formation of citric acid and isocitric acid from *cis*-aconitic acid since part of the enzyme molecules would be occupied by *cis*-aconitate in one configuration and part by *cis*-aconitate in the inverted configuration. Considering the reverse reaction, the direct formation of *cis*-aconitic acid from citric acid and from isocitric acid is apparent and the conversion of citric acid to isocitric acid and *vice versa* seemingly requires *cis*-aconitic acid as an intermediate. While the kinetic and isotopic data[55] show that in part the isocitrate to citrate conversion passes through *cis*-aconitate as an intermediate, they also show that in part the isocitrate to citrate conversion occurs directly. This latter fact is an apparent objection to this new view of the *cis*-aconitase reaction. Conceivably, it may be overcome if in the isocitrate to citrate conversion an intermediate (or *cis*-aconitate) in some instances does not leave the enzyme but rather pivoting on the acetate carboxyl is inverted. On this basis, then, the initial rate of isocitrate to citrate conversion is slow and direct but as the *cis*-aconitate concentration increases return of *cis*-aconitate to the enzyme in the proper configuration is increased and the rate of the isocitrate to citrate conversion would increase. This would account for both the lag period in the isocitrate to citrate (and *vice versa*) conversion and also for the deuterium incorporation into citrate from isocitrate.

If the enzyme–substrate complex is formed as indicated

$$E \cdots \text{Substrate} \cdots Fe^{++} \cdots E$$

then it would be possible for substrate to disengage itself from the complex without leaving the enzyme, for example, by a loosening of the bonds between substrate and Fe^{++}. As a matter of interest, if isocitric acid and citric acid are attached to Fe^{++} as typical α-hydroxy acid 5-membered chelate rings, then transformation of these to *cis*-aconitic acid might loosen the attachment to Fe^{++} and permit *cis*-aconitic acid to assume the other configuration.

Of course, the available experimental evidence does not permit either definitive conclusions or a detailed mechanistic picture of the reaction.

When it is considered that all three enzyme systems operate by a *trans* mechanism, it seems likely that the three enzymes in question would have, at least, common architectural features. Such features might well be a polypeptide chain, which when in combination with substrate is folded in such a way that at the active site there is a *trans* juxtaposition of those specific groups which participate in the reaction and that the substrate when in combination with the enzyme is not at the surface of the protein but rather within, to a greater or lesser extent, the protein. Such an arrangement would be in line with the induced-fit hypothesis of Koshland,[51] would provide a role for tertiary protein structure[56] in maintaining enzyme activity and would also be similar to the accepted picture of the myoglobin molecule in which a heme residue is imbedded in the protein molecule between the adjacent sides of a protein fold.[57]

Acknowledgment.—This investigation was supported by Public Health Service research grant, RG-6245, from the Division of General Medical Sciences, Public Health Service.

(55) See ref. 33 and references contained therein and H. A. Krebs and O. Holzach, *Biochem. J.*, **52**, 527 (1952).

(56) F. M. Richards, *ibid.*, **54**, 207 (1959).

(57) J. C. Kendrew, *Federation Proc.*, **18**, 740 (1959).

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Nucleotides. II.[1] A New Procedure for the Conversion of Ribonucleosides to 2′,3′-*O*-Isopropylidene Derivatives[2]

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Di-*p*-nitrophenyl hydrogen phosphate is an efficient catalyst for the conversion of purine and pyrimidine ribonucleosides to their 2′,3′-*O*-isopropylidene derivatives, and 2,2-dimethoxypropane is highly effective for maintaining the anhydrous conditions necessary for such conversions. The two agents in combination caused quantitative conversion of all nucleosides examined to the respective 2′,3′-*O*-isopropylidene derivatives; previous procedures have been of more restricted usefulness. The products were isolated by a method applicable to all and obtained in purified form in *ca.* 90% yield. The procedure can simplify conversion of ribonucleosides to 5′-phosphate derivatives since the intermediate isopropylidene nucleosides can often be phosphorylated *in situ*.

Purine and pyrimidine ribonucleoside 5′-monophosphates are starting materials for recent chemical syntheses of nucleotide coenzymes,[3–8] and a number of non-natural 5′-nucleotides have become of biological and chemotherapeutic interest as anabolites of anti-neoplastic purine and pyrimidine derivatives,[9] *e.g.*, of 6-mercaptopurine, 2-amino-6-mercaptopurine or 8-azaguanine. The synthesis of nucleoside 5′-phosphates[8] involves conversion

(1) Part I, A. Hampton and M. H. Maguire, *J. Am. Chem. Soc.*, **83**, 150 (1961).

(2) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant CY-3190) and from the Atomic Energy Commission (Contract AT(30-1)-910).

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(5) J. G. Moffatt and H. G. Khorana, *ibid.*, **81**, 1265 (1959).

(6) A. M. Michelson, *Chemistry & Industry*, 1267 (1960).

(7) R. W. Chambers, P Sbapiro and V. Kurkov, *J. Am. Chem. Soc.*, **82**, 970 (1960).

(8) Reviewed by H. G. Khorana, *Federation Proc.*, **19**, 931 (1960).

(9) Reviewed by H. G Mandel, *Pharmacol. Rev.*, **11**, 743 (1959).

of purine or pyrimidine ribonucleosides to their respective 2′,3′-*O*-isopropylidene derivatives and subsequent phosphorylation of these protected nucleosides.

Conversion of the nucleosides to isopropylidene derivatives has sometimes given low or variable yields, and no one method has produced high yields with a wide variety of nucleosides. The procedure presented here has given essentially quantitative conversions with all purine and pyrimidine nucleosides examined, including compounds which have previously given difficulty. This method is also advantageous since it allows subsequent phosphorylation of the isopropylidene nucleoside *in situ*.

A common procedure for the preparation of 2′,3′-*O*-isopropylidene nucleosides consists of treatment of ribonucleosides with a boiling 10–15% solution of zinc chloride in acetone,[10] with satisfactory yields reported for the derivatives of adenosine (84%,[11] 75–85%[12]), guanosine (64%,[13] 70–80%[14]), 9-β-D-ribofuranosylpurine (80%[15]) and cytidine (95%[13] and variable to 80%[14]) but poor yields for the derivatives of inosine (48%[12]) and 6-thioguanosine (2-amino-6-mercapto-9-β-D-ribofuranosylpurine) (40%[1]).

p-Toluenesulfonic acid forms acetone-soluble salts with many nucleosides, and addition of ten molecular equivalents of this catalyst to suspensions of nucleosides in acetone at room temperature has given high yields of the isopropylidene derivatives of 9-β-D-ribofuranosylpurine (90% yield[15]), ribosylthymine (95%[16]), cytidine (95%[7]) and 6-chloro-9-β-D-ribofuranosylpurine (85%[1]). In this Laboratory, isopropylidene adenosine was obtained in 90% yield if a minimum of ten equivalents of *p*-toluenesulfonic acid was employed. Inosine, reacting less readily under the same conditions, gave 60% of the isopropylidene derivative, while 6-thioinosine[1] and 6-thioguanosine[1] did not react unless solution of the nucleosides was promoted by the addition of cupric ions; that modification proved satisfactory for 6-thioinosine (80%) but with 6-thioguanosine gave a yield of only 50%.

Di-*p*-nitrophenyl hydrogen phosphate (pK_a 1.7 in 50% aqueous ethanol[17]) is a sufficiently strong acid to form salts with most purine and pyrimidine nucleosides. Since its lipophilic area is approximately twice that of *p*-toluenesulfonic acid, its salts with nucleosides are correspondingly more soluble in acetone. When four equivalents of di-*p*-nitrophenyl phosphate were substituted for ten of *p*-toluenesulfonic acid in the reactions discussed above and cupric ions were omitted, adenosine, inosine, 6-thioinosine and 6-thioguanosine all dissolved readily at room temperature and analysis of the solutions by means of paper chromatography and electrophoresis showed that essentially complete conversion to the respective isopropylidene derivatives occurred within 5 hr. With cytidine, inosine, guanosine and 6-thioinosine, the minimum amount of catalyst necessary to give high yields of isopropylidene derivatives was 2.2 to 3.5 equivalents.

The presence of traces of water in reactions catalyzed by di-*p*-nitrophenyl phosphate leads to marked reductions in apparent reaction rates and to the establishment of unsatisfactory equilibria between nucleosides and their isopropylidene derivatives. When such condensations are carried out in the presence of 2,2-dimethoxypropane, this ketal undergoes an acid-catalyzed reaction with the water present to yield acetone and methanol. Reaction mixtures containing eight equivalents of the ketal were analyzed by paper chromatography in several solvent systems in which 1% of unchanged ribonucleoside was detectable; after 5 hr. the only components present were the desired isopropylidene nucleoside and di-*p*-nitrophenyl phosphate, which indicated yields of at least 99%. This was confirmed by isolation in high yields of the individual isopropylidene derivatives.

Reaction conditions for each nucleoside are given in Table I. Inclusion of 2,2-dimethoxypropane both accelerated the reactions and reduced, by at least one equivalent, the amount of di-*p*-nitrophenyl phosphate required. With guanosine, for example, 60% conversion to isopropylidene guanosine occurred after 3 hr. using 2.4 equivalents of catalyst, whereas, in the presence of 2,2-dimethoxypropane, 80% conversion occurred after 1 hr. with 1.2 equivalents. The condensation of uridine with acetone, as in Table I, was not influenced when three equivalents of water were added, but a parallel experiment in which 2,2-dimethoxypropane was omitted yielded an equilibrium mixture containing *ca.* 90% uridine and 10% isopropylidene uridine, thus demonstrating both the ready reversibility of the reaction and the efficacy of 2,2-dimethoxypropane for maintaining anhydrous conditions.

TABLE I

CATALYSIS BY DI-*p*-NITROPHENYL PHOSPHATE OF ISOPROPYLIDENE NUCLEOSIDE FORMATION[a]

Nucleoside	Acetone (ml.)	Equivs. of catalyst	Analysis systems[b]	Conversion time (hr.)[d]	Yield of isolated product (%)[e]
Uridine	10	0.10	B, C	0.5	97
Cytidine	10	1.15	B, C	4.0	96
Adenosine	10	1.20	A, D	5.0	87[f]
Guanosine	14	1.20	B, C	5.0	92
Inosine	15	2.10	A, B	3.0	91
6-Thioinosine	24	2.00	B, C, D[c]	3.0	80[f]
6-Thioguanosine	14	1.25	C[c]	5.0	88
8-Azaguanosine	14	1.45	B, C	5.0	90

[a] Reaction mixtures contained 1 mmole of nucleoside and 8 mmoles of 2,2-dimethoxypropane; with 6-thioinosine, 15 mmoles of 2,2-dimethoxypropane was used. [b] See Experimental. [c] Analyzed also by electrophoresis. [d] Time of conversion of at least 99% of nucleoside to its isopropylidene derivative. [e] Products were isolated by the Dowex-1-HCO_3 procedure except where indicated. [f] Isolated by individual procedures.

Only the isopropylidene derivatives of uridine and cytidine dissolved in the acetone without assist-

(10) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **111**, 313 (1935); **121**, 131 (1937).

(11) F. Weygand and O. Trauth, *Ber.*, **84**, 633 (1951).

(12) J. Baddiley, *J. Chem. Soc.*, 1348 (1951).

(13) A. M. Michelson and A. R. Todd, *ibid.*, 2476 (1949).

(14) R. W. Chambers, J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3747 (1957).

(15) A. Hampton and D. I. Magrath, *ibid.*, **79**, 3250 (1957).

(16) B. E. Griffin, A. Todd and A. Rich, *Proc. Natl. Acad. Sci. U. S.*, **44**, 1123 (1958).

(17) N. S. Corby, G. W. Kenner and A. R. Todd, *J. Chem. Soc.*, 1234 (1952).

ance from di-*p*-nitrophenyl phosphate. Uridine (pK_a probably <0.5[18a]) is so weakly basic that 0.1 equivalents of the catalyst can provide sufficient hydrogen ions for the reaction, whereas cytidine (pK_a 4.2[18b]) is appreciably basic and over one equivalent (Table I) of the catalyst is required. Among the remaining nucleosides, inosine (pK_a 1.2[18c]) and 6-thioinosine are also weakly basic but require two equivalents of di-*p*-nitrophenyl phosphate to effect solution of their isopropylidene derivatives in acetone. The marked reactivity of uridine implies that prior protonation of the other nucleosides, either on ring nitrogens or on amino-substituents, may have slowed their reaction rates, possibly by coulombic repulsion of the additional hydrogen ions needed for catalysis.

This procedure for the conversion of ribonucleosides to 2′,3′-*O*-isopropylidene derivatives has proved more convenient than those previously available, since a non-hygroscopic catalyst is employed and precautions for the rigid exclusion of moisture (before and during the reaction) are unnecessary.

A spray technique aided characterization of isopropylidene derivatives on paper chromatograms. These derivatives do not react to the periodate spray test[19] for *cis*-α-glycol systems. Chromatograms could be treated with trichloroacetic acid under conditions causing hydrolysis of the acetone residues of isopropylidene derivatives and allowing the corresponding spots to react subsequently to the periodate spray test. This procedure is applicable to isopropylidene derivatives of other types of *cis*-α-glycols.

Isolation of Isopropylidene Nucleosides.—The suitability for this purpose of various salts of di-*p*-nitrophenyl phosphate was investigated. The sodium and ammonium salts were sufficiently insoluble in chloroform to permit satisfactory isolation of chloroform-soluble isopropylidene derivatives, *e.g.*, those of uridine and adenosine, and sufficiently soluble in water to permit isolation of the relatively insoluble isopropylidene-6-thioinosine. The isopropylidene derivatives of guanosine and 6-thioguanosine, however, had similar solubility properties to the above sodium and ammonium salts in a variety of solvents and invariably coprecipitated with di-*p*-nitrophenyl phosphate from aqueous solutions. Tri-*n*-butylammonium di-*p*-nitrophenyl phosphate, in contrast to most[20] isopropylidene nucleosides, was found to be readily soluble in benzene, and, following addition of tri-*n*-butylamine to reaction mixtures, isopropylidene guanosine and isopropylidene 6-thioguanosine were isolated in 60–75% yield. All the isopropylidene nucleosides are appreciably soluble in methanol, and a more general and quantitative method of isolation consisted of addition of reaction mixtures to an excess of ammonium bicarbonate in aqueous methanol and adsorption of the di-*p*-nitrophenyl phosphate onto the bicarbonate form of an anion-exchange resin. Isopropylidene derivatives containing a nuclear acidic function tended to be retained by the resin but were readily displaced by additional ammonium bicarbonate solution. Yields of purified isopropylidene nucleosides ranging from 88 to 97% were obtained (Table I).

In situ **Phosphorylation of Isopropylidene Nucleosides.**—The most satisfactory synthesis described for guanosine 5′-phosphate[14] involved initial treatment of isopropylidene guanosine in dioxane solution with 1.6 equivalents of tetra-*p*-nitrophenyl pyrophosphate and 0.8 equivalent of di-*p*-nitrophenyl phosphate to yield 95% of isopropylidene guanosine 5′-di-*p*-nitrophenyl phosphate. When the mixture resulting from the reaction of guanosine, di-*p*-nitrophenyl phosphate (1.2 equivalents), acetone and 2,2-dimethoxypropane was treated directly with 2.5 equivalents of tetra-*p*-nitrophenyl pyrophosphate[21] in dioxane, the yield of the above phosphotriester was poor (15%), but similar phosphorylation after removal of acetone and 2,2-dimethoxypropane *in vacuo* gave a yield of 93%. On paper chromatograms, the product was identical with authentic[14] material, and when treated with alkali at room temperature, it liberated the same amount of *p*-nitrophenol. The isopropylidene derivatives of 6-thioinosine and 6-thioguanosine were also phosphorylated *in situ* in high yield by the same reagent, thereby simplifying the conversion[1] of these nucleosides to their 5′-phosphates.

Uridine and cytidine 5′-phosphates are conveniently prepared by phosphorylation of the respective isopropylidene nucleosides with a mixture of phosphoric acid and phosphorus pentoxide.[7,22,23] Cytidine was condensed with acetone (Table I), volatiles removed, and the residual mixture of isopropylidene cytidine and di-*p*-nitrophenyl phosphate was treated with the above reagent. A homogeneous reaction solution was not obtained and paper electrophoretic analysis showed the formation of cytidine 5′-phosphate in 45% yield and a mixture of cytidine 2′- and 3′-phosphates in 8% yield. Phosphorylation of isolated isopropylidene cytidine, which furnishes 65% of cytidine 5′-phosphate,[7] is thus a preferable procedure.

Phosphorylation of isopropylidene nucleosides in the presence of di-*p*-nitrophenyl phosphate may prove practicable in other syntheses of nucleotides. For example, dibenzyl-[24] or di-(β-cyanoethyl)[25] phosphorochloridates, employed in pyridine and lutidine solutions, have given adenosine and guanosine 5′-phosphates in good yield. Under such conditions, di-*p*-nitrophenyl phosphate may be expected to produce P[1]-dialkyl-P[2]-di-*p*-nitrophenyl pyrophosphates. Those, if effective in competition with an excess of the dialkyl phosphorochloridates, could yield the desired dialkyl esters of the nucleo-

(18) (a) The basic pK_a of uracil is *ca.* 0.5 (W. E. Cohn, quoted by G. H. Beaven, E. R. Holiday and E. A. Johnson, in E. Chargaff and J. N. Davidson, "The Nucleic Acids," Vol. I, Academic Press, Inc., New York, N. Y., 1955, p. 500); nucleosides are, in general, less basic than the parent purines or pyrimidines (see pp. 502, 508); (b) P. A. Levene and H. S. Simms, *J. Biol. Chem.*, **65**, 519 (1925); (c) Ref. 18a, p. 508.

(19) J. G. Buchanan, C. A. Dekker and A. G. Long, *J. Chem. Soc.*, 3162 (1950).

(20) Some isopropylidene nucleosides are appreciably soluble in benzene, *e.g.*, 2′,3′-O-isopropylidene-9-β-D-ribofuranosylpurine[15] and its 6-chloro derivative.[1]

(21) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **79**, 3741 (1957).

(22) R. H. Hall and H. G. Khorana, *ibid.*, **77**, 1871 (1955).

(23) A. M. Michelson, *J. Chem. Soc.*, 1957 (1958).

(24) J. Baddiley and A. R. Todd, *ibid.*, 648 (1947).

(25) H. Witzel, H. Mirbach and K. Dimroth, *Angew. Chem.* **72**, 751 (1960).

tides rather than the di-*p*-nitrophenyl esters since phosphorylation of alcohols by mixed pyrophosphates yields phosphotriesters derived from the weaker component acid.[26]

Experimental

Methods.—Paper chromatograms were run on Schleicher and Schuell No. 597 paper by the ascending method. Solvent systems: (A) ethanol–water (7:3), (B) isopropyl alcohol–water–NH_4OH (70:25:5), (C) 1-butanol–acetic acid–water (5:2:3), (D) isopropyl alcohol–water (7:3), (E) system C (4:1:5). Spots were located by inspection of the papers in ultraviolet light (Corning filter No. 9863); R_f values are listed in Table II.

TABLE II

PAPER CHROMATOGRAPHY OF NUCLEOSIDE DERIVATIVES[a]

Compound	R_f values in solvent systems A	B	C	D
Uridine	0.53	0.47	0.55	0.63
2',3'-*O*-Isopropylidene uridine	.67	.70	.79	.82
Cytidine	.50	.55	.51	.55
2',3'-*O*-Isopropylidene cytidine	.66	.75	.77	.80
Adenosine	.47	.57	.60	.58
2',3'-*O*-Isopropylidene adenosine	.59	.75	.80	.78
Guanosine	..	.37	.46	.49
2',3'-*O*-Isopropylidene guanosine	..	.68	.78	.75
Inosine	.49	.50	.46	.52
2',3'-*O*-Isopropylidene inosine	.62	.70	.77	.78
6-Thioinosine	..	.45	.48	.57
2',3'-*O*-Isopropylidene 6-thioinosine	..	.70	.79	.79
6-Thioguanosine	..	.36	.46	..
2',3'-*O*-Isopropylidene 6-thioguanosine	..	.61	.78	..
8-Azaguanosine	..	.40	.47	..
2',3'-*O*-Isopropylidene 8-azaguanosine	..	.67	.81	..
Di-*p*-nitrophenyl hydrogen phosphate	.72	.83	.84	.85

[a] The R_f values for di-*p*-nitrophenyl phosphate, which was run on all chromatograms, are averages; values for the remaining compounds are relative to those of di-*p*-nitrophenyl phosphate.

For characterization of isopropylidene derivatives, the dried chromatograms were sprayed with 2% aqueous trichloroacetic acid, heated for 15 minutes in an oven at 90° and steamed over a bath for 15 minutes. They were suspended for 10 minutes at room temperature in a damp ammonia atmosphere, air-dried and sprayed for *cis*-glycol systems.[19] Spots corresponding to isopropylidene derivatives appeared blue on a light brown background; the sensitivity of detection was of the same order as that for the parent nucleosides.

Paper electrophoresis was carried out on Whatman No. 3MM paper with Model EC451 of the E-C Apparatus Co., Pa. Observed mobilities (cm./hr.) in 0.05 *M* sodium tetraborate (*p*H 9.1) at a gradient of 18 volts/cm. were: di-*p*-nitrophenyl phosphate, 4.3; 6-thioinosine, 6.7; 6-thioguanosine, 5.8; 2',3'-*O*-isopropylidene-6-thioinosine, 3.5; 2',3'-*O*-isopropylidene-6-thioguanosine, 2.9.

Ultraviolet absorption characteristics were examined with the Beckman spectrophotometers, the Model DU for quantitative determinations and the Model DK-2 for over-all characteristics of absorption curves.

Melting points (capillary method) are uncorrected. Microanalyses were by the Schwarzkopf Laboratory, Woodside, New York. Unless otherwise specified, substances were dried at room temperature *in vacuo* over NaOH.

***p*-Toluenesulfonic Acid as Catalyst.** (a) **2',3'-*O*-Isopropylidene Adenosine.**—*p*-Toluenesulfonic acid monohydrate (7.1 g., 37.5 mmoles) was added with exclusion of moisture to a magnetically-stirred suspension of adenosine (1.0 g., 3.75 mmoles; dried over P_2O_5 at 100°, 0.1 mm.) in anhydrous acetone (200 ml.; dried over K_2CO_3 for 3 days, filtered, dried over Drierite for 2 days and distilled from fresh Drierite). The solids dissolved almost immediately. The pale yellow solution, after 1 hr. at 25°, was added to a vigorously-stirred solution of $NaHCO_3$ (6.7 g., 80 mmoles) in ice and water (*ca.* 80 ml.). The mixture was evaporated to dryness under reduced pressure (finally at 50°, 10 mm. for 2 hr.) and the solid residue extracted with acetone in a Soxhlet apparatus. The acetone was evaporated *in vacuo* and the residual solid was dissolved in hot water (20 ml.). The solution was clarified with Celite filter-aid and concentrated *in vacuo* to *ca.* 10 ml. After 2 hr. at 2°, it yielded 1.07 g. (90%) of 2',3'-*O*-isopropylidene adenosine as cream-colored needles, m.p. 216–217°. Recrystallization from water gave 1.03 g., m.p. 217.5–218° (not depressed by admixture with isopropylidene adenosine[12]).

A reaction carried out as above, in which aliquots were removed at intervals, added to an excess of aqueous $NaHCO_3$ and analyzed by paper chromatography in 1-butanol–water (86:14), showed that conversion of the adenosine (R_f 0.23) to isopropylidene adenosine (R_f 0.69) was complete within 40 minutes. It was established that 15% less acetone, or less than 10 equivalents of *p*-toluenesulfonic acid, resulted in incomplete or slow solution of the adenosine.

(b) **2',3'-*O*-Isopropylidene Inosine.**—*p*-Toluenesulfonic acid monohydrate (10 equivalents) was added to a suspension of inosine in 150 parts of acetone. The inosine dissolved after 30 minutes. After 3 hr. at 25°, the solution was added to an excess of aqueous $NaHCO_3$. The mixture was adjusted to *p*H 6 by the addition of *N* HCl,[27] evaporated to dryness *in vacuo* and the residue extracted with acetone in a Soxhlet apparatus. Vacuum-evaporation of the acetone and crystallization of the residue from fifteen parts of water yielded 2'3'-*O*-isopropylidene inosine in 60% yield as white needles, m.p. 266° (reported,[12] 267°). The product gave a single spot on paper chromatograms (solvent systems A, B and C).

Preparations Using Di-*p*-nitrophenyl Phosphate and 2,2-Dimethoxypropane. (a) **Analyses of Reaction Mixtures.**—Aliquots were added to one-third their volume of *N* NH_4OH and 5–8 applications of these solutions chromatogrammed in the solvent systems of Table I. Elution of areas corresponding to nucleosides and their isopropylidene derivatives into 50% aqueous ethanol and spectrophotometric measurement showed (except as noted below) that 1% of unchanged nucleoside was detectable by inspection of the papers in ultraviolet light (260 mμ). Mixtures containing 6-thioinosine or 6-thioguanosine, which absorb weakly at 260 mμ, were analyzed by electrophoresis in the borate buffer, wherein these nucleosides and their isopropylidene derivatives migrated as spots exhibiting an intense green fluorescence in ultraviolet light.

(b) **Preliminary Experiments.**—Stirred, anhydrous suspensions of nucleosides in the volumes of acetone given in Table I were treated at intervals at 25° with di-*p*-nitrophenyl phosphate[21] until a clear solution was obtained and conversion to isopropylidene derivatives occurred at a convenient rate. After a reaction period of 3 hr., conversions were as follows: inosine (3.2 equiv. of catalyst), 90%; 6-thioinosine (3.5 equiv.), 95%; cytidine (2.2 equiv.), 90%; guanosine (2.4 equiv.), 60%.

(c) **Reactions in Aqueous Acetone.**—To a stirred suspension of uridine (97.6 mg., 0.4 mmole, dried 110° (0.1 mm.)) in acetone (4 ml.) containing water (20 mg., 1.1 mmoles) di-*p*-nitrophenyl phosphate (13.6 mg., 0.04 mmole) was added. Little of the uridine dissolved, but analysis of the solution after 2 hr. (solvent B) showed the presence of uridine and isopropylidene uridine in the ratio 10:1. The reaction was repeated with the addition of 2,2-dimethoxypropane (0.4 ml., 3.3 mmoles). The uridine all dissolved within 12 minutes and chromatography (solvents B and C) of an aliquot, taken 25 minutes after the addition of the catalyst, showed that the ratio of uridine to isopropylidene uridine was less than 1:100.

(26) N. S. Corby, G. W. Kenner and A. R. Todd, *J. Chem. Soc.*, 3669 (1952).

(27) Little of the isopropylidene derivative could be extracted by acetone in the presence of $NaHCO_3$. The product presumably forms an acetone-insoluble salt with Na_2CO_3 formed from $NaHCO_3$ before and during the extraction.

(d) **General Procedure.**—Preparations were carried out under the conditions of Table I. 2,2-Dimethoxypropane[28] (b.p. 78–79°) was pipetted into a magnetically-stirred suspension of the nucleoside in anhydrous acetone in a stoppered flask. Di-*p*-nitrophenyl hydrogen phosphate was added and the mixture stirred vigorously until a clear solution resulted (1 hour or less). Glassware and reagents (other than acetone) were not subjected to special drying procedures. Commercial acetone containing 0.1–0.5% water should not require more than 0.8 mmole of 2,2-dimethoxypropane per ml. of acetone.

2',3'-*O*-Isopropylidene 8-Azaguanosine.—A suspension of 8-azaguanosine[29] (102.6 mg., 0.36 mmole) in acetone (5 ml.) containing 2,2-dimethoxypropane (0.4 ml., 3.28 mmoles) and di-*p*-nitrophenyl phosphate (180 mg., 0.53 mmoles) was stirred until solution was complete (1 hr.). After an additional 3 hr., the yellow solution was added at 2°, with stirring, to 50 ml. of 0.25 *M* NH_4HCO_3 in methanol–water (1:1) (freshly prepared by saturating methanolic NH_4OH at 2° with CO_2). A column (9 cm.) of 9 ml. of Dowex-1-HCO_3 ion-exchange resin (200–400 mesh, 8% cross linkage) was prepared from a suspension of the resin in 0.25 *M* NH_4HCO_3 in 50% aqueous methanol, and the solution containing the reaction mixture was percolated under pressure through it. The column was washed with the methanolic NH_4HCO_3 (*ca.* 150 ml.) until the absorbancy of the effluent at 257 mμ had decreased to a value of 1.0; the di-*p*-nitrophenyl phosphate remained as a bright yellow zone near the top of the resin. The combined eluates were evaporated to dryness at 10 mm. on a rotary evaporator (bath at 15°), using methyl-*n*-hexylcarbinol to control frothing. The white residual solid (115 mg.) was dissolved in warm water (4 ml.), and the solution was clarified with Celite filter-aid and concentrated under reduced pressure to *ca.* 1 ml. The solution was warmed to dissolve precipitated solid, adjusted to *p*H 5–6 with *N* acetic acid and after 2 days at 25° 2',3'-*O*-isopropylidene 8-azaguanosine separated as white needles (105 mg., 90% yield), m.p. 228° (gas evolution). On paper chromatograms, the product gave a single spot which reacted negatively to the spray test for *cis*-glycol systems. In 0.01 *M* phosphate buffer, *p*H 6.0, it showed an ultraviolet absorption maximum at 256 mμ, A_{250}/A_{260} 0.94, A_{280}/A_{260} 0.64; 8-azaguanosine had λ_{max} 256 mμ, A_{250}/A_{260} 0.96, A_{280}/A_{260} 0.63.

Anal. Calcd. for $C_{12}H_{16}N_6O_5$: C, 44.44; H, 4.97; N, 25.93. Found (for material dried at 100°): C, 44.28; H, 5.27; N, 26.10.

2',3'-*O*-Isopropylidene Guanosine.—Guanosine (0.4 mmole) was brought into reaction with acetone under the conditions given in Table I, and after 3.5 hr. the product was isolated as described above and dissolved in dilute ammonia. Concentration of the solution *in vacuo* to *ca.* 1 ml. gave white needles of isopropylidene guanosine in 92% yield; m.p. 292° (dec.) alone and in admixture with authentic material. Chromatograms (systems B, C and D) showed a single heavy spot.

Alternatively, tri-*n*-butylamine (0.5 mmole) was added to the mixture after the 3.5 hr. reaction period. The solution was refluxed for several minutes and volatiles removed *in vacuo*. Benzene (5 ml.) was added to the pale yellow gum and after 15 hr. a white gelatinous solid was collected and washed with benzene. The solid contained a small amount of di-*p*-nitrophenyl phosphate in addition to the desired compound; when reprecipitated from its solution in ammonia it gave crystalline isopropylidene guanosine in 75% yield.

2',3'-*O*-Isopropylidene 6-thioguanosine was obtained from 6-thioguanosine[30] as a cream-colored solid in 88% yield by isolation using Dowex-1-HCO_3 followed by reprecipitation from dilute ammonia, as described for guanosine. The ultraviolet absorption spectrum in 0.05 *M* glycine buffer, *p*H 9.0 (maxima at 252 and 322 mμ), was identical with that of material prepared using *p*-toluenesulfonic acid as catalyst.[1] The product decomposed at 242–250° to a brown solid; the material previously prepared melted at 250° with decompn., as did a mixture of *ca.* equal portions of each preparation.

Anal. Calcd. for $C_{13}H_{17}N_5O_4S$: C, 45.99; H, 5.05; N, 20.63. Found: C, 45.66; H, 5.33; N, 20.45.

In a second run, the product was isolated following addition of tri-*n*-butylamine, as described above; the yield after reprecipitation of the product was 60%.

2',3'-*O*-Isopropylidene inosine was isolated as described for 8-azaguanosine. Crystallization from water gave needles, m.p. 266° (91% yield).

2',3'-*O*-Isopropylidene cytidine was isolated in a similar manner[31] as a colorless gum which, when dried by repeated evaporations with absolute ethanol,[7] gave 96% yield of a glass that was chromatographically homogeneous and indistinguishable from authentic isopropylidene cytidine (solvent systems A, B, C and D).

2',3'-*O*-Isopropylidene uridine was obtained by the same procedure as a white crystalline residue (m.p. 163–164°, 97% yield) from evaporation of the aqueous NH_4HCO_3. The product did not depress the m.p. (163.5–164°) of authentic material.

2',3'-*O*-Isopropylidene Adenosine.—The reaction mixture from 0.4 mmole of adenosine was added to 0.1 *M* $NaHCO_3$ (10 ml.). Volatiles were removed under reduced pressure and the residue was extracted twice with boiling chloroform (40, 20 ml.). Evaporation of the chloroform and crystallization of the residue from water gave needles, m.p. 216–217°; yield 87%.

2',3'-*O*-Isopropylidene 6-Thioinosine.—6-Thioinosine[30] (1 g., 3.52 mmoles) was brought into reaction with acetone, and after 4 hr. the pale yellow solution was added to a stirred mixture of ice and water (100 ml.) containing *N* NH_4OH (25 ml.). The solution was concentrated under reduced pressure to *ca.* 80 ml., the *p*H was adjusted to 5 with *N* HCl and the mixture set aside overnight at 3°. The solid was collected by filtration and washed with a small volume of water and dissolved in water (40 ml.) by the addition of *N* NH_4OH (*ca.* 8 ml.). The solution was clarified with Celite filter-aid and evaporated *in vacuo* until separation of solid commenced. The *p*H was adjusted to 5 with *N* HCl and after 1 hr. (25°) the precipitate was collected by filtration and washed with water (2 ml.). A second precipitation gave isopropylidene 6-thioinosine as white fluffy needles (0.91 g., 80% yield), m.p. 235° (alone or in admixture with previously characterized material).[1] The product showed a single spot upon paper chromatography (solvent systems B, C and D) corresponding to the authentic material.

In situ **Phosphorylations of Isopropylidene Derivatives.** (a) **With Tetra-*p*-nitrophenyl Pyrophosphate.**—Guanosine (0.4 mmole) was converted to isopropylidene guanosine (Table I). Volatiles were removed *in vacuo* at *ca.* 20°, air admitted through a drying tube, and the residue was dissolved in anhydrous dioxane (3 ml.). Dioxane was removed *in vacuo* and to the pale yellow gum was added a solution of tetra-*p*-nitrophenyl pyrophosphate prepared *in situ* in 3 ml. of dioxane from di-*p*-nitrophenyl phosphate (2 mmoles) and di-*p*-tolylcarbodiimide (1 mmole).[14] After 15 hr. (25°), the mixture was worked up by the published procedure[14] and the phosphotriester-containing fractions were dissolved in the minimum volume of chloroform and the solution filtered to remove di-*p*-tolylurea. The chloroform was evaporated *in vacuo* and the residue dried for 2 days under high vacuum, giving 283 mg. of a pale yellow resin containing 43 mg. of di-*p*-tolylurea; the maximum yield of isopropylidene guanosine 5'-di-*p*-nitrophenyl phosphate was thus 240 mg. (93%). The product moved as a single spot, R_f 0.94, in solvent system E. To determine the content of alkali-labile *p*-nitrophenol, the resin was dissolved in 50 ml. of dioxane and a portion diluted 250-fold with ethanol. To this solution was added one-half its volume of 0.1 *N* LiOH when the mixture became yellow and exhibited the absorption maximum of 405 mμ characteristic of *p*-nitrophenol. After 4 minutes, A_{405} reached a constant value of 0.38.

Isopropylidene guanosine (0.4 mmole) was treated under the same conditions with tetra-*p*-nitrophenyl pyrophosphate (1 mmole) and di-*p*-nitrophenyl phosphate (0.48 mmole), giving the expected phosphotriester in 95% yield. This material was chromatographically identical with the product described above and in ethanolic LiOH gave, by the same procedure, A_{405} 0.395.

(28) Kindly provided by the Dow Chemical Company, Midland, Michigan.

(29) J. Davoll, *J. Chem. Soc.*, 1593 (1958).

(30) J. J. Fox, I. Wempen, A. Hampton and I. L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).

(31) Aqueous solutions were substituted for aqueous methanolic solutions.

(b) With P_2O_5–H_3PO_4.—Cytidine (199 mg.) was converted to its isopropylidene derivative (Table I) except that 50% less acetone (4 ml.) was used. Paper chromatographic analysis showed that conversion of the cytidine was complete after 3.5 hr. The solution was evaporated under reduced pressure; considerable frothing occurred in the final stages and prevented exhaustive removal of the volatile components. To the residual orange, gummy froth was added 2 ml. of P_2O_5–85% H_3PO_4 (1:1.3, w./w.). The mixture was stirred magnetically for 2 hr. in a bath at 60–62°, with exclusion of moisture. The gum became dark brown and much of it did not dissolve. Water (8 ml.) was added, and the mixture was heated for 30 minutes on a steam-bath, cooled and filtered from dark gummy solid. Inorganic phosphate was removed as lithium phosphate at *p*H 9 as described by Chambers, *et al.*[7] A portion (50 μl.) of the supernate (6.55 ml.) was subjected to paper electrophoresis in 0.05 *M* sodium tetraborate (*p*H 9.1) for 4 hr. at 18 volts/cm. together with appropriate reference compounds. Bands corresponding to cytidine 5′-phosphate, mixed cytidine 2′(3′)-phosphates, *p*-nitrophenol and di-*p*-nitrophenyl phosphate migrated 30.8, 25.2, 22.9 and 16.0 cm., respectively, from the origin. Cytidine had the same mobility as di-*p*-nitrophenyl phosphate and, if present, was not detectable. The band corresponding to cytidine 5′-phosphate was eluted with water (25 ml.); the solution showed an absorption maximum at 271 mμ (A = 0.96), minimum at 249–250 mμ, A_{250}/A_{260} 0.87, A_{280}/A_{260} 0.98; at *p*H 1, maximum at 280 mμ, minimum at 241 mμ, A_{250}/A_{260} 0.49, A_{280}/A_{260} 1.99. These values are closely similar to those recorded for this nucleotide.[32] The yield of cytidine 5′-phosphate was 45% (using $E_{max.}$ = 9.0 × 10^3 at 271 mμ and *p*H 7). The electrophoretic band corresponding to the mixed 2′ and 3′-isomers had similar spectral characteristics and the absorbancy corresponded to a yield of 8.1%.

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(32) Pabst Laboratories, Pabst Brewing Co., Wisconsin, Circular OR-10, "Ultraviolet Absorption Spectra of 5′-Ribonucleotides" (1956); Ref. 18a, p. 531.

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINN.]

The Autoöxidation of 2,3-Diethylindole to 2-Acetyl-3-ethylindole[1]

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Exposure of 2,3-diethylindole to air yielded 2-acetyl-3-ethylindole. This oxidation apparently proceeds *via* 2,3-diethyl-3-hydroperoxyindolenine since this compound is converted to 2-acetyl-3-ethylindole under various reaction conditions. A plausible mechanism for this remarkable transformation is suggested.

When 2,3-diethylindole, which is a colorless crystalline solid melting at 29–30°,[2] was exposed to air and light for several days, it changed to a brownish-yellow viscous liquid. The infrared spectrum of this material had strong absorptions in the 1600 to 1700 cm.$^{-1}$ region indicating the presence of carbonyl groups. Chromatography of this material yielded a colorless crystalline solid melting at 117–118°, having strong absorptions in the infrared at 3330 and 1632 cm.$^{-1}$. The ultraviolet spectrum of this compound in ethanol was very similar to that of 2-acetyl-3-methylindole[3] having maxima at 212, 238 and 314 mμ. It thus seemed probable that this oxidation product of 2,3-diethylindole was 2-acetyl-3-ethylindole and this was confirmed by independent synthesis. The synthesis is analogous to that used by Oddo[4] for the preparation of 2-acetyl-3-methylindole. 3-Ethylindole was added to an ethereal solution of methylmagnesium iodide and the resultant 3-ethylindolylmagnesium iodide treated with acetyl chloride to yield a mixture of 1- and 2-acetyl-3-ethylindole which was readily separated by chromatography.

The formation of 2-acetyl-3-ethylindole from 2,3-diethylindole by atmospheric oxidation is unusual. The only other example of an analogous reaction which has been found in the literature is the autoöxidation of heptahydrocycloöct[b]indole (I) to the ketone II.[5] This was also a very facile oxidation, occurring in a variety of solvents and in the solid state. However, the normal initial prod-

I II

uct of atmospheric oxidation of a 2,3-disubstituted indole is a 3-hydroperoxyindolenine.[5–8] In general these oxidations have been carried out in petroleum ether or ethyl acetate. A solution of 2,3-diethylindole in petroleum ether was thus exposed to air and after 24 hours an almost quantitative yield of 2,3-diethyl-3-hydroperoxyindolenine (III) had separated from the solution. The structure of this product was deduced from its properties, which were analogous to those of 2,3-dimethyl-3-hydroperoxyindolenine.[7] The two compounds had very similar ultraviolet spectra and on reduction the indolenine III yielded 2,3-diethyl-3-hydroxyindolenine (IV). On boiling the indolenine III with water it rearranged to *o*-propionaminopropiophenone (VII). Under similar conditions 2,3-dimethyl-3-hydroperoxyindolenine yields *o*-acetaminoacetophenone.[7] The propiophenone derivative VII was also obtained in very small yield when a solution of the indolenine III in ethyl acetate was

(1) The investigation was supported by a research grant CY-5336 from the National Institutes of Health, Public Health Service.

(2) E. Leete, *Tetrahedron*, in press.

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