DOI: 10.1002/ejoc.200600702

Synthesis and Absolute Configuration of (+)-Pseudodeflectusin: Structural Revision of Aspergione B

Fumiyo Saito,[a] Kouji Kuramochi,[b] Atsuo Nakazaki,[a] Yoshiyuki Mizushina,[c] Fumio Sugawara,[b,d] and Susumu Kobayashi*[a,d]

Keywords: Total Synthesis / Natural products / Aromatic compounds / Carbonyl compounds / Stereochemistry

We report herein the total synthesis and determination of the absolute configuration of (+)-pseudodeflectusin. The total synthesis of (+)-pseudodeflectusin starting from *o*-anisic aid was achieved in 11 total steps with an overall yield of 2.0 %. The ^{1}H- and ^{13}C NMR spectroscopic data of our synthetic pseudodeflectusin was identical to that of the natural compound. The absolute configuration of (+)-pseudodeflectusin was determined by chiral HPLC and X-ray crystallographic analyses. We also synthesized the proposed structure of aspergione B, whose ^{1}H- and ^{13}C NMR spectroscopic data is identical to that of pseudodeflectusin. The ^{1}H- and ^{13}C NMR spectra of our synthetic aspergione B were different from those of the natural compound reported by Proksch et al. Our results confirm that aspergione B and pseudodeflectusin are, in fact, the same compound.

Pseudodeflectusin (**1**), isolated from the culture broth of *Aspergillus pseudodeflectus*, exhibits cytotoxicity against several human cancer cell lines derived from the stomach (NUGC-3), cervix (HeLa-S3), and peripheral blood (HL-60). Pseudodeflectusin is an isochroman derivative, which consists of a cyclic hemiacetal and a methylethylidene group at a furanone ring.[1] Although **1** contains a cyclic hemiacetal, which might exist in a reversible mixture of two isomers, the compound was isolated as a single isomer. However, the absolute configurations of the stereogenic centers at C-7 and C-9 were not determined.

Proksch et al. isolated aspergione B (**2**) from the fungus *Aspergillus versicolor*, as a tricyclic chromone derivative with a dihydropyran ring.[2] Both **1** and **2** (Figure 1) are tricyclic hemiacetals. Compound **1** has a 2-(1-methylethylidene)dihydrofuranone moiety, whereas compound **2** has a 2,3-dimethyl-4-pyrone moiety. The reported NMR spectroscopic data (^{1}H- and ^{13}C NMR spectra) of **2** is in good accordance with that of **1**.

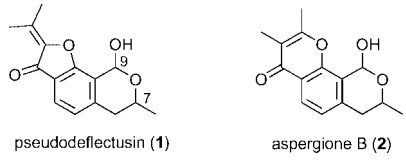


Figure 1. Structure of pseudodeflectusin (**1**) and aspergione B (**2**).

To clarify the structure of **1** and to assign the absolute configurations at C-7 and C-9, we started the synthetic studies of pseudodeflectusin.

Ochratoxin A, which has a similar benzoisochroman skeleton to pseudodeflectusin, has been isolated from some strains of *Aspergillus ochraceus* (Figure 2).[3–5] The absolute configuration at C-3 of ochratoxin A has been determined to be (*R*) by comparison of the optical rotation of the degraded product with (*R*)-(–)-mellein.[4,6] Therefore, we reasoned that the absolute configuration at C-7 of pseudodeflectusin was also (*R*).

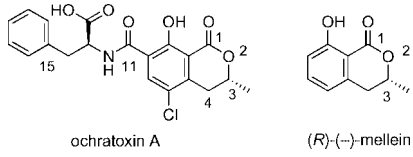


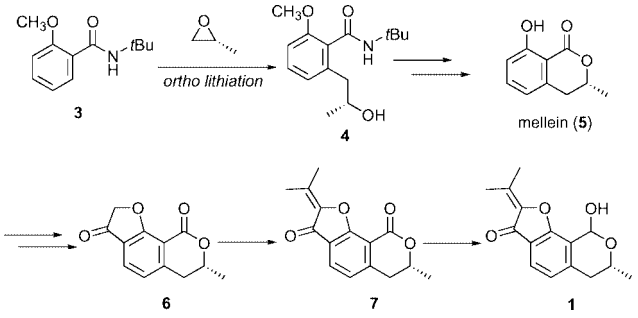
Figure 2. Mellein and ochratoxin A.

Our synthetic approach for the preparation of (+)-pseudodeflectusin is outlined in Scheme 1;[7] mellein (**5**) was the key intermediate for its preparation. *ortho* Lithiation of amide **3** and alkylation of (*R*)-(+)-propylene oxide led to alcohol **4**. Cyclization of **4** and demethylation led to **5**.

[a] Faculty of Pharmaceutical Sciences, Tokyo University of Science,
2641 Yamazaki, Noda, Chiba, 278-8510, Japan
Fax: +81-4-7121-3671
E-mail: kobayash@rs.noda.tus.ac.jp
[b] Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science,
2641 Yamazaki, Noda, Chiba, 278-8510, Japan
[c] Department of Nutritional Science, Kobe-Gakuin University, Nishi-Ku, Kobe, Hyogo 651-2180, Japan
[d] Frontier Research Center for Genome and Drug Discovery, Tokyo University of Science,
2641Yamazaki, Noda, Chiba, 278-8510, Japan

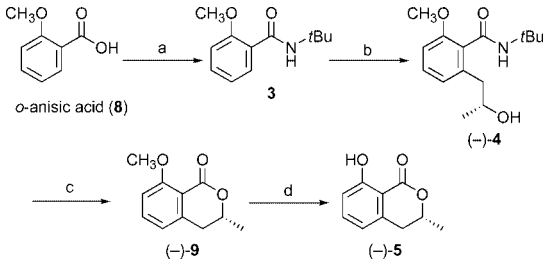
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

Mellein (**5**) was converted into furanone **6**, followed by condensation with acetone to afford **7**. Finally, reduction of **7** generated **1**.



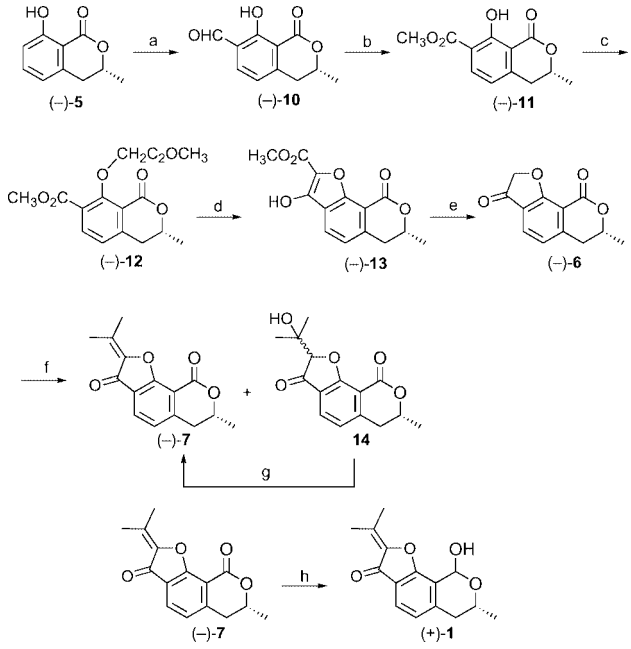
Scheme 1. Synthetic approach for the preparation of pseudodeflectusin.

The synthesis of (–)-mellein (**5**) is shown in Scheme 2. The condensation of *o*-anisic acid (**8**) with *tert*-butylamine and DCC in the presence of DMAP afforded benzamide **3** in 75% yield. Treatment of **3** with two equiv. of *n*BuLi in the presence of TMEDA led to the formation of the orange-colored dianion, which opened (*R*)-(+)-propylene oxide to afford secondary alcohol (–)-**4** in 61% yield.[8,9] Acid-induced ($pTsOH·H_2O$) intramolecular attack of the newly generated alcohol at the amide group of (–)-**4** led to δ-lactone (–)-**9** in 72% yield.[9] The methyl group in (–)-**9** was cleanly removed by the exposure of (–)-**9** to BCl_3 in CH_2Cl_2 to obtain (–)-mellein [(–)-**5**] in 95% yield.



Scheme 2. *Reagents and conditions*: a) $tBuNH_2$, DCC, DMAP/CH_2Cl_2, 75%; b) *n*BuLi, TMEDA, (*R*)-(+)-propylene oxide/THF, –78 °C, 61% (32% recovery of **3**); c) $pTsOH·H_2O$/toluene, reflux, 72%; d) BCl_3/CH_2Cl_2, –78 °C to 0 °C, 95%.

Treatment of (–)-**5** with titanium(IV) chloride and Cl_2CHOCH_3 afforded aldehyde (–)-**10** in 88% yield accompanied by the *p*-formyl isomer in 8% yield (Scheme 3).[10] Aldehyde (–)-**10** was converted in 91% yield into proposed methyl ester (–)-**11** with the use of sodium cyanide and MnO_2 in MeOH according to Corey's procedure.[11] Resulting phenol (–)-**11** was then converted into ether (–)-**12** by means of methyl bromoacetate and potassium carbonate in 98% yield. The cyclization of (–)-**12** with sodium methoxide in MeOH gave β-keto ester (–)-**13**, which was treated with lithium hydroxide in aqueous DMSO at 75 °C to produce furanone (–)-**6** in 80% yield.[12]



Scheme 3. *Reagents and conditions*: a) Cl_2CHOCH_3, $TiCl_4$/CH_2Cl_2, –10 °C, 88%; b) NaCN, MnO_2/CH_3OH, 91%; c) K_2CO_3, $BrCH_2CO_2CH_3$/DMF, 50 °C, 98%; d) $NaOCH_3$/CH_3OH, reflux, 93%; e) $LiOHH_2O$/DMSO, H_2O, 75 °C, 80%; f) $pTsOHH_2O$/acetone, reflux, **7**; 49%, **14**; 24% (9% recovery of **6**); g) MsCl, DMAP/pyridine, 72%; h) DIBAL (2.5 equiv.)/THF, –78 °C, 28% (49% recovery of **7**).

For the preparation of the methylethylidene moiety, the reaction of (–)-**6** with $pTsOH·H_2O$ in acetone afforded lactone (–)-**7** and alcohol **14** in 49% and 24% yield, respectively. Alcohol **14** was converted into (–)-**7** by its reaction with MsCl in pyridine in 72% yield.

Finally, the reduction of lactone (–)-**7** with DIBAL in THF at –78 °C afforded (+)-pseudodeflectusin (**1**) in 28% yield, accompanied by recovered (–)-**7** in 49% yield.[13]

The ^1H- and ^{13}C NMR spectra of synthetic pseudodeflectusin (**1**) agreed with those of natural pseudodeflectusin (**1**) in all respects.[1] However, the optical rotation of synthetic **1** {$[a]_D^{23}$ = +63.7 (*c* = 0.08, MeOH)} was higher than that of natural **1** {$[a]_D^{23}$ = +11 (*c* = 0.18, MeOH)}.[1] Thus, we prepared (±)-pseudofectusin by using (±) propylene oxide according to the same procedure, and used synthetic (±)-**1** as an authentic sample for HPLC analysis. Synthetic (+)-**1**, (±)-**1**, and natural **1** were subjected to chiral HPLC analysis [Daicel Chiralpak OD, 30 °C, 254 nm, hexane/*i*PrOH = 15:1, 0.5 mL/min]. The HPLC analysis revealed that the (7*R*)-isomer [(+)-**1**] (t_R = 55.8 min) eluted after the (7*S*)-isomer [(–)-**1**] (t_R = 36.8 min), which confirmed that natural **1** has the (7*R*)-configuration. The analysis also indicated that the optical purity of our synthetic (+)-**1** was 99% *ee*. Because the (7*S*)-enantiomer was not detected in the sample of natural **1**, the low value for the optical rotation for natural **1** must be due to the presence of impurities in the sample.

The configuration of the C-9 stereocenter was unambiguously determined by X-ray crystallography.[14] Figure 3 shows an ORTEP drawing of (+)-**1**. The *anti* relationship

between the methyl group at C-7 and the hydroxy group at C-9 was established, which indicates that (+)-**1** has the (9*S*)-configuration. As a result, we confirmed that the absolute configuration of natural pseudodeflectusin (**1**) is (7*R*,9*S*).

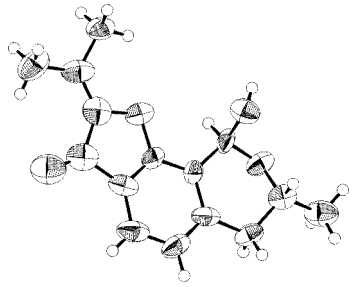
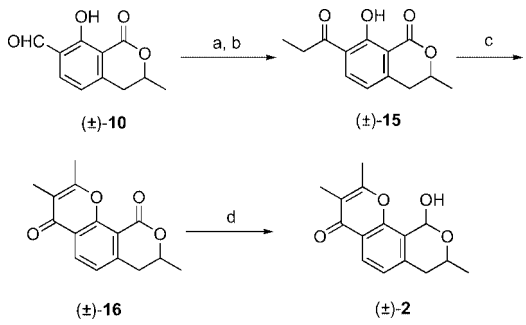


Figure 3. ORTEP drawing of (+)-**1**.

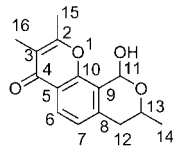
In order to ascertain the structure of natural aspergione B, we prepared (±)-**2** from (±)-**10** (Scheme 4). The addition of ethylmagnesium bromide to aldehyde (±)-**10** gave a 1:1 diastereomeric mixture of the alcohol, which was oxidized by MnO_2 to afford ketone (±)-**15** in 2 steps. Treatment of (±)-**15** with Ac_2O and DBU in pyridine heated at 60 °C produced (±)-**16** in 53% yield.[15] The reduction of the lactone moiety in (±)-**16** with DIBAL in CH_2Cl_2 afforded (±)-**2**, which is the proposed structure of aspergione B.



Scheme 4. *Reagents and conditions*: a) EtMgBr/THF, –5 °C; b) MnO_2/CH_2Cl_2, 52% in 2 steps; c) Ac_2O, DBU, pyridine, 60 °C, 53%; d) DIBAL/CH_2Cl_2, –78 °C, 92%.

The ^{1}H- and ^{13}C NMR spectroscopic data of our synthetic (±)-**2** were different from those of natural **2** reported by Proksch et al. (Table 1).[2] In particular, the chemical shifts of 6-H, 7-H, 15-H, and 16-H were different between the two samples (Table 1).[1,2] In the ^{13}C NMR spectroscopic data, chemical shifts of C-2, C-3, C-10, and C-16 for our synthetic (±)-**2** were quite different from those of natural **2**. In the HMBC spectrum of our synthetic (±)-**2**, HMBC correlations from 15-H to C-2 and C-3, as well as from 16-H to C-2, C-3, and C-4 were observed. Although Proksch et al. reported that the HMBC correlations from 15-H to C-16, and from 16-H to C-15 were observed for natural aspergione B, these correlations were not observed in synthetic (±)-**2**. Taken together, our observations confirmed that the structure of aspergione B is identical to that of pseudodeflectusin.

Table 1. ^{1}H- and ^{13}C NMR spectra of natural aspergione B (**2**) and synthetic aspergione B [(±)-**2**].



Natural aspergione B (**2**)			Synthetic aspergione B ((±)-**2**)		
Assignment	δ_H (*J* [Hz])	δ_C	Assignment	δ_H (*J* [Hz])	δ_C
2		144.5	2		161.3
4		182.5	4		177.7
5		122.7	5		121.0
6	7.61 d (7.8)	122.5	6	8.09 d (8.2)	125.6
7	6.87 d (7.8)	122.0	7	7.10 d (8.2)	124.8
8		142.0	8		140.3
12	2.76 dd (17.4, 3.8) 2.70 dd (17.4, 10.4)	36.5	12	2.82 dd (17.0, 3.4) 2.74 dd (17.0, 11.2)	35.5
13	4.45 ddq (10.4, 3.8, 6.2)	62.2	13	4.51 ddq (11.2, 3.4, 6.2)	62.6
11	6.27 d (4.0)	87.5	11	6.37 d (4.0)	88.1
9		119.1	9		123.1
10		162.5	10		150.3
14	1.39 d (6.2)	21.0	14	1.41 d (6.2)	21.2
3		131.1	3		117.1
15	2.13 s	20.1	15	2.45 s	18.6
16	2.34 s	17.0	16	2.06 s	10.0
OH	2.94 d (4.0)		OH	3.04 br. s	

Conclusion

In conclusion, we have achieved the total synthesis of (+)-pseudodeflectusin (**1**) from *o*-anisic acid in 11 steps with an overall yield of 2.0%. The ^{1}H- and ^{13}C NMR spectroscopic data of our synthetic pseudodeflectusin is identical to that of natural pseudodeflectusin. Chiral HPLC analysis of our synthetic (+)-**1**, (±)-**1**, and natural **1**, together with X-ray crystallographic analysis of synthetic (+)-**1**, confirmed the absolute configuration of natural **1** to be (*7R*,*9S*). Finally, the proposed structure of aspergione B (**2**), whose NMR spectroscopic data were reported to be quite similar to those of pseudodeflectusin, was also synthesized. The NMR spectroscopic data of synthetic (±)-**2** is different from that reported by Proksch et al. for natural **2**. Our results confirm that the structure of aspergione B is identical to that of pseudodeflectusin.

Experimental Section

For details see Supporting Information.

Supporting Information (see footnote on the first page of this article): ^{1}H NMR spectra of natural pseudodeflectusin (**1**) and synthetic (–)-**1**, chiral HPLC analysis of synthetic (+)-**1**, (±)-**1**, and natural **1**, and crystal data and measurement conditions of (+)-**1**. Synthesis of compounds: (–)-**4**, (–)-**5**, (–)-**6**, (–)-**7**, (–)-**9**, (–)-**10**, (–)-**11**, (–)-**12**, (–)-**13**, (+)-**1**, (±)-**15**, (±)-**16**, and (±)-**2**.

[1] A. Ogawa, C. Murakami, S. Kamisuki, I. Kuriyama, H. Yoshida, F. Sugawara, Y. Mizushina, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3539–3543.
[2] W. Lin, G. Brauers, R. Ebel, V. Wray, A. Berg, Sudarsono, P. Proksch, *J. Nat. Prod.* **2003**, *66*, 57–61.
[3] K. J. van der Merwe, P. S. Steyn, L. Fourie, D. B. Scott, J. J. Theron, *Nature* **1965**, *205*, 1112–1113.

[4] K. J. van der Merwe, P. S. Steyn, L. Fourie, *J. Chem. Soc.* **1965**, 7083–7088.
[5] P. S. Steyn, C. W. Holzapfel, *Tetrahedron* **1967**, *23*, 4449–4461.
[6] H. Nishikawa, *J. Agric. Chem. Soc. Jpn.* **1933**, *9*, 772–774.
[7] T. Kumemura, T. Choshi, C. Hirata, M. Sera, Y. Takahashi, J. Nobuhiro, S. Hibino, *Heterocycles* **2003**, *61*, 13–17.
[8] V. Snieckus, *Chem. Rev.* **1990**, *90*, 879–933.
[9] B.-S. Kwon, J. Tae, *Heterocycles* **2004**, *62*, 137–141.
[10] K. van Laak, H.-D. Scharf, *Tetrahedron* **1989**, *45*, 5511–5516.
[11] a) E. J. Corey, N. W. Gilman, B. E. Ganen, *J. Am. Chem. Soc.* **1968**, *90*, 5616–5617; b) E. J. Corey, J. A. Katzenellenbogen, N. W. Gilman, S. A. Roman, B. W. Erickson, *J. Am. Chem. Soc.* **1968**, *90*, 5618–5620; c) G. Lai, W. K. Anderson, *Synth. Commun.* **1997**, *27*, 1281–1287.
[12] Y. Takeuchi, I. Watanabe, K. Misumi, M. Irea, Y. Hirose, K. Hirata, M. Yamato, T. Harayama, *Chem. Pharm. Bull.* **1997**, *45*, 2011–2015.
[13] T. A. Munro, M. A. Rizzacasa, B. L. Roth, B. A. Toth, F. Yan, *J. Med. Chem.* **2005**, *48*, 345–348.
[14] CCDC-614265 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[15] C. Riva, C. De Toma, L. Donadel, C. Boi, R. Pennini, G. Motta, A. Leonardi, *Synthesis* **1997**, 195–201.

Received: August 11, 2006
Published Online: September 11, 2006

