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Synthetic Modifications and Anti-inflammatory Potential of Acetyl β-Boswellic Acid Isolated from *Boswellia serrata*

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ABSTRACT

The gum resin of *Boswellia* serrata[1] is employed in Ayurvedic system of medicine for the treatment of rheumatism and nervous disorder. It has established that said anti-inflammatory[2,3] and anti-arthritis activities are due to the presence of β-Boswellic acid and other related pentacyclic terpenoid acids. So keeping in view some structural activity relationship efforts were made to carryout synthetic modifications of β-Boswellic acid and study its biological activity. The lumps or the granules of dried gum resin of *Boswellia serrata* were extracted with petroleum-ether (60-80). The insoluble fraction was further extracted with (95%) methanol. The methanolic extract was fractionated into acidic and neutral fractions by alkali treatment (10% KOH). A part of the acidic fraction was subjected to column chromatography on silica gel. Acetyl-β-Boswellic acid was isolated and modified into β-hydrogen succinate derivative.

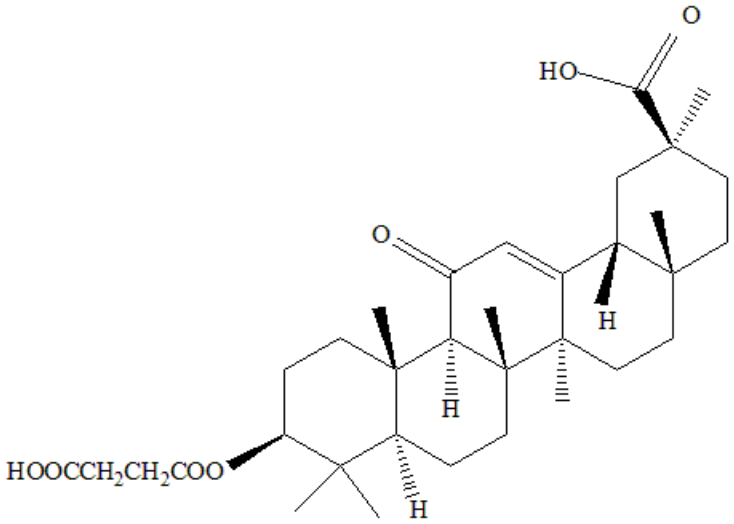
Keywords: *Boswellia serrata*, β-boswellic acid, anti-inflammatory.

INTRODUCTION

Boswellia serrata, commonly known as *Salai guggal*. Genus Boswellia Roxb native to North-East Africa, Southern parts of the Arabian Peninsula and India, consists of ten species. It is widely distributed throughout India .The gum of *B. serrata* is hot, dry with a good flavour but a bitter taste. It is credited to have astringent, stimulant, expectorant, diuretic, diaphoretic, emmenagogue, ecbolic and antiseptic properties. It is reported to be useful in ulcers, tumours, goitre, cystic breast, diarrhoea and dysentery, and piles and skin diseases. It is used in the preparation of an ointment for sores with butter in syphilis. The gum is astringent to the bowels, expectorant, used for boils, scabies, as collyrium in opthalmia, useful in intestinal troubles, bronchitis, asthma, cough, bad throat, heels wounds, strengthen the teeth,

invigorating, may cause vomiting. The gum oleo-resin is recommended in combination with other drugs for the treatment of snake bite and scorpion sting. The non-phenolic fraction obtained from the gum resin of *B. serrata* was found to exhibit marked sedative and analgesic effects.

The gum resin of *Boswellia serrata* is employed in Ayurvedic system of medicine for the treatment of rheumatism and nervous disorder. It has established that said anti-inflammatory and anti-arthritis[4,5] activities are due to the presence of β-Boswellic acid and other related pentacyclic terpenoid acids[6]. 3-(3-carboxy-1-11-oxoolean-12-en-30-oic acid hydrogen succinate i.e. glycrrhetic acid (1) effective anti-inflammatory glucocorticoid. So, keeping in view some structural activity relationship efforts were made to carryout synthetic modifications of β-Boswellic acid and study its anti-inflammatory activity.



(1)

MATERIALS AND METHODS

The chemicals used in the present project work were of A.R Grade and L.R Grade, purchased from Nice, BDH and CDH labs.

Analytical technique

Physical Data:

Melting point of the compounds was taken on capillary melting point apparatus.

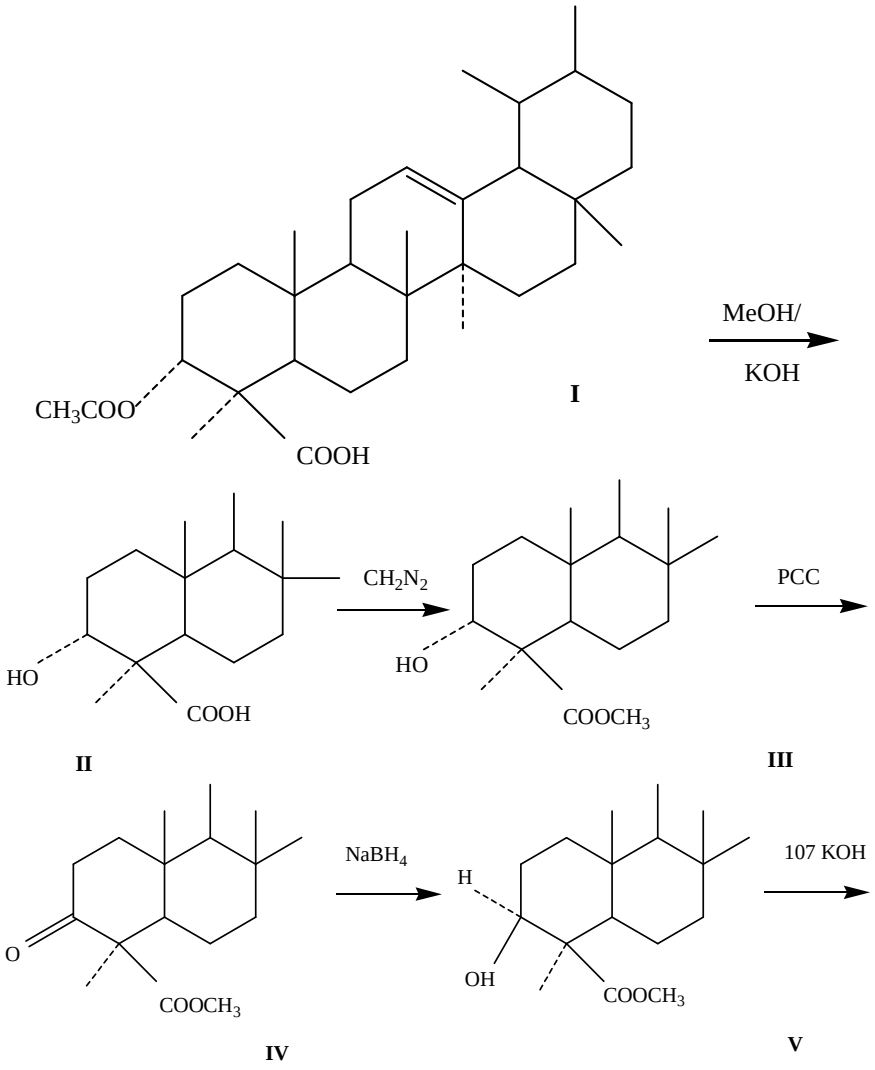
Chromatography:

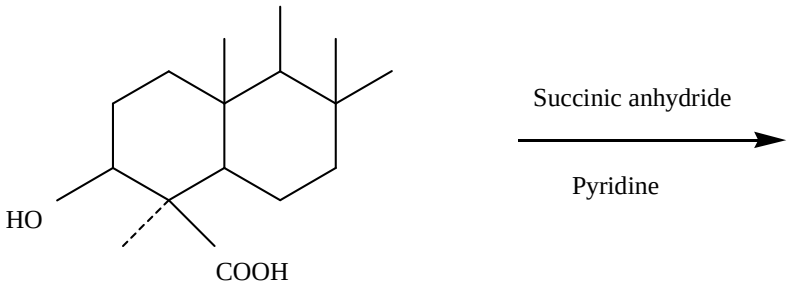
Compounds were isolated using column chromatography using silica gel G as stationary phase and various combination of n-hexane-ethylacetate as mobile phase.

INSTRUMENTATION

The compounds isolated using column chromatography was modified synthetically by conventional methods. The technique employed for the characterisation of the compounds was IR Spectra and HNMR. The IR spectra of the compounds were recorded on a Fourier Transform IR spectrometer using KBr pallet. HNMR spectra were recorded in different solvents on Jeol FX250 spectrometers using TMS as internal standard.

REACTION SCHEME





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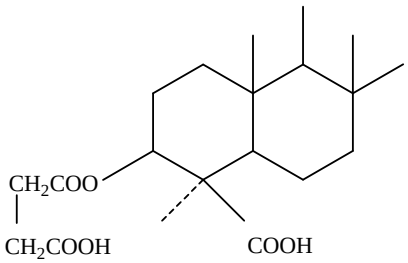

VII

EXPERIMENTAL PROCEDURE

Isolation of Acetyl β-Boswellic acid (I)

The lumps or the granules of dried gum resin of *Boswellia serrata* were extracted with petroleum-ether (60-80). The insoluble fraction was further extracted with (95%) methanol. The methanolic extract was fractionated into acidic and neutral fractions by alkali treatment (10% KOH). A part of the acidic fraction was subjected to column chromatography on silica gel. Acetyl β-Boswellic acid was isolated using hexane:ethylacetate gradient. The structure of Acetyl- β-Boswellic acid was identified by comparing them with authentic samples.

Synthesis of 3β-hydroxy- urs-12-en-24-oic acid hydrogen succinate (VII)

The various steps involved in the synthesis of 3β-hydroxy- urs-12-en-24-oic acid hydrogen succinate are depicted in scheme.

Synthesis of Boswellic acid (II)

Acetyl-β-Boswellic acid (i) (5g) was dissolved in methyl alcohol (150 ml) and to it potassium hydroxide (0.358) dissolved in water was added. The reaction mixture was refluxed on water bath for two hours. The reaction mixture was then acidified with dilute hydrochloric acid and the methanol was removed under pressure. The remaining solution was extracted with chloroform dried over sodium sulphate and solvent evaporated. The solid left behind afforde β-Boswellic acid identified by comparing them with authentic samples.

M.P. 228-30 ^{0}C
IRV$_{max}$(KBr) 3400 cm^{-1} (-OH), 1700 cm^{-1} (-C=O-OH) other bands 1450, 2920 cm^{-1}. ^{1}H NMR : ($CDCl_3$) δ 0.81 ,0.9, 1.11 (21H, 23, 25, 26, 27, 28, 29 & 30-CH_3), 4.1(s, 1H, -C<u>H</u>OH), 5.16 (t, 1H, H-12).

Methyl β-boswellic ester (III)

β-Boswellic acid (5g) in ether was treated with 5% ethereal diazomethane(500ml.) and the mixture was kept overnight at room temperature. The ester left after evaporation of ether crystallized from methanol in needles. M.P. 185-90 ^{0}C.
IRV$_{max}$(KBr): 3410 cm^{-1} (OH), 1720 cm^{-1}(-CO-OCH_3) other bands 2922, 1452 cm^{-1}
^{1}H NMR : ($CDCl_3$) δ 0.80 ,0.93, 1.1, 1.36 (21H, 23, 25, 26, 27, 28, 29 & 30-C<u>H</u>$_3$), 3.68(s, 3H, -OC<u>H</u>$_3$), 4.1(s, 1H-C<u>H</u>OH), 5.13 (t, 1H, <u>H</u>-12).

Methyl 3-oxo-urs-12-en-24-oate (IV)

To 1 gm. Of methyl 3α-hydroxy-urs-12-en-24-oate in dichloromethane was added pyridinium chloride chromate (1gm). After two hours the reaction was completed and furnished Methyl 3-oxo-urs-12-en-24-oate(iv).
M.P. 158-59 ^{0}C
IRV$_{max}$ (KBr) 1725 cm^{-1} (-C=O-OCH_3), 1702 cm^{-1} (C=O) other bands 1455, 1385, 1235 cm^{-1}.
^{1}H NMR ($CDCl_3$) δ, 0.83, 0.90, 1.10, 1.36 (21 H, 23, 25, 26, 27, 28, 29 and 30-CH_3), 3.68, (s, 3H, -O-C<u>H</u>$_3$) 5.13 (t, 1H, <u>H</u>-12).

Methyl 3β hydroxyurs-12-en-24-oate (V)

3-oxo-urs-12-en-24-oate in methanol was treated with sodium borohydride and allowed to stand at room temperature for 1.5 hrs. The reaction mixture was then diluted with water and extracted with chloroform. The chloroform extract was washed with water till neutral, dried over sodium sulphate and evaporated. The residue on crystallisation from aqueous methanol yielded Methyl 3β- hydroxyurs-12-en-24-oate(v).
M.P. 115-17 ^{0}C
IRV$_{max}$(KBr) 3400 cm^{-1} (-OH), 1722 cm^{-1} (-COCH_3) other bands, 2922, 1450 cm^{-1}
^{1}H NMR ($CDCl_3$); δ 0.82, 0.91, 1.1, 1.39 (21 H, 23, 25, 26, 27, 28, 29 and 30, -C<u>H</u>$_3$), 3.16, (m, 1H, -C<u>H</u>OH), 3.70 (s, 3H, -OC<u>H</u>$_3$), 5.13 (t, 1H, <u>H</u>-12)

3β-hydroxyurs-12-en-24-oic-acid (VI)

Methyl 3β hydroxyurs-12-en-24-oate was dissolved in 10% KOH in pressure vessel. This vessel was kept in oven at 110^o temperature for 12 hrs. The reaction mixture was neutralized with dilute HCl and extracted with chloroform. The chloroform extract washed with water, dried over anhydrous sodium sulphate and evaporated. The residue on crystallization yielded 3β hydroxyurs-12-en-24-oic-acid(vi).
M.P. 165-67^{0}C
IRV$_{max}$ (KBr) 3400 cm^{-1} (-OH) other bands 1700, 1450, 1380, 1234 cm^{-1}

^{1}H NMR ($CDCl_3$) δ 0.81, 0.91, 1.0, 1.1, 1.2, (21H, 23, 25, 26, 27, 28, 29 and 30 –C$\underline{H}_3$), 3.83 (t, 1H, C$\underline{H}$OH), 5.09 (bs, 1H, $\underline{H}$-12).

3β- hydroxyurs-12-en-24-oic-acid hydrogen succinate (VII)

3β-hydroxyurs-12-en-24-oic-acid(VI) was dissolved in 5 ml Pyridine. To this added succinic anhydride. The reaction mixture was heated for half an hour and kept overnight. The reaction mixture was poured on crushed ice when it furnished white precipitates. These precipitates on crystallisation yielded 3β hydroxyurs-12-en-24- oic-acid hydrogen succinate(VII). M.P. 255 ^{0}C

^{1}H NMR ($CDCl_3$) δ 0.9, 1.15, 1.44, 1.59 (21 H, 23, 25, 26, 27, 28, 29 and 30, -C$\underline{H}_3$), 2.8 (m, 4H, C=O-(C$\underline{H}_2$)$_2$-C=O), 4.8 (m, 1H, C$\underline{H}$-OH), 5.2 (bs, 1H, H-12).

Sodium salt of 3β- hydroxyurs-12-en-24-oic-acid hydrogen succinate (VIII)

3β- hydroxyurs-12-en-24-oic-acid hydrogen succinate (1 g) was dissolved to the solution of sodium (0.05 g) in anhydrous methanol (2 ml) under anhydrous condition and kept overnight. Methanol is then removed under reduced pressure leaving behind colourless disodium salt of (vii).

Anti-inflammatory activity: Anti-inflammatory activity of β-hydrogen succinate and its sodium salt was evaluated in rats by carrageenin induced paw odema. Compounds I, VI, VII have been investigated for anti-inflammatory activity. Albino rats weighing between 100-300 gm of either sex were selected for the bioassay. The animals were housed in polyacrylic cages (5 animals per cage), for one week at standard laboratory conditions [(25ºC ±2ºC), relative humidity 60 ± 5%]. The diet was standard pellets and water was given *ad libitum*. The rats were divided into five groups consisting of 5 animals in each group, two groups for negative (received 5% Gum acacia 5 ml/kg) and positive controls (received standard drug ibuprofen) respectively and three groups others (received respective compounds).

RESULTS AND DISCUSSION

The lumps or the granules of dried gum resin of *Boswellia serrata* were extracted with petroleum-ether (60-80).The insoluble fraction, further extracted with (95%) methanol. The methanolic extract was fractionated into acidic and neutral fractions by alkali treatment (10% KOH).A part of the acidic fraction was subjected to column chromatography on silica gel. Acetyl-β-Boswellic acid was isolated using hexane:ethylacetate. The structure of Acetyl-β-Boswellic acid was identified by comparing them with authentic samples. The various steps involved in the synthesis of 3β-hydroxy- urs-12-en-24-oic acid hydrogen succinate are depicted in scheme (I). The structures of the products formed at different steps are characterised through IR, NMR and Mass spectroscopy. Acetyl β-Boswellic acid was isolated and hydrolyzed to β-Boswellic acid which was converted into its methyl ester by the treatment of diazomethane. Methyl-β-Boswellic acid, thus obtained was oxidised to methyl 3-oxo-urs-12-en-24-oate by Jones reagent. Methyl-3-oxo-urs-12-en-24-oate on sodium borohydride

reduction in methanol yielded methyl-3β-hydroxy-urs-12-en-oate which on alkaline hydrolysis i.e. KOH in methanol under pressure furnished 3β-hydroxy-12-en-24-oic acid. 3β-hydroxy-12-en-24-oic acid on treatment with pyridine and succinic anhydride furnished 3β-hydroxy- urs-12-en-24-oic acid hydrogen succinate. The two moles of sodium methoxide and succinate derivative in methanol gave disodium salt of 3β-hydroxy-urs-12-en-24-oic acid hydrogen succinate. Anti-inflamatory activity of β-hydrogen succinate and its sodium salt was evaluated in rats by carrageenin induced paw odema.at the dose of 100 mg/kg p.o. These compounds could not retain the potency of parent compound i.e. β-Boswellic acid. It has shown on an average 21.3% activity.

CONCLUSION

Salai guggal has shown to possess marked anti-inflammatory and anti-arthritic activities when tested against carragenin induced paw odema, adjuvant arthritis in rats. It has now been established that said anti-inflammatory and anti-arthritic activities are due to the presence of β-Boswellic acid and other pentacyclic triterpenoid acids isolated from *B. serrata*. So keeping in view the structural relationship of compound (1) which is also an effective inflammatory and compound (viii) that we prepared from β-Boswellic acid, when tested for its anti-inflammatory activity it could not retain the potency of β-Boswellic acid. It has shown on an average 21.3% activity.

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