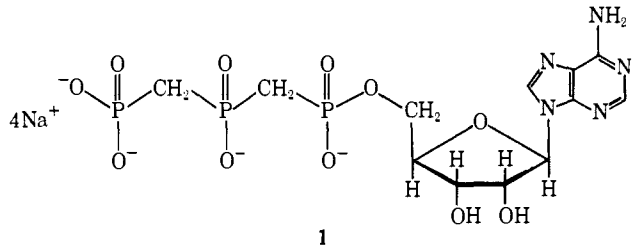
Adenosine 5′-Bis(dihydroxyphosphinylmethyl)-phosphinate, the α,β:β,γ-Bismethylene Analog of Adenosine 5′-Triphosphate

Sir:

We wish to report the synthesis of the tetrasodium salt of adenosine 5′-bis(dihydroxyphosphinylmethyl)phosphinate (**1**), which is the α,β:β,γ-bismethylene analog of adenosine 5′-triphosphate (ATP) and is the most hydrolytically stable analog of ATP reported to date.



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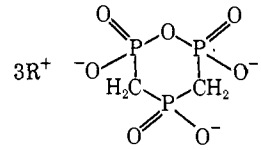
For the synthesis the pentaethyl ester of bis(dihydroxyphosphinylmethyl)phosphinic acid was prepared by the procedure of Maier.[1] *Anal.*[2] Calcd for $C_{12}H_{29}O_8P_3$: C, 36.55; H, 7.41; P, 23.57. Found: C, 36.24; H, 7.51; P, 23.22. This pentaethyl ester (2.0 g) was heated on a steam bath with 30 ml of 48% HBr for 24 hr. After removal of the aqueous HBr, the free bis(dihydroxyphosphinylmethyl)phosphinic acid[1a,b] remained as a highly viscous, hygroscopic oil in nearly quantitative yield. Investigation of the ^1H nmr spectrum[3] (D_2O), which corresponded to that reported by Maier,[1b] confirmed the complete absence of any ester linkages. The crystalline tricyclohexylammonium salt of this acid was prepared by Maier's method for comparison purposes, mp 200–202° dec (lit.[1b] 205° dec). A portion of the acid was converted without further purification into the tricyclohexylammonium salt of the cyclic bismethylene analog of trimetaphosphoric acid[4] (**2a**) by the use of dicyclohexylcarbodiimide (DCC). The bis(dihydroxyphosphinylmethyl)phosphinic acid (560 mg), anhydrous tri-*n*-butylamine (2.5 g), and DCC (2.0 g) were dissolved in 10 ml of anhydrous pyridine, and the solution was left at room temperature for 3 days. After filtering to remove the dicyclohexylurea,



2a, $R^+ = C_6H_{11}NH_3^+$
b, $R^+ = [CH_3(CH_2)_3]_3NH^+$

the crude product mixture was poured into water and the resulting solution was extracted with three 10-ml portions of ether. Most of the water was removed from the aqueous layer and the solution was passed through a 1.5 × 45 cm Dowex 50 (H^+) column to give the free acid which was quickly neutralized with cyclohexylamine. After removal of the water, the tricyclohexylammonium salt **2a** was recrystallized from MeOH–CH_3CN. *Anal.* Calcd for $C_{20}H_{46}N_3O_7P_3 \cdot H_2O$: C, 43.55; H, 8.77; N, 7.62; P, 16.85. Found: C, 43.90; H, 9.10; N, 7.58; P, 17.12. The compound showed principal infrared bands (Nujol) at 3.00, 6.12, 6.50, 8.35 (broad), 9.40, 9.73, 10.75 (broad), 12.08, and 12.50 μ. The tris(tri-*n*-butylammonium) salt **2b**, a viscous oil, was prepared from the tricyclohexylammonium salt (**2a**) by a similar ion-exchange process. A 180-mg portion of the salt **2b** was then placed in a 50-ml flask equipped with a reflux condenser, a $CaCl_2$ drying tube, and a nitrogen purge. Anhydrous dimethylformamide (2 ml), anhydrous methanesulfonic acid (17 mg), and 2′,3′-isopropylideneadenosine (430 mg; Aldrich Chemical Co.) were added and the solution was heated at reflux (*ca.* 153°) for 4 hr. The primary alcohol group of the 2′,3′-isopropylideneadenosine presumably solvolyzed the relatively weak P–O–P linkage of **2b** to give the expected product, which was isolated as follows. After removal of the dimethylformamide, the crude product mixture was dissolved in the minimum amount of water and passed through a 1.5 × 45 cm Dowex 50 (H^+) column. At this stage the strongly acidic aqueous solution was allowed to stand at room temperature for 3 hr to remove the isopropylidene protective group and to hydrolyze any possible side product containing an acid-labile P–N linkage. The product was then subjected to chromatography on a 2.5 × 40 cm DEAE-cellulose column using stepwise elution with 200-ml portions each of 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 *N* triethylammonium bicarbonate.[8] The ATP analog, observed by monitoring samples at 259 nm, emerged at the 0.05 and 0.06 *N* salt concentrations. After removal of the water and the volatile salt, the crude product was redissolved in 1 ml of water and subjected to further chromatography on a 1.5 × 45 cm Dowex 50 (H^+) column. Water was used as the eluent and 1-ml fractions were collected. Fractions 15–20 contained the product.[9] After neutralization with dilute NaOH solution and removal of the water, the product (**1**) was obtained as the glassy tetrasodium salt (23 mg, 16% yield based on the amount of salt **2a** used). *Anal.* Calcd for $C_{12}H_{16}N_5O_{11}P_3Na_4 \cdot H_2O$: C, 23.65; H, 2.98; N, 11.50. Found: C, 23.91; H, 3.01; N, 11.03. The uv spectrum (H_2O) showed λ_{max} 259 nm (ε 1.54 × 10^4). The product showed chromatographic behavior similar to that of

(1) (a) L. Maier, *Angew. Chem., Int. Ed. Engl.*, **7**, 384 (1968); (b) L. Maier, *Helv. Chim. Acta*, **52**, 827 (1969).

(2) Microanalyses were carried out by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif.

(3) Nmr spectra were taken on either a Varian Model T-60 or HA-100 spectrometer with tetramethylsilane as an internal or external standard.

(4) The tentative assignment of the six-membered ring structure to this intermediate is predicated on the following arguments: (a) inorganic triphosphoric acid can be converted using condensing agents in high yield to the analogous, well-characterized trimetaphosphoric acid;[5] (b) when a portion of salt **2a** was passed through a Dowex 50 (H^+) column, the free acid which emerged was shown by potentiometric titration with 0.100 *N* NaOH solution to contain only strongly acidic OH groups, corresponding to 3.0 equiv/mole; (c) a single spot, visualized with molybdate spray,[6] was observed on polyethylenimine cellulose thin layer chromatography[7] using 0.3 *N* LiCl as the eluent (R_f 0.75); under the same chromatographic conditions the bis(dihydroxyphosphinylmethyl)phosphinic acid traveled slower (R_f = 0.24); (d) in the presence of a strong acid and a large excess of methanol, compound **2a** can be converted nearly quantitatively into the monomethyl ester of the bis(dihydroxyphosphinylmethyl)phosphinic acid under rather mild conditions, whereas further esterification, as determined by nmr spectroscopy, is much slower (D. Trowbridge, unpublished results).

(5) E. Thilo, I. Grunze, and H. Grunze, *Monatsber. Deut. Akad. Wiss. Berlin*, **1**, 40 (1959).

(6) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(7) K. Randerath and E. Randerath, *J. Chromatog.*, **16**, 111, 126 (1964).

(8) J. Moffatt, *Can. J. Chem.*, **42**, 599 (1964).

(9) In separate experiments both bis(dihydroxyphosphinylmethyl)-phosphinic acid and its cyclic counterpart emerged from the Dowex 50 column considerably earlier than the product, whereas free adenosine adhered tightly to the column.

ATP itself. Thus it showed a single ultraviolet-visible spot on ascending paper chromatography in both an acidic solvent system[10] (R_f 0.44) and in a basic solvent system[11] (R_f 0.32) and a single spot on polyethylenimine cellulose thin layer chromatography using 0.5 *N* LiCl as the eluent (R_f 0.39).[7] The spots could also be visualized using the periodate–benzidine spray of Viscontini, *et al.*,[12] which is diagnostic for the presence of the *vic*-dihydroxyl group in the ribose ring.

The proton nmr spectrum taken at 100 MHz was consistent with the assigned structure. The spectrum (D_2O) showed δ 2.25 (2 H, apparent triplet, J_{P-H} = 20 Hz), 2.33 (2 H, apparent triplet, J_{P-H} = 20 Hz), 4.20 (5 H, multiplet), 6.25 (1 H, doublet, J_{H-H} = 6 Hz), 8.28 (1 H, singlet), 8.44 (1 H, singlet). Upon decoupling of the phosphorus (irradiation at 40.5 MHz; NMR Specialties heteronuclear decoupler) the pair of apparent triplets centered at δ 2.29 collapsed to a single broad peak, with a width at half-height of 18 Hz. Moreover, a broad peak which constituted the major component of the multiplet centered at δ 4.20 also partially collapsed. This peak, presumably arising from the methylene hydrogens on the 5′ carbon of the ribose moiety, changed from having a width at half-height of 12 Hz to having a width at half-height of 6 Hz.

Unlike either ATP or its known α,β- and β,γ-monomethylene analogs,[13-15] the bismethylene analog, which contains no labile P–O–P linkages, is stable to strongly acidic aqueous solutions for long periods of time. The stability of the ester linkage in the bismethylene analog is similar to that of a monoester of a phosphonic acid.[16] Investigations into the potential inhibitory properties and use as a substrate analog in enzymatic systems requiring ATP (*e.g.*, S-adenosylmethionine synthetase) are in progress.

Acknowledgments. This work was supported in part by the U. S. Public Health Service, Grant AM 13529. We wish to thank Mr. Richard Neese for his help with the phosphorus decoupling experiment.

(10) O. Pfrengle, *Z. Anal. Chem.*, **158**, 88 (1957); dioxane–2-propanol–trichloroacetic acid–acetic acid–concentrated ammonia–water (30:26.3:1.9:0.6:0.9:29.6, v/v).

(11) G. Biberacher, *Z. Anorg. Allg. Chem.*, **285**, 88 (1956); 2-propanol–dimethylformamide–2-butanone–concentrated ammonia–water 20:20:20:1:39, v/v).

(12) M. Viscontini, D. Hoch, and P. Kaiser, *Helv. Chim. Acta*, 38, 642 (1955).

(13) T. C. Myers, K. Nakamura, and J. Flesher, *J. Amer. Chem. Soc.*, **85**, 3292 (1963); T. C. Myers, U. S. Patent 3,238,191 (March 1, 1966).

(14) L. Simon, T. C. Myers, and M. Mednicks, *Biochim. Biophys. Acta*, 103, 189 (1965).

(15) J. W. B. Hershey and R. E. Monro, *J. Mol. Biol.*, 18, 68 (1966).

(16) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley & Sons, Inc., New York, N. Y., 1950.

(17) National Institutes of Health Predoctoral Fellow, 1967–1969.

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Received December 31, 1969

Preparation of Potassium Trihydridomagnesiate, $KMgH_3$

Sir:

The importance of complex metal hydrides of aluminum and boron (*e.g.*, $LiAlH_4$ and $NaBH_4$) in both organic and inorganic chemistry is well known.[1] Complex metal hydrides of alkali metals with magnesium, although highly sought after, are unknown. For example, in an attempt to prepare $LiMgH_3$, Tanaka[2] reported that hydrogenolysis of a mixture of methyllithium and dimethylmagnesium at elevated temperatures formed a mixture of LiH and MgH_2. Coates[3] recently reported that pyrolysis of the *n*-butyllithium adduct of dimethylmagnesium (presumably $LiMg(CH_3)_2C_4H_9$) formed a mixture of LiH and dimethylmagnesium.

We wish to report the first successful synthesis of a complex metal hydride of an alkali metal and magnesium. The compound $KMgH_3$ (potassium trihydridomagnesiate) was prepared by the hydrogenolysis of $KMg(sec\text{-}C_4H_9)_2H$ in benzene solution.[4] The solubility

$$KMg(sec\text{-}C_4H_9)_2H \xrightarrow{H_2} KMgH_3 + 2C_4H_{10} \quad (1)$$

of $KMg(sec\text{-}C_4H_9)_2H$ in benzene is unique and avoids the necessity of hydrogenolysis in more basic solvents, such as ethers, thus eliminating the competition between solvent and hydride ion for coordination sites in the expected product. In addition, secondary butyl groups bonded to relatively electropositive metals are known to undergo hydrogenolysis under relatively mild conditions. This factor allows reduction of this compound at room temperature where it is known to exist as an authentic $KMgR_2H$ complex. This is an important point since high-temperature hydrogenation of Lewis acid–base complexes of this type might be preceded by extensive dissociation at the higher temperatures, followed by reduction, forming a mixture of alkali metal hydride and magnesium hydride, according to

$$KMgR_2H \rightleftharpoons KH + MgR_2 \xrightarrow{H_2} KH + MgH_2 + 2RH \quad (2)$$

Hydrogenolysis of a 0.5 *M* benzene solution of potassium di-*sec*-butylhydridomagnesiate (K:Mg:butyl:H = 1.0:1.0:1.9:0.95) under 3000 psi of hydrogen pressure at 25° for 4 hr resulted in quantitative precipitation of a yellow solid which reacted violently when exposed to the atmosphere. This solid was analyzed for alkali metal (by flame photometry), magnesium (by EDTA titration), and hydrogen (by gas evolution analysis). Analysis gave a potassium, magnesium, and hydrogen ratio of 1.0:1.0:3.0. *Anal.* Calcd for $KMgH_3$: K, 58.7; Mg, 36.8; H, 4.52. Found: K, 58.9; Mg, 36.2; H, 4.56. No butane was produced on hydrolysis, indicating complete reduction and formation of a $KH:MgH_2$ species. These analytical data are also consistent with the formation of a physical mixture of KH and MgH_2. However, X-ray powder analysis (Table I) revealed a unique diffraction pattern, different from the patterns for KH and MgH_2, indicating that the reaction product is not a physical mixture. The strongest line for KH (at 3.30 Å) and strongest lines for MgH_2 (at 3.19, 2.495, 1.67, and 1.59 Å) are clearly absent from the $KMgH_3$ pattern. Preliminary studies on $KMgH_3$ indicate that it is insoluble in common hydrocarbon and ether solvents, stable to disproportionation, and

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(2) J. Tanaka and R. Westgate, Abstracts, 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 1969, No. INOR 155.

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