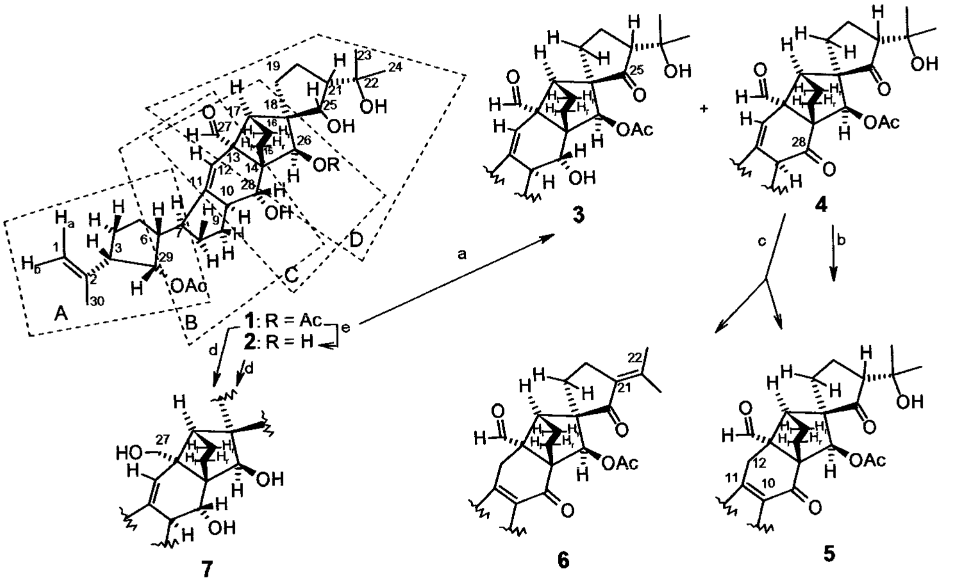
Metabolites with a Novel C_{30} Backbone from Marine Ciliates[**]

Graziano Guella, Fernando Dini, and
Francesco Pietra[*]

In memory of Antonino Fava

Unicellular organisms in the phylum Ciliophora (the ciliates) are a recent addition to marine microbes that are of growing interest as sources of secondary metabolites, particularly unusual sesqui- and diterpenoids.[1] We describe here two compounds with an unusual C_{30} backbone isolated from tropical strains of *Euplotes vannus*, an interstitial ciliate which plays a fundamental role in matter recycling within the marine ecosystem, which demonstrate the diverse nature of compounds produced by these organisms.



Scheme 1. Structure of vannusal A (**1**) and vannusal B (**2**) and their transformation products. a) PCC in CH_2Cl_2; b) slow on standing in $CDCl_3$ at RT; c) fast in CH_2Cl_2 containing catalytic *p*-toluensulfonic acid; d) $NaBH_4$ (3 equiv) at RT, 20 min; e) KOH/MeOH, RT, fast.

The structures of the new compounds, vannusal A (**1**) and vannusal B (**2**), isolated in this order of abundance from strain Sil21, and in the reverse order of abundances from strain BUN3 of *E. vannus*, and those of their derivatives **3**–**7** (Scheme 1), were fully assigned, with the exception of the absolute configuration, by a combination of mass spectrometry (MS) and NMR spectroscopy (Table 1).

With **1**, fast-atom bombardment (FAB) MS showed a protonated molecular ion ($[M+H]^+$ m/z 585) and a fragment ion m/z 507 ($[M+H-H_2O-CH_3COOH]^+$), whereas high resolution electron-impact MS measurements on the highest mass peak m/z 506 ($[M-H_2O-CH_3COOH]^{\cdot+}$) established the composition $C_{34}H_{48}O_8$ and thus the degree of unsaturation and the number of rings, which were in agreement with the NMR spectra. These allowed us to identify four structural blocks, A–D, which are partly superimposed and which define the entire molecule. Block A is characterized by the observations that the pivotal H29 is ^3J heterocoupled with C29–O–*C*=O, C4, and C5, and homocoupled and NOE-related with H3 and H6. Block B is defined by ^3J couplings of H7 with C10 and C12, H12 with C7 and C14, H27 with C14, and H28 with C9, C11 and C13. Moreover, a large coupling of H6 with H7 infers the dominance of a *trans*-diaxial conformation, which is in agreement with the lowest energy conformation calculated by molecular-mechanics (Figure 1). The allylic positions of H7 and H10 are confirmed both by their deshielding and ^4J coupling with H12. The pseudoaxial position for C28–OH is supported by a small coupling $J_{10,28}$. Further support for the linkage between blocks A and B is given by NOESY maps for H29 with H_β8, H7 with H_α8 and H10, H12 with $H_2$5, and H28 with H10 and H_β9. Block C, which is critical because of the high density of quaternary stereocenters[2] at C13, C14 and C18 (which poses as many problems for assignment as it will in any attempt at the total synthesis of vannusal A) and problems arising from the spin isolation of C11, is defined by the coupling pattern of H26 (^3J heterocoupling with C26–O–*C*=O, C15, and C28, and ^4J homocoupling with H_l15), the coupling of H_l15 with both protons at C16, and, for H17, ^3J homocoupling with H_l16 and ^3J heterocoupling with C14, C15, and C26. The pseudoequatorial position of C26–OAc and the bridgehead configuration are supported by NOE enhancements for H27 with H17 and

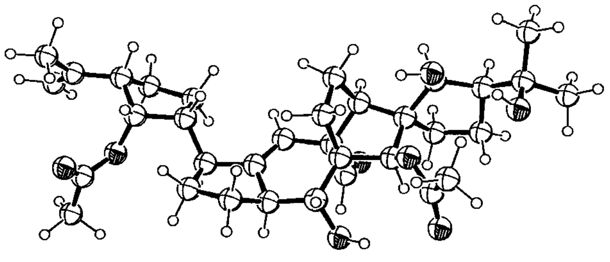


Figure 1. Lowest energy conformation of vannusal A (**1**) from molecular mechanics calculations.

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[*] Dr. F Pietra
Centro Linceo Interdisciplinare "Beniamino Segre"
Accademia Nazionale dei Lincei
via della Lungara 10, I-00165 Rome (Italy)
Fax: (+390) 0583-469277
E-mail: fpietra@discat.unipi.it
Dr. G. Guella
Laboratorio di Chimica Bioorganica
Facoltà di Scienze MFN, Università di Trento (Italy)
Dr. F. Dini
Dipartimento di Etologia, Ecologia ed Evoluzione
Università di Pisa, Pisa (Italy)

[**] We thank L. Bongiorni for collecting sand samples for this work in Indonesia, A. Sterni for running the mass spectra, and MURST and CNR, Rome, for financial support.

Table 1. NMR spectral data for vannusal A (**1**) in CD_3OD and C_6D_6 (^{1}H at 600 MHz, ^{13}C at 150 MHz).

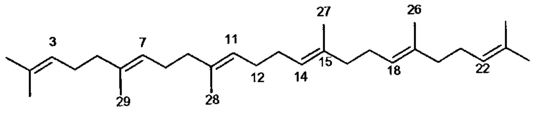
Atom	In CD_3OD		In C_6D_6	
	^{1}H	^{13}C	^{1}H	^{13}C
1	α 4.757 dq (1.4, 0.9) β 4.825 q (1.4)	111.45 t	α 4.881 dq (1.4, 0.9) β 4.951 q (1.4)	111.60 t
2	–	144.78 s	–	144.32 s
3	2.547 br. ddd (3.3, 7.9, 11.4)	52.11 d	2.197 br. ddd (3.5, 7.4, 11.8)	51.74 d
4	α 1.99 m β 1.78 m	26.61 t	α 1.98 m β 1.62 m	26.30 t
5	α 1.93 β 1.74	28.97 t	α 1.45 m β 1.65 m	28.76 t
6	2.009 dq (3.3, 9.7)	50.76 d	1.384 dq (3.5, 10.0)	50.27 d
7	2.477 dddd (1.8, 6.6, 7.8, 9.7)	45.20 d	2.385 dddd (1.3, 6.6, 8.4, 10.0)	44.53 d
8	α 1.475 tdd (6.6, 11.8, 13.0) β 1.859 dddd (1.8, 6.6, 7.8, 13.0)	31.05 t	α 1.209 tdd (6.6, 11.9, 12.6) β 1.817 dddd (1.8, 6.6, 8.4, 12.6)	30.63 t
9	α 2.248 dddd (1.8, 6.6, 8.1, 13.0) β 1.309 dddd (1.8, 7.8, 11.8, 13.0)	33.65 t	α 2.131 dtd (1.8, 8.4, 12.7) β 1.142 dtd (7.2, 11.9, 12.7)	33.62 t
10	2.278 dddd (1.8, 2.4, 7.8, 8.1)	51.82 d	2.448 dddd (1.8, 3.2, 7.2, 8.4)	51.23 d
11	–	157.08 s	–	156.54 s
12	5.555 dd (1.8, 2.4)	114.72 d	5.302 dd (1.3, 3.2)	114.32 d
13	–	69.21 s	–	68.78 s
14	–	57.59 s	–	57.34 s
15	left 1.041 ddt (2.4, 2.8, 12.7) right 1.975 ddd (5.7, 9.0, 12.7)	25.41 t	left 1.026 ddt (1.3, 2.5, 12.7) right 1.943 ddd (5.9, 9.0, 12.7)	25.30 t
16	left 1.529 ddt (3.8, 5.7, 12.7) right 2.493 ddd (2.8, 9.0, 12.7)	22.59 t	left 1.441 ddt (3.8, 5.9, 12.7) right 2.753 ddd (2.5, 9.0, 12.7)	22.45 t
17	2.635 d (3.8)	51.67 d	2.417 d (3.8)	50.74 d
18	–	56.78 s	–	55.38 s
19	α 1.463 m β 1.545 ddd (6.3, 7.8, 12.6)	39.87 t	α 1.373 m β 1.635 ddd (6.3, 7.8, 12.6)	38.47 t
20	α 1.634 dddd (3.1, 6.3, 7.8, 12.6) β 1.345 dddd (6.6, 10.6, 11.4, 12.6)	24.12 t	α 1.082 dddd (3.1, 6.3, 7.8, 12.6) β 1.405 m	23.61 t
21	1.808 ddd (6.6, 7.8, 11.4)	60.55 d	1.680 ddd (7.8, 8.3, 10.7)	57.43 d
22	–	71.90 s	–	72.30 s
23	1.238 s	29.24 q	1.118 s	30.35 q
24	1.160 s	28.18 q	1.004 s	26.33 q
25	4.162 d (6.6)	76.54 d	4.189 d (8.3)	77.56 d
26	5.160 d (2.4)	79.40 d	5.552 d (2.4)	79.62 d
27	9.690 s	200.76 d	9.690 s	198.07 d
28	3.537 d (1.8)	71.06 d	3.784 dd (1.8, 8.9)	70.40 d
29	5.433 t (3.3)	78.06 d	5.433 t (3.5)	76.69 d
30	1.762 br. t (0.9)	24.02 q	1.876 br. t (0.9)	24.45 q
C26−O*C*(Me)=O	–	172.10 s	–	169.75 s
C26−OC(*Me*)=O	1.972 s	20.94 q	1.689 s	21.12 q
C29−O*C*(Me)=O	–	173.00 s	–	171.63 s
C29−OC(*Me*)=O	2.084 s	21.53 q	1.781 s	21.34 q
HO-22			1.72 br. s	
HO-25			2.03 br. s	
HO-28			4.608 d(8.9)	

H26, H_l15 with H_l16 and H28, and H28 with C28−OH. The W relationship between H_l15 and H28 rests on their mutual weak coupling, while the C25−OH ⋯ O=C(Me)O−C26 hydrogen bond, unusually strong for an eight-membered ring, is reflected in the independence of the C25−OH resonance from the sample concentration in C_6D_6. Finally, block D is characterized by ^3J heterocorrelations of H25 with C19, C22, and C26, H17 with C19, both $H_3$23 and $H_3$24 with C21, and by ^2J heterocorrelations of C18 with H25, H26, and $H_2$19. The relative configurations at both C18 and C25 are established by NOE enhancements for H25 with both H_r16 and H17, H26 with H_α19, and H17 with H_β19.

The structure of vannusal B (**2**) rests on similar spectral evidence, and further structural support for the structures of both **1** and **2** was obtained from their interconversion and from the MS and NMR spectra of their chemical transformation products (Scheme 1). Thus, the secondary alcoholic groups of vannusal A (**1**) were oxidized by pyridinium chlorochromate (PCC) to give **4** as the major product together with minor amounts of **3** (route a). The former underwent isomerization to the α,β-conjugated enone **5** (route b), slowly on standing in $CDCl_3$, but more rapidly on addition of *p*-toluenesulfonic acid, which also induced dehydration to give a mixture of **5** and **6** (route c). Reduction of the aldehyde group of **1** to a primary alcohol with $NaBH_4$/MeOH (route d) was crucial as it allowed us to locate the cryptic environment of the aldehyde group from the differential chemical shifts in **7** (largest $\Delta\delta(^1H)$ at H12 (+0.25), H17 (−0.34), H7 (−0.10), H10 (+0.07) and $\Delta\delta(^{13}C)$ at C13 (−14), C12 (−11), and C11 (−11)). Notably, reduction of the aldehyde group of **1** was accompanied by regiospecific hydrolysis of the C26 ester group. This found parallels in the facile hydrolysis of **1** to **2** in basic methanol (route e) and in the clean reduction of **2** to **7** in $NaBH_4$/MeOH (route d).

No evidence could be obtained for microbial symbionts of *E. vannus* that could explain the formation of **1** or **2**, which are not produced by the organism food, *Dunaliella salina*. Therefore, both **1** and **2** must be products of the ciliate.

Disconnections at C3–C29, C7–C11, and C21–C25 of both vannusal A (**1**) and vannusal B (**2**) reveal the C1–C9 and C20–C25 portions of the squalene (**8**) backbone, which, however, cannot be obtained in its entirety by further bond disconnections. A route to **1** and **2** may be envisaged to occur from a hypothetical squalene-type precursor[3] which involves unprecedented C15–C28 and C12–14 bond formations followed by C13 extrusion. More appealing, particularly in the light that abzymes may contravene chemical rules,[4] is an unorthodox abstraction of the disfavored primary allylic proton in the squalene pathway[5] to close the central ring of **1** or **2** through bond C28–C14. Whether these are real possibilities is left to challenging biosynthetic experiments.



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In summary, the molecular diversity described here suggests that prolific ciliates such as strains of *E. vannus* are border-zone species[6, 7] that deserve particular care in the planning of biodiversity.[6]

Experimental Section

Stocks Sil21 and BUN3 of *E. vannus* (Müller, 1786) were collected in August 1996 along the coasts of the Siladen and Bunaken Islands, respectively, northwest of Menado, Indonesia, in the Celebes Sea. Their membership to *E. vannus*, comprising the cluster of the marine single dargyrome cirrotype 10 forms, *E. vannus-crassus-minuta*, was defined according to the classical (morphological) taxonomic practice.[8] To obtain mass cultures, stocks Sil21 and BUN3 were grown separately in large, shallow 30 liter tanks and fed the green microalga *Dunaliella salina*. Cultures were maintained at two fissions per day by feeding excess food and were kept at 23 ± 1 °C while exposed to alternate light and darkness with a rhythm of 12 h. Centrifuged, closely-packed cells of the Sil21 strain (14 mL; about 2×10^8 individuals) were suspended in ethanol which was then evaporated. The residue was subjected to high-performance liquid chromatography (HPLC) on a Merck Lichrosphere RP18 25 × 1 cm column, eluant CH_3CN/H_2O (7/3, 5 mL min^{-1}) to yield vannusal A (**1**) and vannusal B (**2**). **1**: $t_R = 8.7$ min, 9.3 mg; m.p. (water): 95 °C; $[\alpha]_D^{20} = -17$, $[\alpha]_{365}^{20} = -287$ ($c = 0.3$, MeOH); CD (MeOH): λ[nm] ($\Delta\varepsilon$ [deg L mol^{-1} cm]) = 229 (+6.2), 304 (−5.4); UV (MeOH): λ_{max} [nm] (ε [M^{-1} cm^{-1}]) = 229 sh (6600), 304 (1300); **2**: $t_R = 6.2$ min, 1.4 mg; amorphous powder; $[\alpha]_D^{20} \approx 0$, $[\alpha]_{365}^{20} = -85$ ($c = 0.05$, MeOH); CD (MeOH): λ[nm] ($\Delta\varepsilon$ [deg L mol^{-1} cm]) = 228 (+5.4), 308 (−3.1); UV/Vis (MeOH): λ_{max} [nm] (ε [M^{-1} cm^{-1}]) = 229 (5100), 301 (1100). NMR spectra were recorded with Bruker AMX-600 and Varian XL-300 spectrometers equipped for inverse detection; chemical shifts are reported relative to residual solvent signal (for CD_3OD $\delta(^1H) = 3.310$ and $\delta(^{13}C) = 49.00$; for C_6D_6 $\delta(^1H) = 7.150$ and $\delta(^{13}C) = 128.50$) and J in Hz at probe temperature 20 °C. A conformational space search was carried out with the program GMMX (steric energy minimization program based on the MMX force field by Serena Software, Bloomington, IN) and minima were refined with the MM3(96) program (by N.L. Allinger et al., distributed by QCPE, Indiana University, 17/1, February 1997) by the block diagonal Newton-Raphson energy minimization method with default parameters.

Received: September 22, 1998
Revised version: December 10, 1998 [Z12441IE]
German version: *Angew. Chem.* **1999**, *111*, 1217–1220

Keywords: ciliates · molecular modeling · natural products · NMR spectroscopy · terpenoids

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Molecular Recognition within a Self-Assembled Cylindrical Host**

Thomas Heinz, Dmitry M. Rudkevich, and
Julius Rebek, Jr.*

We recently reported the synthesis and characterization of the capsular assembly **1** (Figure 1).[1] It is a cylinder-shaped dimer held together by eight bifurcated hydrogen bonds in apolar organic solvents. The dimer has the capacity to select one large molecule or two small molecules from the bulk solution and encapsulate them reversibly. Further expressions of the capsule's unique behavior have emerged from the use of rigid, flexible and complex guests, and we relate them here.

Rigid structures such as stilbenes **2**–**5** offer a set of "rulers" for measuring the interior dimensions of the capsule (Figure 2). The (*E*)-4,4′-dimethylstilbene **2** is indeed readily-

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[*] Prof. J. Rebek, Jr., Dr. T. Heinz, Prof. D. M. Rudkevich
The Skaggs Institute for Chemical Biology and
The Department of Chemistry
The Scripps Research Institute, MB-26
10550 North Torrey Pines Rd., La Jolla, CA 92037 (USA)
Fax: (+1) 619-784-2876
E-mail: jrebek@scripps.edu

[**] We are grateful to the Skaggs Foundation and the National Institutes of Health for support. T. H. thanks the Swiss National Science Foundation and the Ciba-Geigy-Jubiläums-Stiftung for fellowships. Dr. A. Lützen is acknowledged for helpful discussions.