# A NEW ANTIBIOTIC, OKICENONE

## II. PHYSICO-CHEMICAL PROPERTIES AND STRUCTURE ELUCIDATION

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The structure of a new cytocidal antibiotic, okicenone was elucidated to be 3,4-dihydro-4,6,9-trihydroxy-8-methyl-1(2*H*)-anthracenone on the basis of spectroscopic methods.

In the course of a screening program for novel antibiotics showing cytocidal activity, okicenone (**1**) was isolated from the culture broth of *Streptomyces* sp. KO-3599 which had been isolated from a soil sample collected in Okinawa Prefecture, Japan. The isolation procedure and physico-chemical properties of the new antibiotic together with the taxonomic studies of the producing strain were reported in the preceding paper[1]. This paper deals with the structure elucidation of okicenone (**1**).

## Materials and Methods

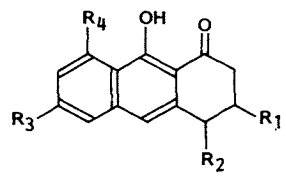
### General Experimental Procedures

MP's were determined using a Yanagimoto MP-3 hot stage microscope and are uncorrected. UV spectra were recorded on a Shimadzu model UV-200S spectrophotometer. IR spectra were recorded on a Jasco model A-102 interferometer. MS were obtained with a Jeol model DX-300 mass spectrometer. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian XL-400 instrument. Kieselgel 60 (Merck), Diaion HP-20 (Mitsubishi Chemical Industries) and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for column chromatography and DC-Fertigplatten Kieselgel 60 (Merck) was used for TLC analysis and for preparative TLC.

## Results and Discussion

### Structure of Okicenone

Physico-chemical properties of okicenone (**1**) are summarized in Table 1. The UV and IR absorption spectra of **1** are shown in Figs. 1 and 2, respectively. The antibiotic is soluble in $CHCl_3$, EtOAc and MeOH but practically insoluble in $H_2O$. Okicenone gave a positive color reaction with iodine, 50% sulfuric acid and $FeCl_3$ and was negative to DRAGENDORFF's reagent and ninhydrin. The mo-




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| **1** | H | OH | OH | $CH_3$ |
| **2** | OH | H | OH | $CH_3$ |
| **3** | H | OH | $CH_3$ | OH |
| **4** | OH | H | $CH_3$ | OH |

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Table 1. Physico-chemical properties of okicenone (**1**).

| | | | |
|---|---|---|---|
| Appearance | Pale yellow needles | IR $\nu_{max}$ $cm^{-1}$ | Fig. 2 |
| MP | 209~210°C | EI-MS (*m/z*) | 258 ($M^+$), 240, 202, 201 |
| Optical rotation | | HREI-MS (*m/z*) | |
| $[\alpha]_D^{18}$ | +20° (*c* 0.1, acetone) | Obsd | 258.0879 |
| TLC (silica gel) | | Calcd for $C_{15}H_{14}O_4$ | 258.0892 |
| $CHCl_3$-$CH_3OH$ (15:1) | 0.29 | Color reaction | |
| $CHCl_3$-$CH_3OH$ (9:1) | 0.65 | Positive | 50% $H_2SO_4+\Delta$, iodine, $FeCl_3$ |
| Hexane-acetone (1:1) | 0.42 | Negative | Ninhydrin reagent, DRAGENDORFF's reagent |
| Molecular formula | $C_{15}H_{14}O_4$ | | |
| MW | 258 | | |
| UV $\lambda_{max}$ nm | Fig.1 | | |

Fig. 1. UV spectrum of okicenone (**1**).

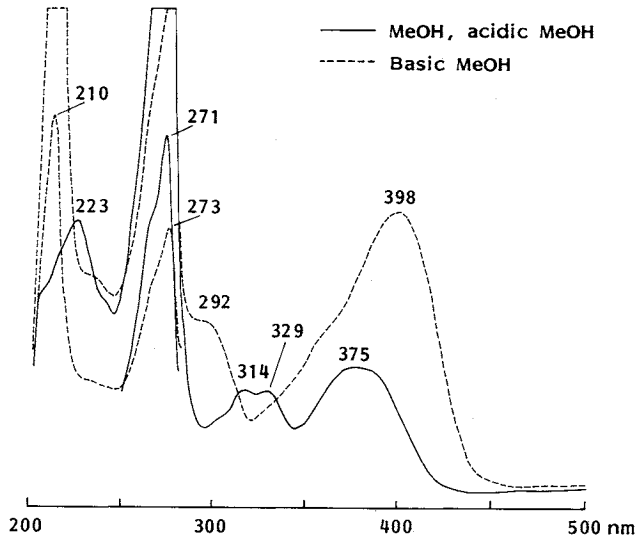
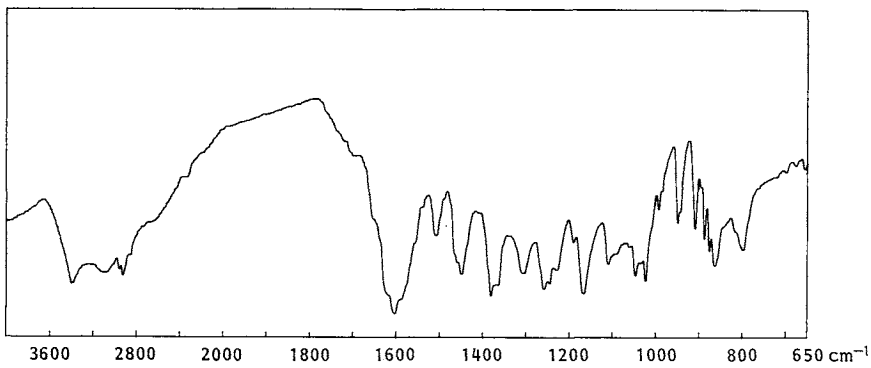



Fig. 2. IR spectrum of okicenone (**1**).



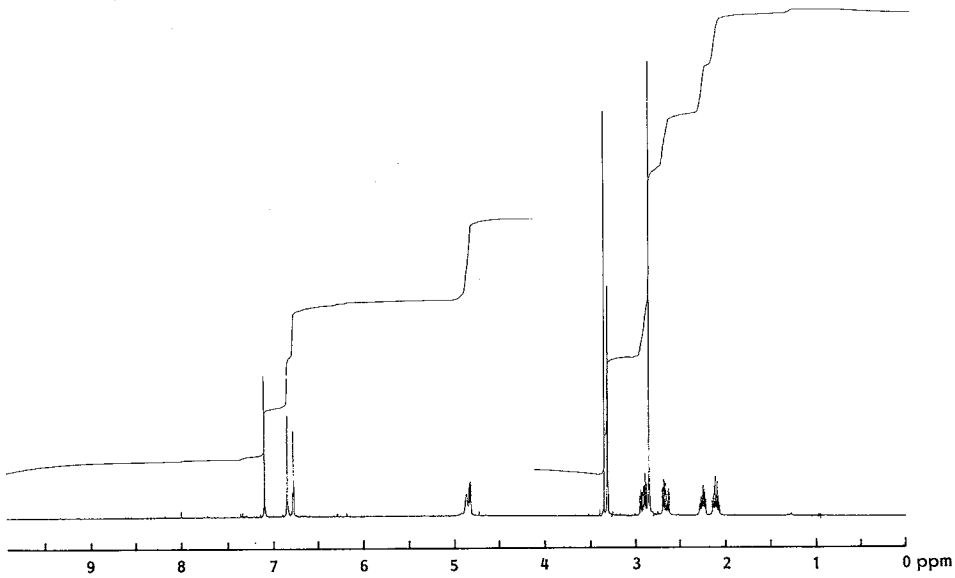

lecular formula of **1** was determined to be $C_{15}H_{14}O_4$ by HREI-MS (*m/z* 258.0879, $\Delta$ −1.3 mmu). The UV spectrum (Fig. 1) showed absorption maxima at 223, 271 and 375 nm. These absorptions appeared similar to those of the aureolic acid group such as chromomycins[2], and indicative of an anthracenone moiety contained in the molecule of okicenone (**1**).

Table 2. $^1H$ and $^{13}C$ NMR chemical shifts of okicenone (**1**) in $CD_3OD$ and protons to which an NOE was observed.

| Position | $^{13}C$ | $^1H$ | NOE | | Position | $^{13}C$ | $^1H$ | NOE | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 205.3 s | | | | 7 | 121.5 d | 6.77 dq | 8-$CH_3$ | ( 2%) |
| 2 | 35.7 t | (ax) 2.66 ddd | | | 8 | 143.1 s | | | |
| | | (eq) 2.91 ddd | | | 8-$CH_3$ | 25.4 q | 2.84 d | 7-H | (14%) |
| 3 | 32.5 t | (ax) 2.10 dddd | | | 8a | 119.0 s | | | |
| | | (eq) 2.25 dddd | | | 9 | 168.2 s | | | |
| 4 | 68.9 d | 4.83 ddd | 10-H | ( 6%) | 9a | 109.8 s | | | |
| 4a | 141.7 s | | | | 10 | 117.0 d | 7.10 d | 5-H | (13%) |
| 5 | 109.7 d | 6.84 d | 10-H | (10%) | 10a | 143.8 s | | | |
| 6 | 160.7 s | | | | | | | | |

*J* (H,H) in Hz: $2_{ax},2_{eq}=18$; $2_{ax},3_{ax}=8$; $2_{ax},3_{eq}=5$; $2_{eq},3_{ax}=5$; $2_{eq},3_{eq}=8$; $3_{ax},3_{eq}=13$; $3_{ax},4=8$; $3_{eq},4=3.5$; $4,10=1$; $5,7=2.5$; $7,8\text{-}CH_3=1$.

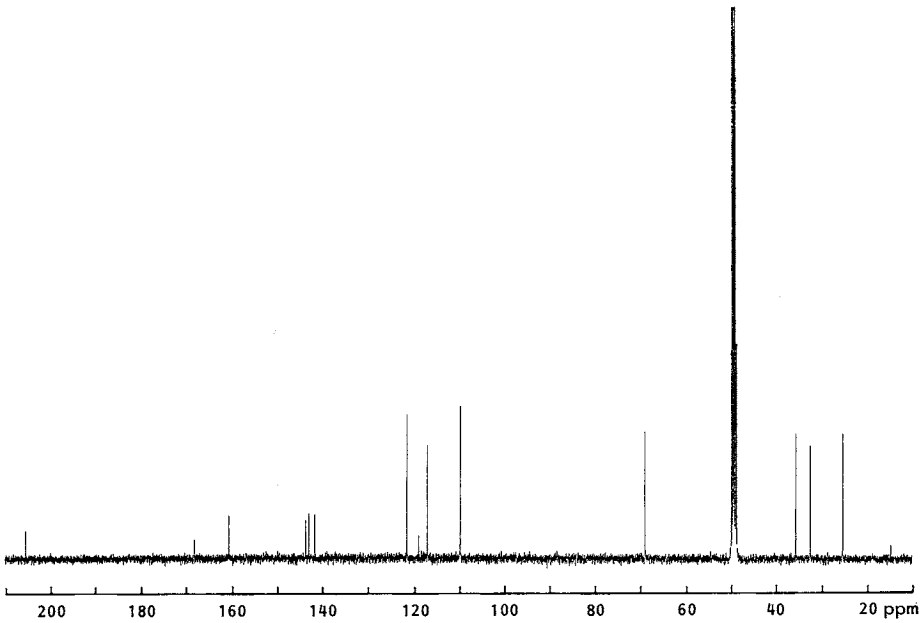
Fig. 3. $^1H$ NMR spectrum of okicenone (**1**).




$^1H$ and $^{13}C$ NMR spectra of **1** (Table 2, Figs. 3 and 4) along with DEPT experiments revealed the presence of one carbonyl, three $sp^2$ methines, seven $sp^2$ quarternary carbons, one $sp^3$ oxymethine, two $sp^3$ methylenes and one methyl group. This accounts for 15 carbons and 11 protons. The presence of three hydroxyl groups was inferred from the molecular formula $C_{15}H_{14}O_4$. The $^1H$-$^1H$ COSY spectrum indicated a proton network: X–$CH_2$–$CH_2$–CH(OH)–X. Since consideration of the unsaturation number implied that three rings were contained in the molecule of **1**, all the observations described above indicated a 3,4-dihydro-1(2*H*)-anthracenone nucleus with one methyl and two hydroxyl groups on the aromatic rings and a hydroxyl group on the C-2 or C-4 position. To determine the location of the hydroxyl and methyl groups, NOE experiments were carried out as follows (Fig. 5).

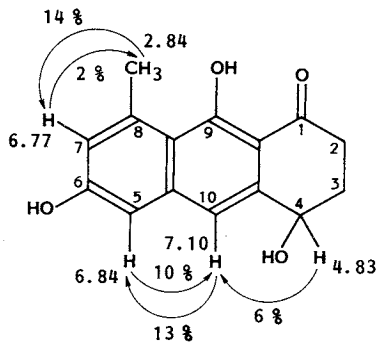
Upon irradiation of the $sp^3$ oxymethine proton at $\delta$ 4.83, a significant NOE (6%) was observed with the aromatic proton at $\delta$ 7.10, indicating the hydroxyl group at C-4 and the $\delta$ 7.10 proton at 10-H. Irradiation of the 10-H signal ($\delta$ 7.10) resulted in a 13% NOE in the aromatic proton at $\delta$ 6.84, which

Fig. 4. $^{13}C$ NMR spectrum of okicenone (1).




was threfore assigned to 5-H. Converse irradiation of 5-H yielded a 10% NOE in the 10-H signal. Since the 5-H signal appeared as a doublet with a typical *J*-value (2.5 Hz) for *meta*-coupling, the third aromatic proton ($\delta$ 6.77) was assigned to 7-H; accordingly two hydroxyl and one methyl substituents had to be placed at the remaining C-6, C-8 and C-9 positions.

Fig. 5. NOE experiments of okicenone (1).




Irradiation of the methyl protons ($\delta$ 2.84) caused a strong NOE (14%) in the 7-H signal ($\delta$ 6.77) but an NOE was not observed in the 5-H signal ($\delta$ 6.84). This observation clearly showed that the methyl group was on C-8 and the hydroxyl group on C-6. The remaining hydroxyl group was therefore deduced to be on C-9. Additional proof for these results was provided by the study on the $^{1}H$-$^{13}C$ long-range couplings through long range selective proton decoupling experiments to show the following connectivities: 4-H/C-4a, 4-H/C-10, 10-H/C-10a, 10-H/C-5, 5-H/C-10, 5-H/C-6, 7-H/C-6, 7-H/C-8, 8-$CH_3$/C-7 and 8-$CH_3$/C-8. Thus the structure of okicenone was concluded to be 3,4-dihydro-4,6,9-trihydroxy-8-methyl-1(2*H*)-anthracenone (**1**).

For those bearing structural similarity to okicenone (**1**), aloesaponols II (**2**)[3], III (**3**)[4] and germichrysone (**4**)[5~7] have been reported having the same molecular formula and very similar chemical properties.

Acknowledgment

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