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Synthesis and Biological Evaluation of Betulonic Acid Derivatives as Antibacterial Agents

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ABSTRACT: Betulonic acid is pentacyclic triterpenes of the lupane type, which were isolated from birch bark. Betulonic acid and its derivatives can serve as interesting structural plan of action for the advancement of new therapeutic agents because of its high accessibility and a wide scope of natural activities. It has significant organic properties, for example, antiviral, antitumor, calming, antimicrobial, hepatoprotective, just as immunostimulant exercises. A series of novel synthetic and semisynthetic derivatives of BetA have been synthesized using different synthetic techniques and subjected to various testes and investigated for their therapeutic activities. Amino acid alkyl esters were prepared using amino acids-glycine. Presence of amino acids enhances the anti-inflammatory activity. Amino acids possess anti-inflammatory activity therefore the derivative of betulonic acid via amino acid esterification enhances the antiinflammatory activity of the synthesized compound. The synthesized betulonic acid derivatives were subjected to biological screening to evaluate their antimicrobial and anti-inflammatory effects.

KEYWORDS: Antineoplastic, Bhojpatra, Betulinic Acid Antibacterial, Anti-inflammatory etc.

I. INTRODUCTION

[1-2]Lupane triterpenoids of plant origination, for example, betulin and betulinic and betulonic acids show an assortment of organic movement and are fascinating as beginning materials for synthetic and biocatalytic changes

Betulin is commonly isolated from the bark of birch tree (Betula utilis) plant species. Birch tree is familiar for a long time for its curing properties; the oil obtained from birch bark was used as medicament for the remedy of skin diseases like dermatitis and psoriasis. The bark of the tree was used to prepare teas for treating infections of digestive tract by Native Americans, so it is regarded that the use of this medicinal plant as a source for the isolation of active constituents could be a vital source for curing many ailments. The major reason behind selecting this research work is that betulonic acid has better solubility than betulin. As well as the presence of amino acids enhances the anti-inflammatory activity. [3]Amino acids possess anti-inflammatory activity therefore the derivative of betulonic acid via amino acid esterification enhances the antiinflammatory activity of the synthesized compound.

Betulonic acid—a potent leading biological moiety

[4]Betulonic acid [lup-20(29)-en-3-oxo-28-oic] has valuable biological properties such as antiviral Antitumor, anti-inflammatory, antimicrobial, hepatoprotective, as well as immunostimulant activities . Until the 2000s interest to betulonic acid was basically because of its role as the precursor for synthesis of betulinic acid, which is a fruitful drug in case of human melanoma.

[5]The antitumor action of BetA has drawn in the consideration of the pioneers who aim to evolve novel antitumor agents. Betulonic acid is the one of the major successful parts of numerous customary Chinese medications.

[6]It is also established as an intermediate in synthesis of several triterpenoid derivatives with anti-inflammatory, antiviral, and antiproliferative properties. Being an auxiliary metabolite of birches betulonic acid was found in particular as minor component in bud extracts .Today's flow interest for medicinal chemistry is synthesis of betulonic acid peptide derivatives, since they show high antiviral activity and can act as inhibitors of the tumor cell growth.

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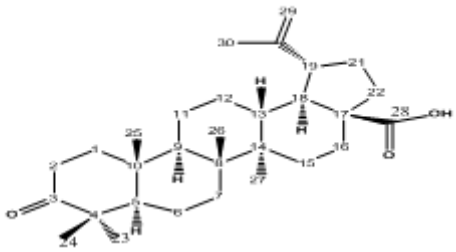

Structure of Betulonic acid

II. MATERIALS AND METHODS

Extraction and Isolation of Betulin Present research project work is based on the approach of semi-synthetic work on natural products. Lot of research work has been conducted on bhojpatra adopting both natural and synthetic approaches. But here, we have consolidated both natural as well as synthetic work. In this reference few reported extraction and isolation procedures were carried out which are as follows:

- Initially 15 gm of bhojpatra was boiled in methanol for about 10-15 minutes in iodine flask along with few porcelain chips. Then its solid part was separated from solvent part. The solvent front was then concentrated to the maximum and
- kept overnight in fridge. This procedure was found to be the simplest and provides with maximum yield of betulin in impurified form.
- When impurified betulin obtained, its recrystallisation process was carried out with butanol or isopropanol and water.
- TLC of the synthesized compounds was carried out in order to ascertain its purity.

Mobile phase for TLC: -

Ethyl acetate: benzene: formic acid (36:12:5 **Detecting agent:** Anisaldehyde (0.5mL) + H_2SO_4(1 mL 97%) + Glacial acetic acid (50 mL)

Result ⇨ Less violet spot- Betulin
More violet spot- Betulonic acid

Preparation of Betulonic acid

Preparation of Jone's Reagent

Jone's reagent was produced by dissolving chromium trioxide (70 gm, 0.70 moles) in 100 ml of water placed in a 500 ml beaker. The beaker was then kept in an ice bath. 18 ml of sulphuric acid (61 ml, 1.10 mole) and then 200 ml of water was further added on with constant manual stirring. The above mixture was then cooled to 0ºC - 5ºC.

Preparation of Betulin to Betulonic Acid:-

Jone's reagent was added drop by drop to a betulin (1 gm, 2.26 mmol) solution mixture in 50 ml acetone cooled to 0ºC,. Continuous stirring the above resulting mixture was done around 1.5 hrs at a temperature of 0º C, a further addition of 25 ml methanol was done, then for next 5 minutes the solution was stirred. 40 ml of water was further added. Vacuum was applied to remove the acetone and by using 40 ml of ethyl acetate the aqueous residue was extracted. The next step involved the separataion of aqueous layers from the ethyl acetate layer. Washing of ethyl acetate layer was done first with 20 ml of water followed by washing with 15 ml of brine. Magnesium sulphate was employed for drying ethyl acetate layer, filtered and further removal of ethyl acetate layer was done under vacuum. Then column chromatography of residue was carried out using 60-200 mesh silica gel employing petroleum ether/ ethyl acetate (4:1) to produce betulonic acid (770 mg), whose melting point was detected to be in the range 247º C- 249ºC. The reaction resulted in a 75% yield of betulonic acid.

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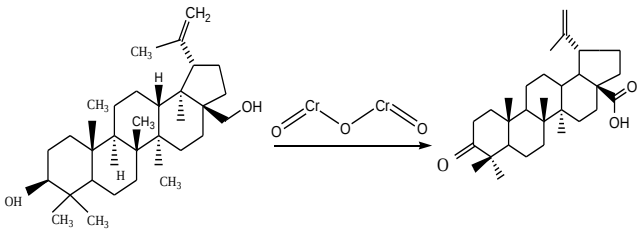
Chemical reaction:



Betulin Betulonic acid

General Procedure for Preparation of Amino Acid Alkyl Ester Hydrochlorides Derivatives of Betulonic Acid

In a round bottom flask 0.1 mol amino acid was placed. Newly prepared distilled chlorotrimethylsilane (0.2 mol) was added steadily to this under continuous stirring with a magnetic stirrer. 100 ml of methanol was then added to the above mixture. The resulting solution was then mixed at 25ºC. The resulting mixture was concentrated with the help of rotary evaporator as the reaction completed, to yield the product amino acid ester hydrochloride.Following are the above mentioned method amino acid alkyl ester derivatives which were synthesized:

I. Glycine methyl ester
II. Glycine ethyl ester
III. Glycine propyl ester

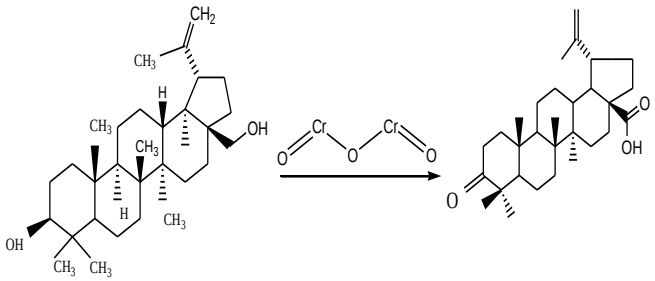
Now, the next step consisted the chemical conjugation of synthesized amino acid alkyl ester derivatives with betulonic acid so as to derivitize biologically potent derivatives

Procedure for the Conjugation of Amino Acid Alkyl Esters with Betulonic Acid to Give Potent Derivatives

Dissolved Betulonic acid, amino acid alkyl ester and triethyl amine in tetrahydrofuran at room temperature. To this solution mixture added DCC and DMAP, stirred this for 48 hrs. Filtered the precipitate (Dicyclohexyl urea). Evaporated the filtrate to will remove THF. After evaporation, remaining was dissolved in ether/ethyl acetate (2:1). 100 ml water and HCl poured in separating funnel. Organic layer collected then dried on $MgSO_4$

Synthetic Scheme

1. Betulin oxidised by chromium oxide to Betulonic acid



2. Formation of amino acid alkyl esters conjugates of betulonic acid

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3. Formation of amino acid salt conjugate of betulonic acid

Synthetic Scheme for the Amino Acid Alkyl Ester Conjugates of Betulonic Acid

I Glycine methyl ester of betulonic acid

II Glycine ethyl ester of betulonic acid

III Glycine propyl ester of betulonic acid

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III BIOLOGICAL ACTIVITY

Antibacterial activity

These are chemotherapeutic agent that destroy bacteria or hinders its growth. Synthetic antibacterial compounds are classified into two significant classes, topical and systemic compounds.

Antimicrobial activity:

An antimicrobial agent is whatever that can kills microscopic organism or hinders its growth. Antibacterial synthetics are classified in three general classes like antibacterial medicaments, antiseptics and disinfectants. They are utilized in low fixations in or upon the bodies of organisms to forestall or treat explicit bacterial sickness without harming the host living being. Antimicrobial movement is examined depending upon the invitro action in unadulterated cultures. In-vitro vulnerability test are finished by the accompanying techniques:

- Tube dilution method
- Agar dissemination technique

- **Tube dilution method**:

The antimicrobial agent dilutions will be favored in growth medium with the end goal that the medication concentration achieves the desired clinical significance range. An equivalent amount of broth consisting 105-106 microbes/ml will be then added on to every tube and also to a control tube in which no microbial antagonist is present. Noticeable turbidity will be inspected in the tubes for after an overnight incubation. This strategy is utilized for detecting susceptibility of microbes in liquid media.

- **Agar diffusion method:**

In this procedure by employing pouring method Petri dishes of agar will be made. Inoculation of the agar will be done with microorganisms. In agar dilution method for the two; aerobes and anaerobes diverse antibiotic concentrations will be utilized in to an agar culture. For 24 hours at 37 ºC temperature the plates are brooded. The microbial antagonist disperses through the agar and forms an inhibition zone. The diameter of the zone can be determined and an assessment of the level of action of the microbial antagonist can be acquired.

Material and Methodology

The microbiological testing of the subordinates was finished by agar well diffusion method.

Standard drug--- Ciprofloxacin

Media--- Nutrient agar media were utilized for the reason, which contains the constituents as introduced in. (table-1)

S.NO	Constituents	Quantity Required
1.	Peptic Digest	5gm/liter
2.	Yeast Extract	1.5gm/liter
3.	Beef Extract	1.5gm/liter
4.	Sodium Chlolride	5gm/liter
5.	Agar	15gm/liter
6.	Distilled Water	1 liter

Experimental procedure:

Agar-diffusion method was utilized for the determination of preliminary bacterial antagonist activities. The agar well diffusion test was carried out using nutrient agar medium, according to the system set out by Magaldi et al. 2004 and at 15 lbs pressure (121°C) this agar medium was autoclaved for 15 minutes and afterward it was cooled instantly to 50- 55°C in ice-bath. This medium was filled in petridishes to a static 4 mm depth; this is proportional to round about 40mL in a 90mm plate. The culture was then inoculated on the surface of medium after the medium had solidified. These were performed in a laminar air flow. The germ free swab was utilized on the outer surface of the nutrient agar culture to guarantee a uniform distribution. Then the petridishes were settled for few minutes to ensure abundant moisture absorption. Germ free plug borer (7mm) was utilized for making agar wells, and the

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concentrations of the 25, 50, 75, 100 and 200 µg/ml of the diluted stock solutions were set in each wells(Indian Pharmacopoeia, 1996). The level of inhibition can be determined utilizing the equation:

$$\% \text{ Inhibition} = \frac{\text{I (diameter of inhibition zone in mm)}}{\text{90(diameter of Petri-plates in mm)}}$$

Antibacterial activity

Test strains

For the current work, effectiveness of the test compounds was resolved against following bacterial strains:

Gram +ve bacterial strains

1-Bacillus pumilus 2- Staphylococcus aureus Gram -ve bacterial strains- 1- Escherichia coli 2- Klebsiella pneumonia

IV. RESULT AND DISCUSSION

S.No	Compound Code	Conc.	Inhibition zone in mm			
		(µg/ml)	S.aureus	B.pumilus	E.coli	K.pneumoniae
1.	AS-1	1000	17	16	15	18
2.	AS-2	1000	18	17	16	18
3.	AS-3	1000	19	17	19	20
4.	Ciprofloxacin	1000	30	35	38	35

The in-vitro antibacterial activities of newly synthesized compounds (AS1 to AS3) were carried out by Agar Diffusion Method counter the micro organisms viz. gram positive (Bacillus pumilus, Staphyococcus aureus) gram negative (Escherichia coli, Klebsiella pneumonia).

All three derivatives were screened for antibacterial activities at 1000µg/mL concentrations. The inhibition zone (in mm) was estimated for each compound accompanying ciprofloxacin as standard drug and results were presented in **table 2**

Results demonstrated that compound **AS1** showed maximum activity (inhibition zone in mm) against Klebsiella pneumonia, least action against Escherichia coli.

Compound **AS2** showed maximum activity (inhibition zone in mm) against Klebsiella pneumonia, Staphylococcus aureus and minimal activity against Escherichia coli.

Compound **AS-3** showed maximum action (inhibition zone in mm) against Klebsiella pneumonia and minimum activity against Bacillus pumilus.

V. CONCLUSION

The basic purpose of the present research work seems to be served and appreciably fulfilled as encouraging results have been obtained both in terms of establishment of structural features as well as the spectrum of the biological activities of the synthesized compounds. The results of the antibacterial study revealed that all three compounds (AS1, AS2 and AS3) exhibited medium to good antibacterial activity when juxtaposed with the standard drug.

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