THE STRUCTURE OF SS-228Y, AN ANTIBIOTIC FROM *CHAINIA* SP.

TAKEJI KITAHARA, HIROSHI NAGANAWA, TAKAO OKAZAKI,
YOSHIRO OKAMI and HAMAO UMEZAWA

Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo, Japan

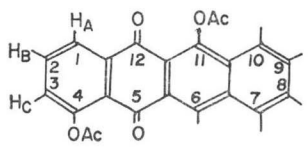
(Received for publication December 25, 1974)

SS-228Y, an antibiotic produced by *Chainia* sp., has been established as a benz(a)-anthraquinone derivative by spectroscopic evidence.

As reported in a previous paper[1], SS-228 Y is an antibiotic produced by an actinomycete, *Chainia* sp. The antibiotic is active against Gram-positive bacteria and inhibits dopamine β-hydroxylase. In this paper, the structural studies on SS-228Y and a derivative, SS-228 R obtained from its photolysis or thermolysis are described.

SS-228 Y (**I**) is an orange powder [m.p. 256~266°C, dec., $[\alpha]_D$—85° (*c* 1.0, acetone)] having a molecular formula of $C_{19}H_{14}O_6$ (mol. wt. 338, elemental analysis and mass spectrum, *m/e* 320, M^+-H_2O[1]). The ultraviolet and visible absorption spectra of **I** are as follows: $\lambda_{max}^{80\%MeOH}$(nm) 218 (ε 22000), 228sh (ε 21800), 415 sh (ε 4000), 440~460 (ε 4100), and $\lambda_{max}^{0.2N\,NaOH-80\%MeOH}$ (nm) 269 (ε 17900), 408 (ε 6400), 552 (ε 4400). The IR spectrum of **I** indicated the presence of an α,β-unsaturated carbonyl (1680 cm^{-1}), a quinone carbonyl (1665 cm^{-1}) and a chelated quinone carbonyl (1630 cm^{-1}). The NMR spectrum (d_4-methanol) of **I** displayed the following signals: δ 1.93 (3H, s, sharpened on irradiation at δ 6.12, $\begin{matrix}CH_3\\ \diagdown\\ \quad C=C\\ \diagup\\ \quad H\end{matrix}$), δ 2.68 (2H, broad s), δ 6.12 (1H, m), δ 6.47 (1H, d, J=10.0), δ 6.96 (1H, d, J=10.0), δ 7.24 (1H, dd, J=7.5, 2.0), δ 7.45~7.85 (2H, m). The NMR pattern observed for the aromatic protons of **I** is similar to that recorded for both rabelomycin[2] and tetrangomycin[3]. Color tests for **I** were positive with magnesium acetate[4] and sodium dithionite test[5]. The above results indicate the presence of a *peri*-hydroxyquinone moiety.

As reported in the previous paper[1], **I** is very labile to light and heat, being converted into SS-228 R (**II**) [m.p. 256~266°C, $C_{19}H_{12}O_5$ (M^+320)]. The IR spectrum of **II** indicated the presence of a chelated quinone carbonyl (1635 sh cm^{-1}) and an aromatic double bond (1605 cm^{-1}). Color tests with magnesium acetate and sodium dithionite were positive. The UV spectrum of the reaction product derived from the zinc-dust distillation of **II** is indicative of a naphthacene[6,7,8]. These experiments permit the formulation of **II** as a naphthacenequinone derivative in which both quinone carbonyls are chelated by phenolic hydroxyls. Acetylation of **II** with acetic anhydride in pyridine at room temperature gave a triacetate (**III**). Methylation of **II** with diazomethane followed by acetylation gave a monomethyl diacetate (**IV**). The IR spectrum of **III** indicated the presence of an acetoxy group (1775 cm^{-1}) together with a non-chelated quinone carbonyl group (1675 cm^{-1}) in place of the chelated quinone carbonyl (1635 cm^{-1}) of **II**. The NMR spectrum



-OAc
-CH3
-3xH
III

of **III** in $CDCl_3$ is characterized by three acetyl groups (δ 2.52, 2.54, 2.65, 3H each, s), an aromatic methyl group (δ 2.58, 3H, s) and three aromatic protons at δ 8.26 (1H, dd, J=8.0, 1.0), δ 7.78 (1H, t, J=8.0) and δ 7.42 (1H, dd, J=8.0, 1.0). On irradiation at δ 8.26 (H_A), the doublet of doublets at δ 7.42 (H_C) changed to a doublet (J=8.0). This data leads to the part structure shown for **III**. The placement of the remaining acetoxy group and the methyl of **III** followed from a series of nuclear OVERHAUSER effect (NOE) experiments (see Table 1). Irradiation at δ 2.58 (8-CH_3) resulted in enhancements of 22% and 13% of the integrated area of 7-H at δ 7.80 and 9-H at δ 7.37, respectively, thereby demonstrating an *ortho* relationship of this aromatic methyl group to these two protons. Irradiation of the 7-H (δ 7.80) gave an increase (13~19%) of the integrated area of the 6-H (δ 8.74) signal which was assigned *peri* to the carbonyl group by virtue of its low chemical shift. Spin decoupling experiments also indicated the presence of long-range coupling constants; $J_{6,7}$=1.0, $J_{7,9}$=1.0, $J_{7,8\text{-}CH_3}\leqslant 1$ and $J_{9,8\text{-}CH_3}\leqslant 1$, respectively. The results of those NOE experiments performed on the monomethyl diacetate (**IV**) of **II** are shown in Table 2. Since saturation of the methoxy group at δ 4.02 led only to an increase in the integrated area of 9-H at δ 6.80, it may be considered to be *peri* to the C-11-acetoxy group. Saturation of the aromatic methyl group (δ 2.52) resulted in 8% and 14% enhancements of the integrated areas at δ 6.80 (9-H) and δ 7.41 (7-H), respectively. Saturation at δ 7.41 (7-H) resulted in enhancement (26%) of the integrated area at δ 9.04 (6-H).

Table 1. NOE of **III** in $CDCl_3$

Proton signal observed	Proton signal saturated	% increase in integrated area of signal
6-H (δ 8.74)	7-H (δ 7.80)	13~19
7-H (δ 7.80)	8-CH_3 (δ 2.55)	22
9-H (δ 7.37)	8-CH_3	13

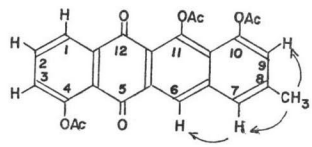
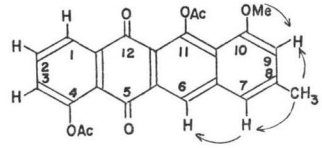
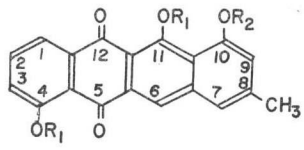


Table 2. NOE of **IV** in $CDCl_3$

Proton signal observed	Proton signal saturated	% increase in integrated area of signal
9-H (δ 6.80)	10-OCH_3(δ 4.02)	20
9-H	8-CH_3 (δ 2.52)	8
7-H (δ 7.41)	8-CH_3	14
6-H (δ 9.04)	7-H (δ 7.41)	26



From these results, the structure of **II** was deduced as 4, 10, 11-trihydroxy-8-methylnaphthacene-5, 12-quinone.



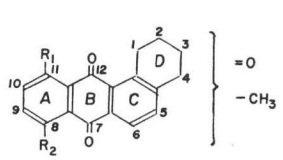
II $R_1 = R_2 = H$

III $R_1 = R_2 = COCH_3$

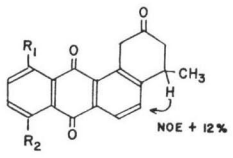
IV $R_1 = COCH_3$, $R_2 = CH_3$

Hydrogenation of **I** over platinum oxide in methanol at room temperature gave a tetrahydro derivative ($C_{19}H_{16}O_6$, M^+ 342, **V**). The UV spectrum of **V** showed λ_{max}^{MeOH} (nm) 250 (ε 10100), 274 (ε 10600), 410 sh (ε 4300), 425 (ε 4400) similar to that of 5-hydroxy-1, 4-naphthoqui-

none[9] The IR spectrum of **V** indicated the presence of a saturated carbonyl group (1720cm^{-1}, six-membered ring ketone, displaced from 1680 cm^{-1} in **I**, α, β-unsaturated ketone). The UV absorption at 228 nm (sh) in **I** no longer present within **V** may be accounted for by the saturation of the double bond of an α, β-unsaturated, β-alkyl-substituted enone system of a six membered ring (calcd. value 227 nm)[10]. The NMR spectrum of **V** no longer displays the two proton pattern at δ 6.47 and δ 6.96 seen in the NMR of **I** and this together with the hypsochromic shift in its UV spectrum indicated that the double bond conjugated to the *peri*-hydroxynaphthoquinone in **I** is saturated in **V**. The NMR spectrum of **V** in $CDCl_3$ is characterized by the following resonances; methyl doublet (δ 1.00, 3H, J=6.0), one methine proton (δ 1.28, 1H, m), eight methylene protons containing an alcoholic hydroxyl (δ 1.65~3.05, 9H, m), one alcoholic hydroxyl (δ 5.10, 1H, disappears on addition of D_2O), three aromatic protons (δ 7.2~7.7, 3H, m) and a chelated phenolic hydroxyl (δ 11.94, 1H, s, disappears on addition of D_2O). Treatment of **V** with *p*-toluenesulfonic acid in refluxing xylene led to **VI** ($C_{19}H_{14}O_4$, M^+, 306) by the loss of two moles of water. The UV spectrum of **VI** is very similar to that of tetrangomycin[3], indicating the presence of a *peri*-hydroxyanthraquinone moiety. The IR spectrum of **VI** showed a saturated carbonyl (1700 cm^{-1})*, non-chelated quinone carbonyl (1665 cm^{-1}) and chelated quinone carbonyl (1630 cm^{-1}). The NMR spectrum indicated the presence of a methyl group (δ 1.21, 3H, d, J=6.0), a methine proton (δ 2.43, 1H, m) (on irradiation of this signal, the methyl signal

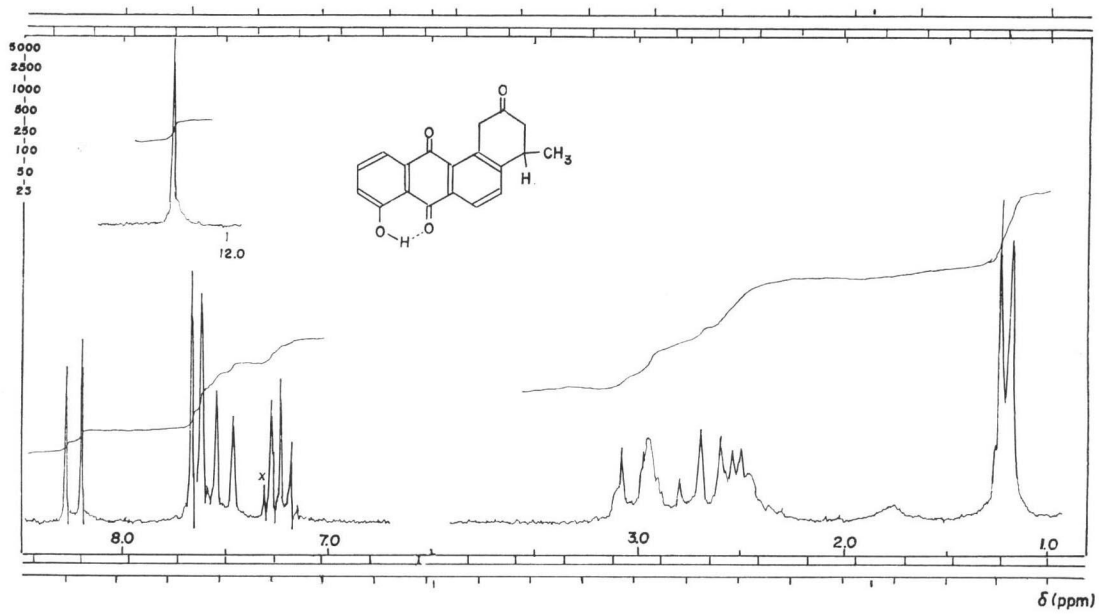


VIa R_1 = H, R_2 = OH
b R_1 = OH, R_2 = H



VIa' R_1 = H, R_2 = OH
b' R_1 = OH, R_2 = H

Fig. 1. NMR spectrum of **VI** (100 Mhz, in $CDCl_3$)

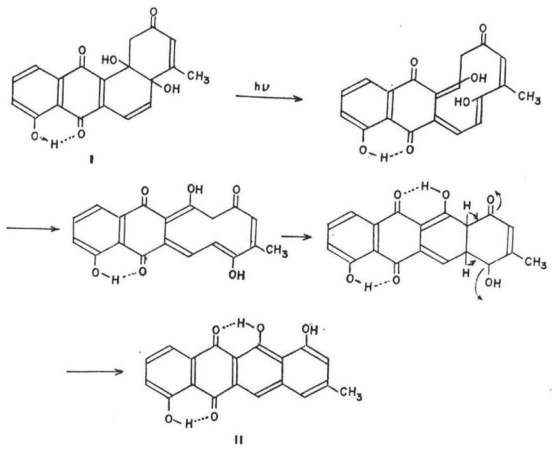


* This value is somewhat low compared with that for a normal saturated ketone. An NMR analysis and the structure of **II** are, however, consistent with a saturated carbonyl group as described later.

at δ 1.21 collapsed to a singlet), two methylenes (δ 2.5~3.1, 4H, m), the aromatic protons of ring *A* of the *peri*-hydroxyanthraquinone (δ 7.24, 1H, t, J=5.0; δ 7.65, 2H, d, J=5.0), aromatic protons at δ 7.51 and δ 8.21 (1H each, d, J=8.0) and one phenolic hydroxyl group (δ 12.25, 1H, s), (Fig. 1). The molecular formula and the spectral data of **VI** suggest that a six membered ring is fused to the *peri*-hydroxyquinone moiety. Furthermore, the two proton pattern at δ 7.51 and δ 8.21 indicates that **VI** is a benz(a)anthraquinone derivative (**VIa** or **VIb**). The signal at lower field of this two proton pattern is assigned to a position *peri* to the quinone carbonyl. On irradiation at δ 2.43 (4-H, methine), an increase in the integrated area at δ 7.51 (5-H, 12%) is observed. This result is consistent with the C-methyl group being *peri* to the proton at C-5 of **VIa** or **VIb**. This in turn in the light of earlier arguments leads to the structure **VIa'** or **VIb'** for **I**.

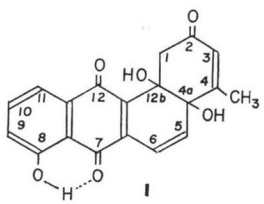
Conformation for the placement of the keto-carbonyl at C-2 on ring *D* was obtained through measurement of the NMR spectrum ($CDCl_3$) of **VI** in the presence of the shift reagent $Eu(fod)_3$. With the addition of $Eu(fod)_3$, the methylene signals at δ 2.5~3.1 were gradually

Chart 1. Rearrangement of I by light



resolved and a doublet of doublets (δ 3.33, dd, J=16.0, 4.5) was revealed ($Eu(fod)_3$:Vi = 1:11, mole ratio). On irradiation of the 4-H proton (δ 2.43), the doublet of doublets at δ 3.33 collapsed to a doublet (J=16.0). This large coupling constant (J=16.0) is consistent with geminal coupling of the C-3-methylene protons. The smaller coupling constant (J=4.5) may be attributed to vicinal coupling between the C-3-methylene and the C-4-methine proton. As the C-3-methylene proton does not couple further, the C-2-position must be substituted by some group (ketone) lacking a proton.

The NMR spectrum of **V** in d_6-dimethylsulfoxide showed two alcoholic hydroxyls at δ 4.59 and δ 4.92 and one phenolic hydroxyl at δ 12.30 (all of these signals were a singlet and disappeared on addition of D_2O). These two alcoholic hydroxyl groups should also be present in **I**. Since the 5-H (δ 6.47) and the 6-H (δ 6.96) protons are observed as doublets in



I, a hydroxyl group may be placed at the C-4a-position. The evidence for the placement of the remaining hydroxyl at C-12b in **I** follows then from the structures of **II** and aquayamycin[8] which possesses a chromophore similar to that of **I**, and which is reported to undergo a structural rearrangement on exposure to light or heat[6]. If the molecular rearrangement of **I** proceeds by a mechanism similar to that proposed for aquayamycin, the relationship between **I** and **II** may be explained according to the flow sheet of Chart 1.

From these results, the structure of **I** is elucidated as 4a,12b-dihydro-4a,8,12b-trihydroxy-4-methylbenz(a)anthracene-2,7,12 (1H)-trione.

Experimental

SS-228 R (**II**): (**a**) 10 mg of **I** was dissolved in 5 ml of methanol, and exposed to sunlight or a UV lamp (20 W, 20 cm distance) at room temperature. After a few days red needles gradually separated. The crystals were collected after 5 days (8 mg) and recrystallized from methanol-acetone (6 mg, m.p. 256~266°C, dec.). Anal. Calcd. for $C_{19}H_{12}O_5 \cdot CH_3OH$ (m.w. 320); C 68.18, H 4.58, O 27.25. Found: C 68.28, H 4.44, O 27.42. Mass; *m/e* 320 (M^+, base peak). UV; $\lambda_{max}^{30\%MeOH}$(nm) 264 (ε 34000), 295 sh (ε 7300), 306 sh (ε 4800) and 495 (ε 8600), $\lambda_{max}^{30\%MeOH-0.2N\ NaOH}$ (nm) 267 (ε 39000), 560 (ε 9200), NMR (100 MHz, d_5-pyridine); δ 7.94 (1H, dd, J=8.5, 1.5, H_A), δ 7.63 (1H, t, J=8.5, H_B), δ 7.34 (1H, dd, J=8.5, 1.5, H_C), δ 9.22 (1H, s, 6-H), δ 7.95 (1H, d, J=1.0, 7-H or 9-H), δ 2.35 (3H, s, 8-CH_3), δ 7.58 (1H, d, J=1.0, 9-H or 7-H). (**b**) 10 mg of **I** was packed in a glass tube and heated at 150°C for 5 hours *in vacuo*. After cooling, the brownish red residue was crystallized from methanol-acetone as red needles (8 mg). Identity of these crystalls with **II** was proved by mixed m.p. and by comparison of UV, IR and NMR spectra.

Zinc-dust distilation of **II**: A mixture of **II** (15 mg) and dried Zn dust (300 mg) was packed in a round-bottomed tube (13 mm) and additional Zn dust (1 g) was added to cover the mixture. The tube was heated to nearly red heat with a Bunsen burner. After cooling, the distilled oil was extracted with cyclohexane. After removal of the solvent, the residue was purified on an alumina column (Woelm neutral, 1×10 cm) using benzene as an eluant to give a yellowish oil. UV; $\lambda_{max}^{cyclohexane}$(nm) 276.5, 294, 376, 397, 415, 441, 471.

Triacetate of SS-228 R (**III**): A solution of **II** (30 mg) in pyridine (1 ml) and acetic anhydride (1 ml) was allowed to stand overnight at room temperature and then poured into ice water to give a yellow crystalline-like ppt., which was crystallized from $CHCl_3$-MeOH, giving **III** (25 mg) as yellow needles; m.p. 235~238°C, dec. UV; $\lambda_{max}^{CHCl_3}$ (nm) 252 (ε 44600), 280 sh (ε 36000), 291 (ε 40000), 302 (ε 44000), 410 (ε 12500), 425 sh (ε 11000). Mass; *m/e* 446.1057 (M^+, calcd. for $C_{25}H_{18}O_8$, 446.1002), 404 (M^+-CH_2CO), 362 (M^+-2CH_2CO), 320 (M^+-3CH_2CO, base peak). NMR (100 MHz, $CDCl_3$); δ 8.26 (1H, dd, J=8.0, 1.0, H_A), δ 7.78 (1H, t, J=8.0, H_B), δ 7.42 (1H, dd, J=8.0, 1.0, H_C), δ 2.52, 2.54, 2.65 (3H each, s, 4-OAc, 10-OAc, 11-OAc), δ 8.74 (1H, d, J=1.0, 6-H), δ 7.80 (1H, m, $J_{6,7}$=1.0, $J_{7,8-CH_3} \leqslant 1$, $J_{7,9}$=1.0, 7-H), δ 2.58 (3H, s, 8-CH_3), δ 7.37 (1H, m, $J_{7,9}$=1.0, $J_{9,8-CH_3} \leqslant 1$, 9-H). Addition of Eu(fod)$_3$ (100 MHz, $CHCl_3$, Eu(fod)$_3$: III=1:1.8); δ 8.35 (1H, dd, J=8.0, 1.0, H_A), δ 7.81 (1H, t, J=8.0, H_B), δ 7.56 (1H, dd, J=8.0, 1.0, H_C), δ 8.98 (1H, d, J=1.0, 6-H), δ 7.94 (1H, m, 7-H), δ 2.59 (3H, s, 8-CH_3), δ 7.55 (1H, m, 9-H), δ 2.74, 2.84, 2.87 (3H each, 4-OAc, 10-OAc, 11-OAc).

Monomethyl diacetate of SS-228 R (**IV**): A solution of **II** (53 mg) in acetone (15 ml) was treated with an excess of ethereal diazo-methane and allowed to stand overnight. The solvent was removed *in vacuo* to give a red residue, which was acetylated with pyridine (2 ml) and acetic anhydride (5 ml) at room temperature for 10 hours and then poured into ice water. The solution was extracted with $CHCl_3$. The extract was washed with water and dried over Na_2SO_4 and concentrated to obtain an orange residue, which was purified by silica gel chromatography (benzene-methanol, 99:1) and crystallized from $CHCl_3$ to give orange yellow needles (23 mg);

m.p. 214~216°C, dec., Mass; *m/e* 418 (M^+), 376 (M^+-CH_2CO), 334 (M^+-2CH_2CO, base peak), 319 ($M^+-2CH_2CO-CH_3$). UV; $\lambda_{max}^{CHCl_3}$(nm) 262, 280 sh, 293, 305, 370, 438. IR; 1760 cm^{-1} (acetyl carbonyl of phenolic OH), 1665 cm^{-1} (non-chelated quinone carbonyl), 1618, 1590 cm^{-1} (aromatic C=C). NMR (100 MHz, $CDCl_3$); δ 8.23 (1H, dd, J=7.0, 1.0, H_A), δ 7.74 (1H, t, J=7.0, H_B), δ 7.37 (1H, dd, J=7.0, 1.0, H_C), δ 2.49, 2.61 (3H each, s, 4-OAc, 11-OAc), δ 9.04 (1H, d, J=1.0, 6-H), δ 7.41 (1H, m, $J_{6,7}$=1.0, $J_{7,8-CH_3} \leqslant 1$, $J_{7,9}$=1.0, 7-H), δ 2.51 (3H, s, 8-CH_3), δ 6.80 (1H, m, $J_{7,9}$=1.0, $J_{9,8-CH_3} \leqslant 1$, 9-H), δ 4.02 (3H, s, 10-OMe).

Tetrahydro SS-228 Y (V): A solution of **I** (100 mg) in MeOH (30 ml) was hydrogenated over PtO_2 (50 mg) at room temperature and atmospheric pressure for 6 hours. The catalyst was removed by filtration and the filtrate was evaporated to give a yellow powder, which was chromatographed on a silica gel column (1×40 cm) using benzene-ethylacetate (9:1) as an eluant. The eluate was concentrated to give a yellow powder (53 mg); m.p. 109~112°C, dec. Mass; *m/e* 342.1122 (M^+, calcd. for $C_{19}H_{18}O_6$, 342.1142), 324 (M^+-H_2O), 306 (M^+-2H_2O), 296, 278, 264 (base peak). IR; 1720 cm^{-1} (saturated CO), 1660 cm^{-1} (non-chelated quinone CO), 1635 cm^{-1} (chelated quinone CO), 1610, 1575 cm^{-1} (aromatic C=C).

Dehydration product of **V** (**VI**): A mixture of **V** (60 mg) and a trace of *p*-toluenesulfonic acid was dissolved in xylene (2 ml) and refluxed for 3 hours. After elimination of the solvent, the residue was purified by preparative TLC (Kieselgel) using benzene-ethyl acetate as a developing solvent and crystallized from benzene-*n*-hexane to give yellow needles; m.p. 163~165°C. Mass; *m/e* 306.0892 (M^+, calcd. for $C_{19}H_{14}O_4$, 306.0893), 278 (M^+-CO), 264 (M^+-CH_2CO, base peak), 236, 208. UV; λ_{max}^{MeOH}(nm) 266 (ε 49000), 400 (ε 7800).

Acknowledgement

The authors wish to thank Drs. S. KONDO and M. OHNO for valuable suggestions and advice.

References

1) OKAZAKI, T.; T. KITAHARA & Y. OKAMI: Studies on marine microorganisms. IV. A new antibiotic SS-228 Y produced by *Chainia* isolated from shallow sea mud. J. Antibiotics 28: 176~184, 1975.
2) LIU, W.; W.L. PARKER, D.S. SLUSARCHYK, G.L. GREENWOOD, S.F. GRAHAM & E. MEYERS: Isolation, characterization and structure of rabelomycin, a new antibiotic. J. Antibiotics 23: 437~441, 1970
3) KUNTSMANN, M.P. & L.A. MITSCHER: The structural characterization of tetrangomycin and tetrangulol. J. Org. Chem. 31: 2920~2925, 1966
4) SHIBATA, S.: Rhodocladonic acid, a pigment from a lichen of genus *Cladonia*. (in Japanese) Yakugaku Zasshi 61: 320~325, 1941
SHIBATA, S.; M. TAKITO & O. TANAKA: Paper chromatography of anthraquinone pigments. J. Am. Chem. Soc. 72: 2789~2790, 1950
5) FEIGL, F. & V. ANGER: Spot tests in organic analysis. p. 336, Elsevier Publishing Co., Amsterdam, 1966
6) SEZAKI, M.; S. KONDO, K. MAEDA, H. UMEZAWA & M. OHNO: The structure of aquayamycin. Tetrahedron 26: 5171~5190, 1970
7) BROCKMANN, H. & P. BOLDT: Rhodomycine. V; Antibiotica aus Actinomyceten. XLVI. ε-Isorhodomycinon. Chem. Ber. 94: 2174~2187, 1961
8) BROCKMANN, H. & B. FRANK: Rhodomycinone und Iso-rhodomycinone, Rhodomycine. IV. Chem. Ber. 88: 1792~1818, 1955
9) SINGH, I.; R.T. OGATA, R.E. MOORE, C.W.J. CHANG & P.J. SCHEUER: Electronic spectra of substituted naphthoquinones. Tetrahedron 24: 6053~6073, 1968
10) SILVERSTEIN, R.M. & G.C. BASSLER: Spectrometric identification of organic compounds. p. 160. John Wiley & Sons Inc., New York, 1967