

## Niveulone, a Heterocyclic Spiro Terpenoid from the Ascomycete *Dasyscyphus niveus*

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**Abstract** The new heterocyclic spiro terpenoid niveulone (**1**) was isolated from the cultural fluid of the ascomycete *Dasyscyphus niveus*, and its chemical structure and relative configuration were determined by spectroscopic techniques.

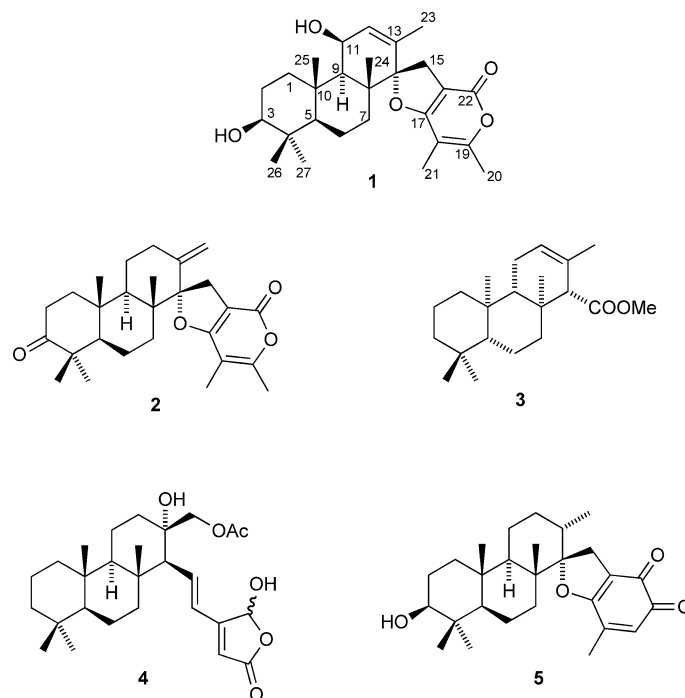
**Keywords** niveulone, heterocyclic spiro terpenoid, *Dasyscyphus niveus*

Niveulone (**1**) was isolated as one of several biologically active metabolites of the ascomycete *Dasyscyphus niveus*, as described in a preceding paper [1]. It is weakly cytotoxic towards human cell lines, and has an interesting as well as challenging structure with an interesting carbon skeleton. The structure was elucidated by spectroscopic techniques. The 1D NMR data recorded in CDCl<sub>3</sub> as well as in C<sub>6</sub>D<sub>6</sub> are presented in Table 1. MS experiments with FAB ionization revealed that the molecular weight of niveulone (**1**) is 442, as a molecular ion of 443 (M+H) was obtained under normal conditions while 465 (M+Na) and 481 (M+K) appeared when NaOAc or KOAc was added to the sample. High resolution experiments suggested that the elemental composition is C<sub>27</sub>H<sub>38</sub>O<sub>5</sub>, as the correct exact masses were obtained for M+H, M+Na, and M+K. This composition is in perfect agreement with the NMR data which show the presence of 27 carbons and 36 hydrogens bound to carbons. This gives **1** 9 unsaturations, and although the number of double bonds was difficult to determine based on the 1D NMR data alone it was obvious that the compound contains several rings.

The structure of niveulone (**1**) (see Fig. 1) was essentially determined from 2D NMR spectroscopy (COSY, HMQC and HMBC) while the relative stereostructure was determined based on NOESY data (see Fig. 2 for a summary of the pertinent HMBC and NOESY correlations observed). A critical partial structure to address was the pyranone moiety. HMBC correlations from 20-H<sub>3</sub> to two carbons only, C-18 and C-19, and from 21-H<sub>3</sub> to C-17, C-18 and C-19, suggested that the two methyls are vicinal and positioned on a highly polarized C-18/C-19 carbon–carbon double bond substituted with a heteroatom (oxygen) at C-19 and adjacent to the unsaturated carbon C-17. HMBC correlations from 15-H<sub>2</sub> to C-16, C-17 and C-22 reveal the remaining carbons of the pyranone, that have the expected high field (C-16 and C-18) and low field (C-17, C-19 and C-22) resonances. The distinction between a 2-pyranone (with C-22 as the carbonyl carbon) and a 4-pyranone (with C-17 as the carbonyl carbon) was facilitated by a synthetic study of the pyranoditerpene lygodinolide (**2**) [2], that produced both variants. Comparing the carbon resonances obtained for the pyranone moiety of niveulone (**1**) with those reported for the spiro furo-2-pyranone and the spiro furo-4-pyranone derivatives [2] clearly show that **1** is a 2-pyranone. C-15 is obviously also attached to C-14, as 15-H<sub>2</sub> gives HMBC correlations also to C-8, C-13 and C-14, while the methyl groups C-23 (attached to C-13) and C-24 (attached to C-8) both give HMBC correlations to C-14 without being geminal. In addition, 23-H<sub>3</sub> gives HMBC correlations to C-12 and C-13, a carbon–carbon double bond, while 24-H<sub>3</sub> also correlates with C-7, C-8 and C-9. With three substituents bound by carbon–carbon single bonds C-14 is obviously not unsaturated, although its <sup>13</sup>C

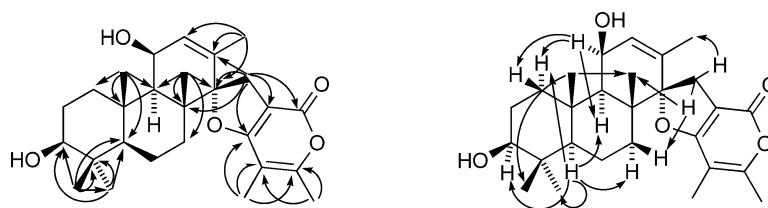
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**Fig. 1** The structure and relative configuration of the pyranoditerpenes niveulone (**1**) and lygodinolide (**2**).

The previously reported products methyl isocopalate (**3**), spongianolide A (**4**) and stypoldione (**5**) are shown with the absolute configuration.



**Fig. 2** Pertinent HMBC (left) and NOESY (right) correlations observed with niveulone (**1**).

NMR shift (99 ppm) is lower than expected if the fourth substituent is an oxygen. The clear COSY as well as HMBC correlations between 9-H and 11-H as well as 11-H and 12-H, and between 12-H and C-9, close the C-ring. HMBC correlations from the remaining methyl protons, from 25-H<sub>3</sub> to C-1, C-5, C-9 and C-10, from 26-H<sub>3</sub> to C-3, C-4, C-5 and C-27, and from 27-H<sub>3</sub> to C-3, C-4, C-5 and C-26 reveal a spin system that comprises almost all remaining carbons. Cosy correlations between 2-H<sub>2</sub> and 1-H<sub>2</sub> as well as 3-H, and HMBC correlations from 1-H<sub>2</sub> and 3-H to C-2 close the A-ring, while COSY correlations between 6-H<sub>2</sub> and 5-H as well as 7-H<sub>2</sub> together with HMBC correlations from 5-H and 7-H<sub>2</sub> to C-6 close the B-ring. With all atoms incorporated in the structure and 8 of the 9 unsaturations accounted for, nothing but closing the five-membered ring with the oxygen between C-14 and C-17 is possible.

Niveulone (**1**) consequently contains a 2,3-dihydrofuro[3,2-*c*]pyran-4-one moiety that is linked to a more traditional terpenoid part in a heterocyclic spiro compound.

The relative configuration of niveulone (**1**) was determined from NOESY correlations. Correlations between 25-H<sub>3</sub> and 24-H<sub>3</sub> as well as 26-H<sub>3</sub>, and between 5-H and 1-H $\alpha$  (1-Hb in Table 1), 3-H, 7-H $\alpha$  (7-Hb in Table 1), 9-H, and 27-H<sub>3</sub> show that the connections between rings A/B and B/C are as usual, *trans*. NOESY correlations for 11-H were observed to both 1-H $\alpha$  and 1-H $\beta$  as well as to 9-H, but not to 25-H<sub>3</sub>. This indicates that 11-H is  $\alpha$  as well as equatorial and that 11-OH consequently is  $\beta$  and axial. This is consistent with the relatively small coupling constant observed between 9-H and 11-H, 4.7 Hz in CDCl<sub>3</sub>. The relative configuration of the spiro carbon could be determined by the NOESY correlations between 15-H $\alpha$  and

**Table 1**  $^1\text{H}$  (500 MHz) ( $\delta$ ; multiplicity;  $J$ ) and  $^{13}\text{C}$  (125 MHz) ( $\delta$ ; multiplicity) NMR data for niveulone

C	$\text{CDCl}_3$		$\text{C}_6\text{D}_6$	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
1a	2.03; ddd; 3.5, 3.9, 12.8	38.2; t	1.84; ddd; 3.4, 3.6, 12.9	38.8; t
1b	1.26; m		1.00; m	
2a	1.72; m	27.0; t	1.58; m	28.0; t
2b	1.72; m		1.44; m	
3	3.26; dd; 6.5, 9.6	78.8; d	3.03; dd; 4.5, 11.7	79.0; d
4	—	38.7; s	—	39.4; s
5	0.72; dd; 4.3, 8.7	55.7; d	0.71; dd; 2.9, 10.8	56.7; d
6	1.59; m	18.3; t	1.43; m	19.0; t
7a	1.50; ddd; 3.1, 3.3, 12.9	32.9; t	1.41; m	33.5; t
7b	1.38; m		1.27; m	
8	—	40.6; s	—	41.2; s
9	1.55; d; 4.7	51.1; d	1.55; d; 4.8	52.0; d
10	—	38.0; s	—	38.7; s
11	4.49; dd; 4.7, 4.9	65.3; d	4.18; t; 4.8	65.6; d
12	5.70; d; 4.9	129.6; d	5.27; d; 4.8	130.4; d
13	—	133.7; s	—	134.0; s
14	—	99.4; s	—	99.8; s
15a	3.04; d; 15.9	29.3; t	3.01; d; 15.9	30.6; t
15b	2.98; d; 15.9		2.94; d; 15.9	
16	—	99.6; s	—	100.3; s
17	—	161.9; s	—	170.7; s
18	—	102.8; s	—	102.4; s
19	—	160.5; s	—	160.7; s
20	2.22; s	17.2; q	1.59; s	17.0; q
21	1.92; s	9.7; q	1.45; s	9.6; q
22	—	171.0; s	—	161.0; s
23	1.73; s	18.8; q	1.52; s	19.0; q
24	1.21; s	19.5; q	1.02; s	19.9; q
25	1.38; s	17.7; q	1.37; s	18.2; q
26	0.84; s	15.6; q	0.83; s	16.2; q
27	0.98; s	28.4; q	1.01; s	29.2; q

The spectra were recorded in  $\text{CDCl}_3$  and  $\text{C}_6\text{D}_6$ , and the solvent signals (7.26 and 7.16 ppm for  $^1\text{H}$  NMR, and 77.0 and 128.4 ppm for  $^{13}\text{C}$  NMR) were used as reference. The chemical shifts ( $\delta$ ) are given in ppm and the coupling constants  $J$  are given in Hz. The multiplicities of the carbon signals were determined indirectly from HMQC experiments.

24- $\text{H}_3$  as well as 7- $\text{H}\beta$ , which is equatorial. As expected, 15- $\text{Hb}$  correlates with 23- $\text{H}_3$ , although this is not informative.

Niveulone (**1**) is a new compound, but shares the carbon skeleton with the pyranoditerpene lygodinolide (**2**) reported from the fern *Lygodium flexuosum* [3]. It shares the basic part with the spongian diterpenes, e.g. the isocopalanes (e.g. methyl isocopalate (**3**) in Fig. 1) which are marine diterpenoids [4], but has a 2-pyranone moiety attached to C-15. There is some resemblance with the cheilanthanes

[5~7], e.g. spongianolide A (**4**), and the stypoldione type of terpenoids (e.g. stypoldione (**5**) [8]), although they have different carbon skeletons.

## Materials and Methods

$^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) spectra were recorded at room temperature with a Bruker DRX500 spectrometer with an inverse multinuclear 5 mm probehead

equipped with a shielded gradient coil. The spectra were recorded in  $\text{CDCl}_3$  and  $\text{C}_6\text{D}_6$ , and the solvent signals (see Table 1 for details) were used as reference. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for  $^1J_{\text{CH}}=145$  Hz and  $^nJ_{\text{CH}}=10$  Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101). Mass spectra (HRFAB) were recorded with a Jeol SX102 spectrometer. The UV and the IR spectra were recorded with a Varian Cary 2290 and a Perkin Elmer 298 spectrometer, and the optical rotations were measured with a Perkin-Elmer 141 polarimeter at  $22^\circ\text{C}$ .

Niveulone was obtained as a yellowish oil from fermentations of the ascomycete *Dasyscyphus niveus*, as described in the preceding paper [1].  $[\alpha]_{\text{D}}^{20} -23$  (*c* 0.5,  $\text{CHCl}_3$ );  $\lambda_{\text{max}}$  ( $\epsilon$ ) in MeOH: 295 nm (8,100);  $\nu_{\text{max}}$  (liquid film) 3435, 2930, 1700, 1575, 1445, 1275, 1115, 1060, 980, 920, 850 and  $750\text{ cm}^{-1}$ ; *m/z* HRMS (FAB,  $\text{M}+\text{H}^+$ ) found 443.2791.  $\text{C}_{27}\text{H}_{39}\text{O}_5$  requires 443.2797. See Table 1 for  $^1\text{H}$  and  $^{13}\text{C}$  NMR data.

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