ml.) and with saturated ammonium sulfate solution (once, 100 ml.). Evaporation of the chloroform solution gave 5 g. of a very pale yellow oil which solidified on standing at room temperature. The residue was sublimed directly at 50° and 0.2 mm. to provide 4.35 g. (85%) of white crystals, m.p. 62.5–63.5°. Thin layer chromatography on silica gel (ether–hexane, 1:1) indicated the sample was homogeneous, R_f 0.70. The analytical sample was obtained by two additional sublimations: m.p. 65.5–66.0°; ν_{max} 3040, 3070 (w), 2940, 2870, 2820, 2790 (s), 1703 (mw), 1660 (s), a cluster of maxima between 1460 and 1355 (m), 1220 (s), 1140 (s), 1100, 1040, 1000 (ms), and 780 (s) cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.80; H, 8.31.

1,2,3,4,5,6,7,8-Octahydronaphthalene-2,7-dione (V).—Under a nitrogen atmosphere 0.292 g. (0.00152 mole) of 2,7-dimethoxytetrahydronaphthalene (IV) was added to a previously deoxygenated solution of 0.250 g. (0.00200 mole) of oxalic acid dihydrate in 1.5 ml. of water and 20.0 ml. of methanol. The mixture was warmed gently on the steam bath until the solid had dissolved. The solution was allowed to stand for 15 min. at room temperature, then 0.210 g. (0.00200 mole) of sodium carbonate was added, and the solvent was removed with a rotary evaporator. The crude product was dissolved in 25 ml. of chloroform, the organic phase was dried with anhydrous magnesium sulfate, and on evaporation there was produced 0.278 g. of a yellow oil which solidified on standing. The material was sublimed directly at 58° and 0.2 mm. to give pale yellow crystals. The analytical sample was obtained by two additional sublimations: m.p. 62.5–63.5°; R_f 0.30 (same solvent system); ν_{max} 2700, 1700 (broad), 1605, several maxima between 1450 and 1300, 1200, 1185, 1140, 1000, and 850 cm.$^{-1}$; δ 2.80 (singlet, 4H, protons which are both allylic and on a carbon atom α to a carbonyl group) and 2.55 (singlet, 8H, protons which are either allylic or on a carbon atom α to a carbonyl group).

Anal. Calcd. for $C_{10}H_{12}O_2$: C, 73.14; H, 7.37. Found: C, 73.22; H, 7.57.

7-Hydroxy-1,2,3,7,8,9-hexahydronaphthalen-2-one (VI).—During the purification of 1,2,3,4,5,6,7,8-octahydronaphthalene-2,7-dione (V), a significant portion of a dark yellow substance remained unaffected at 58°. This material was induced to sublime at 135° and 0.2 mm. and afforded a yellow solid, m.p. 179.0–179.5°. The analytical sample was crystallized from water: m.p. 179.0–179.5°; R_f 0.12 (same solvent system); ν_{max} 3400–2500 (very broad), 2950, 1595 (vs), 1530 (vs), 1345, 1335, and 1165 (ms) cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{12}O_2$: C, 73.14; H, 7.37; neut. equiv., 164. Found: C, 72.94; H, 7.34; neut. equiv., 164.

7-Methoxy-1,2,3,7,8,9-hexahydronaphthalen-2-one (VIII).—A solution of 0.500 g. (0.00260 mole) of 2,7-dimethoxytetrahydronaphthalene (IV) in 0.1 ml. of concentrated hydrochloric acid, 2.0 ml. of water, and 35.0 ml. of methanol was allowed to stand for 2 hr. at room temperature. The solvent was removed with a rotary evaporator and the residue was sublimed at 65° and 1 mm. to yield 0.418 g. (89%) of white crystals, m.p. 86.5–87.5°. The analytical sample was obtained by two additional sublimations: m.p. 92.0–92.2°; ν_{max} 3010 (w), 2930 (s), 1650 (s), 1605 (s), 1575 (s), and 875 (s) cm.$^{-1}$; δ 5.72, 5.43 (1H each, two nonequivalent vinyl protons), 3.70 (3H, vinyl methoxy), and 2.60–1.4 (9H, remaining allylic protons or protons on carbon atom α to the carbonyl group).

Anal. Calcd. for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.96; H, 7.94.

A reddish brown derivative formed when 2,4-dinitrophenylhydrazine solution was added to a methanolic solution of 2,7-dimethoxytetrahydronaphthalene (IV). After chromatographing through alumina (neutral, activity I) the dinitrophenylhydrazone was crystallized from ethyl acetate–ethanol (1:1) as short, red needles, m.p. 215.0–215.5°, $\lambda_{max}^{CHCl_3}$ 422.5 mμ (log ϵ 4.65) and 317 mμ (log ϵ 4.33).

Anal. Calcd. for $C_{17}H_{18}N_4O_5$: C, 56.98; H, 5.06; N, 15.63. Found: C, 56.49; H, 5.18; N, 15.80.

Reactions Attempted with 1,2,3,4,5,6,7,8-Octahydronaphthalene-2,7-dione (V).—Freshly prepared dione IV was added in turn to triphenylmethylphosphonium bromide and methylmagnesium bromide solutions. In each case a yellow precipitate formed as soon as the two phases were mixed together. Aliquots of each solution were analyzed for the presence of volatile products by gas chromatography. With reaction times varying from 2 to 4 hr., no evidence was seen for the formation of compound IX or related olefins.

Synthesis of Phospho Diester Using Phosphoryl Chloride

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It has been known that the phosphorylation of 2′,3′-O-isopropylidene nucleoside with phosphoryl chloride affords phosphorylated products in addition to expected nucleoside monophosphate. Levene and Tipson[1] have reported the formation of adenosine N^6,5′-diphosphate in addition to the 5′-monophosphate by the reaction of 2′,3′-O-isopropylideneadenosine with phosphoryl chloride, but not the formation of any other phosphorylated products in the case of 2′,3′-O-isopropylideneinosine.[2] Recently Khorana,[3] *et al.*, attempted to apply this method to 2′,3′-O-isopropylideneguanosine and obtained a mixture which consists of guanosine 5′-monophosphate and a small amount of phosphorus-containing products, such as guanosine 5′-diphosphate, 5′-triphosphate, and higher phosphates.

In the present work phosphorylation of 2′,3′-O-isopropylideneinosine (I) with 1.4 equiv. of phosphoryl chloride in anhydrous pyridine and hydrolytic removal of the isopropylidene group gave a mixture of two products. Crystallization of the sodium salt of the mixture afforded a precipitate of the major component which was identified as inosine 5′-monophosphate (II). The minor component was obtained in pure state from the mother liquors by fractional crystallization of the barium salt. It showed the ultraviolet absorption spectrum characteristic of hypoxanthine and accounted for 20% of the ultraviolet-absorbing material in the crude product. Potentiometric titration with acid suggested the product was diinosine 5′-phosphate (III), since no basic group corresponding to a phosphate dianion was observed. The barium salt of the compound had a molecular formula, $C_{20}H_{22}O_{12}N_8P \cdot 0.5Ba$, and consumed 1.8 moles of periodate, which further confirm the structure assignment. As expected the product was resistant to venom 5′-nucleotidase.

Further proof of the structure was obtained by comparison of the product with authentic samples of diinosine phosphate, which was prepared by two methods. The first method involves the reaction of *p*-nitrophenyl phosphorodichloridate (IV)[4] with I in pyridine and hydrolytic removal of the protective groups. III was isolated in 31% yield. The second method involves the condensation of 2′,3′-di-O-acetylinosine (VII) with 2′,3′-di-O-acetylinosine 5′-phosphate pyridinium salt (VIII) by the aid of dicyclohexylcarbodiimide[5] followed by deacetylation with sodium ethoxide. VII was prepared from 5′-tritylinosine (V) by acetylation to diacetate (VI) and subsequent detritylation. VIII was synthesized by acetylation of inosine 5′-phosphate pyridinium salt with acetic anhydride in pyridine.

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In order to determine the optimum conditions for the formation of the diester, the effect of ratio of reactants and order of addition of the reagents on the yield of the diester was investigated. The slow addition of 1 mole of I to 2 moles of phosphoryl chloride gave no III, but to 1.2–1.4 moles of phosphoryl chloride gave III in 20–22% yield. Addition of 1 mole of phosphoryl chloride to 0.5–2 moles of I gave an improved yield of III (30–70%). The presence of a trace amount of moisture in the reaction mixture promoted the formation of III and lowered markedly the yield of II.

Experimental

Paper chromatography was undertaken with two solvent systems: solvent A, *n*-propyl alcohol–aqueous ammonia–water (20:12:3); solvent B, isopropyl alcohol–saturated aqueous ammonium sulfate–water (2:79:19).

Preparation of Diinosine Phosphate (III) from 2′,3′-O-Isopropylideneinosine (I) and Phosphoryl Chloride.—To a solution of I (100 g.) in dried pyridine (1.7 l.) cooled to −10° was added a solution of phosphoryl chloride (15 ml.) in dried pyridine (200 ml.) previously cooled to −10° with stirring in 7 min. After being kept for 1 hr. at −5°, the reaction mixture was poured into ice and water with vigorous stirring. After the pH of the solution was adjusted to 9 with aqueous sodium hydroxide, pyridine was removed under reduced pressure. Then the residual solution was acidified to pH 1.5 with hydrochloric acid and the acidic solution was warmed for about 1 hr. at 70° to remove the isopropylidene group. The resultant mixture was analyzed by paper chromatography. The per cent yields of II and III were estimated to be 30 and 70%, respectively, by the optical density at 250 mμ. The R_f values of II and III were 0.11 and 0.12 (solvent A) and 0.55 and 0.28 (solvent B), respectively. After neutralization with sodium hydroxide, the solution was concentrated to 500 ml., and most of II was separated by addition of an equal volume of ethyl alcohol. The filtrate was evaporated to 300 ml. and addition of 2 vol. of ethyl alcohol to the residue gave the precipitate which contained a large amount of III. The precipitate was dissolved in water, sodium ions were removed with IRC-50 (H^+ form) resin, and the solution was treated with barium hydroxide. The barium salt was recrystallized from water–ethyl alcohol repeatedly: $\lambda_{max}^{0.1\,N\,HCl}$ 250.4 mμ (ϵ_{250} 21,000), $\lambda_{max}^{0.1\,N\,NaOH}$ 255.0 mμ (ϵ_{250} 25,600), pK_a 4.01.

Anal. Calcd. for $C_{20}H_{22}O_{12}N_8P\cdot 0.5Ba$: C, 35.23; H, 3.51; N, 16.39; P, 4.1. Found: C, 35.85; H, 4.09; N, 16.34; P, 4.4.

Synthesis of Diinosine Phosphate from 2′,3′-O-Isopropylideneinosine (I) and *p*-Nitrophenyl Phosphorodichloridate (IV).—To a solution of I (2 g.) in pyridine (50 ml.), IV (1 g.) was added, and the mixture was kept overnight at room temperature. After the reaction mixture was poured into water (100 ml.), the pH of the solution was adjusted to 8.0, and pyridine was removed under reduced pressure with repeated addition of water. The concentrated solution (about 50 ml.) was acidified to pH 1.5 with 1 *N* hydrochloric acid and held for 1.5 hr. at 70° to remove the isopropylidene group. After the acidic solution was neutralized with sodium hydroxide, an additional 250 mg. of sodium hydroxide was added and the alkaline solution was heated for 2 hr. at 100° to remove the *p*-nitrophenyl group. The brownish solution was again slightly acidified with dilute hydrochloric acid and the liberated *p*-nitrophenol was extracted three times with ethyl acetate (50 ml.). Inosine, II, and III were detected by paper chromatography in the aqueous solution. After neutralization and concentration of the aqueous solution to 50 ml., 100 ml. of ethyl alcohol was added to precipitate II and III as sodium salts. I was purified as the barium salt, yield 1.35 g. (31%). The product was identical with the sample which was obtained by phosphorylation of I with phosphoryl chloride, by paper chromatography, paper electrophoresis, and infrared spectra.

5′-Tritylinosine (V).—Trityl chloride (6 g.) and anhydrous pyridine (50 ml.) were added to a solution of anhydrous inosine (5 g.) in dimethylformamide (100 ml.), and the mixture was heated for 2 hr. at 40°. After cooling to 0° the reaction mixture was poured into 500 ml. of ice and water with vigorous stirring. The precipitate was filtered, washed with benzene to remove trityl alcohol, and dried; yield 1.45 g. (crude 15.2%). Recrystallization from ethyl alcohol gave the pure crystal which melted at 207–210°. R_f value of V on paper chromatogram was 0.83 (solvent A).

Anal. Calcd. for $C_{27}H_{26}N_4O_5$: C, 68.22; H, 5.13; N, 10.97. Found: C, 67.91; H, 5.44; N, 10.98.

5′-Trityl-2′,3′-di-O-acetylinosine (VI).—Trityl chloride (6 g.) and anhydrous pyridine (50 ml.) were added to a solution of anhydrous inosine (5 g.) in dimethylformamide (100 ml.), and the mixture was heated for 2 hr. at 50°. To the resultant pale yellow solution was added acetic anhydride (15 ml.); then the mixture was kept for 18 hr. at room temperature. After the reaction mixture was poured into 500 ml. of ice and water, the deposited precipitate was filtered and washed with benzene to remove trityl alcohol; yield 3.7 g. (33%). Recrystallization from ethyl alcohol gave the pure crystal, m.p. 174°.

Anal. Calcd. for $C_{33}H_{30}N_4O_7$: C, 66.65; H, 5.09; N, 9.42. Found: C, 66.81; H, 5.26; N, 9.32.

The infrared spectrum of VI showed absorption band at 1240 cm.$^{-1}$ due to acetyl group.

2′,3′-Di-O-acetylinosine (VII).—A solution of VI (5 g.) in 80% acetic acid (100 ml.) was boiled for 30 min. After cooling, the precipitate of trityl alcohol was filtered off. The filtrate was concentrated to dryness, and the residue was extracted with chloroform. The residual sirup obtained by concentration of the solution of chloroform was crystallized from ethyl alcohol–petroleum ether; yield 0.6 g. (23%). Recrystallization from ethyl alcohol–petroleum ether gave pure crystal which melted at 215°.

Anal. Calcd. for $C_{14}H_{16}N_4O_7$: N, 15.92. Found: N, 15.75.

Reaction of 2′,3′-Di-O-acetylinosine (VII) with 2′,3′-Di-O-acetylinosine 5′-Phosphate Pyridinium Salt (VIII).—The pyridinium salt of II, which was prepared from the sodium salt of II (1 g.) by use of IR-120 (pyridinium form),[5] was dissolved in 40 ml. of dried pyridine and 5 ml. of acetic anhydride. The homogeneous solution was allowed to stand overnight and evaporated to dryness under reduced pressure in the presence of pyridine. To the residue 10 ml. of 90% pyridine in water was added to hydrolyze excess acetic anhydride. The pyridinium acetate produced was removed by lyophilization. The product was dissolved in pyridine and evaporated to dryness, and this procedure was repeated to remove traces of water. The resultant substance VIII, VI (1 g.), and dicyclohexylcarbodiimide (6 g.) were dissolved in 50 ml. of pyridine, and the solution was kept overnight with shaking. After addition of 100 ml. of water, precipitated dicyclohexylurea was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was suspended in ethyl alcohol and a piece of metallic sodium was added to remove acetyl groups. In this solution II, III, inosine, and a trace of unknown spot (R_f 0.40 in solvent B) were found by paper chromatography (III could not be isolated in a pure state from the mixture).

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Enolic Concentrations in β-Keto Esters. Correlation of Bromometric and Ultraviolet Absorption Data[1]

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In an earlier paper,[3] we reported ultraviolet spectral data for a series of cyclic β-keto esters and appro-

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