# Isocyanides and Cyanides as Natural Products

PAUL J. SCHEUER

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

The unique character of the isocyano function, as the only stable entity whose carbon is bonded to a single atom, coupled with the foul odor of its volatile representatives has been responsible for its notoriety in the laboratory and its appeal for theoretical and synthetic chemists. Discovery of the isocyano function is ascribed to Lieke,[1] who reacted allyl iodide with silver cyanide and obtained a vile-smelling liquid distinctly different from the anticipated allyl cyanide. Firm recognition of this new isomeric cyano function came several years later by Hofmann[2] and by Gautier.[3] The first report of a naturally occurring isocyano compound, xanthocillin (1), isolated from *Penicillium notatum*, was published in 1957,[4] nearly 100 years later. The structure of only a second microbial isocyano compound, trichoviridine (2) (a k a dermadin), isolated from *Trichoderma* spp. and unrelated to xanthocillin, was elucidated nearly 20 years later, in 1976.[5] In the interim,
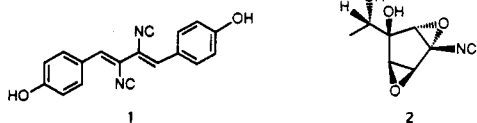


however, a number of isocyanoterpenes were reported, most of them from marine sponges. In sharp contrast, the familiar cyano function, well established in the laboratory and widely occurring in terrestrial biota, has so far only rarely been encountered in marine organisms. In this Account, I will examine both isocyano and cyano natural products, marine and terrestrial, and attempt to highlight some unresolved questions about these two

functions. An excellent review of naturally occurring isocyanides by Edenborough and Herbert was published in 1988.[6] This Account therefore will emphasize the more recent isocyano literature.

## Background

Interestingly, in this age of sophisticated instrumentation, two biological properties of the mucus of a marine snail, a "strong unusual smell" and its lethality to fish and crustaceans,[7] led to our discovery of isocyanoterpenes in the mucous secretion of the nudibranch (an opisthobranch mollusk) *Phyllidia varicosa*.[8] Our first small collection of *P. varicosa* in the fall of 1972 served only to confirm the earlier observation[7] of a volatile ichthyotoxic secretion with a strong unidentifiable odor. GC–MS experiments were inconclusive. A year later, two events helped us realize that we were dealing with isocyano-substituted hydrocarbons.

The first was an observation by B. J. Burreson. While collecting *P. varicosa* at Pupukea, O'ahu, he observed that the nudibranchs were feeding on an off-white sponge, subsequently identified as *Ciocalypta* sp. This discovery provided us with an adequate source of the same odorous compounds that we had isolated in minute amounts from the mollusks. The second event was the correct interpretation of infrared spectral bands by C. Christophersen, who at that time was studying the

Paul Scheuer is Professor of Chemistry Emeritus at the University of Hawaii, Manoa. He has studied natural products, predominantly marine, since 1950 after receiving his Ph.D. degree with R. B. Woodward at Harvard University. His graduate study, interrupted by service in the U.S. Army, followed his B.S. degree in chemistry from Northeastern University in 1943. He arrived in the United States in 1938 via England and Central Europe from Germany, where he was born in 1915.

(1) Lieke, W. *Liebigs Ann. Chem.* 1859, *112*, 316–321.
(2) Hofmann, A. W. *Liebigs Ann. Chem.* 1867, *144*, 114–120.
(3) Gautier, A. *Liebigs Ann. Chem.* 1868, *146*, 119–124. [The earlier paper, *Liebigs Ann. Chem.* 1867, *142*, 289–294, betrays no clear recognition of the isomeric nature of the cyano functions.]
(4) Hagedorn, I.; Tonjes, H. *Pharmazie* 1957, *12*, 567–580; *Chem. Abstr.* 1958, *52*, 6362c.
(5) Nobuhara, M.; Tazima, H.; Shudo, K.; Itai, A.; Okamoto, T.; Iitaka, Y. *Chem. Pharm. Bull.* 1976, *24*, 832–834.
(6) Edenborough, M. S.; Herbert, R. B. *Nat. Prod. Rep.* 1988, *5*, 229–245.
(7) Johannes, R. E. *Veliger* 1963, *5*, 104–105.
(8) Burreson, B. J.; Scheuer, P. J.; Finer, J.; Clardy, J. *J. Am. Chem. Soc.* 1975, *97*, 4763–4764.

constituents of a *Halichondria* sp. sponge in our laboratory. As soon as we linked the previously unidentified odor to IR bands at 2160, 2120, and 2100 cm⁻¹, we knew that the volatile constituents of the sponge and of the mollusk bore isocyano functions. By the time the *Phyllidia*[8] and the *Halichondria*[9,10] research was completed, Fattorusso[11] and Minale[12] had already reported on sponge isocyano sesquiterpenes isolated from two Mediterranean sponges, *Axinella cannabina* and *Acanthella acuta*.

## Marine Isocyanides

Marine-derived isocyano compounds have been terpenoid, sesqui- or diterpenes, some with previously described and some with new carbon skeletons. Among the isocyano sesquiterpenes we find representatives of the known carbon framework of gorgonene (3), eudesmane (4), cadinane (5), e.g., the recently described halipanicine (6), and aromadendrane (7) (Chart I). Newly described sesquiterpene types include the axanes (8), the spiroxanes (9), the tricyclic pupukeananes (10), and neopupukeananes (11). Frequently, though not invariably, the isocyanoterpene was accompanied by lesser amounts of an isothiocyano or a formamido analog. Occasionally, as in the monocyclic bisabolenes (12), amino and urea derivatives[14] have been reported. We isolated an aminobisabolene from *Ciocalypta* sp., along with an unprecedented isocyanate (13).[15] Our clue was an IR band at 2260 cm⁻¹ of the crude hexane extract of the sponge. Purification of the trace amounts of isocyanate was achieved by complexing the major isocyano component with silver ion. Two unique phenylthiourea derivatives of the known aromadendrane (7) and maaliane (14) sesquiterpenes were reported from the sponge *Epipolasis kushimotoensis*.[16] Yet another derivative of the isocyano function, a dichloro carbimide, has been reported in a group of sesquiterpenes, acyclic, monocyclic, and bicyclic, from the sponge, *Pseudaxinsyssa pitys*.[17] An acyclic farnesyl derivative 15 will serve as an example.[17] Notably, these compounds are polyfunctional, bearing chloro and occasionally hydroxy substituents.



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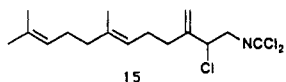
Among the first marine isocyano sesquiterpenes to be described were the tricyclic pupukeananes (10).[8] Except for the rearranged *neo*pupukeananes (11),[18] no

(9) Burreson, B. J.; Christophersen, C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1975**, *97*, 201–202.

(10) Burreson, B. J.; Scheuer, P. J. *J. Chem. Soc., Chem. Commun.* **1974**, 1035–1036.

(11) Cafieri, F.; Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. *Tetrahedron* **1973**, *29*, 4259–4262.

(12) Minale, L.; Riccio, R.; Sodano, G. *Tetrahedron* **1974**, *30*, 1341–1343.

(13) Nakamura, H.; Deng, S.; Takamatsu, M.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Agric. Biol. Chem.* **1991**, *581*–583.

(14) Sullivan, B. W.; Faulkner, D. J.; Okamoto, K. T.; Chen, M. H.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5134–5135.
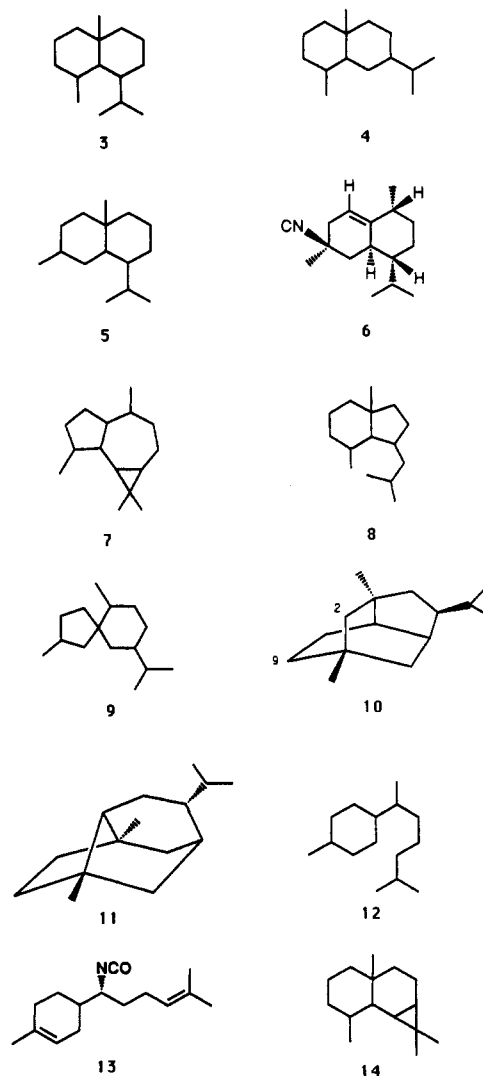
(15) Gulavita, N. K.; deSilva, E. D.; Hagadone, M. R.; Karuso, P.; Scheuer, P. J.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5136–5139.

(16) Tada, H.; Yasuda, F. *Chem. Pharm. Bull.* **1985**, *33*, 1941–1945.

(17) Wratten, S. J.; Faulkner, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 7367–7368.

(18) Karuso, P.; Poiner, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 2095–2097.

**Chart I**



other tricyclic sesquiterpenes have been reported. Both pupukeanane isomers, 2- and 9-isocyano, are present in the nudibranch *Phyllidia varicosa* and in the sponge *Ciocalypta* sp. We had indirect evidence that each isomer is in fact a mixture of two epimers, but at the time we were unable to effect their separation.[19] 9-*epi*-Isocyanopupukeanane from a nudibranch, *Phyllidia bourgini*, was recently reported by Fusetani and co-workers,[20] who used sophisticated HPLC techniques. Interestingly, 9-*epi*-isocyanopupukeanane proved to be the more active isomer in ichthyotoxicity assays.

In addition to an acyclic isocyano geranyllinalool,[10] a number of tri- and tetracyclic isocyano diterpenes have been described. The tricyclic kalihinols constitute a large family of richly functionalized compounds.[21] Biogenetically, perhaps the most interesting recent additions were reported by Fusetani et al.[22] from *Acanthella klethra*. The two new compounds, kalihinene (16) and isokalihinol B (17), suggest that 16

(19) Hagadone, M. R. M.S. Thesis, University of Hawaii, Honolulu, HI, 1978.

(20) Fusetani, N.; Wolstenholme, H. J.; Matsunaga, S. *Tetrahedron Lett.* **1990**, *31*, 5623–5624.

(21) Chang, C. W. J.; Patra, A.; Baker, J. A.; Scheuer, P. J. *J. Am. Chem. Soc.* **1987**, *109*, 6119–6123.

(22) Fusetani, N.; Yasumuro, K.; Kawai, H.; Natori, T.; Brinen, L.; Clardy, J. *Tetrahedron Lett.* **1990**, *31*, 3599–3602.

might be a precursor of **17** and of kalihinol B, in which the sites of vicinal hydroxyl and isocyano functions are reversed.
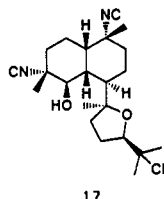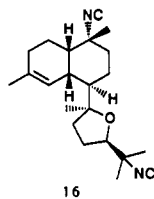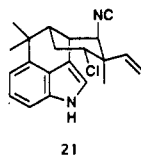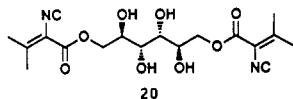



## Terrestrial Isocyanides

In striking contrast to the terpenoid character of the marine isocyano compounds, all terrestrial representatives are amino acid-derived and none have been accompanied by isothiocyano or formamido analogs. In fact, as is the case with xanthocillin (**1**), by far the predominant amino acid is tyrosine. A recently isolated variant of the xanthocillin type is antibiotic MK 4588 (**18**).[23] In the hazimycins, e.g., hazimycin factor 5 (**19**) from *Pseudomonas* sp.,[24] the tyrosine dimers are biphenyls rather than 1,4-diphenylbutanes.
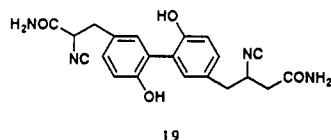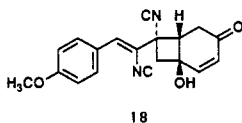



The second major group of terrestrial isocyanides is structurally related to trichoviridine (**2**) and seemingly unrelated to a known amino acid. Yet ingenious biosynthetic research by Baldwin et al.[25] has shown that these compounds are also biosynthesized from tyrosine.

Two additional terrestrial isocyano compounds are noteworthy, antibiotic A32390A (**20**)[26] and hapalindole A (**21**).[27] Both are the result of mixed biosynthesis: tryptophan and a linear monoterpene for **21**, which was isolated from the cultured blue-green alga *Hapalasiphon fontinalis*, and valine and mannitol in the case of **20**, which was produced by *Pyrenochaeta* sp.




## Isocyanide Biosynthesis

Successful incorporation experiments were reported by Moore and co-workers[27] into hapalindole A (**21**) in cultures of the terrestrial blue-green alga *Hapalosiphon fontinalis*. Clearly, the cyanobacterium does utilize C$_1$ sources glycine, serine, formate, and methionine,

which are associated with tetrahydrofolate metabolism, for synthesis of the isocyano function. The organism also incorporates cyanide. In all cases, hapalindole A (**21**) was degraded to the corresponding amine with essentially total loss of activity. Glycine and serine were incorporated more efficiently than methionine or formate. Experiments with [2-$^{13}$C,$^{15}$N]glycine moreover proved that both atoms are utilized for isocyano biosynthesis. This contrasts with the results obtained earlier with xanthocillin (**1**),[28] which serves as a reminder that it is hazardous to base predictions on results obtained with an entirely different organism.

Biosynthetic studies of marine isocyanides differ from those of their terrestrial counterparts in two important aspects: All known terrestrial isocyano natural products are amino acid-derived, while the marine isocyanides are terpenoid. Secondly, all terrestrial producers of isocyano compounds are microorganisms, thus allowing experiments to be performed under controlled conditions of laboratory culture, while marine sponges are invertebrate animals which are difficult to keep alive in the laboratory. Biosyntheses of marine metabolites in general[29] and of marine isocyanides[30] have been subjects of recent reviews and hence will not be discussed here.

## Terrestrial Cyano Compounds

**Flowering Plants.** Since marine sponges have the capability to utilize cyanide for in vivo generation of isocyano groups,[30] it is desirable to discuss cyano natural products. Cyanogenic glycosides of flowering plants are a widely distributed and well-understood group of natural products.[31] More than 1000 cyanogenic species in 100 plant families elaborate cyanogenic glycosides. Perhaps the best known of these plants cassava (*Manihot esculentus*, Family Euphorbiaceae), which is a valuable food crop in the tropics and known in western countries as tapioca, and various *Prunus* spp. (apricots, peaches, etc., Family Rosaceae), whose seeds contain the purported anticancer drug laetrile. Structurally, all are β-glycosides of 2-hydroxy nitriles (cyanohydrins). D-Glucose is by far the most common sugar, and most of the aglycones are derived from only a few amino acids: valine, isoleucine, phenylalanine, or tyrosine. Lotaustralin (**22**) is one of the most common cyanogenic glycosides. It occurs in manihot and other plant families and is derived from isoleucine. Amygdaline is a glycoside made up of a phenylalanine and the disaccharide gentiobiose. It is responsible for the bitter almond taste of peach or plum seeds. The widely distributed prunasin (**23**) differs from amygdalin only in the sugar moiety—glucose instead of gentiobiose. A few cyanogenic aglycones may be derived from cyclopentenylglycine. Gynocardin (**24**), a constituent of *Gynocardia odorata* (Family Flacourtiaceae), is the best studied example of this group.

Degradation of the cyanogenic glycosides to the sugar, the aglycone, and hydrogen cyanide takes place on heating with dilute acid or by enzymatic action when

(23) Itoh, J.; Takeuchi, Y.; Gomi, S.; Inouye, S.; Mikawa, T.; Yoshikawa, N.; Ohkishi, H. *J. Antibiot.* **1990**, *43*, 456–461.

(24) Kim Wright, J. J.; Cooper, A. B.; McPhail, A. T.; Merrill, Y.; Nagabhushan, T. L.; Puar, M. S. *J. Chem. Soc., Chem. Commun.* **1982**, 1188–1190.

(25) Baldwin, J. E.; Bansal, H. S.; Chondrogianni, J.; Field, L. D.; Taha, A. A.; Thaller, V.; Brewer, D.; Taylor, A. *Tetrahedron* **1985**, *41*, 1931–1938.

(26) Marconi, G. G.; Molloy, B. B.; Nagarajan, R.; Martin, J. R.; Deeter, J. B.; Occolowitz, J. L. *J. Antibiot.* **1978**, *31*, 27–32.

(27) Bornemann, V.; Patterson, G. M. L.; Moore, R. E. *J. Am. Chem. Soc.* **1988**, *110*, 2339.

(28) Achenbach, H.; Grisebach, H. *Z. Naturforsch.* **1965**, *20B*, 137–140.

(29) Garson, M. J. *Nat. Prod. Rep.* **1989**, *6*, 143–170.

(30) Chang, C. W. J.; Scheuer, P. J. *Comp. Biochem. Physiol.* **1990**, *97B*, 227–233.

(31) Conn, E. E. In *Secondary Plant Products*; Bell, E. A., Charlwood, B. V., Eds.; Springer: Berlin, 1980; pp 461–492.

22    23    24

**Scheme I**
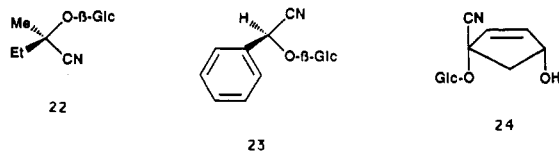


the plant is crushed. Cassava, for example, becomes safe to eat after the crushed roots of *Manihot* sp. are heated.

The biosynthesis, primarily the stepwise conversion of an amino acid to a hydroxy nitrile, has been studied extensively.[32] Recent research has shown that the biosynthesis of dhurrin, which is the cyanogenic glycoside of *Sorghum bicolor* (Family Gramineae), proceeds[33] from *N*-hydroxytyrosine to *p*-hydroxyphenylacetaldehyde oxime via the aci tautomer of 1-nitro-2-(*p*-hydroxyphenyl)ethane (Scheme I).

The cyanolipids are another sizable class of cyanobearing compounds. They occur exclusively in seed oils of members of the plant family Sapindaceae. Although the cyanogenic property of kusum seed oil from the sapindaceous tree *Schleichera trijuga* has been known for many years, definitive chemistry has been rather recent, despite the fact that cyanolipids constitute more than 50% of kusum seed oil.[34] The chain length of the fatty acids varies from $C_{14}$ to $C_{22}$, of which $C_{18:1}$ and $C_{20:1}$ predominate, but the structural types are few. All cyanolipids that are known so far are mono- or diesters of mono- or dihydroxy nitriles (25–28), all derivable from leucine, which after decarboxylation provides the carbon framework. It is worth noting that after hydrolysis to the alcohols only the $\alpha$-hydroxy nitriles 26 and 28 are cyanogenic.



25    26

27    28
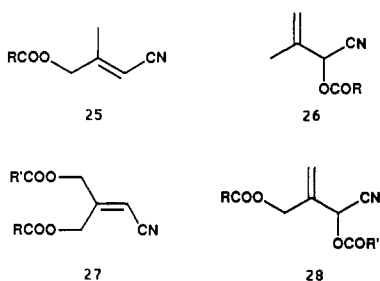
As is true for many natural products, there has been much speculation and relatively little research into their ecological function. A clue may lie in a report by Seigler and Price[35] that the cyanolipids of *Ungnadia speciosa* seeds disappear within 3 days of germination.

**Other Terrestrial Biota.** Two volumes of conference proceedings on the role of cyanide in biological systems provide a good overview and some detail of the occurrence and biosynthesis of cyano compounds.[36,37] Bacteria, cyanobacteria, microalgae, and fungi include some cyanogenic species. There appears to be no common pattern for their occurrence or origin.

*Chromobacterium violaceum* and many strains of *Pseudomonas* spp. are cyanogenic. It has been shown that glycine, serine, and threonine, stimulated by glutamate or methionine, are precursors of hydrogen cyanide in these bacteria.[36] In several cyanobacteria, such as *Anacystis nidulans, Nostoc muscorum,* and *Plectonoma boryanum,* and the unicellular green alga *Chlorella vulgaris,* hydrogen cyanide is produced from D-histidine (29) and an oxidase/peroxidase system. Fungi, especially basidiomycetes and ascomycetes, resemble bacteria in that cyanide is produced by decarboxylation of glycine.



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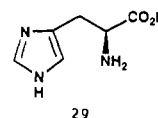
Cyanogenesis is also known from arthropods, specifically from Chilopoda (centipedes), Diplopoda (millipedes), and Insecta (insects). The millipede Order Polydesmida is a prominent cyanide producer, as is the Order Lepidoptera among the insects. There is good evidence that insects use cyanogens for defense. They may acquire them from their plant diet, but they are also capable of de novo biosynthesis. This was demonstrated for *Zygaena* and *Heliclonius* spp.,[38] whose larvae incorporate valine and isoleucine to biosynthesize linamarin (36) and lotaustralin (22), respectively. Both species feed on cyanogenic plants during their larval stage. *Zygaena* spp. feed on *Lotus corniculatus* (Family Fabaceae), which contains linamarin (30) and lotaustralin (22), while *Heliclonius* spp. feed on Passifloraceae which do not contain these cyanogens.



30
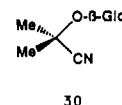
Likewise, in the millipede *Harpaphe haydeniana* it was shown[39] that mandelonitrile (31) is biosynthesized from L-phenylalanine. The millipede stores it in a chamber from which it is squeezed when needed for defense. Upon release it meets a lyase which generates hydrogen cyanide. What happens to excess hydrogen cyanide that is not needed to deter or kill a predator? The predominant mechanism appears to be detoxification by production of thiocyanate. Additionally, detoxification occurs by reaction of HCN with serine or cysteine to produce $\beta$-cyanoalanine, which in turn is converted to asparagine (32).

A few cyano natural products are unrelated to the cyanogenic glycosides or the cyanolipids. Several of them are 3-cyanopyridines. The best known of these is ricinine (33), a toxic alkaloid which occurs in the seeds and leaves of the castor plant, *Ricinus communis*

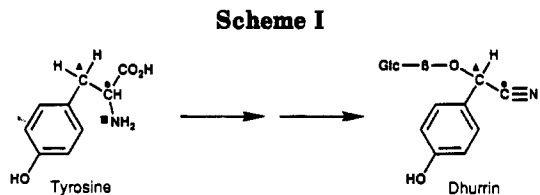(32) Conn, E. E. *Naturwissenschaften* 1979, 66, 28–34.
(33) Halkier, B. A.; Moeller, B. L. *J. Biol. Chem.* 1990, 265, 21114–21121.
(34) Mikolajczak, K. L. *Prog. Chem. Fats Other Lipids* 1977, 15, 97–130.
(35) Seigler, D. S.; Price, P. W. *Am. Nat.* 1976, 110, 101–105.
(36) *Cyanide in Biology;* Vennesland, B., Conn, E. E., Knowles, C. J., Westley, J., Wissing, F., Eds.; Academic Press: London, 1981.
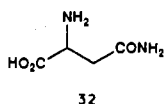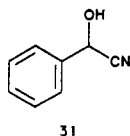(37) *Cyanide Compounds in Biology;* Ciba Foundation Symposium 140; Wiley-Interscience: Chichester, England, 1988.
(38) Davis, R. H.; Nahrstedt, A. *Insect Biochem.* 1987, 17, 689–693.
(39) Duffey, S. S. In ref 36, pp 385–414.

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(Family Euphorbiaceae). The cyano group of ricinine is derived from the corresponding carboxylic acid via the amide.
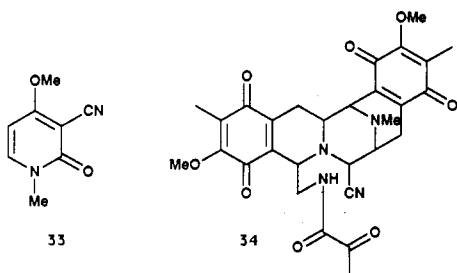
Another cyano-bearing compound is the antibiotic saframycin A (**34**), a metabolite of *Streptomyces lavendulae*.[40] In this case the origin of the cyano group is neither obvious nor known.

## Marine Cyano Compounds

Sponges of the Order Verongida elaborate a broad spectrum of compounds derived from dibromotyrosine.[41] Among the earliest of these compounds was amide **35**, which Sharma and Burkholder[42] isolated from *Verongia cauliformis*. A few years later Fattorusso et al.[43] reported isolation from *Aplysina aerophoba* the same compound and a cyano derivative **36**, which was named aeroplysinin-1. The cyano function clearly is a product of amide dehydration concomitant with hydration of an intermediate arene epoxide.
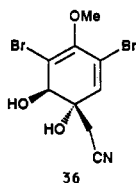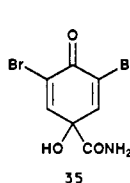
Somewhat parallel to saframycin A (**34**) bearing a cyano group of no readily discernible provenance are the calyculins, metabolites of the sponge *Discodermia calyx*.[44] Calyculin A (**37**) is a potent tumor promoter and inhibitor of protein phosphatases.[45]

## Thiocyanates and Isothiocyanates

The mechanism mentioned earlier by which the millipede *H. haydeniana* renders cyanide nontoxic to itself, i.e., by transformation to thiocyanate, is somewhat surprising as the thiocyano group—in contrast to the
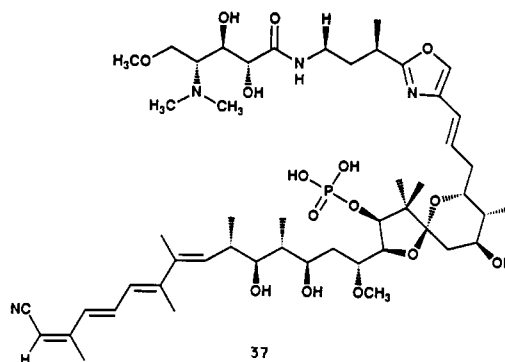
isothiocyano function—is exceedingly rare in nature. Only three such compounds are known from terrestrial higher plants: allyl, benzyl, and 4-(methylthio)butyl.[46] In a well-documented case it was shown[47] that freshly crushed *Eruca sativa* (Family Cruciferae) plants yield 4-(methylthio)-1-thiocyanatobutane, while *E. sativa* seeds furnish the corresponding isothiocyanate, a so-called mustard oil. The mustard oils are produced from glucosinolates (vide infra), which are commonly found in the Cruciferae as well as in other plant families.[48] The enzymes, myrosinases, which generate isothiocyanates from glucosinolates, are well characterized, but we do not know how thiocyanates arise, whether directly from glucosinolates or by isomerization of isothiocyanates.

Occurrence of the thiocyano group in marine biota is also uncommon. Only three such compounds, all sesquiterpenes, have been reported so far. The first is a bicyclic cadinene **38**, functionalized at C-4, isolated from a sponge, *Trachyopsis aplysinoides*.[49] The other two are regioisomers of the tricyclic neopupukeanane skeleton: compound **39** from the Okinawan sponge *Phycopsis terpnis* and **40** from an unidentified Pohnpei sponge.[50]
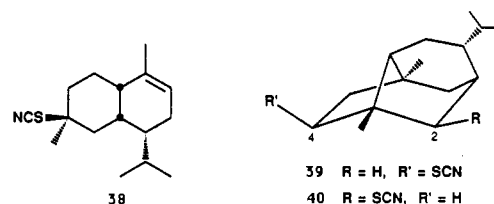
The terrestrial glycosinolates and their degradation products, the mustard oils, have been well studied.[48] In the general formula **41** of the glucosinolates, the sugar is invariably D-glucose, the cation is almost always potassium, and the geometry of the R group and sulfate is anti. Unlike the rather monotonous structural features of cyanohydrins and cyanolipids, and R groups of the glucosinolates exhibit considerable variation and include ω-(methylthio)alkyl and ω-(methylsulfoxy)alkyl homologs. 1-Isothiocyanato-4(R)-(methylsulfinyl)butane (sulforaphane) (**42**), isolated from broccoli (*Bras-*

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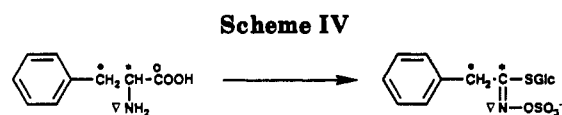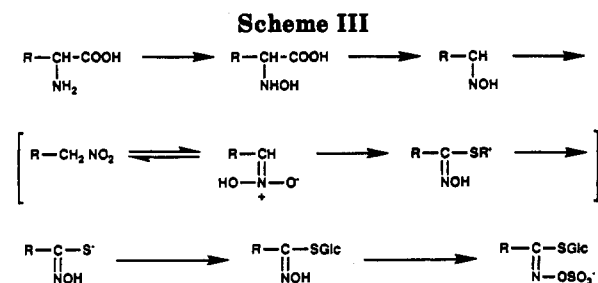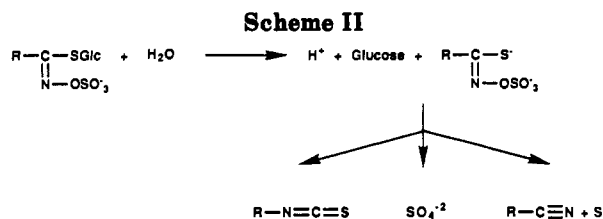
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**Scheme II**

R—C—SGlc + H₂O ⟶ H⁺ + Glucose + R—C—S⁻
|| ||
N—OSO₃⁻ N—OSO₃⁻

R—N=C=S     SO₄⁻²     R—C≡N + S

**Scheme III**

R—CH-COOH ⟶ R—CH-COOH ⟶ R—CH ⟶
| | |
NH₂ NHOH NOH

[ R—CH₂NO₂ ⇌ R—CH ⟶ R—C—SR' ⟶ ]
|| |
HO—N—O⁻ NOH
+

R—C—S⁻ ⟶ R—C—SGlc ⟶ R—C—SGlc
|| || ||
NOH NOH N—OSO₃⁻

**Scheme IV**

C₆H₅—CH₂-CH-COOH ⟶ C₆H₅—CH₂-C—SGlc
| ||
NH₂ N—OSO₃⁻

*sica oleracea italica*, Family Cruciferae), was recently shown to induce anticarcinogenic protective enzymes.[51]

R—C—S—Glc                    compound 42 structure with NCS
|
N—OSO₃⁻ K⁺

41                                  42
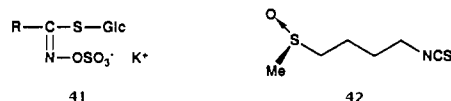
The plants that contain glucosinolates contain a hydrolytic enzyme, a myrosinase. The action of the enzyme on the glucosinolate is shown in Scheme II. The aglycone, under neutral conditions, decomposes into sulfate and by a Lossen-type rearrangement into an isothiocyanate. Under weakly acidic conditions or in the presence of Fe²⁺, a nitrile and elemental sulfur are produced.

Biosynthetic studies have shown that the R groups of the glucosinolates are derived from amino acids by a common pathway (Scheme III), involving loss of the carboxyl group, stepwise oxidation of the amino group to an aldoxime, *aci*-nitro compound, reaction with a thiol anion, which exchanges its R group with glucose, and finally reaction of the oxime with sulfate. In benzylglucosinolate, for example, it could be shown that the carbons and nitrogen of L-phenylalanine, except for the carboxyl carbon, were incorporated into benzylglucosinolate (Scheme IV).

**The Final Four**

Even though our knowledge and understanding of the biosynthetic origin of some of the compounds which we have discussed are far from complete, all of them, with the exception perhaps of a few cyano and thiocyanato compounds, fall into well-defined categories. There are a few compounds that are not readily categorized, which will be discussed next.

Emerin (43) is the cyano analog of the dimethyl ether of xanthocillin (1). It was isolated in 1975 from *Aspergillus nidulans*,[53] and despite an intriguing relationship to xanthocillin (1), it appears to have received no further attention. Admittedly, the two compounds are products of two different microorganisms, *Penicillium* sp. and *Aspergillus* sp. Yet the occurrence of an isocyano function in one and a cyano function in the other, with both skeletons being those of tyrosine, invites comparative biosynthetic studies that might shed new light on the origin of the isocyano-cyano functions. This lacuna may well have been caused by the unspectacular biological activity of emerin. As is often the case, chemical studies are driven by a bioactivity engine.

While emerin (43) and xanthocillin (1) are produced by different organisms yet are close chemical relatives, bursatellin (44) is a single compound with a cyano group and a latent isocyano derivative in the same molecule. Bursatellin was first isolated by Gopichand and Schmitz[54] from the sea hare *Bursatella leachii pleii* collected in Puerto Rico. From the mantle of the animals, the Oklahoma researchers isolated an aromatic compound, bursatellin, to which structure 45 was assigned. It was an unprecedented dicyano compound, only the second such natural product, the other being emerin (43).

43     44

45     46
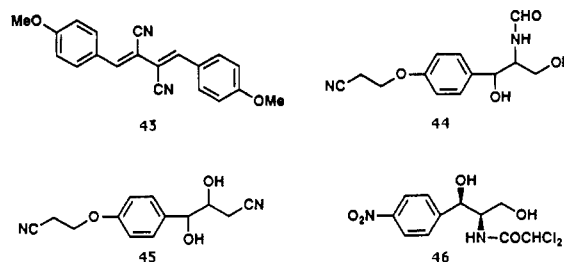
Subsequently, Cimino et al.[55] collected two Mediterranean species of *Bursatella*, *B. leachii leachii* and *B. leachii savignyana*. From the mantles of both species and from the digestive glands of *B. leachii savignyana*, the Italian workers extracted a compound with spectral properties nearly identical to those reported earlier.[54] Comparison and reinterpretation of the earlier data led to revised structure 44.[55] While the earlier formulation 45 bears no obvious relationship to an amino acid precursor, the new representation 44 contains the elements of tyrosine. It is formylated at the amino nitrogen and the phenolic oxygen is alkylated with propionitrile, thus incorporating the elements of cyano and isocyano in one molecule. The authors[55] note that the bursatellin side chain is reminiscent of the microbial metabolite chloramphenicol (46), which advantageously became the starting compound for a bursatellin synthesis.[56]

Since natural products from sea hares, which are herbivorous opisthobranch mollusks, reflect their algal diet, it was of interest to learn of the dietary habits of

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*Bursatella* spp. A 1982 report[57] on the foraging strategy in the sea hare *Bursatella leachii* was uninformative.

More informative, though not necessarily helpful, is a Ph.D. dissertation by Paige.[58] Paige's study found that *Bursatella* "feeds as a generalist, lapping the mud and sand surfaces and scraping epiphytes off seagrasses and algae. Its preferred foods include species of blue-green algae from the family Oscillatoriaceae that are common inhabitants of shallow water, mud and grass communities all around the Gulf of Mexico." This explains our finding[59] that collections of *Bursatella* from Puerto Rico and Florida and of blue-green algae from two locations in Florida failed to show traces of bursatellin (44), thus short-circuiting for the time a biosynthetic study of this compound which incorporates cyano and cryptic isocyano functions in one molecule.

We have recently[60] encountered in a Verongid sponge a cyano compound, 15-cyanopuupehenol (47), which is not related to tyrosine. It is in fact an HCN adduct of



47
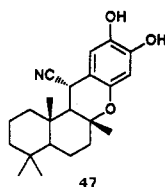
the known[61] puupehenone, from which it can be generated in the laboratory by HCN addition at 0 °C.[62] Puupehenone belongs to a well-recognized class of sponge constituents constructed of a sesquiterpene—usually a drimane—and a C_6 shikimate moiety. However, these compounds have not previously been found

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in Verongid sponges.[41] Adding to the dichotomy is the fact that the sponge, in addition to the puupehenones, does contain substantial concentrations of typical dibromotyrosine derivatives.[62] When the freshly collected sponge is broken apart, hydrogen cyanide can be detected by its odor and by conventional qualitative analytical procedures.[62] We have not yet discovered the HCN generator.

Finally, there is another set of compounds that does not follow a general pattern: the long-chain aliphatic isothiocyanates which we reported a few years ago from a Fijian sponge, *Pseudaxinyssa* sp.[63] All of them are $\alpha,\omega$-functionalized, and they are unaccompanied by isocyanides or formamides. The three types, 48–40, differ by the number of double bonds, by chain length, and in the series 50 by a formyl instead of an isothiocyanate terminus. These compounds represent a different and unknown biogenetic pattern.
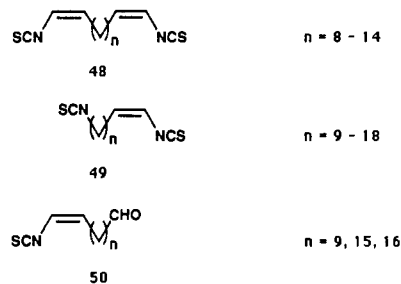


**Concluding Remarks.** Isocyano- and cyano-bearing natural products occur on land and in the ocean. The major terrestrial representatives, cyanogenic glycosides, cyanolipids, and glucosinolates, have been well studied, and only a few puzzles remain. Among the marine representatives the situation is reversed: our understanding of their biogenesis is spotty and rudimentary. They offer many opportunities for rewarding research.

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