A Pair of Novel Sulfonyl-Containing N-Acetyldopamine Dimeric Enantiomers From Aspongopus chinensis

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Abstract
A pair of novel sulfonyl-containing N-acetyldopamine dimer enantiomers, (±)-aspongamide E (I), a new ester 2-aminoethyl (E)-hex-2-enoate (2), along with 3 known compounds (3-5) were isolated from Aspongopus chinensis. Their structures were determined by spectroscopic methods. Compound 1 is a racemic mixture, chiral high-performance liquid chromatography separation followed by electronic circular dichroism calculations assigned the absolute configurations of 2 enantiomers of 1. Compounds 3-5 were isolated from A. chinensis for the first time. The biological activity of the selected new compounds against renal fibrosis was evaluated.

Keywords
Aspongopus chinensis, insect, N-acetyldopamine dimer, ECD, renal fibrosis

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Medicinal insect has been widely used in the clinic since ancient times. Proteins and peptides are usually considered as the major active components of medicinal animals. However, non-peptidal small molecules are increasingly characterized by insect sources. Cantharidin, a well-known small-molecule defensive substance from Mylabris (blister beetle), has been found to have potent anticancer properties.1,2 N-acetyldopamine (NADA) derivatives, known as compounds present in the cuticle, have been characterized by diverse beetles.3,4 We have become interested in small molecules from insects in recent years. As a result, structurally diverse substances have been identified from beetles such as ants, Blapt japanensis, and Periplaneta americana.5-7 We have embarked on an investigation on Aspongopus chinensis, which has been used as a traditional Chinese medicine for the treatment of chronic kidney disease, renal fibrosis, resulting in the isolation of NADA trimer, 4 adenosine analogs,8 and sesquiterpenoids,9 etc. In the continuous study of this insect, 5 compounds including a pair of novel sulfonyl-containing NADA dimer, (±)-aspongamide E (I), and a new ester consisting of a (E)-hex-2-enoic acid and a 2-aminoethan-1-ol were isolated and structurally identified (Figure 1). In vitro renal protection of these isolates in transfecting growth factor beta 1 (TGF-β1) induced rat renal proximal tubular cells were explored.

(±)-Aspongamide E (I) was isolated as a yellowish solid and was assigned the molecular formula of C21H24N2O8S on the basis of its high-resolution electrospray ionization mass spectrometry (HRESIMS) at m/z 465.1320 [M + H]+ (calcd for C21H25N2O8S, 465.1326), 13C nuclear magnetic resonance (NMR), and distortionless enhancement by polarization transfer (DEPT) spectra, having 11 degrees of unsaturation. The 1H NMR spectrum of 1 indicates an ABX aromatic proton system (δH 6.87, d, J = 8.3 Hz, H-5; δH 6.79, dd, J = 8.3, 2.0 Hz, H-6; δH 6.84, d, J = 2.0 Hz, H-8), 2 aromatic protons (δH 7.06, s, H-2; δH 7.46, s, H-5′), 2 oxygenated methines (δH 5.73, d, J = 6.8 Hz, H-2; δH 5.75, d, J = 6.8 Hz, H-3), 2 methylenes (δH 2.71, t, J = 7.2 Hz, H-9; δH 3.37, t, J = 7.2 Hz, H-10), and 3 methyls (δH 1.88, s, H-3b; δH 1.90, s, H-10b; δH 3.18, s, H-7). The 13C NMR and DEPT spectra (Table 1) display 21 carbons ascribed to 3 methyls, 2 methylenes, 7 methines (including 2 oxygenated aliphatic and 5 olefinic), and 9 nonprotonated carbons (2 amide...
carbonyls and 7 olefinic including 4 oxygenated). The NMR data of 1 are similar to those of (2R,3)-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(N-acetyl-2″-aminoethyl)-1,4-benzodioxane, indicating that they might be analogs. The only difference between them is that a methylsulfonyl group is present in 1; this is mainly due to the molecular formula of 1 and chemical shifts of H-7′ (δH 3.18) and C-9 (δC 46.2), the extra signals should be ascribed to a methylsulfonyl group, which is positioned at C-6′ by the observation of rotating frame Overhauser effect spectroscopy (ROESY) correlations of H-7′/H-5′, H-2, corresponding to the observation of 2 proton singlets (H-2′ and H-5′). For structure identification of NADA derivatives, 1 key point is to clarify whether the side chain is attached to C-6 or C-7. Weak ROESY correlations of H-8 (δH 6.84 [d, J = 2.0 Hz, H-8]) and C-7′, H-2, and an heteronuclear multiple bond correlation of H-6/C-4a, in combination

Table 1. 1H (600 MHz) and 13C NMR (150 MHz) Data of 1 in Methanol-d$_4$ (δ in ppm, J in Hz).

| No. | δH         | δC         | δH         | δC         |
|-----|-------------|------------|-------------|------------|
| 1   |             |            | 5.95 (dt, 15.4, 1.6) | 124.8     |
| 2   | 5.73 (d, 6.8) | 73.2       | 6.77 (dt, 15.4, 7.0) | 145.6     |
| 3   | 5.75 (d, 6.8) | 78.3       |             |            |
| 3a  |             | 173.1      |             |            |
| 3b  | 1.88 (s)    | 22.7       |             |            |
| 4   |             |            | 2.17 (m)    | 35.1       |
| 4a  |             |            | 144.1       |            |
| 5   | 6.87 (d, 8.3) | 118.2     | 1.49 (m)    | 22.7       |
| 6   | 6.79 (dd, 8.3, 2.0) | 123.7     | 0.94 (t, 7.4) | 14.0     |
| 7   | 134.6       |            |             |            |
| 8   | 6.84 (d, 2.0) | 118.0      |             |            |
| 8a  | 2.71 (t, 7.2) | 42.1       |             |            |
| 9   | 142.0       |            |             |            |
| 10  | 3.37 (t, 7.2) | 35.8       |             |            |
| 10a |             | 173.3      |             |            |
| 10b | 1.90 (s)    | 22.5       |             |            |
| 1′  |             |            | 130.7       | 61.6       |
| 2′  | 7.06 (s)    | 117.0      | 3.35 (t, 3.8) | 43.0     |
| 3′  |             | 147.3      |             |            |
| 4′  |             | 152.1      |             |            |
| 5′  | 7.46 (s)    | 117.6      |             |            |
| 6′  |             | 128.6      |             |            |
| 7′  | 3.18 (s)    | 46.2       |             |            |
with the diagnostic chemical shifts of C-7 ($\delta_C$ 134.6), C-4a ($\delta_C$ 144.1), C-8a ($\delta_C$ 142.0), C-2 ($\delta_C$ 73.2), and C-3 ($\delta_C$ 78.3), evidently indicate the side chain is at C-7. Eventually, the planar structure of 1 was deduced. The relative configurations of 1 at C-2 and C-3 were determined as trans by the coupling constant of $J_{2,3} = 6.8$ Hz. Generally, NADAs were isolated as racemic mixtures. In the current study, compound 1 was also racemic; subsequent chiral separation on high-performance liquid chromatography (HPLC) afforded their antipodes. The absolute configurations of enantiomers were assigned by electronic circular dichroism (ECD) calculations at B3LYP/6-31G (d,p) level. The results show that the calculated ECD curves of $2R,3S$-1 and $2R,3S$-1 agree well with the experimental ones for (+)−1 and (−)−1, respectively, allowing to assign the absolute configurations of enantiomers of 1 (Figure 2).

Compound 2 was isolated as a yellowish solid and has the molecular formula C$_8$H$_{15}$NO$_2$ deduced from its HREIMS at $m/z$ 158.1177 [M + H]$^+$ (caled for C$_8$H$_{16}$NO$_2$, 158.1176), $^{13}$C NMR, and DEPT spectra, having 2 degrees of unsaturation. The $^1$H NMR spectrum of 2 exhibits 2 sp$^2$ methines ($\delta_H$ 5.93, dt, $J = 15.4$, 1.6 Hz, H-2; $\delta_H$ 6.77, dt, $J = 15.4$, 7.0 Hz, H-3), 4 methylenes ($\delta_H$ 3.61, t, $J = 5.8$ Hz, H-1;$\delta_H$ 3.35, t, $J = 5.8$ Hz, H-2; $\delta_H$ 2.17, m, H-4; $\delta_H$ 1.49, m, H-5), 1 methyl ($\delta_H$ 0.94, t, $J = 7.4$ Hz, H-6). The $^{13}$C NMR and DEPT spectra (Table 1) display resonances for 8 carbons including 1 methyl, 4 methylenes (1 oxygenated), 2 olefinic methines, and 1 ester carbonyl. To deduce the structure of 2, two-dimensional NMR experiments were utilized. The $^1$H–$^1$H correlation spectroscopy (COSY) spectrum (Figure 3) shows correlations of H-2/H-3/H-4/H-5/H-6 and H-1'/H-2', suggesting the fragment of the C-2–C-6 and C-1’–C-2’, which was assembled via HMBC (Figure 3) of H-2, H-3, H-1’/C-1 ($\delta_C$ 169.1). Apart from the above fragment, the remaining signal is corresponding to an amino group on the basis of the chemical composition of 2 and the chemical shift of C-2’ ($\delta_C$ 43.0). In addition, the presence of

![Figure 2](image-url)  
**Figure 2.** Comparison between the experimental spectrum for (+)−1 and (−)−1 with the calculated electronic circular dichroism (ECD) spectra for (2R,3S)-1 in methanol at B3LYP/6-31G (d,p) level. $\sigma = 0.30$ eV; shift = 25 nm.

![Figure 3](image-url)  
**Figure 3.** Figure 2 correlation spectroscopy (COSY) and key heteronuclear multiple bond correlation (HMBC), and rotating frame Overhauser spectroscopy (ROESY) correlations of 1 and 2.
an amino group was also secured by the observation of a positive reaction after spraying ninhydrin reagent on thin-layer chromatography plate.

Of note, NADAs are common in the insect cuticles. However, such compounds bearing a methylsulfonyl group are rare in nature. Such a moiety has ever been found in insect natural products such as polyrhadopamine C from Chinese black ants\(^\text{11}\) and pipaipajin A from Blaps japonensis,\(^\text{12}\) indicating that the methylsulfonyl group might be a characteristic of insect natural products. In addition, we noted that there is an ethanolamine and a (E)-hex-2-enoic acid residue in the structure of 2. The former moiety is usually coexisted with choline and phospholipid, whereas the latter group so far has been found in insects exemplified as Asponguanine A and Aspongester A,\(^\text{8}\) indicating its significance in the insects.

The 3 known compounds were identified as (E)-hex-2-enoic acid (3),\(^\text{13}\) cyclo (L-Pro−L-Val) (4),\(^\text{14}\) and cyclo (L-Ala−L-Phe) (5),\(^\text{15}\) by comparison of their spectroscopic data with the literature.

The new compounds (+)−1, (−)−1, and 2 were evaluated for their renal protection in TGF-β1 induced rat renal proximal tubular cells (NRK-52E) by using extracellular matrix components such as fibronectin and collagen I as well as α-smooth muscle actin as indicators since they are the hallmarkers of renal fibrosis.\(^\text{4}\) Unfortunately, none of them exhibits inhibitory activities in these assays even at 40 μM. Besides, the Cell Counting Kit-8 assay was carried out to exclude possible cellular toxicity effects. The results show that all the tested 3 compounds are not toxic toward NRK-52E cells.

**Experimental**

**General**

Optical rotations were measured on a Bellingham + Stanley ADP 440 + digital polarimeter (Bellingham & Stanley, Kent, UK). Ultraviolet (UV) spectra were obtained on a Shimadzu UV-2600 spectrometer (Shimadzu Corporation, Tokyo, Japan). Circular dichromism (CD) spectra were measured on a Chirascan instrument (Agilent Technologies, Santa Clara, CA, USA). NMR spectra were recorded on a Bruker AV-600 spectrometer (Bruker, Karlsruhe, Germany) with tetramethylsilane as an internal standard. HRESIMS of new compounds 1, 2 were collected by a Shimadzu LC-20 CE AB SCIEX triple TOF 5600 + MS spectrometer (Shimadzu Corporation, Tokyo, Japan). MCI gel CHP 20P (75-150 μm, Mitsubishi Chemical Industries, Tokyo, Japan), RP-18 (40-60 μm, Daiso Co., Tokyo, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Semipreparative HPLC equipment was a SEP LC-52 with a MWD UV detector (Separation Technology Co. Ltd., Beijing, China) equipped with an YMC-Pack ODS-A column (250 mm×10 mm, i.d., 5 μm). Chiral separation was carried out using an Agilent 1260 liquid chromatograph equipped with a Daicel Chiralpak AD-H column (250 mm × 4.6 mm, i.d., 5 μm).

**Insect Material**

Dry whole bodies of A. chinensis were purchased from Hunan Corporation of Chinese Materia Medica (Changsha) of China, in December 2013. The material was identified by Professor Xiao-Jiang Zhou at the Hunan University of Chinese Medicine and a voucher specimen (CHYX-0633) is deposited at the School of Pharmaceutical Sciences, Shenzhen University Health Science Center, China.

**Extraction and Isolation**

The dried powders of whole bodies of A. chinensis (6 kg) were extracted 3 times with methanol (60 L, 24 hours) at room temperature to get a crude extract (600 g) by concentration under vacuum. The crude extract was suspended in water (H₂O) and partitioned with n-butanol (BuOH) for 3 times to afford a n-BuOH extract (150 g). The n-BuOH soluble extract was divided into 6 parts (Fr.A−Fr.F) by a MCI gel CHP 20P column (methanol (MeOH)/H₂O, 10%-100%). Fr.C (7.6 g) was subjected to MCI gel CHP 20P CC (MeOH/H₂O, 30%-65%) to yield 5 fractions (Fr.C.1−Fr.C.5). Fr.C.3 (1.4 g) was separated by using Sephadex LH-20 to yield 4 fractions (Fr.C.3.1−Fr.C.3.4). Fr.C.3.4 (37 mg) was subjected to semipreparative HPLC (MeOH/H₂O, 43%) to give compound 1 (2.1 mg, \(t_R = 12.7\) minutes, 3 mL/min). Compound 1 is a racemate which was submitted to semipreparative HPLC on a chiral phase by equipping with a Daicel Chiralpak AD-H (n-hexane/2-propanol, 80%) to afford (+)−1 (\(t_R = 12.8\) minutes, 0.32 mg, 1.0 mL/min) and (−)−1 (\(t_R = 16.7\) minutes, 0.52 mg, 1.0 mL/min). Fr.D (3.1 g) was separated by Sephadex LH-20 (MeOH) to afford 6 portions (Fr.D.1−Fr.D.6). Fr.D.3 (940 mg) was submitted to a MCI gel CHP 20P column washed with gradient MeOH/H₂O (10%-90%) to afford 4 fractions (Fr.D.3.1−Fr.D.3.4). Fr.D.3.4 (168 mg) was subjected to semipreparative HPLC (MeOH/H₂O, 35%) to give compound 2 (2.1 mg, \(t_R = 12.0\) minutes, 1.0 mL/min). Compound 2 is a racemate which was submitted to semipreparative HPLC on a chiral phase by equipping with a Daicel Chiralpak AD-H (n-hexane/2-propanol, 80%) to afford (+)−2 (\(t_R = 12.8\) minutes, 0.32 mg, 1.0 mL/min) and (−)−2 (\(t_R = 16.7\) minutes, 0.52 mg, 1.0 mL/min). Fr.D (3.1 g) was separated by Sephadex LH-20 (MeOH) to afford 6 portions (Fr.D.1−Fr.D.6). Fr.D.3 (940 mg) was submitted to a MCI gel CHP 20P column washed with gradient MeOH/H₂O (10%-90%) to afford 4 fractions (Fr.D.3.1−Fr.D.3.4). Purification of Fr.D.3.1.2 (72 mg) by semipreparative HPLC with 30% aqueous MeOH led to the isolation of compound 2 (1.3 mg, \(t_R = 11.5\) minutes, 3 mL/min) and 4 (11.5 mg, \(t_R = 22.6\) minutes, 3 mL/min). Likewise, compound 3 (1.1 mg, \(t_R = 15.5\) minutes, 3 mL/min) was purified from Fr.D.3.1.3 (39 mg) washed with aqueous MeOH (25%). Fr.D.3.4 (208 mg) was subjected to Sephadex LH-20 (MeOH) to give 3 fractions (Fr.D.3.4.1−Fr.D.3.4.3). Among them, compound 5 (1.4 mg, \(t_R = 15.0\) minutes, 3 mL/min) was purified from Fr.D.3.4.3 (37 mg) and washed with aqueous MeOH (35%).

\((\pm)\)-Aspongamide E (1)

Yellowish gum.

\([\alpha]_D^{25}\): +9.6 (c 0.013, MeOH); CD (MeOH) \(\Delta \varepsilon_{204} +14.80, \Delta \varepsilon_{221} -9.18, \Delta \varepsilon_{257} -1.30\); (+)-aspongamide E; \([\alpha]_D^{25}\): −8.0 (c 0.032, MeOH); CD (MeOH) \(\Delta \varepsilon_{204} -15.12, \Delta \varepsilon_{221} +6.09, \Delta \varepsilon_{257} +0.64\); (−)-aspongamide E.

UV (MeOH) \(\lambda_{\text{max}}\) (logε): 283 (3.46), 203 (4.45) nm.

\(^{1}\)H and \(^{13}\)C NMR: Table 1.

ESIMS \(m/z\) 465 [M + H\(^{+}\)].
HRESIMS \(m/z\) 465.1320 [M + H]\(^{+}\) (calcd for C\(_{21}\)H\(_{24}\)N\(_2\)O\(_8\)S, 465.1326).

2-Aminoethyl (E)-Hex-2-Enoate (2)

Yellowish gum.

ESIMS \(m/z\) 158 [M + H]\(^{+}\).

HRESIMS \(m/z\) 158.1177 [M + H]\(^{+}\) (calcd for C\(_8\)H\(_{15}\)NO\(_2\), 158.1176).

ECD calculations

The ECD spectrum of \((7R, 8S)-1\) was calculated as a previously described method.\(^{16}\)

Conclusion

Structurally diverse NADA derivatives have been found in many insects.\(^{11, 17-19}\) In this article, 1 novel sulfonyl-containing NADA derivative was characterized which will add a new facet to the research, authorship, and/or publication of this article: this study was financially supported by Shenzhen Government’s Plan of Science Foundation of China (21272241), National Science Fund for Distinguished Young Scholars (81525026), National Science Fund for Nonpeptidal Small Molecule Chemical Profiling of A. chinensis, and their biological evaluation. Fitoterapia. 2017;120:58-60.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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