

Biomimetic Synthesis of Macahydantoin A and B from *Lepidium meyenii*, and Structure Revision of Macahydantoin B as a Class of Thiohydantoin with a 4-Methyl-hexahydropyrrolo[1,2-*c*]imidazole Skeleton

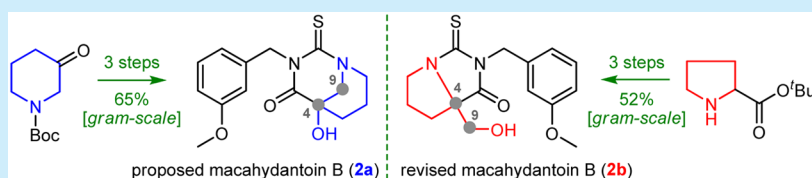
Min Zhou,^{*,†,‡,§} Hang-Ying Ma,^{†,§} Huan-Huan Xing,[†] Ping Li,[†] Gan-Peng Li,[†] Hui-Chun Geng,^{*,‡} Qiu-Fen Hu,^{*,†} and Guang-Yu Yang[§]

[†]Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission and Ministry of Education, Yunnan Minzu University, Kunming 650031, Yunnan People's Republic of China

[‡]Institute of Quality Standard and Testing Technology, Yunnan Academy of Agricultural Science, Kunming 650223, Yunnan People's Republic of China

[§]Key Laboratory of Tobacco Chemistry of Yunnan Province, China Tobacco Yunnan Industrial Co., Ltd, Kunming 650231, Yunnan People's Republic of China

S Supporting Information



ABSTRACT: Phytochemical investigation on *Lepidium meyenii* led to the discovery of macahydantoin C (3), a new thiohydantoin with a 1,3-diazabicyclo[3.3.1]nonane core, the spectral properties of which indicate a potential structural misassignment of its previously reported analogue, macahydantoin B (2a). To probe this hypothesis, a concise, scalable, and biomimetic synthesis of the originally proposed 2a and its revised structure (2b) was efficiently accomplished using the modified Edman degradation as the key step from commercially available materials in 65% (three steps) and 52% (three steps) overall yields, respectively. These synthetic endeavors undoubtedly reassigned the structure of macahydantoin B as an unreported type of thiohydantoin featuring a 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole scaffold.

Recently, (\pm)-macahydantoin A (1) and B (reported 2a), a novel class of thiohydantoin with a 1,3-diazabicyclo[3.3.1]nonane core linking to a benzyl moiety, were both isolated as racemic mixtures from the roots of *Lepidium meyenii* by Qiu and co-workers (Figure 1).¹ Their structures, including absolute

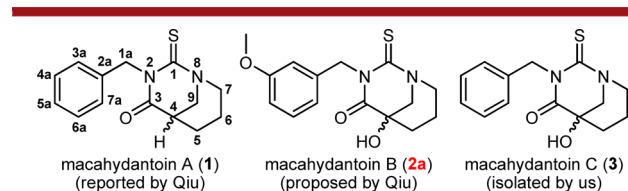


Figure 1. Structures of macahydantoin 1, 2a, and 3.

configurations, were elucidated using a combination of NMR spectroscopy and ECD calculations. Moreover, macahydantoin A was further confirmed by a five-step synthesis route with an overall yield of 23% from benzylamine. According to the close spectroscopic data, Qiu's group suggested that the structure of 2a was similar to that of 1, except for the presence of an additional methoxy group located at C-4a and one more hydroxy group located at C-4, respectively. Interestingly, in our ongoing

research for bioactive sulfur-containing derivatives from the roots of the title plant collected from the Yunnan province of China,² we also obtained a new analogue, macahydantoin C (3) (Figure 1). The only structural difference between the proposed 2a with 3 is that 3 is lacking the additional methoxy group at C-4a.

However, the respective ¹³C NMR signals belonging to their 1,3-diazabicyclo[3.3.1]nonane nucleus of 2a and 3 were distinctly different, indicating a potential problem with one of the structures. Herein, we disclose the concise biomimetic synthesis of the originally proposed structure for macahydantoin B (2a) reported by Qiu and its revised structure (2b) (Figure 2). On the basis of these findings, the published 2a with the 1,3-diazabicyclo[3.3.1]nonane skeleton has been revised to 2b possessing an unreported 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole framework.

In this study, macahydantoin C (3), a new thiohydantoin with a previously reported 1,3-diazabicyclo[3.3.1]nonane framework,¹ was isolated as a racemic mixture from the lipidic fraction

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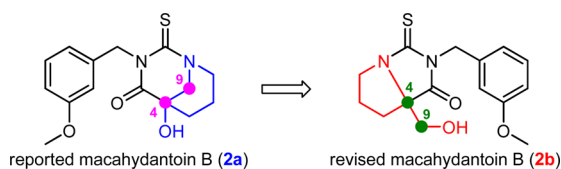


Figure 2. Structures of the published 2a and revised 2b.

of *Lepidium meyenii*. The absolute configurations of (+)-3 and (–)-3 were elucidated as 4*S* and 4*R* by comparison of their experimental and calculated ECD spectra, respectively (see Supporting Information, Figure S1). The only structural difference between 3 and 2a was the lacking of a methoxy group at C-4a of the benzyl moiety of 3. However, except for the signals of the 3-methoxybenzyl moiety and benzyl moiety in their ¹³C NMR spectra, the chemical shifts belonging to the heterocyclic moiety were significantly different (chemical shift differences up to 9.6 ppm): especially for C-1 ($\Delta\delta = -3.9$ ppm), C-4 ($\Delta\delta = +5.5$ ppm), C-5 ($\Delta\delta = -6.7$ ppm), C-7 ($\Delta\delta = -8.7$ ppm), and C-9 ($\Delta\delta = +9.6$ ppm) (Table 1). Thus, we concluded

Table 1. Comparison of the ¹³C NMR Data of the Reported Structure 2a by Qiu and Our Isolated 3 in CDCl₃ (δ ppm)

no.	2a	3	$\Delta\delta$	no.	2a	3	$\Delta\delta$
1	188.1 s	192.0 s	-3.9	2a	137.0 s	136.6 s	+0.4
3	175.6 s	174.5 s	+1.1	3a	113.5 d	128.0 d	-14.5
4	74.8 s	69.3 s	+5.5	4a	159.6 s	128.6 d	+31.0
5	27.8 t	34.5 t	-6.7	5a	113.4 d	127.6 d	-14.2
6	25.4 t	23.0 t	+2.4	6a	129.5 d	128.6 d	+0.9
7	48.6 t	57.3 t	-8.7	7a	120.3 d	128.0 d	-7.7
9	63.9 t	54.3 t	+9.6	OMe	55.2 q	-	-
1a	45.2 t	48.7 t	-3.5				

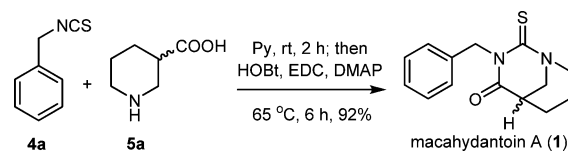
that one of their structures could be misassigned. To tackle this problem, we first proceeded to design a concise biomimetic strategy to construct the 1,3-diazabicyclo[3.3.1]nonane nucleus.

Previously, Qiu's laboratory suggested that macahydantoin A (1) and B (reported structure, 2a) might be biosynthetically derived from nicotinamide and benzoic acid derivative.¹ This hypothetical pathway involved a series of condensation, hydrogenation, thionation, and oxidation reactions. Considering the fact that natural isothiocyanates occur commonly in cruciferous plants,³ we hypothesized that the 2-thioxo-1,3-diazabicyclo[3.3.1]nonan-4-one core of macahydantoin A–C could be more smoothly constructed by a simple Edman degradation reaction^{2,4} between related isothiocyanates (4a or

4b) with piperidine-3-carboxylic acid (5a) or 4-hydroxypiperidine-3-carboxylic acid (5b) (Scheme 1).

Guided by this biosynthetic consideration, we have developed a gram-scale one-pot synthetic method (a single-step process, 92% yield) for the synthesis of macahydantoin A (1) using readily available starting materials, benzyl isothiocyanate (4a) and piperidine-3-carboxylic acid (5a) (Scheme 2). Briefly,

Scheme 2. One-Pot Method for Synthesizing Macahydantoin A



exposure of 4a to 5a in pyridine at room temperature for 2 h, followed by condensation with HOBT, EDC, and DMAP at 65 °C for an additional 6 h, provided the desired macahydantoin A (1), whose spectral data were identical in all respects to those for the natural substance.¹ This structure was further confirmed by a single crystal X-ray diffracton analysis (CCDC 1566874) (Figure 3).

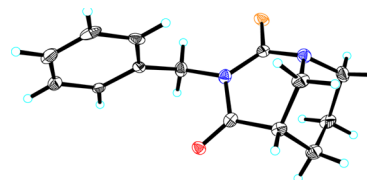
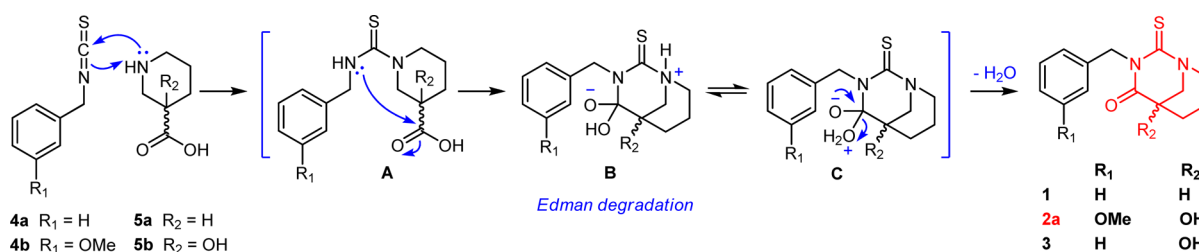


Figure 3. ORTEP diagram of synthetic 1.

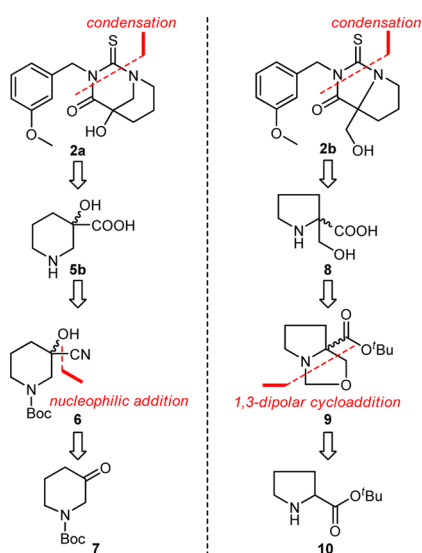
With the above-mentioned strategy in mind, we envisioned that the proposed 2a would be incorporated from 3-methoxybenzyl isothiocyanate (4b) and 4-hydroxypiperidine-3-carboxylic acid (5b) in a similar manner, as depicted in our retrosynthetic plan (Scheme 3). Further analysis indicated that the key intermediate 5b could be readily accessible by acid hydrolysis of the cyano group from cyanohydrin 6, which would in turn arise by nucleophilic addition of cyanide from commercially available Boc-protected piperidine-3-carboxylic acid (7).⁵

The preparation of the published 2a is summarized in Scheme 4. Lewis acid-catalyzed nucleophilic addition of starting material 7 with trimethylsilyl cyanide (TMSCN) in DCM afforded cyanohydrin 6, which was hydrolyzed in refluxing concentrated HCl at 85 °C for 12 h to give the desired hydroxy acid hydrochloride 5b.⁵ With the key intermediate 5b in hand, attention was turned to the key condensation reaction. After

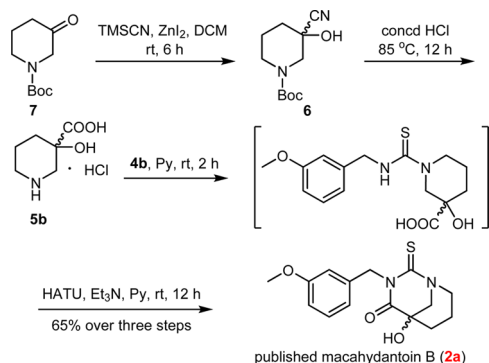
Scheme 1. Putative Biogenetic Pathway of Macahydantoin A (1), B (Reported Structure, 2a) and C (3)



Scheme 3. Retrosynthetic Plan for the Reported 2a and Revised 2b



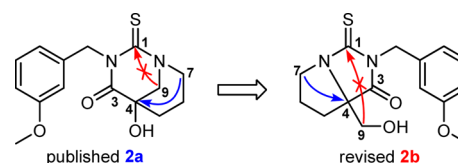
Scheme 4. Concise Biomimetic Synthesis of the Reported 2a



extensive optimization of the reaction conditions, we found that **5b** was successfully coupled with 3-methoxybenzyl isothiocyanate (**4b**) in the presence of HATU and Et₃N to provide the target compound **2a** on a gram scale (a three-step route, an overall yield of 65%).

As expected, the ¹³C NMR spectrum of the synthetic **2a** exhibited noticeable similarity to those of the naturally occurring macahydantoin C (**3**). However, the synthetic **2a** has some odd inconsistent ¹³C NMR data (chemical shift differences up to 9.6 ppm) compared to those of the published **2a** assigned by Qiu's group,¹ particularly for C-1 ($\Delta\delta = -3.9$ ppm), C-4 ($\Delta\delta = +5.5$ ppm), C-5 ($\Delta\delta = -6.6$ ppm), C-7 ($\Delta\delta = -8.6$ ppm), C-9 ($\Delta\delta = +9.6$ ppm), and C-1a ($\Delta\delta = -3.6$ ppm). Additionally, we further carefully reexamined the 2D NMR data of the published **2a** in the literature,¹ and observed the appearance of an unreasonable HMBC correlation from H₂-7 to C-4 (a four-bond correlation) and the absence of the key HMBC correlation from H₂-9 to C-1 (a three-bond correlation) (Figure 4). These observations led us to consider a radical structural revision for macahydantoin B as **2b**. Instead of the 1,3-diazabicyclo[3.3.1]nonane core as originally proposed, we hypothesized that a novel 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole skeleton (**2b**) was in fact the correct structure of the natural macahydantoin B.

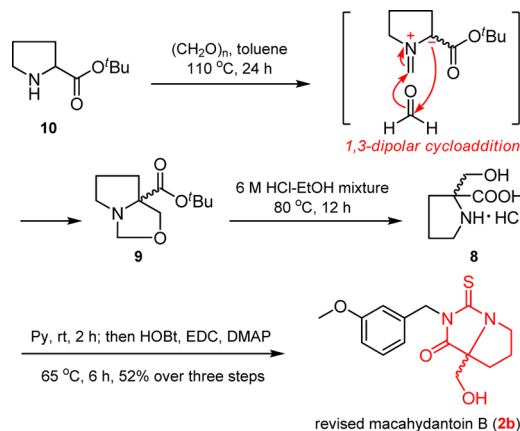
To add further evidence in favor of this assumption, we set out to synthesize the postulated structure **2b**, the retrosynthetic analysis of which is shown in Scheme 3. We rationalized that the

Figure 4. Reexamination of the key HMBC correlations in the published structure **2a** and the reassigned structure **2b**.

hexahydropyrrolo[1,2-*c*]imidazole core in **2b** would be available from α -hydroxymethylproline hydrochloride (**8**) and 3-methoxybenzyl isothiocyanate (**4b**) by means of modified Edman degradation.² In turn, intermediate **8** could be constructed from bicyclic hexahydropyrrolo[1,2-*c*]oxazole derivative (**9**), which might be readily prepared by a 1,3-dipolar cycloaddition between commercially available proline *t*-butyl ester (**10**) and paraformaldehyde.⁶

Initially, a racemic mixture of proline *t*-butyl ester (**10**) was treated with paraformaldehyde in toluene at 110 °C to afford bicyclic hexahydropyrrolo[1,2-*c*]oxazole derivative (**9**) in approximately 90% isolated yield, which was subsequently transformed into α -hydroxymethylproline hydrochloride **8** by acid hydrolysis in a refluxing 6 M HCl–EtOH mixture (Scheme 5).⁵ Without further purification, HOBT/EDC/DMAP coupling

Scheme 5. Concise Biomimetic Synthesis of the Revised 2b



of the key intermediate **8** with 3-methoxybenzyl isothiocyanate **4b** under mild conditions furnished the presumed (\pm)-macahydantoin B (**2b**) on a gram scale (a three-step route, an overall yield of 52%) (Scheme 5).

To our delight, the NMR spectral data of the revised structure **2b** were in full agreement with those reported for the natural (\pm)-macahydantoin B (Figure 5).¹ On the basis of this positive result, the absolute configurations of its enantiomers (+)-macahydantoin B and (–)-macahydantoin B were unambiguously established as 4*S* and 4*R* by computational evidence, respectively (see Supporting Information, Figure S1). This evidence strongly corroborates the hypothesis that the natural (\pm)-macahydantoin B represents a new class of thiohydantoin with a unique 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole skeleton. Biosynthetically, this novel nucleus might be formed by a combination of Edman degradation⁴ and Aldol condensation⁷ from the related proline (**11**) and isothiocyanate **4b** (Scheme 6).

Compounds **1**, **2a**, **2b**, and **3** were tested for their cytotoxic activity against a panel of human cancer cell lines using the MTT method.⁸ However, no significant activities were detected for these compounds at the concentrations up to 40 μ M.

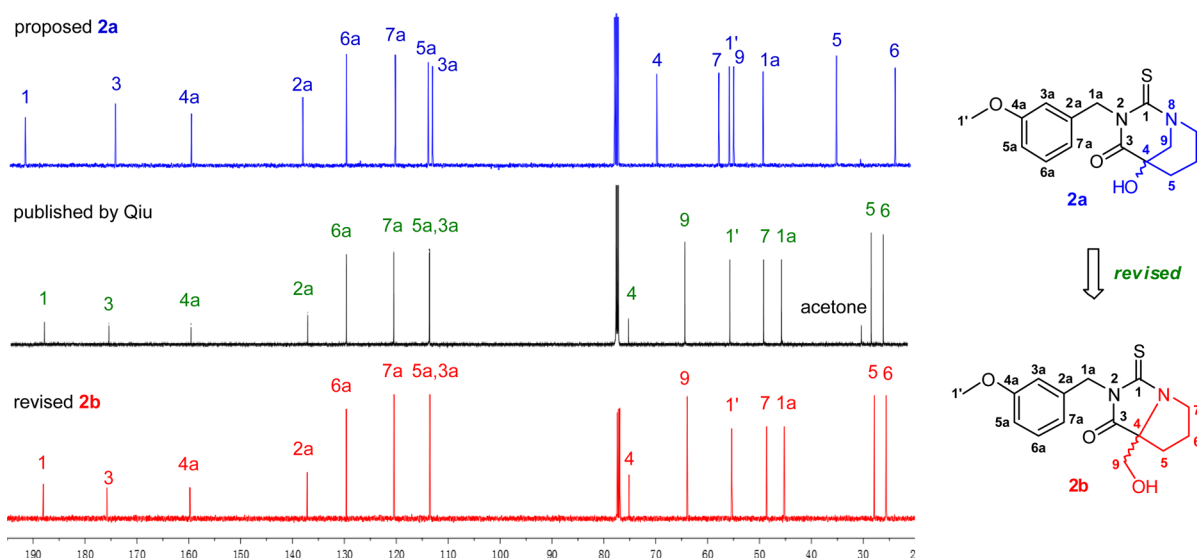
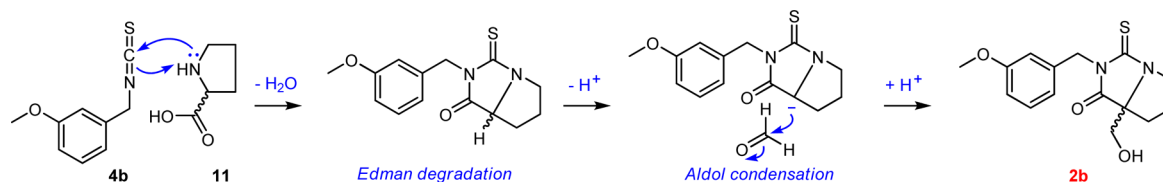


Figure 5. Comparison of the ^{13}C NMR spectra of the synthetic **2a**, the published **2**, and the synthetic **2b** in CDCl_3 (δ ppm).

Scheme 6. Putative Biogenetic Pathway of Macahydantoin B (Revised Structure, **2b**)



In summary, a concise, scalable, and biomimetic synthesis of the originally proposed structure for macahydantoin B (**2a**) and its revised structure **2b** has been efficiently achieved using commercially available materials in 65% (three steps) and 52% (three steps) overall yields, respectively. The key transformations include a 1,3-dipolar cycloaddition and an modified Edman degradation process. On the basis of their spectral properties, we strongly suggest that the published **2a** with a 1,3-diazabicyclo[3.3.1]nonane core should be revised as **2b** possessing a novel 4-methyl-hexahydropyrrolo[1,2-*c*]imidazole scaffold. We are investigating the biological function of the novel molecules. These results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.orglett.7b02433](https://doi.org/10.1021/acs.orglett.7b02433).

Experimental procedures, spectral and other characterization data of isolated or synthetic compounds (PDF) X-ray data for **1** (CIF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: zhouminynun@163.com.

*E-mail: huichungeng@163.com.

*E-mail: ygy1110@163.com.

ORCID

Min Zhou: 0000-0003-1896-9832

Author Contributions

#M.Z. and H.-Y.M. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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