

AN ANTIMICROBIAL SESTERTERPENE FROM A PALAUAN SPONGE

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Abstract An unidentified sponge from Palau contained the antimicrobial sesterterpene 1 that was identified by interpretation of spectral data and conversion into a degradation product of illimaquinone.

As part of a continuing search for new classes of antimicrobial agents from marine organisms, we have screened the crude methanolic extract of over 200 sponges from Palau, Western Caroline Islands, against eight microorganisms. Approximately half of the crude extracts inhibited the growth of one or more microorganisms. One of the extracts that showed activity against *Bacillus subtilis* and *Staphylococcus aureus* had resulted from a mixture of at least three sponges that had been inadvertently combined.¹ One of the major secondary metabolites from the mixture of sponges has been identified as the sesterterpene 1, that we have called palauolide.

The ethyl-acetate soluble material from a methanolic extract of the sponge sample (80-029) was chromatographed on Sephadex LH-20 using methanol as eluant then on silica gel using 60:40 ethyl acetate/hexane as eluant to obtain palauolide (1) as a yellow oil (3.7% of extract). Palauolide (1), $[\alpha]_D^{25} 1.5^\circ$ ($c = 0.2$, CHCl_3) had the molecular formula $\text{C}_{25}\text{H}_{36}\text{O}_3$. Infrared bands at 3500, 1740 and 1620 cm^{-1} suggested the presence of hydroxyl, ester or lactone carbonyl and olefinic groups. Treatment of palauolide (1) with acetic anhydride in pyridine gave a monoacetate 2 with infrared bands at 1780, 1750 and 1620 cm^{-1} that were assigned to an unsaturated γ -acetoxylactone group. The ultraviolet spectrum of 1 contained an absorption at 322 nm ($\epsilon 17,000$) indicating that the γ -hydroxybutenolide was further conjugated to two olefinic groups.² The ^{13}C NMR spectrum contained a lactone carbonyl signal at $\delta 172.2$ (s), a hemiacetal carbon signal at 99.85 (d) and olefinic signals at 162.4 (s), 160.2 (s), 150.2 (s), 137.3 (d), 124.3 (d), 118.8 (d), 114.0 (d).

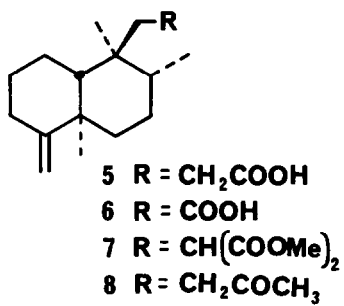
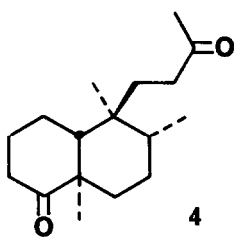
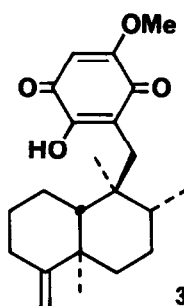
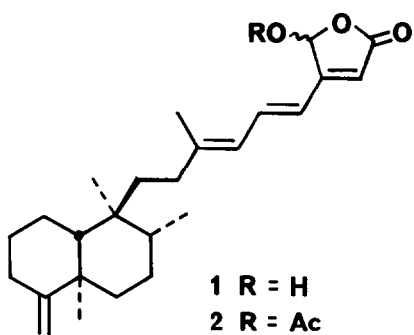
and 102.4 (t) due to the conjugated triene and an isolated exocyclic methylene group. The ^1H NMR spectrum contained signals at δ 5.95 (d, 1 H, $J = 11$ Hz), 7.16 (dd, 1 H, $J = 15.5, 11$ Hz) and 6.28 (d, 1 H, $J = 15.5$ Hz) due to protons on conjugated trisubstituted and *E*-disubstituted olefinic bonds, at 5.83 (s, 1 H) for the olefinic proton in the butenolide ring, at 6.26 (s, 1 H) due to the hemiacetal proton and at 1.86 (s, 3 H) assigned to the methyl group on the conjugated trisubstituted olefin. The *E*-stereochemistry about the trisubstituted olefinic bond was inferred from the ^{13}C NMR signal at δ 15.8 (q) due to the methyl group on the trisubstituted olefin.

Comparison of the remaining signals³ in the ^{13}C and ^1H NMR spectra of palauolide (1) with those of illimaquinone (3) indicated that both molecules had the same bicyclic ring system. Ozonolysis of palauolide acetate (2) in ethyl acetate solution at -78°C , followed by reduction of the ozonolysis product with dimethyl sulfide, gave a diketone 4 that was then synthesized from illimaquinone (3).⁴ We were therefore able to establish a structure of palauolide (1) for which the relative stereochemistry was known for all chiral centers except the hemiacetal carbon.

Oxidation of illimaquinone (3) with basic hydrogen peroxide followed by acidification gave the acid 5 as the major product. The acid 5 is the homologue of the product 6 reported by Luibrand *et al* for the same reaction.⁵ By varying the conditions for this reaction, we were able to isolate, after methylation with diazomethane, mixtures of the methyl esters of acids 5 and 6 together with the dimethyl malonate 7. The major oxidative degradation pathway for illimaquinone must proceed through decarboxylation of a malonic acid to obtain the homologue 5 of the acid 6 expected in this reaction. Treatment of the acid 5 with excess methyl lithium in ether gave the methyl ketone 8 that was ozonized to obtain a sample of the diketone 4, identical in all respects to the ozonolysis product of palauolide (1).

The carbon skeleton of palauolide (1) has not been encountered previously although the rearranged drimane skeleton that comprises the bicyclic portion of the molecule is relatively common among sponge metabolites. Palauolide (1) inhibited the growth of *B. subtilis* and *S. aureus* at 10 $\mu\text{g}/\text{disc}$.

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References and Notes

1. This sample was collected by Dr. W. Fenical during an expedition of R/V Alpha Helix. The sponges were inadvertently combined by the ship's crew when being transferred from one freezer to another.
2. Calculated value = 323 nm, using γ -hydroxybutenolide (227 nm) as the parent base.
3. ^1H NMR (CDCl_3) δ 0.74 (s, 3 H), 0.82 (d, 3 H, $J = 7$ Hz), 1.05 (s, 3 H), 1.86 (s, 3 H), 4.51 (s, 2 H), 5.83 (s, 1 H), 5.95 (d, 1 H, $J = 11$ Hz), 6.26 (s, 1 H), 6.28 (d, 1 H, $J = 15.5$ Hz), 7.16 (dd, 1 H, $J = 15.5, 11$ Hz); ^{13}C NMR (CDCl_3) δ 172.2 (s), 162.4 (s), 160.2 (s), 150.2 (s), 137.3 (d), 124.3 (d), 118.8 (d), 114.0 (d), 102.4 (t), 99.9 (d), 48.5 (d), 39.8 (s), 39.2 (s), 37.1 (d), 36.5 (t), 36.2 (t), 33.6 (t), 32.9 (t), 28.5 (t), 27.3 (t), 21.5 (t), 20.6 (q), 17.9 (q), 17.6 (q), 15.8 (q).
4. R. T. Luijbrand, T. R. Erdman, J. J. Vollmer, P. J. Scheuer, J. Finer and J. Clardy, Tetrahedron **35**, 609 (1979).
5. This is confusing since the spectral data reported matches that of the methyl ester of acid 5 while the structure drawn in the paper implies that acid 6 had been formed. We could isolate both compounds.

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