Tetrahedron Letters,Vol.25,No.9,pp 931-934,1984
Printed in Great Britain

0040-4039/84 $3.00 + .00

BIOACTIVE MARINE SPONGE NORDITERPENE AND NORSESTERTERPENE PEROXIDES

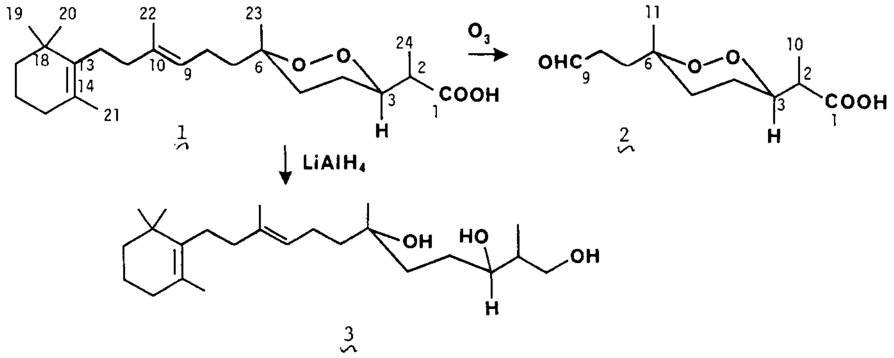
Lawrence V. Manes+, Gerrald J. Bakus# and Phillip Crews*+

+ Thimann Laboratories and Center for Marine Studies, University of California,
Santa Cruz, California 95064, and # Allan Hancock Foundation, University of
Southern California, Los Angeles, Ca. 90007

SUMMARY: Peroxyterpenoids have been isolated which are: methyl nuapapuanoate (4), a norditerpene; and muqubilin (1), a norsesterterpene.

In 1980 and 1983 we collected a rather large soft drab sponge[1] from the Tongan coral reefs in connection with our study of sponges which appear to be unmolested by fish.[2] We set out to explore its chemistry because the crude CH_2Cl_2 extract caused 32% inhibition of sea urchin egg cell division at 16 μg/ml.

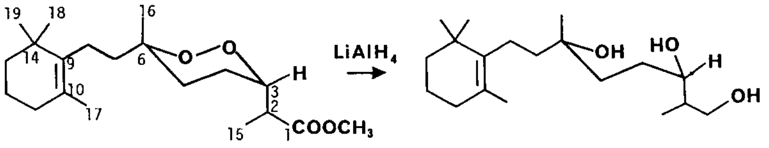
Two compounds, 1 and 4, were isolated from the methanol fraction obtained by partitioning the crude CH_2Cl_2 extract between hexanes/ methanol (anticell division activity; hexanes:35%, methanol:71%). Muqubilin 1 ($C_{24}H_{40}O_4$), $[\alpha_D] = +31.6^0$ (c=0.18M, $CHCl_3$) was a major polar component of the methanol fraction. Its gross structure, first reported by Kashman,[3] was based in part on the biogenetic isoprene rule. Sokoloff[4] later suggested stereochemical details for 1 without accompanying substantiation. Our analysis of spectroscopic data of both 1 and its transformation products 2 and



3 provides confirmation for the original gross structure, but requires revision of its C-6 stereochemistry, and its C-2 stereochemistry can not be defined.

The subunit of C-8 to C-22 in 1 was quickly recognized from both ^{13}C NMR peaks[5,6] and the mass spectrum base peak at m/z=137, while ^{1}H NMR double resonance experiments showed a $-CH_2CH(O)CH(CH_3)CO_2-$ (A) array. The remaining features could be summarized as a cyclic peroxide, δ81.0(d) & 80.2(s), flanked by geminal CH_3/CH_2R and geminal H/R ring substituents, but deciding between a 5, 6, or 7 membered ring required additional data. Firstly, the five atom ring possibility was ruled out by ^{1}H NMR double resonance: in 1 irradiation at δ4.15 (H-3) simplified the multiplet at H-4 but not to a clean AB pattern. Secondly, the seven atom ring was also ruled out by NMR double resonance: in 2[7] irradiation at δ9.81 (H-9) transformed the pattern for H-8a into a dt, J=8.0,4.6,4.6 Hz. Moreover, comparison of 1 to triol 3[8] showed 3-7 PPM ^{13}C NMR shifts for C-2 to C-7, Me-23 & Me-24. Characteristic ^{1}H NMR J's in 1 at H-3 ($J_{3,4a+3,4e}$)=12Hz[9] and ^{13}C NMR shifts at C-22 =δ16.1 and C-23 =δ20.8[12] and similar data in 2 ($J_{3,4a+3,4e}$=13Hz, and C-11 =δ20.8) justified the stereochemistry shown in 1.

The ester 4 ($C_{19}H_{34}O_4$), $[\alpha_D]$=+53.7° (c=0.13M, $CHCl_3$) was a minor component of the methanol fraction. Comparison to diagnostic ^{13}C NMR resonances in 1[6] showed the tetrasubstituted cyclohexene ring (C-9 to C-14, C-17 to C-19),[13] while ^{1}H NMR double resonance established the sub-unit A for C-1 to C-3, C-15. The peroxide, δ81.1(d) 79.9(s) was flanked by the same ring substituents as in 1, and appearance of both H-8(δ2.05,A=1) and H-4,4' (δ1.6-1.7 with spin decoupling at H-3) as complex non-AB multiplets required a six atom peroxide ring. Also consistent with this was the comparison of the



4 5

^{13}C NMR of 4 to transformation product 5[14] showing 3-7 PPM shifts for C-2 to C-7 and Me-16. Finally, parallel arguments to those above supported stereochemical assignments, Me-16 as axial (δ23.6) and H-3 as equatorial (δ4.25, $J_{3,4a+3,4b}$ =8Hz)[9].

Compound 4[15] is, to the best of our knowledge, the first norditerpene observed from a marine sponge.[16] Norditerpenes are apparently also rare from other marine invertebrates, since only one other example can be found in the literature.[17] By contrast, marine sponge norsesterterpene peroxides (eg.1) are more common.[3,4,10] Compound 1 appears to be responsible for the bioactivity of the crude extract, because at 16 µg/mL it exhibits 100% inhibition of cleavage of fertilized sea urchin eggs.[18] The hexane fraction, which shows no 1 by ^{13}C NMR, is now under study for additional examples of compounds with anticell division activity.

REFERENCES

1. This sponge is an undescribed Prianos species, and its appearance matches that outlined in Ref. 3. A voucher sample or underwater photo is available from PC.

2. (a) Bakus, G. J.; Thun, M. Colloq. INT. C.N.R.S. **1979**, 291 417; (b) Myers, B. L.; Crews, P. J. Org. Chem. **1983**, 48, 3583.

3. Kashman, Y.; Rotem. M. Tetrahedron Lett. **1979**, 1707.

4. Sokoloff, S.; Halevy, S.; Usieli, V.; Colorni, A.; Sarel, S. Experientia **1982**, 38, 337.

5. See manoalide: DeSilva, E. D.; Scheuer, P. J. Tetrahedron Lett. **1980**, 21, 1611.

6. 1:^{13}C NMR, n.o.e. suppressed & integrated, ($CDCl_3$,25.1MHz):180.4(C-1); 137.1(C-13);136.5(C-10);126.9(C-14);123.3(C-9);81.0(C-3)80.2(C-6);43.0(C-2); 40.3(C-17);39.9((C-11);39.7(C-5);35.0(C-18);32.8(C-15);32.0(C-4);28.7(C-19, C-20);27.9(C-12);23.5(C-7);21.7(C-8);20.8(C-23);19.9(C-21);19.6(C-16);16.1 (C-22);13.2(C-24).

7. 2:^{1}H NMR ($CDCl_3$,300MHz):9.81(1H,t,J=3,H-9);4.15(1H,m,J<2,3>=8.0, J<3,4a+3,4e>=13.2,H-3);2.74(1H,m,H-2);2.55(1H,ddt,J=9.0,6.5,6.5,3.0,H-8a); 2.0(1H,m,H-8b);1.8(4H,m,H-5,7);1.70(2H,m,H-4),1.30(3H,s,Me-11);1.27(3H,d, J=6.8,Me-10). Carbon NMR:20.8(C-11). Additional C-13 data in agreement with Ref. 3.

8. 3:^{13}C NMR ($CDCl_3$,25.1MHz):137.1(C-13);136.0(C-10);126.8(C-14);123.8 (C-9);74.2(C-3);72.4(C-6);66.1(C-1);42.9(C-5);40.3(C-17);39.9(C-11);39.7 (C-2);38.6(C-4);35.0(C-18);32.8(C-15);28.7(C-19,C-20);28.4(C-7);27.9(C-12); 25.9(C-23);22.7(C-8);19.8(C-21);19.6(C-16);16.0(C-22);10.5(C-24). M.S. (20

eV): m/z=362(M+),347,329,303($-C_3H_7O$),245($-C_6H_{13}O_2$),137(base, $C_{10}H_{17}$).

9. Compare to other six membered ring peroxides[10,11]: sigmosceptrellin-A with Ja,a=7Hz and Ja,e=4Hz; or plakortin and epi-plakortin with Ja,a=9.5Hz, Ja,e=5.4Hz.

10. Albericci, M.; Braekman, J. C.; Daloze, D.; Tursch, B. Tetrahedron **1982**, 1881.

11. (a) Crews, P., unpublished results; (b) Stierle, D. B.; Faulkner, D. J. J. Org. Chem. **1980**, 45, 3396.

12. Me_{ax} in plakortin = δ21[11]; see also: Crews, P.; Kho-Wiseman, E. Tetrahedron Lett. **1978**, 38, 2483.

13. 4: ^{13}C NMR, n.O.e. suppressed & integrated ($CDCl_3$,25.1MHz);174.1(C-1); 136.8(C-9);127.0(C-10);81.1(C-3);79.9(C-6);42.7(C-2);40.0(C-13);34.9(A=2, C-5,C-14);32.8(A=2,C-4,C-11);28.6(A=2,C-18,C-19),23.6(C-16);22.6(C-7or8); 22.2(C-8or7);19.6(C-17);12.5(C-15). ^1H NMR ($CDCl_3$,300MHz)4.25(1H,m, J<2,3>=7,J<3,4ae>=8,H-3)3.7(3H,s,Me-20);2.58(1H,pent,H-2);2.05(1H,m,H-8); 1.9(3H,m.H-11,11',8');1.76(1H,m,H-5);1.5-1.7(7H,4,4',5',7,7',12,12');1.61 (Me17);1.4(2H,m,H-13,13');1.15(3H,s,Me16),1.13(3H,d,Me15);1.0(3H,s);0.99 (3H,s). IR:1734. M.S.(20eV):m/z=338(M+),137(base peak,$C_{10}H_{17}$).

14. 5: ^{13}C NMR ($CDCl_3$,25.1MHz):136.9(C-9);127.0(C-10);77.4(C-3);72.9(C-6); 67.7(C-1);42.2(C-5);40.0(C-13);39.9(C-2);37.0(C-4);35.2(C-14);32.9(C-11); 29.3(C-7);28.9(C-18,C-19);26.7(C-16);23.0(C-8);19.9(C-12);19.7(C-17);13.9 (C-15). M.S.(20eV):m/z=294(M+,$-H_2O$),137($C_{10}H_{17}$),136(base peak).

15. Because this sponge abounds in the reefs off Nuapapu Is., Tonga, we suggest the name nuapapuane for the 4 carbon framework with the numbering shown.

16. For recent examples of sponge diterpenes see: Cimino, G.; Morrone,R.; Sadono, G. Tetrahedron Lett. **1982**, 23, 4139.

17. Bowden, B. F.; Coll, J.,C.; Mitchell, S. J.; Mulder, J.; Stokie, G. J. Aust. J. Chem. **1978**, 31, 2049.

18. We thank Prof. R. Jacobs and his research group at UCSB for this data.

ACKNOWLEDGEMENT This work is partially a result of grant to PC from NOAA, National Sea Grant College Program, Department of Commerce, University of California project number R/MP-33. The U.S. Government is authorized to produce and distribute reprints for governmental purposes. LVM was a Sea Grant Trainee (1983-84). A grant to PC from the University Research Expedition Program supported our field work in Tonga. We thank Mr. Jim Loo for assistance with NMR experiments.

(Received in USA 28 November 1983)