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# The Defensive Secretion of the Opisthobranch Mollusc *Onchidella binneyi*[1]

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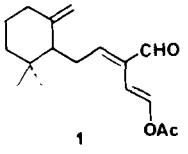
Opisthobranch molluscs of the family Onchidiacea have been reported to employ a chemical deterrent as a protection against predators. A single lipid-soluble compound, onchidal, has been isolated from the defensive secretion of *Onchidella binneyi*. The structure of onchidal was determined from spectral data and from chemical degradation studies.

## INTRODUCTION

*Onchidella binneyi* (*1*) is an opisthobranch mollusc which inhabits the rocky intertidal zone at Bahia de los Angeles in the Gulf of California (*2*). Like other opisthobranchs, the Onchidiacea do not have the protection of an external shell. They rely instead on the production of a defensive secretion. When the animal is molested, the defensive secretion is expelled from apical pores in papillae situated around the edge of the mantle (Fig. 1). Arey and Crozier (*3*) demonstrated that the secretion of *O. floridanum*, a closely related organism, acted as a deterrent to several potential predators, including fish and crabs. We wish to describe the isolation and structural elucidation of onchidal (**1**),[3] the major lipid component of the defensive secretion of *O. binneyi*.

The defensive secretion was obtained in the field by squeezing the mollusc and collecting the mucus discharge in capillary tubes. The capillary tubes were stored in acetone. To obtain larger quantities of material, an acetone extract of intact animals was prepared. Thin-layer silica gel chromatography of the acetone extract of the defensive secretion revealed the presence of a single nonpolar component. The ether-soluble portion of the acetone extracts of intact animals was chromatographed on a silica gel column, using hexane as eluant, to obtain the same nonpolar component, onchidal (**1**), as a mobile oil (0.23 mg/animal).

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[1] This paper is dedicated to Professor W. S. Johnson on the occasion of his 65th birthday.

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[3] Bold faced numbers in parentheses refer to corresponding structures presented in this paper.



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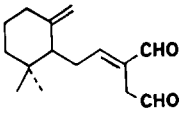
FIG. 1. Collection of the defensive secretion. The onchid, held with the foot toward the camera, produced a defensive mucus (larger white spots) at apical pores of papillae situated around the edge of the mantle. The mucus was collected in a capillary tube.

Onchidal (**1**) was shown to have the molecular formula $C_{17}H_{24}O_3$ by high-resolution mass measurement. The mass spectrum contained signals due to loss of ketene (*m/e* 234) and loss of acetic acid (*m/e* 216) from the molecular ion (*m/e* 276), suggesting that onchidal (**1**) was a sesquiterpene acetate. The infrared spectrum contained a band at 1757 $cm^{-1}$, which was assigned to an enol acetate, as well as bands at 2725 and 1685 $cm^{-1}$ due to an unsaturated aldehyde functionality. The $^1$H-nmr spectrum contained three methyl singlets at $\delta$ 0.89, 0.99, and 2.05 (acetate) and signals at 4.50 and 4.81 due to exocyclic methylene protons, at 6.10 (dd, $J$ = 14, 1 Hz) and 8.26 (d, $J$ = 14, Hz) due to protons on a *trans*-enol acetate double bond, at 6.41 (t, $J$ = 7 Hz) for the $\beta$-proton on an $\alpha,\beta$-unsaturated aldehyde, and at 9.40 (d, $J$ = 1 Hz) due to the aldehyde proton. The aldehyde proton signal was shown to be W-coupled to the olefinic proton signal at 6.10 ppm. The $^{13}$C-nmr spectrum contained signals at 193.3 (d) and 167.5 (s) for the aldehyde and acetate carbonyl carbons at 156.6 (d), 148.2 (s), 141.2 (d), 135.2 (s), 109.8 (t), and 105.1 (d) for the carbons of the three olefinic bonds, and only one additional quaternary carbon at 35.2 ppm. Onchidal (**1**) was therefore monocyclic and must contain a gem-dimethyl group.

The observed coupling between the aldehyde and olefinic protons, together with the chemical shift values of the olefinic protons of the enol acetate, suggested that both the aldehyde and the enol acetate were conjugated to the same olefinic bond. This was confirmed by hydrolysis of the enol acetate with sodium carbonate in methanol to obtain a 1,4-dialdehyde (**2**), which was reacted with hydrazine hydrate to obtain a 1,2-diazine (**3**). The $^1$H-nmr spectrum contained signals at $\delta$ 9.03 (2H) and 7.29, assigned to the protons on a 4-substituted 1,2-diazine ring.

Ozonolysis of onchidal (**1**) in ethyl acetate solution at −70°C, followed by a

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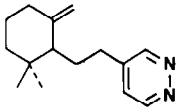


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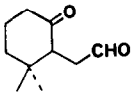
reduction using zinc in acetic acid, gave a keto-aldehyde (**4**). The infrared spectrum contained bands at 1710 and 1720 $cm^{-1}$, suggesting that the ketone was part of a six-membered ring. Based on the amassed spectral data, we concluded that the keto-aldehyde (**4**) had resulted from the ozonolysis of a monocyclofarnesane derivative and assigned the structure of onchidal (**1**) accordingly.

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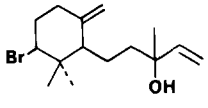
The carbon skeleton was confirmed by interrelationship of onchidal (**1**) with β-snyderol (**5**), a known monocyclofarnesane derivative (*4*). β-Snyderol (**5**) was treated with lithium in liquid ammonia to obtain debromosnyderol (**6**) in high yield. Comparison of the $^{13}C$-nmr spectra of compounds **6** and **1** showed excellent agreement

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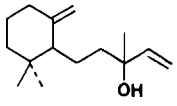


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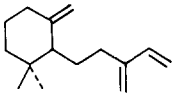
for the chemical shifts of the carbon atoms which are common to both compounds. Treatment of debromosnyderol (**6**) with phosphorus oxychloride in pyridine at 0°C caused dehydration to obtain a 1:1 mixture of trienes **7** and **8**. Ozonolysis of the



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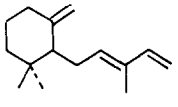

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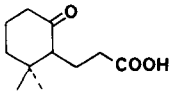
mixture of trienes, followed by reductive workup, gave an acid (**9**) and an aldehyde which was shown by combined gas chromatography–mass spectrometry to be identical to the keto-aldehyde (**4**).

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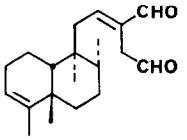
The stereochemistry about the trisubstituted olefinic bond was difficult to assign. The chemical shift of the olefinic proton (6.74 ppm) in the dialdehyde (**2**) was closest to the theoretical value (*5*) for the *Z* stereochemistry (*Z* = 6.67 ppm, *E* = 6.40 ppm). This was, however, in conflict with the stereochemical assignment presented by Kitagawa *et*

*al.* (**6**) for linaridial (**10**), in which the olefinic proton signal was observed at 6.75 ppm. We therefore decided to determine the stereochemistry of onchidal (**1**) by performing a lanthanide-induced shift (LIS) experiment on a suitable derivative.

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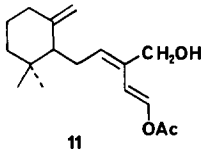


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Reduction of onchidal (**1**) with lithium tri-*t*-butoxyaluminum hydride in ether at room temperature gave the corresponding alcohol (**11**). The $^1$H-nmr spectrum of the alcohol (**11**) was measured after each addition of 0.1 equiv. of $Eu(fod)_3$, and the induced shifts were determined for selected protons. Since the alcohol (**11**) exhibited a diene chromophore at 243 nm, we chose to compare models in which the diene system was planar. The LIS data indicated that the protons at C-1 and C-15 were closest to and almost equidistant from the europium atom. Since the induced shift of the proton at C-4 is greater than that of the proton at C-2, the C-4 proton must be *cis* with respect to the C-15 hydroxymethylene group. Onchidal (**1**) is therefore (1E,3E)-1-acetoxy-1,3,7(14)-monocyclofarnesatriene-15-al.

We were unable to test onchidal (**1**) or the crude secretion as chemical deterrents in field assays. However, the deterrent properties of the secretion from *Onchidella* species have been well documented. In laboratory assays, onchidal (**1**) inhibited the growth of *Staphylococcus aureus*. The minimum inhibitory concentration was between 0.21 and 0.63 $\mu$g/ml, implying that onchidal is a potent inhibitor of gram-positive bacteria. Since we have shown that onchidal (**1**) has potent antibacterial activity, and since we were unable to locate any other secondary metabolites in the defensive secretion, we wish to propose that onchidal (**1**) is the chemical deterrent responsible for the protection of *O. binneyi*.

## EXPERIMENTAL

$^1$H-nmr spectra were recorded on a Varian HR-220 spectrometer, $^{13}$C-nmr spectra were recorded on a Varian CFT-20 spectrometer, infrared spectra were recorded on a Perkin–Elmer Model 700 spectrophotometer, and optical rotations were measured on a Perkin–Elmer Model 141 polarimeter using a 10-cm microcell. Low-resolution mass spectra were recorded on a Hewlett–Packard 5930A mass spectrometer. High-resolution mass measurements were supplied by the Analytical Facility at California Institution of Technology. Melting points were measured on a Fisher–Johns apparatus and are reported uncorrected. All solvents used were either spectral grade or distilled from glass prior to use.

### *Collection and Extraction of Onchidella binneyi: Isolation of Onchidal* (**1**)

*Onchidella binneyi* were collected by hand from beneath rocks in the intertidal region at Bahia de los Angeles, Baja California (29°00′ N, 113°33′ W) in April and June 1977. The mucus secretion was collected in capillary tubes and stored in acetone

solution. Whole animals (~1000) were allowed to soak in acetone for 7 days at 5°C. The acetone was decanted and evaporated *in vacuo* to obtain an oily residue, which was partitioned between diethyl ether and water. The ether extract was dried over anhydrous sodium sulfate and evaporated to obtain an oil (4.5 g). The oil (4.0 g) was applied to a column (30 cm × 2.5 cm diameter) of silica gel. Elution with 5% ether in hexane gave onchidal (**1**) (200 mg, 0.23 mg/animal): $[\alpha]_D^{22}$ 17.2° (*c* 1.01, $CHCl_3$); ir ($CHCl_3$) 2930, 2725, 1757, 1685, 1385, and 1370 $cm^{-1}$; $\lambda_{max}$ 235 and 260 nm; $^1$H-nmr ($CDCl_3$) $\delta$ 0.89 (s, 3H), 0.99 (s, 3H), 1.26 (m, 2H), 1.57 (m, 3H), 2.05 (m, 2H), 2.16 (s, 3H), 2.57 (m, 2H), 4.50 (s, 1H), 4.81 (s, 1H), 6.10 (dd, 1H, $J$ = 14, 1 Hz), 6.41 (t, 1H, $J$ = 7 Hz), 8.26 (d, 1H, $J$ = 14 Hz), and 9.41 (d, 1H, $J$ = 1 Hz); $^{13}$C-nmr ($CDCl_3$) $\delta$ 193.3 (d), 167.5 (s), 156.6 (d), 148.1 (s), 141.2 (d), 135.2 (s), 109.5 (t), 105.8 (d), 53.5 (d), 37.4 (t), 35.2 (s), 33.5 (t), 28.6 (q), 26.7 (q), 24.8 (t), 23.6 (t), and 20.6 (q); mass spectrum, *m/e* 276, 234, 216, 123, and 81 (base peak); high-resolution mass measurement 276.172, $C_{17}H_{24}O_3$ requires 276.173.

*The Dialdehyde*(**2**)

Sodium carbonate (2 mg, 0.019 mmol) was added to a stirred solution of onchidal (**1**) (10 mg, 0.036 mmol) in methanol (10 ml). After 20 min of stirring at room temperature, the methanol was evaporated and the residue was partitioned between ether and dilute hydrochloric acid. The ether extracts were dried over anhydrous sodium sulfate and the solvent was evaporated to obtain the dialdehyde (**2**) (4 mg, 47% theoretical): $[\alpha]_D^{22}$ 12.7° (*c* 0.52, $CHCl_3$); ir ($CHCl_3$) 1725, 1685, and 1645 $cm^{-1}$; $\lambda_{max}$ 232 nm; $^1$H-nmr ($CDCl_3$) $\delta$ 0.86 (s, 3H), 0.96 (s, 3H), 1.25 (m, 3H), 1.57 (m, 3H), 2.05 (m, 3H), 2.45 (m, 1H), 3.39 (d, 1H, $J$ = 14 Hz), 3.43 (d, 1H, $J$ = 14 Hz), 4.52 (s, 1H), 4.82 (s, 1H), 6.74 (t, 1H, $J$ = 7 Hz), 9.40 (s, 1H), and 9.59 (s, 1H); mass spectrum, *m/e* 234, 219, 205, 190, 123, and 81 (base peak); high-resolution mass measurement 234.161, $C_{15}H_{22}O_2$ requires 234.162.

*The Diazine* (**3**)

Sodium carbonate (2 mg, 0.019 mmol) was added to a stirred solution of onchidal (**1**) (12 mg, 0.043 mmol) in methanol (10 ml). After 1 min, hydrazine hydrate (500 $\mu$l, 10.2 mmol) was added to the solution, which was stirred for 20 min at room temperature. The resulting mixture was partitioned between ether and water. The ether extract was dried over anhydrous sodium sulfate and the solvent was evaporated to yield the diazine (**3**) (8 mg, 79% theoretical): $[\alpha]_D^{22}$ 7.1° (*c* 0.46, $CHCl_3$); $\lambda_{max}$ 245, 252, and 256 nm; $^1$H-nmr ($CDCl_3$) $\delta$0.84 (s, 3H), 0.91 (s, 3H), 1.25 (m, 2H), 1.55 (m, 2H), 1.75 (m, 2H), 2.08 (m, 2H), 2.41 (m, 1H), 2.61 (m, 1H), 4.59 (s, 1H), 4.86 (s, 1H), 7.29 (s, 1H), and 9.03 (bs, 2H); mass spectrum, *m/e* 230, 215, 187, 173, 159, 107, and 94 (base peak); high-resolution mass measurement 230.176, $C_{15}H_{22}N_2$ requires 230.178.

*Ozonolysis of Onchidal* (**1**)

A stream of ozone in oxygen was bubbled into a solution of onchidal (16 mg, 0.058 mmol) in ethyl acetate (10 ml) which had been cooled to −78°C. After 2 min, excess ozone was removed in a stream of nitrogen while the solution was allowed to warm to room temperature. The ethyl acetate was evaporated *in vacuo* to obtain an ozonide, which was dissolved in acetic acid (5 ml) and reduced by addition of zinc dust (20 mg)

to the stirred solution. After 1 hr at room temperature, ether was added to the solution and the zinc salts were removed by filtration. The solvents were removed *in vacuo* to obtain the keto-aldehyde (**4**) (8 mg, 80% theoretical); ir ($CHCl_3$) 1720 and 1710 $cm^{-1}$; $^1$H-nmr ($CDCl_3$) $\delta$ 0.75 (s, 3H), 1.05 (s, 3H), and 9.83 (s, 1H); mass spectrum, *m/e* 168, 153, 140, 126, and 121.

*Debromosnyderol* (**6**)

Lithium wire (20 mg, 2.86 mmol) and ether (10 ml) were placed in a 100-ml three-necked flask equipped with a dry ice–acetone condenser, a nitrogen bubbler, and a rubber septum. The flask was cooled to −78° and ammonia (10 ml) was added. A solution of $\beta$-snyderol (**5**) (375 mg, 1.25 mmol) in ether (10 ml) was added dropwise to the stirred reaction mixture. One hour after the addition was complete, ammonium chloride (~100 mg) was added to the solution until it remained white. After being warmed to room temperature, the solution was washed with water and the ether layer was dried over anhydrous sodium sulfate. Evaporation of the ether gave debromosnyderol (**6**) (215 mg, 78% theoretical): $[\alpha]_D^{22}$ −16.2° (*c* 1.62, $CHCl_3$); $^1$H-nmr ($CDCl_3$) $\delta$ 0.82 (s, 3H), 0.90 (s, 3H), 1.25 (s, 3H), 4.51 (s, 1H), 4.73 (s, 1H), 5.03 (d, 1H, $J$ = 10 Hz), 5.18 (d, 1H, $J$ = 16 Hz), and 5.88 (dd, 1H, $J$ = 16, 10 Hz); $^{13}$C-nmr ($CDCl_3$) $\delta$ 149.1, 145.1, 111.4, 108.9, 73.2, 54.2, 40.8, 36.1, 34.9, 32.3, 28.3, 27.9, 26.2, 23.5, and 20.2; mass spectrum, *m/e* 207 (M-15), 204, 189, 123, 109.

*Degradation of Debromosnyderol* (**6**) *to Keto-aldehyde* (**4**)

Phosphorus oxychloride (150 $\mu$l) was added to a stirred solution of debromosnyderol (50 mg, 0.22 mmol) in pyridine (0.5 ml) at 0°C. The reaction mixture was allowed to warm to room temperature and was stirred for 24 hr. The excess reagent was carefully hydrolyzed with water, and the reaction mixture was then partitioned between ether and hydrochloric acid. The ether extracts were dried over anhydrous sodium sulfate and evaporated to give a 1:1 mixture (26 mg) of two trienes (**7** and **8**). The mixture of trienes **7** and **8** (16 mg) was ozonized in ethyl acetate solution according to the procedure used for the ozonolysis of onchidal to obtain a mixture of an acid (**9**) and the keto-aldehyde (**4**), which was identical in all respects, except optical rotation, with the previous sample.

*Reduction of Onchidal* (**1**) *to an Alcohol* (**11**)

Lithium tri-*t*-butoxyaluminum hydride (30 mg, 0.11 mmol) was added to a stirred solution of onchidal (26 mg, 0.09 mmol) in anhydrous ether (10 ml). The reaction mixture was stirred under nitrogen at room temperature for 1 hr. Excess reagent was carefully destroyed with water (10 ml). The ether layer was dried over anhydrous sodium sulfate and the solvent was evaporated to give the alcohol (**11**) (20 mg, 76% theoretical): ir ($CHCl_3$) 3600 and 1750 $cm^{-1}$, $\lambda_{max}$ 234 nm; $^1$H-nmr ($CDCl_3$) $\delta$ 0.85 (s, 3H), 0.95 (s, 3H), 1.84 (dd, 1H, $J$ = 12, 5 Hz), 2.05 (m, 2H), 2.16 (s, 3H), 2.27 (m, 2H), 4.21 (s, 2H), 4.50 (s, 1H), 4.77 (s, 1H), 5.52 (t, 1H, $J$ = 7 Hz), 6.29 (d, 1H, $J$ = 14 Hz), and 7.79 (d, 1H, $J$ = 14 Hz); mass spectrum, *m/e* 278, 260, 236, 200, 185, and 123.

*Lanthanide-Induced Shifts for Alcohol* (**11**)

The lanthanide-induced shifts corresponding to the addition of 1 equiv. of $Eu(fod)_3$ reagent were obtained by extrapolation from the measured data. The shifts are listed in

order of magnitude, with the initial chemical shift and proton assignment in parentheses; [$\Delta\delta$ ($\delta$, proton at C-$X$)]: $CDCl_3$, 10.2 (7.79, C-1), 9.4 (4.21, C-15), 4.6 (5.52, C-4), 3.8 (6.29, C-2), 2.1), 2.1 (2.27, C-5), 1.8 (1.84, C-6), 1.5 (4.50, C-14), and 1.3 (2.16, –OAc).

## ACKNOWLEDGMENTS

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