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STRUCTURES OF DUOCARMYCINS, NOVEL ANTITUMOR ANTIBIOTICS PRODUCED BY *STREPTOMYCES SP.*

Tohru Yasuzawa, [a] Takao Iida,[a] Ken'ichi Muroi,[b] Michio Ichimura, [b] Keiichi Takahashi, [b] and Hiroshi Sano*, [a]

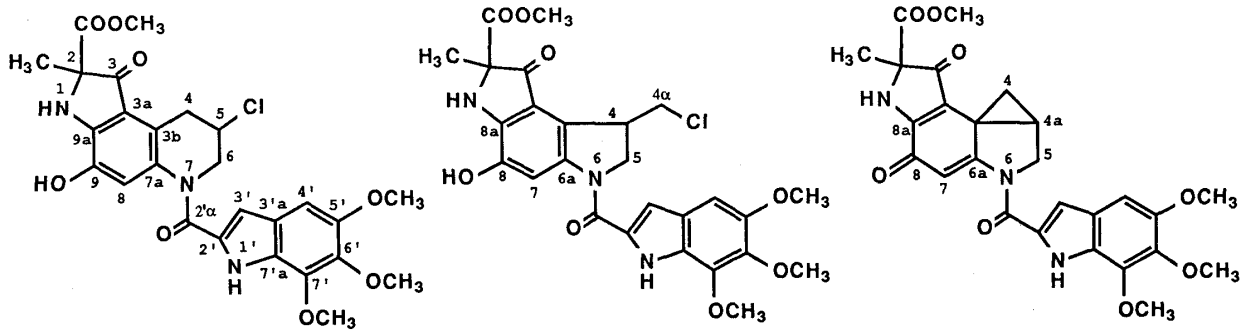
Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., [a] 3-6-6 Asahimachi, Machida-shi, Tokyo 194, Japan and Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., [b] 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan.

The structures of new antitumor antibiotics, Duocarmycin A, C_1 and C_2, isolated from the culture broth of *Streptomyces sp.*, have been determined on the basis of chemical and physicochemical evidence.

KEYWORDS ——— *Streptomyces sp.* ; antitumor antibiotic ; COLOC ; Duocarmycin A ; Duocarmycin C_1 ; Duocarmycin C_2

Duocarmycin A,[1] C_1[2] and C_2 produced by *Streptomyces sp.* are novel antitumor antibiotics which are effective against murine lymphocytic leukemia p388 and murine Sarcoma 180 in mice. In this report, we describe the structural determination of the Duocarmycins.

Duocarmycin C_1 (**1**) [3] was obtained as an optically active yellow powder, $[\alpha]_D^{27}$-126° in methanol. High-resolution electron impact mass spectrum and ^{13}C NMR spectrum indicated that **1** had the molecular formula $C_{26}H_{26}ClN_3O_8$. The ^1H NMR spectrum revealed the presence of three aromatic protons, four methoxyl groups, one *tert.* methyl group, two methylene groups, one methine proton and three protons which disappeared in the presence of D_2O.



Duocarmycin C_1 (**1**) Duocarmycin C_2 (**2**) Duocarmycin A (**3**)

Fig. 1. Structures of Duocarmycin C_1, C_2 and A

IR carbonyl absorption at 1740 cm^{-1}, two ^{13}C signals at 169.2 and 52.6 ppm, and a mass spectral fragmentation which lost a $C_2H_3O_2$ unit indicated that **1** had a methoxycarbonyl group. The ^{13}C NMR signal at 197.8 ppm and the IR absorption at 1695cm^{-1} established the presence of a ketone group which conjugated with an aromatic ring. Since the *tert.* methyl protons at 1.47 ppm were bound by long range coupling to the ester carbonyl at 169.2 ppm, the ketone at 197.8 ppm and a quaternary carbon at 70.1 ppm, and an amino proton at 7.39 ppm was bound by long range coupling to the latter two carbons, the partial structure **I** was established as shown in Fig. 2.

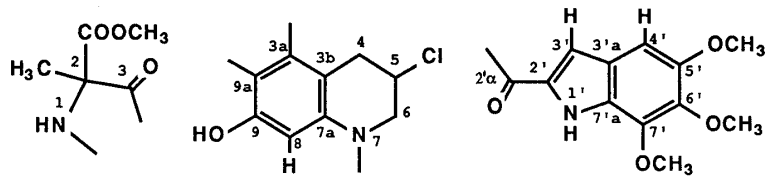


Fig. 2. Partial Structures of Duocarmycin C_1 (**1**)

The benzylic methylene (3.53 and 3.25 ppm) was coupled to a chlorine-bearing methine (4.76 ppm) which in tern was coupled to another methylene attached to nitrogen (4.35 and 3.96 ppm). The amino proton at 7.39 ppm was long-range coupled to each of the aromatic carbons C-3a, C-9 and C-9a. The ^1H-^{13}C long range coupling from those protons , an aromatic proton at 6.91 ppm and a phenolic OH proton at 9.87 ppm to aromatic carbons indicated the presence of a tetrahydroquinoline moiety (**II**). The occurrence of NOE between this NH and the phenolic OH confirmed the attachment of N-1 to C-9a and C-3 to C-3a in the partial structure **I** and **II**.

The attachment of the amino group between C-2 and C-9a, and of the phenolic OH group to C-9 were also established by a COLOC experiment with a permethylate **6**, where the long range couplings from *N*-methyl protons at 3.24 ppm and *O*-methyl protons at 3.67 ppm occurred. Treatment of **1** with triethylamine in methanol at room temperature in the presence of Ag_2CO_3 produced a quinoline derivative **4** and carboxylic acid methyl ester **5**. The aromatic proton signals of **4** at 8.61, 7.33 and 8.53 ppm (H-4, H-5 and H-6, respectively) had chemical shifts and coupling constants that were characteristic of the protons on C-4, C-3 and C-2 of quinoline. This indicated the presence of nitrogen linked to C-6 and C-7a. Alkaline treatment of **6** led to a dehydrochloride **7**. The ^1H NMR spectrum showed that two vinyl protons at 7.49 and 6.07 ppm (H-4 and H-5, respectively) were coupled to the nitrogen-bearing methylene (4.61 and 4.53 ppm), thereby confirming the attachment of the chlorine to C-5.

The long range couplings from two aromatic protons (6.87 and 6.57 ppm) and an amino proton (11.54 ppm), and a mass spectral fragment ion peak at *m/z* 234 ($C_{12}H_{12}NO_4$) established the presence of an indolecarboxylic acid moiety (**III**). The NH proton at 11.54 ppm was long-range coupled (J=1.8 Hz) to the aromatic proton at 6.57 ppm (H-3'), which in tern exhibited long range coupling to a carbonyl carbon (163.3 ppm), indicating that this carbonyl was bonded to C-2'. The remaining aromatic proton at 6.87 ppm was assigned to H-4' by the occurrence of NOE between those two aromatic protons. The presence of three methoxyl groups at C-5', C-6' and C-7' were confirmed by the long range couplings from each OCH_3 group. The partial structure **III** was also defined by isolation of the alkaline degradation product **5**.

Finally, IR *tert.* amide absorption at 1620 cm^{-1} and long range couplings between 6-H_2 (4.35 and 3.96 ppm) and C-2'α determined by an LSPD experiment established the connection of C-2'α to N-7, thus confirming the whole structure of **1** as shown in Fig. 1.

The structure of Duocarmycin C_2 (**2**), [4] $C_{26}H_{26}ClN_3O_8$, yellow needles, mp 256-257 °C, $[\alpha]_D^{24}$ -50.4° (MeOH), is different from that of **1** with respect to the chlorine-containing moiety. The ^1H NMR spectrum

showed that chloromethyl protons (4.03 and 3.86 ppm) were coupled to a methine (3.99 ppm), which in turn was coupled to a nitrogen-bearing methylene (4.62 and 4.32 ppm). This indicated that **2** had a dihydroindole skeleton on the corresponding partial structure **II** of **1**.

The structural differences between Duocarmycin A (**3**), [5] $C_{26}H_{25}N_3O_8$, yellow powder, $[\alpha]_D^{22}$+282° (MeOH), and Duocarmycin C_1 are also related to the partial structure **II**. Methylene signals at 2.24 and 1.29 ppm ($J_{g\,em}$ = 4.1 Hz) and ^{13}C signals at 22.0, 22.3 and 30.6 ppm indicated the presence of a cyclopropane ring system. The long range couplings from the methylene (2.24 and 1.29 ppm) to C-3a and C-6a, and from a vinyl proton at 7.17 ppm to C-8a and C-3b, and a ^{13}C signal at 179.7 ppm revealed the structure of **3** having dienone conjugated with the cyclopropane ring. Treatment of **3** with hydrochloric acid in acetone gave **1** and **2** in the ratio *ca*. 1 : 4. This provided the evidence for the structures of Duocarmycins and meant that **1** and **2** seemed to be artifact products of **3**.

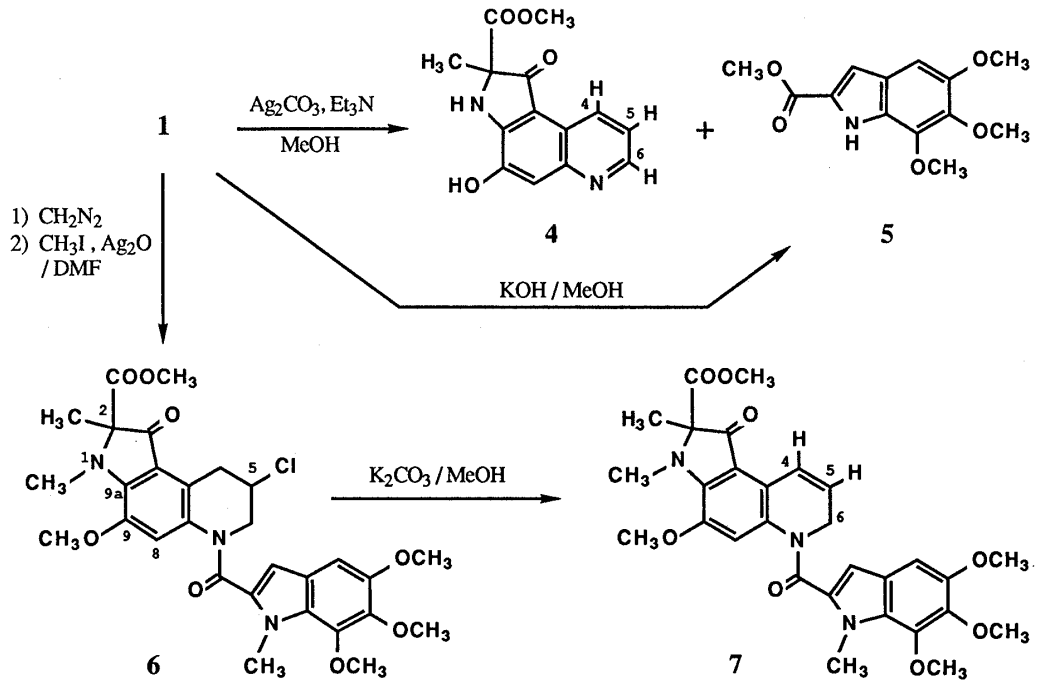


Fig. 3. Chemical Convertion of **1**

REFERENCES AND NOTES

1) I. Takahashi, K. Takahashi, M. Ichimura, M. Morimoto, K. Asano, I. Kawamoto, F. Tomita and H. Nakano, *J. Antibiot.*, in preparation.

2) M. Ichimura, K. Muroi, K. Asano, I. Kawamoto, F. Tomita, M. Morimoto and H. Nakano, *J. Antibiot.*, in press.

3) **1**: IR ($CHCl_3$) : 3450, 3300, 1740, 1695, 1620 cm^{-1}, UV λ_{max}^{MeOH} nm (ε): 323 (23000), 415 (4000), MS *m/z* : 543 (M+), 507, 310, 272, 251, 234, 213 (base peak), high-resolution MS Calcd for $C_{26}H_{26}ClN_3O_8$: 543.1406;

Found: 543.1422, ^{1}H NMR (400MHz, DMSO-d_6, δ): 11.54 (1H, d, J=1.8Hz, 1'-NH), 9.87 (1H, s, 9-OH), 7.39 (1H, s, 1-NH), 6.91 (1H, br s, H-8), 6.87 (1H, s, H-4'), 6.57 (1H, d, J=1.8Hz, H-3'), 4.76 (1H, m, H-5), 4.35 (1H, dd, J=13.4, 4.6 Hz, Ha-6), 3.96 (1H, br d, J=13.4Hz, Hb-6), 3.92 (3H, s, 7'-OCH_3), 3.77 (6H, s, 5'-OCH_3 and 6'-OCH_3), 3.61 (3H, s, 2-$COOCH_3$), 3.53 (1H, dd, J=19.2, 5.7Hz, Ha-4), 3.25 (1H, dd, J=19.2, 2.8Hz, Hb-4), 1.47 (3H, s, 2-CH_3), ^{13}C NMR (100MHz, DMSO-d_6, δ): 197.8 (C-3), 169.2 (2-*CO*OCH_3), 163.3 (C-2'α), 152.2 (C-9), 149.1 (C-5'), 141.2 (C-9a), 139.5 (C-6'), 139.0 (C-7'), 130.7 (C-2'), 128.4 (C-7a), 125.4 (C-7'a), 122.7 (C-3'a), 117.2 (C-8), 114.8 (C-3a), 114.7 (C-3b), 106.6 (C-3'), 97.9 (C-4'), 70.1 (C-2), 61.0 (7'-OCH_3), 60.9 (6'-OCH_3), 55.9 (5'-OCH_3), 54.7 (C-5), 52.6 (2-COO*C*H_3), 51.2 (C-6), 32.9 (C-4), 20.2 (2-CH_3).

4) **2**: IR ($CHCl_3$): 3450, 3325, 1740, 1700, 1585 cm^{-1}, UV λ_{max}^{MeOH} nm (ε): 250 (17000, sh.), 300 (19000), 337(26000), 435 (4000), MS *m/z*: 543 (M+), 507, 310, 234 (base peak), high-resolution MS Calcd for $C_{26}H_{26}ClN_3O_8$: 543.1406; Found: 543.1427, ^{1}H NMR (400MHz, DMSO-d_6, δ): 11.34 (1H, d, J=2.0Hz, 1'-NH), 10.20 (1H, s, 8-OH), 8.03 (1H, br s, H-7), 7.33 (1H, br s, 1-NH), 6.97 (1H, d, J=2.0Hz, H-3'), 6.95 (1H, s, H-4'), 4.62 (1H, br t, J=11.0Hz, Ha-5), 4.32 (1H, dd, J=11.0, 4.0Hz, Hb-5), 4.03 (1H, dd, J=10.0, 3.0Hz, Ha-4α), 3.99(1H, m, H-4), 3.92 (3H, s, 7'-OCH_3), 3.86 (1H, dd, J=10.0, 6.9Hz, Hb-4α), 3.81 (3H, s, 5'-OCH_3[a]), 3.79 (3H, s, 6'-OCH_3[a]), 3.61 (3H, s, 2-$COOCH_3$), 1.47 (3H, s, 2-CH_3), ^{13}C NMR (100MHz, $CDCl_3$, δ): 196.6 (C-3), 169.6 (2-*CO*OCH_3), 160.5 (C-2'α), 150.4 (C-5' *b*), 150.1 (C-8 *b*), 144.2 (C-8a), 140.9 (C-6'), 138.7 (C-7'), 137.7 (C-6a), 129.1 (C-2'), 126.0 (C-7'a), 123.5 (C-3'a), 119.5 (C-3a *c*), 115.6 (C-3b *c*), 112.5 (C-7), 107.9 (C-3'), 98.0 (C-4'), 71.2 (C-2), 61.5 (6'-OCH_3), 61.2 (7'-OCH_3), 56.4 (5'-OCH_3), 55.0 (C-5), 53.4 (2-COO*C*H_3), 46.4 (C-4α), 42.3 (C-4), 22.0 (2-CH_3). *a*, *b* and *c* are exchangeable assignment.

5) **3**: IR ($CHCl_3$): 3600, 3450, 3300, 1740, 1684, 1630 cm^{-1}, UV λ_{max}^{MeOH} nm (ε): 310 (18000), 358 (28000), 425 (8000), MS *m/z*: 507 (M+), 274, 234 (base peak), high-resolution MS Calcd for $C_{26}H_{25}N_3O_8$: 507.1639; Found: 507.1624, ^{1}H NMR (400MHz, $CDCl_3$, δ): 9.49 (1H, br s, 1'-NH), 7.17 (1H, s, H-7), 6.94 (1H, d, J=2.3Hz, H-3'), 6.78 (1H, s, H-4'), 6.36 (1H, s, 1-NH), 4.45 and 4.41 (AB in ABX, J_{AB}=10.3Hz, J_{AX}=4.7Hz, J_{BX}=*ca.* 1Hz, 5-H_2), 4.06 (3H, s, 7'-OCH_3), 3.93 (3H, s, 6'-OCH_3), 3.88 (3H, s, 5'-OCH_3), 3.74 (3H, s, 2-$COOCH_3$), 3.05 (1H, m, H-4a), 2.24 (1H, dd, J=7.6, 4.1Hz, Ha-4), 1.67 (3H, s, 2-CH_3), 1.29 (1H, dd, J=4.7, 4.1Hz, Hb-4), ^{13}C NMR (100MHz, $CDCl_3$, δ): 194.8 (C-3), 179.7 (C-8), 168.0 (2-*CO*OCH_3), 165.1 (C-6a), 164.4 (C-8a), 161.2 (C-2'α), 150.6 (C-5'), 141.3 (C-6'), 138.9 (C-7'), 128.2 (C-2'), 126.6 (C-7'a), 123.3 (C-3'a), 113.2 (C-7), 112.0 (C-3a), 108.2 (C-3'), 97.7 (C-4'), 71.3 (C-2), 61.5 (6'-OCH_3), 61.2 (7'-OCH_3), 56.3 (5'-OCH_3), 55.3(C-5), 53.4 (2-COO*CH*$_3$), 30.6 (C-3b), 22.3 (C-4a), 22.0 (C-4), 21.1 (2-CH_3).

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