peaks, this residue showed absorption peaks for all of the individual peaks of the respective compounds.

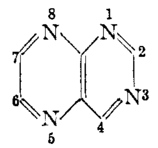
Baker Laboratory of Chemistry
Cornell University
Ithaca, New York

## Spectrophotometric Determination of the Ionization Constants of Pteridine, 2-Aminopteridine and 4-Aminopteridine

By DeLos F. DeTar,[1] C. K. Cain and B. Spencer Meeks, Jr.[2]

Received May 5, 1953

Interest in the chemistry of pteridine (I) and its derivatives has been especially great since the discovery that the pteridine nucleus is an important part of the folic acid molecule. Until very re-



cently pteridine itself and its simple monofunctional derivatives were largely unknown, a situation perhaps stemming from the fact that all of the interesting naturally occurring derivatives were tri- and tetra- substituted pteridines.

A few years ago Jones[3] made a preliminary report of the preparation of pteridine. More recently the chemistry of the simple pteridines has been admirably worked out by Albert, Brown and Cheeseman.[4] The present investigation was undertaken before the report of this work was available. Albert, Brown and Cheeseman measured the $pK$ of the acid forms ($BH^+$) of the pteridines by potentiometric titration. In this work the $pK^*$ values have been measured spectrophotometrically by the general procedure described by Sager, Schooley, Carr and Acree.[5] The results are presented in Table II.

Table I

Spectrophotometric Data for Calculation of the Ionization Constant of 2-Aminopteridine

| $p$H of buffer (0.02 $M$) | Extinction coefficients $\times 10^{-4}$ at various wave lengths, mμ 260 | 265 | 290 | 300 | 305 |
|---|---|---|---|---|---|
| 6.98 | 0.751 | 0.681 | 0.086 | 0.076 | 0.080 |
| 7.14 | .748 | .681 | .084 | .075 | .079 |
| 7.27 | .738 | .675 | .084 | .075 | .078 |
| Av. $\epsilon_B$[a] | .746 | .679 | .085 | .075 | .079 |
| 1.90 | 0.130 | 0.176 | 0.646 | 0.769 | 0.745 |
| 1.98 | .133 | .175 | .645 | .770 | .744 |
| 2.11 | .135 | .178 | .646 | .771 | .748 |
| Av. $\epsilon_i$[a] | .133 | .177 | .645 | .770 | .746 |
| 4.19 | 0.382 | 0.383 | 0.439 | 0.513 | 0.500 |
| 4.30 | .410 | .406 | .406 | .474 | .463 |
| 4.68 | .549 | .517 | .266 | .300 | .296 |

[a] See text for definitions.

(1) Department of Chemistry, University of South Carolina, Columbia, S. C.

(2) From the Ph.D. thesis of B. Spencer Meeks, Jr.

(3) W. G. M. Jones, *Nature*, **162**, 524 (1948).

(4) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 474 (1951); 1620, 4219 (1952); A. Albert, *Quart. Rev.*, **6**, 197 (1952).

(5) E. E. Sager, M. R. Schooley, A. S. Carr and S. F. Acree, *J. Research Natl. Bur. Standards*, **35**, 521 (1945).

Table II

Calculated Values of $pK^*$

| $p$H | $pK^*$ calcd. at 275 mμ | 285 mμ | | | |
|---|---|---|---|---|---|
| Pteridine: av. $pK^*$ 4.15 ± 0.06[a] (4 samples) (4.12 ± 0.05)[b] | | | | | |
| 3.85 | 4.23 | 4.13 | | | |
| 4.21 | 4.28 | 4.09 | | | |
| 4.30 | | 4.12 | | | |
| 4.67 | | 4.07 | | | |
| | 305 | 320 | 325 | | |
| 4-Aminopteridine: av. $pK^*$ 3.514 ± 0.020[a] (3 samples) (3.56 ± 0.08)[b] | | | | | |
| 3.45 | 3.48 | 3.48 | 3.50 | | |
| 3.62 | 3.49 | 3.50 | 3.50 | | |
| 3.93 | 3.57 | 3.55 | 3.56 | | |
| $p$H | 260 | 265 | 290 | 300 | 305 |
| 2-Aminopteridine: av. $pK^*$ 4.386 ± 0.020[a] (3 samples) (4.29 ± 0.03)[b] | | | | | |
| 4.19 | 4.35 | 4.35 | 4.42 | 4.42 | 4.42 |
| 4.30 | 4.38 | 4.38 | 4.43 | 4.43 | 4.43 |
| 4.68 | 4.35 | 4.36 | 4.36 | 4.36 | 4.36 |

[a] For error limits see text. [b] $pK$ by titration as reported by Albert, Brown and Cheeseman.[4] Error figures represent maximum deviations from the calculated titration curve and apparently are derived from a single determination.

The agreement between the two sets of $pK$ values is very good.

### Experimental Part

**Pteridines.**—The pteridine and 2-aminopteridine samples were prepared by a method similar to that described by Albert, Brown and Cheeseman[4] but independently worked out in this Laboratory. The samples were sublimed and recrystallized several times until further purification gave no change in the ultraviolet spectra. The 4-aminopteridine was a sample prepared by Mr. N. T. Gehshan.[6]

**Buffer Solutions.**—The following buffers were used: $p$H 0.5–1.5, hydrochloric acid; 1.5–3.5, citrate; 3.5–5.5, acetate; 5.5–8.0, phosphate; 8.0–12.0, borate; 12.0, sodium hydroxide. Buffer concentrations were 0.02 $M$, and $p$H values were read using a Leeds and Northrup $p$H meter with calomel and glass electrodes standardized at $p$H 4 and $p$H 7 against Bureau of Standards potassium acid phthalate and Leeds and Northrup phosphate buffer.

**Spectrophotometric Measurements.**—A Beckman model DU spectrophotometer was used in this work. In obtaining the extinction coefficients such as those given in Table I two sets of absorbance readings were taken on each sample, one set of readings with solvent in cell 1 and solution in cell 2, and another set with solvent in cell 2 and solution in cell 1. The two absorbance values were averaged for each wave length. This averaging procedure increases the accuracy by cancelling out bias between the two cells, it provides increased precision, and it eliminates gross errors.

**Equations for Calculation of $pK^*$.**—For a compound $BH^+$, the acid strength that is determined spectrophotometrically is defined by eq. 1

$$K = C_B a_{H_3O^+}/C_{BH^+} \quad (1)$$

The ionization constant is rather insensitive to ionic strength since $a_{H_3O^+}$ and $a_{BH^+}$ tend to be changed to about the same extent by the ionic environment. Since the buffer concentrations used were low (about 0.02 $M$), the $pK^*$ values reported in Table II are probably equal to $pK_a$ within the experimental error.

The values of $pK^*$ were calculated using eqs. 2 and 3

$$pK^* = pH + \log [\alpha/(1 - \alpha)] \quad (2)$$

$\alpha$ = fraction of the compound which is in the form $BH^+$. In terms of spectrophotometrically measured quantities $\alpha$ is given by eq. 3

$$\alpha = (\epsilon - \epsilon_B)/(\epsilon_i - \epsilon_B) \quad (3)$$

where $\epsilon_B$ is the extinction coefficient of the basic form, $\epsilon_i$ of

(6) N. T. Gehshan, M.S. Thesis, Cornell University, 1948.

the acidic form $BH^+$ and $\epsilon$ the extinction coefficient of the mixture of the two forms.

**Procedure.**—The first step was to obtain the absorption spectra curves of the compound in a series of buffers in order to ascertain regions in which the acidic and basic forms of the compound differ significantly. The second step was to select suitable wave lengths for a more careful study using a much wider series of buffer solutions. A plot of the extinction coefficients against $p$H served to locate the $p$H regions at which the absorption of the pure acidic and basic forms may be obtained. Such plots also showed that multiple ionization was absent in the $p$H range covered.

Typical spectrophotometric data are given for 2-aminopteridine in Table I in order to illustrate the type of precision attainable. The calculation of $pK^*$ is illustrated in Table II. The variance ($s^2$) of a single value of $pK^*$ is 0.00114 as estimated from the deviation of the individual values from the average. The expected variance can also be estimated from the data in Table I using the principle of propagation of error. The contribution to the variance of $pK^*$ due to spectrophotometric errors is about 0.0005, and that due to variance of $p$H determinations is about 0.0009, giving a total variance of 0.0014. The two variance estimates are in excellent agreement. Simple averaging of the $pK^*$ values is permissible since it can be shown that all the values have nearly the same weight. The actual number of independent determinations of $pK^*$ is three, since three samples of 2-aminopteridine were used for each $p$H range (a separate sample at each $p$H value of Table I). Thus the variance of the average is 0.00038, corresponding to a standard deviation of the average of 0.020 $pK$ unit.

Pteridine itself offered considerable difficulties. It was found that spectrophotometer readings drifted and it was necessary to obtain a series of readings over a period of about an hour. The zero time reading was obtained by extrapolating a plot of log $\epsilon$ against time back to zero time. The instability of the spectral data resulted in rather larger uncertainty in $pK^*$ for this compound. A "weighted" average of 4.17 was calculated, based on variance estimates of individual $pK^*$ values due to spectrophotometric errors. This differs very little from the straight average, and there are an insufficient number of measurements to justify the use of weighting.

DEPARTMENT OF CHEMISTRY
CORNELL UNIVERSITY
ITHACA, NEW YORK

## The Selective Demethylation of Homoveratrylamine[1]

BY K. E. HAMLIN AND F. E. FISCHER

RECEIVED JULY 2, 1953

During the course of another investigation,[2] substantial quantities of isovanillin or a compound leading to 3-hydroxy-4-methoxyphenethylamine were required. Synthesis of isovanillin by various literature methods including the application of a variety of demethylating agents to veratraldehyde[3] has not been satisfactory. The experiments of Birch[4a,b] involving the demethylating action of sodium and liquid ammonia on various phenol ethers suggested a possible solution to the problem. Significantly, by this reaction, Birch was able to convert 3,4-dimethoxytoluene to 4-methoxy-*m*-cresol in 70% yield.

Accordingly, the action of sodium and liquid ammonia on a group of veratryl compounds was studied. The application of this method to veratraldehyde, veratric acid and homoveratronitrile was singularly unfruitful. In these cases, guaiacol was the only identifiable product and was found only in trace amounts. However, when homoveratrylamine was treated with sodium and liquid ammonia, a selective demethylation occurred, and 3-hydroxy-4-methoxyphenethylamine was obtained in excellent yields. Inasmuch as homoveratrylamine is readily available, it may now serve as a convenient source of isovanillyl derivatives.

(1) Presented before the Medicinal Chemistry Division at the 124th Meeting of The American Chemical Society, Chicago, Illinois, 1953.

(2) M. B. Moore, H. B. Wright, M. Freifelder and R. K. Richards, *J. Am. Pharm. Assoc.*, in press.

(3) M. B. Moore, personal communication.

(4) (a) A. J. Birch, *J. Chem. Soc.*, 102 (1947); (b) George W. Watt, *Chem. Revs.*, **46**, 339 (1950).

### Experimental

**3-Hydroxy-4-methoxyphenethylamine.**—With stirring, 63.4 g. (0.35 mole) of 3,4-dimethoxyphenethylamine was added in a slow stream to a solution of 26.2 g. (1.14 atoms) of sodium in 700 cc. of liquid ammonia. The resulting mixture was allowed to stand for six hours until it reached room temperature and was then decomposed by cautious addition of ice. This material was extracted with ether to remove unreacted amine, and the aqueous phase was aerated to remove excess ammonia and was finally made acidic while cooling. Following ether extraction, the acid layer was treated with excess sodium bicarbonate solution and the amine thus released was extracted by means of butanol. The butanol extract was dried over anhydrous magnesium sulfate and was then treated with ethereal hydrogen chloride. The crystalline material thus formed was recrystallized from methanol-ether. In this manner, 61 g. (85% yield) of 3-hydroxy-4-methoxyphenethylamine hydrochloride was obtained melting at 204–205.5°; reported[5] m.p. 201–203°.

*Anal.* Calcd. for $C_9H_{13}NO_2 \cdot HCl$: N, 6.88. Found: N, 6.85.

**N-Acetyl-3-hydroxy-4-methoxyphenethylamine.**—A solution of 20.4 g. (0.1 mole) of 3-hydroxy-4-methoxyphenethylamine hydrochloride in 15.3 g. (0.15 mole) of acetic anhydride was stirred while adding portionwise 27 g. (0.32 mole) of sodium bicarbonate. The mixture was then heated on a steam-bath for one hour. After cooling, the resulting crystalline material was filtered, washed with water and dried. After recrystallization from a mixture of absolute ethanol and heptane, the N-acetyl-3-hydroxy-4-methoxyphenethylamine melted at 124–125°; yield 12.9 g., 53%.

*Anal.* Calcd. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N 6.70. Found: C, 63.30; H, 7.04; N, 6.74.

**Acknowledgment.**—We are indebted to E. F. Shelberg, Chief Microanalyst, and his staff for the analytical data.

(5) F. A. Ramirez and A. Burger, THIS JOURNAL, **72**, 2781 (1950).

ABBOTT LABORATORIES
NORTH CHICAGO, ILLINOIS

## The Reaction of Cyclohexene with Bromine, Iodine Monobromide and Iodine Monochloride

BY ALEXANDER GERO, JOSEPH J. KERSHNER AND RICHARD E. PERRY

RECEIVED JUNE 21, 1953

As a preliminary to studies on enol titration, we have investigated the relative rates of addition of various halogens to an olefin. Rate studies on such additions have been performed[1] but we know of no comparative studies on the rates of addition of various halogens to the same olefin, under the same conditions.

Cyclohexene was chosen as the standard olefin because it is easily available in good purity, sufficiently high-boiling for easy handling and non-

(1) For literature, see A. E. Remick, "Electronic Interpretations of Organic Chemistry," 2d ed., John Wiley and Sons, Inc., New York, N. Y., p. 432 ff.