Photocyclization of γ-Chlorotiglyl-L-tryptophan Methyl Ester Yields Azocinoindole and Azepinoindole Derivatives

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We aimed to synthesize ten-membered lactams ring-closed at C-4 of the indole by photocyclization reaction of γ-chlorotiglyl L-tryptophan methyl ester 5a for structure–activity study of medium-ring lactams related to indolactams having tumor-promoting activity. However, the ^{13}C-NMR spectra of the products showed that two eight-membered lactams ring-closed at C-4 of the indole, (4*S*,7*S*)-1,3,4,5,6,7-hexahydro-4-methoxycarbonyl-7-methyl-6-oxo-7-vinylazocino[4,5,6-*cd*]indole (6a) and its (4*S*,7*R*)-epimer 7a, and a seven-membered lactam ring-closed at C-2 of the indole, (2*S*,5*S*)-1,2,3,4,5,6-hexahydro-2-methoxycarbonyl-5-methyl-4-oxo-5-vinylazepino[4,5-*b*]indole (8a), were produced rather than the expected ten-membered lactams 3a and 4a. That is, this photocyclization linked the carbon atom adjacent to the amide-carbonyl groups to the indole ring.

Keywords indole; tryptophan; photochemical reaction; medium ring lactam; azocino[4,5,6-*cd*]indole; azepino[4,5-*b*]indole

Teleocidins (such as teleocidin B-4 **1**) exhibit potent tumor-promoting activity as well as many important biological activities connected with cell proliferation and cell differentiation. Recent research[1-4] has shown that (−)-indolactam-V **2** is the minimum essential portion of teleocidins required for tumor-promoting activity. Although structure-activity studies of indolactams are quite well developed and have revealed substitution effects on the biological activities,[5-10] all the studies were confined to the nine-membered lactam structure. In order to determine whether the nine-membered lactam structure is requisite for the promoting activity, we undertook to design ring-expanded indolactams, *i.e.*, a ten-membered lactam moiety fused with indole, and bearing a hydroxymethyl group. We found a report describing the synthesis of ten-membered lactams **3a** and **4a** by photocyclization, by Anderson and Lawton[11] (see also ref. 12). We anticipated that the new indolactams **3b** and **4b** would be easily obtained by reduction of **3a** and **4a**, respectively.

The starting material for the photocyclization reaction, γ-chlorotiglyl L-tryptophan methyl ester **5a** was prepared in 73% yield from γ-chlorotiglic acid[13] and L-tryptophan methyl ester, as previously described.[11] the photocyclization reaction was carried out at the concentration of 30 mM in dry acetonitrile at room temperature (18 °C), with irradiation at 254 nm through a Vycor filter. After irradiation for 16 h, we obtained three products; the least polar **6a** (17%), **7a** (17%) and the recovered amide **5a**, and the most polar **8a** (3%). In the ^{1}H-NMR spectra the chemical shifts and coupling modes of the indole moieties of the products **6a** and **7a** indicated ring-closure at C-4 of the indole, and similarity of the two structures (the chemical shifts are summarized in Table I). The chemical shifts of **6a** and **7a** are essentially similar to those reported for **3** and **4**, respectively.

In the ^{13}C-NMR spectra of **6a** and **7a**, however, two

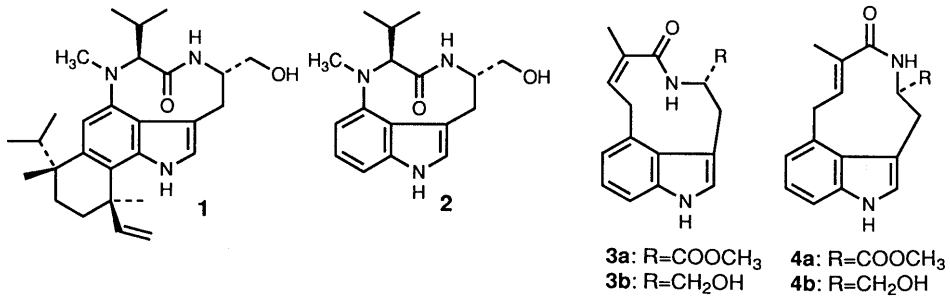

Fig. 1

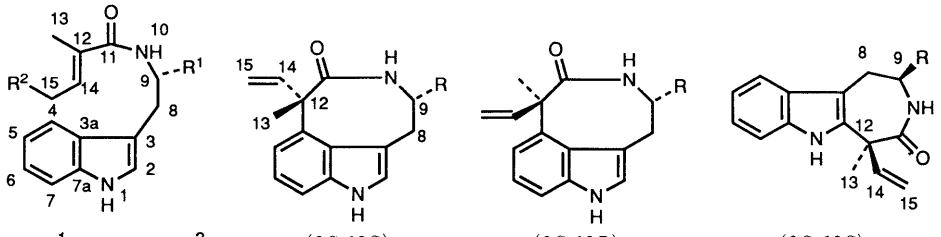

Fig. 2

TABLE I. ^{1}H-NMR Spectral Data in $CDCl_3$ (400 MHz)[a]

Proton	6a	7a	7b[b]	8a
1-H	8.15 br s	8.12 br s	8.21 br s	7.83 br s
2-H	6.98 d (2.6)	6.97 d (1.5)	7.04 br s	—
4-H	7.52 dd (7.5, 0.8)	—	—	7.47 ddd (7.9, 0.9, 0.7)
5-H	7.12 ddd (7.6, 7.5, 0.7)	7.07 dd (7.8, 1.0)	7.08 d (7.7)	7.12 ddd (7.9, 7.4, 0.7)
6-H	7.20 ddd (7.6, 7.6, 0.8)	7.17 dd (8.0, 7.8)	7.18 dd (8.0, 7.7)	7.19 ddd (7.9, 7.4, 0.9)
7-H	7.37 dd (7.6, 0.7)	7.28 dd (8.0, 1.0)	7.30 d (8.0)	7.29 ddd (7.9, 0.7, 0.7)
8a-H	3.36 dd (14.7, 5.2)	3.01 d (15.8)	3.33 br	3.04 dd (15.8, 12.0)
8b-H	3.39 dd (14.7, 5.3)	4.08 dd (15.8, 7.2)	3.75 br	3.52 dd (15.8, 1.9)
9-H	4.98 ddd (8.4, 5.3, 5.2)	4.77 dd (15.8, 7.2)	4.44 br	4.76 ddd (12.0, 5.2, 1.9)
10-H	6.29 d (8.4)	6.40 d (8.3)	6.28 d (7.3)	6.92 d (5.2)
13-H	1.84 d (0.5)	1.67 s	1.78 s	1.80 s
14-H	6.33 tq (7.6, 0.5)	6.28 dd (17.1, 10.5)	6.33 dd (17.2, 10.4)	6.38 dd (17.6, 10.6)
15a-H	4.08 d (2H, 7.6)	5.20 d (17.1)	5.07 d (17.2)	5.48 d (17.6)
15b-H	—	5.33 d (10.5)	5.23 d (10.4)	5.56 d (10.6)
17-H	3.72 s	3.70 s	3.77 s	3.52 s

a) Chemical schifts are shown in δ, and coupling constants J in Hz are given in parentheses. *b*) These spectral data were measured at 55 °C, because some of the peaks were too broad to observe at room temperature (23 °C).

TABLE II. ^{13}C-NMR Spectral Data in $CDCl_3$ or CD_3OD (100 MHz)[a]

Carbon	5a	6a	7a[b]	8a
2-C	122.7 d	124.9 d	124.8 d	131.5 s
3-C	109.8 s	109.8 s	111.0 s	108.0 s
3a-C	127.6 s	124.8 s	125.2 s	128.4 s
4-C	118.4 d	140.2 s	139.0 s	118.0 d
5-C	119.7 d	122.6 d	124.9 d	119.7 d
6-C	122.2 d	122.0 d	122.1 d	122.6 d
7-C	111.4 d	110.7 d	110.9 d	110.7 d
7a-C	136.1 s	138.7 s	138.8 s	134.3 s
8-C	27.4 t	31.3 t	31.0 t	29.9 t
9-C	53.4 d	55.2 d	55.5 d	53.8 d
11-C	167.8 s	181.8 s	182.6 s	173.8 s
12-C	135.0 s	55.7 s	52.4 s	49.5 s
13-C	12.6 q	25.4 q	25.4 q	23.8 q
14-C	130.2 d	142.2 d	142.5 d	140.2 d
15-C	39.1 t	116.2 t	113.9 t	114.3 t
16-C	172.2 s	171.9 s	171.8 s	171.1 s
17-C	52.4 q	52.5 q	52.4 q	53.3 q

a) Chemical shifts are shown in δ. *b*) This compound was measured in CD_3OD at 40 °C. The others were measured in $CDCl_3$ at 23 °C.

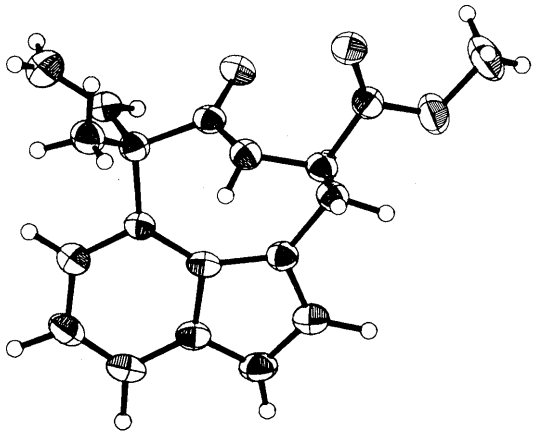

Fig. 3. ORTEP Drawing of **6a**

unexpected signals (55.7 ppm (s) and 116.2 ppm (dt) in the case of **6a**; and 55.5 ppm (s) and 115.3 ppm (t) in the case of **7a**) were observed (Table II), strongly indicating that these products are not the reported structures **3** and **4** but the eight-membered lactam structures showed in Fig. 2. The quaternary carbon signal at 55.7 or 55.5 ppm is reasonably assigned to carbon adjacent to the indole (C-12), and the methylene carbon signal at 116.2 or 115.3 ppm is assigned to the exo-methylene carbon of the vinyl group (C-15). The ^{13}C-NMR spectrum of **7a** indicates that **7a** possesses an eight-membered lactam ring, like **6a**, and suggests that **6** and **7** are diastereomeric. Irradiation presumably causes cleavage of the C-15–Cl bond of **5a**, and the resultant allyl radical couples at C-12 (adjacent to the amide-carbonyl group) with the indole ring (C-4 or C-2), rather than at C-15. These assignments were confirmed by the 2D CH shift correlation spectrum. In addition, lower field absorptions in the ^{1}H-NMR spectra of **6a** and **7a** (5.20 and 5.33 ppm in the case of **6a**; 5.07 and 5.23 ppm in the case of **7a**) can also be reasonably assigned to the vinyl methylene protons (15-H) rather than allylic methylene ones (in **3** and **4**).[11,14,15] The structure and stereochemistry of **6a** were confirmed by X-ray crystallographic analysis; the *S* chirality at C-9 of **6a** was postulated to be maintained during the photocyclization from L-tryptophan methyl ester. The crystal of **6a** belongs to orthorhombic space group $P2_12_12_1$, with cell constants $a=10.048$, $b=17.335$, $c=8.565$ Å and $z=4$. The ORTEP drawing of the structure of **6a** is shown in Fig. 3.

On the other hand, the ^{1}H-NMR spectrum of **8a** clearly indicates ring-closure at C-2 of the indole. Such an azepinoindole derivative was also reported to be formed in the photocyclization reaction of *N*-chloroacetyl indolylethylamine.[16,17] In the ^{13}C-NMR spectrum of **8a**, the quaternary carbon signal at 49.5 ppm (s) and the exo-methylene signal at 114.3 ppm indicate the structure **8a** in Fig. 2. In proton nuclear Overhauser effect (^{1}H-NOE) measurement, saturation of the methyl proton (13-H) results in characteristic enhancement of the H-9 signal (22%). This clearly shows that the absolute configuration of **8a** is (*S*,*S*).

The lactam esters **6a** and **8a** were treated with $LiBH_4$ in tetrahydrofuran (THF) to give the corresponding alcohols (**6b**, 87%; **8b**, 77%). In this reduction step, **8a** is most reactive and **6a** is least among the three esters **6a**, **7a**, and **8a**. Similarly, the mixture of **7a** and **5a** was treated with

$LiBH_4$ to give three easily separable alcohols: **7b** (77% yield from **7a**) and two alcohols (**5b** and **5c**) derived from **5a**.

Finally, tumor-promoting activity was evaluated for the ring-contracted indolactam-related compounds **6b**, **7b**, and **8b**.[18] However, all the lactam alcohols **6b**, **7b**, and **8b** were inactive below the concentration of 10^{-5} M. (−)-Indolactam-V **2** was proposed to exist predominantly in two conformations, sofa and twist structures in solution.[3] The contraction of the lactam ring in the indolactams **6b**, **7b**, and **8b** would have induced significant conformational change, leading to a conformation different from the proposed active conformation.

Experimental

General Remarks Melting points were obtained on a Yanagimoto micro hot stage apparatus and are uncorrected. ^{1}H-NMR spectra were measured with a JEOL JMN-GX-400 spectrometer (400 MHz), with tetramethylsilane (TMS) as an internal standard, and the chemical shifts are given in ppm as δ values from TMS. ^{13}C-NMR spectra were measured with a JEOL JMN-GX-400 spectrometer (100 MHz), with $CDCl_3$ or CD_3OD as an internal standard (77.0 or 49.8 ppm). Mass spectra were recorded on a JEOL JMS-D-300 instrument for DI-Mass and JMS-DX-300 for high-resolution analysis. IR spectra were recorded with a Shimadzu IR-408. UV spectra were recorded on a Shimadzu UV-200S. Flash column chromatography was performed on silica gel (Merck 9385).

Starting Material for the Photocyclization Reaction γ-Chlorotiglyl L-tryptophan methyl ester **5a** was prepared in 73% overall yield from γ-chlorotiglic acid and L-tryptophan methyl ester, according to the literature.[11,13] mp (dichloromethane–*n*-hexane) 130.5—131.5 °C. IR (KBr): 1730 (s), 1665 (s), 1630 (s), 1515 (s) cm^{-1}. UV (95% aqueous ethanol) λ_{max} nm (log ε): 276 (3.83), 283 (3.85), 292 (3.79). *Anal.* Calcd for $C_{17}H_{19}ClN_2O_3$: C, 60.99; H, 5.72; N, 8.37. Found: C, 60.75; H, 5.70; N, 8.18.

Photocyclization The light source for cyclization was a 160W low-pressure mercury lamp (Riko-Kagaku-Sangyo UVL-160LA) with a Vycor filter. Dry acetonitrile was prepared by distillation with calcium hydride after standing for one night over Molecular Sieve 4A, and its was stocked over Molecular Sieve 3A.

A colorless solution of γ-chlorotiglyl L-tryptophan methyl ester **5a** (2.00 g) in dry acetonitrile (200 ml) was divided into two quartz flasks, and irradiated at room temperature (18 °C) for 16 h with stirring. Acetonitrile was distilled off *in vacuo*, and the residue was purified by flash column chromatography using benzene–ethyl acetate (6 : 1—4 : 1) to give 300.7 mg (17%) of (4*S*,7*S*)-1,3,4,5,6,7-hexahydro-4-methoxycarbonyl-7-methyl-6-oxo-7-vinylazocino-[4,5,6-*cd*]indole (**6a**) as a brown oil, 620.4 mg of a mixture of (4*S*,7*R*)-1,3,4,5,6,7-hexahydro-4-methoxycarbonyl-7-methyl-6-oxo-7-vinylazocino[4,5,6-*cd*]indole (**7a**) (17%) and the starting material **5a** (1 : 1 estimated on integration of ^{1}H-NMR) as a pale orange amorphous powder, and 46.9 mg (3%) of (2*S*,5*S*)-1,2,3,4,5,6-hyxahydro-2-methoxycarbonyl-5-methyl-4-oxo-5-vinylazepino[4,5-*b*]indole (**8a**) as colorless crystals.

Compound **6a** was recrystallized from dichloromethane–methanol–*n*-hexane as white fine needles. mp 200.5 °C. IR (KBr): 1745 (s), 1695 (m), 1675 (s), 1520 (s) cm^{-1}. UV (95% aqueous ethanol) λ_{max} nm (log ε): 281 (3.74), 286 (3.79), 294 (3.71). MS *m/z*: 298 (M^+): *Anal.* Calcd for $C_{17}H_{18}N_2O_3$: C, 68.43; H, 6.08; N, 9.39. Found: C, 68.31; H, 6.17; N, 9.13. *m/z* of **7a** purified by preparative TLC (this sample contained less than 20% **5a**) 298 (M^+).

Recrystallization of **8a** from dichloromethane–*n*-hexane gave white flaskes. mp 201.5 °C. IR (KBr): 1735 (s), 1645 (s) cm^{-1}. UV (95% aqueous ethanol) λ_{max} nm (log ε): 275 (3.89), 283 (3.91), 291 (3.84). MS *m/z*: 298 (M^+). *Anal.* Calcd for $C_{17}H_{18}N_2O_3$: C, 68.43; H, 6.08; N, 9.39. Found: C, 68.71; H, 6.14; N, 9.11. The ^{1}H- and ^{13}C-NMR data for the products **6a**, **7a** (including **5a**), and **8a** are listed in Tables I and II.

Reduction of 6a $LiBH_4$ (54.0 mg, min. assay 90%) was added to a solution of 150.6 mg of **6a** in 30 ml of THF at 0 °C with stirring, and the mixture was kept at 0 °C. After 2 h, more $LiBH_4$ (44.8 mg) was added, and after another 2.5 h, $LiBH_4$ (55.2 mg) was again added. The mixture was stirred for 7 h from the beginning, then the reaction was quenched by addition of 3 ml of water. After removal of the solvent *in vacuo*, the resulting white residue was partitioned between 50 ml of ethyl acetate and 50 ml of water, and extracted four times with 200 ml of ethyl acetate. The organic layer was washed with 30 ml of saturated brine, dried over $MgSO_4$, and concentrated to give 146.3 mg of white powder. The dichloromethane-insoluble portion (87.1 mg) of this powder was pure (4*S*,7*S*)-1,3,4,5,6,7-hexahydro-4-hydroxymethyl-7-methyl-6-oxo-7-vinylazocino[4,5,6-*cd*]-indole (**6b**). After filtration, the filtrate was concentrated and purified by flash column chromatography using benzene–ethyl acetate (6 : 1—1 : 2) to give 9.3 mg of recovered **6a** and 31.2 mg of **6b** as a white powder. This is, we obtained 118.3 mg of the alcohol **6b** (87%) in total.

Recrystallization of **6b** from ethyl acetate–*n*-hexane gave white fine plates. mp 200.0—200.5 °C. The ^{1}H-NMR data are listed in Table I. IR (KBr): 3500 (s), 3250 (s), 1690 (s), 1660 (s), 1525 (m) cm^{-1}. MS *m/z*: 270 (M^+). *Anal.* Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 70.82; H, 6.69; N, 10.09.

Reduction of 7a and 5a The procedure was similar to that used for preparation of **6b**. $LiBH_4$ (102.4 mg) was added to a solution of 304.7 mg of the mixture of **7a** and **5a** (1 : 1.4 estimated from ^{1}H-NMR) in 60 ml of THF at 0 °C with stirring, and the mixture was kept at 0 °C. After 2 h, more $LiBH_4$ (118.2 mg) was added. The reaction mixture was stirred for 4 h from the beginning, then the reaction was quenched by addition of 5 ml of water. After removal of the solvent *in vacuo*, the resulting white residue was partitioned between 50 ml of ethyl acetate and 50 ml of water, and extracted four times with 200 ml of ethyl acetate. The organic layer was washed with 30 ml of saturated brine, dried over $MgSO_4$, and concentrated to give 302.0 mg of a white amorphous powder. This crude powder was dissolved in a minimum quantity of ethyl acetate, and the cyclized alcohol, (4*S*,7*R*)-1,3,4,5,6,7-hexahydro-4-hydroxymethyl-7-methyl-6-oxo-7-vinylazocino[4,5,6-*cd*]indole (**7b**) was reprecipitated alone with ether, as a white powder (54.6 mg). After filtration, the filtrate was concentrated and purified by flash column chromatography using ether to give 33.0 mg of **7b** as a colorless oil less polar than the two uncyclized alcohols (**5b** and **5c**) derived from **5a**. That is, we obtained 87.6 mg of the alcohol **7b** (77%) in total.

Recrystallization of **7b** from ethyl acetate–*n*-hexane gave white prisms. mp 186.0—186.5 °C. IR (KBr): 3220 (br s), 1660 (s) cm^{-1}. MS *m/z*: 270 (M^+). *Anal.* Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.14; H, 6.70; N, 10.15.

Reduction of 8a The procedure was similar to that used for preparation of **6b**. $LiBH_4$ (3.5 mg) was added to a solution of 8.5 mg of **8a** in 1.7 ml of THF at 0 °C with stirring, and the mixture was stirred at 0 °C for 2.5 h, then 5 drops of water were added. After removal of the solvent *in vacuo*, the resulting residue was partitioned between 5 ml of ethyl acetate and 5 ml of water, and extracted four times with 20 ml of ethyl acetate. The organic layer was washed with 3 ml of saturated brine, dried over $MgSO_4$, and concentrated to give 8.0 mg of a colorless oil. This oil was purified by flash column chromatography using ether–ethyl acetate (3 : 1—1 : 1) to give 5.9 mg of the alcohol, (2*S*,5*S*)-1,2,3,4,5,6-hexahydro-2-hydroxymethyl-5-methyl-4-oxo-5-vinylazepino[4,5-*b*]indole (**8b**) (77%), as colorless oil.

As this alcohol **8b** is liable to decompose during heating over 40 °C, crystallization is difficult. mp (as a white powder) 90.0—93.0 °C. *m/z* by high-resolution mass spectroscopy Calcd for $C_{16}H_{18}N_2O_2$: 270.1367 (M^+), Found: 270.1379.

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