128. From Epiraikovenal, an Instrumental Niche-Exploitation Sesquiterpenoid of Some Strains of the Marine Ciliated Protist *Euplotes raikovi*, to an Unusual Intramolecular *tele*-Dienone-Olefin [2+2] Photocycloaddition[1])

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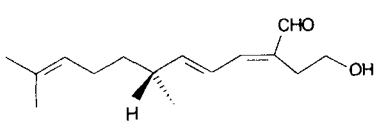
It is shown that strains of the marine ciliate *Euplotes raikovi* are subtly variable in their production of secondary metabolites. Strains GA8 and 39W from Mediterranean and SB8 from Californian coasts produce the sesquiterpenoid epiraikovenal (**3**), while strains GA8 and SB8 also produce secoepiraikovenal (**4**), which play an instrumental niche-exploitation role and have also taxonomic significance. Comparison of **3** and **4** with raikovenal (**2**) and its putative biogenetic precursor **1**, which have similar roles in the conspecific strain Morl from Casablanca coast in the Atlantic Ocean, inspired us the first case of intramolecular *tele*-dienone-olefin [2+2] photocycloaddition, exemplified here by the transformation of **1** into *ent*-**3**. This served also to unequivocally clarify the stereochemical relationship between **3** and **2**.

1. Introduction. – We have recently described raikovenal (**2**) and its putative biogenetic precursor, preraikovenal (**1**), two sesquiterpenoids co-occurring in the ciliate *Euplotes raikovi* AMAGALIEV, 1966, strain Morl, from the Casablanca coast [1]. Results of bioassays suggested an implementing niche-exploitation role for **2** [1]. We report here evidence for an intraspecific variation in closely related sesquiterpenoids that



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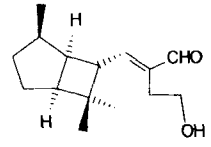

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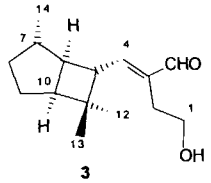

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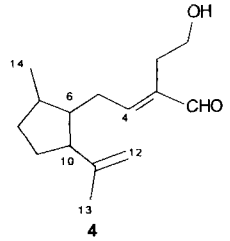

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[1]) Presented in part by *F. P.* in a lecture at the School of Chemistry, University of Melbourne, on April 28, 1995.

are all instrumental in supporting a similar ecological strategy: some strains of *E. raikovi*, representative of populations collected in marine coastal regions far apart from the Morl collecting site and from each other, use an epimer of **2**, complemented in some cases by its seco-counterpart, as functional alternatives. These observations bring the focus on the possibility that in ciliated protists, which are important components of the marine food web, a secondary metabolic system may undergo an intraspecific evolutionary process, partitioning variations closely similar in form and function among morphologically indistinguishable populations.

The peculiar structures of the molecules involved in these affairs inspired us the first case of intramolecular *tele*-dienone-olefin [2+2] photocycloaddition.

2. Results and Discussion. – 2.1. *New Sesquiterpenoids from* E. raikovi *Strains.* Raikovenal (**2**) [1] was not detected in strains SB8, GA8, and 39W of *E. raikovi* of geographic areas far apart from each other (*Table*). These ciliates proved instead to contain epiraikovenal (**3**) and, limited to the SB8 and GA8 strains, also secoepiraikovenal (**4**). Although yields of the isolated products proved to be somewhat dependent on the physiological state of cells, as well as on culture and workup conditions, the various strains were about equally productive, 0.2–0.4 mg of sesquiterpenoids per 10^8 cells (*Table*).

It should be noted that strains collected along Thyrrenian and Adriatic coasts of Italy, in the same Mediterranean basin, differ not only in the mating-group membership but

Table. *Secondary Metabolites Produced by the Different Strains of the Protistan Ciliate Morphospecies* Euplotes raikovi *and Their Biological Effectiveness*

Metabolite	Biological effectiveness[b])	Strains[a])			
		Mating group I		Mating group II	
		Morl	39W	GA8	SB8
Preraikovenal (**1**)[c])	0	+			
Raikovenal (**2**)[c])	50	+			
Epiraikovenal (**3**)	100		+	+	+
Secoepiraikovenal (**4**)	17			+	+
1/**2** or **3**/**4** relative ratios		1:20		4:1	1:1
Total amount produced (mg per 10^8 cells)		0.42	0.21	0.21	0.34
Geographic origin of the strains		Casablanca, Morocco, Atlantic Ocean, July 1990	Porto Recanati, Italy, Adriatic Sea, June 1979	Gaeta, Italy, Thyrrenian Sea, July 1987	Santa Barbara, California, Pacific Ocean, May 1985

[a]) The four strains belong to two 'mating, groups': mating, that is the occurrence of a (putative) gene exchange, may occur only between strains of the same group, whereas representatives of the two groups do not mate, thus meeting the definition of 'biological species', morphological indistinguishability between strains of the two groups notwithstanding.

[b]) Evaluated in terms of killing ability against individuals of the predacious ciliate *Litonotus lamella*, treated with each substance at doses corresponding to consecutive steps in concentration; lethality towards *L. lamella* in terms of dead (d) or alive (a) cells/tested cells for the given metabolite at doses 20 μg/ml (**3** 3d/3, **2** 2d/3, **4** 1d/3) or 10 μg/ml (**3** 3d/3, **2** 1a/3, **4** 3a/3).

also in metabolite production (*Table*). Also noticeable is that strains comprising the same mating group may produce different compounds (*Table*). Anyway, the overall production of terpenoids was similar for all strains.

Structures **3** and **4**[2]) were established as follows. The EI-MS of epiraikovenal (**3**) proved to be practically superimposable to that of raikovenal (**2**) [1], while only slight differences were observed in the δ(H) values for H–C(5), H–C(6), and Me–C(7), and in the coupling pattern of H–C(10) (*cf. Exper. Part*). This suggested a stereoisomeric relationship between the two compounds. In fact, the epimeric relationship at C(7) was suggested for **3**[3]) by positive NOE's between 3 H–C(12) and both H–C(4) and H–C(10), and between 3 H–C(13) and H–C(5), and by the lack of the strong NOE between 3 H–C(14) and H–C(5) observed for **2** [1]. Confirming evidence was derived from a $\Delta\delta$(C) 5.5 ppm downfield shift of the C(14) and C(5) signals for **3** with respect to **2**, attributable to lack of the γ-gauche interactions of the latter [1].

MM Calculations were in accordance with these attributions, suggesting for **2** a dominant conformer with *cisoid* 3 H–C(14)/C(8) and for **3** a slightly predominant conformer with *transoid* 3 H–C(14)/C(8). These calculations also suggested that δ and J values are averaged by rapid flipping of the five-membered ring, which is reflected in crowding of the proton resonances and a change of the coupling pattern of the five-membered ring, in contrast with the neatly resolved corresponding resonances for **2** [1]. This rationalizes the appearance of H–C(10) as either a br. *dd* for **2** [1] or a br. *q* for **3**.

The clue to the C connectivities in **4** was a *t* in place of the *d* arising from H–C(4) of **3** and replacement of the gem-dimethyl signals of the latter [1] by the signals for an isopropylidene group (*Exper. Part*). Similarity of the C(6), C(10), and C(14) δ(C) values for **4** and **3** suggested the same relative (*cis*) configuration at the C(6)–C(10) bond, as expected from common biogenesis of the two metabolites. Unfortunately, this stereochemical conclusion could not be put on a more solid basis because of the small amount of **4**, and crowding of its ^{1}H-NMR signals which prevented carrying out reliable NOE experiments. Luckily, however, this is immaterial for the main conclusions of this study.

2.2. *Biological Activities and Predator-Prey Interaction.* The morphospecies randomly selected to assess the biological activity of epiraikovenal (**3**) and secoepiraikovenal (**4**; *Exper. Part*) represent a fair sample of the diversity occurring within ciliate communities that are sand dwellers of the sea's intertidal zone. No killing effect on such ciliates by either **3** or **4** was observed even at the highest dosage tested (20 μg/ml), except in strain Li of the predacious ciliate *Litonotus lamella,* killed by **3** even at concentrations as low as 10 μg/ml. Despite its failure to kill *Euplotes* and *Diophrys* strains at the highest dosages tested, **3** proved 100% effective on depressing the fission rate of several of these strains, namely CM4 and VH3 of *Euplotes vannus*, SicAA of *Euplotes rariseta*, and PR5 of *Diophrys oligothrix*, which are characterized by a generalized sensitivity to aldehydic sesquiterpenoids of either protozoological [1] [2] or algal [3] origin. Secoepiraikovenal (**4**) showed a lower cytotoxicity on *L. lamella* (*Table*), in the decreasing order of effectiveness of the metabolites isolated from *E. crassus*, **3** > **2** > **4** > **1** (*Table*). On incubating

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[2]) The raikovane numbering [1] is used throughout except for retrieval purposes (see *Exper. Part*).

[3]) These data are also consistent with the enantiomer of **3**. That this is not a trivial remark will become clear later with the photochemical experiments and the biogenetic hypothesis. Anyway, no absolute-configuration meaning has to be attributed to any of the structural formulae displayed in this paper.

L. lamella with a 1:1 mixture **3**/**4**, in a series of consecutive steps in concentration, the effects observed were those expected for **3** alone at the concentration present in the mixture. This argues against a synergistic effect involving **3** and **4**.

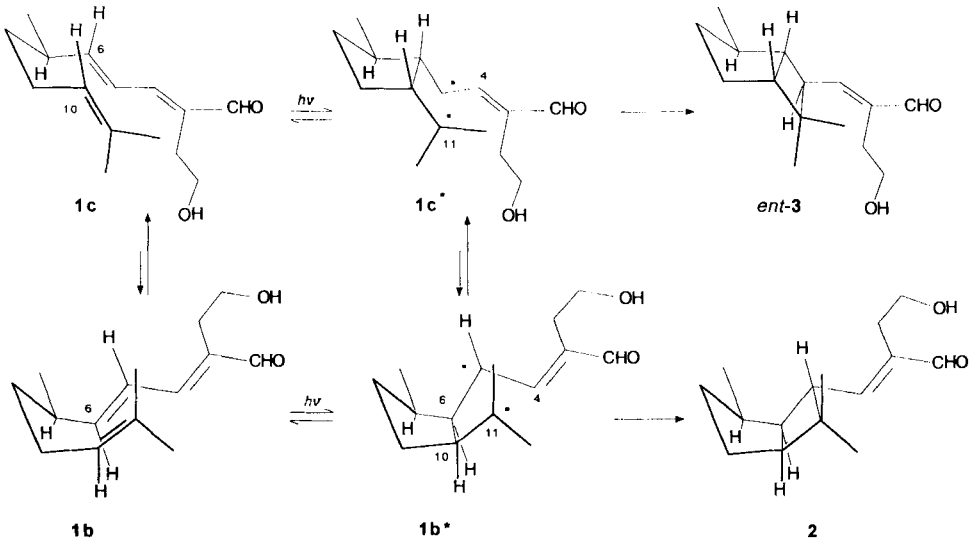
To get insight into the ecological implications of the detrimental effects established for **3** and **4**, we focused our attention on the interspecific interaction between the raptorial *L. lamella* and the potential prey *E. raikovi*. In predator-prey mixtures (*Exper. Part*), representatives of the strains GA8 and SB8 of *E. raikovi* did not appreciably decrease in number within 5 d, while well-fed *L. lamella* cells did not increase in number or increased by only a few units, indicating that cells of these *E. raikovi* strains were not palatable for the predator *L. lamella*, at least under our experimental conditions. Such a situation is utterly comparable to that observed on substituting strains GA8 and SB8 with Mor1 as potential prey. In contrast, *ca.* 99% of *E. raikovi* cells, strain 39W, disappeared within 5 d in mixtures comprising either well-fed or starved *L. lamella* which markedly increased in number. This suggests that cells of the 39W strain of *E. raikovi* are edible for the predator.

The combination of epiraikovenal-secoepiraikovenal in strains GA8 and SB8 of *E. raikovi* recalls that of preraikovenal-raikovenal in conspecific strain Mor1 in representing an efficient feeding-deterrent system against strain Li of *L. lamella*. The presence of epiraikovenal alone in strain 39W of *E. raikovi* appeared insufficient to deter feeding by this predator. However, when 39W cells were presented to *L. lamella* together with cells of the strain CL3 of *E. crassus*, the number of these last decreased rapidly. Although strain 39W of *E. raikovi* lacked protection by its single-terpenoid chemical system (epiraikovenal) when presented alone to *L. lamella*, the outcome was different when this 39W strain was presented together with the CL3 strain of *E. crassus* to this predator: *E. crassus* was preferred. Even if *E. raikovi* cells are smaller in size than *E. crassus* cells, there is circumstantial evidence against the possibility that such discrimination is the consequence of a predation by a size-selective predator. It may be thus concluded that the secondary-metabolite systems represented by the preraikovenal-raikovenal and epiraikovenal-secoepiraikovenal sets play a role in helping *E. raikovi* representatives in niche-exploitation.

2.3. *Photochemical Synthesis.* The structure of preraikovenal (**1**) struck us for its functionalities suitably positioned for a *tele*-dienone-olefin [2+2] photocycloaddition leading to the bicyclo[3.2.0]heptane backbone of the sesquiterpenoids isolated from *E. raikovi*. We felt that, should preraikovenal react in chair-like conformation, the C(7)-epimer of raikovenal (or its enantiomer) would be obtained. MM Calculations were encouraging, suggesting a folded, chair-like favored conformation **1c** for preraikovenal, where Me–C(7) occupies an equatorial position, *exo* with respect to ring closure (*Scheme 1*). MM Calculations also suggested that the boat-like conformation **1b** (which can be viewed to lead to raikovenal (*Scheme 1*) [1] with Me–C(7) in the equatorial position, though *endo* with respect to ring closure) is by 0.18 kcal mol^{-1} higher in energy, and that the extended conformation, roughly represented by **1**, is by 0.65 kcal mol^{-1} higher in energy. For an intramolecular [2+2] photocycloadditions to the γ,δ bond of $\alpha,\beta,\gamma,\delta$-unsaturated enone system[4]), expectations are that **1** will afford *ent*-**3** and **2** *via* the respective 1,4-biradical intermediates **1c*** and **1b*** (*Scheme 1*). This finds analogy with the amply documented enone-olefin [2+2] photocycloadditions [5]. Once the five-membered ring is

[4]) Intermolecular [2+2] photocycloaddition of maleic anhydride or, more rarely, olefins to the γ,δ bond of $\alpha,\beta,\gamma,\delta$-unsaturated steroids are documented in [4].

Scheme 1



closed, as in **1b***, repulsive interactions between Me–C(7) and H–C(5), destabilizing **1b** with respect to **1c**, should become particularly serious, thus further raising the activation energy for the pathway to **2** *vs.* that to *ent*-**3**. In these movements, preferred closure to a bicyclo[3.2.0]heptane system is expected from the 'rule-of-five', Me–C(7) taking the *exo*-position in analogy with the high regio- [5b, d] and stereoselectivity of classical enone-olefin [2+2] intramolecular photocycloadditions [5c, d].

On the above premises, we decided to sacrifice to photochemistry the exiguous amount of preraikovenal (0.4 mg) obtained from the cultures of *E. raikovi*. We were amply rewarded by obtaining *ent*-**3** in high quantum and chemical yield (*Scheme 1*). The enantiomeric relationship between *ent*-**3** and **3** was demonstrated by CD spectra (*Exper. Part*), thus proving that natural **3** is the C(7)-epimer of **2**[5]).

The new photocyclization described here may offer a new dimension to enone-olefin photochemistry in the construction of bicyclo[3.2.0]heptane systems. The potential for subsequent elaboration by four-membered ring opening [5e] [7] makes this route worth of exploration.

3. A Biogenetic and Evolutionary Outlook. – While biogenesis of raikovenal (**2**) may be imagined to proceed through the disfavored boat form of **1** (*Scheme 2, a*) [1], the above observations suggest that epiraikovenal (**3**) is formed biogenetically through the favored chair form of *ent*-**1** (*Scheme 2, b*). Different metabolic pathways are implied.

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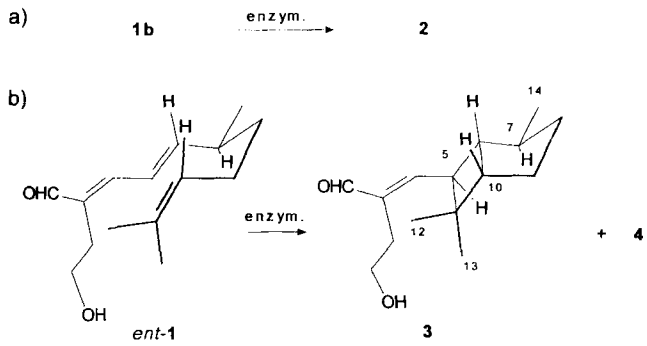
[5]) It should be noted that the [2+2] photocyclization of γ-curcumene, although originally described to afford italicene and C(7)-epimeric isoitalicene [6], must follow instead a course similar to that described here from **1** to *ent*-**3**; *i.e.*, the photoproducts of the reaction of γ-curcumene, deriving from its chair-like or boat-like reacting 1,5-diene form, must be in *ent*-epi-interrelationship.

Scheme 2



This may be regarded as the result of an evolutionary process by which variations upon the same chemical and functional theme are partitioned among conspecific, morphologically-indistinguishable populations of *E. raikovi*. An opposite situation has recently been found in the ciliated morphospecies *E. crassus*, where virtually all strains produce the same secondary metabolites, implying the same enzyme system throughout [1] [2]. These metabolites, named euplotins, can thus be used straightforwardly to define species membership of new collected strains. What about *E. raikovi* from this viewpoint? Despite its interpopulation diversification in metabolic pathways, the terpenoids produced by *E. raikovi* represent a unique whole that serves as a reliable taxonomic tool at the morphospecies level, as the euplotins of *E. crassus* do [1] [2]. Additionally, such heterogeneity might perhaps be used to discriminate intraspecific population clusters that may have resulted from a speciation process. Whether this is really allowed is perhaps too early to be answered, requiring more extensive sampling. Anyway, the use of euplotins as well as raikovenal/epiraikovenal systems as taxonomic criteria at the morphospecies level is reliable.

We thank Prof. *P. Luporini* for strains 39W, GA8, and SB8 and Prof. *M. A. Gates* for strain Morl of *E. raikovi*, Prof. *F. Verni* for strain Li of *L. lamella*, Mr. *A. Sterni* for recording the mass spectra, and *MURST* (Progetti 40% and 60%) and *CNR*, Roma, for financial support.

Experimental Part

1. *General.* All evaporations were carried out at reduced pressure. Yields are given on reacted compounds. TLC: *Merck Kieselgel 60 PF_{254}* plates. Flash-chromatography (FC): *Merck Si-60*, 15–25 μm, and reversed-phase FC on *Merck LiChrosorb RP18*, 20–50 μm. HPLC: *Merck LiChrosorb Si-60* (7 μm), hexane/i-PrOH. For prep. HPLC 25 × 1 cm column, solvent flux 6 ml min^{-1}, and UV monitoring at λ 245 nm. Polarimetric data: *JASCO DIP-181* polarimeter; $[\alpha]_D$ in dm^{-1} deg ml g^{-1}. CD: *JASCO-J-710* spectropolarimeter; $\Delta\varepsilon(\lambda)$. UV: *Perkin-Elmer Lambda 3* spectrophotometer. NMR: δ in ppm rel. to internal Me_4Si (= 0 ppm), *J* in Hz; *Varian XL-300* spectrometer (^1H at 299.94 MHz; ^{13}C at 75.43 MHz), multiplicities from DEPT experiments [8]; $^1H,^1H$ [9] and $^1H,^{13}C$ assignments from one-bond [10a] and long-range $^1H,^{13}C$ COSY experiments [10b]. HMBC *via* the heteronuclear multiple-quantum coherence pulse sequence [11a], using a dedicated probe [11b]. Selective, differential NOE (obtained with 5-s preirradiation): irradiated proton→ % NOE on the observed proton(s). EI-MS (*m/z* (%)): *Kratos MS80* mass spectrometer with home-built data system.

2. *Collection and Isolation.* The collecting location information of the *E. raikovi* strains is given in the *Table*. Taxonomic identification was established by *M. A. Gates* (State University of Cleveland, USA) for Morl, and by

P. Luporini (Università di Camerino, Italy) for the 39W, GA8, and SB8 strains. We confirmed the membership of these strains to the morphospecies of *E. raikovi*. Strains were grown at 23 ± 1° on sterilized, defined artificial sea water (*Allen*'s formula [12]) inoculated with *Dunaliella tertiolecta* BUTCHER, 1959 (Chlorophyceae, Dunaliellales) as food organism. This alga was cultured in aerated 5-l *Erlenmeyer* flasks in sterilized, defined, sea water enriched with *Walne*'s formula ingredients [12] and was kept at 20° with an 11 h light/13 h dark cycle. Pellets, obtained by centrifugation of mass cultures of *E. raikovi* strains 39W, GA8, and SB8, contained *ca.* 6.2×10^8, 2.4×10^8, and 1.3×10^9 cells, resp. They were soaked in the minimum volume of absolute EtOH. In the case of strain SB8 the filtrate was evaporated and the residue was extracted with hexane/AcOEt 9:1 to give 0.32 g of residue that was subjected to FC (*RP 18*; MeCN) to give five fractions of *ca.* 10 ml each. *Fr. 2* and *3* were subjected to HPLC (*Si60*, with hexane/i-PrOH 97:3; monitoring by UV at λ 245 nm) to yield epiraikovenal (**3**; t_R 9.7 min, 2.3 mg) and secoepiraikovenal (**4**; t_R 11.0 min, 2.2 mg). Cultures of *Euplotes raikovi* GA8 from Golfo Aranci in Sardinia and 39W from Porto Recanati in the Adriatic Sea were similarly carried out to give either **3** (0.4 mg) and **4** (0.1 mg), or **3** alone (1.3 mg), resp.

3. *Cytotoxicity Assays and Predator-Prey Interaction.* Experimental protocols and techniques used for cytotoxic assays were already described in [1] [2]. The test terpenoids were dissolved in abs. EtOH at *ca.* 1 mg/ml concentration. Conveying them into sea water from such solns., the incubation medium for the ciliate cells resulted in EtOH content ranging from 0.05 to 2%, the lowest and highest EtOH values corresponding to the lowest and highest values of terpenoid concentration, respectively. Controls included solvent-treated and untreated cells and were run simultaneously with terpenoid-treated cells.

The following ciliate strains were randomly selected for bioassays: Mor1, 39W, GA 8, and SB8 of *E. raikovi* AMAGALIEV, 1966, TB6, CM4, and VH3 of *Euplotes vannus* (MÜLLER, 1786), SSt22, CL3, and SL2 of *Euplotes crassus* (DUJARDIN, 1841), CpM2 and Kling4 of *Euplotes minuta* YOCOM, 1930, SicAA of *Euplotes rariseta* CURDS, WEST, and DORAHY, 1974, Galv 1 of *Euplotes charon* (MÜLLER, 1783) CA1 of *Euplotes parkei* CURDS 1973, PR5 of *Diophrys oligothrix* BORROR, 1965, and Li of *Litonotus lamella* SCHEWIAKOFF, 1896. The strains were incubated for 16 h with different dosages of **3** or **4** in a set of consecutive steps in concentration. For interspecific cell-to-cell interactions, predator-prey mixtures were set up as follows. *Ca.* 1.5×10^3 cells of the strains 39W, GA8, and SB8 of *E. raikovi* were separately placed in 2 ml of sterilized sea water with four cells of *L. lamella*, strain Li, and the number of these last was counted in each mixture after 5 d. The experiment was run in duplicate using *L. lamella*, either well fed on *E. crassus*, strain CL3, or starved from 6 d, since the last feeding with the same prey. Strain CL3 of *E. crassus* proved to be one of the most palatable preys for *L. lamella*. Palatability was estimated in terms of reproductive or division rate of *L. lamella*, representing a reliable measure of the ciliates' viability which, in turn, proved to depend from the metabolic and genetic capabilities for maximizing the materials and energy gains from the engulfed prey.

4. *Raikovenal* (= *(1S*,4R*,5R*,6R*,E)-4-Hydroxy-2-[(4,7,7-trimethylbicyclo[3.2.0]hept-6-yl)methylidene]butanal*; **2**). Additional data [1]: CD (hexane): $\Delta\varepsilon_{max}(243) = -1.6$. ^{1}H-NMR ($CDCl_3$): 3.61 (*q*, $J(1,2) = J(1,OH) = 6.5$, 2 H–C(1)); 2.51, 2.45 (2*dt*, $J_{gem} = 13.5$, $J(2,1) = 6.5$, 2 H–C(2)); 6.63 (*d*, $J(4,5) = 10.6$, H–C(4)); 2.67 (*dd*, $J(5,4) = 10.6$, $J(5,6) = 7.0$, H–C(5)); 2.55 (*td*, $J(6.5) \approx J(6,7) = 7.8$, $J(6,10) = 7.0$, H–C(6)); 1.89 (*pseudo-sept.*, $J = 6.8$, H–C(7)); 1.88, 1.47 (2*m*, 2 H–C(8)); 1.75, 1.40 (2*m*, 2 H–C(9)); 2.27 (br. *t*, $J(10,6) = J(10,9a) = 7.0$, H–C(10)); 1.09 (*s*, 3 H–C(12)); 0.91 (*s*, 3 H–C(13)); 0.83 (*d*, $J(14,7) = 6.8$, 3 H–C(14)); 9.39 (*s*, CHO), 1.98 (*t*, $J(1,OH) = 6.5$). ^{13}C-NMR ($CDCl_3$): 61.95 (*t*, C(1)); 28.29 (*t*, C(2)); 139.62 (*s*, C(3)); 159.88 (*d*, C(4)); 40.92 (*d*, C(5)); 45.76 (*d*, C(6)); 34.16 (*d*, C(7)); 36.71 (*t*, C(8)); 27.19 (*t*, C(9)); 46.68 (*d*, C(10)); 38.89 (*s*, C(11)); 26.87 (*q*, C(12)); 24.08 (*q*, C(13)); 13.83 (*q*, C(14)); 196.26 (*d*, C(15)).

5. *Epiraikovenal* (= *(1S*,4S*,5R*,6R*,E)-4-Hydroxy-2-[(4,7,7-trimethylbicyclo[3.2.0]hept-6-yl)methylidene]butanal*; **3**). Oil. $[\alpha]_D^{20} = -13$ ($c = 0.1$, EtOH). UV (MeOH) 245 (7000). CD (hexane): $\Delta\varepsilon_{max}(244) = -1.0$). ^{1}H-NMR ($CDCl_3$): 3.58 (*q*, $J(1,2) = J(1,OH) = 6.5$, 2 H–C(1)); 2.47 (*t*, $J(2,1) = 6.5$, 2 H–C(2)); 6.70 (*d*, $J(4,5) = 10.3$, H–C(4)); 2.53 (*dd*, $J = 5,4) = 10.3$, $J(5,6) = 6.0$, H–C(5)); 2.28 (*q*, $J(6,5) \approx J(6,7) \approx J(6,10) = 7.0$, H–C(6)); 1.87 (*pseudo-sept.*, $J = 6.9$, H–C(7)); 1.68–1.47 (*m*, 2 H–C(8)); 1.68–1.95 (*m*, 2 H–C(9)); 2.32 (*q*, $J(10,6) \approx J(10,9b) \approx J(10,9a) = 7.0$, H–C(10)); 1.12 (*s*, 3 H–C(12)); 0.91 (*s*, 3 H–C(13)); 0.78 (*d*, $J(14,7) = 6.9$, 3 H–C(14)); 9.40 (*s*, CHO); 1.87 (*t*, $J(1,OH) = 6.5$). ^{13}C-NMR ($CDCl_3$): 62.06 (*t*, C(1)); 28.13 (*t*, C(2)); 140.05 (*s*, C(3)); 159.08 (*d*, C(4)); 46.57 (*d*, C(5)); 47.88 (*d*, C(6)); 33.52 (*d*, C(7)); 37.88 (*t*, C(8)); 24.55 (*t*, C(9)); 45.80 (*d*, C(10)); 34.31 (*s*, C(11)); 27.12 (*q*, C(12)); 24.32 (*q*, C(13)); 19.66 (*q*, C(14)); 196.07 (*d*, C(15)). MS: 236 (32, $M^{+\cdot}$), 221 (11, $[M - Me]^+$), 218 (4, $[M - H_2O]^{+\cdot}$), 205 (7, $[M - CH_2OH]^+$), 203 (9, $[218 - Me]^+$), 193 (33, $[M - C_3H_7]^+$), 175 (37, $[193 - H_2O]^+$), 165 (94), 125 (76), 109 (36), 95 (47), 81 (59), 69 (80), 55 (71), 41 (100). HR-MS: 236.1782 ± 0.004 ($[C_{15}H_{24}O_2]^{+\cdot}$; calc. 236.1776); 211.1549 ± 0.004 ($[C_{14}H_{21}O_2]^+$; calc. 221.1541);

218.1669 ± 0.006 ($[C_{15}H_{22}O]^{+\cdot}$; calc. 218.1671); 205.1578 ± 0.003 ($[C_{14}H_{21}O]^+$; calc. 205.1592); 203.1433 ± 0.003 ($[C_{14}H_{19}O]^+$; calc. 203.1436); 193.1228 ± 0.003 ($[C_{12}H_{17}O_2]^+$; calc. 193.1228); 175.1131 ± 0.003 ($[C_{12}H_{15}O]^+$; calc. 175.1123); 165.0915 ± 0.003 ($[C_{10}H_{13}O_2]^+$; calc. 165.0.915).

6. *Secoepiraikovenal* (= *2-(Hydroxyethyl)-4-(2-isopropylidene-5-methylcyclopentyl)but-2-enal*; **4**). Oil. $[\alpha]_D^{20} = -18$ ($c = 0.1$, EtOH). UV (MeOH): 238 (7000). ^{1}H-NMR ($CDCl_3$): 3.64 (*t*, *J*(1,2) = 6.5, 2 H–C(1)); 2.47 (br. *t*, *J*(2,1) = 6.3, 2 H–C(2)); 6.66 (*t*, *J*(4,5) = 7.3, H–C(4)); 2.40, 2.28 (*m*, 2 H–C(5)); 1.60 (*m*, H–C(6)); 2.20 (*m*, H–C(7)); 1.85–1.40 (*m*, 2 H–C(8)); 2 H–C(9), H–C(10)); 4.73 (*q*, *J*(12,13) = 0.9, 2 H–C(12)); 1.67 (*t*, *J*(13,12) = 0.9, 3 H–C(13)); 0.85 (*d*, *J*(14,7) = 7.2, 3 H–C(14)); 9.39 (*s*, CHO). ^{13}C-NMR ($CDCl_3$): 62.58 (*t*, C(1)); 27.97 (*t*, C(2)); 157.54 (*d*, C(4)); 29.06 (*t*, C(5)); 45.78 (*d*, C(6)); 32.76 (*d*, C(7)); 35.29 (*t*, C(8)); 29.33 (*t*, C(9)); 51.10 (*d*, C(10)); 111.03 (*t*, C(12)); 15.59 (*q*, C(13)); 18.88 (*q*, C(14)); 196.06 (*d*, C(15)). MS: 236 (4, $M^{+\cdot}$), 221 (3, $[M - Me]^+$), 218 (4, $[M - H_2O]^{+\cdot}$), 205 (22, $[M - CH_2OH]^+$), 203 (4, $[218 - Me]^+$), 193 (6), 187 (16), 175 (9), 165 (10), 123 (71), 107 (56), 95 (49), 81 (100), 69 (43), 55 (88), 41 (100). HR-MS: 236.1778 ± 0.004 ($[C_{15}H_{24}O_2]^{+\cdot}$; calc. 236.1776).

7. *Photocyclization of Preraikovenal* (**1**). The reaction was carried out by irradiating **1** with a very-low-output *RS55* semimicro photochemical reactor from *Applied Photophysics*, London. In a first experiment, **1** (10^{-4} mol l^{-1}) was irradiated in a quartz cuvette in N_2-flushed hexane until complete conversion (1.5 h). In a second experiment with **1** (4×10^{-3} mol l^{-1}), hexane was replaced by $CDCl_3$, using a standard, 5-mm *Pyrex* NMR tube and limiting the conversion to 50% (25 min). The latter was a cleaner reaction affording *ent*-**3** and **2** in 90% and 5% yields, resp., on reacted substrate. Pure compounds were easily obtained by HPLC with hexane/i-PrOH 97:3 (t_R 9.6 or 9.0 for *ent*-**3** or **2**, resp.).

Data for ent-**3** (ent-*Epiraikovenal*). Oil. UV (MeOH): 245 (7000). CD /hexane): $\Delta\varepsilon_{max}(244) = +1.0$. ^{1}H-NMR superimposable to that for **3**.

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