Selective Deacetylation of Zaluzanin D Using Transformed *Escherichia coli* Cultures

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The use of conventional and unconventional reaction methodology for the hydrolysis of the acetate group in zaluzanin D (**1**) resulted in hydration of the 11,13 exocyclic bond along with deacetylation. But the microorganism *E. coli* selectively cleaved the acetate group to yield zaluzanin C (**4**).

Key words: Zaluzanin, *E. coli*, Unconventional Reactions

Introduction

Sesquiterpene lactones have always been of interest to natural product chemists for their wide range of biological activities (Wedge *et al.*, 2000) especially in the field of pharmaceutical chemistry (Fraga, 1997). Literature reports indicate that the activity of the compounds lie in the α,β-enone system present in the lactone ring (Lamare and Furtoss, 1990). *In vitro* studies have also proved the capability of this functional group to form a Michael adduct with proteins (Mabry and Gill, 1979).

In our pursuit to gain further insight into the correlation between structure and biological activity of the sesquiterpene lactone zaluzanin D (**1**) several modifications of this compound were carried out (Krishna Kumari *et al.*, 2003a). For this purpose phytopathogenic fungi were used as biocatalysts as they were known to carry out selective transformations in systems involving multiple functionalities (Hashimoto *et al.*, 2001). The products obtained were tested for their antifungal activity against fungi of commercial importance and the results compared (Table I).

The products 11,13-dihydrozaluzanin D (**2**), 11,13-dihydrozaluzanin C (**3**) and zaluzanin C (**4**) (Fig. 1) showed decreased activity against phytopathogenic fungi compared to that of **1** (Table I). In case of compounds **2** and **3** the decrease in activity was expected as the 11,13 exocyclic double bond had been reduced, but surprisingly compound **4** also showed reduction in activity even though in this case the double bond was intact.

Table I. Growth inhibition of plant pathogenic fungi* tested against compounds **1**, **2**, **3** and **4** by poison food technique (Krishna Kumari *et al.*, 2003b).

	Growth inhibition (%) 20 mg/100 ml media of compound			
Fungus	**1**	**2**	**3**	**4**
Botrytis cinerea	77	32	11	49
Curvularia lunata	75	26	9	61
Colletotrichum lindemuthianum	59	16	5	28
Fusarium oxysporum	58	14	0	31
Rhizoctonia solani	100	23	0	87

* Obtained from Mycology Laboratory of TRG CNP, Spic Science Foundation.

This might be attributed to an increase in polarity of the molecule. To confirm this we wanted to introduce a ketone functionality at C-3 position, thereby reducing the polarity of the molecule and creating another α,β-enone system which could result in increasing the activity of the molecule.

To introduce the ketone functionality, the acetyl group at C-3 position had to be hydrolysed. Initial attempts to carry out this selective hydrolysis to yield **4** using *Sclerotinia sclerotiorum* was sluggish and time consuming (10 d). Hence, base hydrolysis of the ester was carried out under different conditions but in all the cases the product obtained was 11-hydro-13-hydroxy-zaluzanin C (**5**). Consequently a culture of *E. coli* was used to carry out the selective deacetylation of **1** to yield **4** in good quantities in a short time (24 h).

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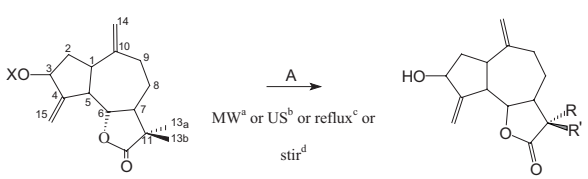
Materials and Methods

General experimental procedures

Melting points were determined with a Mettler-Toledo melting point apparatus and are uncorrected. ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on a Bruker DPX200 spectrometer with TMS as internal standard. IR spectra were recorded on a Bruker FT-IR instrument. Reactions were carried out using a modified microwave oven (2450 MHz) and an ultrasonicator (38 kHz).

*Synthesis of compound **5***

Compound **1** (40 mg) was dissolved in THF (10 ml) to which an equimolar amount of base (Fig. 1) in 10 ml of water was added. This reaction mixture was subjected to experimental conditions and observed for disappearance of starting material. The solvent was then evaporated and the product extracted with EtOAc. This resulted in a single polar product (more than 70% in all cases), which was subjected to various spectroscopic analyses. During the course of the reaction an intermediate was observed whose colour (in 10% H_2SO_4/MeOH spray) and R_f (0.3) in TLC (EtOAc/hexane 2:3 v/v) matched with that of **4**. However, at no time during the reaction this product was formed in any sufficient amount (< 15%).



	X	13a	13b
1	Ac		$=CH_2$
2	Ac	CH_3	H
3	H	CH_3	H
4	H		$=CH_2$

	R	R′
5	CH_2OH	H
4		$=CH_2$

Fig. 1. Structures of zaluzanin D (**1**), 11,13-dihydrozaluzanin D (**2**), 11,13-dihydrozaluzanin C (**3**), zaluzanin C (**4**), and synthesis of 11-hydro-13-hydroxy-zaluzanin C (**5**). A, NEt_3 or NH_3 or KOH or NaOH or $NaHCO_3$, *E. coli*. [a] Microwave irradiation (max. 2 min). [b] Ultrasound (max. 1 h). [c] Reflux (more than 6 h). [d] Stir (more than 12 h).

Culture medium

Luria Bertani (LB) medium (Sambrook *et al.*, 1989) containing NaCl (10 g/l), yeast extract (10 g/l) and tryptone (5 g/l) was dissolved in 100 ml of distilled water and pH adjusted to 7 using NaOH. The mixture was filled into a cotton-plugged 250 ml conical flask, autoclaved and used for the experiments.

*Incubation of compound **1** with transformed E. coli*

Sterilized LB medium was inoculated (100 μl) with starter culture (overnight grown) of transformed *E. coli* (Sambrook *et al.*, 1989) DH-5α+ and BL-21-DE(3)+ (commercially available). Both these strains contain the plasmid pRSET B (commercially available) that has an ampicillin resistant gene. This helped to prevent contamination by unwanted microorganisms as the broth also contained ampicillin (100 μg/ml). The flasks were then incubated at 37 °C and 120 rpm. The growing cultures were monitored for absorption at 600 nm until they reached an optical density in the range of 0.5 – 1 (log phase). 40 mg of **1** dissolved in 2 ml of acetone were added to the flask under shaking and on completion after 24 h the incubation was stopped, and the medium saturated with NaCl. The broth was then extracted with EtOAc (100 ml), dried over Na_2SO_4 and concentrated under vacuum. The crude extract (45 mg) was purified by silica gel column chromatography using *n*-hexane/EtOAc (100:0–0:100). The yield in all the cases was around 70%.

Zaluzanin D (**1**): M.p. 103–104 °C. – $[a]_D^{25}$ +21.43° ($CHCl_3$; *c* 0.28). – UV ($CHCl_3$): λ_{max} = 246 nm (ε 25302). – IR (KBr): ν_{max} = 1756, 1732 cm^{-1}. – ^{1}H, ^{13}C NMR: see Table II. – MS: *m/z* = 289 $[M + 1]^+$.

Zaluzanin C (**4**): M.p. 95–96 °C. – $[a]_D^{25}$ +50° ($CHCl_3$; *c* 0.1). – UV ($CHCl_3$): λ_{max} = 242 nm (ε 59290). – IR (KBr): ν_{max} = 3200, 1748 cm^{-1}. – ^{1}H, ^{13}C NMR: see Table I. – MS: *m/z* = 247 $[M + 1]^+$.

11-Hydro-13-hydroxy-zaluzanin C (**5**): $[a]_D^{25}$ +78.33° ($CHCl_3$; *c* 0.22). – UV ($CHCl_3$): λ_{max} = 248 nm (ε 31345). – IR (KBr): ν_{max} = 3187, 1745 cm^{-1}. – ^{1}H, ^{13}C NMR: see Table II. – MS: *m/z* = 265 $[M + 1]^+$.

Results and Discussion

The IR of compound **5** showed absence of an acetyl carbonyl peak which was also confirmed by

Table II. ^{13}C and ^{1}H NMR chemical shift values [ppm (coupling constant, *J* in Hz)] of compounds **1**, **4** and **5.**

	1		**4**		**5**	
C H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	44.5		44.1		43.5	
2	36.4		39.0		38.5	
3	74.6	5.56 m	73.5	4.57 tt (7.5, 3)	73.5	4.63 m
4	147.7		153.0		153.4	
5	45.2		45.5		49.5	
6	83.7	4.07 t (9.3)	83.8	4.10 t (9.2)	83.9	3.91 t (9.2)
7	50.2		49.9		43.1	
8	30.5		30.5		32.3	
9	34.4		34.1		35.9	
10	148.0		147.9		148.8	
11	139.5		139.6		42.9	
12	169.8		169.9		178.9	
13a	120.2	6.22 d (3.5)	120.1	6.21 d (3.5)	58.6	3.69 dq (3, 12)
13b		5.51 d (3.5)		5.49 d (3.5)		
14a	113.4	4.95 s	114.3	5.00 s	113.5	4.86 s
14b				4.94 s		4.79 s
15a	114.3	5.49 t (2)	111.2	5.46 t (1.8)	111.0	5.46 t (1.8)
15b		5.29 t (1.9)		5.33 t (1.8)		5.33 t (1.8)
Me–*C*O	170.6					
Me–CO–	21.1	2.11 s				

the disappearance of a signal at d 170.6 in the ^{13}C NMR spectrum (Table II). Upfield shift in the range of the C-3 proton from δ 5.56 to δ 4.54 clearly established the hydrolysis of the acetyl group to a hydroxyl group. Further scrutiny of the ^{1}H NMR spectrum revealed that the methylene protons of the 11,13 exocyclic double bond have been replaced, as shown by a multiplet at δ 3.69. This correlated with the ^{13}C NMR spectrum, which showed a new signal at δ 58.6 accounting for a methylene signal. Thus the compound was suspected to be **5** resulting from the hydroxylation of the 11,13 double bond. Perusal of literature (Wedge *et al.*, 2000) showed that the spectral values of **5** matched with the reported values, thus confirming the compound to be 11-hydro-13-hydroxy-zaluzanin C.

The spectral values of **4** matched with the literature reports of zaluzanin C (Krishna Kumari *et al.*, 2003a) confirming the product formation.

Similar experiments were also carried out with the non-transformed *E. coli* cultures DH-5α and BL-21 to investigate the possibility of deacetylation being carried out by the action of β-lactamase present in the transformed cultures due to vector introduction. In all the cases the product was found to be compound **4**.

Encouraged by the results, other substrates like swietenine (Anand Solomon *et al.,* 2003) with an ester functionality were incubated with *E. coli*, but the microorganism failed to modify the substrate. However this environmentally benign method has an edge over the usual synthetic techniques for its selectivity.

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Anand Solomon K., Malathi R., Rajan S. S., Narasimhan S., and Nethaji M. (2003), Swietenine. Acta. Cryst. E **59**, 1519–1521.

Fraga B. M. (1997), Natural sesquiterpenoids. Nat. Prod. Rep. **14**, 145–162.

Hashimoto T., Noma Y., and Asakawa Y. (2001), Biotransformation of terpenoids from the crude drugs and animal origin by microorganisms. Heterocycles **54**, 529–559.

Krishna Kumari G. N., Masilamani S., Ganesh M. R., and Aravind S. (2003a), Microbial transformation of zaluzanin-D. Phytochemistry **62**, 1101 –1104.

Krishna Kumari G. N., Masilamani S., Ganesh M. R., Aravind S., and Sridhar S. R. (2003b), Zaluzanin D: A fungistatic sesquiterpene from *Vernonia arborea*. Fitoterapia **74**, 479 –482.

Lamare V. and Furtoss R. (1990), Bioconversion of sesquiterpenes. Tetrahedron **46**, 4109–4132.

Mabry T. J. and Gill J. E. (1979), Sesquiterpene lactones and other terpenoids. In: Herbivores: Their Interaction with Secondary Plant Metabolites (Rosenthal G. A. and Janzen D. H., eds.). Academic Press, New York, pp. 502–538.

Sambrook J., Fritsch E. F., and Maniatis T. (1989), Molecular Cloning – A Laboratory Manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y., USA.

Wedge D. E., Galindo J. C. G., and Macias F. A. (2000), Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. Phytochemistry **53**, 747 –757.