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A simple acromelic acid analog potentially useful for receptor photoaffinity labeling and biochemical studies ☆

Kyoji Furuta,[a] Guang Xing Wang,[a] Toshiaki Minami,[b] Mikio Nishizawa,[c] Seiji Ito[c] and Masaaki Suzuki[a,*]

[a]*Regeneration and Advanced Medical Science, Graduate School of Medicine, Gifu University, Gifu 501-1193, Japan*
[b]*Department of Anesthesiology, Osaka Medical College, Takatsuki 569-8686, Japan*
[c]*Department of Medical Chemistry, Kansai Medical University, Moriguchi 570-8506, Japan*

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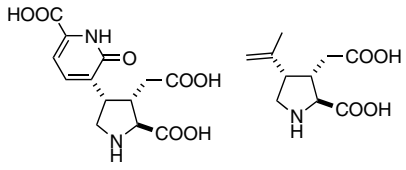
Abstract—A novel acromelic acid analog possessing an azido-functionalized phenyl group was designed and synthesized as a biochemical probe for studies on kainoid receptors. The analog exerted a biological activity equivalent to natural acromelic acid A, suggesting that both compounds possibly bind to the same target biomolecule. In order to utilize the probe in photoaffinity labeling experiments, a procedure for the introduction of radioactive iodine into the molecule was established.

Acromelic acid A (**1**) is a potent neuroexcitatory amino acid isolated from a toadstool, *Clitocybe acromelalga*, by Shirahama and co-workers[1] It belongs to a class of so-called kainoids bearing a pyrrolidine dicarboxylic acid structure represented by kainic acid (**2**).[2] The kainoids include a structure like that of glutamic acid, a major excitatory neurotransmitter in the human central nervous system, and therefore, are considered to exert their biological activities through glutamate receptors.[3] Glutamate receptors are currently classified into two categories: ionotropic and metabotropic receptors, both of which are further comprised of three and eight subtypes, respectively.[4] It has been shown that kainoids bind to and activate kainate and AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors, but not NMDA (*N*-methyl-D-aspartate) subtype among the ionotropic glutamate receptors.[3] Acromelic acid, like kainic acid, causes strong depolarization of neurons, but its in vivo behavioral and pathological effects are reportedly different from those of kainic acid,[5] suggesting the existence of distinct types of kainoids receptors. Therefore, the actual receptor for acromelic acid and its signaling pathway are yet to be determined. Neuronal damages induced by kainoids share several features with serious neuronal injuries.[6] Thus the elucidation of the molecular mechanism behind the neuro-toxicity of acromelic acid and associated receptor functions will contribute to the development of studies on serious neuronal diseases. In this letter, we present the design and synthesis of an azido-functionalized acromelic acid analog with a simplified structure and biological activities comparable to natural acromelic acid A. The compound is not only applicable to the identification of acromelic acid receptors by photoaffinity labeling technique, it may also be utilized in receptor signaling analysis as a substitute for difficult to obtain acromelic acid.



1, acromelic acid A **2**, kainic acid

In general, photoaffinity probes require a photo-activatable unit, such as azido group, and a radio- or fluorescent-detectable functional group in the structure, as well as high binding affinity for a target biomolecule. The molecular structure of acromelic acid, however, makes the design of such photoaffinity labeling probes difficult because its pyrrolidine dicarboxylic acid

Keywords: Acromelic acid; Photoaffinity labeling; Biochemical probe.
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* Corresponding author. Tel.: +81-58-293-2635; fax: +81-58-293-2635; e-mail: suzukims@biomol.gifu-u.ac.jp

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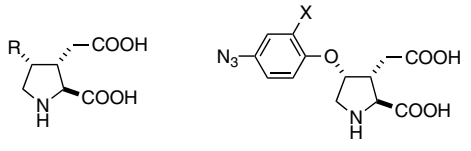
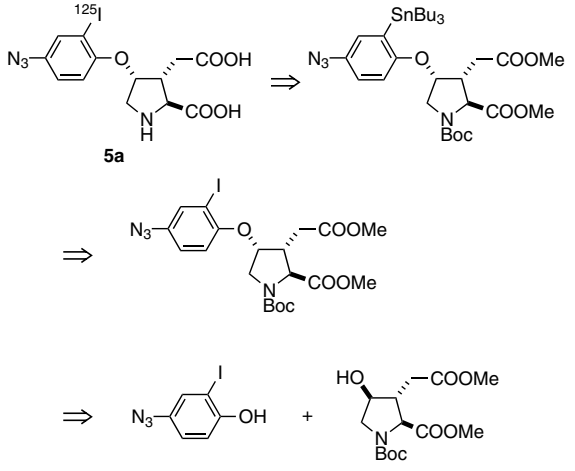
structure seems to be essential for the kainoid activity, therefore opposing further modification, and the synthetic construction of a highly functionalized pyridone ring connected directly to the pyrrolidine skeleton is not an easy task. Shirahama and co-workers, who first synthesized acromelic acid,[1b] elaborated MFPA (**3**), through precise structure–activity studies,[7] as a simplified analog with depolarization activity against neurons more potent than acromelic acid. More recently, Baldwin et al. reported the design and synthesis of 4-arylsulfanyl type analogs **4**, though their biological activities were not described.[8] Such structurally simplified compounds inspired us to design a novel azido-functionalized 4-phenoxy type analog **5a** as a photoaffinity labeling probe for targeting acromelic acid receptors. In this analog, the sulfur atom of **4** was replaced with an oxygen atom to avoid the oxidation of the sulfanyl moiety at the iodination step during radio-labeling and the synthetic facility of the azidophenoxy part, as well.



3, R = 2-MeOPh (MFPA)
4, R = ArS

5a, X = ^{125}I
5b, X = I

Our retrosynthetic pathway is illustrated in Scheme 1. For the introduction of radioactive iodine in **5a**, we planned to adopt the tin–iodine exchange reaction of an aromatic stannane via in situ oxidation of iodide anion.[9] Deprotection of the *N*-Boc group and hydrolysis of methyl esters in the resulting radio-labeled intermediate can be carried out under mild conditions without destruction of the azido group. The synthesis of the stannylated precursor is considered feasible from the corresponding iodo derivative by a palladium-catalyzed stannylation.[10] Regarding the incorporation of the azido-functionalized iodophenoxy moiety into the pyrrolidine ring, we envisioned the use of the Mitsunobu reaction of azidoiodophenol and the pyrrolidinol derivative. The protected pyrrolidinol with necessary functional groups and stereochemistries can be prepared from the known compound, 3-carboxymethyl-4-hydroxyproline.[8]

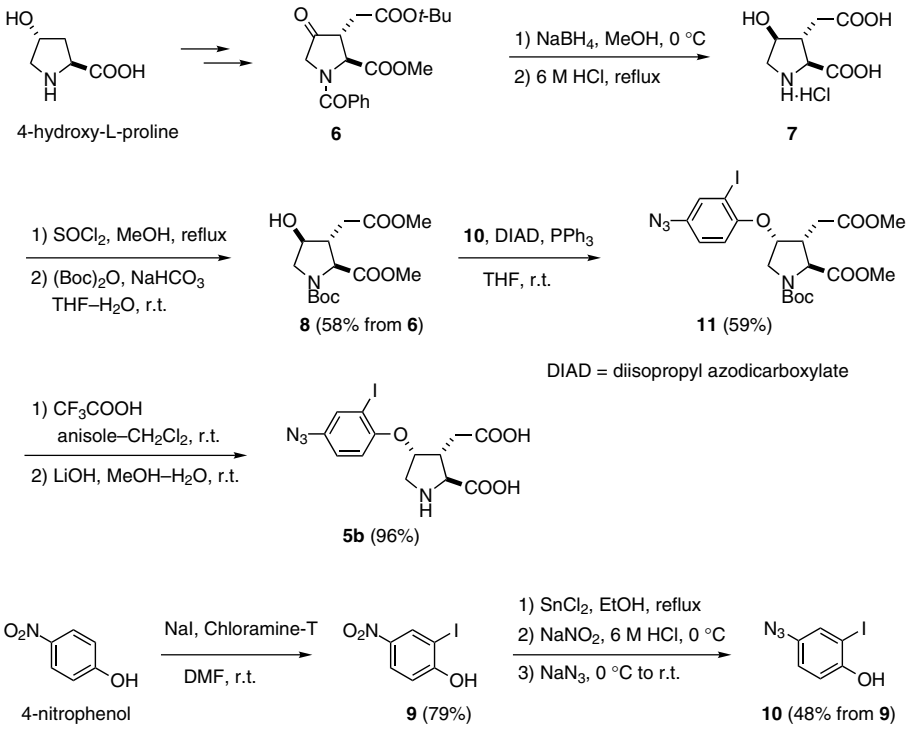


Scheme 1.

Prior to the synthesis of **5a**, we decided to prepare and evaluate the biological activities of a nonradioactive molecule **5b**. To start with, optically active 4-hydroxyproline derivative **7** was prepared from *trans*-4-hydroxy-L-proline according to the previously reported procedure[8,11] with a slight modification.[12] Subsequent esterification of the acid moieties and Boc protection of the amino group in **7** afforded the intermediate pyrrolidinol **8**. Although the protection of the amino group in the proline derivative by Boc instead of benzoyl at an early stage of this synthesis appears to be straightforward to obtain **8**, *N*-Boc protection resulted in the formation of **6** as a mixture of inseparable isomers at C-3. Accordingly, we pursued a relatively roundabout, but secure, route for the synthesis of **8** as demonstrated in the reaction scheme. The coupling partner, 4-azido-2-iodophenol (**10**), was conveniently synthesized from 4-nitrophenol by the iodination–nitro group reduction—Sandmeyer azidation sequence.

Mitsunobu coupling of **8** and the phenol derivative **10** was achieved by adding diisopropyl azodicarboxylate and **10**, in that order, to a mixture of **8** and triphenylphosphine in THF. Thus the coupling product **11** was obtained in 59% yield, as expected. The ^{1}H NMR spectrum of **11** represented the characteristic pattern of 3,4-*cis*-configuration as judged by accumulated data of analogs.[8,13] Removal of the *N*-Boc group of **11** with trifluoroacetic acid and hydrolysis of methyl esters by treatment with lithium hydroxide in methanol–water afforded the desired compound **5b** as a pale yellow amorphous solid in 96% yield, after ion-exchange chromatography and lyophilization (Scheme 2).

A preliminary bioassay of **5b** for the induction of mechanical allodynia (tactile pain)[14] was conducted as an index of the effectiveness of the designed molecule. Interestingly, acromelic acid is proved to provoke marked allodynia by intrathecal injection into the subarachnoid space of conscious mice.[15] By contrast, kainic acid does not induce allodynia. This fact strongly suggests the existence of a different signaling pathway for this biological activity, which is possibly mediated through a novel receptor specifically activated by acromelic acid.[15] Fortunately, the administration of our compound **5b** resulted in the prominent induction of allodynia at almost the same potency as acromelic acid A (Fig. 1), proving the validity of our molecular design. It seems reasonable to assume that both **5b** and acromelic acid possibly share one of the target receptors, although further biological evaluation is necessary.[16] Thus **5b** may be a powerful candidate for photoaffinity labeling probes that target acromelic acid receptors. Despite the substantial differences in structure and spatial arrangement of the phenyl ring between **5b** and acromelic acid, this complete preservation of the biological activity is particularly intriguing. While the existence of a novel acromelic acid receptor is still hypothetical, and no information about the chemical interaction between the receptor and acromelic acid



Scheme 2.

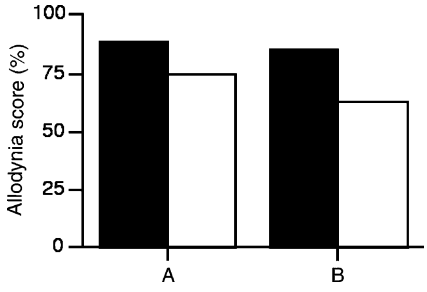
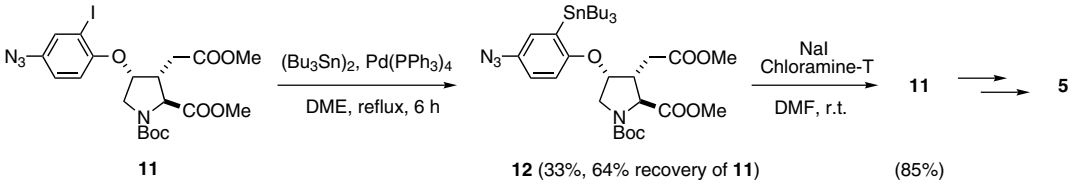


Figure 1. Induction of allodynia by acromelates. Acromelic acid A (A) or the azido analog (B) was injected (solid bar, 1 fg/kg; open bar, 10 fg/kg) into the subarachnoid space of conscious mice. Data are expressed as a percent of the maximum possible cumulative score of six mice.[15]

exists, we speculate that the phenyl ring or appropriate hydrophobic substituent at C-4 of the pyrrolidine ring plays a pivotal role for specific binding.

Considering this promising result, we next intended to establish a route for preparing radioactive probe **5a**. The synthesis started with the preceding iodide **11** (Scheme 3). Thus the palladium-catalyzed stannylation of **11** with hexabutylditin under standard reaction conditions gave the stannylated precursor **12** in 33% yield, with a 64% recovery of starting **11**. The incorporation of an iodine atom into the molecule was verified by the reaction under cold conditions. Accordingly, the stannane **12** was reacted with ICl, generated in situ from nonradioactive sodium iodide and Chloramine-T in DMF at ambient temperature, to produce the desired iodide **11** in 85% yield. Deprotection of **11** has already been described above. Consequently, the use of [^{125}I]NaI will provide the radioactive probe **5a** applicable to actual photoaffinity labeling experiments.

In conclusion, we elaborated a simple acromelic acid analog **5b** as a biochemical probe with biological activities comparable to natural acromelic acid. The compound is stable[17] and readily synthesized in large quantities. Thus, it is usable not only as a photoaffinity labeling probe, but also as a biochemical tool for acromelic acid receptor signaling analysis. The simplicity of the synthesis would allow diverse structural modification for further investigations on kainoid activities. Incorporation of ^{125}I and studies to identify the acromelic acid receptor are now in progress.



Scheme 3.

Acknowledgements

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16. Biological assays, such as the measurements of electrophysiological responses and intracellular Ca^{2+} increase in neurons, are currently underway and detailed descriptions will be reported separately.
17. The compound can be handled for routine purpose of biochemical experiments without special care under laboratory conditions, and kept for months unchanged in the refrigerator unless exposed to UV light.