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Selenium Dioxide Oxidation of Oxime derivative of Lupanone and Antimicrobial activity of the Oxidized Products

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Abstract

Oxime derivative (1a) of lupanone (1) on oxidation with hydrogen peroxide and selenium dioxide in tertiary butanol furnishes a δ-lactone, 4,23,24-tri-nor-lupan-3→5-olide (2) and 1-carboxy-2,3-seco-lupan-3→1—olide (3). The structures of these compounds have been fully established on the basis of spectral data (IR, MS, PMR, and 13-C NMR) and by comparison with reported data. Both the compounds showed better antimicrobial activity against all the microorganisms studied compared to the parent natural product, lupanone.

Keywords: Oxime derivative, selenium dioxide, triterpenoid, biological activity.

Introduction

Exploration of the chemical constituents of plants and pharmacological screening of phytoconstituents is the basis for developing new lead molecules in natural product drug discovery research[1-3]. Search for bioactivity among the naturally occurring compounds is well documented in literature [4]. People have also reported the presence of triterpenoids in wide variety of medicinal plants [5]. But limited reports are available on the bioactivity of either the parent molecule or on their derivatives. Keeping this view in mind the present investigation is based on the transformative reaction on the triterpenoid oxime and the biological screening of the new derivatives. Although studies on oxidation of triterpenoid ketones with hydrogen peroxide and selenium dioxide have been reported[6-8] but no such work on the oxime derivative of triterpenoid ketones with this reagent has been reported so far. Thus in continuation of our studies on the transformative reactions on pentacyclic triterpenoids of lupane and friedelin skeleton[9-14] and in order to examine the nature of the products formed on the oxidation of oxime derivatives of 3-ketotriterpenoids having gem dimethyl group at C4, the oxidation of keto oximes of lupanone with hydrogen peroxide and selenium dioxide has been taken up and characterisation of the products (**A**, B) along with the evaluation of their preliminary biological activity are presented in this paper.

Material and Methods

Chemical part: M.ps are uncorrected. Petrol used had b.p. 60-80°. PMR spectra (Chemical shifts in δppm; TMS as internal standard) and 13-C NMR spectra were recorded in $CDCl_3$ on Bruker WH-400 and BrukerWH-270 with DEPT programme; IR spectra in nujol in Pye Unichem-300 spectrometer and mass spectra on a Varian MAT 711 at 70 eV. Column chromatography was performed over silica gel (60-80 mesh) and TLC on silica gel G.

***Oxidation of oxime* (1):** A solution of oxime derivative (**1**) (0.5g) in tertiary butanol (100ml) was refluxed with selenium dioxide (0.5g) and hydrogen peroxide (20ml, 25%) for 60 hrs. The completion of the reaction was indicated by deposition of black selenium metal. After recovery of the solvent by distillation, the residue was extracted with ether and separated into neutral and acid parts by usual method. The neutral part was chromatographed over silica gel column. The acid part was esterified with diazomethane and the reaction product chromatographed over silica gel column. Elution of the column with solvents of increasing polarities was performed and residue of the same polarity and same Rf value in TLC were combined together and purified by crystallizations.

Oxidation products of lupanone oxime (4): Characterisation of 4,23,24-tri-nor-lupan-3→5-olide (2): Compound **C** [0.14g; petrol-benzene (2:3)] was crystallized from $CHCl_3$-MeOH to furnish white solid, m.p. 250-251°; IR: 1750 cm^{-1} (δ-lactone); PMR: δ0.80, 0.87,0.90 and 1.08 (4s, 12H, 4 x t-CH_3), 0.75 (d, 3H, J = 7.5 Hz, sec-Me), 0.80 (d,3H,J = 7.5Hz, sec-Me), 2.6(m, 2H, -O-C-CH_2), 3.9 (t, 1H, J = 8Hz, -CO-O-CH-CH_2); MS: 400 [M^+], 384, 357, 219, 209, 206, 195, 179, 165, 163 (base), 149, 135, 123, 121, 119, 109, 107, 95, 93, 81 [Found: C, 80. 8; H, 11.0. Calc. for $C_{27}H_{44}O_4$: C, 80.94; H, 11.07%].

1-Carbomethoxy-2,3-seco-lupan-3→1-olide (3a): Compound **D'** [0.04g, petrol-benzene (2:3)] was crystallized from $CHCl_3$-MeOH mixture to afford white crystals, m.p. 202-203° C; IR: 1740 cm^{-1}(-COOMe), 1775 cm^{-1} (δ-lactone carbonyl); PMR: δ 0.76, 0.95, 1.11, 1.12 and 1.3 (6s, 18H, 6 x t-CH_3) , 0.78 (d, 3H, J = 6.5Hz, Me), 0.84 (d, 3H, J = 6.5Hz, -Me), 3.74(s, 3H, -$COOCH_3$), 4.45 (s, 1H, -O-CH)**;** MS: m/z 486 [M^+], 443, 397, 369, 191, 163, 123, 107, 93, 81, 69, 55 (base peak); [Found: C, 75.2; H, 9.74. $C_{31}H_{50}O_4$ requires: C, 76.5; H, 10.35%].

Antimicrobial activity: Fresh outer bark of *Xanthoxylum budrunga* collected from Sukna belt of foothills of Darjeeling in an early summer. The plants collected were shade dried at room temperature and mechanically reduced to coarse powder. The prepared powdered leaves were then used for further studies. The powdered plant material (2 gm) was extracted with toluene in a soxhlet apparatus for 72 h. The solvent was recovered at reduced pressure, which yielded a deep brown gummy residue (1.2 gm). This crude toluene extract of the plant was then fractionated over a column of silica gel of 60-120 mesh using petroleum ether and ethyl acetate with increasing concentration as eluent.

In the present study, five different fungal pathogens (*Colletotrichum camelliae*, *Fusarium equisiti*, *Alternaria alternata*, *Curvularia eragrostidis* and *Colletotrichum gloeosporioides)* were used for *in vitro* antifungal assay[15-16]. 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 triterpenoids against bacterial and fungal pathogens have been presented in Table 1 and 2 respectively. The antifungal and antibacterial media used are as follows. For nutrient agar 28 gm 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 (121^0C for 15 min.). The nutrient agar contained peptic digest of animal tissue (5 gm), sodium chloride (5 gm), beef extract (1.5 gm), yeast extract (1.5 gm), agar (15 gm) and dissolved water (1000 ml). p^H 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 dissolved 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 (121^0C for 15 min.). Composition of the media was peeled potato (400 gm), dextrose (20 gm), agar (20 gm) and dissolved water (1000 ml). P^H was adjusted to 6.0. DMSO (Dimethyl sulfoxide) was used as solvent to prepare different concentrations of the triterpenoids. Solvent control (DMSO) was also maintained throughout the experiment. All experiments were performed in Petri dishes and were incubated at 37 ^{o}C 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 µl/well) was poured in the well or cup. We compared the antifungal activities of these compounds with that of Bavistan and antibacterial activity with that of Ampicillin, a β-lactam antibiotic.

Seeds of rice (*Oriza sativa*), wheat (*Triticum aestirium*) and pea (*Pisum sativum*) were collected from local market. The assay seeds were shorted for uniformity of size and all damaged seeds were discarded. Before the bioassay seeds were washed with tap water and the surface were sterilized using NaCl (10% v/v) for 10 min followed by several washes in sterile distilled water. For testing phytotoxicity dehydrated ethanol was used as control. Bioassays were carried out using petridishes (90 mm diameter) containing a sheet of Whatman 1 filter paper as support. Test solutions (5 ml) was added to the filter paper in the petridish and dried completely *in vaccuo* at 40^0C. Five seeds from each category were placed on the filter paper and incubated for 7 days at 25^0C in the dark. The effects of the pure compounds were determined by measuring the elongation of roots and average for each concentration.

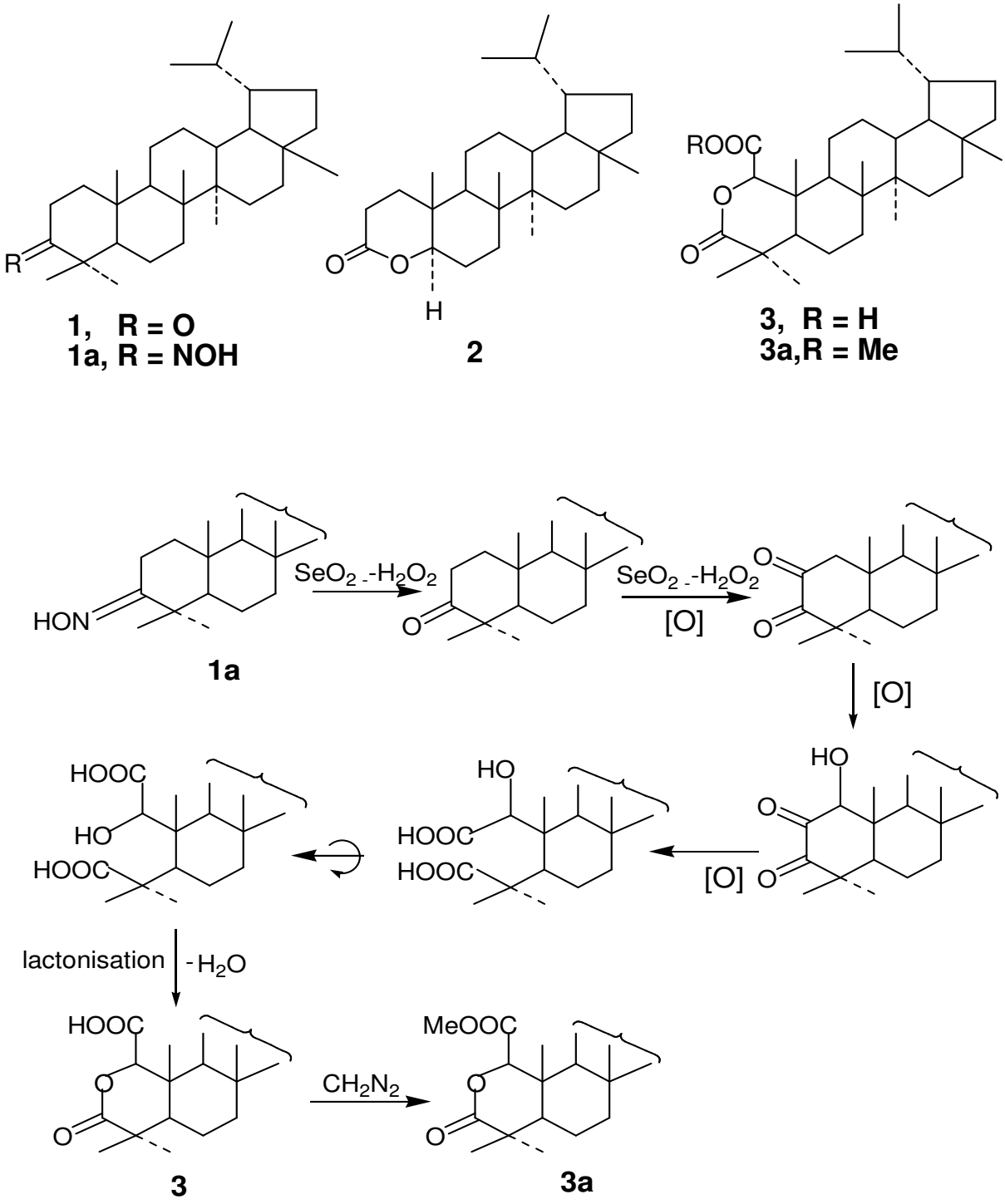
Results and Discussion

The oxime derivative **1a** in tertiary butanol was refluxed with selenium dioxide and hydrogen peroxide. The residue obtained after recovery of solvent by distillation was extracted with ether and separated into neutral and acid parts by usual method.

The neutral part of the reaction product of 1a on chromatography furnished a single compound A, m.p. 250-51°. It was analyzed for $C_{27}H_{44}O_2$, (M^+ 400) and IR spectroscopy showed a sharp absorption band at 1750 cm^{-1} indicating the presence of a δ -lactone carbonyl group. Its PMR spectrum showed sharp similarity with 4,23, 24-tri-nor-lupan- 3→5-olide[6] (2) that was confirmed by direct comparison (m.m.p., co-IR, and co-TLC) with an authentic sample.

The acid part on esterification with diazomethane afforded the methyl ester B that was analyzed for $C_{31}H_{50}O_4$, m.p. 202-03°C. Its IR showed absorption band at 1740 cm^{-1} and 1775cm^{-1} that indicated the presence of a methoxyl carbonyl group and the lactonic carbonyl group respectively. MS showed its molecular ion peak at m/z 486 (M^+). The structure of **B** was forthcoming from a study of its PMR spectrum which showed the presence of eight methyls that resonated in the region δ 0.76 – 1.3 ppm of which the peaks at δ 0.78 and 0.84 are doublets with J value 6.5Hz showing the presence of two secondary methyls and six tertiary methyls. The singlet at δ 3.78 ppm integrated for three protons is definitely due to carbomethoxyl protons and another singlet at δ 4.45 integrated for a single proton is attached to the carbon bearing the lactonic oxygen; further since there is no absorption peak in the region between δ 1.8 to 3.7 ppm, the carbon of methoxycarbonyl group is attached to the same carbon to which the lactonic oxygen is also attached, causing a downfield shift of the protons of carbomethoxyl group from the usual position of δ 3.68 ppm. Thus assuming that the carbonyl group of the lactone is formed by the hydrolysis of the oxime, the structure of the compound can be assigned as 1-carbomethoxy-2,3-seco-lupan-3→1-olide (3a), thus the corresponding acid B formed is 1-carboxyl-2,3-seco-lupan-3→1-olide (3).

In contrary to the earlier observation[17] that the nitrogen of the oximes remains intact during its reaction only with SeO_2, in the present case the addition of H_2O_2 has been found to remove the nitrogen during the course of the reaction. The presence of H_2O_2 along with SeO_2 may have oxidized the oxime 1a first to the ketone 1 which then undergo usual oxidation reaction to furnish compounds A and B. The formation of the compound 3 probably follows the pathway as shown in the scheme – 1



Scheme - 1

Antimicrobial activity: Although the compounds (1-3a), do not show any significant phytotoxicity when tested on a number of specimens (table 3) but showed prominent antimicrobial activities against the tested fungal and bacterial pathogens as evident from the experimental data tables 1 and 2. Compound 2 and 3a showed better activity against all the microorganisms compare to the parent natural product, lupanone (1) and their activity is found comparable to that of Ampicillin against *E. coli* and *Enterobactor*. The activity of compound 2 and 3a was nearly comparable to that of Bavistan, when tested against *Colletotrichum gloeosporioides* and *Colletotrichum camelliae*. Finally it can be concluded that the present study will be extremely helpful to enrich the present knowledge about triterpenoids of lupane skeleton and also help the researchers to develop newer generation of drugs based on such information about triterpenoids of lupane skeleton.

Conclusion

Elimination of nitrogen atom takes place during the SeO_2 – H_2O_2 oxidation of lupanone oxime to yield two different lactones. Both of them showed better antimicrobial activity against all the microorganisms studied compared to the parent natural product, lupanone.

Table-1
MICs of 1 to 3a against different bacteria

Compounds	MIC in µg/ml against different bacterial strains			
	EC	**BS**	**SA**	**EB**
1	100	130	150	150
2	150	100	200	130
3a	130	<150	100	100
Ampicillin	128	64	64	128

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

Table-2
MICs of 1 to 3a against different fungi

Compounds	MIC in µg/ml against different fungal strains				
	CG	**FE**	**CE**	**AA**	**CC**
1	5.00	20.0	35.0	20.0	39.0
2	4.87	19.5	40.0	19.5	<5.0
3a	<5.0	20.0	40.0	10.0	<5.0
Bavistan	3.50	3.50	3.70	4.00	4.20

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

Table-3
Phytotoxicity of the Compounds Based on the Length (in cm) of Roots after 7 Days

Compounds	Concentration (µg/ml)	Rice	Wheat	Pea
1	Control	0.5	1.10	1.53
	100	0.5	11.2	1.53
	250	0.5	1.13	1.53
	500	0.5	1.12	1.53
2	100	0.5	1.21	1.62
	250	0.5	1.22	1.64
	500	0.5	1.25	1.65
3a	100	0.5	1.09	1.65
	250	0.5	1.09	1.64
	500	0.5	1.11	1.63

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