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Reaction mechanism of the macrophomate synthase: experimental evidence on intermediacy of a bicyclic compound

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Abstract

In the reaction of methyl coumalate **4** and oxalacetate with the macrophomate synthase, an aberrant product **5** was formed instead of the expected benzoate **6**. The structure of **5** strongly indicates intermediacy of a bicyclic compound **10** in the extraordinary five-step transformation catalyzed by the macrophomate synthase. © 2000 Elsevier Science Ltd. All rights reserved.

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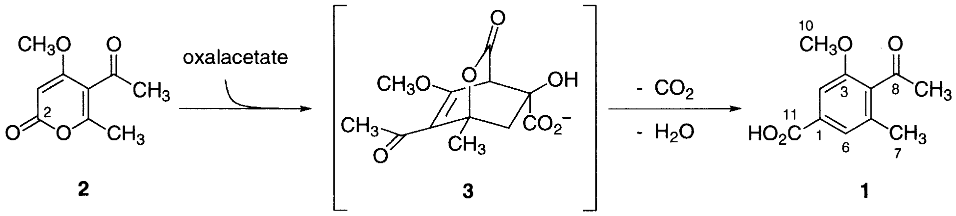
Recently, we have purified the macrophomate synthase[1] which catalyzes extraordinary multistep transformation from oxalacetate and 2-pyrone **2** to macrophomic acid **1** as shown in Scheme 1. This enzyme accepted various 2-pyrones which were converted to the corresponding benzoates.[2] To our knowledge, dehydroquinate synthase[3] and mechanistically related 2-deoxy-*scyllo*-inosose synthase[4] are only examples on catalyzing different types of chemical reactions. This type of enzymatic reaction is of great interest to not only enzymology but also bio-organic chemistry. Previously, we proposed that the 2-pyrone **2** was first transformed into a bicyclic intermediate **3** on the basis of observation that bicyclic compounds mimicking the plausible intermediate **3** effectively inhibited the enzymatic reaction.[1] In this report, we describe experimental evidence that supports our proposed reaction mechanism shown in Scheme 1.



Scheme 1. Transformation catalyzed by the macrophomate synthase

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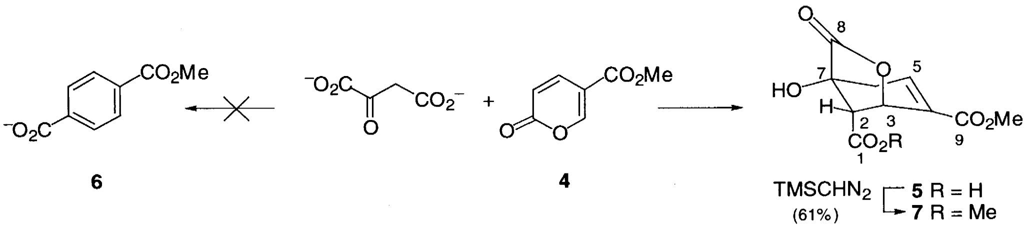
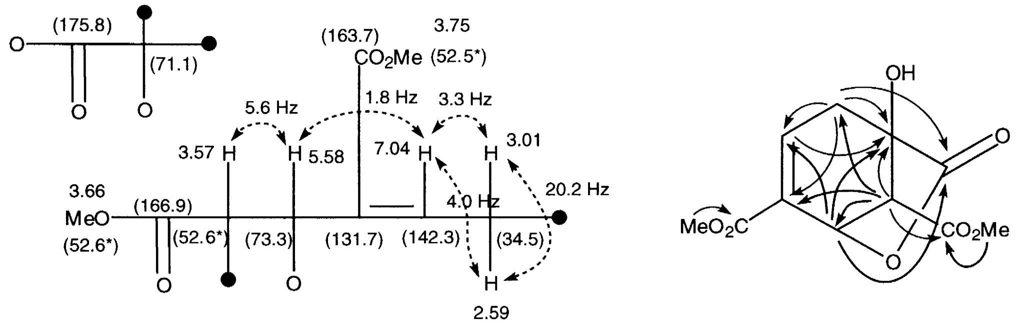
During the study on the substrate selectivity of the macrophomate synthase,[2] we found that a simple 2-pyrone, methyl coumalate **4**, was consumed very rapidly to afford an aberrant product **5**[5] which is totally different from the expected benzoate **6** (Scheme 2). Under standard incubation conditions (10 min, 30°C), 10% of **2** was converted to the corresponding **1** while **4** was completely consumed within 10 min. Among 14 substrates tested,[2] **4** revealed the highest conversion rate.



Scheme 2. Enzymatic reaction with methyl coumalate **4** and oxalacetate

Based on the molecular formula $C_{10}H_{10}O_7$ obtained from HR-MS, it was suggested that **5** is an adduct of **4** and oxalacetate with concomitant loss of carbon dioxide. Since the aberrant product **5** was relatively unstable, its structure was elucidated after conversion to the corresponding dimethyl ester **7**[6] (Scheme 2). The NMR data including ^{1}H, ^{13}C NMR, COSY and HSQC spectra allowed us to deduce the partial structure of **7** and then the gross structure was determined by HMBC analysis as shown in Fig. 1.



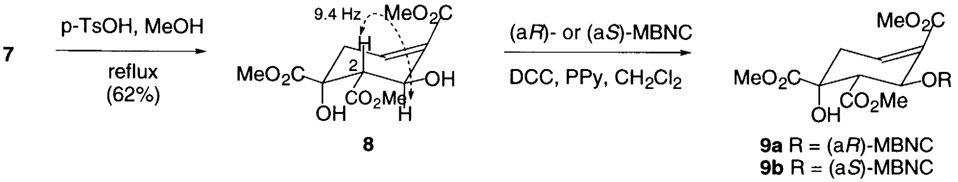
The values δ_H (δ_C) are chemical shifts in the NMR spectra of **7**.
* The values are interconvertible.

C-C conectivities obtained from the HMBC spectra of **7**.

Fig. 1.

In the NOED experiment with **7**, no NOE was detected between C_2-H and either of unequivalent methylene protons C_6-H[7] while the large coupling constant (9.4 Hz) between C_2-H and C_3-H was observed in the ^{1}H NMR spectrum of the trimethyl ester **8** derived from **7** by methanolysis of the lactone moiety (Scheme 3). These data unambiguously confirmed the relative stereochemistry of **5**, **7** and **8**. In order to determine the absolute configuration of **8**, we chose the method[8] developed by Fukushi due to the steric hindrance of the secondary alcohol at C_3. Thus, trimethyl ester **8** was converted to the corresponding (a*R*)- and (a*S*)-2′-methoxy-1′,1′-binaphthyl-2-carboxylic acid (MBNC) esters **9a** and **9b**, respectively. The calculated $\Delta\delta$ values ($\Delta\delta=\delta(aS)-\delta(aR)$) in Fig. 2 established the absolute configuration of **8** as shown in Scheme 3.

Formation of the aberrant product **5** in the reaction of **4** would be reasonably explained by conversion from the plausible intermediate **10** via lactone ring opening with concomitant allylic rearrangement and the subsequent lactonization with C-8 carboxylate as shown in Scheme 4. These conversions might occur in the active site of the enzyme because no quenched product of the corresponding cation was detected. Relatively large differences (6.6 kcal/mol) on the steric energy between **5** and **10** supports



PPy = 4-pyrrolidinopyridine
MBNC = 2'-methoxy-1',1'-binaphthyl-2-carboxylic acid

Scheme 3.

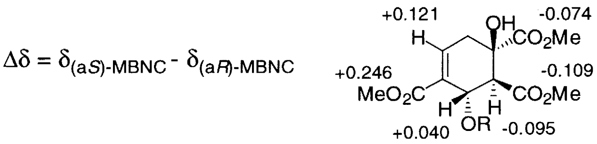
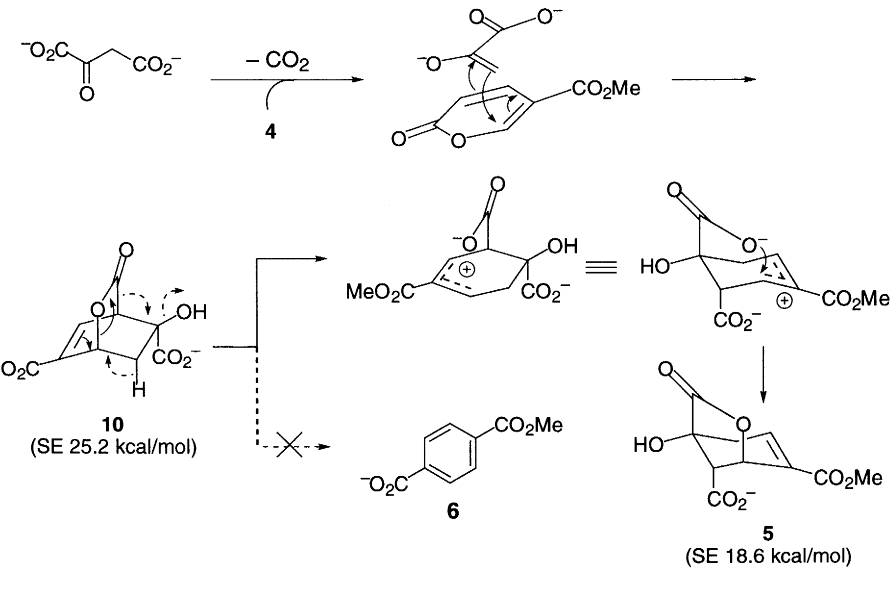


Fig. 2.

that these reactions are favorable. The reason why the reaction with **4** provided the lactone **5** instead of the formation of normal product **6** might be ascribed to flexibility of the plausible intermediate **10** in the active site of the enzyme. Since the bicyclic intermediate **10** has only one substituent compared with another intermediate **3** which possesses three substituents, this conformational freedom allowed **10** to escape the usual dehydrative decarboxylation process. The C–C bond formations described in Scheme 4 are possibly regarded as a Michael addition–aldol reaction although a possibility involving a Diels–Alder reaction cannot be rigorously ruled out. Currently, we are working on elucidating the full reaction sequence in this extraordinary enzymatic transformation.



Scheme 4. The formation of an aberrant product **5** in the enzymatic reaction with **4**. The steric energies (SE) were calculated by MM2

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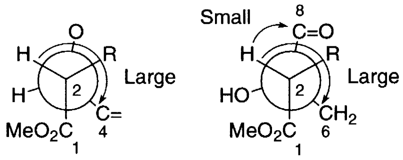
5. Compound **5**: $[\alpha]^{22}_{D}$=+25.5 (*c* 0.17, MeOH); IR ($CHCl_3$) ν_{max} 2924, 1790, 1716, 1645, 1441, 1261, 1047, 955, 801 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.14 (m, 1H, C_5-*H*), 5.65 (dd, *J*=5.9 and 1.5 Hz, 1H, C_3-*H*), 3.82 (s, 3H, OCH_3), 3.72 (d, *J*=5.9 Hz, 1H, C_2-*H*), 3.11 (dd, *J*=20.2 and 3.0 Hz, 1H, C_6-*H*), 2.69 (dd, *J*=20.2 and 2.7 Hz, 1H, C_6-*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.3 (C_8), 171.5 (C_1), 164.1 (C_9), 142.6 (C_5), 131.9 (C_4), 73.2 (C_7), 70.9 (C_3), 52.3 (C_2), 52.2 (C_{10}), 34.2 (C_6); MS (FD^+) *m/z* (relative intensity) 243 (M^++H, 100); FDHRMS for $C_{10}H_{11}O_7$ (M^++H) calcd: 243.0505; found: 243.0487.
6. Compound **7**: $[\alpha]^{22}_{D}$=+18.9 (*c* 0.80, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2956, 2332, 1733, 1645, 1436, 1258, 966, 916, 732 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.04 (m, 1H, C_5-*H*), 5.56 (dd, *J*=5.4 and 1.8 Hz, 1H, C_3-*H*), 3.75 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.57 (d, *J*=5.6 Hz, C_2-*H*), 3.01 (dd, *J*=20.2 and 3.3 Hz, 1H, C_6-*H*), 2.59 (dd, *J*=20.2 and 4.0 Hz, 1H, C_6-*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8 (C_8), 166.9 (C_1), 163.7 (C_{10}), 142.3 (C_5), 131.7 (C_4), 73.3 (C_3), 71.1 (C_7), 52.6 (C_2), 52.6 (OCH_3), 52.5 (OCH_3), 34.5 (C_6); MS (FD^+) *m/z* (relative intensity) 257 (M^++H, 100); FDHRMS for $C_{11}H_{13}O_7$ (M^++H) calcd: 257.0661; found: 257.0652.
7. The relative stereochemistry of **7** was also confirmed by relative intensities of the cross peaks originated from ^3J (C,H) correlation in the HMBC spectrum optimized for ^3J (C,H)=7 Hz. See: Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.

$^3J_{CH}$ obtained from HMBC spectrum of **7**



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