STUDIES ON THE ISOTETRACENONE ANTIBIOTICS III. A NEW ISOTETRACENONE ANTIBIOTIC, GRINCAMYCIN

Sir:

During the course of our screening program for new antitumor antibiotics, an actinomycete identified as *Streptomyces griseoincarnatus* was found to produce a new antibiotic, which was named grincamycin. This substance contains a modified benz[a]anthraquinone chromophore which is characteristic of the isotetracenone antibiotics.[1,2]

The producing organism was cultivated on a rotary shaker at 27°C for 3 days in 500-ml Erlenmeyer flasks containing a medium consisting of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and calcium carbonate 0.4% (pH 7.0). The cultured broth (1 liter) was filtered with the aid of Celite and the mycelial cake was extracted with Me_2CO. After being evaporated *in vacuo*, the extract was partitioned between EtOAc and water. The organic layer was concentrated to dryness and then subjected to Toyopearl HW-40 column chromatography. The active fraction eluted with MeOH was evaporated *in vacuo* and applied to a silica gel

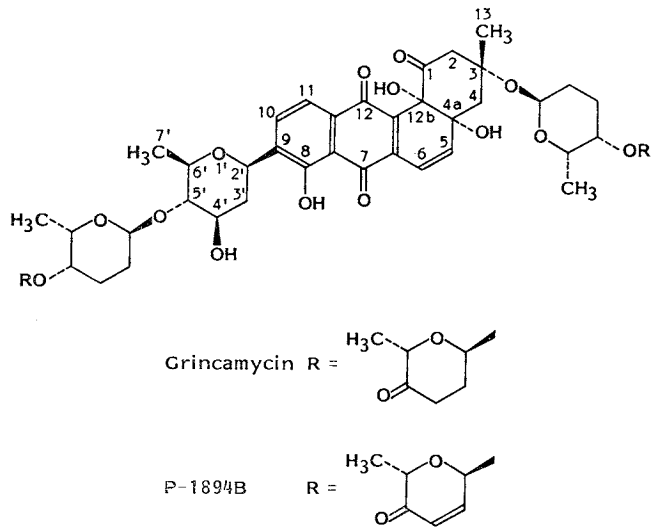
Table 1. ^{13}C and ^{1}H NMR spectral data for grincamycin in $CDCl_3$.

	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
Aquayamycin			Rhodinose 1		
1	204.0 s		1	99.3 d	4.99 br s
2	50.2 t	3.19 dd (13.2, 2.7), 2.51 d (13.2)	2	25.3 t	2.13 m, 1.70 m
3	82.3 s		3	24.8[a] t	2.10 m, 1.91 m
4	44.4 t	2.29 dd (15.0, 2.7), 1.84 d (15.0)	4	74.5 d	3.69 br s
4a	79.8 s		5	67.9 d	4.22 q (6.4)
5	145.1 d	6.44 d (9.8)	6	17.3[b] q	1.29[d] 3H, d (6.4)
6	117.1 d	6.91 d (9.8)	Rhodinose 2		
6a	138.3 s		1	92.3 d	5.26 br s
7	187.5 s		2	24.9 t	2.02 m, 1.48 m
8	157.5 s		3	24.6[a] t	2.10 m, 1.84 m
9	138.1 s		4	74.5 d	3.67 br s
10	133.2 d	7.88 d (7.8)	5	67.1 d	4.22 q (6.4)
11	119.3 d	7.61 d (7.8)	6	17.1[b] q	1.27[d] d (6.4)
11a	130.0 s		Cinerulose A 1		
12	181.5 s		1	98.9 d	5.08 dd (5.8, 5.0)
12a	138.4 s		2	28.5 t	2.39 m, 2.10 m
13	25.6 q	1.41 3H, s	3	33.6 t	2.50 2H, m
2′	70.9 d	4.87 d (10.7)	4	209.9[c] s	
3′	38.8 t	2.51 dd (13.0, 5.7), 1.38 ddd (13.0, 10.7, 10.7)	5	71.0 d	4.32[e] q (6.9)
4′	71.3 d	3.81 ddd (10.7, 8.3, 5.7)	6	15.0 q	1.27 3H, d (6.9)
5′	88.7 d	3.06 dd (8.8, 8.3)	Cinerulose A 2		
6′	74.4 d	3.55 dq (8.8, 5.9)	1	98.8 d	5.08 dd (5.8, 5.0)
7′	18.6 q	1.36 3H, d (5.9)	2	28.5 t	2.39 m, 2.10 m
8-OH		13.31 s	3	33.6 t	2.50 2H, m
-OH		4.97 s	4	210.1[c] s	
-OH		4.61 s	5	71.0 d	4.29[e] q (6.9)
-OH		4.37 s	6	15.0 q	1.27 3H, d (6.9)

Assignments are based on chemical shift data, decoupling experiments and two-dimensional C-H correlation spectral analysis.

[a~e] Assignments of these signals may be interchanged.

Fig. 1. Structures of grincamycin and P-1894B.



column. Development of the column with $CHCl_3$ - MeOH (50 : 1) gave a yellow band, which was collected and concentrated to dryness to give a yellow powder (75 mg) of grincamycin in pure form.

The physico-chemical properties of grincamycin are as follows: MP 153~158°C; $[\alpha]_D^{22}$ −48° (*c* 0.1, $CHCl_3$); *Anal* Calcd for $C_{49}H_{62}O_{18}$: C 62.68, H 6.65, O 30.67; found: C 62.62, H 6.60, O 30.78; fast atom bombardment mass spectra *m/z* 961 $(M+Na)^+$; UV λ_{max} nm ($E_{1cm}^{1\%}$) 219 (312), 316 (59), 421 (66) in MeOH; 227 (361), 318 (113), 390 (36), 553 (61) in 0.01 N NaOH - MeOH; IR ν_{max} (KBr) cm^{-1} 3430, 2980, 1730, 1640.

The ^{13}C and ^1H NMR spectral data for grincamycin (Table 1) indicate that this antibiotic consists of 1 mol of aquayamycin,[3] 2 mol of rhodinose[4] and 2 mol of cinerulose A.[5] Among the isotetracenone antibiotics containing an aquayamycin moiety, these properties are similar to those of P-1894B[6] (vineomycin A_1),[7] which contains 2 mol of aculose[8] in place of cinerulose A. The tetrahydro derivative of P-1894B was prepared by catalytic hydrogenation with 5% Pd-$BaSO_4$ at room temp for 5 minutes and compared with grincamycin. These two compounds showed good accordance in their chromatographic and spectral behaviors. Therefore, the structure of grincamycin was determined as shown in Fig. 1.

Grincamycin inhibited the growth of P388 murine leukemia cells (IC_{50} 13 ng/ml) and showed antimicrobial activity against Gram-positive bacteria. MIC values as determined by the agar dilution method on Mueller-Hinton agar were 50 μg/ml for *Staphylococcus aureus* FDA 209 P, 25 μg/ml for *Micrococcus luteus* ATCC 9341 and 50 μg/ml for *Bacillus cereus* IAM 1729. Grincamycin had no antimicrobial activity against Gram-negative bacteria (*Escherichia coli* NIHJ, *Salmonella typhimurium* IID 971, *Pseudomonas aeruginosa* NCTC 10490), yeasts (*Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* Yu 1200) and fungi (*Aspergillus fumigatus* IFO 4400, *Penicillium chrysogenum* ATCC 10002, *Trichophyton mentagrophytes*) tested at maximum dose of 100 μg/ml.

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