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FURTHER STUDIES ON PALYTOXIN. I.

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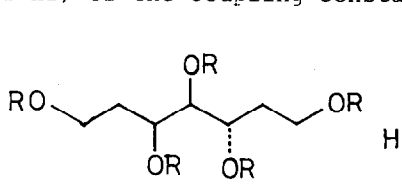
Summary: The structures of two degradation products of N-(p-bromobenzoyl)palytoxin were elucidated, and then the sequences of those fragments were determined.

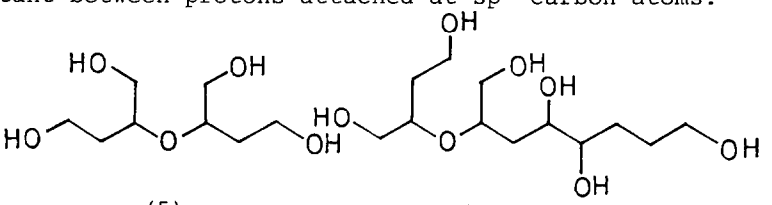
Palytoxin has been known because of its physiological activities and unusual properties.[1] Studies on the structure of palytoxin have been advancing by simple protection[2,3] of the terminal amino group, and especially, by determining the molecular weight by means of ^{252}Cf-PDMS, which is very useful method[3] for molecular weight determination of non-volatile and biologically active substances. Now, we wish to report further studies toward the elucidation of the full structure of palytoxin by determining the structures of the oxidation products obtained from its derivative, N-(p-bromobenzoyl)palytoxin.

We described in previous reports[4,5] that the mild ozonolysis of eight double bonds which were present in palytoxin was very suitable for the structural elucidation of palytoxin. Further investigation gave the following results. Procedure of ozonolysis was done according to the method reported by Pappas et al.[6] because of the increased solubility of N-(p-bromobenzoyl)palytoxin in methanol at -78° compared with palytoxin. After ozone was passed through the MeOH solution of N-(p-bromobenzoyl)palytoxin at -78° for 10 min, this solution was allowed to stand at the same temperature for 20 min. Then the system was flushed with nitrogen at -78° and dimethyl sulfide was added. This solution was stirred at room temp for 3 hr, and concentrated under reduced pressure. The residue was dissolved again in methanol and reduced with $NaBH_4$. The solvent was evaporated to give an oily material that was separated by the use of a TSK G3000S (polystyrene gel) column. The aqueous eluate was further treated with Dowex 50W-X4 (H^+ form), and then concentrated. After the removement of boric acid, resulting products were acetylated to give three compounds (1), (2) and (3). The structure of compound (1) was determined by the analysis of the spectral

data: MS, m/e 331 (M^+-59); ^{1}H-NMR ($CDCl_3$) 1.8-2.0 (4H, m), 2.05 (12H, s), 2.15 (3H, s), 4.04 (4H, t, J= 7Hz), 4.9-5.4 (3H, m); ^{13}C-NMR ($CDCl_3$) 20.8 (q), 29.2 (t), 30.2 (t), 60.1 (t, 2xC), 67.7 (d), 67.9 (d), 73.3 (d), 169.7 (s, 2xC), 169.8 (s, 2xC) and 170.4 (s). Compound (2) was converted to an alcohol (4) by hydrolysis. It was then oxidized with $NaIO_4$ followed by $NaBH_4$ treatment to yield two products (5)[4] and (6). The structure of compound (6) was confirmed by the spectral data of its acetate [MS, m/e 535 (M^+-59), 345; ^{1}H-NMR (C_6D_6) 3.57, 3.72, 5.20 and 5.31 ppm (1H each)] as well as by its chemical conversion to compound (5) by $NaIO_4$ oxidation followed by $NaBH_4$ reduction. Consequently, the structure of compound (2) was determined as shown in the structure 2 by above-mentioned chemical means and by the reasonable assignment of each signal in 270 MHz ^{1}H-NMR spectrum of 2 which was performed with the aid of decoupling experiments. The chemical shifts and coupling constants of each proton are shown in the figure. On the other hand, compound (3) was converted to compound (2) by ozonolysis followed by reduction with $NaBH_4$ and then by acetylation with Ac_2O/py. The double bond present in 3 is Z-configuration, judging from the value (12 Hz) of the coupling constant between protons attached at sp^2 carbon atoms.

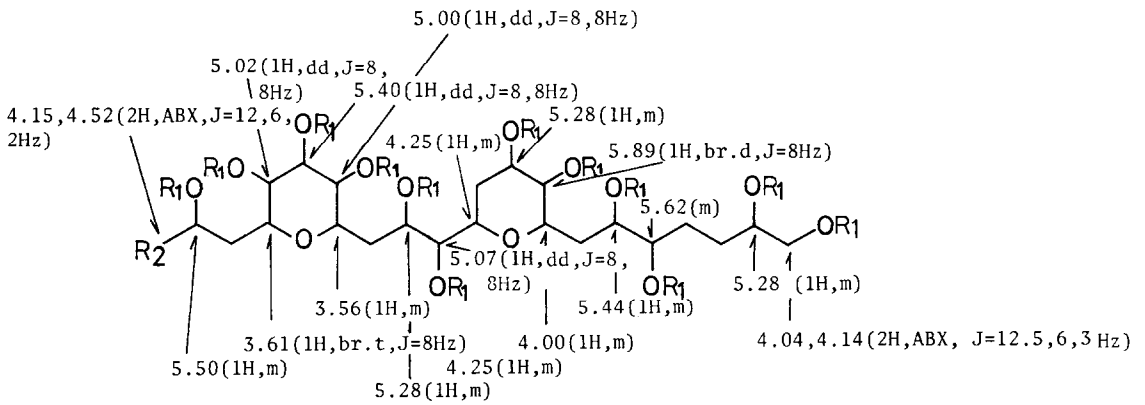


(1) R= $COCH_3$
(11) R= H



(5)

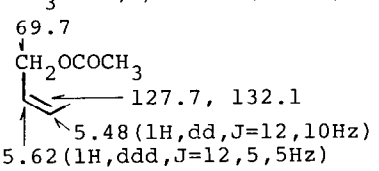
(6)

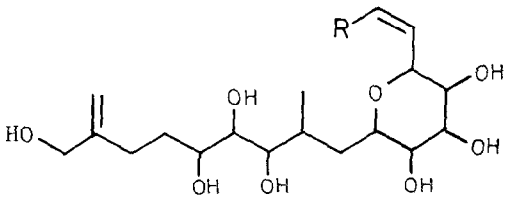


(2) R_1= $COCH_3$, R_2= CH_2OCOCH_3
:MS, m/e 1047 (M^+-59)

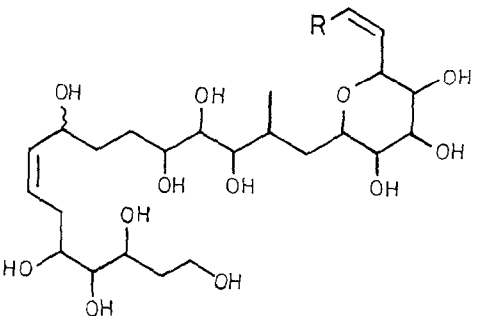
(3) R_1= $COCH_3$:MS,m/e 1073(M^+-59)

(4) R_1= H, R_2= CH_2OH





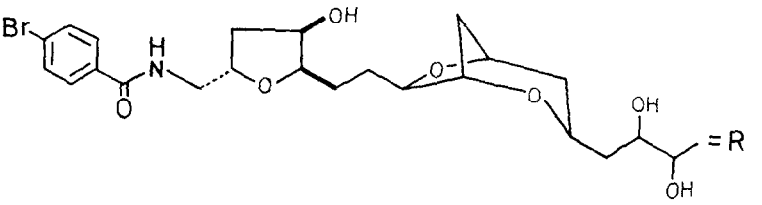
(7)

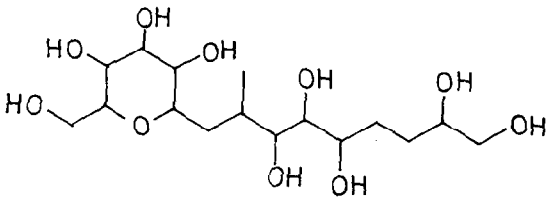


(8)

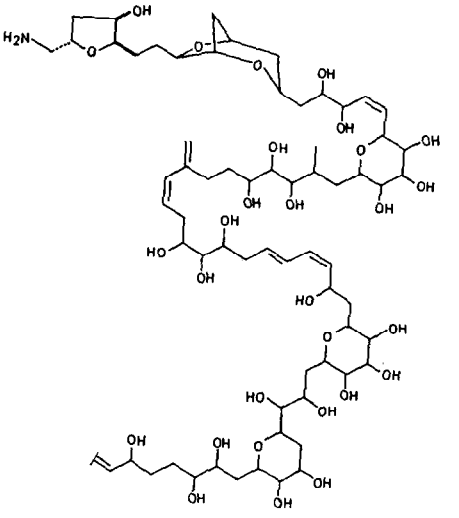
$R-CH_2OH$

(9)

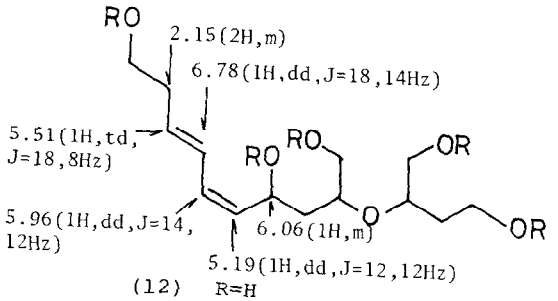




(10)



(14)



(12) R=H

(13) $R=COCH_3$: MS, m/e 500(M^+), 458, 398

The 50% EtOH eluate from the polystyrene gel column afforded two fragments (7) and (8)[4] containing the terminal amino moiety, and the segments[7] containing the intramolecular acetal moiety. Compounds (8) [FDMS, 1061 and 1059 $(M+H+Na)^+$] which were recognized as the mixture of two epimers have four olefinic protons [5.4-5.75 ppm (4H, m)] and four olefinic carbon atoms (135.1, 134.7 and 134.4, 131.6 and 129.3, and 129.0 ppm). Ozonolysis of the two epimers followed by reduction with dimethyl sulfide and then with $NaBH_4$ gave compounds (9)[4] and (10)[4] which were obtained by the same treatment of compound (7), and yielded additionally compound (11). Therefore, the structures of the epimers (8) were suggested by those results.

Six double bonds in palytoxin have been already recognized in several segments obtained by degradation with $NaIO_4$.[2,8] It is suggested that two remaining double bonds constitute a conjugated diene which is present in palytoxin as the second λ233 nm chromophore by the formation of compound (3). We obtained fragment (12) containing this second diene by the treatment of N-(p-bromobenzoyl)palytoxin with $NaIO_4$ and then with $NaBH_4$. The structure of its acetate (13) was determined by the analysis of spectral data. Recently, this conclusion was also reported by Moore's group.[9] Consideration of the aforementioned results would suggest that the sequence starting from the terminal amino group in palytoxin itself is to be as shown in structure 14.

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