## Notes

# STRUCTURE OF DUOCARMYCIN SA, A POTENT ANTITUMOR ANTIBIOTIC

TOHRU YASUZAWA, YUTAKA SAITOH, MICHIO ICHIMURA†, ISAMI TAKAHASHI and HIROSHI SANO*,††

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 3-6-6 Asahimachi, Machida-shi, Tokyo 194, Japan
†Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan

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Recently, we reported the structures of the duocarmycins[1,2], a unique antitumor antibiotics constituted with two structural moieties, a substituted pyrroloquinoline or its chemical equivalent cyclopropanopyrroloindole and a trimethoxyindolylcarbonyl group[3]. In this communication we wish to report the structure of duocarmycin SA (**1**), a stable compound structurally related to the duocarmycin A (**2**). Duocarmycin SA (**1**) produced by an unidentified *Streptomyces* sp. is a novel and potent antitumor antibiotic, which is effective against murine lymphocytic leukemia P388 and murine sarcoma 180[4]. The structure determination was accomplished by extensive 2D NMR experiments in addition to comparison of its spectra with those of duocarmycin A (**2**).

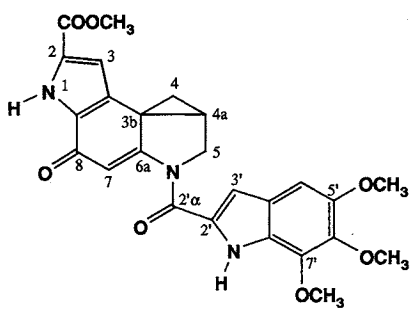
Physico-chemical properties were summarized in Table 1. $^{1}H$ and $^{13}C$ chemical shifts for **1** and **2** were shown in Tables 2 and 3, respectively. A battery of 2D techniques including COSY, $^{13}C$-$^{1}H$ COSY, and correlation spectroscopy *via* long range coupling[5] (COLOC) experiments were used to determine all proton and carbon line assignments. $^{13}C$ multiplicity data were derived from DEPT experiments. All the proton and carbon chemical shifts were related by $^{13}C$-$^{1}H$ COSY experiment. The assignments of chemical shifts of **1** are based on analysis of 2D experiments (described below) and correlation with **2**.

HR-MS of **1** (Table 1) exhibited the molecular formula $C_{25}H_{23}N_3O_7$ which was smaller by $CH_2O$ than **2**. Comparison of the NMR spectra of **1** and **2**, revealed the existence of trimethoxyindolylcarbonyl group in both compounds. It was also confirmed by the mass fragment at *m/z* 234 (HR-MS, 234.0784; for $C_{12}H_{12}NO_4$, err +1.8 mmu), produced by

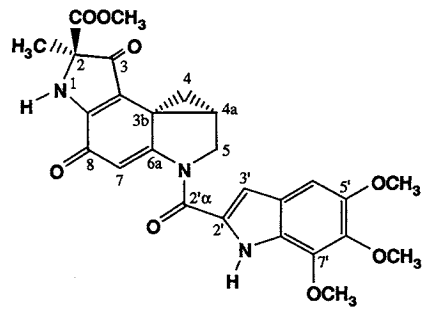
Table 1. Physico-chemical properties of duocarmycin SA (**1**).

| | |
|---|---|
| Appearance | Yellow powder |
| Molecular formula | $C_{25}H_{23}N_3O_7$ |
| EI-MS (*m/z*) | 477 $(M)^+$, 234 |
| HR-MS (*m/z*) Calcd: | 477.1535 |
| Found: | 477.1522 |
| $[\alpha]_D^{24}$ (*c* 0.1, MeOH) | +180° |
| UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$) | 235 (sh, 21,000), 316 (16,000), 367 (27,000) |
| IR ($CHCl_3$) $\nu$ $cm^{-1}$ | 3460, 1714, 1642, 1619, 1258 |

Fig. 1. Structures of duocarmycin SA (**1**) and duocarmycin A (**2**).




Duocarmycin SA (**1**)




Duocarmycin A (**2**)

†† Present address: Shimizu Research Laboratories, Marine Biotechnology Institute Co., Ltd., 1900 Sodeshi-cho, Shimizu-shi, Shizuoka 424, Japan.

Table 2. $^1$H NMR data[a] of duocarmycin SA (**1**) and duocarmycin A (**2**).

| Proton | 1 | 2 |
|---|---|---|
| 1-NH | 10.25 (br s) | 6.36 (s) |
| 2-$CH_3$ | — | 1.67 (s) |
| 2-$COOCH_3$ | 3.88 (s) | 3.74 (s) |
| 3-H | 6.56 (d, $J$=2.2 Hz) | — |
| 4-$H_a$ | 1.71 (dd, $J$=7.6, 4.7 Hz) | 2.24 (dd, $J$=7.6, 4.1 Hz) |
| 4-$H_b$ | 1.54 (t, $J$=4.7 Hz) | 1.29 (dd, $J$=4.7, 4.1 Hz) |
| 4a-H | 2.77 (d, t, $J$=7.6, 4.7 Hz) | 3.05 (m) |
| 5-$H_a$ | 4.46 (dd, $J$=10.6, 4.9 Hz) | 4.45 (dd, $J$=10.3, 4.7 Hz) |
| 5-$H_b$ | 4.37 (d, $J$=10.6 Hz) | 4.41 (br d, $J$=10.3 Hz) |
| 7-H | 7.02 (s) | 7.17 (s) |
| 1'-NH | 9.50 (br s) | 9.49 (br s) |
| 3'-H | 6.92 (d, $J$=2.2 Hz) | 6.94 (d, $J$=2.3 Hz) |
| 4'-H | 6.76 (s) | 6.78 (s) |
| 5'-$OCH_3$ | 3.87 (s) | 3.88 (s) |
| 6'-$OCH_3$ | 3.92 (s) | 3.93 (s) |
| 7'-$OCH_3$ | 4.03 (s) | 4.06 (s) |

[a] Measured on Bruker AM400 spectrometers with TMS (0 ppm) as an internal standard in $CDCl_3$.

Table 3. $^{13}$C NMR data[a] for duocarmycin SA (**1**) and duocarmycin A (**2**).

| Carbon | 1 | 2 | Carbon | 1 | 2 |
|---|---|---|---|---|---|
| C-2 | 126.9 | 71.3 | 2-$COOCH_3$ | 52.1 | 53.4 |
| C-3 | 107.5 | 194.8 | C-2' | 128.5 | 128.2 |
| C-3a | 130.0 | 112.0 | C-2'α | 161.2 | 161.2 |
| C-3b | 31.3 | 30.6 | C-3' | 107.8 | 108.2 |
| C-4 | 26.1 | 22.0 | C-3'a | 123.2 | 123.3 |
| C-4a | 23.6 | 22.3 | C-4' | 97.8 | 97.7 |
| C-5 | 54.9 | 55.3 | C-5' | 150.5 | 150.6 |
| C-6a | 161.6 | 165.1 | C-6' | 141.1 | 141.3 |
| C-7 | 112.5 | 113.2 | C-7' | 138.9 | 138.9 |
| C-8 | 177.9 | 179.7 | C-7'a | 126.4 | 126.6 |
| C-8a | 131.6 | 164.4 | 5'-$OCH_3$ | 56.3 | 56.3 |
| 2-$CH_3$ | — | 21.1 | 6'-$OCH_3$ | 61.4 | 61.5 |
| 2-$COOCH_3$ | 161.0 | 168.0 | 7'-$OCH_3$ | 61.1 | 61.2 |

[a] Measured on Bruker AM400 spectrometers with TMS (0 ppm) as an internal standard in $CDCl_3$.

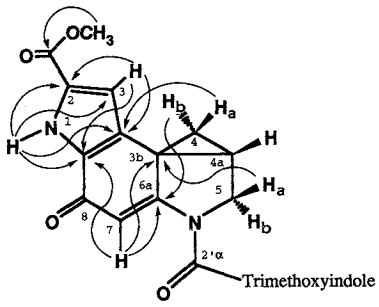
cleavage of amide bond.

In $^1$H NMR spectrum of **1** (Table 2), one vinyl doublet ($\delta$ 6.56, $J$=2.2 Hz) coupled with the 1-NH proton (confirmed by $^1$H homo decoupling experiment) was observed instead of the 2-methyl singlet observed in the spectrum of **2**. In the comparison of $^{13}$C NMR spectra of **1** and **2** (Table 3), the 2-methyl ($\delta$ 21.1) and 3-keto ($\delta$ 194.8) signals of the latter were lacking and trisubstituted double bond carbon signals ($\delta$ 126.9 and 107.5) were now present in **1**. These NMR data suggested the difference around the 2, 3 position.

Remaining protons were $CH_2$-CH-$CH_2$ five spin system (position 4, 4a, 5), a methoxy group, and an isolated aromatic proton. $^{13}$C-$^1$H coupling constant of a methylene ($\delta_C$ 26.0; $J_{CH}$=166 Hz) in the five spin system suggests that it consists a part of cyclopropane ring system. Other connectivities of quaternary carbons were established by COLOC experiment (polarization delay is 50 mseconds). The diagnostic results were shown in Fig. 2.

Cross peaks between $\delta_H$ 1.54 (4-$H_b$), 7.02 (7-H) and $\delta_C$ 161.6 (C-6a); $\delta_H$ 4.46 (5-$H_a$), 7.02 (7-H) and $\delta_C$ 31.3 (C-3b); $\delta_H$ 1.71 (4-$H_a$) and $\delta_C$ 130.0 (C-3a); and $\delta_H$ 7.02 (7-H) and $\delta_C$ 131.6 (C-8a) indicate the cyclopropanoindole ring. No long range coupling was observed on $\delta_C$ 177.9 carbonyl carbon in gated decoupling experiment and no cross peak was observed on it in the COLOC experiment, but consideration of chemical shifts indicated the

Fig. 2. COLOC experiment of **1**.




location of the carbonyl group at 8-position. The methoxy group was shown to connect to carbonyl group, but location of methoxycarbonyl group was not indicated by this experiment. Cross peaks were observed from NH (1-H) proton and vinyl proton (3-H) to all the pyrrole carbons.

The orientation of pyrrole ring and the location of methoxycarbonyl group were determined by an NOE experiment on **1**, where a 2.0% NOE from the vinyl proton to 4a-H, and 4.3% and 6.2% NOE from 4a-H and 4-$H_a$ to the vinyl proton, respectively, were observed. Whereas no NOE was observed between methoxycarbonyl group and 4-$H_a$, 4a-H protons. Thus the position of the vinyl proton at $\delta$ 6.56 was established at 3 position. These NOE also indicated the configuration of 4a-H and 4-$H_a$ to be $\beta$, supposed the cyclopropane ring to be $\alpha$. Coupling constant between 4a-H and 5-$H_a$ indicate that they are in *cis* configuration. Thus the relative structure of **1** was determined as shown in Fig. 1.

**1** seems to be an intermediate in duocarmycin biosynthesis from its structural features.

Acknowledgment

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