FEATURE ARTICLE

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Cyclopeptide alkaloids: chemistry and biology

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Recent progress in the synthesis and investigation of the biological activities of cyclopeptide alkaloids is reviewed. New strategies have been devised to overcome some of the synthetic challenges inherent in the formation of strained paracyclophanes. However, issues remain which offer opportunities for the application of catalytic enantioselective organometallic reactions. Members of this class of natural products have been isolated from various parts of a wide variety of plants and researchers will likely continue to show great interest in their formation and function. The biological properties of certain members of this class warrant further investigation. To gain additional insight into these areas, continuing development of synthetic methodology will be essential.

The cyclopeptide alkaloids are natural products that have been isolated from the leaves, stem bark, root bark, and seeds of a wide variety of plant species throughout the world. They are distinguished by their structural similarity and possess a 13, 14, or 15-membered cycle containing an aromatic ring. The remainder of the macrocycle consists of a peptide unit which is connected to the benzene ring in either a 1,4 or a 1,3-orientation. A system of nomenclature has been developed which classifies cyclopeptide alkaloids by the number of amino acid constituents (four or five) and the size of the macrocycle (Fig. 1). The vast majority of these compounds possess a styrylamine unit as well as an alkyl-aryl ether, but exceptions to this generalization do exist (members of the pandamine or mucronine classes). Cyclopeptide alkaloids possessing a 14-membered macrocycle represent the largest subgroup of natural products and have been the focus of the majority of research in the area.

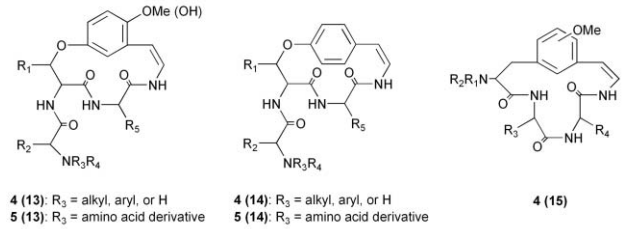

Fig. 1 The generalized structures of the cyclopeptide alkaloids.

Madeleine M. Joullié was born in Paris, France, and raised in Brazil. She received her B.Sc. from Simmons College in Boston and her M.Sc. and Ph.D. degrees from the University of Pennsylvania under the guidance of Professor Allan R. Day. Following her dissertation, she joined the faculty at Penn, where she is currently the Class of 1970 Professor of Chemistry. Her research interests are in the areas of heterocyclic, medicinal, and natural products chemistry.

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The adouetines and zizyphines (Fig. 2) represent the first reported members of this class of natural products and were described independently by Menard and Goutarel over forty years ago.[1,2] Nevertheless, isolation of new compounds remains an active field of research. To date well over two hundred structures are known, including thirty-one structures that have been described[3–16] since publication of the most recent comprehensive review on the subject.[17] The present discussion will describe recent reports related to the biological properties and syntheses of these compounds.

Biological activity

The biological properties of cyclopeptide alkaloids, as well as their physiological role within plants, have been the subject of extensive investigation. This is due in part to the fact that a number of compounds have been isolated from plant species which have historically been used for medicinal purposes for treatment of a variety of ailments. Literature reviews which include descriptions of the sedative, antibacterial, and antifungal activities of these compounds are available.[17–20]

The most investigated group of cyclopeptide alkaloids with respect to their sedative properties has been the sanjoinines. Han and co-workers have identified these agents as some of the principal components responsible for the sedative activity of plants of the *Zizyphus* species which have found use in traditional Chinese herbal medicine as treatments for insomnia.[21] More recent work by this group has provided a possible mechanism of action. Naturally occurring sanjoinine A (also known as frangufoline) and sanjoinine G2, along with synthetically derived sanjoinine AH-1 and sanjoinine A dialdehyde, are reported to be effective inhibitors of calmodulin-induced activation of Ca^{2+} ATPase. In addition, sanjoinine D was shown to act as an inhibitor of calmodulin-induced activation of phophodiesterase.[22] Notably, the extent to which the four above compounds inhibited calmodulin-induced activation of Ca^{2+} ATPase was found to correlate well with their sedative properties. Studies by Lee and co-workers have also shown that the 13-membered cyclopeptide alkaloids paliurines A and F

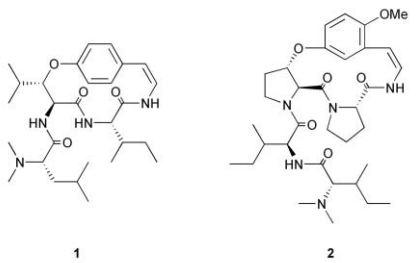

Fig. 2 The structures of adouetine-X (**1**) and zizyphine-A (**2**).

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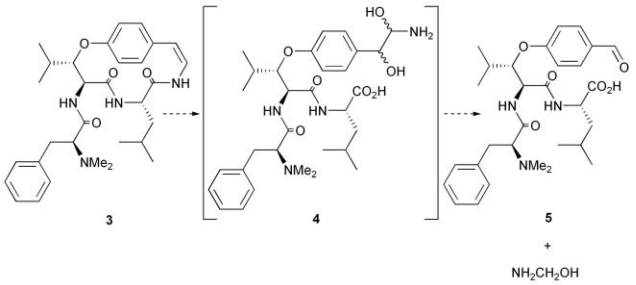
acted as sedatives in a mouse model. The closely related nummularine H, however, acted as a stimulant under identical conditions and resulted in shortened periods of induced sleep,[12] indicating the significant effect of minor structural modifications. The same researchers reported that the paliurines possessed immunostimulant activity.[10]

Examination of the antibacterial properties of a number of 14-membered compounds has recently been conducted by Morel and co-workers.[15] The results of these inhibition studies showed that condaline A and scutianine B limited growth of three Gram positive and three Gram negative bacteria to relatively equal degrees at the microgram dosage level. However, the related 14-membered compounds adouetine Y′ and scutianine C showed no activity. Though significantly more work will be required for development of an SAR profile, the compounds that showed activity possessed two phenylalanine moieties, while those which were inactive contained one phenylalanine and one isoleucine constituent. The issue is complicated by the fact that scutianine C was reported to possess activity against another strain of Gram positive bacteria, *Bacillus subtilis*.[23]

The ecological role of cyclopeptide alkaloids in plants remains unexplained. A study on the insecticidal properties of the vignatic acids showed that they possessed only minor activity.[4] In addition, the gene responsible for protection against a certain species of bean weevil was shown to be distinct from that which encodes vignatic acid production. The conclusion was therefore made that while it is possible these compounds may contribute to this defense, they are not the primary agents responsible for this property.

Cyclopeptide alkaloids may function as ionophores in plants and may be involved in the process of nutrient and metal absorption. Based upon data obtained by analysis of circular dichroism spectra, frangulanine demonstrated selective binding for rubidium and potassium ions over sodium or lithium.[24] In another study by Rapoport and co-workers, the natural product ceanothine B exhibited binding with Mg^{2+}, Ca^{2+}, and lithium but not sodium.[25] Both compounds possess 14-membered rings and β-hydroxyleucine moieties adjacent to the aromatic system. The recently isolated ramosines and hemsines, which contain β-hydroxyproline or β-hydroxyphenylalanine at these positions, have shown no apparent interaction with sodium, magnesium, calcium, or potassium.[16] Analysis of a wider variety of natural products will be required to definitively establish ion-binding trends.



Scheme 1 Proposed metabolic pathway of frangufoline in rodents.

Recent progress has been reported in the study of the metabolism of the cyclopeptide alkaloid frangufoline (**3**). Cleavage of the macrocycle has been observed both *in vitro* and *in vivo* in rodents to produce a linear compound containing a benzaldehyde and carboxylic acid (**5**) (Scheme 1).[26] It was proposed that this reaction occurs *via* concerted enamide oxidation and amide bond cleavage. This data represents the first metabolic study of this large class of compounds. The same authors demonstrated that the analogous reaction may be promoted by chemical methods by mild heating with aqueous hydrochloric acid under an air atmosphere.[27] It is of note that in many synthetic routes, the point of attachment of the side chain to the macrocycle was often protected by a carbamate that required treatment under acidic conditions for removal. The yields of this step were consistently low, which may be explained by this recent observation. Modification of the deprotection protocol has been successful in improving reaction efficiency.[28]

Synthetic chemistry

Synthetic chemistry related to the cyclopeptide alkaloids has also seen significant advancements through the late 1990s and the early part of this decade. As will be discussed, the first syntheses of four natural products have been reported during this period—only five

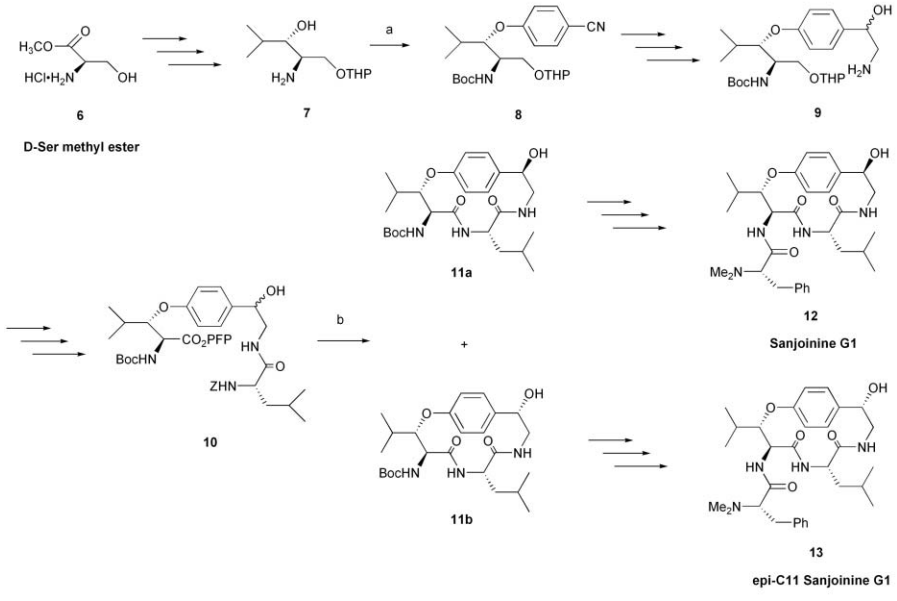
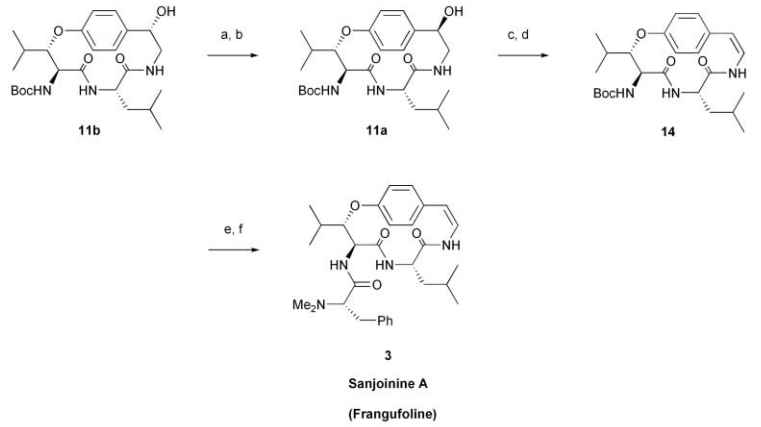


Fig. 3 Summary of the Joullié synthesis of sanjoinine G1 and epi-C11 sanjoinine G1.



Reagents and Conditions: a) Dess-Martin reagent, CH_2Cl_2, 87%; (b) BH_3•THF, (R)-Me CBS catalyst, THF, -78 °C to RT, 96%; c) 2-nitrophenylselenocyanate, Bu_3P, THF; (d) H_2O_2, pyr., CH_2Cl_2, 68% for two steps; (e) TMSOTf, 2,6-lutidine, CH_2Cl_2; (e) *N,N*-dimethylphenylalanine, HATU, K_2CO_3, DMF, 60% for two steps.

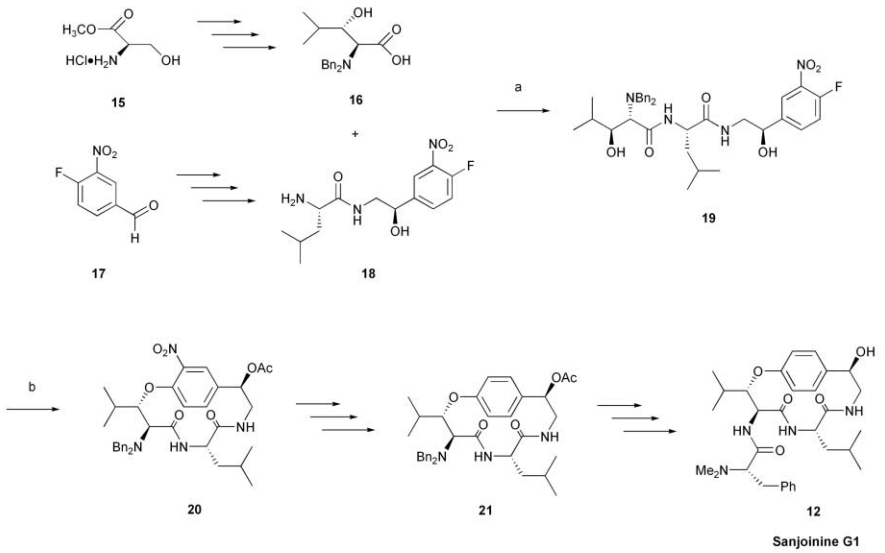
Scheme 2 The synthesis of sanjoinine A by Joullié and co-workers.

compounds (zizyphine A,[29] mucronine B,[30] frangulanine,[31] sanjoinine G1,[32] and nummularine F[33]) had been produced prior to this time. The primary synthetic challenges that must be overcome in such an endeavor are formation of the alkyl-aryl ether, introduction of unsaturation, and macrocyclization.

Among the most studied of these compounds have been the 14-membered ring sanjoinines. Since the first synthesis of sanjoinine G1 by Han and co-workers,[32] two other synthetic routes to this molecule have been described. In 1998, the Joullié group reported the production of this alkaloid from D-serine methyl ester (**6**) (Fig. 3).[34] From the amino acid starting material, amino alcohol **7** was prepared by appropriate protection and treatment with Grignard reagent, utilizing a known method.[35] Formation of the alkyl-aryl ether (**8**) was accomplished by S_NAr reaction with 4-fluorobenzonitrile. The resultant nitrile group was then reduced to the benzaldehyde, treated under Henry conditions, and reduced to provide the requisite amine (**9**). Following elongation to the linear precursor (**10**), cyclization to the macrocycles (**11a,11b**) was achieved using the Schmidt procedure[36] by hydrogenation of a benzyl carbamate in the presence of a pentafluorophenyl ester. At this stage, the epimeric benzylic alcohols could be efficiently separated by chromatographic methods. Removal of the *tert*-butyl carbamate, followed by BOP-mediated coupling to *N,N*-dimethylphenylalanine, completed this route and resulted in efficient formation of both sanjoinine G1 (**12**) and its C-11 epimer (**13**).

The synthetic route summarized above also allowed for formation of sanjoinine A, or frangufoline (**3**), a related cyclopeptide alkaloid possessing the styrylamine moiety (Scheme 2).[28] Joullié and co-workers utilized both epimers of the macrocyclic alcohols **11a** and **11b**. While the β-epimer underwent selenenylation, oxidation, and elimination to the unsaturated system **14** in good yield, reaction of the α-epimer led to only trace amounts of product. Therefore, the α-epimer was oxidized with Dess–Martin reagent and enantioselectively reduced using the Corey–Bakshi–Shibata chiral oxazaborolidine catalyst[37] to yield the reactive β alcohol **11a**. Efficient conversion of the styrylamine derivative to the free amine required *in situ* generation of the silyl carbamate by treatment with TMSOTf. Coupling of the side chain led to the isolation of sanjoinine A (**3**).

The strategy of Zhu and co-workers was successfully applied to



Reagents and Conditions: a) (i) EDC, C_6F_5OH, CH_2Cl_2; (ii) DMF, 60 °C;
b) TBAF, DMSO, 85 °C, then Ac_2O, Et_3N, DMAP, CH_2Cl_2, 45 %.

Fig. 4 Summary of the Zhu synthesis of sanjoinine G1.

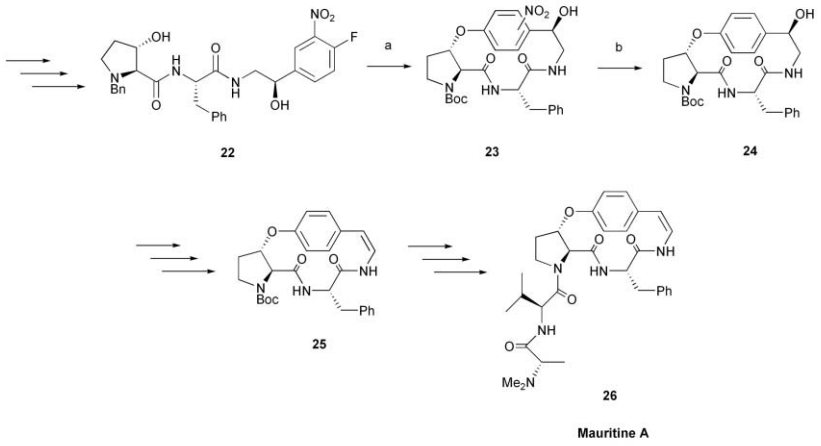


Fig. 5 Summary of the Zhu synthesis of mauritine A.

the concise assembly of sanjoinine G1 (Fig. 4).[38,39] (2*S*, 3*S*)-*N,N*-Dibenzylhydroxyleucine **16** and the aryl fluoride derivative **18** were prepared from D-serine[35] (**15**) and 4-fluoro-3-nitrobenzaldehyde[5] (**17**) respectively. Coupling of these two fragments was accomplished by way of the pentafluorophenyl ester to yield a linear precursor (**19**). Upon treatment of this compound with TBAF in DMSO at 85 °C, the key S_NAr cyclization occurred to provide a macrocyclic compound (**20**). A two-step, one-pot reductive deamination procedure produced the disubstituted aryl macrocycle (**21**), and deprotection and side chain coupling provided the natural product.

The macrocyclization protocol developed by Zhu and co-workers was also utilized in the synthesis of the cyclopeptide alkaloid mauritine A (**26**), a 14-membered ring compound containing a hydroxyproline moiety (Fig. 5).[40] Formation of the linear precursor (**22**) was achieved by utilization of methods similar to those employed in the sanjoinine G1 synthesis. Cyclization was achieved by treatment with TBAF in DMSO to yield a mixture of nitrobenzene derivative atropisomers (**23**). Following reduction and deamination, a sequence of protecting group conversion, selenenylation, and oxidative selenoxide elimination successfully generated the requisite macrocyclic core (**25**). Deprotection and side chain coupling completed the synthetic effort.

Another approach to construction of the cyclopeptide alkaloid ring system has been developed by Lipshutz and co-workers. The concept of utilizing oxazolophanes as dipeptide equivalents in the construction of cyclophane ring systems was first proposed by this research group a number of years ago and has been used for the construction of model systems (Fig. 6).[41,42] Following elaboration to nosylate linear precursor **28**, ring closure was achieved by treatment with excess sodium hydride in THF. Exposure of the oxazole intermediate (**29**) to trifluoroacetic acid provided concomitant ring opening to the diamide and removal of the *tert*-butyl carbamate yielded the 14-membered macrocycle (**30**). More recent developments have resulted in the establishment of a method for ring closure by intramolecular Nozaki–Kishi reaction.[43] Utilization of this chemistry has produced preliminary results in which a 14-membered ring containing a benzyl alcohol (**32**) has been prepared. Though the generality and scope of this methodology remains to be explored, the route provides a novel alternative to previously developed ring closure methods.

The synthesis of two cyclopeptide alkaloids containing hydroxyproline systems, mauritine D (**33**) and amphibine E (**35**), as well as their epimers (**34**, **36**), has been recently disclosed by Han and co-workers.[44,45] The structures of these natural products and their analogs are shown in Fig. 7. Formation of the alkyl-aryl ether linkage was accomplished by application of either Mitsunobu or S_NAr reaction. Macrocyclization was achieved through a

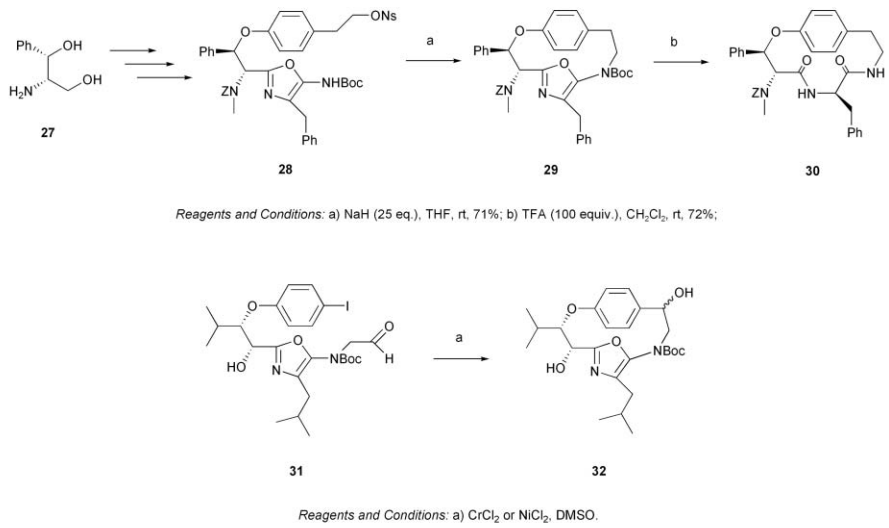


Fig. 6 Summary of the Lipshutz oxazolophane approach towards 14-membered ring cyclopeptide alkaloids.

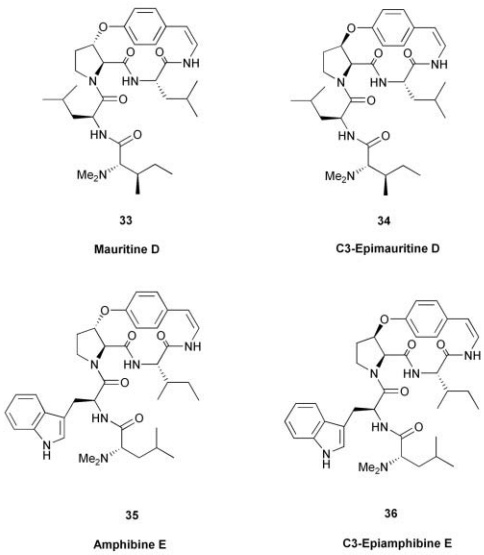


Fig. 7 Natural product cyclopeptide alkaloids and their epimers recently synthesized by Han and co-workers.

lactamization approach by derivatization of the appropriate linear precursor to its pentafluorophenyl ester or acid fluoride. Completion of the target molecules was achieved by TFFH mediated peptide coupling with the side chain carboxylic acid.

In conclusion, significant contributions to both the study of the biological properties and the synthetic chemistry of cyclopeptide alkaloids continue to be made. Despite this fact, the development of a structure–activity relationship capable of predicting the antibacterial and anti-fungal properties of these compounds remains elusive. Recent reports related to the ecological role of these natural products have yet to produce consistent theories. As is the case for many natural products, plant sources cannot provide sufficient quantities of material for extensive investigations. Additional insight into these very interesting issues will therefore be dependent upon the continuing creative contributions from a number of synthetic chemists.

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