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Aspeverin, a New Alkaloid from an Algicolous Strain of *Aspergillus versicolor*

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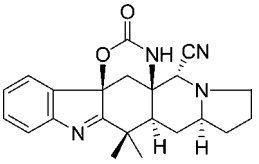
ABSTRACT



A novel carbamate- and cyano-containing alkaloid, aspeverin (1), was isolated from the culture of an algicolous *Aspergillus versicolor* strain (dl-29). The structure and absolute configuration were unambiguously identified by NMR, IR, ECD, and mass spectrometric methods as well as quantum chemical calculations. 1 exhibited potent bioactivities against some marine-derived organisms.

Aspergillus species of diverse origins have been proven to be prolific sources of structurally interesting secondary metabolites, such as alkaloids, peptides, polyketides, and terpenes, and some of them exhibited intriguing biological activities.[1−6] Among these, indolic alkaloids with unusual skeletons often occurred,[1−3] which also attracted much attention for total syntheses, biomimetic syntheses, and biosyntheses.[7−9] Here, our efforts on an endophytic strain (dl-29) of *A. versicolor*, isolated from the marine green alga *Codium fragile*, led to the isolation and identification of a novel carbamate- and cyano-containing alkaloid, aspeverin (**1**). The main subjects of this paper are the isolation, structure elucidation, and bioactivity of aspeverin (**1**).

The whole culture of *A. versicolor* dl-29 was extracted with EtOAc after a static fermentation (15 L) for 30 days. Then, the extract was separated by repeated column chromatography on silica gel and Sephadex LH-20 as well as preparative TLC to yield compound **1** (Supporting Information).

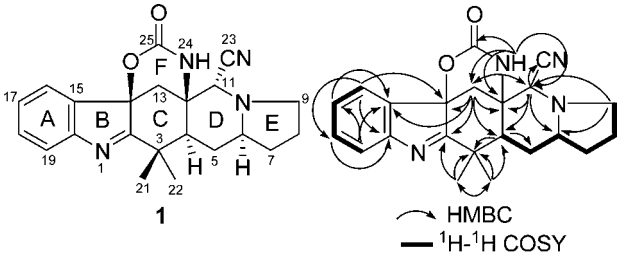


Figure 1. Structure and key ^{1}H−^{1}H COSY (solid lines) and HMBC (arrows) correlations of **1**.

Compound **1** was obtained as a colorless gum with $[\alpha]^{23}_{D}$ +17.8 (*c* 0.18, MeOH). The positive electrospray ionization mass spectrum (ESIMS) showed a quasimolecular ion peak at *m*/*z* 377 $[M + H]^+$, and the negative ESIMS gave a quasimolecular ion peak at *m*/*z* 375 $[M - H]^-$, which suggested a molecular weight of 376. A molecular formula of $C_{22}H_{24}N_4O_2$ was assigned by HREIMS

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(m/z 376.1857 $[M]^+$, calcd for $C_{22}H_{24}N_4O_2$, 376.1899), requiring 13 degrees of unsaturation.

Table 1. ^1H and ^{13}C NMR Data for **1**[a]

	δ_H, mult. (J in Hz)		δ_C	
pos.	in DMSO-d_6	in $CDCl_3$	in DMSO-d_6	in $CDCl_3$
2			186.0, qC	184.4, qC
3			39.3, qC	39.3, qC
4	1.65, dd (12.6, 2.9)	1.82, dd (12.5, 3.4)	49.4, CH	49.0, CH
5a	1.56, m	1.50, m	26.9, CH_2	27.4, CH_2
5b	1.91, m	2.00, m		
6	2.26, m	2.48, m	58.5, CH	58.4, CH
7a	1.55, m	1.54, m	30.0, CH_2	30.1, CH_2
7b	1.94, m	2.07, m		
8a	1.74, m	1.88, m	21.5, CH_2	21.4, CH_2
8b	1.84, m	1.93, m		
9a	2.31, ddd (8.7, 8.7, 8.7)	2.54, ddd (8.8, 8.8, 8.8)	50.2, CH_2	50.6, CH_2
9b	3.00, ddd (8.7, 8.7, 2.6)	3.00 ddd (8.8, 8.8, 2.8)		
11	4.45, s	3.93, s	60.7, CH	61.4, CH
12			54.4, qC	54.6, qC
13a	1.78, dd (13.5, 1.4)	1.99, dd (13.6, 1.6)	36.8, CH_2	38.0, CH_2
13b	2.79, d (13.5)	2.48, d (13.6)		
14			85.8, qC	86.2, qC
15			136.4, qC	135.2, qC
16	7.55, d (7.3)	7.45, d (7.4)	123.7, CH	123.0, CH
17	7.32, ddd (7.3, 7.4, 1.0)	7.29, dd (7.2, 7.4)	127.0, CH	126.9, CH
18	7.47, ddd (7.4, 7.6, 1.2)	7.44, dd (7.2, 8.0)	131.2, CH	131.1, CH
19	7.56, d (7.6)	7.58, d (8.0)	121.2, CH	121.3, CH
20			153.1, qC	152.7, qC
21	1.12, s	1.26, s	22.1, CH_3	22.0, CH_3
22	1.38, s	1.49, s	27.4, CH_3	27.2, CH_3
23			114.4, qC	113.0, qC
24	7.58, s	6.29, s		
25			151.4, qC	151.6, qC

[a] Recorded at 500 and 125 MHz for ^1H and ^{13}C, respectively.

The ^1H NMR spectrum (Table 1) displayed signals for four aromatic protons in the downfield region, which along with $^1H-^1H$ COSY data (Figure 1) and an IR absorption at 756 cm^{-1} indicated the presence of an ortho-disubstituted phenyl group (ring A). On the other hand, the HMBC correlations from H-13 to C-2, C-4, C-12, and C-14 and from H-21 and H-22 to C-2, C-3, and C-4 suggested the presence of a six-membered ring C. The connectivity between rings A and C was established by HMBC correlations from H-13 to C-15 and from H-16 to C-14 and by comparison of ^{13}C NMR data (Table 1) with those reported for a synthetic intermediate of brevianamide B.[8] Moreover, this tricyclic moiety was further extended from C-4 to C-9 by $^1H-^1H$ COSY correlations, where C-6 and C-9 were connected to C-11 through N-10 by the relatively downfield ^1H NMR signals of H-6, H-9, and H-11 and HMBC correlations from H-11 to C-6 and from H-9 to C-6 and C-11. Based on the HMBC correlation from H-11 to C-23, a cyano group, indicated by ^{13}C NMR data (C-23) and an IR absorption at 2233 cm^{-1}, was bonded to C-11,[10] which was attached to C-12 by HMBC correlations from H-11 to C-4, C-12, and C-13. Furthermore, a carbonyl group (C-25) indicated by a strong IR absorption at 1712 cm^{-1} was deduced to be linked to C-12 and C-14 through a nitrogen and an oxygen atom, respectively, to form a carbamate group by analysis of HMBC correlations from H-24 to C-12 and C-25 and by comparison of ^{13}C NMR data with those of similar units as well as by inspection of the elemental composition of **1**.[8,11] The above data evidenced the planar structure of **1**, which was further supported by the other HMBC correlations as illustrated in Figure 1.

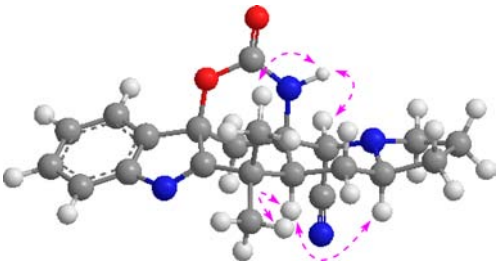

Figure 2. Energy-minimized conformer and key NOESY correlations (dashed arrows) of **1**.

The relative configuration of compound **1** was determined by a NOESY experiment. H-11, Me-21, and ring F were located on the same face by NOE correlations of H-24 with H-11 and H-21, while H-4, H-6, and Me-22 were oriented on the other side by NOE correlations of H-4 with H-6 and H-22 (Figure 2). Additionally, eight possible conformers of **1** were generated by the Dreiding force field in MarvinSketch[12] and further optimized using density function theory (DFT) at the gas-phase B3LYP/6-31G(d) level via Gaussian 09 software[13] to give just one conformer (Figure 2) within a 3 kcal/mol energy threshold from the

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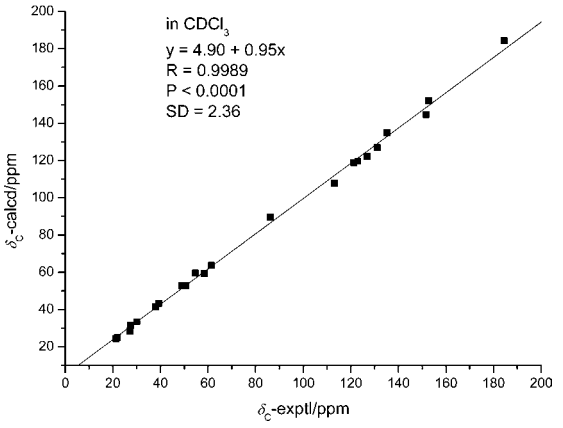

Figure 3. Correlation between experimental and calculated ^{13}C NMR data for **1**.

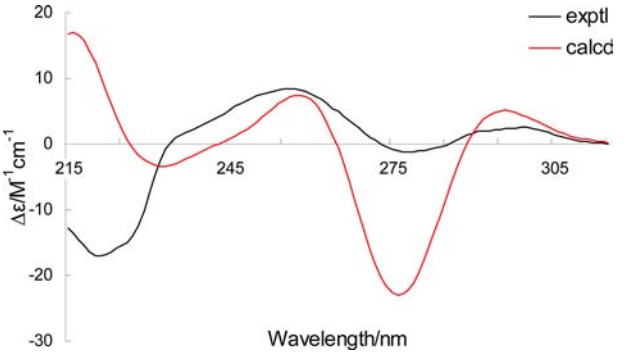

Figure 4. Experimental and calculated ECD spectra of **1**.

global minimum, which matched well with the above NOESY data. Thus, the relative configuration of **1** was established.

To confirm the structure and relative configuration of compound **1**, we tried to crystallize it for an X-ray single crystallographic analysis but failed at various conditions. Alternatively, the ^{13}C NMR data of **1** were tentatively predicted by quantum chemical calculations using the gauge-independent atomic orbital (GIAO) method at the gas-phase B3LYP/6-31+G(d,p) level with tetramethylsilane (TMS) as a reference.[14,15] The calculated ^{13}C NMR data with a standard deviations (SD) of 2.36 ppm (in $CDCl_3$) for **1** were in good agreement with the experimental ones (Figure 3), which further corroborated the complicated structure of **1**.

Quantum chemical calculations of electronic circular dichroism (ECD) spectra have been proven to be reliable tools in deducing the absolute configurations of organic molecules.[16] To establish the absolute configuration of compound **1**, its ECD spectrum was determined and computed with the time-dependent density function theory (TD-DFT) method at the gas-phase B3LYP/6-31G(d) level. The calculated ECD spectrum was produced by SpecDis software,[17] which was in good accordance with the experimental one (Figure 4). Thus, the absolute configuration was suggested to be 4*S*, 6*S*, 11*R*, 12*R*, and 14*R*, and **1** was trivially named aspeverin.

In order to evaluate the biological activity of aspeverin (**1**), it was assayed for growth inhibition against marine zooplankton (*Artemia salina*) and phytoplankton (*Heterosigma akashiwo*) as well as four bacteria (*Vibrio ichthyoenteri*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Bacillus cereus*) isolated from seawater.[18,19] The results (Table S1) showed that **1** inhibited growth of *H. akashiwo* with the EC_{50}'s of 6.3 and 3.4 μg/mL for 24 and 96 h, respectively, and weak inhibitory activities against *A. salina*, *P. mirabilis*, *E. cloacae*, and *B. cereus*.

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Supporting Information Available. Experimental details; NMR, MS, and IR spectra; and Cartesian coordinates of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.