251. Defense Allomones of the Nudibranch *Phyllidia varicosa* Lamarck 1801

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Summary

The defensive secretion of the nudibranch *Phyllidia varicosa* consists of two isocyanosesquiterpenes: the previously described 9-isocyanopupukeanane (**1**), and its 2-isomer (**4**), for which we report the structure and properties. The mixture originates with a sponge, *Hymeniacidon* sp., where it is produced in varying proportions. CD. measurements and X-ray diffraction data establish the absolute configuration of the two metabolites.

Nudibranchs (sea slugs) are marine mollusks which lack an external shell and which are frequently associated with sessile marine invertebrates, *e.g.* sponges, bryozoans, or coelenterates, in a grazer-prey or in a symbiotic relationship [1]. We recently [2] elucidated the molecular basis of such a relationship between the nudibranch *Phyllidia varicosa* and its prey, a sponge, *Hymeniacidon* sp.[3]), and we reported the structure of 9-isocyanopupukeanane (**1**), the defense allomone of the mollusk, which the mollusk in turn obtains from its preferred diet, a sponge. The mollusk accumulates the allomone, which is the active constituent of its mucous skin secretion that is lethal to fish and crustaceans [3].

We recognized early in our investigation that the isonitrile fractions of the mollusk and of the sponge were identical binary *mixtures* of isonitriles. However, the two compounds proved inseparable by chromatography or complexation. On the other hand, degradation of the mixture to 9-pupukeanone (**2**) [2], or derivatization of 9-methylaminopupukeanane (**3**) with phenylisothiocyanate [2] led in each instance to isolation of a single compound, having a sesquiterpene skeleton corresponding to **1**. These observations indicated that either isomerization of one of the constituents had occurred, or that one constituent had been eliminated during reaction or work-up. We now report the identity and properties of the second iso-

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nitrile, 2-isocyanopupukeanane (**4**), and an explanation of its loss during degradation of the natural mixture.

Isonitrile fractions from the nudibranch or sponge gave single spots on TLC. IR. ($\tilde{\nu}_{max}^{CH_2Cl_2}$ 2120 cm^{-1}) and EI. mass spectra were identical and indicative of pure compounds. A molecular formula of $C_{16}H_{25}N$ was suggested by high resolution mass spectral data. NMR. spectra, however, revealed the presence of a mixture. The ^{13}C-NMR. spectrum had nearly 32 instead of the anticipated 16 signals. The ^{1}H-NMR. spectrum, which had few interpretable features, exhibited two sets of signals for the proton attached to the carbon atom bearing the isocyano function. In one set of signals for (**1**), the single proton is a broadened (by adjacent nitrogen) poorly defined doublet ($J = 8$ Hz) centered at 3.20 ppm, while in the other (for **4**) the informative proton gives rise to a broadened singlet at 3.03 ppm. Integration of these signals gives the ratio of the two constituents which is highly variable from one sponge collection to another. Individual sponge specimens occasionally might elaborate nearly pure isomers.

These sets of signals in the diagnostic region of an otherwise featureless spectrum were fortuitous, indeed, in that they placed the isocyano function of one constituent (**1**) at a methine carbon atom with vicinal proton(s) (3.20 ppm), while the other constituent was characterized by a secondary isocyano function that was flanked by two fully substituted carbon atoms (3.03 ppm). When the mixture of isocyanides was defunctionalized with lithium in ethylamine [4] [5], a single tricyclic hydrocarbon was obtained. The ^{13}C-NMR. spectrum of this hydrocarbon, pupukeanane (**5**), exhibited 15 signals between 18.6 and 54 ppm. The ^{1}H-NMR. spectrum of the hydrocarbon clearly showed signals arising from two methyl and one isopropyl group. This experiment proved that, barring an unlikely rearrangement during the lithium-ethylamine reaction, the two isonitriles possessed identical carbon skeletons. Once the structure of the major isomer was secured by X-ray analysis of a thiourea derivative [2], the most likely structure of the second isomer would be 2-isocyanopupukeanane (**4**), since C(2) is the only methine carbon atom with two fully substituted neighboring carbon atoms. X-Ray diffraction analysis of **4** *(vide infra)* bore out this prediction.

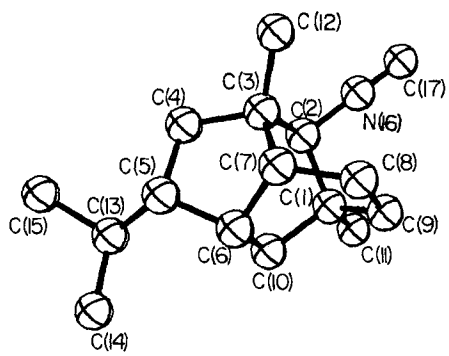


Figure. *A computer-generated perspective drawing of 2-isocyanopupukeanane* (**4**). Hydrogen atoms are omitted for clarity and no absolute configuration is implied

Our unsuccessful attempts to separate the two constituents included complexation with silver nitrate, TLC. separation on silver nitrate impregnated silica gel, on alumina, Florisil, or kieselguhr, preparative GC., and adsorption chromatography on BioSil impregnated with silver nitrate. Fortunately upon long standing of a sample in the refrigerator, **4** crystallized as long needles which could be separated from oily **1** by filtration and recrystallized from aqueous methanol, suitable for X-ray diffraction.

The result of a single crystal X-ray diffraction study of 2-isocyanopupukeanane **4** is summarized in *Figure 1*, which is a computer generated perspective drawing of the final X-ray model. The carbon skeleton is the anticipated tricyclo[4.3.1.$0^{3,7}$]-decane. The configuration is conveniently discussed with reference to the bicyclo-[3.2.1]octane substructure composed of atoms 1–7 and 10. The isonitrile substituent at C(2) and the methyl substituent at C(3) are *exo* while the isopropyl group at C(5) and the methyl at C(1) are *endo*. The X-ray experiment defined only the relative configuration which is (1*S**), (2*S**), (3*S**), (5*S**), (6*R**), and (7*R**). All six membered rings are forced to adopt a boat conformation because of the bicyclo[2.2.2]-octane substructure (C(1)–C(3), C(6)–C(10)). The five-membered ring (C(3)–C(7)) adopts an envelope conformation with the first 4 carbon atoms planar and C(7) removed 0.71 Å from this plane. Further metric details can be found in *Tables 2* and *3*. In general all bond distances and angles agree well with generally accepted values.

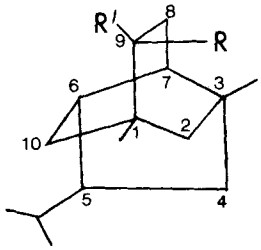
With crystalline 2-isocyanopupukeanane (**4**) in hand, we attempted to degrade this compound to 2-pupukeanone (**6**) in order to measure its CD. curve. We soon discovered that **4** undergoes only two reactions readily: defunctionalization to the hydrocarbon **5** *(vide supra)* and hydrolysis in moist glacial acetic acid to the formamide **7**. This formamide when subjected to 6 N HCl at 100° yielded only about 25% of the desired amine **8** plus much unreacted formamide. Further reaction of the amine with *N*-chlorosuccinimide yielded not the expected chloramine but a complex mixture in poor overall yield. LAH reduction of **4** or **7** resulted only in recovered starting material.

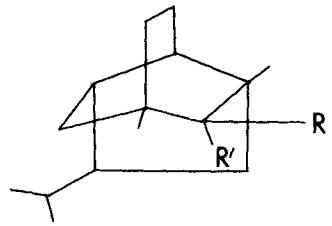
This extreme inertness of **4** or **7** can be rationalized by the hindered nature of C(2), which is a bisneopentyl carbon atom. This also explains our earlier results when we degraded the natural mixture of **1** and **4** to a single ketone and to a single phenylthiourea, both functionalized at C(9). In the reaction sequence leading to the ketones we showed that pure **4** leads to the 2-amino compound **8** in only very low yield. We further showed that this amine is virtually not extractable into aqueous acid from organic solvents. Hence no 2-ketone **6** was ever prepared form the mixture of isonitriles, nor did we succeed in preparing it from pure **4**. In the LAH reduction of the formamides **9** and **7** to the methylamines only the C(9) isomer reacts. Any 2-amino compound that might be formed remains in the neutral fraction. Thus when we examined the unreacted methylamine fraction that had remained after reaction with phenylisothiocyanate and removal of the thiourea, we found that it contained in fact only 2-formamidopupukeanane (**7**) and not the 2-methylamino derivative **10**. In contrast to this lack of reactivity of the 2-isocyano isomer **4**, the 9-isocyano compound **1** is rather unstable and readily isomerizes to

the cyano derivative on standing, as evidenced by change in odor and IR. absorption.

As we were unable to prepare 2-pupukeanone (**6**), we measured the CD. curve of 9-pupukeanone (**2**), which displayed negative ellipticity. From the octant rule and relative configuration established by X-ray diffraction, coupled with the assumption that configuration was retained during derivatization, the absolute configuration must be (1*R*, 3*S*, 5*R*, 6*S*, 7*S*)-9-pupukeanone, with a 9*R*-configuration for the NC functional group. Since the 2-isomer possesses the identical carbon skeleton and its relative configuration was secured by X-ray diffraction, the absolute configuration of 2-isocyanopupukeanone (**4**) is 1*R*, 3*S*, 5*R*, 6*S*, 7*S*, 2*R*.

Structural Formulas





1, 4	R = NC R' = H
2, 6	RR' = O
3, 10	R = NHMe R' = H
5	RR' = H_2
9, 7	R = NHCHO R' = H
8	R = NH_2 R' = H

When we began this research in 1973, only one naturally occurring isonitrile had been described: xanthocillin, a metabolite of the microorganism *Penicillium notatum* [6]. More recently [7], xanthocillin has also been isolated from *Eupenicillium egyptiacum.* Xanthocillin is formally derivable from a dimeric tyrosine precursor. Another microbial isocyano metabolite, which possesses an unbranched ethylcyclopentane skeleton is trichoviridin isolated from *Trichoderma* sp. [8]. However, by far the largest number of natural isocyano compounds have been reported from marine sponges [9–20], all of terpenoid biogenesis. The sponge metabolites are frequently accompanied in nature by formamido and isothiocyanato derivatives. The biosynthesis of these compounds, including the mode and sequence of functionalization, raises intriguing and as yet unanswered questions. These uncommon natural products have stimulated interest in laboratory syntheses. Two total syntheses of 9-isocyanopupukeanane **1** have been reported [21] [22].

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Experimental Part

General Remarks. UV. spectra were taken on a *Beckman* Acta CIII UV.-visible spectrophotometer using 1 cm silica cells in methanol, cyclohexane, or hexane. IR. spectra were recorded on a *Beckman* IR-10 or a *Perkin-Elmer* 467 grating IR. spectrometer. The spectra were obtained as solutions in CH_2Cl_2 with a cell path length of 0.1 mm, or as neat films (cm^{-1}). Circular dichroism was measured on a *Cary* CD-61 recording spectrometer using cyclohexane as the solvent. ^{1}H-NMR. spectra were obtained

on *Varian* HA-100 and *Varian* XL-100 FT spectrometers using tetramethylsilane (TMS) or a deuterium lock, respectively, as the internal reference. All spectra were recorded in the frequency sweep mode. All ^{13}C-NMR. spectra were recorded on a *Varian* XL-100-FT spectrometer using a deuterium lock as the internal reference. Chemical shifts are in the δ units from TMS. Mass spectra were obtained on a *Varian* Mat 311 high resolution spectrometer operating between 20 and 70 eV.

2-Isocyanopupukeanane (**4**). The mixture of isocyanides as isolated from *Hymeniacidon* sp. [2] upon rigorous solvent removal and prolonged refrigeration deposited crystals of **4**. Recrystallization from MeOH/H_2O 90:10 furnished translucent needles, m.p. 81–82°. – UV.: end absorption. – IR. (CH_2Cl_2): 2120, 1460, 1380, 1370. – ^{1}H-NMR. ($CDCl_3$): 3.03 (br. *s*, 1H); 1.99–1.26 (complex, 12 H); 1.15 (*s*, 3 H); 0.91 (*s*, 3 H); 0.81 (*d*, *J* = 5, 6 H). – ^{13}C-NMR. ($CDCl_3$): 156.2, 72.4, 49.6, 48.4, 43.6, 40.8, 38.0, 31.7, 31.2, 29.2, 27.2, 26.2, 22.8, 21.2 (2), 16.8. – MS. (70 eV): *m/z* 231, 216, 204, 188, 161, 160, 148, 133, 119, 107, 105, 95, 94, 93, 91, 81, 79, 77, 67, 55, 33, 31 (100%).

2-Formamidopupukeanane (**7**). To 5 ml HOAc and one drop H_2O were added 6 mg **4**. After 15 h at RT. solvent was removed *in vacuo*. The residue in CH_2Cl_2 was washed with 10% aq. $NaHCO_3$-solution and dried (Na_2SO_4). Slow solvent evaporation at RT. left 5 mg translucent crystals, m.p. 170–174°. – IR. (solid): 1675. – MS. (70 eV): *m/z* 249 (100%), 206, 191, 161, 135, 105, 93, 81, 69, 59, 43, 41.

2-Aminopupukeanane (**8**). 2-Isocyanopupukeanane (**4**) (42 mg) was allowed to stand at RT. with 7 ml 6N HCl for 10 h. The mixture was then heated for 2 h at 50°, and finally for 4 h at 100°. The reaction mixture was extracted with 3 × 5 ml CH_2Cl_2 and the aq. layer was brought to pH 8 with 50% KOH-solution. The aq. phase was extracted with 5 × 5 ml CH_2Cl_2. Concentration *in vacuo* and drying (Na_2SO_4) yielded 3.9 mg of oil with a fishy odor. Chromatography of the CH_2Cl_2 extract of the acidic reaction mixture on silica gel yielded 6 mg of amine **8** and 12 mg of formamide **7**. The amine was chromatographically pure (*Hewlett-Packard* 5708 A GC, 6′ × 1/8″ ss column, 3% OV-17 on ABS Chromosorb W; column t 210°, injection 250°, detector 250°; N_2 33 ml/min, H_2 30 ml/min, air 200 ml/min). – ^{1}H-NMR. ($CDCl_3$): 3.94 (br. *s*, 2 H); 2.30 (br. *s*, 1H); 2.10–1.10 (complex); 1.05 (*s*, 3 H); 0.85 (*d*, 6 H); 0.84 (*s*, 3 H). – MS. (70 eV): 221 (100%), 204, 178, 161, 147, 107, 106, 105, 93, 70, 43, 41, 30.

9-Formamidopupukeanane (**9**). From 15 mg of **1** by the method described above we isolated 11 mg of amorphous white solid. – ^{1}H-NMR. ($CDCl_3$): 8.15 (*d*, *J* = 2, 1 H); 7.98 (*d*, *J* = 12, 1 H); 6.20 (br. *d*, 1 H); 5.92 (br. *d*, 1 H); 3.75 (br. *m*, 1 H); 3.00 (br. *m*, 1 H); 0.95 (*s*, 3 H); 0.86 (*d*, *J* = 6, 6 H); 0.71 (*s*, 3 H).

9-Pupukeanone (**2**). 9-Isocyanopupukeanane (**1**) was degraded by the previously described sequence [2]. The resulting ketone was purified on BioSil A ($CHCl_3$), then on silica gel G (hexane/$CHCl_3$, 1:1). – IR. ($CHCl_3$): 1700. – CD. (*c* = 0.002M, cyclohexane, 25°): $[\theta]_{340} = 0$, $[\theta]_{294} = -2050$, $[\theta]_{240} = 0$. – ^{1}H-NMR. ($CDCl_3$): 2.34 (*d*, *J* = 3, 2 H); 1.80–1.10 (complex, 10 H); 1.01 (*s*, 3 H); 0.88 (*s*, 3 H); 0.86 (br., 6 H). – MS. (70 eV): 220, 204, 189, 149, 58, 55, 44, 43, 42, 41, 29, 28, 27, 18 (100%), 15.

2-Isocyanopupukeanane (**4**) formed large, rectangular crystals. Preliminary *Weissenberg* and precession photographs indicated the orthorhombic crystal class. Accurate cell constants, determined by least-squares fitting of 15 moderate ($35° \leq 2\theta \leq 45°$) angle reflections, were $a = 6.98$ (2), $b = 9.696$ (2) and $c = 21.113$ (4) Å. Systematic extinctions conformed to the common chiral space group $P2_12_12_1$ which was consistent with the known optical activity of 2-isocyanopupukeanane (**4**). A measured and calculated ($z = 4$) density of ~ 1.05 g/ml was taken to mean that one molecule of composition $C_{16}H_{25}N$ formed the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were explored with an ω-scan technique on a *Syntex* $P2_1$ diffractometer using graphite monochromated Cu*Ka* (1.54178 Å) X-rays. The ω-scans had a 1° width and were collected at a variable rate with 1°/min being the slowest. Backgrounds of one-half the scan time were collected at each end of the scan. Intensities of 3 standard refelctions were monitored periodically (once an hour) during data collection and showed a linear decline of intensity with time. At the end of data collection the standard intensities had fallen 38%. The intensity data were rescaled to correct for this decay. After further correction for *Lorentz*, polarization and background effects, 911 (76%) of the 1195 reflections surveyed were judged observed ($Fo^2 \geq 3\sigma(Fo^2)$).

A trial set of phases was uneventfully arrived at with a multiple solution, weighted tangent formula procedure. An E-synthesis from this procedure showed most of the non-hydrogen atoms. The remaining non-hydrogen atoms were located in subsequent F_o-syntheses and hydrogen atoms were located (with some difficulty) in a ΔF-synthesis [23]. Full-matrix least-squares refinements with

Table 1. *Fractional coordinates and temperature factors for 2-isocyanopupukeanane* (**4**). Standard deviations of the least significant figures are given in parentheses. Hydrogen atoms are assigned the same numbers as the heavy atoms to which they are bonded

Atom	*X*	*Y*	*Z*	B11 or B	B 22	B 33	B12	B13	B 23
C(1)	0.1534 (6)	0.2817 (3)	0.2918 (1)	0.0247 (10)	0.0114 (4)	0.0021 (1)	−0.0005 (6)	−0.0008 (3)	0.0005 (1)
C(2)	0.3480 (6)	0.3424 (3)	0.3107 (1)	0.0227 (9)	0.0099 (4)	0.0024 (1)	0.0016 (7)	0.0006 (3)	0.0007 (1)
C(3)	0.3815 (6)	0.3329 (3)	0.3841 (1)	0.0245 (9)	0.0101 (4)	0.0022 (1)	0.0009 (6)	−0.0004 (2)	−0.0001 (1)
C(4)	0.4978 (6)	0.2039 (4)	0.3981 (2)	0.0283 (11)	0.0124 (4)	0.0025 (1)	0.0030 (7)	−0.0008 (3)	0.0005 (2)
C(5)	0.3523 (7)	0.0974 (3)	0.4208 (1)	0.0358 (13)	0.0113 (4)	0.0020 (1)	0.0012 (7)	0.0005 (3)	0.0004 (1)
C(6)	0.1624 (6)	0.1551 (4)	0.3968 (1)	0.0265 (10)	0.0127 (4)	0.0023 (1)	−0.0023 (7)	0.0007 (3)	−0.0000 (1)
C(7)	0.1842 (6)	0.3030 (3)	0.4140 (1)	0.0246 (10)	0.0117 (4)	0.0021 (1)	0.0018 (6)	0.0007 (3)	−0.0000 (1)
C(8)	0.0227 (6)	0.3920 (4)	0.3897 (2)	0.0247 (10)	0.0157 (5)	0.0029 (1)	0.0026 (7)	0.0013 (3)	0.0002 (2)
C(9)	−0.0083 (6)	0.3686 (4)	0.3187 (2)	0.0210 (9)	0.0132 (4)	0.0034 (1)	−0.0001 (7)	−0.0013 (3)	0.0010 (2)
C(10)	0.1412 (6)	0.1440 (3)	0.3243 (1)	0.0285 (10)	0.0106 (3)	0.0026 (1)	−0.0022 (7)	−0.0011 (3)	0.0002 (1)
C(11)	0.1352 (8)	0.2675 (3)	0.2203 (1)	0.0432 (14)	0.0152 (4)	0.0023 (1)	−0.0010 (8)	−0.0022 (3)	0.0007 (1)
C(12)	0.4767 (7)	0.4579 (3)	0.4117 (2)	0.0313 (12)	0.0127 (4)	0.0034 (1)	−0.0010 (7)	−0.0020 (3)	−0.0006 (2)
C(13)	0.4039 (8)	−0.0469 (4)	0.4038 (2)	0.0454 (15)	0.0109 (4)	0.0029 (1)	0.0034 (8)	−0.0008 (3)	0.0007 (2)
C(14)	0.2412 (11)	−0.1425 (5)	0.4242 (3)	0.0669 (25)	0.0110 (5)	0.0063 (2)	0.0000 (10)	−0.0001 (6)	0.0011 (2)
C(15)	0.5961 (10)	−0.0874 (5)	0.4332 (2)	0.0614 (23)	0.0163 (6)	0.0043 (1)	0.0132 (11)	−0.0011 (5)	0.0011 (2)
N(16)	0.3572 (5)	0.4799 (3)	0.2883 (1)	0.0258 (8)	0.0127 (4)	0.0030 (1)	−0.0006 (6)	0.0006 (3)	0.0015 (1)
C(17)	0.3616 (8)	0.5878 (5)	0.2701 (2)	0.0347 (14)	0.0160 (5)	0.0047 (1)	−0.0022 (9)	−0.0005 (4)	0.0035 (2)
H(2)	0.459 (5)	0.292 (3)	0.287 (1)	1.7 (6)					
H(4A)	0.603 (7)	0.225 (4)	0.431 (2)	5.0 (9)					
H(4B)	0.564 (5)	0.170 (3)	0.357 (1)	2.6 (7)					
H(5)	0.343 (5)	0.103 (3)	0.468 (1)	2.0 (6)					
H(6)	0.048 (6)	0.119 (3)	0.417 (1)	2.9 (7)					
H(7)	0.201 (5)	0.316 (3)	0.462 (1)	1.3 (6)					
H(8A)	−0.094 (6)	0.381 (4)	0.412 (2)	4.2 (8)					
H(8B)	0.057 (6)	0.489 (4)	0.399 (2)	3.7 (9)					
H(9A)	−0.002 (7)	0.460 (4)	0.295 (2)	4.9 (9)					
H(9B)	−0.126 (5)	0.321 (3)	0.311 (1)	2.0 (6)					
H(10A)	0.237 (5)	0.082 (3)	0.306 (1)	1.5 (6)					
H(10B)	0.011 (5)	0.110 (3)	0.318 (1)	2.1 (6)					
H(11A)	0.234 (6)	0.205 (4)	0.203 (2)	4.3 (9)					
H(11B)	0.144 (7)	0.348 (3)	0.200 (1)	4.2 (8)					
H(11C)	0.004 (8)	0.230 (5)	0.209 (2)	6.1 (11)					
H(12A)	0.613 (6)	0.481 (4)	0.388 (1)	3.2 (8)					
H(12B)	0.509 (5)	0.438 (3)	0.456 (1)	2.6 (7)					
H(12C)	0.394 (8)	0.528 (5)	0.405 (2)	6.8 (12)					
H(13)	0.416 (5)	−0.052 (3)	0.356 (1)	2.7 (7)					
H(14A)	0.251 (8)	−0.137 (5)	0.474 (2)	6.3 (12)					
H(14B)	0.287 (10)	−0.227 (6)	0.409 (2)	9.0 (15)					
H(14C)	0.105 (10)	−0.122 (6)	0.405 (2)	9.6 (16)					
H(15A)	0.700 (8)	−0.031 (5)	0.416 (2)	7.7 (12)					
H(15B)	0.620 (10)	−0.185 (6)	0.427 (3)	9.1 (16)					
H(15C)	0.575 (8)	−0.085 (5)	0.482 (2)	6.1 (11)					

anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for hydrogen atoms have converged to an unweighted crystallographic residual (R) of 0.048 for the observed data. *Table 1* contains the fractional coordinates and thermal parameters for the X-ray model and *Table 4* contains the observed and calculated structure factors. The derived metric details, bond distances and angles, can be found in *Tables 2* and *3* respectively. The final difference electron density synthesis showed no substantial residual electron density and there were no anomalously short intermolecular contacts.

Table 2. *Bond distances of 2-isocyanopupukeanane* (**4**). The standard deviation of the least significant figure of each distance is given in parentheses

C(1)–C(2)	1.540 (5)	C(5) –C(6)	1.531 (6)
C(1)–C(9)	1.532 (5)	C(5) –C(13)	1.525 (5)
C(1)–C(10)	1.537 (4)	C(6) –C(7)	1.526 (5)
C(1)–C(11)	1.521 (4)	C(6) –C(10)	1.543 (4)
C(2)–C(3)	1.570 (4)	C(7) –C(8)	1.523 (6)
C(2)–N(16)	1.452 (4)	C(8) –C(9)	1.532 (5)
C(3)–C(4)	1.549 (5)	C(13)–C(14)	1.544 (8)
C(3)–C(7)	1.544 (5)	C(13)–C(15)	1.532 (8)
C(3)–C(12)	1.527 (5)	N(16)–C(17)	1.142 (5)
C(4)–C(5)	1.546 (6)		

Table 3. *Bond angles of 2-isocyanopupukeanane* (**4**). The standard deviation of the least significant figure of each angle is given in parentheses

C(2) –C(1)–C(9)	109.4 (3)	C(4) –C(5) –C(6)	102.0 (3)
C(2) –C(1)–C(10)	106.5 (3)	C(4) –C(5) –C(13)	114.9 (4)
C(2) –C(1)–C(11)	111.6 (3)	C(6) –C(5) –C(13)	118.8 (3)
C(9) –C(1)–C(10)	107.4 (3)	C(5) –C(6) –C(7)	101.5 (3)
C(9) –C(1)–C(11)	111.1 (3)	C(5) –C(6) –C(10)	112.6 (3)
C(10)–C(1)–C(11)	110.8 (3)	C(7) –C(6) –C(10)	108.3 (3)
C(1) –C(2)–C(3)	111.3 (3)	C(3) –C(7) –C(6)	100.3 (3)
C(1) –C(2)–N(16)	109.0 (3)	C(3) –C(7) –C(8)	114.3 (3)
C(3) –C(2)–N(16)	111.9 (3)	C(6) –C(7) –C(8)	114.2 (3)
C(2) –C(3)–C(4)	108.5 (3)	C(7) –C(8) –C(9)	110.1 (3)
C(2) –C(3)–C(7)	106.3 (3)	C(1) –C(9) –C(8)	110.2 (3)
C(2) –C(3)–C(12)	113.1 (3)	C(1) –C(10)–C(6)	111.9 (2)
C(4) –C(3)–C(7)	103.3 (3)	C(5) –C(13)–C(14)	110.1 (4)
C(4) –C(3)–C(12)	112.1 (3)	C(5) –C(13)–C(15)	111.1 (4)
C(7) –C(3)–C(12)	113.0 (3)	C(14)–C(13)–C(15)	111.6 (4)
C(3) –C(4)–C(5)	106.6 (3)	C(2) –N(16)–C(17)	178.8 (4)

Table 4. *Observed and calculated structure factors for 2-isocyanopupukeanane* (**4**)

K	L	FO	FC	K	L	FO	FC	K	L	FO	FC	K	L	FO	FC	K	L	FO	FC
H=0				1	1	66	86	1	12	6	6	2	3	45	49	2	15	4	4
0	2	33	38	1	2	1	1	1	13	27	27	2	5	8	8	2	17	5	4
0	4	71	82	1	3	70	82	1	14	9	9	2	6	12	12	2	18	3	3
0	6	100	118	1	4	66	71	1	15	6	7	2	7	2	2	3	2	14	13
0	8	56	54	1	5	27	27	1	16	2	2	2	8	3	2	3	3	25	24
0	10	91	94	1	6	17	17	1	18	1	2	2	9	24	25	3	4	25	25
0	12	6	7	1	7	6	6	1	20	1	1	2	10	12	12	3	5	16	14
0	14	6	5	1	8	20	20	1	21	2	1	2	11	15	14	3	6	3	3
0	16	15	15	1	9	12	13	2	0	18	19	2	12	10	9	3	7	3	4
0	20	1	1	1	10	30	31	2	1	48	49	2	13	6	6	3	8	12	12
0	22	2	1	1	11	45	43	2	2	24	26	2	14	2	2	3	9	1	1

Table 4 (continued)

K	L	FO	FC	K	L	FO	FC	K	L	FO	FC	K	L	FO	FC	K	L	FO	FC
3	10	2	2	6	13	2	3	0	18	3	4	4	1	27	27	7	5	7	7
3	11	7	7	6	14	11	11	0	19	4	4	4	2	28	28	7	6	8	8
3	12	9	9	6	15	14	14	0	20	4	3	4	3	17	17	7	7	5	5
3	13	5	5	6	16	3	4	1	0	12	13	4	4	8	8	7	8	4	4
3	14	12	13	6	17	4	4	1	1	67	76	4	5	18	17	7	9	3	4
3	15	2	2	6	19	1	1	1	2	38	41	4	6	17	16	7	10	3	3
3	16	2	1	7	1	7	7	1	3	12	13	4	7	17	16	7	11	8	8
3	17	3	3	7	2	2	2	1	4	33	35	4	8	9	9	7	12	5	5
3	18	4	4	7	3	7	7	1	5	31	30	4	9	10	10	7	13	4	4
3	19	4	4	7	4	3	3	1	6	8	8	4	10	10	10	7	14	4	3
3	20	3	3	7	5	4	4	1	7	6	5	4	11	6	6	7	15	2	2
3	21	3	3	7	7	9	9	1	8	9	9	4	2	6	5	7	16	4	4
4	0	41	41	7	9	9	10	1	9	33	31	4	13	14	15	8	0	24	24
4	1	10	10	7	10	1	2	1	10	16	15	4	14	6	6	8	1	12	12
4	2	18	17	7	11	3	3	1	11	22	21	4	15	10	10	8	2	4	4
4	3	34	34	7	12	3	4	1	12	17	17	4	16	11	11	8	3	6	6
4	4	30	29	7	14	4	4	1	13	1	1	4	17	5	4	8	4	8	8
4	5	8	8	8	1	8	8	1	14	8	8	4	18	6	6	8	5	2	2
4	6	9	9	8	2	7	7	1	15	2	3	4	19	6	5	8	6	9	10
4	7	13	13	8	3	5	5	1	18	2	2	4	20	3	3	8	7	8	8
4	8	13	14	8	4	4	5	2	0	2	2	5	0	33	31	8	8	4	4
4	10	3	3	8	5	3	3	2	1	42	45	5	1	11	11	8	9	3	2
4	12	15	15	8	6	9	9	2	2	19	20	5	2	7	8	8	10	12	12
4	13	9	9	8	7	4	4	2	3	6	6	5	3	11	10	8	11	2	2
4	14	18	18	8	8	6	6	2	4	20	20	5	4	6	6	8	13	6	5
4	16	16	16	8	9	6	6	2	5	30	30	5	5	18	18	8	14	4	3
4	17	10	10	8	12	2	2	2	6	13	14	5	6	11	11	8	15	4	3
4	20	5	5	8	13	2	2	2	7	2	1	5	7	4	4	9	0	3	3
4	21	4	5	9	1	2	2	2	8	10	10	5	8	11	11	9	1	8	8
5	1	20	19	9	3	6	6	2	9	20	20	5	10	13	13	9	2	4	3
5	2	20	19	9	4	3	3	2	10	12	12	5	11	4	4	9	3	11	11
5	3	32	31	9	5	11	11	2	12	10	10	5	13	14	15	9	4	2	1
5	4	6	7	9	9	2	2	2	13	9	8	5	14	8	8	9	5	7	6
5	6	23	22	9	10	2	1	2	14	4	4	5	15	12	13	9	6	4	4
5	7	8	7	9	12	2	1	2	15	16	16	5	16	3	4	9	7	2	3
5	8	10	9	10	0	3	3	2	16	3	3	5	17	3	3	9	8	2	2
5	9	2	2	10	1	5	5	2	17	5	5	5	18	3	3	9	9	3	3
5	10	13	13	10	3	4	4	2	19	2	2	6	0	7	7	9	10	4	4
5	11	11	11	10	4	3	2	2	21	3	3	6	1	12	11	9	11	6	5
5	12	2	2	10	5	3	3	3	0	6	5	6	2	17	18	9	12	4	4
5	13	13	13	10	6	3	3	3	1	19	20	6	3	13	12	10	0	4	3
5	14	4	4					3	2	34	34	6	4	5	5	10	2	3	3
5	15	8	8					3	3	24	23	6	5	8	8	10	3	2	2
5	16	16	16					3	4	6	6	6	6	4	4	10	6	3	2
5	17	11	11	H = 1				3	5	10	10	6	7	10	10	10	7	4	4
5	19	1	1	0	1	11	11	3	6	6	5	6	8	5	5				
6	0	11	11	0	2	98	123	3	7	20	19	6	9	6	6				
6	1	19	19	0	3	60	64	3	8	11	11	6	10	2	3				
6	2	4	4	0	4	9	10	3	9	4	3	6	11	13	13	H = 2			
6	3	1	1	0	5	37	39	3	10	7	7	6	12	7	7	0	0	24	21
6	4	17	17	0	6	13	12	3	11	8	8	6	13	5	5	0	1	2	1
6	5	11	11	0	7	35	35	3	12	8	9	6	14	3	3	0	2	7	7
6	6	12	12	0	8	53	53	3	13	6	6	6	15	3	2	0	3	26	27
6	7	5	5	0	9	3	4	3	15	5	6	6	17	5	5	0	4	32	35
6	8	2	2	0	10	11	11	3	16	13	13	7	0	14	14	0	5	24	24
6	9	11	12	0	11	5	5	3	17	9	9	7	1	14	14	0	6	1	0
6	10	12	12	0	12	24	23	3	18	3	3	7	2	10	10	0	7	22	22
6	11	3	3	0	14	7	7	3	20	3	3	7	3	6	6	0	8	13	13
6	12	2	2	0	15	8	8	4	0	22	22	7	4	4	3	0	9	28	27

Table 4 (continued)

K	L	FO	FC	K	L	FO	FC	K	L	FO	FC	K	L	FO	FC	K	L	FO	FC
0	10	8	8	3	11	5	5	7	3	4	4	1	6	8	8	4	7	6	6
0	11	11	11	3	12	2	2	7	4	8	8	1	7	3	2	4	8	3	3
0	13	2	2	3	13	14	14	7	5	5	5	1	8	13	13	4	9	8	8
0	14	15	15	3	14	17	17	7	6	5	5	1	9	14	15	4	10	6	5
0	18	5	5	3	15	17	17	7	8	5	5	1	11	4	4	4	11	7	8
0	19	3	4	3	16	4	4	7	9	12	12	1	12	7	7	4	12	5	5
0	20	2	2	3	18	4	4	7	10	3	4	1	13	14	14	4	13	3	2
1	0	30	31	3	19	3	3	7	11	8	9	1	14	10	10	4	14	11	11
1	1	47	48	3	20	4	4	7	12	11	11	1	15	3	4	4	15	4	4
1	2	41	43	4	1	22	21	7	13	3	3	1	17	9	9	4	16	6	6
1	3	26	28	4	2	20	19	7	16	2	2	1	18	9	9	4	17	3	4
1	4	16	17	4	3	30	29	8	2	20	20	1	19	6	6	5	1	6	6
1	5	14	13	4	4	3	3	8	3	3	3	2	0	24	22	5	2	10	10
1	6	11	10	4	5	9	9	8	5	3	4	2	1	37	38	5	3	10	10
1	7	11	11	4	6	11	11	8	6	5	5	2	2	17	17	5	5	5	5
1	8	8	8	4	7	12	12	8	7	5	5	2	3	9	10	5	6	3	3
1	9	8	7	4	8	3	2	8	8	11	11	2	4	26	27	5	7	5	4
1	10	12	12	4	9	14	13	8	9	3	3	2	5	15	15	5	10	6	5
1	11	7	6	4	11	2	2	8	10	2	2	2	6	19	19	5	12	5	5
1	12	8	9	4	12	5	6	8	12	5	5	2	7	16	16	5	13	5	5
1	13	7	7	4	13	6	6	8	13	4	4	2	8	13	13	5	14	4	4
1	14	3	3	4	14	4	4	9	1	8	8	2	9	19	19	5	15	4	4
1	15	7	7	4	15	7	7	9	2	4	4	2	10	14	14	5	16	2	2
1	16	14	15	4	16	8	8	9	4	5	5	2	11	10	10	5	18	2	2
1	17	4	4	4	18	9	9	9	7	3	3	2	12	5	5	6	0	13	13
1	18	4	5	4	19	5	5	9	8	3	3	2	13	11	11	6	1	8	7
1	19	6	5	4	20	2	2	9	9	4	4	2	14	22	22	6	2	7	7
1	20	2	2	5	0	11	10	9	11	2	3	2	15	10	10	6	3	13	14
1	22	2	2	5	1	3	4	10	1	2	1	2	16	8	8	6	4	5	4
2	0	25	25	5	2	21	21	10	4	2	1	2	17	6	7	6	5	3	3
2	1	26	27	5	3	7	7	10	5	3	2	2	18	8	8	6	6	4	3
2	2	23	24	5	4	3	2	10	6	3	3	2	19	3	3	6	7	10	10
2	3	10	10	5	5	9	9					2	20	3	3	6	8	4	5
2	4	37	38	5	6	5	5					3	0	8	8	6	10	5	5
2	5	10	10	5	8	11	10					3	1	20	20	6	11	7	7
2	6	18	18	5	10	5	5	H=3				3	2	19	18	6	12	3	2
2	7	22	21	5	11	4	4	0	1	10	10	3	3	25	26	6	13	8	8
2	8	8	8	5	12	10	10	0	2	21	20	3	4	7	7	6	14	5	5
2	9	8	8	5	15	3	3	0	3	18	17	3	5	12	12	7	1	11	12
2	10	7	6	5	16	2	2	0	4	12	12	3	6	5	5	7	2	10	10
2	11	10	10	6	0	8	9	0	5	3	3	3	7	9	8	7	3	12	12
2	12	22	21	6	1	10	10	0	6	11	12	3	8	9	9	7	4	13	13
2	13	8	8	6	2	4	4	0	7	11	11	3	9	5	4	7	5	5	5
2	14	7	7	6	3	3	4	0	8	15	15	3	10	5	5	7	6	5	6
2	15	6	7	6	4	7	7	0	9	4	4	3	11	15	16	7	7	7	8
2	16	12	11	6	5	7	7	0	10	6	6	3	12	9	9	7	8	4	4
2	17	11	11	6	6	4	3	0	12	13	12	3	13	13	12	7	10	7	7
2	18	3	3	6	8	7	7	0	13	7	6	3	14	3	2	7	11	9	9
2	19	3	3	6	9	3	3	0	15	10	10	3	15	8	8	7	13	4	4
3	0	28	26	6	10	6	6	0	16	4	3	3	16	4	4	7	14	5	5
3	1	18	17	6	11	5	5	0	17	10	10	3	17	10	10	8	0	16	16
3	2	6	6	6	12	6	6	0	18	2	3	3	18	2	2	8	1	4	5
3	3	25	24	6	13	10	10	0	19	4	4	4	0	12	12	8	2	9	10
3	4	14	13	6	14	3	3	1	0	18	18	4	1	18	18	8	3	4	4
3	5	28	27	6	15	8	8	1	1	18	17	4	2	4	4	8	4	3	3
3	6	12	12	6	17	2	2	1	2	11	11	4	3	5	4	8	7	4	3
3	7	5	4	7	0	3	4	1	3	37	37	4	4	14	14	8	8	6	6
3	8	22	21	7	1	19	19	1	4	16	17	4	5	4	4	8	9	5	5
3	10	4	4	7	2	18	19	1	5	25	25	4	6	5	5	8	10	5	5

Table 4 (continued)

K	L	FO	FC
8	11	2	1
8	12	2	2
9	1	3	2
9	2	3	2
9	4	2	3
9	5	2	1
9	6	4	4
9	8	4	3
H=4			
0	0	32	30
0	1	8	8
0	2	21	21
0	3	2	2
0	4	10	10
0	5	13	13
0	6	5	4
0	8	4	4
0	10	5	5
0	11	9	9
0	12	6	6
0	14	7	7
0	15	5	5
0	17	3	3
1	0	16	16
1	1	8	8
1	2	11	11
1	3	8	7
1	4	15	14
1	5	6	6
1	6	11	11
1	7	3	3
1	8	3	3
1	9	4	4
1	10	13	14
1	11	8	8
1	12	4	4
1	13	4	4
1	14	5	5
1	15	6	6
1	16	4	5
1	17	3	4
1	18	2	1
2	1	8	7
2	2	4	3
2	3	5	5
2	4	6	6
2	6	5	5
2	7	6	7
2	9	5	4
2	10	2	2
2	11	8	8
2	12	6	6
2	13	7	7
2	14	4	4
2	16	5	5
3	0	9	9
3	1	5	5
3	2	4	4
3	3	5	5
3	4	12	12
3	5	5	5
3	6	8	9
3	8	5	4
3	10	7	6
3	12	3	3
3	13	5	5
3	14	3	2
3	15	5	5
3	16	4	4
4	0	2	2
4	1	10	11
4	2	6	7
4	3	3	2
4	4	6	6
4	5	2	3
4	7	10	9
4	10	3	3
4	11	5	6
4	12	7	7
4	14	3	4
5	0	3	3
5	1	12	13
5	2	14	15
5	3	4	4
5	4	5	5
5	5	9	9
5	6	3	3
5	7	4	4
5	8	7	7
5	11	6	5
5	12	7	7
5	14	4	4
5	15	3	4
6	0	3	3
6	1	16	16
6	2	9	9
6	4	7	7
6	5	9	9
6	6	7	6
6	7	2	2
6	8	6	5
6	9	6	6
6	10	5	5
6	11	5	5
6	12	5	5
6	14	6	6
7	1	6	6
7	2	4	5
7	3	8	7
7	4	6	6
7	5	5	6
7	6	3	4
7	9	8	7
7	11	3	3
7	12	4	4

K	L	FO	FC
8	1	3	3
8	3	2	1
8	4	4	4
8	6	3	3
8	7	3	3
8	8	3	2
8	9	2	3
9	1	3	3
9	4	2	1
H=5			
0	1	12	13
0	2	15	15
0	3	7	7
0	4	13	13
0	6	10	10
0	8	5	6
0	9	4	4
0	12	4	4
0	14	3	1
0	15	2	2
0	17	2	1
1	0	3	3
1	1	16	16
1	2	14	14
1	3	7	7
1	4	10	10
1	5	4	4
1	6	3	3
1	7	3	3
1	9	9	9
1	11	4	5
1	12	6	6
1	13	3	3
1	14	2	3
1	17	3	3
2	0	14	14
2	1	7	7
2	2	6	7
2	3	3	3
2	4	10	9
2	5	8	8
2	6	6	5
2	10	5	5
2	12	6	6
2	13	4	4
2	14	4	4
2	15	4	4
3	1	11	11
3	3	7	7
3	4	5	6
3	5	8	8
3	6	3	2
3	7	5	5
3	8	3	3
3	9	3	3
3	10	2	2

K	L	FO	FC
3	11	6	6
3	12	4	4
3	13	3	3
3	14	3	3
3	15	4	4
3	16	2	2
4	0	6	6
4	1	8	8
4	2	5	6
4	3	4	5
4	4	4	4
4	5	3	3
4	6	3	3
4	7	4	4
4	9	2	2
4	10	5	5
4	11	4	4
4	12	2	2
4	13	2	2
4	15	3	3
5	0	10	10
5	1	5	6
5	2	8	9
5	3	5	5
5	4	4	4
5	5	6	6
5	6	4	5
5	7	3	4
5	8	4	5
5	9	3	3
5	10	6	6
5	12	5	5
5	13	7	6
6	1	2	3
6	2	6	6
6	3	3	3
6	4	5	6
6	6	4	4
6	7	4	3
6	9	3	3
7	1	4	3
8	2	2	2
H=6			
0	0	24	23
0	1	5	6
0	2	8	7
0	3	3	3
0	4	3	3
0	5	3	3
0	7	4	4
0	8	5	5
0	9	8	7
0	10	11	12
0	14	3	3
1	1	12	12
1	2	7	7

K	L	FO	FC
1	3	8	8
1	4	7	6
1	6	5	5
1	7	6	6
1	8	3	3
1	9	3	2
1	11	9	9
1	12	2	0
1	13	2	3
2	0	6	6
2	2	6	6
2	3	5	5
2	4	3	2
2	5	5	5
2	6	4	3
2	7	5	5
2	10	4	4
2	12	4	4
3	1	4	5
3	3	7	7
3	4	4	4
3	5	3	3
3	6	4	4
3	7	2	3
3	8	2	2
3	9	2	2
3	10	3	3
3	11	2	2
3	12	3	3
4	0	3	3
4	1	3	3
4	2	5	5
4	4	3	4
4	5	4	4
4	6	3	3
4	7	3	3
4	9	3	3
5	0	2	3
5	3	3	4
5	6	3	5
5	9	3	3
6	5	3	3
H=7			
0	2	4	4
0	4	3	3
0	5	4	3
0	7	3	3
0	8	5	5
1	3	3	3
1	4	4	4
1	5	3	2
1	6	3	2
2	1	3	3
2	3	4	4
2	7	2	1
3	4	2	2

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