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Transformative reaction on triterpenoids: action of hydrogen peroxide in presence of selenium dioxide on oxime derivative of taraxerone and antimicrobial activity of isolated compounds

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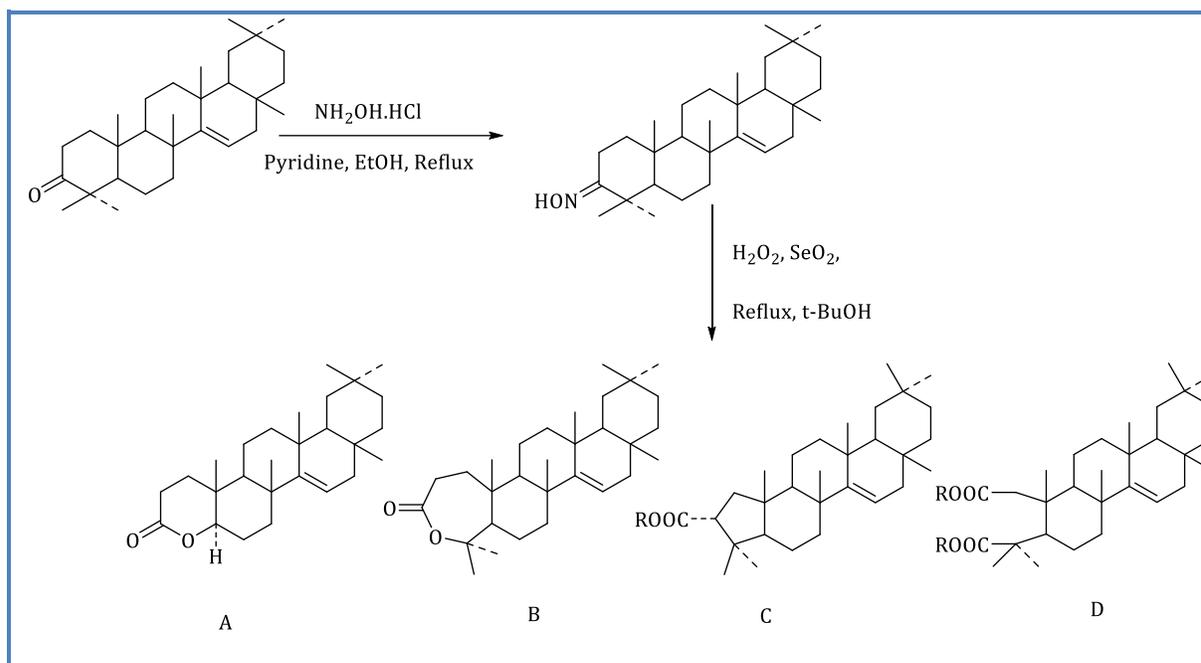
ABSTRACT

Although studies on oxidation of triterpenoid ketones with hydrogen peroxide and selenium dioxide have been reported, literature reports on the effect of the oxidizing agent on the oxime derivative of triterpenoid ketones are scanty. Thus in continuation of our previous studies on the transformative reactions on pentacyclic triterpenoids of lupane and friedelin skeleton and in order to examine the nature of the products formed on the oxidation of oxime derivatives of 3-keto-triterpenoids having gem dimethyl group at C4 and a double bond at ring D (between C14-C15), the oxidation of keto-oximes of taraxerone with hydrogen peroxide and selenium dioxide was taken up and characterisation of the products (A -D) along with the evaluation of their preliminary biological activity were studied in this work. The oxime derivative of taraxerone (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 using usual method.

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Graphical Abstract



Introduction

Plants derived triterpenoids natural products have attracted a great deal of attention for their efficient biological activity. These triterpenoids are consisted of five rings with some methyl groups in their skeleton. So, basically these are of C₃₀-isoprenoid compounds with few functional groups in their skeleton [1]. Triterpenoids are the secondary metabolites widely present in plants and are traditionally used as medicines [2]. They show many biological activities including, antioxidant, antimicrobial, antiviral, antiallergic, antipruritic, antiangiogenic, and spasmolytic activity [3, 4]. To exploit the therapeutic potential of the natural triterpenoids or their derivatives, intense pharmacological and mechanistic studies have been carried out. Antitumor, antiviral, anti-inflammatory, antidiabetic, antiparasitic, antimicrobial, cardio-hepato and gastro-protective, analgesic and wound-healing effects are included in these bioactivities. So, we carried out the transformative reaction on the

oxime derivative of taraxerone and antimicrobial activity of the isolated compounds.

Materials and Methods

Plant Material

Plants of *Sapium baccatum* ROXB used in this experiment were collected from North Bengal, India in May, 2016. We submitted specimens of *Sapium baccatum* with the tag numbers to Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal, Darjeeling, India. After collection, all the plants were washed thoroughly by plenty of water and stem bark were separated by simple cutting through a knife in wet condition and separated these from the rest parts. The plant's materials were shade dried and cut into small pieces. It was then grinded in small lots in a mechanical grinder and used for the extraction process. IR spectra were recorded on KBr disc at the range of 4000-

400 cm^{-1} on Shimadzu FT-IR 8300 Spectrometer. ^1H NMR and ^{13}C NMR were recorded on 500 and 300 MHz Bruker Avance FT-NMR Spectrometer using TMS as internal standard.

Isolation of Taraxerone from *Sapium baccatum* ROXB:

Dried and powdered stem bark of *Sapium baccatum* ROXB (2 kgs) 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 and was kept aside. This was identified as 3, 3'-di-o-methyl ellagic acid. From the clear filtrate, toluene was distilled off and the residual gummy solid (30 gms) was taken up in ether (2 ltrs). A cloudy precipitate which remained in the ether extract was separated by filtration. The clear ether solution was washed with 10% aqueous sodium hydroxide solution for three to four times and then washed with cold water till washings were neutral and dried over anhydrous Na_2SO_4 .

The solvent was evaporated when the neutral material (11 gms) was obtained as a yellow gummy solid, which after chromatography and crystallisation from chloroform-methanol mixture gave shining crystals (1.3 gm) m.p 238-240 $^\circ\text{C}$, $[\text{D}]_D^{25} + 10.80$ identical in all respect

with authentic sample of taraxerone (mixed m.p. Co-1R; Co-TLC). Other compounds isolated were 1-hexacosanol, taraxerol and baccatin.

Preparation of Oxime Derivative of Taraxerone

To a solution of taraxerone (3 g) dissolved in pyridine (30 mL) was added hydroxylamine hydrochloride (3 g) and ethanol (150 mL). The mixture was then refluxed in a water bath for 4 h. It was then cooled and poured over ice-cold water when white solid separated out. It was washed with water filtered through suction and dried. The dried mass was crystallised several times with chloroform-methanol mixture when taraxerone oxime m.p 250 $^\circ\text{C}$ was obtained.

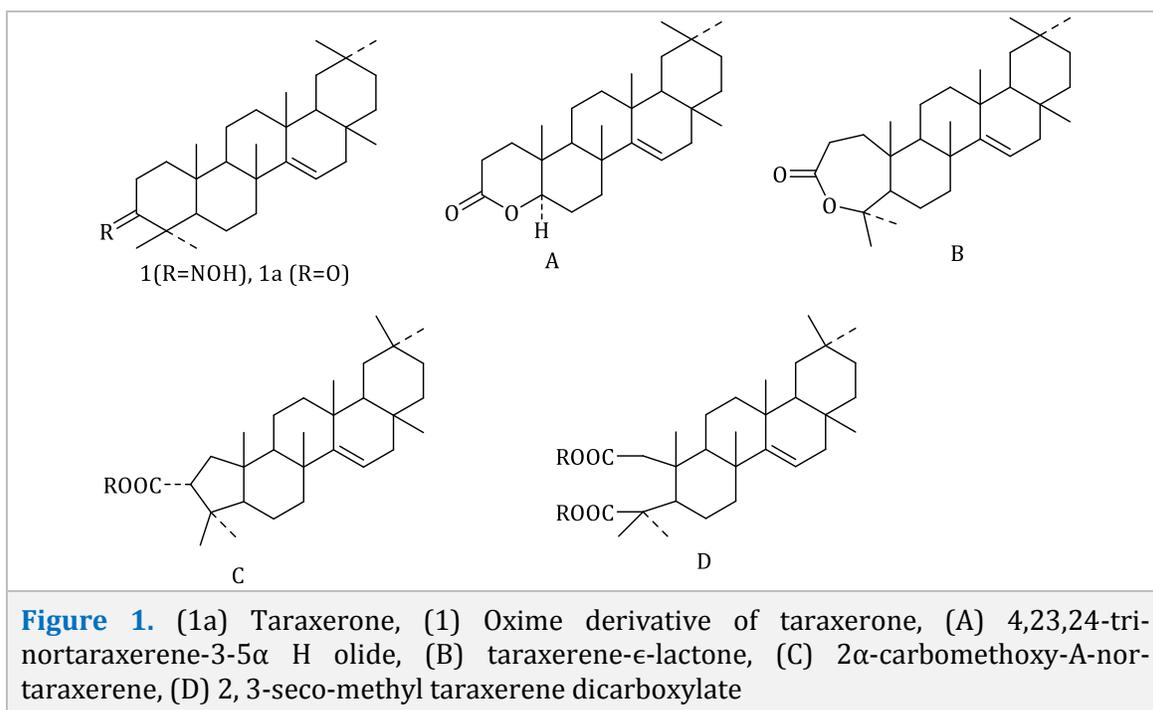


Figure 1. (1a) Taraxerone, (1) Oxime derivative of taraxerone, (A) 4,23,24-trinortaraxerene-3-5 α H olide, (B) taraxerene- ϵ -lactone, (C) 2 α -carbomethoxy-A-nor-taraxerene, (D) 2, 3-seco-methyl taraxerene dicarboxylate

Reaction of Taraxerone oxime (1) with molar proportion of hydrogen peroxide and catalytic amount of selenium-dioxide in tertiary butanol

Taraxerone oxime (1) prepared from taraxerone (1a) was subjected to oxidation with molar proportion of hydrogen peroxide and catalytic amount of selenium-dioxide in tertiary butanol by refluxing over water-bath for 20 h. The completion of the reaction was indicated by the precipitation of black selenium metal. After recovery of solvent by distillation, the residue was extracted with ether and separated into neutral and acid parts by the usual method.

The neutral part was chromatographed over silica-gel, the column on elution with petroleum ether: ethylacetate (2:3) mixture afforded a solid which on fractional crystallisation from chloroform-methanol afforded two solid compound A and B. Compound A was analysed for $C_{27}H_{42}O_2$ and compound B was analysed for $C_{30}H_{48}O_2$.

Structure Elucidation of Compound A

Compound A was recrystallized from chloroform-methanol mixture m.p 228-30 °C. It was analysed for $C_{27}H_{42}O_2$. Its IR-spectroscopy showed sharp absorption peak at 1750 cm^{-1} indicating the presence of a lactone carbonyl group and the other at 810 cm^{-1} indicating the presence of trisubstituted double bond that was supported by TNM test. Elemental analysis showed the molecular formula of compound A found to be $C_{27}H_{42}O_2$ which is in agreement with its mass-spectrum. It showed molecular ion peak at $m/z\ 398(M^+)$ with the other fragment of prominence appeared at $m/z\ 383(18.45)$; $274(100)$; 259 ; $204(75)$. The PMR-spectrum of Compound A showed the presence of a lactonic proton ($-\text{CO}-\text{O}-\text{CH}-\text{CH}_2-$) at 3.92 ppm as a quartet (q, $J_{\alpha\beta}=5\text{ Hz}$; $J_{\alpha\gamma}=12\text{ Hz}$) the appearance of multiplet at 2.26 ppm due to presence of methylene proton adjacent(alpha) to the

carbonyl group (m, 2H, $-\text{O}-\text{CO}-\text{CH}_2-\text{CH}_2-$), the multiplet centered at 5.57 ppm was due to the presence of trisubstituted double bond proton of olefinic proton (m, 1H) and six tertiary methyls as singlet from 0.83 to 1.12 (6s, 18H, 6xt- CH_3) ppm. The high J-value showed the lactonic proton to be axially oriented with one axial and another equatorial neighbours. Thus, from the study of IR, mass-spectrum and PMR-spectrum, the structure of the Compound A was assigned as 4, 23, 24-tri-nor-taraxerene-3-5 α H olide (A).

Structure Elucidation of Compound B

Compound B was recrystallized from chloroform-methanol mixture m.p 218 °C. It was analysed for $C_{30}H_{48}O_2$. Its IR-spectroscopy showed sharp peak at 1720 cm^{-1} indicating the presence of a ϵ -lactone carbonyl group and the other at 810 cm^{-1} indicating the presence of trisubstituted double bond. The presence of the olefinic double bond is confirmed by the generation of yellow colour in TNM test. Elemental analysis revealed that the molecular formula of the compound B was $C_{30}H_{48}O_2$ which is in agreement with its mass-spectrum. It showed molecular ion peak at $m/z\ 440(M^+)$ and the other fragments of prominence at $m/z\ 425$, 316 , 301 , 205 , 204 , and 189 . Thus, from the study of IR-spectrum and mass spectral analysis, the structure of the compound B was assigned as taraxerene- ϵ -lactone.

The genesis of the ion fragment $m/z\ 316$, 301 , 204 and 189 of the compound B is explained in [Figure 2](#) shown below which stands as support for the structure (B).

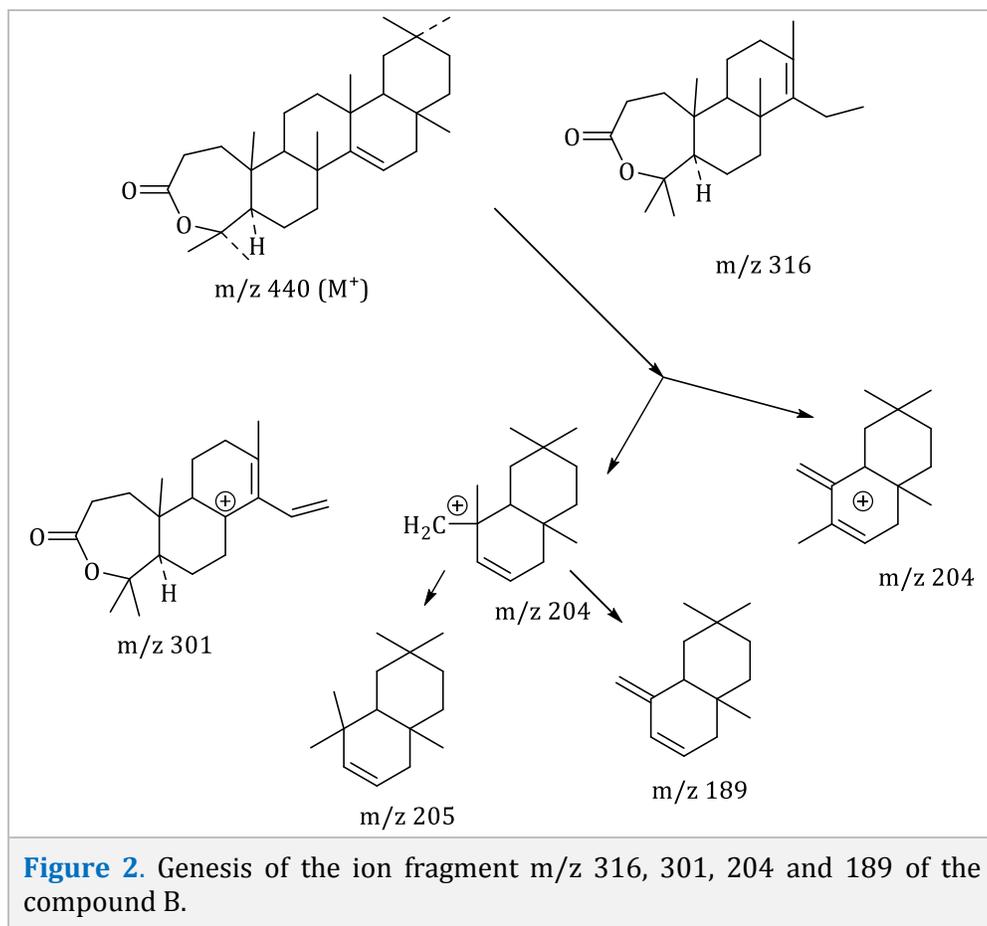
The acid part showed two spots on chromatoplate. The gummy mass was esterified with diazomethane and the products were separated by column chromatography on a deactivated alumina column. Elution with pet.ether (b.p 60-80 °C) furnished compound C which was crystallised from chloroform-

methanol mixture and analysed for $C_{31}H_{50}O_2$ m.p. 161-163 °C. Further elution of the column with pet.ether:ethylacetate (1:1) gave a white solid compound D which was crystallised from chloroform-methanol mixture and analysed for $C_{32}H_{52}O_4$, m.p. 151 °C.

Structure Elucidation of Compound C

Compound C was recrystallized from chloroform-methanol mixture m.p 161-163 °C. It was analysed for $C_{31}H_{50}O_2$. Its IR-

spectroscopy showed peak at 1735 cm^{-1} indicating the presence of carbomethoxy group, at 1155 cm^{-1} for $-C-O$ stretching vibration of the ester group and at 815 cm^{-1} indicating the presence of trisubstituted double bond. Elemental analysis showed the molecular formula of compound C is $C_{31}H_{50}O_2$ which in agreement with its mass-spectrum. It showed molecular ion peak at $454(M^+)$ (32) and the other fragments at $439(13)$; $330(14)$; $315(10)$; $287(20)$; $204(75)$.



The 1H NMR-spectrum of compound C demonstrated the presence of eight tertiary methyl-group which resonated at 0.82; 0.85; 0.90; 0.92 ; 0.95; 0.99; 1.08 and 1.12 (8s;24H;8xt- CH_3) (ppm). A three proton singlet at 3.6 ppm shows the presence of carbomethoxy group in the compound (s, 3H;- $COOCH_3$). A

quartet centered at 2.75 ppm integrable for one proton may be assigned to the hydrogen atom germinal to the carbomethoxy group (q; 1H; $J_{ae}=5\text{ Hz}$, $J_{aa}=11\text{ Hz}$; $-CH_2-C-CO-O$). The J value also indicate that the proton is axially oriented with one axial and one equatorial neighbours. The multiplet at 5.54 ppm integral for one

proton indicate the presence of trisubstituted olefinic proton (m, 1H -C=CH). Thus, on the basis of spectral data the structure of compound has been assigned as 2 α -carbomethoxy-A-nor-taraxerene(C).

Structure Elucidation of Compound D

Compound D was recrystallized from chloroform-methanol mixture m.p. 151 °C. It was analysed for C₃₂H₅₂O₄. Its IR-spectroscopy showed absorption peaks at 1725 cm⁻¹ and 1730 cm⁻¹ indicating the presence of two carbomethoxy group. Stretching frequency at 810 cm⁻¹ indicates the presence of trisubstituted double bond. The absorption at 1440 cm⁻¹ and 1140 cm⁻¹ are due to -CH₃ vibration of ester group and -C-O stretching vibration of the ester group respectively.

The ¹H NMR-spectrum of the compound D showed eight singlet tertiary-methyl groups which resonated in the range 0.81-1.25 (8s; 24H; 8x t-CH₃) ppm. Two three proton singlet each 3.60 ppm and 3.65 ppm indicate the presence of two carbomethoxy group in the compound. The two proton multiplet centred at 2.3 is attributed to a methylene group alpha to a carbomethoxy group. The multiplet centred at 5.54 ppm is due to the presence of trisubstituted double bond. The mass-spectrum of the compound D showed

molecular ion peak at m/z 500 (M⁺), 485(6), 470(7), 468(9), 440(6), 399(7), 376(7), 361(2), 344(20), 316(12), 287(17), 204(100). All these spectral data analysis of the compound D assigned the structure as 2, 3-seco-methyl taraxerene dicarboxylate (D).

Biocidal Activity of Isolated Compounds

In this present work the in vitro antifungal, antibacterial activities and the phytotoxicity of isolated taraxerone (1a), 4,23,24-tri-nor-taraxerene-3-5 α H olide [A], taraxerene- ϵ -lactone [B], 2 α -carbomethoxy-A-nor-taraxerene [C], 2, 3-seco-methyl taraxerene dicarboxylate [D], have been studied. Five different fungal pathogens namely, Colletrichum gloeosporioides, Fusarium equisetiae, Curvularia eragrostidies, Alternaria alternata and Colletotrichum camelliae were used for the antifungal study. For antibacterial study Escherichia Coli, Bacillus subtilis, Staphylococcus aureus, Enterobacter were used as bacterial pathogens. Suitable strains of these organisms were procured from the microbiology laboratory of our institute (for details see experimental). MICs (minimum inhibitory concentrations) of the triterpenoids against bacterial pathogens are presented in [Table 1](#) and [2](#).

Table 1. MICs of 1a to D against different bacteria

| Compounds | MIC in $\mu\text{g/mL}$ against different bacterial strains | | | |
|-------------------|---|------|------|-----|
| | EC | BS | SA | EB |
| 1a | 100 | 100 | 98 | 96 |
| A | 130 | <150 | 100 | 100 |
| B | 150 | 100 | 200 | 100 |
| C | 200 | 170 | 130 | 200 |
| D | 150 | 150 | <150 | 100 |
| Ampicillin | 128 | 64 | 64 | 128 |

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

Table 2. MICs of 1a to D against different fungi

| Compounds | MIC in $\mu\text{g/mL}$ against different fungal strains | | | | |
|-----------------|--|------|------|------|------|
| | CG | FE | CE | AA | CC |
| 1a | <5.0 | 20.0 | 40.0 | 10.0 | <5.0 |
| A | 5.00 | 20.0 | 35.0 | 20.0 | 39.0 |
| B | 4.50 | 19.0 | 35.0 | 20.0 | 40.0 |
| C | 4.00 | 15.0 | 32.5 | 15.5 | 35.0 |
| D | 3.70 | 10.5 | 30.5 | 15.0 | 32.5 |
| 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.

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