

7,8 β -Dihydroponasterone A, a New Phytoecdysteroid from the Needles of the Japanese Yew, *Taxus cuspidata*

Qing-Wen Shi,^{*,a} Mei Dong,^a Chang-Hong Huo,^a Xiao-Hui Su,^a Xing Li,^a Teiko Yamada^b and Hiromasa Kiyota^{*,b}

^aDepartment of Natural Product Chemistry, School of Pharmaceutical Sciences, Hebei Medical University, 361 Zhongshan East Road, 050017, Shijiazhuang, Hebei Province, P. R. China

^bLaboratory of Applied Bioorganic Chemistry, Division of Biocience & Biotechnology for Future Bioindustry, Graduate School of Agricultural Sciences, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, Japan

O novo ecdisteróide 7,8 β -di-hidroponasterona A e a ponasterona A, foram isolados a partir do extrato metanólico das agulhas do teixo japonês *Taxus cuspidata*. As estruturas foram elucidadas por meios espectroscópicos, nomeadamente, RMN ¹³C e ¹H, COSY ¹H-¹H, NOESY, HMQC e HMBC, e confirmadas por HR-FABMS.

A new plant ecdysteroid 7,8 β -dihydroponasterone A, together with ponasterone A, were isolated from the methanol extract of the needles of the Japanese yew, *Taxus cuspidata*. Their structures were elucidated on the basis of spectroscopic analysis including ¹H NMR, ¹³C NMR, ¹H-¹H COSY, NOESY, HMQC and HMBC and confirmed by high-resolution FABMS data.

Keywords: *Taxus cuspidata*, yew, phytoecdysteroid, ponasterone A, 7,8 β -dihydroponasterone A

Introduction

Phytoecdysteroids are steroidal molecules firstly recognized as hormones controlling moulting and metamorphosis in insects. Today it is realized that these steroids are present at all stages of insect development, regulating many biochemical and physiological processes.¹ Ecdysteroids and their analogs are also of great interest for medicinal application.² To date, more than 200 phytoecdysteroids were identified,^{3,4} however, only four of them were isolated from the genus *Taxus* (yew trees).⁵ The Japanese yew, *Taxus cuspidata* (Taxaceae), is a low trailing bush or tree very popular in Hokkaido and Tohoku regions, widely used as ornamental plant in Japan. Previous phytochemical studies on this species led to the isolation of taxanes with various skeleton, and other classes of compounds.^{5,6} Here we report on the isolation and identification of a new phytoecdysteroid, 7,8 β -dihydroponasterone A (**2**), as well as the known ponasterone A (**1**) from the needles of *T. cuspidata*.

Results and Discussion

A methanolic extract of the needles of *Taxus cuspidata* was processed as described in the experimental section to afford compounds **1** and **2** (Figure 1). Combined analysis of NMR⁷ and FAB-MS data of compound **1**, obtained as a white amorphous solid, established its structure as ponasterone A, which has been previously isolated from the bark of *T. brevifolia*,⁸ *T. cuspidata*,⁹ and *T. yunnanensis*.¹⁰

Compound **2** was obtained as a white amorphous solid with a molecular formula C₂₇H₄₆O₆ calculated from the peaks at *m/z* 489.3193 (M+Na)⁺ and 505.2921 (M+K)⁺ in the HR-FABMS. The spectroscopic data of **2** (Table 1) closely resembles those of **1**, with the exceptions that in compound **2** the olefinic carbons and the olefinic proton were absent, the ketone resonance was shifted from δ_c 206.4 to 215.1 ppm, and its UV spectrum lacked the characteristic absorption for the α,β -unsaturated ketone at 242 nm.^{11,12} Based on these features it was concluded that the structure of **2** corresponded to the 7,8-dihydro derivative of **1**.

The relative stereochemistry of **2** was deduced from NOESY data and ¹H-¹H coupling constants of vicinal protons (Figure 2). *cis*-Fused chair conformation of the

*e-mail: shiqingwen@hebmh.edu.cn; kiyota@biochem.tohoku.ac.jp

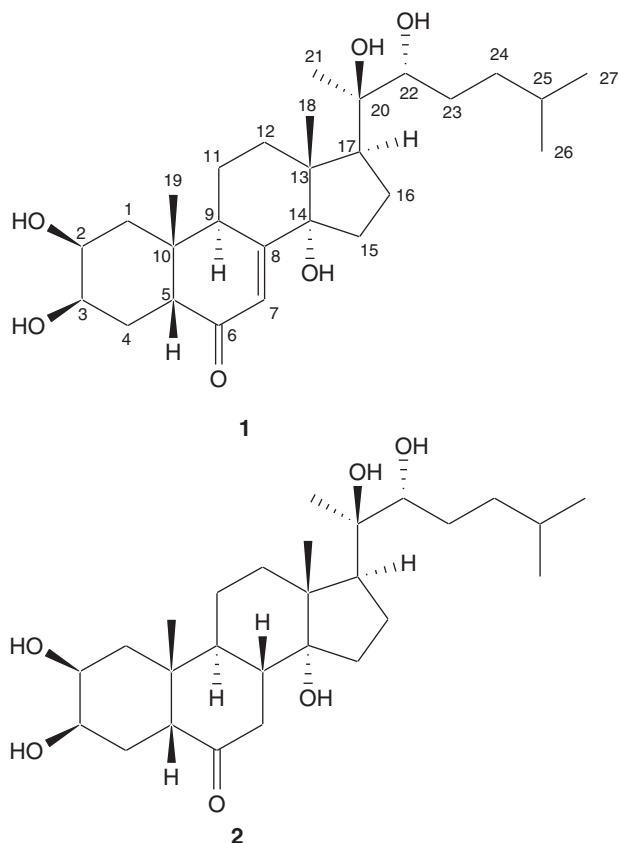


Figure 1. Ponasterone (**1**) and 7,8 β -dihydroponasterone (**2**) isolated from *Taxus cuspidata*.

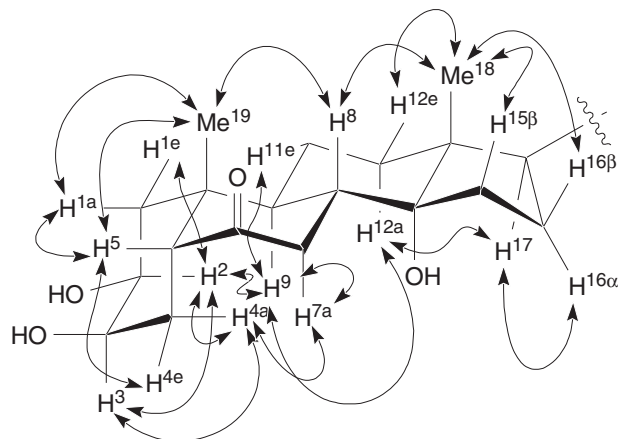


Figure 2. Relative stereochemistry of 7,8 β -dihydroponasterone A (**2**). The arrows denote selected NOESY correlations.

AB rings was elucidated from the coupling constants ($J_{1ax,2} = 12.3$ Hz, and $J_{3,2} = J_{3,4ax} = J_{3,4eq} = 2.9$ Hz), and the NOESY correlations of H-2 to H-4 α , H-2 to H-9, H-5 to H-19, and H-4 α to H-7 α . The hydroxy groups at C-2 and C-3 were to be β -oriented, judging from the small coupling constant between H-2 and H-3. The NOE correlations between H-7 α to H-9, H-8 to H₃-18, H-8 to H₃-19, and H-9 to H-12 α demonstrated the BC ring was *trans*-fused. *trans*-Fusion of the CD-rings was

deduced from the similarity of the ^1H NMR chemical shifts and coupling constant values to **1** and the NOESY correlation of H-15 β to H₃-18. The β -orientation of the side chain C-20-C-27 was deduced from the NOE between H-12 α to H-17. C-20-OH and C-22-OH were assigned to be β - and α -oriented, respectively, by comparing with the analogous compound.¹³ Consequently, the compound **2** was determined to be 7,8 β -dihydroponasterone. The corresponding 8 α -diastereomer of **2** was previously synthesized by stereoselective catalytic hydrogenation of **1**.¹⁴

Compound **2** was formerly reported as a hypothetical CoMFA model compound to investigate QSAR of ecdysteroids.¹⁵ Phytoecdysteroids appeared to have agonistic activity at the insectsteroid receptor complex,^{15,16} and participate in the defense of plant against non-adapted phytophagous invertebrates.^{1,17} The fact that yew trees exhibited a very strong resistance to insect pests was considered to be caused by taxanes such as 10-deacetylbaccatin III and 10-deacetylbaccatin V.¹⁸ The presence of **1** and **2** in the needles of the Japanese yew would also be responsible for insecticidal activity.¹⁹

Experimental

General experimental procedure

Optical rotations: a JASCO DIP-370 digital polarimeter. Flash chromatography: Silica gel 60 (230-400 mesh EM Science). Thin layer chromatography: Silica Gel 60 F₂₅₄ pre-coated TLC plates (0.25 mm or 0.5 mm, EM Science). Analytical HPLC: a Waters 600 FHU delivery system and a PDA 996 detector, and Whatman partisol 10 ODS-2 (4.6 \times 250 mm) column. Preparative HPLC: a Waters Delta Prep 3000 instrument and a UV 486 detector (210 nm, Waters), and Whatman partisol 10 ODS-2 Mag-9 (9.4 \times 250 mm) or partisol 10 ODS-2 MAG-20 (22 \times 500 mm) column, eluent: a 50 min linear gradient of acetonitrile (25 to 100%) in water (18 mL min⁻¹). NMR: a Bruker Avance-500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C). The solvent was used as an internal reference. FAB-MS: a Vacuum Generators ZAB-HS double-focusing instrument.

Plant material

The needles of *Taxus cuspidata* were collected in the autumn of 2000 at Tsutsuzigaoka park, Sendai city in the north-east of Japan. The botanical identification was made by Professor T. Oritani of Toyama Prefectural University, Toyama, Japan. Several voucher specimens have been deposited in the Laboratory of Applied Bioorganic

Table 1. ^1H and ^{13}C NMR data of **2** in CDCl_3 (500 MHz for ^1H , 125 MHz for ^{13}C)

2				
Position	δ (^1H) mult.	J (Hz)	δ (^{13}C)	HMBC (H \rightarrow C)
1-ax	1.38 (m)			
1-eq	1.81 (m)		37.2	
2	3.82 (dt)	12.3, 3.8	67.1	3
3	4.04 (ddd)	2.9	67.8	
4-ax	1.99 (m)		31.9	
4-eq	1.84 (m)			
5	2.42 (dd)	13.5, 3.9	53.3	4, 6, 9, 10, 19
6			215.1	
7-ax	2.55 (dd)			
	2.06 (dd)	14.3, 12.0	37.0	8, 14
8	2.14 (m)	14.3, 3.6	39.1	
9	2.18 (m)		32.8	8, 10, 11, 12, 19
10			40.0	
11-ax	1.46 (m)			
11-eq	1.79 (m)		20.2	
12-ax	1.84 (m)			
12-eq	1.60 (m)		32.2	
13			47.8	
14			84.7	
15 β	1.84 (m)			
15 α	1.35 (m)		32.3	
16 β	1.99 (m)			
16 α	1.65 (m)		20.4	
17	2.21 (t)	9.3	49.3	13, 15, 16, 18, 20, 21
18	1.00 (s)		17.4	12, 13, 14, 17
19	0.91 (s)		22.9	1, 5, 9, 10
20			76.9	
21	1.18 (s)		20.3	17, 22
22	3.38 (br.d)	8.6	76.6	21, 24
23a	1.46 (m)			
23b	1.22 (m)		29.3	
24a	1.44 (m)			
24b	1.21 (m)		36.3	
25	1.54 (m)		27.9	
26	0.91 (d)	6.4	22.9	24, 27
27	0.90 (d)	6.4	22.5	24, 26

Chemistry, Graduate School of Agricultural Sciences, Tohoku University, Japan.

Extraction and isolation

Air-dried needles of *Taxus cuspidata* were ground (3.0 kg) and extracted with 8 L of methanol for one week at room temperature. The plant material was filtered, and extracted again with fresh solvent for another two times (each time with 4 L solvent, total 16 L) in seven days. The combined organic extracts were evaporated under reduced pressure. Water (2 L) was added, and lipids were removed by stirring the mixture with hexane (3×2 L). The aqueous phase was then salted (NaCl, 150 g) and extracted with CH_2Cl_2 (4×1 L). The combined CH_2Cl_2 extracts were dried with anhydrous sodium sulfate, filtered and evaporated yielding a dark green extract (45 g). A

portion of the methylene chloride extract (38 g) was absorbed onto silica gel (45 g) and packed on a wet column chromatography (silica gel, 820 g). Successive elution with CH_2Cl_2 -MeOH gradient with increasing amounts of methanol from 5% to 45% (total 10 L) yielded 32 fractions (Fr_{D-1} to Fr_{D-32}). Fr_{D-27} to Fr_{D-30} were combined (14 g) and chromatographed over silica gel (470 g), being eluted with a stepwise hexane-acetone gradient (1:1, 3:4, 2:3) to yield 28 fractions (Fr_{D-27-1} to Fr_{D-27-28}). The fractions Fr_{D-27-16}, Fr_{D-27-17} and Fr_{D-27-18} (640 mg) were pooled and re-chromatographed over silica gel (50 g) eluting with hexane-EtOAc (1:2) to yield 17 fractions (Fr_{D-27-16-1} to Fr_{D-27-16-17}). The fractions Fr_{D-27-16-14} (18 mg) and Fr_{D-27-16-15} (21 mg) were combined and applied to preparative HPLC to yield **1** (7 mg, $t_R = 22.32$ min). Fr_{D-27-19} to Fr_{D-27-28} (1.59 g) were absorbed onto 3.0 g silica gel and re-chromatographed over silica gel, being eluted using stepwise gradients of CH_3CN in

CH₂Cl₂ to give 25 fractions (Fr_{D-27-19-1} to Fr_{D-27-19-25}). Fr_{D-27-19-24} (170 mg) was subjected to preparative HPLC. The material eluted at $t_R = 20.18$ min was collected and concentrated (25 mg), and further purified by preparative TLC with EtOAc-EtOH (100:6) elution, to yield **2** (3.5 mg, $R_f = 0.40$).

Ponasterone A (**1**)

Amorphous solid; $[\alpha]_D^{22} +68^\circ$ (c 0.20, MeOH).

7,8β-Dihydroponasterone A (**2**)

Amorphous solid; $[\alpha]_D^{22} +76^\circ$ (c 0.20, MeOH); ¹H and ¹³C NMR spectral data, see Table 1. HRFABMS: m/z 489.3193 [M+Na]⁺ (Calc. for C₂₇H₄₆O₆Na, 489.3192), 505.2921 [M+K]⁺ (Calc. for C₂₇H₄₆O₆K, 505.2931).

Acknowledgments

We thank Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China and the Foundation for Researching New Drugs of China for the financial support in part. No: 2003AA2Z3527.

Supplementary Information

Supplementary data of compound **1** are available free of charge at <http://jbcs.sbc.org.br>, as PDF file.

References

- Dinan, L.; *Phytochemistry* **2001**, *57*, 324.
- Akhren, A. A.; Kovganko, N.V.; *Ekdisteroidy: Khimia I Biologicheskaya Aktivnost (Ecdysteroids: Chemistry and Biological Activity)*, Nauka I Tekhnika: Minsk, 1989.
- Nakanishi, K.; Koreeda, M.; Sasaki, L.; Chang, M. L.; Hsu, H. Y.; *J. Chem. Soc., Chem. Commun.* **1966**, 915.
- Lafont, R.; Wilson, I. D.; *The Ecdysone Handbook*, 2nd ed., The Chromatographic Society: Nottingham, UK, 1996; <http://ecdybase.org/>, accessed in February 2006.
- Parmar, V. S.; Jha, A.; Bish, K. S.; Taneja, P.; Sight, S. K.; Kumar, A.; Poonam, J. R.; Olsen, C. E.; *Phytochemistry* **1999**, *50*, 1267.
- Baloglu, E.; Kingston, D. G. I.; *J. Nat. Prod.* **1999**, *62*, 1448; Kobayashi, J.; Shigemori, H.; *Med. Res. Rev.* **2002**, *22*, 305 and references cited therein; Shi, Q. W.; Kiyota, H.; *Chem. Biodivers.* **2005**, *2*, 1597; Shi, Q. W.; Oritani, T.; Horiguchi, T.; Sugiyama, T.; Murakami, R.; Yamada, T.; *Biosci., Biotechnol., Biochem.* **1999**, *63*, 924; Shi, Q. W.; Oritani, T.; Sugiyama, T.; Horikuchi, T.; Murakami, R.; Zhao, D.; Oritani, T.; *Tetrahedron* **1999**, *55*, 8365.
- Vokác, K.; Budesinsky, M.; Harmatha, J.; Kohoutová, J.; *Phytochemistry* **1998**, *49*, 2109.
- Rao, K. V.; Bhakuni, R. S.; Hanuman, J. B.; Davies, R.; Johnson, J.; *Phytochemistry* **1996**, *41*, 863.
- Imai, S.; Nakanishi, K.; Koreeda, M.; Kurokawa, T.; *Steroids* **1967**, *10*, 557; Ripa, P. V.; Martin, E. A.; Cocciolone, S. M.; Adler, J. H.; *Phytochemistry* **1990**, *29*, 425; Takemoto, T.; Hikino, Y.; Jin, H.; Hikino, H.; *Yakugaku Zasshi* **1968**, *88*, 359.
- Li, S. H.; Zhang, H. J.; Yao, P.; Niu, X. M.; Xiang, W.; Sun, H. D.; *J. Asian Nat. Prod. Res.* **2002**, *4*, 147.
- The UV absorption was checked by the UV detector for HPLC.
- Costantino, V.; Carmela D.; Fattorusso, E.; Mangoni, A.; *Steroids* **2000**, *65*, 138.
- Ruan, B.; Wilson, W. K.; Schroepfer Jr., G. J.; *Steroids* **1999**, *64*, 385.
- Suksamrarn, A.; Tanachatchairatana, T.; Sirigarn, C.; *Tetrahedron* **2002**, *58*, 6033.
- Dinan, L.; Hornann, R. E.; Fujimoto, T.; *J. Comput. Aid. Mol. Des.* **1999**, *13*, 185.
- Dinan, L.; Savchenko, T.; Whiting, P.; Sarker, S.D.; *Pestic. Sci.* **1999**, *55*, 331.
- Slama, K.; Lafont, R.; *Eur. J. Entomol.* **1995**, *92*, 355.
- Daniewski, W. M.; Gumulka, M.; Anczewski, W.; Masnyk, M.; Bloszyk, E.; Gupta, K. K.; *Phytochemistry* **1998**, *49*, 1279.
- Horn, D. H. S. In *Naturally Occurring Insecticides*; Jacobson, M.; Crosby, D. G., eds.; Marcel Dekker: New York, 1971, p. 333.

Received: November 1, 2006

Web Release Date: August 10, 2007

7,8 β -Dihydroponasterone A, a New Phytoecdysteroid from the Needles of the Japanese Yew, *Taxus cuspidata*

Qing-Wen Shi,^{*,a} Mei Dong,^a Chang-Hong Huo,^a Xiao-Hui Su,^a Xing Li,^a Teiko Yamada^b and Hiromasa Kiyota^{*,b}

^aDepartment of Natural Product Chemistry, School of Pharmaceutical Sciences, Hebei Medical University, 361 Zhongshan East Road, 050017, Shijiazhuang, Hebei Province, P. R. China

^bLaboratory of Applied Bioorganic Chemistry, Division of Biocience & Biotechnology for Future Bioindustry, Graduate School of Agricultural Sciences, Tohoku University, 1-1 Tsutsumidori-Amamiya, Aoba-ku, Sendai 981-8555, Japan

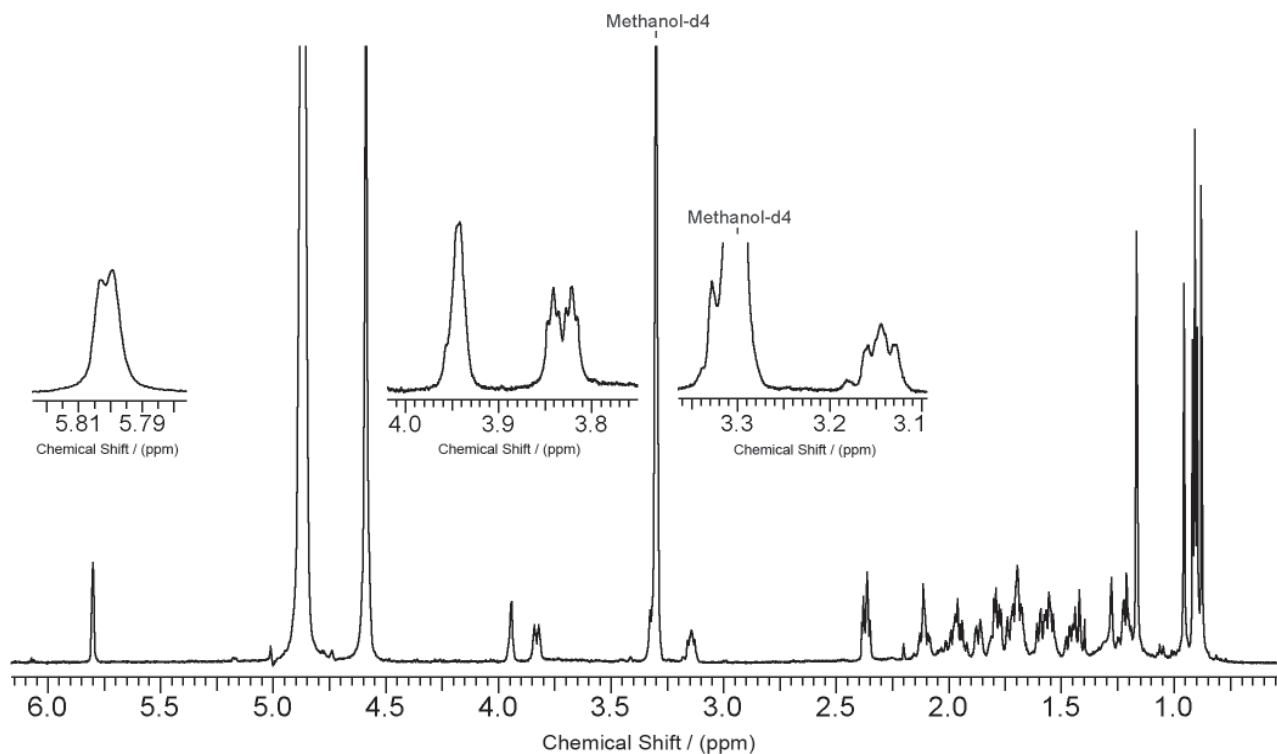


Figure S1. ¹H NMR data of compound 1 in CDCl₃ (500 MHz).

*e-mail: shiqingwen@hebmh.edu.cn; kiyota@biochem.tohoku.ac.jp

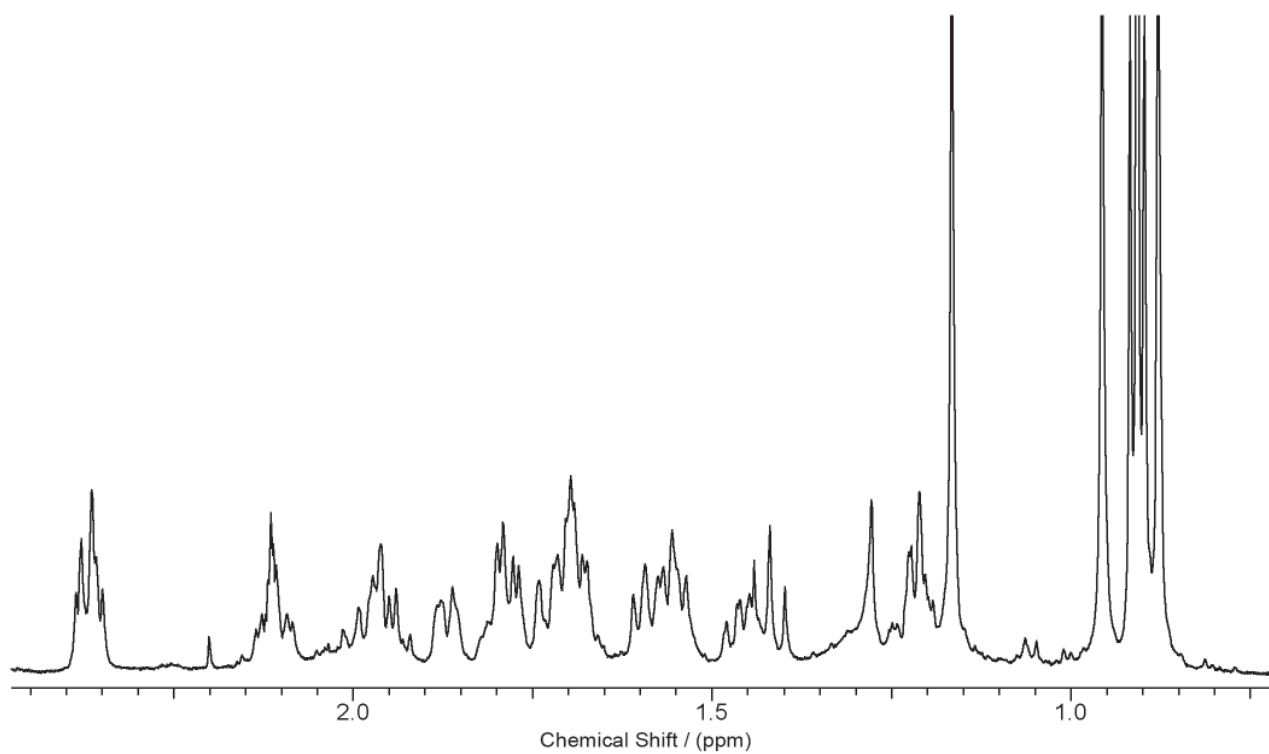


Figure S2. ^1H NMR data of compound **1** in CDCl_3 (500 MHz).