FUNGAL MEDIATED BIOTRAMSFORMATION OF E-GUGGULSTERONE

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ABSTRACT

Biotransformation of E-Guggulsterone pregna-4, 17(20)-cis diene 3, 16 dione(I) by Cunnignhamella elegans resulted in the formation of four new hydroxyl derivatives identified as 7- â hydroxy pregn 4,17(20)-trans diene 3,16-dione (2), 7- â hydroxy pregn 4,17(20)-cis diene 3,16-dione (3), 7-â hydroxy pregn 4,ene 3,16-dione (4) and 7- â, 15 â Dihydroxy pregn 4 -ene 3,16-dione (5).the structure of these compounds were elucidated on the basis of H^1 and C^{13} NMR spectroscopic technique.

Key words: E-Guggulsterone, Cunnignhamella elegans, Microbial transformation

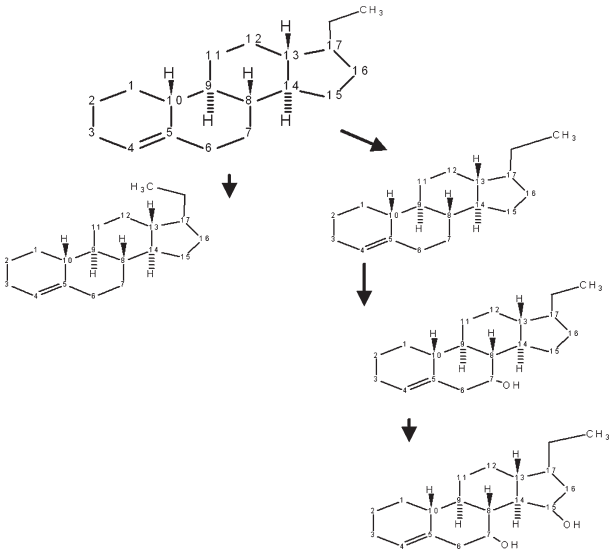
1. INTRODUCTION

The pregnane type of steroid, E-guggulsterone (I) has been isolated from the gum resins of *Commiphora mukul* [Atal CK, 1975]. The gum resin of C.mukul has been used in the treatment of ulcers, helminthes, rheumatoid arthritis, epilepsy and hyperlipemia the ancient Indian system of medicines [Medicinal Plants of India, 1987]. The gum extract of *C.mukul* called guggul lipid has been found to be safe and effective lipid lowering agent comparable in efficiency of other marketed drugs [Pandy VN, 1992]. Further pharmacological studies on the pure constituents of gum resin revealed that compound I has pronounced hypolipemic and hypocholesterimic activity. It also exhibited cytotoxic activity against the human lung cancer.

Microbial transformation of E-guggulsterone pregna-4, 17(20)-cis diene 3, 16 dione (I) with various fungal organisms like *Cunnignhamella elegans*, *Pencilium notatum* and *Trematus versicolor* carried out in an effort to obtain new metabolites[Patil VD, 1992]. From the screening experiments *C.elegans* was selected as best yielding microorganism. The metabolites 2 exhibited antibacterial and antifungal activity against P.putida, E.coli where compound has not shown any activity [P Fernandes, 2003].

2. RESULTS AND DISCUSSIONS:

The incubation of compound I with C. elegans for 8 days produced a crude organic extract that was subjected to TLC to obtain more polar metabolites 2-5, metabolites 2and 3 are inseparable due to paucity of sample and exhibited (m/z 328) molecular ion peak at m/z 328 (C21H28O2). The H^1 NMR spectrum of the mixture of 2 and 3 revealed that it was Z and E isomeric mixture of hydroxyl derivatives of substrate I. The assignment of the stereochemistry of the hydroxyl group at ä 3.46 was based on the comparison of spectral data of metabolites 4 and 5.



1. Microbial transformation of E-Guggulsterone (I) by C.elegans

Compound (4) C21H30O3 exhibited molecular ion at m/z 330.21.determined by GCMS. The IR spectrum exhibited the absorption band at 3400cm-1 indicating the presence of the free hydroxyl group.

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The H^1 NMR spectrum of compound 4 was remarkably different from the compound I in several aspects. First, the signal at the ä 6.45 corresponding to H-20 of exocyclic double bond in compound I was not observed in derivative 4. The up fields' 3-H signals at ä 1.01 resonating as a triplet, was assigned to the C-21 methyl in compound 4 (which appeared as doublet at ä 1.86 in compound I). In addition down field signals at ä3.40 was assigned to the methine H-7 geminal to the hydroxy group. Large coupling constants and peak pattern of H-7 hydroxyl indicated the â stereochemistry of the C-7 hydroxyl group.

The position of the C-7 was also confirmed by C^{13} NMR. The down fields at ä 75.0 was assigned to C-7 in compound 4 after comparison with that of I based on the broad band decoupled C^{13} NMR experiments. The H-6 methylene protons (ä 2.49 and 2.81) and H-8 methine proton resonating at ä 1.79 exhibited heteronuclear interaction with C-7 (ä75.00). Further more, the methyl protons resonated at ä 1.01 (C-21) and ä 0.75 (C-18) indicating the 3j heteronuclear interaction with C-17 at ä64.5. In addition, the C-20 methylene protons at ä1.29and j22 showed 3j interaction with C-21 (ä 13.8) as well as with carbonyl carbon resonating at ä 218.0(C-160) , hence exhibiting close proximity to the carbonyl carbon.

The GC-MS of the compound 5 exhibited the M^+ at m/z 346.21 corresponding to the molecular formula C21H34O4. An IR absorption band was observed at 1510cm-1(OH). The HNMR spectrum of compound 5 closely resembled that of compound 4 with no additional signals. At ä 4.20 which could be assigned to H-15 on the basis of observe HNMR coupling pattern of H-15 (J=7.0H2). The signal at ä3.60 was due to H-7 geminal to OH group structure 5 was further investigated by ^{13}C NMR. (HMQC and HMBC experiment) H-15 (ä 4.20) showed 4jhetero nuclear coupling with C-16 carbonyl resonating at ä 217.6 and 3j interaction with C-13 (ä42,.8) the H-20 methylene protons also showed heteronuclear correlation with closely lying C-36 (ä217.6) .

3. EXPERIMENTAL SECTION:

General experimental procedure: Optical rotation was determined in methanol on a polarimeter. UV spectra were obtained on UV spectrophotometer (Nanodrop) IR spectra were recorded on NICOLET-AVATAR 330 FTIR spectrophotometer. The HNMR spectra (äppm J in H2) was recorded in Bruker Avance II 400 NMR spectrometer, ^{1}HNMR, ä (CDCl3 400.1324MHz) spectrometer whereas ^{13}C NMR recorded in the same solvents on Bruker AM-400MHz instrument at 100 MHz . Instrument at 75 MHz. Mass were recorded on high resolution mass spectrometer equipped with data system in combination with GC under the following condition. Capillary column (30M lenth), 0.25mmID, initial column temperature 80^0 C programmed to 250^0 C at the rate of 10^0 C/ mnt. The helium as carrier gas with flow rate 1ml/mnt. The purity of the compound was checked on TLC (Si gel pre coated plates, Merck PF254 20*20 0.25mm)

Isolation of compound E-guggulsterone from the gum resin of Commiphora Mukul:

The gum resin (5kg) was purchased from the Udupi Ayurveda herbal store. The gum resin extracted with hexane. The hexane extract was concentrated, concentrated extract saponified with 40% of Potassium hydroxide for 24 hours at 60^0C . Further methanol layer evaporated to dryness. To the saponified mass equal amount of water was added this mixture has been extracted with methylene dichloride or dichloromethane. The MDC layer washed with water until pH becomes neutral. Evaporate the DCM layer, re crystallized with the hexane to get pure compound. Melting point of the isolated compound is 230 to 234^0C, $[á]^{25}$D- 66^0(e 0.03: in MeOH), ^{1}HNMR ä (CDCl3 400.1324 MHz)6.45, 5.71, 1,86. GCMS 312 M^+, 298, 297, 270, 255, 227, 189.

Fermentation Procedure:

Sabouraud Dextrose Broth

(EMD Cat. No. 1.08339.0500/5000, © Merck KGaA) Typical Composition (g/liter), Peptone from meat 5.0; peptone from casein 5.0; D(+)glucose 20.0Preparation: Suspend 30 g/liter, if necessary dispense into smaller vessels, autoclave (15 min at 121 °C). pH: 5.6 ± 0.2 at 25 °C.

Stock cultures of selected species were cultured on PDA medium and incubated at 27^o C temperature under aerobic conditions. The fungus was subcultured regularly within 30 days and stored at 4^oC temperature. The slant cultures were used for suspension culture preparation. All media were prepared with distilled water and pH was adjusted to 5.6, prior to sterilization at 121ÚC for 15 minutes.

Time course of experiment:-

In each transformation experiment the fungus was grown on a shake culture at 28^0C for 3 days in 8 conical flasks (250ml), each containing sterile medium (100ml). Compound I (100mg) in acetone (16ml) was distributed equally among the flasks and the organisms was cultured for 8 more days. Everyday, one flask of the culture medium was harvested , filtered , saturated with NaCl and extracted with ethyl acetate and then the organic solvents was evaporated under vacuum . The crude concentrated extract was monitored by TLC.

Large scale fermentation:

The following procedure was for the each fungus was grown on shake flasks culture (100ml of medium in 50ml of conical flask 250ml) and incubated for the 3 days at 28^0 C. The substrate 1gm (500mg) was dissolved in acetone (20ml) and the solution was evenly distributed among 100 conical flasks. The incubation was allowed to continue for further 8 days. Then the broth was filtered and extracted with ethyl acetate the mycelium was also washed with ethyl acetate and the total extract was combined and dried over anhydride sodium sulphate and concentrated to a brown gum. The ethyl acetate extract obtained from the each fungus was purified by TLC using 60% of ethyl acetate and petroleum ether as developing solvent

COMPOUNDS 2 AND 3:-

Oily 9mg UV (methanol) ëmax 278, IR (CHCl3) 3351cm^{-1}, 1752 cm^{-1}, Bruker Avance II 400 NMR spectrometer, ^{1}HNMR, ä (CDCl3 400.1324MHz) 5.62/6.52(1H.QJ=7.5 Hz, H-20), 5.72 (1H br.s.H4) 3.62 (1H .m..H-7)2.08/1.8 (3H.d.J=7.5Hz H-21) ^{13}C NMR ä (CDCl3 100.6228MHz). GCMS m/z 328. M+, 313 (72) 310 (100) 295 (24)228 (60) 175 (72) 136 (77).

COMOUND 4:-

Oil 10mg [á] D^{25} -80^0 (C 0.04 MeOH) UV (MeOH) ëmax (lower) 240, FTIR (CHCl3) 3390cm^{-1}, 1624cm^{-1}. ^{1}HNMR, ä (CDCl3 400.1324MHz), ^{13}C NMR ä (CDCl3 100.6228MHz) see table no 1. Mass (m/z relation) 330M^+ GCMS 330$M^{+,}$ 312, 315, 177, 124, 55.

COMPOUND 5:-

15mg [á] D^{25} -122^0 (C 0.05 MeOH) UV (MeOH) ëmax (lower) 237 , FTIR (CHCl3) 3510cm^{-1}, 1634cm^{-1}. ^{1}HNMR, ä (CDCl3 400.1324MHz), ^{13}C NMR ä (CDCl3 100.6228MHz) see table no 1. Mass (m/z relation) 346M^+ GCMS 330$M^{+,}$ 330, 328, 313,257, 196, 83.

Table-1

List of C13NMR:		
	4	5
C1	36.3	36.6
C2	34.5	32.9
C3	198	198.0
C4	125	125.0
C5	168	168.0
C6	43.3	42.0
C7	75.0	74.8
C8	42.8	37.0
C9	51.7	50.9
C10	38.9	38.1
C11	21.2	20.8
C12	38.5	37.9
C13	43.8	42.3
C14	19.8	56.0
C15	12.3	72.0
C16	218.0	217.0
C17	64.5	64.0
C18	13.6	15.9
C19	17.6	17.2
C20	18.1	16.9
C21	12.3	13.9

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