

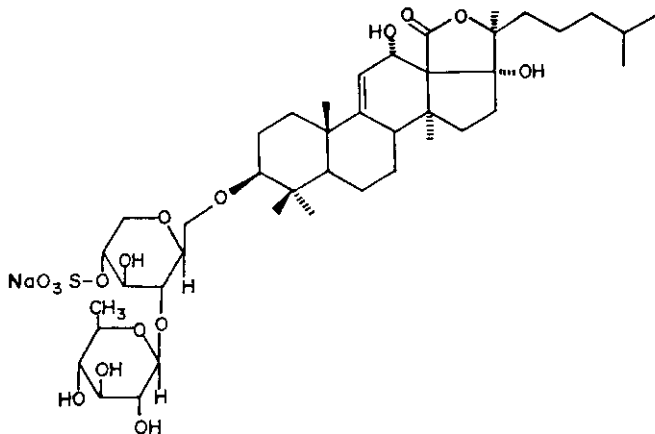
Echinocide-B from the Lakshadweep sea cucumber *Actinopyga mauritiana* (Quoy and Gaimard)†

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Brine-shrimp assay guided purification of the methanolic extract of the sea cucumber *Actinopyga mauritiana* yields echinocide-B as a major toxic constituent. The compound has been characterized based on its spectral data.



Sea cucumber glycosides, commonly known as holothurins are extremely toxic to fish and animals¹, besides exhibiting antimicrobial², hemolytic and piscicidal³ activities. During our studies the methanolic extract of the sea cucumber *Actinopyga mauritiana* was found to be extremely toxic to mice (LD₅₀ = 0.825 mg/kg), fish (*Sarotherodon* sp., LD₅₀ = 7.5 ppm) and brine shrimps (LD₅₀ = 1.03 mg/ml, 105 min.) and also antibiotic as well as hypotensive (*in vitro*, unpublished results).

The purification from the sea cucumber extract, monitored by the brine-shrimp bioassay yielded the active fraction as a single spot on TLC (SiO₂, CHCl₃-MeOH = 76:24, R_f = 0.5); LD₅₀ = 0.293 mg/ml, 90 min; m.p. 203°. Its spectral data (IR, UV, PMR, CMR and MS) were compatible with those of echinocide-B² (Experimental). Further confirmation to the structure was provided by the formation of the heteroannular diene upon acid hydrolysis and formation of a mixture of penta and tetra acetate upon acetylation.

Experimental

The animals (5 kg) were collected from Kavaratti island in the Lakshadweep sea. They were immediately cut into pieces and soaked in MeOH and transported to the laboratory. After two months, the MeOH was filtered off and concentrated to dryness (187 g); LD₅₀ = 1.03 mg/ml, 105 min. The extract was resuspended in methyl alcohol and toxicity studies showed that active principles (64 g) have gone into solution (LD₅₀ = 0.66 mg/ml, 120 min. LD₅₀ = 0.33

mg/ml, 240 min). This fraction was further chromatographed over a short celite column using CHCl₃ with increasing amounts of MeOH. The toxic principles were eluted with CHCl₃-MeOH (1:1 and 1:2) (10 g) (LD₅₀ = 0.344 mg/ml, 10 min, all dead within 50 min).

TLC comparison as well as preliminary spectral studies showed them to be a mixture of a number of glycosides. Further chromatographic purification of this active fraction yielded echinocide-B from the mixture of more polar glycosides (R_f = 0.6, SiO₂, 24% MeOH/CHCl₃, LD₅₀ = 0.293 mg/ml, 90 min). The isolation and purification of the more toxic constituents from the above fractions are under progress.

Echinocide-B, 203°, IR (KBr): 3400, 1730, 1625, 1230, 1050 and 830 cm⁻¹; UV (MeOH): Transparent above 210 nm; PMR (Py-d₅): δ 5.616 (1H, d, J = 5 Hz, 11-H), 5.01 (1H, d, J = 5 Hz, 12B-H), 4.816 [1H, d, J = 7.5 Hz, 1-H (Xyl)], 4.746 [1H, d, 1-H (Qui)], 4.316 (1H, br.t, 3-H), 4.086 (2H, m), 3.766 (2H, m), 3.366 (1H, m), 3.156 (1H, m), 1.76 (3H, s, 21-H), 1.68 (3H, s, 28-H), 1.38 (3H, s, 19-H), 1.30 (3H, s, 29-H), 1.14 (3H, s, 30-H) and 0.846 (6H, d, J = 7 Hz, 26 and 27-H); ¹³C NMR (Py-d₅): δ 174.87 (s, C-18), 154.12 (s, C-9), 115.67 (d, C-11), 105.94 (d, Xyl-1), 105.33 (d, Qui-1), 89.39 (s, C-17), 88.71 (d, C-3), 87.26 (s, C-20), 83.40 (d, Xyl-2), 77.66 (d, Qui-3), 76.90 (d, Xyl-3), 76.70 (d, Qui-4), 75.95 (d-Qui-2), 75.52 (d, Xyl-4), 73.44 (d, Qui-5), 71.34 (d, C-12), 64.29 (t, Xyl-5), 58.67 (s, C-13), 52.79 (d, C-5), 46.46 (s, C-140), 40.85 (d, C-8), 40.13 (s, C-4), 39.78 (s, C-10), 38.93 (t, C-24), 36.7 (t, C-22), 36.32 (t, C-1), 35.88 (t, C-16), 28.42 (t, C-25), 28.17 (t, C-16), 28.07 (q, C-29), 27.05 (t, C-2), 23.12 (q, C-21), 22.81 (q, C-26), 22.71 (q, C-28), 22.66 (q, C-27).

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22.36 (t, C-23), 21.20 (t, C-6), 20.16 (q, C-19), 18.67 (q, Qui-6) and 16.74 (q, C-30); FABMS: 907 ($M_{Na} + K$)⁺, 891 ($M_{Na} + Na$)⁺, 869 ($M_{Na} + H$)⁺, 865 ($891-H_2O-CO_2$)⁺, 789 ($891-NaSO_3 + H$)⁺, 771 ($891-NaHSO_4$)⁺, 610 (891-sugar)⁺ etc; EIMS: 488 (Nil, Aglycone, M⁺), 470 (24%, M⁺-H₂O), 452 (51%, M⁺-2H₂O), 437 (100%, M⁺-2H₂O-CH₃), 411 (50%, M⁺-H₂O-CO₂-CH₃), 393 (82%, 437-44), 383 (44%, M⁺-ring A), 375 (17.6%, 393-H₂O)⁺, 350 (65%, 383-H₂-CH₃), 323 (16.5%, M⁺-2H₂-S.C.), 297 (40.47%, M⁺-H₂-S.C.-CO₂-CH₃), 281 (38.39%, M⁺-S.C.-CO₂-2H₂O-CH₃+H) etc.

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References

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- 2 Kitagawa I, Kobayashi M, Inamoto T, Fuchida M & Kyogoku Y, *Chem pharm Bull*, 33 (1985) 5224.
- 3 Nigrelli R F, *Zoologica*, 37 (1952) 89.

Corrigendum

Paper entitled, "Three new labdane diterpenes from leaves and twigs of *Juniperus pseudosabina* Hook and 2D-NMR studies of 3-acetoxymanol", *Indian J Chem*, 29B (1990) 911-917.

The correct names of compounds **1, 2, 3** and **6**, reported in the above paper are as follows: 15-acetoxy-3 α -hydroxy-8(17)-13-labdadiene (**1**), 3 α -acetoxy-13 β -hydroxy-8(17)-14-labdadiene (**2**), 3 α ,13 β -diacetoxy-8(17)-14-labdadiene (**3**) and 3 α ,15-diacetoxy-8(17)-13-labdadiene (**6**) and not as printed in the paper.