Ulosantoin, a Potent Insecticide from the Sponge *Ulosa ruetzleri*[1]

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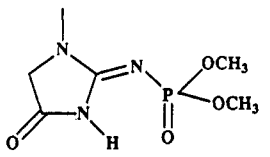
Ulosantoin (2), a phosphorylated hydantoin, has been isolated from organic soluble extracts of the sponge *Ulosa ruetzleri* and found to exhibit marked insecticidal activity against tobacco hornworm larvae and cockroaches. The structure was determined by X-ray diffraction analysis. A structurally related compound, dimethyl N^2-creatininylphosphate (1), was also isolated from the sponge extracts, but was inactive in the insecticidal screens.

Our laboratory has been investigating the potential of marine natural products to serve as insect control agents via mechanisms of toxicity, interference with molting or metamorphosis, and feeding deterrence. Our previous reports of sponge sesquiterpenes[3] and diterpenes from gorgonians[4] and sea pens,[5] while demonstrative of the validity of the premise that the marine biosphere might be a source of new insecticides, did not disclose compounds of commercially significant potency against important insect pests. In this report, we describe a simple, but novel sponge metabolite with striking activity against not only our primary assay insect, the tobacco hornworm, but also one of the more intrasigent insect pests, the cockroach.

The organic extracts of *Ulosa ruetzleri*, collected from the inshore waters of Harrington Sound, Bermuda, in June of 1983, significantly inhibited weight gain of the larvae of the tobacco hornworm, *Manduca sexta*. Solvent–solvent partitioning of the extracts provided insecticidal chloroform and ethyl acetate soluble fractions. Gel permeation chromatography (BioBeads S-X4, then Sephadex LH-20) provided a mixture of two compounds which appeared, by NMR, to be closely related. They were successfully resolved by a second Sephadex LH-20 chromatography (see Figure 1).

HRMS provided molecular formulas for 1 ($C_6H_{12}N_3O_4P$) and 2 ($C_5H_9N_2O_5P$). The very similar ^{1}H-NMR spectra



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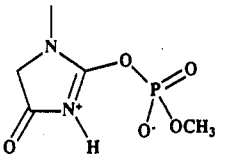

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were distinguished by the absence of any proton–proton coupling. Each had a broad singlet near δ 9.7 for an NH proton, a two proton singlet near δ 3.90, and an *N*-methyl singlet near δ 3.05. Each also had a doublet ($^3J_{PH}$ = 11) for an *O*-methyl group attached to phosphorus; in the case of 1, the signal integrated for six protons, while there was a single methoxyl group in 2. The ^{13}C-NMR spectra of 1 and 2 were also similar; in addition to the expected signals for *N*-methyl, *O*-methyl, and *N*-methylene groups, there were two sp^2 resonances which were assigned to imine ($\sim\delta$ 156, $^2J_{PC}$ = 7–8) and amide ($\sim\delta$ 168) carbons.

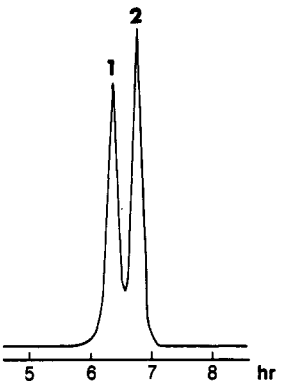

Figure 1. Sephadex LH-20 separation of 1 and 2: column 2.7 × 193 cm; eluant MeOH–CH_2Cl_2 (1:1), monitored by UV ca. 254 nm; total run time 7.5 h.

To distinguish the several structural possibilities unequivocally and to preserve the scant supplies of the compounds for biological testing, the structures of 1 and 2 were secured by X-ray diffraction analyses (see Figure 2). Except for some disorder in the phosphates, the solutions were unremarkable. Compound 1, is, then, dimethyl N^2-creatininylphosphate, while 2, a phosphorylated hydantoin, has been named ulosantoin. To our knowledge, neither compound was previously known.

Since the original extraction and partitioning work was done with methanol, there was some concern that 1 and 2 might be artifacts produced during methanolysis of some larger metabolites. To test this hypothesis, a second collection, made in 1984, was extracted sequentially with acetone and dichloromethane; the total extract was partitioned between dichloromethane and water. Further extraction of the aqueous phase with ethyl acetate provided a small fraction enriched in 2, confirming that ulosantoin was indeed a natural product.

Followup testing of 1 and 2 against tobacco hornworm larvae at 250 ppm (in agar-based diet) revealed that 1 was

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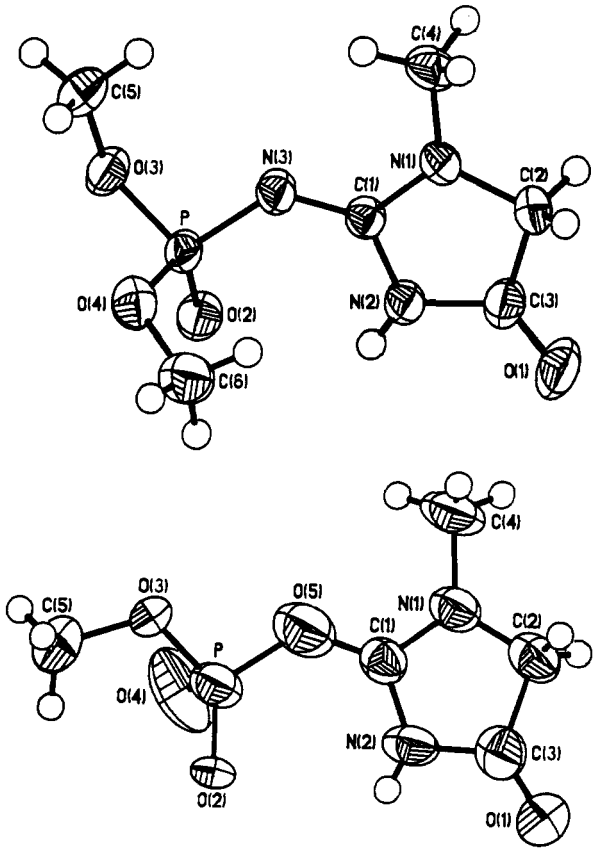

Figure 2. Computer generated Ortep drawings of dimethyl N^2-creatininylphosphate (1) (upper) and ulosantoin (2) (lower).

devoid of activity, while 2 caused 100% mortality within 24 h. Dose–response studies disclosed an LD_{50} value of 6 ppm for ulosantoin over the normal 5–7 day experimental run[6]. Ulosantoin was fully lethal in topical applications of 0.2 and 2.0 μg, respectively, to the Mexican bean beetle and the southern armyworm. Ulosantoin (2) was exceptionally toxic to the American cockroach (*Periplaneta americana*), whether applied topically (LD_{100} = 1.0 μg) or intrathoracically (LD_{100} = 0.2 μg). Ulosantoin was equipotent with Paraoxon, a phosphate-type AChE inhibitor, in inhibiting acetylcholinesterase, with IC_{50} values of 10^{-7}–10^{-8} M. Ulosantoin, then, would seem to be the most potent insecticide isolated from the marine biosphere since nereistoxin,[7] which served as a model for the commercial insecticide Padan.

Experimental Section

Collection and Extraction of *Ulosa ruetzleri*. *U. ruetzleri* was collected from the inshore waters, of Harrington Sound, Bermuda, in June of 1983, and stored in acetone at −5 °C prior to extraction. The acetone was removed by filtration and the sponges were homogenized in MeOH. After filtration, the MeOH extracts were combined with the acetone wash and reduced to an aqueous suspension. The remaining marc was soaked three times with CH_2Cl_2 for 8 h each time. Each CH_2Cl_2 extract was subsequently equilibrated with the aqueous suspension and separated into water and organic solubles. Reduction of the organic solubles yielded 53.0 g (9.4% dry weight) of a dark oil. Lyophilization of the water solubles yielded 102.7 g (18.1% of dry weight) of a tan solid.

The organic extract (52.3 g) was dissolved in 500 mL of 10% aqueous MeOH and and extracted three times with 300 mL of hexane. The remaining aqueous phase was then increased in polarity to 20% H_2O and extracted three times with 300 mL of CCl_4. The remaining aqueous phase was increased further in polarity to 40% H_2O and extracted three times with 300 mL of $CHCl_3$. The aqueous MeOH phase was then partially evaporated to remove MeOH and the residual aqueous suspension was extracted three times with an equal volume of ethyl acetate. The individual partitions were reduced, in vacuo, to give 47.3 g (hexane), 1.6 g (CCl_4), 0.77 g ($CHCl_3$), 0.16 g (EtOAc), and 0.47 g (H_2O) fractions.

Isolation of 1 and 2. The $CHCl_3$ solubles (770 mg) were first fractionated by permeation through Bio-Beads S-X4 (4 × 95 cm) using *n*-hexane–CH_2Cl_2–EtOAc (4:3:1). Fraction 10 (60 mg), of 10 fractions collected, was further chromatographed through Sephadex LH-20 (2.7 × 193 cm) using MeOH–CH_2Cl_2 (1:1). The second fraction (35 mg), of seven fractions collected, was further permeated through Bio-Beads S-X8 (2.5 × 115 cm) using CH_2Cl_2–C_6H_{12} (3:2). Fraction 5 (24 mg), of six fractions collected, was rechromatographed through the aforementioned Sephadex LH-20 column to afford two fractions. Fraction 1 (11 mg) contained dimethyl N^2-creatininylphosphate (1), a crystalline solid: mp 154–155 °C: UV λ_{max} (MeOH) 223 nm (ε 5200); IR ν_{max} ($CDCl_3$) 3294 (br), 2990, 2950, 2930, 2850, 1778, 1754, 1670, 1495, 1450, 1410, 1315, 1220, 1185, 1060, 1040 cm^{-1}; MS (EI) *m/z* (rel inten) 221 (M^+, 100), 191 (20), 163 (12), 150 (47), 120 (38), 109 (48), 95 (14), 79 (36); HRMS (EI) found 221.0563 (calcd for $C_6H_{12}N_3O_4P$, 221.0561); ^{1}H-NMR ($CDCl_3$) δ 9.8 (1 H, br s), 3.90 (2 H, s), 3.70 (6 H, d, $^3J_{PH}$ = 11), 3.03 (3 H, s); ^{13}C-NMR ($CDCl_3$) δ 168.8, 156.2 (d, $^2J_{PC}$ = 7), 53.3 (d, $^2J_{PC}$ = 6), 52.1, 30.3. Fraction 2 (11 mg) contained 1-methyl-2-[(methoxyphosphinyl)oxy]imidazolin-4(5*H*)-one (ulosantoin 2), an amorphorous solid. Recrystallization from acetone–isooctane afforded crystals: mp 127–128, °C; UV λ_{max} (CH_3OH) 224 nm (ε 5000); IR ν_{max} ($CDCl_3$) 3320 (br), 2955, 2930, 2855, 1783, 1760, 1660, 1500, 1455, 1320, 1296, 1250, 1240, 1190, 1070, 1050 cm^{-1}; MS (EI) *m/z* (rel inten) 209 (MH^+, 100), 179 (11), 165 (11), 151 (12), 139 (52), 108 (25), 97 (14), 81 (13); HRMS (EI) found 209.0326 (calcd for $C_5H_{10}N_2O_5P$, 209.0325); ^{1}H-NMR ($CDCl_3$) δ 9.6 (1 H, br s), 3.94 (2 H, s), 3.82 (3 H, d, $^3J_{PH}$ = 11), 3.05 (3 H, s); ^{13}C-NMR ($CDCl_3$) δ 168.3, 156.9 (d, $^2J_{PC}$ = 8), 54.4 (d, $^2J_{PC}$ = 5), 52.2, 30.5.

X-ray Structure Determinations. Intensity data for both compounds were collected on a Nicolet R3mE automated diffractomer at 25 °C with graphite monochromated Mo Kα radiation (0.71069 Å). Unit cell dimensions were refined by least-squares using 25 centered reflections. Data reduction included corrections for Lorentz and polarization effects, and the data sets were corrected for absorption by Gaussian integration using measured dimensions between indexed crystal faces. Phosphorus positions were obtained from Patterson maps, and all other atoms, including hydrogens, were located by difference maps. The structures were refined by least-squares with anisotropic thermal parameters and statistical weighting of the data. Hydrogen atoms were included in calculated positions with fixed isotropic thermal parameters. No corrections for extinction were needed. All crystallographic calculations were made with the SHELXTL program package by G. M. Shaldrick, Nicolet Instrument Corp., Madison, WI. The ulosantoin structure (2, $C_5H_9N_2O_5P$) showed partial disorder of the PO_2OCH_3 group by rotation around the bridging O–P bond. The disorder was modeled with a major (≃74%) and two minor orientations (≃13% each). The minor orientations were given fixed isotropic thermal parmaters and geometric constraints based on the refined values for the major orientation. Crystal and structure refinement data are as follows: $C_6H_{12}N_3O_4P$, pale yellow crystal, 0.4 × 0.6 × 0.6 mm, triclinic, *P*1, *a* = 7.885(1) Å,, α = 104.27 (1)°, β = 94.26 (1)°, γ = 116.03 (1)°, *V* = 493.9 (1) Å^3, *Z* = 2, *F*(000) = 232, *d*(calcd) = 1.49 g/cm^3, μ (Mo Kα) = 2.8 cm^{-1}, omega scans, 3° < 2θ < 65°, 2604 observed data ($I > 3\sigma[I]$), 130 parameters, R = 0.044, R_w = 0.050; $C_5H_9N_2O_5P$, pale yellow crystal, 0.08 × 0.25 × 0.45 mm, monoclinic, $P2_1/c$, *a* = 8.186 (1) Å, *b* = 8.186 (1) Å, *b* = 12.441 (2) Å, *c* = 8.816 (1) Å, β = 93.89 (1)°, *V* = 895.8(3) Å^3, *Z* = 4, F(000) = 432, *d*(calcd) = 1.54 g/cm^3, μ (Mo Kα) = 3.1 cm^{-1}, θ/2θ scans, 4° < 2θ < 55°, 716 observed data ($I > 2\ ^1/_2\sigma[I]$), 151 parameters, R = 0.071, R_w = 0.074.

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Supplementary Material Available: 250 MHz ^{1}H-NMR spectra and EIMS data for compounds **1** and **2** (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information. The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.