Tetrahedron. Vol. 31, pp. 2513 to 2515. Pergamon Press 1975. Printed in Great Britain

PUKALIDE, A FURANOCEMBRANOLIDE FROM THE SOFT CORAL *SINULARIA ABRUPTA*

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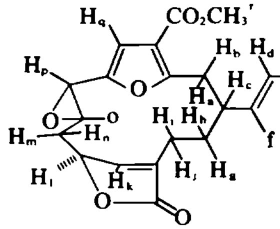
(*Received in USA 25 February 1975; Received in UK for publication 23 April 1975*)

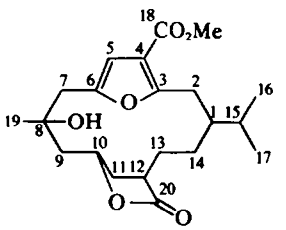
Abstract—We have assigned structure **1a** to pukalide, which is a constituent of an alcyonarian, on the basis of spectral data. Pukalide is diterpenoid with a cembrane skeleton, bearing butenolide, epoxide, isopropenyl, and an unprecedented α,α-disubstituted furan-β-carboxylate function.

The serendipitous discovery of prostaglandins in extracts of a gorgonian (*Plexaura homomalla*) by Weinheimer and Spraggins[1] conferred instant recognition in the chemical community to the relatively obscure marine invertebrate subclass Alcyonaria (or Octocorallia), to which the gorgonians belong. That event sparked a number of investigations into constituents of the Gorgonacea (horny corals) and other Alcyonaria. Remarkably, isolation of the C_{20} fatty acid-derived prostaglandins from *P. homomalla* has remained unique, while C_{20} diterpenoids belonging to the 14-membered cembrane ring system are emerging as characteristic constituents of the octocorals.[2-7]

Chance collection of a locally abundant octocoral *Sinularia abrupta* Tixier-Durivault 1970§ off a pocket beach near the Blowhole on the SE coast of Oahu and ethanol extraction of the animals, followed in turn by partition between ether and water and chromatography; resulted in the isolation of a new highly functionalized cembranolide, which we have named pukalide,‖ and for which we have deduced structure **1a**. Recrystallization from methanol of chromatographic fractions, or occasionally of the solid ether residue, afforded colorless crystalline pukalide, m.p. 204-206°, $[\alpha]_D^{CHCl_3}$ 44°, $C_{21}H_{24}O_6$, in yields of 0·03–0·05%.

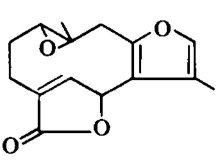


1a



1b

The functional groups and olefinic sites of this highly oxygenated diterpene became apparent from the spectral characteristics of pukalide (**1a**) and of hexahydropukalide (**1b**), m.p. 212·5–213·5°, $[\alpha]_D^{CHCl_3}+43°$. An α,β-unsaturated 5-membered lactone, which is a recurring characteristic of marine cembranolides, was readily deduced from IR absorption at $1760\,cm^{-1}$, shifted to $1770\,cm^{-1}$ in the hexahydroderivative. The sesquiterpene neolinderane (**2**) with corresponding IR bands at 1750, shifted to $1760\,cm^{-1}$ in the dihydroderivative[8] and the related linderalactone (**3**) with IR absorption at 1762 and $1769\,cm^{-1}$,[9] respectively, proved to be satisfactory analogs with their remarkably parallel functionalities. Corroborative evidence for the lactone moiety was derived from a broadened singlet PMR signal at δ 7·06, which was assigned to the β-proton of the α,β-unsaturated lactone (H_k), in excellent analogy with corresponding protons (H_x) in the gorgonian constituent ancepsenolide (**4**), which exhibit a chemical shift of δ 7·05.[10]



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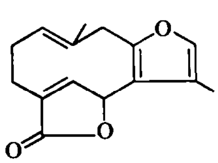

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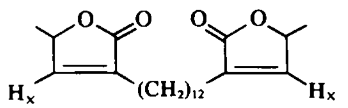

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Another relatively familiar feature is the epoxide. Our assignment was based on a series of IR bands (3020, 1270, 905, $830\,cm^{-1}$) and on NMR data. A 3-proton singlet at δ 1·0 (H_o), identical with the corresponding Me chemical shift in **2**,[8] was assigned to the Me group at C-8. A one-proton singlet at δ 4·04 (H_p) suggested the allylic nature of this proton by comparison with corresponding protons in styrene oxide (δ 3·8) and propylene oxide (δ 2·9). In agreement with this assignment are CMR frequencies for C-7 (55·0, d) and C-8 (57·0, s). In hexahydropukalide (**1b**), obtained from **1a** by hydrogenation over Pd/C in isopropyl alcohol, the epoxide has been hydrogenolized; as a consequence, the C-8 Me resonance

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[§] Family Alcyoniidae, order Alcyonacea (soft corals), subclass Alcyonaria, class Anthozoa, phylum Coelenterata; identified by J. Verseveldt through the courtesy of D. M. Devaney, B. P. Bishop Museum, Honolulu.

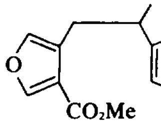
[‖] *Puka* is Hawaiian for hole; the geographical landmark near the collection point is the Blowhole, which is a hole in the lava rock that gives rise to intermittent surf spouts.

has shifted to δ 1·3 and **1b** has a new broad IR band at 3500 cm^{-1}.

The third unexceptional structural part of pukalide is the isopropenyl. Our evidence rests on an IR absorption at 890 cm^{-1} and on PMR signals at δ 1·75 (3 H, s) for the allylic Me (H_f) and broad singlets at δ 4·91 and 5·20 for the terminal methylene (H_d, H_e). In **1b** these PMR signals become overlapping doublets (6 H, J = 7 Hz) at δ 0·9. CMR frequencies (singlet at 145·8 and triplet at 112·9 ppm are in agreement with the methylene carbons of an isopropylidene.

The isopropenyl function received additional confirmation by irradiation of the allylic Me at δ 1·75 (H_f), which collapses the terminal methylene signals to doublets, J = 2 Hz.

The remaining structural feature of pukalide (**1a**), an α,α'-dialkyl methyl furan-β-carboxylate, is so far without precedent among the marine cembranolides.[2-6] Infrared bands at 3140, 1580 and 870 cm^{-1} were indicative of the furan and comparable with corresponding bands at 3120, 1605 and 1540 cm^{-1} in neolinderane (**2**) and at 1595, 1540 and 885 cm^{-1} in the fungal sesquiterpenoid **5**.[11]



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A lone PMR singlet at δ 6·33 (H_q) is compatible with a β-proton in a furan system. This assignment is strengthened by a CMR doublet at 106·4 ppm. The remaining CMR frequencies assigned to the furan (113·9, 148·2 and 160·0 ppm) are singlets in the off-resonance spectrum. The high field frequency of the doublet at 106·4 ppm shows that the lone proton must be on a β-carbon.[12] Finally, an IR band at 1715 cm^{-1}, a 3-proton singlet at δ 3·75 (H_r) and a CMR singlet at 163·8 ppm (the corresponding carbon of methyl acrylate is observed at 164·5 ppm[12]) are consistent with a conjugated methyl carboxylate. UV absorption of **1a** at 248 nm (ε 5200) seemed reasonable when compared with that of **5** (243 nm, ε 2300; no α-substituents) although we could find no close analogs for an α,α'-dialkylfuran-β-carboxylic ester. In hexahydropukalide (**1b**) the furan system remains intact as evidenced by unchanged IR and NMR signals. Surprisingly, though, the UV maximum shifts bathochromically to 255 nm (ε 3500). A plausible explanation for this change may be that hydrogenolysis of the epoxide may have relaxed the geometry of the absorbing system and thereby eased the electronic transition.†

All functional groups of pukalide were thus securely established. In **1a**, 3 rings, 4 olefinic linkages, and two carbonyl oxygens accounted for all but one unsaturation. All evidence, including comparison with hexahydropukalide (**1b**), required the presence of one more ring. While **1a** represented a reasonable structure for a marine cembranolide on biogenetic grounds, we had little direct evidence for the arrangement within the cembrane system of 4 methylene groups [triplets at 40·0, 32·5(2) and 22·8 ppm] and one methine (doublet at 40·7 ppm). The disposition of the functional groups in the framework of the 14-membered ring was eventually secured by a series of double irradiation and NOE experiments.

In the PMR spectrum (100 MHz) of pukalide (**1a**) the signals for the terminal methylene (H_d, H_e) and the lactone methine (H_l) protons accidentally overlap. The broad singlet at δ 4·91 seemed reasonable for H_l, and the broad singlet at 5·20 appeared compatible with H_d and H_e, although we were puzzled by the lack of multiplicity in the δ 4·91 signal. However, when we measured the PMR spectrum of pukalide (**1a**) in deuteriobenzene, it became clear that the lactone methine (H_l) did not give rise to the resonance at δ 4·91 ($CDCl_3$) as originally assigned, but was hidden under the δ 5·20 signal, and the δ 4·91 resonance was one of the signals of the terminal methylene (H_d, H_e). In deuteriobenzene, the lactone methine (H_l) was shifted up-field to δ 4·3. Irradiation of this proton caused the signal at δ 7·06 (H_k) to sharpen, thereby confirming the butenolide in **1a** unambiguously. By comparison, the resonance of the lactone methine in **4** is observed at δ 5·07, while in **2**, where the corresponding methine is doubly allylic, the signal is seen at δ 5·75.

For interpretation of the high-field (δ 2·0–3·7) region we used a 220 MHz spectrum. A one-proton multiplet at δ 3·55 (H_c), which appears to be a triplet of doublets (J = 3·5, 11 Hz), is coupled to a pair of doublets at δ 3·0 (H_f, J = 11, 18 Hz) and to another pair of doublets at δ 2·9 (H_a, H_b, J = 3·5, 18 Hz). These low field chemical shifts indicate that methine H_c and the methylene (H_a, H_b) must be allylic and that a single methylene group is interspersed between the furan and the methine carbon (C-1) bearing the isopropenyl.

The 220 MHz PMR spectrum also shows two one-proton doublets of doublets at δ 2·5 and 2·2 (J = 3·5, 15 Hz). Decoupling at 100 MHz proved that geminal coupling between H_m and H_n gives rise to the 15 Hz splitting, while the 3·5 Hz coupling is due to interaction with lactone methine H_l. Again, only a single methylene group should be placed between the epoxide and lactone functions.

Two unassigned methylene groups remain. They can only be placed between C-1 and the α-carbon of the butenolide (C-12). The methylene adjacent to C-12 (H_i, H_j) can be demonstrated by irradiating the multiplet at δ 2·35, which results in sharpening of the signal at δ 7·06 (H_k) to a doublet, J = 2 Hz.

In an attempt to clarify the stereochemistry of pukalide (**1a**) we irradiated the epoxide methyl (H_o), but observed no NOE enhancement on H_p, thus showing a *trans* relationship. Conversely, H_k, the β-proton of the butenolide system experienced an enhancement of 11%. Dreiding models confirm the proximity of these groups in a *trans* epoxide.

In a recent publication Uchida and Kuriyama[13] showed that the stereochemistry of α,β-unsaturated-γ-lactones may be deduced from the sign of the CD curve at the π→π* transition. The CD curve of pukalide (**1a**) displayed a weak negative Cotton effect at the n→π* transition [$(\Phi)_{265}$ − 62·5, (θ) − 232, Δε = − 0·071] and a stronger positive effect at the π→π* transition [$(\Phi)_{225}$ + 1020, (θ) + 3800, Δε = + 1·15]. These results are consistent with α-configuration of H_l, which happens to be identical with the configuration of the close analog neolinderane (**2**).

Mass spectra of **1a** and **1b** (Experimental) are fully consistent with the assigned structures.

EXPERIMENTAL

Ir spectra were recorded on a Beckman IR-10 instrument in CH_2Cl_2. PMR and NOE experiments were performed on a Varian

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†One referee suggested that elimination of the αβ-unsaturated lactone band at ~ 215 nm may cause the observed shift.

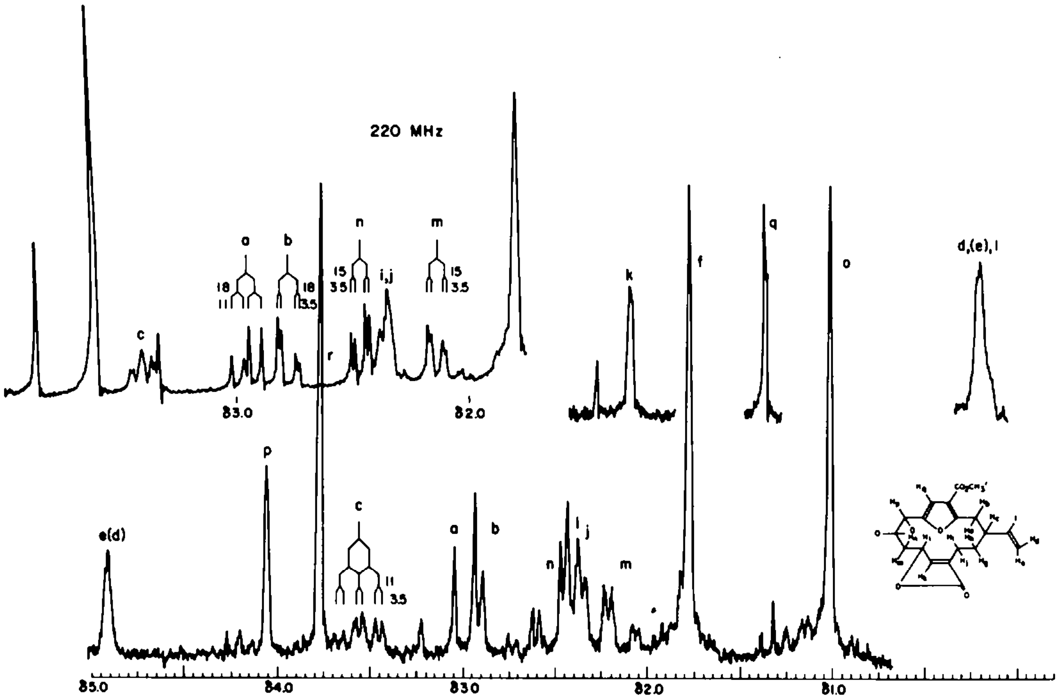

Fig. 1. PMR spectra of pukalide (**1a**). The main trace is recorded at 100, the upper trace at 220 MHz. Chemical shifts for isolated signals (100 MHz): d(e), 1, δ 5·20; q, 6·33; k, 7·06.

HA-100 spectrometer in $CDCl_3$ except as noted, using TMS as internal reference with $\delta = 0$, s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. CMR spectra were determined on a Varian XL-100 spectrometer in $CDCl_3$ with TMS as internal reference in ppm, the off-resonance multiplicities are shown in parentheses. UV spectra were recorded on a Cary 14 spectrophotometer in 95% EtOH and the CD spectra on a Cary 61 spectropolarimeter in MeOH. Optical rotations were measured in $CHCl_3$ on a Bendix Ericsson ETL-NPL automatic polarimeter type 143A. High resolution mass spectra were recorded on a JEOL JMS-01SG-2 instrument and low resolution on a Hitachi-Perkin Elmer RMU-6D. Elemental analyses were performed by the University of California Analytical Services, Berkeley, Calif. Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Bio-Sil A 200–325 mesh (Bio-Rad Laboratories) was used for column chromatography and 0·25 mm precoated silica gel plates (60-F-254), EM Laboratories) were used for TLC.

Isolation of pukalide (**1a**). Freshly collected animals (6·9 kg dry wt after extraction) were soaked 3x in 95% EtOH for 3 d each. The solvent was evaporated under reduced pressure and the aq residue extracted with ether. The dried ($MgSO_4$) ether layer was taken to dryness to give an oily, or occasionally a solid, residue, 59 g. Ten g of this oil was chromatographed on a silica gel column (500 g) eluting with methylene chloride; 300 ml fractions were collected. Fractions 4 and 5 yielded a solid on evaporation, which after two recrystallizations from MeOH gave pure pukalide (600 mg), which gave a single spot on TLC (silica gel, 5% MeOH in CH_2Cl_2, visualization by sort wavelength UV light) at R_f 0·8; m.p. 204–206°, $[\alpha]_D + 44$ (c 1·1, $CHCl_3$). (Found: C, 67·50; H, 6·58; Calcd for $C_{21}H_{24}O_6$: C, 67·73; H, 6·50). UV: λ_{max}^{EtOH} 248 (5200). IR: ν_{max} 3140, 3020, 1760, 1715, 1580, 1270, 1230, 1080, 905, 890, 870, 830 cm^{-1}; NMR: for PMR see Fig. 1. CMR (ppm): 173·7 (s) C-20, 163·8 (s) C-18, 160·0 (s) C-3, 148·2 (s) C-6, 148·2 (d) C-11, 145·8 (s) C-15, 137·3 (s) C-12, 113·9 (s) C-4, 112·9 (t) C-16, 106·4 (d) C-5, 77·8 (d) C-10, 57·0 (s) C-8, 55·0 (d) C-7, 51·2 (q) C-21, 40·7 (d) C-1, 40·0 (t), 32·5 (2 carbons (t), 22·8 (t), 19·8 (q), 18·7 (q).

Ms: *m/e* 372·1564 (M^+, 34%), 340 (8%–CH_3OH), 315 (13), 276 (15) 208·0737 ($C_{11}H_{12}O_4$, 100), 204 (54), 168 (88), 165 (87).

Hexahydropukalide (**1b**). Pukalide (100 mg) was hydrogenated in isopropyl alcohol (100 ml) with 10% Pd/C (20 mg) at ambient pressure for 4 hr when 3·2 eq H_2 had been taken up. The suspension was filtered, the solvent removed, and the residue recrystallized from MeOH to give **1b** (60 mg) which gave one spot on TLC (silica gel, 5% MeOH in CH_2Cl_2, UV) at R_f 0·5; m.p. 212·5–213·5°. UV: λ_{max}^{EtOH} 255 (3500). IR: ν_{max} 3500, 1770, 1715, 1615, 1570, 1230, 1080 cm^{-1}. PMR: δ 0·93 d and 9·96 d J = 7 Hz (isopropyl), 1·36 s (CH_3–C–OH), 1·88 d J = 7 Hz (H_9), 2·75 d and 3·05 d J = 15 Hz (H_7), 3·79 s (carbomethoxy), 4·72 bm (lactone methine), 6·50 s (furan proton). CMR (PPM): 179·2 (s) C-21, 164·0 (s) C-18, 161·2 (s) C-3, 149·5 (s) C-6, 114·3 (s) C-4, 109·0 (d) C-5, 76·3 (d) C-10, 70·8 (s) C-8, 51·3 (q) C-19, 45·4, 43·6, 39·9, 32·4, 31·4, 30·4, 30·2, 30·0, 28·8, 28·7, 19·6 (q), 18·8 (q); *m/e* (90%), 278 (15%, M^+), 346 (89%–MeOH), 328 (19%–H_2O from 346), 303 (100%–isopropyl from 346).

Acknowledgements—We are grateful to Dr. William Fenical for the 220 MHz PMR spectrum of pukalide and to Dr. Peter Roller for the high resolution mass spectral data.

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