

Short Communication

Isolation of 2-deoxyecdysterone, a novel oxytocic agent, from a marine *Zoanthus* sp.*†§

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Abstract

The insect-moulting hormone 2-deoxyecdysterone (2-deoxy-20-hydroxyecdysone 1) has been isolated for the first time and in relatively high yield (0.016%) from a marine *Zoanthus* sp. The compound exhibited promising oxytocic activity in guinea pig uterus assay. The ¹H and ¹³C NMR assignments of the compound aided by COSY, TOCSY, HMQC and HMBC data are reported here for the first time.

Keywords: *Zoanthus* sp, ecdysone, ecdysterone, oxytocin, prostaglandin $F_{2\alpha}$, moulting hormone.

Ecdysone and its analogs are well known as 'insect-moulting hormones'. They are present in many terrestrial plants and animals, albeit in very low concentrations.¹ These compounds have also been isolated from a few marine organisms such as the barnacle *Balanus balanoides*, Zoanthids: *Gerardia savaglia*, *Palythoa australiae* and *Parazoanthus* sp.¹⁻³ One of the most active steroidal hormones is 2-deoxyecdysterone (2-deoxy-20-hydroxy ecdysone, 1), which is as potent as ecdysone in effecting insect moulting.⁴ 2-Deoxyecdysterone (1) has been reported from such diverse sources as a marine crayfish *Jasus lalandei*,⁵ a fern *Blechnum minus*² and the terrestrial plants *Silene brahuica*^{5,6} and *Silene praemixta*.⁷ But isolation and purification of this compound were always very difficult as it was only a minor constituent, co-occurring with other ecdysone analogs of similar structure.

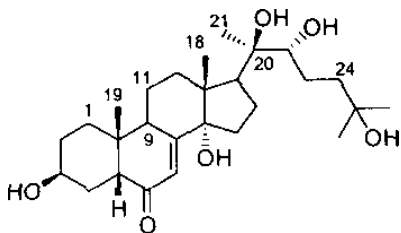
Surprisingly, this compound was isolated as a major, if not the exclusive steroidal hormone from a *Zoanthus* sp. (Coelenterata, Zoanthidea) collected from Goa for the first time. Pharmacological screening of the compound revealed its promising oxytocic activity which was compared to that of clinical standards, oxytocin and prostaglandin $F_{2\alpha}$. Details of these studies will be published elsewhere. The present communication deals with the isolation and structure determination including the detailed NMR assignments carried out with the help of 2D NMR spectral data: COSY, TOCSY, HMQC and HMBC, in both $Py-d_5$ and CD_3OD .

The methanol extract of the animal (5 kg wet wt) exhibited promising oxytocic activity in the guinea pig uterus assay besides inhibiting nicotine and serotonin-induced contractions

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2-Deoxyecdysterone (1)

during guinea pig ileum assay. Solvent-solvent partitioning of the extract followed by bioassay indicated the oxytocic principle to be almost exclusively present in the chloroform fraction (5 g). Purification of this fraction *on silica gel* (petroleum ether-acetone gradient) and Sephadex LH-20 (acetone) columns yielded about 800 mg (0.016%) of the active metabolite, identified as 2-deoxyecdysterone (1).

Compound 1, $[\alpha]_D^{25} = 75.11$ ($c = 1.2$, MeOH), had IR peaks at 3400 and 1640 cm^{-1} , indicating the presence of hydroxyl and unsaturated carbonyl groups. The UV absorption at 243 nm ($\epsilon = 19,600$) and the ^1H and ^{13}C NMR signals at δ 203.18 (C-6), 6.27/121.5 (C-7) and 166.7 (C-8) also indicated the presence of an α,β -unsaturated carbonyl group. Its molecular weight was found to be 464 by FABMS while elemental composition was determined to be $\text{C}_{27}\text{H}_{48}\text{O}_6$ (observed: 465.3229 against the calc: 465.3216 for $[\text{M}+\text{H}]^+$) by HRFAB MS. Its NMR spectra revealed the presence of 5 methyl groups, 9 methylenes, 3 methines, 2 quaternary carbons, 2 oxymethines, 3 oxygenated quaternary carbons and an α,β -unsaturated carbonyl group in agreement with elemental composition. These results, especially the NMR signals at δ 4.08 (1H, m)/64.12 (d, C-3), 1.15 (3H, s)/17.90 (C-18) and 1.00 (3H, s)/24.38 (C-19) and the α,β -unsaturated carbonyl group indicated it to be a steroidal molecule. Comparison of these values with those of different ecdysone analogs confirmed its structure to be 2-deoxyecdysterone (1).⁵⁻⁹ The assignment of carbon and proton signals in both CD_3OD and pyridine- d_5 are given in Table I, while the COSY and TOCSY data are given in Table II.

In pyridine- d_5 , the methyl protons appeared at δ 1.15 (3H, s, 18-H), 1.00 (3H, s, 19-H), 1.54 (3H, s, 21-H) and 1.33 (6H, s, 26 and 27-H), the methylene protons at δ 1.59-1.67 (2H), 1.70-1.86 (9H), 1.98-2.14 (4H), 2.23, 2.37 and 2.51 (1H each) and the tertiary hydrogens, 5-H, 9-H and 17-H, at δ 2.87, 3.48 and 2.92, respectively. In CD_3OD solution the methyl signals appeared at δ 0.64 (18-H), 0.74 (19-H), 0.96 (21-H) and 0.98 (6H, 26 and 27-H), while the methylenes were seen at δ 1.07 (1H), 1.23 (2H), 1.35-1.41 (6H), 1.50-1.55 (5H), 1.61 (1H), 1.74 (2H) and 1.86 (1H) and the tertiary hydrogens at δ 2.21, 2.93 and 2.13, respectively. The second oxymethine proton at δ 3.80 (d, 8.7 Hz) (δ 3.12 in CD_3OD) was assigned to 22-H. HMQC experiment revealed the corresponding carbon signals (Table I). The above ^{13}C NMR assignments were supported by HMBC experiment which revealed the linkages: 18-H (δ 1.15) to C-12, 13, 14 and 17 (δ 32.3 t, 48.59 s, 84.42 s and 50.17 d), 19-H (δ 1.00) to C-1, 5 and 10 (δ 29.50 t, 51.75 d and 37.0 s), 17-H (δ 2.92) to C-12, 13, 16 and 18 (δ 32.3 t, 48.59 s, 21.7 t and 17.9 q), 16-H (δ 2.37) to C-17, 20, 14 and 15 (δ 50.17 d, 76.9 s, 84.42 s and 31.63 t), 21-H (δ 1.54) to C-17, 20 and 22 (δ 50.17 d, 76.9 s and 77.6 d) and 26 and 27-H (δ 1.33) to C-24 and C-25 (δ 42.63 t and 69.58 s, respectively). Similar results were obtained with the CD_3OD solution as well. The stereochemistry of the 20 and 20-OH groups was assigned R(β), R(α), respectively, in agreement with other naturally occurring ecdysone analogs.⁵⁻⁹

Table I
¹H and ¹³C NMR assignments and HMBC correlations of 2-deoxyecdysterone (1) in pyridine-d₅ solution*

| Carbon no. | ¹³ C NMR | ¹ H NMR | HMBC correlations |
|------------|---------------------------|--|---|
| 1 | 29.50 t; (28.32) | 1.61 (1H, m) 1.67 (1H, m); (1.23-1.41) | 64.12 |
| 2 | 27.5 t; (25.66) | 1.76 (1H)& 1.74 (1H); (1.41, 1.52) | |
| 3 | 64.12 d; (64.04) | 4.08 (br); (3.78, br) | |
| 4 | 33.20 t; (31.87) | 1.83 (1H) & 1.85 (1H); (1.37, 2H) | |
| 5 | 51.75 d; (50.60) | 2.87 (br); (2.21, dd, 12.1, 3.8 Hz) | |
| 6 | 203.18 s; (205.6) | | |
| 7 | 121.5 d; (120.8) | 6.16 (d, 2.1 Hz); (5.60) | 84.42,51.75,33.20 |
| 8 | 166.7 s; (167.0) | | |
| 9 | 34.3 d; (33.5) | 3.48 br; (2.93, br) | |
| 10 | 37.0 s; (36.3) | | |
| 11 | 21.7 t; (20.14) | 1.84 (1H, m) and 1.74 (1H, m); (1.74,1.55) | 1.74/50.17 |
| 12 | 32.31; (31.2) | 2.51 (1H, dt, 12.6 and 4.8 Hz); (1.86), 2.01 (1H,m); (1.61) | 2.51/21.70, 17.90 |
| 13 | 48.59 s (49.45) | | |
| 14 | 84.42 s; (84.3) | | |
| 15 | 31.63 t; (30.45) | 2.11 (1H, m), (1.74, 1H, m), 1.80 (1H, m); (1.35, 1H,m) | 2.11/21.70 |
| 16 | 21.7 t; (20.14) | 2.37 (brq, 9.6 Hz); (1.35) 2.03 (1H,m); (1.07) | 2.37/50.17, 76.9, 84.42, 31.63; 2.03/84.42 |
| 17 | 50.17 d; (48.88) | 2.92 (1H, t, 9 Hz); (2.13, brt, 8.7 Hz) | 32.3,48.59,21.57, 17.9 |
| 18 | 17.9 q; (17.0) | 1.15 (3H, s); (0.64) | 32.3,48.59,84.42,50.17 |
| 19 | 24.38 q; (23.5) | 1.00 (3H, s); (0.74) | 29.50,37.00,51.75 |
| 20 | 76.9 s; (76.56) | | |
| 21 | 21.57 q; (20.06) | 1.54 (3H, s); (0.96) | 50.17,76.9,77.6 |
| 22 | 77.6 d; (76.76) | 3.80 (1H, d, 8.7 Hz); (3.12, d, 10.9 Hz) | 21.7,42.63,76.9 |
| 23 | 29.12 t; (27.5) | 2.08 (1H, m); (1.54, 2H, m) 1.79 (1H, m); | 2.08/42.63; 1.79/76.9, 77.6 |
| 24 | 42.63 t; (40.66) | 2.23 (1H, m); (1.53) 1.76 (1H, m); (1.23) | 2.23/21.57, 29.12, 69.58, 30.04, 31.1; 1.73/27.5, 69.58, 30.04, 31.1 |
| 25 | 69.58 s; (70.34) | | |
| 26/27 | 30.1 q/30.04 q (28.61) | 1.33 (6H, s); (0.98, 6H, s) | 69.58, 42.63 |

* Values in parenthesis are for CD₃OD solution.

The oxytocic activity was compared with those of the clinical standards, oxytocin and PGF_{2α} at equivalent concentrations. Thus, the activity of compound 1 was 80.8, 92.3 and 118% of that of oxytocin at concentrations 50, 100 and 200 μg/ml, respectively. Against PGF_{2α}, these values were found to be 69.4%, 82.3% and 114.1%, respectively, at similar dose levels. These preliminary results indicate its possible clinical use in future for inducing labour or for therapeutic abortion. In addition, 2-deoxyecdysterone may also be useful in effecting uterine contraction after caesarean surgery and in controlling postpartum uterine atony and hemorrhage.

Adequate supply of the active compounds for biological studies was always a challenging task to chemists. For example, the yield of crustecdysone (ecdysterone or 20-hydroxy-

ecdysone) from different sources such as the barnacle *Balanus balanoides*, the crayfish *Jasus lalandei* and the lobster *Homarus americanus* were only 1, 2 and 6 $\mu\text{g}/\text{kg}$, respectively,¹ while 4-280 $\mu\text{g}/\text{kg}$ was obtained from the crab *Callinectes sapidus* depending upon its moult stage.¹ The crayfish *Jasus lalandei* also yielded 0.2 $\mu\text{g}/\text{kg}$ of 2-deoxyecdysterone.¹ In another study, about 20 mg ecdysone and 200 μg of 2-deoxyecdysterone were isolated from 3 tons of shrimp *Crangon vulgaris*.² With improvements in separation methods, much higher yield of ecdysterone has now been achieved.^{3,9} Also, two related ecdysones, Palythoalone A and B have been isolated in 0.014% and 0.0006%, respectively, from a marine zoanthid *Palythoa australiae*.³ The fact that several ecdysone analogs of closely resembling structures co-occur in many plants and animals further complicates the efforts to isolate the individual compounds in the pure state. This is more the case with compound 1, which has so far been found to occur only as a minor metabolite. This is the first report of occurrence of 2-deoxyecdysterone in a zoanthid species and that too as a major metabolite. Its oxytocic activity is also being reported for the first time. The ready availability, useful biological property and the relative stability of the molecule should make it the compound of choice in the treatment of uterine disorders and as an abortifacient in future.

Experimental

The animal (5 kg wet wt) was collected from the beach in Anjuna, Goa, during October 1998. They were cleaned of the associated rock particles, washed with water and soaked in MeOH (5 l). After two days, the supernatant was drained off and concentrated under vacuum. The extraction was repeated three times and the combined extract (25 g) was resuspended in 90% aqueous MeOH and petroleum ether. The aqueous layer from the above was then extracted with chloroform (5 g). The chloroform-soluble fraction was purified by chromatography over silica gel (petroleum ether-acetone, gradient elution), the fraction eluting between 40% and 60% acetone being predominantly richer in compound 2. Separation of the above impure mixture on Sephadex LH-20 column (eluant: acetone) yielded about 800 mg of a white crystalline solid giving a single spot on TLC analysis (R_f - 0.72, acetone-pet.ether 1:1).

2-Deoxyecdysterone (1): $[\alpha]_D^{25} = 75.11$ ($c = 1.2$, MeOH); IR (KBr) ν_{max} : 3373, 2937, 2869, 1640, 1448, 1380, 1275, 1261, 1226, 1151, 1048, 959 and 897 cm^{-1} ; UV (MeOH) λ_{max} 243 nm ($\epsilon = 19,600$); FAB MS (m/z): 505 $[\text{M}+\text{Na}+\text{H}_2\text{O}]^+$, 487 $[\text{M}+\text{Na}]^+$, 465 $[\text{M}+\text{H}]^+$, 447 $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$, 429 $[\text{M}+\text{H}-2\text{H}_2\text{O}]^+$, 411 $[\text{M}+\text{H}-3\text{H}_2\text{O}]^+$, 393 $[\text{M}+\text{H}-4\text{H}_2\text{O}]^+$, 347, 329, 307 and 289. HRFAB MS (m/z): 465.3229 $[\text{M}+\text{H}]^+$, calc. for $\text{C}_{27}\text{H}_{45}\text{O}_6$, 465.3216. For ^1H and ^{13}C NMR as well as HMQC and HMBC and COSY, and TOCSY data, see Tables I and II.

Table II
COSY and TOCSY data of 2-deoxyecdysterone in pyridine- d_5

| Chemical shift of proton | COSY connectivities | TOCSY connectivities |
|--------------------------|------------------------|------------------------------------|
| 6.16 (7-H) | 3.48, 2.87, 1.74, 1.84 | 3.48, 2.87, 2.51, 2.01, 1.74, 1.84 |
| 4.08 (3-H) | 2.87, 1.74, 1.76, 1.82 | 2.87, 1.82, 1.74, 1.76, 1.61, 1.67 |
| 3.80 (22-H) | 2.23, 2.08, 1.79, 1.72 | 2.23, 2.08, 1.72, 1.79 |
| 3.48 (9-H) | 2.51, 2.01, 1.84, 1.74 | 2.51, 2.01, 1.54, 1.74 |
| 2.92 (17-H) | 2.37, 2.11, 2.03, 1.85 | 2.37, 2.11, 2.03, 1.85 |

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