trated sulfuric acid was heated under reflux with occasional stirring for 3 hours. The resulting clear solution was evaporated to dryness under reduced pressure and the residue digested with water and then filtered to yield 3.5 g. (68%) of the crude acetylated product. Recrystallization from 95% ethanol yielded pale yellow crystals, m.p. 144–146° dec.; $\lambda_{max}^{0.1\ N\ NaOH}$ 220.5, 261.5, 363 mμ; log ε 4.34, 4.06, 4.17.

Anal. Calcd. for $C_{14}H_{15}N_5O_8$: C, 44.1; H, 4.0; N, 18.4. Found: C, 44.1; H, 4.2; N, 18.1.

2-Amino-4-hydroxy-7-keto-8-(D-1-sorbityl)-7,8-dihydropteridine-6-carboxylic acid (XXV) was prepared in 63% yield from 2-amino-4-hydroxy-5-phenylazo-6-D-glucaminopyrimidine by the procedure described in method A above. The crude product was recrystallized from water containing a few drops of glacial acetic acid to give a pale yellow microcrystalline solid, m.p. 343° dec. Aqueous solutions of this material exhibited a strong blue fluorescence; $\lambda_{max}^{0.1\ N\ NaOH}$ 263.5, 369 mμ; log ε 3.91, 4.04.

Anal. Calcd. for $C_{13}H_{17}N_5O_9$: C, 40.3; H, 4.4; N, 18.1. Found: C, 40.3; H, 4.6; N, 18.0.

2-Amino-4-hydroxy-7-keto-8-(D-1-ribityl)-7,8-dihydropteridine-6-carboxylic acid (XXVI) was prepared in 35% yield from 2-amino-4-hydroxy-5-phenylazo-6-D-ribaminopyrimidine by the procedure described above. The crude product was recrystallized from dilute acetic acid to yield a pale yellow microcrystalline solid, m.p. 345° dec. Aqueous solutions of this material exhibited a strong blue fluorescence; $\lambda_{max}^{0.1\ N\ NaOH}$ 263.5, 369 mμ; log ε 4.08, 4.22.

Anal. Calcd. for $C_{12}H_{15}N_5O_8$: C, 40.3; H, 4.2; N, 19.6. Found: C, 39.9; H, 4.2; N, 19.4.

2-Amino-4-hydroxy-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic Acid (XXVII).—Zinc amalgam was prepared by shaking manually a mixture consisting of 10 g. of mossy zinc, 0.75 g. of mercuric chloride, 0.5 ml. of concentrated hydrochloric acid and 12 ml. of water. After 5 minutes, the liquid was decanted and the amalgam covered with 7.5 ml. of water and 10 ml. of concentrated hydrochloric acid. At once 4.0 g. of 2-amino-4-hydroxy-7-keto-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic acid was added and the reaction mixture was heated under reflux for 20 minutes. The resulting yellow-green solution was cooled, adjusted to *p*H 9 with ammonium hydroxide and cooled at 5–10° for 2 days. Filtration yielded 2.62 g. (65%) of the ammonium salt of 2-amino-4-hydroxy-8-(2,3-dihydroxypropyl)-7,8-dihydropteridine-6-carboxylic acid. The product was purified by dissolution in dilute ammonium hydroxide followed by reprecipitation with glacial acetic acid, and finally by recrystallization from dilute acetic acid. It was obtained as a cream-colored microcrystalline solid, m.p. 332° dec. Aqueous solutions of this material exhibited a strong blue fluorescence; $\lambda_{max}^{0.1\ N\ NaOH}$ 260, 342 mμ; log ε 3.99, 4.15.

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.4; H, 4.6; N, 24.7. Found: C, 42.3; H, 4.6: N, 24.9.

2-Amino-4-hydroxy-8-(D-1-sorbityl)-7,8-dihydropteridine-6-carboxylic acid (XXVIII) was prepared in 53% yield from 2-amino-4-hydroxy-7-keto-8-(D-1-sorbityl)-7,8-dihydropteridine-6-carboxylic acid by the method described above. It was obtained in the form of a pale yellow microcrystalline solid, m.p. 346° dec. upon recrystallization of the crude product from very dilute acetic acid; $\lambda_{max}^{0.1\ N\ NaOH}$ 260, 356 mμ; log ε 4.01, 4.07.

Anal. Calcd. for $C_{13}H_{19}N_5O_8$: C, 41.8; H, 5.1; N, 18.8. Found: C, 41.6; H, 5.4; N, 18.9.

2-Amino-4-hydroxy-8-(D-1-ribityl)-7,8-dihydropteridine-6-carboxylic acid (XXIX) was prepared in 22% yield from 2-amino-4-hydroxy-7-keto-8-(D-1-ribityl)-7,8-dihydropteridine-6-carboxylic acid by the method described above. It was obtained in the form of a pale yellow microcrystalline solid, m.p. 354° dec. upon recrystallization from very dilute acetic acid. The identity of the product was established by comparison of its ultraviolet absorption spectrum with that given by the 8-(D-1-sorbityl) derivative above; $\lambda_{max}^{0.1\ N\ NaOH}$ 259, 353 mμ; log ε 4.04, 4.13.

PRINCETON, N. J.

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Pteridines XX. 3-Amino-4(3*H*)pteridinone[1,2]

BY EDWARD C. TAYLOR, O. VOGL AND PAULA K. LOEFFLER

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3-Amino-4(3*H*)-pteridinone (VI) has been prepared by cyclization with ethyl orthoformate and acetic anhydride of the isopropylidene (II) and benzylidene (III) derivatives of 2-aminopyrazine-3-carboxyhydrazide (I), followed by very mild acid cleavage of the protecting groups. Because of the great ease with which VI may be hydrolyzed both by acid and by base to regenerate I, it would appear that pteridine intermediates of this type are unsuited for the preparation of pteridine "pseudo" glycosides.

There is an increasing body of evidence which indicates that purine[3–7] and pyrimidine[8–11] antimetabolites may be more effective as ribosides or ribotides than as the free bases, and that, indeed, prior ribosidation or ribotidation[12,13] *in vivo* may be a prerequisite first step in their biological utilization. Although pteridine glycosides have neither been prepared nor isolated from nature, their possible presence in biological systems is strongly suggested by a number of considerations which have been summarized in an accompanying paper.[14] As a part of a program directed toward the synthesis of pteridine glycosides, it was thought that "pseudo" glycosides in which the sugar grouping was attached through a substituent amino group rather than directly to the ring might be of interest as potential antifolic acid compounds. We report in this paper the preparation and properties of a

(1) For the previous paper in this series, see O. Vogl and E. C. Taylor, THIS JOURNAL, **81**, 2472 (1959).

(2) This investigation was supported by a grant (C-2551) to Princeton University from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(3) J. A. Johnson, Jr., H. J. Thomas and H. J. Schaeffer, THIS JOURNAL, **80**, 699 (1958).

(4) M. P. Gordon, O. M. Intrieri and G. B. Brown, *J. Biol. Chem.*, **229**, 641 (1957).

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

(6) J. Baddiley, J. G. Buchanan and G. O. Osborne, *ibid.*, 1651 (1958).

(7) R. E. F. Matthews, in "Chemistry and Biology of Purines," ed. by G. E. W. Wolstenholme and C. M. O'Connor, J. and A. Churchill, Ltd., London, 1957, p. 270.

(8) R. Schindler and A. D. Welch, *Science*, **125**, 548 (1957).

(9) R. Schindler and A. D. Welch, *Proc. Am. Assoc. for Cancer Research*, 3 (1957).

(10) F. Sorm and H. Keilova, *Experientia*, **14**, 215 (1958).

(11) R. E. Handschumacher, *Biochim. et Biophys. Acta*, **23**, 428 (1957).

(12) R. W. Brockman, M. C. Sparks and M. S. Simpson, *ibid.*, **26**, 671 (1957).

(13) H. M. Kissman and M. J. Weiss, THIS JOURNAL, **80**, 5559 (1958).

(14) E. C. Taylor and H. M. Loux, *ibid.*, **81**, 2474 (1959).

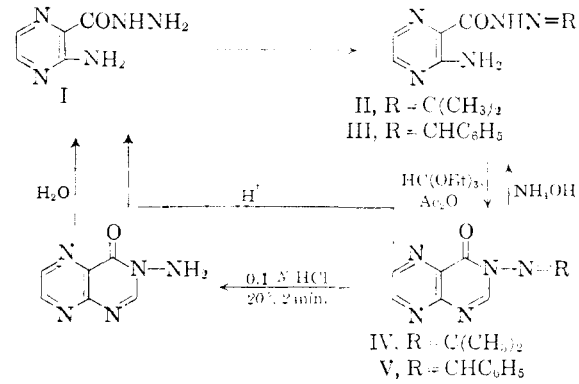
simple pteridine "aglycone" of this type, 3-amino-4(3*H*)pteridinone (VI).

2-Aminopyrazine-3-carboxyhydrazide[15] (I) was prepared by treatment of 2-aminopyrazine-3-carboxamide[1,15,16] or methyl 2-aminopyrazine-3-carboxylate[15,16] with hydrazine. Initial attempts to effect direct cyclization of this compound to a pteridine indicated that prior blocking of the $-NH_2$ portion of the hydrazide grouping would be required. Thus, the reaction of I with formic acid yielded N'-formyl-2-aminopyrazine-3-carboxyhydrazide, which could be recovered unchanged even upon heating for 30 minutes with formamide. The action of a mixture of ethyl orthoformate and acetic anhydride on I yielded a mixture of impure products which could not be characterized. Therefore, compound I was treated with acetone and with benzaldehyde to give isopropylidene and benzylidene derivatives of 2-aminopyrazine-3-carboxyhydrazide (II and III), respectively. Subsequent reaction with ethyl orthoformate and acetic anhydride brought about smooth cyclization to the isopropylidene and benzylidene derivatives of 3-amino-4(3*H*)pteridinone (IV and V), respectively.

Initial attempts to remove the blocking groups from the derivatives IV and V revealed that the pyrimidine ring of this pteridine system was extremely labile to hydrolysis. For example, warming V with dilute ammonium hydroxide resulted in rapid cleavage to III, while warming with dilute acetic or hydrochloric acid led to very rapid cleavage to I. The occurrence of ring cleavage in these hydrolysis attempts was readily apparent by the appearance of a deep yellow color characteristic of the pyrazine derivatives I, II and III. Successful removal of the benzylidene and isopropylidene blocking groups was achieved, however, by treatment of IV and V for a few minutes with cold 0.1 *N* hydrochloric acid followed by immediate dilution of the reaction mixture with ethanol and chilling to 0°. 3-Amino-4(3*H*)pteridinone (VI) was isolated in the form of white needles, m.p. 242–245°. It was rapidly cleaved by dilute acid or dilute alkali to 2-aminopyrazine-3-carboxyhydrazide (I). Reaction of VI with acetone and with benzaldehyde readily reconstituted the isopropylidene and benzylidene derivatives IV and V, respectively.

The lability of VI toward alkaline hydrolysis parallels previous observations on the instability to alkali of pteridines lacking electron-donating substituents and an enolizable hydrogen atom.[17–19] The ease with which VI undergoes ring cleavage in acid is probably a result of ready protonation of the 3-amino group, which serves to decrease even further the electron density of the pteridine nucleus.

It would appear from these results that the instability of 3-amino-4(3*H*)pteridinone (VI) would preclude consideration of this type of intermediate for the preparation of "pseudo" pteridine glycosides.



Experimental[20]

2-Aminopyrazine-3-carboxyhydrazide (I).—A mixture of 42 g. of 2-aminopyrazine-3-carboxamide, 100 ml. of 85% hydrazine hydrate and 500 ml. of ethanol was heated under reflux for 10 hours. Cooling caused the separation of a yellow crystalline solid which was collected by filtration; yield 31 g. (67%), m.p. 200–209°. Recrystallization from ethanol yielded yellow crystals, m.p. 210–211°. The reported[15] melting point for this compound is 207–209°.

N'-Formyl-2-aminopyrazine-3-carboxyhydrazide.—A solution of 1.0 g. of 2-aminopyrazine-3-carboxyhydrazide in 2 ml. of 98–100% formic acid was heated to boiling for 3–5 minutes, cooled slightly and diluted with 20 ml. of methanol. Cooling caused the separation of 0.95 g. (81%) of light yellow silky needles, m.p. 227–229°. The product could be recrystallized from water without a change in the melting point.

The formyl group was assigned to the terminal nitrogen of the hydrazide grouping rather than to the 2-amino grouping, since the compound was recovered unchanged after prolonged heating with benzaldehyde.

Anal. Calcd. for $C_6H_7N_5O_2$: C, 39.8; H, 3.9. Found: C, 39.8; H, 3.6.

Isopropylidene Derivative of 2-Aminopyrazine-3-carboxyhydrazide (II).—A suspension of 2.0 g. of 2-aminopyrazine-3-carboxyhydrazide in 150 ml. of acetone was heated under reflux until complete solution had been achieved (approximately 6 hours). The solution was evaporated to dryness to give 2.35 g. (93%) of a yellow solid, m.p. 162–164°. Recrystallization from acetone–petroleum ether yielded yellow needles, m.p. 166–167°.

Anal. Calcd. for $C_8H_{11}N_5O$: C, 49.7; H, 5.7; N, 36.3. Found: C, 49.9; H, 5.6; N, 36.7.

Benzylidene Derivative of 2-Aminopyrazine-3-carboxyhydrazide (III).—A suspension of 3.0 g. of 2-aminopyrazine-3-carboxyhydrazide in 50 ml. of ethanol containing 3 ml. of freshly distilled benzaldehyde was heated under reflux for 2 hours. The resulting clear yellow solution was concentrated under reduced pressure to about 10 ml. volume and chilled. Filtration then yielded 3.3 g. (70%) of yellow needles, m.p. 175–177°, which were recrystallized from ethanol.

Anal. Calcd. for $C_{12}H_{11}N_5O$: C, 59.7; H, 4.6; N, 29.0. Found: C, 60.0; H, 4.7; N, 29.6.

Isopropylidene Derivative of 3-Amino-4(3H)-pteridinone (IV).—A mixture of 2.5 g. of the isopropylidene derivative of 2-aminopyrazine-3-carboxyhydrazide, 20 ml. of ethyl orthoformate and 20 ml. of acetic anhydride was heated under reflux for 1 hour. The resulting dark yellow solution was diluted with 30 ml. of ethanol and chilled at 0°. Filtration yielded 1.8 g. of light gray crystals, m.p. 210–212°. An additional 0.6 g. of product was obtained by concentration of the mother liquor; total yield 2.4 g. (91%). Recrystallization from methanol yielded colorless crystals but did not alter the melting point.

Anal. Calcd. for $C_9H_9N_5O$: C, 53.2; H, 4.5; N, 34.5. Found: C, 53.2; H, 4.3; N, 34.7.

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

(16) R. C. Ellingson, R. L. Henry and F. G. McDonald, THIS JOURNAL, **67**, 1711 (1945).

(17) A. Albert, *Quart. Rev.*, **6**, 197 (1952).

(18) A. Albert in "Progress in the Chemistry of Organic Natural Products," ed. by L. Zechmeister, Springer-Verlag, Vienna, Vol. 11 1954, p. 350.

(19) For a general review of ring cleavage reactions of pteridines, see E. C. Taylor in "Chemistry and Biology of Pteridines," ed. by G. E. W. Wolstenholme and M. P. Cameron, J. and A. Churchill, Ltd., London, 1954, p. 2.

(20) We are indebted for the microanalyses to Drs. G. Weiler and F. B. Strauss, Oxford, England. All melting points are corrected.

Benzylidene Derivative of 3-Amino-4(3H)pteridinone (V).—A mixture of 2.0 g. of the benzylidene derivative of 2-aminopyrazine-3-carboxyhydrazide, 15 ml. of ethyl orthoformate and 15 ml. of acetic anhydride was heated under reflux for 2 hours, diluted with 50 ml. of ethanol and cooled to give 2.07 g. (quantitative) of light tan fluffy needles, m.p. 202–203°, with preliminary softening at 199°. Recrystallization from ethanol yielded colorless needles, m.p. 203–204°.

Anal. Calcd. for $C_{13}H_9N_5O$: C, 62.1; H, 3.6; N, 27.9. Found: C, 62.3; H, 3.9; N, 27.9.

3-Amino-4(3H)pteridinone (VI).—To a solution of 3.0 g. of the isopropylidene derivative of 3-amino-4(3*H*)pteridinone in 50 ml. of water at room temperature was added, with shaking, 5 ml. of 0.1 *N* hydrochloric acid. The reaction mixture was allowed to stand for 2 minutes and was then diluted with 300 ml. of ethanol and chilled to 0°. Filtration after 1 hour yielded 2.0 g. (83%) of white needles, m.p. 240–245°. Recrystallization from aqueous ethanol raised the melting point to 242–245°; λ_{max}^{EtOH} 240, 311 mμ; log ε 3.94, 3.73; $\lambda_{max}^{H_2O}$ 238, 311 mμ; log ε 4.00, 3.78; $\lambda_{max}^{0.1\ N\ HCl}$ 239, 311 mμ; log ε 3.98, 3.75; $\lambda_{max}^{0.1\ N\ NaOH}$ 272, 338 mμ; log ε 4.19, 3.74.

Anal. Calcd. for $C_6H_5N_5O$: C, 44.2; H, 3.1; N, 42.9. Found: C, 44.5; H, 3.1; N, 42.4.

The same compound could be obtained in much lower yield by hydrolysis of the benzylidene derivative of VI as follows: A suspension of 0.5 g. of V in 40 ml. of ethanol containing 10 ml. of 0.5 *N* hydrochloric acid was stirred at room temperature for 10 minutes, by which time complete solution of the starting material had taken place. The resulting pale yellow solution was diluted with 200 ml. of ether and chilled to give 0.05 g. (15%) of white needles, m.p. 240–245°, identical with the product obtained by hydrolysis of the isopropylidene derivative as described above.

Solutions of 3-amino-4(3*H*)pteridinone in dilute acid or dilute alkali upon warming rapidly turned bright yellow. Cooling then yielded 2-aminopyrazine-3-carboxyhydrazide in good yield.

PRINCETON, N. J.

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT OF CIBA PHARMACEUTICAL PRODUCTS, INC., THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, AND THE DEPARTMENTS OF CHEMISTRY OF IOWA STATE COLLEGE AND THE UNIVERSITY OF WISCONSIN]

The Stereochemistry of Reserpine, Deserpidine and Related Alkaloids[1,2]

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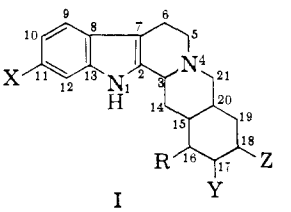
Chemical and physical data are presented which allow the rigorous assignment of stereoformulas XXXIc and XXXIb to the alkaloids reserpine and deserpidine, respectively. The stereochemistry of related natural products is discussed in light of these data.

By early 1955, intensive structure studies on reserpine and deserpidine, two important hypotensive and sedative alkaloids of the Rauwolfia species, by Schlittler and his co-workers[7] had led to structures Ia and Ib, respectively, for these medicinally valuable natural products. The task of elucidating the relative spatial configuration of the six asymmetric centers C-3, 15, 16, 17, 18 and 20 in both compounds remained, although the accumulated evidence[7] already pointed to certain definite stereochemical features. Both alkaloids had been shown to possess a thermodynamically unstable environment at C-3 and a *cis* relationship of the C-16 and -18 substituents, while deserpidine, whose chemical reactivity paralleled in every respect that of reserpine, had been interrelated with rauwolscine (α-yohimbine) (Ic) and 3-epi-α-yohimbine (Ic), whose stereochemistry had been assigned only partially. It appeared that a critical study of the basic ring systems and of the behavior of the individual skeletal substituents present in the alkaloids would lead to the information necessary for complete stereochemical assignment.



I

a, R = CO_2Me, X = Y = OMe, Z = $OCOC_6H_2(OMe)_3$
b, R = CO_2Me, X = H, Y = OMe, Z = $OCOC_6H_2(OMe)_3$
c, R = CO_2Me, X = Z = H, Y = OH
d, R = CO_2Me, X = Y = OMe, Z = $OSO_2C_7H_7$
e, R = CH_2OH, X = Y = OMe, Z = H
f, R = CH_2OH, X = Z = H, Y = OMe
g, R = CO_2Me, X = Z = H, Y = $OSO_2C_7H_7$
h, R = CO_2Me, X = Y = OMe, Z = Br
i, R = CO_2Me, X = Y = OMe, Z = OH
j, R = CO_2H, X = Y = OMe, Z = OH

(1) The work contained herein, representing the independent efforts of four laboratories, was written as a joint publication at the suggestion of the Editor. Presented in part by E. W. to the 7th Summer Seminar in the Chemistry of Natural Products, University of New Brunswick, Fredericton, Canada, August 16–20, 1955.

(2) For preliminary accounts of part of this work *cf.*; (a) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, THIS JOURNAL, **77**, 2028 (1955); (b) E. E. van Tamelen, P. D. Hance, K. V. Siebrasse and P. E. Aldrich, *ibid.*, **77**, 3930 (1955); (c) C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F. St. André, *Experientia*, **11**, 303 (1955); (d) E. Wenkert and L. H. Liu, *ibid.*, **11**, 302 (1955); (e) C. F. Huebner and E. Wenkert, THIS JOURNAL, **77**, 4180 (1955); (f) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, *ibid.*, **77**, 4687 (1955); (g) E. E. van Tamelen and P. D. Hance, *ibid.*, **77**, 4692 (1955); (h) C. F. Huebner, M. E. Kuehne, B. Korzun and E. Schlittler, *Experientia*, **12**, 249 (1956); (i) C. F. Huebner and D. F. Dickel, *ibid.*, **12**, 250 (1956); (j) E. Wenkert, E. W. Robb and N. V. Bringi, THIS JOURNAL, **79**, 6570 (1957).

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(7) (a) H. B. MacPhillamy, L. Dorfman, C. F. Huebner, E. Schlittler and A. F. St. André, THIS JOURNAL, **77**, 1071 (1955), and preceding papers. For the full articles pertaining to this communication *cf.*: (b) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, *ibid.*, **77**, 4335 (1955); (c) C. F. Huebner, A. F. St. André, E. Schlittler and A. Uffer, *ibid.*, **77**, 5725 (1955).