

Phytochemical investigation of *Sapium baccatum*: Identification of 3 α -hydroxy-1 α ,2 α -epoxy lupan

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Phytochemical investigation was carried out on the crude toluene extract of the stem bark of *Sapium baccatum*. The compounds were isolated and purified by column chromatography followed by crystallization. The structure of all compounds was established by chemical characterization and by the analysis of spectroscopic (IR, NMR and Mass) data and in some cases by comparison with authentic sample. Five compounds, namely 3,3'-di-*o*-methyl ellagic acid, taraxerone, 1-hexacosanol, taraxerol, baccatin along with a new compound 3 α -hydroxy-1 α ,2 α -epoxy lupan were identified.

Keywords: *Sapium baccatum*, phytochemical investigation.

Introduction

Natural products play a major role in drug discovery, and nearly half of all newly introduced drugs into the market in the past two decades are natural products or their derivatives¹⁻³. Many medicinal plants have been found in Darjeeling foothills. Local people in this place are used these plants as their traditional folklore medicine. Now, our interest is to collect the medicinal plant from the Darjeeling foothills, isolate the products, characterization of the isolated product and investigate their biocidal activities. For this we collect the stem bark of *Sapium baccatum* ROXB from Darjeeling foothills, isolate the products, characterization of the isolated product and investigate their biocidal activities.

Isolation process:

Dried and powdered stem bark of *Sapium baccatum* ROXB (2 kg) was extracted with toluene in a soxhlet apparatus for 20 h. On cooling the toluene extract, a yellow insoluble compound separated out, this was collected by filtration. This was purified and identified as 3,3'-di-*o*-methyl ellagic acid⁴. From the clear filtrate, toluene was distilled off and the residual

gummy solid (30 g) was taken up in ether (2 L). The clear ether solution was washed with cold water till washings were neutral and dried over anhydrous Na₂SO₄.

The solvent was evaporated when the neutral material (11 g) was obtained as a yellow gummy solid, which after chromatography and crystallization from chloroform-methanol mixture gave shining crystals (1.3 g) having m.p. 238–240°C and found identical in all respect with authentic sample of taraxerone⁵ (mixed m.p. Co-IR; Co-TLC). Other compounds isolated were 1-hexacosanol⁶, taraxerol⁵ and baccatin⁷ along with the new compound F. 1-Hexacosanol, taraxerol and baccatin were identified by comparison (mixed m.p. Co-IR; Co-TLC) with respective authentic samples.

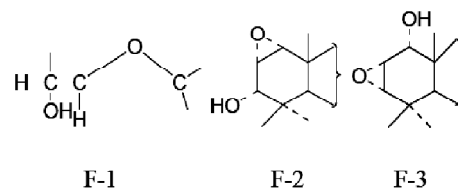
Characterization of compound F: Identification of 3 α -hydroxy-1 α ,2 α -epoxy lupan:

Compound F was purified by crystallization from chloroform-methanol mixture to afford white crystal of m.p. 203–204°C. IR absorption peak at 3530 cm⁻¹ and 880 cm⁻¹ indicated the presence of hydroxyl group and epoxide ring in F. It did not res-

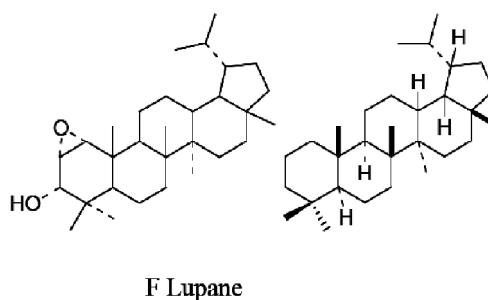
pond to TNM test for the presence of unsaturation and the absence of any hydrogen was also indicated by Beilstein test. UV spectrum showed no absorption in the region 220–300 nm. The mass spectral analysis indicate the molecular ion peak at 442 $[M]^+$ with other peaks at m/z 427 $[M-Me]^+$, 424 $[M-H_2O]^+$, 409 $[427-H_2O]^+$, 406 $[424-H_2O]^+$, 399 $[M-CHMe_2]^+$, 381 $[399-H_2O]^+$, 331, 272, 231, 217, 191, 169, 163, 149, 55 (base peak). From elemental analysis and mass spectral data the compound F was shown to have the molecular formula $C_{30}H_{50}O_2$. 1H NMR spectrum of compound F shows the presence of eight methyls in the region 0.75 to 1.17 ppm. It shows a doublet of triplet centred at 1.88 ppm and another at 2.04 ppm each integrate for one proton, it further showed two doublets centred at 2.31 (J 3.5 Hz) and 3.05 (J 3.5 Hz) ppm, a sextet (ddd) centred at 3.53 ppm (J 3.5 and 4.47 Hz), another doublet of doublet at 3.64 ppm with J 6.04 and 8.7 Hz all integrated for a single proton each. The nature and relationship of these protons were established by carrying out the following experiments.

D_2O exchange experiments caused collapse of the doublet at 2.31 ppm and the doublet of doublet at 3.64 ppm appeared as a doublet with J value of 6 Hz. This clearly indicates that the doublet at 2.31 ppm is due to hydroxyl proton of the grouping $-CH-OH$ and the methine proton germinal to $-OH$ group has a neighbouring proton with small coupling constant of 6 Hz. This proton (at 3.64 ppm) on irradiation caused the appearance of a singlet at 2.31 ppm and collapse of the doublet of a doublet at 3.53 ppm to a broadened singlet, thus this proton (i.e. at 3.64 ppm) is α to the $-CH-OH$ proton (F-1); irradiation at 3.53 ppm caused collapse of the quartet at 3.64 ppm to a flattened doublet, and the doublet at 3.05 ppm to a sharp singlet. Hence, the proton centred at 3.05 ppm is related to that centred at 3.53 ppm. Thus the above experiments indicate the presence of partial skeleton F-1 in F. Two such structures of ring-A can be drawn as F-2 and F-3.

Construction of drying models showed that the



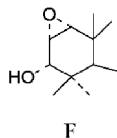
epoxide ring at $1\alpha,2\alpha$ -position with the $-OH$ group at C-3 position as quasi-equatorial is the most probable conformation with least interaction. Thus the structure of compound F has been assigned as lup- $1\alpha,2\alpha$ -epoxy- 3α -ol,7.



HETCORR NMR spectrum showed that the carbon at 67.56 ppm is attached to proton at 3.64 ppm, carbon at 56.06 ppm is related to the proton at 3.53 ppm and that 65.06 ppm is attached to proton appearing at 3.05 ppm. ^{13}C NMR spectrum displayed the presence of 30 carbons in the region 14.6 to 67.6 ppm and the DEPT experiment showed the presence of 8- CH_3 as quartets, 8- CH_2 as triplets 9-doublets (for $-CH$ carbon) and 5 singlets (for $-C-$ carbon). The ^{13}C NMR signals are assigned on the basis of assignments to lupane skeleton and the present XH correlation spectrum (Table 1).

Construction of drying model showed that the epoxide ring with the least interaction is only possible if it is attached to the ring-A in its boat form at $1\alpha,2\alpha$ -position. In this conformation the C- 4β methyl is quasi-equatorial and the C- 10β methyl is quasi axial with flag pole interaction with C- 3β hydrogen. The 1,3-methyl-methyl interactions between C-24 and C-25 methyl groups become minimize in this arrangement. It is further apparent that in order to have minimum flag pole interaction the substituent at C-3 should be quasi equatorial. Thus it is assumed that

the compound F will have the epoxide ring in ring-A of lupane skeleton and should have the structure as given below:



The structure F is proved by LAH reduction of lup-1 α ,2 α -epoxy-3-one [prepared by treatment of lup-1(2)-en-3 one with alkaline H₂O₂] which furnished a compound identical with F (¹H NMR, Co-TLC, CO-IR, m.m.p.).

Table 1. Comparison of carbon-13 NMR of 7 with lupane

Carbon	F	Lupane
1	56.1(d)	40.5(t)
2	65.1(d)	18.8(t)
3	67.6(d)	42.3(s)
4	40.4(s)	33.3(s)
5	43.2(d)	56.5(d)
6	18.8(t)	18.8(t)
7	33.1(s)	34.6(s)
8	40.6(s)	41.3(s)
9	40.4(d)	50.4(d)
10	32.8(s)	37.6(s)
11	21.4(t)	21.0(t)
12	24.7(t)	27.1(t)
13	38.1(d)	38.1(d)
14	43.5(s)	43.3(s)
15	27.5(t)	27.5(t)
16	35.5(t)	35.8(t)
17	43.2(s)	43.2(s)
18	47.6(d)	47.8(d)
19	44.8(d)	44.9(d)
20	27.9(d)	29.5(t)
21	22.2(t)	22.0(t)
22	40.1(q)	40.5(q)
23	21.9(q)	33.4(q)
24	29.3(q)	21.7(q)
25	16.3(q)	16.1(q)
26	15.9(q)	16.2(q)
27	14.6(q)	14.6(q)
28	18.1(q)	18.2(q)
29	15.1(q)	15.2(q)
30	23.0(q)	23.0(q)

Biocidal activity of the isolated compounds:

In the present study, five different fungal pathogens were choosen (*Colletotrichum camelliae*, *Fusarium equisiti*, *Alternaria alternata*, *Curvularia eragrostidis*, *Colletotrichum gloeosporioides*) for *in vitro* antifungal assay. Antibacterial assay were performed against four bacterial pathogens (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobactor*).

Suitable strains of these organisms were procured from the Microbiology Laboratory of our Institute. MICs (Minimum inhibitory concentration) of the 3 α -hydroxy-1 α ,2 α -epoxy lupan (F) against bacterial and fungal pathogens have been presented in Tables 2 and 3, respectively. The antifungal and antibacterial media used are as follows. For nutrient agar (NA) 28 g of media (HiMedia) was suspended in 1000 ml of distilled water according to the manufacturer's protocol. It was boiled to dissolve the medium completely at sterilized by autoclaving at 15 lbs pressure (121° for 15 min). The nutrient agar contained peptic digest of animal tissue (5 g), sodium chloride (5 g), beef extract (1.5 g), yeast extract (1.5 g), agar (15 g) and distilled water (1000 ml). pH was adjusted to 7.2. For preparation of PDA (potato-dextrose-agar) peeled potato was cut into small pieces and boiled in required volume of distilled water. The mixture was filtered through muslin cloth and the extract was mixed with dextrose and agar. The resultant mixture was heated in order to dissolve. Finally the media was sterilized at 15 lbs (121° for 15 min). Composition of the media was peeled potato (400 g), dextrose (20 g), agar (20 g) and distilled water (1000 ml). pH was adjusted to 6.0. DMSO (dimethyl sulfoxide) was used as solvent to prepare different concentrations of the 3 α -hydroxy-1 α ,2 α -epoxy lupan (F). Solvent control (DMSO) was also maintained throughout the experiment. All experiments were performed in Petri dishes and were incubated at 37° for 48 h. The required media (either PDA or NA) was poured in a Petri dish and allowed for solidification. After solidification wells or cups

were made by inserting a cork borer in the media. The numbers of wells were made according to the requirement of the experiment. Fungal spores were suspended on the PDA media before well or cup formation. Test solution (100 ml/well) was poured in the well or cup. Additionally slide germination method was also used for determination of antifungal activity (Table 4). We compared the antifungal activities of these compounds with that of Bavistan and antibacterial activity with that of ampicillin, a β -lactam antibiotic.

Table 2. MIC of isolated compounds against different bacteria

Compounds	MIC in mg/ml against different bacterial strains			
	EC	BS	SA	EB
F	100	100	98	96
Ampicillin	128	64	64	128

BS: *Bacillus subtilis*, EC: *Escherichia coli*, SA: *Staphylococcus aureus*, EB: *Enterobacter*, MIC: Minimum inhibitory concentration.

Table 3. MIC of isolated compounds against different fungi

Compounds	MIC in mg/ml against different fungal strains				
	CG	FE	CE	AA	CC
F	7	19	38	7	6
Bavistan	3.5	3.5	3.7	4.0	4.2

CG: *Colletotrichum gloeosporioides*, FE: *Fusarium equiseti*, CE: *Curvularia eragrostidis*, AA: *Alternaria alternata*, CC: *Colletotrichum camelliae*.

Table 4. Antifungal properties of triterpenoids against five fungal pathogens by spore germination bioassay after 48 h of incubation

Fungal pathogen	3 α -Hydroxy-1 α ,2 α -epoxy lupan (F)		
	PG ^a	PI	AL ^b (mm)
CC	00	100	00
FE	00	100	00
AA	00	100	00
CG	00	100	00
CE	05	96	6.0

CG: *Colletotrichum gloeosporioides*, FE: *Fusarium equiseti*, CE: *Curvularia eragrostidis*, AA: *Alternaria alternata*, CC: *Colletotrichum camelliae*. PG: Percent germination, PI: Percent inhibition, AL: Average germ tube length, ^aBased on 200 spores, ^bBased on 25 germ tubes.

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