NON-ENZYMIC AND ENZYMIC OXYGENATIONS OF A DIBENZOCYCLOOCTADIENE LIGNAN, (±)-DEOXYSCHIZANDRIN: IMPLICATIONS FOR BIOSYNTHESIS OF THE CORRESPONDING LIGNANS

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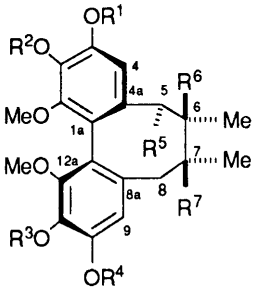
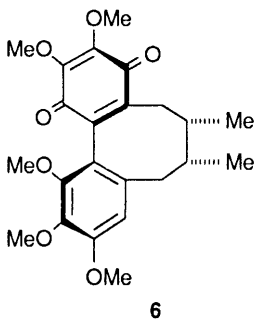
Non-enzymic and enzymic oxygenation reactions of (±)-dibenzocyclooctadiene lignan, (±)-deoxyschizandrin (**1**) using a simple model system for mono-oxygenases $Fe(MeCN)_6^{2+}$-Ac_2O-H_2O_2 and rat liver S9 mix were investigated in connection with mammalian and plant metabolisms of the corresponding lignans. The non-enzymic reaction of **1** gave the two phenol acetates **4a** and **5a** and a quinone **6**, and the enzymic reaction of **1** afforded several compounds from which three compounds characterized as **7**, **8**, and **9** were isolated. The latter result has implications for biosynthesis of these lignans.

KEYWORDS oxygenation; non-enzymic; enzymic; lignan; (±)-deoxyschizandrin; metabolism

More than three dozen dibenzocyclooctadiene lignans, deoxyschizandrin (**1**), gomisin A (**2**), and schizandrin (**3**), etc., have been isolated from the fruits of *Schizandra chinesis* Baillon (Schizandraceae) since 1961,[1e] and several studies on the synthesis[1] and biological activities[1e] of these lignans have also been reported to date. These lignans bear the dibenzocyclooctane ring (DBCO ring) as a common structural unit having a *twist-boat-chair* form (TBC form) except for gomisin R, in which it has a *twist-boat* form (TB form).[2] The variation of these lignans mainly arises from a difference of oxygen functional group on DBCO ring. Additionally, studies were published recently on the metabolism of gomisin A (**2**) utilizing rat liver S9 mix and oral administration to rats, in connection with an interest in the metabolic fate of these lignans; several metabolites were yielded, including oxygenated products.[3]



1: R^1=R^2=R^3=R^4=Me, R^5=R^6=R^7=H
2: R^1+R^2=CH_2, R^3=R^4=Me, R^5=R^6=H, R^7=OH
3: R^1=R^2=R^3=R^4=Me, R^5=R^6=H, R^7=OH
4a: R^1=R^2=R^4=Me, R^3=Ac, R^5=R^6=R^7=H
4b: R^1=R^2=R^4=Me, R^3=R^5=R^6= R^7=H
5a: R^1=R^3=R^4=Me, R^2=Ac, R^5=OAc, R^6=R^7=H
5b: R^1=R^3=R^4=Me, R^2=R^6=R^7=H, R^5=OH
7: R^1=R^2=R^3=R^4=Me, R^5=R^7=H, R^6=OH
8: R^1=R^2=R^4=Me, R^3=R^5=R^6=R^7=H
9: R^1=R^2=R^3=Me, R^4=R^5=R^6=R^7=H



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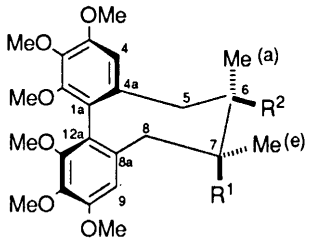

(twist-boat-chair form)

3: R^1=OH, R^2=H
7: R^1=H, R^2=OH

Chart 1

The above reports prompted us to investigate oxygenation reactions of (±)-**1**, one of the simple DBCO lignans, by both non-enzymic and enzymic methods, to obtain further information on the mechanism on mammalian and plant metabolisms of these lignans.

The non-enzymic oxygenation reaction of **1** was investigated utilizing a simple model system for mono-oxygenases $Fe(MeCN)_6^{2+}$-Ac_2O-H_2O_2 [4] under various conditions according to the procedure described in the previous reports.[4c] Three

products, **4a**, **5a**, and **6**, were obtained from the above reaction, and the product yields varied with the reaction conditions used as shown in Table I.

TABLE I. Oxygenation of (±)-Deoxyschizandrin (**1**)

Run	Molar ratio			Product (Yield, %)				Recovery(%)
	1 :	Fe^{2+} :	H_2O_2	Total	**4a**	**5a**	**6**	
1	1	0.1	3.0	18.5	0.5	-	18.0	45.7
2	1	0.2	1.2	7.1	2.9	-	4.1	90.0
3	1	0.2	6.0	17.3	0.8	1.9	16.5	24.0
4	1	0.4	2.4	16.3	5.2	8.5	11.1	7.2
5	1	0.5	1.5	21.5	4.4	11.0	6.1	55.0
6	1	0.5	3.0	29.4	6.9	8.7	14.7	7.2
7	1	0.5	3.5	6.0	2.5	-	3.5	15.0
8	1	1.0	1.5	18.5	5.6	5.0	7.9	45.0
9	1	1.0	2.25	16.5	3.3	8.5	4.7	37.0
10	1	1.0	3.0	12.5	2.7	6.0	3.8	30.5
11	1	1.0	0.0	-	-	-	-	100.0

The analyses of all physical data of **4a** show that **4a** is a mono-acetate of the O-demethylated product of **1**, and the position of O-acetyl moiety may be located at C(11) as was postulated from ^{13}C-NMR comparison of **1** and **4a**, in which the signals at δ 139.1 and 140.1 due to C(8a) and C(11) in **1** [5] were replaced by the signals at δ 141.9 and 130.6 in **4a** shifted to +2.8 and -9.5 ppm by the substitution effect. The second product, **5a**, was similarly revealed to be a diacetate, as shown in Chart 1. The position of phenol acetate in **5a** was assigned to be situated at C(2) based on the fact that the signals depending on C(4a) and C(2) in the ^{13}C-NMR of **5a** were observed at δ 136.4 and 133.1 shifted +2.5 and -6.6 ppm from the signals of δ 133.9 and 139.7 in **1**. Another acetoxyl group in **5a** was assigned to be located at C(5)-α position on the DBCO ring by the analyses of ^{1}H-NMR and ^{1}H-Nuclear Overhauser effect (^{1}H-NOE) experiments. That is, when the signal of C(4)-H of **5a** at δ 6.48 was irradiated, 11% increment of the C(5)-H at δ 5.72 was observed, and the signal of the C(5)-H was observed as doublet J=9.2 Hz. Therefore, the dihedral angle between C(5)-H and C(6)-H may be about 0°, and the C(5)-H may possess a β configuration.

The third product **6** was assigned to have quinone structure from the IR absorption spectrum at 1653 and 1606 cm^{-1}, and the quinone carbonyl positions were assigned to be C(1) and C(4) because the signals of C(1) and C(4) in **6** were observed at δ 183.2 and 184.1 rather than the signals due to C(1) and C(4) in **1** in the ^{13}C-NMR spectrum.[5]

In another study, we investigated the reaction of **1** with rat liver S9 mix (the 9000 x g supernatant fraction of rat liver homogenate) [4] to give several compounds from which the three compounds **7**, **8**, and **9** (in order of product amount) were isolated. When oxygenation reaction takes place at tertiary carbon atoms of **1**, two isomers may be possible, such as **3** and **7** which have TBC conformation. We found that the major metabolite **7** is an oxygenated product at C(6) on the DBCO ring by direct comparison of the physical data with the reported ones.[1e] **8** was identified by direct comparison with the compound **4b** obtained by KOH hydrolysis of the reaction product by the model enzyme system **4a**. **9** was elucidated by comparison with the ^{1}H-NMR spectrum of gomisin K$_1$.[6]

These results are of interest from the following viewpoints. First, oxidative O-dealkylation of alkyl aryl ethers is one of the major metabolic reactions catalyzed by cytochromes P-450.[7] The formation of the demethylated derivatives **4a** and **5a** (the precursor of **6** may also be a demethylated compound) by a simple model system for mono-oxygenase $Fe(MeCN)_6^{2+}$-Ac_2O-H_2O_2 is interesting in connection with the enzymic reaction. Although several reaction mechanisms for oxidative demethylation

reactions by the enzyme and the model systems have been proposed,[8] they can be generally classified into two types: one involves the hemiacetal intermediate that is collapsed to yield the phenols, and the other involves the *ipso*-hydroxylated intermediate which is aromatized to give the phenols. The present investigation does not reveal the reaction mechanism, but clearly this reaction is not a Lewis acid-catalyzed reaction (Run 11 in Table I). Thus, further investigation to clarify the corresponding reaction mechanism is now under way.

Second, formation of **7** by the treatment of **1** with rat liver S9 mix has an implication for biosynthesis of DBCO lignans in plants. Among the 40 kinds of DBCO lignans isolated from nature, almost half, 18 species, have no hydroxyl group at C(7). Therefore, the precursor of these lignans may be non-hydroxylated compounds such as **1** or hydroxylated compounds such as **2** and **3**. Although the enzyme used for our present study does not originated from plants, the described results may have an implication for the biosynthesis of DBCO lignans -- that is, the primary compound of the lignans may not be a hydroxylated one. When the oxygenation reaction takes place at tertiary carbon atoms of DBCO, the two isomers **3** and **7**, oxygenated at C(7) and C(6) respectively, may be produced. Needless to say, schizandrin **3** is a natural product, but **7**, obtained by the present experiment, is not natural. This difference may depend on the structure of the peptide part of iron-porphyrin dependent oxygenases, and suggests that mammalian mono-oxygenase has a particular structure that attacks the C(6) carbon atom. This suggests the necessity to investigate oxygenation reaction of **1** using mono-oxygenase originating from the plant kingdom.

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(Received May 25, 1994; accepted June 17, 1994)