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STRUCTURE OF SWINHOLIDE-A, A NEW MACROLIDE FROM THE MARINE SPONGE <u>THEONELLA SWINHOEI</u>

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Abstract: Swinholide-A (1a) a novel 22-membered macrolide has been isolated from a Red Sea sponge. The structure of the tetraformate (1b) has been determined on basis of spectral data using 2D-NMR correlations.

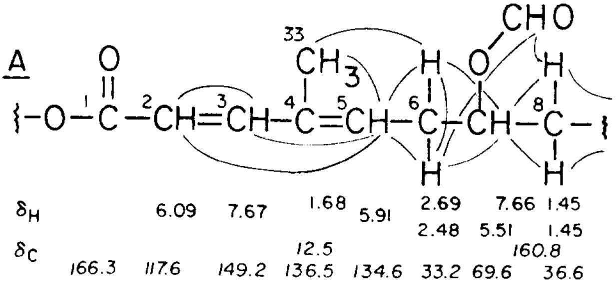
In our continuing search for physiologically active marine metabolites[1] we have tested several marine sponge extracts from the Gulf of Eilat, The Red Sea, for antibacterial and antifungal activities.

In this communication we report isolation and structure elucidation of a new macrolide polyketide which possesses in vitro antifungal activity.

Frozen Theonella swinhoei (Gray)[2] was lyophilized and successively extracted with petrol-ether and CH_2Cl_2/MeOH(8:2). The latter extract was chromatographed on Sephadex LH-20(MeOH/$CHCl_3$1:1) and on silicagel (petrol-ether, ether, ethyl acetate). The ethyl acetate fractions gave a microcrystalline compound designated swinholide-A(1a), m.p.102°, $C_{39}H_{66}O_{10} \cdot H_2O$(elemental analysis), responsible for the activity of the extract.

Swinholide-A showed no molecular ion in the mass spectrum (EI,CI,FD, SIMS or even with the FAB technique), however, the EI spectrum presented two important fragments of m/e 129 and 285 (see below).

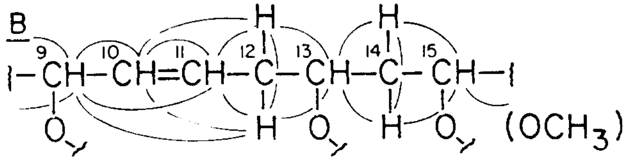
The ^{13}C NMR spectrum of 1a and its tetraformate (1b)[3,4] revealed the following groups: $6xCH_3$, $9xCH_2$, 4xCH, $2xOCH_3$, 11xCH-O, 5x=CH, 1 =C and 1-CO_2-. This accounts for 39 C-atoms and 62 protons. The four missing protons belong to 4 hydroxyls which were confirmed by the formylation of 1a to the tetraformate 1b[4]. Out of the 11 methinoxy signals four belong to the latter secondary hydroxyls, one to a lactone (confirmed by its opening to the corresponding hydroxy methyl ester[5]), and two additional signals to the 2 methoxy bearing C-atoms. The remaining four CH-O groups have to form two ethereal bridges which will account for two out of the 7 unsaturations of 1a; the 5 others being 3 double bonds and a lactone moiety.



Analysis of the 360MHz ^1H NMR spectrum of compound 1b in $CDCl_3$ and C_6D_6, with the aid of proton spin decoupling, suggested the following five partial structures A-E.

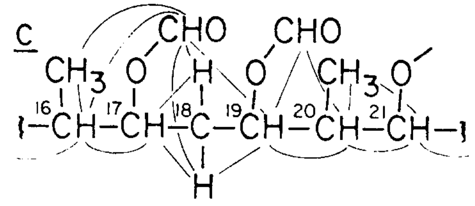
Each of the five segments possesses a characteristic functionality which served as a starting point for its NMR structure elucidation: an α,γ-diunsaturated ester in A, an allyl ether in B, a 1,3-dialcohol in C, a -CH(Me)CH(OH)$CHCH_3$- moiety in D, and a

B



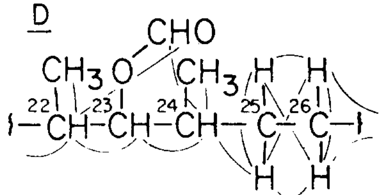
δ_H	4.22	5.35	5.64	1.80 1.58	3.16	1.37 1.77	3.64	(3.25)
δ_C	68.9	129.7	124.5	31.7	63.9	35.7	75.6	(56.4)

C



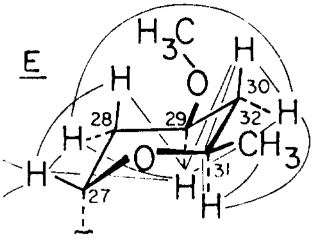
δ_H	0.85 1.50	8.52 5.47	2.01 2.01	7.82 5.18	0.91 2.46	5.50
δ_C	8.7 40.5	161.2 71.57	34.0	160.5 70.0	9.9 35.9	71.7

D



δ_H	1.17 2.24	7.95 4.94	0.81 1.90	1.50 1.30	1.70 0.97
δ_C	10.4 35.7	161.1 78.8	17.1 33.8	26.7	29.4

E



	27	28	29	30	31	32	OMe
δ_H	3.84	1.62 1.62	3.28	1.19 1.77	3.50	1.18	3.12
δ_C	70.8	35.7	73.6	38.9	69.9	22.0	55.1

tetrahydropyrane (THP) ring in E.

The IR and UV spectra of 1a are in agreement with the α,γ-unsaturated ester which is further supported by the doublet of the β-proton (H-3) at δ 7.67[6]. Irradiations of Me-33 and H-3 in a NOE experiment[7], caused enhancements of H-2 and H-5 respectively, establishing the E,E configuration of the diene. Of special importance in B was the finding of a 10(11)Z-configuration ($J_{10,11}$=9Hz) essential for the formation of a dihydropyrane (DHP) ring between C9 and C13, vide infra. Most significant also were the δ and especially the J-values[8] of E clearly pointing to a 1,3,5-trisubstituted THP ring. However, the coexistence of the resonance lines of 9 methylenes and 4 methines (total 22H) in the 1.5-2.4 ppm region results in a high degree of overlapping and thence to uncertainly in the proton sequencing and connections between the various fragments.

After laborious attempts to prepare a crystalline derivative of 1a failed we turned to 2D-NMR experiments[9], i.e. to H-H and C-H (short and long range) shift correlation spectra[10].

First, the C-H shift correlations have been established[11] (see partial structures A-E for summary of all H-H connectivity patterns). The latter correlations not only established the relationships within structures A-E, but, also the connections between all partial structures.

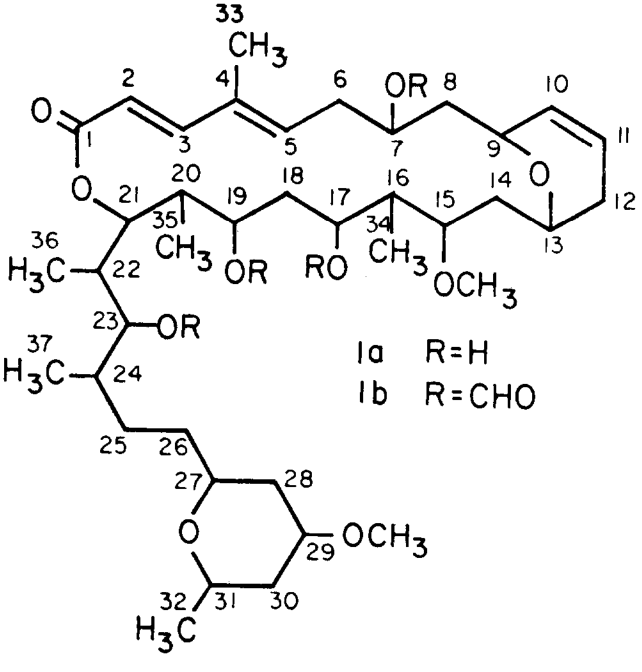
Among others, the proposed 22 membered macrolide was unequivocally confirmed by the observation of a $^3J_{COCH}$ coupling (∿10 Hz)[12] between C1, the lactonic carbonyl and H-21(δ 5.55-the lactonic methinoxy group which remains unchanged in 1b). The THP ring on the end of the C21-side chain was confirmed by long range J_{HH}-couplings between various protons of the THP ring (see fragment E).

At last the differentiation between the remaining four CH-O groups (C9,C13,C15 & C29) had to be accomplished. Two of the groups were expected to carry methoxyls while the other two have to form an ethereal bridge. A gated (^{13}C) decouoling experiment revealed that one of the two methoxy groups possessed a ∿5Hz coupling constant with a neighbour proton[13]. Furthermore, the same experiment also exhibited long range couplings of C15 and C29 with vicinal protons[13]. Based on this information a C-H shift correlation 2D experiment with the emphasis on small couplings (5 Hz) has been undertaken[10]. Indeed, small couplings between one of the OMe groups (δ_C 56.44) and H-15 (δ_H 3.64) as well as couplings between C15 & C29[13] and the two methoxyls, δ_H3.25 & δ_H3.12, (3H each) respectively, could be revealed (see B & E), thus, establishing the location of the two OMe groups.

The NMR evidence for the C29-OMe group is also in full agreement with the above mentioned two mass spectrum fragmentations, i.e. with a. the $C_7H_{13}O_2$ (129.0911, 15%) fragment which agrees best with a $^{+}\overline{\text{O=CHCH}_2\text{CH(OCH}_3\text{)CH}_2}\text{CHCH}_3$ ion due to cleavage of C26∥C27 α-to the THP ring and b. the $C_{17}H_{33}O_3$ (285.2449, 14%) peak, which most likely, is obtained by C19∥C20 fragmentation accompanied by H-migration and disconnection of the lactone. Additional support for the C9 to C13 DHP ring was obtained from the $J_{11,12}$, $J_{11,12'}$, $J_{12,13}$ and $J_{12',13}$ values (2, 5.7, 3 and 10 Hz, respectively) which are in good agreement with the expected ones from a DHP ring (a ring in which the oxygen and C13 extend from both sides of the double bond plane)[14]. Finally, also from the biogenetic point of view the C9 to C13 DHP ring (with the 10, 11 Z - bond) is expected to be most preferred.

Swinholide-A may be considered as a 22-membered macrolide ring. Embedded in the macrolide is a DHP ring and a second THP ring is located on the terminus of the C21 side chain. The longest chain of carbon atoms is 32 and has been used in the proposed numbering system. The oxygen substitution pattern, augmented by the presence of five methyl groups (at C4, C16, C20, C22 and C24- all in proper locations for propionate precursors) suggest a polyketide biosynthesis for swinholide-A.

The structure elucidation of other accompanying swinholides as well as the stereochemistry study of compound 1, is under progress.



Acknowledgements

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References and Notes

1. Y. Kashman, A. Groweiss, S. Carmely, Z. Kinamoni, D. Czarkie and M. Rotem, Pure & Appl. Chem., 54, 1995-2010 (1982).
2. The sponge was collected several times during 1979-83 in the southern parts of the Gulf of Eilat.
3. The ^{1}HNMR spectra were recorded on a WH-360 MHz instrument in $CDCl_3$, C_6D_6 and d_6-DMSO.
4. Swinholide-A was formylated by leaving its solution in HCO_2H at r.t. for 48 hrs; an oil, no molecular ion, $\nu_{max}^{CHCl_3}$ 2920, 1720, 1710, 1690, 1445, 1355, 1300, 1270 cm^{-1}, for NMR data, in C_6D_6, see text fragments A - E.
5. The 21-hydroxy methyl ester was obtained following treatment of 1a with 1% NaOMe in abs. MeOH solution for 2 hrs; an oil $\delta(CDCl_3)$ 4.06brd (J=10.3, H-21), 3.73s (CO_2CH_3).
6. All the ^{1}H NMR data in the discussion refer to the spectrum of 1b in C_6D_6.
7. Except for protons in the double bonds vicinity, only very weak NOEs could have been observed.
8. $J_{27,28}=4.5$, $J_{27,28'}=4.5$, $J_{27,29}<1.0$, $J_{28,29}=7.6$, $J_{28',29}=7.6$, $J_{28,30}=1.0$, $J_{29,30}=4.5$, $J_{29,30'}=$ $=9.0$, $J_{30,30'}=12.9$, $J_{30,31}=3.0$, $J_{30',31}=9.0$ and $J_{31,32}=6.0$.
9. The 2D-experiments became possible after two years of work, when we obtained a Bruker AM-360 instrument.
10. 360 MHz shift correlation spectrum of 0.25M 1b in C_6D_6, at 295 K, have been performed with a ^{1}H delayed COSY 90 sequence (the fixed delay has been adjusted for J=10 and 2.5Hz). The H-C shift correlated experiments of the above sample have been performed with a heteronuclear shift correlated sequence (polarization transfer via J_{CH}). In order to detect all the various CH_n (n=1,2,3) group correlations, suitable time development for polarization transfer (τ_1= 1/2J) and proper refocusing time for antiphase CH multiplet components (τ_2=1/4J) have been selected. Accordingly, three different experiments have been performed by adjusting τ_1 and τ_2 to give maximum polarization transfer for J_{CH} values of 144, 10 and 5Hz, respectively.
11. It is interesting to note that coupling constants up to ^6J could have been revealed in the COSY experiment. Furthermore, the formyls were found to be very useful for establishing long range couplings with vicinal protons.
12. J.L. Masshall, "Carbon-Carbon and Carbon-Proton NMR Couplings" Verlag Chemie, Florida 1983.
13. The following coupling constants have been measured: δ56.44dq(4.4, 139.6,OMe), 55.13q(139.6,OMe), 75.61dq(140.1,5.0,C15) and 73.63dq (139.9,4.8,C29).
14. A.E. de Jesus, P.S. Steyn, F.R. van Heerden, R. Vleggaar, P.L. Wessels and W.E. Hull, J. Chem. Soc. Per I, 1857 (1983).

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