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STRUCTURE OF PHOLIPOMYCIN

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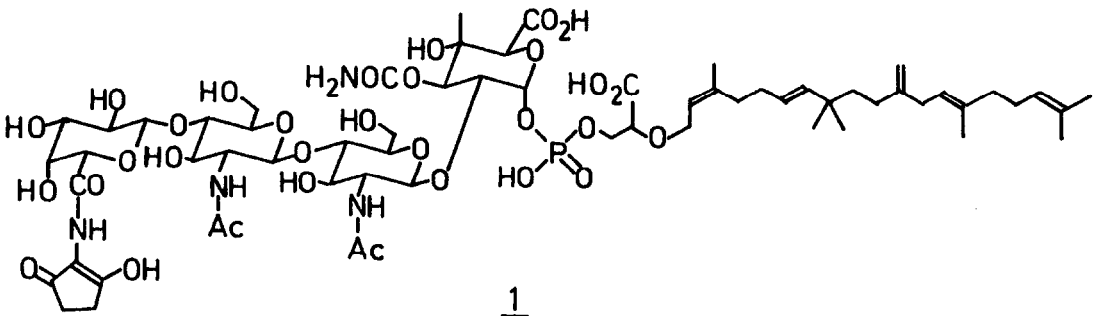
Pholipomycin (1) is a new member of phosphoglycolipid antibiotics. The structure of pholipomycin was proposed by chemical degradation products and FAB-MS and ^{1}H and ^{13}C-NMR spectra.

In the previous papers[1a,b,c], one of the authors (M.A.) have reported isolation, physico-chemical and biological properties of pholipomycin, a new member of the phosphoglycolipid antibiotics. Pholipomycin could be differentiated from the other members by the acid hydrolysis products as follows; the presence of glucosamine, a chromophore (2-amino-3-hydroxy-Δ^2-cyclopentenone) and moenocinol-type C_{25}lipids, but the absence of glucose, quinovosamine and glycine. In the present paper we report the total structure of pholipomycin(1).

1($C_{63}H_{96}O_{31}N_4P$, M.W.1435) was hydrogenated with 10% Pd-C in 50% aq.MeOH to give perhydropholipomycin($C_{63}H_{106}O_{31}N_4P$, m/z 1445 by FAB-MS, vide infra; anal. found: C: 46.91, H: 7.50, N: 4.86, P: 2.00; uv: 258 nm in MeOH). Degradation of perhydropholipomycin was conducted by heating (70°C, 4 hrs) it in CF_3COOH, followed by separation of an acidic lipophilic fraction extracted into ethyl acetate and a water-soluble part. The structure of the former compound (2; $C_{31}H_{63}O_7P$, m/z 578) was determined by GC/MS analysis(2% OV-17, 150°→270°C, 10°C/min.) of the esterified derivative and was found to be identical with the compound obtained by the same treatment of perhydromoenomycin[2].



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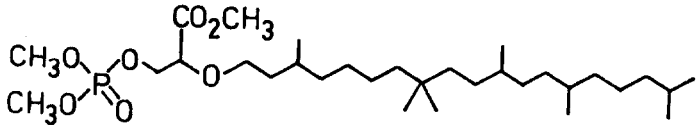

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After lyophilization, the latter was dissolved in MeOH followed by methylation with diazomethane and then acetylation with acetic anhydride in pyridine. Preparative TLC of this fraction gave three sugar derivatives 3a, 3b and 4, which contained the chromophore part. The ^{1}H-NMR spectra of 3a, 3b and 4 were fully analyzed as described in Table.

Table: 400MHz ^{1}H-NMR Data of 3a, 3b ($CDCl_3$) and 4 ($CDCl_3$+CD_3OD)

3a: glucosamine: 1'-H,6.02,d,2'-H,4.08,m,3'-H,5.28,q,4'-H,3.91,t,5'-H,3.72,m, 6a'-H,4.13,q,6b'-H,4.52,q,NH,4.42,d,OCH_3,3.48,s;$J_{1',2'}$=9.5,$J_{2',3'}$=9.5, $J_{3',4'}$=8.5,$J_{4',5'}$=8.5,$J_{5',6a'}$=5.5,$J_{5',6b'}$=2.5,$J_{6a',6b'}$=12.0,$J_{2',NH}$=8.5, galactouronic acid: 1"-H,4.72,d,2"-H,5.16,q,3"-H,5.12,q,4"-H,5.75,q, 5"-H,4.38,d; $J_{1",2"}$=7.5,$J_{2",3"}$=10.5,$J_{3",4"}$=3.0,$J_{4",5"}$=1.5, chromophore: 4-CH_2,2.76,m,5-CH_2,2.50,m,OCH_3,4.02,s.

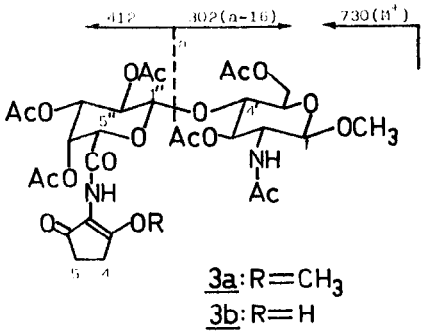
3b: glucosamine: 1'-H,6.23,d,2'-H,4.05,m,3'-H,5.28,q,4'-H,3.94,t,5'-H,3.82,m, 6a'-H,4.12,q,6b'-H,4.49,q,NH,4.46,d,OCH_3,3.45,s; galactouronic acid: 1"-H,4.79,d,2"-H,5.16,q,3"-H,5.11,q,4"-H,5.72,q,5"-H,4.39,d;chromophore: 4-CH_2,2.61,m,5-CH_2,2.55,m. J values are same as 3a.

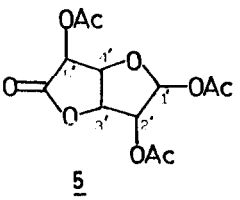
4: moenouronic acid: 1'-H,5.50,s,2'-H,ol,3'-H,ol,5'-H,ol,4'-CH_3,1.72,s; glucosamine:1"-H,4.62,d,2"-H,3.9,ol,3"-H,5.13,ol,4"-H,3.9,ol,5"-H,3.63,m,6a"-H, 4.14,q,6b"-H,4.44,q;$J_{1",2"}$=9.0,$J_{2",3"}$=ca. 9.0,$J_{3",4"}$=ca. 9.0, $J_{4",5"}$= ca. 9.0,$J_{5",6a"}$=5.5,$J_{5",6b"}$=2.5,$J_{6a",6b"}$=12.0, glucosamine: 1'"-H,4.55,d, 2'"-H,ca.3.90,m,3'"-H,5.08,q,4'"-H,3.73,t,5'"-H,3.67,m,6a'"-H,q,OCH_3,3.44,s; galactouronic acid: 1'""-H,4.69,d,2'"-H,ca.5.1,q,3'"-H,ca.3.1,q,4'"-H,5.72,q, 5'"-H,4.34,d;chromophore: 4-CH_2,2.79,m,5-CH_2,2.52,m,OCH_3,4.01,s, in addition to same as 3a.

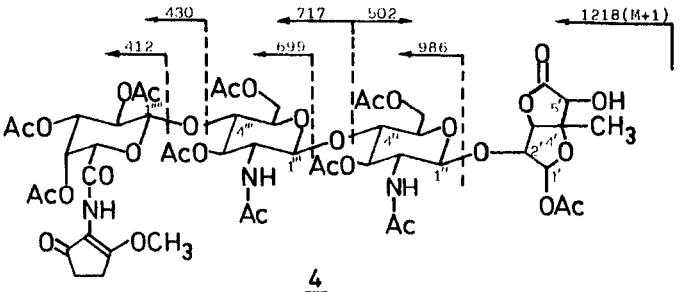
chemical shifts are in ppm relative to internal TMS; s: singlet, d: doublet, t: triplet, q: quarter, m: multiplet, ol: overlap.

From these assignments, the structures of 3a, 3b and 4 have been deduced as shown below. The sequence and comformation of the tetrasacchride in 4 -- moenouronic acid(lactone form) (2'→1")glucosamine(4"→1"')glucosamine(4"'→1"")galactouronic acid -- were completely analyzed by 400 MHz ^{1}H-NMR and the results summarized in Table indicated its structure to be analogous to the tetrasacchride in moenomycin[3], which contained quinovosamine instead of N-Ac glucosamine. In addition, the lactone form[4] of moenouronic acid in 4 was supported by the same form of hydrolysis product (5) of D-glucuronic acid obtained after the same treatment as perhydropholipomycin. 5 showed the following spectrum data, m.p. 178-180°C, $C_{12}H_{14}O_9$, m/z 260(M^+-42), δ_{ppm}: 6.19(1'-H,s), 5.30(2'-H,s), 5.25(4'-H, q, $J_{3',4'}$=4.5, $J_{4',5'}$=7.0), 5.13(5'-H,d), 5.04(3'-H,d) in PMR and 169.9, 169.0, 168.8, 168.4, 98.5, 81.5, 78.0, 76.2, 68.0, 20.7, 20.4, 19.8 in CMR. The mass spectra of 3a and 3b showed the molecular ion at m/z 730 and 716 and fragment ions at m/z 412, 310,302 and 250 and m/z 302, 296 and 236, respectively. These data strongly supported the

sequence of chromophore→galactouronic acid→glucosamine[5]. Also the mass spectrum of 4 showed the molecular ion at m/z 1218 $(M+1)^+$ and fragment ions at m/z 986, 717, 699, 502, 430 and 412 by FAB-MS spectrum.







The ^{1}H-NMR spectrum of perhydropholipomycin revealed the linkage between compound 2 and 4. The anomeric proton of moenouronic acid at 5.85 ppm appeared as a broad signal coupled with 2'-proton(J=ca. 3.5Hz) at 3.73 ppm and further with a phosphorous (J=6.8Hz). The 2'-proton was coupled with 3'-proton at 5.01 ppm (J=10.0Hz), having 0-carbamoyl group. These observation, in comparison with those of moenomycin[6], indicated that the structure of pholipomycin was assumed to have a linkage between 1' of moenouronic acid and compound 2 through phosphoric ester. Finally, an evidence for the proposed structure of pholipomycin was obtained from FAB-MS and ^{13}C-NMR analyses. The ^{13}C-NMR spectrum by INEPT technique (Fig. 1) revealed the following 63 carbons, C=0(ketone, 200.2), C=0 (carbonyl, x6, 175.1, 175.0, 174.7, 174.5, 173.2, 170.5), SP^2(without proton, x6, 158.9, 149.8, 142.4, 136.6, 131.5, 111.3; with proton, x5, 141.2, 126.2, 125.1, 122.8, 121.4; endomethylene, 109.5), anomeric carbons (x4, 103.5, 102.9, 102.3, 95.3), oxycarbons(x15, 79.9, 78.4, 78.2, 78.1, 75.7, 75.6, 75.3, 75.2x2, 73.7, 73.2x2, 72.9, 71.2, 69.7), oxymethylene carbons(x4, 67.4, 66.8, 60.8, 60.6), amino-carbons(x2, 56.3, 55.8) methylene carbons(x9, 42.3, 40.4, 35.5, 32.9, 32.1 x2, 30.9x2, 27.3), quaternary carbon (36.0), and methyl carbons(x9, 27.9x2, 26.3, 24.1, 23.3, 23.1, 18.0, 16.5, 15.9).

The FAB-MS spectrum of perhydropholipomycin showed the molecular ion species at m/z 1446 $(M+H^+)$, 1468 $(M+Na^+)$, 1484 $(M+K^+)$, and 1491 $(M+2Na^+)$. In conclusion we

propose a molecular formula of $C_{63}H_{96}O_{31}N_4P$ (M.W. 1435) and structure (1) for pholipomycin.

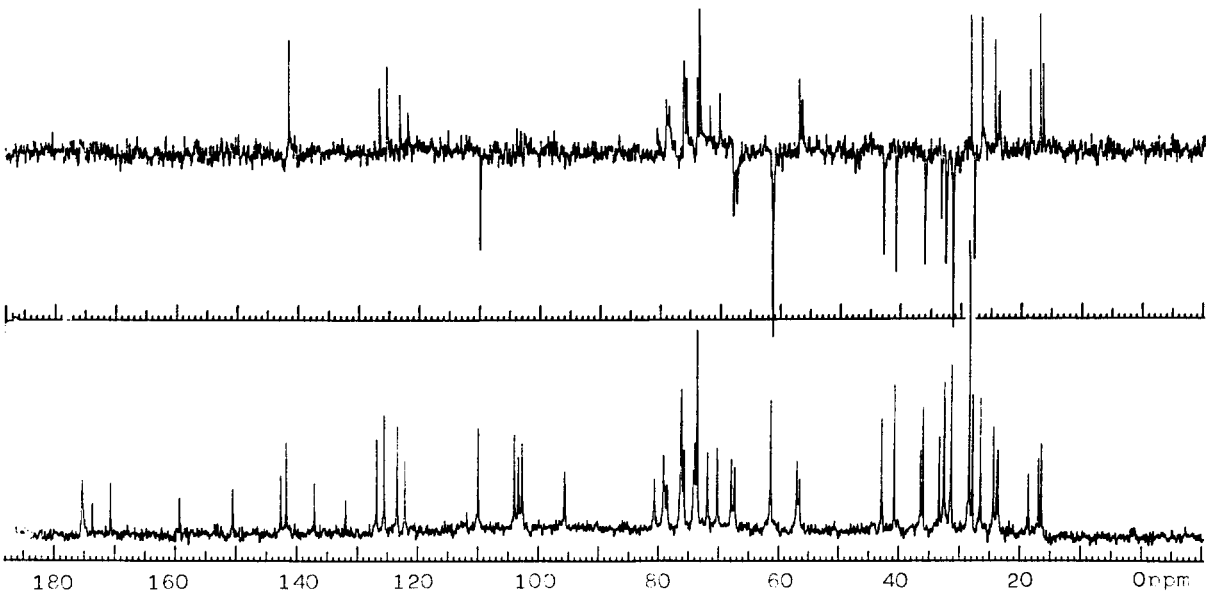


Fig. 1. ^{13}C-NMR (lower, complete proton-decoupled) and INEPT (upper, $\Delta=\frac{3}{4J}$, J was set to 125 Hz) spectra of pholipomycin (1) (D_2O, 70°C)

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