Available online at www.sciencedirect.com

ScienceDirect

Tetrahedron Letters 49 (2008) 3376–3379

Tetrahedron Letters

Oldhamine A, a novel alkaloid from *Daphniphyllum oldhami*

Chengjian Tan[a,b,c], Yingtong Di[a], Yuehu Wang[a], Ye Wang[c], Shuzhen Mu[c], Suo Gao[a], Yu Zhang[a], Ningchuan Kong[a], Hongping He[a], Jianxin Zhang[c], Xin Fang[a], Chunshun Li[a], Yang Lu[d], Xiaojiang Hao[a,*]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China*
[b] *Graduated School of Chinese Academy of Sciences, Beijing 100039, PR China*
[c] *The Key Laboratory of Chemistry for Natural Product of Guizhou Province and Chinese Academy of Science, Guiyang 550002, PR China*
[d] *Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, PR China*

Received 1 January 2008; revised 25 March 2008; accepted 26 March 2008
Available online 29 March 2008

Abstract

Oldhamine A, a novel alkaloid with an unusual cyclopentadienyl anion in the fused-pentacyclic skeleton was isolated from the twigs of *Daphniphyllum oldhami*. The structure of oldhamine A was elucidated based on the spectroscopic data and the single-crystal X-ray diffraction crystallography. It is the first X-ray diffraction of the *Daphniphyllum* alkaloids with nitrogen-containing two pentacyclic rings. The inter-conversion between cyclopentadienyl anion and conjugated enolic unit was also conducted.

Keywords: Daphniphyllaceae; *Daphniphyllum oldhami*; Oldhamine A; Internal salt; Cyclopentadienyl anion; Nortriterpenoid alkaloid

Daphniphyllum alkaloids are a family of diversified and complex polycyclic natural products elaborated by the trees of the genus *Daphniphyllum*.[1,2] We previously reported several novel alkaloids from the *Daphniphyllum* species.[3] In our continuing search for alkaloids of this genus, one new *Daphniphyllum* alkaloid named oldhamine A with an unusual cyclopentadienyl anion in the fused-pentacyclic skeleton, together with two alkaloids, deoxycalyciphylline B and deoxyisocalyciphylline B,[2c] were isolated from the extract of the twigs of *Daphniphyllum oldhami* collected in Songtao County of Guizhou Province, People's Republic of China.

The powdered twigs (20 kg) of *D. oldhami* were percolated three times with 75% EtOH to give a crude extract (500 g). The extract was concentrated to dryness under reduced pressure, followed by the partitioning between EtOAc and 3% tartaric acid. The aqueous phase was then adjusted to pH 10 with a saturated Na_2CO_3, and extracted with $CHCl_3$ to give crude alkaloids (18 g). The crude alkaloids were subjected to a silica gel column eluted with $CHCl_3$/MeOH (1:0–0:1), in which a fraction eluted with $CHCl_3$/MeOH (5:1) was chromatographed over a silica gel column with $CHCl_3$/MeOH (10:1) and then RP-18 (10% MeOH) to afford oldhamine A (15 mg).

Oldhamine A was isolated as an optically active ($[\alpha]_D^{27}$ +118.3, *c* 0.48 $CHCl_3/CH_3OH$ 1:1) light yellow crystal ($CHCl_3/MeOH/H_2O$, 5:1:0.04).[6] The molecular formula was established as $C_{21}H_{23}NO_4$ by HR-ESI-MS (*m*/*z* 354.1698, $[M+H]^+$, calcd: 354.1705), indicating 11° of unsaturation. The IR absorption spectrum showed the presence of conjugated carbonyl (1663 and 1618 cm^{-1}) functionality. The compound was dissolved in CD_3OD and then NMR experiments were carried out. All the carbon atoms in the molecule were resolved in the ^{13}C NMR spectra (Table 1) as 21 carbon signals comprising of eight sp^2 and one sp^3 quaternary carbons, four methines, six methylenes, and two methyls. Among them, two

---

[*] Corresponding author. Tel.: +86 871 522 3263; fax: +86 871 521 9684.
E-mail address: haoxj@mail.kib.ac.cn (X. Hao).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.03.126

Table 1
^{1}H, ^{13}C NMR and HMBC correlations of oldhamine A in CD_3OD

No.	δ_C[a]	δ_H[b]	HMBC (H→C)
1	196.8 s	—	—
2	58.6 d	3.00 (1H, d, 7.9)	1, 4, 13, 20
3α	27.2 t	2.69 (1H, m)	1, 2, 5, 18
3β		2.60 (1H, m)	1, 2, 4, 5
4	79.5 d	4.19 (1H, br d, 7.9)	2, 3, 5, 8, 18, 21
5	51.4 s	—	—
6	50.0 d	2.62 (1H, m)	4, 5, 7, 8, 12, 21
7α	63.1 t	2.83 (1H, m)	18
7β		4.10 (1H, m)	4, 5
8	137.6 s	—	—
9	124.2 s	—	—
10	204.5 s	—	—
11α	39.5 t	2.49 (1H, dd, 17.6, 5.2)	6, 9, 10, 12
11β		2.88 (1H, m)	6, 10, 12
12α	26.6 t	2.12 (1H, m)	5, 10, 11
12β		1.35 (1H, br d, 11.9)	6, 7, 10, 11
13	121.7 s	—	—
14	111.7 s	—	—
15	144.2 s	—	—
16α	25.9 t	2.74 (1H, m)	14, 15
16β		3.10 (1H, m)	14, 15, 17
17α	68.7 t	4.29 (1H, m)	15, 16, 22
17β		4.41 (1H, m)	15, 16, 22
18	75.0 d	4.06 (1H, m)	1, 3, 7, 20
20	20.8 q	1.55 (3H, d, 6.8)	2, 18
21	32.7 q	1.51 (3H, s)	4, 5, 6, 9
22	168.0 s	—	—

[a] Measured in 100 MHz.

[b] Measured in 400 MHz.

methines (δ_C 79.5 and 75.0) and one methylene (δ_C 63.1) were ascribed to those bearing a nitrogen atom. Since eight sp^2 quaternary carbons accounted for five degrees of the unsaturation, oldhamine A was inferred to possess six rings.

The ^{1}H–^{1}H COSY spectrum of oldhamine A indicated connectivity of three protonated fragments: **a** (C-2 to C-4 and C-18 to C-20), **b** (C-6 to C-7 and C-11 to C-12), and **c** (C-16 to C-17) drawn with bold bonds in Figure 1. Comprehensive analyses of the HMBC spectrum indicated the connection of the above-mentioned substructures (**a**–**c**), as well as heteroatoms and quaternary carbon atoms. The connectivity of C-4, C-7, and C-18 through a nitrogen atom was assigned by the HMBC correlations of H-7α to C-18 (δ_C 75.0) and H-7 β to C-4 (δ_C 79.5). HMBC correlations of H_3-21 to C-4, C-5, and C-6 implied that C-4, C-6, and C-21 were attached to C-5 (δ_C 51.4), and the correlations of H-4 and H-6 to C-8 (δ_C 137.6) indicated the connection between C-5 and C-8. The connections of C-8 to C-9 and C-14 and C-15 were suggested by the HMBC correlations of H_3-21 to C-9 (δ_C 124.2), H_2-16 to C-14 (δ_C 111.7) and C-15 (δ_C 144.2), H_2-17 to C-15 (δ_C 144.2), respectively. The connections of C-17 to C-22 and C-16 to C-15 were confirmed by the HMBC correlations of H_2-17 to C-15 (δ_C 144.2) and C-22 (δ_C 168.0). HMBC cross peaks from H_2-11 and H_2-12 to C-10 (δ_C 204.5), which indicated the signal of conjugated ketone, were observed. Furthermore, the attachment of C-1 to C-2 was established by the HMBC correlation between H-2 and C-1. However, the resonance of δ_C 196.8 rooted in C-1 is not a convincing evidence to distinguish between the enolic carbon and conjugated ketone here,[2d] therefore, two proposed planar structures (A and B) with mainly difference at C-1 were established as shown in Figure 1.

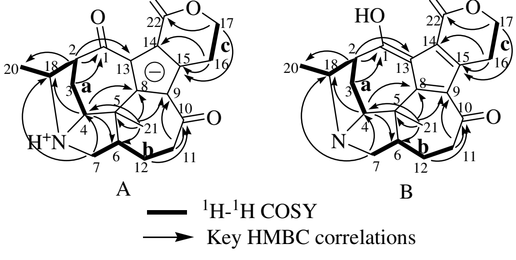


Fig. 1. Selected 2D NMR correlations for A and B.

The single crystal of this compound was obtained from $CHCl_3/MeOH/H_2O$ (5:1:0.04) and an X-ray diffraction experiment (Fig. 2)[5] was realized. The very similar bond lengths (1.424 ± 0.026 Å) of C-8 to C-9, C-9 to C-15, C-15 to C-14, C-14 to C-13, and C-13 to C-8 acquired from the X-ray diffraction analysis supported the presence of cyclopentadienylium anion in the system. Thus, the structure of A was unveiled. Compared to the other two cyclopentadienylium zwitterion compounds macropodumine B and daphnicyclidin L,[4] two five-membered rings and one seven-membered ring contained the same N-atom. Furthermore, the β configuration at C-2, C-4, C-6, and C-18, which was deduced from ROESY correlations of H3-21/H-3β, H-3 β/H-2, H3-21/H-4, H3-21/H-6, H-3α/H-18, was further confirmed by the results of single-crystal X-ray diffraction analysis.

To study whether inter-conversion could be happened between cyclopentadienyl anion and conjugated enolic unit, NMR experiments were performed after acid and alkali were subsequently added. Firstly, one drop of TFA

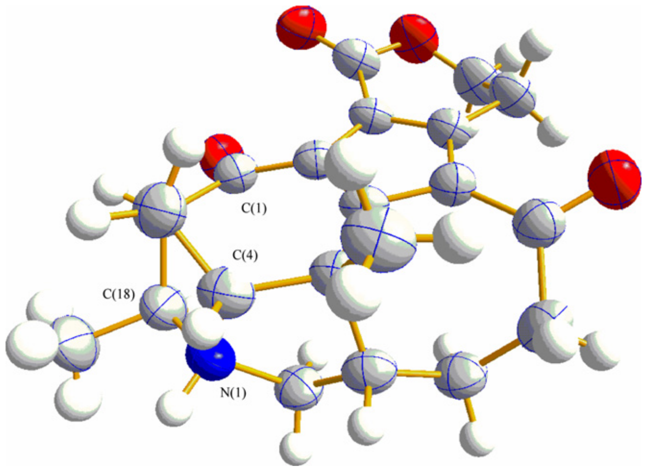


Fig. 2. ORTEP drawing of oldhamine A. Thermal ellipsoids are shown at the 50% probability level.

Table 2
^{1}H, ^{13}C NMR and HMBC correlations of oldhamine B in CD_3OD and ^{13}C NMR of oldhamine C in $CD_3OD/CDCl_3$ (9:1)

No.	δ_C[a]	δ_H[b]	HMBC (H→C)	δ_C[a]
1	189.3	—	—	201.9
2	55.1	3.26 (1H, br d, 8.2)	1, 3, 4, 13, 18, 20	59.7
3α	28.5	2.75 (2H, m)	1, 2, 4, 5	28.1
3β				
4	79.2	4.25 (1H, br d, 6.5)	2, 3, 5, 8, 18, 21	79.3
5	51.8	—	—	52.3
6	50.0	2.65 (1H, m)	4, 5, 7, 8, 12, 21	50.0
7α	63.0	2.89 (1H, m)	12, 18	64.9
7β		4.14 (1H, m)	4, 5, 18	
8	145.8	—	—	141.0
9	132.7	—	—	123.8
10	205.1	—	—	205.6
11α	39.1	2.56 (1H, dd, 16.8, 5.6)	6, 9, 10, 12	40.4
11β		2.85 (1H, m)	6, 10, 12	
12α	25.5	2.12 (1H, m)	5, 6, 11	27.8
12β		1.42 (1H, m)	6, 7, 10, 11	
13	117.0	—	—	122.4
14	113.2	—	—	111.6
15	150.3	—	—	143.2
16α	23.8	2.75 (1H, m)	9, 14, 15, 17, 22	26.3
16β		3.32 (1H, m)	9, 14, 15, 17, 22	
17α	70.6	4.55 (2H, m)	15, 16, 22	68.2
17β				
18	75.8	4.20 (1H, m)	1, 2, 3, 7, 20	73.7
20	20.8	1.59 (3H, d, 7.0)	2, 18	25.2
21	32.0	1.55 (3H, s)	4, 5, 6, 8, 9	33.2
22	171.7	—	—	167.7

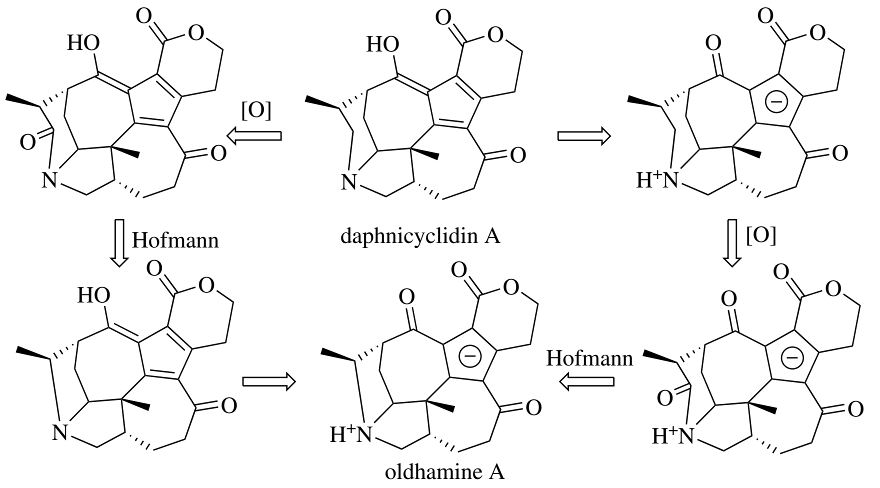
[a] Measured in 125 MHz.

[b] Measured in 500 MHz.

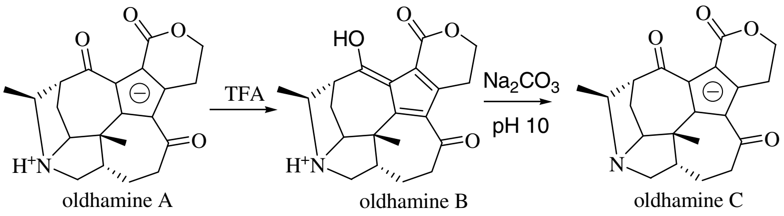
was added in CD_3OD solution of oldhamine A. In its ^{13}C NMR spectra, 21 carbon signals were observed, including six sp^2 carbon atoms, two carbonyl groups, and 13 sp^3 carbon atoms (1 × C, 4 × CH, 6 × CH_2 and 2 × CH_3), which were same as those of oldhamine A. By a detailed comparison of their ^{13}C NMR data, partial of them, especially C-1, C-8, C-9, C-13, and C-15 shifted up or downfield up to about 8 ppm, which were similar to those in daphnicyclidin A, whose structure was confirmed by an X-ray diffraction experiment.[2d] Therefore, oldhamine B (**2**)[7] was implied to possess partial structure of daphnicyclidin A, including conjugated cyclopentadiene moiety (C-8–C-9 and C-13–C-15) and enolic carbon at C-1 as shown, and 2D-NMR experiments were further confirmed by this hypothesis (Table 2). Secondly, the above-mentioned CD_3OD solution was directly adjusted to pH 10 with a saturated Na_2CO_3, and subjected by Sephadex LH-20 (CH_3OH) to yield oldhamine C (**3**).[8] Except that the ketone carbonyl at C-1 and methyl at C-20 downfield shifted about 5 ppm, the close similarity of the other ^{13}C NMR data of oldhamine C to those of oldhamine A indicated that the structure of the former should be analogous to the latter. Under basic condition, the downfield shift of C-20 was due to the lack of γ-gauche effects between H–N and CH_3-20, which existed in oldhamine A. So, we concluded that oldhamine C should be non-HN form of oldhamine A as shown. 2D-NMR experiments further confirmed this character. It seemed that the inter-transformation between conjugated cyclopentadiene moiety and cyclopentadienyl anion can be conducted in a pH dependent chemical environment as shown in Scheme 2.

Biogenetically, oldhamine A might be derived from daphnicyclidin A by two different pathways. The main distinctions were focused on the sequences of the Hofmann degradation of C-19 and the isomerization of enolic carbon and conjugated ketone at C-1 as described in Scheme 1.

The natural product with cyclopentadienyl carbanions, stabilized as a zwitterion by an inner salt is structurally unique in the class of alkaloids.[4b] In general, this kind of alkaloid has high solubility in H_2O and showed high polarity. The cyclopentadienyl anion present in oldhamine A is not only stabilized by the formation of an inner salt, but also by the electron-withdrawing effect of the three adjacent C=O groups.[5] The unique structural feature of macropodumine B, daphnicyclidin L, and oldhamine A represents a new type of alkaloids in the genus *Daphniphyllum*, which is also a source of challenging subjects on total and biosynthetic synthesis.



Scheme 1. Biogenetic pathway proposed for oldhamine A.



Scheme 2. Chemical correlations between cyclopentadienyl anion and conjugated enolic unit.

The cytotoxicity assays showed that oldhamine A was not active against the acute myelogenous leukemia (HL-60) or human lung cancer (A-549) cell line ($ED_{50} >$ 10 μg/ml).

Acknowledgments

The work was financially supported by the National Natural Science Foundation of China (No. 20672120) and the Foundation from the Ministry of Science and Technology (No. 2006CB708518). The authors thank Dr. Xun Chen of the Guizhou Academy of Science for the identification of the plant materials and the reviewers for good suggestions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.03.126.

References and notes

1. For a review of *Daphniphyllum* alkaloids see: Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 2003; Vol. 60, pp 165–205 and references cited therein.
2. (a) Saito, S.; Kubota, T.; Fukushi, E.; Kawabata, J.; Zhang, H. p.; Kobayashi, J. *Org. Lett.* **2007**, *9*, 1207–1209; (b) Yang, S. P.; Yue, J. M. *Org. Lett.* **2004**, *6*, 1401–1404; (c) Yang, S. P.; Yue, J. M. *J. Org. Chem.* **2003**, *68*, 7961–7966; (d) Kobayashi, J.; Inaba, Y.; Shiro, M.; Yoshida, N.; Morita, H. *J. Am. Chem. Soc.* **2001**, *123*, 11402–11408.
3. (a) Li, C. S.; Di, Y. T.; He, H. P.; Gao, S.; Wang, Y. H.; Lu, Y.; Zhong, J. L.; Hao, X. J. *Org. Lett.* **2007**, *9*, 2509–2512; (b) Di, Y. T.; He, H. P.; Wang, Y. S.; Li, L. B.; Lu, Y.; Gong, J. B.; Fang, X.; Kong, N. C.; Li, S. L.; Zhu, H. J.; Hao, X. J. *Org. Lett.* **2007**, *9*, 1355–1358; (c) Zhang, Y.; He, H. P.; Di, Y. T.; Mu, S. Z.; Wang, Y. H.; Wang, J. S.; Kong, N. C.; Gao, S.; Hao, X. J. *Tetrahedron Lett.* **2007**, *48*, 2737–2740; (d) Mu, S. Z.; Li, C. S.; He, H. P.; Di, Y. T.; Wang, Y.; Wang, Y. H.; Zhang, Z.; Lu, Y.; Zhang, L.; Hao, X. J. *J. Nat. Prod.* **2007**, *70*, 1628–1631; (e) Li, C. S.; He, H. P.; Di, Y. T.; Wang, Y. H.; Mu, S. Z.; Li, S. L.; Gao, S.; Gao, Z. L.; Hao, X. J. *Tetrahedron Lett.* **2007**, *48*, 2737–2740; (f) Kong, N. C.; He, H. P.; Wang, Y. H.; Gao, S.; Di, Y. T.; Hao, X. J. *Helv. Chim. Acta* **2007**, *90*, 972–976; (g) Li, L.; He, H. P.; Di, Y. T.; Gao, S.; Hao, X. J. *Tetrahedron Lett.* **2006**, *47*, 6259–6262; (h) Di, Y. T.; He, H. P.; Liu, H. Y.; Du, Z. Z.; Tian, J. M.; Yang, X. W.; Wang, Y. H.; Hao, X. J. *Tetrahedron Lett.* **2006**, *47*, 6259–6262; (i) Mu, S. Z.; Wang, Y.; He, H. P.; Yang, X. W.; Wang, Y. H.; Di, Y. T.; Lu, Y.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1065–1069; (j) Di, Y. T.; He, H. P.; Li, C. S.; Tian, J. M.; Mu, S. Z.; Li, S. L.; Gao, S.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1745–1748; (k) Di, Y. T.; He, H. P.; Lu, Y.; Yi, P.; Li, L.; Wu, L.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1074–1076; (l) Mu, S. Z.; Yang, X. W.; Di, Y. T.; He, H. P.; Wang, Y.; Wang, Y. H.; Li, L.; Hao, X. J. *Chem. Biodivers.* **2007**, *4*, 129–137.
4. (a) Gan, X. W.; Bai, H. Y.; Chen, Q. G.; Ma, L.; Hu, L. H. *Chem. Biodivers.* **2006**, *3*, 1255–1259; (b) Zhang, W.; Guo, Y. W.; Krohn, K. *Chem. Eur. J.* **2006**, *12*, 5122–5127.
5. *X-ray diffraction of oldhamine A crystal data*: $C_{21}H_{23}NO_4$, $M_W = 353.42$, orthorhombic system, space group $P2_12_12_1$, crystal cell parameters: $a = 9.896(1)$ Å, $b = 10.445(2)$ Å, $c = 18.749(1)$ Å, $V = 1938.0(3)$ Å^3, $Z = 4$, $d = 1.352$ g/cm^3. A colorless, block crystal of dimensions 0.10 × 0.20 × 0.30 mm was used for X-ray measurements on a MAC DIP-2030K diffractometer with a graphite-monochromated Mo Kα radiation; the $2\theta_{max}$ value was set at 50.0°. The total number of independent reflections measured was 1661 of which 1978 were considered to be observed ($|F|^2 \geqslant 2\sigma|F|^2$). The crystal structure of oldhamine A was solved by the direct method SHELXS-97[9] and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[10] and full-matrix least-squares calculations. H atoms were fixed at calculated positions. The final indices were $R_1 = 0.0575$, $wR_2 = 0.1509$, $S = 1.082$. Crystallographic data for the structure in this Letter have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC 671960. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
6. *Oldhamine A*: A light yellow crystal ($CHCl_3/MeOH/H_2O$, 5:1:0.04); $[\alpha]_D^{27}$ +118.3, (*c* 0.48 $CHCl_3/CH_3OH$ 1:1); R_f 0.41 ($CHCl_3$/MeOH 7:3); dissolved in CH_3OH and H_2O; IR (KBr) ν_{max} 3431, 2926, 1663, 1618 and 1458 cm^{-1}; UV ($CHCl_3$/MeOH 1:1) λ_{max} 236.5 nm (ε 4886), 296 nm (ε 18337), 360 nm (ε 9388); ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 354 $[M+H]^+$; HRESIMS *m/z* 354.1698 (M+H; calcd for $C_{21}H_{23}NO_4$, 354.1705).
7. *Oldhamine B*: A light yellow solid; $[\alpha]_D^{20}$ +27.5, (*c* 0.23 CH_3OH); UV (CH_3OH) λ_{max} 192.5 nm (ε 4210), 201.5 (ε 4873), 294.5 nm (ε 7564); 360 nm (ε 4454); ^1H and ^{13}C NMR data (Table 2); FAB$^+$ *m/z* 354 $[M]^+$.
8. *Oldhamine C*: A light yellow solid; $[\alpha]_D^{20}$ +24.4, (*c* 0.43 CH_3OH); UV (CH_3OH) λ_{max} 229 (ε 1467), 295 nm (ε 5224); 360 nm (ε 2676); ^{13}C NMR data (Table 2); FAB$^-$ *m/z* 352 $[M]^-$.
9. Sheldrick, G. M. *SHELX-97, Program for Crystal Structure Refinement*; University of Gottingen: Gottingen, Germany, 1997.
10. Lu, Y.; Wu, B. M. *Chin. Chem. Lett.* **1992**, *3*, 637–640.