chloride with trimethylamine was studied. Eastman Kodak Company White Label trimethylamine (vapor pressure at 0°, 676 mm.; calcd., 681 mm.[11]) was employed in the reaction. The course of this reaction, which was studied by means of a graph similar to that of Fig. 1, indicated that a one-to-one addition compound was formed. However, in the vicinity of a mole ratio of one, the straight line was rounded off and the pressure dropped only to about 20 mm. Inasmuch as this pressure is greater than the vapor pressure of diethylaminoboron dichloride, dissociation of the addition compound is indicated. The melting point of the trimethylamine adduct was observed to be about 20° with decomposition.

When the solid adduct was treated with water at about 10°, rapid hydrolysis took place, as indicated by the formation of an instant precipitate of silver chloride when the solution was treated with silver nitrate. Because of the low stability of the addition compound, it was impossible to obtain an elementary analysis for this substance.

(11) A. Simon and J. Huter, *Z. Elektrochem.*, **41**, 28 (1935).

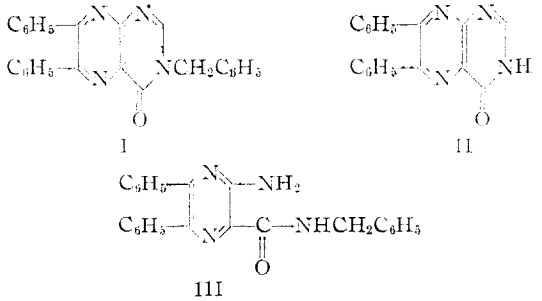
MALLINCKRODT CHEMICAL LABORATORIES
DEPARTMENT OF CHEMISTRY
HARVARD UNIVERSITY
CAMBRIDGE 38, MASSACHUSETTS

Pteridines. IX. Hydrolytic Ring Cleavage of 3-Benzyl-6,7-diphenyl-4(3*H*)-pteridinone

BY E. C. TAYLOR, JR.

RECEIVED NOVEMBER 23, 1951

A project at present under investigation in this Laboratory is the conversion of N-substituted amides of 3-aminopyrazinoic acids into 3-substituted 4(3*H*)-pteridinones. One compound of the latter type, 3-benzyl-6,7-diphenyl-4(3*H*)-pteridinone (I), has already been reported.[1] In an attempt to prepare I by an alternate route, 6,7-diphenyl-4(3*H*)-pteridinone (II) was treated with benzyl chloride and potassium hydroxide in methanol solution. Upon treatment of the reaction solution with dilute alkali, a yellow crystalline solid separated which proved to be 3-amino-N-benzyl-5,6-diphenylpyrazinamide (III) rather than the expected pteridinone (I). In order to test the hypothesis that this product arose by hydrolytic cleavage of I formed initially, an authentic sample of 3-benzyl-6,7-diphenyl-4(3*H*)-pteridinone (I) was treated with potassium hydroxide and 90% methanol. III was formed smoothly in 87.5% yield. If the reaction of 6,7-diphenyl-4(3*H*)-pteridinone



I

II

III

(II) with benzyl chloride and potassium hydroxide in stock methanol was worked up after a shorter reaction period and the final addition of aqueous alkali was avoided, it was possible to isolate unreacted II, a small amount of I and some III from the reaction mixture. No III was formed under the same conditions when freshly prepared, absolute methanol was used as a solvent rather than stock methanol; under these conditions only a poor yield of pure I could be isolated from the reaction mixture. Attempts to synthesize I from 6,7-diphenyl-4(3*H*)-pteridinone (II) and benzyl chloride in the absence of alkali were unsuccessful, as were attempts to effect ring cleavage of II with dilute alkali alone.

The stability of II toward ring cleavage in dilute alkali is undoubtedly due to ready distribution of the negative charge of the anion over the pteridine ring system. Such a stabilization is impossible with I, as it cannot form a simple anion, and hydrolytic attack at C_2 followed by ring cleavage occurs with ease. A more general study of the hydrolytic cleavage of 3-substituted 4(3*H*)-pteridinones is under investigation.

These experiments support the generalization recently made by Albert, *et al.*,[2] that substituents on the pteridine nucleus are best introduced before ring closure.

Experimental[3]

6,7-Diphenyl-4(3*H*)-pteridinone (II).—A suspension of 15.0 g. (0.059 mole) of 5,6-diamino-4-hydroxy-2-mercaptopyrimidine sulfate[4] in 300 ml. of boiling water was treated with 20% sodium carbonate solution until all the suspended solid had dissolved. The *p*H was adjusted to 10 by the addition of dilute hydrochloric acid and 80 g. of wet Raney nickel added in small portions. After the violent evolution of gas had ceased, the mixture was heated under reflux for four hours. The reaction mixture was then allowed to cool, the nickel removed by filtration, and 12.4 g. (0.059 mole) of benzil dissolved in a mixture of 100 ml. of ethyl methyl ketone and 350 ml. of ethanol added to the filtrate. The resulting mixture was heated under reflux for eight hours. Acidification of the hot yellow solution and cooling caused the separation of colorless platelets which were collected by filtration and recrystallized from aqueous dimethylformamide; yield 13.2 g. (75%); m.p. (dec.) 297–298°.

Anal. Calcd. for $C_{18}H_{12}N_4O$: C, 72.0; H, 4.0; N, 18.7. Found: C, 72.0; H, 4.1; N, 18.6.

3-Amino-N-benzyl-5,6-diphenylpyrazinamide (III). Method A.—A mixture of 0.50 g. (0.00167 mole) of 6,7-diphenyl-4(3*H*)-pteridinone, 30 ml. of methanol, 0.2 ml. (0.00174 mole) of benzyl chloride and 0.16 g. (0.00286 mole) of potassium hydroxide was heated under reflux for two hours. Addition of 15 ml. of 2 *N* sodium hydroxide and warming caused the immediate separation of yellow needles. The reaction mixture was allowed to cool to room temperature, the crystals collected by filtration and recrystallized from ethanol; yield 0.483 g. (76%); m.p. 188.5–189°.

Method B.—To a solution of 75 mg. of 3-benzyl-6,7-diphenyl-4(3*H*)-pteridinone in 30 ml. of methanol was added 5 ml. of water containing 0.1 g. of potassium hydroxide and the solution heated under reflux for 10 minutes. The reaction mixture rapidly turned yellow with the simultaneous separation of yellow needles. Addition of 5 ml. of water and cooling gave 64 mg. (87.5%) of III in the form of long, yellow needles; m.p. 188.5–189°.

Mixed melting points of the products obtained by Methods A and B with an authentic sample of 3-amino-N-benzyl-5,6-diphenylpyrazinamide[1] showed no depression; infrared spectra of all three samples were identical.

3-Benzyl-6,7-diphenyl-4(3*H*)-pteridinone (I).—A mixture of 1.0 g. (0.00333 mole) of 6,7-diphenyl-4(3*H*)-pteridinone, 0.186 g. (0.00332 mole) of potassium hydroxide, 3.8 ml. (0.00332 mole) of benzyl chloride and 30 ml. of stock meth-

(1) E. C. Taylor, Jr., THIS JOURNAL, **74**, 1651 (1952). It was prepared from 3-amino-N-benzyl-5,6-diphenylpyrazinamide (III) and formic acid in the presence of acetic anhydride.

(2) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 474 (1951).

(3) All melting points are corrected.

(4) W. Traube, *Ann.*, **331**, 73 (1904).

anol was heated under reflux for one hour. Three milliliters of glacial acetic acid was added to the warm, yellow reaction solution, followed by sufficient hot water to induce crystallization. After cooling, the solution was filtered and the light yellow crystalline precipitate recrystallized from absolute ethanol. 3-Benzyl-6,7-diphenyl-4(3*H*)-pteridinone (I) (0.26 g., 25%) separated as colorless platelets from the warm ethanol; m.p. 248°. A mixed melting point with an authentic sample of I[1] showed no depression. Addition of a small amount of water to the ethanol filtrate and further cooling caused the separation of 0.19 g. of 3-amino-N-benzyl-5,6-diphenylpyrazinamide (III); m.p. 187°.

The mother liquor from the original reaction mixture above was diluted with an equal volume of water. A heavy, tacky yellow solid separated which was collected by filtration and extracted with 20 ml. of hot 1 *N* sodium hydroxide. Acidification of the filtrate precipitated 0.195 g. of unreacted 6,7-diphenyl-4(3*H*)-pteridinone (II), while repeated recrystallizations of the base-insoluble solid yielded an additional 0.11 g. (total yield 29%) of pure III.

In a second experiment, a mixture of 40 ml. of freshly prepared, anhydrous methanol, 0.793 g. (0.00264 mole) of 6,7-diphenyl-4(3*H*)-pteridinone, 0.301 ml. (0.00262 mole) of benzyl chloride and 0.148 g. (0.00264 mole) of potassium hydroxide was heated under reflux for 24 hours. By the end of this time, the reaction mixture was only faintly basic. Addition of a few drops of acetic acid to acidity followed by water caused the crystallization of light yellow crystals; yield 0.530 g.; m.p. 230–238°. Repeated recrystallizations from methanol gave 0.21 g. (20%) of pure I melting sharply at 248°.

NOYES CHEMICAL LABORATORY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

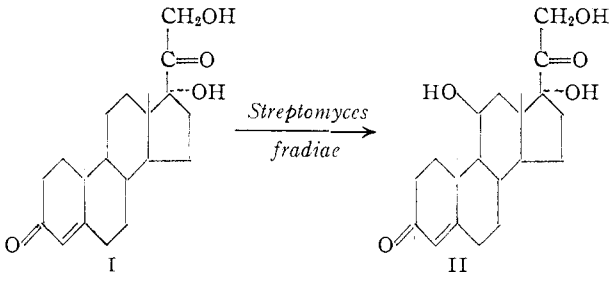
COMMUNICATIONS TO THE EDITOR

A PARTIAL MICROBIOLOGICAL SYNTHESIS OF ADRENAL CORTEX HORMONES

Sir:

It appears that either a hydroxyl group, having the *beta* configuration, or a ketone at the 11-position of the steroid nucleus is an obligatory structural requirement for the so-called carbohydrate-regulating hormone activity of the adrenal steroids, corticosterone, 11-dehydrocorticosterone, 17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone. Since both 11-desoxycorticosterone and 11-desoxy-17-hydroxycorticosterone are essentially devoid of this type of bioactivity, as measured by the Rat Liver Glycogen Deposition Assay,[1] it is possible to detect the introduction of an 11-β-hydroxyl group or an 11-keto group into these compounds by means of this assay.

We wish to report evidence for the microbiological oxygenation of these latter two steroids, with particular emphasis on the conversion of 11-desoxy-17-hydroxycorticosterone (Reichstein's compound S) (I) to 17-hydroxycorticosterone (Kendall's compound F, hydrocortisone) (II) by *Streptomyces fradiae*, Waksman's strain 3535.



Several species of *Streptomyces* were incubated with 100-mg. quantities of 11-desoxycorticosterone and 11-desoxy-17-hydroxycorticosterone. The quantitative measurement of glycogen deposition activity in the resulting beers[2] and calculation of this bioactivity in terms of a theoretical conversion to corticosterone and 17-hydroxycorticosterone gave values which varied from 1.4 to 5.8%.

In an experiment of somewhat larger scale 5.0 g. of I was incubated with *Streptomyces fradiae*, strain 3535, for 7 hours at 24° in rotary shaker flasks, using a medium containing dextrose, soybean meal and distillers' solubles. The total volume of the beer was 15 liters. A neutral hormone concentrate which was obtained from the beer by a standard procedure,[3,4] weighed 4.86 g. and possessed total bioactivity equivalent to 140 mg. of 17-hydroxycorticosterone. Evaluation of this material by paper chromatography[5,6,7] indicated the presence of II, a trace of 11-dehydro-17-hydroxycorticosterone and unreacted I.

One-half of the neutral hormone concentrate (2.43 g.) was subjected to automatic partition column chromatography.[8] The three adrenal steroids mentioned above were found in individual bands in the resulting chromatogram. The "17-hydroxycorticosterone band" weighed 110 mg. First crop crystals from acetone (22.8 mg.) were identified as II. Evidence for this characterization was afforded by long-term paper chromatography in which fermentation product moved at a rate identical with authentic II. In addition, a mixture of the fermentation product and authentic II could not be resolved under any of several conditions. Furthermore, the data from infrared spectroscopy,[9] as shown in Fig. 1, provided additional evidence for the identification of the crystalline product from

(1) M. L. Pabst, R. Sheppard and M. H. Kuizenga, *Endocrinology*, **41**, 55 (1947).

(2) We are indebted to Dr. K. J. Olson and his staff for the bioassays reported herein.

(3) M. H. Kuizenga, A. N. Wick, D. J. Ingle, J. W. Nelson and G. F. Cartland, *J. Biol. Chem.*, **147**, 561 (1943).

(4) W. J. Haines, R. H. Johnson, M. P. Goodwin and M. H. Kuizenga, *ibid.*, **174**, 925 (1948).

(5) A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).

(6) R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, **188**, 763 (1951).

(7) W. J. Haines and N. A. Drake, *Fed. Proc.*, **9**, 180 (1950).

(8) W. J. Haines, N. A. Drake, C. D. Alway and M. P. Brunner, *Abstracts of Papers*, 118th Meeting Am. Chem. Soc., Chicago, Illinois, Sept. 1950, p. 11-M.

(9) We are indebted to Dr. J. L. Johnson and his staff for the infrared data reported herein.