

FUSIDIC ACID FROM CORCHORUS AESTUANS L.

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ABSTRACT

The fusidic acid together with known compounds β -sitosterol, 2-methylanthraquinone and scopoletin from the leaf extract of *Corchorus aestuans*. From these isolates β -sitosterol, 2-methylanthraquinone, scopoletin and fusidic acid were reported for the first time from *C.aestuans*. The triterpenoid antibiotic fusidic acid was obtained earlier from a fungi (*Fusidium coccineum*), is now reported from the plant *C.aestuans*.

INTRODUCTION:

Corchorus aestuans L. a *Tiliaceae* member is an erect to procumbent annual herb, grow up to 20 cm high cm long. Most of the compounds isolated from this genus are sterols, terpenes, phenolic compounds, tannins, saponins and cardiac glycosides. The *C. aestuans* was reported to possess anti-inflammatory, in the treatment of pneumonia, antiviral, anti-inflammatory, plasma-cholesterol lowering activities and anticancer activity against epidermal

carcinoma of nasopharynx in tissue culture [1-5].

PLANT MATERIAL:

The plant material, *Corchorus aestuans*, was collected from Warangal in September 2007(2kg). The plant was authenticated by Prof. V.S. Raju , Department of Botany, Kakatiya University, Warangal. A specimen was deposited in the herbarium (Voucher specimen number (CAL/07) roots were collected from the plant and dried under shade.

EXTRACTION:

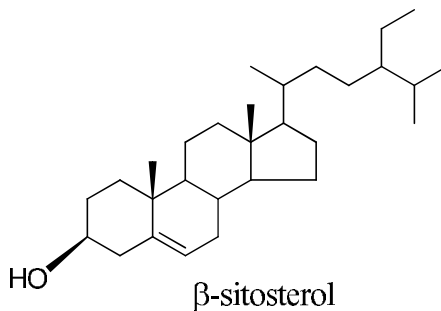
The leaves (2kg) of *Corchorus aestuans* were air dried and coarsely powdered in a Wiley mill and successively extracted with petroleum ether (3×3 l), chloroform (3×3 l) and methanol (3×3 l) and concentrated under reduced pressure. The petroleum ether, chloroform extracts of *Corchorus aestuans* roots shown similar spots on TLC (1:1 Benzene: Chloroform) and hence combined and column chromatographed over silica gel (Acme 100 mesh), which afforded two compounds designated as CAL-1 and CAL-2, The methanolic extracts showed positive tests for terpenoids and cardiac glycosides. On column chromatography the methanolic extract gave two compounds CAL-3 and CAL-4.

Characterization of the isolated compounds:

CAL-1: (β -sitosterol, 30mg)

The compound was crystallized from petroleum ether as a colorless needles, m.p

136-138°C. It showed color reaction for sterols with Liebermann-Burchard test. The UV (MeOH) λ_{max} 205 nm; EIMS m/z 414 [M]⁺(calc. for C₂₉H₅₀O). ¹H NMR (CDCl₃, 400 MHz): δ H 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). ¹³C NMR (CDCl₃, 100 MHz): δ C 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). 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.) [6-7]

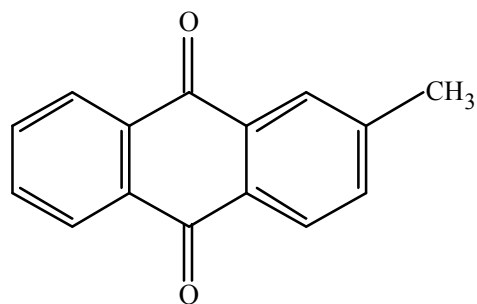


CAL-2: (2-methylanthraquinone, 25mg)

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It was obtained as an yellow solid, crystallized as needles in methanol: chloroform mixture. mp 170 - 173° C and answered with Kedde's reagent, negative with LB reaction. Rf 0.72 in CDCl₃ : MeOH (19:1) and Rf 0.37 in petroleum ether : acetone : acetic acid (75:25:1.5) and ¹H-NMR (250 MHz, CDCl₃) : 2.47 (3H, s, -CH₃), 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, CDCl₃): 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 (CH₃). The data corresponded well with that of 2-methylantraquinone, and further confirmed by comparison with an authentic sample by m.m.p and co-TLC.[8-9]. This is the first report of this compound from *C.aestuans* and particularly from the genus *Corchorus*.

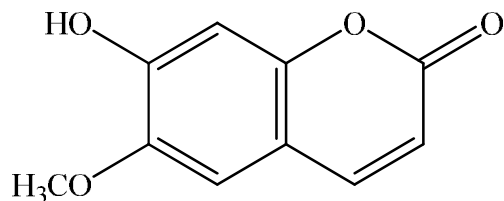


2-Methylantraquinone

CAL-3: (scopoletin, 80mg)

This compound was obtained as yellow crystal, mp: 202-204 °; IR (KBr) λ max cm⁻¹: 3340, 3106, 3031, 2990, 1710, 1600; UV max (MeOH) nm: 230, 254, 260, 298, 346 ; FAB-MS *m/z*:193 [M-H]⁻ ; ¹H-NMR and ¹³C-NMR spectral data were in accord with the molecular formula C₁₀H₈O₄. The ¹H-NMR spectrum revealed the presence of two doublets at δH 7.90 (H-4, *J* = 6.0 Hz) and 6.25 (H-3, *J* = 6.0 Hz). The two singlet signals appeared at δ H 6.80 and 7.25 were assigned for the two protons H-5 and H-8,

respectively. ¹H-NMR: (CD₃OD): d 3.90 (3H, s, 6-OCH₃), 6.19 (1H, d, *J* = 9, H-3), 6.75 (1H,s, H-8), 7.09 (1H, s, H-5), 7.84 (1H, d, *J* = 9, H-4). ¹³ C-NMR : (CD₃OD): d 56.8 (OMe), 104.0 (C-8), 109.9 (C-5), 112.6 (C-3 and C-4a), 146.1 (C-4), 147.1 (C-6), 151.4 (C-8a), 153.0 (C-7), 164.1 (C-2).[10-15] Based on the spectral data and chemical tests, the compound was identified as scopoletin.



Scopoletin

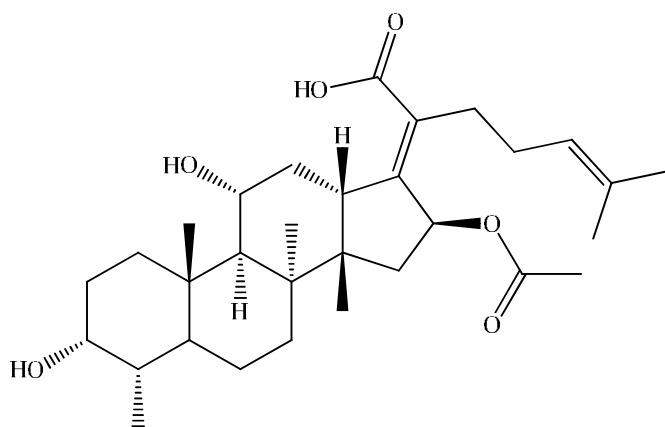
CAL-4: (fusidic acid, 60mg)

It was obtained as colourless substance, m.p.190-192⁰C . The ¹H and ¹³C NMR spectra of the compound showed signals for five methyl singlets, one methyl doublet, four olefinic carbons and a carbonyl of a carboxylic acid (*d*, C 173.8), were indicative of a fusidane of the fusidic acid skeleton. Assuming that the methyl doublet was C-28 of the fusidane skeleton, the protons of this group coupled to a methine proton (*d*,H 1.54, H-4) in the COSY spectrum. H-4 formed part of a spin system with a deshielded methane (*d*,H 3.73, H-3) and two methylene groups (at C-2 and C-1). In the HMBC spectrum, C-1 was coupled to by the protons of methyl-C19 (*d*,H 0.95) which showed further couplings to C-10 (2J), C-9 (3J) and C-5 (3J). In the COSY spectrum, H- 5 (*d*,H 2.31 m) coupled to both protons of a methylene moiety (C-6, *d*,H 1.45, 1.67), Inspection of the HMBC spectrum showed that the carbon associated with this deshielded proton was coupled to by the protons of a further angular methyl singlet (C-30), which showed additional

couplings to a methine carbon (C-9) and two quaternary carbons (C-8, *d*C 45.6 and C-14, *d* C 49.6). This completed the resonances for the A and B rings of compound. Inspection of the COSY spectrum showed that the proton associated with C-9 (H-9) formed part of a CH–CH–CH₂–CH spin system which allowed identification of positions C-9, C-11, C-12 and C-13, respectively. C-11 was deshielded (*d*, C 68.7, *d*,H 4.37) indicating that an oxygen should be placed here. H-13 was also deshielded (*d*,H 3.05) suggesting that it was allylic and that an olefinic carbon (C-17) should be placed at the neighbouring carbon, which is typical for fusidic acid .The protons of a methyl group (C-18) coupled to C-13 (3J), C-14 (2J) and to a methylene carbon (C-15, 3J). CH₂-15 coupled to a deshielded allylic methylene group (*d*,H 2.68, 2.86 (CH₂- 16)) which again was supportive of being alpha to an olefinic carbon (C-17, *d*C 160.4). This completed rings C and D of 1. H-13 and H2-16 both gave a 2J coupling to C-17 and a 3J coupling to C-20, suggesting a C-17, 20

double bond. In the HMBC spectrum C-17 was also coupled to by the protons of an allylic methylene (C-22, d,H 2.44) which also coupled to a carbonyl carbon of a carboxylic acid group (C-21) and an olefinic methine carbon (C-24, dC 124.0). A further methylene (C-23) could be placed between C-22 and C-24 by couplings observed in the COSY spectrum. Finally, two deshielded geminal methyl groups could be placed on

an olefinic carbon (C-25) via their HMBC correlations to this carbon and to the olefinic partner C-24 finalising the C-24–C-25 double bond. These resonances completed the eight carbon chain of the fusidane triterpene skeleton. Chemical shift of the C-21 carbon, a carboxylic acid must be placed at C-21 and this is identical to the fusidic acid. [16-48]



Fusidic acid

RESULTS AND DISCUSSION:

The chemical examination of the leaves of *C.aestuans* on conventional extraction and a sequence of chromatographic methods afforded four compounds.. These are characterized as β -sitosterol, 2-methylanthraquinone, scopoletin, and

fusidic acid. Out of these compounds, 2 -methyl anthraquinone and fusidic acids were new to the genus *Corchorus* and reported for the first time from *C.aestuans*. Occurance of coumarins in *Corchorus* genus are rare, but the author could isolate scopoletin, a coumarin from this species.

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