A novel antimicrobial triterpenic acid from the leaves of *Ficus benjamina* (var. *comosa*)

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- (9,11), (18,19)-Disecoolean-12-en-28-oic acid

**Abstract**
The chloroform extract of the leaves of *Ficus benjamina* (var. *comosa*) (Moraceae) afforded a new triterpenic acid named as (9,11), (18,19)-disecoolean-12-en-28-oic acid (1) along with β-amyrin (2). Their structures were established on the basis of chemical and physical evidences (IR, 1H NMR, and MS data). The compound 1 exhibited significant antimicrobial activity against *Salmonella typhimurium* (MTCC-98), *Candida albicans* (IAO-109), *Staphylococcus aureus* (IAO-SA-22), *Escherichia coli* (K-12) and low activity against *Aspergillus niger* (lab isolate ICAR) and *Aspergillus brassicola*.

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**1. Introduction**

*Ficus benjamina* L. (var. *comosa*) (Moraceae), commonly known as kabra, is a moderate sized, evergreen epiphytic tree (Brandis, 1971). It is used as folk medicine for the treatment of certain skin and respiratory disorders (Dhar et al., 1968; Dhawan et al., 1977). The fruit extract of *F. benjamina* possessed antitumor activity and significant antibacterial activity (Mousa, 1994; Werfel et al., 2001; Diez-Gomez et al., 1998; Axelesson et al., 1990). Medicinal importance of this plant encourages us to carry out the comprehensive investigation of the leaves of *F. benjamina* (var. *comosa*). Now we are reporting the isolation and characterization of a new triterpene, (9,11), (18,19)-disecoolean-12-en-28-oic acid (1) along with β-amyrin (2). The compound 1 has been found to show significant antimicrobial activity.

**2. Experimental**

**2.1. General experimental procedure**

The melting points were taken on a Kofler block and are uncorrected. 1H NMR and 13C NMR were recorded on Bruker DRX-300 and Bruker Avance 400 MHz with TMS as an
internal standard. IR spectra were taken on Shimadzu IR-408 Perkin–Elmer 1800 (FTIR). The MS were measured in both EI Mode and Jeol D-300 and FAB mode on Jeol SX 102/DA-6000 mass spectrometers.

2.2. Plant material

The leaves of *F. benjamina* (var. *comosa*) were collected from the Botanical garden, AMU, Aligarh, India and identified by Prof. Wazahat Hussain, Taxonomist, Department of Botany, AMU, Aligarh.

2.3. Extraction and isolation

The leaves of *F. benjamina* (var. *comosa*) were dried under shade and crushed to make powder (1 kg). The dried leaves were defatted with light petroleum ether (60–80 °C). The residue was exhaustively refluxed with chloroform. The solvent was removed by distillation. The chloroform extract was concentrated under reduced pressure to yield a greenish gummy mass. The concentrate was subjected to column chromatography over silica gel. Elution of the column with petroleum ether–benzene in ratio 9:1–1:1 and benzene gave various fractions which upon repeated column chromatography and fractional crystallization afforded two compounds marked as I and 2.

2.4. (9,11), (18,19)-Disecoolean-12-en-28-oic acid (1)

The fraction obtained by the elution of the column with petroleum ether–benzene (2:3) eluents was crystallized with chloroform–methanol to yield white crystals of 1 (35 mg) m.p. 195 °C; IR νmax (KBr) cm−1: 3459, 2929, 2864, 1719, 1642, 1461, 1383, 1202, 1111, 995, 878, 723; MS m/z: 426 [M]+. C30H52O2 (43.6), 427 (10.5), 414 (16.7), 314 (28.7), 299 (9.8), 274 (74.1), 234 (30.0), 219 (12), 193 (100), 150 (14.5), 136 (81.9), 124 (9.7), 123 (16.8), 121 (13.7), 111 (21.3), 109 (19.8), 97 (43.7), 83 (28.1), 69 (73.9), 55 (76.2), 43 (52.6). Calc for C30H52O2: % C, 81.9; H, 12.4. Found: % C, 81.1; H, 12.6. IR (KBr) cm−1: 3461, 1719, 1642, 1461, 1383, 1202, 1111, 995, 878, 721; MS: 444 [M]+. C30H52O2 (43.6), 427 (10.5), 414 (16.7), 314 (28.7), 299 (9.8), 274 (74.1), 234 (30.0), 219 (12), 193 (100), 150 (14.5), 136 (81.9), 124 (9.7), 123 (16.8), 121 (13.7), 111 (21.3), 109 (19.8), 97 (43.7), 83 (28.1), 69 (73.9), 55 (76.2), 43 (52.6). Calc for C30H52O2: 1H NMR (400 MHz, CDCl3) (Table 1).

2.5. β-Amyrin (2)

The compound 2 was obtained from the column with petroleum ether–benzene (1:4) eluate and crystallized from chloroform–methanol as white crystalline solid (40 mg); m.p. 198 °C, Rf = 0.63 (benzene–chloroform, 8:2) (lit. m.p. 197–198 °C). It gave positive Liebermann–Burchard test IR νmax (KBr) cm−1: 3360 (OH), 2960, 2880, 1650, 1465 (C=O), 1040 and 980 MS m/z: 426 [M]+; 1H NMR (400 MHz, CDCl3): δ: 0.78 (3H, s), 0.83 (3H, s), 0.88 (6H, s), 0.95 (3H, s), 0.98 (3H, s), 1.0 (3H, s), 1.14 (3H, s), 1.08, 2.01 (–CH2 and –CH protons of cyclic system and side chain), 3.01 (1H, dd, J = 9.0 Hz and 7.0 Hz), 4.88 (brs, 1H, OH proton), 5.21 (1H, m, olefinic proton).

2.6. Acetylation of compound (2)

Compound 2 (25 mg) was acetylated by heating it with acetic anhydride (1 ml) and pyridine (0.5 ml) on a boiling water bath for 1 h. The reaction mixture was cooled at room temperature and poured over crushed ice. The solid obtained was washed well with water and dried. On crystallization from chloroform–methanol, it gave colorless crystals m.p. 241–242 °C, [α]D23 + 68.9° (lit. m.p. 241 °C).

2.7. Antimicrobial activity

The in vitro antibacterial activity was carried out against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus subtilis* and in vitro antifungal activity was carried out against *C. albicans*, *Fusarium oxysporum*, *Penicillium notatum*, *Aspergillus niger* and *Trichoderma viridae*. The agar well diffusion method (Pervez et al., 1990; Ahmad and Beg, 2001) was used. About 0.1 ml of diluted inoculum (105 CFU/ml) of test organism was spread on Mueller Hinton Agar plates (Hi-Media Pvt. Ltd., Mumbai, India). The wells of 8 mm diameter were punched into the agar medium and filled with 100 μl of Fe-1 of 1 mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C overnight. The antibiotic (Chloramphenicol) and antifungal disc (Nystatin) of 30 mcg potency each were used in the test system as positive controls. Zone of inhibition of bacterial and fungal growth around each well was measured in millimeter.

3. Results and discussion

The air dried and powdered leaves of the plant after being defatted with light petroleum ether (60–80 °C) were exhaustively extracted with chloroform. The chloroform extract was concentrated under reduced pressure to yield a greenish gummy mass. It was chromatographed over silica gel col-
umn. Elution of the column with petroleum ether–benzene in different ratios gave various fractions which upon fractional crystallization afforded two TLC homogenous compounds marked as 1 and 2. The compound 1 has been characterized as a novel triterpene characterized as (9,11), (18,19)-disecoolean-12-en-28-oic acid with sufficient support of spectral studies and comparison with closely related reported compounds. The compound 2 has been identified as β-amyrin by comparison of TLC, co-TLC, m.p, co-m.p. and spectral data with that of authentic samples (Inoue et al., 1978).

The compound 1 was obtained as white crystalline solid m.p. 195 °C from light petroleum ether–benzene (2:3) eluates. Elemental analysis along with molecular ion peak at m/z 444 agreed with the molecular formula C_{30}H_{52}O_{2}. Considering the carboxylic group and one double bond in the structure along with the fact that molecular composition indicated five double bond equivalents, it was considered to be a tricyclic compound. Its IR spectrum showed characteristic absorption bands at 3459, 1719, and 1642 cm\(^{-1}\) corresponded to carboxyl group which was further confirmed by the appearance of effervescences with NaHCO\(_3\) and the fragment ion peak at m/z 427 (characteristic of carboxylic group). The carboxylic group did not undergo esterification under normal conditions showing the tertiary nature. The prominent ion peaks generated at m/z 55 [C\(_{3,4}\)-C\(_{5,10}\)-C\(_{5,6}\) fission]\(^+\), 69 [C\(_{3,4}\)-C\(_{5,10}\)-C\(_{6,7}\) fission]\(^+\), 83 [C\(_{3,5}\)-C\(_{5,10}\)-C\(_{7,8}\) fission]\(^+\), 97 [C\(_{2,3}\)-C\(_{5,10}\)-C\(_{7,8}\) fission]\(^+\), 111 [C\(_{2,3}\)-C\(_{5,10}\)-C\(_{7,8}\) fission], 124 [C\(_{5,6}\)-C\(_{9,10}\) fission]\(^+\) suggested saturated nature of rings A and B and devoid of any functional group in these rings. The base peak at m/z 193 was formed due to cleavage of C\(_8\)-C\(_{14}\) linkage supporting seco nature of ring C. The ion peaks at m/z 234 [C\(_{13,14}\)-C\(_{14,15}\) fission]\(^+\), 219 [234-\text{Me}]\(^+\), 314 [C\(_{16,17}\)-C\(_{17,18}\) fission]\(^+\) and 299 [314-\text{Me}]\(^+\) indicated saturated nature of ring D and seco pattern of ring E (Ali, 2001) (Scheme 1). A characteristic band at 1461 cm\(^{-1}\) in IR spectrum exhibited the presence of double bond. On the basis of composition and above spectral data it was anticipated to be a triterpenic compound with two incomplete rings [ring C and E] and the structure 1 was tentatively proposed which satisfied the mass fragmentations and the NMR spectrum. The \(^1\)H NMR spectrum of 1 (Table 1) exhibited a one-proton broad signal at \(\delta\) 5.63 assigned to vinylic H-12 (Ageta and Arai, 1983). A three-proton broad signal at \(\delta\) 1.56 was ascribed to C-11 (methyl) protons attached to C-12 vinylic carbon. Eight broad signals at \(\delta\) 1.36, 1.26, 1.17, 1.10, 1.06, 0.90, 0.89, and 0.87 all integrated for three protons each, can be ascribed to C-23, C-30, C-27, C-26, C-29, C-19, C-25, and C-24 tertiary methyl signals. In the light of the foregoing discussion the compound 1 can best be characterized as (9,11), (18,19)-disecoolean-12-en-28-oic acid (1), which is being reported for the first time.

Scheme 1  Mass fragmentation pattern of compound 1.
The compound 2 was obtained as colourless crystalline mass from light petroleum ether–benzene (1:4) eluates and crystallized from chloroform–methanol as white crystalline solid m.p. 198 °C. \([\alpha]_{D}^{19} + 88.4^\circ\) (CDCl₃). It gives positive Leibermann–Burchard test (Irvine, 1961). The infrared spectrum showed the bands at 3360 cm\(^{-1}\) (OH), 2960 cm\(^{-1}\), 2880 cm\(^{-1}\), 1650 cm\(^{-1}\), 1465 cm\(^{-1}\), (C=C), 1040 and 980 cm\(^{-1}\) indicating the presence of hydroxy and olefinic group. Its structure was established as \(\beta\)-amyrin by comparison of m.p., m.m.p., \(R_f\) value and spectral data with that of authentic sample (Inoue et al., 1978).

3.1. Antimicrobial activity

The compound 1 found to be showing significant antimicrobial activity against \(S.\ typhimurium\) (MTCC-98), \(C.\ albicans\) (IAO-109), \(S.\ aureus\) (IAO-SA-22), \(E.\ coli\) (K-12) and low activity against, \(A.\ niger\) (lab isolate ICAR), \(A.\ brassicola\) (Table 2).

### References