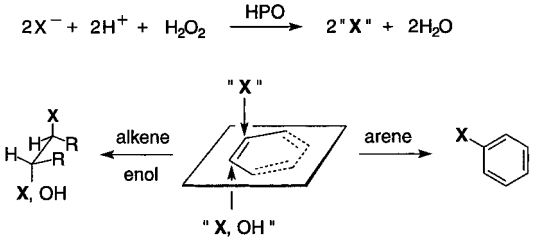
The Biosynthesis of Barbamide—A Radical Pathway for "Biohalogenation"?

Jens Hartung*

In natural product research naturally occurring organohalogens have evolved within the last few years from exotics to firmly established compounds—and there are good reasons for this! Halogenated organic compounds occur widely in the biosphere, they are structurally diverse, and some of these compounds are formed in significant quantities. Fluorine, chlorine, bromine, and iodine, the four most important halogens, exist in natural products which act as numerous important physiological agents, for instance as thyroid hormones, as repellents, and as toxins.[1] Halogens are deposited in nature as minerals or are dissolved as negatively charged ions in oceans. They can be activated from these sources by organisms for biosynthetic purposes. A multitude of so called "biohalogenations" is achieved by haloperoxidases (classification number for haloperoxidases: EC 1.11.1.X, halide: hydrogen peroxide oxidoreductases; for example, chloroperoxidase: EC 1.11.1.10). Haloperoxidases are enzymes that utilize hydrogen peroxide as an electron acceptor. According to the maximally oxidizable halide they are subdivided into chloroperoxidases, bromoperoxidases, and iodoperoxidases. Some haloperoxidases require iron porphyrin complexes or peptide-bound vanadium(v) as cofactors, while others function without transition metal catalysts.[2] In general, haloperoxidases allow the activation of halides by hydrogen peroxide and their preferential incorporation into substrates with electron rich C–C double bonds (Scheme 1).

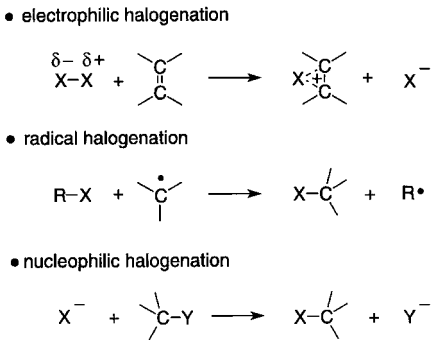


Scheme 1. Haloperoxidase reaction: enzymatic oxidation of halides by hydrogen peroxide and the incorporation of the halogen in a substrate with electron-rich C–C double bonds. X = Cl, Br, I. HPO = haloperoxidase. "X" = $^1/_2X_2$ or X^+ in HOX.

[*] Dr. J. Hartung
Institut für Organische Chemie der Universität
Am Hubland, D-97074 Würzburg (Germany)
Fax: (+49) 931-888-4606
E-mail: hartung@chemie.uni-wuerzburg.de

The oxidational power of haloperoxidases is solely dependent on the nature of the active centre. Chloroperoxidases of all three types (iron-porphyrin-containing, vanadium-dependent, and transition metal-free) are known.[1, 2] In spite of intensive research, the nature of the halogenating species in haloperoxidase reactions is not fully established. Experimental results point to elemental halogens or hypohalite (for example, hypobromous or hypochlorous acid) in a free or coordinatively bound form as the halogen source in "biohalogenations".[1–3] However, the formation of monohalogenomethanes from *S*-adenosyl methionine,[4] as well as reactions of NADH-dependent halogenases,[5] seem to be easier to rationalize because such processes lead to the incorporation of halides into organic residues through nucleophilic substitution. It is likely that the last mentioned methods also account for the synthesis of fluorometabolites in vivo, because elemental fluorine or reagents that contain positively polarized fluorine cannot be generated under physiological conditions.[6]

Laboratory synthesis takes profit from three mechanisms for halogenation: electrophilic, radical, or nucleophilic processes (Scheme 2). In terms of mechanistic considerations,



Scheme 2. Mechanisms for the synthesis of organohalogen compounds.

electrophilic halogenations correspond to haloperoxidase reactions. Alternatively, well elaborated free radical halogenation methods became available in the last few years and can be efficiently applied in synthesis.[7] Nucleophilic incorporation of halogens into target compounds can be achieved by substitution reactions that utilize halides as nucleophiles.

New results obtained by Gerwick and co-workers indicate, however, that the picture that has been drawn about "biohalogenation" may not yet be complete.[8] The authors

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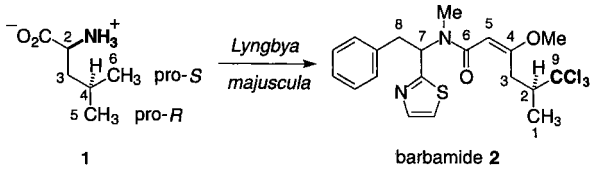
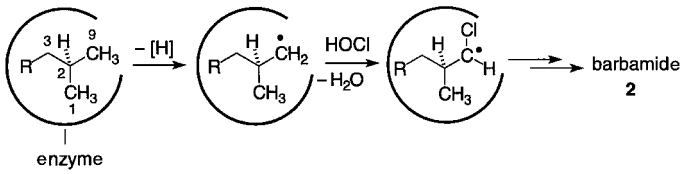
studied constituents of a species of cyanobacteria. These organisms are photosynthetically active procaryontic blue green algae that lack cellular organelles. Before their studies, cyanobacterial metabolites attracted attention as a result of the tricyclic guanidines, cyclic peptides, and a large number of structurally diverse isonitriles that were isolated from these organisms.[9] Gerwick and co-workers, however, purified a trichloromethyl substituted metabolite barbamide (**2**) from a lipophilic fraction of extracts of cyanobacterium *Lyngbya majuscula*. This result afforded valuable information about the symbiosis between sponges and cyanobacteria.

Further, by using isotopically labelled precursors the authors were able to show that carbon atoms 1–4 and 9 of the lipophilic moiety of **2** originate from the amino acid L-leucine (**1**) and correspond to atoms 2–6 (Scheme 3). The



Scheme 3. Biosynthesis of barbamide (**2**) from L-leucine (**1**).

carbon atom of the carboxylic acid group of **1** is not incorporated into **2**. The C5 and C6 atoms in **2** originate from an acetate molecule.[10] While determination of the absolute geometry at C7 is still under investigation, the 2-*S*-configuration at C2 was unambiguously determined. Gerwick and co-workers prepared two stereoselectively labelled amino acids **1** in order to determine which methyl group from **1** is transformed in the course of the biosynthesis of **2** into a trichloromethyl group. The first sample contained a ^{13}C label at the pro-*R*-methyl group, the second one at the pro-*S*-methyl substituent. These precursors were fed in two parallel sets of experiments over a certain period of time to culture media of *L. majuscula*. After ten days the organisms were harvested and metabolite **2** was isolated from both batches. Results from ^{13}C NMR spectroscopy surprisingly indicated that only the pro-*S*-methyl group of **1** is chlorinated in the course of the barbamide synthesis! By what mechanism could such a chlorination reaction take place? The feeding of perdeuterated **1** afforded a new labelled product **2**. ^{2}H NMR spectroscopy indicated that the ratio of deuterium in position 3 and 2 taken together with respect to the non-chlorinated perdeuteromethyl group C1 was 2.77:3.00. These experiments seem to rule out any oxidation reactions of **1** or derivatives thereof in the course of catabolism of **1**. In other words: no temporary C–C double bond should be formed between C2 and C3 in the course of the barbamide synthesis. These findings however led to new questions: Which enzyme in *L. majuscula* is able to stereoselectively chlorinate a nonactivated methyl group? Does this generally rule out a common electrophilic chlorination reaction using a chloroperoxidase? Gerwick and co-workers proposed a free-radical chlorination for this step of the biosynthesis without going further into details of this reaction. At a closer look it becomes clear that such a radical chlorination must be exceptional in two ways. The required stereoselectivity of hydrogen abstraction from **1** or a derivative thereof is the first issue and the energetics of this process the second. If free-radical chlorination of 2-methylbutane were taken as an example for the chlorination that leads to barbamide (**2**) it is clear from bond dissociation energies[6] that hydrogen abstraction from tertiary hydrogen bonds ($BDE_{C-H_{tert}} = 404 \pm 2\ kJ\,mol^{-1}$) is favored over homolytical cleavage of primary C–H bonds ($BDE_{C-H_{prim}} = 423 \pm 2\ kJ\,mol^{-1}$). A simple example illustrates the problem. In the gas-phase chlorination of 2-methylbutane by elemental chlorine at $T = 300\,^{\circ}C$, the chlorine atoms attack the tertiary C–H bond of the substrate 4.4 times faster than the C–H bonds from methyl groups (namely 5-H or 6-H in **1**). A hypothetical decrease of the reaction temperature from $T = 300\,^{\circ}C$ to physiological conditions will even further increase the preference for abstraction of tertiary hydrogen in 2-methylbutane that corresponds to 4-H in **1**. [1,2] Migrations of atoms via radical intermediates are forbidden reactions because they are 3-center-3-electron processes. Thus, the pro-*S*-methyl group should also be the location of the initial hydrogen abstraction. The answer to the question of the chlorination mechanism that is part of the biosynthesis of **2** remains to be uncovered by new decisive experiments. This topic however is reminiscent of the principle of negative catalysis by enzymes.[11] This principle states that a pathway of a reaction between an organic compound and a reactive intermediate in solution can be completely altered by the binding of the substrate to an enzyme. If applied to the biosynthesis of **2** the first step of the chlorination reaction should be the binding of a biosynthetic precursor of **2** to an enzyme in such a way that the pro-*R*-methyl group and the tertiary hydrogen are sterically shielded. Only the pro-*S*-methyl group would then be exposed to attacking reagents (Scheme 4). If hypochlorous acid (chloroperoxidase?) is generated in the vicinity of the coordinated



Scheme 4. Proposed pathway for the stereoselective chlorination of a barbamide-precursor using free radical chemistry.

substrate, spontaneous or induced homolysis of the weak O–Cl bond in HOCl ($BDE_{HO-Cl} = 251 \pm 13\ kJ\,mol^{-1}$)[6] would afford chlorine atoms and hydroxyl radicals. Further, only the hydrogen atoms of the nonshielded methyl group would be available for hydrogen abstraction. The newly formed alkyl radical would be trapped by additional HOCl to afford a carbon–chlorine bond and a hydroxyl radical. The latter would have to be detoxified or could be used for further hydrogen abstraction from the CH_2Cl group.

The challenge for further studies in this field of research will be to verify the radical nature of intermediates in the chlorination reaction in the biosynthesis of barbamide (**2**) by using mechanistic probes and to answer the following

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questions: Which chlorinating enzyme is available in *L. majuscula* and which cofactor is required? Will other amino acids except **1** be suitable substrates for a stereoselective free-radical "biochlorination"? If these assumptions were confirmed, a second enzyme-induced radical process besides coenzyme B_{12} mediated reactions would have been uncovered. The new halogenation reaction could have a significant influence on modern radical chemistry comparable to the role of the alkyl cobalamines,[11] and should stimulate important new research in the field of stereo- and regioselective free radical chemistry.

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Monovalent Group 13 Organometallic Compounds: Weak Association to Monomeric, Versatile Two-Electron Donors**

Ramaswamy Murugavel* and Vadapalli Chandrasekhar*

Dedicated to Professor S. S. Krishnamurthy on the occasion of his 60th birthday

There is currently an immense enthusiasm among chemists to stabilize low-valent, multiply bonded compounds containing low-coordinate elements from Groups 13, 14, and 15. Especially the last few years have been an exciting period in which reports concerning the synthesis of several unusual compounds have appeared in the literature. To name a few, a) a tetrasila-1,3-butadiene,[1a] b) the first silaarene,[1b] c) compounds with unsupported Bi=Bi[2a] and Sb=Sb double bonds,[2b] d) silylium cations,[3a] e) a cyclotrigermenium cation with a 2π-electron system,[3b] f) an Al_{77} cluster ion with concentric spheres of Al atoms,[4] g) compounds containing a discrete metal – chalcogen double bond such as Sn=Se,[5] and h) multiply bonded species involving gallium[6] have been discovered.

[*] Dr. R. Murugavel
Department of Chemistry
Indian Institute of Technology
Powai, Bombay-400 076 (India)
Fax: (+91) 22-578-3480
E-mail: rmv@chem.iitb.ernet.in
Prof. Dr. V. Chandrasekhar
Department of Chemistry
Indian Institute of Technology
Kanpur-208 016 (India)
Fax: (+91) 512-597436
E-mail: vc@iitk.ac.in

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During this period another exciting facet of main group chemistry has been unraveled, and there have been important developments in the stabilization of monovalent Group 13 organometallic compounds. These efforts have resulted in the isolation of compounds of the type M^IR (M = Group 13 metal), which, while being monomeric in the gas phase or in solution, show interesting aggregation properties in the solid state. Recently in an important breakthrough Schnöckel and co-workers have shown that although [GaCp*] is hexameric in the solid state, the Ga ··· Ga distances are very long and only weakly influence the aggregation.[7] Instead, these clusters are believed to be formed as a result of van der Waals interactions of the organic envelope around the metal. In another development, Power, Niemeyer, and Haubrich have succeeded in preparing examples of unassociated Group 13 monoalkyl compounds that exist as monomers in the solid state.[8] Continuing their studies on the use of In^I compounds as ligands, Uhl et al. have reported an unusual Ni^0 complex bearing only monomeric indium moieties as ligands.[9] Also Jutzi and co-workers have prepared a variety of metal carbonyl clusters containing monomeric [GaCp*] groups as terminal as well as bridging ligands.[10] This article is aimed to highlight these new results and to provide a brief summary of other important results in Group 13 M^I – organometallic chemistry obtained recently.

While subvalency is common among the inorganic and organometallic compounds of heavier Group 13 elements

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