227. Two Furanosesquiterpene Marine Metabolites with Antifeedant Properties[1])

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Summary

Two new sesquiterpenoids, nakafuran-8 (**1**) and -9 (**8**), have been isolated from the marine sponge *Dysidea fragilis* and from its prey, the nudibranchs *Hypselodoris godeffroyana* and *Chromodoris maridadilus*. The structures were established by spectral analysis and chemical transformations. Both compounds possess antifeedant properties when assayed in the laboratory against common reef fishes, *Chaetodon* spp.

Gastropod mollusks of the order Nudibranchia (subclass Opisthobranchia) are softbodied, unprotected, and often beautifully colored and delicately shaped members of coral reef ecosystems. They owe their survival to various stratagems, which include ingestion of functional cnidarian stinging cells or secretion of strong acids and other offensive substances [1]. We showed earlier that the nudibranch *Phyllidia varicosa* employs a defensive secretion which contains a mixture of two isocyanosesquiterpenes, and that this mollusk sequesters these compounds from its specific prey, a sponge, *Hymeniacidon* sp. [2] and accumulates them in its mantle.

While collecting specimens of the sponge *Dysidea fragilis* in Kaneohe Bay, Oahu, in connection with other research [3], one of us (G.S.) observed nudibranchs, identified as *Hypselodoris godeffroyana*, grazing on *D. fragilis*. On a subsequent field trip, another nudibranch, *Chromodoris maridadilus*, was observed while it was feeding on *D. fragilis*. Further investigation proved that two furanosesquiterpenes, which we have named nakafuran-8 and nakafuran-9[2]), are the two principal metabolites common to all three animals and that these compounds indeed repel frequently encountered reef fishes in laboratory assays[3]). We now report the

[1]) A preliminary account of this work was presented at the International Colloquium CNRS-ORSTOM on Natural Products of Biological Interest of the Pacific, in Noumea, New Caledonia, September 1979.

[2]) *Naka* is the Hawaiian word for sea creature; it is often used as a general prefix for various invertebrates [4]. The suffixed numerals indicate the carbocyclic ring size.

[3]) Both furanosesquiterpenes were subsequently isolated from an unidentified sponge collected at Canton Island, Phoenix Island group, by Dr. *D.J. Faulkner* (personal communication).

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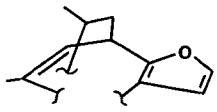
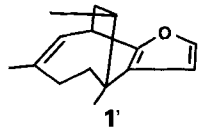
structure of nakafurans-8 and -9, which are bicyclo[4.2.2] and bicyclo[4.3.1]decane-based furanosesquiterpenes. They were extracted from *D. fragilis*, *H. godeffroyana* and *C. maridadilus* (see experimental part).

Freshly collected *D. fragilis*[4]) was immediately frozen and subsequently freeze-dried (909 g). Consecutive solvent extraction with petroleum ether, dichloromethane, and 2-propanol furnished 4.7 g of petroleum ether-soluble residue. Chromatography on silica gel and elution with hexane/dichloromethane (95:5) yielded nakafuran-8 (**1**, 1.7 g) and nakafuran-9 (**8**, 250 mg) as colorless oils, sensitive to air exposure and possessing the characteristic odor of the animal. One large nudibranch, *H. godeffroyana*[5]), (1.5 g) was briefly extracted with methanol. The residue was taken up in dichloromethane and filtered, leading to 52 mg of an orange oil. This oil furnished *via* HPLC. on *μ-Partisil* 32 mg of **1** and 5 mg of **8**. Similarly, one specimen of *C. maridadilus*[5]) weighing 35 mg yielded 2 mg of methanol extract that contained virtually pure nakafuran-8 and -9 in a ratio of 7:1.

The composition of nakafuran-8 (**1**), $C_{15}H_{20}O$, and its UV. spectrum strongly suggest a furanosesquiterpenoid structure. ^{1}H-NMR. data confirmed the furan moiety and proved in addition the presence of one isolated double bond and three methyl groups (secondary, tertiary and vinylic), thus implying two carbocyclic rings. Double resonance experiments established details of two contiguous methylenes and of a C_{11} moiety possessing partial structure **a**, which together with the above-mentioned tertiary methyl group accounted for all 15 carbon atoms, fully compatible with ^{13}C-NMR. data (see *Table 1*).



a



1'

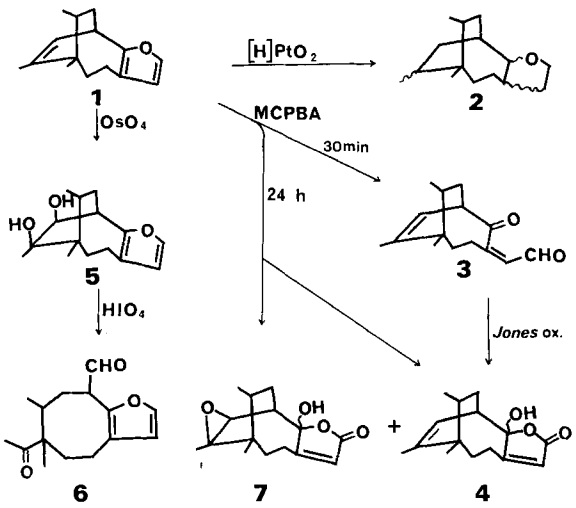
Only two structures remain for nakafuran-8, **1** and **1'**, both bicyclo[4.2.2]octanes. Prominent loss of propene in the mass spectrum of **1**, arising from a reverse *Diels-Alder* reaction could not be reconciled with **1'**. Structure **1** was also supported by heteronuclear decoupling of all carbon atoms. The decoupling data when extrapolated to the minor constituent nakafuran-9 (**8**) *(vide infra)* provide evidence for its assigned structure. A series of chemical transformations, summarized in *Scheme 1*, eliminated **1'** and proved structure **1** for nakafuran-8. Nakafuran-8 is inert to Pd/C catalyzed hydrogenation, but yields the hexahydro derivative **2** over *Adams'* catalyst. Treatment of **1** with *m*-chloroperbenzoic acid (MCPBA) for 30 min furnished γ-keto enal **3**. The aldehydic proton of **3** (δ 9.6, *d*, $J=8$ Hz) is coupled to an olefinic H-atom at δ 5.95 (*d*×*d*, $J=8$ and 1 Hz), distinct from the olefinic H-atom in **1** (δ 5.95, *d*×*d*, $J=7$ and 1 Hz). UV. and IR. data support structure **3**. The ^{1}H-NMR. signal for the tertiary methyl *s* at δ 1.10, virtually unchanged from the starting material (1.06), helped to exclude structure **1'**. *Jones* oxidation of **3** resulted in hydroxybutenolide **4**. Comparison of the ^{1}H-NMR. signals for H–C(9)

[4]) Identified by Dr. *P. Bergquist.*

[5]) Identified by *Scott Johnson.*

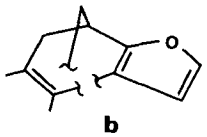
(δ 3.45, $d\times d\times d\times d$, J=7.4, 3.5 and 1 Hz, 1 H) and 2 H–C(4) (δ 2.45, $d\times d\times d$, J=14.7 and 3.5 Hz, 1 H; 2.25, $d\times d\times d$, J=14.4 and 4 Hz, 1 H) in nakafuran-8 (**1**) with their corresponding shifts of δ 2.9 ($d\times d\times d$, J=7.6 and 3 Hz, 1 H), 2.6 ($d\times d\times d$, J=14.5 and 5 Hz, 1 H) and 2.15 ($d\times d\times d$, J=14.14 and 5 Hz, 1 H) in hydroxybutenolide **4** supports the geometrical orientation of the furan in structure **1**. Compound **4**, incidentally, was also isolated from the methylene chloride extract of the animal, but probably as an artifact of furan autooxidation [5]. Osmylation of **1** led to a complex mixture of products from which diol **5** was isolated in low yield. Spectral data ($\tilde{\nu}_{max}$ 3620, 3520 cm^{-1}) provided evidence for the diol (IR., ^{1}H-NMR.) and for the intact furan moiety (UV., ^{1}H-NMR.). Periodic acid cleavage of **5** produced an unstable cyclooctane derivative, presumably **6**.

Scheme 1



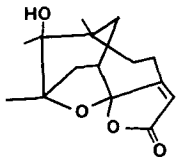
The minor nudibranch-sponge metabolite nakafuran-9, $C_{15}H_{20}O$, isomeric with **1**, appeared to possess a carbon skeleton different from that of **1**, as its mass spectrum bore no evidence of a reverse *Diels-Alder* cleavage. A disubstituted furan was readily established from the UV. (λ_{max} 219 nm, ε 4290), ^{1}H- (δ 7.15, *d*, J=2 Hz, 1 H; 6.06, $d\times d$, J=2, 1 Hz, 1 H) and ^{13}C-NMR. (156.3 *s*, 138.4 *d*, 118.5 *s*, 113.3 *d*) spectral data. The ^{1}H-NMR. spectrum of nakafuran-9 (**8**) was devoid of olefinic proton signals, but displayed signals for two vinylic methyls. Two singlets in the ^{13}C-NMR. spectrum at δ 129.6 and 126.4 (see *Table 1*) placed these two methyl groups on the same tetrasubstituted double bond. Proton double resonance experiments in (D_6) benzene and in (D) chloroform accounted for all atoms in the molecule by providing evidence for two contiguous methylenes, a tertiary-methyl, and a C_{11} part structure **b**. The remaining ambiguities were eliminated by the reactions shown in *Scheme 2*. Hydrogenation of **8** over *Adams'* catalyst furnished a hexahydro derivative **9**. Oxidation of **8** with MCPBA transformed the furan moiety into a keto enol in analogy with **1** (λ_{max} 239 nm, ε 3600; ν_{max} 1700 cm^{-1}; δ 9.5,

d, $J=7.5$, 1 H). The isolated double bond of **8**, which had remained intact when **1** was treated under identical conditions, was epoxidized, as seen by the signals of the methyl groups, which resonated as singlets at δ 1.30 and 1.22 in compound **10**.



b

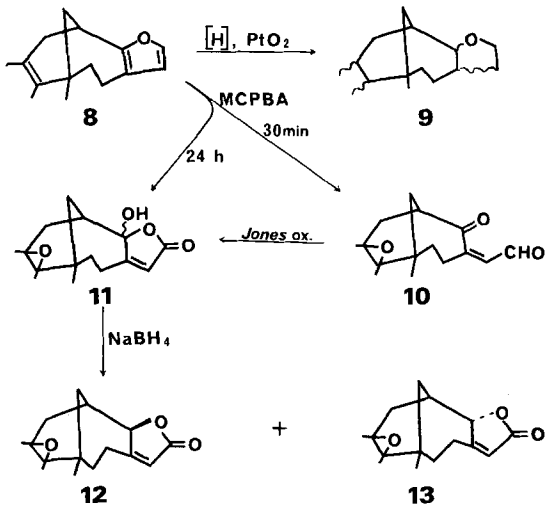
The third methyl group was seen as a singlet at δ 1.06, only slightly changed from δ 0.97 in the starting material **8**. Oxidation of **10** furnished hydroxybutenolide **11** formulated in accordance with its spectral properties. Conceivably, oxidation of **10** might have resulted in **11'**. This alternate structure could be eliminated by sodium borohydride reduction of **11** (or **11'**), which furnished epimeric epoxylactones **12** and **13** in combined quantitative yield. The same compound **11** could be obtained in a single step by carrying out the MCPBA reaction for 24 h. These reaction sequences fully confirm structure **8** for nakafuran-9.



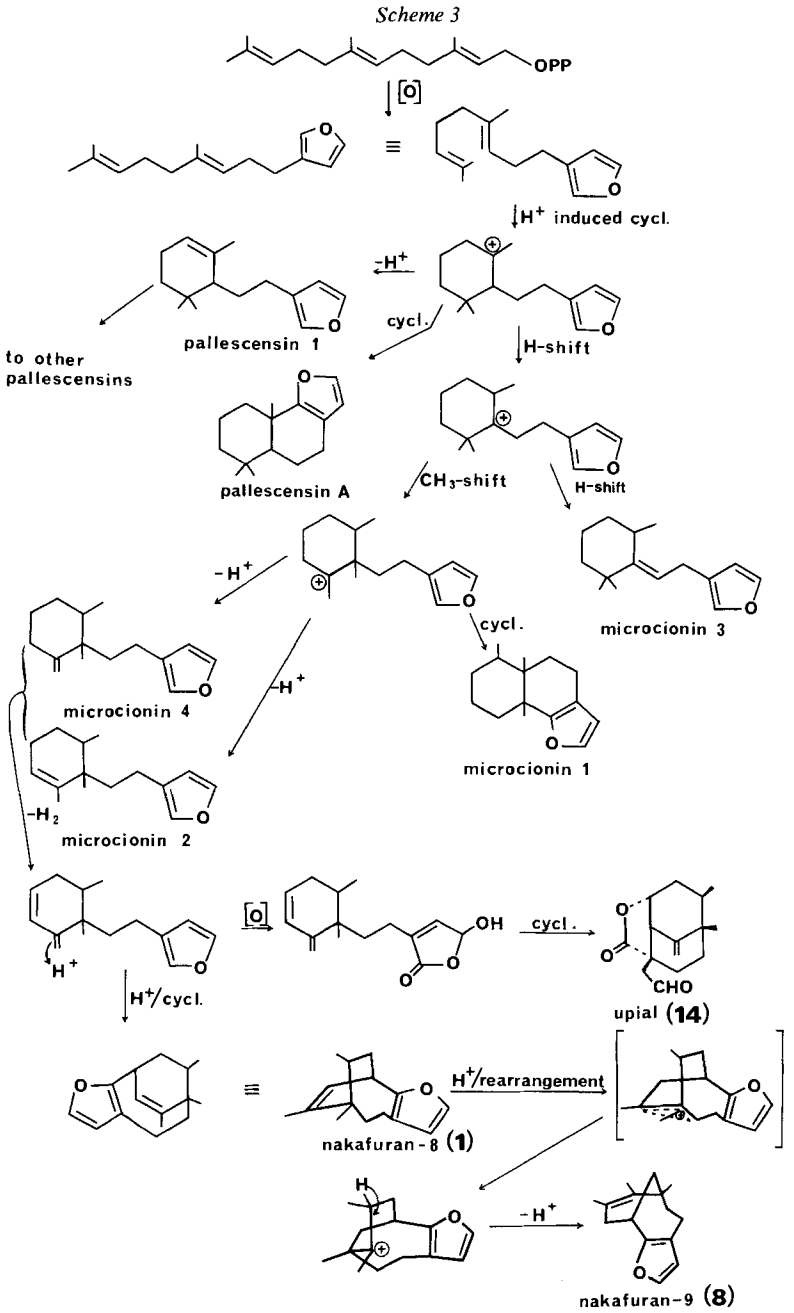
11'

It will be recalled that nakafuran-8 (**1**) yielded no epoxide when treated only briefly with MCPBA. An epoxybutenolide **7** was obtained as the predominant product in addition to **4** when nakafuran-8 reacted with MCPBA for 24 h *(Scheme 1)*.

Scheme 2

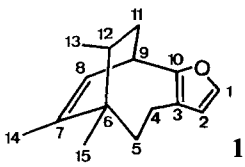


Scheme 3

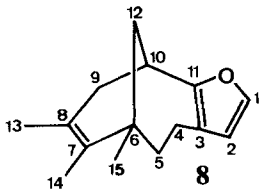


The stereochemistry of the secondary methyl group in nakafuran-8 was evident from the deshielding effects of the epoxide on the secondary methyl protons in epoxybutenolide **7** (δ 1.08, *d*, $J = 7$ Hz, 3 H) shifted from δ 0.89. This assignment is

Table. *^{13}C-Chemical shifts and ^{1}H-heteronuclear decoupling of nakafurans*
(δ values in ppm relative to TMS = 0)



1



8

Nakafuran-8 (**1**)			Nakafuran-9 (**8**)	
C	Carbon shift (multiplicity)	Heteronuclear decoupling	C	Carbon shift (multiplicity)
1	138.4(*d*)		1	138.4(*d*)
2	113.8(*d*)		2	113.3(*d*)
3	118.5(*s*)		3	118.5(*s*)
4	23.4(*t*)	2.32	4	23.3(*t*)
5	48.3(*t*)	1.84 and 1.73	5	41.6(*t*)
6	38.8(*s*)		6	37.9(*s*)
7	141.1(*s*)		7	129.6(*s*)[a])
8	124.6(*d*)		8	126.4(*s*)[a])
9	35.0(*d*)	3.44	10	32.0(*d*)
10	151.0(*s*)		11	156.3(*s*)
11	39.3(*t*)	1.30	12	38.8(*t*)[a])
12	36.8(*d*)	1.73 and 1.84	9	38.5(*t*)[a])
13	19.0(*qa*)	0.91	13	30.0(*qa*)[a])
14	20.5(*qa*)	1.73	14	31.0(*qa*)[a])
15	24.6(*qa*)	1.09	15	20.1(*qa*)[a])

[a]) Values for carbons 7 and 8, 9 and 12, and 13 and 14 are interchangeable.

supported by the sluggish reactivity of the isolated trisubstituted double bond and by the absence of NMR. effects resulting from chemical manipulations of the furan moiety.

The sponge genus *Dysidea* has been the source of diverse organic metabolites, which have included bromophenols [6] and thiazole derivatives [7] in addition to numerous sesquiterpenoids [8]. Although the nakafuran skeletons have not previously been encountered, they can be readily related by established biogenetic transformations *(Scheme 3)* to known sesquiterpenes from *Dysidea* and from other sponges. Our suspicion that these two compounds, nakafuran-8 (**1**) and -9 (**8**), which were common to the sponge and to its prey, the nudibranchs *Hypselodoris godeffroyana* and *Chromodoris maridadilus*, might be retained by the mollusk for its own defense against predators, was confirmed by simple laboratory assays. In feeding experiments with two species of reef fishes, *Chaetodon* spp., the nakafurans **1** and **8** repelled the fish, while another *Dysidea* sesquiterpene, upial (**14**), which is not present in the nudibranchs, had no repellent properties. Details of these experiments will be described elsewhere.

We thank Mr. *James Loo* for assistance with NMR. measurements and Mr. *Norman Liu* with mass spectral determinations. We also thank Dr. *W. Fenical*, Ms. *M. Bandurraga*, Mr. *B. Gerwick* and the UCSD NMR. facility (NIH Grant RR-00708) for 220 MHz ^{1}H-NMR. spectra. Our gratitude is extended to Professor *P. Bergquist* for sponge identification and to Mr. *S. Johnson* for his untiring efforts to help us collect and identify nudibranchs, and the *National Science Foundation* for financial support.

Experimental Part

General remarks. UV. spectra were recorded on a *Beckman* Acta CIII spectrophotometer. IR. spectra were recorded on *Perkin-Elmer* 467 or *Beckman* IR-10 spectrophotometers. NMR. spectra (δ, J = Hz) were determined on *Varian* model HR-220, XL-100, or T-60 spectrometers. MS. were recorded on a *Varian* MAT 311 instrument. Optical rotations were measured on a *Bendix Ericsson* ETL-NPL polarimeter.

Collection. – *Dysidea fragilis* may be found throughout Kaneohe Bay, Oahu, but is abundant near the lighthouse pier at Coconut Island. Equally large populations of the sponge may be found in the Ala Wai Canal, Oahu.

The nudibranchs *Hypselodoris godeffroyana* and *Chromodoris maridadilus* may also be found in these areas, with larger populations in the Ala Wai Canal.

Collections of *D.fragilis, H.godeffroyana,* and *C.maridadilus* from all areas were made at 3 m depth by snorkeling.

Isolation of odoriferous constituents. – A small (125 g) portion of freshly collected *D.fragilis* was freeze-dried into a cold trap at −78°. The characteristic odor of the freshly collected sponge was present in the trapped frozen concentrate. Separation of the odoriferous constituents from the aqueous solution was easily accomplished by bubbling N_2 through the solution in a gas washing bottle. The gas carried the volatiles to an adsorbent trap filled with *Tenax*-GC[6]) support. Elution of the *Tenax* GC support with hot N_2 and condensation on a cold finger at −78° yielded 2.3 mg of odoriferous constituents, which by ^{1}H-NMR. proved to be a 7:1 mixture of nakafuran-8 (**1**) and -9 (**8**).

Large sponge collections (>1 kg), which proved chemically identical from all sites, were frozen, then freeze-dried. Separation was achieved by consecutive extraction with petroleum ether, CH_2Cl_2, and 2-propanol.

Nudibranchs were extracted with MeOH for 30 min. The extract was concentrated and the residue diluted with CH_2Cl_2. Filtration and solvent removal yielded an orange oil. From *H.godeffroyana* one large animal (1.5 g, wet) yielded 52 mg of oil. Three specimens of *C.maridadilus* (1.18 g, wet) supplied 23 mg of orange colored oil.

Nakafuran-8 (**1**) *and -9* (**8**). The petroleum ether extract (4.7 g) of the sponge was chromatographed on silica gel and eluted with hexane/CH_2Cl_2, 95:5. Nakafuran-8 (**1**, 1.7 g) preceded nakafuran-9 (**8**, 250 mg). Both compounds were isolated under similar conditions from the nudibranch extracts. From *H.godeffroyana* chromatography of 52 mg extract hexanes/CH_2Cl_2 95:5 afforded **1** (32 mg) and **8** (5 mg) by HPLC. on *μ-Partisil*[7]) with hexane/CH_2Cl_2, 95:5. Extract (23 mg) of *C.maridadilus* yielded 16 mg of **1** and 3 mg of **8**.

Data of Nakafuran-8 (**1**). $[\alpha]_D^{25} = +24.2°$ (c = 2.65, $CHCl_3$). – UV. (hexane): 228 nm (ε 6100). – IR. (CH_2Cl_2): 3020, 2920, 1550, 1455, 1250, 1165 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 7.13 (*d*, J = 1.5, 1 H); 6.07 (*d*×*d*, J = 1.5 and 1, 1 H); 5.95 (*d*×*d*, J = 7 and 1, 1 H); 3,45 (*d*×*d*×*d*×*d*, J = 7.4, 3.5 and 1, 1 H), 2.45 (*d*×*d*×*d*, J = 14.7 and 3.5, 1 H); 2.25 (*d*×*d*×*d*, J = 14.4 and 4, 1 H); 2.0 (*d*×*d*×*d*, J = 14.4 and 3.5, 1 H); 1.9 (*qa*×*d*×*d*, J = 7.6 and 6, 1 H); 1.8 (*d*×*d*×*d*, J = 14.7 and 4, 1 H); 1.75 (*d*, J = 1, 3 H); 1.28 (*d*×*d*×*d*, J = 14.6 and 3.5, 1 H); 1.24 (*d*×*d*×*d*, J = 14.6 and 4, 1 H); 1.06 (*s*, 3 H); 0.89 (*d*, J = 7, 3 H). – ^{13}C-NMR. ($CDCl_3$): 151(*s*), 141.1(*s*), 138.4(*d*), 124.6(*d*), 118.5(*s*), 113.8(*d*), 48.3(*t*), 39.3(*t*), 38.8(*s*), 36.8(*d*), 35.0(*d*), 24.6(*qa*), 23.4(*t*), 20.5(*qa*), and 19.0(*qa*). – High resolution EIMS. (*m/z*): 216.14912 (M^+, $C_{15}H_{20}O$ requires 216.151419); 201 ($M^+ - CH_3$); 174 (M^+-propene).

Data of Nakafuran-9 (**8**). $[\alpha]_D^{25} = -106.1°$ (c = 0.33, $CHCl_3$). – UV. (hexane): 219 nm (ε 4290). – IR. (CH_2Cl_2): 3060, 2880, 1540, 1260, 1180 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 7.15 (*d*, J = 2, 1 H); 6.06 (*d*×*d*, J = 2 and 1, 1 H); 3.16 (*t*×*d*×*d*×*d*, J = 4, 5.5, 2 and 1, 1 H); 2.34 (*d*×*d*×*d*, J = 14.10 and 4, 1 H); 2.3 (*d*×*d*, J = 14 and 5.5, 1 H); 2.25 (*d*×*d*×*d*, J = 14.7 and 4, 1 H); 1.91 (*d*×*d*×*d*, J = 14.2 and 1, 1 H); 1.81 (*d*×*d*×*d*, J = 14.4 and 4, 1 H); 1.75 (*d*, J = 4, 2 H); 1.60 (*d*, J = 1, 3 H); 1.60 (br. *s*, J < 0.5, 3 H); 1.35 (*d*×*d*×*d*, J = 14.10 and 7, 1 H), and 1.06 (*s*, 3 H). – ^{1}H-NMR. (C_6D_6): 7.10 (*d*, J = 2, 1 H); 6.04 (*d*×*d*, J = 2 and 1, 1 H); 3.27 (*t*×*d*×*d*×*d*, J = 4, 5.5, 2 and 1, 1 H); 2.32 (*d*×*d*×*d*, J = 14.7 and 4, 1 H); 2.26 (*d*×*d*×*d*, J = 14.10 and 4, I H); 2.25 (*d*×*d*, J = 14 and 5.5, 1 H); 2.00 (*d*×*d*×*d*, J = 14.2 and 1, 1 H); 1.79 (*d*×*d*×*d*, J = 14.4 and 4, 1 H); 1.64 (br. *d*, J = 4, 2 H); 1.50 (*d*, J = 1, 3 H); 1.40 (br. *s*, J < 0.5, 3 H); 1.25

6) *Tenax*-GC is a trademark of AKZO Research Laboratories, Holland, and is supplied by Applied Sciences Laboratories, State College, PA.

7) *Partisil* is a trademark of *Whatman Inc.*, Clifton, New Jersey.

($d\times d\times d$, J = 14.10 and 7, 1 H); 0.97 (*s*, 3 H). – ^{13}C-NMR. ($CDCl_3$): 156.3(*s*), 138.4(*d*), 129.6(*s*), 126.4(*s*), 118.5(*s*), 113.3(*d*), 41.6(*t*), 38.8(*t*), 38.5(*t*), 37.9(*s*), 32.0(*d*), 31.0(*qa*), 30.0(*qa*), 23.3(*t*), and 20.1(*qa*). – High resolution EIMS. (*m/z*): 216.14890 (M^+, $C_{15}H_{20}O$ requires 216.151419); 201 ($M^+ - CH_3$).

Hexahydronakafuran-8 (**2**). A solution of nakafuran-8 (**1**, 48.6 mg) in 15 ml of dry ether was stirred under H_2 in the presence of PtO_2 at RT. After 3 h the reaction mixture was filtered through 3 g silica gel with dry ether, and the solvent was evaporated. Silica gel chromatography of the total crude material 47.8 mg with petroleum ether/ether 95:5, provided 33.2 mg (68%) of **2**. – IR. (CH_2Cl_2): 2950, 1455, 1090, 1065 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 3.8 (complex, 3 H); 2.0–1.0 (complex); 0.94 (*s*, 3 H); 0.90 (*d*, J = 7, 3 H); 0.82 (*d*, J = 7, 3 H). – EIMS. (*m/z*): 222 (M^+, $C_{15}H_{26}O$).

γ-Keto enal **3**. A solution of nakafuran-8 (**1**, 11.4 mg) in 500 µl of $CDCl_3$ was added in an NMR. tube to 1 equiv. of MCPBA (*Aldrich*, techn.) (10.7 mg). Monitoring the reaction by ^{1}H-NMR. showed that after 30 min only the furan had reacted, while the alkene remained unchanged. Addition of an excess MCPBA did not cause further change after 1 h. Silica gel chromatography or basic work-up caused decomposition of the product. Partial separation with a SEP-PAK C_{18} cartridge (*Waters Assoc.*) gave 11.2 mg of a mixture of which **3** was the major and MCPBA the minor component. – UV. (MeOH); 242 nm (ε 2100). – IR. (CH_2Cl_2): 2920, 1685 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 9.6 (*d*, J = 8, 1 H); 5.95 ($d\times d$, J = 8 and 1, 1 H); 5.52 ($d\times d$, J = 6.5 and 1.5, 1 H); 3.30 (*m*, 1 H); 2.6–1.7 (complex); 1.70 (*d*, J = 1.5, 3 H); 1.10 (*s*, 3 H); 0.86 (*d*, J = 7, 3 H). – EIMS. (*m/z*): 232 (M^+, $C_{15}H_{20}O_2$).

Hydroxybutenolide **4**. Two drops of Jones' reagent [9] were added to a 2 ml acetone solution of **3** (11.2 mg, mostly **3**). The solution was stirred at 0° for 10 min, then allowed to warm up slowly to RT. and stirred for an additional 30 min. Excess reagent was quenched with 2-propanol. The solution was dried ($MgSO_4$) and filtered. The crude material was chromatographed by HPLC. on *LiChrosorb* Si-60[8]) with CH_2Cl_2/EtOAc/HOAc 9.5:9.5:1. Chromatography yielded 7.4 mg of pure **4** which decomposes slowly on silica gel (it easily forms the γ-methoxybutenolide with MeOH catalyzed by silica gel). – UV. (MeOH): 233 nm (ε 2400); ($MeOH + OH^-$): 236 nm (ε 2500), 246 (ε 500). – IR. (CH_2Cl_2): 3040, 2930, 1790, 1775 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 5.85 (*s*, 1 H); 5.80 ($d\times d$, J = 7 and 1, 1 H); 2.9 ($d\times d\times d$, J = 7, 6 and 3, 1 H); 2.6 ($d\times d\times d$, J = 14.5 and 5, 1 H); 2.15 ($d\times d\times d$, J = 14.14 and 5, 1 H); 1.80 ($d\times d\times d$, J = 14.5 and 5, 1 H); 1.70 (*d*, J = 1, 3 H); 1.65 (complex, 2 signals, 2 H); 1.56 ($d\times d\times d$, J = 14.14 and 5, 1 H); 1.25 ($d\times d\times d$, J = 14.6 and 2, 1 H); 1.06 (*s*, 3 H); 0.85 (*d*, J = 7, 3 H). – EIMS. (*m/z*): 248 (M^+, $C_{15}H_{20}O_3$).

Diol **5**. Osmium tetroxide (50 mg) was added slowly to a 2 ml solution of **1** (41 mg) in pyridine and the mixture was stirred at RT. After 2 h a solution of $NaHSO_3$ (200 mg) in 12 ml of water/pyridine, 5:1, was added [10]. Cleavage of the osmate ester was complete after stirring for 20 min as indicated by a color change from dark brown to orange. The aqueous solution was extracted with CH_2Cl_2 (3 × 50 ml). Evaporation of the solvent after washing with 5% hydrochloric acid and brine, and drying ($MgSO_4$), yielded 49 mg of crude products. Chromatography of 34.3 mg of crude material on silica gel using a gradient of ether in petroleum ether afforded 11.2 mg (34%) of pure diol **5**. – UV. (MeOH): 218 nm (ε 5600). – IR. (CH_2Cl_2): 3620, 3520, 2930, 1460, 1050 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 7.18 (*d*, J = 2, 1 H); 6.08 ($d\times d$, J = 2 and 1, 1 H); 4.04 (*d*, J = 1.5, 1 H); 3.12 ($d\times d\times d\times d$, J = 4.2, 1.5 and 1, 1 H); 2.42 ($d\times d\times d$, J = 14.14 and 6, 1 H); 2.40 ($d\times d\times d$, J = 14.4 and 4, 1 H); 1.80 ($d\times d\times d$, J = 14.14 and 4, 1 H); 1.7–1.1 (complex); 1.28 (*s*, 3 H); 1.08 (*s*, 3 H); 0.90 (*d*, J = 7, 3 H). – EIMS. (*m/z*): 250 (M^+, $C_{15}H_{22}O_3$).

Furocyclooctane **6**. To a stirred solution of diol **5** (11.2 mg) in 4 ml of ether, HIO_4 (20 mg) was slowly added. After continued stirring at RT. for 1.5 h silica gel TLC. indicated absence of starting material. Evaporation of the ethereal solution and immediate spectroscopic determination provided limited results for the extremely sensitive product **6**. – ^{1}H-NMR. (C_6D_6): 9.1 (*d*, J = 2, 1 H); 7.24 (*d*, J = 1.5, 1 H); 6.1 (*d*, J = 1.5, 1 H); 2.0 (*s*, 3 H); 1.1 (*s*, 3 H), 1.0 (*d*, J = 7, 3 H). – EIMS. (*m/z*): 248 (M^+, $C_{15}H_{20}O_3$).

Epoxy-γ-hydroxybutenolide **7**. A solution of nakafuran-8 (**1**, 13.1 mg) in 5 ml of CH_2Cl_2 was stirred at RT. for 24 h in the presence of excess MCPBA (42.1 mg). Chromatography of the crude reaction mixture by HPLC. on *LiChrosorb* Si-60 with CH_2Cl_2/EtOAc/HOAc 9.5:9.5:1 furnished pure **4** (2.8 mg) and **7** (6.3 mg). – UV. (MeOH): 235 nm (ε 1400); ($MeOH + OH^-$): 236 nm (ε 2500), 245 nm (ε 500). – IR. (CH_2Cl_2): 3520, 2910, 2850, 1780 sh, 1770, 1200, 1090 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 5.90 (*d*, J = 0.5, 1 H); 3.29 (*d*, J = 4.5, 1 H); 2.98 (*m*, 1 H); 2.65 (*m*, 1 H); 2.62 (*m*, 1 H); 2.0 → 1.3 (complex); 1.3 (*s*, 3 H);

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[8]) *LiChrosorb* Si-60 is a trademark of *E. Merck*, Darmstadt, West Germany. The column was prepared by Dr. *H. Knauer* and distributed by *Unimetrics*, Anaheim, Ca.

1.12 (*s*, 3 H); 1.08 (*d*, *J*=7, 3 H). – EIMS. (*m/z*): 264 (M^+, $C_{15}H_{20}O_4$); 249 ($M^+ - CH_3$); 246 ($M^+ - H_2O$); 231 ($M^+ - CH_3 - H_2O$); 221 ($M^+ - CH_3CO$); 131 (base); 43 (CH_3CO^+).

Hexahydronakafuran-9 (**9**). A solution of nakafuran-9 (**8**, 33.4 mg) in 10 ml of dry ether was stirred under H_2 in the presence of a catalytic amount of PtO_2 at RT. The starting material was completely consumed after 2 h (TLC., silica gel). The reaction mixture was filtered through 3 g of silica gel with dry ether. Solvent exaporation yielded 25.8 mg of crude material, silica gel chromatography of which with petroleum ether/ether 95:5 provided pure **9** (18.6 mg, 54%). – IR. (CH_2Cl_2): 2960, 1445, 1070, 1060 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 3.80 (complex, 3 H); 2.1–1.1 (complex); 1.0 (*s*, 3 H); 0.88 (*d*, *J*=7, 3 H); 0.84 (*d*, *J*=7, 3 H). – EIMS. (*m/z*): 222 (M^+, $C_{15}H_{26}O$).

Epoxyketo enal **10**. A solution of nakafuran-9 (**8**, 7.6 mg) in 500 µl $CDCl_3$ was added to one equivalent of MCPBA (7.1 mg) in an NMR. tube. After 30 min the furan and tetrasubstituted alkene functions were reacting and starting material was still present (^{1}H-NMR.). An additional equivalent of MCPBA was added to the reaction mixture. After 30 min there was complete consumption of starting material and emergence of one major product (^{1}H-NMR.). As was the case with compound **3**, epoxyketo enal **10** was extremely sensitive to silica gel or basic work-up. Partial purification with a SEP-PAK C_{18} cartridge yielded 7.4 mg of partially pure **10** (MCPBA was the only contaminant). – UV. (MeOH, 239 nm) (ε 3600). – IR. (CH_2Cl_2): 2940, 1700, 1250 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 9.5 (*d*, *J*=7.5, 1 H); 6.0 (*d*×*d*, *J*=7.5 and 1, 1 H); 2.7 (complex *m*, 1 H); 2.6–2.1 (complex); 1.92 (*d*×*d*, *J*=15 and 4, 1 H); 1.52 (*d*×*d*, *J*=15 and 10, 1 H); 1.3 (*s*, 3 H); 1.22 (*s*, 3 H); 1.06 (*s*, 3 H). – EIMS. (*m/z*): 248 (M^+, $C_{15}H_{20}O_3$).

Epoxy-γ-hydroxybutenolide **11**. Two drops of *Jones'* reagent were added to a 2 ml acetone solution of impure **10** (7.4 mg) while stirring at 0°. After addition, the mixture was warmed to RT. and stirred for an additional 30 min. Two drops of 2-propanol were added to quench excess reagent. The solution was dried ($MgSO_4$), filtered, and evaporated to a colorless oil. Compound **11** slowly decomposes on silica gel. Chromatography of the products by HPLC. on *LiChrosorb*-Si 60 with CH_2Cl_2/EtOAc/HOAc 9.5:9.5:1 cleanly separated compound **11** (5.9 mg). – UV. (MeOH): 226 nm (ε 4400); (MeOH + OH^-) 226 nm (ε 3500), 248 (ε 500). – IR. (CH_2Cl_2): 3420, 2960, 1790, 1770 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$): 5.8 (*d*, *J*=1.5, 1 H); 2.75 (*d*×*d*×*d*, *J*=14, 13 and 4, 1 H); 2.5–1.1 (complex); 1.26 (*s*, 3 H); 1.19 (*s*, 3 H); 1.09 (*s*, 3 H). – EIMS. (*m/z*): 264 (M^+, $C_{15}H_{20}O_4$); 249 ($M^+ - CH_3$); 246 ($M^+ - H_2O$); 231 ($M^+ - CH_3 - H_2O$); 221 ($M^+ - CH_3CO$); 43 (CH_3CO^+, base).

Epimeric epoxybutenolides **12** *and* **13**. A 2 ml EtOH solution of $NaBH_4$ (5.8 mg) was slowly added to a 5 ml EtOH solution of **11** (10.3 mg) and stirred at RT. After 20 min, silica gel TLC. showed complete consumption of starting material (**11**) and the appearance of a single, less polar, spot. Three drops of glacial HOAc were added followed by 1 ml of H_2O. The aqueous solution was extracted with CH_2Cl_2 (3×8 ml). Evaporation and then lyophilization produced **12** and **13** in overall quantitative yield (9.8 mg) in a 2:3 ratio. – UV. (MeOH): 233 nm (ε 1500). – IR. (CH_2Cl_2): 2920, 2820, 1755, 1150, 1020 cm^{-1}. – ^{1}H-NMR. ($CDCl_3$) of **12**: 5.79 (*d*×*d*, *J*=1.5 and 1, 1 H); 4.80 (*d*×*d*, *J*=2 and 1, 1 H); 2.8→1.4 (complex); 1.37 (*s*, 3 H); 1.30 (*s*, 3 H); 1.02 (*s*, 3 H). – ^{1}H-NMR. ($CDCl_3$) of **13**: 5.89 (*d*×*d*, *J*=2 and 1, 1 H); 4.98 (*d*×*d*, *J*=2 and 1, 1 H); 2.8–1.4 (complex); 1.24 (*s*, 3 H); 1.18 (*s*, 3 H); 1.11 (*s*, 3 H). – EIMS. (*m/z*): 248 (M^+, $C_{15}H_{20}O_3$); 233 ($M^+ - CH_3$); 230 ($M^+ - H_2O$); 215 ($M^+ - CH_3 - H_2O$); 205 ($M^+ - CH_3CO$); 43 (CH_3CO^+, base).

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