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The Formation of Cyclic Peroxide from Guaia-6,9-diene as a Model for Hanalpinol Biosynthesis

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Autooxidation and photosensitized oxidation converted guaia-6,9-diene into hanalpinol, both compounds are contained in the rhizomes of *Alpinia japonica*. This process may be considered to be a biomimetic conversion. Guaia-6,9-diene may be regarded as an immediate precursor of hanalpinol, and a biogenetic pathway has been proposed. The results of molecular orbital calculation (modified neglect of differential overlap (MNDO) method) supported this proposal and provided a basis for conformational analysis.

Keywords——*Alpinia japonica*; guaia-6,9-diene; hanalpinol; biosynthesis; molecular orbital calculation; MNDO; autooxidation; photosensitized oxidation; conformational analysis

Introduction

Polyunsaturated fatty acids possessing 1,4-diene moieties are known to be converted into their hydroperoxides by a group of enzymes known as lipoxygenase.[1] The nonenzymatic conversion by means of a radical-initiated reaction has also been thoroughly examined.[2]

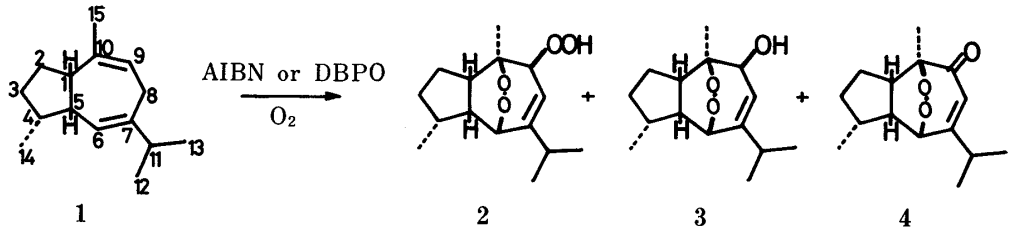
The electrons occupying the highest and lowest orbitals are referred to as frontier electrons and are known to play a decisive role in the chemical reactivity of molecules.[3] The frontier electron density in the ground states of molecules predicts the chemical reactivity of the molecules in radical reactions and is useful to elucidate the reaction mechanism.

The structures of many eudesmanes, agarofurans, eremophilanes, guaianes, and seco-guaianes isolated from *Alpinia japonica* have already been disclosed.[4] It seems probable that the biosynthetic pathway to those guaianes having a cyclic peroxide linkage is similar to that of prostaglandins, though no report has so far suggested that pathway.

From the rhizomes of *A. japonica*, we newly isolated guaia-6,9-diene (having a 1,4-diene moiety), which is considered to be a biogenetic precursor of hanalpinol and its isomers. We describe here the biomimetic conversion of guaia-6,9-diene into hanalpinol and propose a novel biogenetic conversion pathway, which is supported by the results of frontier electron theory calculations.

Results and Discussion

Fresh rhizomes of *A. japonica* were extracted with methanol. The extraction was carried out with shielding from light under an argon atmosphere at 0 °C to avoid autooxidation by atmospheric oxygen. The methanolic extract was shaken with *n*-hexane. The chromatography of the *n*-hexane-soluble fraction on silica gel and $AgNO_3$–silica gel gave guaia-6,9-diene (**1**), the spectral data and optical rotation of which were identical with those reported in the



1 2 3 4

Chart 1

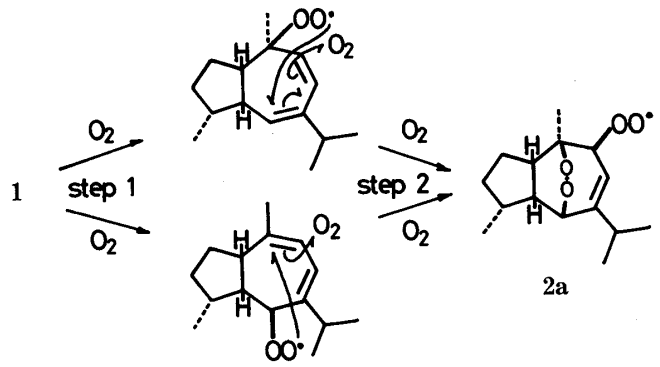


Chart 2

literature.[5]

When treated with an appropriate free radical initiator, α,α′-azobisisobutyronitrile (AIBN) or dibenzoyl peroxide (DBPO), **1** was converted into hanalpinol peroxide (**2**), hanalpinol (**3**) and hanalpinone (**4**)[4] as shown in Chart 1.

For the biological conversion of **1** to **2**, a biosynthetic process involving peroxy radicals (shown in Chart 2) has been suggested. This process is analogous to be the biosynthetic route to prostaglandin, which has been examined nonenzymatically by the oxidation of arachidonic acid.[6] The conversion of **1** into hanalpinol involves the introduction of four oxygen atoms into one guaia-6,9-diene molecule.

Two consecutive radical cyclization reactions are considered to be involved in the proposed biosynthetic conversion pathway. In the first step, two oxygens were introduced at either C-10 or C-6 to form the hydroperoxide, and then in the second step, another two oxygens were introduced to form the cyclic peroxide and to introduce another hydroperoxide moiety. The radical generated by the removal of a hydrogen from C-8 is a stable pentadienyl radical which may trap molecular oxygen at either the C-6 or C-10 position. The cyclization of the peroxy radical at C-6 or C-10 with the double bond at C-10 or C-6, respectively, produces either of the allylic radicals, which trap another oxygen to give the radical, **2a**. Then, **2a** may be converted into **3** or **4** by a radical termination reaction.[7]

To predict the reactivity of **1** in step 1, conformational analysis was conducted. Two boat-type conformers (A and B) of the seven-membered ring are considered to be possible (Fig. 1). The heat of formation of conformer A, calculated by the modified neglect of differential overlap (MNDO) method (molecular orbital calculation),[8] is smaller than that of B by about 2 kcal/mol (25 °C in the gas phase). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, 7.5% nuclear Overhauser effect (NOE) was observed between H-14 and H-6, which shows that the distance between these hydrogens is 2.86 Å and that the seven-membered ring adopts the A conformation. When the temperature of the NMR measurement was changed from 50 °C to −70 °C, the signals of the two possible conformers were not separated and unchanged chemical shifts were observed, so the molecule is also considered to be present as conformer A in the liquid phase ($CDCl_3$).

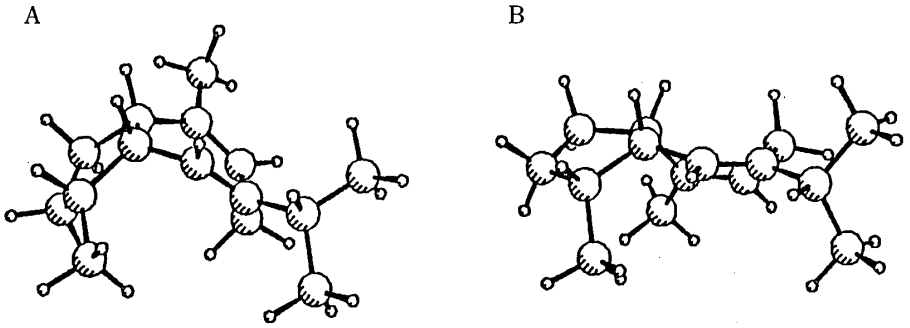


Fig. 1. Heat of Formation

A, −3.0535 kcal/mol; B, −1.0591 kcal/mol.

TABLE I. Frontier Electron Densities of Conformers A—D Calculated by the MNDO Method

Conformers	A		B		C	D		A	B
C	$f_r^{(E)}$	$f_r^{(R)}$	$f_r^{(E)}$	$f_r^{(R)}$	$f_r^{(R)}$	$f_r^{(R)}$	H	$f_r^{(R)}$	$f_r^{(R)}$
1	0.02412	0.01562	0.01268	0.00731	0.00736	0.00570	1	0.00842	0.02487
2	0.00285	0.01405	0.00044	0.00035	0.02370	0.00168	2	0.00013	0.00004
3	0.00513	0.00272	0.00093	0.00069	0.00109	0.00044	2′	0.00031	0.00014
4	0.06522	0.04635	0.03556	0.02251	0.00048	0.00100	3	0.00003	0.00019
5	0.02922	0.01747	0.03761	0.02750	0.00915	0.01874	3′	0.00171	0.00018
6	0.76832	0.61011	0.51240	0.44250	0.00484	0.00543	4	0.00144	0.00057
7	0.74535	0.57676	0.52127	0.42629	0.54901	0.55697	5	0.00804	0.02998
8	0.01679	0.01514	0.01388	0.01231	0.35231	0.35912	6	0.00026	0.00170
9	0.10093	0.28494	0.34506	0.45071	0.36891	0.36248	8	0.04036	0.03122
10	0.07441	0.26817	0.32039	0.42955	0.55036	0.54543	8′	0.00087	0.00156
11	0.02247	0.01200	0.01768	0.00962	0.00657	0.00641	9	0.00242	0.00213
12	0.03800	0.02361	0.02078	0.01430	0.02149	0.01370	11	0.00062	0.00155
13	0.02692	0.01816	0.02228	0.01464	0.00666	0.01583	12	0.00032	0.00020
14	0.00136	0.00075	0.00288	0.00150	0.00024	0.00025	12′	0.00032	0.00002
15	0.00727	0.00572	0.01056	0.00778	0.00640	0.00613	12″	0.00188	0.00174
							13	0.00045	0.00030
							13′	0.00163	0.00079
							13″	0.00005	0.00026
							14	0.00069	0.00074
							14′	0.00002	0.00007
							14″	0.00001	0.00007
							15	0.00370	0.01585
							15′	0.00165	0.00048
							15″	0.01308	0.01822

$f_r^{(E)} = 2(C_{HOMO,r})^2$; $f_r^{(R)} = (C_{HOMO,r})^2 + (C_{LUMO,r})^2$.

The frontier electron density ($HOMO^2 + LUMO^2$) for radical reactions in the ground state of both conformers was calculated by the MNDO method.[8] The frontier electron densities of the carbon and hydrogen atoms are shown in Table I. It was revealed that by the removal of H-8, the reactivity of C-6 becomes larger than that of C-10, suggesting that the radical reaction mechanism of step 1 is possible. It is also suggested that conformer A shows higher selectivity for reaction at C-6 and the stereoselectivity at C-6 is considered to be controlled by the steric hindrance of conformer A (basket-like conformer).

On the other hand, the reactive site in an ene-type reaction[9] with a singlet oxygen is considered to be at C-6 because of the higher density of the frontier electrons [$2 \times (HOMO)^2$] (Table I) and the steric hindrance of the isopropyl moiety at C-7. For the purpose of obtaining

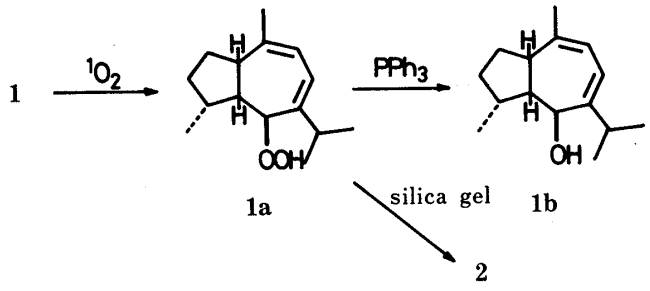


Chart 3

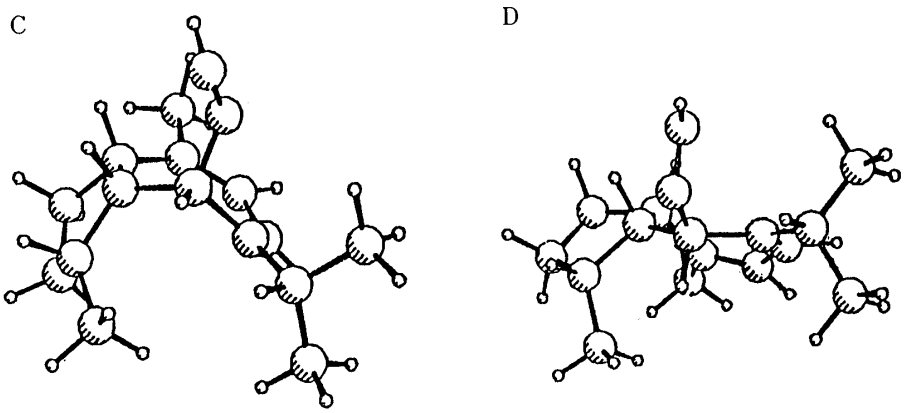


Fig. 2. Heat of Formation
C, −11.9206 kcal/mol; D, −1.2798 kcal/mol.

the reaction intermediate, the dye-sensitized oxygenation of **1** was carried out by using hematoporphyrin as a dye-sensitizer. However, the hydroperoxy compound (**1a**) produced was very unstable and was converted to **2** during the purification by silica gel column chromatography. To determine the position of the hydroperoxide, the hydroperoxide group was converted to a hydroxyl group with triphenylphosphine immediately after the oxidation (Chart 3). Compound **1b** was obtained as colorless needles (mp 56.0—57.0 °C) having the molecular formula, $C_{15}H_{24}O$, according to the mass spectrum (MS). The presence of a secondary hydroxyl group was indicated by the infrared (IR) (3634 cm^{-1}) and ^{1}H-NMR (δ 4.24, 1H, d, J=8.4 Hz) spectra. The signals of a methyl group attached to the double bond (δ 1.89, 3H, br s) and two olefinic protons which exhibited a long-range coupling to the methyl group (δ 5.70, 1H, br d, J=3.6 Hz and 5.55, 1H, br d, J=4.2 Hz) were observed in the ^{1}H-NMR spectrum. Furthermore, the presence of a diene moiety was indicated by the ultraviolet (UV) spectrum (251 nm, ε 7260). The stereoselectivity of this reaction at C-6 is also considered to be influenced by the steric hindrance caused by the C-14 methyl group and other groups in conformer A.

As regards the conformers of **1a**, there are two more possible stable conformers (C and D) (Fig. 2). The result of MNDO calculation of both conformers shows that conformer C is more stable than conformer D by about 10.6 kcal/mol. In the ^{1}H-NMR spectrum, irradiation of H-14 in **1b** caused a 10.0% increase of the integrated intensity of H-6: H-14 and H-6 must be at the van der Waals distance from each other, because the van der Waals radius of the hydrogen atom is about 1.2 Å and the distance between them in conformer C is 2.40 Å.

The radical reactive site of **1a** was deduced from the frontier electron density ($HOMO^2+LUMO^2$) in the ground state of both conformers C and D. To produce hanalpinol, attack at C-10 by the hydroperoxy oxygen is necessary. It was revealed that the

reactivity at C-10, particularly in conformer C is higher than that of the other double bond, as shown in Table I.

As regards the biogenetic route to hanalpinol, the two possibilities described below may be considered. As hanalpinol has a 1,4-diene group, a characteristic of unsaturated lipoacids, it may be biosynthesized by lipoxigenase-like enzymes. A large amount of heavy metals, in particular, Mn and Fe, was contained in the rhizomes of *A. japonica* and *A. intermedia* as described in a previous paper,[10] and these heavy metals are known to catalyze autooxidation.[11] It may also be catalyzed by a trace amount of active oxygen as in the case of the autooxidation described in this paper. Therefore, hanalpinol may be concluded to be biosynthesized from guaia-6,9-diene, which may be biosynthesized from farnesyl pyrophosphate *via* germacrene C.

Experimental

Spectral data were obtained on the following instruments; optical rotation on a JASCO DIP-4, IR on a JASCO A-302, UV on a Hitachi 557, NMR on a Brucker AM 400, and MS on a Hitachi M-80. High-performance liquid chromatography (HPLC) was carried out on a CIG column system (Kusano Scientific Co., Tokyo) with Iatrobeads (60 μ silica gel, IATRON Co., Tokyo) as the stationary phase. Molecular orbital calculations were carried out with the MNDO program in MOPAC distributed by Quantum Chemical Program Exchange (QCPE) using a HITAC M-280H computer at the Computer Centre, the University of Tokyo.

Autooxidation of 1 to Give 2, 3 and 4——Compound **1** was heated with α,α'-azobisisobutyronitrile or dibenzoyl peroxide under oxygen under the conditions given in Table II. The product was subjected to HPLC (*n*-hexane : ethyl acetate = 13 : 1 and benzene : ethyl acetate = 12 : 1) to give **2**, **3** and **4**.[4]

Table II

1 (mmol)	Radical initiator (mmol)	Temperature (°C)	Time (h)	**2**	**3**	**4** (mmol)
1.142	AIBN 0.030	60	6	0.072	0.027	0.008
1.217	DBPO 0.031	60	6	0.213	0.148	0.013

Photooxygenation of 1 to Give 1b——A 30 mm quartz tube enclosed by a 100 W mercury lamp was charged with a solution of 200 mg of **1** and 40 mg of hematoporphyrin in 30 ml of benzene under ice-cold water. Oxygen was admitted into the tube through its gas dispersion tube, and the mixture was irradiated for 1 h. The removal of the solvent under reduced pressure gave a crude product, which was immediately treated with triphenylphosphine in ether for a few minutes at room temperature. Then the solvent was evaporated off and the residue was subjected to HPLC (*n*-hexane : ethyl acetate = 9 : 1), $AgNO_3$-HPLC[12] (*n*-hexane : ethyl acetate = 9 : 1) and ODS-HPLC (methanol) to give **1b** as colorless needles (30 mg). **1b**: mp 56.0—57.0 °, $[\alpha]_D$ −181.8 ° (c = 0.12, $CHCl_3$). MS m/z (%): 220 (M^+, 40), 177 (100), 159 (75), 121 (90), 105 (92), 91 (98), 81 (88). IR (CCl_4) cm^{-1}: 3634, 2961, 2932, 2872, 1463, 1381, 1261, 1094, 1056, 1030. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.00 (3H, d, J = 7.1 Hz), 1.09 (3H, d, J = 6.8 Hz), 1.14 (3H, d, J = 6.8 Hz), 1.89 (3H, br s), 2.14 (1H, m), 2.59—2.67 (2H, m), 2.83 (1H, dt, J = 6.6, 8.4 Hz), 4.24 (1H, d, J = 8.4 Hz), 5.55 (1H, br d, J = 4.2 Hz), 5.70 (1H, br d, J = 3.6 Hz). ^{13}C-NMR ($CDCl_3$) δ ppm: 15.8 (q), 21.6 (q), 24.0 (q), 24.4 (q), 28.8 (t), 30.9 (d), 34.0 (t), 36.5 (d), 43.1 (d), 68.1 (d), 72.2 (d), 118.8 (d), 122.8 (d), 144.6 (s), 153.8 (s). UV λ_{max}^{EtOH} nm (ε): 251 (7260).

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