

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

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We report herein the total synthesis and determination of the absolute configuration of (+)-pseudodefectusin. The total synthesis of (+)-pseudodefectusin starting from *o*-anisic acid was achieved in 11 total steps with an overall yield of 2.0 %. The ¹H- and ¹³C NMR spectroscopic data of our synthetic pseudodefectusin was identical to that of the natural compound. The absolute configuration of (+)-pseudodefectusin was determined by chiral HPLC and X-ray crystallographic analyses. We also synthesized the proposed structure of as-

pergione B, whose ¹H- and ¹³C NMR spectroscopic data is identical to that of pseudodefectusin. The ¹H- and ¹³C 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 pseudodefectusin are, in fact, the same compound.

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Pseudodefectusin (**1**), isolated from the culture broth of *Aspergillus pseudodefectus*, exhibits cytotoxicity against several human cancer cell lines derived from the stomach (NUGC-3), cervix (HeLa-S3), and peripheral blood (HL-60). Pseudodefectusin 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 (¹H- and ¹³C NMR spectra) of **2** is in good accordance with that of **1**.

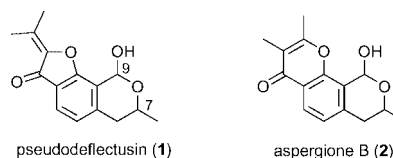


Figure 1. Structure of pseudodefectusin (**1**) and aspergione B (**2**).

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

Ochratoxin A, which has a similar benzoisochroman skeleton to pseudodefectusin, 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 pseudodefectusin was also (*R*).

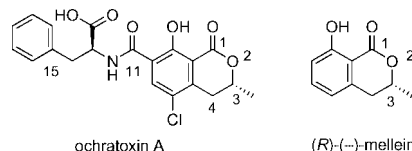


Figure 2. Mellein and ochratoxin A.

Our synthetic approach for the preparation of (+)-pseudodefectusin 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**.

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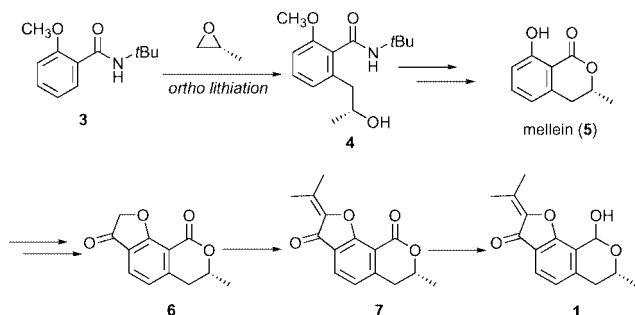
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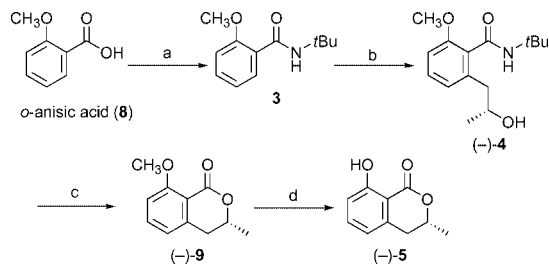
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Mellein (**5**) was converted into furanone **6**, followed by condensation with acetone to afford **7**. Finally, reduction of **7** generated **1**.



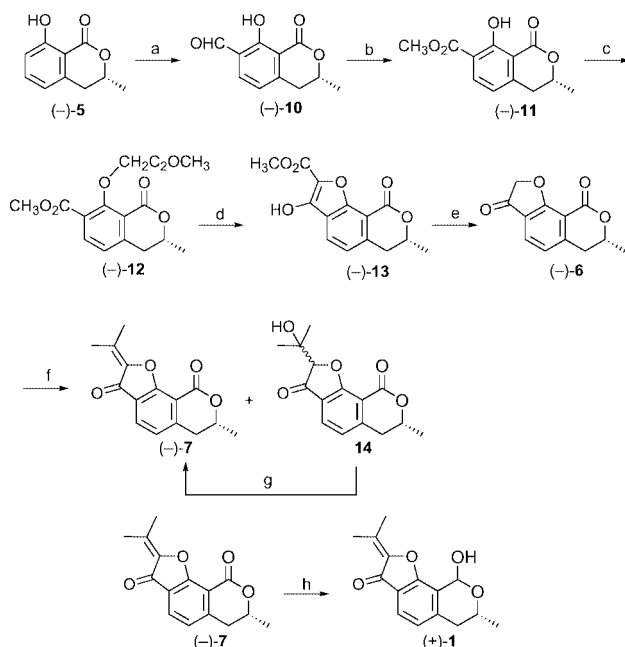
Scheme 1. Synthetic approach for the preparation of pseudodeflectusin.

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 (*p*TsOH·H₂O) 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 BCl₃ in CH₂Cl₂ to obtain (–)-mellein [(–)-**5**] in 95% yield.



Scheme 2. Reagents and conditions: a) *t*BuNH₂, DCC, DMAP/CH₂Cl₂, 75%; b) *n*BuLi, TMEDA, (*R*)-(+)-propylene oxide/THF, –78 °C, 61% (32% recovery of **3**); c) *p*TsOH·H₂O/toluene, reflux, 72%; d) BCl₃/CH₂Cl₂, –78 °C to 0 °C, 95%.

Treatment of (–)-**5** with titanium(IV) chloride and Cl₂CHOCH₃ 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 MnO₂ 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) Cl₂CHOCH₃, TiCl₄/CH₂Cl₂, –10 °C, 88%; b) NaCN, MnO₂/CH₃OH, 91%; c) K₂CO₃, BrCH₂CO₂CH₃/DMF, 50 °C, 98%; d) NaOCH₃/CH₃OH, reflux, 93%; e) LiOH·H₂O/DMSO, H₂O, 75 °C, 80%; f) *p*TsOH·H₂O/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 *p*TsOH·H₂O 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 ¹³C NMR spectra of synthetic pseudodeflectusin (**1**) agreed with those of natural pseudodeflectusin (**1**) in all respects.^[1] However, the optical rotation of synthetic **1** {[α]_D²³ = +63.7 (*c* = 0.08, MeOH)} was higher than that of natural **1** {[α]_D²³ = +11 (*c* = 0.18, MeOH)}.^[1] Thus, we prepared (±)-pseudodeflectusin 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*P-rOH = 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 pseudodefectusin (**1**) is (7*R*,9*S*).

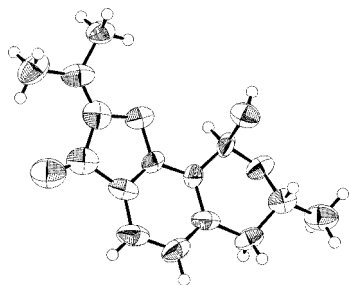
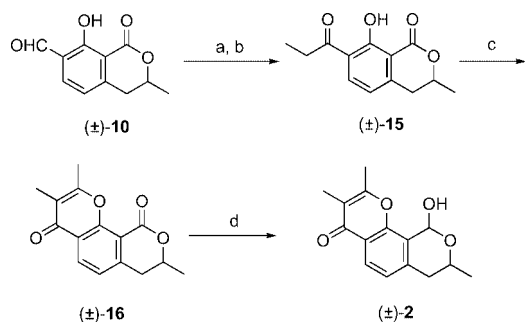


Figure 3. ORTEP drawing of (+)-**1**.

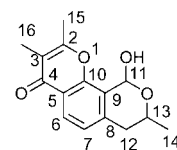
In order to ascertain the structure of natural aspergione B, we prepared (±)-**2** from (±)-**10** (Scheme 4). The addition of ethylmagnesium bromide to aldehyde (±)-**10** gave a 1:1 diastereomeric mixture of the alcohol, which was oxidized by MnO₂ to afford ketone (±)-**15** in 2 steps. Treatment of (±)-**15** with Ac₂O 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 CH₂Cl₂ afforded (±)-**2**, which is the proposed structure of aspergione B.



Scheme 4. Reagents and conditions: a) EtMgBr/THF, −5 °C; b) MnO₂/CH₂Cl₂, 52% in 2 steps; c) Ac₂O, DBU, pyridine, 60 °C, 53%; d) DIBAL/CH₂Cl₂, −78 °C, 92%.

The ¹H- and ¹³C 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 ¹³C 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 pseudodefectusin.

Table 1. ¹H- and ¹³C 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)	36.5	12	2.82 dd (17.0, 3.4)	35.5
	2.70 dd (17.4, 10.4)			2.74 dd (17.0, 11.2)	
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 (+)-pseudodefectusin (**1**) from *o*-anisic acid in 11 steps with an overall yield of 2.0%. The ¹H- and ¹³C NMR spectroscopic data of our synthetic pseudodefectusin is identical to that of natural pseudodefectusin. 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 (7*R*,9*S*). Finally, the proposed structure of aspergione B (**2**), whose NMR spectroscopic data were reported to be quite similar to those of pseudodefectusin, 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 pseudodefectusin.

Experimental Section

For details see Supporting Information.

Supporting Information (see footnote on the first page of this article): ¹H NMR spectra of natural pseudodefectusin (**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**.

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