PHOSPHOGLYCERATE, A BUILDING BLOCK OF MOENOMYCIN

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Dedicated to Professor H. RUSCHIG in honour of his 65 th birthday

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Moenomycin contains phosphorus which is present in diester linkage. During hydrolysis a phosphoric monoester, P_4, is obtained which is converted into the polyhydroxycarboxylic acid D_4 by enzymatic dephosphorylation. The structure of P_4 has been identified as D-phosphoglycerate. The position of the phosphate group is still not clear. D_4 is identical with D-glyceric acid.

The antibiotic moenomycin (Flavomycin®)[1,2,3] isolated from *Streptomyces bambergiensis* and other strains is a surface-active phosphorus-containing glycolipid that contains an UV-chromophore, 2-amino-cyclopentane-dione-1,3[4], a lipid portion, moenocinol[5] a carbohydrate moiety[6] consisting of several sugars (D-glucose, D-glucosamine, D-quinovosamine), and phosphorus linked in ester form. The present study is concerned with the phosphoric ester fraction obtained on hydrolysis of moenomycins A and C.

As reported in a earlier communication[2], phosphorus is present in moenomycin in the form of a diester, stable to alkalis. On hydrolysis with dilute HCl the phosphorus is split off as the phosphoric monoester P_4. The phosphoric esters P_1 (*m*-inositol phosphate), P_2 (ribitol phosphate) and P_3 (anhydroribitol phosphate) previously described are now recognized as being foreign substances, whilst P_4 has been identified as a structural constituent of moenomycins A and C.

Isolation of P_4

P_4 is liberated by hydrolysis of moenomycin with 0.5 N HCl (4 hours at 100°C). It is detected by paper chromatography: *n*-propanol - conc. NH_3 - H_2O, 60 : 30 : 10 (system A), Rf = 0.25; *n*-propanol - pyridine - acetic acid - water, 15 : 10 : 3 : 12 (system B), Rf = 0.44. Blue spots are obtained with phosphate spray reagent.

Besides P_4 the "acid fraction" of the hydrolysate contains a polyhydroxycarboxylic acid, X, (system B, Rf = 0.44, silver nitrate), the structure of which is still unknown.

The HCl hydrolysate is extracted with chloroform to remove the lipid portion. After evaporation of the HCl, the "acid fraction" containing P_4, inorganic phosphate and the unidentified acid X, can be purified on an anion-exchanger column (*e.g.* Dowex-2, Lewatit MP-500) followed by elution with 0.1 N HCl and fractionation *via* the barium salts. Whilst barium phosphate is precipitated directly during neutralization with barium hydroxide, the barium salt of X precipitates only with an excess of ethanol.

Better separation is obtained on a column of Dowex-1. Here, X is first eluted with 5% formic acid, then P_4 with 1 N HCl. After decoloration with activated

charcoal a fairly pure preparation of P_4 is obtained that can be further purified by reprecipitation as the barium salt. On paper chromatography the product is found to be a single compound.

Dephosphorylation and Identification as Glyceric Acid

P_4 is readily dephosphorylated by alkaline phosphatase (calf intestinal). The reaction product is an acid, D_4, which migrates to the anode in high voltage electrophoresis (pyridine acetate buffer at pH 6.5, staining with silver nitrate or periodic acid-benzidine), and has an Rf=0.45 in thin-layer chromatogram (TLC; chloroform - acetic acid - water, 6 : 7 : 1).

For purification D_4 is chromatographed on a silica gel column with the same chloroform - acetic acid - water mixture, remainders of X being removed in the process. The pure D_4 is converted with Dowex-50 (H-form) into the syrup-like free acid. The sodium salt is a white, amorphous, hygroscopic powder. D_4 is a highly polar carboxylic acid, that is soluble in water, methanol and glacial acetic acid, and sparingly soluble or insoluble in other organic solvents. The preparation contains a single component by paper chromatography and paper electrophoresis. The compound produces a strong reaction with periodic acid-benzidine and is gradually stained with ammoniacal silver nitrate solution, a behavior characteristic of polyhydroxycarboxylic acids. No other functional groups are detectable.

Elementary analysis shows the molecular formula to be $(C_3H_6O_4)_n$. There is slight optical activity: $[\alpha]_D^{25}$ $-1.8°$ (c 5, H_2O), but no UV absorbance. The IR-spectrum shows strong bands in the carboxyl and C-O valency region. NMR and mass spectra are not characteristic. Reaction with benzylthiuronium chloride and ω-bromacetophenone does not produce any crystalline derivatives.

Comparisons with a number of hydroxymono- and dicarboxylic acids by paper chromatography, paper electrophoresis and TLC demonstrate that D_4 behaves like D-glyceric acid $C_3H_6O_4$ in the following systems:

1. Paperchromatography: n-butanol - pyridine - water, 4 : 6 : 3 (system C), Rf=0.12; system B, Rf=0.38; *sec.* butanol - acetic acid - water, 4 : 1 : 1 (system D) Rf=0.25.
2. Paper electrophoresis (1,500 V, 75 min.): pyridine acetate buffer pH 6.5, anodic migration 10.5 cm.
3. TLC (Silica gel "Merck"): chloroform - acetic acid - water, 6 : 7 : 1, Rf=0.45.

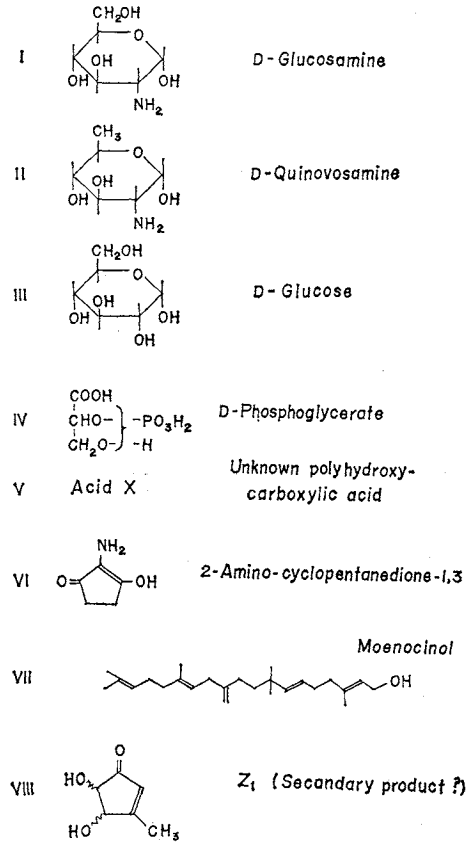
Molecular formula, IR-spectrum, optical activity (D-glyceric acid $[\alpha]_D$ $-1.99°$) and other characteristics are also identical with those of glyceric acid.

Several reactions were carried out and evaluated by paper chromatography to confirm identity. D_4 and glyceric acid both have an Rf=0.12 in system C. Both compounds behaved similarly in subsequent tests. On leaving the acid form to stand for a few days a new band (Rf=0.19) positive to silver nitrate is gradually produced. This probably represents an intermolecular ester for it yields D_4 when heated with acid. Reaction with diazomethane produces a methyl ester (Rf=0.72) that in turn forms a nitrogen-free product of unknown structure (Rf=0.40) with methanol/ammonia instead of the expected amide.

Position of the Phosphate Group

As the dephosphorylation product D_4 has been identified as glyceric acid the phosphoric ester P_4 contained in moenomycin must be considered phosphoglycerate (PG) formed at an intermediate stage in glycolysis. In PG the phosphate group can be linked in ester form at either the 2- or 3-position of the glyceric acid (**IV**, Fig. 1). To clarify its position in P_4 and in moenomycin, cleavage experiments with periodic acid and lead tetraacetate were carried out on intact moenomycin. Unfortunately the reactions did not take a clearly defined course.

Fig. 1. Building blocks of moenomycin.



Attempts were then made to determine the position of the phosphate group in P_4 and in moenomycin. On comparing P_4 with authentic 2- and 3-PG, identical behavior of all 3 compounds was observed in paper chromatography systems A and B, as well as in paper electrophoresis pH 6.5. Consequently no assignment of structure can be made based on these results. It is known that with the aid of an enzymatic assay both 2-PG and 3-PG can be determined separately[7]. The method was applied to P_4 and moenomycin hydrolysate (1 N HCl, 1 and 2 hours at 100°C) with the results obtained in Table 1.

To our surprise the determination revealed a ratio of 86 : 14 for both 3-PG and 2-PG. Authentic 2- and 3-PG hydrolyzed under the same conditions gave similar ratios. Apparently during hydrolysis migration of the phosphate occurs leading to the results observed. These results agree with the observation of BALLOU[8] who found that during hydrolysis of 2-PG with 1 N HCl a balance of 3-PG/2-PG =80.5 : 19.5 is obtained after 30 minutes at 100°C.

Table 1. Enzymatic determination of 2-phosphoglycerate (2-PG) and 3-phosphoglycerate (3-PG) from P_4, moenomycin hydrolysate and after acid hydrolysis of 2-PG and 3-PG. (Percent 2- and 3-PG in reaction mixture)

	Hydrolysis (hours)	2-PG	3-PG
Isolated P_4	—	10	90
Moenomycin, hydrolysate	1	14	86
Moenomycin, hydrolysate	2	13	87
2-PG, hydrolysate	1	15	85
3-PG, hydrolysate	1	12	88

Thus it is not possible by this method to determine the position of the phosphate group in P_4 or in moenomycin. However, the test does furnish additional proof for the structure of P_4 as phosphoglycerate which due to the stereospecifity of the enzymes apparently must be present in the D-form. The position of the

phosphate needs to be clarified in further experiements.

Discussion

Through the identification of P_4 as D-phosphoglycerate one of the two phosphoric ester linkages in moenomycin has been established. However, since the phosphoric acid is present in the molecule as the diester, the question as to the second ester link remains open. No other phosphoric monoester has so far been detected in hydrolysates.

Phosphoglycerate has been identified as still another structural element of moenomycin arising from primary microbial metabolites. The building blocks of moenomycin known at the present time are listed in Fig. 1. They include D-glucose (**III**), D-glucosamine (**I**) and D-quinovosamine (**II**). The last has only been detected in microorganisms to date[9,10]. Similarly the UV-chromophore, the "amino-reductone" 2-amino-cyclopentane-dione-1,3 (**VI**) has only been detected as a constituent of metabolic products from streptomycetes (flavensomycinic acid[11], limocrocin[12]). The lipid portion of moenomycin., *i. e.* the C_{25}-lipid alcohol moenocinol (**VII**), is very unusual. Z_1 (3-methyl-4,5-dihydroxy-cyclopentene-2-one-1) (**VIII**) formed on prolonged acid hydrolysis (5 hours, 100°C) seems to be a secondary product. Experiments are being conducted in order to discover the linkages of the structural elements.

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