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ISOLATION AND CHARACTERIZATION OF SESQUITERPENE LACTONES FROM THE GENUS AMBERBOA

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ABSTRACT:

Amberboa divaricata Kuntze (syn. *Amberboa ramosa* (Roxb.) Jafri = *Volutarella divaricata* Benth. et Hook.f. = *Tricholepis procumbens* Wight) belongs to the family Asteraceae and tribe-Cynareae. Asteraceae is the second largest family of the flowering plants and comprises of about 1100 genera and more than 20,000 species. Various sesquiterpene lactones especially guaianolides have been isolated from this genus. Some of these have α-methylene-γ-lactone moiety which is essential for cytotoxic activity. The present work deals with the chemical investigation of the aerial parts of *Amberboa divaricata* and reports the isolation of lupeol acetate, stigmasterol, β-sitosterol, lupeol, aguerin-B, cynaropicrin, desacylcynaropicrin, betulinic acid and β-sitosterol-D-glucoside.

KEYWORDS: ***Amberboa divaricata Kuntze*** *; Asteraceae , Triterpenoids , Steroids.*

INTRODUCTION:

Amberboa divaricata Kuntze (syn. *Amberboa ramosa* (Roxb.) Jafri = *Volutarella divaricata* Benth. et Hook.f. = *Tricholepis procumbens* Wight) belongs to the family Asteraceae and tribe-Cynareae. It is an annual, dichotomously branched, straggling stiff weed and troublesome from its hard head with spiny involucral bracts. Asteraceae is the second largest family of the flowering plants and comprises of about 1100 genera and more than 20,000 species. The plants of this family are distributed over most of the earth and for intangible, physiological and structural reasons they seem to be well adapted to even adverse habitats such as deserts, warren lands, etc. In India, about 100-200 genera are reported, of which

few are of great medicinal importance[1]. This plant is used as an aperient, deobstruent, tonic, febrifuge, slightly mucilaginous and used in cough[5]. A perusal of literature revealed that six species of genus *Amberboa* have been examined phytochemically[6]. Various sesquiterpene lactones especially guaianolides have been isolated from this genus. Some of these have □-methylene-□-lactone moiety which is essential for cytotoxic activity[7].

Amberboa divaricata Kuntze is commonly known as Badaward. It is mostly found in dry and waste places on the warren land. The young plants are used as fodder. Several species of *Amberboa* have been reported to possess cytotoxic and antibacterial activity[2].

Earlier phytochemical work on this species led to the isolation of a number of sesquiterpene lactones[2-6]. Some of these have □-methylene-□-lactone moiety which is essential for cytotoxic activity[8,9].

We describe here the isolation and structure elucidation of Stigmasterol, Lupeol acetate , Aguerin-B , Betulinic acid, Cynaropicrin and Desacylcynaropicrin[10].

MATERIALS AND METHODS:

Experimental:

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus and are uncorrected. column chromatography (CC): silica gel (Merck 60-120 mesh). Prep.TLC: Merck silica gel 60 F_{254} precoated glass plates, UV spectra: Hitachi U-200 spectrophotometer, IR spectra: FT-IR Nicolet Magna 550 and Shimadzu QP-5000 spectrophotometer . ^{1}H and ^{13}C NMR spectra: JEOL AL-300 MHz and Bruker Avance DRX 500 FT NMR spectrometers, MS: JEOL JMS-SX 102A and JEOL D-300 spectrometers.

Identity of the compounds were confirmed, unless otherwise stated, by comparison of their melting points and spectral data with literature values and also by mixed m.p., co-TLC and co-IR with the authentic samples.

The UV spectra were recorded on Beckmann recording spectrophotometer DB and Perkin-Elmer model 202 automatic recording spectrophotometer.

Plant material: The plant material were collected from Jaipur, district of Rajasthan and identification was done with the help of Botany Department, University of Rajasthan, Jaipur and specimen deposited at RUBL Herbarium, Jaipur.

Extraction and Isolation:

The air-dried and coarsely powdered aerial parts (4 kg) of *A. divaricata* were extracted with ether-petroleum ether (1:2) mixture at room temperature for 24 hours. The extract was concentrated under reduced pressure over water bath and furnished greenish semi-solid mass (12 gm). The resulting semi-solid mass was dissolved in methanol and left overnight at 0° in order to precipitate out the bulk of fatty mass. It was then filtered and filtrate was again concentrated and chromatographed over silica gel with solvents of increasing polarity.

Fractions thus obtained were examined on TLC plates using several combinations of solvents to find out the number of compounds present in them. Fractions 1 and 3 gave unworkable amounts with trailing on TLC plates and seemed to be a mixture of several compounds. Hence could not be investigated further.

However, fractions 2, 4 and 5 were found to be a mixture of 3 to 4 compounds each. Hence these fractions were subjected to repeated preparative TLC after selecting suitable solvent systems for individual fraction and following compounds were obtained in pure form.

Stigmasterol colourless needles, 100 mg, m.p. 166-67^{o} and lupeol as colourless needles, 180 mg, m.p. 210-211^{o} .

fraction 3 on qualitative TLC examination revealed the presence two compounds. Separation by preparative TLC gave following compounds: Aguerin-B as pale yellow viscous oil, from fraction 4 and Betulin is crystallized as colourless needles, 100 mg, m.p. 316-318°. Fraction No. 5 gave Cynaropicrin as colourless crystals, 270 mg, m.p. 230-231° and Desacylcynaropicrin as colourless gum, 570mg. Fraction 6 and 7 showed trailing on TLC plate and seemed to be a mixture of several compounds and were discarded.

RESULTS AND DISCUSSION:

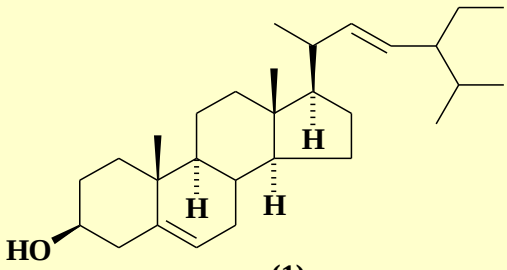
(1) Characterization of Stigmasterol:

It was isolated as colourless shining flakes, m.p. 166-167^{o} and displayed single spot on TLC-plate. It responded positive Liebermann-Burchard and Noller tests for sterols. It also gave positive test for unsaturation.

It has one hydroxyl group and two double bonds. The presence of hydroxyl group was ascertained by the appearance of a broad absorption band at 3400-3200 cm^{-1} in IR-spectrum.

The ^{1}H NMR spectrum in $CDCl_3$ displayed a broad triplet at δ5.34 for olefinic H-6 proton and a pair of double doublets at δ5.04 and δ5.12 for H-22 and H-23 olefinic protons respectively. Large coupling constants of order of 16 Hz in double doublets indicated their *trans* geometry. A multiplet centered at δ3.52 was explainable to H-3 methine proton under oxygen function. A triplet at δ0.81

corresponded to C-29 methyl protons, while a doublet at δ0.91 (J = 7 Hz) and singlets at δ0.79, 0.88 ppm were due to C-21, C-18 and C-19 methyl protons respectively. A doublet at δ1.16 was observed for C-27 methyl protons.



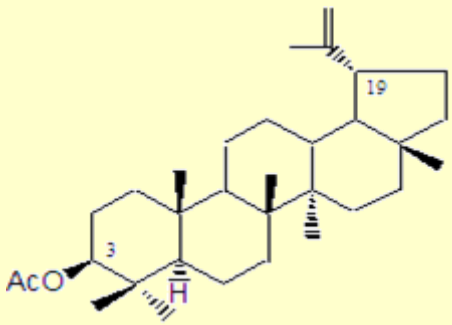
(1)

In mass spectrum , molecular ion peak $[M]^+$ was observed at m/z 412 corresponding to its molecular formula $C_{29}H_{48}O$. An intense peak at m/z 397 was due to the loss of methyl radical from 412. The other important peaks were observed at m/z 328, 302, etc.

(2) Characterization of Lupeol acetate :

It was isolated as colourless crystals, m.p. 213-14° and belongs to lupane series of triterpenoids. This compound gave single spot on TLC plate and responded positive Liebermann-Burchard11 and Noller tests12 characteristic of triterpenoids. It was first isolated from the leaves of *Carphephorus odoratissimus*13,14. Its molecular formula C32H52O2 was established from mass spectrometry. The IR spectrum showed the presence of a strong absorption band at 1720 cm–1 for an acetate moiety.

Its 1H NMR spectrum (Fig. 1) in CDCl3, displayed a pair of broad singlets at □ 4.69 and 4.57 corresponded to the vinylic protons. An olefinic methyl showed a broad singlet at □□□□69. Proton under acetoxy function (H-3□) shifted to downfield and appeared at □ 4.38 (J = 12, 5 Hz) as double doublet. Six tertiary methyl groups appeared as singlets in the upfield region at □ 0.76, 0.79, 0.83, 0.94, 0.97 and 1.03. A multiplet centered at □ 2.38 was attributed to H-19 proton of cyclopentane ring. A characteristic resonance for an acetyl group appeared at □ 2.10 as a singlet.



(2)

The mass spectrum displayed a prominent parent ion peak at m/z 468 indicating its molecular formula as C32H52O2 along with peaks at m/z 453 [M-Me]+, 408 [M–AcOH]+, 242, 240, 231 and 213.

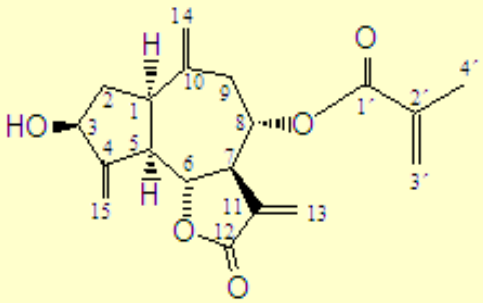
Its identity was confirmed by above spectral data and by comparison with an authentic sample.

(3) Characterization of Aguerin-B :

It was obtained as pale yellow viscous oil. It showed homogeneous behaviour on TLC plate. High resolution mass spectrometry established its molecular formula as C19H22O5.

The appearance of broad absorption bands in its IR spectrum at 3450 cm–1 and 1720 cm–1 revealed the presence of hydroxyl and unsaturated ester functions in the molecule. A strong absoption band at 1760 cm–1 assigned to □-lactone moiety.

Its 1H NMR spectrum (Fig. 5) in CDCl3, showed three pairs of downfield absorptions at δ 6.22 (J = 3.5 Hz) and 5.63 (J = 3 Hz) as doublets for H-13 and H-13′; δ 5.53 (J = 1 Hz) and 5.39 (J = 1.5 Hz) as triplets for H-14 and H-14′ and δ 5.17 and 4.97 as broad singlets for H-15 and H-15′ exomethylene protons respectively. A pair of doublet of double doublets at δ 2.25 (J = 15, 8, 1.5 Hz) and 1.77 (J = 15, 8, 5 Hz) were assigned to geminally coupled H-2 δ and H-2 δ protons. H-9 δ and H-9 δ protons exhibited a pair of double doublets at δ 2.39 (J = 14.5, 4 Hz) and 2.72 (J = 14.5, 6 Hz). A doublet of doublet at δ 4.25 (J = 11, 9 Hz) was attributed to H-6 proton. Proton under hydroxyl group (H-3 δ) and H-1 proton exhibited doublet of double doublets at δ 4.58 (J= 8, 8, 1.5 Hz) and 3.00 (J = 10, 8, 8 Hz) respectively. Proton under ester function (H-8 δ) gave a downfield three fold doublet at δ 5.12 (J = 7, 6,4 Hz). A double doublet and a four fold doublet at δ 2.87 (J = 11, 10 Hz) and 3.20 (J = 9, 7, 3.5, 3 Hz) were assigned to H-5 and H-7 protons respectively. Olefinic protons of a methacrylate moiety exhibited a pair of downfield triplets at δ 6.19 and 5.69 (J = 1 Hz, each) along with an olefinic methyl signal at δ 2.01 as broad singlet.



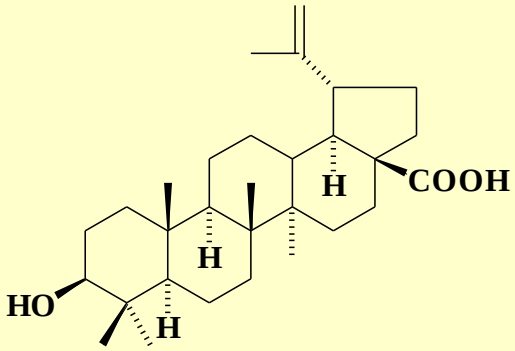
(3)

In high resolution mass spectrum a molecular ion peak was observed at m/z 330.147, which established its molecular formula C19H22O5. The other distinguishable peaks were observed at m/z 244, 226 and 198 due to the loss of RCOOH, H2O and CO from molecular ion successively. These spectral studies were consistent with the proposed structure of aguerin-B and it was confirmed by comparison with an authentic 1H NMR spectrum.

(4) Characterization of Betulinic acid [3β-Hydroxylup-20(29)-en-28-oic acid]:

It was obtained as colourless crystals, m.p. 316-18° and belongs to lupane series of triterpenes. It was analysed for $C_{30}H_{48}O_3$ as its molecular composition. It developed yellow colour with TNM indicating unsaturation in the molecule and gave positive Liebermann-Burchard and Noller tests characteristic of triterpenoids. Its IR spectrum revealed the presence of broad absorptions bands at 3325-2800 and 1715 cm^{-1} for hydroxyl and carboxyl functions respectively.

The ^1H NMR spectrum in $CDCl_3$ displayed singlets at δ 0.76, 0.78, 0.82, 0.96 and 1.03 for five tertiary methyl groups. Two broad singlets at δ 4.56 and 4.68 and a broad singlet at δ 1.68 were discernible as those of two vinylidene protons and an olefinic methyl group of the side chain respectively. Multiplet at δ 2.30 and double doublet 3.27 were assigned to H-19 and H-3α protons.



(4)

Its mass spectrum exhibited a prominent parent ion peak at m/z 456 $[M]^+$ corresponding to its molecular formula $C_{30}H_{48}O_3$. Fragment ion peaks at m/z 438 and 411 were appeared due to loss of H_2O and COOH radical from molecular ion respectively.

Above mentioned spectral data were in close agreement with literature value of betulinic acid and its identity was confirmed by preparing its methyl ester, m.p. 222-23°.

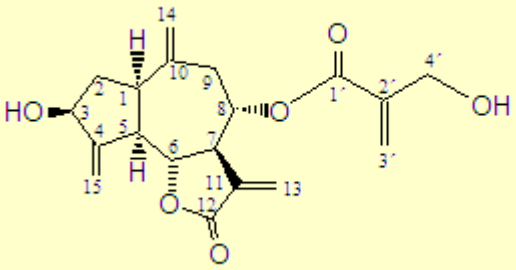
(5) Characterization of Cynaropicrin :

It was isolated as colourless crystals15, m.p. 230-31° and showed homogeneous behaviour on TLC plate. High resolution mass spectrometry established its molecular composition as C19H22O6. In

its IR spectrum the presence of □-lactone moiety was ascertained by a strong absorption band at 1775 cm-1 along with the absorptions at 3450 cm–1 for hydroxyl group and 1720 cm–1 for an unsaturated ester moiety in the molecule.

The 1H NMR spectrum in CDCl3, displayed a pair of characteristic downfield doublets at □ 6.23 (J = 3.5 Hz) and 5.64 (J = 3 Hz) for exomethylene H-13 and H-13′ protons. A pair of triplets at □ 5.51 (J = 1.5 Hz) and 5.38 (J = 1.5 Hz) was assigned for H-14 and H-14′ olefinic protons. Broad singlet at □ 5.18 and a doublet at 4.97 (J =1.5 Hz) were assigned for H-15 and H-15′ exomethylene protons. H-1□, H-2□, and H-2□ appeared as doublet of double doublets at □□2.98 (J = 10, 8, 8 Hz), 2.25 (J = 15, 8, 1.5 Hz) and 1.76 (J = 15, 8, 5 Hz) respectively. A pair of double doublets at □ 2.73 (J = 14, 5 Hz) and 2.42 (J = 14, 3.5 Hz) exhibited for H-9 and H-9′ protons. H-5□ and H-6□ protons appeared as double doublets at □ 2.85 (J = 10, 9 Hz) and 4.27 (J = 10, 9 Hz) respectively. Proton at C-7 displayed a four fold doublet at □ 3.20 (J =10, 7, 3.5, 3.0 Hz).

Proton under oxygen function (H-3□) gave a doublet of double doublet at □ 4.57 (J = 5, 1.5, 1.5 Hz). A three fold doublet at □ 5.17 (J = 7, 5, 3.5 Hz) was assigned to proton under an ester function at C–8 position. A broad singlet at □ 4.39 was attributed to –CH2OH protons of hydroxy methacrylate ester moiety. A singlet at □ 6.33 and a triplet at □ 5.93 (J = 1.5 Hz) were shown by olefinic protons of ester moiety.



(5)

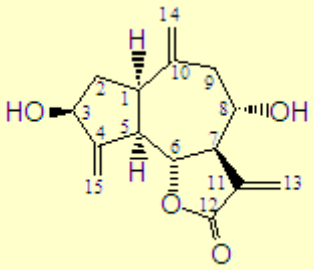
In mass spectrum a molecular ion peak was observed at m/z 346.141 corresponding to its molecular formula C19H22O6. The other distinguishable peaks observed at m/z 224, 226 were due to the loss of hydroxy methacrylic acid and water molecules from the molecular ion peak successively.

On the basis of above spectral studies and comparison of its 1H NMR spectrum with an authentic sample it was characterized as cynaropicrin.

(6) Characterization of Desacylcynaropicrin :

It was obtained as colourless gum16 after purification with preparative TLC. It showed homogeneous behaviour on TLC plate. Accurate mass measurements established its molecular composition as C15H18O4. Strong absorption bands in the region of 1780 cm–1 and 3500 cm–1 indicated the presence of □-lactone and hydroxyl functions in its IR spectrum.

The presence of hydroxyl groups was further confirmed by the loss of two H2O molecules successively from parent ion peak at 244 [262-H2O]+ and 226 [244-H2O]+ in the mass spectrum . Its molecular formula C15H18O4 was followed from its mass spectrum.



(6)

The 1H NMR spectrum (Fig. 7) in CDCl3 of the compound was similar to that of cynaropicrin (**5**) except the signals of hydroxy methacrylate moiety. The comparative upfield shift of H-8□ proton (□ 3.98) in this lactone suggested that it was under a hydroxyl function rather than the ester residue. On the basis of above spectral evidences, it was characterized as desacylcynaropicrin which was finally confirmed by comparison17 with an authentic sample.

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