Raikovenal, a New Sesquiterpenoid Favouring Adaptive Radiation of the Marine Ciliate *Euplotes raikovi*, and its Putative Biogenetic Precursor, Preraikovenal

Graziano Guella,[a] Fernando Dini,[b] Fabrizio Erra[b] and Francesco Pietra*[a]

[a] *Istituto di Chimica, Università di Trento, 38050 Povo-Trento, Italy*
[b] *Dipartimento Scienze dell'Ambiente e del Territorio, Sezione Protistologia, Università di Pisa, 56126 Pisa, Italy*

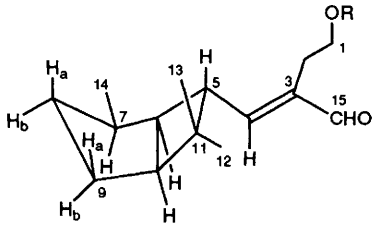
The new-skeleton sesquiterpenoid raikovenal **1a**, isolated from the marine ciliate *Euplotes raikovi* and likely to originate biogenetically from co-existing preraikovenal **2a**, can be seen to favour its adaptive radiation by selectively killing the predacious ciliate *Litonotus lamella*.

We have recently shown that stocks of the marine ciliate *Euplotes crassus* (Dujardin, 1841) contain euplotins which are highly-strained acetalic sesquiterpenes,[1] for which an ecological role as factors favouring niche exploitation has been proposed upon following the disruptive effects of exposing euplotin-non-producing ciliates to these metabolites.[1a] An exception and a dramatic variant have now emerged. A recognized specific predator of *Euplotes* spp., the carnivorous ciliate *Litonotus lamella* (Schewiakoff, 1896), is insensitive to euplotins and preys regularly on euplotin-producing *E. crassus*, but typically avoids stocks of the euplotin-non-producing *Euplotes raikovi* (Agamaliev, 1966). While predation may reflect acquired immunization, here we present data suggesting that avoidance results from the presence in *E. raikovi* of a terpenoid that is specifically active against *L. lamella* and is co-existent with its likely biogenetic precursor.

E. raikovi, stock Mor1, collected along Atlantic coasts of Morocco, near Casablanca, was grown as described for *E. crassus*,[1] obtaining 9.3 cm^3 of pellets, *ca.* 2×10^9 cells, which were soaked in absolute ethanol, filtered and evaporated to give 0.85 g of residue. This was extracted with hexane–EtOAc (9:1) to give 0.52 g of residue which was subjected to Si-60 flash chromatography with hexane–EtOAc gradient elution, collecting 13 × 30 ml fractions. The residue from fractions 4–6 was subjected to RP-18 HPLC with MeCN–H_2O (7:3) (solvent flow, as for all other HPLC experiments = 5 ml min^{-1}), and led to the isolation of raw raikovenal **1a**,† t_R 6.2 min, 2.8 mg. Acetylation of 1.6 mg of this material with Ac_2O, followed by Si-60 HPLC with *n*-hexane–Pr^{i}OH (99:1), gave raikovenal acetate **1b** (t_R 5.4 min, 1.3 mg, 91%) and preraikovenal acetate **2b**‡ (t_R 6.1 min, 0.2 mg). On aq. K_2CO_3 hydrolysis, **1b** gave pure raikovenal **1a**§ (85%) that, on treatment with Ag_2CO_3/Celite in *n*-hexane at reflux for 3 h followed by Si-60 HPLC with *n*-hexane–Pr^{i}OH (98:2), gave butenolide **3** (t_R 7.6 min, 83%).¶ Based on this experience, the remaining crude raikovenal was subjected to silica gel HPLC with hexane–Pr^{i}OH (97:3) directly obtaining pure raikovenal **1a**, t_R 9.0 min, and preraikovenal, **2a**,‖ t_R 12.2 min, in relative ratio of *ca.* 95:5.

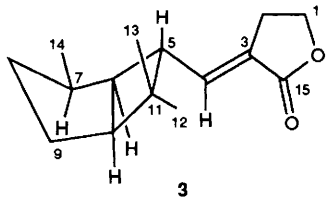
^{13}C and ^1H NMR and HR–MS data for raikovenal **1a** revealed the composition $C_{15}H_{24}O_2$, *i.e.* four unsaturations, for both the enal functionality and, on account of the primary alcoholic group, two carbocycles. The side chain and the three methyl groups were revealed, in accordance with the MS fragmentation, from DDS,[2a] HOM2DJ,[2b] COSY,[2c] HMQC and inverse-detection HMBC,[2d] thus establishing the bicyclo[3.2.0]heptane system. The position of the C-5 side chain was based on long-range ^1H–^{13}C correlation of 5-H with C-4, C-11 and C-3, while the position of the *gem* methyl groups was established by their heterocorrelation with C-5, C-10 and C-11 and that of 14-Me by heterocorrelation with C-7, which proved also to be correlated with H-6. *cis*-Fusion was based on both coupling (*J* 8.6 Hz) and NOE enhancement between 10-H and 6-H, and the transoid position of the C-5 side chain with respect to the five-membered ring at $J_{5,6}$ 6.8 Hz. (*E*)-C^3=C^4 was established by +13% NOE between CHO and 4-H, and 12-Me was located by +5% NOE with 4-H, while the spatial position of 14-Me was based on NOE with 5-H (well defined by NOE with 13-Me also, while 12-Me showed +9% NOE with 10-H). Clearly, the predominant conformer must have anticoplanar 4-H and 5-H, as represented by **1a**. Molecular mechanics (MM) calculations** proved in agreement with these conclusions by indicating interproton coupling patterns and distances matching observed *J* and NOE values and suggesting dominance of conformer **1a** by 99:1 over the one with C-8 moved down by five-membered ring flipping. Sharp disagreement was obtained instead for the MM-simulated, hypothetical stereoisomer deriving from joining C-5 with C-11 and C-6 with C-10 in the chair-like conformer of preraikovenal. Thus, the biogenesis of raikovenal **1a** may be seen from the boat-like conformer **2a** of preraikovenal in terms of C-10 conjugated attack to C-6 followed by electron-rich C-5 joining to electron-poor C-11.

The observed ineffectiveness of the widely toxic euplotins[1] on *L. lamella* contrasts with the effectiveness of raikovenal, which, assayed by the methodology previously described for the euplotins,[1] proved to kill 100% of tested *L. lamella* cells at low dosages in the concentration range 10–20 μg cm^{-3} (LD100). Such a susceptibility of *L. lamella* has no counterpart in other ciliate species. At these dosages, raikovenal proved 100% effective on the fission rate of 4 out of 11 ciliate species tested: *Euplotes magnicirratus* (Carter, 1972), *Euplotes rariseta* (Curds, West and Dorohy, 1974), *Euplotes vannus* (Müller, 1786) and *Diophrys oligothrix* (Borror,



2a R = H
2b R = Ac

1a R = H
1b R = Ac



3

1975). This may confer some advantage to *E. raikovi* for adaptive radiation.

The specificity of these biological effects of raikovenal suggests that fine-tuned recognition phenomena are involved with ciliated cells and their strategic metabolites. Since ciliates constitute a fundamental ring of the marine trophic chain, receptor studies are eagerly solicited.

Raikovenal **1a** deviates from the structural resemblance of the euplotins and preuplotin to defensive terpenoids of tropical green seaweeds in the families Caulerpaceae and Udoteaceae.[1]

We thank Professor M. Gates for a gift of the Morl stock, Mr A. Sterni for recording the mass spectra, and MURST (Progetti 40% and 60%) and CNR, Roma, for financial support.

Received, 12th August 1994; Com. 4/04965I

Footnotes

† No absolute configuration meaning is implied by any of the structural formulae in this paper.

‡ Data and structural elucidation for **2b**: ^{1}H NMR ($CDCl_3$, *J*/Hz, 299.94 MHz) δ 4.09 (t, $J_{1,2}$ 6.7, 1-H_2), 2.68 (t, $J_{2,1}$ 6.7, 2-H_2), 6.92 (d, $J_{4,5}$ 11.1, 4-H), 6.51 (dd, $J_{5,4}$ 11.1, $J_{5,6}$ 15.1, 5-H), 6.19 (dd, $J_{5,6}$ 15.1, $J_{6,7}$ 8.1, 6-H), 2.35 (m, H-7), 1.41 (m, 8-H_2), 2.00 (m, 9-H_2), 5.07 (br t, *J* 6.9, 10-H), 1.68 (br s, 12-Me), 1.57 (br s, 13-Me), 1.06 (d, $J_{14,7}$ 6.6, 14-Me), 9.41 (s, CHO); EI–MS, *m/z* 278 ($M^{\cdot+}$, 12%), 263 ([M − Me]$^+$, 3), 235 ([M − MeCO]$^+$, 6), 218 ([M − AcOH]$^{+\cdot}$, 24), 207 ([235 − CO]$^+$, 28), 203 ([218 − Me]$^+$, 8), 193 (5), 43 (100). HREI–MS, *m/z* 278.1875 ± 0.004, calc. for $C_{17}H_{26}O_3$ 278.1882, which indicates five unsaturations (AcO, CHO and three C=C). The connectivities were derived from *J* couplings and COSY, while (*E*)-C^5=C^6 was based on $J_{5,6}$ 15.1 and (*E*)-C^3=C^4 on the relatively high field δ_{CHO} 9.41 (as for both **1a** and **1b**, where there is independent NOE evidence).

§ *Data for* **1a** (new skeleton raikovane numbering): Oil; $[\alpha]_D^{20}$ −40 (MeOH, *c* 0.1); UV (MeOH) λ_{max} 245 nm (ε 7000 dm^3 mol^{-1} cm^{-1}); ^{13}C NMR (C_6D_6, 75.43 MHz, Me_4Si) δ 61.66 (t, C-1), 28.64 (t, C-2), 140.35 (s, C-3), 157.86 (d, C-4), 40.92 (d, C-5), 45.76 (d, C-6), 34.31 (d, C-7), 36.96 (t, C-8), 27.11 (t, C-9), 46.86 (d, C-10), 38.68 (s, C-11), 27.05 (q, C-12), 23.86 (q, C-13), 13.87 (q, C-14), 194.90 (d, C-15); ^{1}H NMR (C_6D_6 *J*/Hz, 299.94 MHz) δ 3.51 (t, $J_{1,2}$ 6.5, 1-H_2), 2.50 and 2.38 (2dd, J_{gem} 13.5, $J_{2,1}$ 6.5, 2-H_2), 6.16 (d, $J_{4,5}$ 10.5, 4-H), 2.53 (dd, $J_{5,4}$ 10.5, $J_{5,6}$ 6.9, 5-H), 2.20 (dt, $J_{6,10}$ 8.6, $J_{6,5} \approx J_{6,7}$ 6.9, 6-H), 1.68 (pseudo spt, *J* 6.9, 7-H), 1.30–1.10 (m, 8-H_2), 1.58 (br dt, J_{gem} 12.8, $J_{9a,8a} \approx J_{9a,8b}$ 5.8, 9-H_a), 1.37 (ddt, J_{gem} 12.8, $J_{9b,10}$ 7.2, $J_{9b,8b} \approx J_{9b,8a}$ 9.0, 9-H_b), 2.01 (ddt, $J_{7,11}$ 8.6, $J_{10,6b}$ 7.2, $J_{10,9a} \approx J_{10,5}$ 1.2, 10-H), 0.87 (s, 12-Me), 0.75 (s, 13-Me), 0.78 (d, $J_{14,7}$ 6.9, 14-Me), 9.24 (s, CHO); EI–MS, *m/z* 236 ($M^{\cdot+}$, 30%), 221 ([M − Me]$^+$, 10), 218 ([M − H_2O]$^{\cdot+}$, 2.5), 205 ([M − CH_2OH]$^+$, 3.5), 203 ([218 − Me]$^+$, 7), 193 (28), 175 (30), 165 (75), 125 (65), 109 (26), 95 (36), 81 (46), 69 (78), 55 (64), 41 (100); HREI–MS, *m/z* 236.1773 ± 0.005, calc. for $C_{15}H_{24}O_2$ 236.1776; 221.1536 ± 0.007, calc. for $C_{14}H_{21}O_2$ 221.1541; 218.1666 ± 0.007, calc. for $C_{15}H_{22}O$ 218.1671; 175.1124 ± 0.005, calc. for $C_{12}H_{15}O$ 175.1123; 165.0912 ± 0.005, calc. for $C_{10}H_{13}O_2$ 165.0915.

¶ *Data for* **3**: ^{13}C NMR (C_6D_6) δ 64.36 (t, C-1), 25.27 (t, C-2), 140.97 (d, C-4), 42.83 (d, C-5), 45.11 (d, C-6), 34.40 (d, C-7), 36.44 (t, C-8), 27.14 (t, C-9), 46.67 (d, C-10), 27.07 (q, C-12), 24.36 (q, C-13), 13.97 (q, C-14). ^{1}H NMR (C_6D_6, *J*/Hz) δ 3.48 (*t*, $J_{1,2}$ 7.3, 1-H_2), 2.04 and 1.70 (two ddd, J_{gem} 16.6, $J_{2,1}$ 7.3, $J_{2,4}$ 2.9, 2-H_2), 6.92 (dt, $J_{4,5}$ 10.4, $J_{4,2}$ 2.9, 4-H), 2.02 (dd, $J_{5,4}$ 10.4, $J_{5,8}$ 7.8, 5-H), 2.21 (br q, $J_{6,7} \approx J_{6,10}$ 7.3, 6-H), 1.64 (pseudo spt, *J* 6.9, 7-H), 1.30–1.10 (m, 8-H_2), 1.60 (m, 9-H_a), 1.36 (m, 9-H_b), 1.95 (br t, $J_{10,11} \approx J_{10,9b}$ 7.8, 10-H), 0.95 (s, 12-Me), 0.73 (s, 13-Me), 0.75 (d, $J_{14,7}$ 6.4, 14-Me); EI–MS, *m/z* 234 ($M^{\cdot+}$, 20%), 219 ([M − Me]$^+$, 4), 191 (100).

‖ Except for differences in δ_H (but not in *J* couplings) for 1-H_2, 2-H_2, 4-H and CHO (3.66, 2.63, 6.94 and 9.43, respectively) all other signals proved superimposable with those for **2b**.

** By 'PCMODEL 4.0', MMX force field, Serena Software, Bloomington, IN.

References

1 (*a*) F. Dini, G. Guella, P. Giubbilini, I. Mancini and F. Pietra, *Naturwissenschaften*, 1993, **80**, 84; (*b*) G. Guella, F. Dini, A. Tomei and F. Pietra, *J. Chem. Soc., Perkin Trans. 1*, 1994, 161.

2 (*a*) J. K. M. Sanders and J. D. Mersch, *Progr. NMR Spectrosc.*, 1982, **15**, 353; (*b*) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, 1971, **54**, 301; (*c*) A. Bax and R. Freeman, *J. Magn. Reson.*, 1981, **44**, 542; (*d*) M. F. Summers, L. G. Marzilli and A. Bax, *J. Am. Chem. Soc.*, 1986, **108**, 4285; L. Müller, *J. Am. Chem. Soc.*, 1979, **55**, 301; G. Gray, *Magn. Moments*, 1987, **3**, 6.