Products of Peroxidase Catalyzed Oxidation of Indolyl-3-acetic Acid

Yoshikatsu SUZUKI and Akira KAWARADA

*The Institute of Physical and Chemical Research,
Wako-shi, Saitama 351, Japan*

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Six new products of oxidation of indolyl-3-acetic acid catalyzed by horseradish peroxidase were isolated, along with four known ones, 3-hydroxymethyloxindole (1), 3-methyleneoxindole (2), indolyl-3-aldehyde (4), and 3,3-diindolylmethane (10). Based on spectroscopic and chemical evidence, the new products were identified as 3-acetoxyindole (3), 3-(indol-3-ylmethyl)-oxindole (6), 3-[(2-indol-3-ylmethyl)indol-3-ylmethyl]oxindole (9), the 3-hydroxymethyl compounds of 6 and 9 (5 and 7), and 2-(indol-3-ylmethyl)indolyl-3-acetic acid (8), respectively.

The presence of peroxidase-like enzyme systems catalyzing the oxidative inactivation of indolyl-3-acetic acid (IAA) in higher plants has long been recognized. Recently two IAA oxidases lacking peroxidase activities have also been reported. To clarify the physiological significance of *in vivo* IAA inactivation by these enzyme systems, much effort¹⁾ has been devoted to the study of *in vitro* oxidative IAA decomposition by horseradish peroxidase (HRP) or by other systems, as well as of *in vivo* metabolisms in plants. Hinman and Lang²⁾ proposed a reaction scheme for the oxidation of IAA by HRP in which IAA is converted to either indolyl-3-aldehyde or 3-methyleneoxindole, *via* a common intermediate to both. In a corn IAA oxidase system, another pathway that 3-hydroperoxymethylindole is a key intermediate in oxindole formation has also been proposed.³⁾ However, these proposals have not fully substantiated because the formation of any of the hypothetical key intermediates have not been confirmed. We have recently reported the isolation of oxindolyl- and dioxindolyl-3-acetic acids from rice bran⁴⁾ and matured corn seeds,⁵⁾ which seemed to suggest the possibility of the presence of a different oxidative route of IAA to dioxindoles *via* oxindoles, at least in these plants. A part of a similar route has been found in a fungus, *Hygrophorous conicus*, in which an oxidase system converts IAA to oxindolyl-3-acetic acid.⁶⁾

The previous works on enzymatic oxidation of IAA have not been very thorough in regard to the product identification; 3-methyleneoxindole,⁷⁾ indolyl-3-aldehyde,⁸⁾ 3,3-diindolylmethane, 3-methylene-indolenine, and the indolylmethyl ester of indolyl-3-acetic acid³⁾ have been ultimately confirmed and 3-hydroxymethyloxindole^{9,2)} has been tentatively identified. This paper describes a more extended isolation and identification of *in vitro* HRP oxidation products of IAA, carried out to provide basis for the establishment of the oxidation routes and mechanism.

Isolation of enzymatic oxidation products of IAA with HRP

The course of the enzymatic oxidation of IAA has been known highly dependent on the concentration of each reaction component, *i.e.*, substrate, enzyme, cofactor, and buffer, as well as pH of the medium. The main product of reaction with IAA concentration below 2×10^{-4} M is known to be 3-methyleneoxindole, while it is an unidentified neutral indole with IAA concentration up to 10^{-2} M.²⁾ Thus, the initial IAA concentration of 2×10^{-4} M that permits possibly straightforward enzymatic reaction was used in the present study.

The UV spectral change of the reaction is shown in Fig. 1. During the first 10 min the rapid decrease of the indole absorption (280~288 nm) with simultaneous increase of the

FIG. 1. UV Spectra of the Ether Extract of the IAA/HRR Reaction Mixture Taken at Intervals. (— 0, ---- 10, — 15, ---- 30 min).

FIG. 2. UV Spectra of IAA Oxidation Products in MeOH.

A: IAA and oxindolyl-3-acetic acid (1:1), B: (2:1).

TABLE I. YIELDS AND *R_f* VALUES OF PRODUCTS

Product ^a	Yield (mg)	<i>R_f</i> Value ^b	
		a	b
1	80 ^c	0.42	0.15
2	32 ^c	0.62	0.43
3	5.0	0.69	0.62
4	8.0	0.49	0.33
5	8.0	0.44	0.19
6	19.0	0.61	0.35
7	4.2	0.51	0.27
8	4.0	0.62	0.12
9	9.1	0.62	0.44
10	5.4	0.73	0.63

^a The sum of the substrate added corresponds to 420 mg as free IAA.

^b a: CHCl₃-EtOAc-HCO₂H (35:55:10),
b: MeOH-CHCl₃ (7:93).

^c Yields of crude materials.

oxindole absorption (250 nm region) was observed, and the decrease of the former absorption indicated the consumption of about a half

of the substrate at this point. The reaction slowed down after 10 min, and came to virtual standstill after 15 min. An incubation time of 30 min for a large scale reaction was therefore selected in order to minimize the possibility of further reactions.

A preparative scale reaction was carried out in a similar manner. Ten products which are summarized in the Table could be separated by gel filtration on Sephadex LH 20, followed by either preparative thin-layer chromatography (TLC) on silica gel and/or crystallization.

Identification and structural elucidation

Ten products thus isolated may be classified into the following 5 groups on the basis of their UV spectral features; 1) oxindoles: 1 and 2, 2) indoles: 3, 8, and 10, 3) oxindoles with one indole unit: 5 and 6, 4) oxindoles with two indole units: 7 and 9, 5) others: 4. Among these ten, two were readily identified by their characteristic UV spectra as 3-methylene-oxindole (2) and indolyl-3-aldehyde (4) which have been established as the products in IAA-peroxidase reactions.⁷⁻⁸⁾

1) Group 1 (1 and 2)

Product 1, *m/e* 163 (*M*⁺), was identified as 3-hydroxymethyloxindole²⁾ by the ready change of 1 to 3-methyleneoxindole (2). This substance has been reported first as an inhibitor of cell growth in photo-oxidation products of IAA⁹⁾ and subsequently as an immediate precursor leading to 2 in the HRP oxidation of IAA.²⁾ However, only a tentative structure

FIG. 3. PMR Spectrum of 3-Hydroxymethyloxindole (1) (CD₃SOCD₃, 100 MHz).

for this substance has been presented because of its insufficient purity due to its lability. The PMR spectrum of **1**, shown in Fig. 3, ultimately confirmed this.

2) Group 2 (**3**, **8**, and **10**)

Products **3**, **8**, and **10**, the latter two of which turned deep red during purification procedure, showed exclusively an UV absorption characteristic to indole compounds. The absorption intensities showed the presence of single indole chromophore in one molecule of **3**, and two, in **8** and **10**.

Product **3**, m/e 175 (M^+), gave a singlet (3H) at δ 2.30 that is ascribed to the methyl of an acetyl group, a multiplet (5H, aromatic protons) at δ 7.00~7.50 in the PMR spectrum, and also an enolacetate band (ν 1750 cm^{-1}) in the IR spectrum. These data suggested that **3** is 3-acetoxyindole, being identified by comparison with an authentic sample.

Product **8**, m/e 304 (M^+ , $C_{19}H_{16}O_2N_2$), showed a carbonyl band (ν 1700 cm^{-1}) in the IR spectrum. In addition the fragment ions at m/e 259 ($M-\text{CO}_2\text{H}$) and 245 ($M-\text{CH}_2\text{CO}_2\text{H}$) in the MS spectrum indicated the presence of the partial structure of $-\text{CH}_2\text{CO}_2\text{H}$, the methylene signal of which was observed as a singlet at δ 3.74 in the PMR spectrum. The presence of this group was also supported by the MS data of the methyl ester (by diazomethane) and of the reduction product (by lithium aluminum hydride) of **8**. The PMR spectrum showed another singlet (2H) at δ 4.25 ascribed to the methylene bearing two olefins. These data are consistent with an indolyl-3-acetic acid with an indolylmethyl substituent, which is attached to one of available positions excluding the 1-position of the indole ring, since two NH were observed in the PMR spectrum. The position of the attached methylene group, although not fully evidenced, is probably between the C-2 of IAA and the C-3 of indole, on the basis of its probable formation mechanism discussed later.

Product **10**, m/e 246 (M^+), was identified as 3,3-diindolylmethane from the MS and PMR data. This compound has been reported as the reaction product in a corn IAA oxidase

system.³⁾

3) Group 3 (**5** and **6**)

Product **5** and **6** have characteristic UV absorption presumably consisting of bands of indole and oxindole chromophores, as shown in Fig. 2. In fact an UV spectrum of an equimolar mixture solution of IAA and oxindolyl-3-acetic acid (Spectrum A) showed close resemblance to that of **5** or **6**. In addition, the absorption intensities showed the presence of each one unit of indole and oxindole chromophore in either of the molecules.

Product **6**, m/e 262 (M^+), showed carbonyl and NH bands (ν 1700 and 3400 cm^{-1}) in the IR spectrum. The PMR spectrum gave signals of an *ABX* type of splitting at δ 3.10 and 3.45, and 3.78, and a multiplet (9H, aromatic protons) at δ 6.70~7.60. These data suggested 3-(indol-3-ylmethyl)oxindole. The fragment ion at m/e 130 (base peak) is consistent with this structure. Reduction of **6** (by lithium aluminum hydride) afforded the expected substance **10**.

Product **5**, m/e 292 (M^+ , $C_{18}H_{16}O_2N_2$), gave signals of an *AB* type quartet centered at δ 3.15, a singlet (2H) at δ 3.78, and a multiplet (9H, aromatic protons) at δ 6.50~7.50 in the PMR spectrum. The singlet at δ 3.78, which shifted to an *AB* type quartet centered at δ 4.59 when diacetylated, was assigned as the methylene bearing a hydroxy group. These data are consistent with the 3-hydroxymethyl derivative of **6**. The presence of the fragment ions at m/e 262 ($M-\text{CH}_2\text{O}$) and 130 (C_9H_8N , base peak) supported the elucidated structure.

4) Group 4 (**7** and **9**)

Products **7** and **9** have a characteristic UV absorption as shown in Fig. 2, that is similar to that of a synthetic 2:1 mixture of IAA and oxindolyl-3-acetic acid. Also the absorption intensities showed the presence of two units of indole and one unit of oxindole chromophores in its molecule.

Product **9**, m/e 391 (M^+ , $C_{26}H_{21}ON_3$), gave an *ABX* type of splitting at δ 3.14, 3.64, and 3.82 similar to those of **6**, and a singlet (2H) at δ 4.14 that was assigned as the methylene bearing two olefins. These suggested **9** as a

3-(indol-3-ylmethyl)oxindole (**6**) with an indolylmethyl substituent. In the MS spectrum of **9**, the presence of the fragment ions at m/e 259 (M-132) and 260 (M-131) indicated that the substitution of the indole residue occurs on the indole ring of **6**. If it does on the oxindole side, the fragment ions at m/e 261 (M-130) and 262 (M-129) will be expected. The assumption that the linkage is at the C-2 of **6** seems to be most probable, considering the usual high reactivity at this position in indole ring.

Product **7**, m/e 421 (M^+), gave signals of AB type quartet centered at δ 3.40, a singlet at δ 4.00 (2H) similar to those of **5**, and a singlet at δ 4.10 (2H) assigned to the methylene bearing two olefins. The fragment ions at m/e 391 and 259 corresponding to M-CH₂O and M-CH₂O-132 also indicated the presence of the same system as **5**. Thus **7** was a 3-hydroxymethyl derivative of **9**. The MS data of the diacetate of **7** also supported this structure.

Routes of formation

Under the condition employed, 3-hydroxymethylindole (**1**) and 3-methyleneoxindole (**2**) were the main products, and indolyl-3-aldehyde (**4**), 3-acetoxyindole (**3**) and six of the dimeric and trimeric adducts were the minor ones, among those isolated and charac-

terized. Figure 4 shows a possible formation route of these products.

The first product of the HRP-catalyzed oxidation of IAA appears to be an IAA free radical (**I**) cited by Hinman and Lang³⁾ or its equivalent, from a consideration of the formation mechanism of **3** and six of the adducts, as follows. When the radical **I** rearranges with the cleavage between the C-3 carbon and the side chain as shown in Route a, **3** will be produced. When decarboxylation occurs at this stage as shown in Route b, 3-methyleneindolenine (**II**), which have been found as a reaction product of IAA in a corn IAA oxidase system,³⁾ will be produced. Also, when a Michael type addition of **1** occurs to **II**, the dimer **5** will be produced, and subsequently will lead to **6** via a reverse aldole type reaction. The formation of the adducts, **7**, **8**, and **9**, can also be speculated by the reaction mechanism similar to that of **5**. When the C-2 carbon of the indole ring of **5**, IAA, or **6** couples with **II**, each corresponding dimer or trimer will be produced. The product **10** will be formed through the pathway proposed in the corn IAA oxidase system³⁾; 3-hydroxymethylindole (**III**), which will be formed reversibly by hydration of **II**, couples with **II**, and the resulting adduct **IV** is then converted into **10** by release of formaldehyde. In our scheme speculated here, the most significant point is that the free

FIG. 4. A Possible Formation Route of Products.

====>Hinman's proposed route. -----> BeMiller's proposed route.

radical I or its equivalent could give rise to the two routes a and b, rather than the conventionally accepted route of the oxygen addition.

Among ten products isolated, each of **5** and **7** has an asymmetric carbon, which, once formed, cannot racemize through enolization at the C-3 of the oxindoles. Both compounds, however, were optically inactive. This fact suggested that these two adducts are formed nonenzymatically. The same logic may be given for the other adducts, when referred to their possible formation route shown in Fig. 4. On the other hand, the hydration process from **1** to **2** have been already proved to be a post-enzymatic step.³⁾ As to this problem, a mechanism in which IAA oxidation by HRP occurs mainly through a free radical chain reaction has been also proposed,¹⁰⁾ on the basis of the spectroscopic observation of the enzymatic reaction. Therefore, the determination of the reaction process where HRP enzyme participate directly, along with that of the forming pathway of **1** and **4**, are remaining problems to be clarified. This is under current investigation in parallel with that of photo-oxidation of IAA.

EXPERIMENTAL

Melting points were uncorrected. UV spectra were determined with a Shimadzu UV 200 spectrometer. IR spectra were measured with a JASCO IRA-1, PMR spectra with Varian HA-100 or JEOL JNM-PMX 60 instruments using TMS as an internal standard, and MS spectra with Hitachi RMU-6MG (20 eV, low resolution MS spectra) and JEOL-01SG-2 (70 eV, high resolution MS spectra) spectrometers using a direct inlet. ORD and optical rotations were recorded with a JASCO J-20 spectrometer.

Pre-coated silica gel F₂₅₄ plates (0.25 mm thickness) for TLC and silica gel PF₂₅₄ for preparative TLC (0.5 mm thickness) were used.

HRP oxidation

a) *Time course experiment.* The reaction mixture composed of IAA potassium salt (4.2 mg) and HRP (1.0 mg; Boehringer Mannheim Ltd., Reinheitszahl: 0.6) in 100 ml of 5×10^{-2} M acetate buffer (pH 5.0) was stirred magnetically at 25°C. Two ml of the reaction mixture was taken out at zero min and after 10, 15, and 30 min of incubation and extracted with 3 ml of Et₂O.

The UV spectra of each Et₂O layer were measured in quartz cuvetts of 1-cm light path.

b) *Preparative scale reaction.* Three liters of the reaction mixture including 128 mg of IAA potassium salt was incubated for 30 min in similar manner. The reaction mixture was extracted two times with EtOAc (2, 1 liters, each), which were combined and washed with H₂O. The extract, after drying over anhyd. Na₂SO₄, was concentrated *in vacuo*. The combined concentrated extract of the four batches was subjected immediately to gel filtration on Sephadex LH 20 (1.9 × 90 cm) with MeOH. The eluate was examined by TLC using CHCl₃-EtOAc-HCO₂H (35:55:10, Solvent a) and grouped into 8 fractions; A (80 mg), B (32 mg), C (50 mg), D (40 mg), E (10.5 mg), F (14.7 mg), G (22.9 mg), and H (9.2 mg). Among these, C fraction was a mixture of **3**, **4**, and **5** and the unconsumed IAA. The other fractions were consisted of almost one component on TLC.

3-Hydroxymethyloxindole (1)

Fraction A was crystallized rapidly from EtOH to afford pale yellow fine needles (**1**, 45 mg); mp 126~129°C. Attempts to purify fraction A by silica gel TLC (Solvent a) or slower crystallization than one day at -20°C in EtOH were unsuccessful, being accompanied with small amounts of **2** derived from **1**. $[\alpha]_D^{20} \pm 0.0$ ($c=1.0$, MeOH); MS *m/e* (rel. int.): 163 (M⁺, 18), 145 (M-H₂O, 34), 133 (M-CH₂O, 100), 117 (44); UV λ_{max}^{MeOH} nm (ϵ): 280 (1150), 249 (7100), 207 (23000); PMR (CD₃SOCD₃, Fig. 3) δ : 3.42 (1H, t, $J=5$ Hz), 3.64~4.06 (2H, m), 4.80 (1H, t, $J=5$ Hz, OH), 6.75~7.32 (4H, m); IR ν_{max}^{KBr} cm⁻¹: 3450, 3200, 1690.

3-Methyleneoxindole (2)

Fraction B, which was almost homogeneous with TLC (Solvent a), gave several spots at lower *R_f* than that of **2** together with MeOH insoluble substances. The yield and the absorption intensity of the UV spectrum were estimated on the basis of the amount of pale yellow solid obtained by removal of the solvent. UV λ_{max}^{MeOH} nm (ϵ): 278 (3800), 254 (23000), 247 (35000); MS *m/e* (rel. int.): 145 (M⁺, 100), 130 (78), 117 (32), 90 (17), and 290 (43), 162 (9), 161 (9), 176 (15), 158 (7) due to the dimers of **2**.

3-Acetoxyindole (3), indolyl-3-aldehyde (4), and 3-hydroxymethyl-3-(indol-3-ylmethyl)oxindole (5)

Fraction C was purified by preparative TLC (Solvent a). The respective band at *R_f* 0.69, 0.55, 0.49, and 0.44 regions which were visible under UV light was eluted with MeOH followed by gel filtration on Sephadex LH 20 (0.7 × 20 cm) with MeOH, affording **3** (5.0 mg), IAA (7.1 mg), **4** (8.0 mg), and **5** (8.0 mg).

3, plates (EtOH), mp 127~130°C; MS *m/e* (rel. int.):

175 (M^+ , 23), 133 (100); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 289 (3600), 280 (4300), 220 (22900); PMR (CD_3OD) δ : 2.30 (3H, s), 7.00~7.50 (5H, m); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3350, 1750.

4, plates (H_2O), mp 195~199°C; MS m/e (rel. int.): 145 (M^+ , 83), 144 (100), 116 (34), 89 (29); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 294 (8000), 260 (7600), 242 (9300), 212 (17000); PMR (CD_3COCD_3) δ : 7.20~8.30 (5H, m), 10.02 (1H, s), 11.10 (1H, bs, NH).

5, prisms (MeOH), mp 178~181°C (dec.); $[\alpha]_D^{20} \pm 0.0$ ($c=0.67$, MeOH); MS m/e (rel. int.): 292.1220 (M^+ , $\text{C}_{18}\text{H}_{16}\text{O}_2\text{N}_2$, 3), 262.1116 ($M-\text{CH}_2\text{O}$, 10), 130.0655 ($\text{C}_8\text{H}_8\text{N}$, 100), 116.0491 ($\text{C}_8\text{H}_8\text{N}$, 12); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 290 (4400), 282 (5100), 250 (8000), 223 (25600), 210 (37000); PMR (CD_3SOCD_3) δ : 3.15 (2H, ABq, $J=14$ Hz), 3.78 (2H, s), 6.50~7.50 (9H, m), 10.00 and 10.62 (each 1H, bs, NH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1700. 5 (7.0 mg) was acetylated with Ac_2O and pyridine (each 0.2 ml) at room temperature. Removal of the excess of the reagents afforded the diacetate of 5, R_f 0.71 with Solvent a. MS m/e (rel. int.): 376 (M^+ , 2), 172 (3), 145 (5), 130 (100), 117 (3); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 290, 280, 275, 219; PMR (CD_3SOCD_3) δ : 1.80 (3H, s), 2.28 (3H, s), 3.35 (2H, bs), 4.59 (2H, ABq, $J=10$ Hz), 6.50~7.90 (9H, m), 10.80 (1H, bs, NH).

3-(Indol-3-ylmethyl)oxindole (6)

Fraction D afforded pale red amorphous powder 6 (8.0 mg) by preparative TLC ($\text{MeOH}-\text{CHCl}_3$ (7:93), Solvent b) and gel filtration (MeOH). $[\alpha]_D^{20} \pm 0.0$ ($c=2.2$, MeOH); MS m/e (rel. int.): 262 (M^+ , 10), 130 (100); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 290 (4900), 282 (6000), 250 (9000), 220 (35000), 217 (37000); PMR (CD_3SOCD_3) δ : 3.10, 3.45, and 3.78 (each 1H, ABX type of splitting, $J=15$, 8, and 5 Hz), 6.70~7.60 (9H, m), 10.24 and 10.72 (each 1H, bs, NH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1700. 6 (22.1 mg) was treated with an excess of lithium aluminum hydride (10 mg) in dry tetrahydrofuran (3 ml) with refluxing for 30 min. After adding EtOAc saturated with H_2O (10 ml) to the reaction mixture, the EtOAc extract was concentrated and passed through Sephadex LH 20 with MeOH to afford a main product (4.7 mg), identified (PMR, UV, MS, and R_f values on TLC) as 10.

3-Hydroxymethyl-3-[2-(indol-3-ylmethyl)indol-3-ylmethyl]oxindole (7)

Fraction E was purified by preparative TLC (Solvent b), affording pale red amorphous powder 7 (4.2 mg). MS m/e (rel. int.): 421 (M^+ , 0.1), 391 (10), 259 (100), 133, 130, 117; UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 292 (12500), 284 (14500), 276 (13500), 260 (13000), 225 (61000), 215 (56000); PMR (CD_3COCD_3) δ : 3.40 (2H, ABq, $J=14$ Hz), 4.00 and 4.10 (each 2H, s), 6.70~7.50 (17H, m), 9.10, 9.40, and 9.90 (each 1H, bs, NH). 7 (1.2 mg) was acetylated in similar manner to afford the diacetate of 7, R_f 0.68 with Solvent a. MS m/e (rel. int.): 505 (M^+ ,

0.5), 259 (100), 145 (17), 130 (5), 117 (7).

2-(Indol-3-ylmethyl)indolyl-3-acetic acid (8)

Fraction F was purified by preparative TLC (Solvent b), affording a pale red solid 8 (4.0 mg). MS m/e (rel. int.): 304.1217 (M^+ , $\text{C}_{19}\text{H}_{16}\text{O}_2\text{N}_2$, 100), 259.1227 ($M-\text{CO}_2\text{H}$, 43), 245.1083 ($M-\text{CH}_2\text{CO}_2\text{H}$, 93), 187.0631 ($M-117$, 12), 130.0634 ($\text{C}_8\text{H}_8\text{N}$, 12), 117.0601 ($\text{C}_8\text{H}_7\text{N}$, 6); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 290 (13200), 282 (15200), 224 (31000); PMR ($\text{CD}_3\text{OOCDC}_3$) δ : 3.74 (2H, s), 4.25 (2H, s), 6.90~7.60 (9H, m), 9.56 and 9.95 (each 1H, bs, NH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1700. 8 (0.7 mg) was treated with ethereal diazomethane at 0°C for 30 min to afford the methyl ester of 8, MS m/e (rel. int.): 318 (M^+ , 93), 259 245 (100), 201 (6), 130 (7), 117 (2). 8 (0.7 mg) was treated with an excess of lithium aluminum hydride (3 mg) in dry tetrahydrofuran (3 ml) at room temperature for 1 hr to afford the reduction product of 8, MS m/e (rel. int.): 290 (M^+ , 54), 259 (100), 245 (5), 130 (10).

3-[(2-Indol-3-ylmethyl)indol-3-ylmethyl]oxindole (9)

Fraction G was also purified by preparative TLC (Solvent b), affording a pale red solid 9 (9.1 mg). $[\alpha]_D^{20} \pm 0.0$ ($c=0.80$, MeOH); MS m/e (rel. int.): 391.1718 (M^+ , $\text{C}_{26}\text{H}_{21}\text{ON}_3$, 9), 259.1219 ($M-\text{C}_8\text{H}_8\text{ON}$, 100), 260.1314 ($M-\text{C}_8\text{H}_8\text{ON}$, 26), 133, 130, 117 (10); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 290 (12300), 282 (14200), 260 (13200), 250 (14000), 225 (56300), 215 (58300); PMR (CD_3COCD_3) δ : 3.14, 3.64, and 3.82 (each 1H, ABX type of splitting, $J=14$, 9, and 4 Hz), 4.14 (2H, s), 6.70~7.60 (13H, m), 9.30, 9.70, and 10.00 (each 1H, bs, NH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1700.

3,3-Diindolylmethane (10)

Fraction H was purified similarly by preparative TLC (Solvent b) to afford 10 (5.4 mg), pale yellow plates (MeOH), mp 162~163°C; MS m/e (rel. int.): 246 (M^+ , 100), 245 (75), 130 (10), 117 (5); PMR (CD_3COCD_3) δ : 4.22 (2H, s), 6.90~7.65 (10 H, m), 9.90 (2H, bs, NH); UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 290 (12600), 283 (14500), 275 (14000), 225 (78500); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400.

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