

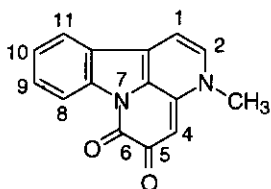
SYNTHESIS AND ANTITUMOR ACTIVITY OF CANTHIN-5,6-DIONE DERIVATIVES

Kazuo Koike, Hiroshi Yoshino, and Tamotsu Nikaido*

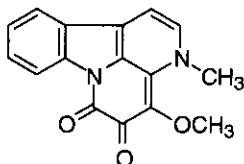
Department of Pharmacognosy, School of Pharmaceutical Sciences,
Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

Abstracts- Syntheses of canthin-5,6-dione derivatives have been achieved *via* one step route starting from their respective β -carbolines. Their synthetic compounds showed antitumor activities against P-388 murine leukemia cells and PC-6 human lung carcinoma cells.

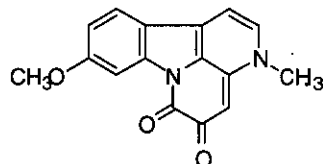
The picrasidine L (**1**),^{1,2} picrasidine O (**2**)³ and eurycomine E (**3**)⁴ are novel canthin-5,6-dione alkaloids isolated from the Simaroubaceae plants, *Picrasma quassioides* Bennet and *Eurycoma longifolia* Jack. Their structures have been proposed on the basis of interpretation of spectral data and partial synthesis of their parent 5-hydroxycanthin-6-one derivatives.^{2,3} In order to search for new lead compounds having potential for antitumor activity, we are interested in development of a facile synthesis of new canthin-5,6-diones which are a subclass of β -carboline alkaloid with diketonic D-ring. In this paper, we describe the one-pot synthesis and antitumor activity *in vitro* of canthin-5,6-dione derivatives.



Picrasidine L (**1**)



Picrasidine O (**2**)

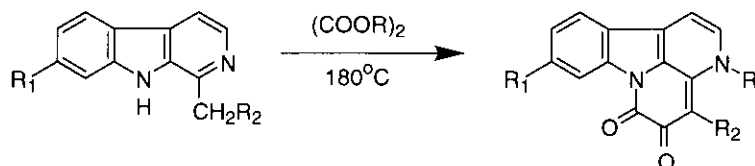


Eurycomine E (**3**)

Synthesis

Canthin-5,6-diones (**1-9**) were synthesized by the route shown in Scheme 1. Cycloaddition of β -carbolines (**10-12**) with alkyl oxalates for 20-60 min at 180°C yielded *N*₃-alkylcanthin-5,6-dione derivatives (**1-9**). The cycloaddition proceeded easily to give only canthin-5,6-dione derivatives without side reactions. However, the yield decreased with the increase in the carbon number of alkyl group of the alkyl oxalates. To confirm the cycloadducts, we conducted X-Ray crystallographic analysis of the synthetic sample of **2**. The crystal structure of **2** established the canthin-5,6-dione formation (Figure 1). The UV-VIS spectra of **2** showed a hypsochromic shift on the addition of acid, but was unchanged by

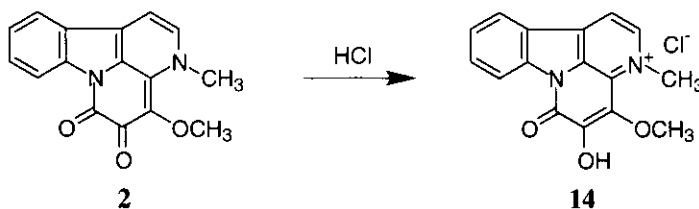
base. The hypsochromic shift of absorption maxima was very similar to that of 4-methoxy-5-hydroxycanthin-6-one (**13**).⁵ In the ¹H-NMR spectrum of **2** in the presence of TFA, the chemical shifts were also similar to those of **13** in TFA.⁵ All the spectral data indicated that protonation converted the 5-keto form (**2**) to the 5-enol form (**14**) in the acid condition (Scheme 2). Finally, the structure of the enol formation of **2** in an acidic medium was established by X-Ray crystallographic analysis of the hydrochloric acid salt of **2** (Figure 2).



	R ₁	R ₂		R	R ₁	R ₂
10	H	H	1	CH ₃	H	H
11	H	OCH ₃	4	CH ₂ CH ₃	H	H
12	OCH ₃	H	5	CH ₂ CH ₂ CH ₂ CH ₃	H	H
			2	CH ₃	H	OCH ₃
			6	CH ₂ CH ₃	H	OCH ₃
			7	CH ₂ CH ₂ CH ₂ CH ₃	H	OCH ₃
			3	CH ₃	OCH ₃	H
			8	CH ₂ CH ₃	OCH ₃	H
			9	CH ₂ CH ₂ CH ₂ CH ₃	OCH ₃	H

Reagents: (COOR)₂: R=CH₃, CH₂CH₃, CH₂CH₂CH₂CH₃.

Scheme 1.



Scheme 2.

Table 1. $^1\text{H-NMR}$ spectral data for compound (2) and (13) in $\text{DMSO-}d_6$ (323 K)

H	2	2+TFA	13	13+TFA
1	7.43 (d, 7.0)	8.60 (d, 6.6)	7.98 (d, 5.0)	8.31 (d, 5.5)
2	7.91 (d, 7.0)	8.92 (d, 6.6)	8.71 (d, 5.0)	8.80 (d, 5.5)
8	8.44 (d, 8.2)	8.55 (d, 8.2)	8.38 (d, 7.7)	8.51 (d, 8.2)
9	7.66 (t, 8.2)	7.93 (t, 8.2)	7.67 (t, 7.7)	7.81 (t, 8.2)
10	7.50 (t, 8.2)	7.70 (t, 8.2)	7.49 (t, 7.7)	7.61 (t, 8.2)
11	8.15 (d, 8.2)	8.50 (d, 8.2)	8.14 (d, 7.7)	8.40 (d, 8.2)
3- CH_3	3.82 (s)	4.68 (s)		
4- OCH_3	4.26 (s)	4.11 (s)	4.28 (s)	4.22 (s)
5-OH			5.77 (s)*	

*Disappeared with the addition of D_2O or TFA. Coupling constant in Hz.

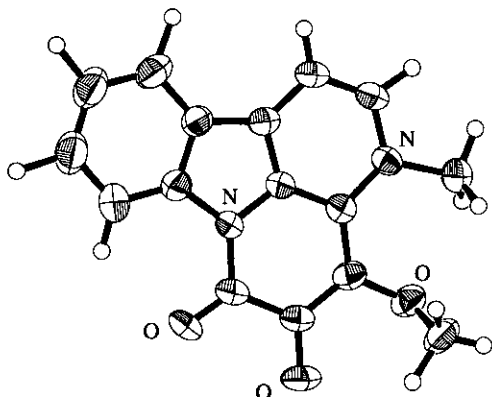


Figure 1. An ORTEP drawing of 2.

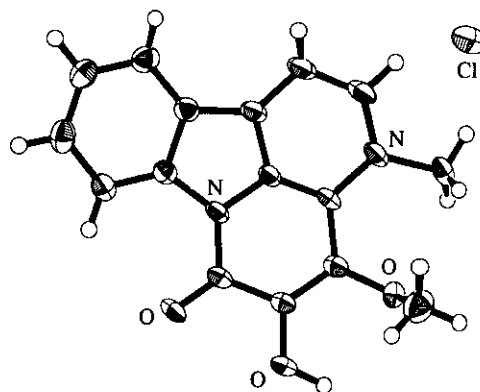


Figure 2. An ORTEP drawing of 14.

Biological Results

A study of the biological properties of nine canthin-5,6-dione derivatives (1-9) was carried out in P-388 murine leukemia cells and PC-6 human lung carcinoma cells *in vitro*. All compounds tested exhibited weaker antitumor activity than cisplatin. However, the antitumor activity increased as the carbon number of the alkyl chain was increased from the activity-structure relationship, indicating that the alkyl chain was a requisite functional group for the antitumor activities against P-388 and PC-6 cell lines *in vitro*. Further synthetic work aimed at structural modification is in progress to find compounds which show more potent antitumor activity than cisplatin.

Table 2. Antitumor activities against P-388 and PC-6 cell lines (GI₅₀ μg/mL)

compound	P-388	PC-6	compound	P-388	PC-6
1	27.9	27.2	3	>50	>50
4	10.6	16.2	8	10.3	30.7
5	5.9	8.5	9	4.8	13.8
2	42.6	38.5	cisplatin	0.02	0.28
6	33.8	28.9			
7	9.5	16.0			

EXPERIMENTAL

General Procedures. All melting points were measured on a Yanagimoto micromelting point hot-stage type apparatus without being uncorrected. UV-VIS spectra were recorded with a Hitachi 340 spectrophotometer. IR spectra as KBr pellets were recorded with a JASCO D300 FT-IR spectrophotometer. The ¹H-NMR spectra were recorded with a JEOL EX-400 (¹H: 400 MHz) spectrometer. Chemical shifts are given on the δ scale (ppm) with tetramethylsilane as an internal standard, and coupling constants are given in Hz. EIMS and HRMS spectra were conducted using JEOL D-300 and DX-303 mass spectrometers, respectively. Column chromatography was performed on silica gel (Merck).

General Procedure of Preparation for β-Carbolines. These compounds were prepared from tryptamine or *dl*-tryptophan by the Pictet-Spengler reaction with the appropriate aldehyde in an acidic medium, according to the procedure in the literature.⁶ 7-Methoxy-1-methyl-β-carboline (**12**) was purchased from Sigma.

1-Methyl-β-carboline (10)⁷: Yield 88.7%, colorless needles, mp 232°C (acetone). IR (KBr) cm⁻¹: 3300, 1530, 1500, 1450, 1380, 1330, 1310, 1240. MS *m/z*: 182 (M⁺). ¹H-NMR (DMSO-*d*₆, 323K) δ: 2.76 (3H, s, CH₃), 7.23 (1H, t, *J*=8.2 Hz, H-6), 7.53 (1H, t, *J*=8.2 Hz, H-7), 7.60 (1H, d, *J*=8.2 Hz, H-8), 7.92 (1H, d, *J*=5.3 Hz, H-4), 8.19 (1H, d, *J*=8.2 Hz, H-5), 8.20 (1H, d, *J*=5.3 Hz, H-3), 11.53 (1H, brs, NH). MS *m/z*: 182 (M⁺). *Anal.* Calcd for C₁₂H₁₀N₂: C 79.10, H 5.53, N 15.37, Found: C 79.07, H 5.48, N 15.18.

1-Methoxymethyl-β-carboline (11)⁸: Yield 65.3%, colorless needles, mp 123-125°C (acetone). IR (KBr) cm⁻¹: 3310, 1610, 1550, 1225, 1150, 1070. ¹H-NMR (CDCl₃) δ: 3.49 (3H, s, CH₂OCH₃), 5.04 (2H, s, CH₂OCH₃), 7.23 (1H, t, *J*=7.2 Hz, H-6), 7.45 (1H, t, *J*=7.2 Hz, H-7), 7.64 (1H, d, *J*=7.2 Hz, H-8), 8.05 (1H, d, *J*=6.6 Hz, H-4), 8.08 (1H, d, *J*=6.6 Hz, H-3), 8.12 (1H, d, *J*=7.2 Hz, H-5), 11.33 (1H, brs, NH). MS *m/z*: 212 (M⁺). *Anal.* Calcd for C₁₃H₁₂N₂O: C 73.56, H 5.70, N 13.20, Found: C 73.79, H 5.56, N 13.19.

3-Methylcanthin-5,6-dione (picrasidine L, 1): A mixture of 1-methyl- β -carboline (**10**, 0.8 g, 4.4 mmol) and dimethyl oxalate (1.0 g, 8.5 mmol) was heated for 20 min at 180°C. The red reactant was recrystallized from MeOH to give red needles. Yield 0.82 g (75.0%), mp >300°C. IR (KBr) cm^{-1} : 1688, 1651, 1541, 1508, 1486, 1447, 1409, 1337, 1310, 1218. UV λ max (MeOH) nm (log ϵ): 225 (4.24), 245 (4.33), 250 (4.31), 273 (3.98), 288 (4.07), 353 (sh, 3.63), 363 (sh, 3.60), 450 (4.08). UV λ max (MeOH+NaOH) nm (log ϵ): 225 (4.25), 245 (4.34), 250 (4.33), 273 (3.98), 288 (4.01), 353 (3.67), 450 (4.10). UV λ max (MeOH+HCl) nm (log ϵ): 254 (4.30), 324 (3.90), 332 (sh, 3.92), 380 (4.04), 397 (4.04), 450 (3.40). $^1\text{H-NMR}$ (DMSO- d_6 , 323K) δ : 3.91 (3H, s, CH_3 -3), 6.00 (1H, s, H-4), 7.47 (1H, d, $J=7.0$ Hz, H-1), 7.54 (1H, t, $J=8.1$ Hz, H-10), 7.7 (1H, t, $J=8.1$ Hz, H-8), 8.03 (1H, d, $J=7.0$ Hz, H-2), 8.22 (1H, d, $J=8.1$ Hz, H-8), 8.47 (1H, d, $J=8.1$ Hz, H-11). EIMS m/z (%): 250 (M^+ , 48), 236 (5), 222 (69), 193 (100). HRMS m/z : Calcd for $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}_2$, 250.0740. Found: 250.0733 [M^+]. *Anal.* Calcd for $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}_2 \cdot \text{H}_2\text{O}$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.62; H, 4.25; N, 10.10.

3-Ethylcanthin-5,6-dione (4): A mixture of 1-methyl- β -carboline (**10**, 0.5 g, 2.7 mmol) and diethyl oxalate (2.0 g, 13.7 mmol) was heated for 30 min at 180°C. The red reactant was chromatographed on silica gel eluting with CHCl_3 -MeOH (9:1). The product was recrystallized from CHCl_3 -MeOH (7:3) to give red needles. Yield 0.20 g (28.2%), mp >300°C. IR (KBr) cm^{-1} : 1697, 1648, 1614, 1591, 1548, 1520, 1455, 1368, 1338, 1325, 1311, 1208, 1183. UV λ max (MeOH) nm (log ϵ): 216 (3.98), 224 (4.06), 227 (4.05), 268 (3.69), 290 (3.82), 352 (sh, 3.36), 364 (sh, 3.34), 451 (3.82). UV λ max (MeOH+NaOH) nm (log ϵ): 216 (3.98), 224 (4.06), 227 (4.05), 268 (3.69), 290 (3.82), 352 (sh, 3.36), 364 (sh, 3.34), 451 (3.82). UV λ max (MeOH+HCl) nm (log ϵ): 249 (4.03), 292 (3.37), 323 (3.70), 335 (sh, 3.67), 381 (3.78), 400 (3.78), 450 (3.00). $^1\text{H-NMR}$ (DMSO- d_6 , 323K) δ : 1.44 (3H, t, $J=7.2$ Hz, CH_2CH_3 -3), 4.34 (2H, q, $J=7.2$ Hz, CH_2CH_3 -3), 6.11 (1H, s, H-4), 7.53 (1H, d, $J=7.0$ Hz, H-1), 7.55 (1H, t, $J=7.3$ Hz, H-10), 7.72 (1H, t, $J=7.3$ Hz, H-9), 8.06 (1H, d, $J=7.0$ Hz, H-2), 8.23 (1H, d, $J=7.3$ Hz, H-8), 8.47 (1H, d, $J=7.3$ Hz, H-11). EIMS m/z (%): 264 (M^+ , 64), 250 (3), 236 (57), 207 (100). HRMS m/z : Calcd for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_2$, 264.0896. Found: 264.0845 [M^+]. *Anal.* Calcd for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_2$: C, 72.72; H, 4.58; N, 10.60. Found: C, 72.58; H, 4.57; N, 10.52.

3-Butylcanthin-5,6-dione (5): A mixture of 1-methyl- β -carboline (**10**, 0.5 g, 2.7 mmol) and dibutyl oxalate (1.0 g, 5 mmol) was heated for 30 min at 180°C. The red reactant was chromatographed on silica gel eluting with CHCl_3 -MeOH (9:1). The product was recrystallized from MeOH to give yellow needles. Yield 0.05 g (6.3%), mp 278-280°C. IR (KBr) cm^{-1} : 1695, 1646, 1614, 1595, 1551, 1364, 1272. UV λ max (MeOH) nm (log ϵ): 228 (4.29), 245 (4.37), 248 (4.36), 273 (4.01), 287 (4.13), 348 (sh, 3.70), 360 (sh, 3.65), 450 (4.12). UV λ max (MeOH+NaOH) nm (log ϵ): 228 (4.26), 244 (4.33), 252 (4.32), 270 (3.99), 285 (4.09), 348 (sh, 3.68), 360 (sh, 3.63), 450 (4.09). UV λ max (MeOH+HCl) nm (log ϵ): 250 (4.35), 320 (4.04), 225 (sh, 4.00), 275 (4.07), 294 (4.07), 450 (3.42). $^1\text{H-NMR}$ (DMSO- d_6 , 323K) δ : 0.95 (3H, t, $J=7.5$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ -3), 1.40 (2H, sextet, $J=7.5$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ -3), 1.79 (2H, quintet, $J=7.5$ Hz, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ -3), 4.28 (2H, t, $J=7.5$ Hz, $\text{H-CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ -3), 7.49 (1H, d, $J=7.0$ Hz, H-1), 7.53 (1H, t, $J=8.4$ Hz, H-10), 7.69 (1H, t, $J=8.4$ Hz, H-9), 8.04 (1H, d, $J=7.0$ Hz, H-2), 8.21 (1H, d, $J=8.4$ Hz, H-11), 8.27 (1H, s, H-4), 8.46 (1H, d, $J=8.4$ Hz, H-8). EIMS m/z (%): 292

(M^+ , 75), 264 (13), 250 (8), 235 (13), 222 (100). HRMS m/z : Calcd for $C_{18}H_{16}N_2O_2$, 292.1208. Found: 292.1205 [M^+]. *Anal.* Calcd for $C_{18}H_{16}N_2O_2 \cdot 1.5H_2O$: C, 73.95; H, 5.52; N, 9.58. Found: C, 73.48; H, 5.43; N, 9.24.

3-Methyl-4-methoxycantin-5,6-dione (picrasidine O, 2): A mixture of 1-methoxymethyl- β -carboline (**11**, 1.5 g, 7.1 mmol) and dimethyl oxalate (1.4 g, 12 mmol) was heated for 20 min at 180°C. The red reactant was recrystallized from MeOH to give red needles. Yield 1.51 g (75%). mp 274°C (decomp). IR (KBr) cm^{-1} : 1682, 1642, 1549, 1500, 1450, 1282, 1108. UV λ max (MeOH) nm (log ϵ): 242 (4.28), 250 (sh, 4.27), 290 (4.20), 360 (3.54), 480 (4.04), 500 (4.00). UV λ max (MeOH+NaOH) nm (log ϵ): 242 (4.28), 250 (sh, 4.27), 290 (4.20), 360 (3.54), 480 (4.04), 500 (4.00). UV λ max (MeOH+HCl) nm (log ϵ): 242 (4.14), 258 (sh, 4.11), 320 (3.84), 328 (sh, 3.81), 378 (3.76), 416 (sh, 3.52). 1H -NMR: Table I. EIMS m/z (%): 280 (M^+ , 54), 273 (3), 265 (70), 250 (6), 235 (4), 209 (39), 193 (4), 181 (100). HRMS m/z : Calcd for $C_{16}H_{12}N_2O_3$, 280.0845. Found: 280.0838 [M^+]. *Anal.* Calcd for $C_{16}H_{12}N_2O_3$: C, 68.59; H, 4.22; N, 9.99. Found: C, 68.46; H, 4.21; N, 9.93.

3-Ethyl-4-methoxycanthin-5,6-dione (6): A mixture of 1-methoxymethyl- β -carboline (**11**, 0.8 g, 3.8 mmol) and diethyl oxalate (1.0 g, 7 mmol) was heated for 1 h at 180°C. The red reactant was chromatographed on silica gel eluting with $CHCl_3$ -MeOH (9:1). The product was recrystallized from $CHCl_3$ -MeOH (7:3) to give red powder. Yield 0.32 g (28.6%), mp 248-250°C. IR (KBr) cm^{-1} : 1694, 1636, 1541, 1505, 1468, 1455, 1357, 1116. UV λ max (MeOH) nm (log ϵ): 247 (4.46), 294 (4.34), 350 (sh, 3.86), 361 (sh, 3.80), 481 (4.14). UV λ max (MeOH+NaOH) nm (log ϵ): 247 (4.43), 294 (4.30), 350 (sh, 3.85), 361 (sh, 3.79), 481 (4.11). UV λ max (MeOH+HCl) nm (log ϵ): 246 (4.41), 257 (4.38), 325 (4.18), 335 (sh, 4.16), 380 (4.11). 1H -NMR (DMSO- d_6 , 323K) δ : 1.47 (3H, t, $J=7.2$ Hz, CH_2CH_3 -3), 3.87 (3H, s, OCH_3 -4), 4.67 (2H, q, $J=7.2$ Hz, CH_2CH_3 -3), 7.51 (1H, $J=7.0$ Hz, H-1), 7.51 (1H, t, $J=8.3$ Hz, H-10), 7.67 (1H, t, $J=8.3$ Hz, H-9), 8.02 (1H, d, $J=7.0$ Hz, H-2), 8.18 (1H, d, $J=8.3$ Hz, H-11), 8.44 (1H, d, $J=8.3$ Hz, H-8). EIMS m/z (%): 294 (M^+ , 53), 279 (85), 265 (67), 251 (7), 236 (13), 223 (47), 207 (3), 195 (63), 179 (6), 167 (100). HRMS m/z : Calcd for $C_{17}H_{14}N_2O_3$, 294.1001. Found: 294.0983 [M^+]. *Anal.* Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.68; H, 4.91; N, 9.22.

3-Butyl-4-methoxycanthin-5,6-dione (7): A mixture of 1-methoxymethyl- β -carboline (**11**, 1.0 g, 4.7 mmol) and dibutyl oxalate (2.0 g, 9.9 mmol) was heated for 1 h at 180°C. The red reactant was chromatographed on silica gel eluting with $CHCl_3$ -MeOH (9:1). The product was recrystallized from $CHCl_3$ -MeOH (7:3) to give red powder. Yield 0.051 g (3.4%), mp 248-249°C. IR (KBr) cm^{-1} : 1685, 1635, 1609, 1585, 1352, 1275, 1149, 1123. UV λ max (MeOH) nm (log ϵ): 246 (4.56), 296 (4.45), 350 (sh, 3.98), 364 (sh, 3.93), 488 (4.24). UV λ max (MeOH+NaOH) nm (log ϵ): 245 (4.52), 294 (4.40), 350 (sh, 3.95), 364 (sh, 3.90), 481 (4.19). UV λ max (MeOH+HCl) nm (log ϵ): 244 (4.53), 255 (4.50), 321 (4.29), 379 (4.21). 1H -NMR (DMSO- d_6 , 323K) δ : 0.95 (3H, t, $J=7.3$ Hz, $CH_2CH_2CH_2CH_3$ -3), 1.40 (2H, sextet, $J=7.3$ Hz, $CH_2CH_2CH_2CH_3$ -3), 1.84 (2H, quintet, $J=7.3$ Hz, $CH_2CH_2CH_2CH_3$ -3), 3.86 (3H, s, OCH_3 -4), 4.61 (2H, t, $J=7.3$ Hz, $CH_2CH_2CH_2CH_3$ -3), 7.50 (1H, d, $J=7.0$ Hz, H-1), 7.51 (1H, t,

$J=8.3$ Hz, H-10), 7.67 (1H, t, $J=8.3$ Hz, H-9), 8.01 (1H, d, $J=7.0$ Hz, H-2), 8.18 (1H, d, $J=8.3$ Hz, H-11), 8.44 (1H, d, $J=8.3$ Hz, H-8). EIMS m/z (%): 322 (M^+ , 88), 307 (100), 293 (17), 275 (5), 265 (94), 251 (96), 237 (16), 220 (11). HRMS m/z : Calcd for $C_{19}H_{18}N_2O_3$, 322.1313. Found: 322.1347 [M^+]. *Anal.* Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.97; H, 5.64; N, 8.64.

3-Methyl-9-methoxycanthin-5,6-dione (eurycomine E, 3): A mixture of 1-methyl-7-methoxy- β -carboline (**12**, 0.8 g, 3.7 mmol) and dimethyl oxalate (1.0 g, 8.5 mmol) was heated for 30 min at 180°C. The red reactant was recrystallized from MeOH to give red needles. Yield 0.83 g (79.8%), mp >300°C. IR (KBr) cm^{-1} : 1692, 1649, 1616, 1591, 1553, 1272, 1232, 1217, 1175, 1153, 1099, 1073. UV λ max (MeOH) nm (log ϵ): 450 (4.19), 347 (4.26), 279 (4.39), 255 (4.24), 230 (4.45). UV λ max (MeOH+NaOH) nm (log ϵ): 450 (4.17), 347 (4.22), 279 (4.35), 255 (4.20), 230 (4.40). UV λ max (MeOH+HCl) nm (log ϵ): 400 (4.05), 358 (4.17), 303 (4.35), 260 (4.24), 223 (4.46). 1H -NMR (DMSO- d_6 , 323K) δ : 3.89 (3H, s, CH_3 -3), 3.93 (3H, s, OCH_3 -9), 7.14 (1H, dd, $J=2.6$ and 8.4 Hz, H-10), 7.40 (1H, d, $J=7.0$ Hz, H-1), 8.00 (1H, d, $J=7.0$ Hz, H-2), 8.01 (1H, d, $J=2.6$ Hz, H-8), 8.10 (1H, d, $J=8.4$ Hz, H-11). EIMS m/z (%): 280 (M^+ , 60), 266 (4), 252 (100), 237 (11), 223 (74), 209 (29). HRMS m/z : Calcd for $C_{16}H_{12}N_2O_3$, 280.0845. Found: 280.0837 [M^+]. *Anal.* Calcd for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99. Found: C, 68.38; H, 4.40; N, 9.84.

3-Ethyl-9-methoxycanthin-5,6-dione (8): A mixture of 1-methyl-7-methoxy- β -carboline (**12**, 0.8 g, 3.7 mmol) and diethyl oxalate (1.25 g, 8.5 mmol) was heated for 1 h at 180°C. The red reactant was recrystallized from MeOH to give red needles. Yield 0.30 g (52.6%), mp >300°C. IR (KBr) cm^{-1} : 1696, 1646, 1544, 1517, 1464, 1439, 1277. UV λ max (MeOH) nm (log ϵ): 235 (4.18), 264 (3.95), 284 (4.11), 345 (3.99), 425 (3.91). UV λ max (MeOH+NaOH) nm (log ϵ): 235 (4.26), 263 (4.04), 284 (4.20), 350 (4.08), 450 (4.01). UV (MeOH+HCl) nm (log ϵ): 227 (4.21), 265 (3.92), 285 (4.04), 362 (4.05), 402 (3.73). 1H -NMR (DMSO- d_6 , 323K) δ : 1.42 (3H, t, $J=7.2$ Hz, CH_2CH_3 -3), 3.93 (3H, s, OCH_3 -9), 4.30 (2H, q, $J=7.2$ Hz, CH_2CH_3 -3), 6.02 (1H, s, H-4), 7.13 (1H, dd, $J=2.6$ and 8.8 Hz, H-10), 7.39 (1H, d, $J=7.0$ Hz, H-1), 7.98 (1H, d, $J=7.0$ Hz, H-2), 8.02 (1H, d, $J=2.6$ Hz, H-8), 8.08 (1H, d, $J=8.8$ Hz, H-11). EIMS m/z (%): 294 (M^+ , 100), 280 (11), 266 (78), 252 (14), 237 (97), 223 (37), 210 (24). HRMS m/z : Calcd for $C_{17}H_{14}N_2O_3$, 294.1001. Found: 294.0978 [M^+]. *Anal.* Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 68.85; H, 4.81; N, 9.46.

3-Butyl-9-methoxycanthin-5,6-dione (9): A mixture of 1-methyl-7-methoxy- β -carboline (**12**, 0.5 g, 2.3 mmol) and dibutyl oxalate (1.0 g, 4.9 mmol) was heated for 1 h at 180°C. The red reactant was chromatographed on silica gel eluting with $CHCl_3$ -MeOH (9:1). The product was recrystallized from MeOH to give orange needles. Yield 0.031 g (4.2%), mp 270-272°C. IR (KBr) cm^{-1} : 1695, 1647, 1582, 1543, 1509, 1490, 1453, 1359, 1340, 1206. UV λ max (MeOH) nm (log ϵ): 235 (3.88), 260 (3.65), 277 (3.80), 350 (3.69), 452 (3.60). UV λ max (MeOH+NaOH) nm (log ϵ): 234 (3.94), 260 (3.74), 282 (3.87), 348 (3.74), 450 (3.65). UV λ max (MeOH+HCl) nm (log ϵ): 227 (3.92), 263 (3.65), 287 (3.75), 360 (3.77), 400 (3.45). 1H -NMR (DMSO- d_6 , 323K) δ : 0.94 (3H, t, $J=7.3$ Hz, $CH_2CH_2CH_2CH_3$ -3), 1.39 (2H, sextet, $J=7.3$ Hz, $CH_2CH_2CH_2CH_3$ -3), 1.79 (2H, quintet, $J=7.3$ Hz, $CH_2CH_2CH_2CH_3$ -3), 3.93

(3H, s, OCH₃-9), 4.28 (2H, t, $J=7.3$ Hz, CH₂CH₂CH₂CH₃-3), 6.04 (1H, s, H-4), 7.14 (1H, dd, $J=2.6$ and 8.43 Hz, H-10), 7.43 (1H, d, $J=7.0$ Hz, H-1), 8.01 (1H, d, $J=2.6$ Hz, H-8), 8.02 (1H, d, $J=7.0$ Hz, H-2), 8.11 (1H, d, $J=8.4$ Hz, H-11). EIMS m/z (%): 322 (M⁺, 86), 294 (14), 280 (11), 266 (16), 252 (100), 237 (32), 224 (14), 210 (41). HRMS m/z : Calcd for C₁₉H₁₈N₂O₃, 322.1313. Found: 322.1359 [M⁺]. Anal. Calcd for C₁₉H₁₈N₂O₃: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.84; H, 5.64; N, 8.66.

Crystal Data of 3: Crystallized from methanol and belonging to monoclinic space group *P*1 (#1). Lattice constants and intensity data were measured on a Rigaku AFC-5R diffractometer with a device for graphite-monochromated CuK α radiation. Crystal data: C₁₆H₁₂N₂O₃, $a=6.1954(28)$, $b=11.113(1)$, $c=4.9527(5)$ Å, $Z=1$, $\alpha=97.600(7)$, $\beta=105.674(8)$, $\gamma=101.419(9)^\circ$, $D_{\text{calc}}=1.475$ g/cm³, CuK α $\lambda=1.54178$ Å. A total of 925 independent reflections with $I>3\sigma(I)$ was used for structure analysis. The structure was solved by the direct method (MULTAN88)⁹ and expanded using Fourier techniques.¹⁰ The structure was then refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen and isotropic atoms for hydrogen atoms to an *R* factor of 0.028 ($R_w=0.038$).

Crystal Data of 14: Crystallized from methanol and belonging to monoclinic space group *P* $\bar{1}$ (#2). Lattice constants and intensity data were measured on a Rigaku AFC-7R diffractometer with a device for graphite-monochromated CuK α radiation. Crystal data: C₁₈H₁₃N₂O₃Cl, $a=8.807(2)$, $b=12.446(3)$, $c=7.437(4)$ Å, $\alpha=100.77(3)$, $\beta=95.65(3)$, $\gamma=99.38(2)^\circ$, $Z=2$, $D_{\text{calc}}=1.445$ g/cm³, CuK α $\lambda=1.54178$ Å. A total of 2215 independent reflections with $I>3\sigma(I)$ was used for structure analysis. The structure was solved by the direct method (SIR88)¹¹ and expanded using Fourier techniques.¹⁰ The structure was then refined by full-matrix least squares with anisotropic temperature factors for non-hydrogen and isotropic atoms for hydrogen atoms to an *R* factor of 0.062 ($R_w=0.103$).

In Vitro Cytotoxicity: To examine the direct growth-inhibitory effects of test compounds against P-388 against murine leukemia cells and PC-6 human lung carcinoma cells, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed and the concentration giving a growth inhibition of 50% (GI₅₀) was calculated according to a published procedure.¹²

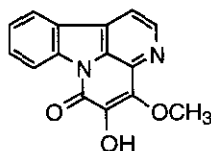
ACKNOWLEDGMENT

We are grateful to Daiichi Pharmaceutical Co., Ltd. for the biological assay.

REFERENCES AND NOTES

1. T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, 1982, **30**, 1204.
2. T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, 1985, **33**, 3847.
3. T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, 1985, **33**, 4901.
4. K. Mitsunaga, K. Koike, T. Tanaka, Y. Ohkawa, Y. Kobayashi, T. Sawaguchi, and T. Ohmoto, *Phytochemistry*, 1994, **35**, 799.

5. T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, 1984, **32**, 3579.
 4-Methoxy-5-hydroxycanthin-6-one (nigakinone, **13**): UV λ max (MeOH) nm (log ϵ): 246 (4.60), 262 (sh, 4.50), 286 (4.25), 340 (4.01), 350 (4.00). UV λ max (MeOH+NaOH) nm (log ϵ): 254 (4.53), 286 (4.44), 318 (3.95), 4.20 (4.19). UV λ max (MeOH+HCl) nm (log ϵ): 248 (3.65), 322 (4.00), 360 (4.25), 274 (4.25), 234 (3.94).

**13**

6. W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, 1936, **113**, 759; A. Brossi, A. Focella, and S. I. Teitel, *J. Med. Chem.*, 1973, **16**, 418; J. M. Bobbit and J. P. Willis, *J. Org. Chem.*, 1980, **45**, 1978; H. R. Synder, C. H. Hansch, L. Katz, S. M. Parmerter, and E. C. Spaeth, *J. Am. Chem. Soc.*, 1948, **70**, 219.
7. M. Cain, R. Mantei, and J. M. Cook, *J. Org. Chem.*, 1982, **47**, 4933.
8. K. Wakabayashi, T. Yamamoto, K. Tsuji, H. Zenda, and T. Kosuge, *Yakugaku Zasshi*, 1978, **98**, 898; M. R. Prinsep, J. W. Blunt, and M. H. G. Munro, *J. Nat. Prod.*, 1991, **54**, 1068.
9. T. Debaerdemaeker, G. Germain, P. Main, L. S. Refaat, C. Tate, and M. M. Woolfson, Computer programs for the automatic solution of crystal structures for X-Ray diffraction data 1988, University of York, U. K.
10. P. T. Beurskens, G. Admiraal, G. Beurskens, W. P. Bosman, R. de Gelder, R. Israe, and J. M. M. Smits. The DIRDIF94 program system 1994, Technical Report of the Crystallography Laboratory., University of Nijmegen, The Netherlands.
11. M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, G. Polidori, R. Spagna, and D. Viterbo, *J. Appl. Cryst.*, 1989, **22**, 389.
12. I. Mitsui, E. Kumazawa, Y. Hirota, M. Aonuma, M. Sugimori, S. Ohsuki, K. Uoto, and A. Ejima, *Jpn. J. Cancer Res.*, 1995, **86**, 776.

Received, 7th October, 1998