Communications to the Editor

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PARTIAL PURIFICATION AND SOME PROPERTIES OF EMODIN-O-METHYLTRANSFERASE FROM (+)-GEODIN PRODUCING STRAIN OF ASPERGILLUS TERREUS

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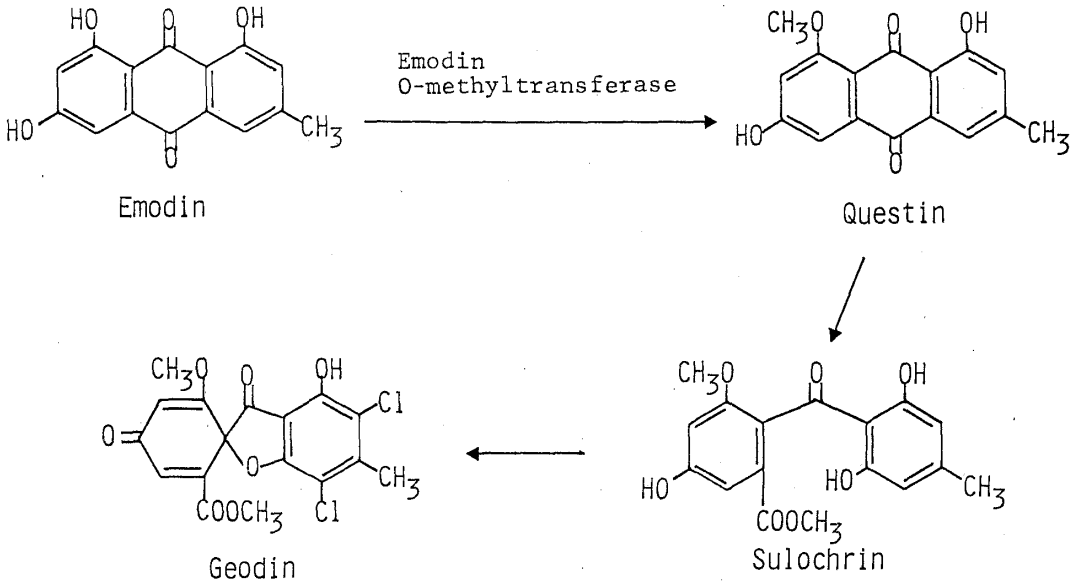
Emodin-1-O-methyltransferase (E-OMT), an enzyme catalysing methylation of emodin and involved in the biosynthesis of (+)-geodin, was purified upto 89 folds by two-step purification with Blue Sepharose and Sepharose 6B column chromatography. The molecular weight of E-OMT was estimated to be 3.4×10^5, and Km values for emodin and S-adenosylmethionine (SAM) were 3.3×10^{-7} M and 3.1×10^{-6} M, respectively. Substrate specificity of E-OMT was extremely strict, and the enzyme seems to recognize whole the molecule of substrates.

KEYWORDS —— O-methyltransferase; emodin; S-adenosylmethionine; questin; (+)-geodin; Aspergillus terreus; Blue Sepharose

Extensive studies on the biosynthesis of natural products in the past decade have clarified the detailed biosynthetic pathways of a great number of natural products.[1] Most of the results of the biosynthetic studies were obtained by incorporation experiments with appropriate labelled precursors in intact organisms. Enzymes involved in the biosynthesis of natural products are not well understood except for those relating to terpenoid and steroid biosynthesis.[2] Chalcone



Biosynthetic Scheme of (+)-Geodin

synthase, which has been purified and characterized, may be a rare example of an enzyme involved in the biosynthesis of phenolic poliketides.[3] (+)-Geodin (1) and related benzophenones such as sulochrin (2) are seco-anthraquinones[4] derived from an octaketide anthraquinone, emodin (3), which is distributed widely in fungi and higher plants.[5] A substrate for Baeyer-Villiger type ring cleavage has been proved to be questin (4), 1-O-methylemodin, by a feeding experiment with ^{14}C-labelled questin (4), which was prepared enzymatically from [CH_3-^{14}C]-S-adenosylmethionine (SAM) and emodin(3) with cell-free extract of Penicillium frequentans.[4b] A variety of reactions involved in this biosynthetic pathway drew our attention. Purification and characterization of enzymes responsible for the reactions were extensively studied, and some of the results have already been reported.[6] This communication deals with the purification and characterization of emodin-1-O-methyltransferase (E-OMT) from Aspergillus terreus IMI 16043, which produces (+)-geodin (4) as the main metabolite.

An assay mixture to determine E-OMT activity contained [CH_3-^{14}C]-SAM (6.0×10^4dpm; 25 n mol), emodin (5 n mol), enzyme solution, Tris-HCl (pH 8.0; 100 mol) in a total volume of 500 µl. The reaction was initiated by the addition of emodin (3) dissolved in ethyleneglycol monomethylether, performed at 30°C for 15 min and terminated by the addition of 5N HCl (200 µl). The reaction mixture was extracted with toluene (2 ml), and the radioactivity of the toluene layer that corresponded to questin (4) formed in the enzyme reaction was measured with a liquid scintillation counter. Crude extracts of mycellia of A. terreus grown in Czapek-Dox medium (shake culture) showed E-OMT activity. The enzyme was extremely unstable and lost more than 90% activity within 48 h when kept at 4°C in 0.05 M Tris-HCl buffer (pH 8.0). The enzyme was considerably stabilized by the addition of glycerol to the buffer solution (15% V/V); however, about 40% of activity was lost within 5 days at 4°C. The purification procedure for E-OMT was therefore designed to utilize rapid and effective chromatography. Affinity chromatography with Blue Sepharose column was a very efficient step. E-OMT was adsorbed on Blue Sepharose column equilibrated with 0.05 M Tris-HCl buffer solution (pH 8.0) containing glycerol (15%) and ammonium sulphate (30% saturation). After the column was washed with the same buffer solution, the enzyme was eluted with a continuous down gradient of ammonium sulphate concentration (30% to 0% saturation). E-OMT was purified 24-fold with 86% recovery by this step. This is because SAM, one of the substrates for E-OMT, contains adenosine moiety so that the enzyme has an affinity to nucleotide fold of Cibacron Blue F3G-A, a ligand of Blue Sepharose.[7] By further purification with Sepharose 6B column, E-OMT was finally purified up to 88.8-fold with 31.3% recovery by two-step purification as shown in Table I.

The molecular weight of E-OMT was estimated to be 3.4×10^5 by gel filtration with Sephadex

Table I. Summary of Purification Procedures for Emodin-O-methyltransferase

Step	Volume (ml)	Total Protein (mg)	Total Activity (unit)	Specific Activity (unit/mg)*	Recovery (%)
Crude Extract	160	333.6	7646	22.9	100
Blue Sepharose	54	12.2	6596	541	86.3
Sepharose 6B	14	1.18	2394	2029	31.3

*One unit of activity is defined as the amount of enzyme yielding 1 n mol of questin at 30°C.

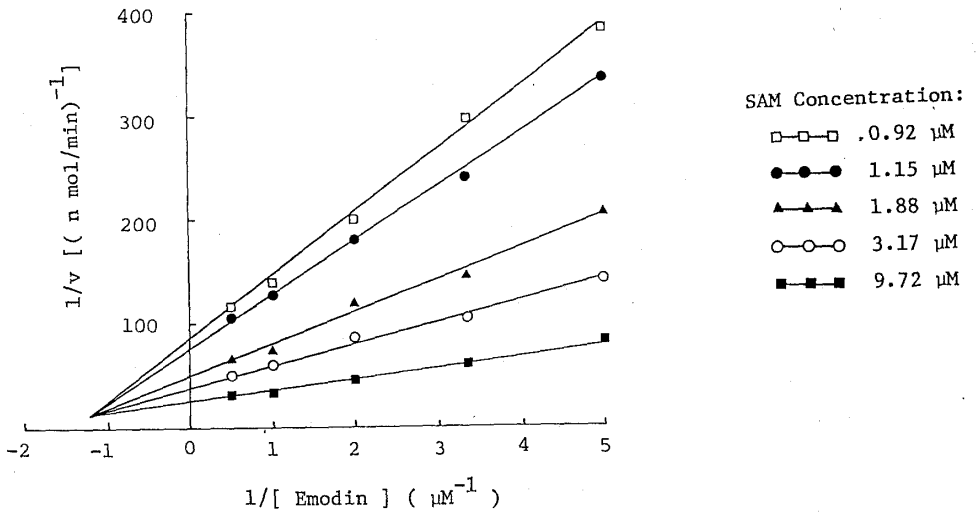


Fig. 1. Double Reciprocal Plots of E-OMT Reaction with Varied Concentrations of SAM

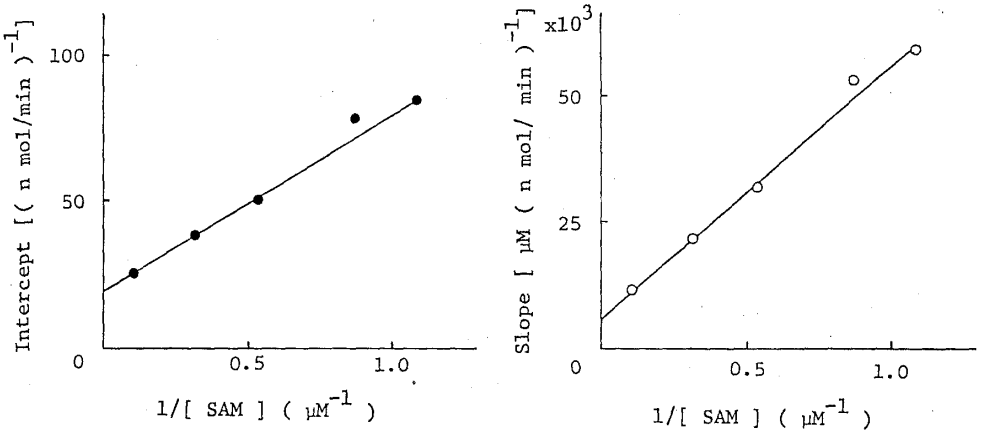
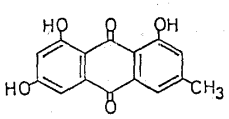
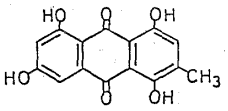


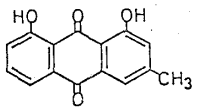
Fig. 2. Secondary Plots for E-OMT Reaction

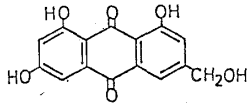
G-200, and optimum pH was pH 7-8. Michaelis constants for emodin (3) and SAM were obtained from steady-state kinetic studies,in which the composition of an assay mixture was modified from that mentioned above so as to fit to kinetic studies. A double reciprocal plot of 1/V against 1/[emodin] with at a series of concentrations of SAM gave the primary plot shown in Fig. 1. The lines have a common point of intersection above the horizontal axis, indicating that the enzyme and the two substrates form a ternary complex in the course of the reactoin by a random or ordered mechanism.[8] The values of slopes and vertical intercepts were replotted to give a secondary plot as shown in Fig. 2. Km values for emodin (3) and SAM were 3.3×10^{-7} M and 3.1×10^{-6} M, respectively. In order to clarify the substrate specificity of E-OMT, fifteen anthraquinone derivatives were tested for their activity as substrates. The results, summarized in Table II, indicate that the enzyme recognizes whole the substrate molecule and that its specificity is extremely high. Low Km value for emodin (3) and strict specificity of the enzyme suggest that emodin (3) formed in cells is readily converted into questin (4), which serves as a substrate for the next Baeyer-Villiger oxidation. The results so far obtained may shed light on the properties of enzymes involved in secondary metabolism, which hitherto have not been extensively investigated.

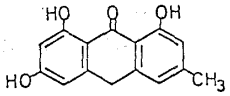
Table II. Substrate Specificity of E-OMT

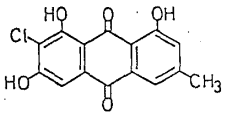

Emodin 100%

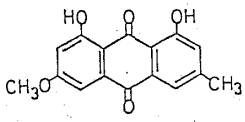

Catenarin 80%

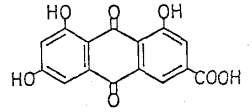

Chrysophanol 1%


ω-OH Emodin 22%


Emodinanthrone 5%


2-Cl Emodin 18%


Parietin 0%


Emodic acid 0%

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