cdot H_2O$: C, 60.74; H, 8.92; O, 30.34). As **1** differs from **2** by having one more oxygen atom, it appears possible that **1** is hydroxy A28695A.

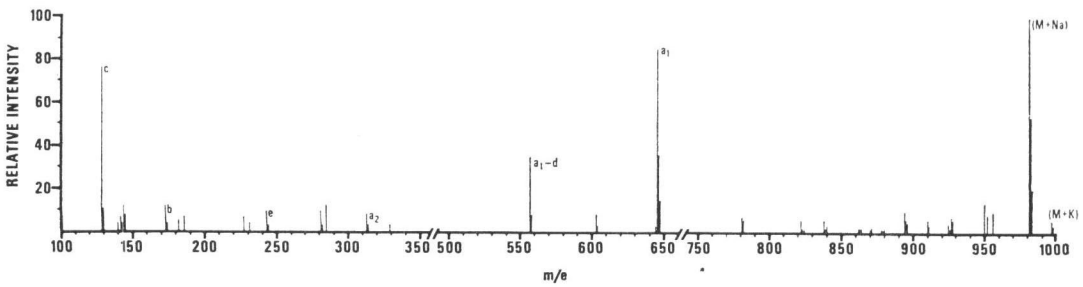
Previous experience[6] with the polyether antibiotics A204 and K-41, which are structurally similar to **2**, indicates that the FD spectra of derivatives of **1** would be useful in its structure determination. Treatment of **2** with MeOH for 16 hours, followed by diazomethane, yielded A28695A methyl ether methyl ester (**3**) molecular weight 942 by FDMS. Using emitter currents beyond the best anode temperature (BAT)[7], the FDMS of **3** showed the structurally significant peaks a_1 (*m/e* 645), a_2 (*m/e* 297), b (*m/e* 157), c (*m/e* 129), e (*m/e* 227), and (a_1-d) (*m/e* 557). When **2** was treated with CD_3OD, followed by diazomethane, A28695A deuteromethyl ether methyl ester (**4**) was obtained. The FDMS of **4** taken beyond the BAT had a_2, b, and e shifted to higher mass by 3 MU, supporting the assignments shown.

Treatment of **1** with MeOH, then diazomethane, yielded A28695B methyl ether methyl ester (**5**) whose FD spectrum showed (Fig. 1): $(M+Na)^+$ (*m/e* 981), a_1 (*m/e* 645), a_2 (*m/e* 313), b (*m/e* 173), c (*m/e* 129), e (*m/e* 243), and (a_1-d) (*m/e* 557). These data indicate that apart from an extra oxygen on ring A, **1** is the same as **2**. Further confirmation that ring A bears an extra oxygen came from the FDMS of **6**, where a_2, b, and e were shifted 3 MU to higher mass. Additionally, ion b had the expected composition, $C_9H_{14}D_3O_3$ (Found 176.1369, Calcd. 176.1366).

The above mass spectral data, coupled with the facts that **1** and **2** have similar properties and are produced by the same organism, suggest that **1** is an F-ring OH substituted A28695A. However, it was necessary to use other methods to support this proposal. Such support came from the ^{13}C and ^1H NMR spectra of the sodium salts of **1** and **2** which are quite similar, differing only in resonances that can be assigned to ring F (Tables 1 and 2). The spectra showed that **1** and **2** contain the same number of Me groups and that the multiplicity of the Me resonances in the ^1H spectra are the same, ruling out the possibilities that **1** is hydroxylated at C-26, C-28, or any of the Me groups on ring F. The ^{13}C NMR spectrum of **1** contained one more OCH and one less CH_2 resonances than did that of **2**. This result exclude the possibility of hydroxyla-

Fig. 1. Field desorption mass spectrum of A28695B methyl ether methyl ester (**5**).

All mass spectra were determined with a Varian-MAT 731 mass spectrometer using carbon dendrite deposited on 10 μM tungsten wires. Emitter currents were in the range of 10~15 mA. The peak matching experiment which determined the accurate mass of the cationized molecular ion of **1** was made using a co-field desorbed mass standard of A204 methyl ether methyl ester.



tion at C-25, and indicate that **1** is a 27-hydroxy A28695A.

Various observations; *viz.*, the reluctance of **1** to form an acetate, the absence of a free OH stretch in its IR spectrum measured in dilute CCl_4 solution, and chemical-shift differences at

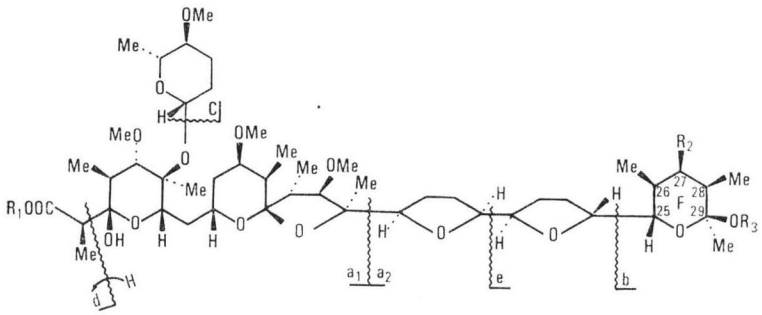


Table 1. ^{13}C chemical shifts (δ) of A28695 factors in $CDCl_3$[a].

Carbon No.[b]	Multiplicity[c]	Sodium salts		Sodium salts, methyl ethers		Carbon No.[b]	Multiplicity[c]	Sodium salts		Sodium salts, methyl ethers	
		A (2)	B (1)	A (2)	B (1)			A(2)	B (1)	A (2)	B (1)
C-1	s	180.5	180.6	180.3	180.4	C-2	d	45.4	45.5	44.7	44.6
C-13	s	106.6	106.7	106.7	106.6	C-4	d	40.7	40.6	40.7	40.5
C-3	s	99.5	99.5	99.8	99.8	C-28	d	39.6	42.2	40.2	43.0
C-29	s	96.8	99.1	99.1	101.8	C-12	d	36.9	36.8	37.0	36.8
C-1′	d	96.5	96.5	96.5	96.4	C-27	t(d)[d]	36.8	74.8	36.5	74.1
C-15	d	94.7	94.6	95.0	94.8	C-26	d	32.7	38.1	32.5	37.5
C-5	d	88.7	88.6	89.1	89.0	C-8[e]	t	32.5	32.3	32.2	32.3
C-17	d	83.3	83.3	82.8	82.7	C-2′[e]	t	31.8	31.7	31.8	31.7
C-16	s	83.2	83.3	83.8	83.7	C-10[e]	t	31.1	31.1	31.1	31.1
C-24	d	80.4	80.4	81.2	81.1	C-22[e]	t	29.2	29.1	28.4	28.3
C-4′	d	80.2	80.1	80.2	80.1	16-Me	q	28.4	28.3	28.4	28.2
C-6	s	80.2	80.1	80.1	80.1	C-3′	t	27.7	27.6	27.7	27.7
C-11	d	79.6	79.6	81.1	81.1	29-Me	q	26.4	26.2	21.7	21.5
C-21	d	79.2	79.2	79.3	79.2	C-19[e]	t	25.6	25.5	26.0	26.0
C-20	d	79.1	79.0	79.0	78.6	C-23[e]	t	24.2	23.9	24.6	24.4
C-25	d	75.4	70.6	78.4	74.1	C-18[e]	t	23.0	22.9	23.3	23.4
C-5′	d	74.3	74.2	74.2	74.1	5′-Me	q	18.5	18.4	18.5	18.3
C-7	d	67.4	67.5	67.2	67.2	26-Me	q	17.3	13.6	17.5	13.8
5-OMe	q	61.2	61.6	61.6	61.5	28-Me	q	16.9	13.6	16.3	13.0
C-9	d	61.5	61.4	62.0	61.9	12-Me[f]	q	12.6	12.6	12.7	12.6
15-OMe	q	60.1	60.0	60.0	60.0	4-Me[f]	q	12.0	11.9	11.9	11.9
11-OMe	q	58.9	58.7	58.6	58.5	2-Me[f]	q	11.5	11.4	11.6	11.5
4′-OMe	q	56.8	56.7	56.7	56.6	14-Me[f]	q	11.5	11.4	11.6	11.5
29-OMe	q	—	—	48.4	48.6	6-Me	q	9.9	9.9	9.8	9.8
C-14	d	46.0	46.0	46.4	46.3						

[a] ^{13}C FT NMR spectra were recorded on a Varian FT-80 and/or an XL-100-12A spectrometer at 20 and 25.160 MHz, respectively. Typical FT conditions on the latter are: spectral width, 6016 Hz; acquisition time, 0.8 sec; pulse width, 8 μsec (flipping angle, 19°); number of data points, 9625.

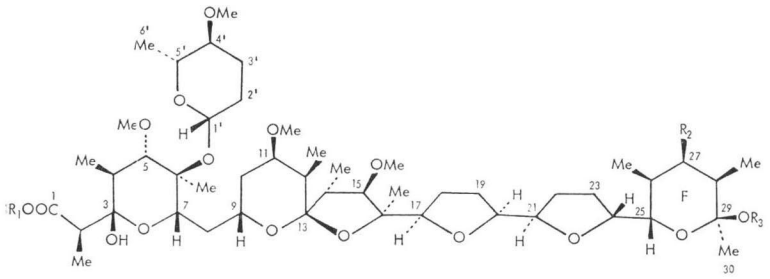
[b] Signal assignments were carried out by ^1H single-frequency off-resonance and selective decoupling techniques and by chemical-shift comparisons with other polyethers of known structures whose chemical-shift assignments are reported,[9] as well as with the A28695 factors.

[c] Abbreviations s, d, t, and q are singlet, doublet, triplet or double doublet, and quartet, respectively.

[d] The multiplicity in parenthesis shows that of B (**1**).

[e, f] Tentatively assigned and may be interchanged.

H-25, OH-29, C-25, and C-29, indicate that the hydroxyl at C-27 has an axial configuration. This proposal was confirmed by the ^1H NMR spectrum of **1** in $CDCl_3$ (Table 2). This spectrum included a doublet at δ 3.73 which was exchangeable with D_2O. The proton is coupled (9 Hz) to



	R_1	R_2	R_3
1:	H	OH	H (A28695B)
2:	H	H	H (A28695A)
3:	Me	H	Me
4:	Me	H	CD_3
5:	Me	OH	Me
6:	Me	OH	CD_3

Table 2. ^1H chemical shifts (δ) of A28695 factors in $CDCl_3$[a].

Assignment[b]	Sodium salts		Free acids		Assignment[b]	Sodium salts		Free acids	
	A(2)	B(1)	A(2)	B(1)		A(2)	B(1)	A(2)	B(1)
H-2	2.465	2.456	2.623	2.630	H-21	4.558	4.562	4.247	4.272
2-Me	1.057	1.058	1.098	1.106	H-22	1.93, 1.38	~1.93, ~1.48	1.97, 1.4	1.98, 1.4
H-4	1.76	1.76	1.73	~1.73	H-23	2.17, 1.80	2.16, 1.80	2.03, 1.78	2.05, 1.77
4-Me	1.005[c]	1.013[d]	0.987	0.987	H-24	4.302	4.318	4.195	4.215
H-5	3.335	3.333	3.35	~3.36	H-25	3.82	4.052	3.847	4.139
6-Me	1.217	1.215	1.222	1.220	H-26	1.32	1.346	1.35	1.403
H-7	3.785	3.779	3.762	3.752	26-Me	0.817	0.943	0.839	0.960
H-8a	1.78	1.80	1.77	~1.77	H-27	[e]	3.44	[e]	3.468
H-8b	1.48	1.48	1.54	~1.54	27-OH	—	4.172	—	3.73
H-9	3.994	3.970	3.983	3.963	H-28	1.48	~1.53	1.53	1.603
H-10 ax	1.16	1.16	1.084	~1.08	28-Me	0.899	1.132	0.926	1.153
H-10 eq	2.15	2.16	2.05	~2.05	29-OH	6.22	7.191	5.881	5.80
H-11	~3.35	~3.37	3.35	~3.35	H-30	1.275	1.261	1.314	1.308
H-12	1.76	1.76	1.73	~1.73	OMe	3.35, 3.407, 3.470, 3.540	3.35, 3.41, 3.43, 3.54	3.316, 3.339, 3.408, 3.522	3.297, 3.356, 3.428, 3.538
12-Me	0.981[c]	0.987[d]	0.999	0.996					
H-14	2.12	2.127	1.98	~1.98	H-1′	4.864	4.864	4.837	4.836
14-Me	0.996	1.004	0.962	0.960	H-2′ ax	1.55	1.581	1.58	~1.60
H-15	3.544	3.546	3.53	3.539	H-2′ eq	1.84	1.84	1.84	[e]
16-Me	1.624	1.615	1.488	1.478	H-3′ ax	1.32	1.32	1.32	[e]
H-17	3.699	3.716	3.57	~3.54	H-3′ eq	2.217	2.217	2.21	[e]
H-18	1.93, 1.77	1.93, 1.78	~1.78	~1.8	H-4′	2.810	2.816	2.82	2.823
H-19	~1.72	~1.78	1.73, 1.22	1.82, 1.3	H-5′	3.327	3.329	3.31	3.308
H-20	3.918	3.928	3.78	3.824	H-6′	1.263	1.261	1.22	1.264

[a] ^1H FT NMR spectra were recorded on a Bruker WH-360 and/or a Varian XL-100-12A spectrometer. Accuracies of chemical shifts δ and coupling constants J are $\pm$0.05 ppm and $\pm$0.5 Hz, respectively.

[b] Assignments are based on ref. 8. On the basis of our spectra, the assignments given[8] for H-5 and H-7, H-4 and H-28, 4-Me and 28-Me, and 16-Me and 29-Me (30) should be reversed.

[c, d] Assignments may be reversed.

[e] Not ascertained.

a resonance at δ 3.468, examination of which showed that spin couplings between H-27 and adjacent protons were very small. If the conformation of ring F of **1** is similar to that of **2**[8], then this result requires that the 27-hydroxyl group should be axial.

By selective ^{1}H-decoupled ^{13}C spectral measurements, the assignments of the key ^{13}C signals indicating structure **1** were confirmed, *i.e.*, those of C-27 (δ 74.8), C-25 (70.6), C-26 (38.1), C-28 (42.2), 26-Me (13.6), and 28-Me (13.6). The downfield shifts of the C-26 and C-28 signals (β-effects) and the upfield shifts of the C-25, 26-Me, and 28-Me signals (steric γ-effects) from **2** to **1** are well explained. The downfield shift of the C-29 signal is attributable to the hydrogen bonding between 27-OH and 29-OH. Thus, we conclude that A28695B is 27(R)-hydroxy A28695A.

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