Pergamon

Tetrahedron, Vol. 53, No. 6, pp. 2303-2308, 1997
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0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)01131-3

New Polypropionates from *Siphonaria pectinata*

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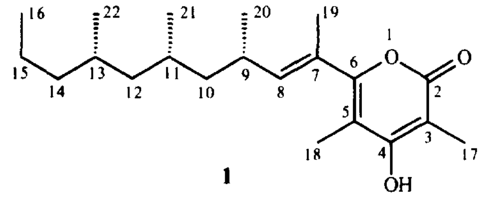
Abstract: The marine pulmonate *Siphonaria pectinata* contains the known polypropionates pectinatone (**1**), siphonarienone (**6**), a mixture of C-4 epimers of siphonarienedione (**7**), siphonarienolone (**8**), two independent mixtures of C-2 epimers of siphonarienfuranone (**9**) and the corresponding *Z* isomers (**10**), together with four new polypropionates, norsiphonarienone A (**2**), isosiphonarienolone (**3**), 2-deoxysiphonarienfuranone (**4**) and isopectinatone (**5**). The structures of the new compounds were elucidated by interpretation of spectral data. The polypropionates isolated from *S. pectinata* showed mild cytotoxicity against four tumor cell lines. © 1997, Elsevier Science Ltd. All rights reserved.

The chemistry of marine pulmonates of the genus *Siphonaria*, generally known as false limpets, is dominated by the presence of polypropionate metabolites.[1] These natural products belong to the polyketide group and their biosynthesis implies condensation of propionate units.[2-4] The taxonomic distribution of polypropionates in the marine enviroment is not only restricted to pulmonate but also to opisthobranchia molluscs, and in fact, the first accounts of this type of compounds in the late seventies were reported from the opisthobranchia sacoglossans *Tridachiella diomedea*[5] and *Tridachia crispata*.[6]

In the course of our investigations directed towards the search of bioactive compounds from marine organisms we obtained specimens of the pulmonate *Siphonaria pectinata* collected in the intertidal zone of La Caleta (Cádiz, Spain). A collection of *S. pectinata* from Key Biscayne, Florida, had been reported[7] to contain pectinatone (**1**), an antibiotic polypropionate with a γ-hydroxy-α-pyrone ring. Our specimens contained, in addition to pectinatone (**1**), four new polypropionates (**2-5**) together with the known compounds siphonarienone (**6**),[8] a mixture of the C-4 epimers of siphonarienedione (**7**),[8-10] siphonarienolone (**8**),[8,9] and two mixtures of the C-2 epimers of siphonarienfuranone (**9**),[8] and the corresponding *Z* isomers (**10**).[8]

Specimens of *Siphonaria pectinata* were collected by hand and immediately frozen. The less polar material of an acetone extract was chromatographed on Si gel. Further purification using both normal and reversed phase HPLC allowed isolation of the following compounds: norsiphonarienone A (**2**, $3\cdot10^{-4}$ mg/animal), isosiphonarienolone (**3**, $5\cdot10^{-4}$ mg/animal), 2-deoxysiphonarienfuranone (**4**, $2\cdot10^{-3}$ mg/animal), isopectinatone (**5**, $2\cdot10^{-3}$ mg/animal), together with the known compounds above mentioned (**1**, **6-10**).

Norsiphonarienone A (**2**) was isolated as a colorless oil. The molecular formula, $C_{16}H_{30}O$, was deduced from the high resolution mass measurement. Both the UV and IR absorptions together with the ^1H and ^{13}C NMR data closely resembled those of siphonarienone (**6**). Thus the signals at δ 0.81 (d, J=6.6 Hz, 3H), 0.84



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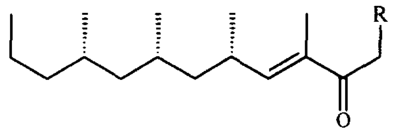

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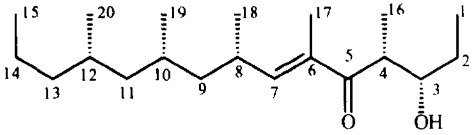
6 R = CH_3



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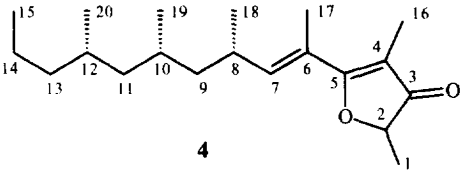

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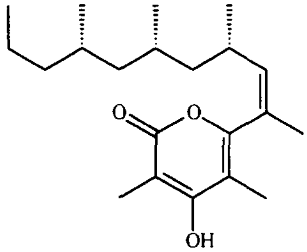

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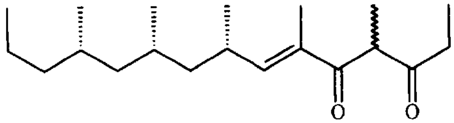

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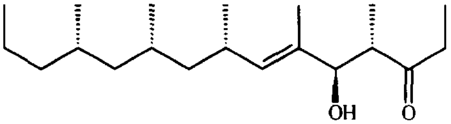

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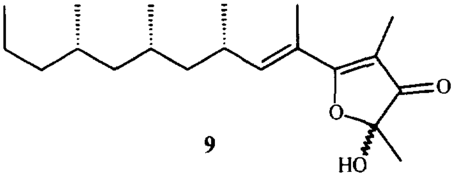

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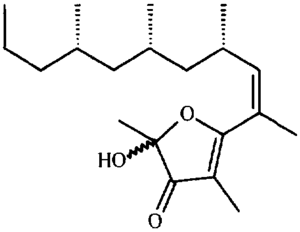

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(d, J=6.4 Hz, 3H), 0.87 (t, J=7.1 Hz, 3H) and 1.02 (d, J=6.6 Hz, 3H) were assigned to four methyl groups attached to saturated carbon atoms. The signal at δ 1.02 showed a cross peak in the COSY spectrum with the methine proton signal at δ 2.77 (m, 1H) which was also coupled with the olefinic proton signal at δ 6.35 (dq, J=10.0 and 1.3 Hz, 1H). This latter signal was correlated in the HETCOR experiment with the doublet at δ 157.9 and presented allylic coupling with the signal at δ 1.85 (d, J=1.3 Hz, 3H) of a vinylic methyl group which in the ^{13}C NMR spectrum gave rise to a quartet at δ 10.5 indicating an *E* geometry for the double bond. These data defined the presence of a 1,3,5,7-tetramethyldec-1-enyl moiety in compound **2** as that of siphonarienone (**6**). Since the small amount isolated of **2** prevented degradative reactions from being performed the absolute configuration in the chiral centers was proposed as *S* similarly to other polypropionates of the genus *Siphonaria* with a related structure[8,9,11,12] and to the known compounds isolated from *S. pectinata* (**1**, **6-10**) whose absolute stereochemistry is well known.[8,9,11]

The tetramethyldecenyl moiety above mentioned accounted for fourteen of the sixteen carbons of the molecule. The two carbons remaining gave rise to a singlet at δ 200.0 due to an α,β-unsaturated carbonyl group and to a quartet at δ 27.0 due to a methyl group that must be attached to the carbonyl. It was therefore proposed structure **2** for norsiphonarienone A.

Isosiphonarienolone (**3**) was obtained as a colorless oil. The molecular formula, $C_{20}H_{38}O_2$, was obtained from the high resolution mass measurement and indicated that **3** was an isomer of the known compound siphonarienolone (**8**).[8,9] The analysis of both ^{1}H and ^{13}C NMR spectra indicated that **3** possessed the 1,3,5,7-tetramethyldec-1-enyl moiety as norsiphonarienone A (**2**) above described. The olefinic proton signal appeared at δ 6.37 (dq, J=9.8 and 1.3 Hz, 1H) indicating the presence of a carbonyl conjugated with a double bond as ascertained by the UV absorption at 242 nm. In addition the ^{1}H NMR spectrum showed two methyl protons signals at δ 1.18 (d, J=7.1 Hz, 3H) and 0.95 (t, J=7.4 Hz, 3H) which were correlated in the COSY spectrum with the methine proton signal at δ 3.32 (qd, J=7.1 and 5.1 Hz, 1H) and with the methylene protons signal at δ 1.45 (m, 2H), respectively. These two signals were also coupled to a common signal at δ 3.59 (m, 1H) assigned to a proton attached to a carbon bearing a hydroxyl group. These connectivities defined the 2-hydroxy-1-methylbutyl unit linked to the α,β-unsaturated carbonyl group.

The stereochemistry 4*R*, 8*S*, 10*S*, 12*S* depicted in formula **3** was proposed based on a similar rationale as that followed for norsiphonarienone A (**2**). The stereochemistry at C-3 was proposed as *S* upon observation of $J_{3,4}$ = 5.1 Hz in contrast with that exhibited by siphonarienolone (**8**) in which a diastereomeric orientation of the corresponding protons led to a coupling constant of 10.1 Hz.[8]

2-Deoxysiphonarienfuranone (**4**) was isolated as a colorless oil and readily decomposed. This fact prevented the high resolution mass measurement from being obtained. However, the EIMS showed the molecular ion peak at *m/z* 306 u.m.a. and the ^{13}C NMR accounted for twenty carbon atoms.

The ^{1}H and ^{13}C NMR were basically similar to those of siphonarienfuranone (**9**)[8] indicating the presence of the polypropionate side chain, the *E* trisubstituted double bond and a furanone ring modified with respect to that of **9**. Thus the ^{13}C NMR signals at δ 79.7 (d) and 16.6 (q) were correlated in the HETCOR experiment with the mutually coupled proton signals at δ 4.43 (q, J=7.1 Hz, 1H) and 1.43 (d, J=7.1 Hz, 1H) indicating that **4** lacked the hydroxyl group in the heterocyclic ring whose structure was therefore proposed as 2,3-dihydro-2,4-dimethylfuran-3-one. This rationale indicated that **4** was the 2-deoxy derivative of siphonarienfuranone (**9**).

Isopectinatone (**5**) was isolated as a colorless oil. The molecular formula, $C_{21}H_{34}O_3$, was obtained from the high resolution mass measurement and indicated that **5** was an isomer of pectinatone (**1**). Furthermore, the ^1H and ^{13}C NMR signals suggested the presence in **5** of the polypropionate side chain, the trisubstituted olefinic system and the 3,5-dimethyl-4-hydroxy-2*H*-pyran-2-one ring. The ^{13}C NMR signals of the double bond carbons appeared at δ 126.4 (s) and 140.8 (d). This latter signal was correlated in the HETCOR experiment with the olefinic proton signal at δ 5.33 (dq, J=10.4 and 1.5 Hz, 1H) which showed couplings with the allylic proton signal at δ 2.16 (m, 1H) and the vinylic methyl signal at δ 1.90 (d, J=1.5 Hz, 3H) in the COSY spectrum. This methyl signal was correlated in the HETCOR experiment with the carbon signal at δ 21.9 (q) indicating a *Z* geometry of the double bond. These data indicated that **5** was the *Z* isomer of pectinatone (**1**).

The biological activities of polypropionate compounds are mainly antibiotic and cytotoxic. The known compounds isolated from *S. pectinata* (**1**, **6-10**) had been reported[8] to exhibit antimicrobial activity against gram (+) bacteria. However, since the cytotoxicity of these compounds had not been evaluated we tested both the known and the new compounds[13] isolated from *S. pectinata* against P388 mice lymphoma, A549 human lung carcinoma, HT29 human colon carcinoma and MEL28 human melanoma tumor cell lines to detect *in vitro* cytotoxicity. The results of ED_{50} (μg/mL) were 10 or over for compounds **3**, **6** and **7**. Compounds **1** and **9** showed ED_{50} values of 5 μg/mL against the four cell lines tested. The highest inhibition was exhibited by compounds **5**, **8** and **10** with ED_{50} values of 2.5 μg/mL in all cases.

EXPERIMENTAL SECTION

General: IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer and UV spectra were recorded on a Philips PU 8710 spectrophotometer. ^1H NMR and ^{13}C NMR were made on a Varian Unity 400 at 400 MHz and 100 MHz respectively, using $CDCl_3$ as solvent. The resonance of residual chloroform at δ_H 7.26 and δ_C 77.0 were used as internal reference for ^1H and ^{13}C spectra, respectively. Mass spectra were recorded on a VG 12250 or on Kratos MS 80RFA spectrometer. In High Performance Liquid Chromatography separations LiChrospher Si 60 and LiChrosorb RP-18 were used in normal and reversed phase modes, respectively, using a differential refractometer. Assignments with identical superscripts may be interchanged. All solvents were spectral grade or were distilled from glass prior to use.

Collection, Extraction and Purification: A total of 3637 specimens of *Siphonaria pectinata* were collected by hand in the intertidal zone of La Caleta (Cádiz, Spain) in June 1995 and inmediately frozen. The animals were extracted exhaustively with acetone at room temperature. The filtered Me_2CO solution was evaporated under reduced pressure and the aqueous residue was extracted with Et_2O. The solvent was evaporated to give an oil residue (21.4 g) which was chromatographed on a SiO_2 column eluting with solvents of increasing polarity from hexane to diethyl-ether and, subsequently, chlorofom-methanol (8:2). Fractions eluted with 5-10% ether in hexane were recromatographed on a SiO_2 column using solvents of increasing polarity from hexane to 10% ether in hexane. Selected fractions were purified by normal phase HPLC to yield siphonarienone (**6**, $4.3 \cdot 10^{-3}$ mg/animal), norsiphonarienone (**2**, $3 \cdot 10^{-4}$ mg/animal) and a mixture of the C-4 epimers of siphonarienedione (**7**, $9.3 \cdot 10^{-2}$ mg/animal). Fractions of the general cromatography eluted with 20% ether in hexane were subjected to reversed phase HPLC separation eluting with THF/acetonitrile (75:25), to obtain isosiphonarienolone (**3**, $5 \cdot 10^{-4}$ mg/animal) whose final purification was accomplished by HPLC on normal phase mode using as solvent hexane/EtOAc (95:5). Fractions of the general cromatography eluted with 30% ether in hexane were further separated by normal phase HPLC eluting with 10% EtOAc in hexane to yield 2-deoxysiphonarienfuranone (**4**, $2 \cdot 10^{-3}$ mg/animal) and siphonarienolone (**8**, $1.5 \cdot 10^{-2}$ mg/animal). From the medium polar fractions of the general cromatography sterols were precipitated with acetonitrile and the supernatant was subjected to SiO_2 column cromatography. Selected fractions were purified by normal phase HPLC (hexane/EtOAc, 8:2) yielding two independent mixtures of the C-2 epimers of siphonarienfuranone (**9**, $3.4 \cdot 10^{-2}$ mg/animal) and its Z isomer (**10**, $8.2 \cdot 10^{-3}$ mg/animal). Polar fractions of the general cromatography were crystallized from CH_2Cl_2 yielding pectinatone (**1**, 1.23 mg/animal). The mother liquors were purified by HPLC on normal phase mode using 30% EtOAc in hexane to obtain isopectinatone (**5**, $2 \cdot 10^{-3}$ mg/animal).

Norsiphonarienone A (2): Colorless oil; $[\alpha]_D^{25}$ +12.0 (c = 0.10, $CHCl_3$); IR (film) 2923, 1658, 1628 cm^{-1}; UV ($CHCl_3$) λ_{max} 242 (ε 2064) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, 3H, *J*=6.6 Hz, H-15), 0.84 (d, 3H, *J*=6.4 Hz, H-16), 0.87 (t, 3H, *J*=7.1 Hz, H-12), 0.95 and 1.15 (m, 1H each, H-8),[a] 1.02 (d, 3H, *J*=6.6 Hz, H-14), 1.05 and 1.22 (m, 1H each, H-10)[a], 1.12 (m, 1H, H-6), 1.25 (m, 2H, H-11), 1.35 (m, 1H, H-6), 1.36 (m, 1H, H-9), 1.46 (m, 1H, H-7), 1.85 (d, 3H, *J*=1.3 Hz, H-13), 2.37 (s, 3H, H-1), 2.77 (m, 1H, H-5), 6.35 (dq, 1H, *J*=10.0, 1.3 Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.5 (q, C-13), 14.4 (q, C-12), 19.9 (q, C-16) 19.9 (t, C-11), 20.2 (q, C-14), 20.5 (q, C-15), 27.0 (q, C-1), 28.3 (d, C-9), 29.6 (d, C-7), 31.9 (d, C-5), 39.3 (t, C-10), 44.1 (t, C-8), 45.5 (t, C-6), 131.3 (s, C-3), 157.9 (d, C-4), 200.0 (s, C-2). EIMS (70 eV) *m/z* (rel. int.) 239 (M^++1, 0.4), 223 (M^+-CH_3, 46), 139 (8), 125 (11), 111 (54), 97 (37), 83 (36), 69 (100); HREIMS Obsd. *m/z* = 223.2070 (M^+-CH_3), $C_{15}H_{27}O$ requires *m/z* = 223.2061.

Isosiphonarienolone (3): Colorless oil; $[\alpha]_D^{25}$ +17.6 (c = 0.17, $CHCl_3$); IR (film) 3400, 2922, 1660, 1648, 1027; UV ($CHCl_3$) λ_{max} 242 (ε 4615) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.80 (d, 3H, *J*=6.6 Hz, H-19)[a], 0.83 (d, 3H, *J*=6.6 Hz, H-20)[a], 0.87 (t, 3H, *J*=7.2 Hz, H-15), 0.94 and 1.15 (m, 1H each, H-11),[b] 0.95 (t, 3H, *J*=7.4 Hz, H-1), 1.00 and 1.23 (m, 1H each, H-13),[b] 1.02 (d, 3H, *J*=6.8 Hz, H-18), 1.15 (m, 1H, H-9), 1.18 (d, 3H, *J*=7.1 Hz, H-16), 1.25 (m, 2H, H-14), 1.36 (m, 2H, H-9 and H-12), 1.45 (m, 2H, H-2), 1.48 (m, 1H, H-10), 1.80 (d, 3H, *J*=1.3 Hz, H-17), 2.72 (m, 1H, H-8), 3.10 (d, 1H, *J*=7.7 Hz, -OH), 3.32 (qd, 1H, *J*=7.1, 5.1 Hz, H-4), 3.59 (m, 1H, H-3), 6.37 (dq, 1H, *J*=9.8, 1.3 Hz, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.3 (q, C-17)[c], 11.4 (q, C-1)[c], 14.4 (q, C-15), 16.4 (q, C-16), 20.0 (t, C-14), 20.1 (q, C-19)[d], 20.4 (q, C-18)[d], 20.6 (q, C-20)[d], 28.4 (t, C-2), 28.4 (d, C-12), 29.7 (d, C-10), 31.5 (d, C-8), 39.3 (t, C-13), 43.0 (d, C-4), 44.6 (t, C-11), 45.6 (t, C-9), 76.0 (d, C-3), 135.7 (s, C-6), 149.9 (d, C-7), 208.8 (s, C-5); EIMS (70 eV) *m/z* (rel. int.) 310 (M^+, 9), 252 (M^+-C_3H_7O, 5), 241 (33), 223 (M^+-$C_5H_{11}O$, 40), 125 (53), 111 (100), 97 (65), 83 (52); HREIMS Obsd. *m/z* = 310.2887, $C_{20}H_{38}O_2$ requires *m/z* = 310.2872.

2-Deoxisiphonarienfuranone (4): Colorless oil; $[\alpha]_D^{25}$ +40.0 (c = 0.10, $CHCl_3$); IR (film) 2921, 1701, 1656, 1105 cm^{-1}; UV ($CHCl_3$) λ_{max} 300 (ε 3252), 235 (ε 2344) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, 3H, J=6.6 Hz, H-19), 0.86 (d, 3H, *J*=6.4 Hz, H-20), 0.87 (t, 3H, *J*=7.1 Hz, H-15), 0.96 and 1.18 (m, 1H each, H-11),[a] 1.02 (d, 3H, *J*=6.6 Hz, H-18), 1.04 and 1.25 (m, 1H each, H-13),[a] 1.12 (m, 1H, H-9), 1.30 (m, 2H, H-14), 1.36 (m, 1H, H-9), 1.43 (d, 3H, *J*=7.1 Hz, H-1), 1.45 (m, 1H, H-12), 1.48 (m, 1H, H-10), 1.82 (s, 3H, H-16), 1.96 (d, 3H, *J*=1.3 Hz, H-17), 2.72 (m, 1H, H-8), 4.43 (q, 1H, *J*=7.1 Hz, H-2), 6.00 (dq, 1H, *J*=10.0, 1.3 Hz, H-7); ^{13}C NMR (100 MHz, $CDCl_3$) δ 7.5 (q, C-16), 13.4 (q, C-17), 14.4 (q, C-15), 16.6 (q, C-1), 19.9 (q, C-19), 20.1 (t, C-14), 20.4 (q, C-20), 21.0 (q, C-18), 28.3 (d, C-12), 29.7 (d, C-10), 30.7 (d, C-8), 39.3 (t, C-13), 44.7 (t, C-11), 45.6 (t, C-9), 79.7 (d, C-2), 108.4 (s, C-4), 125.8 (s, C-6), 146.0 (d, C-7), 182.9 (s, C-5), 206.5 (s, C-3); EIMS (70 eV) *m/z* (rel. int.) 306 (M^+, 3), 223 (5), 179 (M^+-C_9H_{19}, 4), 149 (27), 123 (16), 69 (56), 43 (100).

Isopectinatone (5): Colorless oil; $[\alpha]_D^{25}$ + 35.0 (*c* = 0.10, $CHCl_3$); IR (film) 3200, 2925, 1656, 1577, 1545, 1231, 1126; UV ($CHCl_3$) λ_{max} 294 (ε 6596), 238 (ε 2879) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (d, 3H, *J*=6.6 Hz, H-22), 0.79 (d, 3H, *J*=6.6 Hz, H-21), 0.86 and 1.06 (m, 1H each, H-12),[a] 0.87 (t, 3H, *J*=7.3 Hz, H-16), 0.88 (m, 1H, H-10), 0.95 (d, 3H, *J*=6.6 Hz, H-20), 1.00 and 1.20 (m, 1H each, H-14),[a] 1.22 (m, 3H, H-10 and H-15), 1.42 (m, 1H, H-13), 1.43 (m, 1H, H-11), 1.90 (d, 3H, *J*=1.5 Hz, H-19), 1.92 (s, 3H, H-17), 2.00 (s, 3H, H-18), 2.16 (m, 1H, H-9), 5.33 (dq, 1H, *J*=10.4, 1.5 Hz, H-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.4 (q, C-17)[b], 10.7 (q, C-18)[b], 14.4 (q, C-16), 20.0 (t, C-15), 20.0 (q, C-21)[c], 20.4 (q, C-22)[c], 21.2 (q, C-20), 21.9 (c, C-19), 27.7 (d, C-13), 29.7 (d, C-11), 32.0 (d, C-9), 39.4 (t, C-14), 44.8 (t, C-12), 45.5 (t, C-10), 98.8 (s, C-3)[d], 106.4 (s, C-5)[d], 126.4 (s, C-7), 140.8 (d, C-8), 156.9 (s, C-6), 163.6 (s, C-4), 165.4 (s, C-2); EIMS (70 eV) *m/z* (rel. int.) 334 (M^+, 5), 278 (4), 263 (M^+-C_5H_{11}, 2), 221 (M^+-C_8H_{17}, 7), 207 (M^+-C_9H_{19}, 5), 181 (60), 168 (100), 152 (14), 123 (17); HREIMS Obsd. *m/z* = 334.2507 (M^+), $C_{21}H_{34}O_3$ requires *m/z* = 334.2508.

Acknowledgements. This research was supported by grants from C.I.C.Y.T. (research project SAF94-1383) and Junta de Andalucia (PAI 1081). Cytotoxicity assays were performed through a Cooperative Agreement with Instituto BioMar S.A.

REFERENCES

1. Faulkner, D.J. *Nat. Prod. Rep.* **1996**, *13*, 75-125 and previous reviews of this series.
2. Manker, D.C.; Garson, M.J.; Faulkner D.J. *J. Chem. Soc., Chem. Commun.* **1988**, 1061-1062.
3. Garson, M.J.; Jones, D.D.; Small, C.J.; Liang, J.; Clardy, J. *Tetrahedron Lett.* **1994**, *35*, 6921-6924.
4. Garson, M.J.; Goodman, J.M.; Paterson, I. *Tetrahedron Lett.* **1994**, *35*, 6929-6932.
5. Ireland, C.; Faulkner, D.J.; Solheim, B.A.; Clardy, J. *J. Am. Chem. Soc.* **1978**, *100*, 1002-1003.
6. Ireland, C.; Faulkner, D.J.; Finer, J.S.; Clardy, J. *J. Am. Chem. Soc.* **1979**, *101*, 1275-1276.
7. Biskupiak, J.E.; Ireland, C.M. *Tetrahedron Lett.* **1983**, *24*, 3055-3058.
8. Norte, M.; Cataldo, F.; González, A.G.; Rodríguez, M.L.; Ruiz-Pérez, C. *Tetrahedron* **1990**, *46*, 1669-1678.
9. Norte, M.; Cataldo, F.; González, A.G. *Tetrahedron Lett.* **1988**, *29*, 2879-2880.
10. For an example of a mixture of epimers as result of enolization of a β-diketone system see: Szabo, C.M.; Nakao, Y.; Yoshida, W.Y.; Scheuer, P.J. *Tetrahedron*, **1996**, *52*, 9681-9686.
11. Garson, M.J.; Small, C.J.; Skeleton, B.W.; Thinapong, P.; White, A.H. *J.Chem. Soc., Perkin Trans. 1* **1990**, 805-807.
12. Norte, M.; Fernández, J.J.; Padilla, A. *Tetrahedron Lett.* **1994**, *35*, 3413-3416.
13. The small amount available of norsiphonarienone A (**2**) and the high unstableness of 2-deoxysiphonarienfuranone (**4**) prevented cytotoxicity assays from being carried out.

(*Received in UK* 18 *November* 1996; *accepted* 5 *December* 1996)