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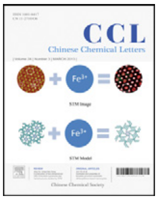

Original article

Bishyoscyamine, one unusual dimeric tropane alkaloid from *Anisodus acutangulus*

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ABSTRACT

One unusual dimeric tropane alkaloid, bishyoscyamine, was isolated from the roots of *Anisodus acutangulus*, whose structure including the absolute stereochemistry was unambiguously determined based on extensive 1D NMR and 2D NMR, HR-ESI-MS $[\alpha]_D$ and CD spectroscopic analyses. To our knowledge, bishyoscyamine is the first example of tropane alkaloid dimer condensed by a C–N bond.

1. Introduction

Tropane alkaloids with the 8-azabicyclo[3.2.1]octane skeleton represent an important class of natural products possessing atropine-like effects. Most of the natural tropane alkaloids are tropinol carboxylic esters, mainly distributed in the families of Solanaceae, Erythroxylaceae, Proteaceae, Euphorbiaceae, Rhizophoraceae, Convolvulaceae and Cruciferae, *etc.* [1,2]. Generally, tropane alkaloids possess two stable conformations: (I) boat–chair form, C-3 and C-6/7 are located at the same side of the C-1/2/4/5 plane; (II) chair–boat form, C-3 and C-6/7 are located at the different sides of the C-1/2/4/5 plane, and thus, two groups of signals for each H and C might be observed in their NMR spectra [1]. In addition to the normal monomeric tropane alkaloids, several unusual dimers, such as schizanthines A and B (from *Schizanthus pinnatus*), schizanthines C–E (from *Schizanthus grahamii*), schizanthines Y and X (from *Schizantus porrigens*), α/β-scopodonnines (from *Datura inoxia*), subhirsine (from *Convolvulus subhirsutus*), and mooniines A and B (from *Erythroxylum moonii*), *etc*., and a trimer, named grahamine (from *S. grahamii*) have been isolated from natural sources [3–9]. Structurally, all these tropane alkaloid dimers and trimer are constructed by condensing carboxylic acids with the alkaline parts through ester linkages. Therefore, it will be very interesting to search new tropane alkaloid oligomers with different condensation modes.

Anisodus acutangulus (Solanaceae) with the Chinese name San-Fen-San is a perennial herb mainly distributed in Yunnan and Sichuan provinces of China [10]. *A. acutangulus* is rich in tropane alkaloids, however no tropane alkaloid oligomers has been isolated from this plant [11,12]. In order to clarify its alkaline constituents, we recently investigated *A. acutangulus* and discovered one unprecedented dimeric tropane alkaloid, namely bishyoscyamine, which was condensed by a N–C bond instead of an ester connection, clearly different from those disclosed in previous reports.

2. Experimental

1D NMR and 2D NMR spectra were recorded on a Bruker Avance III-600 spectrometer (Bruker, Bremerhaven, Germany). HR-ESI-MS data were collected on LCMS-IT-TOF apparatus (Shimadzu, Kyoto, Japan). IR (KBr) spectra were recorded on a Bio-Rad FTS-135 infrared spectrometer (Bio-Rad, Hercules, CA, USA). UV data were collected on a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotations were measured on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). CD spectra were performed on an Applied Photophysics Chirascan spectrometer (Agilent, Palo Alto, USA). Silica gel (200–300 mesh) for column chromatography and TLC plates were purchased from Qingdao Makall Chemical Company (Makall, Qingdao, China). Fractions were monitored by TLC analysis sprayed with Dragendorff reagent.

The roots of *A. acutangulus* were purchased from Ju-Hua-Cun medicinal herb market, Kunming, Yunnan Province, China, in July 2010, and authenticated by Prof. Li-Gong Lei, Kunming Institute of Botany, CAS. A voucher specimen (No. 201007S) was deposited in the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany.

Extraction and isolation: The dried and powdered roots of *A. acutangulus* (2.0 kg) were extracted with EtOH (10 L × 3) at room

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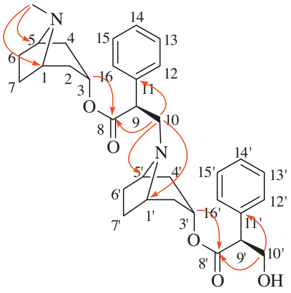

Fig. 1. Structure of bishyoscyamine (**1**), and the key HMBC correlations (→).

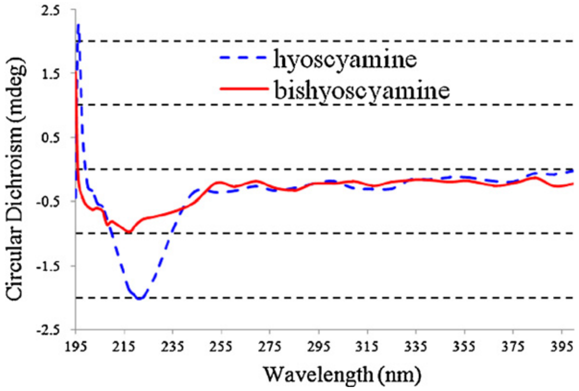

Fig. 2. The CD spectra of bishyoscyamine (**1**) and hyoscyamine.

temperature, and the combined EtOH solvent was evaporated in vacuo. This residue was dissolved in aqueous HCl (1%), subsequently extracted with $CHCl_3$, then, the aqueous portion was basified to pH 9 using aqueous ammonia. The basic solution was partitioned with $CHCl_3$ to afford the total alkaloids. The total alkaloids (100 g) were subjected to silica gel column chromatography (Si-CC) eluting with $CHCl_3$–MeOH system to afford four fractions (I–IV). Fraction I (10 g) was further purified by repeated Si-CC eluting with petroleum ether/acetone/diethyl-amine (9:1:0.1) to yield bishyoscyamine (**1**) 30 mg (Fig. 1).

Bishyoscyamine (**1**): Colorless oil. $[\alpha]_D^{24}$ −20.1 (*c* 0.11, MeOH); CD (218 μmol/L, MeOH) λ_{max} ($\Delta\varepsilon$) 219 (−0.89) nm; UV (MeOH) λ_{max} (log ε) 205 (4.23) nm; IR (KBr, cm^{-1}): ν_{max} 3440, 3425, 2956, 2924, 2853, 1726, 1630, 1606, 1464, 1377, 1157, 1030, 699; ^{1}H NMR and ^{13}C NMR data, see Table 1; HR-ESI-MS (*m/z*): 547.3157 $[M+H]^+$ (calcd. for $C_{33}H_{42}N_2O_5$, 547.3166).

3. Results and discussion

Bishyoscyamine (**1**) was obtained as a colorless oil with a molecular formula of $C_{33}H_{44}N_2O_5$ from the positive HR-ESI-MS, which showed the $[M+H]^+$ ion at *m/z* of 547.3157 (error 1.64 ppm). The IR spectrum indicated the presence of hydroxyl (3440 cm^{-1} and 3425 cm^{-1}), carbonyl (1726 cm^{-1}) and phenyl (1630, 1606, 1464 cm^{-1}) groups. Its ^{13}C NMR (DEPT) spectrum displayed two carbonyl groups and twelve aromatic carbons in the down-field region, a series of *O* or *N* containing methines and methylenes between δ_C 69 and 53, one *N*-methyl (δ_C 40.7), and two groups of methylenes in the up-field region. In the ^{13}C NMR spectrum, most of the carbon signals were present in pairs indicating that two closely related moieties should be in its structure. Extensive analyses of its ^{13}C NMR (DEPT) data suggested two hyoscyamine-like parts were present except that one methylene was shifted up-field from δ_C 64 to 57 and one *N*-substituted methyl was absent. Based on the above deduction, compound **1** was reasonably proposed to be a hyoscyamine dimer, and the key for its structural determination was to verify its connectivity and stereochemistry. The HMBC correlations of H-10 with C-1′/5′ clearly suggested the linkage of C-10/N/C-1′/5′, which was in good agreement with a gap of a CH_4O unit between its molecular formula ($C_{33}H_{44}N_2O_5$) with that of two molecules of hyoscyamine (2× $C_{17}H_{23}NO_3$).

Its absolute stereochemistry was determined to be identical with that of hyoscyamine based on their close $[\alpha]_D$ values and similar negative Cotton effects near 220 nm in their CD spectra

Table 1
The 1D NMR and key 2D NMR data of bishyoscyamine (**1**) in CD_3OD.

Position	^{1}H NMR (δ_H, mult, *J* in Hz)[a]	^{13}C NMR (δ_C)[b]	COSY	HMBC
1/5[⊥]	3.05, 2H, m, overlapped	60.9, d	H-2/4, H-6/7	C-3, NMe
2/4[*]	1.99, 2H, m 1.57, 2H, m	37.2, t	H-1/5, H-3	
3	4.93, 1H, m	68.8, d	H-2/4	C-1/5, C-8
6/7[⊥]	1.84, 4H, m, overlapped	26.4, t	H-1/5	
8	–	174.3, s		
9	3.73, 1H, m, overlapped	53.9, d	H-10	C-12/16
10	3.07, 1H, m, overlapped 2.59, 1H, td, 13.0, 5.0	57.0, t	H-9	C-8, C-11, C-1′/5′
11	–	139.0, s		
12/16	7.29, 2H, m, overlapped	130.0, d		C-9
13/15	7.29, 2H, m, overlapped	129.4, d		
14	7.27, 1H, m, overlapped	128.9, d		
1′/5′[*]	3.08, 2H, m, overlapped	59.2, d	H-2′/4′, H-6′/7′	C-10, C-3′
2′/4′[*]	1.99, 2H, m 1.57, 4H, m	37.3, t	H-1′/5′, H-3′	
3′	4.93, 1H, m	69.7, d	H-2′/4′	
6′/7′[*]	1.53, 4H, m	27.3, t	H-1′/5′	
8′	–	173.4, s		
9′	3.75, 1H, m, overlapped	56.4, d	H-10′	C-12′/16′
10′	4.14, 1H, td, 12.1, 5.7 3.74, 1H, m, overlapped	64.8, t	H-9′	C-8′, C-11′
11′	–	137.5, s		
12′/16′	7.29, 2H, m, overlapped	130.0, d		C-9′
13′/15′	7.29, 2H, m, overlapped	129.3, d		
14′	7.27, 1H, m, overlapped	128.8, d		
NMe	2.22, 3H, s	40.7, q		C-1/5

[a] ^{1}H NMR data were measured in 600 MHz.
[b] ^{13}C NMR data were measured in 150 MHz.
[⊥] The δ_C values were the mean of four closed data.
[*] The δ_C values were the mean of two closed data.

(Fig. 2). This deduction was also consistent with their biogenetic precursors of L-phenylalanine and ornithine. Therefore, the structure of compound **1** was solved and it was named as bishyoscyamine. It should be noted that the carbons (1/5, 2/4, 6/7, 1′/5′, 2′/4′ and 6′/7′) were split as groups of close peaks in the ^{13}C NMR spectrum (150 MHz), which might be due to the interconversion between the boat–chair and chair–boat conformations [1].

4. Conclusion

To our knowledge, bishyoscyamine is the first example of tropane alkaloid dimer directly condensed by a N–C bond. This investigation will provide valuable information for further understanding of tropane alkaloid dimers and the chemical constituents of *A. acutangulus*.

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