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Oryzalexin S Structure: A New Stemarane-type Rice Plant Phytoalexin and its Biogenesis.

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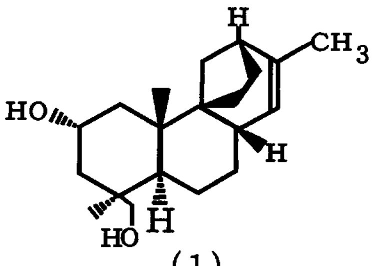
Abstract: Details of the structural assignment of oryzalexin S, a novel rice plant phytoalexin obtained from 2-D NMR experiments, are presented. This is the first phytoalexin with the stemarane skeleton, and it indicates a characteristic biogenesis.

INTRODUCTION

We isolated 1) a new antifungal diterpene, oryzalexin S 2) (1) that differs from known rice plant labdadiene type phytoalexins and reported a proposed structure 2) based on a comparison with characteristic signals of stemaran type compounds 3). Oryzalexin S is the first stemarane type phytoalexin to be reported. We here describe in detail the assignment of the ^1H and ^{13}C NMR spectra and the relative stereochemistry of oryzalexin S on the basis of various 2-D NMRs (^{13}C-^1H COSY, ^1H-^1H COSY, ^{13}C-^1H HOHAHA, ^{13}C-^1H COLOC and NOESY). The characteristic biogenesis of oryzalexin S from GGPP is discussed and compared with the biogenesis of other labdadiene type phytoalexins.



(1)
oryzalexin S

)

STRUCTURE ELUCIDATION

Properties of oryzalexin S were as follows : mp 173 ° C, $[\alpha]_D^{26.5}$ +32(c 1.0, $CHCl_3$), molecular formula $C_{20}H_{32}O_2$ (HR-EIMS m/z 304.2404 requires 304.2386). The ^{13}C-NMR spectrum of the compound shows peaks for 20 carbons (Table 1). The multiplicity of signals was established using the DEPT pulse sequence. The DEPT experiments also established the presence of 30 protons bonded to these carbons. Interpretation of the ^{13}C-NMR data indicates that two oxygenated carbons (C2, C19) and two olefinic carbons (C13, C14) are present. Moreover, taking the presence of these 30 protons and two oxygenated carbons (C2, C19), the molecular formula ($C_{20}H_{32}O_2$) suggests the presence of two hydroxy groups. The following carbon types therefore are present: $3CH_3$, $7CH_2$, $1CH_2OH$, 3CH, 1CHOH, 1C=C, and 3C.

Table 1. Carbon and proton NMR assignments for oryzalexin S in $CDCl_3$.

position	δ_C	δ_H	position	δ_C	δ_H
1	41.92 (t)	1.32(m) 1.84(dt 12, 4)	11	29.99 (t)	1.46(dd 11, 4) 1.65#
2	64.96 (d)	3.93(tt 12, 4)	12	43.14 (d)	2.21(m)
3	44.56 (t)	0.88(dt 1, 12) 2.20(br dt 12, 4)	13	138.89 (s)	
4	40.48 (s)		14	123.49 (d)	4.95(dd 4, 1)
5	49.42 (d)	1.24#	15	33.21 (t)	1.53(m) 1.63#
6	21.76 (t)	1.23# 1.64#	16	31.77*(t)	1.72# 1.75(br t 12)
7	31.80*(t)	1.20# 1.22#	17	21.90 (q)	1.62(br s)
8	43.81 (d)	1.99(m)	18	27.27 (q)	1.01(s)
9	51.05 (s)		19	65.91 (t)	3.42(dd 11, 1) 3.70(d 11)
10	40.56 (s)		20	18.70 (q)	0.98(s)

#: Chemical shift obtained from the cross section of ^{13}C-^{1}H COSY.

*: Assignments are interchangeable.

Because of the severe overlap of resonance in ^{1}H-NMR, it was necessary to use 2-D NMR techniques to determine the complete assignments. A standard hetero nuclear correlation ^{13}C-^{1}H COSY experiment showed the C-H connectivities (Table 1). The proton chemical shifts marked # were deduced from the cross section of the ^{13}C-^{1}H COSY spectrum. The broad band decoupling ^{1}H-^{1}H COSY[4] correlations and ^{13}C -^{1}H HOHAHA[5] correlations gave the three partial structures (A), (B), and (C) in Fig. 1. In the ^{1}H-^{1}H COSY experiment, as broad band decoupling simplified the resulting 2-D NMR spectrum, the spin couplings could be readily assigned. Vicinal couplings are shown in Fig. 1 (H1-H2, H2-H3, H5-H6, H7-H8, H8-H14, H11-H12, H12-H15, and H15-H16). The C6-C7 connectivity in (B) was validated showing the correlations between C6 and its neighboring protons in ^{13}C -^{1}H HOHAHA. Other correlations in the ^{13}C -^{1}H HOHAHA also validated the fragment (C).

The combinations of these fragments together with the other connectivities deduced from the observed two and three bond correlations in ^{13}C-^{1}H COLOC6) led to the fragments (D) and (E) in Fig. 2. In particular, the assignments of the quaternary carbons (C4, C10) were relied upon to detect the ^{13}C-^{1}H COLOC correlations. The correlations between C12 and its neighboring protons in ^{13}C-^{1}H COLOC validated the assignment of fragment (E). These fragments (D) and (E) were linked together through C14 to give the fragment (F) shown in Fig. 3. Moreover, correlations between the residual quaternary carbon, C9, and its neighbors also appeared in ^{13}C-^{1}H COLOC made clear the total structure of oryzalexin S including the stereochemistry of the double bond (Fig. 4).

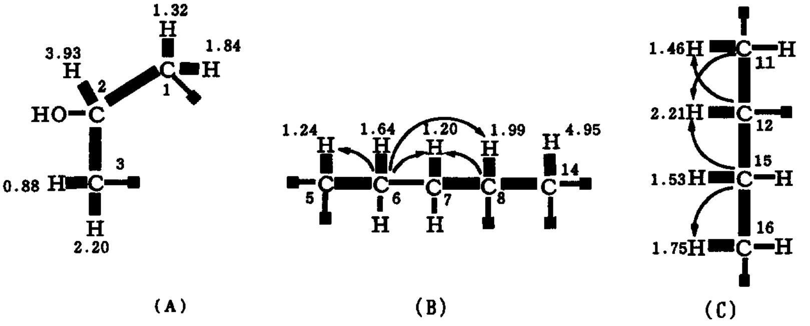


Fig.1. Partial fragments deduced from the 2D-NMR experiments.

→ ^{13}C-^{1}H HOHAHA ▬ ^{1}H-^{1}H COSY

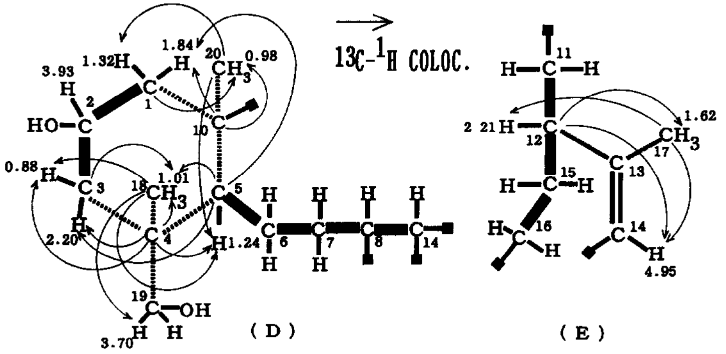


Fig.2. Fragments (D) and (E) deduced by ^{13}C-^{1}H COLOC.

Stereochemistry of the double bond is not elucidated(——).

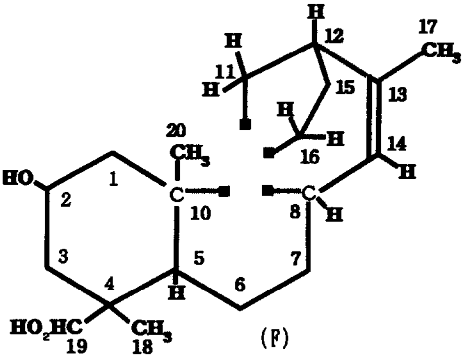


Fig.3 . Fragment (F).

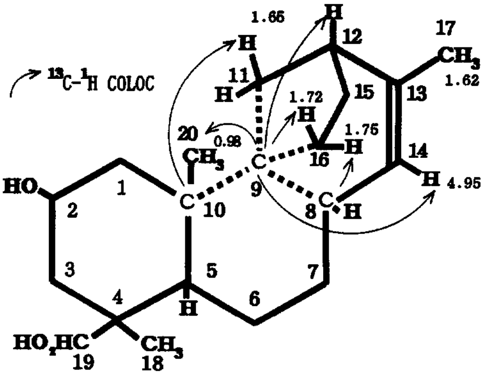


Fig.4. Structure of oryzalexin S.

Corroboration of the conformation and relative stereochemistry of oryzalexin S was made from phase-sensitive NOESY spectra. NOEs were seen between H20 (0.98)- equatorial H1 (1.84); H20(0.98)- H2 (3.93); H20 (0.98)-H19 (3.42 and 3.70); H20 (0.98)-H8 (1.99); axial H1 (1.32)-H11 (1.46); and equatorial H1 (1.84)-H16 (1.72 and 1.75). The axial orientation of the bridge head methyl at C10 was confirmed by its correlation with the axial proton at C2. The NOEs between this axial bridge head methyl at C10 and H8 as well as the NOEs between axial proton H1 and methylene proton H11 suggest a conformation of the rings A, B, and C. It is evident that the hydroxy methyl side chain at C4 has a axial orientation. The structure and relative stereochemistry of oryzalexin S are shown in Fig.5 .

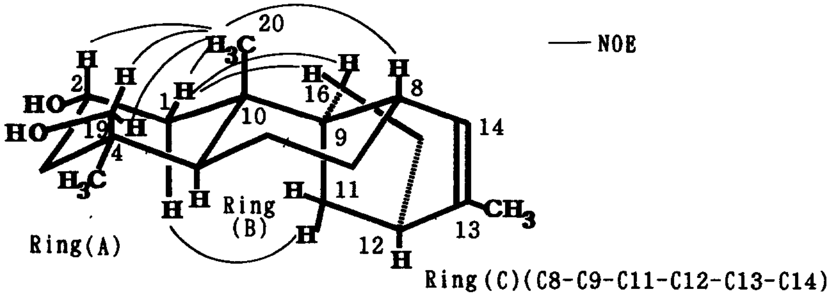
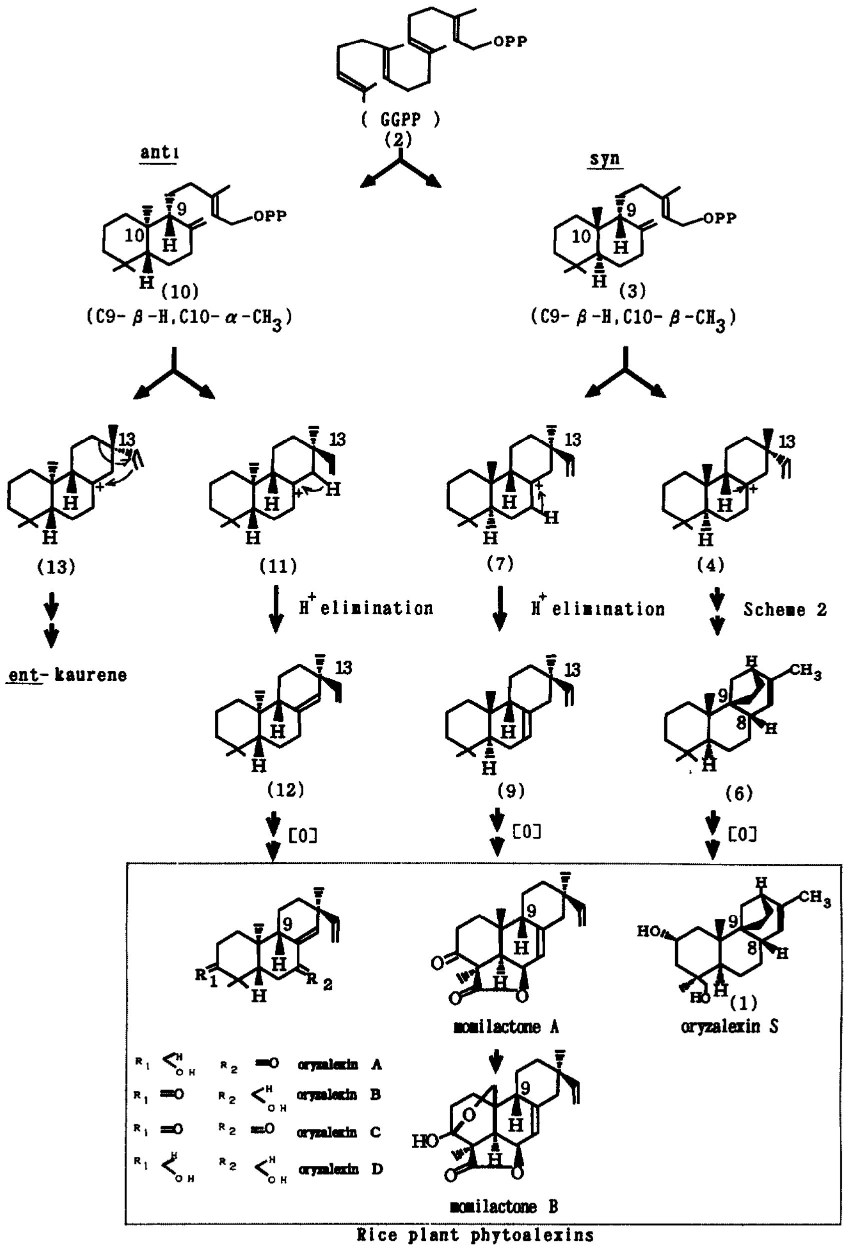


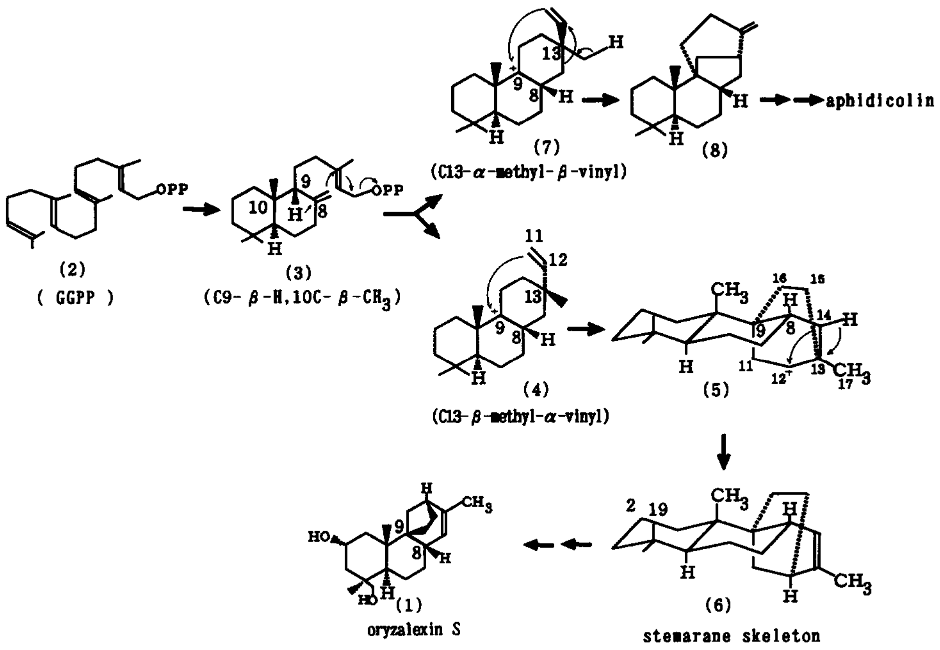
Fig.5. NOESY data.

SPECULATION ON THE BIOGENESIS OF ORYZALEXIN S

Oryzalexin A-D 7)~9) and momilactone A,B 10) are phytoalexins isolated from rice plants. These diterpenoid compounds contribute to the plant's chemical defence against microbial invasion. We isolated and characterized these diterpenoid phytoalexins which indicate a characteristic biogenesis that starts from geranyl geranyl pyrophosphate (GGPP) (Scheme 1). Stereochemistry is important for determining the derivations in the biogenesis of these phytoalexins . This biogenesis has two branches: The first is dependent on the conformation, *anti* or *syn* with H9 and 10-methyl in the labdadienyl pyrophosphate (3) and (10). The second is dependent on the configuration at C13 in the pimarenyl cations (4), (7), (11) and (13). We show the biogenesis of oryzalexin S from GGPP (Scheme 2). Since oryzalexin S is a diterpenoid phytoalexin from the rice plant, its precursor also should be GGPP. Structure (1) shows that oryzalexin S has neither a proton at C9 nor a vinyl group; therefore, its biogenesis includes hydride rearrangement from C9 to C8. The cation at C9 is attacked by the vinyl group at C13 to give the next intermediate (5). This rearrangement has been proposed in the biosynthesis of aphidicolin11) from (7) which has the conformation at the C13-α-methyl-β-vinyl. A similar rearrangement with (4) which has the conformation at C13-β-methyl-α-vinyl, leads to intermediates (5) and (6) which then lead to oryzalexin S via oxidative reactions at C2 and C19. Oryzalexin S is a tetracyclic diterpenoid that is related structurally to stemarane diterpenoid,8) but it is a new type of a rice plant phytoalexin that differs from known labdadiene type phytoalexins (Scheme 1.).



Scheme 1. Biogenesis of diterpenoid phytoalexins.



Scheme 2. Biogenesis of oryzalexin S .

Fortunately, we found this biogenetically unique compound which is distinguishable from other phytoalexins (momilactones and oryzalexin A-D) because of its biogenetic route. The finding and classification of other new phytoalexins will provide help in proposing the systematic biogenesis of phytoalexins.

EXPERIMENTAL

A 7 mg sample of oryzalexin S dissolved in 0.5 ml of $CDCl_3$ was used. All NMR measurements were made at 35 ℃ on a JEOL GSX-400 spectrometer with a $^1H/^{13}C$ 5 mm dual prove. The respective ^1H 90° and ^{13}C 90° pulses were 12.5 and 8.5 μsec . The $^1H-^1H$ COSY was completely decoupled along the F1 axis (program run by Professor K. Hikichi). Phase sensitive $^{13}C-^1H$ HOHAHA and NOESY were used. The mixing time of HOHAHA was 22.5 msec and that of NOESY 500 msec. In the COLOC experiment the maximum coupling constant (J_{C-H}) used was 7 Hz.

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