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EMENIVEOL; A NEW POLLEN GROWTH INHIBITOR FROM THE FUNGUS, *EMERICELLA NIVEA*

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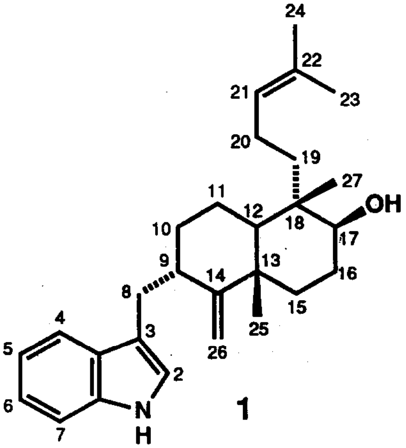
Summary *Emeniveol (1), a new pollen growth inhibitor, was isolated from the fungus, Emericella nivea. The structure was elucidated from the X-ray crystallographic analysis as well as homonuclear decoupling, COSY, COLOC and HMBC experiments, and chemical reaction.*

Pollen, the gametophyte of plants, is a physiologically important organ to cross-breed with a pistil. During the course of our screening program for new pollen growth regulators among the metabolites of fungi, an active compound named emeniveol (1) was isolated from the fungus, *Emericella nivea.*



1

The fungus was statically cultured in a malt extract medium at 24 °C for 21 days. The mycelial mats obtained after filtration were extracted with acetone. After evaporating the solvent, the acetone soluble fraction was subjected to a silica gel (bezene-acetone) column and subsequently purified on a Sephadex LH-20 (methanol) column chromatography. The active principle **1** thus obtained was recrystallized from methanol to afford pure needles in a yield of 7 mg per liter of the culture broth.

Emeniveol (**1**) was the following physicochemical properties: colorless needles (from MeOH); mp 179 °C; $[\alpha]_D^{20}$ -91 °(c 1.0, MeOH); $C_{28}H_{39}NO$ (M^+ obsd., *m/z* 405.3034, calcd., 405.3030); Anal. found, C: 82.59, H:9.72, N: 3.47 %, calcd., C: 82.91, H: 9.69, N 3.45 %. A positive coloration with the Ehrlich reagent (green red) and the strong fragmentation at *m/z* 130 in the EI-MS together with the ^{13}C chemical shifts (C-2, 121.93; C-3, 115.40; C-3a, 127.89; C-4, 118.92; C-5, 119.08; C-6, 121.76; C-7, 111.04; C-7a, 113.15) suggested the presence of an indole-3-yl methyl group (A) in **1**.

On hydrogenation of **1** by PtO_2, tetrahydroemeniveol[1] reduced of two double bonds was obtained. One of the double bonds was exomethylene (C-14, C-26) and the other was isohexenyl one (C-21, C-22).

Acetylation with Ac_2O-pyridine afforded a monoacetyl derivative[2]. Since the signal at δ_H 7.98 due to $N\underline{H}$ was remained in the ^1H-NMR spectrum, the presence of a hydroxyl group was indicated.

^1H-NMR and ^{13}C-NMR spectra indicated the presence of partial structure of F and G, further decoupling experiments, COSY and HMBC spectra together with chemical reactions accounted for the presence of the partial structures of B-G.

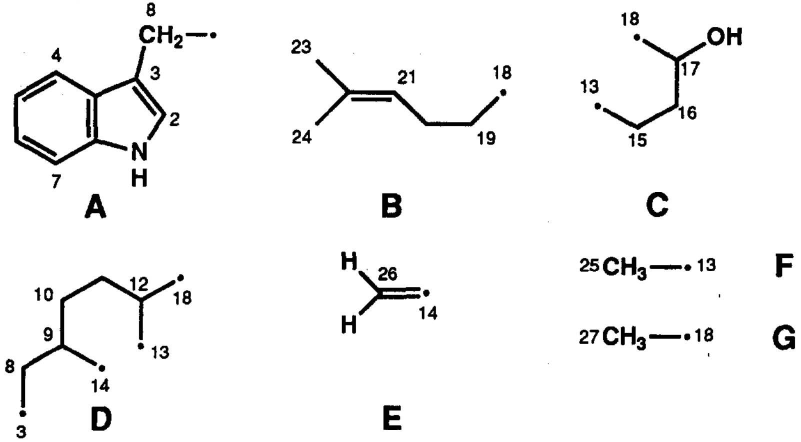


Fig. 1. Partial structures of emeniveol (**1**)

Considering the connections of the partial structures A-F just mentioned, and combining with the unsaturation degrees (ten units) from the molecular formula, Emeniveol (**1**) must have had a bicyclic skeleton except for indole moiety. In order to clarify the whole structure, an X-ray crystallographic analysis was carried out. Emeniveol (**1**) crystallized in the orthorhombic space group $P2_12_12_1$ with lattice constants, determined from a least-squares fit of diffractometer measured 2θ-values, of *a*=6.612 (1), *b*=31.689 (2), *c*=11.433 (2) Å and one molecule of composition $C_{28}H_{39}NO$ in the asymmetric unit. All diffraction maxima with $2\theta \leq 45°$ were collected using MoKα radiation and variable speed ω-scans. Of the 1845 reflections collected in this fashion, 1543 (84 %) were judged observed ($|Fo| \geq 4\sigma(|Fo|)$ after correction for Lorentz, polarization, and background effects. A phasing model was found routinely using the SHELXTL implementation of direct methods, and the structure was refined using full-matrix least squares refinements with anisotropic nonhydrogen atoms and fixed isotropic hydrogens. The final discrepancy index was 0.056 for the observed reflections[3].

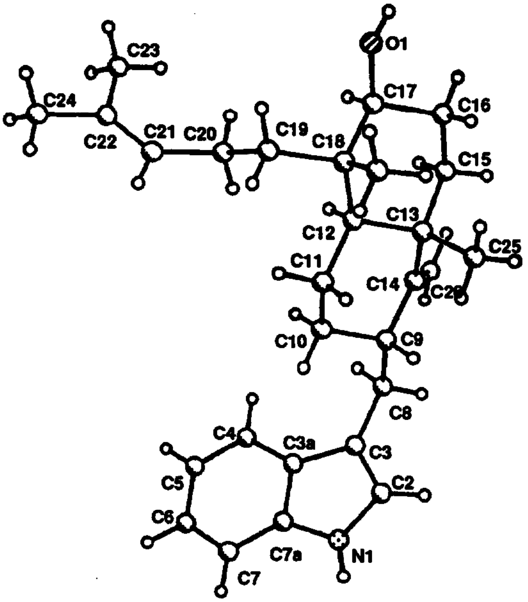


Fig. 2. A computer generated perspective drawing of the final x-ray model of emeniveol. No absolute stereochemistry is implied.

A computer generated perspective drawing of the final X-ray model is given in Figure 2. The indole fragment is planar within experimental error, and the decalin fragment has a chair chair conformation. The indolyl, isohexenyl and hydroxyl substituents are equatorial, and both methyls (C-25, C-27) are axial.

Although the chemical shifts at δ_C 162.97 in **1** due to C-14 was rather lower than that of other exomethylene resonance, the irradiations at C-9H, C-10H and C-25H in the COLOC spectra confirmed the assignment by appearing cross peaks.

Indole diterpenes, emindoles DA[4,5], DB[5], and SA[4,6], aflavinine derivatives[7], and nomine[8], have been previously isolated from the fungi as biologically active substances.

Compound **1** at concentration of 100 mg/L inhibited pine pollen germination and tea pollen growth by about 35.5 % over the control.

Table 1. ^{1}H and ^{13}C-NMR assignments of Emeniveol.

Carbon number	δ_C	δ_H	Carbon number	δ_C	δ_H
2	121.93 d	6.93 (1H, d, 1.95)	15	35.70 t	1.65 (2H, m)
3	115.40 s		16	27.72^{a} t	1.60 (1H, m) 1.77 (1H, m)
3a	127.89 s		17	73.36 d	3.52 (1H, dd, 4.98, 5.12)
4	118.92 d	7.56 (1H, dd, 8.05, 1.22)	18	41.52 s	
5	119.08 d	7.10 (1H, ddd, 8.05, 7.81, 0.98)	19	37.24 t	1.22 (1H, m) 1.45 (1H, m)
6	121.76 d	7.18 (1H, ddd, 8.05, 7.81, 1.22)	20	21.24^{a} t	1.80 (2H, m)
7	111.04 d	7.33 (1H, dd, 8.05, 0.98)	21	124.72 d	5.04 (1H, dd, 6.34, 1.46)
7a	136.15 s		22	131.15 s	
8	28.87 t	2.56 (1H, m)	23	17.61 q	1.57 (3H, br. s)
		3.11 (1H, dd, 13.78, 8.00)	24	25.66 q	1.65 (3H, br.s)
9	38.94 d	2.58 (1H, m)	25	21.22 q	1.10 (3H, s)
10	34.59 t	0.91 (1H, m), 1.77 (1H, m)	26	100.60 t	4.73 (1H, br. s)
11	21.65^{a} t	1.48 (2H, m)			4.75 (1H, br. s)
12	47.93 d	1.12 (1H, m)	27	16.73 q	0.78 (3H, s)
13	40.10 s			$\mathrm{N\underline{H}}$	7.98 (br. s)
14	162.97 s				

Chemical shifts are expressed as δ ppm from TMS in $CDCl_3$.

Coupling constants are shown by Hz in parenthesis.

^{a}: may be exchanged.

Acknowledgements

JC acknowledges the NIH (Grant CA24487) for financial support.

References and Notes

1. EIMS: *m/z* 409; Although the exact chemical shifts could not be assigned, the observed signals for C-26 at δ_C 100.60 ppm, C-14 at 162.97, C-21 at 124.72 and C-22 at 131.15 were moved to higher field.
2. EIMS: *m/z* 447; CH_3COO-, δ_H 2.02 (3H, s), δ_C 21.65 (q), δ_C 170.50 (s)
3. Archival crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lens field Road, Cambridge CB2 1EW, UK. Please give a complete literature citation when ordering.
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