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Microbial transformation of zaluzanin-D

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Abstract

Microbial transformation of zaluzanin-D using different fungi gave 11,13-dihydrozaluzanin-C, zaluzanin-C, 4,16,11,13 - tetrahydro zaluzanin-C, estafiatone, dihydroestafiatol and dihydroestafiatone.

Keywords: *Vernonia arborea*; Zaluzanin; Estafiatone; *Rhizoctonia solani*; *Botrytis cinerea*; *Curvularia lunata*; *Colletotrichum lindemuthianum*; *Botryodiplodia theobromae*; *Fusarium equiseti*; *Fusarium oxysporum*; *Alternaria alternata*; *Sclerotinia sclerotiorum*; *Phyllosticta capsici*

1. Introduction

Sesquiterpene lactones are known to possess insect antifeedant, insect growth regulatory, antifungal, cytotoxic, and anti-tumor activities (Mabry and Gill, 1979; Wedge et al., 2000).

Biotransformation of naturally occurring sesquiterpene lactones using fungi were attempted in order to obtain regio and stereo selective modifications to enhance the activity (Becher et al., 1981) and to develop structure activity models. A number of biotransformations of sesquiterpene lactones using fungi has been attempted earlier (Aleu et al., 1999; Barrero et al., 1999).

In pursuit of sesquiterpene lactones that possess insect antifeedant activity, phytochemical investigation of plants belonging to Indian verbenaceae and compositae were attempted in this laboratory. *Vernonia arborea* (Compositae), a species endemic to southern India, yielded considerable quantitites of zaluzanin-D (**1**), a bioactive sesquiterpene lactone of guaianolide skeleton. Biotransformation of zaluzanin-D was attempted in the present investigation utilizing several fungal plant pathogens and the results are presented.

2. Results and discussion

Zaluzanin-D was incubated with the liquid cultures under normal laboratory light conditions (i.e. 12 h light/12 h dark). Culture filtrates were withdrawn at different intervals, extracted with ethyl acetate and the organic extract was analyzed by TLC. Fungal cultures in the medium alone were maintained under similar conditions and extracted for comparison. Chemical structures of transformed compounds were deduced from comparison of their spectroscopic data with that of zaluzanin-D. The yield of products formed with different micro organisms were presented in Table 1.

Hydrogenation of the double bonds was the common transformation observed with all the fungi tested (Scheme 1). *Rhizoctonia solani* was the only fungus, which afforded oxidation products. Out of the 3 exocyclic double bonds in zaluzanin-D (**1**), the 10–14 double bond present in the 7-membered ring, being non-conjugated to a carbonyl group was found to be resistant to reduction.

Botrytis cinerea was able to reduce the exocyclic double bond conjugated to γ-lactone stereospecifically to give the major product 11,13-dihydrozaluzanin-D (**2**), with 11,13-dihydrodeacetylatedzaluzanin-D (dihydrozaluzanin-C (**3**)) as minor product. Six fungi effected total conversion of zaluzanin-D to **3** (Scheme 1). With *Sclerotinia sclerotiorum* only deacetylation was observed giving **4** as an exclusive product without effecting any reduction. With *Rhizoctonia solani*, in addition to the reduction of double bonds, further oxidation products were obtained, the products obtained being 11,13-dihydrozaluzanin-C (**3**), dihydroestafiatone (**5**), dihydroestafiatol (**6**) and estafiatone (**7**).

Comparison of ^{1}H NMR spectrum (Table 2) of **2** with that of zaluzanin-D (**1**) indicated the hydrogenation of

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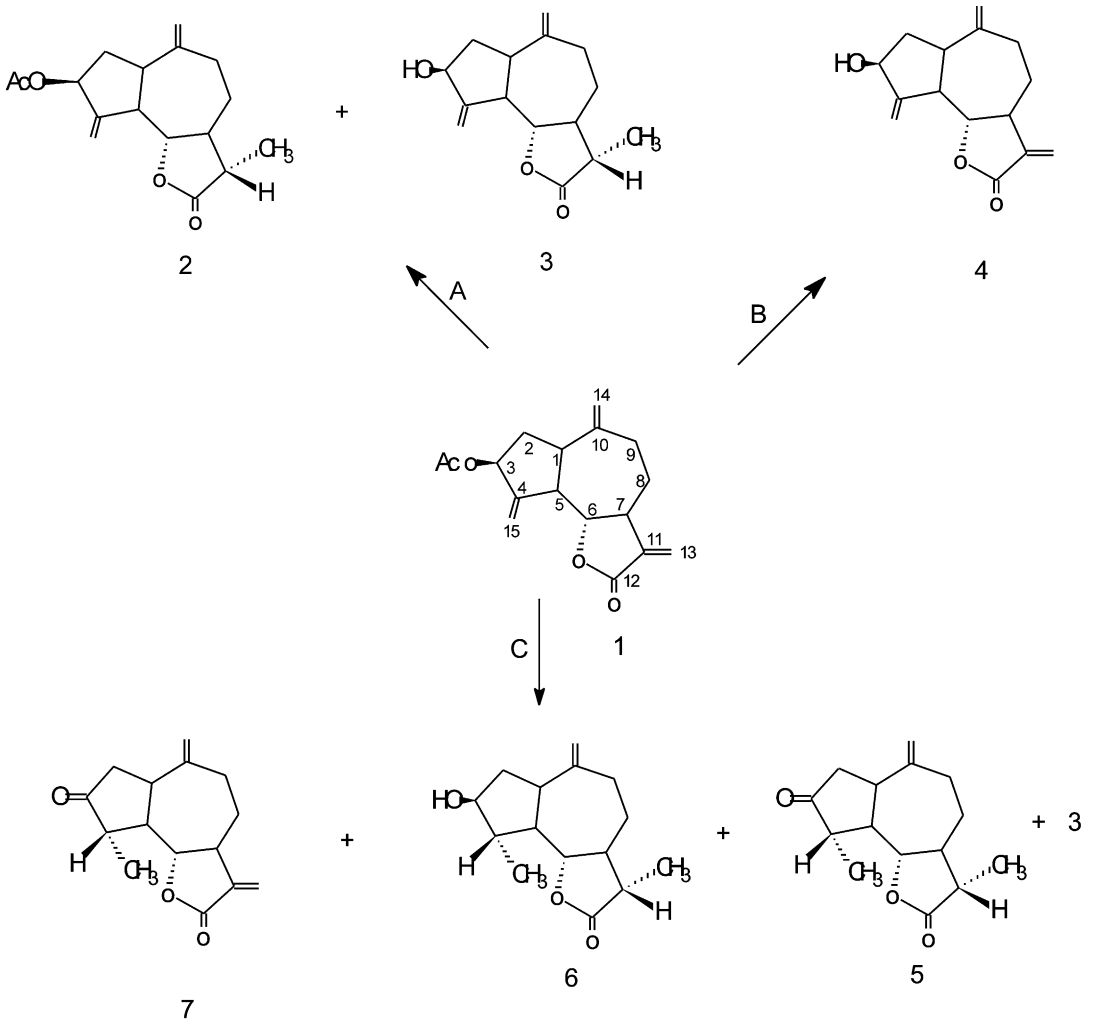
Table 1
Yields of products obtained

	Name of the fungus	Age of the culture (days)	Fermentation time (days)	Product (% yield)
A	*Botrytis cinerea*	3	2	**2** (85) + **3** (15)
	Fusarium equiseti	5	4	**2** (33) + **3** (66)
	Curvularia lunata	3	3	**3** (96)
	Collet. lindemuthianum	3	3	**3** (94)
	Botryodiplodia theobromae	3	3	**3** (94)
	Fusarium oxysporum	3	3	**3** (93)
	Alternaria alternata	5	5	**3** (86)
	Phyllosticta capsici	3	5	**3** (88)
B	*Sclerotinia sclerotiorum*	5	5	**4** (98)
C	*Rhizoctonia solani*	3	24	**3** (<1) + **5** (42) + **6** (47) + **7** (6)

11,13- double bond as shown by the presence of one secondary methyl at δ 1.24 (*d*, $J = 6.9$ Hz) and the absence of olefinic protons at δ 6.22 and δ 5.51 (corresponding to 13-H). This was further confirmed by replacement of the quaternary carbon, C-11, in **1** at δ 139.5 by the methine carbon at δ 42.0 in the ^{13}C NMR spectrum of **2**. The orientation of the 13-methyl group was assigned as α by the NOE observed between 6-H and 11-H and also from the coupling constant of 11 Hz between 7-H and 11-H. Thus compound **2** was identified as 11,13-dihydrozaluzanin-D.

^{1}H NMR spectrum of compound **3** showed the absence of acetyl methyl signal. Also the upfield shift of signal at δ 5.56 corresponding to –C**H**OAc in **1** to δ 4.54 in **3** indicated deacetylation. This was further supported by the absence of acetyl carbonyl signal at δ 170.6 in the C-13 spectrum. In addition, the secondary methyl signal at δ 1.23 (*d*, $J = 6.9$ Hz) showed the hydrogenation of 11–13 double bond. The 13-methyl orientation was confirmed as α as in **2**. Thus compound **3** was identified as 11,13-dihydrozaluzanin-C. Both the products were found to be formed simultaneously (by TLC) ruling out the possibility of initial formation of **2** and its subsequent conversion to **3**.

Compound **4** was identified as zaluzanin-C from the ^{1}H and ^{13}C NMR data (Tables 2 and 3), which is deacetyl derivative of zaluzanin-D.



Scheme 1. Transformation of zaluzanin-D (**1**) by different fungi (**A**, **B** and **C** mentioned in Table 1).

Table 2
^{1}H NMR spectral data for compounds **1**–**7** (200 MHz, values in $CDCl_3$)

H	1	2	3	4	5	6	7
1					3.05 *m*		3.05 *m*
3	5.56 *m*	5.55 *dd* (3.5, 5.9)	4.54 *t* (7.4)	4.57 *tt* (7.5, 3)		3.71 *m*	
6	4.07 *t* (9.3)	3.99 *t* (9.4)	4.02 *t* (9.5)	4.10 *t* (9.2)	3.96 *t* (9.2)	3.93 *t* (10)	3.97 *t* (8.6)
11	–	2.12 *dq* (11, 6.9)	2.14 *qd* (11, 6.9)	–	2.12 *m*	2.12 *m*	–
13 a	6.22 *d* (3.5)	1.24 *d*[a] (6.9)	1.23 *d*[a] (6.9)	6.21 *d* (3.5)	1.23 *d*[a] (6.7)	1.21 *d*[a] (6.8)	6.29 *d* (3.2)
13 b	5.51*d* (3.5)			5.49 *d* (3.5)			5.57 *d* (3.2)
14 a	4.95 *s*	4.93 *s*	4.96 *s*	5.00 *s*	4.98 *s*	4.95 *s*	5.01 *s*
14 b		4.91 *s*	4.93 *s*	4.94 *s*	4.66 *s*		4.68 *s*
15 a	5.49*t* (2)	5.41 *t* (2.1)	5.38 *t* (1.9)	5.46 *t* (1.8)	1.26 *d*[a] (6.1)	1.24 *d*[a] (6.3)	1.24 *d*[a] (6.3)
15 b	5.29 *t* (1.9)	5.27 *t* (2.1)	5.29 *t* (1.9).	5.33 *t* (1.8)			
CH_3-CO-O	2.11 *s*	2.10 *s*	–	–	–	–	–

[a] Methyl doublets integrating for 3 H.

Table 3
^{13}C NMR spectral data for compounds **1**–**6** (50 MHz, values in $CDCl_3$)

Carbon no.	1	2	3	4	5	6
1	44.5	43.9	43.5	44.1	39.7	42.1
2	36.4	36.2	38.7	39.0	44.0	38.3
3	74.6	74.6	73.5	73.5	219.2	78.3
4	147.7	148.6	153.2	153	47.2	47.0
5	45.2	49.8	49.5	45.5	50.9	50.6
6	83.7	83.6	83.7	83.8	88.5	85.9
7	50.2	50.4	50.8	49.9	48.6	52.9
8	30.5	32.2	32.3	30.5	32.9	32.7
9	34.4	36.1	35.9	34.1	39.0	37.0
10	148.0	148.2	148.8	147.9	149.1	149.2
11	139.5	42.0	42.0	139.6	41.7	42.0
12	169.8	178.3	179.8	169.9	178.2	178.6
13	120.2	13.1	13.1	120.1	13.3	13.0
14	113.4	113.2	113.5	114.3	112.5	112.5
15	114.3	113.4	111.0	111.2	14.0	14.1
CH_3–CO–O–	170.6	170.7	–	–	–	–
CH_3–CO–O–	21.1	21.1	–	–	–	–

With *Rhizoctonia solani* two major products **5** and **6** were obtained in addition to the minor products **3** and **7**.

Compound **5** had the molecular formula $C_{15}H_{20}O_3$. ^{1}H NMR of **5** showed absence of acetyl methyl signal and presence of two secondary methyl signals at δ 1.23 (*d*, $J=6.7$ Hz), δ 1.26 (*d*, $J=6.1$ Hz) indicating deacetylation as well as reduction of two exocyclic double bonds. ^{13}C NMR of compound **5** also confirmed the absence of acetyl methyl and acetyl carbonyl. Instead, a signal at δ 219.2 indicated the presence of a 5-membered ring ketone. IR band at 1738 cm^{-1} supported this. Upfield shift of the 5-H signal to δ 2.2 (which appeared at δ 2.78 in **1**) suggested the reduction of a 4–15 double bond. The NOE observed between 6-H, 4-H and 11-H indicated the α orientation of the methyls at C-4 and C-11. Thus compound **5** was identified as dihydroestafiatone, which was earlier reported from *Centaurea webbiana* (Antonia et al., 1972).

Relative to **5**, compound **6** contained two additional protons ($C_{15}H_{22}O_3$) as determined by EIMS. The lack of 5-membered ring ketone carbon signal at δ 219.2 in the ^{13}C NMR and the presence of additional methine carbon bearing –OH at δ 78.3, suggested the possibility of a secondary hydroxyl group at position 3. This was further confirmed by the characteristic hydroxyl absorption at 3200 cm^{-1} in the IR spectrum. Hence compound **6** was identified as dihydroestafiatol.

^{1}H NMR of compound **7** was similar to that of compound **5** which showed the absence of peak at δ 5.41 and δ 5.27 corresponding to H-15. The presence of secondary methyl signal at δ 1.24 (*d*, $J=6.8$ Hz) confirmed the reduction of a 4–15 double bond. But the methylene protons (C-13) seen at δ 6.29 and δ 5.57 suggested that the 11–13 double bond was intact. Thus **7** was identified as estafiatone.

3. Experimental

Leaves of *Vernonia arborea* (50 kg) were collected from Kolli hills, Tamilnadu, during November 1999. Their identity was confirmed by Dr. K.V.Krishnamurthy, Department of Botany, Bharathidasan University, and a voucher specimen (No. BDU 556) was deposited in their herbarium. The dried and crushed material was extracted exhaustively with n-hexane and methanol. One hundred grams of the residue from the hexane extract was chromatographed over silica gel column using *n*-hexane–EtOAc mixtures (1–100%) as eluents.

3.1. Isolation of compound *1*

Fractions 133–150 on crystallization from hexane–ethyl acetate gave compound **1** (4 g) mp 103–104°;

$[\alpha]_D^{25} + 21.43^\circ$ ($CHCl_3$; *c* 0.28); UV $\lambda_{max}^{CHCl_3}$ nm 246 (ε 25302); IR ν_{max}^{KBr} cm^{-1}; 1756, 1732; ^1H, ^{13}C NMR (Tables 2 and 3); $[M+1]^+$ 289.

3.2. Microorganisms and culture media

Microorganisms *Rhizoctonia solani* (SPICFCC-18), *Botrytis cinerea* (SPICFCC-12), *Curvularia lunata* (SPICFCC-29), *Colletotrichum lindemuthianum* (SPICFCC-38), *Botryodiplodia theobromae* (SPICFCC-41), *Fusarium equiseti* (SPICFCC-31), *Fusarium oxysporum* (SPICFCC-9), *Alternaria alternata* (SPICFCC-15), *Sclerotinia sclerotiorum* (SPICFCC-24), *Phyllosticta capsici* (SPICFCC-46) used in the experiments were obtained from Mycology Department, Centre for Natural Products, SPIC Science Foundation.

Czapek-Dox liquid medium containing $NaNO_3$ (2 g), KH_2PO_4 (1 g), KCl (0.5 g), $MgSO_4.7H_2O$ (0.5 g), Sucrose (30 g) with traces of $FeSO_4.7H_2O$ in 1000 ml of water was used for transformation experiments.

3.3. Biotransformation of zaluzanin-D

The organism was inoculated in sterilized media and kept in a shaker (100 rpm) for 3–5 days (Table 1) at 26 °C. The substrate (40 mg) dissolved in 2 ml of acetone was added to the broth (100 ml) and the incubation continued till complete conversion of starting material. The reaction was monitored using TLC. On completion of the reaction, the broth was filtered from the biomass, which was repeatedly washed with water and EtOAc. The filtrate was then extracted with EtOAc, dried over Na_2SO_4 and concentrated under vacuum. The crude product obtained in each case was purified by Silica-gel column chromatography using *n*-hexane and increasing quantities of EtOAc (1–100%).

3.4. Compound **2**

Mp 126–128°; $[\alpha]_D^{25} + 54.02^\circ$ ($CHCl_3$; *c* 0.06); UV $\lambda_{max}^{CHCl_3}$ nm 246 (ε 129533); IR ν_{max}^{KBr} cm^{-1}; 1771, 1733; ^1H, ^{13}C NMR (Tables 2 and 3); NOE-diff experiments: Proton irradiated (NOEs observed) H-15a (H-15b, H-6), H-6 (H-15a, H-11) $[M+1]^+$ 291.

3.5. Compound **3**

$[\alpha]_D^{25} + 83.33^\circ$ ($CHCl_3$; *c* 0.24); UV $\lambda_{max}^{CHCl_3}$ nm 257 (ε 31225); IR ν_{max}^{KBr} cm^{-1}; 3197, 1747; ^1H, ^{13}C NMR (Tables 2 and 3); $[M+1]^+$ 249.

3.6. Compound **4**

Mp 95–96° ; $[\alpha]_D^{25} + 50o$ ($CHCl_3$; *c* 0.1); UV $\lambda_{max}^{CHCl_3}$ nm 242 (ε 59290); IR ν_{max}^{KBr} cm^{-1}; 3200, 1748; ^1H, ^{13}C NMR (Tables 2 and 3); $[M+1]^+$ 247.

3.7. Compound **5**

Mp 82–83° ; $[\alpha]_D^{25} + 66.67^\circ$ ($CHCl_3$; *c* 0.12); UV $\lambda_{max}^{CHCl_3}$ nm 243 (ε 12052); IR ν_{max}^{KBr} cm^{-1}; 1771, 1738; ^1H, ^{13}C NMR (Tables 2 and 3); NOE-diff experiments: Proton irradiated (NOEs observed) H-6 (H-4, H-11); $[M+1]^+$ 249.

3.8. Compound **6**

$[\alpha]_D^{25} + 25^\circ$ ($CHCl_3$; *c* 0.08); UV $\lambda_{max}^{CHCl_3}$ nm 242 (ε 75927); IR ν_{max}^{KBr} cm^{-1}; 3200, 1738; ^1H, ^{13}C NMR (Tables 2 and 3); $[M+1]^+$ 251.

3.9. Compound **7**

UV $\lambda_{max}^{CHCl_3}$ nm 243 (ε 60507); IR ν_{max}^{KBr} cm^{-1}; 1763, 1737; ^1H NMR (Table 2); $[M+1]^+$ 247.

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