

MINERAL INDUCED FORMATION OF SUGAR PHOSPHATES *

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Abstract. Glycolaldehyde phosphate, sorbed from highly dilute, weakly alkaline solution into the interlayer of common expanding sheet structure metal hydroxide minerals, condenses extensively to racemic aldotetrose-2,4-diphosphates and aldohexose-2,4,6-triphosphates. The reaction proceeds mainly through racemic erythrose-2,4-phosphate, and terminates with a large fraction of racemic altrose-2,4,6-phosphate. In the absence of an inductive mineral phase, no detectable homogeneous reaction takes place in the concentration- and pH range used. The reactant glycolaldehyde phosphate is practically completely sorbed within an hour from solutions with concentrations as low as 50 μM ; the half-time for conversion to hexose phosphates is of the order of two days at room temperature and pH 9.5. Total production of sugar phosphates in the mineral interlayer is largely independent of the glycolaldehyde phosphate concentration in the external solution, but is determined by the total amount of GAP offered for sorption up to the capacity of the mineral. In the presence of equimolar amounts of rac-glyceraldehyde-2-phosphate, but under otherwise similar conditions, aldopentose-2,4-diphosphates also form, but only as a small fraction of the hexose-2,4,6-phosphates.

1. Introduction

This paper describes the outcome of an investigation that has its origin in observations previously made in the two participating laboratories. In Zürich, it had been shown (Müller *et al.* 1990) that the aldolization of glycolaldehyde phosphate in basic aqueous solution produces diastereoselectively rac. hexose-2,4,6-triphosphates in high yield and, in the presence of formaldehyde under otherwise identical conditions, mostly pentose-2,4-diphosphates with rac. ribose-2,4-diphosphate as the major product (Figure 1). In the La Jolla laboratory, on the other hand, the observation had been made (Arrhenius, 1987; Kuma *et al.*, 1989) that common expanding sheet structure metal hydroxide minerals (Table I) pose a high affinity for prebiotically relevant anions, inorganic phosphate among them. Combination of these complementary observations led to a joint experimental study on the aldolization chemistry of glycolaldehyde phosphate in the presence of double layer metal hydroxide minerals. It was to be expected that charge driven sorption of glycolaldehyde phosphate in the interlayers of such sheet structure minerals would accelerate and direct the aldolization to sugar phosphates. Such a behavior would convey additional prebiotic significance to this type of sugar phosphate formation.

* This paper represents communication No. 15 in the series, 'Chemistry of α -Aminonitrile' from the Zürich group; for communication No. 14 see Pitsch and Eschenmoser (1994).

TABLE I
Continued

Mineral name	Interlayer		Anion	Generic name
	Rhombohedral	Hexagonal		
Meixnerite	Mg	Al	OH ⁻	MgAl hydroxide hydroxide
Valerite	Mg	Al	(Fe, Cu)S	MgAl hydroxide Fe, Cu sulfide
Koenerite	Mg	Al	Na ₂ MgCl ₆	MgAl hydroxide NaMg chloride
Wermelandite	Mg	(Al, Fe)	(CaMg)(SO ₄) ₂	MgAl Fe hydroxide CaMg sulfate

¹ Hydrotalcite (Figure 7) with varying proportions in solid solution of other pyroaurite minerals, such as pyroaurite proper, iowaite, chlormagaluminitite, 8.85 Å SO₄ hydrotalcite 3R, and berthierine, occurs as a common product of submarine basalt weathering (Thomassin and Touray, 1979, 1982; Crovisier *et al.*, 1983; Drits *et al.*, 1985, 1987; Zamarreño *et al.*, 1989).

² Pyroaurite structure deformed (monoclinic), due to large size of divalent cation.

³ A variety of ferroferric hydroxide silicates with 1:1 serpentine structures, e.g. greenalite (Bailey, 1980) minnesotaitite (Ahn and Buseck, 1989), occur as important iron ore minerals in the Archean iron formations; they are probably metamorphic (dehydrated-dehydroxylated and partially ordered) green rust silicates.

⁴ Structural comparisons are given in papers by Allmann and collaborators (e.g. Allmann and Lohse, 1966; Allmann, 1968, 1970; Pausch *et al.*, 1986; Frondel (1941); Ingram and Taylor (1967); Taylor (1969, 1973); Bish (1980); Bish and Livingstone, 1981).

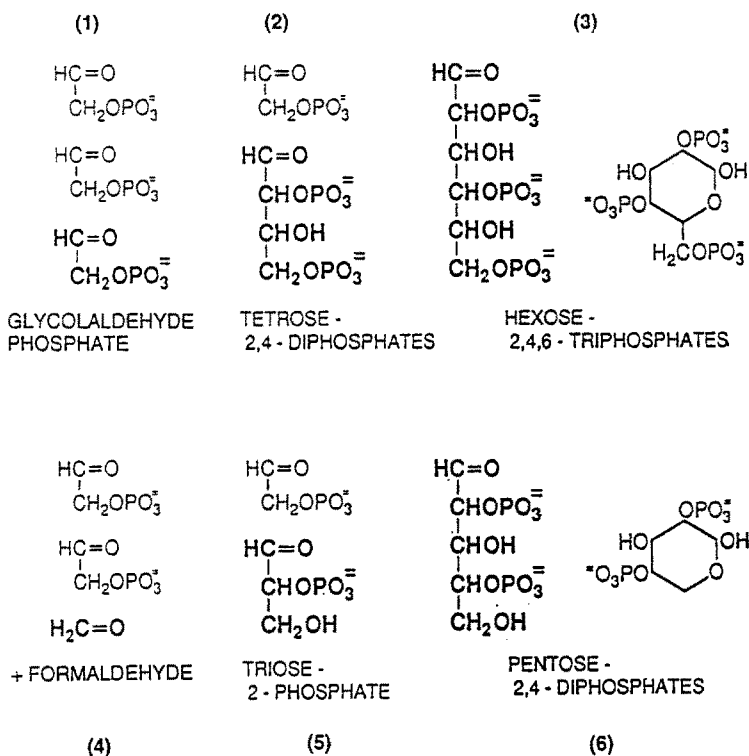


Fig. 1. Aldolization of glycolaldehyde phosphate (1) in alkaline aqueous solution to rac.-tetrose-2,4-diphosphates (2) and rac.-hexose-2,4,6-triphosphates (3) in high yield. A characteristic product distribution is shown in Figure 3. Under identical conditions, except for the additional presence of formaldehyde (4), triose phosphate forms as intermediate product (5), reacting further with glycolaldehyde phosphate to pentose-2,4-diphosphates (6), with rac.-ribose, 2,4 diphosphate as the dominant diastereoisomer. For a characteristic product distribution, see Figure 2b. (From Müller *et al.*, 1990.)

2. Formose Reaction as a Link in Biopoesis

Oligomerization of formaldehyde, the formose reaction, discovered by Butlerow (1861) and further investigated early by Loew (1886) and Fischer (1888) was for a long time considered the only abiotic pathway to the biologically important carbohydrates. The complex, coupled aldol-retroaldol reactions involved, together with aldo-keto isomerization, have been the subject of numerous studies (see e.g. the comprehensive reviews by Ruckert *et al.* (1965), Mizuno and Weiss (1974), and Harsch *et al.* (1977)). These concurrent reactions result in a large number of linear and branched aldoses and ketoses and their derivatives (Fig. 2a).

The complexity of this product mixture has been taken as an argument against a potential role of the formose reaction in molecular evolution (Miller, 1987; Shapiro, 1988). Other reservations against invoking it as a possible link in biopoesis are the

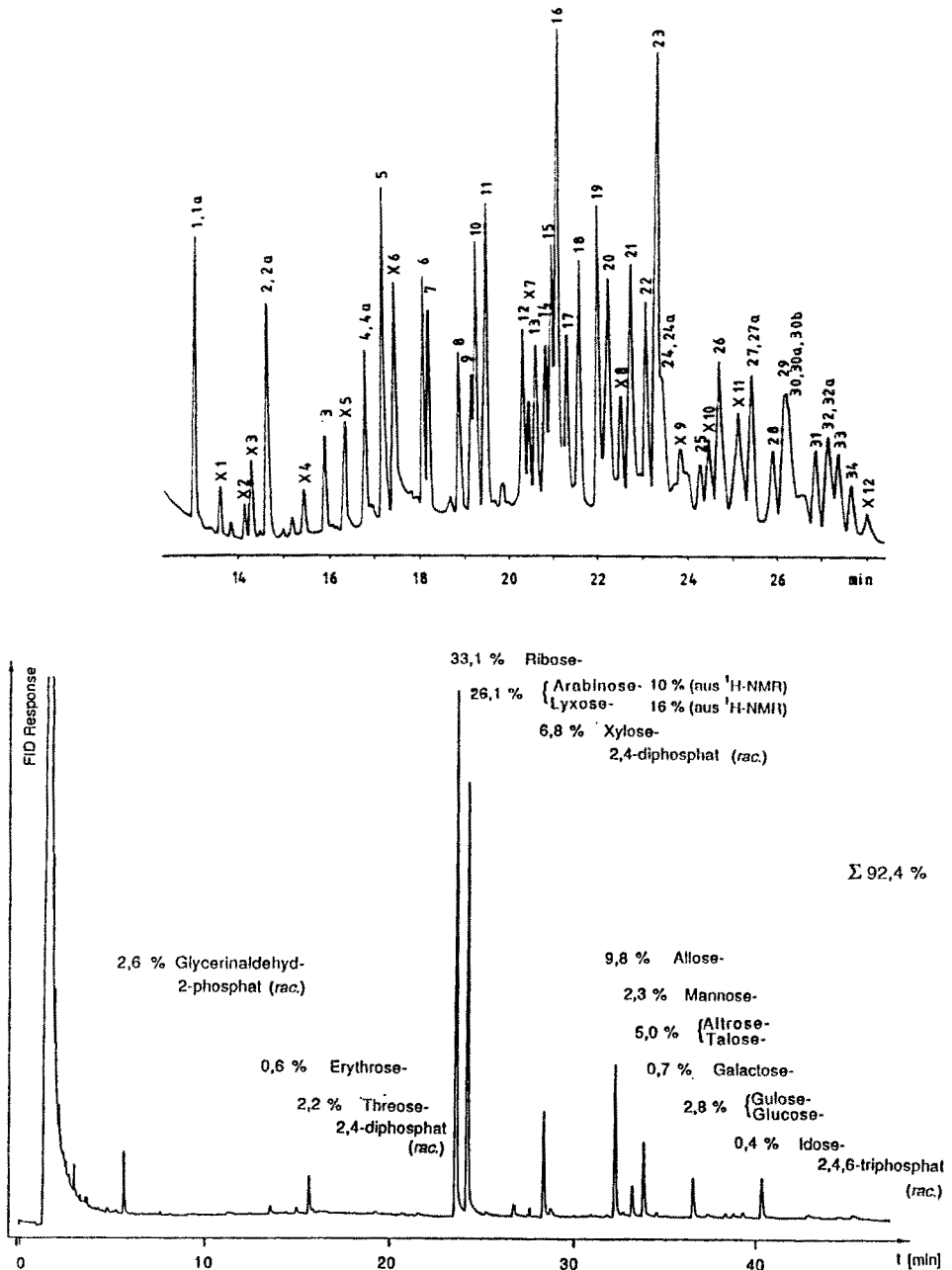


Fig. 2. (a) Gas chromatogram of trifluoroacetylated forms of the n-butyl-oximes of the carbohydrates arising in a *formose reaction*. Peaks 8 and 14 are the ribose derivatives. The dominant production of ketoses and threose derivatives is notable; peak 5 is due to threose; 16 and 23 to threo-pent-2-ulose. From Decker, Schweer and Pohlmann (1982). (b) Gas chromatogram of products from the *glycolaldehyde phosphate reaction* with formaldehyde after enzymic dephosphorylation, reduction, and acetylation, showing the selective production of pentoses, primarily ribopyranose (Müller *et al.*, 1990).

high alkalinity required in the homogeneous reaction, the vanishing net production rate at concentrations less than centimolar, and the instability of the product sugars under the reaction conditions used.

Several attempts have been made to heterogeneous catalysis of the process under conditions expected to occur naturally. Early results are due to A. Cleve-v. Euler and H. v. Euler (Euler and Euler, 1906) using calcium carbonate, with ribulose as the sole discernible product. Mayer and Jäschke (1960), confirming these results found that glycolaldehyde, the initiator of the autocatalytic cycle, and dihydroxyacetone, formed by aldo-keto isomerization, appear as intermediates early in this reaction. The aldolization of glycolaldehyde with and without formaldehyde as a co-reactant has been extensively investigated by Pfeil and Schroth (1952), Pfeil and Ruckert (1961), Ruckert *et al.* (1965), and Harsch *et al.* (1984); see also Noe *et al.* (1994).

Gabel and Ponnampereuma (1967) investigated the aldolization of formaldehyde with microcrystalline synthetic corundum (Al_2O_3) as catalyst at pH 7.4 and demonstrated the formation of pentoses, tetroses and trioses, mainly as ketoses, in yields of the order of 20–40 percent, using high concentration of formaldehyde (0.33 M). Yields of 3–5 percent were obtained with the more commonly occurring low temperature minerals kaolinite and hydromica (illite), and at lower concentration of formaldehyde (centimolar). In millimolar formaldehyde glycolaldehyde seemed to be formed in low yield but no sugars could be detected. Similar results were obtained with kaolinite by Harvey *et al.* (1972).

In a companion paper to Gabel and Ponnampereuma (*op. cit.*), Reid and Orgel (1967) showed that carbonate apatite ($\text{Ca}_5[\text{F}|\text{PO}_4, \text{CO}_3\text{OH}]_3$) in contrast to the inactive carbonate-free mineral, on boiling with 0.5 M formaldehyde gave yields of up to 40 percent sugars, with glycolaldehyde, trioses, tetroses, pentoses (culminating after about 12 h) and hexoses (about 24 h). With centimolar formaldehyde the yield was a few percent, and at millimolar concentration any sugar formation was below the detectability level (1–2 percent). Reid and Orgel expressed the view that these and related experiments do not provide plausible models for the prebiotic accumulation of sugars, because of the high concentration of reactants needed and the rapid decomposition of the sugars formed.

3. The Glycolaldehyde Phosphate Aldomerization Reaction

A selective process that eliminates the objection against indiscriminate distribution of products that are unstable was demonstrated by Müller *et al.* (1990). They showed that if the reactant glycolaldehyde is in its phosphorylated form, aldomerization becomes directed toward a small number of product molecules (Fig. 2b and 3). Specifically, reaction of 0.08 M glycolaldehyde phosphate (GAP) in 2 N sodium hydroxide at room temperature for a week led to the formation of a product mixture containing (racemic) hexose-2,4,6-triphosphates in approximately 80 per-

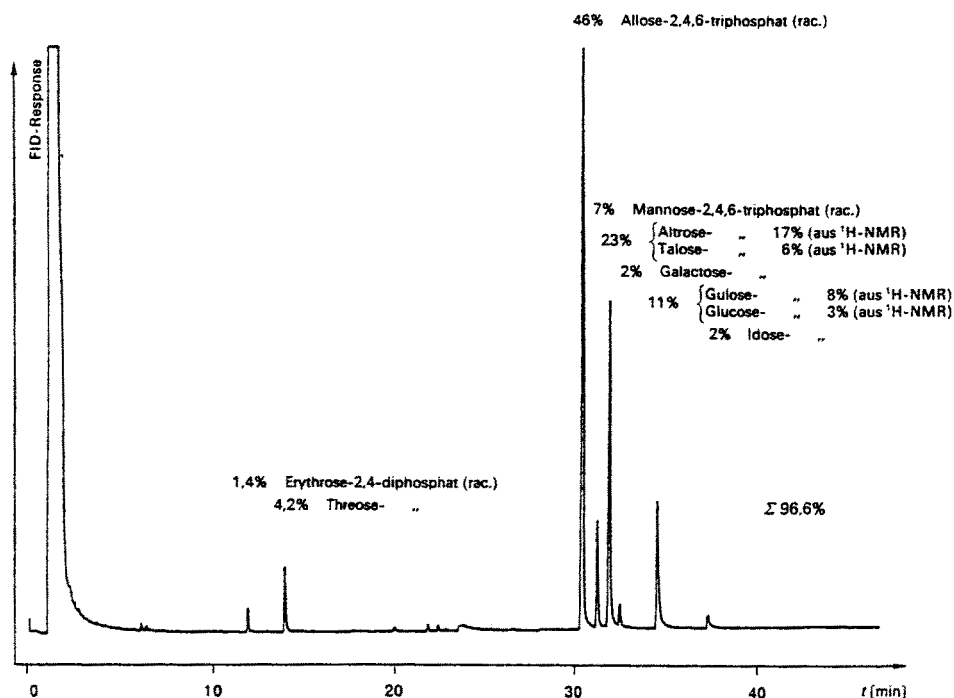


Fig. 3. Gas chromatogram of dephosphorylated, reduced, and acetylated mixture of sugar phosphates, produced by the glycolaldehyde phosphate aldolization reaction in alkaline solution (0.08 M, 2N NaOH, room temperature, one week) in approximately 80 percent yield. (From Müller *et al.*, 1990.)

cent overall yield,* and with the diastereoisomer allose-2,4,6-triphosphate as the dominant component.

Under the same reaction conditions, but in presence of half an equivalent of formaldehyde, glycolaldehyde phosphate aldolizes to a (racemic) mixture of glyceraldehyde-2-phosphate, aldotetrose- and aldopentose-2,4-diphosphates and aldohexose-2,4,6-triphosphates (overall yield approximately 45 percent) in which the aldopentose family strongly dominates and in which ribose-2,4-diphosphate is the main component of the entire sugar phosphate mixture (Figure 2b). These results demonstrate the existence of a facile route to stable sugar phosphates, selectively favoring a single aldohexose diastereoisomer, while blocking entirely the formation of ketoses that dominate the formose reactions.

The glycolaldehyde pathway in the version described above still retains some of the shortcomings of the formose reaction as a possible prebiotic route to the

* In a later experiment (Pitsch, 1993), it was observed that the reaction proceeds in less alkaline (pH 11) but more concentrated solution (0.5 M) slowly, but with even higher diastereoselectivity; after 28 weeks at room temperature a hexose-2,4,6-triphosphate fraction had formed in 51 percent yield beside 15 percent of tetrose-2,4-diphosphate, with the allose derivative dominating the hexose fraction to an extent of 60 percent (Figure 4).

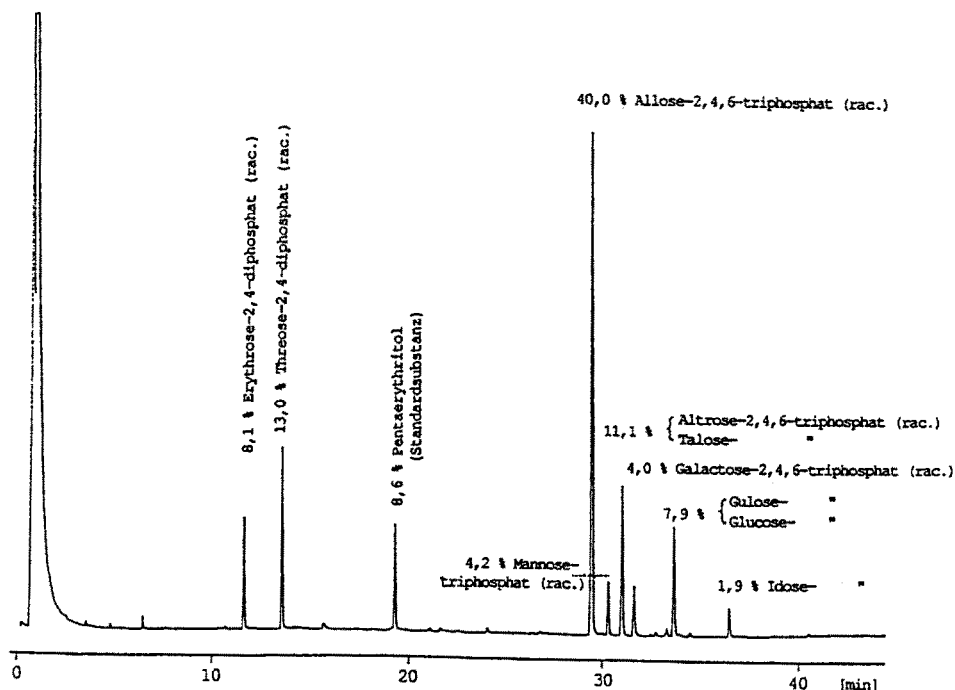


Fig. 4. Gas chromatogram of a reaction mixture obtained in an investigation (Pitsch, 1993) of the minimal reaction conditions for the GAP aldolization in solution to allow the formation of rac.-hexose-2,4,6-phosphates. In this series of experiments, it was observed that, with 5 mM GAP at pH 11.5 and 9.7, only tetose phosphates were formed after 5 weeks at room temperature, in yields of 29 and 2% respectively. In order to increase the imperceptible rate of hexose phosphate formation, the concentration of GAP was increased hundredfold to 500 mM and the reaction carried out at pH 11.0 and room temperature for 28 weeks at which time the reaction had ceased and hexose phosphates had formed in 51% yield. The gas chromatogram shows the composition of the reaction mixture obtained after dephosphorylation, reduction and acetylation. Diastereoselectivity for the allose derivative, typical for the reaction in solution, is even more pronounced under these conditions, with the allose derivative forming about 60% of the total rac.-hexose-2,4,6-triphosphate mixture.

sugar building blocks of ribonucleic acids (high alkalinity of the medium and relatively high concentrations of the reactants). Its major advantage in this context, however, is the potential susceptibility to kinetic and structural control by naturally occurring surface active minerals. This is due to the fact that the pathway is not as in the formose reactions, starting from and leading to neutral compounds, but involves negatively charged species throughout. Most importantly, a charged starting material in a condensation process leading to charged oligomers has the potential for reaching high concentration under natural conditions by ion exchange processes on external and internal solid surfaces.

4. Mixed Valence Double Layer Metal Hydroxide Minerals

Adsorption on solid surfaces or internal sorption in hydrous minerals was proposed as an escape from the dilemma of dilution and activation barriers in the problem of biopoesis by Goldschmidt (1945) and Bernal (1949), who suggested that clay minerals might have been particularly effective. A large number of studies have demonstrated the usefulness of clay minerals as catalysts and mediators of reactions in organic synthesis (see e.g. review by Laszlo, 1987). There are also cases where such sheet structure silicates have been shown specifically to accelerate reactions relevant to the problems of biopoesis. Such studies were initiated by Katchalsky and coworkers (Paecht-Horowitz *et al.*, 1970) using montmorillonite to oligomerize aminoacyladenylates to oligopeptide derivatives of 5' AMP. Oligomerization, possibly more relevant in nature, of underivatized amino acids in heating and drying cycles with clay minerals have since been demonstrated by several authors (see e.g. review and results in Bujdák *et al.*, 1995). Notable success in oligomerization of activated mononucleotide imidazolides was achieved by Ferris *et al.* (1989), Ferris and Ertem (1992, 1993), and Kawamura and Ferris (1994), using montmorillonite as promoter.

Anionic species of potential prebiological importance; carbonate, ferrocyanide, phosphates and phosphate esters, generally carry multiple negative charge, and are therefore efficiently sorbed on or in positively charged minerals. Among several types of such natural solids that have the capacity to sorb anions and which, therefore, are complementary to the cation-binding clay minerals, are the double layer metal hydroxide minerals (Table I). These minerals are natural analogs to a larger class of synthetic compounds, the mixed-valence double-layer hydroxides, first explored by Feitknecht (1942). Their structure can be understood as deriving from simple divalent cation hydroxides $M^{2+}(\text{OH})_2$, typified by $\text{Fe}(\text{OH})_2$ and including minerals such as $\text{Mg}(\text{OH})_2$, brucite, $\text{Mn}(\text{OH})_2$, pyrochroite, and $\text{Ca}(\text{OH})_2$, portlandite.

In these simple hydroxide structures (Figure 5) the octahedra of hydroxyl ions, surrounding the core metal ions, share edges to form continuous, electrically neutral hexagonal sheets, held together in close packing by hydrogen bonding and van der Waals forces. Replacement of a fraction of the divalent ions with trivalent ions such as Fe^{3+} , Al^{3+} or Cr^{3+} (Figure 6) introduces excess positive charge on the hydroxide sheets, forcing them to separate and to attract charge balancing anions, which together with water molecules form a highly concentrated, about 10 M two-dimensional quasi-solution as an interlayer in the resultant double layer structure. Multivalent anions such as S^{2-} , SO_4^{2-} , CO_3^{2-} , PO_4^{3-} and $\text{Fe}(\text{CN})_6^{4-}$ tend to enter the interlayer in some cases together with partially charge balancing cations, forming minerals such as valeriite with $(\text{Cu}, \text{Fe})\text{S}^-$ or wermlandite with $(\text{Ca}, \text{Mg})\text{SO}_4$ interlayers (Allmann, 1970; Rius and Allmann, 1984). The interlayer water may be replaced by other polar molecules such as glycerol (Bish, 1980; Drits *et al.*, 1985). In a thermally dehydrated state the interlayer is open to diffusion of gas molecules,

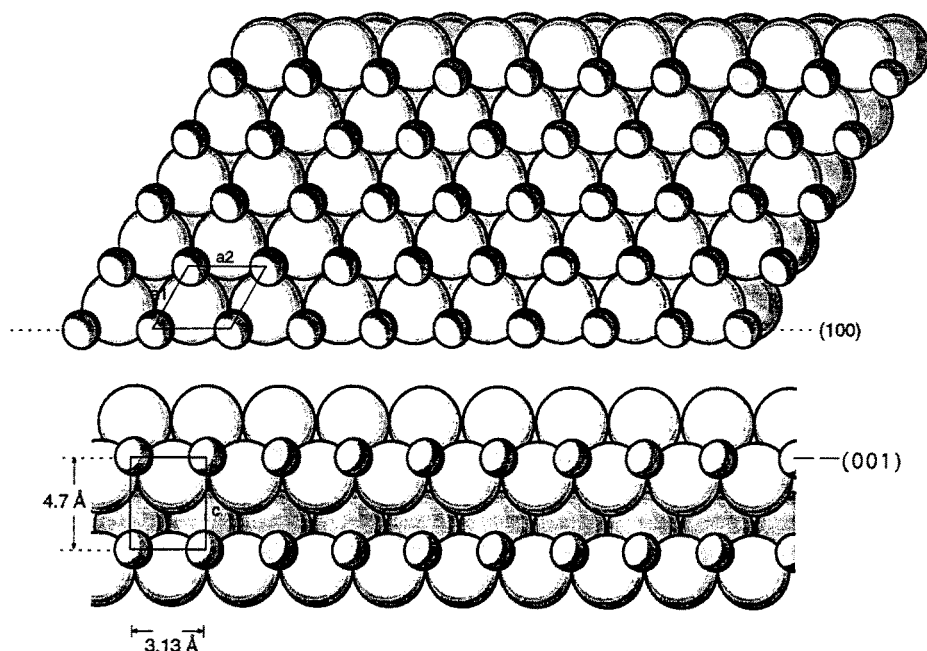
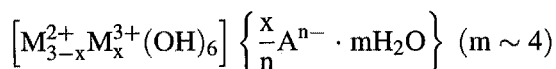


Fig. 5. Layer arrangement in the brucite, $[\text{Mg}(\text{OH})_2]$ type structure, from which the mixed valence double layer hydroxide structure can be conceptually derived by partial replacement of some of the divalent- with trivalent cations. Large spheres represent hydroxyl ions ($r(i) = 1.33 \text{ \AA}$), small spheres divalent metal cations such as Mg, Mn, Fe, Co, Ni or Zn ($0.6 < r(i) < 0.8 \text{ \AA}$). (a) Section in the (001) basal plane through the cation layer in the hydroxide sheet (section marked (001) in Figure 5b). The hexagonal unit cell is outlined in lower left of Figure 5a,b. (b) Cross section through two metal hydroxide sheets along the plane (100) marked in Figure 5a.

and the exposed large internal surface area can be used to catalyze gas reactions in industrial applications (Pinnavaia, 1983; Pinnavaia *et al.*, 1989; Reichle, 1985, 1986; Kwon *et al.*, 1988).

The general formula of the double layer hydroxide minerals can be written as:



where M^{2+} and M^{3+} are (small) divalent and trivalent cations, and the square brackets denote the main hydroxide layer (see also Figure 6). A^- and A^{2-} are mono- and divalent exchangeable anions in the hydrated interlayer, denoted by curly brackets.

As an example, the Mg-Al-hydroxide mineral, hydrotalcite (Figure 7), if crystallized from or anion exchanged in a solution with chloride as dominant anion and

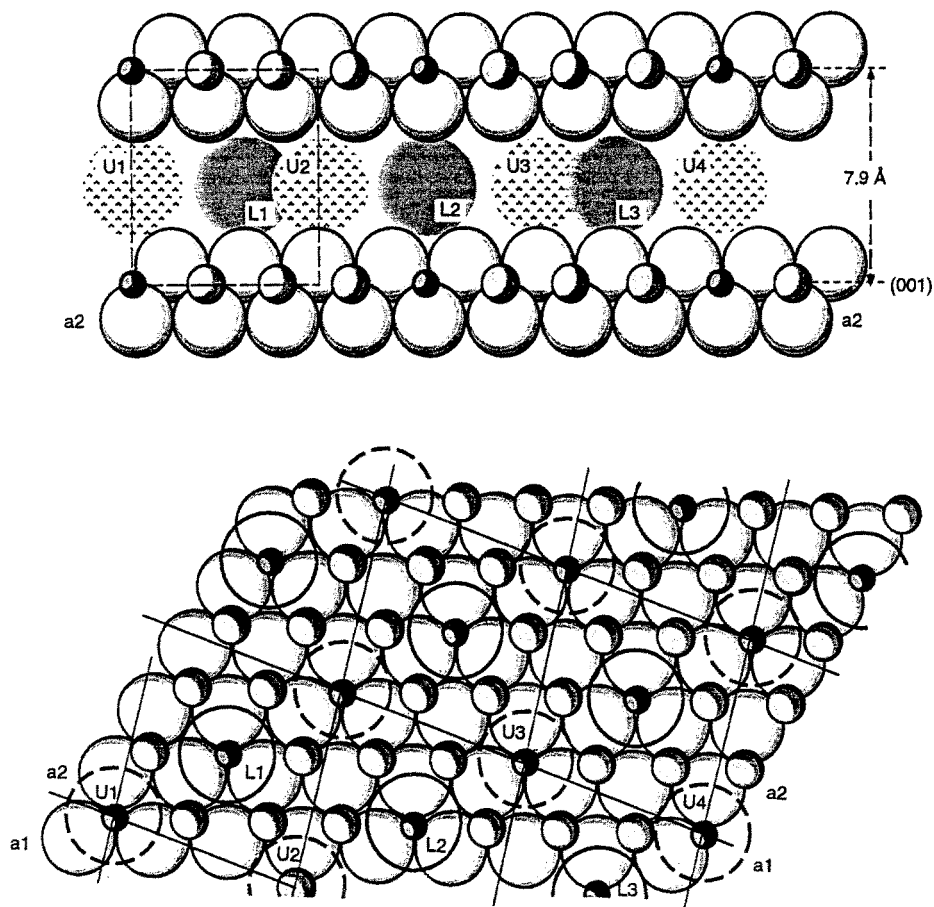


Fig. 6. (a) At partial substitution ($1/4$) of the divalent ions (small grey spheres) in the brucite structure (Figure 5) with trivalent metal ions (black spheres) the latter create excess positive charge on the hydroxide sheets. In the resulting mixed valence structure the layers separate and admit water molecules together with anions such as, in this case, chloride ion, balancing the excess positive charge on the metal hydroxide layer. The figure shows a cross section along the plane (100) through two metal hydroxide sheets and one interlayer. Water molecules in the interlayer (about three to four per anion) are omitted from the graph. The components of the interlayer form a concentrated two-dimensional solution and move by diffusion. The (here ideally) ordered positions of the interlayer chloride anions are at the center of the unit positive charge cells in Figure 6b, in this case adjacent to the counteranions in the main hydroxide layer also shown in idealized ordered positions. The distribution shown thus represents a lowest energy state of order of the metal hydroxide layer and an ordered, 'frozen' hydrous interlayer. (b) Section in the hydroxide sheet plane, with projection of univalent (chloride) ions from the interlayer (Figure 6a) onto the (ordered) cation layer in the central plane (001) of the hydroxide sheet. Chloride ions (U), balanced by positive charge on the upside of the lower sheet (cut along its midplane in this graph), are shown as full circles (L), balanced by charge on the downside of the upper sheet in Figure 6a, are projected as dashed circles. Each of the cells of the two-dimensional monoclinic lattice contains one unit of positive charge; one such cell is thus capable of accommodating a univalent interlayer anion.

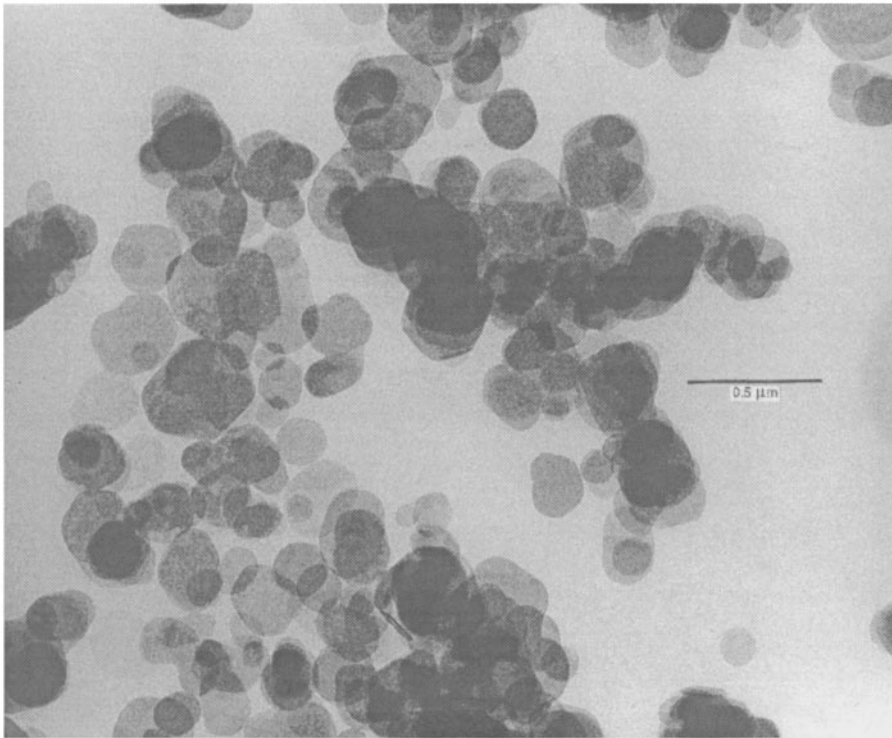
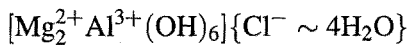


Fig. 7. Thin platy crystals of hydrotalcite (magnesium aluminum hydroxide) a common member of the double-layer hydroxide mineral group (Table I) with prototype structure shown in Figure 6. Hydrotalcite is the mineral most extensively used in the studies of concentration and aldomerization of glycolaldehyde phosphate reported here.

with proportions $Mg^{2+}:Al^{3+}$ chosen as 2:1, would have an end member composition.



1 formula weight of the mineral thus has 1 equivalent of positive charge excess on the main layer. The range of compositions of the main metal hydroxide layer extends from the minimum divalent:trivalent cation ratio 2:1 indicated above, to 3:1 or higher with corresponding variation in the concentration of positive charge on the layer.

In nature the double layer hydroxides are most conspicuous in hydrothermal or metasomatic deposits where relatively large crystals may form. Modern techniques have revealed the common occurrence of the minerals in microcrystalline form as weathering products on marine basalt (Crovisier *et al.*, 1983; Drits *et al.*, 1985; Zamarreño *et al.*, 1989); as precipitates in saline lakes (Drits *et al.*, 1987) and,

as green rust, in anoxic soils (Keller, 1948; Taylor, 1980). Green rust, ferrous hydroxide is considered to have been particularly common in the dysoxic Archean ocean, giving rise by oxidation-dehydration to the widespread deposits of $\text{Fe(II)O} \cdot \text{Fe(III)}_2\text{O}_3$ (magnetite), (Arrhenius *et al.*, 1993). Magnetite formation by bacteria has also been invoked as a source for these deposits (Vali and Kirschvink, 1989).

Sorption isotherms for various anions in hydrotalcite were determined by Miyata (1983) and Sato *et al.*, (1986, 1988); the intercalation of cyanide, hexacyanoferrate, phosphate, sugar-aldol- and alkyl phosphates and nucleotides was demonstrated by Kuma *et al.* (1989). Considering their inferred widespread abundance and their catalytic efficiency in gas reactions (Pinnavaia, 1983; Pinnavaia *et al.*, 1989; Reichle, 1985) the double layer hydroxide minerals appeared as prominent candidates for the concentration function in the glycolaldehyde phosphate aldomerization.

5. Experimental Procedures

5.1. MINERAL SYNTHESIS

Double layer metal hydroxide mineral analogs in chloride form were prepared by a technique similar to Kuma *et al.*, 1989. As sources for the di- and trivalent metal cations forming the main hydroxide layer were used solutions of the corresponding metal chlorides, 0.1 M each, mixed in the desired proportions; under standard conditions applied here, with a ratio of divalent to trivalent ions 3:1. The mixed metal chloride solution (25 ml) was injected through a capillary orifice into a continually stirred 250 ml solution of 0.5 M NaCl at a rate of 5 ml per hour. The recipient solution was maintained constant at pH 9.0 by a pH-stat (Fisher, component models 380, 383, 395), by injecting 1.0 M NaOH in response to the addition of the acidic metal salt solution. Standard preparations were annealed in their source solution at 95° for 12 h to achieve crystal perfection and growth to the size range 0.2–0.8 μm (Figure 7). Structural parameters were measured by X-ray diffraction, using a modified General Electric XRD-5 diffractometer, with monochromatized Cu $K\alpha$ radiation. A typical diffractogram of the product is shown in Figure 8. Reflections (00l) from the layer structure, indicative of the width of the reactive interlayer, were enhanced by preferred orientation of the platy crystals on the flat carrier surface; α -alumina or glass.

5.2. SORPTION EXPERIMENTS

The sorption experiments were undertaken with glycolaldehyde phosphate (GAP) as sodium salt, synthesized by the procedure described by Müller *et al.* (1990) as the calcium salt, followed by replacement of calcium with sodium on Na^+ resin. Exchange- and aldomerization experiments with GAP in concentrations between 0.1 and 80 mM were carried out in an oxygen free nitrogen or argon atmosphere.

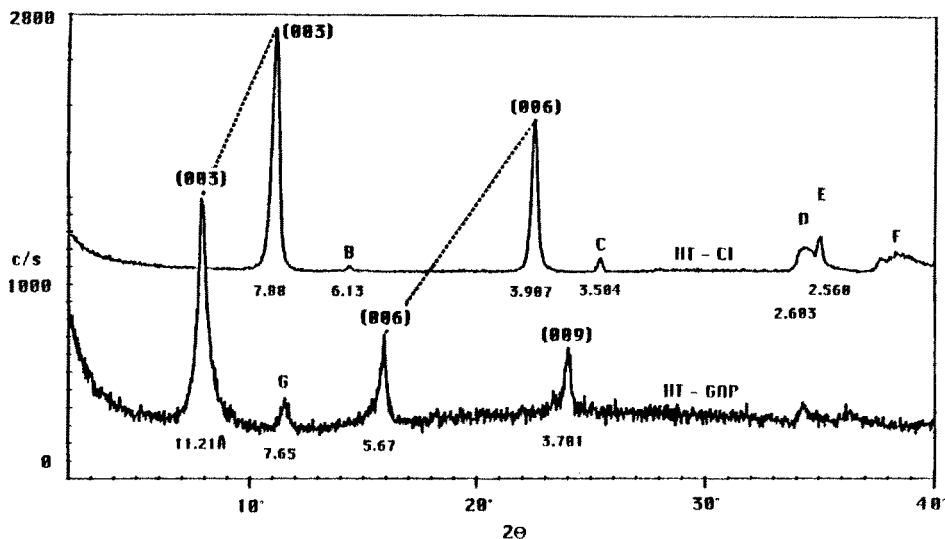


Fig. 8. X-ray powder diffractograms (Bragg angle 2θ vs. photon count) from the double layer magnesium aluminum hydroxide mineral hydrotalcite in two different interlayer anion modifications; chloride (upper) and glycolaldehyde phosphate (lower graph). Miller indices for the reflections are shown in parentheses; the dimension of the corresponding interplanar spacing in Ångström units below the curves. Of particular significance in the present context is the expansion of the aqueous interlayer at exchange of the small chloride ion with the larger glycolaldehyde phosphate anion, providing an accurate measure of the size of the hydrated interlayer complex. The reflection (003) from the basal plane measures the width of the double layer, consisting of the metal hydroxide sheet and the anion containing interlayer, as shown in Figure 6. (The reason why this basal spacing appears as a third order reflection is the lateral stacking displacement in sequence A-B-C-A requiring three layers to identify a unit cell.) In the mineral-chloride complex (upper graph) this spacing (003) is 7.9 Å, the sum of the effective diameter (3.1 Å) of the chloride-water cluster, and the metal hydroxide sheet thickness (4.8 Å; cf. Figure 6). This spacing also gives rise to second (006) and third (009) order reflections. At exchange of the interlayer chloride ion with glycolaldehyde phosphate (lower curve) the basal spacing expands from 7.9 to 11.2 Å, indicating an occupancy width of the GAP complex in the interlayer of 6.4 Å (11.2–4.8), the axial dimension of the GAP ion as determined by molecular modeling (Chem 3D Plus, 1990). In the air-dried state (equilibrium with 50% saturated water vapor at room temperature) the 3–4 water molecules associated with the GAP anions thus appear to be accommodated between and in the plane of these, thus not contributing to the width of the interlayer. This apparent configuration is similar to the one accurately determined by single crystal x-ray structure analysis for the carbonate complex (Allmann, 1970). Condensation of GAP in the interlayer to form tetrose-2,4- and hexose-2,4,6-phosphates does not lead to further expansion of the air-dried structure, indicating an orientation of the hexose phosphate ring coplanar with the mineral sheet. The condensation reaction was carried out in aqueous suspension of the mineral; the water content of the reactive interlayer could, in this state, possibly be higher, and the layer separation thus larger during the reaction. The weak reflections B and C come from small amounts of other phases formed at the low temperature synthesis of the magnesium aluminum hydroxide chloride mineral. B is the (020) reflection from boehmite, γ -Al(OOH); C possibly the strongest reflection from koenigite which has the hydrotalcite structure with an exchangeable interlayer of $[\text{Na}_2 \text{Mg Cl}_6]^{2-}$ (Allmann *et al.*, 1968). D is an unresolved complex containing the third order basal reflection (009); E and F contain the pyramid reflections (012) and (015), which are (purposely) weakly expressed by preferred orientation of the crystallites favoring basal reflections (001). G is the basal reflection from a small fraction of the mineral complex, either with interlayer carbonate ion as a contaminant or with orthophosphate formed at partial dephosphorylation of GAP. The tielines between the basal reflections in the two forms of the mineral emphasize the expansion features at replacement of interlayer chloride ion with GAP.

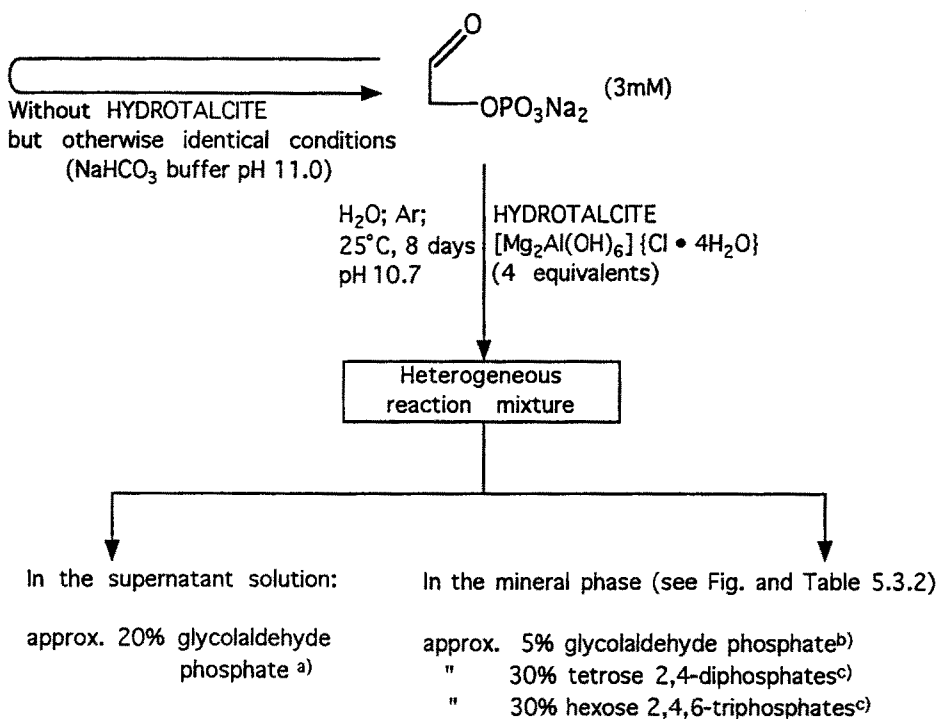
Stock solutions of sugar phosphates were in this set of experiments kept sterile by 1 mM sodium azide.

Phosphate esters were assayed in the solid minerals by microanalysis of carbon, using a Perkin-Elmer Model 2400 CHN Analyzer, and in solution by oxidation to orthophosphate in sulfuric-nitric acid, followed by neutralization and colorimetric determination as the phosphomolybdate blue complex. Individual aldols and aldol phosphates were determined by compound specific techniques, described below and in Pitsch (1993).

5.3. MINERAL INDUCED ALDOLIZATION OF GLYCOLALDEHYDE PHOSPHATE

5.3.1. *Exploratory Experiments: Aldolization to (Rac.) Hexose-2,4,6-Triphosphates*

To investigate the effects of reaction conditions, a standard procedure was adopted where the concentration of glycolaldehyde phosphate in solution was kept constant at 3 mM and the temperature at 25°C, while variables were the amount of mineral (12.5–400 mg; 0.044–1.408 mmol), pH of the solution (9.5–11) and reaction time (2–280 days). Under the standard conditions, hydrotalcite (chloride form; Mg/Al ratio 3:1) was weighed in under nitrogen and suspended in 13.1 to 14.2 ml of water. To the suspension were added 0.2 ml (44 μ mol) of 0.22 M glycolaldehyde phosphate (sodium salt) from a stock solution with concentration controlled by $^1\text{H-NMR}$. The stock solution also contained a known amount of pentaerythritol (PE) as internal standard for yield determination by GC. Aliquots with varying pH were prepared by adding 0.1–1.0 ml (0.01–0.1 mmol) of 0.1 M NaOH (total volume 14.2 ml). The suspensions were kept under nitrogen and shaken about five times daily. All reactions were carried out in duplicate. After completion of the reactions, pH of the supernatant was measured and the reaction mixture shaken with 10 ml of cation exchange resin Amberlyst IR 120 (H^+ -form) until all of the mineral had been dissolved. The sugar phosphates were then converted into the corresponding dephosphorylated and peracetylated polyols by the method described by Müller *et al.* (1990) and Pitsch (1993) and analyzed by GC. The proportions of different diastereoisomers were found to be highly reproducible, while the variation in yield, determined from the ratio between the reaction products and the PE standard, was of the order of $\pm 5\%$. The results from these exploratory experiments are summarized in Figures 9–12 and 15. Some of the experiments were selected to be accompanied by mineral free control reactions, using an NaHCO_3 buffer in place of the mineral. The workup procedure for these solutions was identical to the one used for the supernatant from the mineral suspension experiment. GC analysis of the products from these homogeneous reactions indicated the formation of traces ($< 5\%$) of tetrose derivatives in those experiments that were carried out at pH above 10 and for 7 days or longer, but in no case were any detectable traces of hexoses derivatives found.



a) determined by $^1\text{H-NMR}$

b) from $^1\text{H-NMR}$ spectrum of crude product

c) determined by GC (after derivatization) using pentaerythritol as internal standard

Scheme 1

5.3.2. Preparative Experiments: Aldolization to (Rac.) Hexose-2,4,6-Triphosphates

In the format of the standardized reactions, two experiments were carried out on a preparative scale, one with and one without (Section 3) a mineral component. Both experiments were carried out under otherwise identical conditions (Scheme 1).

For the mineral experiment, a suspension was prepared of 2.50 g (8.8 mmol) hydrotalcite in 646 ml degassed water to which was added 25 ml 0.1 N NaOH solution and 39 ml (2.26 mmol) of a 0.058 M solution of Na-glycolaldehyde phosphate (concentration determined by $^1\text{H-NMR}$). The suspension was kept at

TABLE II

Mineral induced aldolization of glycolaldehyde phosphate (GAP) under conditions outlined in Scheme 1. The percentage yield of sugar phosphates is shown as determined by $^1\text{H-NMR}$ of the products and of the free sugars after dephosphorylation, and by GC after dephosphorylation, reduction and acetylation. Separate analyses were made of the mixture of reaction products, and of the isolated hexose triphosphate and tetrose diphosphate fractions. The distribution of product diastereoisomers is shown graphically in Figure 9.

	Product mixture based on GC	Isolated hexose triphosphate fraction based on:		
		$^1\text{H-NMR}$	$^1\text{H-NMR}$ (free sugars)	GC
Allose-	22.0	24	24	26.0
Mannose-	4.0	4	4	3.0
Altrose-		45	45	
Talose- } Galactose-	54.0 4.5		13 2	} 55.0 4.5
Gulose-		5	8	
Talose- } Idose-2,4,5- triphosphate	11.5 4.0		4 2	} 9.5 3.0
Total	100.0		100	100.0
Isolated tetrose diphosphate fraction				
Erythrose-	67.5	72		69.0
Threose-2,4-	32.5	28		31.0
Total	100.0	100		100.0

25° under argon and shaken five times daily. After 8 days, the colorless supernatant (pH 10.7) was decanted from the now yellow solid, which was analyzed separately. The supernatant was treated with 30 ml ion exchange resin (Amberlyst IR 120; H^+ -form) and the eluent concentrated to a final volume of 150 ml.

A 14.9 ml aliquot of the supernatant was treated with ion exchange resin (Dowex 50 W \times 4, Na^+ -form). Lyophilization yielded 55 mg of a colorless powder, which at $^1\text{H-NMR}$ analysis (200 MHz) in D_2O showed only the signals of glycolaldehyde phosphate. Comparison with the PE standard showed the amount of GAP in the supernatant to be about 20% of the starting material. GC (after derivatization) indicated the presence of tetrose diphosphates with a yield of 1%; no hexoses were detected.

The solid material containing the mineral was suspended in 250 ml water and shaken with 240 ml resin IR 120 (H^+ -form). After one hour when all solid had been dissolved, the resin was removed by filtration and the solution concentrated to

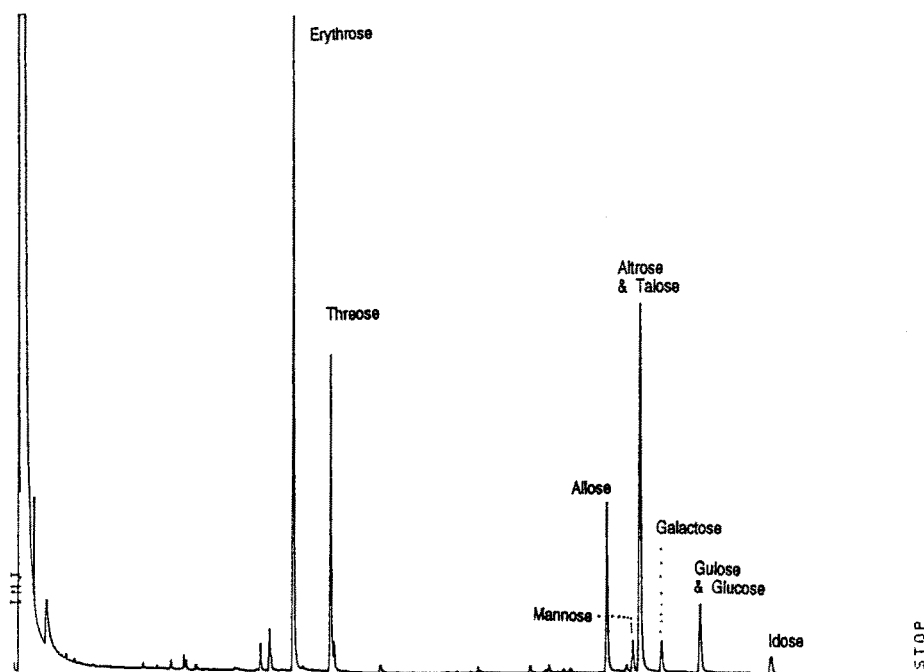


Fig. 9. Mineral-induced sugar phosphate formation from glycolaldehyde phosphate (GAP), sorbed from dilute (3 mM) solution at pH 10.7 into the interlayer of the double layer hydroxide mineral hydroxalcalcite. The two aldotetrose phosphates, dominated by rac.-erythrose-2,4-diphosphate, appear as intermediate products, reacting further with GAP to form hexose-2,4,6-phosphates. In contrast to the homogeneous reaction, which proceeds at measurable rate in this pH range only at two orders of magnitude larger concentration of GAP (Figure 4) and gives mainly allose triphosphate, the mineral induced reaction shown here produces mainly the rac.-altrose-2,4,6-triphosphate diastereoisomer. (GC after dephosphorylation, reduction and acetylation of the reaction product mixture derived from the mineral phase.)

a volume of 150 ml. An aliquot of 14.9 g was treated with Dowex 50 W \times 4 (Na^+ -form) yielding 53 mg of an off-white powder at lyophilization. In the complex ^1H -NMR spectrum (300 MHz, D_2O) could be recognized only the H-C(1)-doublet of erythrose-2,4-diphosphate (5.16 ppm, $J = 2.5$ Hz), the broad H-C(1) singlet of one anomeric form of altrose-2,4,6-triphosphate (5.15 ppm) and the corresponding doublet of β -allopyranose-2,4,6 triphosphate (4.97 ppm, $J = 7.9$ Hz). FAB-MS (neg. mode) showed the signal of hexose-2,4,6-triphosphates at $m/z = 419$ (40%) and of tetrose-2,4-diphosphates at 279 (42%). The results of GC-analysis [after reduction, dephosphorylation and acetylation according to Müller *et al.*, 1990] are shown in Table II and Figure 9; three independent runs against the internal PE standard indicated the amounts of tetrose-2,4-diphosphates and hexose-2,4,6-triphosphates to be 30% each.

The material from the remaining solution derived from dissolution of the mineral phase separated by anion exchange chromatography on Dowex 1 \times 8 resin (HCO_3^- -form; 1 \times 10 cm column), the products were eluted with a linear gradient (0–1 M) of $\text{HNET}_3 \cdot \text{HCO}_3^-$ buffer (1000 ml) and the individual fractions analyzed by tlc (BuOH/ H_2O / AcOH 5:3:2; staining with anisaldehyde/ H_2SO_4 / AcOH / EtOH 5:5:1:90). The fractions which contained the tetrose-2,4-diphosphates (R_f 0.15; yellow-green) were pooled, treated with 40 ml IR 120 resin (H^+ -form), concentrated to 15 ml, treated with 5 ml of the same resin, concentrated to 5 ml and treated with 3 ml Dowex 50 W \times 4 resin (Na^+ -form). Lyophilization gave 62 mg tetrose-2,4-diphosphates (sodium salts) as a colorless powder (yield 21%, determined by GC with PE as internal standard; purity ca. 90% determined by $^1\text{H-NMR}$; the proportions of diastereoisomers are given in Table II). The fractions containing the hexose-2,4,6-triphosphates (R_f 0; dark blue) were evaporated to dryness and treated with Dowex 50 W \times 4 (Na^+ -form). Lyophilization gave 79 mg hexose-2,4,6-triphosphates (sodium salts) as a colorless powder (yield 24%, determined by GC with PE as internal standard; the distribution of diastereoisomers is also shown in Figure 9).

Tetrose-2,4-diphosphate fraction:

- $^1\text{H-NMR}$ (300 MHz, D_2O): 3.70–4.20 ppm (complex multiplet, 4.5 H); 5.00–5.55 ppm (region of anomeric protons, 1.0 H, with the doublets of erythrose-2,4,-diphosphate at 5.15 ppm ($J = 2.48$ Hz, 0.6 H) and of threose-2,4-diphosphate at 5.10 ppm ($J = 4.83$, 0.2 H)); other signals at 5.30–5.55 ppm (0.2 H), 6.47 ppm (0.14 H) and 9.22 (0.14 H).
- $^{13}\text{C-NMR}$ (75 MHz, D_2O): The signals of erythrose-2,4-diphosphate at 68.5 ppm (CH_2 , d, $J = 5$ Hz); 72.6 (CH , t, $J = 7.5$, C(3)); 79.1 ppm (CH , d, $J = 6.0$ Hz); 91.53 ppm (CH , s); and the signals of threose-2,4-diphosphate at 72.2 ppm (CH , t, $J = 8.2$ Hz); 79.4 ppm (CH , d, $J = 6.5$ Hz) and 92.0 (CH ,s).
- FAB-MS (neg. mode): m/z 279 (68%, M-1); 177 (50%); 97 (52%); 79 (100%).
- GC (after derivation): 14.45 min (66.0%, erythrose), 16.22 (29.6%, threose).

Hexose-2,4,6-triphosphate fraction:

- $^1\text{H-NMR}$ (300 MHz, D_2O): 3.80–4.60 (complex multiplet, 6 H); 4.80–5.45 (signals of the anomeric protons, 1 H, see Table III).
- FAB-MS (neg. mode): $m/z = 419$ (100%, M - 1); 177 (46); 97 (50); 79 (57).
- GC (after derivatization): 29.61 min. (26.2%, allose), 30.82 (2.5%, mannose), 31.22 (56%, altrose + talose), 32.21 (3.5%, galactose), 34.09 (8.7%, gulose + glucose), 37.50 (3.1%, idose).

Dephosphorylation of the hexose triphosphate fraction:

- A solution of 32 mg of the hexose triphosphates (from ion exchange chromatography) in 10 ml $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ -buffer (pH 9.8, 0.1 M) was digested for 14 h at room temperature with 0.4 ml alkaline phosphatase solution (Boehringer-Mannheim; 3 mg in 10 ml diethanolamine/HCl buffer, 0.1 M, pH 9.8). After exchange, first with IR 120 (H^+ -form) and then with Amberlyst A 27 (OH -form) the solution was lyophilized, yielding 9.8 mg hexoses (75%)

TABLE III

Source of anomeric proton signal ^a	[ppm]	^J 1,2 [Hz]	integral ^c
α -Allopyranose-	5.21	3.19	0.06
β -Allopyranose-	4.96	8.07	0.16
α/β -Altropyranose- ^b	5.13	br.s	0.26
β/α -Altropyranose- ^b	5.10	br.s	0.18
α -Mannopyranose-	5.28	1.75	0.03
α -Glucopyranose- 2,4,6-triphosphate	5.39	3.92	0.04

^a for assignments see Müller *et al.* (1990).

^b assignment unknown.

^c sum of the anomeric proton region def. as 1.00 H.

TABLE IV

Source of anomeric proton signal ^a	[ppm]	^J 1,2 [Hz]	Integral ^b
α -Allopyranose	5.08	3.7	0.04
β -Allopyranose	4.82	8.2	0.19
β -Gulopyranose	4.80	8.4	0.06
β -Altropyranose	5.05	1.4	0.18
α -Altropyranose	4.92	3.4	0.13
α -Altrofurano	5.19	2.5	0.08
β -Altrofurano	5.22	4.5	0.04
α -Mannopyranose	5.01	1.8	0.01
β -Glucopyranose	4.58	7.9	0.02
β -Talopyranose	4.75	1.0	0.04
α -Talofurano	5.21	1.8	0.03
β -Galactopyranose	4.52	7.9	0.01
β -Idopyranose	5.01	1.0	0.01

^a assignment by comparison with authentic samples.

^b sum of anomeric proton region def. 1.00 H.

as colorless oil. The proportions of diastereoisomers (Table IV) were deduced from the ¹H-NMR spectrum (for details see Müller *et al.*, 1990).

– ¹H-NMR (300 MHz, D₂O;) 3.15–4.30 (complex multiplet, 6 H); 4.50–5.40 (signals of the anomeric protons, 1.0 H, see Table IV).

As indicated in the introduction of this section, a reference experiment was carried out, analogous to the one reported above but without a mineral component. For this purpose a solution was prepared from 25.8 ml degassed water with 1

TABLE V

Aldolization of GAP in the presence of an equimolar amount of rac. glyceraldehyde-2-phosphate (each 2 mM) and hydrotalcite (HT). The table shows reaction conditions and results of GC-analysis of reaction mixtures derived from mineral phase

Time (days)	pH	Ratio (GAP + C ₃)/HT (mol/mol)	Recovery of C ₃ compounds (%)	Product yields in % (based on GAP)*		
				C ₄	C ₅	C ₆
3	9.7	0.17	69	41	6	6
3	9.7	0.17	62	40	6	6
6	9.7	0.17	45	30	6	6
6	9.7	0.17	49	36	7	6
7	11.0	0.20	16	17	7	14
7	10.9	0.20	26	20	7	19
7	11.3	0.20	16	12	7	23
7	11.4	0.20	18	14	7	19
7	11.7	0.20	10	12	7	19
7	11.5	0.25	11	15	7	20
8	9.3	0.25	30	14	2	2

* determined by GC with pentaerythritol as internal standard.

C₃, glyceraldehyde-2-phosphate; C₄, aldotetrose-2,4-diphosphates; C₅, aldopentose-2,4-diphosphates; C₆, aldohexose-2,4,6-triphosphates.

ml 0.1 N NaOH and 1.6 ml of 58 mM glycolaldehyde phosphate, sodium salt (0.093 mmol; concentration determined by ¹H-NMR), and kept under argon at 25° and pH 11.0 for 8 days. The colorless solution was treated consecutively with IR 120 (H⁺-form) and Dowex 50W X 4 (Na⁺-form) and lyophilized. The ¹H-NMR spectrum showed only the signals of glycolaldehyde phosphate. With addition of PE as internal standard to an aliquot, ¹H-NMR spectrometry showed that more than 95% of the original glycolaldehyde phosphate had been recovered. GC (after derivatization) indicated that no more than about 4% of tetrose diphosphates had formed, and no hexose-2,4,6-triphosphate could be detected.

5.3.3. Aldolization of Glycolaldehyde Phosphate in the Presence of (Rac.) Glyceraldehyde-2-Phosphate or Formaldehyde (Exploratory Experiments)

These experiments were carried out, worked up and characterized as described in 5.3.1. A stock solution was used, consisting of a 1:1 mixture of glycolaldehyde phosphate and glyceraldehyde-2-phosphate, the latter prepared by the method described in Müller *et al.* (1990) and with PE as GC-reference. In all experiments, the concentrations of the two sugar phosphates was 2 mM each and all reactions were carried out at 25 °C. Experimental details and results are giv-

TABLE VI

Aldolization of glycolaldehyde phosphate (GAP) together with formaldehyde and hydrotalcite (HT)

GAP (mM)	H ₂ CO (mM)	Time (days)	pH	GAP/HT (mol/mol)	Yield (%)			
					C ₃	C ₄	C ₅	C ₆
10	90	18	9.2	0.33	5	9	1	3
3	80	8	9.3	0.25	30	14	2	2
3	800	8	8.9	0.25	34	15	2	3
3	800	7	9.6	0.25	9	18	2	7
3	80	7	10.4	0.25	6	10	4	12

For key to C₃-C₆, see Table V.

en in Table V. The diastereoisomer distribution of the tetrose and hexose phosphates formed was comparable to that found in corresponding experiments without glyceraldehyde-2-phosphate; the distribution within the (small) pentose-2,4-diphosphates fraction typically was 41% ribose-, 46% arabinose- + lyxose- and 13% xylose-diphosphates.

In a set of analogous experiments, formaldehyde was used instead of (rac.) glyceraldehyde-2-phosphate. Only minute amounts of (C-5) sugars were formed under the conditions used. Reaction conditions and results are presented in Table VI.

5.3.4. Stability of D-Allose-2,4,6-Triphosphate Under the Aldolization Conditions

29 mg (0.1 mmol) hydrotalcite (chloride form) were suspended in a solution made from 0.3 ml 0.1 N NaOH, 5.0 mg (10 μ mol) D-allose-2,4,6-triphosphate (sodium salt) (Müller *et al.*, 1990) and 7.7 ml water; pH of the suspension was 10.6. After 8 days at 25 °C the solid was separated from the supernatant by centrifugation. GC analysis of the derivatized products, using PE as internal standard showed the presence of 6.8 μ mol allose-2,4,6-triphosphate (68% of the original amount of allose triphosphate) in the supernatant solution while the solid yielded 3 μ mol (30%) allose-2,4,6-triphosphate and 0.015 μ mol (0.15%) altrose-2,4,6-triphosphate. No other sugar phosphates could be detected in the gas chromatograms.

6. Exchange Dynamics

The accessibility of the interlayer and the isotherms for sorption of phosphate esters from dilute solution is found to vary substantially between different minerals belonging to the double layer hydroxide structural type. As shown in Figure 10, complete exchange of chloride ion for glycolaldehyde phosphate (GAP) in the anionic interlayer takes place in a few hours in hydrotalcite (magnesium aluminum

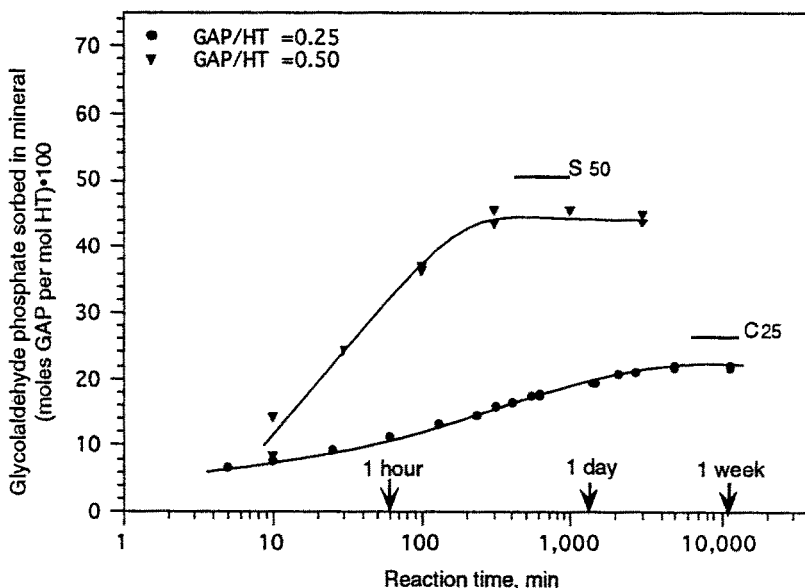


Fig. 10. Two cases of sorption rate for glycolaldehyde phosphate into the interlayer of hydrotalcite. In one experiment (upper curve), equimolar amounts of GAP, initially in 0.3 mM solution, and mineral were exposed in suspension, hence the mineral was offered sufficient amounts of GAP to reach saturation (at molar ratio 0.5 of GAP/HT, marked S₅₀ in graph). This limit is approached within 5 percent after about 3 h. The other experiment (lower curve) employed a molar ratio GAP/HT of 0.25, precluding saturation. Equilibrium was approached within a few percent of this ratio, indicating practically complete uptake of GAP by the mineral but required about 3 days at which time aldolization is about half completed (cf. Figure 16). The first experiment was carried out at pH 8.5, the second at pH 10.6.

hydroxide) at room temperature. A similar behavior was observed for glycerol phosphate (approximately 50% each of -1 phosphate and DL-2-phosphate), and in the isostructural MnAl-, CoAl-, ZnAl-, and Fe(II)Fe(III) double layer hydroxides. In contrast, sorption of GAP was, under the conditions used, imperceptible in the structurally analogous minerals nickel aluminum and nickel ferric hydroxide which, however, expand readily with strongly polar anions such as dodecylsulfate, or higher charge density anions such as $\text{Fe}(\text{CN})_6^{4-}$.

The rate of diffusion of anionic compounds, into or out of the interlayer, is dependent on the total charge and charge distribution on the molecule. Although GAP is replaced by CO_3^{2-} ion from strong (0.1 M) solution at pH 8 in a few hours, such exchange requires several weeks after condensation of the glycolaldehyde phosphate to (rac.) hexose-2,4,6-phosphates in the interlayer (Section 9). Aside from charge, the exchange rate is also dependent on molecular size; while a small molecule such as GAP saturates the interlayer in a few hours from a 0.3 mM solution, polyanions such as ~ 100 -mers of polyglutamate and polyaspartate require

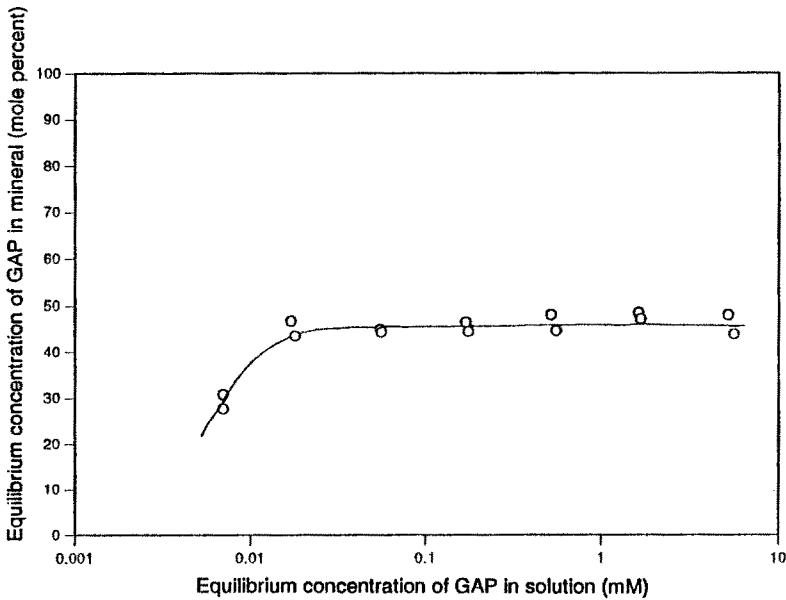


Fig. 11. Sorption isotherm for glycolaldehyde phosphate in hydrotalcite. Identical amounts (1 mmole or, at the three lowest concentrations, 0.1 mmole) of mineral (chloride form) and GAP (Ca-salt) were used with varying volumes of water (50 to 10,000 ml) and thus different initial GAP concentrations ($10 \mu\text{M}$ to 10 mM) in solution in each separate pair of experiments, but with constant total amount of GAP available relative to mineral (twice potential saturation). A constant interlayer concentration of about 0.5 mole GAP per mole of mineral is reached at an equilibrium concentration in solution of $18 \mu\text{M}$, indicating that the mineral is capable of sorption to full saturation of its positive charges with GAP^{2-} anion at all concentrations above this approximate limit.

time of the order of a week for reaching equilibrium from a 0.2 molar solution at room temperature (Lee *et al.*, 1993).

The sorption isotherm for GAP in the hydrotalcite interlayer (Figure 11) shows that saturation of the mineral is achieved in equilibrium with solutions as dilute as $20 \mu\text{M}$. The equilibrium concentration reached in the mineral shows that saturation at equal number of moles of mineral in suspension, and GAP initially in solution, is reached somewhat below 50 mole percent GAP in the solid; this proportion indicates the approximate balancing of one positive charge on the hydroxide main sheet (1 moleivalent of mineral) by 0.5 mol of divalent glycolaldehyde phosphate anion.

Measurements, such as in Figure 8 of the basal spacing in the layer structure mineral – GAP complex make it possible to assess accurately the *c*-dimension of the interlayer occupied by the GAP molecule and associated water. This, together with the mass and molar guest/host proportions, and the water content, determined by chemical analysis, permit evaluation of the average distribution of GAP ions in the mineral interlayer. However, the precise position of individual atoms in the structure can only be determined by structural analysis of single crystals with

size larger than about 50 μm , far exceeding that of the present synthetic material ($\sim 0.5 \mu\text{m}$; Figure 7).

7. Sugar Phosphate Formation in the Mineral Interlayer

7.1. DEPENDENCE ON REACTION CONDITIONS

Condensation of the GAP ions sorbed in the interlayer leads to formation of tetrose 2,4-diphosphates and hexose-2,4,6-triphosphates (Figure 3). With the exception, noted above, of Ni-Al and Ni-Fe³⁺-hydroxides, all of the double layer metal hydroxide minerals studied are effectively inducing aldolization. The highest yields were obtained with magnesium aluminum hydroxide (hydrotalcite), with which the majority of experiments were carried out, also because of the ease of operation in view of the stability of the mineral against oxidation.

The rate of aldolization is characterized by slow formation of hexose triphosphates at the expense of the initially rapidly forming tetrose diphosphates. After about 6 days at room temperature; (Figure 12), the formation of hexose triphosphates ceases in spite of the fact that tetrose diphosphates are still present. The point in time of cessation of hexose phosphate formation depends on pH in the external solution and is reached earlier at higher pH. With extended reaction time, the tetrose phosphate continues to disappear but now without attendant formation of hexose triphosphate, the residual tetrose diphosphate being converted to unidentified products. In the example in Figure 12, hexose triphosphate formation ended after 8 days while 20 percent of tetrose diphosphate remained; after 95 days the latter had completely disappeared, while the hexose phosphate had dropped only 5 percent from the 8–16 day peak value of 50 percent, and after 166 days just another 5 percent, demonstrating the great stability of the hexose-2,4,6-triphosphates sorbed in the mineral interlayer.

The completion of hexose phosphate formation requires a time of the order of days at room temperature and is thus substantially slower than the preceding sorption of glycolaldehyde phosphate from 0.3 mM solution, which is complete after a few hours (Figure 10). When the reaction, as in the set of the experiments represented there, is carried out with insufficient amount of GAP in solution (0.25 moles GAP per mole of hydrotalcite) to occupy all available exchange sites in the mineral (0.5 moles/mole), sorption increasingly slows down as GAP in solution is being exhausted, the rate becoming imperceptible after 8 days, when the ratio 0.25 has, within a few percent, been reached in the mineral.

To further evaluate product formation as a function of concentration of the source molecule, GAP, in external solution, a series of experiments were carried out with stepwise 3-, 9-, 27- and 100-fold dilution of 9 mM GAP solution (Figure 13). The results show that the total production of sugar phosphates in the mineral interlayer is largely independent of the glycolaldehyde phosphate concentration in the external solution, but is determined by the total amount of GAP

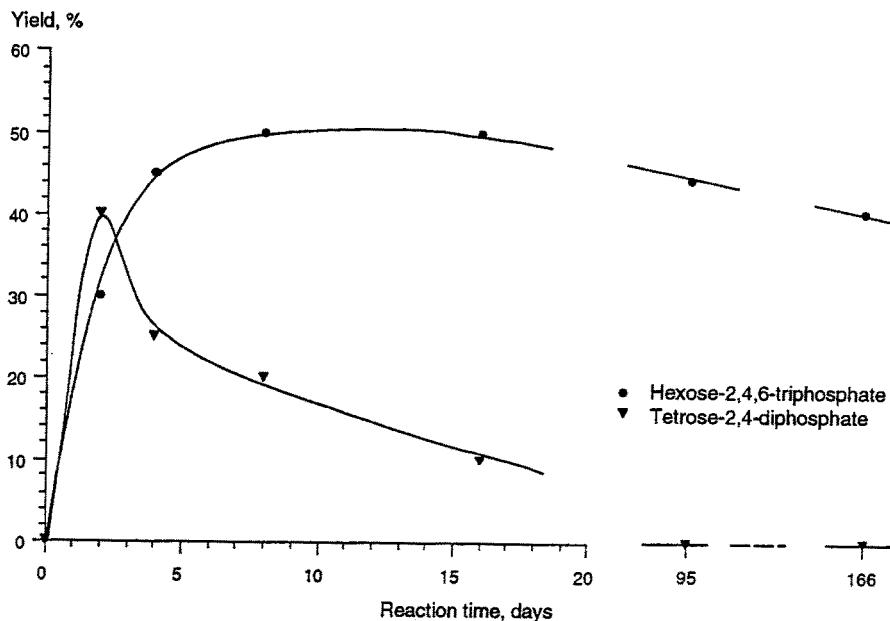


Fig. 12. Yield of tetrose- and hexose phosphates from glycolaldehyde phosphate (GAP) as a function of time of reaction in the mineral interlayer. After a rapid rise in tetrose-2,4-diphosphate production during the first two days, net depletion sets in, initially due to further reaction of this primary aldolization product with GAP to form hexose phosphates. After about a week this reaction stops due to exhaustion of the initial reactant GAP. After this stage the tetrose concentration continues to decrease as a result of a concurrent alteration process, leading to as yet unidentified products. After three weeks the tetrose has entirely disappeared while the hexose product shows a remarkable stability with a decrease of only about 5 percent after almost half a year. Reaction conditions in this experiment were; GAP/HT molar ratio 0.25 (1/2 saturation of GAP in mineral interlayer), pH 11.1 and room temperature.

offered for sorption, up to the capacity of the mineral. The effect of degree of saturation of the mineral with GAP on total yield of product sugar phosphates was investigated by reacting GAP in fixed amount and concentration (10 ml, 3 mM) with varying amounts of mineral. The saturation of 1 mol of mineral requires about 1/2 mol of GAP, in accord with the double negative charge on the ion. Aldolization at saturation (Figure 14) gave about 55 percent hexose triphosphate. This yield was maintained down to about 1/2 of saturation; at lower concentrations of GAP in the interlayer the yield dropped rapidly to 20 percent which, however, remains substantial compared to the complete lack of observable aldolization characterizing the reaction in solution under comparable conditions. These experiments illustrate that the yield of aldolization products depends on the total amount of GAP available and thus extractable from solution, and, as a consequence, on the concentration of GAP attained in the mineral. This reaches a plateau around 0.5 moles of GAP per molequivalent of double layer metal hydroxide mineral. The

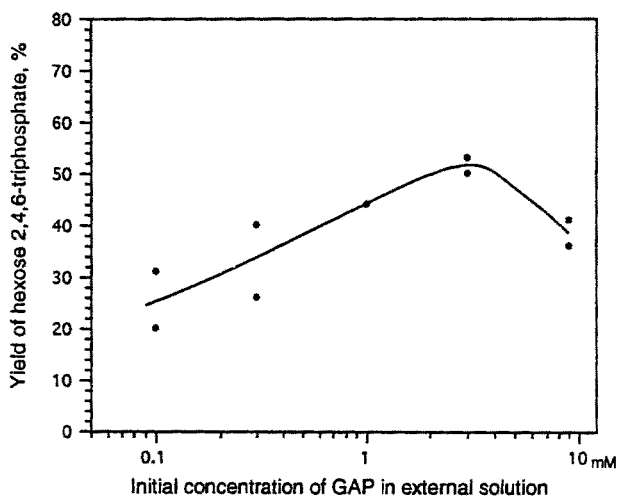


Fig. 13. Effect of initial concentration of glycolaldehyde phosphate (GAP) in external solution on the yield of hexose phosphate, forming from GAP after its sorption into the hydrotalcite interlayer. The experiment was carried out at a constant molar ratio GAP/HT of 0.25, sufficient to half-saturate the interlayer charges with GAP, and under the conditions described in Section 5. The reaction time, 16 days, implies completion of aldolization. (cf. Figure 12). Lowering GAP concentration over two orders of magnitude from 9 mM decreased the hexose yield to 1/2 of the maximum value, attesting to the weak dependence of the interlayer reaction on initial concentration in the source solution. The major factor, controlling the yield, is the internal concentration in the mineral reached at sorption of GAP, and remaining approximately constant in equilibrium with concentrations in the external solution as low as 20 μM .

hexose-2,4,6-triphosphates, sequestered by the mineral, show long-term stability against hydrolysis, retro-aldolization and epimerization. In alkaline solution, they are also much more stable than the unphosphorylated aldoses.

7.2. EFFECT OF pH

The homogeneous glycolaldehyde phosphate aldomerization reaction under the conditions used by Müller *et al.* (1990), requires a basicity of at least pH 11 in order to proceed at a perceptible rate. In contrast, induction of tetrose- and hexose-phosphate formation in the mineral interlayer can proceed at a pH of the external solution as low as 9.5 (Figure 15). The pH effect on hexose yield is more pronounced at a low concentration ($\sim 33\%$ of available sites) of GAP in the interlayer (Figure 15b) than at higher concentration of reactant in the mineral ($\sim 66\%$ of available charge sites occupied by GAP) (Figure 15c). This increased gradient of the pH effect in the phosphate-starved mineral interlayer is likely to be due to partial interlayer occupancy by hydroxyl ion at high pH competing with residual chloride ion for sites not occupied by GAP due to its deficiency in the system. Such an increase in the interlayer hydroxyl ion population may contribute to the rate of the mineral-catalyzed aldolization reaction.

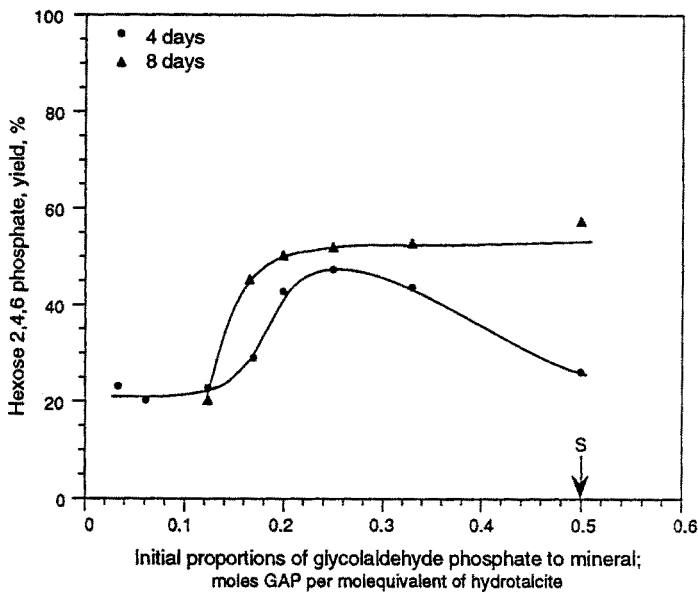


Fig. 14. Yield of hexose-2,4,6-phosphates as function of (constant) initial concentration of glycolaldehyde phosphate in external solution (3 mM; pH 10.8–11.3), relative to the (varied) concentration of hydroxalcite in suspension. Saturation (S) of the excess positive charge on the mineral by GAP^{2-} anions in the interlayer solution occurs at a molar ratio of 0.5, and is attained after a few hours. The half time for completion of the production of hexose phosphate is about 2 days (Figure 12); the 4-day experiment represents an initial stage with ongoing growth of hexose phosphate, forming from tetrose phosphate and GAP. The highest yields of hexose phosphate are reached at approach to saturation of the mineral interlayer; even after a sharp drop around 30–40 percent saturation, substantial yields are maintained at 16- and 32-fold dilution. In homogeneous solution no condensation products could be traced under corresponding conditions of pH-, GAP-concentration and reaction time.

In the absence of sufficient concentration of competing anions, complete charge saturation with hydroxyl ions in the interlayer may be attained, as in the mineral meixnerite (Table I). This situation is conveniently achieved at thermal decomposition of carbonate and hydrogen carbonate ion in the interlayer around 400 °C, leaving a pure anion population of OH^- , which can readily be replaced by higher-charge anions, particularly at low external pH (Kwon *et al.*, 1988).

Although the distribution coefficient for OH^- vs. other univalent anions in the mineral interlayer such as Cl^- , is larger than unity (Miyata, 1983), OH^- is efficiently displaced from the sorption sites by di- and higher valent anions such as HPO_4^{2-} , CO_3^{2-} , SO_4^{2-} , GAP^{2-} and $\text{Fe}(\text{CN})_4^{4-}$; phosphate species retain their competitive strength relative to OH^- through a wide range of pH, since the decrease in net charge due to protonation at increasing hydrogen ion concentration is mitigated by a decrease in hydroxyl ion concentration. The interlayer sites become practically completely occupied with GAP at sorption from $> 100 \mu\text{M}$ solution in suspensions isomolar in GAP and mineral (hydroxalcite), over the entire

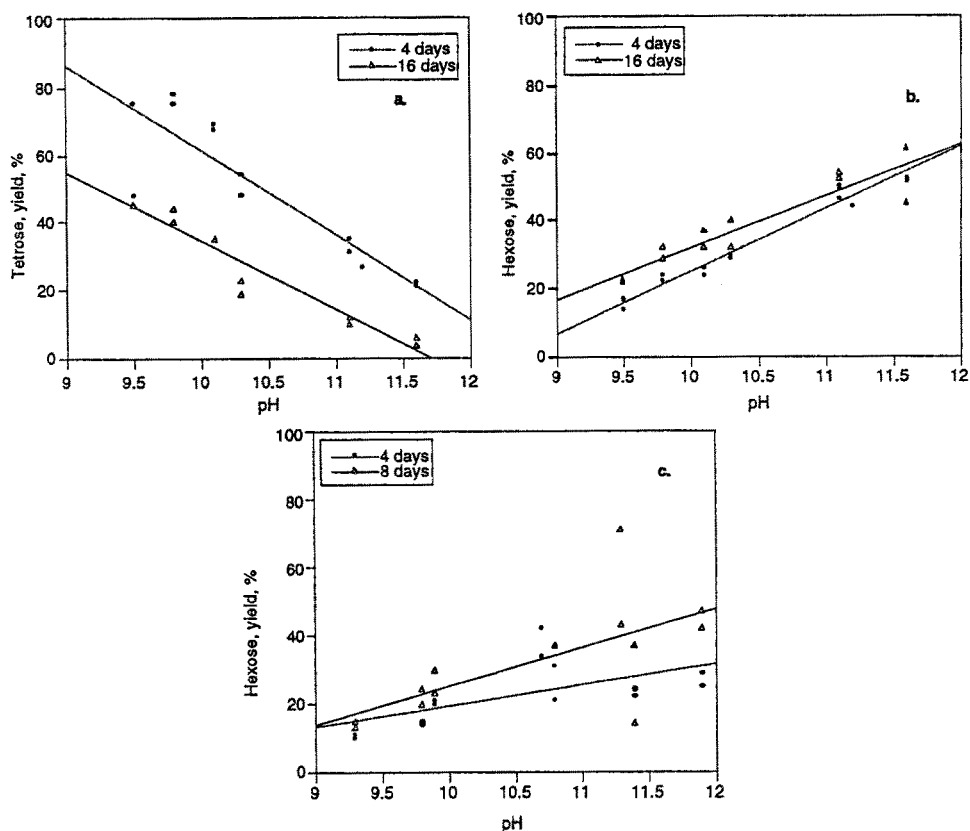


Fig. 15. Yield of (a) tetrose and (b, c) hexose phosphates relative to reactant GAP as function of pH in the external solution, and with amounts of reactant permitting 33% (a, b) and 66% (c) saturation of the mineral interlayer; 100% saturation occurring at approximately 0.5 mole GAP/mole of mineral. The increasing yield of hexose phosphate at higher pH, with a contrast most notable at low GAP occupation of the interlayer sites (33%), as in (b), is probably due to increasing population of the interlayer with hydroxyl ion at high pH when sites are available that are not saturated with glycolaldehyde phosphate. In contrast, the first reaction step, the dimerization of GAP to form tetrose phosphate (a) proceeds rapidly in the low pH range; the decrease in yield with time and increasing pH is at least partly due to progressive transformation of tetrose to hexose.

pH range investigated (pH 7–11). This shows that under these conditions, leading to saturation of the interlayer with GAP, an increased hydroxyl ion concentration in the interlayer can hardly be assumed responsible for the enhanced rate of aldolization in the mineral induced condensation reaction. The limited contributing role of the basicity of the mineral is reflected by the fact that the highest yield of hexose phosphates are found in magnesium aluminum hydroxide (hydrotaalcite) which has an equilibrium pH of about 9.2 in 0.01 M NaCl, (Table VII) while the strongly basic, structurally analogous mineral calcium aluminum hydroxide (hydrocalumite) with equilibrium as high as pH 11.8 shows an order of magnitude lower yield. Interlayer

TABLE VII

pH of 0.01 M NaCl solution in equilibrium with isomolar amounts of double layer M^{2+} Al^{3+} metal hydroxide chlorides in suspension

Divalent metal cation M^{2+} in mineral	pH in external solution
Ca	11.8
Mg	9.2
Mn	8.7
Zn	7.7
Co	7.2
Ni	7.1

aldolization at pH 8.5 is also induced in manganese-, zinc- and cobalt aluminum hydroxides and in ferroferric hydroxide (Pitsch, 1993) characterized by equilibrium pH as low as 7.2 in 0.01 M NaCl solution. In these minerals the yield of sugar phosphates is, however, generally lower than in hydrotalcite; in nickel aluminum hydroxide with pH 7.1 no aldolization was observed under standard conditions.

7.3. DIASTEREOISOMER DISTRIBUTION

In the aldomerization of GAP in solution the molar ratio erythrose/threose was 0.62 and the altrose/allose ratio 0.18 (Müller *et al.*, 1990) or 0.65 and 0.14 respectively (see footnote, Section 3). In contrast the mineral induced reaction at near-saturation of the interlayer, resulted in a threefold increase in the erythrose/threose ratio and an eleven-fold increase in the altrose/allose ratio (Figure 9); hence rac.-allose-2,4,6-triphosphate is the major product in the solution reaction, but rac.-altrose-2,4,6-phosphate in the mineral-induced reaction (Section 5). The test experiments on the stability of allose-2,4,6-triphosphate within the interlayer (see 5.3.4 and 9) do not support the possibility that the altrose/allose ratio in the interlayer aldolization is the result of equilibration.

The increase in erythrose and altrose production in the mineral induced reaction is dependent on and increasing with the concentration of GAP in the interlayer. As shown in Figure 16, the allose yield at low concentration of GAP in the interlayer (1/10 of saturation) approaches that of altrose, and the erythrose proportion of the tetrose fraction is correspondingly lowered. The increase in the fractions of these two sugar phosphates reaches a plateau at about half saturation of the interlayer with the reactant GAP (GAP/HT \sim 0.25). The enhanced selectivity for erythrose-2,4-phosphate among the tetroses and for altrose-2,4,6-phosphate among the hexoses is independent of pH and GAP concentration in solution, and of reaction time.

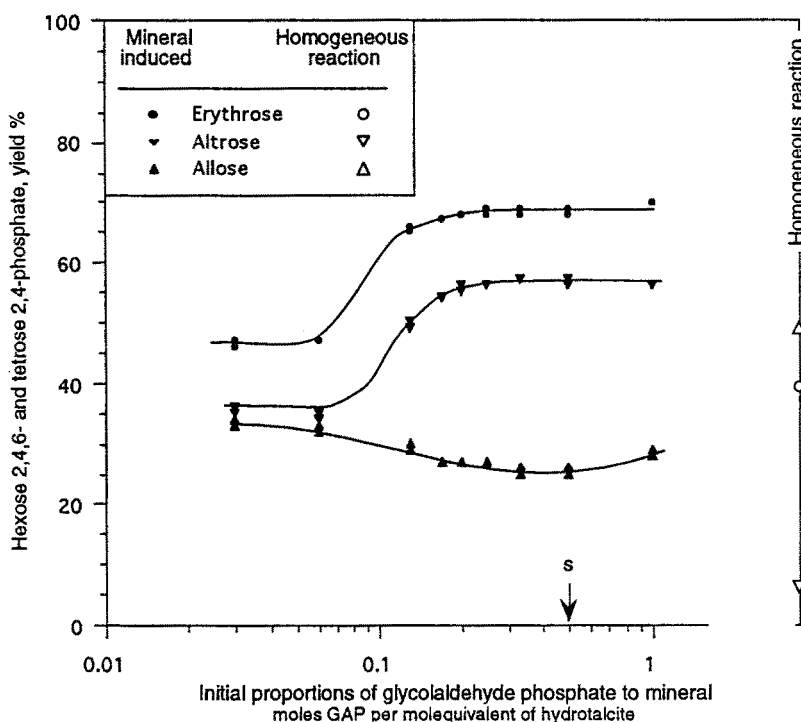


Fig. 16. Selectivity effects at mineral induced aldolization of glycolaldehyde phosphate (data in graph) and in the corresponding reaction in homogeneous solution (on right-hand graph frame). In contrast to the predominant formation of allose phosphate in the solution reaction, the hexose-2,4,6-phosphates formed in the mineral interlayer have altrose phosphate as predominant component (with about 10 percent talose, by $^1\text{H-NMR}$, see 5.3.2). As seen from the graph, the formation of altrose becomes pronounced only as the concentration of GAP in the interlayer solution is allowed to rise toward saturation (S in the graph). This is attained at 0.5 moles of the divalent GAP anion per mole equivalent (= unit positive charge) of the mineral, and corresponds to the order of 10 M concentration of GAP ion in the two-dimensional aqueous quasi-solution that constitutes the hydrotalcite interlayer. Erythrose-p, the tetrose-2,4-diphosphate source of altrose-p, is also increasingly enhanced in the mineral over threose-p when concentration of reactant GAP in the interlayer is increased. The yield values for the hexose phosphate diastereoisomers in the figure indicate their percentage fraction of total hexose phosphates, and for erythrose-2,4-phosphate, its fraction of the tetrose-2,4-phosphates (erythrose + threose). The initial concentration of GAP in the external solution was 3 mM; the degree of interlayer saturation was varied by changing the amount of mineral suspended in GAP solutions of constant concentration. Experiments were conducted at different pH (9.6–11.9) and for times up to 2 weeks. The data shown in the graph represent the yields after 8 days, at which time hexose phosphate formation is completed, and at pH ~ 11.0 , except at the highest interlayer content of GAP (molar ratio GAP/HT 1.0) where pH was 9.6.

8. Formation of Odd-Carbon Sugar Phosphates

One of the prominent features of the homogeneous aldolization process (Müller *et al.*, 1990; Pitsch, 1993) is the formation of rac.-ribopyranose-2,4-phosphate as

the major component in the sugar phosphate mixture formed from glycolaldehyde phosphate and formaldehyde, with the triose *rac.*-glyceraldehyde-2-phosphate as intermediate. In context with the investigations described above, exploratory experiments were undertaken on the formation of pentose-2,4-diphosphate by mineral induced aldolization of glycolaldehyde phosphate in the presence of preformed *rac.*-glyceraldehyde-2-phosphate or formaldehyde. In these experiments, hydrotalcite chloride was used as sorbent phase; the conditions are given in Section 5.3.3, and the observations made are summarized in Tables V and VI. In the experiments, using *rac.*-glyceraldehyde-2-phosphate and GAP as dissolved reactants (Table V), *rac.*-pentose phosphates formed in yields up to 7 percent, with 41 parts ribose-, 46 parts arabinose- + lyxose- and 13 parts xylose-2,4-diphosphate. The experiments with formaldehyde and GAP (Table VI) showed transient formation of triose phosphate, particularly at comparatively low pH (8.9). Pentose phosphate production remained a small fraction (1–4%). It is not clear from these experiments if the observed triose formation took place by reaction of formaldehyde with GAP in the external solution, or in exposed edge regions of the interlayer, after sorption of GAP. In any case, the interlayer reaction of triose phosphate with GAP in the interlayer to form pentose phosphate appears much less efficient than the tetrose reaction with GAP to form hexose phosphates, yet still more efficient than the pentose phosphate production in homogenous solution at the same pH and concentration. It is clear that the important problem of a mineral induced pentose-2,4-diphosphate formation requires further experimentation.

9. Sorption, Desorption and Stability of Diastereoisomers

The notable differences in the diastereoselectivity of hexose-triphosphate formation in the homogeneous aldolization reaction on one hand, and in the mineral-induced process on the other, led us to test the relative retention of the various diastereoisomers in the mineral interlayer. For this purpose, an aldolization mixture of *rac.*-hexose-2,4,6-triphosphates of known composition in solution was exposed to the mineral (hydrotalcite) and the distribution between interlayer and solution determined (by GC after derivatization). According to the trend observed in those experiments, allose-2,4,6-triphosphate seemed to have the lowest interlayer affinity of the hexose-triphosphates. Therefore, the following chromatography experiment was carried out.

A mixture of *rac.*-hexose-2,4,6-triphosphates was exposed to on an eight-fold molar excess of hydrotalcite (200 mg) suspended in the hexose phosphate solution. The proportions in the solution were 60% allose-, 6% mannose-, 11% altrose-, 5% talose-, 4% galactose-, 4% gulose-, 8% glucose- and 2% idose-2,4,6-triphosphate. (The hexose phosphate was obtained in the aldolization experiment at pH 11, as described in Section 3, footnote, and Figure 4.) The solid from this suspension was deposited on a column (1 × 11 cm) that was filled with quartz powder containing

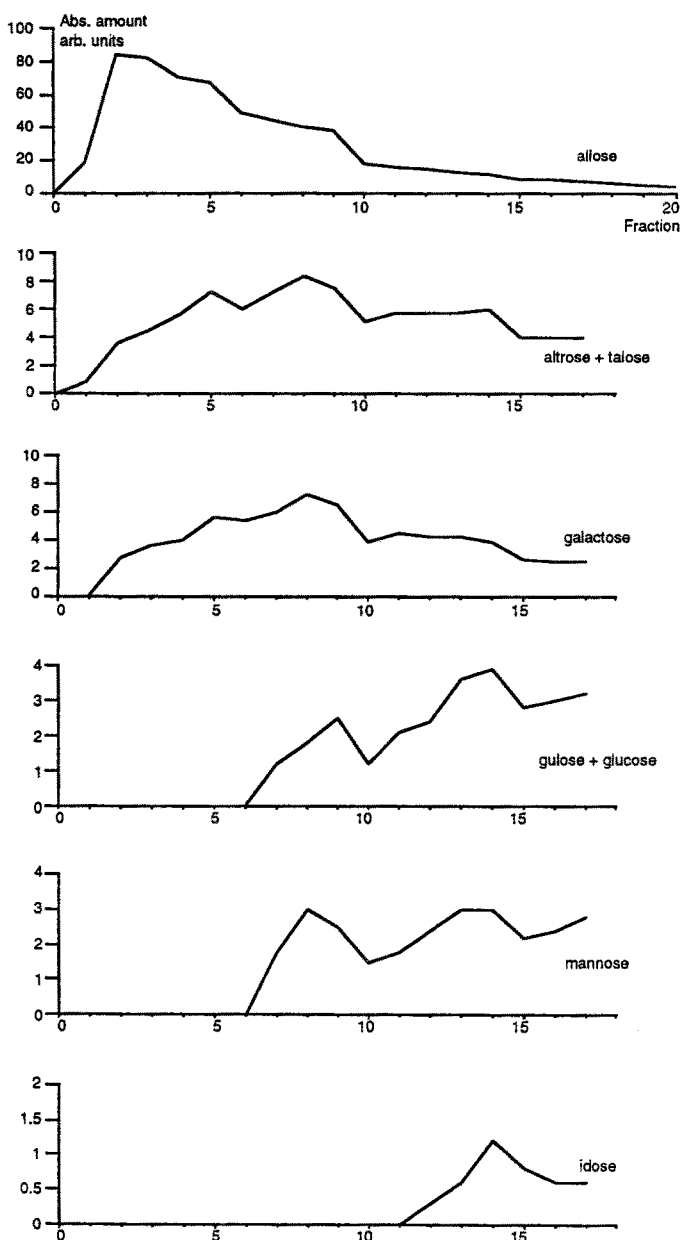


Fig. 17. Chromatography of the diastereoisomers of hexose-2,4,6-phosphate. The column assembly, containing the eight hexose-2,4,6-phosphates (obtained in the aldomerization experiment of 5.3.2) sorbed in hydrotalcite, was eluted at a rate of about 6 ml/h, first 24 h with water, then with 0.02 M, 0.04 and 0.08 M Na_2CO_3 , each 24 h, and finally with 0.1 M $\text{Na}_2\text{CO}_3/0.1$ M NaHCO_3 (pH 10) 10 days. Fractions of each 5 ml were collected. Hexose triphosphates began to appear only after two weeks in the 0.2 M eluent; after 10 days of elution with this medium, 80 percent of the hexose triphosphates had been removed, and the experiment was terminated. The first fractions collected in this part of the experiment, which is shown in the figure, were found to contain rac. allose-2,4,6-triphosphate in about 95 percent purity, followed by the other isomers in the sequence shown, with idose having the longest retention time.

2 g hydrotalcite. The hexose-triphosphates were released by slow elution with a stepwise Na_2CO_3 gradient. After 2 weeks and at a carbonate concentration of 0.2 M, they started to appear and were eluted during another 10 days at this concentration to the extent of 80%. The fractions were separately characterized by GC and quantified by using pentaerythritol as internal standard. Rac.-allose-2,4,6-triphosphate which eluted first (Figure 17) could be isolated in a purity of about 95% (according to GC and $^1\text{H-NMR}$), and in about 50% of the original amount present. The other diastereoisomers were eluted in sequence; galactose- was followed by altrose- and talose-, then mannose- together with gulose- and glucose-, and finally idose-2,4,6-triphosphate. The large differences in retention of the hexose-2,4,6-triphosphate diastereoisomers in the mineral interlayer are likely to reflect the differences in orientation of their (axial or equatorial) phosphate-sugar bonds. These would result in differences in the effective bond strength between the phosphate anions and the localized excess positive charges on the main metal hydroxide sheets, associated with the Al^{3+} substituent cations.

In order to determine whether rac.-allose-2,4,6-triphosphate sorbed in hydrotalcite is epimerizing to rac.-altrose-2,4,6-triphosphate under the conditions of interlayer aldolization, an experiment was carried out in which the mineral was suspended in a solution of D-allose-2,4,6-triphosphate (free of diastereoisomers) under standard aldolization reaction conditions. After 8 days, the proportion sorbed by the solid was retrieved by techniques indicated above and analyzed. Aside from rac.-allose-2,4,6-triphosphate in the mineral and in the supernatant solution, only traces ($\sim 0.5\%$) of D-altrose-2,4,-triphosphate and no other components were found. Therefore, practically no retro-aldolization or α -epimerization took place, neither in the hydrotalcite interlayer nor in the solution in contact with the mineral. In long-term experiments, employing mixtures of hexose phosphates obtained by mineral induced aldolization, such as in Figure 12, a slow α -epimerization of altrose could be observed: the original ratio (altrose + talose)/allose of 2.24 had after 95 days fallen to 1.86, and after 166 days to 1.53. These results indicate that the distribution of diastereomeric hexose-2,4,6-triphosphates in the mineral assisted aldolization of glycolaldehyde phosphate is largely kinetically controlled.

10. Concluding Remarks

The mineral-induced reactions in the double layer hydroxides are likely to be controlled by three important properties of the mineral interlayer reaction space. First, the concentration of the reactant anions in the interlayer is determined by the density of excess positive charge due to the trivalent metal cations in the main hydroxide sheets of the mineral. The dissolved guest anions are attracted to the host lattice by electrostatic charge and presumably by hydrogen bonding. Since these bonds are in the ambient thermal energy range, the sorbed anions can be expected to have a restricted but finite mobility by diffusion and to be in exchange

equilibrium with the external, free solution. This equilibrium is achieved rapidly (in one or a few hours at room temperature) with small, high charge anions (Miyata, 1983; Kuma *et al.*, 1989) and more slowly (weeks or more) with large oligomeric species (Lee *et al.*, 1993).

Second, through the aldomerization process, size and total charge of anions are increasing in the interlayer, establishing a range of diffusion rates. On the time scale of study the tetrose- and trimeric hexose phosphates might be increasingly immobilized while the monomeric glycolaldehyde anions would remain mobile, but are relatively rapidly exhausted by condensation with each other and with the tetroses; the interlayer solution would 'freeze'. In the case of small ions with high charge density, such as SiO_4^{4-} , the freezing results in a two-dimensional crystalline hydrated solid, epitactic on the adjacent hydroxide sheets (Schutz and Biloen, 1987). With large oligomeric anions, such as polypeptides, the interstitial solutes become immobilized in a disordered state, as indicated by their lack of contribution to x-ray reflections from planes parallel to the c-axis. In the case of interlayer solutions containing a range of monomeric and oligomeric species, the 'frozen' system can be regarded as a two-dimensional eutectic or solid solution. The degree of order in the frozen or viscous interlayer solution of sugar phosphates is still under investigation, and would be expected to be (inversely) temperature dependent.

Third, the reduction of dimensionality of the diffusion space from three to two unquestionably represents a major factor that – in addition to the concentration of the reactants (up to the order of 10 M) – enhances the probability for reactive encounters in the interlayer solution. The concept of enhanced diffusion efficiency in a two-dimensional solution was introduced by Polya (1921) and applied to reactive systems by Adam and Delbrück (1968), Delbrück (1970) and Edidin (1974) in models for the cell membrane and for pheromone receptors, and, by Prelog (1950) to the heterogeneous acyloin condensation.

The capability of the double layer hydroxide minerals to effectively sorb glycolaldehyde phosphate anions from concentrations in the micromolar range and to induce their selective aldomerization to higher sugar phosphates deserves attention in the context of the problems of biopoesis. A number of questions arise which would demand further analysis and experimentation. First and foremost, while the production of hexose-2,4,6-triphosphates from glycolaldehyde phosphate in the interlayer seems straightforward, the formation of pentose-2,4-diphosphates is not. The reasons may well be complex; the lack of a negative charge in one of the reaction partners (formaldehyde) is presumably among them, if not the major one. Recent experimental work has shown (Fischer, 1992; Helg, 1994; Groebke, 1993; Eschenmoser, 1993) that hexose-based oligonucleotide systems (in their pyranosyl form) lack the necessary base-pairing properties for a role as potentially prebiological information storage systems. The interest in possible precursors for such systems therefore remains focused on ribose phosphate building blocks (Pitsch *et al.*, 1993; Eschenmoser, 1993, 1994) warranting an extension of the present explorato-

ry study of the mineral-induced formation of ribopyranose phosphate derivatives. Furthermore, processes are to be demonstrated which, under geochemically plausible conditions, yield phosphate esters of glycolaldehyde. Two related types of model reactions have been studied for this purpose, one reported by Wagner *et al.* (1990) and Drenkard *et al.* (1990); the other by Pitsch and Eschenmoser (1994) and Pitsch *et al.* (1994). The latter experiments demonstrated the facile and selective formation of glycolaldehyde phosphate esters from oxiranecarbonitrile in aqueous solution. The oxirane derivative, being isomeric to the ensemble of carbon monoxide and acetonitrile, or of formaldehyde and cyanocarbene, is conjectured to form by a variety of pathways from simple precursors (Pitsch *et al.*, 1994). Current radioastronomical searches aim at establishing the occurrence of oxiranecarbonitrile in interstellar cloud plasmas (Irvine *et al.*, 1994). However, as long as such pathways have not been demonstrated, the concept of glycolaldehyde phosphate originating from oxiranecarbonitrile remains in the necessarily speculative realm that it shares with most prebiotic synthesis schemes.

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