Investigation of a green oligomer of an indolizine

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Abstract
An indolizine-based oligomer was synthesized by stirring a solution of 1-(α-alkoxybenzyl)-indolizine derivative in chloroform at room temperature. The oligomer was characterized as an octamer; it was the main component in the obtained mixture of oligomers. The oligomerization was accelerated by both acid and light. The green color of the oligomer under acidic conditions turned yellowish brown under basic conditions; this color change could be repeated by changing the acidic/basic conditions.

Keywords: Indolizine; pH sensitive behavior; ethynylpyridine; color change; oligomer

Introduction
An indolizine framework consists of an electron-deficient pyridine ring and an [α]-fused electron-rich pyrrole ring, which provides a biased electron density. Because of such an electronic structure, indolizine derivatives exhibit potential biological activities1,2 and optical properties.3–5 Therefore, numerous indolizines have been synthesized by several methods.6–8

Previously, we demonstrated a synthetic method for functionalized indolizines from 2-ethynylpyridines using functionalities; a nucleophilic ring nitrogen and reactive ethynyl group.9 When a solution of indolizine in chloroform-d was stood at room temperature for 1 day, the color of the solution changed from yellowish brown to bluish green. This experimental result prompted us to study this phenomenon; we clarified that an indolizine-based oligomer was formed.10

However, detailed information was not obtained because of poor experimental environment. After our preliminary finding, any report dealing with indolizine-based oligomer has not been found to our best of knowledge during the last quarter century, which prompted us to focus and reinvestigate this phenomenon.
Results and Discussion

Synthesis of indolizines 4 and 5

Indolizine 4a can be efficiently prepared by the three-component cycloaddition of 2-phenylethynylpyridine 1a and dimethyl acetylenedicarboxylate 2a in the presence of a proton source such as methanol 3a which accompanies demethoxycarboxylation of 2a (Scheme 1). The two proton at the 3-position and on the substituent at the 1-position are derived from methanol, which was confirmed by conducting the same reaction using methanol-\textsuperscript{d\textsubscript{1}}, which afforded 4a-\textsuperscript{d\textsubscript{2}}. The deuterium at the 3-position was exchanged to proton leading to 4a-\textsuperscript{d\textsubscript{1}} upon treatment with column chromatography on silica gel. Indolizines 4b, 4c, and 4d were prepared in a similar manner using a combination of 2-(4-methylphenyl)ethynylpyridine (1b) and 2-(4-butylphenyl)ethynylpyridine (1c), diethyl acetylenedicarboxylate 2b, and ethanol 3b. Compared with other established methods using ethynylpyridines,\textsuperscript{11–23} this method is environmentally benign because it is not necessary to use any transition metal catalyst or strong base.

Scheme 1. Three component synthesis of indolizine 4a.

When tert-butyl alcohol 3c was used as a proton source, demethoxycarboxylation did not proceed to afford indolizine 5a. The isolated 5a was quantitatively transformed to 5b upon heating in methanol 3a; however, further change such as demethoxycarboxylation did not occur even though 5b was treated with sodium methoxide in methanol under reflux (Scheme 2).

Scheme 2. Study on the alkoxy exchange of 2,3-bis(methoxycarbonyl)indolizines 5.

On the basis of the abovementioned results, a plausible mechanism for the formation of 4a is illustrated in Scheme 3. This reaction is initiated by [3+2] cycloaddition followed by protonation, affording 1-benzylideneindolizine intermediate 6. Then a counter anion is introduced on the
substituent at the 1-position, and the 3-position of the indolizine is easily protonated to furnish 7. When the formed methoxide anion attacks the carbonyl group, demethoxycarboxylation; subsequent rearomatization of the indolizine framework affords the final product proceeds leading to 4a, which is consistent with the detection of dimethyl carbonate. On the other hand, the bulky tert-butoxide cannot attack the ester function because of steric hindrance, thus only deprotonation at the 3-position occurs to give 5a. Although there is another possibility that bis(methoxycarbonyl)indolizine 5b serves as the intermediate for this reaction, it is excluded by the experimental fact shown in Scheme 2.

Scheme 3. A plausible mechanism for the formation of indolizine 4a.

Oligomerization of indolizine 4
When a solution of indolizine 4 in chloroform-\textit{d} was allowed to stand at room temperature for one day, the color of the solution changed from yellowish brown to bluish green. The $^1$H NMR spectrum of the green solution showed only the signals of methanol. This indicates that a scarcely soluble green material was formed in chloroform without any special treatment, simultaneously eliminating methanol. Similar color change was also observed when indolizine 4a was stood in a silica gel column, or when a solution of 4a in THF was stirred with 10mol% of hydrochloric acid at room temperature. These results indicate that oligomerization of 4a occurs under weak acidic conditions. On the other hand, bis(methoxycarbonyl)indolizines, e.g. 5b, were stable under the same conditions.

The consumption of 4a was monitored by $^1$H NMR using three types of deuterated solvents; benzene–\textit{d}$_6$, chloroform–\textit{d}, and acetic acid–\textit{d}$_4$. Different rates were observed depending on both the solvent and light. Although no change was observed in benzene–\textit{d}$_6$ and chloroform–\textit{d} solutions in the dark, indolizine 4a was consumed in the acetic acid–\textit{d}$_4$ solution (Figure 1, upper). In contrast, the reaction proceeded faster in the light in acetic acid–\textit{d}$_4$ and chloroform–\textit{d} solutions. In the case of benzene–\textit{d}$_6$, indolizine 4a was slightly converted to an unidentified product, but the color of the solution did not change (Figure 1, lower). The initial consumption rate of 4a was $4.3 \times 10^{-4}$ s$^{-1}$ (in acetic acid–\textit{d}$_4$ in the light), $1.8 \times 10^{-4}$ s$^{-1}$ (in chloroform–\textit{d} in the light), and $2.6 \times 10^{-5}$ s$^{-1}$ (in acetic acid–\textit{d}$_4$ in the dark). Chloroform easily decomposes to hydrogen chloride in light. Hence, indolizine 4a may have been consumed by the acid.$^{24,25}$ Because different reaction rates were also observed in acetic acid–\textit{d}$_4$ between under light and dark conditions, this reaction is accelerated by both acid and light; however, the role of light is not well understood.
Figure 1. Consumption of indolizine 4a in the dark (upper) and light (lower), monitored by $^1$H NMR.

Generally, electron-rich heterocyclic compounds such as pyrrole undergo acid catalysed self-condensation to afford polypyrroles.\textsuperscript{26–31} Indolizine is also an electron-rich heterocyclic compound, hence, similar self-condensation is considered to proceed, which is catalyzed by a trace amount of hydrogen chloride generated by decomposition of chloroform,\textsuperscript{24,25} however, direct linkage of bulkier indolizines seems difficult. On the other hand, indolizine 4a has an electrophilic carbon at the 1-position, which serves as a spacer to diminish the steric hindrance between indolizine frameworks and facilitate the oligomerization.
Since the $^1$H NMR showed only the signals of eliminated methanol after the color was changed, the NMR spectrum of the product could not be measured; hardly soluble tiny green particles were homogeneously dispersed in the solvent. Considering the solubility of the monomer 4a, dimer or trimer should be soluble in organic solvents. However, no signal was observed in the monitoring of the reaction by $^1$H NMR because of their extremely small amounts in the reaction mixture. Fortunately, a trace amount of dimer 8 could be isolated by quick treatment with silica gel column chromatography of the reaction mixture stopped in a short reaction time (1 h). The spectral data of 8 indicated this was bimolecularly condensed compounds accompanied by elimination of one molecule of methanol (Figure 2). In the $^1$H NMR, signals of inner protons (H-5, -6, -8', Ph$^b$, COOMe$^b$) were observed in higher fields than the signals of the outer protons or the corresponding signals of monomer 4a. This observation could be explained by that the indolizine rings are torsionally restrained and the protons at the inside positions are located above the other indolizine ring.

Scheme 4. A plausible mechanism for the oligomerization of indolizine 4a.
The dimer 8 was found to be a mixture of isomers (73:27) by $^1$H NMR and coalesced spectrum was not obtained at 70 °C. The $^{13}$C NMR was too complex to be assigned, but six methoxy carbon signals were observed.

As shown in Scheme 1, proton exchange of 4a occurs easily under weakly acidic conditions such as in a silica gel column, which indicates the 3-position has high electron density. In addition, an alkoxy group can be easily eliminated from the α-alkoxybenzyl group at the 1-position only when 5a is heated in methanol as shown in Scheme 2. Considering these structural features, indolizine 4a may have undergone self-condensation in the presence of acid catalyst, forming an oligomeric structure. In particular, a methanol is easily eliminated from the 1-position as a result of protonation affording a stable benzylic cation 9. The cation is attacked by another molecule of 4a leading to dimer 8 via deprotonation of the adduct intermediate 10. Oligomerization is achieved by repeating this sequence.

![Oligomers and gel permeation chromatography results](image)

**Figure 3.** Structures of oligomers and gel permeation chromatography results.

Since indolizine oligomer 4a was not sufficiently soluble to allow measurement of the $^1$H NMR spectrum, a longer alkyl chain was introduced into the indolizine framework to improve the solubility. The chloroform solutions of each indolizine 4b, 4c, and 4d turned green after standing at room temperature for one day; however, no signal was observed in the $^1$H NMR spectra. Then, these four types of indolizines were subjected to gel permeation chromatography (GPC) measurements because the measurable concentration for GPC is lower than that for NMR; the
results are shown in Figure 3. Each oligomer had a similar molecular weight (Mw and Mn), and the degree of oligomerization was between 2 and 6, contrary to our expectation. The insoluble oligomers with a higher degree of oligomerization are removed by filtration during the pretreatment for GPC measurements.

Next, the mass spectrum of the oligomer of indolizine 4b was measured using the MALDI ionization method (Figure 4). The spectrum showed similar peak patterns repeated at an interval of 277 Da. This indicated that a methanol molecule was removed from the indolizine. Hence, the green material was confirmed to be a mixture of oligomers of the indolizine 4b. The mountain range was observed until 6.0 kDa, where the degree of oligomerization was 20; the octamer was the main component in the mixture.

**Figure 4.** Mass spectrum of the oligomer of indolizine 4b. 1.2–10.0 kDa (upper), 2.1–4.0 kDa (lower).

**pH Sensitive behavior of the oligomer**

Although the prepared oligomer was green, it turned yellowish brown by treating with a base such as sodium hydroxide, ammonia and DBU. This color change was reversible; it turned to green again by treating with an acid. This color change was confirmed from the UV–visible spectra of the oligomer (Figures 5). In the UV–visible spectrum, the absorption was observed at 670 nm in the green state. This absorption disappeared, and a new absorption was observed between 500 nm and 600 nm in the brown state. This change of color was reversible and its pK<sub>obs</sub> was 11.1. (Figure 5, right).
Figure 5. UV-visible spectra of the oligomer of indolizine 4b (left) and 4a under different pH conditions (right).

The reversibility of the color change was studied. To a solution of oligomer of 4b (3 mg, corresponding to 10 μmol monomer) in methanol (100 mL), either small amount of concentrated acid or base, 10 M hydrochloric acid or 10 M sodium hydroxide aqueous solution (100–300 μL), was added until the color of the solution changed. The absorbance at 650 nm was plotted in Figure 6, in which the solution was acidified at even runs, and was basified at odd runs.

Figure 6. The change of absorbance at 650 nm. The solution was basified at odd runs, and the solution was acidified at even runs.
In the IR spectrum, although the absorption of the carbonyl group was observed at 1738 cm\(^{-1}\) in the green state, it shifted to 1668 cm\(^{-1}\) in the brown state (Figure 7). Hence, the carbonyl group probably became an enolate under the basic conditions. Based on these spectral changes, the plausible structures of the oligomer in both the states are shown in Scheme 5. As mentioned above, because an indolizine is easily protonated at the 3-position, the oligomer is present in the protonated form under acidic conditions, and in the deprotonated form under basic conditions. It is known that the pyridinium ion serves as an acceptor to form charge-transfer complexes.\(^{32,33}\) Although the similar complex might be formed to produce a green color under acidic conditions, we have not obtained any evidence at present.

![IR spectra](image)

**Figure 7.** IR spectra of the oligomer of indolizine 4b under acidic (upper) and basic (lower) conditions.

## Conclusions

As a result of revisiting the oligomerization of indolizine 4, both acid catalyst and light were found to be crucial for this reaction although the role of the light has not clarified. During the synthesis of indolizines 4 and the oligomer, no metallic reagent\(^ {26-31}\) was necessary. The obtained oligomer shows a green color under acidic conditions and turns to yellowish brown under basic conditions, which is reversible. The application of this oligomer is under investigation, and the results will be reported in due course.
Scheme 5. Structural changes in the oligomer of indolizine 4b under acidic and basic conditions.

Experimental Section

General
All reagents were purchased from commercial sources and used directly without further purification. $^1$H and $^{13}$C NMR spectra were recorded on Bruker DPX-400 spectrometer (400 MHz and 100 MHz, respectively), JEOL FT-NMR GSX spectrometer at 270 MHz and JEOL FT-NMR JMN FX90Q at 90 MHz using TMS and 1,1,2,2-tetrachloroethane as an internal standard. $^1$H NMR data is reported as follows: chemical shift (δ, ppm), (chemical shift multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, coupling constant (Hz). $^{13}$C NMR data is reported in terms of chemical shift (δ, ppm). IR spectra were recorded on Shimadzu IR Affinity-1 Spectrometer, and were reported in frequency of absorption. Mass spectra were obtained on a
Bruker Autoflex II and a JEOL JMS-Q1050GC mass spectrometer using acetonitrile as a solvent and dithranol (1,8-dihydroxyanthracen-9(10H)-one) as a matrix. High resolution mass spectrum was recorded with a JEOL JMS-DX303 mass spectrometer. GPC using Shodex column KF-804F and KF-806L was performed by Shimadzu LC-6A equipped with a Tosoh UV-8020 UV detector and a Jasco RI-2031 refractive index detector. The measurement of GPC was conducted using chloroform as a solvent and polystyrene standard as a molecular weight reference. UV-vis. spectra were measured on a Jasco V-550. Elemental microanalyses were performed using a Yanaco CHN corder. Melting points were recorded with a Yanaco micro-melting points apparatus and were uncorrected.

**General procedure for preparation of indolizines 4**

To a solution of 2-phenylethynylpyridine 1a (179 mg, 1.0 mmol) in dichloromethane (10 mL), dimethyl acetylenedicarboxylate 2a (213 mg, 1.5 mmol) and methanol 3a (0.81 mL, 20 mmol) were added, and the resultant mixture was stirred at 30 °C for 1 d. After removal of the solvent, the residue was treated with column chromatography on silica gel wrapped with aluminium foil to afford indolizine 4a (eluted with dichloromethane, 177 mg, 0.6 mmol, 60%) as a pale yellow oil. Other indolizines were also prepared in a similar manner.

**1-[Methoxy(phenyl)methyl]-2-methoxycarbonylindolizine (4a).** Pale yellow oil. \(^1\)H NMR (400 MHz, C\(_6\)D\(_6\), TMS) δ 2.93 (s, 3H), 3.11 (s, 3H), 5.46 (s, 1H), 6.22 (ddd, J 6.4, 7.2, 1.2 Hz, 1H), 6.40 (ddd, J 8.8, 6.4, 0.8 Hz, 1H), 6.53 (s, 1H), 7.09 (ddd, J 8.8, 1.2, 1.2 Hz, 1H), 7.20 (tt, J 7.2, 1.2 Hz, 1H), 7.34 (dd, J 8.0, 7.2 Hz, 2H), 7.75 (dd, J 8.0, 1.2 Hz, 2H), 8.82 (ddd, J 7.2, 0.8, 1.2 Hz, 1H); \(^13\)C NMR (100 MHz, CDCl\(_3\), TMS) δ 51.5 (CH\(_3\)), 56.2 (CH\(_3\)), 75.0 (CH), 100.2 (CH), 110.6 (CH), 114.6 (C), 118.4 (CH), 118.5 (CH), 126.0 (CH), 127.1 (CH), 128.7 (CH), 130.0 (CH), 132.4 (C), 133.9 (C), 136.5 (C), 169.5 (C); MS (FAB) 295 (M\(^+\), 37), 264 (23), 236 (100), 220 (14), 204 (14). Anal. Calcd. for C\(_{18}\)H\(_{17}\)NO\(_3\): C, 73.20; H, 5.80; N, 4.74. Found: C, 72.87; H, 5.87; N, 4.69 %.

**1-[Methoxy(4-methylphenyl)methyl]-2-methoxycarbonylindolizine (4b).** Pale yellow oil (182 mg, 59%): \(^1\)H NMR (400 MHz, C\(_6\)D\(_6\), TMS) δ 2.20 (s, 3H), 2.96 (s, 3H), 3.12 (s, 3H), 5.51 (s, 1H), 6.23 (ddd, J 6.4, 7.2, 1.2 Hz, 1H), 6.41 (ddd, J 9.2, 6.4, 0.8 Hz, 1H), 6.55 (s, 1H), 7.10 (ddd, J 9.2, 1.2, 1.2 Hz, 1H), 7.17 (d, J 8.0 Hz, 2H), 7.68 (d, J 8.0 Hz, 2H), 8.84 (ddd, J 7.2, 1.2, 0.8 Hz, 1H); \(^13\)C NMR (100 MHz, CDCl\(_3\), TMS) δ 14.2 (CH\(_3\)), 51.5 (CH\(_3\)), 56.2 (CH\(_3\)), 75.1 (CH), 100.2 (CH), 110.5 (CH), 114.5 (C), 118.3 (CH), 118.8 (CH), 126.0 (CH), 129.5 (CH), 129.9 (CH), 132.5 (C), 133.6 (C), 133.9 (C), 136.6 (C), 169.5 (C); MS (EI) 309 (M\(^+\), 13), 250 (100), 234 (24), 218 (16), 204 (16), 59 (12); IR (KBr/cm\(^{-1}\)) 1748. Anal. Calcd. for C\(_{19}\)H\(_{19}\)NO\(_3\): C, 73.77; H, 6.19; N, 4.53. Found: C, 73.49; H, 6.34; N, 4.31 %.

**1-[Ethoxy(4-methylphenyl)methyl]-2-ethoxycarbonylindolizine (4c).** Pale yellow oil (169 mg, 50%): \(^1\)H NMR (400 MHz, C\(_6\)D\(_6\), TMS) δ 0.56 (dd, J 6.8, 6.8 Hz, 3H), 0.72 (dd, J 7.2, 7.2 Hz, 3H), 1.99 (s, 3H), 2.86 (dq, J 8.8, 6.8 Hz, 1H), 3.07 (dq, J 8.8, 6.8 Hz, 1H), 3.52 (dq, J 10.8, 7.2 Hz, 1H), 3.67 (dq, J 10.8, 7.2 Hz, 1H), 5.47 (s, 1H), 6.04 (ddd, J 6.4, 7.2, 1.2 Hz, 1H), 6.21 (ddd, J 9.2, 6.4, 0.8 Hz, 1H), 6.36 (s, 1H), 6.90 (ddd, J 9.2, 1.2, 1.2 Hz, 1H), 6.97 (d, J 8.0 Hz, 2H), 7.52
Monitoring the oligomerization of indolizine 4a by $^1$H NMR

To a solution of indolizine 4a (3 mg, 10 µmol) in chloroform-$d$ (0.5 mL), 1,1,2,2-tetrachloroethane (8 mg) was added as an internal standard, and subjected to the measurements of $^1$H NMR at several-minute intervals. The experiment was conducted in the air, and aluminium foil was used...
to keep in the dark. The experiment in the light was conducted in the ambient atmosphere without irradiation of special light. The reaction rate was calculated by comparison of the integral values of indolizine 4a and tetrachloroethane. Eliminated molecule was confirmed to be methanol by comparison of \(^1\)H and \(^{13}\)C NMR spectra with those of authentic sample.

**Isolation of dimer 8.** A solution of 4a (50 mg, 0.17 mmol) in chloroform (15 mL) was stirred at room temperature for 1 h. The solution was concentrated and the residue was treated with column chromatography on silica gel to give dimer 8 (eluted with hexane/AcOEt = 90/10, 10 mg, 0.018 mmol) with recovery of 70% of 4a.

**Dimer 8.** Brownish yellow solid, mp 41–44 °C; The dimer was found to be a mixture of isomers (73/27) by \(^1\)H NMR. \(^1\)H NMR (270 MHz, C\(_6\)D\(_6\), TMS) the major isomer \(\delta\) 2.97 (s, 3H), 3.05 (s, 3H, COOMe\(^b\)), 3.11 (s, 3H, COOMe\(^a\)), 5.10 (s, 1H, H\(^a\)), 6.0–6.4 (m, 5H, H-6, -7, -6', -7', -8'), 6.33 (s, 1H, H\(^b\)), 6.60 (s, 1H, H-3'), 7.0–7.5 (m, 9H, Ph\(^b\), H-8, -m, -p), 7.74 (dd, J 6.2, 1.1 Hz, 1H, H-o), 8.24 (dd, J 6.2, 1.1 Hz, 1H, H-5), 8.72 (dd, J 5.9, 1.1 Hz, 1H, H-5'), the minor isomer ; \(\delta\) 2.877 (s, 3H, OMe), 2.884 (s, 3H, COOMe\(^b\)), 3.11 (s, 3H, COOMe\(^a\)), 5.21 (s, 1H, H\(^a\)), 6.0–6.4 (m, 5H, H-6, -7, -6', -7', -8'), 6.37 (s, 1H, H\(^b\)), 6.68 (s, 1H, H-3'), 7.0–7.5 (m, 9H, Ph\(^b\), H-8, -m, -p), 7.82 (dd, J 7.3, 1.6 Hz, 2H, H-o), 8.35 (dd, J 6.2, 1.1 Hz, 1H, H-5), 8.84 (dd, J 5.9, 1.1 Hz, 1H, H-5'); MS (EI) 558(M\(^+\), 11), 499 (100); IR (neat/cm\(^{-1}\)) 1740. HRMS Calcd. for C\(_{35}\)H\(_{30}\)N\(_2\)O\(_{5}\) : 558.2156. Found: 558.2158.

**UV-vis spectra of oligomer of 4b**
To a suspension of oligomer of 4b (0.3 mg, corresponding to 1 \(\mu\)mol monomer) in methanol (10 mL) was subjected to the measurement of UV-vis spectrum. The solution turned to yellowish brown when 1 M sodium hydroxide aqueous solution (10 \(\mu\)L, 10 \(\mu\)mol) was added. After drying up, the residue (0.4 mg) was dissolved into methanol (5 mL), and was subjected to the measurement of UV-vis. spectrum.

**UV-vis spectra of oligomer of 4a under different pH conditions**
Several buffer solutions having different pH values were prepared by mixing 0.25 M aqueous solutions of boric acid and sodium hydroxide with different ratio. A solution (suspension) of oligomer 4a (1.33 g/L) in 1,4-dioxane was also prepared. After mixing the buffer solution and dioxane solution (10/1 v/v), pH value and UV-vis. spectrum were measured.

**References**
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