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PLANT GROWTH REGULATORY INDOLES FROM THE SPONGES *DYSIDEA ETHERIA* AND *ULOSA RUETZLERI*[1]

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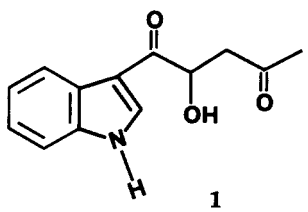
ABSTRACT.—Three indoles, indole-3-acetamide (**2**), indole-3-carboxaldehyde (**3**), and the previously unknown 4-hydroxy-5-(indole-3-yl)-5-oxo-pentan-2-one (**1**), have been isolated from the sponge *Dysidea etheria*. This marks the first isolation of indoles from this sponge genus and of a plant growth regulator from a sponge. Indole-3-acetamide (**2**) is a known auxin; the novel **1** is also active in promoting root growth in the lettuce seedling assay. The diketo-alcohol **1** has also been found in extracts of the sponge *Ulosa ruetzleri*.

In our continuing investigation (1-3) of the secondary metabolites of the Bermudian sponge *Dysidea etheria* de Laubenfels, our attention was directed toward a search for a highly oxidized sterol fraction by a recent report (4) of such a compound from an undescribed species of *Dysidea* from Guam. In the course of purifying the polar sterol fractions from our *D. etheria* extracts, we encountered small quantities of indole derivatives. The activity of *D. etheria* extracts in our plant growth regulatory assay (5) and our recent observation that the bis-indole caulerpin is a root-growth promoter (6) prompted us to identify these indoles and test them for growth regulatory activity. Described herein are the isolation, identification, and assays of these compounds.

The report by Gunasekera and Schmitz (4) of a unique polyoxygenated sterol in the sponge *Dysidea* was of special interest to us because of our developing focus on marine natural products as potential insect control agents (5,7,8). Tlc analyses of the very polar fractions from the Florisil chromatography of the organic soluble extracts of Bermudian *D. etheria* indicated the presence of oxidized sterols. Gel permeation chromatography provided not only major fractions enriched in the steroids but minor amounts of heteroaromatic compounds as well.

Our early collections (1981, 1982) of *D. etheria* provided 7.5 mg of the novel compound **1**. The indole system was indicated by characteristic ^{1}H-nmr signal patterns and the N-H stretch at 3490 cm^{-1} in the ir. The ^{1}H-nmr signal at δ 8.06 was assigned to H-2 and required a strongly deshielding substituent at C-3. Placement of a ketone car-



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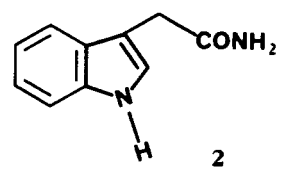

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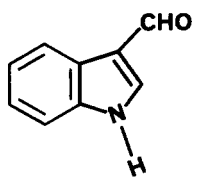

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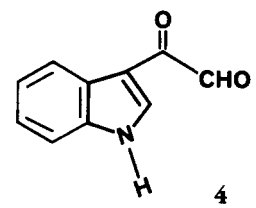

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[1]Contribution No. 1003 from the Bermuda Biological Station.

bonyl at C-3 was deduced from an ir absorption at 1660 cm^{-1} (H-bonded aryl ketone) and a fragment ion at *m/z* 144 (C_9H_6NO) in the mass spectrum.

The side chain could be extended by addition of the -$CHOHCH_2$- fragment indicated by the A_2MX system in the ^1H nmr (δ 2.90, 4.09, and 5.26) and the ir absorption at 3300 cm^{-1}. The hydroxyl-bearing methine was attached to the indolyl-3-carbonyl piece to provide hydrogen bonding to the aryl carbonyl (thus the 1660 cm^{-1} ir absorption). The structure could be completed by adding a methyl ketone (1730, 1375 cm^{-1}; δ 2.25; *m/z* 43) to give **1**, 4-hydroxy-5-(indole-3-yl)-5-oxo-pentan-2-one.

During this period, we initiated a study of marine invertebrates for plant growth regulatory activity and found activity in extracts of *D. etheria* (5). It might seem odd that sponges would possess or produce plant growth promotors, but some sponges overgrow algae without killing them, while others tolerate overgrowth by algae. These sponges might well have plant regulators, and *D. etheria* is a species that overgrows and is overgrown by algae.

Because we had utilized indole-3-acetic acid and some of its analogs as standards in our assay work on the algal pigment caulerpin (6), we thought it appropriate to test the unique indole derivative; **1** proved to be active in promoting root growth in lettuce seedlings. Root lengths were 121-122% those of controls at $10^{-8}/10^{-9}M$ (see Figure 1), not as active as indole-3-acetic acid but slightly more active than indole-3-acrylic and indole-3-pyruvic acids (6).

We were, at first, surprised that **1** exhibited no optical activity, but, because we had previously encountered artifacts formed by condensation of the extraction solvent Me_2CO with aryl aldehydes (9,10), we began to consider the possibility that **1** was formed by condensation of Me_2CO with the keto aldehyde **4**. Neither **1** nor **4** have previously been reported from a natural source, although **4** has been synthesized (11) and used as a synthon for luciferins (12).

A careful reexamination of two additional collections of *D. etheria* yielded no sign of **4**, but the large 1984 collection provided a small quantity of **2** and **3**, which were characterized as a mixture. The key ^1H-nmr signals for the aldehyde proton in **3** (δ 10.06) and the benzylic methylene in **2** (δ 4.78) were present, along with overlapping signals for the various indole protons. The structures were secured by mass spectral analysis;

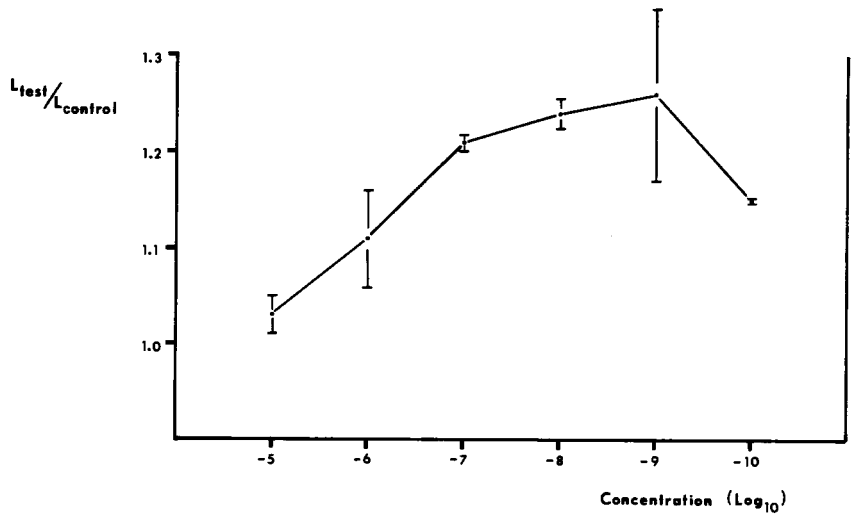

FIGURE 1. Root growth assays of **1**. Points represent the mean of three trials; standard deviation is reflected in the error bars. Root lengths were typically 10-25 mm; concentrations were 10^{-5} to $10^{-10}M$.

the two compounds could be separated in a direct inlet probe on the basis of difference in volatility. Indole-3-acetamide (**2**) is a known plant-growth regulator (13).

In the course of our examination of the toxic extracts of the sponge *Ulosa ruetzleri* Wiedenmayer, we encountered a small quantity of **1**, identical in all respects to the compound isolated from *D. etheria*. This new compound contributes to the plant growth regulatory activity we have observed in the polar extracts of *U. ruetzleri*, but other constituents are involved as well. These other active fractions are under investigation.

In summary, two indole derivatives with plant growth regulatory activity have been isolated from the sponge *D. etheria*, the novel **1** and the known **2**. This is the first report of indolic secondary metabolites from the genus *Dysidea* and the first identification of plant growth regulators from a sponge; **1** has subsequently been found in *U. ruetzleri*. That **1** is an artifact is possible but as yet uncertain. A synthesis of **1** will be undertaken to provide sufficient quantities for a more thorough analysis of its plant growth regulatory activity.

EXPERIMENTAL

The collection, extraction, and initial fractionation of *D. etheria* have been described (1). Fractions 19, 20, and 21 from the Florisil chromatography, eluted with EtOAc-MeOH (4:1 to 3:2), were each permeated through Bio-Beads S-X4 (column 90×4 cm) with hexane-CH_2Cl_2-EtOAc (4:3:1). The last fraction eluted in each run was then permeated through Sephadex LH-20 (column 181×2.5 cm) with CH_2Cl_2-MeOH (1:1). The last fraction in each run was enriched in aromatic compounds. At this point, these three fractions were pooled and permeated once more through Sephadex LH-20, this time with MeOH-MeCN (4:1) (column 123×1.5 cm). Six fractions were obtain; fraction 5 was an off-white solid, **1**, 6.5 mg; ir ν max ($CHCl_3$) 3490, 3300, 1730, 1660, 1375 cm^{-1}; ms *m/z* 231.0897 (M^+, 4%, calcd for $C_{13}H_{13}NO_3$-231.0917), 213 (16), 198 (3), 170 (9), 144 (100), 116 (16), 89 (17), 43 (16); ^1H nmr ($CDCl_3$) δ 8.83 (1H, br s), 8.30 (1H, dd, J=7.8, 3), 8.06 (1H, s), 7.43 (1H, m), 7.33 (2H, overlapping m), 5.26 (1H, dt, J=6.5, 6.1), 4.09 (OH, d, J=6.5), 2.90 (2H, d, J=6.1), 2.29 (3H, s).

Indole-3-acetamide (**2**) and indole-3-carboxaldehyde (**3**) were isolated from the $CHCl_3$-soluble extracts of a 1984 collection of *D. etheria* (3) by gel permeation through Bio-Beads S-X4 and Sephadex LH-20 as described above. A mixture of **2** and **3**, 7.5 mg, was obtained from 1.3 g of extract. The compounds were identified as a mixture; pertinent data: Compound **3**; *m/z* 174.0793 (M^+, 23% calcd for $C_{10}H_{10}N_2O$-174.0793), 130 (100); ^1H nmr ($CDCl_3$) δ 4.78 (2H, s). Compound **2**; ms *m/z* 145.0529 (M^+, 80% calcd for C_9H_7NO-145.0529), 144 (100), 116 (24), 89 (23); ^1H-nmr ($CDCl_3$) δ 10.06 (1H, s).

The lettuce seedling assay has been described (6).

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LITERATURE CITED

1. S.H. Grode and J.H. Cardelina II, *Lipids*, **18**, 889 (1983).
2. S.H. Grode and J.H. Cardellina II, *J. Nat. Prod.*, **47**, 76 (1984).
3. T.J. Schram and J.H. Cardellina II, *J. Org. Chem.*, **50**, 4155 (1985).
4. S.P. Gunasekera and F.J. Schmitz, *J. Org. Chem.*, **48**, 885 (1983).
5. J.H. Cardellina II, *Pure & Appl. Chem.*, **58**, 364 (1986).
6. M.F. Raub, J.H. Cardellina II, and J.G. Schwede, *Phytochemistry*, (in press).
7. S.H. Grode, T.R. James, Jr., J.H. Cardellina II, and K.D. Onan, *J. Org. Chem.*, **48**, 5203 (1983).
8. J.H. Cardellina II, M.F. Raub, and B.C. Van Wagenen, in: "Allelochemicals: Role in Agriculture, Forestry and Ecology." Ed. by G. Waller, American Chemical Society Symposium Series, (in press).
9. J.H. Cardellina II, *J. Nat. Prod.*, **46**, 196 (1983).
10. J.H. Cardellina II, C.J. Graden, B.J. Greer, and J.R. Kern, *Lipids*, **18**, 107 (1983).
11. G. Sanna, *Gazz. Chim. Ital.*, **72**, 363 (1942).
12. Y. Kishi, T. Goto, S. Inoue, S. Sugiura, and H. Kishimoto, *Tetrahedron Lett.*, 3445 (1966).
13. E. Maeda and T.A. Thorpe, *Phytomorphology*, **29**, 146 (1980).

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