Title

The Paradox of Antimalarial Terpenoid Isonitrile Biosynthesis Explained. Proposal of Cyanoformate as an NC Delivery Vector.

Journal

Journal of Natural Products, 88(1)

Author

Molinski, Tadeusz

Publication Date

2025-01-24

DOI

10.1021/acs.jnatprod.4c01295

Peer reviewed

JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp

Note

The Paradox of Antimalarial Terpenoid Isonitrile Biosynthesis Explained. Proposal of Cyanoformate as an NC Delivery Vector

Published as part of Journal of Natural Products special issue "Special Issue in Honor of Sheo B. Singh".

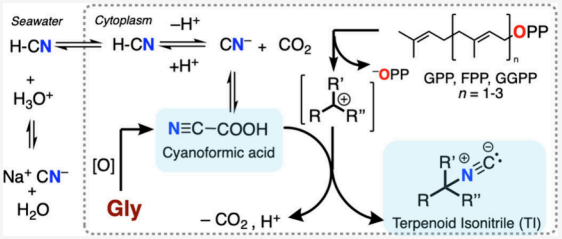
Tadeusz F. Molinski*

Cite This: *J. Nat. Prod.* 2025, 88, 205−210

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Marine sponge diterpenoid isonitriles are exceptional nitrogenous natural products that exhibit antiplasmodial activity. Their biosynthesis presents a biosynthetic puzzle: how do the elements of NC engage terpenyl carbocations in isoprenoid secondary metabolism, and what is the biosynthetic precursor of the NC group? Cyanoformic acid (NC-COOH, **B1**) is proposed as a plausible delivery vehicle of NC that resolves a paradox in the commonly held proposition that an inorganic cyanide anion, CN^-, terminates terpenoid isonitrile (TI) biosynthesis. DFT calculations of NC-COOH and its conjugate base, cyanoformate, NC-COO^- (**B2**), support high nucleophilicity at N and explain bond-forming constitutionality: attack at N and formation of an isonitrile over its nitrile isomer. TI biogenesis is compared to the cyanoformamide-containing ceratamines that arise from oxidation of a terminal *N*-Gly amide precursor. A unifying model links C-NC vs C-CN bond formation and places Gly at the center of both biosynthetic schemes.



Marine natural products from Porifera (sponges) represent an astounding chemically diverse assemblage of compounds with exotic structures. One of the many remarkably unique chemical aspects of sponge-derived terpenoids is the occurrence of mono-, sesqui-, and diterpene isonitriles (R-NC, TIs),[1] containing up to three isocyano groups.[2] The first to be described were axisonitrile-1 (**1**) from the sponge *Axinella cannabina*[3] and acanthellin-1 from *Acanthella acuta*.[4] These nonpolar TIs—found only in Porifera—show highly diversified and potent bioactivities ranging from antibacterial activity, e.g., the diterpene 8-isocyano-l(12)-cycloamphilectene (**2**), from *Haliclona* sp.[5] to antiplasmodial activity. The diterpene 7,20-diisocyanoadociane (**3**) from *Cymbastella hooperi* (revised from *Amphimedon* sp.), the first reported TI,[6] potently inhibits cultured *Plasmodium falciparum* (IC_{50} (clones W2 and D6) = 4.3 and 4.7 ng/mL, respectively).[7] 3D QSAR and molecular docking studies suggest **2** and other TIs inhibit detoxification of human heme, essential to the life-cycle of *Plasmodium*.[8] Accordingly, efforts toward the total synthesis of **2** and other TIs have been pursued vigorously.[9] Classes of TIs, embodying expanded structural complexity, e.g., the diterpene kalihinol A (**4a**) from the sponge *Acanthella* sp., have also been reported since **1**.[10] Most recently, a comprehensive study of the potent antiplasmodial isonitrile MED6-189 (**4b**), with a direct "molecular lineage" to the natural product, **4a**, was disclosed.[11] MED6-189 is a lead compound "effective against drug-sensitive and drug-resistant *P. falciparum* strains in vitro", and may possibly be advanced to clinical trials for therapeutic management of malaria.

While organic nitrile-containing natural products are relatively rare in Nature,[12] *iso*nitriles are rarer still. Studies of bacteria-derived isonitriles have well-established the biosynthetic origins of the NC group: α-amino acids for N (e.g., Tyr, Trp)[13] and a separate carbohydrate source for C, e.g., glucose and ribulose 5-phosphate.[14,15] A pioneering study by Moore and co-workers on isonitriles derived from cultured cyanobacteria suggests involvement of the tetrahydrofolate (THF) pathway: the so-called C-1 pool. Initial pulse labeling studies of the cyanobacterium *Hapalasiphon fontinalis* with [2-^{13}C,^{15}N]-Gly[16] and analysis of the hapalindole (**4c**) by ^{13}C NMR showed incorporation of intact $^{15}N^{13}C$ into **4c** (~1%), although this conflicts with a modern consensus of data that supports the origin of the two atoms of the NC group into the "*cis*-indole isonitrile" core (**4d**) from separate precursors: again, N from Trp and C from ribulose-5-phosphate, respectively.[17] A wealth of evidence shows that **4d** is the common core of most if not all isonitriles from *Hapalasiphon* and *Fischerella* spp; in the latter, vinyl isocyanide **4d** is the product of Amb-I-1 and Amb-I-2 enzymes coded by the cognate biosynthetic gene cluster.[18]

Received: November 14, 2024
Revised: December 3, 2024
Accepted: December 7, 2024
Published: December 20, 2024

https://doi.org/10.1021/acs.jnatprod.4c01295
J. Nat. Prod. 2025, 88, 205−210

Biosynthesis of bacterial- and plant-derived isonitriles has been reviewed.[17,19]

Terpenoid isonitriles are restricted to marine sponges (Porifera). Over 200 TIs have been reported to date,[20] and their biosynthetic origins are different: formally, they arise from classic terpenoid π-cation cascades, beginning with well-known ionization of the C_{10}, C_{15}, and C_{20} allylic pyrophosphates and end with capture of the penultimate carbocation intermediate by cyanide, CN^- (see below). Numerous labeling studies by the Garson and Scheuer groups with live, aerated *Cymbastella* sponges in closed aquaria, containing [^{14}C]-cyanide, show significant ^{14}C incorporation levels into the NC group of the resulting TIs.[21,22] Stable isotope studies with double-labeled Na[^{13}C^{15}N] in TI-producing sponges (TIPS) by the Scheuer group also showed heavy isotope incorporation into TIs with, most tellingly, an intact ^{13}C–^{15}N bond.[23] Direct feeding of sponges with [^{14}C]-Gly failed to show incorporation of any label,[24] likely because this α-amino acid is avidly and rapidly assimilated into protein building, in particular, the collagen-like structural protein spongin, and other reactions within the animal.[25] Finally, TIs (with structures identical or almost identical to sponge-derived TIs) are found in limited genera of nudibranchs that are known specialist predators of TIPSs. For example, the sesquiterpene axisoisonitrile-3 and related secondary metabolic products (e.g., isothiocyanate and *N*-formamide) found in *Phyllidiela pustulosa* were shown by [^{14}C]-labeling studies to originate in the sponge *Acanthella cavernosa* upon which it feeds (dietary transfer).[26]

Beyond incorporation experiments of whole sponges with [^{14}C]- and [^{13}C]-labeled cyanide salts, detailed biosynthetic studies of TIs in sponges are absent. Sponges, despite being the simplest metazoans, are actually quite complex cellular assemblages of eukaryotic cells of the Porifera and the prokaryotic bacteria and archae that inhabit them:[27,28] both obligate symbionts and commensal microbes. Consequently, identifying and culturing TI-producing cell lines are difficult.

Much speculation has been woven into attempts to understand the biosynthesis of TIs. Formally, TIs originate from pathways initiated by S_N1-like dissociation of canonical terpenoid precursors—geranyl- (GPP), farnesyl- (FPP), and geranylgeranyl (GGPP) pyrophosphates—followed by cationic π-addition cascade reactions, sometimes accompanied by Wagner–Meerwein rearrangements, and formal termination by S_N1 capture by cyanide ion (CN^-) at N. The TI-related isothiocyanates, formamides, dichloroimidate (carbonimidic dichloride), and amines (Figure 1) would appear to arise from subsequent reactions (e.g., α-additions of S°, H_2O, "Cl_2", and hydrolysis, respectively).

As neat and compact as this scenario may seem, of inorganic cyanide as the source of the functional group R-NC, there are glaring paradoxes that have been apparent for decades. Cyanide, although an ambident nucleophile ($^-CN \leftrightarrow CN^-$), invariably reacts with electrophiles at the less electronegative C atom to give nitriles (R-CN), not isonitriles (R-NC). Cyanide is a notorious, potent poison of the electron transport chain that is extremely toxic to most organisms and microbes, rendering implausible a scenario where CN^- could serve as a viable "housekeeping" intermediate in secondary metabolism.

Glycine-derived C_1 functional groups are evident in other natural products. Reviewing, for a different purpose, the structures of ceratinamine (**5**)[29] and 7-hydroxyceratinamine (**6**)[30] from the sponges *Pseudoceratina purpurea* and *Aplysinella* sp., respectively (Scheme 1), the terminal cyanoformamide—

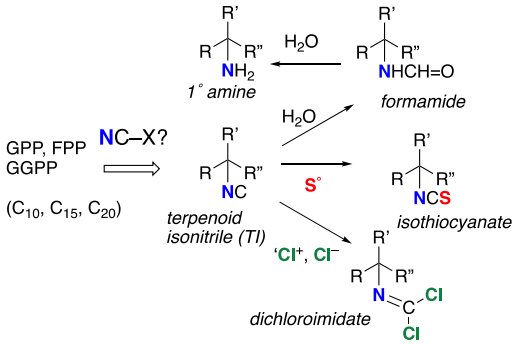
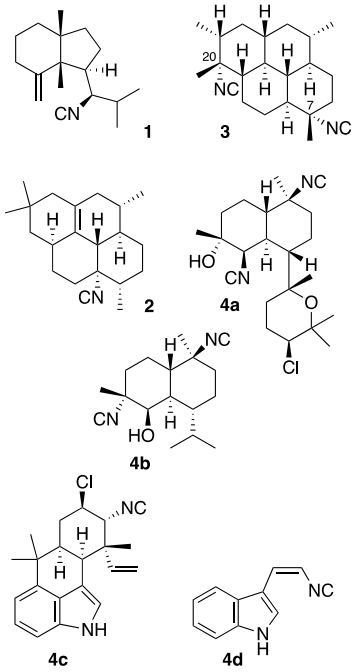


Figure 1. Biosynthetic transformations of geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP) to terpenoid isonitriles (TIs) and related derivatives.



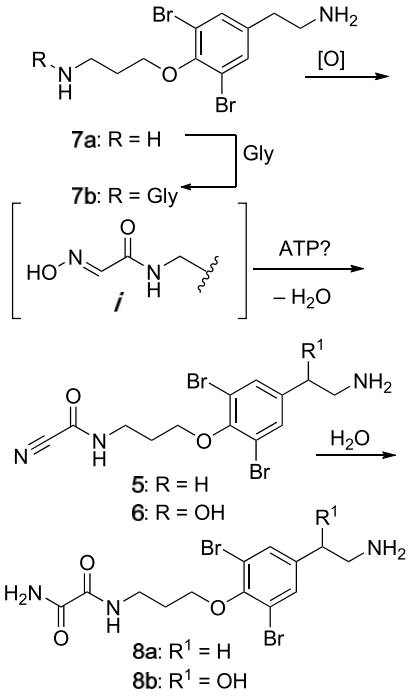
unique in Nature—is seen differently. Unlike alkyl cyanoformate esters that decompose to HCN, CO_2, and ROH in aqueous solution, cyanoformamides are stable and persist in MeOH and even acidic (TFA) aqueous HPLC solvents. The terminal CN group in **5** and **6** suggests a likely oxidative remodeling of a precursor *N*-Gly peptide, perhaps in a manner similar to the isocyano-lipopeptides, INLP-1 and -2, that engage α-ketoglutarate-dependent non-heme iron oxidases of transformation.[31] These two examples demonstrate precedents of oxidative remodeling of Gly derivatives that may have counterparts in the biosynthesis of TIs.

These few foregoing observations led to a re-evaluation of TIs and proposal of a candidate biochemical intermediate for delivery of a nucleophilic CN group in their biosynthesis, cyanoformic acid, NC–COOH (**B1**), derived from Gly, that resolves at least two paradoxes. Compound **B1** is substantially nucleophilic, even more so than HCN, and arises from a shunt reaction in the C-1 pathway from Gly that would engage in the S_N1-like capture of a terpenyl carbocation, R^+, *only* at the N terminus, to create the R-NC group.

Free NC-COOH (**B1**) is unstable, decomposing readily in H_2O to CO_2 and HCN,[32] but its conjugate base, cyanoformate, $NC–COO^-$ (**B2**), is more stable. **B2** has been characterized by

https://doi.org/10.1021/acs.jnatprod.4c01295
J. Nat. Prod. 2025, 88, 205–210

Scheme 1. Proposal for Biosynthesis of the Cyanoformamide Residue in Ceratinamines (5) and 7-Hydroxyceratinamine (6) and the Origin of Oxalamides, 8a,b



IR and NMR spectroscopy and X-ray crystallography (see below). Both **B1** and **B2** may participate in nucleophilic addition reactions, but to simplify discussion, the neutral **B1** will be considered here. In order to more fully appreciate the bonding and electrostatic potential characteristics of **B1**, the optimized geometry and energy of the corresponding *s-trans* and *s-cis* isomers were calculated using DFT methods (ωB97X-D, 6-31G*, in H_2O, Figure 2).[33,34] From examination of partial charges, it can be appreciated that **B1** is amphiphilic: electrophilic at C═O and nucleophilic at N. The calculated dipole moments of both the *s-cis*-**B1** and *s-trans*-**B1** conformers are exceptionally large (μ = 4.47 and μ = 3.90 D, respectively), even more than that of HCN (μ = 3.74 D). Additionally, the C−C bonds are relatively long, implying little charge delocalization (stabilization) through resonance. Thus, **B2**, the conjugate base of **B1**, can be classified as a "hard nucleophile" that better matches the "hard" electrophilicity of terpenoid carbocation intermediates, R^+, with which it may engage.

The calculated dipole moments of hydrocyanic acid (HCN), *s-cis*-**B1**, and *s-trans*-**B1** (see Supporting Information) suggest that the latter two are stronger nucleophiles than HCN. This surprising conclusion finds experimental support. The elusive cyanoformate anion, **B2**, has been characterized crystallographically, spectroscopically, and computationally. Tuononen, Clyburne, and co-workers demonstrated the equilibrium of CO_2 and NC^- to give anionic **B2** and characterized it for the first time as its Ph_4P^+ salt[35,36] (see Supporting Information for the bond parameters of **B1**). The weak C−C bond noted in the X-ray crystal structure of **B2** (*d* C−C = 1.480(9) Å) conforms with the exceptionally long C−C bonds found in **B1**, in the present work, from DFT-calculated structures (**B1**, *s-trans*: 1.477 Å, *s-cis*: 1.481 Å Figure 2 and Supporting Information). Calculations of the reaction profile (eq 1) at the ωB97X-D/aug-cc-pVTZ level of theory show that formation of NC-COO^- (**B2**) is thermodynamically favorable, but only in low-dielectric media, e.g.,

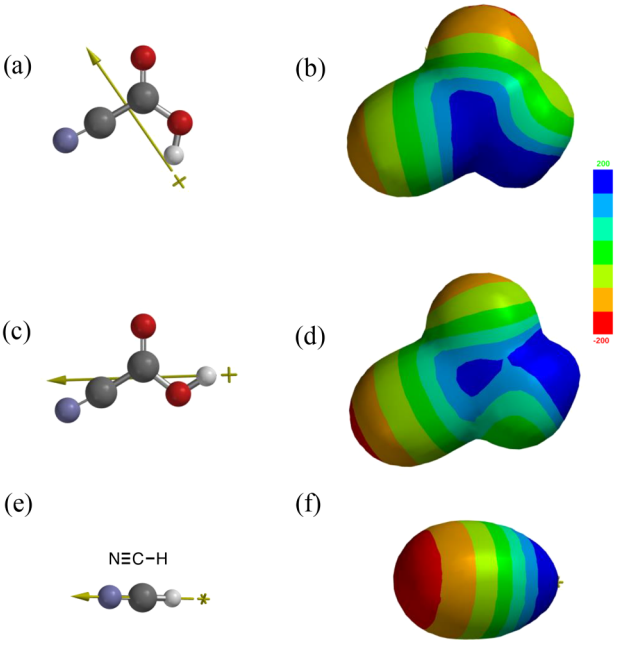
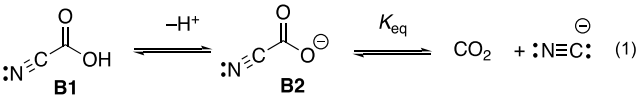


Figure 2. DFT-optimized geometries (ωB97X-D, 6-31G*, in H_2O), surface electrostatic potential maps and dipole moment vectors, μ, of cyanoformic acid, NC-COOH (**B1**) and HCN. Scale bar: −200 to +200 kJ. (a, b) *s-cis*-**B1**, μ = 3.90 D, *d*(C−C) = 1.481 Å. (c, d) *s-trans*-**B1**, μ = 4.47 D, *d*(C−C) = 1.477 Å. (e, f) HCN, μ = 3.74 D, *d*(C−N).



vacuum $\Delta G°$ −35 kJ mol^{-1}, E_A < 1.2 kJ mol^{-1}, and toluene $\Delta G°$ ~ −5 kJ mol^{-1}, K_{eq} = −7.6.[35,37] Using a different level of theory, CCSD(T), Murphy and co-workers calculated the equilibrium constant (K_{eq}, eq 1) in aprotic polar solvent: K_{eq}(DMF, 40 °C) = 0.347. In contrast, NC-COO^- (**B2**) in H_2O is unstable ($\Delta G°$ ~ +55 kJ mol^{-1}) and decomposes to HCN and CO_2 (HCO_3^-) instantly.[35] In low-dielectric media, the **B2** anion is protected from decomposition by a relatively high barrier of dissociation (E_A ~ 40 kJ mol^{-1}), and the half-lives for decomposition of Bu_4N^+**B2** (prepared in an ionic liquid) in aprotic solvents become appreciable: $t_{1/2}$(acetonitrile) = 17 min, $t_{1/2}$(THF) = 55 min, $t_{1/2}$(toluene) = 110 min.[35]

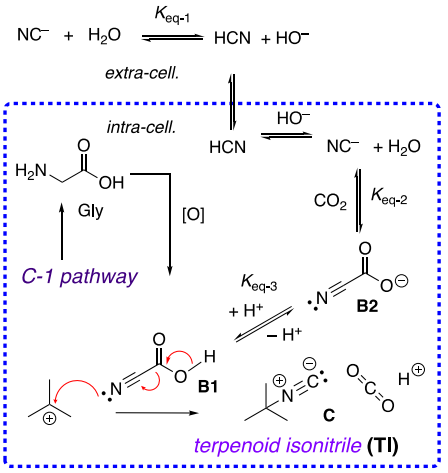
These properties suggest persistent biogenic **B1** and **B2** would be confined to low-dielectric spaces: hydrophobic enzyme active sites, protein pockets, lipid membranes, or vesicles. In the biosynthesis of the plant hormone ethylene from 1-aminocyclopropane-1-carboxylate (ACC) by ACC oxidase (E C 1.14.17.4), **B2** has been proposed as a "cyanide shuttle" that preserves NC^- in a protected form within the water-free Fe(II)-centered active site to prevent "poisoning" of the enzyme until it can diffuse away into bulk H_2O, where it decomposes into CO_2 and CN^-.[36]

The use of **B2** as a cyanide proxy in synthesis has been explored by Lee and co-workers, who showed cyanation of coumarins (Michael addition) by $Et_3N^+CN^-$ was accelerated by the presence of CO_2 and invoked the intermediate **B2**.[38] Here, **B2** acts as a cyanide shuttle: the combination of CO_2 (headspace partial pressure = 1 atm, 40 °C) and $Et_3N^+CN^-$ in DMF solution is more reactive than that of $Et_3N^+NC^-$ alone.

https://doi.org/10.1021/acs.jnatprod.4c01295
J. Nat. Prod. 2025, 88, 205−210

Considering the foregoing data, a unifying model for the biosynthesis of the cyanoformamide group in bromotyrosine natural products **5** and **6** can be proposed that also links the oxidative metabolism of Gly residues to the origin of TIs (Schemes 1 and 2). In cyanoformamide biosynthesis, Gly is

Scheme 2. Proposal for Biosynthesis of Terpenoid Isonitriles (TIs) from Gly-Derived Cyanoformic Acid (B1)



condensed with the 3-aminopropyl ether moloka'iamine (**7a**),[39] followed by oxidative remodeling of the *N*-Gly residue in **7b**, e.g., CYP or α-ketoglutarate-dependent non-heme Fe oxidase (?), likely through the intermediacy of oxime ***i***. Elimination of H_2O (oxime activation by ATP?) delivers cyanoformamides **5** and **6**. Related oxalamides (e.g., moloka'iakitamide, **8a**, and its hydroxyderivative, **8b**, from *Pseudoceratina arabica*[40]) can be explained by formal addition of H_2O to the nitrile group in **5** and **6**, respectively. The stability of the cyanoformamide group, NC(C═O)NH−, within these natural product structures, compared to the lability of cyanoformate esters, needs no special explanation: the usual strong resonance donation by N in the (C═O)−NH bond is sufficient.

A reasonable proposal for TI biosynthesis is now evident (Scheme 2). Gly is directly oxidized to cyanoformic acid (**B1**), which exists in an equilibrium with its conjugate base, **B2**.[41] The cyanoformate anion, **B2**, in turn, is in equilibrium with its decomposition products CO_2 and NC^-. **B1** and **B2** provide a portal through which exogenous inorganic cyanide, transported into a cell or organelle as HCN, intercepts the equilibrium pair **B1**/**B2** and participate in the closing step of TI biosynthesis. Importantly, the higher nucleophilicity of **B1**/**B2** over that of HCN-NC^- ensures that cyanoformate is competitive in S_N1 reactions. It is proposed that the neutral acid form **B1** is the nucleophilic intermediate favored to deliver the elements of NC to the terpenoid cation ***i*** for two reasons: negative charge buildup on O in **B2** renders the latter less nucleophilic at N than **B1**, and neutral **B1** is compatible with the hydrophobic matrices wherein TIs are likely formed. In both cases, capture of the incipient terpenoid carbocation ***i*** by **B1** can proceed *only* at N, directing the reaction outcome to isonitrile, exclusively. In capture of the carbocation R^+ by **B1**, concomitant losses of two small stable species—CO_2 and H^+ (H_3O^+), or from **B2**, CO_2—provide enthalpic and entropic driving forces that are absent in simple capture of R^+ by cyanide ion, NC^-. Indeed, the chemical potential that propels this reaction ultimately derives from the initial oxidation of Gly to produce the reactive intermediate **B1**.

A key to understanding **B1**, a proposed isocyanide delivery vector, is nucleophilicity. Unlike the ambident nucleophile CN^-, putative reaction of **B1** with incipient carbocations arising from π-cation cascades in the terminal step of TI biosynthesis can occur only along a *single* vector (Scheme 2). Unlike NC^-, **B1** is a *unident* nucleophile and can approach R^+ only at N along its diagonal N−C axis. The reaction coordinate only allows S_N1-like capture of the terpenyl carbocation by the lone-pair of N, and the obligatory consequence of this sequence is an isocyanoterpene product rather than the isomeric cyanoterpene.

The incorporation of inorganic cyanide into terpenoid isonitriles by sponge samples incubated in seawater (SW) closed aquaria can now be explained in this new light. Highly soluble cyanide, CN^-, diffuses unimpeded in aqueous solution but would be repelled at the negatively charged lipid bilayers of cell membranes. Neutral HCN (pK_a 9.25) is uniquely able to cross cytoplasmic membranes. Given the buffering capacity of SW and an average pH ~ 8.1,[42] a solution of cyanide in SW comprises ~ 93 mol % [HCN].[43] It is proposed that the reversible dissociation of **B1** to CO_2 and the CN^- (Scheme 2) establishes a cyanide pool at equilibrium, K_{eq}, and a portal for interception by exogenous HCN/NC^- into **B1** at the locus of TI biosynthesis (eq 1).

There is precedent for isocyanide surrogates in the total synthesis of TIs that parallels the canonical capture of a terpenyl carbocation, R^+, by **B1**. In a strategic breakthrough in TI synthesis (Figure 3), Shenvi and co-workers exploited the

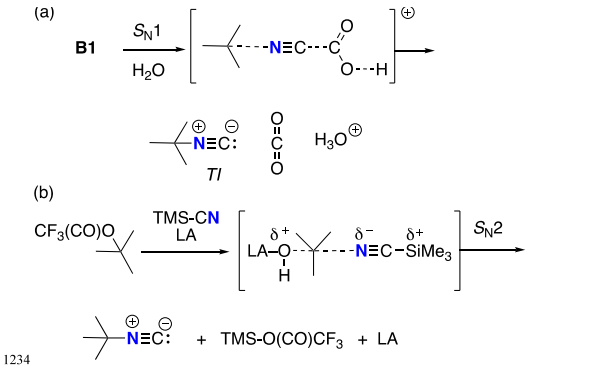


Figure 3. Reaction mechanisms of terpene isonitrile (TI) precursors with isocyanide surrogates: (a) Terpenyl carbocation (R^+) with cyanoformic acid (**B1**). (b) Lewis acid (LA)-promoted S_N2 reaction of *O-tert*-alkyl trifluoroacetate with TMS-CN.[44]

reaction of activated 3° alcohols—trifluoroacetate esters (R-TFA)—with trimethylsilyl cyanide (TMS-CN) in the presence of Lewis acids (e.g., $Sc(OTf)_3$, $Zn(OTf)_2$), in nonpolar solvent, to deliver R-NC.[9,44−46]

How does a sponge tolerate exogenous cyanide? Cyanide is naturally lethal, but the CN group does appear in masked form within certain plants, ranging from ferns to angiosperms, as nontoxic cyanogenic glycosides (e.g., amygdalin).[47] The latter are latent cyanides that serve as a chemical defense:[48] Upon injury, plants release glycosidases that hydrolyze cyanogenic glycosides into glucose and mandelonitrile, which is decomposed by hydroxynitrile lyase (HNL)[49] into HCN and benzaldehyde; consequently, those plants are avoided by herbivorous grazers and other predators. Many higher organisms have adapted to encounters with cyanide by expression of rhodanese, a mitochondrial enzyme that detoxifies NC^- by the addition of S and conversion to benign thiocyanate, NCS^-.

https://doi.org/10.1021/acs.jnatprod.4c01295
J. Nat. Prod. 2025, 88, 205−210

Enhanced levels of sponge-expressed rhodanese would explain two phenomena: first, the side-observation by Garson and others: tolerance of living TIPSs to inorganic cyanide exposure (at least up to some critical threshold concentration) and, second, a need to detoxify adventitious "leakage" of NC^- or spontaneous decomposition of **B1** in the putative C-1 shunt pathway. Elevated rhodanese may be obligatory in cells that deal in the metabolic currency of elevated cyanide and, with it, the vehicle **B1** for delivery of the elements of N and C in TI biosynthesis. Finally, bacterially expressed rhodanese (RhDE) was shown to convert not only cyanide, NC^-, to thiocyanate, NCS^-, but also alkyl isonitriles to their corresponding isothiocyanates with moderate promiscuity.[50]

These interpretations can only allow one to frame hypotheses, to be sure, but they also serve as the basis for design and testing using well-controlled experiments. Can NC-COOH (**B1**) be detected in cultured cells from TIPS, e.g., by Raman microscopy? If Gly incorporation into protein can be blocked (Gly transport inhibition), will [^{14}C]-Gly show incorporation into TIs? Could lipid vesicles carrying stabilized $^{15}N^{13}C-COO^-$ and cognate endogenous terpene cyclase incorporate $^{15}N^{13}C$ into TIs? These and other intriguing questions must await suitable design of experiments and their execution.

In summary, cyanoformic acid, NC-COOH (**B1**), rather than cyanide, NC^-, is proposed as the ultimate source of the isonitrile group in the biosynthesis of TIs in sponges. A long-standing paradox, that cyanide is incorporated into TIs, but only reacts with electrophiles to make nitriles (C−C bond formation), not isonitriles (C−N bond formation), is now resolved. Invocation of the highly reactive *unident* nucleophile, NC-COOH, derived from Gly oxidative metabolism and a dynamic equilibrium between free NC^- and NC-COOH explains the results of earlier isotopic labeling experiments that demonstrated incorporation of [^{14}C]- and ^{13}C]-cyanide into TIs. The possible role of sponge-expressed rhodanese in alkyl isonitrile to isothiocyanate biosynthesis may explain the common co-occurrence of the two related families of compounds.

EXPERIMENTAL SECTION

General Experimental Procedures. DFT Calculations. All DFT calculations were performed using Spartan'20 (Wavefunction, Irvine, CA, USA). Calculations for both *s-cis*-**B1** and *s-trans*-**B1** were carried out under identical conditions using functional ωB97X-D and basis set 6-31G* (in H_2O). Coordinates for the optimized structures and bond parameters can be found in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.4c01295.

Coordinates for optimized *s-cis* and *s-trans* **B1** and bond parameters (PDF)

AUTHOR INFORMATION

Corresponding Author

Tadeusz F. Molinski − *Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California 92093-0358, United States; Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, California 92093-0358, United States;* orcid.org/0000-0003-1935-2535; Phone: +1 858-534-7115; Email: tmolinski@uscd.edu; Fax: +1 (858) 822-0386

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.4c01295

Notes

The author declares no competing financial interest.

ACKNOWLEDGMENTS

I thank R. Berlinck for very helpful discussions and an anonymous reviewer for pointing out rhodanese-promoted alkyl isonitrile−isothiocyanate sulfur transfer. This paper is dedicated to the memory of the late D. John Faulkner, who first encouraged a newly appointed postdoctoral fellow to elucidate structures of novel terpene isonitriles.

REFERENCES

(1) Chang, C. W. J.; Scheuer, P. J. *Comp. Biochem. Physiol* **1990**, *97B*, 227−233.
(2) Patra, A.; Chang, C. W. J.; Scheuer, P. J.; van Duyne, G. D.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem. Soc.* **1984**, *106*, 7981−7983.
(3) Cafieri, F.; Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. *Tetrahedron* **1973**, *29*, 4259−4262.
(4) Minale, L.; Riccio, R.; Sodano, G. *Tetrahedron* **1974**, *30*, 1341−1343.
(5) Molinski, T. F.; Faulkner, D. J.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1987**, *52*, 3334−3337.
(6) Baker, J. T.; Wells, R. J.; Oberhänsli, W. E.; Hawes, G. B. *J. Am. Chem. Soc.* **1976**, *98*, 4010−4012.
(7) König, G. M.; Wright, A. D.; Angerhofer, C. K. *J. Org. Chem.* **1996**, *61*, 3259−3267.
(8) Wright, A. D.; Wang, H. Q.; Gurrath, M.; Konig, G. M.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. *J. Med. Chem.* **2001**, *44*, 873−885.
(9) Lu, H.-H.; Pronin, S. V.; Antonova-Koch, Y.; Meister, S.; Winzeler, E. A.; Shenvi, R. A. *J. Am. Chem. Soc.* **2016**, *138*, 7268−7271.
(10) Chang, W. J.; Patra, A.; Roll, D. M.; Scheuer, P. J.; Matsumoto, G. K.; Clardy, J. *J. Am. Chem. Soc.* **1984**, *106*, 4644−4646.
(11) Chahine, Z.; Abel, S.; Hollin, T.; Barnes, G. L.; Chung, J. H.; Daub, M. E.; Renard, I.; Choi, J. Y.; Vydyam, P.; Pal, A.; Alba-Argomaniz, M.; Banks, C. A. S.; Kirkwood, J.; Saraf, A.; Camino, I.; Castenada, P.; Cuevas, J. C.; De Mercado-Arnanz, J.; Fernandez-Alvaro, E.; Garcia-Perez, A.; Ibarz, N.; Viera-Morilla, S.; Prudhomme, J.; Joyner, C. J.; Bei, A. K.; Florens, L.; Ben-Mamoun, C.; Vanderwal, C. D.; Le Roch, K. G. *Science* **2024**, *385*, 7966−7978.
(12) Scotti, C.; Barlow, J. W. *Nat. Prod. Commun.* **2022**, *17*, 1−24.
(13) Del Rio Flores, A.; Barber, C. C.; Narayanamoorthy, M.; Gu, D.; Shen, Y.; Zhang, W. *Annu. Rev. Chem. Biomol. Eng.* **2022**, *13*, 1−24.
(14) Cable, K. M.; Herbert, R. B.; Knaggs, A. R.; Mann, J. *J. Chem. Soc. Perkin Trans. I* **1991**, 595−599.
(15) Brady, S. F.; Clardy, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 7063−7065.
(16) Bornemann, V.; Patterson, G. M. L.; Moore, R. E. *J. Am. Chem. Soc.* **1988**, *110*, 2339−2340.
(17) Hohlman, R. M.; Sherman, D. H. *Nat. Prod. Rep.* **2021**, *38*, 1567−1588.
(18) Chang, W.-C.; Sanyai, D.; Huang, J.-L.; Ittiamornkul, K.; Zhu, Q.; Liu, X. *Org. Lett.* **2017**, *19*, 1208−1211.
(19) Chena, T.-Y.; Chen, J.; Tang, Y.; Zhou, J.; Guo, Y.; Chang, W.-C. *Chin. J. Chem.* **2021**, *39*, 463−472.
(20) Emsermann, J.; Kauhl, U.; Opatz, T. *Mar. Drugs* **2016**, *14*, 16.
(21) Garson, M. J. *J. Chem. Soc., Chem. Commun.* **1986**, 35−36.
(22) Garson, M. J.; Simpson, J. S. *Nat. Prod. Rep.* **2004**, *21*, 164−179.
(23) Karuso, P.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 2092−2095.
(24) Fookes, C. J. R.; Garson, M. J.; MacLeod, J. K.; Skelton, B. W.; White, A. H. *J. Chem. Soc. Perkin Trans. 1* **1988**, 1003−1011.
(25) Similar reasoning may explain why [^{14}C]-acetate also fails to incorporate significantly into the carbon skeleton of diterpenoid

isonitriles, although utilization of the alternate MEP pathway for isoprenoid biosynthesis in the sponge may also account for this result.
(26) Dumdei, E. J.; Flowers, A. E.; Garson, M. J.; Moore, C. J. *Comp. Biochem. Physiol. A* **1997**, *118*, 1385−1392.
(27) Brantley, S. E.; Molinski, T. F.; Preston, C. M.; DeLong, E. F. *Tetrahedron* **1995**, *51*, 7667−7672.
(28) Preston, C. M.; Wu, K. Y.; Molinski, T. F.; DeLong, E. F. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6241−6246.
(29) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Org. Chem.* **1996**, *61*, 2936−2937.
(30) Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1999**, *62*, 1072−1073.
(31) Harris, N. C.; Sato, M.; Herman, N. A.; Twigg, F.; Cai, W.; Liu, J.; Zhu, X.; Downey, J.; Khalaf, R.; Martin, J.; Koshino, H.; Zhang, W. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 7025−7030.
(32) In contrast, the corresponding esters including commercially available methyl cyanoformate (NC-COOMe, Mander's reagent) are stable. The electrophilic C═O group of NC-COOMe engages lithium enolates of ketones to deliver methyl *β*-ketoesters; here, cyanide acts as a leaving group. Mander, L. N.; Sethi, S. P. *Tetrahedron Lett.* **1983**, *24*, 5425−5428.
(33) Chai, J.-D.; Head-Gordon, M. *J. Chem. Phys.* **2006**, *128*, No. 084106.
(34) Chai, J.-D.; Head-Gordon, M. *Phys. Chem. Chem. Phys.* **2008**, *10*, 6615−6620.
(35) Murphy, L. J.; Robertson, K. N.; Harroun, S. G.; Brosseau, C. L.; Werner-Zwanziger, U.; Moilanen, J.; Tuononen, H. M.; Clyburne, J. A. C. *Science* **2014**, *344*, 75−78.
(36) Hering, C.; von Langermann, J.; Shulz, A. *Angew. Chem. Int. Ed.* **2014**, *53*, 8282−8284.
(37) From $\Delta G^\circ = -RT \ln K_{eq}$ where $\Delta\mathring{G}$ values are estimates from interpolations of the graph in Figure 4 of Murphy et al.[35]
(38) Roy, T.; Kim, M. J.; Yang, Y.; Kim, S.; Kang, G.; Ren, X.; Kadziola, A.; Lee, H.-Y.; Baik, M.-H.; Lee, J.-W. *ACS Catal.* **2019**, *9*, 6006−6011.
(39) Hamann, M. T.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1993**, *58*, 6565−6569.
(40) Badr, J. M.; Shaala, L. A.; Abou-Shoer, M. I.; Tawfik, M. K.; Habib, A-Z.M. *J. Nat. Prod.* **2008**, *71*, 1472−1474.
(41) For simplicity, we represent Gly in the neutral form. From its isoelectric point (pH = 6.082) and average cytoplasmic pH (6.5−8.0), Gly is expected to be largely in its anionic form. Borsook, H.; MacFadyen, D. A. *J. Gen. Physiol.* **1930**, *13*, 509−527.
(42) https://ocean.si.edu/ocean-life/invertebrates/ocean-acidification#section_77.
(43) From the Henderson−Hasselbalch equation, $pH = pK_a + \log[CN^-]/[HCN]$.
(44) Pronin, S. V.; Reiher, C. A.; Shenvi, R. A. *Nature* **2013**, *501*, 195−199.
(45) Shenvi and co-workers further demonstrated that the reaction of TMS-CN with precursor tertiary alcohols, remarkably, follows an S_N2 rate law and proceeds with inversion at the $\mathring{3}$ center![44]
(46) Perhaps more accurately, Davey offers that the reaction proceeds through a contact ion-pair with some inversion and some retention of configuration. Davey, S. *Nat. Chem.* **2013**, *5*, 808.
(47) The biosynthesis of mandelonitrile glycosides from phenylglycine is well-characterized and not unlike that proposed for ceratinamine (Scheme 1).
(48) On the same theme, toxic isonitriles may have evolved in Porifera to afford the IPSs some level of chemical defense.
(49) Kassim, M. A.; Rumbold, K. *Biotechnol. Lett.* **2014**, *36*, 223−228.
(50) Mlotek, M. D.; Bose, B.; Hertweck, C. *ChemBioChem.* **2024**, *25*, No. e202300732.

NOTE ADDED AFTER ASAP PUBLICATION

This article was published ASAP on December 20, 2024. The pH / pKa calculation of cyanide in seawater was corrected. The updated version was reposted on December 31, 2024.

https://doi.org/10.1021/acs.jnatprod.4c01295
J. Nat. Prod. 2025, 88, 205−210