derived from calcium chloride solutions and 4.39 mM assuming calcium ion activities of unity. The present method gives a result in reasonable agreement with the latter value. Dissociation of the $Ca(NANA)^+$ ion increases markedly with temperature; at a total calcium ion concentration of 2.5 mM, 30.7% of the calcium is complexed to $NANA^-$ at 37 °C. The inset in the figure shows the plot of $\log K_{diss}$ as a function of reciprocal temperature, the slope of this plot is $-\Delta H°/2.303\,R$ (Gibbs-Helmholtz equation). The enthalpy of dissociation ($\Delta H°$) is constant at 21.1 kJ $mole^{-1}$. It is clear that at physiological calcium levels the conductance method shows conclusively that NANA binds Ca^{2+} ions.

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26-Hydroxy-β-ecdysone, a metabolite of β-ecdysone in the blowfly, *Calliphora erythrocephala*

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Summary. 26-Hydroxy-β-ecdysone has been identified as a metabolite of the moulting hormone, β-ecdysone in *Calliphora erythrocephala.*

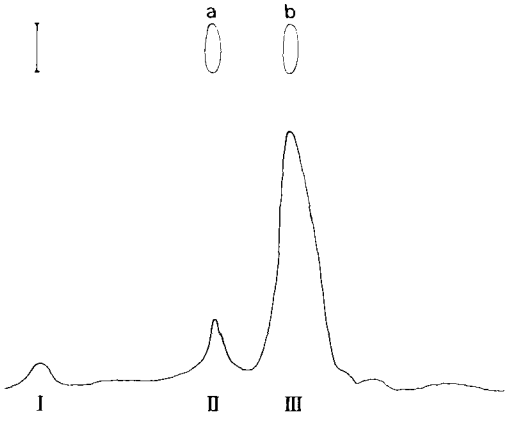
26-Hydroxy-β-ecdysone has been isolated as one of the insect moulting hormones present in the tobacco hornworm, *Manducca sexta*[1-3], and it has also been identified as a metabolite of synthetic 22,25-dideoxy-α-ecdysone[4-6] in this insect. In studies on the metabolism of tritiated α- and β-ecdysone in *Calliphora erythrocephala*[7,8], *Locusta migratoria*[9] and *Choristoneura fumiferana*[10], 26-hydroxy-β-ecdysone has been suggested to occur in the radiochromatograms of the tissue extracts but no positive identifications were made. We have now identified 26-hydroxy-β-ecdysone as a metabolite of β-ecdysone in the blowfly, *Calliphora erythrocephala.*

Late 3rd-instar larvae (115) of *Calliphora erythrocephala* were injected with 5-[^{3}H]-β-ecdysone[8] (1.5 µg/insect, sp. act. 7.2 mCi/mM) and 20 h later, when they had all formed puparia, they were homogenized with methanol and centrifuged. The supernatant representing a 47% recovery of activity (2.76×10^6 dpm), was evaporated to dryness and partitioned between hexane:diethyl ether:methanol:water (8:5:3:1); the aqueous phase was concentrated to remove the methanol and extracted with n-butanol (X3). The n-butanol phase was evaporated to dryness and chromatographed on thin layer plates (Merck precoats, Silica gel HF_{254}) to give 3 radioactive peaks (figure). Peaks II and III co-chromatographed with 26-hydroxy-β-ecdysone[11] and β-ecdysone respectively. The material of peak II was eluted from the plate with methanol and accounted for 10% of the recovered activity (2.74×10^5 dpm). The aqueous phase from the n-butanol extraction accounted for 52% of the activity of the ethanol extract (1.43×10^6 dpm) and contained only ecdysone conjugates[12].

Since the 26-hydroxy-β-ecdysone standard was non-crystalline[13] the identity of the material from peak II was confirmed by the preparation of derivatives. A portion of the material was reacted with acetone (1 ml, 20 °C) in the presence of phosphomolybdic acid under nitrogen[14]. The acetonide mixture was examined by TLC ($CHCl_3$:EtOH, 19:1) and compared with 26-hydroxy-β-ecdysone reacted with acetone under identical conditions. The radiochromatogram showed a pattern of peaks whose R_f values were the same as the components in the 26-hydroxy-β-ecdysone acetonide mixture. The main peak co-chromatographed with the major component, 26-hydroxy-β-ecdysone triacetonide[13].

Acetylation[14] (24 h) of the material from peak II followed by TLC ($CHCl_3$:EtOH, 19:1) also gave a radiochromatogram in which the peaks co-incided with spots in the acetylation mixture of 26-hydroxy-β-ecdysone. The main peak co-chromatographed with the main acetylation product, the tetra-acetate[13].

The co-chromatography of the metabolite and its derivatives with 26-hydroxy-β-ecdysone and its derivatives prepared under identical conditions, confirms the identity of 26-hydroxy-β-ecdysone as a metabolite of β-ecdysone in *Calliphora erythrocephala.*



Thin-layer radiochromatogram of the n-butanol phase of the total ethanol extract of late 3rd-instar *Calliphora erythrocephala* larvae injected with β-5-[^{3}H]-ecdysone. *a* 26-Hydroxy-β-ecdysone, *b* β-ecdysone (vanillin/H_2SO_4 spray reagent). Solvent system: $CHCl_3$:EtOH (2:1, v/v). Peak I is at the origin.

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

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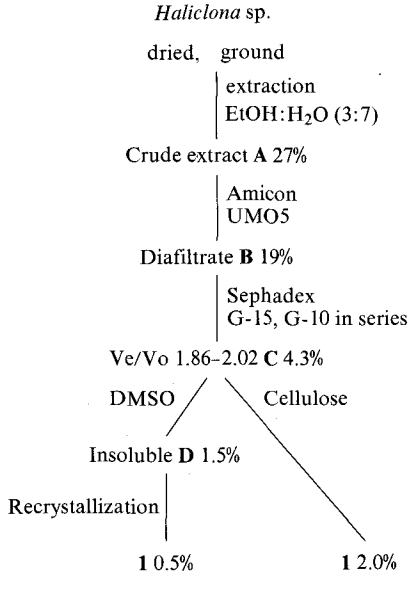
Summary. The aqueous ethanolic extract of 2 related marine sponges *Haliclona* sp. and *Chalinopsilla* sp. displayed antidermatophyte activities specific for *Trichophyton mentagrophytes*. The active constituent of both sponges was isolated and shown to be L-azetidine-2-carboxylic acid.

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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