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A FUNGITOXIC SESQUITERPENE FROM *HANSFORDIA PULVINATA*

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Key Word Index—*Hansfordia pulvinata*; *Cladosporium fulvum*; Hyphomycetes; sesquiterpene; desoxyphomenone; spectral data.

Abstract—A novel antifungal sesquiterpene, 13-desoxyphomenone, was isolated and characterized from a culture filtrate of *Hansfordia pulvinata*, a hyperparasite of *Cladosporium fulvum*.

INTRODUCTION

Our work on the hyphomycete *Hansfordia pulvinata* (Berk. et Curt.) Hughes was initiated to investigate whether this hyperparasite of *Cladosporium fulvum* Cook (syn. *Fulvia fulva*), a foliar parasite of tomato plants [1, 2], could be used in integrated pest control. We observed that *in vitro* cultures of *H. pulvinata* were fungitoxic and we isolated from these cultures a compound which showed fungitoxic activity. In this paper we describe the isolation and structure elucidation of the compound.

RESULTS AND DISCUSSION

The fungitoxic metabolite was isolated from liquid cultures of *H. pulvinata* by solvent extraction, followed by purification through TLC and HPLC. The activity of the fractions obtained was monitored by bioassay. The compound was identified as **1**, the 13-desoxy analogue of phomenone, **2**, a phytotoxin isolated from *Phoma exigua* var. *non oxydabilis* [3, 4].

The structure assignment is based on the following spectral data. The mass spectrum has a strong molecular ion at M^+ 248 (100%) which corresponds with $C_{15}H_{20}O_3$ (the ^{13}C NMR showing 15 carbon atoms). The compound has two double bonds, one in an isopropenyl group [IR ν_{max} cm^{-1}: 3080, 1630 and 904; ^1H NMR: δ 5.11 (2H, $=CH_2$), 1.87 (3H, $-C(=CH_2)\underline{Me}$)], the second in a 3-substituted cyclohex-2-enone [IR ν_{max} cm^{-1}: C=O 1675; ^1H NMR: one vinylic proton δ 5.76 (*d*, J = 2.2 Hz) UV λ_{max}^{EtOH} nm (log ε): 245 (4.34)]. In addition to the carbonyl absorption, the IR spectrum shows a hydroxyl group (3500 cm^{-1}), which is equatorial [IR ν_{max} cm^{-1}: 1020; ^1H NMR: δ 3.63 (1H, *m*, J = 11.4, 10.4 and 4.3 Hz)]. The third oxygen is part of a three-substituted epoxide ring [IR ν_{max} cm^{-1}: 876; ^1H NMR: one proton singlet at δ 3.33; ^{13}C NMR: δ 68.3 (*d*) and 63.5 (*s*)]. In addition to the isopropenyl methyl group, the ^1H NMR shows two methyl groups, one at a quaternary carbon [δ 1.23 (*s*)] and one at a tertiary carbon [δ 1.26 (*d*, J = 6.7 Hz)].

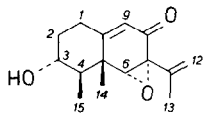
Analysis of the coupling constants and chemical shifts of the protons in the 0–4 ppm region of the 360 MHz spectrum of the compound, and comparison with those published for phomenone [3] and with the spectrum published for the petasol derivative **3** [5, 6], suggest a strong similarity between the three compounds. On the basis of the combined data we propose structure **1** for the *Hansfordia* metabolite.

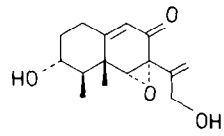
Since 13-desoxyphomenone showed fungitoxic activity, we sought to use it in biological control of parasites on tomato leaves [7]. However, concentrations of 0.25 $\mu g/cm^2$ of leaf induced necrotic lesions. At present we are trying to detect 13-desoxyphomenone *in vivo* and to establish the role of this fungistatic and phytotoxic compound in the tripartite system hyperparasite–parasite–host.

EXPERIMENTAL

Liquid stationary cultures (100 ml 2% malt extract per flask) of *Hansfordia pulvinata* produced the active substance in varying amounts (maximum yield 12 mg/l.). Optimal production of the toxin by *H. pulvinata* was observed after 18–21 days of incubation at 22°. The medium was filtered and the filtrate extracted with an equal vol. of $CHCl_3$. After centrifugation, the organic layer was collected, the solvent removed and the remaining solid dried in vacuum. TLC was carried out on 0.25 or 2 mm layers of Si gel 60 F 254 ($CHCl_3$–MeOH, 95:5). The presence of the inhibitor was monitored by a *Cladosporium herbarum* TLC assay [8] and the antifungal compound was further detected by its quenching property (dark band) at 254 nm. The active band was purified by HPLC (Varian equipment, semi-prep. reverse phase column, eluent MeOH–H_2O 55:45, UV detection). Mp 104–105° (Kofler; uncorr.); $[\alpha]_D^{20}$ +236° (MeOH; *c* 0.5).

Spectral data. ^1H NMR (360 MHz, $CDCl_3$, int. standard TMS): δ5.76 (1H, *d*, J = 2.2 Hz, H-9), 5.11 (2H, *m*, H-12), 3.63 (1H, *m*, J = 11.4, 10.4 and 4.3 Hz, H-3a), 3.33 (1H, *s*, H-6), 2.52 (1H, *m*, J = 14.5, 14.5, 4.9 and 2.2 Hz, H-1a), 2.34 (1H, *m*, J = 14.5, 4.3 and 3.0 Hz, H-1e), 2.15 (1H, *m*, J = 12.4, 4.9, 4.3 and 3.0 Hz, H-2e), 1.87 (3H, *s*, H-13), 1.81 (1H, *dq*, J = 10.4 and 6.7 Hz, H-4a), 1.44 (1H, *m*, J = 14.5, 12.4, 11.4 and 4.3 Hz, H-2a), 1.26 (3H, *d*, J = 6.7 Hz, H-15), 1.23 (3H, *s*, H-14). ^{13}C NMR (25 MHz, $CDCl_3$): δ 192.7 (*s*, C-8), 163.0 (*s*, C-10), 139.0 (*s*, C-11), 121.1 (*d*, C-9), 114.4 (*t*, C-12), 70.9 (*d*, C-3), 68.3 (*d*, C-6), 63.5 (*s*, C-7), 44.3 (*d*, C-4), 41.0 (*s*, C-5), 35.2 (*t*, C-2), 30.9 (*t*, C-1), 19.8 (*q*, C-13), 18.8 (*q*, C-14), 11.3 (*q*, C-15). MS [Kratos MS-80, DS-50, GC/MS, 70 eV, *m/z* (rel. int.)]: 248 $[M]^+$ (100), 189 (99), 161 (93), 91 (66), 123 (43), 105 (42), 133 (41), 107 (38), 233 (28), 204 (26), 175 (25), 176 (23), 230 (22). UV: λ_{max}^{EtOH} nm (log ε): 245 (4.34).





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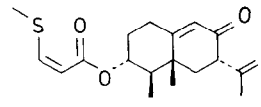

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(+)-8-HYDROXYCALAMENENE: A FISH-POISON PRINCIPLE OF *DYSOXYLUM ACUTANGULUM* AND *D. ALLIACEUM**

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Key Word Index—*Dysoxylum acutangulum*; *D. alliaceum*; Meliaceae; fish-poison; antibacterial activity.

Abstract—A fish-poison principle of *Dysoxylum acutangulum* and *D. alliaceum* has been identified as (+)-8-hydroxycalamenene, a new natural sesquiterpene phenol. This compound shows not only significant toxicity against fish but also antibacterial activity.

INTRODUCTION

Seeds of *Dysoxylum acutangulum* have been traditionally known as fish-poisonous plant material in Sumatera, Indonesia. We have investigated the active principles of this plant by monitoring the toxicity against a species of fish, *Oryzias latipes*, and isolated a phenolic sesquiterpene, **1**, as a major toxic constituent. This compound shows a significant fish-toxicity against *Oryzias latipes* at 5 ppm concentration and moderate antibacterial activity against Gram-positive bacteria, such as *Staphylococcus aureus*, *Candida albicans* and *Trichophyton mentagrophytes*, at 5–20 ppm (MIC). However, it is ineffective against Gram-negative bacteria, such as *Esherichia coli* or *Pseudomonas aeruginosa*.

This article describes the isolation and structure determination of the active principle which is identified as (+)-8-hydroxycalamenene (**1**), a new natural sesquiterpene.

RESULTS AND DISCUSSION

A crude ethanol extract of seeds of *D. acutangulum* showed a significant fish-toxicity, and the activity was always monitored by the bioassay with *Oryzias latipes*. Silica gel CC followed by vacuum distillation afforded a phenolic sesquiterpene (**1**) as a major active constituent. Compound **1**, $C_{15}H_{22}O$, was obtained as a liquid with bp 150–155°/0.1 mm Hg and had a phenolic ring (IR ν_{max} cm^{-1}: 3500, 1620, 1580). The ^{13}C NMR spec-

*Dedicated to Emeritus Professor Takeo Sakan of Osaka City University on the occasion of his 70th birthday.