# Two New Nitrogenous Sesquiterpenes from the Sponge *Axinyssa aplysinoides*

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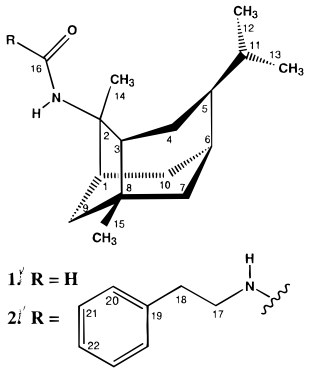
Bioassay-guided fractionation of the EtOAc extract of the Palauan sponge *Axinyssa aplysinoides* yielded two novel alkaloids, **1** and **2**. The structure of 2-(formylamino)trachyopsane (**1**) was determined by X-ray analysis; and the structure of *N*-phenethyl-*N*′-2-trachyopsanylurea (**2**), by interpretation of the spectral data.

Terpenoids are among the most abundant secondary metabolites of marine sponges,[1] but there are relatively few examples that contain nitrogen. Marine sponges of the order Halichondrida often contain mixtures of sesquiterpene isonitriles, isothiocyanates, and formamides.[2] As part of our continuing search for biologically active antitumor agents by screening natural product extracts in a yeast-based screen for DNA damaging agents,[3] we encountered an EtOAc extract of a sponge collected in Palau, *Axinyssa aplysinoides* Dendy, 1922 (Halichondrida), that was found to be active and was therefore selected for fractionation. Bioassay-guided fractionation revealed that the bioactivity was associated with metabolites **1** and **2**. During the preparation of this manuscript, we came across a report on the constituents of *A. aplysinoides*, from which three new and three known sesquiterpenes were isolated.[4]




Specimens of *A. aplysinoides* were collected by hand using Scuba (−20 m) and were immediately frozen. An EtOAc extract of the freeze-dried sponge was chromatographed over an RP-18 column to yield several fractions active in our DNA-damaging assay. Further purification of these fractions by Si gel HPLC led to the isolation of 2-formamido-6-axene[2] and two novel sesquiterpene analogues, 2-formylaminotrachyopsane (**1**) and *N*-phenethyl-*N*′-2-trachyopsanylurea (**2**).

2-(Formylamino)trachyopsane (**1**), $[\alpha]_D$ −67.5°, was isolated as white crystals, and its molecular formula was shown to be $C_{16}H_{27}NO$ by HRFABMS. Its molecular structure was established from a single-crystal X-ray diffraction study at reduced temperature. Two independent rotamers of **1**, differing primarily in the orientation of the formamide group relative to the trachyopsane skeleton, co-crystallized. Thermal ellipsoid diagrams of the two are shown in Figure 1, and the final atomic coordinates are given in Table 3. The IR spectrum exhibited bands at 3279, 1683, and 1655 $cm^{-1}$, suggesting the presence of a formamide carbonyl group. The presence of an ion at *m*/*z* 205 in the ESIMS derived from the loss of formamide. The $^1$H- and $^{13}$C-NMR spectra ($CDCl_3$, 25 °C) clearly showed two sets of resonances (1:2 ratio) indicating the presence of rotational isomers involving the formamide group (Figure 2). Heating the sample to 120 °C (DMSO-$d_6$) caused a reversible coalescence of the rotamer signals yielding a single set of resonances. The $^1$H-NMR spectrum of **1** had two formyl doublets at $\delta$ 8.20 ($J$ = 12.4 Hz, minor) and 7.95 ($J$ = 2.1 Hz, major). All of the other proton resonances were also doubled, the differences in their chemical shifts diminishing with their distance from the formamide group. Similarly, the $^{13}$C-NMR spectrum of **1** showed a double set of peaks in a 1:2 ratio with noticeable differences in the chemical shifts of the two C-16 formamide carbonyls ($\delta$ 163.6 in the minor and 160.1 in the major) and the two quaternary C-2 resonances ($\delta$ 66.4 in the minor and 67.5 in the major). The protonated carbons were assigned by their direct correlations observed in the HMQC spectrum of **1**. In addition, the following correlations were observed in the HMBC spectrum of **1**, which served to assign the quaternary signals and to fix the assignments of the ring junctions and the substituents. The formamide proton doublet ($\delta$ 7.95) correlated to the carbon at $\delta$ 67.5, establishing that the formamide group was attached at C-2. The $CH_3$-14 methyl singlet ($\delta$ 1.51) correlated to C-2, C-1, and C-3 ($\delta$ 67.5, 45.1, and 51.3). The $CH_3$-15 methyl singlet ($\delta$ 0.95) correlated with C-8, C-7, C-9, and C-3 ($\delta$ 38.8, 37.4, 45.0, and 51.3), and the H-6 methine ($\delta$ 1.90) correlated to C-7 and C-10 ($\delta$ 34.6) in one ring and C-5 ($\delta$ 46.6) in another. Finally, the isopropyl methyl doublets correlated to C-11 and C-5, indicating that the isopropyl group was attached at C-5. The relative stereochemistry was defined by analysis of coupling constants and NOE data (Figure 2). The major rotamer displayed a strong NOE between the amide proton and the formyl doublet, which shared a 2.1 Hz coupling. This enhancement and coupling constant were consistent with a *cis* orientation of these protons.[5] In the minor rotamer, in which these same two protons shared a *trans* relationship, no NOE was observed and the coupling constant was a typical 12.4 Hz.

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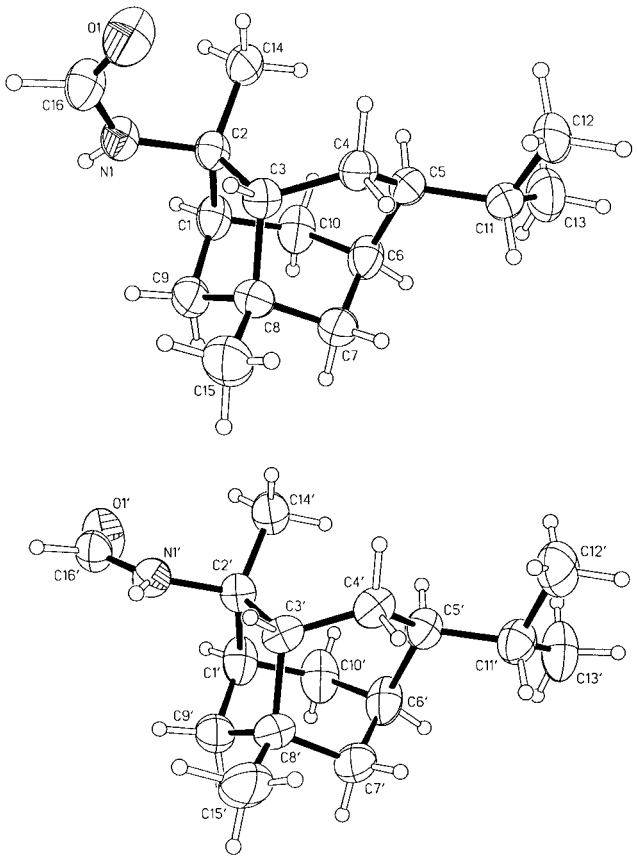


**Figure 1.** Views of the two independent molecules of **1** showing the numbering scheme employed. Anisotropic displacement ellipsoids for non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.

*N*-Phenethyl-*N*′-2-trachyopsanylurea **(2)** was isolated as a clear oil, $[\alpha]_D$ −28.6°, of molecular formula $C_{24}H_{36}N_2O$ (two exchangeable hydrogens), and its IR spectrum possessed a strong band at 1632 $cm^{-1}$ due to the carbonyl group (urea). The ESI tandem mass spectrum of **2** produced peaks at *m*/*z* 105 ($C_8H_9$, phenethyl carbonium ion), *m*/*z* 122 ($C_8H_{12}N$, the protonated phenethylamine fragment), 165 ($C_9H_{13}N_2O$, protonated phenethylurea), and 205 ($C_{15}H_{25}$, from neutral loss of phenethyl urea). The $^1$H-NMR spectrum of **2** (Table 1) had all the signals associated with the trachyopsane moiety of **1**, and in addition, it had five overlapping aromatic multiplets between $\delta$ 7.31 and 7.21. In the COSY spectrum of **2**, these aromatic protons correlated solely to one another, and the benzylic methylene ($CH_2$-18) triplet at $\delta$ 2.81 was coupled to the H-17 methylene at $\delta$ 3.41, which in turn correlated to the broad NH triplet at $\delta$ 4.04. The absence of an H-16 formamide proton at $\delta$ 7.95 in **2** and the emergence of an NH triplet at $\delta$ 4.04 revealed that the phenethylamine group was attached to the trachyopsane moiety via a urea carbonyl. In the $^{13}$C-NMR spectrum (Table 1), the expected 22 signals were observed, which included eight methines, six methylenes, four methyls, and four quaternary carbon signals. Interpretation of the NMR data, including the COSY, HMQC, and HMBC experiments, led to assignment of the resonances associated with the phenethyl urea as well as the sesquiterpene portion, which was found to be identical to that of **1**.

Although the crude extract of *A. aplysinoides* demonstrated selective activity against DNA-repair-deficient yeast mutants, this differential effect was not evident for the purified alkaloids **1** and **2**. These compounds inhibited wild-type and DNA-repair-deficient yeast strains at similar concentrations, as shown in Table 2. The results indicate that these alkaloids do not kill yeast by production of DNA damage.

## Experimental Section

**General Experimental Procedures.** IR spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. All homonuclear and heteronuclear 1D and 2D NMR data were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$. ESIMS were obtained in the negative mode on a Perkin-Elmer Sciex API-III triple quadrupole mass spectrometer, and HRFABMS on a VG-ZAB-4FSE four-sector mass spectrometer. Analytical and preparative TLC were carried out on precoated Si gel plates. A Rainin HPXL solvent delivery system equipped with a refractive index detector, Model 156, was used for HPLC separations employing a Lichrosorb Si 60 column. UV spectra were recorded on a Beckman DV-7 spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter. Reagent grade chemicals (Fisher and Baker) were used throughout.

**Bioassays.** Identical to those described by Bolzani *et al.*[6]

**Collection, Extraction, and Isolation.** The peach-colored sponge, *A. aplysinoides* (voucher sample no. DJF85-085, deposited at SIO Benthic Invertebrate Collection no. P1150), was collected by hand using scuba (−20 m) from Malakal Harbor, Palau, and the specimens were frozen immediately and kept at −20 °C until extraction. The freeze-dried sponge (198 g) was extracted with EtOAc (2 × 1 L) and MeOH (2 × 1 L) to give 6.8 and 26.6 g extracts, respectively. A portion of the active EtOAc extract (2.5 g) was chromatographed on a column of RP-18 (Whatman, ODS-3) using $H_2O$−MeOH (7:93) as a solvent system for elution to yield several fractions that were tested against a wild-type yeast strain, a strain deficient in double-stranded DNA repair (RAD-52), and a strain deficient in both double-stranded DNA repair and topoisomerase I. The residue (231 mg) obtained from the active fractions, which contained polar and non-polar compounds, was applied to a Si gel cartridge and eluted first with EtOAc−hexane (35:65) and then with MeOH−$CH_2Cl_2$ (15:85) to give 181 and 32 mg fractions, respectively. Further purification of the 181-mg fraction by repeated Si gel HPLC (35:65,EtOAc−hexane) yielded 2-formamido-6-axene (24 mg), 2-(formylamino)trachyopsane (**1**, 115 mg), and *N*-phenethyl-*N*′-2-trachyopsanylurea (**2**, 5.3 mg) in pure form.

**2-Formamido-6-axene.** NMR characterization showed this sample to be identical to the literature compound described by He *et al.*[2]

**2-(Formylamino)trachyopsane (1):** colorless crystals; $[\alpha]_D$ −67.5° (*c* 0.56, MeOH); UV(MeOH) $\lambda_{max}$ 354, 215 nm; IR (KBr) $\nu_{max}$ 3279, 3045, 2966, 2752, 1683, 1655, 1535, 1385, 793 $cm^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; ESIMS *m*/*z* 250 $[M + H]^+$, 205; HRFABMS *m*/*z* 250.2090 $[M + H]^+$ calcd for $C_{16}H_{28}NO$, 250.2093.

***N*-Phenethyl-*N*′-2-trachyopsanylurea (2):** clear oil, $[\alpha]_D$ −28.6° (*c* 0.14, MeOH); UV (MeOH) $\lambda_{max}$ 211,

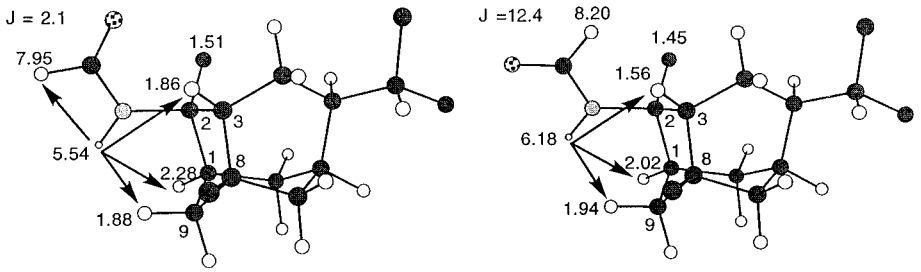


**Figure 2.** The major and minor rotamers of 2-(formylamino)trachyopsane (**1**) with arrows representing NOE enhancements.

**Table 1.** $^1$H- and $^{13}$C-NMR Assignments for 2-(Formylamino)trachyopsane (**1**) and *N*-Phenethyl-*N*′-2-trachyopsanylurea (**2**) in $CDCl_3$

| | compound | | | |
|---|---|---|---|---|
| | compd **1**[a] | | compd **2** | |
| position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 45.1 | 2.28 (1H, m) | 46.0 | 2.15 (1H, m) |
| 2 | 67.5 | | 66.5 | |
| 3 | 51.3 | 1.86 (1H, m) | 51.8 | 1.75 (1H, m) |
| 4 | 24.9 | 1.75 (1H, m) | 25.1 | 1.70 (1H, m) |
| | | 1.17 (1H, m) | | 1.06 (1H, m) |
| 5 | 46.6 | 1.14 (1H, m) | 46.5 | 1.14 (1H, m) |
| 6 | 31.4 | 1.90 (1H, m) | 31.5 | 1.92 (1H, m) |
| 7 | 37.4 | 1.22 (2H, m) | 37.5 | 1.22 (2H, m) |
| 8 | 38.8 | | 39.0 | |
| 9 | 45.0 | 1.88 (1H, m) | 45.1 | 1.92 (1H, m) |
| | | 1.17 (1H, m) | | 1.15 (1H, m) |
| 10 | 34.6 | 1.73 (1H, m) | 34.8 | 1.74 (1H, m) |
| | | 1.46 (1H, m) | | 1.47 (1H, m) |
| 11 | 31.8 | 1.31 (1H, m) | 31.8 | 1.32 (1H, m) |
| 12 | 20.8 | 0.86 (3H, d, 6.5) | 20.9 | 0.87 (3H, d, 6.4) |
| 13 | 21.5 | 0.84 (3H, d, 6.5) | 21.6 | 0.85 (3H, d, 6.4) |
| 14 | 19.6 | 1.51 (3H, s) | 20.4 | 1.46 (3H, s) |
| 15 | 27.7 | 0.95 (3H, s) | 27.9 | 0.97 (3H, s) |
| 16 | 160.1 | 7.95 (1H, d, 2.1) | 156.9 | |
| NH-2 | | 5.54 (1H, b) | | 3.98 (1H, b) |
| NH-16 | | | | 4.04 (1H, bt, 5.8) |
| 17 | | | 41.5 | 3.41 (2H, dt, 5.8, 6.8) |
| 18 | | | 36.3 | 2.81 (2H, t, 6.8) |
| 19 | | | 139.3 | |
| 20 | | | 128.9 | 7.21 (2H, m) |
| 21 | | | 128.6 | 7.31 (2H, m) |
| 22 | | | 126.4 | 7.21 (1H, m) |

[a] The NMR data presented are the chemical shifts and coupling constants of the major rotamer.

**Table 2.** Bioactivity of Alkaloids **1** and **2** $IC_{12}$ ($\mu$g/100 $\mu$L) in Yeast Strain

| compd | wild type | Δ RAD 52 | Δ RAD 52 Δ TOP1 |
|---|---|---|---|
| **1** | 31 | 51 | 4.8 |
| **2** | 14 | 45 | 12 |

315−368 (bs) nm; IR (KBr) $\nu_{max}$ 3400, 3000−3100, 2800, 1632, 1561, 1453, 1384, 748 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; ESIMS *m*/*z* 369 $[M + H]^+$; HRFABMS *m*/*z* 369.2914 $[M + H]^+$ calcd for $C_{24}H_{37}N_2O$, 369.2906, 205.1953 ($C_{15}H_{25}$), 165.1044, ($C_9H_{13}N_2O$), 122.0982 ($C_8H_{12}N$), and 105.0733 ($C_8H_9$).

**Data Collection and X-ray Structure Refinement of 1.** A suitable crystal was flash cooled in a stream of $N_2$ gas to 223(2) K. Lattice parameters were determined from the setting angles of 25 reflections well distributed in reciprocal space measured on an Enraf Nonius CAD-4 diffractometer. Intensity data were collected on the diffractometer using graphite-monochromated Mo Kα radiation and an $\omega - 2\theta$ variable speed scan technique.

**Table 3.** Atomic Coordinates [$\times 10^4$] and Equivalent Isotropic Displacement Parameters [Å$^2 \times 10^3$] for (**1**)

| | *x*/*a* | *y*/*b* | z/c | *U*(eq) |
|---|---|---|---|---|
| O1 | 9093(1) | 1173(2) | 9474(2) | 61(1) |
| N1 | 6982(2) | 2555(2) | 9547(2) | 38(1) |
| C1 | 6213(2) | 4801(2) | 10 119(2) | 43(1) |
| C2 | 7484(2) | 3646(2) | 10 181(2) | 35(1) |
| C3 | 8698(2) | 4676(2) | 9037(2) | 36(1) |
| C4 | 9899(2) | 5158(2) | 9765(2) | 39(1) |
| C5 | 9333(2) | 6089(2) | 10 763(2) | 40(1) |
| C6 | 7889(2) | 6921(2) | 10 210(2) | 47(1) |
| C7 | 8040(2) | 7461(2) | 8492(2) | 52(1) |
| C8 | 7854(2) | 6100(2) | 7999(2) | 47(1) |
| C9 | 6227(2) | 5661(2) | 8429(2) | 51(1) |
| C10 | 6504(2) | 5947(3) | 10 873(3) | 51(1) |
| C11 | 7781(2) | 1443(2) | 9298(2) | 45(1) |
| C12 | 7903(2) | 2713(2) | 11 758(2) | 45(1) |
| C13 | 10 476(2) | 7188(2) | 10 851(2) | 46(1) |
| C14 | 12 009(2) | 6469(3) | 11 049(3) | 58(1) |
| C15 | 10 018(3) | 7794(3) | 12 120(3) | 69(1) |
| C16 | 8350(4) | 6473(3) | 6338(3) | 73(1) |
| O1′ | 3892(1) | 2454(2) | 9383(2) | 66(1) |
| N1′ | 2152(2) | 1301(2) | 8641(2) | 38(1) |
| C1′ | 3374(2) | 2507(2) | 6070(2) | 51(1) |
| C2′ | 2980(2) | 981(2) | 7355(2) | 36(1) |
| C3′ | 1856(2) | 338(2) | 6628(2) | 38(1) |
| C4′ | 2471(2) | −910(2) | 6043(2) | 41(1) |
| C5′ | 3749(2) | −407(2) | 4786(2) | 43(1) |
| C6′ | 3586(3) | 1338(2) | 3942(2) | 54(1) |
| C7′ | 1965(3) | 1743(3) | 3821(2) | 60(1) |
| C8′ | 1245(2) | 1746(2) | 5355(2) | 52(1) |
| C9′ | 1852(3) | 3109(2) | 5605(2) | 58(1) |
| C10′ | 4290(3) | 2293(3) | 4699(3) | 60(1) |
| C11′ | 2653(2) | 1978(2) | 9493(2) | 45(1) |
| C12′ | 4261(2) | −74(3) | 7984(2) | 48(1) |
| C13′ | 3835(2) | −1331(2) | 3726(2) | 50(1) |
| C14′ | 3814(4) | −3037(3) | 4609(3) | 71(1) |
| C15′ | 5194(3) | −951(3) | 2597(3) | 74(1) |
| C16′ | −435(3) | 1772(4) | 5413(3) | 81(1) |

[a] U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor.

Three orientation controls were monitored to assess any crystal movement during the experiment. The intensities of three reflections measured at the beginning, end, and every 3 h of exposure time showed a variation of 5%. Data were corrected for this variation and for Lorentz and polarization effects. Equivalent reflections, but not Friedel mates, were averaged. The structure was solved by direct methods using the SHELXS program[7] and refined using the SHELXL-93 program.[8] Positions for non-hydrogen atoms were eventually refined with anisotropic displacement parameters. The positional and isotropic displacement parameters for the hydrogen atoms were refined. The full-matrix least-squares refinement (on $F^2$) of 542 parameters converged ($\Delta/\sigma_{max} = 0.00$) to values of the conventional crystallographic residuals $R = 0.028$ for 4059 observed data [$I > 2\sigma(I)$] and $R = 0.030$ ($wR_2 = 0.073$) for all 4186 data.

The function minimized was $\sum w(F_o^2 - F_c^2)^2$. Weights, $w$, were eventually assigned to the data as $w = 1/[\sigma^2(F_o^2) + (0.0459P)^2 + 0.0813P]$ where $P = [\mathrm{MAX}(F_o^2,0) + 2F_c^2]/3$. A final difference Fourier map showed residual density between $\pm 0.11$ eÅ$^{-3}$. Values of the neutral atom-scattering factors and real and imaginary dispersion corrections were taken from the *International Tables for X-ray Crystallography*.[9]

**Crystal data of 1:** $C_{16}H_{27}NO$, $M = 249.208$, clear colorless prisms, 0.60 × 0.50 × 0.40 mm, space group $P1$, $Z = 2$, $a = 9.141(2)$ Å, $b = 9.387(2)$ Å, $c = 9.535(2)$ Å, $\alpha = 68.95(3)°$, $\beta = 83.57(3)°$, $\gamma = 86.63(3)°$, $V = 758.6(3)$ Å$^3$, $D$ (calcd) 1.092 Mg m$^{-3}$, $F(000) = 276$, $\mu$(Mo K$\alpha$) = 0.067 mm$^{-1}$.

**Acknowledgment.** We would like to thank Dr. John Faulkner for collection of the sponge, Mary K. Harper for its identification, and the Government of Palau and the staff of the Department of Resources and Development for logistical assistance in the field research program, which was supported by funds from the National Institutes of Health (CA-49084). We also wish to thank Gary Zuber for IR spectra. This work was supported in part by a grant (CA-50771) from the National Institutes of Health.

**Supporting Information Available:** Tables of bond lengths and angles, anisotropic displacement parameters, and hydrogen atom coordinates and isotropic displacement parameters for **1** (6 pages). Ordering information is given on any current masthead page.

## References and Notes

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(10) Atomic coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre and can be obtained upon request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Rd, Cambridge CB2 1EZ, UK.

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