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ACTIVE COMPOUND ANTIASTHMATIC FROM THE STEM OF EUPHORBIA HIRTA (LINN.)

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Abstract

An Anti-asthamatically compound Taraxerol has been isolated from the stem of Euphorbia hirta (linn.) The Anti-asthmatically compounds are very advantages of the medicinal chemistry and its worked very fast about Anti-asthmatically and Antidibatics , cancer etc.

Key words: Euphorbia Hirta; Euphorbiaceae; Triterpenoid; Taraxerol

Introduction

Euphorbia hirta[1-5] (NO – Euphorbiacea) is an annual herbs and grows locally. It has been found to possess various significant medicinal values. Its juice is used in dysentery and coughs. Its decoction is used in bronchial asthma, affections and chronic coughs. The ripe fruits of this plant are used to stop vomiting whereas its extracts are spasmolytic and antihelmintic. The present papers communicates the isolation and identification of triterpenoid characterised as; Taraxerol which has been isolated from the stem of Euphorbia hirta

Materiala and Methods

Plant Materials: The fresh stems of Euphorbia hirta were collected in village area in Jabalpur. Districts and identified by SFRI. Polypather.

Experimental Section

Melting points 1PNMR recoded by Varian unity (300 MHz) spectrometer 13C NMR were determine in open capillary tubes. Infrared (IR) spectra were Recorded FABMS were recorded on VG Auto spec 3000 mass spectroctrometer. Proton Nuclear Magnetic Resonance spectra were recorded on Varian XL (300 MHz).

Extraction and Isolation

The alcoholic extract of the stem (4 kg.) of the Euphorbia hirta was extracted with ethylacetate. The ethylacetate soluble portion was chromatographed over si-gel column and on elution with benzene : $CHCl_3$: ethylacetate (1 :1 :1) ,and on subsequent evaporation of eluants a compound was obtained which showed a single homogeneous spot on TLC[20] over silica gel, using the solvent system n-butanol : acetic acid : water (4 : 1 : 5) and I_2 as detecting agent. It crystallised from methanol.

Compound: Colourless needles which analysed for the m.f. $C_{30}H_{50}O$, m.p. 280-282°C, $[M^+]$ 426, IR υ_{max}^{KBr} cm^{-1} 3422.8 (—OH group), 3018.6 (= C-H str.), 2932.4 (–C–H str. of CH_3), 2856.6 (–C–H str.), 1608.6 (–CH_2–CH group), 1400.0, 1319.5 (triterpenoidal nature), 813 (> C = CH). FABMS m/z 426 $[M^+]$, 408, 393, 284, 269, 204 (base peak), 133.**^{13}C-NMR :** 39.5 (C–1), 33.8 (C–2), 216.8 (C–3), 47.3 (C–4), 54.6 (C–5), 38.6 (C–6), 36.6 (C–7), 36.5 (C–8), 52.7 (C–9), 38.9 (C–10), 51.6 (C–11), 58.1 (C–12), 37.6 (C–13), 156.5 (C–14), 119.2 (C–15), 38.3 (C–16), 35.5 (C–17), 48.2 (C–18), 20.1 (C–19), 28.7 (C–20), 33.2 (C–21), 35.2 (C–22), 21.5 (C–23), 26.2 (C–24), 16.2 (C–25), 26.7 (C–26), 19.5 (C–27), 30.2 (C–28), 33.7 (C–29), 29.9 (C–30).**^{1}H-NMR:** 2.03 (1H, m, H–1a), 2.10 (1H, m, H–1b), 2.38 (1H, ddd, J= 15.2, 6.2, 2.4, H–2a), 2.63 (1H, ddd, J= 16.4, 11.0, 6.6, H–2b), 3.62(1H, t, H-3) , 1.29 (1H, m, H–5), 1.71 (1H, m, H–6), 1.17 (1H, m, H–7a), 1.46 (1H, m, H–7b), 1.02 (1H, d, J= 4.8, H–9), 3.14 (1H, t, J= 4.8, H–11), 2.80 (1H, d, J= 4.8, H–12), 5.53 (1H, dd, J= 8.0, 3.5, H–15), 1.69 (1H, dd, J= 14.6, 8.2, H–16a), 1.89 (1H, dd, J= 14.6, 3.4 H–16b), 1.17 (1H, m, H–18), 1.55 (1H, m, H–19), 1.42 (1H, m, H–19), 0.98 (1H, m, H–22a), 1.07 (1H, m, H–22b), 1.09 (1H, s, H–23), 0.93 (1H, s, H–24), 1.22 (1H, s, H–25), 1.10 (1H, s, H–26), 0.78 (1H, s, H–27), 0.85 (1H, s, H–28), 0.98 (1H, s, H–29), 0.95 (1H, s, H–30).

Result and Discussion

The air-dried, powdered stem of the plant 'Euphorbia hirta' were extracted with ethanol (95%) and the total ethanolic extract was concentrated to light brown viscous mass. The ethylacetate soluble fraction of the ethanolic extract was chromatographed over si-gel G column. On elution with benzene: $CHCl_3$: ethyl acetate (1:1:1), it gave a compound which analysed for m.f. $C_{30}H_{50}O$ $[M^+]$

426 (FABMS), m.p. 282-283°C. The significant peaks which appeared at υ_{max}^{KBr} 1400.0 cm^{-1} and 1319.5 cm^{-1} in the IR spectrum of the compound showed it to be of triterpenoidal nature.[16] It responded to all the characteristic colour reactions of triterpenoid.[6,7]

The IR spectrum of the compound shows a peak at 3422.8 cm^{-1} which implied the presence of –OH group(s) in it. The compound was found to form acetyl derivative, $C_{32}H_{52}O_2$, [M^+] 468, m.p. 304-306°C. Estimation of acetyl group was found to be 9.18% as described by Wiesenberger method[8] as stated by Belcher and Godbert[9] and so impressed the presence of one —OH group in the compound.

The compound on oxidation with CrO_3/pyridine afforded a ketone, of m.f. $C_{30}H_{49}O$, [M^+] 425, m.p. 242-244°C. The compound gave positive Zimmerman test[10] for the C-3 keto group, which confirmed the presence of one —OH group at C-3 and further indicated that this —OH group was secondary in nature.

On the basis of above deliberations it has concluded that the C-3 —OH group of compound was secondary in nature and present at C-3 position.

An IR peak which appeared at υ_{max}^{KBr} 813 cm^{-1} in the IR spectrum of compound indicated the presence of double bond in the compound. On catalytic hydrogenation, with Pd/C, it afforded a dihydro derivative having m.f. $C_{30}H_{52}O$, m.p. 186-187°C. This indicated the presence of one double bond in it. In ^{1}HNMR spectrum of the compound, olefinic proton signal appeared at 5.53 as (dd) at low field, which indicated the presence of vinyl proton at C–15, owing to the double bond between C–14 and C–15. This also suggested that this compound was a taraxerene type triterpene.[11-17]

An IR peak appeared at υ_{max}^{KBr} 2932.4 $cm^{-1,}$ for angular methyl groups, which were estimated by Zeisel's method[19] (21.73%) and confirmed the presence of eight methyl groups in it. Various singlets obtained in the ^{1}H-NMR spectrum at δ 1.09, δ 1.07, δ 1.22, δ 1.10, δ 0.78, δ 0.85, δ 0.98, δ 0.95 indicated the presence of methyl groups. Chemical shift in ^{13}C-NMR spectrum at δ 21.5, δ 26.2, δ 16.2, δ 26.7, δ 19.5, δ 30.2, δ 33.7, δ 29.9, showed methyl groups in it. The compound was further identified by mass spectrum. The base peak at m/z 204 which originated from the fragmentation of ring C and D of an Δ^{14} taraxerene while

another peak observed at m/z 284 originated from retro-Diels-Alder (RDA) fragmentation of ring D which suggested the presence of unsaturation[19] and the various species formed during fragmentation as described below fully confirmed its identity as taraxerol as in Fig. 2.

On the basis of the above deliberations the Triterpenoid was identified as Taraxerol (Fig. 1& Fig. 2).

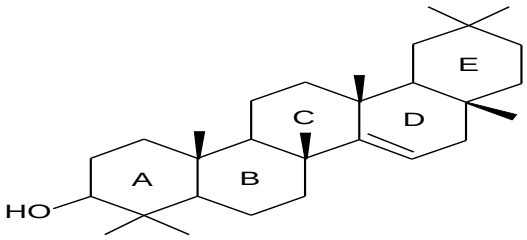

Fig. 1

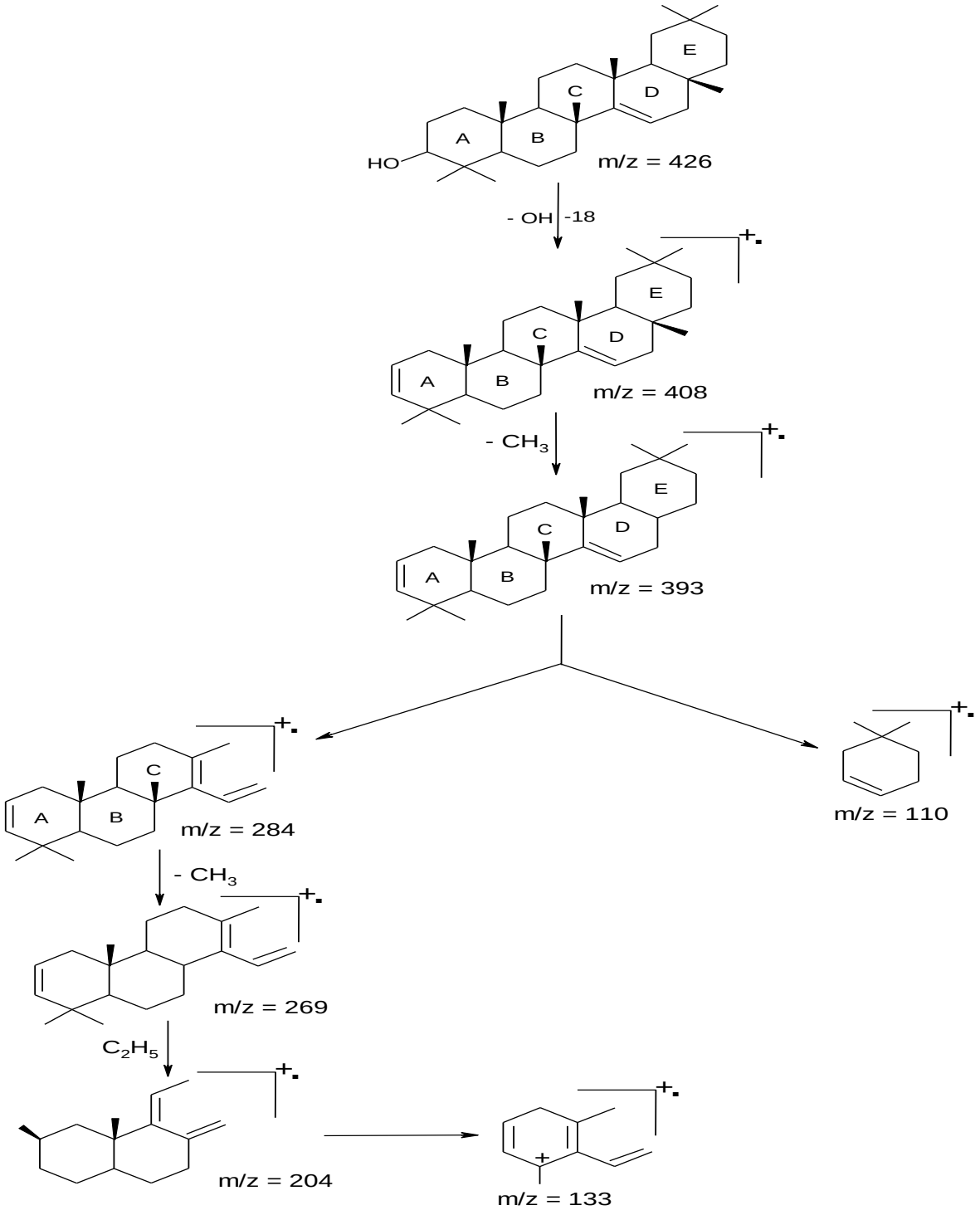
E
C
D
A
B
HO
m/z = 426
- OH -18
m/z = 408
- CH3
m/z = 393
m/z = 284
m/z = 110
- CH3
m/z = 269
C2H5
m/z = 204
m/z = 133

Fig. 2

Screening study of the compound for Anti-asthmatic activity

Anti-asthmatic screening of the isolated compound was carried out on histamine induced bronchonstricted guinea pigs[21-27.]

Guinea pigs of either sex, weighing 250-500 gm of pir bright white strain were used for the biological study. The animals were housed under standard conditions of temperature and humidity for 24 hrs and fasted with fresh leafy vegetables and carrots and with water adlibitum.

Induction of Histamine[28-30]

Animals were divided into five groups i.e. A, B, C, D, E. The guinea pigs of either sex were exposed to histamine aerosol (0.5% histamine diphosphate in saline) in a airtight histamine chamber by using a glass sprayer, to induce the bronchospasm.The guinea pigs after exposure to the histamine aerosol indicated the sign of progressive immobilization and bouts of coughing leading to convulsions when fell on its back.

The time taken by the guinea pigs to fall on its back after exposure to aerosol was called or exposition time which was noted for each animal. Exposition time also known as PCD (pre-convulsive dyspnoea).

After recording the exposition time, the animals were immediately removed from the chamber and placed in a fresh air, so that the animals came back to its normal state.

Drug Treatment Schedule[28-30]

When the histamine was induced, later after 1 hour, the first four group of animals and the fifth group animal (E) was received salbutamol (0.65 mg/kg) by the intraperitoneal route. After 1 hour, the animal was again exposed to histamine and exposition times were noted. The fifth group animal was treated as a standard group of animal.

The animals in a first four group were treated with isolated compounds. Later after 1 hour each of the animals were exposed to aerosol and there exposition time were recorded.

Each time, the animals were fed with fresh green leafy vegetables and tap water and libetum and made them refresh for one week. After then, the animals were ejected intraperitoneally with an increasing doses of (400,200,300 mg/gm) of drugs to A, B, C, D. After 30 min of drugs administration they were again exposed to histamine and the exposition were noted for each animals.

The difference in the exposition time before and after extract administration was taken as a measure of the protective effect of the drugs.

The percentage protection offered by drugs in PCD was calculated by the formula.

$$\text{Percentage protection} = \frac{\text{Eta} - \text{Etb}}{\text{Etb}} \times 100$$

(% Protection offered by the extract)

Where:

Eta is the mean exposition time after administration of drug.

Etb is the mean exposition time before administration of drug.

The results were expressed as mean + SD. The significance between post-treatment and pre-treatment exposition times was analysed by student test. The values are considered to be significantly different when the p value was less then 0.05.

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