A Revised Synthetic Scheme of 6,6′-Dibromoindirubin

Yasuhiro Tanoue*, Akari Hara and Norihisa Kai

Department of Food Science and Technology, National Fisheries University, Nagatahonmachi, Shimonoseki 759-6595, Japan
E-mail: tanoue@fish-u.ac.jp

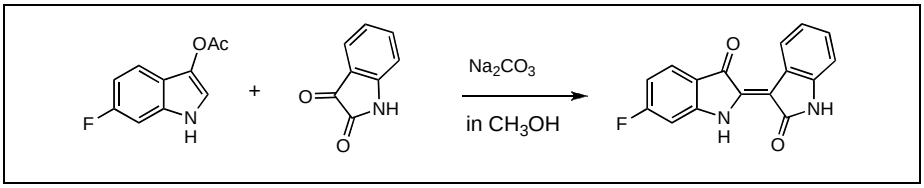
Kazunori Sakata and Mamoru Hashimoto

Department of Applied Chemistry, Faculty of Engineering, Kyushu Insitute of Technology, Tobata-ku, Kitakyushu 804-8550, Japan

Takeshi Nagai

Department of Food Science and Technology, Tokyo University of Agriculture, Abashiri, Hokkaido 099-2493

Received December 22, 2006



The synthetic scheme of 6,6′-dibromoindirubin (**2**) was investigated in detail. The reaction of 6-fluoro-3-acetoxyindole (**7**) with isatin (**8**) in methanol with Na_2CO_3 produced 6′-fluoroindirubin in moderate yields. Its structure determination was mainly undertaken using ^1H NMR spectroscopy. On the basis of this result, the synthetic scheme of **2** reported by Cooksey was revised.

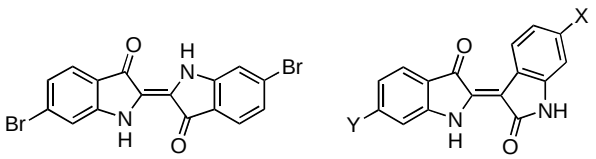
J. Heterocyclic Chem., **44**, 1135 (2007).

INTRODUCTION

Tyrian purple, royal purple and ancient purple are all synonyms for a dye of molluscan origin and were used as valuable purple dyes of garments for ancient exalted persons such as princes and nobles in the districts along the Mediterranean [1-2]. The precursors of Tyrian purple are contained in the hypobranchial glands of various species of gastropods from the families Muricidae and Thaidinae, and converted into Tyrian purple by the action of sunlight and purpurase [3-4]. In 1909, Friedländer isolated 1.4 g of the dye from 12,000 specimens of the gastropod *Murex brandaris* and identified it as 6,6′-dibromoindigo (**1**) [5].

We have already reported a convenient synthesis of **1** [6]. The hypobranchial glands mentioned above contain 6,6′-dibromoindirubin (**2**) and 6-bromo-indiubin (**3**) as minor components [7-10]. Their compounds have become of interest from the standpoint of glycogen synthase kinase (GSK)-3-selective inhibitors [10]. In addition, indirubin (**4**) exhibits strong antitumor [11] and potent aryl hydrocarbon ligand activities [12].

Cooksey [9] reported that the reaction of 6-bromoisatin (**5**) with 6-bromo-3-acetoxyindole (**6**) gave **2** in 76 % yield in methanol at room temperature for 2 h and the synthetic scheme of **2** is as follows (Scheme 1). Judging from this scheme, it was postulated that the moieties A and B of **2** consist of the starting materials **5** and **6**, respectively. As we reported that the hydrolysis of **6** with aqueous ethanol containing sodium hydroxide occurred along with a dimerization which took place at position 2 of **6** to give **1** [6], we doubted whether the scheme is correct. Moreover, it is known that carbon nucleophiles add to isatin and its derivatives at position C-3 in most cases [13].



1

2: X=Y=Br
3: X=Br, Y=H
4: X=Y=H

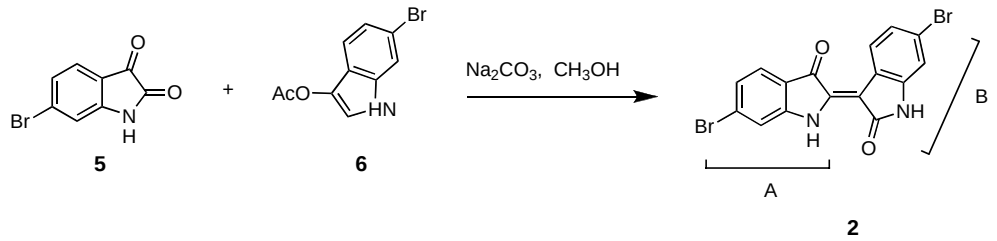
Figure 1

The present paper describes the detailed investigation of the synthetic scheme of 6,6′-dibromoindirubin.

RESULTS AND DISCUSSION

As the F atom strongly couples with protons and the proton - fluorine coupling constants are somewhat higher than those of proton - proton in an NMR spectrum, a compound containing a F atom is useful for a structure

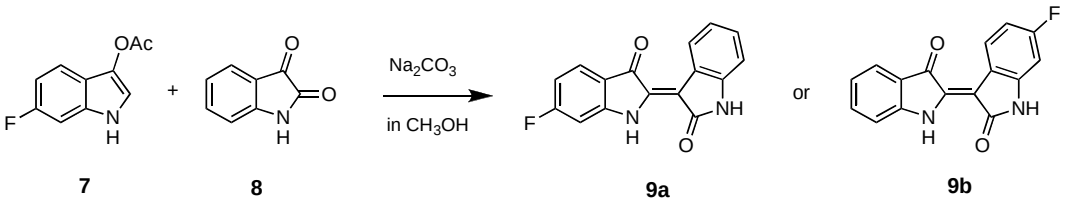
Scheme 1

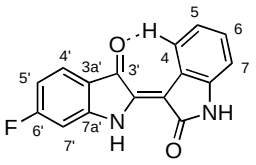


determination. Therefore, 6-fluoro-3- acetoxyindole (**7**) was used as a starting material. The reaction of **7** with isatin (**8**) was carried out in methanol with Na_2CO_3 at room temperature.

The structure of the product was either **9a** or **9b** shown in Scheme 2. The ^{1}H NMR spectrum is shown in Figure 2. The H-4 signal (δ 8.76) showed a remarkable down-field shift relative to the H-4′ signal (δ 7.72). This shift is ascribable to the hydrogen bond between H-4 and the oxygen atom which bonds to C-3′ as shown in Figure 3.

Scheme 2





9a

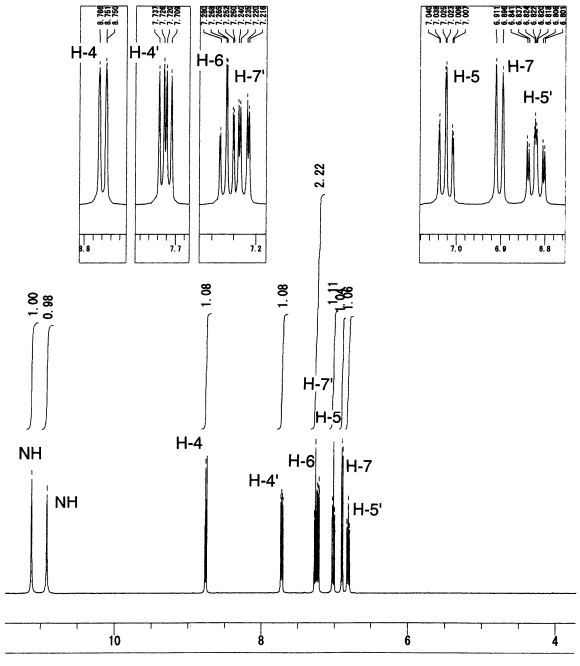


Figure 2. The spectrum of 6'-fluoroindirubin (**9a**)

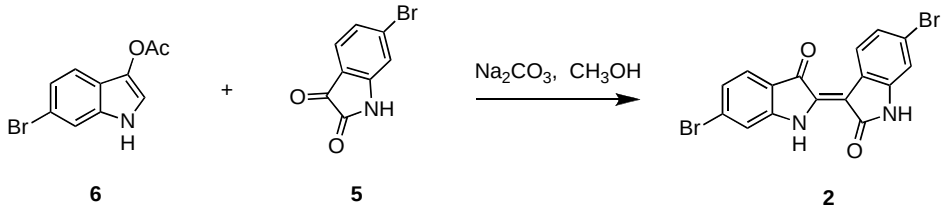
Figure 3. The hydrogen bond between H-4 and oxygen atom

EXPERIMENTAL

The ^{1}H NMR and ^{13}C NMR spectra were obtained using a JEOL JNM-A500 (500 MHz) spectrometer in chloroform-d and dimethyl sulfoxide-d_6 at room temperature. Chemical shifts are given in ppm relative to tetramethylsilane as the internal reference standard. The EI mass spectra were performed using a JEOL JMS-SX 102A mass spectrometer. The infrared spectra were recorded using a Shimadzu IR 470 spectrometer in potassium bromide pellets. The melting points were obtained using a Yanaco MS-S3 micro melting point apparatus (hot- plate type). Elemental analyses were determined using a Yanaco CHN Corder MT-3. For preparative column chromatography, Wakogel C-200 silica gel was employed. Indole, 6-fluoroindole and isatin were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan).

3-Acetoxy-6-fluoroindole (**7**). To a solution of 6-fluoroindole (100 mg, 0.74 mmol) and sodium hydroxide (29.6 mg, 0.74 mmol) in methanol (10 ml) were added iodine (188 mg, 0.74 mmol) and an aqueous solution (1 ml) of potassium iodide (123

Scheme 3



6 **5** **2**

mg, 0.74 mmol). After the mixture was stirred at room temperature for 3 h, water (20 ml) was added. The resulting precipitate was collected by filtration, washed with water, and dried under reduced pressure to obtain 6-fluoro-3-iodoindole (153 mg, 0.584 mmol), which was used for the following reaction without purification because of its lability. Silver acetate (146 mg, 0.876 mmol) was added to a solution of 6-fluoro-3-iodoindole in acetic acid (10 ml). After stirring for 1 h at 90°C, the mixture was cooled to room temperature and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$ to give 3-acetoxy-6-fluoroindole (**7**) (73 mg, 51 % yield). It was recrystallized from hexane as colorless plate crystals, mp 139 – 140 °C; ir (potassium bromide): NH 3380, C=O 1743, 1629, 1598, 1338, 1227, 1122, 806 cm^{-1}; ^{1}H nmr (chloroform-d): δ 2.36 (s, 3H, CH_3), 6.88-6.92 (m, 1H, H-5), 6.96 (dd, 1H, H-7, $J_{7\text{-}F}$ = 9.6, $J_{7\text{-}5}$ = 1.8 Hz), 7.25 (d, 1H, H-2, $J_{2\text{-}1}$ = 2.8 Hz), 7.45 (dd, 1H, H-4, $J_{4\text{-}5}$ = 8.5, $J_{4\text{-}F}$ = 5.2 Hz), 7.91 ppm (br, 1H, NH); ^{13}C nmr (chloroform-d): δ 20.93 (CH_3), 97.62 (d, C-7, $J_{7\text{-}F}$ = 26.9 Hz), 108.92 (d, C-5, $J_{5\text{-}F}$ = 24.8 Hz), 113.52 (C-2), 116.67 (C-9), 118.37 (d, C-4, $J_{4\text{-}F}$ = 9.2 Hz), 130.47 (C-3), 133.00 (d, C-7a, $J_{7a\text{-}F}$ = 12.4 Hz), 160.32 (d, C-6, $J_{6\text{-}F}$ = 238 Hz), 168.77 (C=O); ms: m/z (relative intensity) 193 (M^+, 58 %), 152 (55), 151 (100), 150 (59), 123 (57), 122 (59), 96 (39), 95 (58), 94 (55), 75 (52). *Anal.* Calcd. for $C_{10}H_8FNO_2$: C, 62.18; H, 4.17; N, 7.25. Found: C, 62.19; H, 4.20; N, 7.20.

6′-Fluoroindirubin (**9a**). A solution of **7** (30 mg, 0.155 mmol) and isatin (**8**) (22.8 mg, 0.155 mmol) in methanol (6 ml) was stirred under a nitrogen atmosphere at room temperature for 25 minutes. To the mixture was added anhydrous sodium carbonate (39.3 mg, 0.371 mmol). After the mixture was stirred for 3 h, water was added. The resulting precipitate was collected by filtration, washed with water and aqueous methanol (1:1). The crude product (37.8 mg) was recrystallized from ethyl acetate to give **9a** (28.6 mg, 66 % yield) as a brown – purple powder, mp > 300 °C; ir (potassium bromide): 3270 (NH), NH 3270, C=O 1668, 1620, 1591, 1453, 1291, 1207, 1128, 1090, 1009, 968 cm^{-1}; ^{1}H nmr (dimethyl sulfoxid – d_6): δ 6.82 (m, 1H, H-5′), 6.90 (d, 1H, H-7, $J_{7\text{-}6}$ = 7.5 Hz), 7.02 (m, 1H, H-5), 7.23 (dd, 1H, H-7′, $J_{7'\text{-}F}$ = 9.8, $J_{7'\text{-}5'}$ = 2.3 Hz), 7.27 (m, 1H, H-6), 7.72 (dd, 1H, H-4′, $J_{4'\text{-}5'}$ = 8.5, $J_{4'\text{-}F}$ = 5.5 Hz), 8.76 (d, 1H, H-4, $J_{4\text{-}5}$ = 7.5 Hz), 10.92 (s, 1H, NH), 11.12 (s, 1H, NH); ^{13}C nmr (dimethyl sulfoxide – d_6): δ 100.55 (d, C-7′, $J_{7'\text{-}F}$ = 26.9 Hz), 107.33, 108.80 (d, C-5′, $J_{5'\text{-}F}$ = 24.8 Hz), 109.63, 115.94, 121.21, 121.33, 124.92, 127.04 (d, C-4′, $J_{4'\text{-}F}$ = 12.4 Hz), 129.59, 138.45, 141.12, 154.50 (d, C-7a′, $J_{7a'\text{-}F}$ = 15.6 Hz), 167.73 (d, C-6′, $J_{6'\text{-}F}$ = 252.2 Hz), 170.74, 186.75; ms: m/z (relative intensity) 280 (M^+, 100 %), 252 (52), 223 (29). *Anal.* Calcd for $C_{16}H_9FN_2O_2$: C, 68.57; H, 3.24; N, 10.00. Found: C, 68.40; H, 3.26; N, 10.28.

Acknowledgement. We are grateful to the Center for Instrumental Analysis, Kyushu Institute of Technology for elemental analyses, mass spectra and NMR spectra.

REFERENCES AND NOTES

[1] Baker, J. T. *Endeavour* **1974**, *33*, 11.

[2] McGovern, P. E.; Michel, R. H. *Acc. Chem. Res.* **1990**, *23*, 152.

[3] Baker, J. T.; Sutherland M. D. *Tetrahedron Lett.* **1968**, 43.

[4] Christophersen, C.; Wätjen, F.; Buchardt, O.; Anthoni, U. *Tetrahedron* **1978**, 2779.

[5] Friedländer, P. *Ber. Dtsch Chem. Ges.* **1909**, 765.

[6] Tanoue, Y.; Terada, A.; Sakata, K.; Hashimoto, M.; Morishita, S.; Hamada, M.; Kai, N.; Nagai, T. *Fisheries Science* **2001**, *67*, 726.

[7] Fouquet, H.; Bielig, H-J. *Angew. Chem. Int. Ed. Engl.* **1971**, *10*, 816.

[8] Clark, R. J. H.; Cooksey, C. J. *J. Soc. Dyers, Colour* **1997**, *113*, 316.

[9] Cooksey, C. J. *Molecules* **2001**, *6*, 736.

[10] Meijer, L.; Skaltsounis, A-L.; Magiatis, P.; Polychronopoulous, P.; Knockaert, M.; Leost, M.; Ryan, X. P.; Vonica, C. A.; Brivanlou, A.; Dajani, R.; Crovace, C.; Tarricone, C.; Musacchio, A.; Roe, S. M.; Pearl, L.; Greengard, P. *Chemistry & Biology*, **2003**, *10*, 1255.

[11] Hoessel, R.; Leclerc, S.; Endicott, J. A.; Nobel, M. E. M.; Lawrie, A.; Tunnah, P.; Leost, M.; Damiens. E.; Marie, D.; Marko, D.; Niederberger, E.; Tang, W.; Eienbrand, G. ; Meijer, L. *Nat. Cell Biol.* **1999**, *1*, 60.

[12] Adachi, J.; Mori, Y.; Matsui, S.; Takigami, H.; Fujino, J.; Kitagawa, H.; Miller, CA3rd; Kato, T.; Saeki, K.; Matsuda, T. *J. Biol. Chem.*, **2001**, *276*, 31475.

[13] Silva, J. F. M.; Garden, S. J.; Pinto, A. C.; *J. Braz. Chem. Soc.*, **2001**, *12*, 273.