ESTERS OF DL-α-TOCOPHEROL AND PHOSPHORIC ACID

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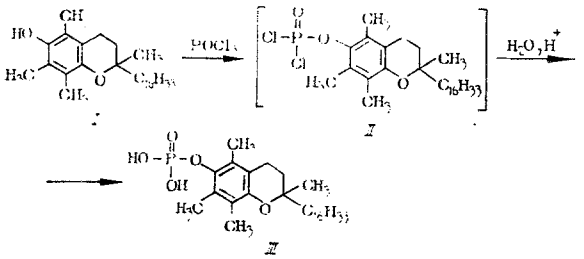
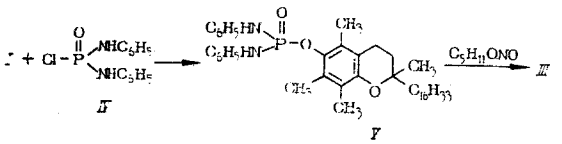
Of the numerous esters of DL-α-tocopherol (I) DL-α-tocopheryl phosphate (III) is of particular interest. Its disodium salt is water soluble. Compound (III) proved to have an influence on enzymic systems. It inhibits the action of fructosidases and amylases [1] and increases the activity of phenylalaninehydroxylase in mammalian liver [2]. Administration of (III) to an animal prolongs the therapeutic action of barbiturates and prefents the agitation phase [3]. It also enhances the action of neopental in animals [4].

Known methods of synthesis of (III) include phosphorylation of (I) with phosphorus oxychloride or phosphorus pentaoxide in various solvents with subsequent acidic hydrolysis of the obtained DL-α-tocopheryl phosphoryl dichloride (II) [5-7].



Phosphorylation of (I) with phosphorus oxychloride [8] is convenient in experimental respects but as a rule is accompanied by side reactions with the possible formation of diesters of phosphoric acid and symmetrical pyrophosphates of (I). Consequently, to obtain phosphate (III) without contaminants it is necessary to use additional purification or to carry out synthesis of it with selectively acting phosphorylating agents such as the chlorophosphate dianilide (IV) which has been applied successfully in the synthesis of nucleoside-5'-phosphates [9] and triphosphoinositol [10]. This reagent was also used by us for the directed phosphorylation of (I) through the stage of forming DL-α-tocopheryl phosphate dianilide (V) with the aim of obtaining (III).



Intermediate (V) is converted into (III) containing no accompanying phosphorus-containing compounds (according to TLC data) by treatment with isoamyl nitrite. The obtained phosphate (III) was characterized by IR, [^{31}P]-NMR, and UV spectroscopy, and by elemental analysis. In spite of the relatively low yield (of the order of 23% calculated on initial I) this method made it possible to prepare samples of (III) suitable for the identification of the corresponding compound obtained by other reactants, particularly phosphorus oxychloride.

On phosphorylating (I) with phosphorus oxychloride by the known method of [11] we noted the formation of a single phosphorus containing byproduct A possessing a high chromatographic mobility in comparison with (III). Analysis of a mixture of (III) with compound A by [^{31}P]-NMR spectroscopy showed the presence of two singlet signals with chemical shifts δ_1 2.9 and δ_2 7.0 ppm. Separation of this contaminant by column chromatography both on silica gel and on silicic acid was accompanied by partial breakdown leading to phosphate (III) (δ_1 2.9 ppm).

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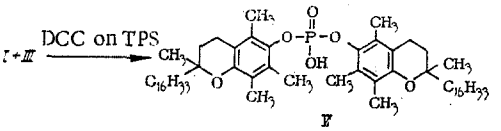
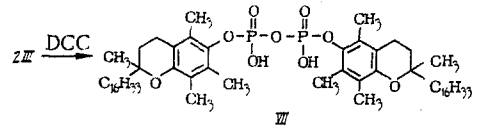
Compound A was obtained in a pure state by treatment of the reaction mixture with an alcohol solution of various alkaline agents, particularly sodium ethylate. In this way (III) was precipitated as the solid disodium salt while compound A remained in the mother liquor. Subsequent acid treatment and preparative TLC led to the preparation of compound A which, according to data of elemental analysis, IR, UV, and [^{31}P]-NMR spectroscopy, may be assigned the structure of bis-(DL-α-tocopheryl) phosphate (VI) or P,P'-bis-(DL-α-tocopheryl) diphosphate (VII).

To establish finally the structure of compound A we effected the synthesis of the possible phosphorus-containing by-products (VI) and (VII) and studied their physicochemical properties



V



VII

Phosphate (VI) obtained by the interaction of (III) with (I) in the presence of DCC, proved to be identical with compound A according to data of TLC and [^{31}P]-NMR spectra. Like compound A it decomposed on attempting to isolate it by column chromatography on silicic acid. Purification of (VI) was achieved by preparative TLC with subsequent gel filtration on Sephadex LH-20. Compound (VI) was also obtained by the interaction of (III) with (I) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS). However, diphosphate (VII) was formed together with (VI) in this way.

The other possible by-product from the phosphorylation of (I) with phosphorus oxychloride, viz., (VII), was synthesized by the known procedure of [12] by the condensation of two molecules of (III) in the presence of DCC. The structure of compound (VII) was confirmed by elemental analysis and by IR, UV, and [^{31}P]-NMR spectra. The presence of a pyrophosphate bond was also demonstrated by acid hydrolysis leading to the initial (III).

Comparison of the data of [^{31}P]-NMR spectra of (VI, VII) and initial (III) made it possible to record that the introduction of an additional DL-α-tocopherol substituent into the molecule of phosphate (III) (δ 2.9 ppm) led to a shift in the signal towards high field (Δ 4.1 ppm) and the formation of a pyrophosphate bond —P—O—P— explaining the shift Δ 10.8 ppm relative to the signal of phosphate (III). The difference in the [^{31}P]-NMR spectra of monophosphate (III), diester (VI), and diphosphate (VII) made it possible to use this method for the identification of a series of phosphate derivatives of (I). At the same time the studied structural changes of phosphate (III) had practically no influence on the position of the absorption maximum in the UV spectrum.

In the course of studying the properties of the given compounds a difference was recorded in the color of their spots on chromatograms (Silufol UV-254 plates) on development with a solution of ammonium molybdate in sulfuric acid. Thus a violet coloration was seen for diphosphate (VII) while monophosphate (III) and diester (VI) were displayed as bright blue spots.

EXPERIMENTAL

Chromatography of reaction products was carried out on columns of silica gel L 40/100 (Czechoslovakia), by gel filtration on columns of Sephadex LH-20 (Sweden), and by preparative chromatography on plates of silica gel L 40/100 (Czechoslovakia) in the solvent system chloroform (system A), chloroform—methanol 4:1 (system B), chloroform—methanol—25% aqueous solution 15:5:1 (system C). System C was used for the TLC of the obtained compounds on Silufol UV-254 plates (Czechoslovakia). IR spectra were taken in thin films with a Perkin-Elmer 257 instrument (West Germany), UV spectra were taken on a Specord UV-VIS spectrophotometer (West Germany) in the 240-350 nm region. [^{31}P]-NMR spectra were described on a WP-60 spectrometer with Fourier transform on a B-NC 28 computer (Brucker—Physik AG, West Germany) at a frequency of 24.28 MHz in deuterochloroform at a concentration of 0.2 M. Chemical shifts are

given relative to 85% phosphoric acid used as internal standard. Spectra were described with wide band suppression of heteronuclear spin–spin coupling of $^{31}P-\{H^1\}$.

Chlorophosphate Dianilide (IV). A solution of freshly distilled aniline (37.20 g, 0.400 mole) in dry ether (50 ml) was added during 45 min with stirring to a cooled (0°C) solution of phosphorus oxychloride (15.75 g, 0.103 mole) in dry chloroform (50 ml). The reaction mixture was kept at 18-20°C for 12 h. The resulting solid was separated and washed with ice water until the absence of a reaction for chloride ion. The solid was dried for 5 h at 18-20°C and recrystallized. Compound (IV) (1.86 g, 15%) was obtained having mp 173-174°C (from chloroboenzene). [^{31}P]-NMR ($CdCl_3$), δ, ppm, 7.2.

DL-α-Tocopheryl Phosphate (III). A solution of recrystallized (IV) (1.86 g, 0.00628 mole) in dry pyridine (10 ml) was added during 30 min to a cooled (–20°C) solution of DL-α-tocopherol (I) (0.80 g, 0.00185 mole) in dry pyridine (10 ml). The reaction mixture was stirred for 30 min at –20°C and then for 30 h at 18-20°C. A solution of potassium acetate (2.00 g, 0.02 mole) in water (80 ml) was then added to the reaction mixture. The mixture was stirred for 30 min and the reaction product extracted with chloroform, the extract was dried with sodium sulfate, and the solvent distilled off. The obtained DL-α-tocopheryl phosphate dianilide (V) was dissolved in a mixture (40 ml) of pyridine and acetic acid (1:1) and isoamyl nitrite (3.30 g, 0.0282 mole) was added. The reaction mixture was stirred for 20 h, evaporated, and the residue dissolved in ether (20 ml). The mixture was acidified with hydrochloric acid (d_4^{20} 1.18) to pH 1.0-2.0 (by universal indicator). The ether solution was separated from the resulting solid, the filtrate evaporated, and dried in vacuum (10-15 mm) at 30°C. Technical (III) (1.27 g) was obtained. Isolation of pure (III) was carried out by column chromatography (diameter 30 mm, 150 g silica gel), contaminants were eluted with system A and phosphate (III) with system B. The eluate was evaporated and the residue dried in vacuum (0.1 mm) at 20°C. Compound (III) (0.22 g, 23%) was obtained. Found, %: C 68.00; H 10.00; P 6.10. $C_{29}H_{51}O_5P$. Calculated, %: C 68.20; H 10.10; P 6.06. UV spectrum ($CHCl_3$), λ_{max}, nm ($E_{1cm}^{1\%}$): 287 (49). [^{31}P]-NMR spectrum ($CDCl_3$), δ, ppm: 2.9. IR spectrum, ν, cm^{-1}: 1260 (–P–O–C–). R_f 0.1 (system C).

Bis-(DL-α-tocopheryl) Phosphate (VI). A. A solution of dichlorohexylcarbodiimide (DCC) (0.49 g, 0.0024 mole) in dry ether (5 ml) was added during 30 min to a mixture of (III) (1.02 g, 0.0020 mole) and (I) (0.86 g, 0.0020 mole) in dry ether (5 ml). The reaction temperature was maintained in the range 0-5°C for 2 h. The precipitate of dicyclohexylurea was separated and washed with ether (4 × 5 ml). The combined filtrate was evaporated in vacuum (10-15 mm) at 20°C. The residue was purified by preparative TLC on plates (20 × 20 cm) of silica gel L 40/100 (Czechoslovakia) in system C, and then by gel filtration on a column (diameter 10 mm, 3.00 g Sephadex LH-20, Sweden) eluting with system A. The eluate was evaporated and dried in vacuum (0.1 mm) at 20°C. Compound (VI) (0.63 g, 33.5%) was obtained. Found, %: C 75.90; H 10.90; P 3.70. $C_{58}H_{99}O_6P$. Calculated, %: C 75.40; H 10.90; P 3.35. UV spectrum ($CHCl_3$), λ_{max}, nm ($E_{1cm}^{1\%}$): 287 (59). [^{31}P]-NMR spectrum ($CDCl_3$), δ: ppm 7.0. IR spectrum, ν, cm^{-1}: 1260 (–P–O–C–). R_f 0.6 (system C).

B. A solution of (I) (0.67 g, 0.00156 mole) in dry pyridine (10 ml) was added during 10 min to a solution of (III) (0.40 g, 0.00078 mole) and 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) (0.71 g, 0.00234 mole) in dry pyridine (30 ml). After 3 h the reaction mixture was poured into ice water (100 ml) and the solution acidified with hydrochloric acid (d_4^{20} 1.18) to pH 1.0 (by universal indicator). The reaction product was extracted with ether. The ether solution was evaporated to a volume of 15 ml, water (5 ml) and hydrochloric acid (d_4^{20} 1.18) (1 ml) were added, and the mixture heated to 30°C for 2 h. The reaction mixture was diluted with ether, the ether layer separated, and evaporated. The residue was purified by preparative TLC on plates (20 × 20 cm) of silica gel L 40/100 (Czechoslovakia) eluting with system C and then by gel filtration on a column (diameter 10 mm, 3.00 g Sephadex LH-20, Sweden) eluting with system A. The eluate was evaporated and dried in vacuum (0.1 mm) at 20°C. Compound (VI) (0.22 g, 30%) was obtained.

P,P'-Bis(DL-α-tocopheryl) Disphosphate (VII). A solution of DCC (0.21 g, 0.001025 mole) in dry ether (5 ml) was added during 30 min to a cooled (0-5°C) solution of (III) (1.05 g, 0.00205 mole) in dry ether (5 ml) under conditions of continuous stirring. The reaction mixture was then stirred for a further 15 h at 0°C. The resulting solid was filtered off and washed with ether. The combined filtrate was evaporated in vacuum (10-15 mm) at 20°C. Technical (VII) (0.53 g) was obtained the purity of which was checked by gel filtration on a column (diameter 10 mm, 3.00 g Sephadex LH-20, Sweden) eluting with system A. Compound

(VII) (0.35 g,34%) was obtained. Found, %: C 70.00; H 10.30; P 6.20. $C_{58}H_{100}O_9P_2$. Calculated, %: C 69.40; H 10.03; P 6.17. UV spectrum ($CHCl_3$), λ_{max}, nm ($E^{1\%}_{1cm}$): 288 (57). [^{31}P]-NMR spectrum ($CDCl_3$), δ, ppm: 13.8. IR spectrum, ν, cm^{-1}: 1260 (—P—O—C—), 960 (—P—O—P—). R_f 0.54 (system C).

Splitting of Pyrophosphate Bond in (VII). Hydrochloric acid (d_4^{20} 1.18) (1 ml) was added to a solution of (VII) (0.10 g) in a mixture of tetrahydrofuran (THF) (10 ml) and water (2 ml) and the mixture was heated at the boiling point for 3 h, then evaporated. The residue was dissolved in ether (50 ml), washed with water, evaporated once again and dried. Compound (III) (0.07 g,70%) was obtained.

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PREPARATION OF DIGITOXIN FROM LEAVES OF ORIENTAL FOXGLOVE (*Digitalis orientalis* LAM.)

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Digitoxin (I) is used as a cardiotonic agent [1]. In 1935 it was shown [2] that in the leaves of purple foxglove, I is formed during the decomposition of purpurea-glycoside A. The preparation of I from *Digitalis ciliata* [3] and *Digitalis ferruginea* [4] is based on the hydrolysis of native plant glycosides.

In 1968 it was shown [3] that the main cardiac glycosides from oriental foxglove are lanatosides A, B, and C.

Since oriental foxglove is characterized by a high content of lanatoside A (II) in the leaves [5], we studied the possibility of obtaining I from this potentially valuable raw material.

EXPERIMENTAL

Air-dried leaves (from 1 and 2 years) of oriental foxglove (*Digitalis orientalis* Lam.) of the Scrophulariaceae family, grown in the Krasnodar region (North-Caucasian Zonal Experimental Station of All-Union Institute of Medicinal Plants (ZOS VILR) and near Moscow (VILR) were provided by the VILR workers D. A. Pakal'nyi, N. F. Bezukladnikova, and V. L. Tikhonova.

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