1-Methoxy-2-amino-3-benzyloxybutane.—The methoxy benzyloxy ketone (13.8 g., 0.066 mole) was treated with 7 g. (0.1 mole) of hydroxylamine hydrochloride, 4.1 g. (0.1 mole) of sodium hydroxide and sufficient ethanol to effect solution. The reaction was heated for 2 hours on a steam-bath, most of the ethanol was evaporated and the remainder of the reaction poured into water. The mixture was extracted with ether and the ether was evaporated and the resulting oil dried under vacuum and then dissolved in dry ether. To this ether solution was added 12.5 g. (0.33 mole) of lithium aluminum hydride. The reduction mixture was then heated with stirring for 7 hours, after which time the excess lithium aluminum hydride was destroyed with water. After a solution of Rochelle salt was added, the layers were separated and the aqueous layer was extracted four times with ether. The combined ether layers were dried over sodium sulfate and the ether was reduced in volume to 200 ml. and then extracted three times with 75-ml. portions of 3 *N* hydrochloric acid. The acid solutions were made basic with sodium hydroxide and the aqueous solution was then extracted three times with 300-ml. portions of ether. The ether solutions were dried over potassium hydroxide pellets, and the ether removed. The remaining material was distilled to yield 8.18 g. (59%) of the amine, b.p. 80° (0.4 mm.), $n^{25}D$ 1.5008, d^{25} 1.014, pK_a' (50% methanol) 8.3, mol. wt. by titration 208 (calcd. 209).

Anal. Calcd. for $C_{12}H_{19}NO_2$: C, 68.87; H, 9.15; N, 6.68. Found: C, 69.08; H, 8.95; N, 6.91.

1-Methoxy-2-acetamide-3-acetoxybutane.—The debenzylative acetylation of 1.56 g. (7.45 millimoles) of the above amine was carried out in acetic anhydride and acetic acid using 0.4 g. 10% palladium-on-charcoal catalyst. After uptake of 0.94 equivalent of hydrogen the reaction mixture was filtered and heated on the steam-bath for 2 hours. Distillation gave 1.01 g. (66%) of the synthetic acetate-amide, whose infrared spectrum had the same bands as those of the natural acetate-amide from Elaiomycin, b.p. 91° (0.1 μ), $n^{25}D$ 1.4543.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.18; H, 8.43; N, 6.89. Found: C, 53.28; H, 8.36; N, 7.08.

Acknowledgment.—The authors wish to thank Dr. J. M. Vandenbelt and associates at Parke, Davis and Co. for the spectral data published here and C. E. Childs and associates of the same company for the analytical determinations.

DETROIT 2, MICH.

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND CHEMISTRY, UNIVERSITY OF WISCONSIN]

Separation and Preliminary Characterization of Oligomycins A, B and C[1]

BY SATORU MASAMUNE, J. M. SEHGAL,[2] E. E. VAN TAMELEN, F. M. STRONG AND W. H. PETERSON

RECEIVED MAY 24, 1958

The antibiotic complex, oligomycin, has been separated into three biologically active components A, B and C. One crystalline preparation contained 67% A, 13% B and 20% C, another 20% A, 80% B and no C. The components were homogeneous and analyzed for the formulas: A, $C_{24}H_{40}O_6$; B, $C_{22}H_{36}O_6$; C, $C_{28}H_{46}O_6$. The oligomycins are neutral, unsaturated, optically active alcohols, soluble in many organic solvents but very insoluble in petroleum ether and water. Comparison of the physical, chemical and biological properties indicates that, while there are differences, the three antibiotics are closely related in structure.

In 1953 a crystalline antibiotic of marked antifungal potency was obtained from culture filtrates of a streptomyces similar to *Streptomyces diastatochromogenes*.[3] Two components were detected in the crystals by paper chromatography and the major one, called A, was obtained by column chromatography substantially free of the minor component. Many of the chemical, physical and biological properties of component A were determined. The antibiotic had no activity toward bacteria but was markedly active against certain fungi, particularly against the human pathogen, *Blastomyces dermatitidis*. Because of this limited activity, the antibiotic complex was named oligomycin.

Later work by Halliday[4] showed that the mycelium contained more oligomycin than the culture filtrate, and, if the *p*H of the medium was controlled, practically all of the antibiotic was retained in the mycelium. All subsequent isolations have been made from the mycelium. By means of selected cultures, improved media and control of aeration, Visser[5] obtained yields of from 1 to 1.3 g. per liter of oligomycin in the broth. Visser also worked out an isolation procedure for pilot plant fermentations and thereby obtained several hundred grams of crystalline oligomycin some of which has been used in this work.

Paper chromatograms showed the crystals contained a predominance of the A component but also revealed the presence of significant amounts of two other components, which later were designated oligomycins B and C. Details on the fermentation and isolation procedures will be published elsewhere. Marty[6] investigated the preparative separation of the three components and provided the basis for a partition chromatographic method which has proved very successful.

In the present study each of the three components has been obtained in sufficient quantity to permit preliminary characterization. Their homogeneity has been established by paper chromatography, constancy of rotations and melting point behavior. Analytical data on the recrystallized compounds and on several derivatives thereof indicate that the molecular formulas are A, $C_{24}H_{40}O_6$; B, $C_{22}H_{36}O_6$; and C, $C_{28}H_{46}O_6$. Oligomycins A and B both exist in polymorphic forms of distinctly different melting points. Although the three com-

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(2) Postdoctorate project associate on a Fulbright travel grant from the University of Delhi, India.

(3) (a) R. M. Smith, Ph.D. Thesis, University of Wisconsin, 1953; (b) R. M. Smith, W. H. Peterson and E. McCoy, *Antibiotics and Chemotherapy*, **4**, 962 (1954).

(4) W. J. Halliday, Ph.D. Thesis, University of Wisconsin, 1955.

(5) J. Visser, M.S. Thesis, University of Wisconsin, 1955.

(6) E. W. Marty, Jr., Ph D. Thesis, University of Wisconsin, 1957

ponents have many different properties and hence are not identical substances, the similarity of the ultraviolet (Fig. 1) and infrared (Fig. 2) spectra indicate that they are closely related in structure.

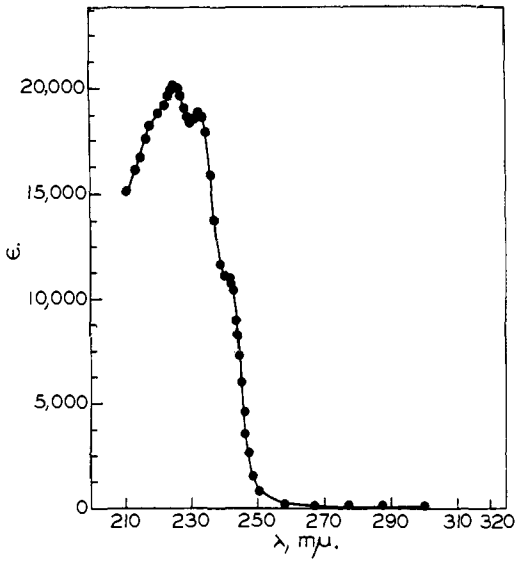

Fig. 1.—Ultraviolet absorption spectra of oligomycin A in absolute ethyl alcohol. The curves of B and C are essentially the same as that of A.

The oligomycins absorb strongly in the ultraviolet. The presence of a high intensity absorption peak at 225 mμ (ε *ca.* 20,000) suggests the presence of a conjugated system in the molecule. At higher concentrations components A and C showed carbonyl absorption evidenced by inflections at 285 mμ. In oligomycin B this carbonyl absorption was shifted slightly to longer wave lengths and appeared as a definite band with a maximum at 295 mμ. These absorptions are not evident at the lower concentrations used in Fig. 1. The infrared absorption band at 1700 cm.$^{-1}$ indicates the presence of carbonyl. A maxima at 3462 cm.$^{-1}$ shows hydroxyl groups. Supplementary evidence for this view is provided by the formation of diacetates. A weak hydroxyl absorption still detectable in the infrared spectra of these products suggests the presence of tertiary hydroxyl. The absorption band at 1638 cm.$^{-1}$ indicates unsaturation.

The oligomycins are neutral, unsaturated, optically active alcohols which may also contain ketone groups.

Biological Activity.—Marty[6] determined the biological activity of the three oligomycins against 40 different fungi. About 30 of these were inhibited, but yeasts and many molds proved to be insensitive. In general, A was more active than B and this in turn was more active than C. For example, the inhibitory concentration (mg./ml. for a 3 day period) against the human pathogen, *B. dermatitidis* was 0.01 for A and 0.1 for both B and C. Against the plant pathogen, *Helminthosporium sativum*, the inhibitory levels were 2, 5 and 50, respectively. One derivative, oligomycin A diacetate, has been tested against *Glomerella cingulata* and found to be inactive. The oligomycins are active against the fungi producing oak-wilt and Dutch elm diseases. Field tests against these and other fungal plant diseases are in progress.

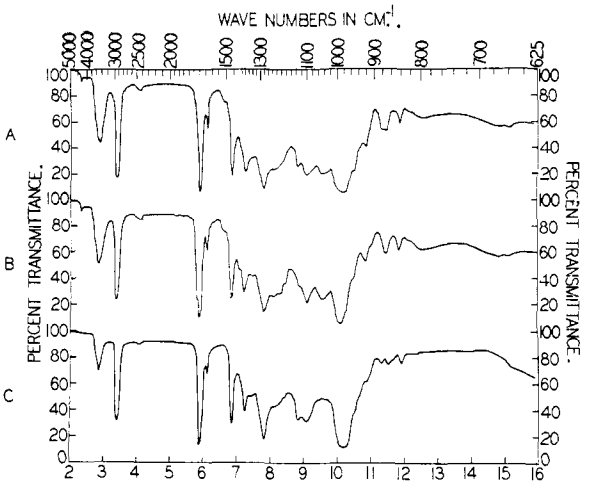

Fig. 2.—Infrared absorption spectra of oligomycins A, B and C.

The toxicity of the components to mice by intraperitoneal administration also was determined by Marty and found to be *LD* 50 (mg./kg. body weight) A 1.5; B 2.9; C 8.3.

Experimental

Chromatographic Separation of Oligomycins.—Redistilled Skellysolve B (purified petroleum ether, b.p. 60–71°, Skelly Oil Co.) (2000 ml.), dioxane dried over potassium hydroxide, refluxed over sodium and redistilled (720 ml.), 95% ethanol (260 ml.) and distilled water (185 ml.) were shaken together for 10 minutes. The mixture was allowed to stand and the organic phase (2400 ml.) was separated from the aqueous phase (735 ml.).

Four kg. of Celite No. 545 (filter aid, Johns-Manville Co.) and 2400 ml. of aqueous phase prepared as above were intimately mixed by stirring for 30 minutes, then flooded with an excess of the organic phase and the stirring continued for 2 hr. The slurry thus obtained was added in six to eight portions to a 9.8 × 130 cm. Pyrex tube[7] fitted with a sintered glass plate at the bottom on which was placed two discs of filter paper. After each addition of the slurry the mixture in the tube was stirred with a large glass rod to eliminate air bubbles, excess solvent drained off, and the Celite mechanically packed with a plunger. Sufficient slurry was added to produce a column 118 cm. high (*ca.* 2500 g. of Celite; equivalent to 0.28 g. of Celite per ml. of bed volume). The top of the packing was carefully leveled and protected with two discs of filter paper. The level of solvent in the tube always was kept slightly above the Celite packing.

A solution of 8 g. of crystalline oligomycin (from pilot plant runs 5 to 11)[5] in 10 ml. of dioxane plus 80 ml. of the organic phase was pipetted onto the top of the column and washed in with four separate 50-ml. portions of the organic phase. The chromatogram then was developed by continuous passage of the organic phase by gravity flow (1.7 liter/hour). Effluent was collected in 250-ml. aliquots until a total of 20 liters of the solvent mixture had passed through the column.

The individual oligomycin components were detected in the effluent fractions by means of a color reaction with concentrated sulfuric acid. Aliquots of 0.5 ml. of each fraction were mixed with an equal volume of concentrated sulfuric acid. On standing a few minutes at room temperature, a yellow, brownish, or wine-red color developed depending on the concentration of oligomycin present. This test is valid only with crystalline preparations of oligomycin

(7) The chromatographic separation was carried out in various sized columns, of which the one described was the largest.

substantially free of organic impurities. The results are summarized in Table I.

TABLE I

PARTITION CHROMATOGRAPHY OF OLIGOMYCIN

Combined effluent fraction	Vol., l.	Color test	Oligomycin component
1	0–6.75	–	None
2	6.75–8.75	+	C
3	8.75–10.25	–	None
4	10.25–14.5	+	A
5	14.5–16.5	–	None
6	16.5–20.0	+	B

The components are named in the order in which they were first detected by paper chromatography, not in accordance with their emergence from the column.

Evaporation of the solvent from the appropriate effluent fractions and drying to constant weight by infrared light at about 50° gave 1.6 g. of oligomycin C, 5.3 g. of A and 1.04 g. of B, total 7.94 g. or 99.3% recovery of the original weight. The corresponding distribution of the components was: A, 66.8%; B, 13.1%; C, 20.1%. This distribution applies only to the product R5-11.[5] Another preparation P-1 (Pfizer product) made with a different culture and processed somewhat differently consisted of about 20% A and 80% B. Factors that affect this difference in composition have not yet been determined.

Paper chromatography of the separated A, B and C fractions by the method of Smith, *et al.*,[3b] showed that each component was homogeneous and free from the others. From 2–4 μg. of the oligomycin complex contained in 0.025 to 0.05 ml. of ethyl alcohol was applied to 1.25 × 40 cm. Eaton–Dykeman, No. 613 paper strips and developed by down-flow with the water–ethyl alcohol–acetic acid mixture (70:24:6 by volume) for 18–20 hours at 30°. In this system B is the fastest moving component and C the slowest. In a typical papergram C moved 13 cm. from the point of application, A, 22 cm. and B, 29 cm.[8] The strips were plated on agar medium seeded with *G. cingulata* and the position and size of the inhibition zones determined. By comparison with A, B and C standards chromatographed at the same time, the components were identified and a fair approximation of the per cent. of each was obtained.

Solubilities.—The approximate solubilities of the fractions from the column were obtained by drying them to constant weight at temperatures below 50° and shaking a weighed portion of the residue with small additions of the appropriate solvent measured from a micro-pipet. The minimum volume of the solvent required to dissolve the weight of oligomycin was taken as a rough measure of the solubility. The results are given in Table II. In some of the solvents the three components have essentially the same solubility but in others, *e.g.*, glacial acetic acid, ethyl alcohol and benzene, they are markedly different. All of the components are conspicuously insoluble in water and in Skellysolve B.

Table II

APPROXIMATE SOLUBILITY OF OLIGOMYCINS AT 25° (g./100 ML. SOLVENT)

Solvent	A	B	C
Acetone	85	86	90
Glacial acetic acid	37.5	9	82
95% Ethyl alcohol	24.1	2.5	30
Absolute ethyl alcohol	25.0	2.5	30
Skellysolve B	0.02	0.02	0.02
Benzene	6	20.5	6
Ether	28	26	27
Water	0.002	0.003	0.003

Qualitative Tests.—All components give a yellow to cherry-red color with concentrated sulfuric acid, the color varying according to concentration. All components give positive tests with Fehling solution and ammoniacal silver nitrate. Several tests each for phenol, steroid, coumarin, flavone and compounds related to flavones were negative.[9-11]

Oligomycin A.—Evaporation of the solvent from effluent fraction 4 (Table I) left the oligomycin A component as an oily mass which formed a semi-solid product when left 24 hours in the refrigerator. This was dissolved in ether, the solution dried over sodium sulfate and Skellysolve B added to incipient opalescence. After 2 hours at room temperature oligomycin A separated as long needles, m.p. 139–141°. After two recrystallizations from the same solvent mixture the melting point remained at 140–141°. It was observed, however, that very slow crystallization from ether–Skellysolve B mixtures or from dilute acetic acid yielded hexagonal crystals, m.p. 150–151° with rapid decomposition. The optical activity of the higher melting compound was $[\alpha]^{23}D$ −54.5 (*c* 4.40, dioxane). The lower melting product was again obtained on recrystallization of the higher melting form from acetone–methanol. For analysis, the substance with m.p. 140–141° was dried at 80° and 0.02 mm. for 12 hours.

Anal. Calcd. for $C_{24}H_{40}O_8$: C, 67.89; H, 9.50; O, 22.61; active hydrogen 4H, 0.94; 5H, 1.18; 5C–CH_3, 17.7; mol. wt., 424.56. Found: C, 67.76, 67.0 (67.80); H, 9.53, 9.50 (9.45); O, 22.30, (22.56); O-alkyl, 0.1; active hydrogen, 1.03; C–CH_3, 16.4; mol. wt. (Rast), 397, 408, (426).[12]

A mixture of 13.4 mg. of oligomycin A, 3 ml. of 95% ethanol and 13.2 mg. of platinum oxide catalyst was shaken at room temperature and pressure with hydrogen for 5 hours, at which time the hydrogen uptake had ceased. A total of 1.36 ml. (STP) of hydrogen was absorbed which is equivalent to 1.92 double bonds per molecule of 424 mol. wt. Attempts to isolate any crystalline product were not successful. The same results were obtained by using glacial acetic acid as solvent.

The degree of unsaturation of oligomycin A as determined by bromination by the method of Rowe, *et al.*,[13] was 2.2 double bonds per 424 molecular weight.

Oligomycin A Diacetate.—A solution of 246 mg. of oligomycin A in 10 ml. of a mixture of one part acetic anhydride and 4 parts pyridine was kept at room temperature 48 hours, the bulk of the reagent removed by distillation and 25 ml. of distilled water added. After 24 hours at room temperature, the amorphous precipitate was collected. Since all attempts at crystallization failed, the precipitate was dissolved in chloroform and chromatographed on a 2 × 12 cm. silicic acid column. The chromatogram was developed with 10% methanol in chloroform. The first fraction of effluent on concentration left a residue which crystallized readily from carbon tetrachloride–petroleum ether mixture (1:1); yield 180 mg. (61%), m.p. 112–113°. The optical rotation was $[\alpha]^{24}D$ −86.1 (*c* 1.74, ethanol). The infrared absorption spectrum showed a new strong band at 1238 cm.$^{-1}$ characteristic of acetates.

Anal. Calcd. for $C_{28}H_{44}O_8$: C, 66.11; H, 8.72; mol. wt., 508.63. Found: C, 66.5; H, 8.6; mol. wt. (Rast), 540.

Oligomycin B.—The material recovered from fraction 6, (Table I) when recrystallized from methanol gave platelets, m.p. 160–161°. On standing for several months the melting point changed to 169–170°. Recrystallization of the higher melting material from methanol reproduced the lower melting point. A determination of the optical rotation gave the following results: $[\alpha]^{23.5}D$ −49.5 (*c* 1.03, methanol); −46.4 (*c* 0.76, dioxane).

A constant optical rotation ($[\alpha]^{23.5}D$ −48.4 and −48.3) was observed for three arbitrary fractions belonging to fraction 6 (Table I). On rechromatographing one fraction in a similar manner except that the relative amount of Celite was 2.7 times as much as described above, an almost symmetrical elution curve was obtained. This was regarded as further evidence of the homogeneity of oligomycin B.

(8) R_f values cannot be stated as the solvent was allowed to drip from the bottom of the paper strips in order to achieve the migrations reported.

(9) F. Feigl, "Spot Tests in Organic Analysis," 5th Ed., Elsevier Publishing Co., New York, N. Y., 1956, pp. 137, 182, 419.

(10) P. B. Hawk, B. L. Oser and W. H. Summerson, "Practical Physiological Chemistry," 13th Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1954, p. 301.

(11) R. C. Elderfield, "Heterocyclic Compounds," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 267.

(12) Data in parentheses from John Dyer, unpublished experiments.

(13) R. G. Rowe, C. C. Furnas and H. Bliss, *Anal. Chem.*, **16**, 371 (1944).

Anal. Calcd. for $C_{22}H_{36}O_6$: C, 66.64; H, 9.15; O, 24.21; active hydrogen 4H, 1.01; 5C–CH_3, 18.9; mol. wt., 396.51. Found: C, 66.68, 66.53; H, 9.04, 9.09; O, 24.34, 24.47; active hydrogen 1.07; C–CH_3, 18.9; mol. wt. (Rast), 416, 394.

Oligomycin B Diacetate.—The diacetate of oligomycin B was prepared in a similar manner to that for oligomycin A. After recrystallization from benzene–cyclohexane (1:1), it melted at 135.5–136.5°. The optical rotation was $[\alpha]^{25}D$ −66.6 (*c* 0.63, dioxane).

A sample was dried at 80°, 0.01 mm., and analyzed.

Anal. Calcd. for $C_{28}H_{40}O_8$: C, 64.98; H 8.39; mol. wt., 480.6. Found: C, 65.29; H, 8.45; mol. wt. (Rast), 488.

Oligomycin C.—The material from fraction 2 (Table I) was recrystallized twice from ether–petroleum ether mixture (1:1). The rod shaped crystals melted at 198–200° and had a rotation, $[\alpha]^{23}D$ −80.7 (*c* 3.70, dioxane).

Anal. Calcd. for $C_{28}H_{46}O_6$: C, 70.26; H, 9.69; 6C–CH_3, 18.8; mol. wt., 478.64. Found: C, 69.9; H, 9.8; C–CH_3, 19.3; mol. wt. (Rast), 496.

Absorption Spectra.—Ultraviolet measurements were made in absolute ethanol on a Cary model 11 automatic recording spectrophotometer. Each component showed two main bands at 225 and 232 ± 0.5 mμ, with inflections at 220, 240 ± 0.5, 285 (A and C) and 295 mμ (B). The corresponding molar extinction values, were, for A: 20200, 18200, 18100, 11100, 64; for B: 18800, 17000, 17400, 10800, 59; for C: 23200, 21600, 21700, 13100, 64, respectively.

The infrared data were obtained in 10% chloroform solution with a Baird IR model B automatic recording spectrophotometer and a sodium chloride prism. Main bands for each component were, for A: 3440–3500, 1700, 1638 cm.$^{-1}$; for B: 3446–3500, 1712, 1690, 1640 cm.$^{-1}$; for C: 3500, 1700 and 1640 cm.$^{-1}$.

NOTE ADDED IN PROOF.—For the purpose of further verifying the molecular weights of the two compounds, single crystal X-ray diffraction measurements were made on oligomycin B and oligomycin A. Oligomycin B is orthorhombic, with lattice constants a = 16.92 Å., b = 26.34 Å., c = 10.25 Å., and a pycnometric density of 1.152 g./cc. The molecular weight calculated for eight molecules per unit cell is 396.2, to be compared with a value of 396.5 based on the formula $C_{22}H_{36}O_6$. Isothermal distillation for the molecular weight determination gave a value of 416 for oligomycin B and 407 for dihydroöligomycin B, using chloroform and acetone as solvent, respectively.

Rotation and Weissenberg pictures of oligomycin A also revealed an orthorhombic unit cell with lattice constants a = 17.7 Å., b = 27.5 Å., and c = 10.1 Å. An observed density of 1.15 g./cc. was obtained by the flotation method. These data together with the assumption of eight molecules per unit cell give a calculated molecular weight of 426 which is in good agreement with the value 424.6 based on the formula $C_{24}H_{40}O_6$. Isothermal distillation data gave a molecular weight of 433.

The similarity of the crystal structures of oligomycin B and oligomycin A as indicated by the lattice constants, symmetry, and the same number of molecules per unit cell (eight) suggests a fairly close relationship both in the carbon skeleton and molecular arrangement of the two compounds in the solid state.

The authors are indebted to R. R. Pfeiffer and A. Van Camp, Eli Lilly and Co., for the above data on oligomycin B and to L. F. Dahl and G. L. Dawes, Chemistry Department, University of Wisconsin for the measurements on oligomycin A.

MADISON 6, WISCONSIN

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, PEARL RIVER LABORATORIES, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

Pteridine Chemistry. III. 2-Amino-1(and 3)6,7-trimethyl-4-pteridones and Some Related Compounds

BY WILLIAM V. CURRAN AND ROBERT B. ANGIER

RECEIVED JUNE 30, 1958

The structure of a previously reported ring methylated pteridine has been shown to be 2-amino-1,6,7-trimethyl-4(1H)-pteridone (I, R = CH_3) by alkaline hydrolysis to the known 2-hydroxy derivative (II, R = CH_3). 2-Amino-3,6,7-trimethyl-4(3H)-pteridone (IV, R = CH_3) has been synthesized by two unequivocal routes. Attempted alkaline hydrolysis of the latter compound led to the rearranged product, 2-methylamino-4-hydroxy-6,7-dimethylpteridine (VIII, R = CH_3).

In connection with another problem under investigation in this Laboratory, it was necessary to synthesize 2-amino-1,6,7-trimethyl-4(1H)-pteridone (I, R = CH_3) and 2-amino-3,6,7-trimethyl-4-(3H)-pteridone (IV, R = CH_3). Roth, *et al.*,[1] have described the synthesis of 2-methylamino-4-hydroxy-6,7-dimethylpteridine (VIII), R = CH_3) and also a ring methylated 2-amino-4-pteridone, the structure of which was not proved. By treating methylguanidine with ethyl cyanoacetate they obtained two pyrimidines which were separated by means of solubility differences. After nitrosation and reduction the resulting 4,5-diaminopyrimidines were condensed with biacetyl to give the corresponding 6,7-dimethylpteridines. On the basis of the infrared and ultraviolet spectra of these compounds, structure VIII (R = CH_3) was assigned to the lower melting pteridine obtained from the more soluble pyrimidine and structure IV (R = CH_3) to the higher melting isomer obtained from the less soluble pyrimidine. The ultraviolet spectrum of VIII (R = CH_3) in 0.1 *N* sodium hydroxide was practically the same as the parent 2-amino-4-hydroxy-6,7-dimethylpteridine except that both maxima were shifted toward longer wave lengths. Subsequent workers[2] have confirmed the fact that alkylation of the 2-amino group in the pteridine series produces a bathochromic shift in the ultraviolet spectrum. The ultraviolet spectrum of the product assigned structure IV (R = CH_3) was completely different from the 2-amino-4-hydroxy-6,7-dimethylpteridine in 0.1 *N* sodium hydroxide.

We reinvestigated the ring methylated isomer and found that by refluxing for five minutes in 2 *N* sodium hydroxide it was converted into a new pteridine. Elemental analyses and the infrared spectrum of this product indicated that the 2-amino group had been hydrolyzed to a 2-hydroxy group. 2-Hydroxy-1,6,7-trimethyl-4(1H)-pteridone (II, R = CH_3) was synthesized by known

(1) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **73**, 2864 (1951).

(2) E. C. Taylor and C. K. Cain, *ibid.*, **74**, 1644 (1952).