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

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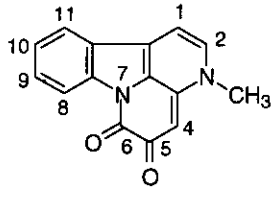
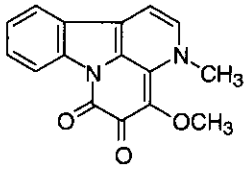
Abstracts- Syntheses of canthin-5,6-dione derivatives have been achieved *via* one step route starting from their respective β-carbolines. Their synthetic compounds showed antitumor activities against P-388 murine leukemia cells and PC-6 human lung carcinoma cells.

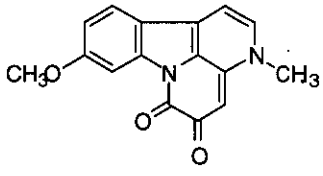
The picrasidine L (**1**),[1,2] picrasidine O (**2**)[3] and eurycomine E (**3**)[4] are novel canthin-5,6-dione alkaloids isolated from the Simaroubaceae plants, *Picrasma quassioides* Bennet and *Eurycoma longifolia* Jack. Their structures have been proposed on the basis of interpretation of spectral 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**)

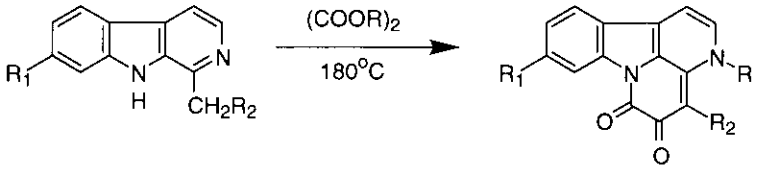


Eurycomine E (**3**)

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 ^{1}H-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).

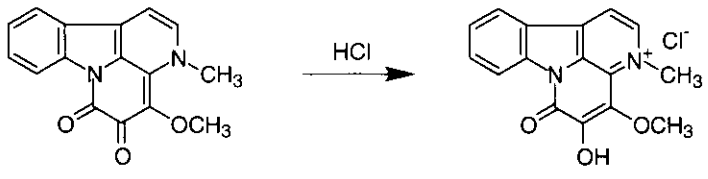


	R_1	R_2
10	H	H
11	H	OCH_3
12	OCH_3	H

	R	R_1	R_2
1	CH_3	H	H
4	CH_2CH_3	H	H
5	$CH_2CH_2CH_2CH_3$	H	H
2	CH_3	H	OCH_3
6	CH_2CH_3	H	OCH_3
7	$CH_2CH_2CH_2CH_3$	H	OCH_3
3	CH_3	OCH_3	H
8	CH_2CH_3	OCH_3	H
9	$CH_2CH_2CH_2CH_3$	OCH_3	H

Reagents: $(COOR)_2$: R=CH_3, CH_2CH_3, $CH_2CH_2CH_2CH_3$.

Scheme 1.



2 **14**

Scheme 2.

Table 1. ^{1}H-NMR spectral data for compound (**2**) and (**13**) in 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.

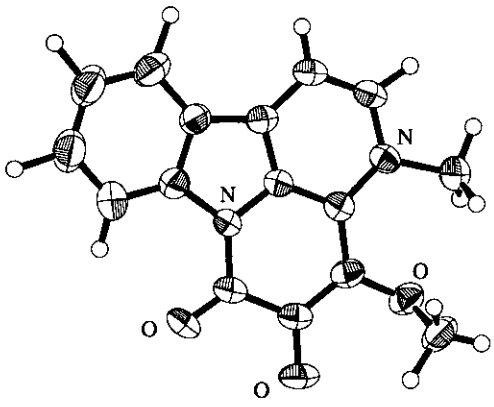


Figure 1. An ORTEP drawing of **2**.

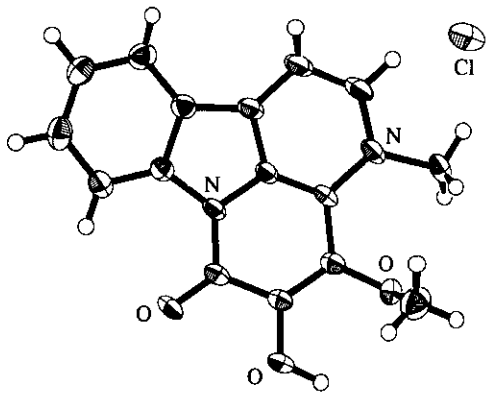


Figure 2. An ORTEP drawing of **14**.

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 (GI_{50} 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 spectrophtometer. IR spectra as KBr pellets were recorded with a JASCO D300 FT-IR spectrophotometer. The ^{1}H-NMR spectra were recorded with a JEOL EX-400 (^{1}H: 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^+). ^{1}H-NMR (DMSO-d_6, 323K) δ: 2.76 (3H, s, CH_3), 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 $C_{12}H_{10}N_2$: 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. ^{1}H-NMR ($CDCl_3$) δ: 3.49 (3H, s, $CH_2OC\underline{H}_3$), 5.04 (2H, s, $C\underline{H}_2OCH_3$), 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 $C_{13}H_{12}N_2O$: 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, 363), 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}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 $C_{15}H_{10}N_2O_2$, 250.0740. Found: 250.0733 [M^+]. *Anal.* Calcd for $C_{15}H_{10}N_2O_2 \cdot H_2O$: 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}H-NMR (DMSO-d_6, 323K) δ: 1.44 (3H, t, *J*=7.2 Hz, $C\underline{H}_2CH_3$-3), 4.34 (2H, q, *J*=7.2 Hz, $CH_2C\underline{H}_3$-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 $C_{16}H_{12}N_2O_2$, 264.0896. Found: 264.0845 [M^+]. *Anal.* Calcd for $C_{16}H_{12}N_2O_2$: 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}H-NMR (DMSO-d_6, 323K) δ: 0.95 (3H, t, *J*=7.5 Hz, $CH_2CH_2CH_2C\underline{H}_3$-3), 1.40 (2H, sextet, *J*=7.5 Hz, $CH_2CH_2C\underline{H}_2CH_3$-3), 1.79 (2H, quintet, *J*=7.5 Hz, $CH_2C\underline{H}_2CH_2CH_3$-3), 4.28 (2H, t, *J*=7.5 Hz, H-$C\underline{H}_2CH_2CH_2CH_3$-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, H11), 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). ^{1}H-NMR: Table 1. 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, 379), 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). ^{1}H-NMR (DMSO-d_6, 323K) δ: 1.47 (3H, t, *J*=7.2 Hz, $CH_2C\underline{H}_3$-3), 3.87 (3H, s, OCH_3-4), 4.67 (2H, q, *J*=7.2 Hz, $C\underline{H}_2CH_3$-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). ^{1}H-NMR (DMSO-d_6, 323K) δ: 0.95 (3H, t, *J*=7.3 Hz, $CH_2CH_2CH_2C\underline{H}_3$-3), 1.40 (2H, sextet, *J*=7.3 Hz, $CH_2CH_2C\underline{H}_2CH_3$-3), 1.84 (2H, quintet, J=7.3 Hz, $CH_2C\underline{H}_2CH_2CH_3$-3), 3.86 (3H, s, OCH_3-4), 4.61 (2H, t, *J*=7.3 Hz, $C\underline{H}_2CH_2CH_2CH_3$-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). ^{1}H-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) . ^{1}H-NMR (DMSO-d_6, 323K) δ: 1.42 (3H, t, *J*=7.2 Hz, $CH_2C\underline{H}_3$-3), 3.93 (3H, s, OCH_3-9), 4.30 (2H, q, *J*=7.2 Hz, $C\underline{H}_2CH_3$-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). ^{1}H-NMR (DMSO-d_6, 323K) δ: 0.94 (3H, t, *J*=7.3 Hz, $CH_2CH_2CH_2C\underline{H}_3$-3), 1.39 (2H, sextet, *J*=7.3 Hz, $CH_2CH_2C\underline{H}_2CH_3$-3), 1.79 (2H, quintet, J= 7.3 Hz, $CH_2C\underline{H}_2CH_2CH_3$-3), 3.93

(3H, s, OCH_3-9), 4.28 (2H, t, J=7.3 Hz, $C\underline{H}_2CH_2CH_2CH_3$-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 $C_{19}H_{18}N_2O_3$, 322.1313. Found: 322.1359 [M^+]. *Anal.* Calcd for $C_{19}H_{18}N_2O_3$: 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 $P1$ (#1). Lattice constants and intensity data were measured on a Rigaku AFC-5R diffractometer with a device for graphite-monochromated CuKα radiation. Crystal data: $C_{16}H_{12}N_2O_3$, a=6.1954(28), b=11.113(1), c=4.9527(5)Å, Z=1, α=97.600(7), β=105.674(8), γ=101.419(9)°, D_{calc}=1.475 g/cm^3, CuKα λ=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 CuKα radiation. Crystal data: $C_{18}H_{13}N_2O_3Cl$, a=8.807(2), b=12.446(3), c=7.437(4) Å, α=100.77(3), β=95.65(3), γ=99.38(2)°, Z=2, D_{calc}=1.445 g/cm^3, CuKα λ=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* Cytotoxity:** 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% (GI_{50}) was calculated according to a published procedure.[12]

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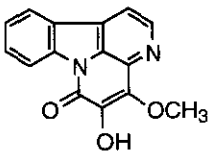
REFERENCES AND NOTES

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).



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6. W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, 1936, **113**, 759; A. Brossi, A. Focella, and S.1 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.

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