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Tetrahedron Letters,Vol.28,No.45,pp 5461-5464,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

REISWIGINS A AND B, NOVEL ANTIVIRAL DITERPENES FROM A DEEPWATER SPONGE

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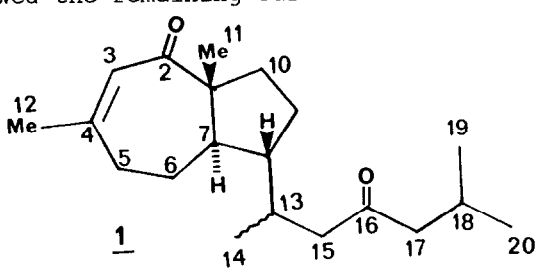
Abstract: The structures of reiswigins A(1) and B(2), new reduced azulene diterpene enones have been determined by combined one and two dimensional NMR and mass spectral techniques.

As part of our continuing program aimed at the isolation of novel antiviral metabolites from deepwater marine organisms we found the diterpenes 1 and 2 in a specimen of Epipolasis reiswigi(Class Demospongiae, Order Halichondria)[1]collected by submersible at 330 meters. Reiswigins A and B show potent in-vitro activity against Herpes simplex type 1 virus and murine A59 hepatitis virus[2], and are novel additions to the vast array of terpene metabolites isolated from marine sponges.[3]

Initial in-vitro assay of a 3:1 methanol/toluene extract of the frozen sponge showed strong antiviral activity against Herpes simplex virus type 1 (HSV-1). Accordingly, lyophilized sponge(55 gr. dry wt.) was extracted with dichloromethane to give 1.3 grams of crude extract. Bioassay guided isolation began by flash chromatography on silica gel with successive elution by heptane, chloroform and finally 1:1 methanol/chloroform. The antiviral fraction was subjected to repeated vacum flash chromatography (silica gel H) followed by HPLC (silica gel, 10,20% ethyl acetate/heptane) to give 1(500mg,0.9% of lyophilized sponge) and 2(40mg,0.07%) as tan oils. Reiswigin A was assigned molecular formula $C_{20}H_{32}O_2$ by HREIMS.[4] The ^{13}C NMR spectrum showed two singlet carbonyl resonances at 210.4 and 206.9 ppm. These, considered along with infrared absorptions at 1700 and 1630 cm^{-1}, and ultra violet absorbance bands at 223nm(ε=2300,heptane) and 205nm(ε=5700) established the presence of ketone and enone functionalities. A DEPT experiment showed the remaining carbon resonances to consist of 2 quaternary(one $sp^2$and one



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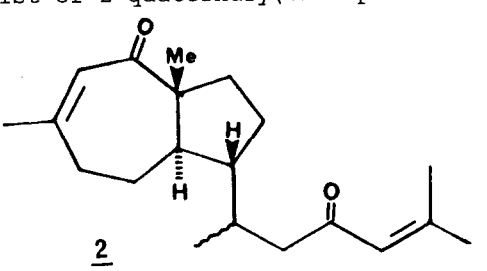

2

sp^3), 5 methine(one sp^2 and four sp^3), 6 methylene and 5 methyl resonances. With 5 sites of unsaturation required by the apparent molecular formula, and 3 accounted for by the C13 data, 1 was determined to be a bicyclic diterpene.

The proton NMR data clearly established the presence of a terminal isopropyl group, observed at 1.0 and 0.98 ppm, a bridgehead methyl resonance at 1.20 ppm, vinyl methyl of an α,β unsaturated ketone observed at 1.98 ppm, along with the α proton resonance of the enone at 5.85 ppm.

With the identity of several key functional elements estabished by the preceding inspection, and the relatively congested nature of the upfield region of the ^1H NMR spectrum, we turned to 2 dimensional methods to establish the overall molecular connectivities. A standard ^1H COSY[6] experiment along with a one bond HETCOR[7] experiment established several proton-proton and one bond proton-carbon connectivities. In addition, several 3 bond correlations were obtained by a long range HETCOR[8]experiment. All correlations observed in these experiments are presented in Table 1. A determination of the complete carbon connectivity network by this method was precluded by substantial overlap of the signals for protons on C-5,C-6, and C-13, and for protons bound to C-8, C-9, and C-10. We subsequently performed a 2D INADEQUATE[9,10]experiment to establish the remaining carbon connectivities. A contour plot of the upfield region of the symmetrical INADEQUATE 2D matrix is presented in Figure 2. Cross peaks delineating the complete connectivity network for all sp^3 carbons are evident. Additional one-bond correlations for C-2,C-3,C-4, and C-16 were observed in the downfield region (not shown).The resulting structure assignment is consistent with the major mass spectral fragmentations, shown in Figure 1. Relative stereochemical relationships between the C-11 methyl group, H-7,and H-8, were assigned by phase sensitive NOESY [11],NOE difference and spin decoupling experiments.

The HREIMS spectrum of reiswigin B(2) indicated a molecular formula of $C_{20}H_{30}O_2$.[5] Intercomparison of ^1H and ^{13}C NMR data with those of (1) showed the presence of an additional double bond in (2), the location of which must be as an isopropenyl terminus, evidenced in the ^1HNMR spectrum by resonances for H-14 at 6.04 ppm and for terminal H-17 and H-16 methyl singlets at 2.13 and 1.88 ppm respectively. All ^1H and ^{13}C NMR assignments for 2 were confirmed by COSY and HETCOR experiments.

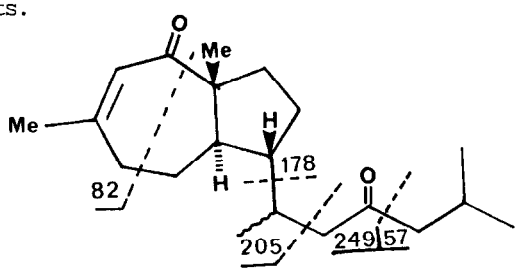

FIGURE 1. Mass Spectral Fragmentation of 1

TABLE 1. NMR Data for Reiswigin A (1) in $CDCl_3$

Carbon #	δC (ppm)	Observed HETCOR Proton Correlations	Observed Longe Range HETCOR Correlations
1	56.5(s)	-	H3, H10, H7, H9, Me11
2	210.4(s)	-	Me11
3	127.1(d)	H3	H5, Me12
4	152.4(s)	-	H5, Me12
5	34.8(t)	H5,H5'	Me12
6	24.7(t)	H6	-
7	45.3(d)	H7	H6, H10, H8, Me11
8	47.9(d)	H8	H10, H7
9	22.2(t)	H9	-
10	35.2(t)	H10	Me11
11	19.8(q)	Me11	-
12	28.5(q)	Me12	-
13	29.6(d)	H13	Me14
14	20.1(q)	Me14	H7, H15
15	45.4(t)	H15	H18, Me19
16	206.9(s)	-	H15, H18
17	52.6(t)	H17	H13, H18
18	24.6(d)	H18	-
19	22.6(q)	Me19	-
20	22.5(q)	Me20	-

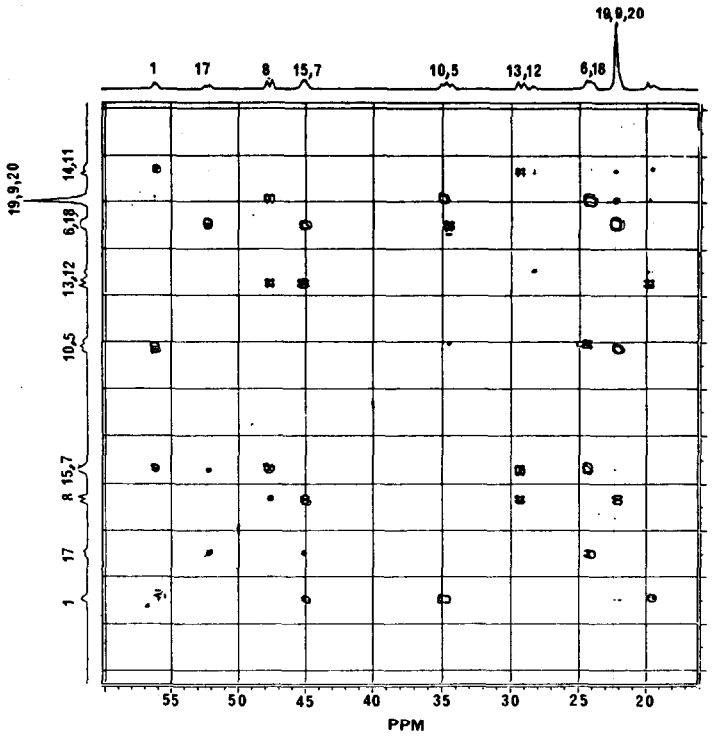

Figure 2. Contour plot of high field region from 2D INADEQUATE experiment of 1.

REFERENCES

1. Taxonomic identification of the sponge was made by S. Pomponi and C. Diaz. M.C. Diaz, B. Alvarez, and R.W.M. VanSoest, New Species of Demospongiae(Porifera) from the National Park "Archipelago de los Roques," Venezuela. Bijdr. Dierk., In Press.
2. Antiviral assays for herpes simplex virus type 1 (HSV-1) and vesicular stomatitis virus(VSV) were plaque reduction tests in infected CV-1 monkey kidney cells. Coronavirus strain A59 inhibitions were measured by reduction in cell fusion and cytopathic effects in NCTC 1469 mouse liver cells. Readings in these assays range from "-" for no observable viral inhibition, to "3+", denoting complete inhibition of virus by the test compound. Reiswigin A(1) was nontoxic at 2µg and completely inhibited HSV-1 and VSV. A59 was partially inhibited at 20µg with a 2+ reading. Reiswigin B(2) showed the same activity as 1 against HSV-1 and A59.
3. D. J. Faulkner, Nat. Prod. Rep.,1-33 (1986).
4. Reiswigin A(1): MS(HREI) m/z 304,$C_{20}H_{32}O_2$, 1.8%, 0.3ppm deviation, ^{1}H NMR(360 MHz,$CDCl_3$): δ 5.85s(1H,H-5), 2.5dt(1H,H-5,J=18.3,4.9), 2.44brd(1H,H-15,J=12.9), 2.38m(2H,H-17), 2.35d(1H,H-13,J=6.6), 2.33m(1H,H-5), 2.27brd(1H,H-15,J=14.5), 2.21sept(1H,H-18,J=6.6,0.6), 1.98s(3H,Me-12), 1.96m(1H,H-10), 1.90m(1H,H-6, 1.85m(1H,H-8), 1.80m(2H,H-6,H-9), 1.74dd(1H,H-10,J=9.6,1.6), 1.68ddd (H7,J=10.2,6.5,2.7),1.39m(1H,H-9), 1.20s(3H,Me-11), 1.0d(6H,Me-19,Me-14,J=6.6), 0.98d(3H,Me-20, J=6.6), IR($CHCl_3$): 2940br, 1700, 1630 cm^{-1}, UV(heptane): 223nm(ε=2300) 205nm(ε=5700), optical rotation $[\alpha]_D^{20}$=-10° (c= 0.1,$CHCl_3$)
5. Reiswigin B(2): MS(HREI) m/z 302,0.5%) $C_{20}H_{30}O_2$, ^{1}H NMR(360 MHz,$CDCl_3$)δ 6.04sht(1H,H-17,J=1.3), 5.82s(1H,H-3), 2.41m(2H,H-15), 2.27m(2H,H-5), 2.20m(1H,H-13), 2.13s(3H,Me-20),1.88s(6H,Me-19,Me-12), 1.82m(1H,H-10) 1.74m(1H,H-8), 1.68m(3H,H-6,H-10), 1.64dt(1H,H-7,J=9.3,1.5), 1.32m(2H,H-9), 1.11s(3H,Me-11), 0.93d(3H,Me-14,J=6.5), ^{13}C NMR(90 MHz, $CDCl_3$):207.9s, 201.3s, 155.6s, 153.2s, 127.8d, 124.8d, 57.1s, 48.6d, 47.0t, 45.8d, 35.7t, 35.3t, 30.8t, 29.1q, 28.2q, 25.2t, 22.8t, 21.2q, 20.6q, 20.4q, IR($CHCl_3$): 2900br, 1650, 1605 cm^{-1}, UV(heptane): 234nm(ε=6700) optical rotation $[\alpha]_D^{20}$=-20° (c= 0.1,$CHCl_3$)
6. K. Nagayama et. al., J. Mag. Res., 40, 321 (1980)
7. A. Bax and G. Morris, J. Mag. Res., 42, 501 (1981)
8. Standard HETCOR pulse sequence with suitable delays to emphasize J_{CH}= 10Hz and 7Hz
9. T.H. Mareci and R. Freeman, J. Mag. Res., 48, 158 (1982)
10. Experimental conditions for INADEQUATE experiment. 300mg sample in 0.4ml $CDCl_3$, Sweep width in F1 and F2 18.5KHz, 256 experiments (t_1 increments) at 256 scans per experiment were recorded. Zero filling in F1 to 1K, no zero filling in F2 to give a total 2D matrix 2K X 2K, Effective resolution in both F1 and F2 9 Hz/point. Transformed 2D matrix was symmetrized about the diagonal.
11. G. Bodenhausen, H. Kogler, R.R. Ernst, J. Mag. Res., 58,370 (1984)

(Received in USA 1 July 1987)