therefore, not much larger and perhaps considerably smaller than that of the hydrolysis of acetylcholine, namely, 3 kcal./mole.[15] The energy of activation for the hydrolysis of acetyl enzyme is evidently (Fig. 1) very low. The value for the estimated asymptote is 1200 cal./mole, and the corresponding entropy of activation is exceedingly high. The very unfavorable entropy of activation suggests a very loose structure for the acetyl enzyme and a high degree of ordering of the protein and the reactant water molecule in the transition state.

(15) S. Hestrin, *Biochim. Biophys. Acta*, **4**, 310 (1950).

The energies of hydrolysis and activation are of only limited help in seeking the chemical identity of the acetylated group because there may well be concomitant changes in the protein structure which very greatly alter the energy values from those corresponding to reactions of the same functional groups in simple compounds. This may be just the role of the protein.

The explanation offered here of decreasing energy of activation with increasing temperature may be of general applicability, particularly in those cases where substrate inhibition is also observed.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Stevioside. III. The Anomeric 2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-D-glucopyranoses and their Behavior with Alkali[1,2]

BY HARRY B. WOOD, JR.,[3] AND HEWITT G. FLETCHER, JR.

RECEIVED JULY 14, 1955

Treatment of 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-β-D-glucopyranose (VI) with alkali gives 1,6-anhydro-β-D-glucopyranose (II, levoglucosan) while the anomeric ester, 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucopyranose (X) yields no detectable quantity of this anhydride. The bearing of these facts on the configuration of the ester-linked D-glucopyranose residue in stevioside is pointed out and the analogy between these substances and the anomeric phenyl D-glucopyranosides with respect to their behavior with alkali is discussed.

In the first paper of this series,[2a] concerned with the structure of the three D-glucose moieties in the remarkably sweet natural glucoside, stevioside, it was shown that one of the D-glucose residues was joined to the large aglucon by esterification at its C_1-position with a highly hindered carboxyl group of the aglucon. At the time of this earlier work there was no evidence bearing upon the configuration of the C_1-carbon involved in the ester linkage; the object of the present paper is to describe further research designed to elucidate this question.

When stevioside (I) is heated with strong alkali the ester-linked D-glucose moiety is split off as levoglucosan (II, 1,6-anhydro-β-D-glucopyranose) and, upon acidification, the remainder of the molecule appears as steviolbioside (III). This transformation is a remarkable one; no other C_1-linked sugar ester of a carboxylic acid has previously, to the authors' knowledge, been cleaved to give a glycosan. Sugar esters normally hydrolyze through nucleophilic attack of the OH^- on the carbon of the carbonyl with acyl–oxygen scission occurring at the O—C=O linkage. In the case of the ester of a sterically hindered acid the carbonyl carbon is relatively inaccessible and the other C–O linkage is broken. If the ester is at C_1 of an aldose, the sugar fragment initially formed might be the same as that which has been postulated[4] as an intermediate in the alkaline cleavage of phenolic β-D-glucopyranosides to levoglucosan. Now it is well known that phenyl β-D-glucopyranoside readily gives levoglucosan when treated with alkali while its anomer, phenyl α-D-glucopyranoside, does not.[5] If the analogy of a C_1 sterically hindered ester to phenyl D-glucoside is a valid one we would predict that such a β-C_1-ester of glucose would give levoglucosan when treated with alkali but its anomer, an α-C_1-ester, would not. To test this point, the simplest and best-studied sterically hindered acid, mesitoic acid (VIII, 2,4,6-trimethylbenzoic acid) was chosen and ways were sought to obtain the two anomeric 1-*O*-mesitoyl-D-glucopyranoses.

When silver mesitoate (V) was condensed with tetra-*O*-acetyl-α-D-glucopyranosyl bromide (IV), a crystalline product giving appropriate analytical values for a 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-D-glucopyranose was obtained. From the mode of preparation and the fact that the rotation of the substance is small ($[\alpha]^{20}D$ +4.3° in chloroform)[6] the product is in all probability the β-anomer VI. Deacetylation with methanolic ammonia gave 1-*O*-mesitoyl-β-D-glucopyranose in crystalline form.

The synthesis of the α-isomer was initially attempted through condensation of tetra-*O*-acetyl β-D-glucopyranosyl chloride with silver mesitoate, but the only product isolated was found, as might be predicted, to be identical with that obtained earlier from the α-halide. Similarly, mesitoylation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucose failed to give a new isomer. Attention was then turned to a method which was devised by Helferich and Schmitz-Hillebrecht[7] for the synthesis of the ace-

(1) Presented before the Division of Carbohydrate Chemistry at the 127th Meeting of the American Chemical Society, Cincinnati, Ohio, March 31, 1955.

(2) (a) Stevioside, I: H. B. Wood, Jr., R. Allerton, H. W. Diehl and H. G. Fletcher, Jr., *J. Org. Chem.*, **20**, 875 (1955); (b) stevioside, II: E. Mosettig and W. R. Nes, *ibid.*, **20**, 884 (1955).

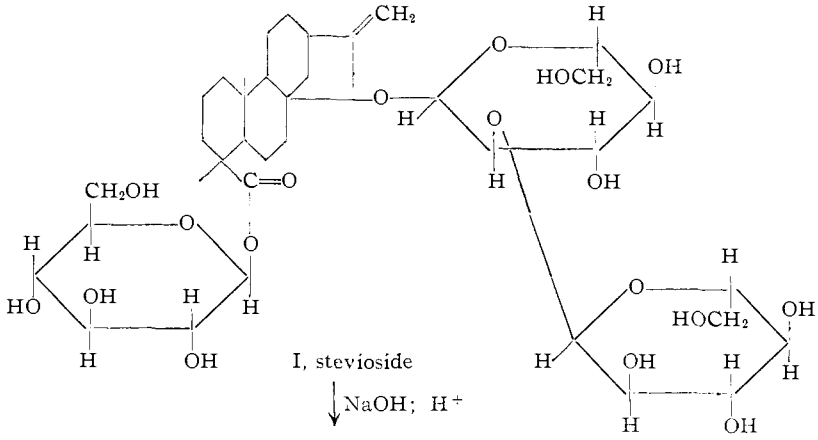
(3) Chemical Foundation Fellow, 1953–1955.

(4) R. U. Lemieux and C. Brice, *Can. J. Chem.*, **30**, 295 (1952).

(5) E. M. Montgomery, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **65**, 3 (1943).

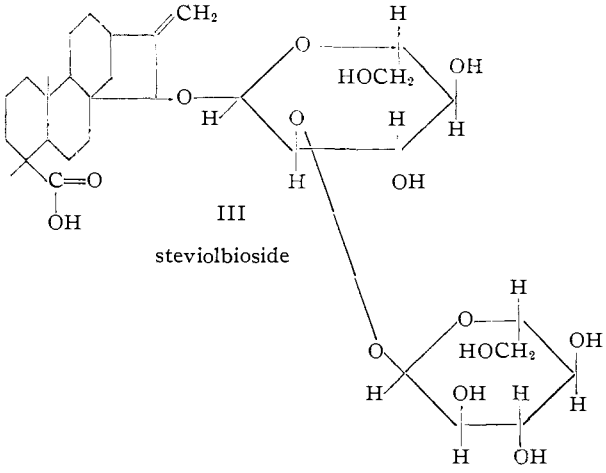
(6) Rotations are specific rotations for the D line of sodium at 20°; concentration is expressed in g. of substance per 100 ml. of solution.

(7) B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, **66**, 378 (1933).



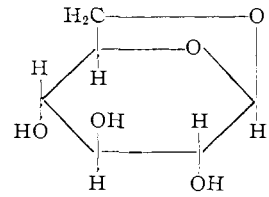
I, stevioside

NaOH; H^+



III

steviolbioside



II, levoglucosan (1,6-anhydro-β-D-glucopyranose)

tates of phenolic α-glycosides. Fusion of a mixture of α-D-glucopyranose pentaacetate (VII), mesitoic acid (VIII) and zinc chloride *in vacuo* at 120–125° gave, in 49% yield, a crude crystalline product which was purified by chromatography on alumina. The mode of preparation and rotation ($[\alpha]^{20}D$ +113° in $CHCl_3$) provide the basis for tentatively considering it to be 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucopyranose (X). That the pyranose ring remained and the mesitoyl group was attached at C_1 was demonstrated through conversion to tetra-*O*-acetyl-α-D-glucopyranosyl bromide. Attempts to deacetylate the compound have thus far failed to provide a well-defined product.

The behavior of the two anomeric 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-D-glucopyranoses[8] toward alkali was now studied. While both substances, when dissolved in alcohol, reduce hot Fehling solution,[9] their behavior when treated with hot aqueous alkali forms a marked contrast. The β-isomer dissolved to give a light amber solution. Acidification of the cooled solution precipitated mesitoic acid; deionization of the filtrate led to the isolation of crude, crystalline levoglucosan in 70% yield. Final identification and purification were made through the nicely crystalline tribenzoate. On the other hand, the α-anomer gave an exceedingly dark solution, indicating extensive destruction of the sugar moiety, and no levoglucosan could be detected. It appears, then, that the analogy between the C_1-ester of a sterically hindered acid and a phenolic glycoside is a valid one and we may conclude with some certainty that the ester-linked glucose residue in stevioside has the β-configuration.

In passing, it is interesting to note that while stevioside is not attacked by emulsin[2a,10] 1-*O*-mesitoyl-β-D-glucose is readily cleaved by this enzyme.

We have speculated that the levoglucosan type of cleavage which plays a major role in the alkaline degradation of stevioside and of 1-*O*-mesitoyl-β-D-glucopyranose tetraacetate may play a minor role in the alkaline destruction of non-hindered esters. However, treatment of relatively large quantities of the two anomeric D-glucopyranose pentabenzoates with hot alkali has led to no detectable quantities of levoglucosan and we must conclude that, if this mode of hydrolysis enters in here, it must do so to an exceedingly small extent.

Experimental[11]

Silver Mesitoate (V).—Ten grams of mesitoic acid, prepared by the method of Barnes,[12] was stirred with dilute ammonium hydroxide until nearly all of the acid had dissolved. The excess of acid was then removed from the neutral solution and 0.1 *M* silver nitrate added until no further precipitate was produced. After standing for one hour at room temperature in subdued light, the precipitate was filtered off and washed successively with water, methanol and ether. The product was dried over phosphorus pentoxide at room temperature and 0.3 mm. pressure; yield 8.52 g.

Anal. Calcd. for $C_{10}H_{11}O_2Ag$: Ag, 39.8. Found: Ag, 39.4.

2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-β-D-glucopyranose (VI).—A mixture of 19.4 g. of silver mesitoate and 19.4 g. of tetra-*O*-acetyl-α-D-glucopyranosyl bromide was suspended in 200 ml. of dry benzene and agitated overnight. The silver bromide was removed and the filtrate concentrated *in vacuo* to a volume of approximately 50 ml.; crystallization was spontaneous upon the addition of 10 ml. of hexane. The product melted at 138–141°; a second crop, m.p. 140–141°, raised the total yield to 20.9 g. (89%, based on the bromide).

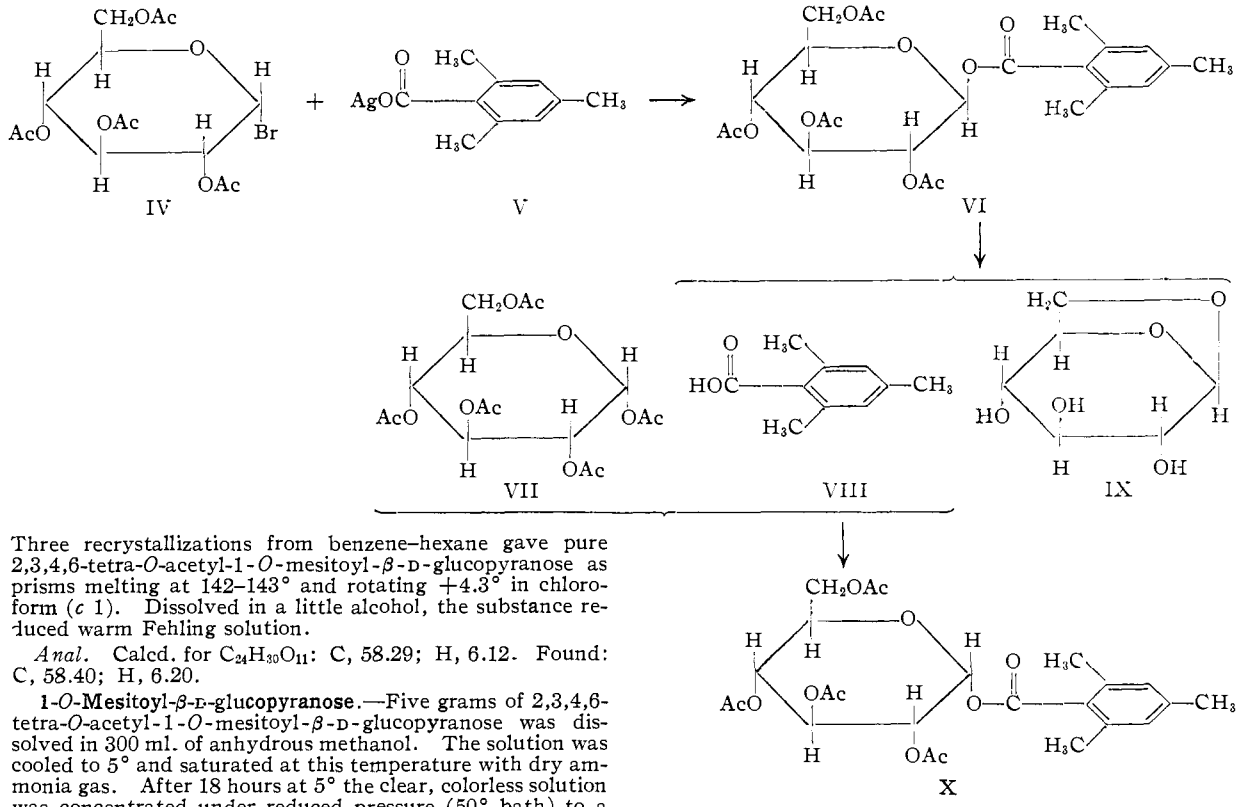
(8) These experiments were conducted with the acetylated products since it is well known that the acetyl groups do not interfere in levoglucosan formation.

(9) As mentioned in the first paper of this series (ref. 2a), even the purest samples of stevioside show a slight reducing power toward Fehling solution and it is probable that a greater or lesser portion of the ester-linked sugar moiety is removed in reducing form when such C_1-esters of sterically hindered acids are treated with alkali.

(10) M. Bridel and R. Lavieille, *Bull. soc. chim. biol.*, **13**, 636 (1931); *J. pharm. chim.*, [8], **14**, 99, 154 (1931).

(11) Melting points are corrected.

(12) R. P. Barnes, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 555.



Three recrystallizations from benzene–hexane gave pure 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-β-D-glucopyranose as prisms melting at 142–143° and rotating +4.3° in chloroform (*c* 1). Dissolved in a little alcohol, the substance reduced warm Fehling solution.

Anal. Calcd. for $C_{24}H_{30}O_{11}$: C, 58.29; H, 6.12. Found: C, 58.40; H, 6.20.

1-*O*-Mesitoyl-β-D-glucopyranose.—Five grams of 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-β-D-glucopyranose was dissolved in 300 ml. of anhydrous methanol. The solution was cooled to 5° and saturated at this temperature with dry ammonia gas. After 18 hours at 5° the clear, colorless solution was concentrated under reduced pressure (50° bath) to a sirup which was then held at 0.02 mm. pressure and 50° to remove the bulk of the acetamide. From 14 ml. of ethyl acetate the product was obtained as plate-like crystals (1.20 g., 36%). Two recrystallizations from 10:1 ethyl acetate–pentane gave pure 1-*O*-mesitoyl-β-D-glucopyranose melting at 160–162° and rotating +6.82° in water (*c* 0.7).

Anal. Calcd. for $C_{16}H_{22}O_7$: C, 58.88; H, 6.80. Found: C, 59.00; H, 6.79; acid (volatile with steam after alkali-treatment and acidification), 1.07 equivalents per mole compound.

A sample (105 mg.) of 1-*O*-mesitoyl-β-D-glucopyranose was acetylated with acetic anhydride in pyridine in the customary fashion to yield 152 mg. (96%) of product melting at 140–142°. After two recrystallizations from ethanol–pentane the substance melted at 143–144°—a range unaffected by the admixture of 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-β-D-glucopyranose.

The Action of Alkali on 2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-β-D-glucopyranose (VI).—Two grams of 2,3,4,6-tetra-*O*-acetyl-1-*O*-mesitoyl-β-D-glucopyranose was added to 40 ml. of 10% aqueous sodium hydroxide and sufficient ethanol then added to effect solution. The mixture, heated on a steam-bath for one hour, changed to light amber in color. After cooling, the solution was treated with 0.15 g. of Daroc G-60 and the filtrate acidified with sulfuric acid. The mesitoic acid was filtered off and the filtrate deionized by passage through Amberlite IR-120(H) and Duolite A-4. Concentration *in vacuo* gave a sirup which was dissolved in *ca.* 12 ml. of absolute ethanol. A small amount of insoluble material was removed by filtration; addition of pentane then gave 0.46 g. (70%) of crude, crystalline levoglucosan. Recrystallization from ethanol–pentane failed to remove the pale yellow color from the material or raise its melting point above 160–165°. Benzoylation of a sample (310 mg.) with benzoyl chloride in pyridine gave, from chloroform–pentane, handsome prisms (786 mg., 87%) melting at 202–204°. Three recrystallizations from the same solvent mixture furnished colorless prisms which, dried at 100° and 0.1 mm. pressure, rotated −36.4° ($CHCl_3$, *c* 1.37),[13] and melted at 202–203°. Mixed with authentic levoglucosan tribenzoate the product melted at 201–202°. The infrared absorption spectrum of the material was identical with that of the authentic sample.

2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucopyranose (X).—Ten grams of mesitoic acid (VIII) and 47.6 g. of α-D-glucopyranose pentaacetate (VII) were melted and thoroughly mixed at 130° (bath temperature). The temperature was then lowered to 120–125°, 20 g. of powdered, freshly-fused zinc chloride added and the mixture, still vigorously stirred, kept at 120–125° and a pressure of *ca.* 25 mm. for 15 minutes. The dark-colored product was dissolved in a mixture of ethylene chloride and water and the organic layer washed thoroughly with water. After a final washing with aqueous sodium bicarbonate, the solution was dried with sodium sulfate, treated with a small amount of bentonite and concentrated *in vacuo*. The sirup was dissolved in 650 ml. of ethanol and the solution partially decolorized with 10 g. of Darco G-60; addition of 600 ml. of water to the filtrate and subsequent seeding[14] gave, after five days at room temperature, 14.8 g. (49%, based on the mesitoic acid) of crude product melting at 114–117°. Since neither decolorizing carbon, bentonite nor recrystallization from the usual solvents appeared to remove the pale yellow color from the product, it was dissolved in 1:1 benzene–ether and adsorbed on a column of activated Alorco alumina. Elution with ether gave six essentially identical fractions[15]; crystallization from absolute alcohol afforded clear prisms melting at 121–122° and rotating +113° ($CHCl_3$, *c* 0.55). Dissolved in a little alcohol, the substance reduced hot Fehling solution.

Anal. Calcd. for $C_{24}H_{30}O_{11}$: C, 58.29; H, 6.12. Found: C, 58.49; H, 6.42; acid (volatile with steam after alkali-

(13) While levoglucosan tribenzoate was prepared by C. Tanret, the discoverer of levoglucosan [*Compt. rend.*, **119**, 158 (1894)], and has been used frequently for the isolation and identification of levoglucosan, its rotation, surprisingly, seems not to have been recorded in the literature. Dr. Nelson K. Richtmyer, of this Laboratory, recently has prepared a pure, authentic sample of levoglucosan tribenzoate and we find this to rotate −36.7° in chloroform (*c* 1.5).

(14) Seeds were obtained in a similar preparation wherein the crude, amorphous product was subjected to chromatography on alumina.

(15) In one experiment the material remaining on the alumina was eluted with methanol and acetylated wth acetic anhydride in pyridine to give a further quantity of nearly pure product. It appears likely, then, that some deacetylation occurred under the relatively vigorous conditions of the fusion.

treatment and acidification), 4.99 equivalents per mole compound.

Tetra-*O*-acetyl-α-D-glucopyranosyl Bromide from 2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucopyranose (X).—2,3,4,6-Tetra-*O*-acetyl-1-*O*-mesitoyl-α-D-glucopyranose (200 mg.) was dissolved in methylene chloride (1.0 ml.) and 0.5 ml. of hydrogen bromide–acetic acid (32% w./w. HBr) added to the solution. After two hours at room temperature the mixture was diluted with a mixture of methylene chloride (15 ml.) and water and the organic layer washed successively with water and aqueous sodium bicarbonate. Moisture was removed with sodium sulfate, the solution concentrated *in vacuo* and the product crystallized from ether–pentane. The fine needles (148 mg., 84%) melted at 88–89° either alone or in admixture with authentic tetra-*O*-acetyl-α-D-glucopyranosyl bromide.

Acknowledgment.—Analyses were carried out by the Institutes' Microanalytical Laboratory under the direction of Dr. W. C. Alford.

BETHESDA 14, MARYLAND

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Pteridines. XIV.[1] Further Studies on a New Approach to Pteridine Synthesis[2]

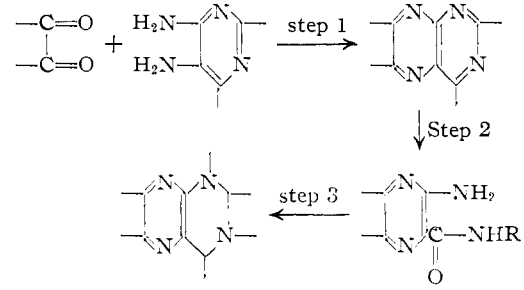
BY E. C. TAYLOR, JR.,[3] ROBERT B. GARLAND AND CHARLES F. HOWELL

RECEIVED MAY 25, 1955

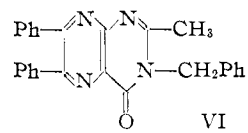
A number of additional cyclizations of 3-aminopyrazinamides and -thiopyrazinamides to pteridines have been carried out, and serve to illustrate further the scope and limitations of this new synthetic approach.

The conventional approach to pteridine synthesis involving the condensation of a 4,5-diaminopyrimidine with an α,β-dicarbonyl compound, an α-halo carbonyl compound, an α-keto alcohol or related derivatives of such intermediates, suffers from a number of disadvantages which are due in large part to limitations encountered in the synthesis of the requisite 4,5-diaminopyrimidines. The number of pteridines variously substituted in the pyrimidine portion of the ring, which are available *via* this approach, is thus severely restricted. The shortcomings of the conventional synthetic approach cannot be adequately compensated for by subsequent alterations of the resulting pteridine, since the latter, as a class, are particularly unsuited for substitution or displacement reactions, and desired substituent groups are best introduced before ring closure.[4,5]

A new approach to pteridine synthesis has been described recently[6] which retains the convenience and versatility of the conventional condensation reaction of a 4,5-diaminopyrimidine with a dicarbonyl reagent with regard to the placement of substituents in the pyrazine ring of the final pteridines, but whose special feature is the varied manner in which the pyrimidine ring may be constructed. The method consists of (a) the synthesis, by the conventional procedure, of a 4-hydroxy- or 2,4-dihydroxypteridine, in which the pyrazine ring carries the substituents desired in the final product; (2) cleavage of the pyrimidine portion of this pteridine, usually by hydrolysis or aminolysis, to give a 3-aminopyrazinamide or a derivative thereof; and (3) ring reclosure of the pyrazinamide to the desired pteridine. The present paper illustrates step 3 with a number of additional cyclization procedures.



The reaction of 3-amino-5,6-diphenylpyrazinamide (I) and 3-amino-N-benzyl-5,6-diphenylpyrazinamide (II) with benzoyl chloride in the absence of a solvent led to the formation of 2,6,7-triphenyl-4(3*H*)-pteridinone (III) and 3-benzyl-2,6,7-triphenyl-4(3*H*)-pteridinone (IV), respectively. Attempts to carry out analogous cyclizations with acetyl chloride or acetic anhydride were not successful, however; the only product isolated in each case was the intermediate 3-acetylamino-5,6-diphenylpyrazinamide (V). Attempts to cyclize these pyrazinamides were likewise unsuccessful; 3-acetylamino-N-benzyl-5,6-diphenylpyrazinamide (V, R = $-CH_2Ph$) was unaffected by ammonia in ethanol or by fusion *in vacuo*, and gave 3-amino-N-benzyl-5,6-diphenylpyrazinamide (II) on treatment with sodium ethoxide in ethanol. It is probable that, in the latter instance, the desired 2-methyl-3-benzyl-6,7-diphenyl-4(3*H*)-pteridinone (VI) was



VI

formed initially but underwent immediate alkaline ring cleavage to the aminopyrazinamide II. This view is substantiated by the observation that 2,6,7-triphenyl-3-benzyl-4(3*H*)-pteridinone (IV) was converted smoothly to II by sodium ethoxide in eth-

(1) For the preceding paper in this series, see E. C. Taylor, Jr., H. M. Loux, E. A. Falco and G. H. Hitchings, THIS JOURNAL, **77**, 2243 (1955).

(2) Abstracted from theses presented by R. B. G. and C. F. H. to the University of Illinois in partial fulfillment for the degree of Bachelor of Science in Chemistry.

(3) Frick Chemical Laboratory, Princeton University, Princeton, N. J.

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

(5) E. C. Taylor, Jr., THIS JOURNAL, **74**, 2380 (1952).

(6) E. C. Taylor, Jr., J. A. Carbon and D. R. Hoff, *ibid.*, **75**, 1904 (1953).