Tetrahedron Letters Vol. 21, pp 4857 - 4860 0040-4039/80/1208-4857$02.00/0

STRUCTURAL STUDIES ON PALYTOXIN, A POTENT COELENTERATE TOXIN

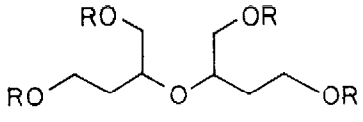
Daisuke UEMURA,[a] Katsuhiro UEDA,[b] and Yoshimasa HIRATA[c]
[a]Faculty of Liberal Arts, Shizuoka University, Ohya, Shizuoka 422, Japan
[b]Department of General Education, University of Ryukyus, Naha 903, Japan
[c]Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan
Chuji KATAYAMA and Jiro TANAKA
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

Summary: The structural elucidation of palytoxin is reported.

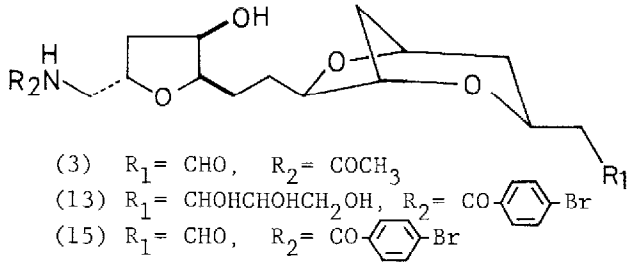
Palythoa tuberculosa (Coelenterata, Zoanthidae), a species of hexacorallia, living on the coral reefs in tropical and subtropical regions, contains palytoxin well known as the most powerful toxin among those obtained from marine animal and plant sources. There has been great interest in elucidating the structure of palytoxin because of its physiological activities and unusual molecular properties.[1] Although palytoxin has not been obtained up to now in crystalline form, some reports[2,3,4] concerning the isolation, properties and partial structures of palytoxin have been published. Now, we wish to describe here the structures of some degradation products.

Recently, Macfarlane and we have reported the molecular weight determination of palytoxin, N-acetylpalytoxin and N-acetylperhydropalytoxins by ^{252}Cf PDMS.[5] The molecular weight of N-acetylpalytoxin is 2723.1 and that of palytoxin, 2681.1 Hydrogenation of N-acetylpalytoxin has resulted in the increase of 16.3 mass units suggesting that the parent molecule contains eight double bonds. Above-mentioned N-acetylpalytoxin was obtained in quantitative yield from palytoxin by the treatment with p-nitrophenyl acetate in pyridine and water. Palytoxin responded positively to the ninhydrin test. However, this N-acetylpalytoxin was negative to the ninhydrin test and showed a dramatic decrease of toxicity.[6] Therefore, we believe that toxicity of palytoxin may depend on its ninhydrin-positive moiety. On the other hand, palytoxin has many hydroxyl groups as shown by its molecular formula[7] and spectral data.[2,4] Oxidation of N-acetylpalytoxin with $NaIO_4$ was carried out on a TSK G3000S polystyrene gel column by considering the smooth separation of products and the clearance of reactions. N-Acetylpalytoxin was charged on this column and treated with an aqueous solution of excess $NaIO_4$. After elution of formic acid and inorganic salts, aqueous eluates were treated with $NaBH_4$ to give compound (1). Its tetraacetate (2) was obtained by acetylation with acetic anhydride in pyridine. The structure of 2 was sug-

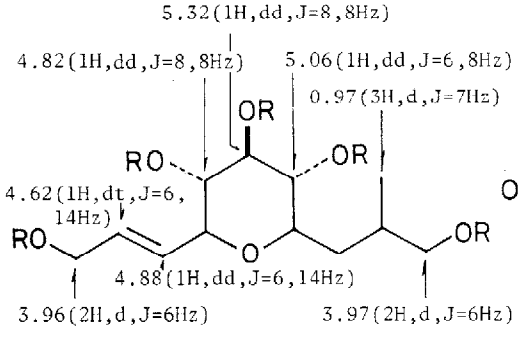
gested by the following spectral data: PMR(δ, $CDCl_3$) 1.86 (4H, dt, J= 6, 6 Hz), 3.78 (2H, m), 4.10 (4H, d, J= 5 Hz), 4.18 (4H, t, J= 6 Hz); Mass, m/e 362 (M^+). The polystyrene gel column was then eluted with ethanol-water, the concentration of the former being gradually increased. The 20% ethanol eluate was treated with $NaBH_4$ to yield three compounds. Their structures were established as structures (3)[8], (4) and (5).[4] Compound (4) was treated with acetic anhydride in pyridine at room temp to give a pentaacetate (6). Its molecular formula is $C_{22}H_{32}O_{11}$ [Mass, m/e 472 (M^+)] and the reasonable assignment of each signal in the PMR spectrum has been performed by decoupling experiments as depicted in the structure (6). The 50% ethanol eluate afforded compound (7).[3] Compounds (5) and (7) contain the 263 nm and 233 nm chromophores, respectively, which are found in palytoxin. However, from the value of the extinction coefficient it can be deduced that palytoxin contains a second 233 nm chromophore. Then $NaBH_4$ reduction of pure ethanol eluate afforded a tetraol (8)[4] and compounds (9) and (10). The structures of 9 and 10 were established by the X-ray crystallographic analysis[9] of compound (11). Among these segments, compounds (3) and (5) are assigned as two terminal moieties present in palytoxin from the consideration of the structure of each fragment and analysis of the CMR spectrum[4] of palytoxin.



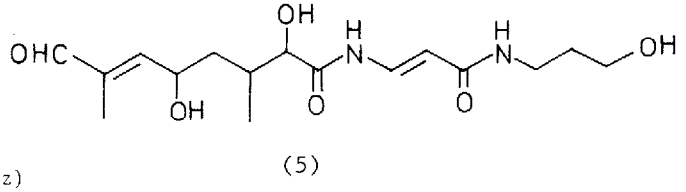
(1) R= H
(2) R= $COCH_3$



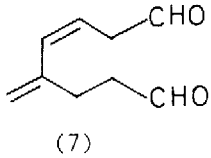
(3) R_1= CHO, R_2= $COCH_3$
(13) R_1= $CHOHCHOHCH_2OH$, R_2= CO-Ph-Br
(15) R_1= CHO, R_2= CO-Ph-Br



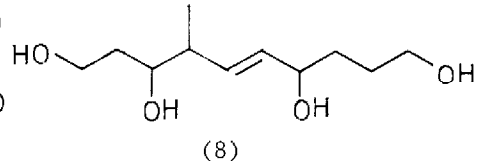
(4) R= H
(6) R= $COCH_3$



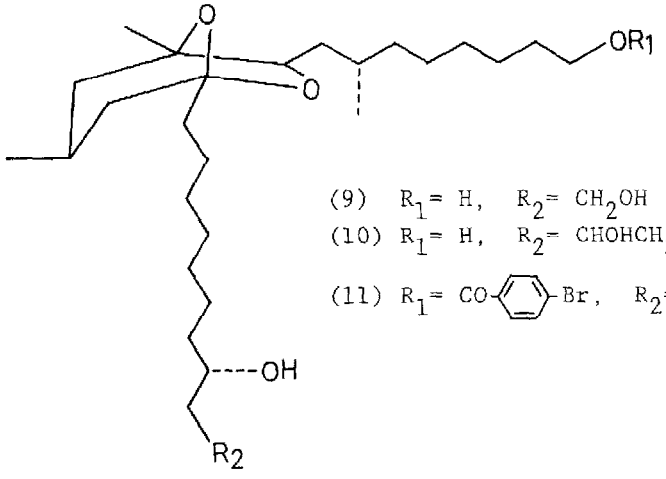
(5)



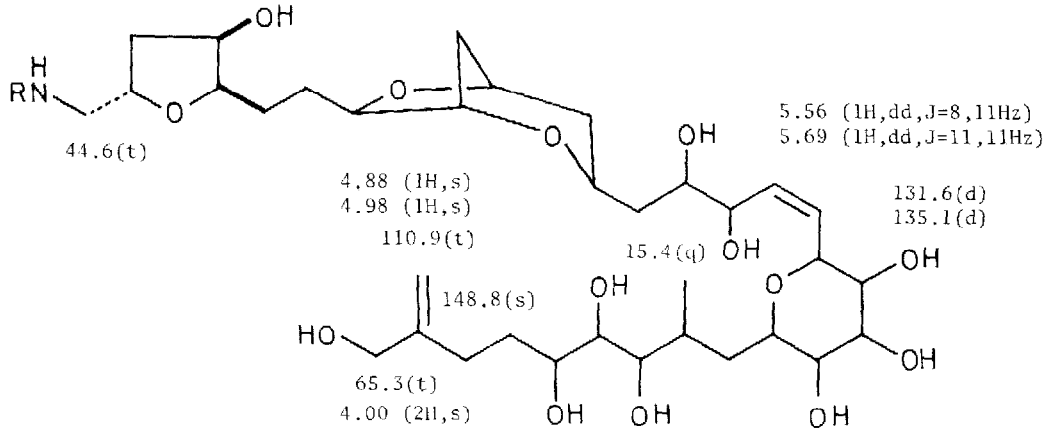
(7)



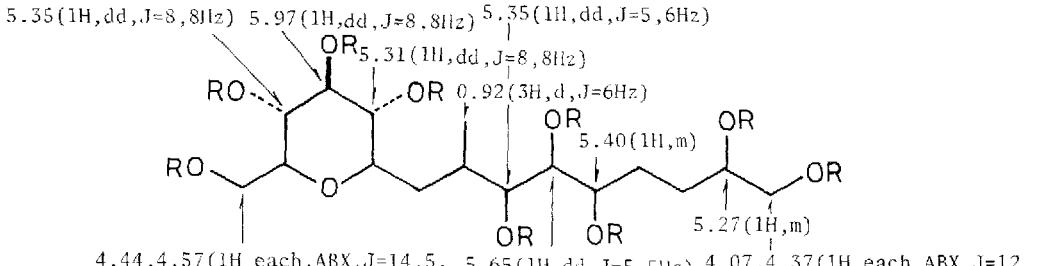
(8)



(9) R_1= H, R_2= CH_2OH
(10) R_1= H, R_2= $CHOHCH_2OH$
(11) R_1= CO-C$_6$H$_4$-Br, R_2= CH(O-CH$_2$-CH$_2$-O)



(12) R= CO-C$_6$H$_4$-Br



(14) R= H (16) R= $COCH_3$

Since we know the presence of eight double bonds in palytoxin, we have attempted ozonolysis of palytoxin, N-acetylpalytoxin and N-(p-bromobenzoyl)palytoxin which was obtained by the treatment of palytoxin with p-nitrophenyl p-bromobenzoate. Expectedly, ozonolysis afforded valuable information concerning the sequence of segments obtained by periodate oxidation. Especially, partial ozonolysis of N-(p-bromobenzoyl)palytoxin led us to the following conclusion. The terminal N-acyl compounds such as 3 were combined with the moiety arising from compounds (4) and (7) as shown in structure (12) of a product[10] which was obtained by ozonolysis under mild condition followed by reduction with $NaBH_4$. The structure of 12 was suggested by the spectral and chemical evidence. Spectral data of 12 are drawn in the figure. Further ozonolysis of 12 followed by reduction with $NaBH_4$ gave two compounds (13) and (14). Molecular formula of 13 was $C_{24}H_{34}NO_8Br$ (m.p. 148-150°), and oxidation of 13 with $NaIO_4$ yielded compound (15). The structure of 14 was deduced by detailed analysis of the 270 MHz PMR spectrum of its acetate (16) [Mass, m/e 703 (M^+-59)]. The chemical shifts and the multiplicities in the PMR spectrum of 16 are shown in the figure.

Consequently, it is found that palytoxin may be composed of poly-methylated and unsaturated fatty acid containing several hydroxyl groups and the various kinds of ether linkages. Further investigations toward the elucidation of the full structure of palytoxin are currently under way.

Acknowledgements: The authors are grateful to the Mitsubishi Foundation, the Foundation for the Promotion of Research on Medical Resources, and the Ministry of Education, Japanese Government (Grant-in-aid, No. 411704), for financial support. We thank Professor Tatsuo Miyazawa, The University of Tokyo, for the measurements of the NMR spectra.

REFERENCES AND NOTES

1) R.E. Moore and P.J. Scheuer, Science, 172, 495 (1971). 2) R.E. Moore, R.F. Dietrich, B. Hatton, T. Higa, and P.J. Scheuer, J. Org. Chem., 40, 540 (1974). 3) R.E. Moore, F.X. Woolard, M.Y. Sheikh, and P.J. Scheuer, J. Am. Chem. Soc., 100, 7758 (1978). 4) Y. Hirata, D. Uemura, K. Ueda, and S. Takano, Pure Appl. Chem., 51, 1875 (1979). 5) R.D. Macfarlane, D. Uemura, K. Ueda, and Y. Hirata, J. Am. Chem. Soc., 102, 875 (1980). 6) Pharmacological studies on palytoxin and its derivatives were carried out by Shibata's group using frog spinal cord. Y. Kubo and S. Shibata, Br. J. Pharmacol., in press (1980). 7) New results recently obtained from chemical degradation and elemental analysis show that most plausible molecular formula of palytoxin is $C_{130}H_{227-229}N_3O_{53}$. 8) The structure of compound (3) was confirmed by the X-ray crystallographic analysis as described in the following paper. 9) X-Ray crystallographic studies of compound (11) is reported in the subsequent paper in this issue. 10) The Z configuration of the double bond in 12 was deduced with the aid of coupling constants between the olefinic protons in its PMR spectrum.

(Received in Japan 22 August 1980)