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# B- SITOSTEROL, LUPEOL, BETULIN, 2-METHYL ANTHRAQUINONES, CORCHOROSIDE-A AND FUSIDIC ACID FROM CORCHORUSAESTUAN ROOTS

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## ABSTRACT

β- sitosterol, lupeol, betulin, 2-methyl anthraquinones, corchoroside-A and fusidic acid from *Corchorusaestuan* roots. Isolation of fusidic acid (CAR-6) from this species is reported for the first time. However, this compound was earlier reported from a *Fusidiumcoccineum* marine organism.The author is now working on the antibacterial activity of CAR-6 on various microorganisms.

**KEY WORDS:** β- sitosterol, lupeol, betulin, 2-methyl anthraquinones, corchoroside-A andfusidic acid from *Corchorusaestuan* roots.

## INTRODUCTION

*Corchorusaestuans L.* a Tiliaceae member is an erect to procumbent annual herb, grow up to 20 cm high cm long.Leaves are 1.5–6.5 x 1–4 cm. Capsule are 1.5–2.7 cm long and seeds are 0.5 mm long. Biologically *Corchorus* species are used as diuretic, chronic cystitis, gonorrhoea and dysuria antihistaminic, anti-inflammatory, antimicrobial, cardiotonic, and also to increase the viscosity of the seminal fluid [1]. Several important bioactive molecules were reported which includes cardiac glycosides, their aglycones and polysaccharides, triterpenoids, phenolics, sterols and fatty acids.[1]

### Collection and Preparation of Plants

The leafs, capsules and root extracts of *Corchorusaestuans* were collected from Warangal in September 2007 (2kg) and was authenticated by Prof.V.S. Raju, Department of Botany, KaKatiya University,Warangal. A specimen was deposited in the Herbarium (Voucher specimen number (CA/07) roots were collected from the plant and dried under shade.

**Extraction**

The roots (2kg) of *Corchorusaestuans* powdered plant material was on extraction in a soxhlet with petroleum ether (3 Lit), chloroform (3 Lit) and methanol (3 Lit) for 18 hrs and concentrated. The petroleum ether, chloroform extract shown similar spots on TLC (1:1 Benzene : Chloroform) and L.B reaction for sterols (pink,blue,green)and triterpenoids and positive test for ferric chloride on column chromatography over silica gel (Acme 100 mesh), which afforded three compounds designated as CAR-1, CAR-2, and CAR-3. The methanolic extracts showed positive tests for terpenoids and cardiac glycosides. On column chromatography the methanolic extract gave three compounds CAR-4, CAR-5 and CAR-6.

**Characterization of the isolated compounds**

**CAR-1 (β-sitosterol, 30mg)**

The compound was crystalized from petroleum ether as a colorless needles, m.p 137°C. It showed color reaction for sterols with Liebermann-Burchard test. The UV (MeOH) *λmax*205 nm; EIMS *m/z* 414 [M]+(calc. for $C_{29}H_{50}O$). $^{1}$H NMR ($CDCI_3$, 400 MHz): *δH*3.52 (1H, *m*, H-3), 5.35 (1H, *m*, H-6), 0.68 (3H, *s*, Me-18), 0.98 (3H, *s*, Me-19), 0.91 (3H, *d*, *J* = 6.4 Hz, Me-21), 0.83 (3H, *d*, *J* = 6.8 Hz, Me-26), 0.81 (3H, *d*, *J* = 6.9 Hz, Me-27), 0.85 (3H, *t*, *J* = 7.8 Hz, Me-29). $^{13}$CNMR (CDCI3, 100 MHz): *δC*37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 29.9 (C-8), 50.3 (C-9), 36.7 (C-10), 21.3 (C-11), 40.0 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.6 (C-19), 36.3 (C-20), 19.0 (C-21), 34.1 (C-22), 26.3 (C-23), 46.0 (C-24), 29.3 (C-25), 20.0 (C-26), 19.2 (C-27), 23.2 (C-28), 12.2 (C-29) [ 2 ].Based on the spectral data the compound was identified as β-sitosterol and the identity was further confirmed by comparison with authentic sample (m.m.p. and Co-TLC.)

**CAR-2 (Lupeol, 20mg)**

The compound was crystallized from hexane as colourless needles with m.p. 212-214$^{0}$C, $[\alpha]_D^{30}$ + 38° (C, 1.12 in chloroform) and analyzed for the formula $C_{30}H_{50}O$. It gave pink colour with L.B. reaction indicating that the compound was a triterpenoid. The IR spectrum showed bands at 3540 cm$^{-1}$– OH absorption, 1380 and 1390 cm$^{-1}$ (*gem*-methyls) and at 890 cm$^{-1}$ (vinyl methylene). $^{1}$H NMR spectrum ($CDCl_3$, 90 MHz, δ) showed peaks at 0.76 (d, 3H); 0.78, 0.80, 0.90, 1.02 (s,15H); 1.63 (s, 3H); 0.91 (s, 6H) and δ 3.18 (m, 1H). From the above properties CAR-2was identified as lupeol and the identity was confirmed by comparison with authentic sample (m.m.p. and co-TLC).[3]

**CAR-3 (Betulin, 40mg)**

The compound was obtained as colorless needles, m.p. 253-255° and showed single spot on TLC. It developed pale-yellow coloration with trinitro methane in chloroform indicating unsaturation. It responded positively to Liebermann-Burchard tests characteristic of triterpenoids. Its infrared spectrum showed characteristic absorption bands at 3460-3400 (broad, OH stretching), 2970-2880 (C-H stretching), 1650 cm-1 (C=C stretching). $^{1}$H-NMR **:** (δ, $CDCl_3$): 4.53 and 4.67 (=$CH_2$), 3.33 and 3.85 (d, *J* = 11 Hz each – $CH_2OH$), 3.18 (dd, *J* = 12, 5Hz H-3α), 2.44 (m, H- 19), 1.67 (s, =C-$CH_3$), 0.75 (s, 3H), 0.85 (s, 3H), 0.96 (s, 3H), 0.98(s, 3H), 1.02 (s, 3H) for five tertiary methyl groups. $^{13}$ C-NMR **:** (δ, $CDCl_3$): 38.8 (C-1), 27.4 (C-2), 79.0 (C-3), 38.3 (C-4), 55.4 (C-5), 18.3 (C-6), 34.3 (C-7), 41.0 (C-8), 50.6 (C- 9), 37.4 (C-10), 20.9 (C11), 25.6 (C-12), 37.0 (C-13), 42.8 (C-14), 27.1 (C-15), 29.3 (C-16), 46.4(C-17), 47.8 (C-18), 48.8 (C-19), 150.3 (C-20), 29.8 (C-21), 34.0 (C-22), 28.0 (C-24), 6.1 (C-25), 6.1 (C-26), 14.7 (C- 27), 60.8 (C-28), 109.6 (C-29), 19.4 (C-30). Based on above data and by comparing with an authentic sample, the compound was identified as betulin [1].

**CAR-4 (2-methylanthraquinone, 25mg)**

It was crystallized as yellow needles in methanol in chloroform mixture, mp 170 - 173º C and gave color reaction with Kedde's reagent, negative with LB reaction and showed a red-orange color under UV 366 nm at Rf 0.72 in $CDCl_3$:MeOH (19:1) and Rf 0.37 in petroleum ether : acetone : acetic acid (75:25:1.5) .The $^{1}$HNMR showed signals at (300 MHz, $CDCl_3$): 2.47 (3H, s, –$CH_3$), 7.53, (1H,d, J=7.8 Hz, H-3), 7.71–7.73 (2H, m, H-5, 8), 8.04 (1H,s ,H-1), 8.14 (1H, d, J = 7.8 Hz, H-4), 8.23–8.25 (2H, m, H-6, 7). $^{13}$ C-NMR**:** (125 MHz, $CDCl_3$): d = 127.8 (C-1), 145.5 (C-2), 135.3 (C-3), 127.7 (C-4), 127.4 (C-5), 134.3 (C-6), 134.2 (C-7), 127.5 (C-8), 183.7 (C-9),183.3 (C-10), 22.1 ($CH_3$). The data and the result correspond with 2-methylanthraquinone, andis in good agreement with that of 2-methylanthraquinoneand further the identity was confirmed by comparison with an authentic sample by m.m.p and co-TLC [3].

**CAR-5 (Corchoroside- A, 20mg)**

The compound was crystallized from methanol-ether as colourless prisms m.p. 167-168$^{o}$C; $[\alpha]_D$ = +19.7$^{o}$ (methanol), it showed positive Kedde and Legal reactions indicating the cardenolide nature of the compound. Based on the UV, NMR spectral data, the compound was identified as corchoroside-A. Which was earlier isolated from *C.capsularis* roots [1]

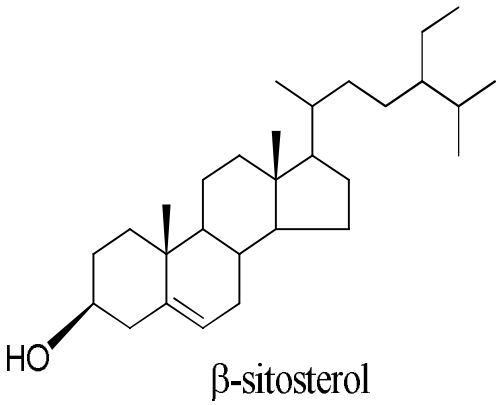
**CAR-6 (Fusidic acid, 40mg)**

It was obtained as a white solid, m.p.190$^0$C . The $^1$H and $^{13}$C NMR spectra and other chemical properties were coincided well with that of fusidic acid, earlier reported from *C.olitorius*[4]. Hence , the compound CAR-6was identified as fusidic acid.

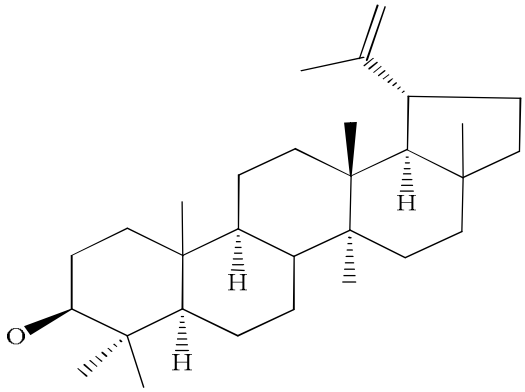
**RESULTS AND DISCUSSION**

The genus *Corchorus* is well known to contain cardiac glycosides. About nine species are available in India and were reported to possess cardiac principles. Besides these compounds, the *g*enus *Corchorus* was also reported to possess saponins, flavonoids and sterols. Chemical examination of the roots of *Corchorusaestuans*afforded corchoroside-A and triterpenesfusidic acid and betulin. Isolation of fusidic acid (CAR-6) from this species is reported for the first time. However, this compound was earlier reported from a *Fusidiumcoccineum* marine organism . The author is now working on the antibacterial activity of CAR-6 on various microorganisms.

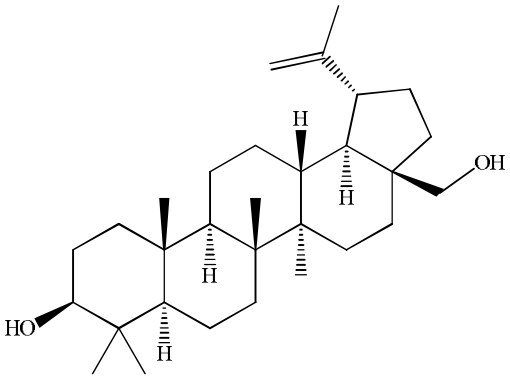
**structures**



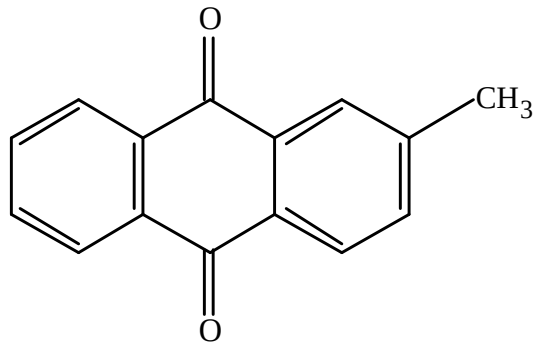

β-sitosterol



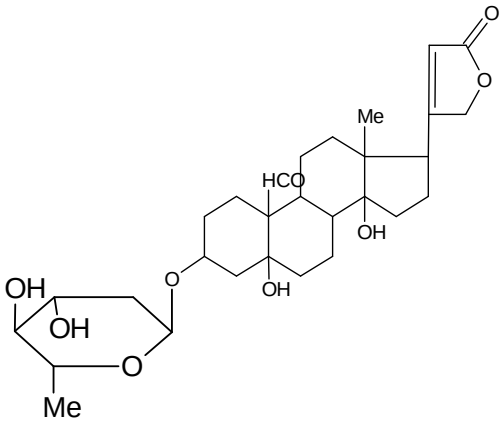

Lupeol

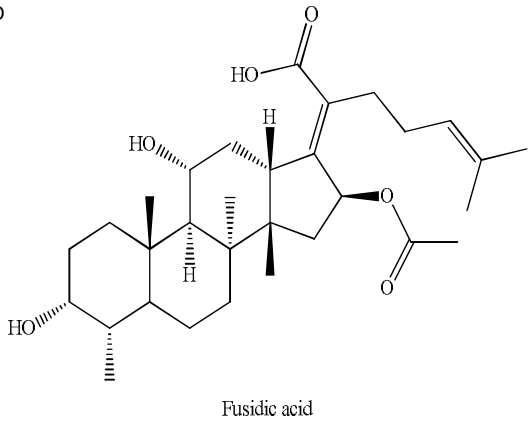



Betulin




2-Methyl anthraquinone




Fusidic acid

Corchoroside-A

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