NPC Natural Product Communications

2006
Vol. 0
No. 0
1 - 5

Synthesis of Hypericin via Emodin Anthrone Derived from aTwo-fold Diels-Alder Reaction of 1,4-Benzoquinone

Jiro Motoyoshiya*, Yusuke Masue, Yoshinori Nishi and Hiromu Aoyama

Department of Chemistry, Faculty of Textile Science and Technology, Shinshu University, Ueda, Nagano 386-8567, Japan

jmotoyo@giptc.shinshu-u.ac.jp

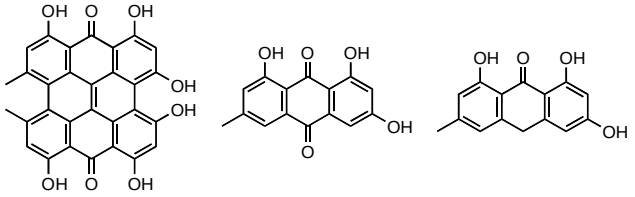
Received: August 14th, 2006; Accepted: XX, 2006

The six-step synthesis of hypericin by the regioselective two-fold Diels-Alder reaction of 1,4-benzoquinone first with (1-methoxy-3-methylbuta-1,3-dienyloxy)trimethylsilane leading to 7-methyljuglone, and next with (1,3-dimethoxybuta-1,3-dienyloxy)trimethylsilane, to give emodin and its *O*-methylated derivative. The reduction of both compounds with tin(II) chloride in acidic media was accompanied by acid hydrolysis that produced emodin anthrone, whose oxidative dimerization with iron (III) chloride hydrate gave the bianthrone in high yield. The oxidation of the bianthrone in the presence of *N*-ethyldiisopropylamine gave protohypericin, which was converted into hypericin upon irradiation.

Keywords: hypericin, emodin, emodin anthrone, Diels-Alder reaction.

Hypericin, 1, 3, 4, 6, 8, 13-hexahydroxy-10,11-dimethylphenanthro(1, 10, 9, 8-opqra)perylene-7, 14-dione (**1**), is isolated from St. John's-wort [1], a traditional medicinal plant distributed throughout the world, which has received much attention because of its various biological activities. Hypericin acts as an anticancer, antiviral, and antiretroviral agent, and induces apoptosis. Since its excited state generated by photo-irradiation produces active species such as singlet oxygen or superoxide ion that are toxic toward organisms, application in photodynamic therapy is considered to be a new use of hypericin [2]. This has prompted numerous reports on its synthesis [3]. However, the straightforward synthesis of hypericin from a simple compound has scarcely been documented because several steps are required to produce the key intermediates, emodin (**2**) [4a,b] or emodin anthrone (**3**) [5a]. The symmetric structure of **1** suggests that the dimerization of **2** or **3** by oxidative coupling is very convenient, and several syntheses are based on this synthetic strategy [5b-e].

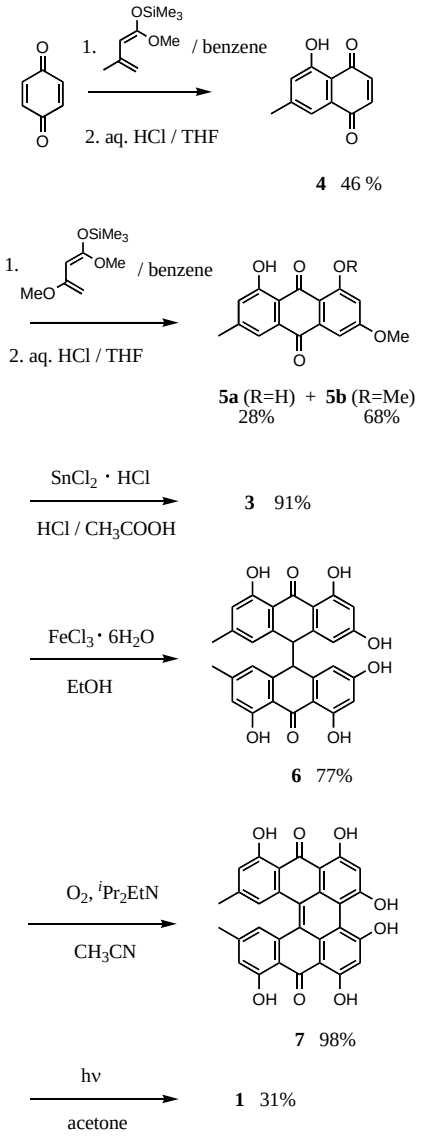
In this paper, we report a short route to hypericin via emodin anthrone starting from 1,4-benzoquinone involving the two-fold Diels-Alder reaction. Our synthetic pathway to hypericin consists of a six-step synthetic procedure as shown in Scheme 1.



1 **2** **3**

Either **2** or **3** is the key precursor of hypericin, and both are anthraquinone derivatives bearing a methyl group at the 6-position and three hydroxyl groups at the 1, 3, and 8-positions. The key problem of their synthesis is the introduction of such functionalities at the suitable positions. Calculations using the frontier molecular orbital theory predict that secondary orbital interaction controls the direction of cycloaddition [6a-c] from 1,4-benzoquinone; namely, 7-methyljuglone formed by the initial Diels-Alder reaction of 1,4-benzoquinone and (1-methoxy-3-methylbuta-1,3-dienyloxy)trimethylsilane [6a,d] followed by acid hydrolysis is expected to react with (1,3-dimethoxybuta-1,3-dienyloxy)-trimethylsilane [7] in a regioselective manner leading to the desired adduct, the emodin precursor.

The Diels-Alder reaction of 1,4-benzoquinone and (1-methoxy-3-methylbuta-1,3-dienyloxy)trimethylsilane was carried out in benzene at 50 ^{o}C and the workup followed by acid hydrolysis afforded 7-methyljuglone



Scheme 1

(**4**) in 46 % yield. The second Diels-Alder reaction of **4** and (1,3-dimethoxybuta-1,3-dienyloxy)trimethylsilane proceeded in benzene at 70°C, and a mixture of physcion (**5a**) [4a] and emodin 6,8-dimethyl ether (**5b**) [4c] in 28 % and 68 %, respectively, was afforded after a similar workup. Anthraquinones **5a** and **5b** are naturally occurring and were readily converted into **2** by acid hydrolysis. The structural elucidation of **5a** and **5b** was done by reduction with tin (II) chloride in acetic acid containing hydrochloric acid to **3**, whose NOE between the protons at the 10-position, chemoselectively introduced by reduction of the carbonyl group, and the two peri-protons at the 4-, and 5-positions unambiguously established its structure. As expected, this second Diels-Alder reaction proceeded in a highly regioselective manner. The reduction was accompanied by acid hydrolysis leading to **3**. Despite the low yield of **4** by the first Diels-Alder reaction and sequential acid hydrolysis, the present synthetic method is not only simple, but also the most practical of those already reported for **2** [3,8] and **3** [5a].

The conversion of **3** into **1** was completed by the previously established procedure [9] with modifications. The oxidative coupling of **3** using iron (III) chloride hydrate gave the bianthrone (**6**) [10] in 77% yield as a mixture of diastereomers, which was oxidized under an oxygen atmosphere in the presence of *N*-ethyldiisopropylamine to yield protohypericin (**7**) [5c,9] in 98% yield. This procedure was much improved by using the tertiary amine in an organic solvent. The final step was the photooxidation of **7** leading to **1**. A solution of **7** in acetone was irradiated under a high-pressure mercury lamp to give **1** in 31% yield after purification by column chromatography. The spectral data are in agreement with those reported. The yield in this step was low, but not inferior to the procedure using **2**. The patent procedure [11] for the oxidation of protohypericin to hypericin leads to the improvement of the yield. Thus, the synthesis of hypericin was accomplished by a six-step synthetic procedure, starting from 1,4-benzoquinone.

Experimental

General: Melting points were determined on a hot stage microscope apparatus (Mitamura). NMR spectra were recorded on a Bruker AVANCE-400 at 400 MHz and 100 MHz respectively. The chemical shifts (δ) are reported in ppm downfield from TMS as internal standard or from the residual solvent peak. Coupling constants (*J*) are reported in Hz. Low resolution mass spectra (MS) were recorded by the JEOL JMS-K9 spectrometers. Elemental analysis was recorded on a Perkin-Elmer 2400CHN elemental analyzer. Analytical TLC was carried out on precoated silica gel 60F-254 plates (E. Merck). Column chromatography was performed on silica gel (E. Merck).

7-methyljuglone (4)

A solution of 1,4-benzoquinone (1.50 g, 13.4 mmol) was added to a solution of (1-methoxy-3-methylbuta-1,3-dienyoxy)trimethylsilane (1.26 g, 6.76 mmol) in benzene (8 ml), and the combined solution was stirred at room temperature for 5 hr and then heated at 50°C overnight. After removal of the solvent, the residue was treated with aqueous hydrochloric acid (5%, 32 ml) in THF (10 ml) at room temperature for 3 hr. The product obtained after removal of the organic solvent

was extracted with chloroform. The organic layer was dried over sodium sulfate and condensed *in vacuo*. The crude product was purified with silica gel column chromatography (chloroform) to afford 7-methyljuglone (581 mg, 46%).

MP. 125-127°C.
^{1}H-NMR ($CDCl_3$): 2.44 (s, 3H, Me); 6.91 (s, 2H, H-2 and 3); 7.08 (brs, 1H, H-6); 7.44 (d, 1H, *J* = 1.3 Hz, H-8); 11.87 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$) 22.0 (CH_3), 112.8(CH), 120.3 (CH), 124.0 (CH), 131.3 (C) , 138.6 (C), 139.1 (C), 148.3 (CO), 161.5 (CO), 184.4;
Anal. Calcd for $C_{16}H_{12}O_5$: C 70.21, H 4.29%, Found : C 70.35, H 4.15%.

Physcion (**5a**) **and 6,8-*O,O'*-dimethylemodin** (**5b**): A solution of **4** (1.06 g, 5.62 mmol) in benzene (30 ml) was added to (1,3-dimethoxybuta-1,3-dienyloxy)-trimethylsilane (2.28 g, 11.2 mmol) in benzene (15 ml) and the resulting solution was stirred at room temperature for 3 hr and then heated at 70°C for a day. The residue obtained after removal of the solvent was treated with aqueous hydrochloric acid (5%, 32 mL) in THF (45 ml) at room temperature for a day. After removal of the organic solvent, the products were extracted with chloroform. The organic layer was dried over sodium sulfate and condensed *in vacuo*. The crude products were purified with silica gel column chromatography (chloroform) to give **5a** (0.45 g, 28%) and **5b** (1.14 g, 68%).

Physcion

MP: 203°C .
^{1}H NMR **(**$CDCl_3$**)**: 2.45 (s, 3H, Me), 3.94 (s, 3H, OMe), 6.69 (d, 1H, *J* = 2.52, H-2), 7.09 (s, 1H, H-7), 7.37 (d, 1H, *J* = 2.78, H-4), 7.63 (s, 1H, H-5), 12.11 (s, 1H, OH), 12.31 (s, 1H, OH);
^{13}C NMR ($CDCl_3$): 22.5 (CH_3), 56.4 (OCH_3), 107.1, 108.5, 114.0, 121.6, 122.4, 124.8, 133.5, 135.6, 148.8 (C), 162.8(C), 165.5 (C), 166.9 (C), 182.2 (CO), 191.1 (CO). Anal. Calcd for $C_{16}H_{12}O_5$: C 67.70, H 4.25%. Found: C 67.74, H 4.16%.

6,8-*O,O'*-dimethylemodin

MP: 211-213°C (Lit. 213-214 °C [4c]).
^{1}H-NMR ($CDCl_3$): 2.43 (s, 3H), 3.98 (s, 3H), 4.03 (s, 3H), 6.79 (d, 1H, J=2.5), 7.08 (s, 1H), 7.47 (d, 1H, J=2.5), 7.57 (s, 1H), 13.08 (s, 1H);
^{13}C-NMR ($CDCl_3$): 22.3, 56.4, 56.9, 104.3, 105.0, 120.3, 123.0, 125.1, 132.7, 138.0, 147.2, 163.0, 163.3, 165.6, 183.3, 187.8. Anal. Calcd for $C_{17}H_{14}O_5$: C 68.45, H 4.73%. Found: C 68.75, H 4.58%.

Emodin anthrone (**3**) **from 5a and 5b**: A warm solution of tin (II) chloride hydrate (1.07 g, 4.78 mmol) in conc. hydrochloric acid (5.5 ml) was added to a suspension of **5a** (136 mg, 0.478 mmol) in acetic acid (10 ml) to give the red-colored solution. After the solution was heated at 120°C one day, it was poured into ice-water and the precipitate, **3**, was collected by filtration and dried (118 mg, 96%). The similar procedure using **5b** (231 mg, 0.774 mmol) in acetic acid (15 ml), tin (II) chloride hydrate (1.07 g, 4.78 mmol) in conc. hydrochloric acid (9.2 ml) gave **3** (189 mg, 95%).

MP: 254-258°C (Lit. 254-258 °C [5a]).
^{1}H-NMR (DMSO-d_6): δ_H: 2.33 (s, 3H, Me), 4.31 (s, 2H, CH2), 6.22 (d, 1H, *J* = 2.2 Hz, H-4), 6.43 (d, 1H, *J* = 2.2, H-2), 6.69 (s, 1H, H-5), 6.79 (s, 1H, H-7), 10.80 (s, 1H, OH), 12.21 (s, 1H, OH), 12.37 (s, 1H, OH);
^{13}C-NMR (acetone-d_6) 22.3 (CH3), 33.6(CH2), 102.4 (CH), 108.4 (CH), 110.1 (CH), 114.4 (CH), 116.6 (C), 121.0 (C),143.2 (C),146.3 (C), 148.5 (C), 163.9 (C),165.9 (C), 166.7 (C), 193.2 (CO).
Anal. Calcd for $C_{16}H_{12}O_5$: C 70.31, H 4.72%. Found: C 70.57, H 4.63%.

Emodin (2) from 5a and 5b: After a solution of **5a** (300 mg, 1.05 mmol) and hydrobromic acid (48%, 59 ml) in acetic acid (59 ml) was heated at 125°C for 12 h, ice-water was added and the precipitate was filtered. The dried product was purified with silica gel column chromatography (chloroform-ethyl acetate) to give **2** (258 mg, 91%). The similar procedure using **5b** (300 mg, 1.01 mmol) and hydrobromic acid (29 mL) in acetic acid (59 mL) afforded **2** (129 mg, 47%) along with **5a** (53 mg, 18%).

MP: 256-258°C (Lit. 257 °C [4a]).
^{1}H-NMR ($CDCl_3$) 2.45 (s, 3H, Me), 5.74(s, 1H, OH), 6.67 (d, 1H, *J* = 2.2 Hz, H-7), 7.09 (d, 1H, *J* = 1.0 Hz, H-5), 7.30 (d, 1H, *J* = 2.5 Hz, H-4), 7.63 (d, 1H, *J* = 1.7 Hz), 12.09 (s, 1H, OH), 12.28 (s, 1H, OH).
Anal. Calcd for $C_{16}H_{12}O_5$: C 66.67, H 3.73%. Found: C 66.42, H 3.82%.

Emodin bianthrone (6) [10] A solution of iron (III) chloride hydrate (127 mg, 0.468 mmol) in ethanol (12 ml) was added drop wise to a solution of emodin anthrone (100 mg, 0.390 mmol) in ethanol (25 mL) within 45 min. After heating at 4 h under reflux, the solution was poured into an aqueous hydrochloric acid (5%, 50 ml) and the product was extracted with ether.

After being dried over sodium sulfate and removal of the solvent, the residue was purified with column chromatography (chloroform:methanol = 20:1) to give **6** (78 mg, 77%) as a diastereomeric mixture.

^{1}H-NMR ($CDCl_3$):2.27, 2.31 (s, 6H, Me), 4.35 (s, 2H, H-10, 10'), 5. 92, 6.02, 6.04, 6.13, 6.29, 6.33 (s, 4H, H-2,2', 5,5', 7,7'), 6.71, 6.68 (s, 2H, H-4,4'), 11.78, 11.83, 12.12, 12.16 (s, 4H, OH).

Protohypericin (7) [5d] A solution of **6** (94 mg, 0.184 mmol) and *N*-ethyldiisopropylamine (0.12 ml, 0.703 mmol) in acetonitrile (50 ml) was bubbled with oxygen gas for 15 min and heated under reflux and an oxygen atmosphere for 2 h. After removal of the solvent, the solution was poured into aqueous hydrochloric acid (10%, 65 ml) and the precipitate was washed with water. The collected precipitate was treated with acetone and the extract was concentrated *in vacuo*. The product was purified with silica gel column chromatography (chloroform and acetone) to give **7** (146 mg, 98%).

^{1}H-NMR (acetone-d_6): 2.11(s, 6H), 6.36 (s, 2H), 6.70 (s, 2H), 7.29 (s, 2H).

Hypericin (1) [2,5e] A solution of **7** (146 mg, 0.288 mmol) in acetone (20 ml) was irradiated under a high-pressure mercury lamp at room temperature for 1 h. After removal of the solvent, the residue was purified with silica gel column chromatography (chloroform : acetone =1:1) to give **1** (45 mg, 31%).

^{1}H-NMR (acetone-d_6) δ: 2.79 (s, 6H, Me), 6.62, 7.36 (s, 4H, ArH); UV (EtOH, c 1.0 x 10^{-5}): l 591 (30940), 547 (15250), 475 (9370), 381 (9290), 329 (20540) nm (ε).

Acknowledgements - This work was partially supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (14B1-30: 21st Century COE Research and 16550122).

References

[1] Brockmann H, Haschad MN, Maier K, Pohl F. (**1939**) Hypericin, the photodynamically active pigment from hypericum perforatum. *Naturwissenshaften*, ***27***, 550.

[2] Duran N, Song PS. (**1986**) Hypericin and its photodynamic action. *Photochemistry and Photobiology*, ***43***, 677-680.

[3] Falk H. (**1999**) From the Photoseisitizer hypericin to photoreceptor stentorin-The chemistry of phenanthroperylene quinones. *Angewandte Chemie International Edition*, ***38***, 3116-3136.

[4] (a) Khan AT, Blessing B, Schmidt RR. (**1994**) An Expedient and efficient synthesis of naturally occurring hydroxy substituted anthraquinones. *Synthesis*, 255-257. (b) Thomson RH. (**1987**) *Naturally Occurring Quinones*, Vol. ***3***, Chapman and Hall: New York, 418-421. (c) *ibid*., 423.

[5] (a) Falk H, Schoppel G. (**1991**) A synthesis of emodin anthrone, *Monatshefte fur Chemie*, ***122***, 739-744. (b) Steglich W, Arnold R. (**1973**) Alkaline condensation of emodin to isohypericin: a simple synthesis of the meso-naphthodianthrone (phenanthro[1,10,9,8-opqra]perylene) system. *Angewandte Chemie International Edition*,***12***, 79. (c) Spizner D. (**1977**) Synthesis of protohypericin from emodin, *ibid.*, ***16***, 46. (d) Rodewald G, Arnold R, Griesler J, Steglich W. (**1977**) Synthesis of hypericin and related meso-naphthodianthrones by alkaline dimerization of hydroxyanthraquinones, *ibid.*, ***16***, 46-47. (e) Gruszecka-Kowalik E, Zalkow LH. (**2000**) An improved synthesis of hypericin and related compounds. *Organic Preparation and Procedures International*, ***32***, 57-61.

[6] (a) Savard J, Brassard P. (**1984**) Reaction of ketene acetals-14, the use of simple mixed vinylketene acetals in the annulation of quinones, *Tetrahedron*, ***40***, 3455-3464. (b) Motoyoshiya J, Kameda T, Asari M, Miyamoto M, Narita S, Aoyama H, Hayashi S. (**1997**) Importance of the role of secondary orbital interaction in the Diels-Alder reaction. Regioselectivity in the catalyzed and uncatalyzed reactions of juglone and aliphatic dienes, *Journal of Chemical Society, Perkin Transaction 2*, 1845-1850. (c) Motoyoshiya J, Masue Y, Iwayama G, Yoshioka S, Nishii Y, Aoyama H. (**2004**) A facile synthesis of the angucyclinone antibiotic (+)-rubiginone B_2 involving the BF_3-mediated Diels-Alder reaction of juglone, *Synthesis*, 2099-2102. (d) Singh SB, Graham PL, Reamer RA, Cordingley MG. (**2001**) Discovery, total synthesis, HRV 3C-protease inhibitory activity, and structure-activity relationships of 2-methoxystypandrone and its analogues. *Bioorganic & Medicinal Chemistry Letters*, ***11***, 3143-3146.

[7] Donner CD, Gill M. (**2002**) Pigments of fungi. Part 68. Synthesis and absolute configuration of thysanone, *Journal of Chemical Society, Perkin Transaction 1*, 938-948.

[8] Ahmed SA, Bardshiri E, Simpson TJ. (**1987**) A convenient synthesis of isotopically labeled anthraquinones, chrysophanol, islandicin, and emodin. Incorporation of [methyl-$^2H_3$]chrysophanol into tajixanthone in aspergillus variecolor, *Journal of Chemical Society, Chemical Communications*, 883-884.

[9] Falk H, Schoppel G. (**1992**) On the synthesis of hypericin by oxidative trimethylemodin anthrone dimerization: isohypericin, *Monatshefte fur Chemie*, ***123***, 931-938.

[10] Cameron DW, Edmonds JS, Raverty WD. (**1976**) Oxidation of emodin anthrone and stereochemistry of emodin bianthrone, *Australian Journal of Chemistry*, ***29***, 1535-48.

[11] Mazur Y, Bock H, Lavie D. (1992) *Can. Pat. Appl.* CA 2.029.993. CAN **116:**6343.