The same compound was made starting with $\Delta^{1(6)}$-tetrahydrojulolidinium bromide (XIX, Br^- in place of ClO_4^-) made by adding anhydrous hydrogen bromide to an ethereal solution of 0.976 g. (5.5 mmoles) of tetrahydrojulolidine[17] until no more salt precipitated. The colorless hydrobromide was washed with ether, dissolved in methylene chloride and treated with excess diazomethane in ether. After standing overnight the solution was filtered and the filtrate was evaporated to dryness. The residue was recrystallized from 2-propanol, the crystals were dissolved in ether, the ethereal solution was filtered, and the ether was evaporated. The crystals gave a positive Beilstein test and an immediate precipitate upon addition of silver perchlorate in acetone. Infrared maxima were observed (10% CCl_4) at 2925, 2863, 2810, 2778, and 2690 cm.$^{-1}$. The band at 2863 cm.$^{-1}$ was more intense than the comparable band for XXI and XXIII.

The **perchlorate** of 13-bromomethyl-1-azatricyclo[7.3.1.$0^{5,13}$]-tridecane (XXIV) was formed in ether using 1:1 aqueous perchloric acid–ethanol. The precipitate (92% yield) was washed with ether, m.p. 263–265° dec.; ν_{max}^{Nujol} *ca.* 3100 cm.$^{-1}$ (—$\overset{+}{N}$—H) (Infracord); n.m.r. τ value (CH_3CN): 5.87 (singlet).

Anal. Calcd. for $C_{13}H_{23}BrClNO_4$: C, 41.89; H, 6.22; N, 3.76. Found: C, 42.31; H, 6.06; N, 3.68.

Treatment of 0.232 g. (0.85 mmole) of 13-bromomethyl-1-azatricyclo[7.3.1.$0^{5,13}$]tridecane (XXIV) in acetone with an acetone solution of 0.219 g. (0.98 mmole) of silver perchlorate monohydrate yielded silver bromide, which was separated by filtration. The filtrate was evaporated to dryness *in vacuo* on a rotary evaporator leaving 0.216 g. (87%) of crude product which was recrystallized from ethyl methyl ketone. Colorless needles, m.p. 150–151° dec., 0.097 g. (39%), were obtained which did not depress the melting point of **1-azoniatetracyclo[7.3.2.0.^{1,13}0^{5,13}] tetradecane perchlorate (XX)** previously prepared.

N-(1-Bromocyclohexylmethyl)pyrrolidine (XXV).—A solution of 3.0 g. (34.6 mmoles) of dry lithium bromide in 70 ml. of anhydrous acetonitrile was filtered to remove a trace of residue. To the clear solution 2.0 g. (7.55 mmoles) of 5-azoniadispiro-[4.0.5.1]dodecane perchlorate (I) was added, and the resulting solution was extracted continuously with pentane overnight. The pentane extracts were evaporated to dryness leaving 1.42 g. (77% yield) of free base, $n^{24}D$ 1.5150. This material was distilled, 73–75° (0.02 mm.), to give 0.93 g. of clear distillate, $n^{24}D$ 1.5156, together with 0.157 g. of pot residue, crude m.p. 190–208°. The nature of this residue was not determined. Several attempts were made to obtain a satisfactory analysis on the free base; however, despite repeated distillation, each analysis gave a high per cent of carbon, indiating that dehydrobromination had occurred. Each distillation was accompanied by salt formation, and each time the clear distillate turned turbid after standing a short time. N.m.r. τ values were observed (neat) at: 7.05 (singlet), 7.28 (multiplet), and broad complex absorption near 8.30, in a ratio of areas of 2:4:14; (benzene): 7.08 (singlet, 2 protons), 7.35 (complex multiplet, 4 protons), ring protons at higher field.

Addition of 1:1 perchloric acid–ethanol to an ethereal solution of the free base afforded the **perchlorate,** colorless plates, m.p. 165–166° dec. The perchlorate was recrystallized from isopropyl alcohol, m.p. now 162.5–163° dec.; ν_{max}^{Nujol} 3100 cm.$^{-1}$ (—$\overset{+}{N}$—H); n.m.r. τ values ($CDCl_3$): *ca.* 2.3 ($\overset{+}{N}H$); 6.24 (doublet, J = 5.0 ± 0.5 c.p.s.) plus complex signals both sides of doublet (total of 6 protons); complex absorption at high field (14 protons).

Anal. Calcd. for $C_{11}H_{21}BrClNO_4$: C, 38.10; H, 6.11; N, 4.04. Found: C, 38.09; H, 6.02; N, 3.90.

A solution of 1.6 g. of N-(1-bromocyclohexylmethyl)pyrrolidine in benzene was filtered to remove some crystals which had formed and evaporated *in vacuo*. The residue was dissolved in 50 ml. of acetone and to this solution at −33° was added an acetone solution of 1.4 g. of silver perchlorate. An immediate precipitate of silver bromide formed and the temperature rose to −5°. The silver bromide was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved quickly in absolute ethanol; the solution was filtered and cooled in a Dry Ice–isopropyl alcohol bath. The colorless needles were removed by filtration and washed with ether to afford 0.70 g. (40%) of I, m.p. 127–128.5°; m.m.p. with authentic 5-azoniadispiro-[4.0.5.1]dodecane perchlorate, 131.5–132°; authentic I, m.p. 132–133°. The infrared spectrum of the compound was identical with that of pure I except for very weak bands at 3400 cm.$^{-1}$ and 1700 cm.$^{-1}$.

1-N-Pyrrolidylcyclohexanemethanol.—This compound, previously described,[1] showed n.m.r. signals (CH_2Cl_2) at τ values 6.45 (2 protons), 6.95 (1 proton, O$\underline{H}$), 7.35 (4), 8.29 (4), 8.50 (10). The chemical shift of the hydroxyl hydrogen was assigned by addition of a small amount of acetic acid.

Polynucleotides. I. Synthesis of Uridylyl-(3′ → 5′)-uridine and Uridylyl-(3′ → 5′)-6-azauridine

ROSS H. HALL AND ROOSEVELT THEDFORD

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo 3, New York

Received January 14, 1963

2′,5′-Di-*O*-trityluridine serves as a convenient starting point for the synthesis of phosphate dinucleosides containing uridine. This compound was readily phosphorylated with cyanoethylphosphate and, after removal of the cyanoethyl group, the resultant blocked nucleotide was used to phosphorylate 2′,3′-isopropylideneuridine and 2,3′-isopropylidene-6-azauridine. After removal of blocking groups, the compounds listed in the title were isolated in good yield from ion-exchange columns.

The synthesis of (3′ → 5′) linked diribonucleoside phosphates by present techniques requires extensive use of multiple blocking groups.[1,2] The major problem in these syntheses arises from the need for a suitably blocked derivative of a nucleoside with a free 3′ hydroxyl group. Blocking requirements alternatively may be minimized by synthesizing diribonucleoside phosphates consisting of mixed (2′ → 5′) and (3′ → 5′) linked isomers.[3] The mixed isomers in most cases can be separated by ion exchange chromatography. Both these synthetic approaches are applicable to general synthesis in this field; however, there may be special cases where opportune use of a particular compound can avoid many of the intermediate steps. Such a case is exemplified by 2′,5′-di-*O*-trityluridine which is readily synthesized and makes a useful starting point for synthesis of diribonucleoside phosphates containing uridine.

Tritylation of uridine with an excess of trityl chloride gives a product containing two trityl groups. This compound, first synthesized by Levene,[4] was identified

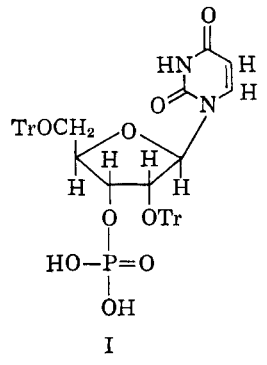
(1) M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 430 (1962).

(2) D. H. Rammler and H. G. Khorana, *ibid.*, **84**, 3112 (1962).

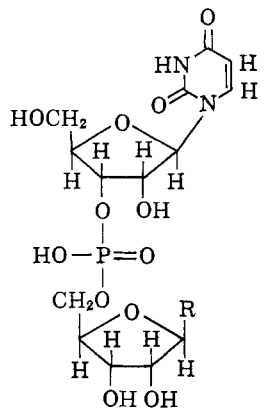
(3) A. M. Michelson, *J. Chem. Soc.*, 3655 (1959).

(4) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **105**, 419 (1934).

by Fox[5] as 2′,5′-di-*O*-trityluridine. Proof of structure rests on the fact that when the free hydroxyl at position-3′ is inverted, only the xylose derivative is obtained. 2′,5′-Di-*O*-trityluridine was phosphorylated readily with cyanoethyl phosphate[6] to produce 2′,5′-ditrityluridine-3′ cyanoethyl phosphate which upon removal of the cyanoethyl group by alkaline treatment produced compound I. The product as obtained from the reaction mixture was contaminated with salts and organic material. It was purified by partition chromatography on a column of Celite according to the general method of Hall.[7] Compound I represents a key intermediate and would be expected to react with a number of suitably protected nucleosides which contain an open 5′ position. Two such syntheses are presented here. Reaction of I with the 2′,3′-isopropylidene derivatives of uridine and 6-azauridine afforded, after removal of blocking groups with 80% acetic acid, uridylyl-(3′ → 5′)-uridine (II), and uridylyl-(3′ → 5′)-6-azauridine (III), respectively.



I



II. R = uracil
III. R = 6-azauracil

Uridylyl-(3′ → 5′)-uridine at this stage was obtained in a yield of 33% when the ratio of nucleoside intermediate to nucleotide intermediate was 2:1. This result compares with a yield of 50% reported for this dinucleoside phosphate when the 2′ position of the nucleotide intermediate was blocked with a dihydropyranyl group.[1] The bulkier trityl group thus appears to reduce the yield at the phosphorylation step but this is more than offset by a considerable reduction in number of steps in the over-all synthesis. The yield can be increased to 44% by using a 5:1 ratio of nucleoside intermediate to nucleotide intermediate. Uridylyl-(3′ → 5′)-uridine was readily purified by chromatography on a column of DEAE-cellulose using a buffer of triethylammonium carbonate (pH 8.6). It was obtained as the triethylammonium salt, analytically pure, in a yield of 29% based on compound I.

Uridylyl-(3′ → 5′)-6-azauridine was also purified on a DEAE-cellulose column and was isolated as the chromatographically homogeneous triethylammonium salt. Great care had to be exercised in the final isolation due to lability of this product. For example an aqueous solution of the free acid standing at room temperature decomposed within twelve hours to uridylic acid and 6-azauridine in contrast to uridylyl-(3′ → 5′)-uridine which can stand several days under these conditions without decomposing. An aqueous solution of the neutral salt which stood at room temperature for one day showed considerable amount of free nucleoside. The synthesis of compound III by another method has been reported[8] but authors of this paper did not suggest that the product so obtained was particularly labile.

Both products were degraded to uridine-3′ phosphate and nucleoside by ribonuclease, with about 1% of each product being resistant to the action of the enzyme which indicates slight contamination with the 2′ → 5′ isomer. This result agrees with that of Smith and co-workers[1] who found that treatment of such reaction products with acetic acid resulted in a small amount of isomerization. The fact that these dinucleoside phosphates were hydrolyzed with ribonuclease constitutes independent proof that the starting material in these syntheses is indeed 2′,5′-ditrityluridine.

2′,5′-Ditrityluridine-3′ phosphate offers a convenient starting point for the preparation of diribonucleoside phosphates containing a uridine residue and the procedure reported in this paper is suitable for scaling up to larger quantities.

Experimental

Preparation of 2′,5′-Di-*O*-trityluridine-3′ Phosphate.—To a solution of 5 g. (6.86 mmoles) of 2′,5′-di-*O*-trityluridine[5] in 75 cc. of anhydrous pyridine was added (13.72 mmoles) of cyanoethyl phosphate.[6] The mixture was concentrated to a sirup on a rotary evaporator (*in vacuo*) and again dissolved in 75 cc. of pyridine and evaporated (repeated three times). After dissolving the resulting gum in 100 cc. of anhydrous pyridine, 10 g. of dicyclohexylcarbodiimide (DCC) was added and the mixture was shaken a few minutes to effect solution. The mixture was tightly stoppered and allowed to stand at room temperature for 3 days after which water was added and the reaction mixture was allowed to stand at room temperature overnight. Insoluble dicyclohexylurea was removed by filtration. Concentration of the filtrate yielded more of the urea. The final filtrate was evaporated to dryness and the residue was washed several times with anhydrous ether to remove any remaining DCC and dicyclohexylurea. The slightly colored material was placed in a flask and 125 cc. of 1 *N* sodium hydroxide was added and, after refluxing the mixture for 40 min., it was filtered and the filtrate concentrated to dryness. The entire product mixture was dissolved in water and the pH was adjusted to 9.0 with IR-120 resin (H^+). It was necessary to add ethanol occasionally to the mixture because of its tendency to gel. After removal of the resin and other solid particles by filtration, the filtrate was lyophilized. The product was purified by partition chromatography on a Celite column according to the following general method of Hall.[7] The lyophilized material was dissolved in 15 cc. of the lower phase of a *n*-butyl alcohol–water (3:1) solvent mixture and mixed with 30 g. of Celite-545 which was then packed on top of a previously packed Celite column (130 g., 1 in. × 36 in.). The upper phase of the *n*-butyl alcohol–water solvent mixture was passed through the column at a rate of 175 cc./hr. The product began to come off the column immediately after the solvent front had appeared and elution was complete when 700 cc. of solvent had passed through. Upon concentration of this fraction a solid precipitated which was collected and air dried (4.51 g., 73%). Paper chromatography in solvent A showed a single spot (R_f 0.86).

Anal. Calcd. for $C_{47}H_{39}O_9PNa_2 \cdot 3H_2O$: C, 62.40; H, 4.98; N, 3.09; P, 3.41. Found: C, 62.55; H, 5.00; N, 3.22; P, 3.11.

Uridine-3′ Phosphate.—2′,5′-Di-*O*-trityluridine-3′ phosphate (sodium salt 0.5 g.) was dissolved in a minimum amount of water and filtered to remove the suspended particles. After addition of 24 cc. of 80% acetic acid, the solution was heated at 100° for 1.5 hr. The solution was cooled and filtered to remove triphenylcarbinol, after which the filtrate was concentrated to dryness and excess acid was removed by azeotroping with

(5) N. C. Yung and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 3060 (1961).
(6) G. M. Tener, *ibid.*, **83**, 159 (1961).
(7) R. H. Hall, *J. Biol. Chem.*, **237**, 2282 (1962).
(8) J. Smrt and F. Sorm, *Collection Czech. Chem. Commun.*, **27**, 73 (1962).

water and ethanol. The remaining solid material was dissolved in a minimum amount of water, and two volumes of ethanol were added. After cooling the solution in an ice bath for 30 min., fine crystalline needles began to come out of solution. Uridine-3′ phosphate (disodium salt) was collected by filtration and dried over phosphorus pentoxide in a desiccator (yield 230 mg., 95%).

Anal. Calcd. for $C_9H_{11}O_9N_2PNa_2 \cdot 3H_2O$: C, 25.60; H, 4.04; N, 6.64; P, 7.34. Found: C, 25.42; H, 4.04; N, 6.57; P, 7.27.

Preparation of 2′,3′-Isopropylidene-6-azauridine.—This method is based on that of Hampton.[9] Five grams (20.3 mmoles) of 6-azauridine and 6.3 g. (20.3 mmoles) of di-*p*-nitrophenol phosphate were dissolved in 200 cc. of acetone containing 20.8 g. (200 mmoles) of 2,2-dimethoxypropane. The mixture was shaken for 30 min. on a mechanical shaker at which time a clear solution had resulted. After standing at room temperature for 4.5 hr., 24 g. of acetone washed Dowex-2 resin (OH^-) was added and the mixture was shaken for several minutes until the solution was neutral. After filtering, a clear solution was obtained which contained only one ultraviolet absorbing spot upon paper chromatography in systems A and B. The solution was lyophilized and the residue upon shaking with anhydrous ether became a dry powder (wt. 5.2 g., yield 85%) which gave the correct analysis.

Anal. Calcd. for $C_{11}H_{15}O_6N_3$: C, 46.31; H, 5.33; N, 14.73. Found: C, 46.25; H, 5.37; N, 14.47.

Synthesis of Uridylyl-(3′→5′)-Uridine.—The sodium salt of 2′-5′-di-*O*-trityluridine-3′ phosphate (90.6 mg., 0.1 mmole) was dissolved in a small quantity of water. This was passed through a short column of Dowex-50 (pyridinium form). The column was washed with a few milliliters of water and the combined filtrates were lyophilized. 2′,3′-Isopropylideneuridine (0.2 mmole) dissolved in 2 cc. of anhydrous pyridine was added and the mixture was concentrated to a gum on a flash evaporator. Evaporation with pyridine was repeated three times in order to render it completely anhydrous. In order to protect the mixture from the atmospheric moisture, dry nitrogen was released into the evaporator after each evaporation. After dissolving the resulting gum in 4 cc. of anhydrous pyridine, DCC (4 mmoles) was added, after which the reaction flask was flushed with dry nitrogen and tightly stoppered. The mixture was allowed to stand at room temperature for 3 days at which time more DCC (2 mmoles) was added and the mixture was allowed to stand at room temperature for two additional days. On the fifth day, water (8 cc.) was added and after remaining at room temperature for 24 hr. the reaction mixture was concentrated to dryness. Excess pyridine was removed by azeotroping with water then ethanol and the resulting solid product mixture was treated with 80% acetic acid (40 cc.) for 30 min. at 100°. The acid solution was cooled and filtered to remove dicyclohexylurea and triphenylcarbinol. Concentration of the filtrate yielded a gummy material which was evaporated repeatedly with water to remove excess acetic acid. The product was dissolved in the minimum amount of water, a small portion of which was applied to Whatman no. 3MM paper (acid washed). Development of the chromatogram in solvent I, indicated three bands, corresponding to uridylic acid, uridylyl-(3′→5′)-uridine, and uridine, respectively. Quantitative elution of the dinucleoside phosphate band indicated a yield of 33% at this point. Increased yields were obtained by using a larger ratio of 2′,3′-isopropylideneuridine to 2′,5′-di-*O*-trityluridine-3′ phosphate as shown.

Nucleoside : nucleotide	Yield, %
2:1	33
5:1	44
10:1	50

Isolation of Uridylyl-(3′→5′)-uridine by Ion Exchange Chromatography.—DEAE-Cellulose resin was suspended in an excess of water and stirred for several minutes. After allowing the material to stand for a while, the water was decanted, after which the washing procedure was repeated until the wash water became clear. The cellulose was washed with 1 l. of 0.5 *N* sodium hydroxide and then washed with more water until it was free of base, after which it was suspended in 0.1 *M* ammonium carbonate (pH 8.6) and packed, under pressure (4 lb.), in a 1 × 24 cm. column. One liter of the above ammonium carbonate solution was passed through the column, followed by 1 l. of a 0.01 *M* ammonium carbonate solution (pH 8.6). Finally, the column was washed with 1 l. of a 0.01 *M* triethylammonium carbonate solution (pH 8.6). The pH of a solution containing the reaction mixture was adjusted to 8.6 with ammonium hydroxide and the solution run into the column. A linear gradient with respect to triethylammonium carbonate at pH 8.6 (0.01 *M* → 0.1 *M*, total volume 2 l.) was applied to the column at a flow rate of 24 cc. per hour. All operations were conducted at 4°. Uridylyl-(3′→5′)-uridine appeared in the fraction of the effluent between 910 cc. and 1200 cc. This fraction was lyophilized and the residue was dissolved in water and relyophilized. This process was repeated a total of four times after which 20 mg. of product (29% based on compound I) was obtained as the triethylammonium salt.

Anal. Calcd. for $C_{24}H_{38}O_{14}N_5P \cdot 2H_2O$: C, 41.9; H, 6.15; P, 4.49; N, 10.2. Found: C, 41.85; H, 6.49; P, 3.51; N, 9.98.

Uridylyl-(3′→5′)-6-azauridine.—Synthetic procedure was the same as that preceeding 0.2—mmole of nucleoside and 0.1 of nucleotide being used. The product was purified on a DEAE-cellulose column described previously with a slight modification because direct lyophilization of the column effluent resulted in some hydrolysis of the product. Dowex-50 (H^+) was added carefully to the collected effluent until a pH 7.5 was reached, after which it was lyophilized. The residue weighed 22 mg. and moved as a single spot upon chromatography in solvent systems A and B and upon electrophoresis at pH 3.0. It was 95% pure, estimated spectrophotometrically which means a final yield of 31% based on compound I. Ribonuclease hydrolyzes this product to one equivalent each of uridylic acid and 6-azauridine.

Ribonuclease Treatment.—One milligram of the dinucleoside phosphate was dissolved in 0.5 cc. of water and the pH was adjusted to 7.5. Magnesium sulfate (5 λ of 1 *M* solution), and 0.1 mg. of crystalline ribonuclease were added. After 6 hr. at room temperature the entire sample was streaked on Whatman 3MM paper which was developed in solvent B. The quantitative elution and spectrophotometric analysis showed that each

TABLE I

Compound	R_f value, Solvent A	R_f value, Solvent B
Uridine	0.62	0.44
6-Azauridine	.59	.47
2′,3′-Isopropylideneuridine	.81	.77
2′,3′-Isopropylidene-6-azauridine	.79	.78
Uridine-3′ phosphate	.50	.09
Uridylyl-(3′ → 5′)-uridine	.39	.15
Uridylyl-(3′ → 5′)-6-azauridine	.29	.16

TABLE II

Compound	Distance moved toward anode, cm.
6-Azuridine	2.6
Uridine	2.3
Uridylyl-(3′ → 5′)-uridine	18.5
Uridylyl-(3′ → 5′)-6-azauridine	23.2
Uridine-3′ phosphate	24.5

TABLE III

SPECTROPHOTOMETRIC DATA

Compound	pH	Max	E_{max}
Uridine-3′ phosphate	2.0	262	10.0
	11.5	261	7.0
Uridylyl-(3′ → 5′)-uridine	2.0	260	19.2
	9.0	260	17.6
Uridylyl-(3′ → 5′)-6-azauridine	2.0	259	16.3
	10.0	254	12.7
2′,3′-Isopropylidene-6-azauridine	6.3	258	6.5
	11.5	254	7.4
2′,5′-Di-*O*-trityluridine-3′-phosphate	6.3	260	7.2
	10.5	260	6.2

(9) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3640 (1961).

dinucleoside phosphate had produced one equivalent each of nucleoside and uridylic acid. A residue (1.3%) of each dinucleoside phosphate still remained.

Paper Chromatography.—Solvent A: isopropyl alcohol–1% aqueous ammonium sulfate (2:1). Solvent B: isopropyl alcohol–concentrated ammonium hydroxide–water (7:1:2). (See Table I.)

Paper Electrophoresis.—Electrophoresis was conducted in a Gilson Electrophorator for 1 hr., using a buffer of 0.01 *M* ammonium formate (pH 3.0) with a voltage gradient of 100 volts per cm. (See Tables II and III.)

Acknowledgment.—The authors thank the National Cancer Chemotherapy Screening Center, U. S. Public Health Service for a generous gift of 6-azauridine. This research was supported in part by a grant (CA-05697) from the U. S. Public Health Service.

Pteridine Chemistry. X. Methylation Studies. III. Steric Effects of Phenyl Groups at C-6 and C-7[1]

ROBERT B. ANGIER

Organic Chemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

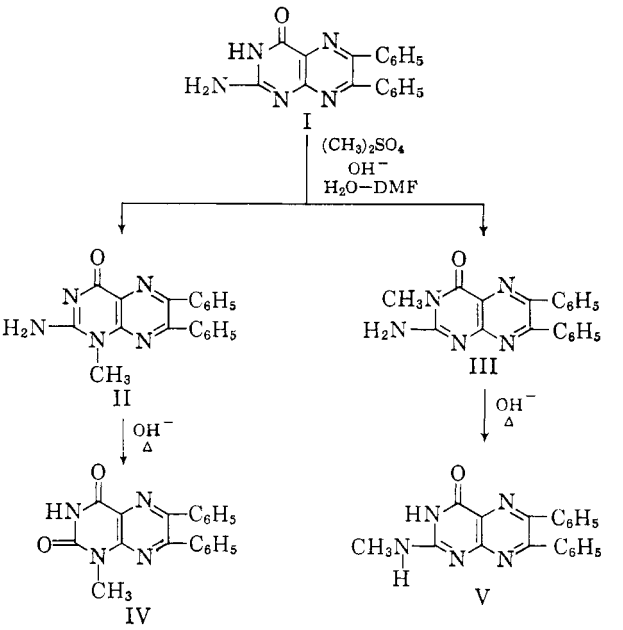
Received December 26, 1962

The methylation of 2-amino-3-methyl-6,7-diphenyl-4(3*H*)-pteridinone (III) with dimethyl sulfate in dimethylformamide–acetic acid occurred predominantly at N-1 and only slightly at N-8 to give the 2-imino-1,3-dimethyl derivative VII (63% yield) and the 2-imino-3,8-dimethyl derivative VIII (10% yield). Contrastingly, when the C-7 substituent was hydrogen rather than phenyl, *i.e.*, 2-amino-3-methyl-6-phenyl-4(3*H*)-pteridinone (XIIa), the relative amounts of N-1 and N-8 methylated products (XIIIa and XIVa) were reversed. However, when the C-6 substituent was hydrogen, *i.e.*, 2-amino-3-methyl-7-phenyl-4(3*H*)-pteridinone (XIIb), methylation occurred only at N-1. The varying yields of 8-methyl derivatives are ascribed to steric effects. Additional related methylations also are discussed.

The nature of the products obtained from the methylation of 2-amino-4-hydroxypteridines has been found to be dependent not only upon the conditions of the reaction[2a] but also upon the substituents located at positions 6 and 7 of the pteridine ring. Thus, 2-amino-4-hydroxypteridine-6-carboxylic acid treated with dimethyl sulfate in an aqueous alkaline solution at pH 8–11.5 gave a 30% yield of a crude mixture of the 1-methyl and 3-methyl derivatives and a 25–30% yield of the 3,8-dimethyl derivative. The isomeric 2-amino-4-hydroxypteridine-7-carboxylic acid, however, gave only the 1-methyl and 3-methyl derivatives.[1]

In order to continue the study of the effect of substituents upon this methylation reaction 2-amino-4-hydroxy-6,7-diphenylpteridine (I) was treated with dimethyl sulfate in a water–dimethylformamide[2b] solution at pH 8–11.5. There was isolated from this reaction the 1-methyl derivative II (52% yield) and the 3-methyl derivative III (10% yield). (A very small amount of the 3,8-dimethyl derivative VIII was detected by paper chromatography but was not isolated.) The comparatively high yield of the 1-methyl isomer II in this reaction is somewhat different from the results of the previous methylation studies[2a,3] where any preponderance of one isomer over the other was, in each case, in favor of the 3-methyl derivative.

The structures of the 3-methyl III and 1-methyl II derivatives were proved by well established methods.[2a,4] The 3-methyl derivative III upon treatment with 1.0 *N* sodium hydroxide rearranged to the 2-methylamino derivative V while the 1-methyl derivative II under the same conditions was hydrolyzed to the 1-methyl-2,4-pteridinedione IV. Furthermore, the 3-methyl III and 1-methyl II compounds also were synthesized unequivocally by the reactions of benzil with 3-methyl-2,5,6-triamino-4(3*H*)-pyrimidinone[4] and 1-methyl-2,5,6-triamino-4(1*H*)-pyrimidinone,[4,5] respectively.



A previous report[1] also has shown that 2-amino-3-methyl-4-pteridinones can be methylated in nonbasic solvents and that the substituents in the pyrazine ring again have some influence upon the course of the reaction. Thus the methylation of the 3-methyl derivative of 2-amino-4-hydroxypteridine-6-carboxylic acid with dimethyl sulfate in a boiling dimethylformamide–acetic acid solution gave, as the only major product, the 3,8-dimethyl derivative. In contrast, the isomeric 7-carboxylic acid derivative under the same conditions gave no detectable dimethyl derivative.[1]

In the present investigation 2-amino-3-methyl-6,7-diphenyl-4(3*H*)-pteridinone (III) when treated with

(1) Presented in part at the IIIrd International Pteridine Symposium: Stuttgart, Germany, September 12–15, 1962.

(2) (a) R. B. Angier and W. V. Curran, *J. Org. Chem.*, **27**, 892 (1962). (b) The use of dimethylformamide was necessitated by the low solubility of the 6,7-diphenyl derivative I in water.

(3) R. B. Angier and W. V. Curran, *J. Org. Chem.*, **26**, 2129 (1961).

(4) W. V. Curran and R. B. Angier, *J. Am. Chem. Soc.*, **80**, 6095 (1958).

(5) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, *ibid.*, **73**, 2864 (1951).