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Abstract

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PYROPHOSPHORIC ESTER OF 3-METHYLBUTEN-2-OL-1

(Presented by Academician M. I. Kabachnik on 8 VI 1962)

The stepwise enzymatic synthesis of squalene from acetate includes the formation of mevalonic acid and the cleavage of its 5-pyrophosphate to the pyrophosphoric ester of 3-methylbuten-3-ol-1 (isopentenyl alcohol) (II) ([1]). Also described is the enzyme isopentenyl pyrophosphate isomerase ([2,8]), which catalyzes the isomerization of (II) into the pyrophosphoric ester of 3-methylbuten-2-ol-1 (dimethylallyl alcohol) (I). Condensation of two pyrophosphoric esters under the influence of yeast or liver enzymes to give geranyl pyrophosphate (III) ([3]) begins a series of biochemical syntheses based on the addition of allyl pyrophosphates to an isolated methylene group with elimination of pyrophosphoric acid.

$$\text{(I)} \qquad \text{(II)} \longrightarrow \text{(III)} + H^+ \quad \text{etc.}$$

This new type of formation of a C–C bond, without the participation of carbonyl groups, is regarded as a consequence of the reactivity of an allylic cation of the type formed upon cleavage of pyrophosphate,

$$[\ >C\cdots C\cdots C<\ ]^+$$

with strongly electrophilic properties ([3–6]). However, *in vitro* the formation of such cations requires the presence of acid. V. Johnson and R. Bell ([7]) believe that the alkylation step is facilitated by the participation of a nucleophilic phosphate grouping

$$\text{I} + \begin{array}{c} CH_2{=}C(CH_3)-CH_2 \\ O^- \\ O \leftarrow P - O \\ OPO_3H_2 \end{array} \longrightarrow \begin{array}{c} (CH_3)_2C{=}CH-CH_2-CH_2-C(CH_3)-CH \\ O \leftarrow P - O \\ OPO_3H \end{array} \longrightarrow \text{(III)}$$

(IV)

Fig. 1

Figure 1: Fig. 1

In reality, however, the residue of the first dissociation step of phosphoric acid has only a very weak tendency toward nucleophilic attack–

to a carbon atom. A detailed study of the chemical properties of 3-methylbut-2-en-1-ol pyrophosphate (I) is complicated by the difficulty of obtaining it in pure form. By chemical means this compound was obtained [8,9] in the form of a Ba salt containing impurities. Plieninger et al. [10] were unable to synthesize sufficiently concentrated samples of deuterated I.

Fig. 1

The starting 3-methylbut-2-en-1-ol was obtained by us by cleavage of 4,4-dimethylmetadioxane [11] and, according to its IR spectrum, was completely free from impurities of isomeric 3-methylbut-3-en-1-ol. Phosphorylation by Kramer' s method [12] gives a mixture of the mono- and pyrophosphate (I), which could be separated and obtained in an analytically pure state as cyclohexylammonium salts. Figure 1 gives the IR spectra of the dicyclohexylammonium salts: *a* –3-methylbut-2-en-1-ol monophosphate; *b* – 3-methylbut-2-en-1-ol pyrophosphate.

Pyrophosphate (I) is less mobile than the corresponding monophosphate in paper chromatography. The double bond in the allylic position greatly increases the tendency toward elimination of mineral phosphate. For samples that had been subjected even to short-term storage in a vacuum desiccator, the spot of the substance becomes somewhat blurred both in the direction of the chromatogram front and toward the starting line.

Experimental part

3-Methylbut-2-en-1-ol [11]. B.p. 140—140.5° at 750 mm. n_D^{20} 1.4410, d_4^{20} 0.8640, MR_D found 26.27 for $C_5H_{10}O$. MR_D calculated 26.348. IR spectrum: 780 (medium), 840 (medium), 895 (very weak), 990–1020 (strong), 1042 (strong), 1118 (medium), 1190 (medium), 1250 (medium), 1310 (medium), 1385 (medium), 1450 (strong), 1678 (medium), 2735 (weak), 2880 (strong), 2925 (strong), 2975 (strong), 3250–3450 (strong) cm^{-1}.

Mixture of phosphates. To 20.0 g (0.23 mole) of 3-methylbut-2-en-1-ol in 100.4 g (0.69 mole) of trichloroacetonitrile, a solution of 76.1 g (0.255 mole) of di-(triethylammonium) orthophosphate, prepared from 29.5 g of orthophosphoric acid (85%) in 280 ml of dry acetonitrile and 52 g of dry triethylamine, is added dropwise at 25—30°. The mixture is stirred for 3 hours at 18—20°, 100 ml of water and 200 ml of ether are added, the ether layer is separated, and the aqueous layer is additionally extracted with benzene (4 × 75 ml). From the combined extracts, 51 g (0.315 mole) of trichloroacetamide with m.p. 140—141°

is obtained. To the aqueous layer 56.0 ml of cyclohexylamine is added; the mixture is twice successively evaporated to 1/3 of the initial volume at no higher than 40°; the precipitates that separate, which according to paper chromatography contain 3-methylbut-2-en-1-ol monophosphate and mineral phosphates, are removed.

3-Methylbut-2-en-1-ol monophosphate dicyclohexylammonium salt. The crystalline substance obtained is dissolved in dry

with methanol, separated from the precipitate of mineral phosphates, precipitated with acetone, and 13.6 g is obtained. Recrystallized from acetone with water (4:1). M.p. 189–190°, R_f 0.47 (ascending chromatogram on Whatman No. 1 paper, system *n*-propanol–25% ammonia–water 15:9:1; developer: a mixture of solutions of 2 g of ammonium molybdate in 50 ml of water and 0.3 g of quinine sulfate in 4 ml of conc. and 20 ml of 1:1 diluted nitric acid).

$C_{17}H_{37}O_4N_2P \cdot H_2O$.
Found, %: C 53.34, 53.28; H 10.24, 10.25; P 8.25, 7.98
Calculated, %: C 53.38; H 10.28; P 8.10

Dicyclohexylammonium salt of 3-methylbut-2-en-1-ol pyrophosphate. After separation of the precipitates containing predominantly monophosphate (see above), the water is completely evaporated. To the viscous residue, 300 ml of dry acetone is added. The crystalline precipitate formed contains (paper chromatography) mineral phosphates, pyrophosphates, and orthophosphates of the organic alcohol. It is treated with 75 ml of an ethyl acetate–methanol mixture (3:2), and the insoluble impurities (about 9 g) are separated. From the solution the substance (4.35 g) is precipitated with acetone and recrystallized from an ethyl acetate–methanol–acetone mixture. Colorless crystals. M.p. 172–175° (with decomp.). R_f 0.336 (under the same conditions as for the monophosphate).

$C_{17}H_{38}O_7N_2P_2$.
Found, %: C 45.71, 45.68; H 8.46, 8.98; P 13.88, 13.06; N 6.81, 6.52
Calculated, %: C 45.94; H 8.56; P 13.96; N 6.3

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Note: Figure translations are in progress. See original paper for figures.