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Assignment of ^{13}C and ^{1}H Chemical Shifts of Cyclovirobuxinum D

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Absract: Cyclovirobuxinum D was isolated from traditional Chinese medicine *Buxus icrophylla Sieb. et Zucc*, for treating coronary heart disease and arrhythmias. High performance liquid preparation chromatography was used for separation and purification. ^{13}C and ^{1}H chemical shifts of the compound were assigned using 1D and 2D NMR techniques including ^{1}H NMR, ^{13}C NMR, COSY, DEPT, HMQC and HMBC. The structure of the compound was determined from the NMR data.

Key words: NMR, chemical shift, 2D NMR, cyclovirobuxinum D, Buxus alkaloids
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Introduction

A number of *Buxus* alkaloids from *Buxus microphylla Sieb. et Zucc*, such as one of the oral preparations known as huang-yangning tablet in traditional Chinese medicine, are used to treat coronary heart disease and arrhythmias[1-4]. Previously, we have examined raw cyclovirobuxinum D brought from market by HPLC method[5], the content of which is between 55% to 85%. In fact, it was the Buxus alkaloids extractive (Huangyangning), not a pure compound. Here, we obtained pure cyclovirobuxinum D (the purity>98%), through high performance preparation liquid chromatography. 2D NMR experimental techniques including ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC and HMBC[6, 7], were used to study its structure and complete assignments of its ^{1}H and ^{13}C

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chemical shifts are reported for the first time. The molecular structure is depicted in Fig. 1.

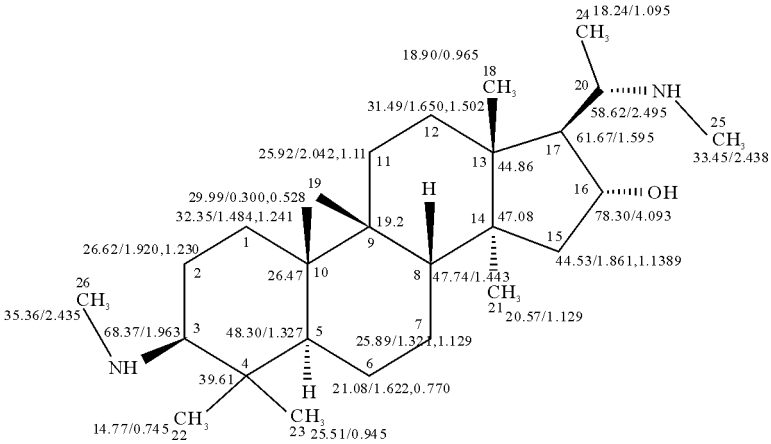

Fig. 1 Structure of cyclovirobuxinum D

1 Experiment

1.1 Material

Buxus alkaloids extractive (Huangyangning) was provided by xiaoying pharmacy factory. All the solvents used were commercially available reagents (AR).

1.2 Purification

We have established a high performance preparation liquid chromatography method. Cyclovirobuxinum D was purified on a Lichrospher-SiO_2 (250 mm×18 mm, 5 μm) column with a mobile phase of methoth- hexamethylene- acetone- ethanediamine(100∶50∶50∶2)and a flow rate of 5 mL·min^{-1} with the ELSD detection.

1.3 Purity test method

HPLC method was performed using Agilent 1100 series, Lichrospher-NH_2 (250 mm×4.6 mm, 5 μm) column with a mobile phase of acetonitrile-0.4% dipotassium hydrogen phosphate solution(70∶30) and a flow rate of 1 mL·min^{-1} at 40 ℃ with the UV detection at 210 nm.

1.4 Sample preparation and Apparatus

20 mg Cyclovirobuxinum D was dissolved in 0.5 mL $CDCl_3$, which was used as the internal reference at the same time. in a 5 mm NMR tube for experiments. All the experiments were performed on a Bruker advance DRX500 spectrometer equipped with TBI tube, a temperature control system.

1.5 NMR Experiment

All the NMR experiments were conducted at room temperature, the main parameters are listed in Table 1.

Table 1　Experimental parameters

Experiment	SW (kHz)	TD	SI	NS
^{1}H NMR	4.5	32 k	32 k	8
^{13}C NMR	31.5	32 k	32 k	2 k
DEPT	31.5	32 k	32 k	2 k
COSY	4.5/4.5	1 024×256	512×512	4
NMQC	4.5/31.5	1 024×128	512×512	8
HMBC	4.5/31.5	1 024×128	512×512	8

2　Results and Discussion

The cyclovirobuxinum D obtained is some what white crystalline powder. The molecular formula $C_{26}H_{46}N_2O$ was determined 402.359 4 by TOF-MS, which was supported by ^{13}C NMR spectrum. Its purity was 98.65%.

From ^{1}H NMR spectrum, it can be concluded that the cyclovirobuxinum D has forty-three protons in sixteen different circumstances, expect for solvent peaks. The ratio of their integral is (from high magnetic field to low magnetic field) 1∶1∶6∶1∶3∶3∶3∶1∶2∶2∶4∶4∶6∶4∶1∶1. From ^{1}H NMR and D_2O exchanged ^{1}H NMR spectra, we can conclude that the cyclovirobuxinum D has three active protons 16-OH(δ 4.1), 3-NH(δ 1.4) and 20-NH(δ 1.9). The ^{13}C NMR spectrum of cyclovirobuxinum D displayed signals of twenty-six carbons in twenty-six different circumstances, expect for solvent peaks. The DEPT spectrum indicated that the cyclovirobuxinum D has seven methyl carbons(δ 35.36, δ 33.45, δ 25.51, δ 20.57, δ 18.90, δ 18.24, δ 14.77), eight methylene carbons(δ 44.53, δ 32.35, δ 31.49, δ 29.99, δ 26.62, δ 25.92, δ 25.89, δ 21.08), six methane carbons(δ 78.30, δ 68.37, δ 61.67, δ 58.62, δ 48.30, δ 47.74), and five quaternary carbons(δ 47.08, δ 44.86, δ 39.61, δ 26.47, δ 19.20). Total carbon number and the carbon's type agreed with the cyclovirobuxinum D structure. All direct connections between protons and carbons were identified with HMQC spectra (see Table 2).

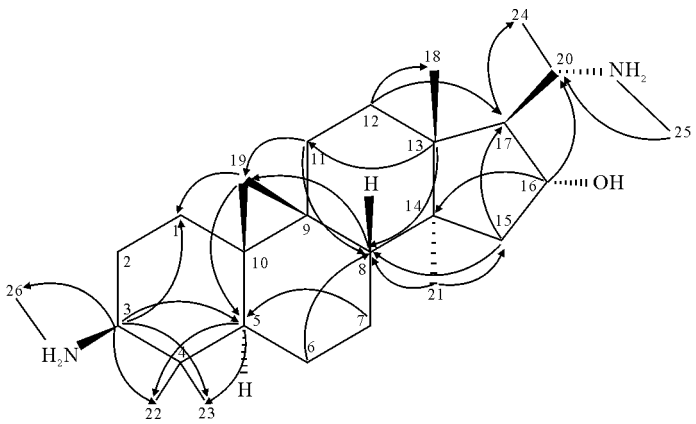

Fig. 2　Main HMBC of cyclovirobuxinum D

Table 2 NMR data and ^{13}C-^{1}H correlations of cyclovirobuxinum D (in $CDCl_3$)

Position	δ_C	DEPT	Related proton		
			δ_H(J_{Hz}) (HMQC)	^{1}H-^{1}H COSY	HMBC
1	32.35	CH_2	1.48 (d, 12), 1.24(d, 12)	H-2	H-19
2	26.62	CH_2	1.92, 1.28(m)	H-1, H-3	H-11, H-8
3	68.37	CH	1.96(m)	H-2	H-22,H-23,H-5,H-26,H-2,H-1
4	39.61	C	/	/	H-22, H-23, H-5, H-3
5	48.3	CH	1.33(d,8, overlapped)	H-6	H-22, H-23
6	21.08	CH_2	1.62, 0.77(m)	H-5, H-7	H-5, H-7, H-8
7	25.89	CH_2	1.33(m, overlapped) 1.11(m, overlapped)	H-6, H-8	H-8, H-5, H-6
8	47.74	CH	1.44(m)	H-7	H-7, H-18, H-21
9	19.2	C	/	/	H-19, H-11, H-8
10	26.47	C	/	/	H-5
11	25.92	CH_2	2.04(m), 1.11(m, overlapped)	H-12	H-19, H-8, H-12
12	31.49	CH_2	1.65, 1.50(m)	H-11	H-18, H-17, H-11
13	44.86	C	/	/	H-12, H-18, H-17, H-11, H-8
14	47.08	C	/	/	H-15, H-8
15	44.53	CH_2	1.39(d, 9), 1.86(m)	H-16	H-17, H-8
16	78.3	CH	4.09(m)	H-15, H-17	H-20, H-17, H-15
17	61.67	CH	1.59(m)	H-20, H-16	H-18, H-20, H-24
18	18.9	CH_3	0.96(s)	/	H-17, H-12
19	29.99	CH_2	0.30(d, 10), 0.53(s)	/	H-5, H-8
20	58.62	CH	2.49(m)	H-17, H-24	H-17, H-25, H-16, H-24
21	20.56	CH_3	1.11(s, overlapped)	/	H-15, H-8
22	14.77	CH_3	0.74(s)	/	H-23, H-5, H-3
23	25.51	CH_3	0.94(s)	/	H-22, H-3
24	18.24	CH_3	1.09(m)	H-20	H-17
25	33.45	CH_3	2.44(m, overlapped)	/	H-20
26	35.36	CH_3	2.44(m, overlapped)	/	H-3

From the structure of cyclovirobuxinum D, δ_C 78.3(δ_H 4.093, m)methane carbon in lowest magnetic field (proved by DEPT) belongs to C-16 obviously, because it is connected with the strongest electron drawing group (-OH). δ 68.37(δ 1.963, methane carbons)in the low magnetic field belongs to C-3 connected with NH, obviously.

References:

[1] Jiangsu Institute of Botany, CAS(中国科学院江苏省植物研究所). Compendium of New China (Xinhua) Herbal (新华本草纲要)[M]. Shanghai(上海): Science and Technology Press(上海科学技术出版社), 1988. 816－817.

[2] Liu Ru-ying(刘如英). Clinical application of huangyangning tablets(黄杨宁片的临床应用)[J]. Journal of Clinical Internal Medicine(临床内科杂志), 1997, 14(2): 112－115.

[3] Zhang Kai-shan(张开山), Wu Yi-yuan(吴义元). Summarization of clinical application of huangyangningtablets (黄杨宁片临床应用概述)[J]. Primary Journal of Chinese Materia Medica(基层中药杂志), 2000, 14(4): 52－54.

[4] Chinese Pharmacopoeia Commission(中国药典委员会). ChP 2000 Vol I(中国药典 2000 年版 一部) [S]. Beijing (北京): Chemical Industry Press(化学工业出版社), 2000. 153.

[5] Liu Jie(刘洁), Hang Tai-jun (杭太俊), Zhang Zheng-xing(张正行). Determination of cyclovirobuxinum D and its related alkaloids in huangyangning by HPLC-UV(HPLC 法测定黄杨宁中环维黄杨星 D 和有关生物碱含量) [J]. Chin J Pharm Anal (药物分析杂志), 2006, 26(4): 446－449.

[6] Jiang Gao-xi(蒋高喜), Li Qin(李勤), Liu Xue-hui(刘雪辉), *et al*. Structure elucidation for some drugs using NMR technics(一些药物分子的核磁共振应用研究)[J]. Chinese J Magn Reson(波谱学杂志), 2003, 20(4): 393－401.

[7] Li Xiang(李想), Li Min-yi(李敏一), Zhen Yi-nan(郑毅男), *et al*. Structure elucidation of taraxerone isolated from mangrove excoecaria agallocha(红树植物海漆中 Taraxerone 化合物的结构解析) [J]. Chinese J Magn Reson(波谱学杂志), 2006, 23(4): 451－455.

环维黄杨星 D ^{13}C 和 ^{1}H 化学位移的全归属

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摘　要: 环维黄杨星 D 为自黄杨木中提取精制的有效生物碱, 临床上已长期用于心血管疾病的治疗. 本文用制备高效液相色谱提取、分离并纯化环维黄杨星 D, 用 1D, 2D NMR 技术 (COSY, DEPT, HMQC 和 HMBC)对其结构进行研究, 并且首次对环维黄杨星 D 的 ^{1}H NMR 和 ^{13}C NMR 信号进行了全归属, 同时通过 NMR 数据确证了环维黄杨星 D 的结构.

关键词: NMR; 归属; 2D NMR; 环维黄杨星 D; 黄杨宁生物碱

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