PHOLIPOMYCIN, A NEW MEMBER OF PHOSPHOGLYCOLIPID ANTIBIOTICS

II. PHYSICO-CHEMICAL PROPERTIES AND COMPARISON WITH OTHER MEMBERS OF THIS FAMILY OF ANTIBIOTICS

MAMORU ARAI, RUMI NAKAYAMA, KAYOKO YOSHIDA, MICHIKO TAKEUCHI, SUSUMU TERAMOTO and AKIO TORIKATA

Fermentation Research Laboratories, Sankyo Co., Ltd.
2–58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo 140, Japan

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Physico-chemical characterization of pholipomycin revealed that this antibiotic is a new member of phosphoglycolipid antibiotics. Pholipomycin was differentiated from other members by the products formed on acid hydrolysis as follows: the presence of glucosamine, a 257 nm chromophore and moenocinol-type C_{25} lipids, but the absence of glucose, 6-deoxyglucosamine and glycine.

As described in the preceding paper,[1] pholipomycin was produced by a new species of streptomycete designated as *Streptomyces lividoclavatus*. Fermentation, isolation and brief characterization indicated that the antibiotic is a new member of a family of phosphoglycolipid antibiotics. In the present report, the physico-chemical properties of pholipomycin, as well as comparative studies of the antibiotic with other known members of this family, are described.

Physico-chemical Properties

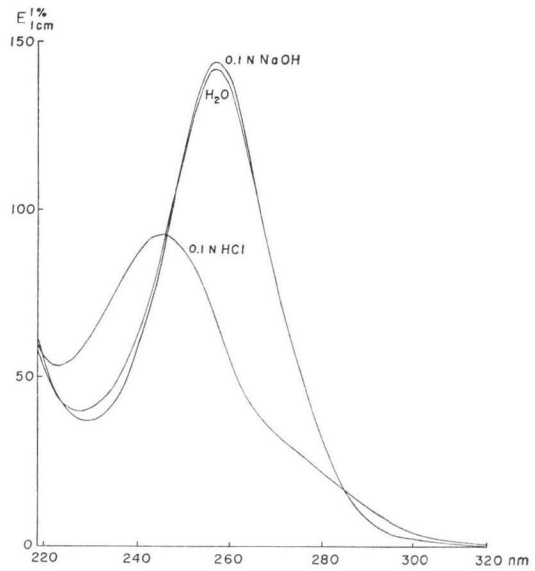
Pholipomycin was obtained as an amorphous powder as its ammonium salt. It is an acidic substance with pKa′ of 4.37 and 9.43 indicating a neutral equivalent of 896. It exhibited no sharp melting point but decomposed with gradual brown coloring at 250°C or higher. The elementary analysis of pholipomycin was as follows: Found: C, 50.15; H, 7.14; N, 5.48; P, 2.33%. Calculated for $C_{56}H_{94}N_3O_{29}P$ (MW 1332): C, 50.47; H, 7.11; N, 5.25; O, 34.82; P, 2.32%. The approximate molecular weight of 5100 (high, probably due to aggregation) was determined by distribution analysis of pholipomycin on Sephadex G–100 in a 0.05 M, pH 7.0, phosphate buffer. A tentative molecular formula for pholipomycin, arrived at by elementary analysis and molecular weight determination mentioned above, is $C_{224}H_{376}N_{20}O_{116}P_4$ (MW 5328). Pholipomycin was optically active, $[\alpha]_D^{20}$ +6.0° (*c* 1, H_2O). The UV spectrum of pholipomycin showed strong absorption at 257 nm ($E_{1cm}^{1\%}$ 142) in H_2O and 258 nm ($E_{1cm}^{1\%}$ 144) in 0.1 N NaOH. The maximal peak shifted to 245 nm ($E_{1cm}^{1\%}$ 93) in 0.1 N HCl (Fig. 1). The infrared spectrum in KBr pellet exhibited strong absorption bands at 3500 (–OH, –NH), 1740 (–C=O), 1650 (–CO–NH–) and 1565 (–CO–NH–) cm^{-1}, as shown in Fig. 2.

Pholipomycin is soluble in water, methanol and aqueous acetone, but insoluble in acetone, ethyl acetate, chloroform and benzene.

Color reactions were positive for TOLLENS, false positive for ELSON-MORGAN, but negative for ninhydrin, BENEDICT, biuret, BIAL's, ferric chloride and anthrone. When heated with pyridine, the ninhydrin reaction changed to positive. The stability in buffer solution is presented in Fig. 3. It was stable in the pH range between pH 4.0 to 10.0 even after heating at 60°C for 120 minutes.

By thin-layer chromatography (ascending method) on Cellulose Chromagram Sheet 6065 and on Silica gel Chromagram Sheet 6060 (both manufactured by Eastman Kodak Co., U.S.A.) using *n*-propanol - 2 N NH_4OH (7 : 3) for developing, Rf values were 0.74 and 0.25, respectively. Pholipomycin was detected by bioautography against *Staphylococcus aureus* FDA 209 P JC-1 or by inspection with short wave UV light.

Fig. 1. Ultraviolet absorption spectra.



Degradation Studies

Acid hydrolysis of pholipomycin under various conditions has yielded various degradation products as with other members of this family of antibiotics. Hydrolysis in 1 N H_2SO_4 at 105°C for 4 hours followed by neutralization with IRA 400 (HCO_3^-) was carried out to determine the sugar components. Paper chromatography of the hydrolysate in the system, pyridine - *n*-butanol - water (4: 6: 3), by the descending method, indicated the presence of glucosamine and small amounts of other slow-moving reducing substances but no glucose nor 6-deoxyglucosamine. The absence of glucose was also confirmed by quantitative analysis by Glucostat.

Fig. 2. Infrared absorption spectrum

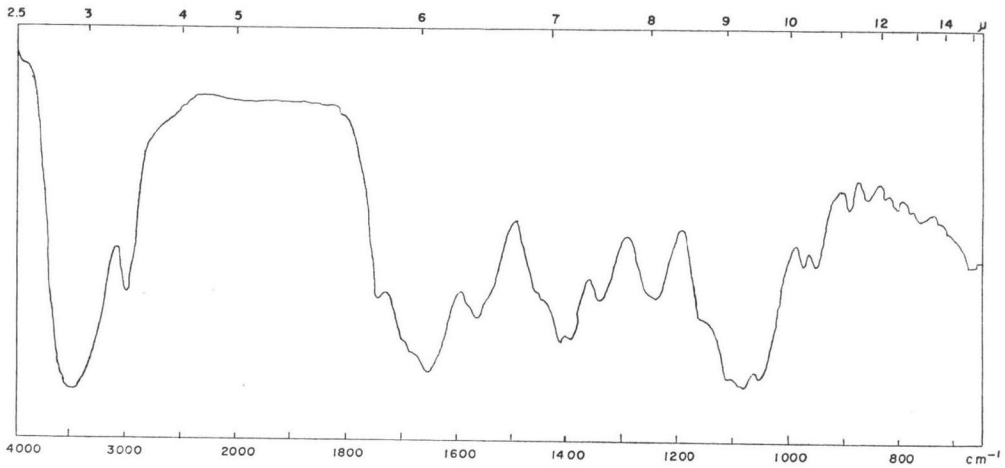
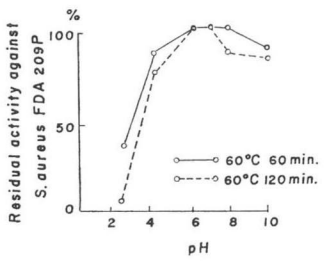


Fig. 3. Heat stability of pholipomycin at various pH values.



Quantitative determination of glucosamine in the hydrolysate (2 N HCl, at 105°C, 3 hours) indicated 18.4% by the method of ELSON-MORGAN[2]. STEIN-MOORE analysis of the

hydrolysate (6 N HCl, 100°C, 22 hours) indicated glucosamine, ammonia and one more ninhydrin-positive substance with an elution volume identical to threonine. This unknown substance, however, was differentiated from threonine by TLC on Chromagram 6060. DNP derivatives of the hydrolysate (6 N HCl, 110°C, 24 hours), had an Rf of 0.07 in the solvent system, benzene-pyridine-acetic acid (80: 20: 2), whereas the Rf value of DNP-threonine was 0.17 under identical conditions. Acid hydrolysis (1 N HCl, 105°C, 35 minutes) of pholipomycin (300 mg) yielded a chloroform-soluble oil (45.6 mg) that could be resolved by preparative TLC (Silica gel plate F_{254}, Merck) to give three lipid components (Rf 0.29, 0.43 and 0.69 in the system benzene - methanol (98.5: 1.5), as in the moenomycin[3] and diumycin antibiotics[4].

As shown in Fig. 4, the NMR spectrum (C_6D_6) of these three lipid components allowed proton assignments identical to those of moenocinol, isomoenocinol and moenocene, respectively. Two proton signals at *ca* 4.9 ppm, characteristic of a terminal methylene, and two methyl protons at *ca* 1.0

Fig. 4. NMR spectra of C_{25} lipids from pholipomycin.

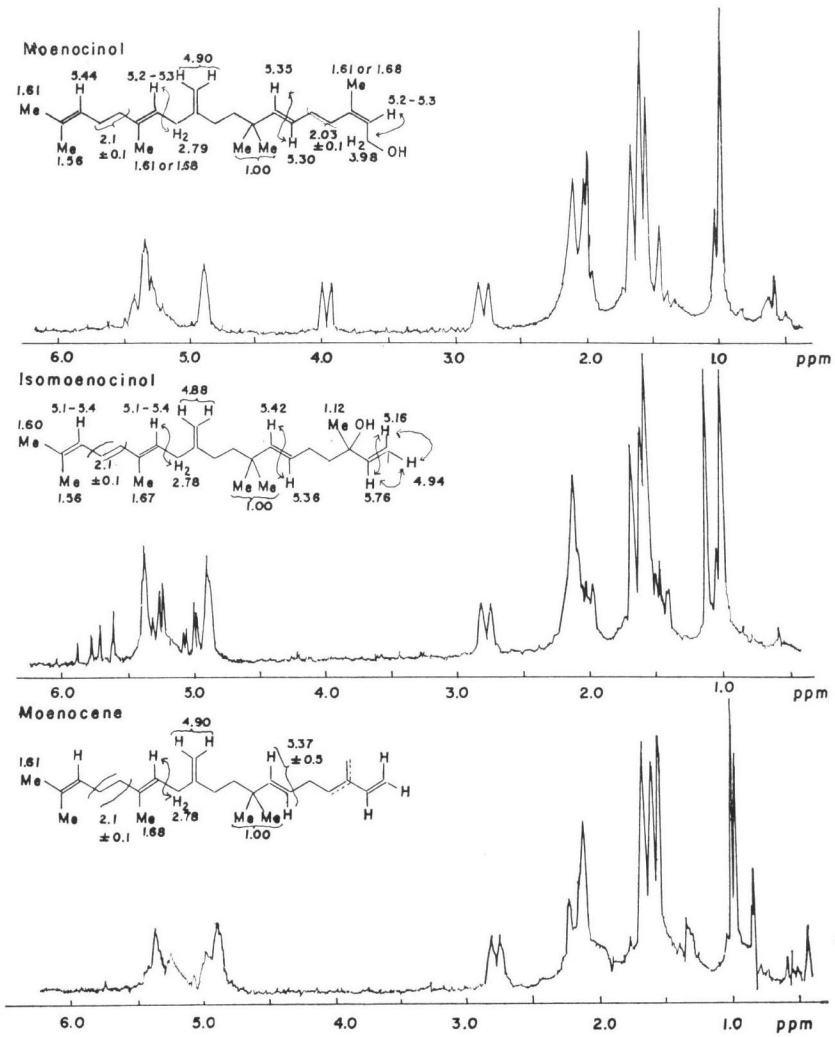


Table 1. Chemical components of phosphoglycolipid antibiotics

Antibiotic	Component					
	Glucosamine	6-Deoxy-glucosamine	Glucose	Glycine	UV 257 nm	Lipid
Moenomycin A	+	+	+	−	+	M*
C	+	+	+	?	+	M
D	+	+	+	−	+	M
E	+	+	+	−	−	M
F	+	+	+	+	−	M
G	+	−	+	−	−	M?
H	+	+	+	+	−	M
8036 RP (Quebemycin)	+	−	?	+	−	D**
11837 RP	+	+	+	+	−	M
19402 RP	+	−	?	−	+	D
Prasinomycin A	+	+	+	+	−	M
B	+	+	+	−	+	M
C	+	+	+	−	−	M
Diumycin A	+	−	+	−	+	D
A′	+	−	−	−	+	D
B	+	−	+	−	−	D
B′	+	−	−	−	−	D
Macarbomycin	+	−	+	−	+	D
Ia	+	−	+	−	−	?
Ib	+	−	+	±	±	?
II	+	−	+	−	±	?
III	+	−	+	−	+	?
Pholipomycin	+	−	−	−	+	M

M*: Moenocinol type, D**: Diumycinol type

corresponding to C-CMe_2C were consistent with those of three lipids from the moenomycins but were distinguished from those from the diumycins in which two terminal methylene and four C-methyls at the same region as mentioned above were indicated.

Comparative Studies

A comparison of the chemical components of the family of the phosphoglycolipid antibiotics[5~14] are shown in Table 1. It appears that the gross structural differences among these antibiotics are the presence or absence of 6-deoxyglucosamine, glucose, glycine and a 257 nm chromophore. Two different sets of isomeric C_{25} lipids, the moenocinol and the diumicinol types, have been obtained from these antibiotics. Pholipomycin was closely related to diumycin A′ in most of its chemical components but clearly distinguished from that in its lipid set. Therefore, pholipomycin was characterized as a new member of this family.

Two more antibiotics of this family prenomycin[15] produced by *S. ambofaciens* and ensanchomycin[16] produced by *S. cinnamonensis* and *S. melanogenes* reported in the patent literature could not be compared with those described in Table 1, because of a lack of detailed description of the chemical components.

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