AURAMYCINS AND SULFURMYCINS, NEW ANTHRACYCLINE ANTIBIOTICS: CHARACTERIZATION OF AGLYCONES, AURAMYCINONE AND SULFURMYCINONE

Sir:

In the screening program for new antitumor anthracycline antibiotics, anthracycline glycosides with new aglycones were isolated from the culture of *Streptomyces* sp. OBB-111, which was classified as *S. galilaeus*.

The strain OBB-111 was isolated from a soil sample collected in Bayern, West Germany. The strain was shaken cultured in 500 ml flasks containing 100 ml medium of the following composition: Glucose 2%, soluble starch 2%, S-3 meat (Ajinomoto Co., Ltd.) 0.5%, yeast extract 0.25%, K_2HPO_4 0.01%, $MgSO_4 \cdot 7H_2O$ 0.01%, NaCl 0.3% and $CaCO_3$ 0.3%. The cultivation was continued for 7 days on a rotary shaker operating at 180 rpm at 27°C. Yellow antibiotics were extracted with a solvent mixture of chloroform and methanol (1:1, v/v) from the culture and were recovered in chloroform layer. Analyses on tlc plates indicated that the crude antibiotics contained many components as detected by yellow color and bioautography against *Sarcina lutea*. Two major components were purified by silica gel column chromatography and thin-layer chromatography and identified to be aclacinomycin A and B[1]. This was confirmed by direct comparison of physicochemical properties with authentic sample of aclacinomycin A (gener-

Fig. 1. Separation of three aglycones by HPLC. Column: μBondapak C_{18} (Waters); mobile phase: methanol-water (60:40, v/v) with PIC B-7; flow-rate: 1 ml/minute; detection: absorbance at 430 nm.

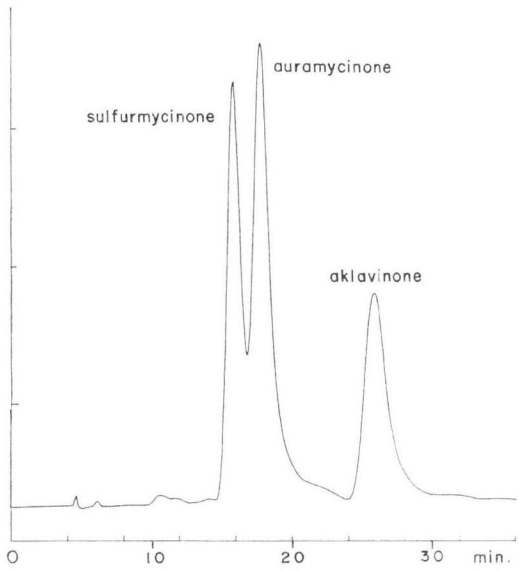
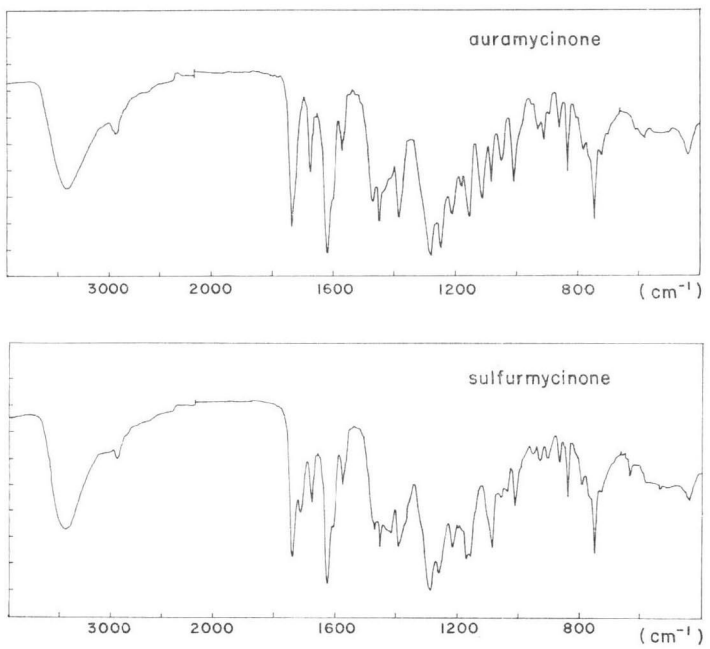


Fig. 2. IR Spectra of auramycinone and sulfurmycinone (KBr).



ous gift of Sanraku-Ocean Co., Ltd.). Besides aclacinomycin A and B, minor quantities of their analogues, MA144-M1, -N1, -S1 and -T1[2] were also produced by the strain OBB-111.

Mass fragmentation of anthracyclinones was well studied by H. BROCKMANN Jr. and his colleagues, who reported that the base peak of aklavinone at *m/z* 376 originated in bisanhydroaklavinone[3]. While characterizing all the components of the antibiotics in the culture by mass fragmentation, we observed several components with two different fragmentation patterns from that of alkavinone glycosides. Thus, the presence of anthracyclines with two different aglycones was suggested. We designated those glycosides auramycins and sulfurmycins, respectively. In order to characterize the aglycones, all the glycosides as a mixture were hydrolyzed and the aglycones separated from each other by tlc on silica gel plates. Aklavinone and two unidentified aglycones, auramycinone and sulfurmycinone, were obtained as yellow powder. Auramycinone: m.p. 153.5°C; $[\alpha]_D^{20}+178°$ (*c* 0.1, $CHCl_3$); *Anal.* calcd for $C_{21}H_{18}O_8$, C 63.33, H 4.56, O 32.11; found, C 63.05, H 4.51, O 32.44. Sulfurmycinone: m.p. 159°C; $[\alpha]_D^{20}+232.2°$ (*c* 0.1, $CHCl_3$); *Anal.* calcd for $C_{23}H_{20}O_9$, C 62.71, H 4.58, O 32.71; found, C 62.84, H 4.51, O 32.65.

Chromatographic behavior of the three aglycones is shown in Fig. 1. UV spectra at neutral as well as alkaline pH's were almost identical among the three, indicating that they have the same chromophore in common. IR spectra of auramycinone and sulfurmycinone are shown in Fig. 2.

Molecular weight (398) and moleuclar formula ($C_{21}H_{18}O_8$) of auramycinone showed that it has the structure lacking $-CH_2-$ from that of aklavinone (MW 412, $C_{22}H_{20}O_8$). ^{1}H-NMR spectrum of auramycinone had a strong resemblance to that of aklavinone. Only difference was that instead of the signals at δ 1.10 (3H, t) and δ 1.60 (2H, q) for C_2H_5- in the spectrum of aklavinone, a sharp singlet appeared at δ 1.43 (3H, s) in the spectrum of auramycinone. This clearly indicated that auramycinone has a methyl group instead of ethyl group at C-9 position. This was confirmed by ^{13}C-NMR spectrum (see Table 1).

From the molecular formula of sulfurmycinone ($C_{23}H_{20}O_9$), it is shown that it has an additional CO to the formula of aklavinone. IR spectrum (see Fig. 2) also shows extra carbonyl band at 1,710 cm^{-1}, corresponding to aliphatic ketone. In ^{1}H-NMR spectrum, signals of methyl and methylene of the ethyl side chain of aklavinone disappeared and a new signal at δ 2.27 (3H) as a singlet and 2 doublets at δ 2.68 (1H) and δ 2.91 (1H) appeared, the latter two being coupled with the coupling constant of 17 cps. These signals explain $-CH_2-CO-CH_3$ as a side chain. ^{13}C-NMR spectrum of sulfurmycinone showed an additional signal at 209.6 ppm, confirming the presence of an additional carbonyl carbon atom (see Table 1).

Table 1. ^{13}C-Chemical shift (δ) of auramycinone and sulfurmycinone.

Assignment*	Auramycinone	Sulfurmycinone	Aklavinone**
C-5	192.4	192.4	192.8
C-12	180.9	181.1	181.3
C-15	171.3	170.7	171.5
C-4	162.4	162.4	162.8
C-6	161.0	161.6	161.4
C-10a	142.3	140.8	142.9
C-2	137.4	137.3	137.7
C-12a	133.4	133.4	133.6
C-6a	132.6	133.3	133.1
C-11a	132.5	132.4	132.7
C-3	124.8	124.8	125.0
C-11	121.1	121.0	121.5
C-1	120.2	120.1	120.4
C-4a	115.5	115.7	115.8
C-5a	114.5	114.6	114.7
C-9	69.9	71.9	71.9
C-7	62.5	61.7	62.5
C-10	57.9	55.4	56.7
C-16	52.5	52.7	52.6
C-8	37.0	36.8	34.9
C-13	27.5	50.5	32.5
C-14		209.6	6.7
C-14a		31.7	

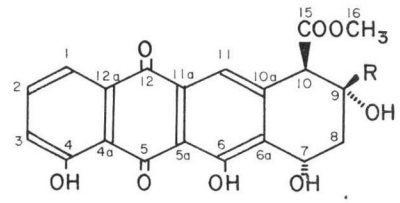
^{13}C-FT NMR spectra were recorded on JEOL FX-100.

* Carbon number is given in Fig. 3.

** The data were taken from the report by T. OKI.[10]

All the data support the structures of auramycinone and sulfurmycinone as shown in Fig. 3. The absolute configuration at C-7, C-9 and C-10 was determined from ORD and CD spectrum to be the same as that reported for aklavinone[4].

Fig. 3. Structures of auramycinone and sulfurmycinone.



$R = \overset{13}{CH_3}$ Auramycinone

$R = \overset{13}{CH_2}\overset{14}{C}O\overset{14a}{CH_3}$ Sulfurmycinone

($R = \overset{13}{CH_2}\overset{14}{C}H_3$ Aklavinone)

There have been known many anthracycline antibiotics which can be classified into three groups according to the grouping attached to C-9 position of their aglycones[5]: 1) daunomycin-adriamycin group with $-COCH_3$ or related moiety at C-9, 2) aclacinomycin-rhodomycin group with ethyl group at C-9 position and 3) others with methyl group at C-9, such as steffimycin[6], nogalarol[7], and SM-173B[8]. Auramycinone and sulfurmycinone described in this report are unique in that they have a methyl group and an acetonyl group, respectively, at C-9 together with a carbomethoxy group at C-10. It is also interesting that such side chains at C-9 have recently been reported in feudomycinone b and a[9], daunomycinone-type aglycones.

Acknowledgement

We thank Drs. B. WEGRZYNSKI and V. TOOME, Hoffmann La-Roche, Nutley, New Jersey, U.S.A., for the determination of the absolute configuration and Mr. K. WATANABE for the strain identification.

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