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L-Azetidine-2-carboxylic acid, the antidermatophyte constituent of two marine sponges

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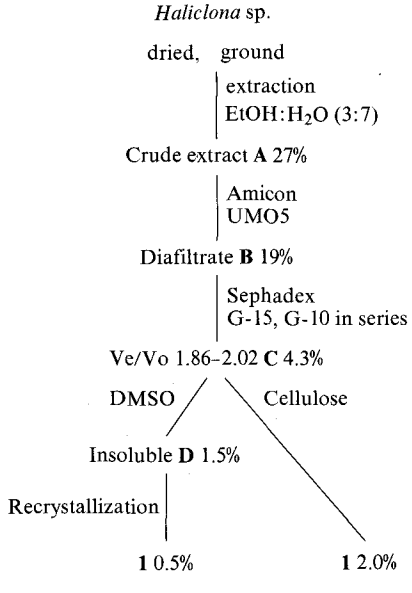
Summary. The aqueous ethanolic extract of 2 related marine sponges *Haliclona* sp. and *Chalinopsilla* sp. displayed antidermatophyte activities specific for *Trichophyton mentagrophytes*. The active constituent of both sponges was isolated and shown to be L-azetidine-2-carboxylic acid.

Routine bioassays of aqueous ethanolic extracts of marine organisms revealed that the extracts of 2 sponges of the order Haplosclerida had specific in vitro activity against the dermatophyte *Trichophyton mentagrophytes*. The crude extracts, A and F, of *Haliclona* sp.[1] and *Chalinopsilla* sp.[1] displayed minimum inhibitory concentrations (m.i.c.) in vitro of 0.625 and 2.5 $\mu g \cdot ml^{-1}$ respectively, against *T. mentagrophytes*. The standard, griseofulvin, had a m.i.c. of 2.5 $\mu g \cdot ml^{-1}$.

Isolation of the active constituent was achieved by monitoring the fractionation of the extract for in vitro activity against *T. mentagrophytes*. An aqueous solution of **A** (28 g) was diafiltered through a membrane having a cut-off at 500 a.m.u. A portion of the diafiltrate **B** (4.0 g) was chromatographed in water on columns of Sephadex G-15 and G-10 connected in series and the active constituent **C** (0.9 g) was eluted between Ve/Vo 1.86 and 2.02. Trituration of **C** (1.19 g) with dimethylsulphoxide and recrystallisation of the insoluble solid **D** (0.4 g) with methanol: water (95:5) gave colourless crystals of L-azetidine-2-carboxylic acid **1** (141 mg)[α_D^{21} -105°(C = 1.8, H_2O). Trituration of the dimethylsulphoxide soluble material **E** (520 mg) with methanol afforded a colourless solid **2** (65 mg) which was shown to be taurine **2**. Both **1** and **2** were identical (^{13}C-NMR, m.p., m.s., TLC) with the respective authentic samples.



Separation scheme for isolation of the antifungal constituent from *Haliclona* sp.

High pressure (1300 kPa) partition chromatography on cellulose of **C** (37 mg) in n-butanol:acetic acid:water (3:1:1) separated **1** (17.3 mg, Ve/Vo 3.0–3.6) from the minor constituents and allowed unequivocal assignment of **1** as the only active constituent. A similar procedure was adopted for the fractionation of *Chalinopsilla* sp. and **1** was isolated as the only active constituent.

1 is known to act as a L-proline analogue and thereby inhibit the growth of *Escherichia coli*[2,3] and become incorporated into proteins of higher animals[4–6]. Low concentrations of **1** were found to be active in vitro against recent clinical isolates of the fungal dermatophytes *T. mentagrophytes, Epidermophyton floccosum* and *Microsporum audouini*. **1** has systemic activity against s.c. infections of mice by *T. mentagrophytes*, with an ED_{50} of 82 mg kg^{-1} s.c. and 400 mg kg^{-1} p.o. Although **1** was tolerated by mice when administered as a single s.c. dose of 1000 mg kg^{-1}, chronic toxicity was observed at 400 mg kg^{-1}/day s.c. after 3 days of treatment. Topical application of **1** failed to cure guinea pigs with experimental skin infections of *T. mentagrophytes*[7]. These results indicate that **1** does not have any therapeutic value as a topical or systemic agent against fungal dermatophytes. **1** was also inactive when tested against a broad spectrum of other fungi and protozoa[7].

1 RRIMP Museum Specimen Numbers FN 1156 *Haliclona* sp. and FN 0834 *Chalinopsilla* sp. We thank I.G. Skinner for sponge taxonomy.
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