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# Indigoferabietone, a novel abietane diterpenoid from *Indigofera longeracemosa* with potential antituberculous and antibacterial activity

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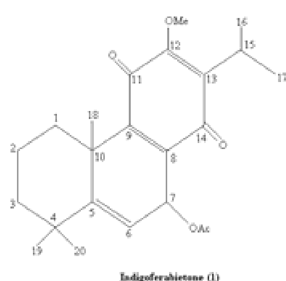
From the stems of *Indigofera longeracemosa*, a novel abietane diterpenoid, indigoferabietone was isolated. The structure was established by spectral techniques. The inhibitory activity of indigoferabietone (**1**) tested against *Mycobacterium tuberculosis* and *Candida albicans*, and the antibacterial activity tested against *Staphylococcus aureus*, *Proteus vulgaris*, and *Escherichia coli* were found to be significant.

## 1. Introduction

*Indigofera longeracemosa* Boiv. ex Baill. (Fabaceae), a slender shrub with woody branches and leaves with rather small very thin leaflets, commonly known as *karunelli* in *Tamil* language grows in the Western Ghats region of Tamil Nadu, preferentially on sloping soils near the streams [1, 2]. The plant is reported to be useful in tribal medicine as antidote for all snake poisons and the root extract of this plant is showing potential antiulcerogenic activity in rats [3]. Even though, no previous phytochemical study of this species has been reported. The present paper deals with the isolation and structural elucidation of a novel abietane diterpenoid, indigoferabietone from the stems of this plant.

## 2. Investigations, results and discussion

Compound **1** had a molecular formula of $C_{23}H_{30}O_5$, as deduced from its HREIMS (*m/z* 386.2123), indicating nine degrees of unsaturation, of which two were assigned to two quinoid carbonyl groups, and three degrees of unsaturation were accounted for by three double bonds in the molecule. The IR and $^{13}$C NMR spectra indicated the presence of quinoid carbonyl groups at 1689 cm$^{-1}$ and δ 183.2, 181.4, respectively. The observed signals at δ 3.81 (3 H, s, OMe), 5.35 (1 H, brs, H-7), 2.03 (3 H, s, $CH_3$) and 6.92 (1 H, brs, H-6), indicates the presence of OAc and OMe. Examination of the structure proposed for **1** indicated an angle nearly 90° between H-6 and H-7, thus supporting the presence of singlets for H-6, and H-7. The $^{13}$C NMR spectrum displayed the presence of seven methyl quartets as six signals, including one methoxyl and one acetyl methyl, along with three methylene triplets, three methine doublets, and ten carbon singlets. The presence of an additional carbonyl signal at δ 172.6 and an acetyl methyl signal at δ 22.3 verified the presence of the acetyl group (Table 1). The structure of **1** was therefore assigned as 7-acetyl-12-methoxy-11,14-dioxoabieta-5,8,12-triene, to which we have accorded the trivial name, indigoferabietone.



Indigoferabietone (1)

The compound **1** showed strong antituberculous activity against *Mycobacterium tuberculosis* and moderate fungicidal activity against *Candida albicans* when compared to standards (Table 2). It is also showing strong antibacterial

**Table 1: $^1$H NMR and $^{13}$C NMR data of indigoferabietone (1)$^@$**

| Position | δH | δC |
|---|---|---|
| 1α | 1.48 br dd (12.8, 6.0) | 37.5 t |
| 1β | 2.69 td (14.2, 13.1) | |
| 2α | 1.59 m | 18.2 t |
| 2β | 2.55 t (14.0, 5.5) | |
| 3α | 1.37 t (14.0, 6.0) | 40.7 t |
| 3β | 2.16 m | |
| 4 | | 33.4 s |
| 5 | | 127.6 s |
| 6 | 6.29 brs | 108.0 d |
| 7 | 5.35 brs | 76.9 d |
| 8 | | 124.8 s |
| 9 | | 142.6 s |
| 10 | | 41.2 s |
| 11 | | 183.2 s |
| 12 | | 154.4 s |
| 13 | | 129.5 s |
| 14 | | 182.0 s |
| 15 | 3.92 sept (6.8) | 25.9 d |
| 16 | 1.21 d (6.8) | 21.5 q |
| 17 | 1.18 d (6.8) | 21.5 q |
| 18 | 1.05 s | 22.0 q |
| 19 | 1.05 s | 33.0 q |
| 20 | 1.08 s | 20.2 q |
| OMe | 3.18 s | 55.9 q |
| C=O | | 172.6 q |
| $CH_3$ | 2.03 s | 22.3 q |

@*J* values are given in parentheses (Hz)

**Table 2: Activity of indigoferabietone (1) against selected fungal and tuberculous pathogens**

| Organisms | Minimum Inhibitory Concentration (μg/μl) | |
|---|---|---|
| | Indigoferabietone (1) | Standards* |
| *Aspergillus fumigatus* | NA | NT |
| *Aspergillus niger* | NA | NT |
| *Candida albicans* | 9.72 | 5.0 |
| *Fusarium oxysporum* | NA | NT |
| *Mycobacterium tuberculosis* | 0.38 | 2.0 |

* Kanamycin for *M. tuberculosis* and clotrimazole for *C. albicans*
NA – not active
NT – not tested

**Table 3: Activity of Indigoferabietone (1) against selected bacterial pathogens**

| Organisms | Minimum Inhibitory Concentration (μg/μl) | |
|---|---|---|
| | Indigoferabietone (1) | Standards* |
| *Bacillus subtilis* | NA | NT |
| *Escherichia coli* | 1.46 | 3.0 |
| *Klebsiella pneumoniae* | NA | NT |
| *Proteus vulgaris* | 0.89 | 2.0 |
| *Staphylococcus aureus* | 0.48 | 2.0 |

* Clindamycin for *B. subtilis* and *S. aureus*; tetracycline for *E. coli*; gentamicin for *P. vulgaris*; and cefotaxime for *K. pneumoniae*
NA – not active
NT – not tested

activity against *Staphylococcus aureus, Proteus vulgaris* and moderate activity against *Escherichia coli* which are comparable with the respective standards [4] (Table 3).

## 3. Experimental

### *3.1. General experimental procedures*

IR spectra were recorded on Perkin-Elmer 983 spectrophotometer in $CHCl_3$. UV spectra were measured on a Varian Techtron 635 instrument using MeOH as solvent. DEPT, SINEPT and HMBC experiments were carried out in a Bruker AMX 500 MHz NMR spectrometer, and all other spectra were recorded on a Bruker AC 200 L instrument in $CDCl_3$. HRMS were measured on a VG ZabSpec mass spectrometer. Also used were Kieselgel 60 F 254 precoated A1 sheets for TLC (0.2 mm, Merck), silica gel (70–200 mesh) for column chromatography (VLC) and Sephandex LH-20 (Fluka) for gel permation chromatography.

### *3.2. Plant material*

The stems of *Indigofera longeracemosa* Boiv. ex Baill. (Fabaceae ) were collected from the forest vicinity of Kalakkad Mundanthurai Tiger Reserve, in Tirunelveli District of Tamil Nadu, India in August 1997 and identified by the co-author Dr. M. B. Viswanathan. A voucher specimen was deposited in the Department Herbarium of Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India.

### *3.3. Extraction and isolation*

The powdered stems of *Indigofera longeracemosa* (600 g) were extracted with acetone in a Soxhlet apparatus. The extract was evaporated *in vacuo* to give 25 g of residue. The residue was fractionated by CC on a silica gel column, eluted with petroleum ether, followed by a gradient of EtOAc up to 100% and then with EtOH. After TLC analysis, the combined fractions were purified by vaccum-liquid chromatography (VLC), eluting with petroleum ether and ethy1 acetate to yield compound **1** (12 mg).
Indigoferabietone (**1**) was obtained as an amorphous powder; 12 mg, m.p. 168–170 °C; UV (MeOH) $\lambda_{max}$ (log ε) 395 (2.6), 285 (3.1), 230 (4.1) nm; IR ($CHCl_3$) $\nu_{max}$ 2950, 2870, 1720, 1689, 1610, 1500, 1463, 1410, 1350, 1260, 1240, 1110, 970, 885, 850, 740 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (200 MHz in $CDCl_3$) (Table 1); EIMS m/z 386 $[M]^+$ (5), 344 $[M-42]^+$ (60), 327 $[M-59]^+$ (95), 296 $[M-60-30]^+$ (40), 270 (50), 213 (40), 171 (18), 128(5); HREIMS *m/z* 386.2123 $[M]^+$ calcd for $C_{23}H_{30}O_5$, 386.2094.

### *3.4. Antifungal and antituberculous activity tests*

The broth microdilution method was used [5, 6]. The fungal pathogens (MTCC), *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum* and *Mycobacterium tuberculosis* were subcultured into Middlebrook 7H9 broth in screw-capped tubes and incubated at 37 °C for 3 weeks. After observation of the growth, the tubes were left at room temperature for 30 min for sedimentation of solid particles. The homogenous suspension at the upper part of each tube was transferred into sterile screw-capped plastic tubes and the inoculam size was adjusted with Middlebrook 7H9 broth to approximately $10^8$ cfu/M1 according to the MacFarland No. 0.5 turbidity standard. This suspension was further diluted 10-fold and used for MIC determinations. Microdilution susceptibility testing was performed in clear 96-well polystyrene plates containing 50 μl of liquid medium in each well. Compound **1** was dissolved in chloroform. Drug solutions (50 μl) were dispensed into the microplates beginning from the first well. Two-fold dilutions were made for compound **1**. The last well was used for control. The inocula (5 μl) were dispensed into the wells, and the plates were tested in plastic bags and incubated at 37 °C in a humid atmosphere for 21 days. The same test was carried out with chloroform as control. MIC values were determined as the lowest concentration of the compound for which no visible growth was observed.

### *3.5. Antibacterial activity tests*

The disc-diffusion method [4, 7] was used to determine the inhibition zones of the compound **1**. Inhibition zones greater than 7 mm were selected for the tube dilution tests [8] to determine the antibacterial activity quantitatively as minimum inhibitory concentrations (MIC). The standard bacterial strains (MTCC) used were *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Staphylococcus aureus*.

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