employed in these studies. Six 4-liter Pyrex bottles, each containing 3 liters of minimal medium[12] were prepared and sterilized by autoclaving. After cooling, the medium was inoculated and maintained at 30° with forced aeration for 4 days. At the end of this period, 500 mg. of desoxycorticosterone in 2.5 ml. of propylene glycol was added to the grown culture in each bottle, using aseptic technique. The propylene glycol solution had previously been sterilized by heating to 100° for 15 minutes. After the addition of the steroid the incubation with forced aeration was continued for a further 48 hours.

Following the incubation period, the medium was separated from the mold by straining through gauze, and extracted with ethyl acetate. The cells were extracted by homogenization in a Waring blendor with acetone. The acetone extract was combined with the ethyl acetate extract, dried over sodium sulfate, and taken down to a sirup *in vacuo*. The combined residue was taken up in benzene and chromatographed on silica gel. A crystalline compound was eluted using a benzene–ethyl acetate mixture (1:1). Several recrystallizations from acetone–petroleum ether furnished needles, m.p. 182–184°, $[\alpha]_D$ +163°, λ_{max}^{EtOH} 241 mμ, log ε 4.17. The infrared spectrum (kindly determined by Dr. T. F. Gallagher, Sloan-Kettering Institute for Cancer Research) in chloroform solution showed the presence of an associated hydroxyl group(s), 20-ketone (1706 cm.$^{-1}$) and the Δ^4-3-keto function (1668 and 1618 cm.$^{-1}$). In a comparative paper chromatogram with corticosterone with benzene on formamide-impregnated paper,[13] the product was found to be slightly more polar than corticosterone, a behavior which also conforms in the case of 14α-hydroxydesoxycorticosterone.[14]

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.50; H, 8.70.

Acetylation with acetic anhydride–pyridine (room temperature, 16 hours) followed by recrystallization from petroleum ether–acetone yielded the **21-monoacetate**, m.p. 200–205°, $[\alpha]_D$ + 185°, which in its paper chromatographic behavior in benzene or hexane–benzene on formamide-impregnated paper[12] was indistinguishable from corticosterone 21-acetate. The infrared spectrum in chloroform solution (courtesy of Dr. T. F. Gallagher) still showed the presence of a free hydroxyl group as well as the characteristic bands for the 20-keto-21-acetoxy (1748 and 1725 cm.$^{-1}$) and Δ^4-3-keto (1668 and 1617 cm.$^{-1}$) functions.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.33; H, 8.58.

The crystalline monoacetate was recovered unchanged after standing for 3 hours at 15° with chromium trioxide in 80% acetic acid.

x-Hydroxy-Δ^4-3-ketoetienic Acid.—A solution of 40 mg. of x-hydroxy-desoxycorticosterone in 10 cc. of 50% acetic acid was oxidized at room temperature for 30 minutes with 1.0 g. of sodium bismuthate. After processing in the conventional manner[8] and recrystallizing from petroleum ether–acetate, the etioacid, exhibited m.p. 254–260°, $[\alpha]_D$ +218° (pyridine).

Anal. Calcd. for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49. Found: C, 72.14; H, 8.69.

(12) G. W. Beadle and E. L. Tatum, *Am. J. Bot.*, **32**, 678 (1945).

(13) *Cf.* A. Zaffaroni and R. B. Burton, *J. Biol. Chem.*, **193**, 749 (1951), and earlier papers.

(14) Private communication from Dr. A. Zaffaroni, Syntex, S. A., Mexico, D. F.

JOINT CONTRIBUTION FROM
THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASS., AND
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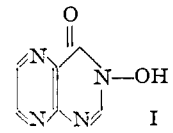
The Preparation of 3-Hydroxy-4-pteridinone

BY W. B. WRIGHT, JR., AND J. M. SMITH, JR.

RECEIVED MARCH 4, 1955

In recent years, a large number of hydroxy- and polyhydroxypteridines have been synthesized and characterized,[1] but no cyclic hydroxamic acid derivative has been described in the literature. In view of the antibacterial activity of cyclic hydroxamic acid derivatives of other heterocyclic systems[2] we have prepared such a derivative of pteridine.

The compound, 3-hydroxy-4-pteridinone (I), was



I

prepared by a modification of the method of Albert[3] for the preparation of 4-hydroxypteridine. A mixture of 3-aminopyrazinehydroxamic acid, acetic anhydride and ethyl orthoformate was heated at reflux and the acetate which was formed was hydrolyzed to I by heating with alkali.

3-Hydroxy-4-pteridinone decomposes at about 290°, the exact temperature depending somewhat upon the rate of heating. It can be recrystallized from hot water but is poorly soluble in cold water, acetone or hot ethanol. It dissolves easily in aqueous bicarbonate and is reprecipitated by the addition of acid. The *p*H of a saturated aqueous solution is 3, and the solution gives a green color with cupric salts and an amber color with ferric chloride. The ultraviolet spectra have been determined: in 0.1 *N* NaOH (λ_{max} 274 mμ, log ε 4.41) and in 0.1 *N* HCl (λ_{max} 240 mμ, log ε 4.12; λ_{max} 310 mμ, log ε 3.78).

Experimental

3-Aminopyrazinehydroxamic Acid.—A mixture of 15.3 g. of methyl 3-aminopyrazinoate, 10.4 g. of hydroxylamine hydrochloride and 250 ml. of 1 *N* sodium hydroxide was warmed for two hours at 35–50°. The clear solution was cooled to 30° and then was treated with 20 ml. of 5 *N* hydrochloric acid. The precipitate which separated was filtered and dried to yield 14.1 g. (92%). When this product was recrystallized from 35 parts of water, light yellow crystals which decomposed at 196° were obtained.

Anal. Calcd. for $C_5H_6N_4O_2$: C, 39.0; H, 3.92; N, 36.3. Found: C, 38.9; H, 4.07; N, 36.3.

3-Hydroxy-4-pteridinone (I).—A mixture of 11.0 g. of 3-aminopyrazinehydroxamic acid, 100 ml. of acetic anhydride and 100 ml. of ethyl orthoformate was heated under reflux for two hours. The clear solution was concentrated under reduced pressure to a brown residue and this was warmed at 70–75° for three minutes with 120 ml. of 1 *N* sodium hydroxide. After cooling in an ice-bath, the reaction mixture was treated with activated carbon and clarified. The filtrate was acidified to *p*H 2.5, and the precipitate was filtered, washed with a little water and then with acetone, and dried at 70°. The yield of 3-hydroxy-4-pteridinone, m.p. 287° dec., was 67%. Recrystallization from 50 parts of hot water raised the decomposition point to 290°.

Anal. Calcd. for $C_6H_4N_4O_2$: C, 43.9; H, 2.46; N, 34.1. Found: C, 44.1; H, 2.54; N, 34.0.

Acetate of 3-Hydroxy-4-pteridinone.—When the brown residue, obtained by concentrating the reaction mixture after the initial reflux period, was recrystallized twice from absolute alcohol, a crystalline product which melted at 172–

(1) A. Albert, *Fortsch. Chem. org. Naturstoffe*, **11**, 350 (1954).

(2) (a) J. D. Dutcher and O. Wintersteiner, *J. Biol. Chem.*, **155**, 359 (1944); (b) G. Dunn, *et al.*, *Nature*, **164**, 181 (1949); (c) S. R. Safir and J. H. Williams, *J. Org. Chem.*, **17**, 1298 (1952); (d) E. Shaw, THIS JOURNAL, **71**, 67 (1949); (e) A. Lott and E. Shaw, *ibid.*, **71**, 70 (1949); (f) G. T. Newbold and F. S. Spring, *J. Chem. Soc.*, 1864 (1948).

(3) A. Albert, D. J. Brown and G. Cheeseman, *ibid.*, 474 (1951).

173° was obtained. The elementary analysis indicated that it was an acetate of 3-hydroxy-4-pteridinone.

Anal. Calcd. for $C_8H_6N_4O_3$: C, 46.6; H, 2.93; N, 27.2. Found: C, 46.5; H, 2.93; N, 27.2.

Acknowledgment.—We wish to express our gratitude to Mr. O. Sundberg and his associates for the microanalyses and to Mr. J. Morath for the photometric data.

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COMMUNICATIONS TO THE EDITOR

THE STRUCTON NUMBER RULE

Sir:

A theory according to which many properties of solids and liquids can be related to the numbers and properties of the "structons" present, recently has been outlined.[1] A "structon" is defined as an atom or ion or molecule or group of atoms of a given kind, surrounded in a specified manner. A "structon number rule," relating the minimum number of structon types (S) to the number of degrees of composition freedom (F), was presented.

Application was made specifically to sodium silicate glasses. When the theory is extended to other systems, including liquid solutions, it appears advisable to express the structon number rule somewhat differently. One can still use the same equation, but now C denotes the number of types of contact between unlike structon centers. In the

$$S = C + F + 2 \qquad (1)$$

Na_2O–SiO_2 system, C is two, there being only Na–O and Si–O contacts. In a solution composed of two molecular species, forming strong contacts with each other (*e.g.*, by hydrogen-bonding), C is one, regardless of whether or not *like* molecules also form strong contacts.

To determine the number of each of S types of structons requires S equations. For each of the C types of contact between different types of structon centers (A, B), there is one equation, equating the number of contacts between A-type structon centers and B neighbors to the number of contacts between B-type structon centers and A neighbors. Thus, in the high-silica region of the sodium silicate system

$$2N_{O(2Si)} + 2N_{O(2Si,Na)} + N_{O(Si,3Na)} = 4N_{Si(4O)} \qquad (2)$$

$$N_{O(2Si,Na)} + 3N_{O(Si,3Na)} = 6N_{Na(6O)} \qquad (3)$$

There is one normalizing equation. In the sodium silicate example, it expressed the fact that the total number of oxygen-centered structons equals unity (since the quantity of glass being considered was that containing a single atom of oxygen)

$$N_{O(2Si)} + N_{O(2Si,Na)} + N_{O(Si,3Na)} = 1 \qquad (4)$$

In molecular solutions, the normalizing equation may show that the sum of the mole fractions equals unity.

(1) M. L. Huggins, *J. Phys. Chem.*, **58**, 1141 (1954).

There is also another equation, in many cases, expressing the over-all neutrality or valence-balancing requirement; *e.g.*

$$N_{Na(6O)} + 4N_{Si(4O)} = 2[N_{O(2Si)} + N_{O(2Si,Na)} + N_{O(Si,3Na)}] \qquad (5)$$

The number of degrees of composition freedom gives the number of additional equations required to fix the numbers of all structons present. This, with the other relationships just given, leads to eq. (1).

In molecular solutions, the neutrality equation is no longer of use, hence eq. (1) must be replaced by

$$S = C + F + 1 \qquad (6)$$

This is also the equation to use if all the structon charges (see ref. 1) are zero, since then the neutrality equation is not independent; it can be obtained by appropriate addition of the structon-contact equations, such as eqs. (2) and (3). This applies, for example, to pure silica.

Application of structon theory and the structon number rule to molecular solutions will be made in another paper.

RESEARCH LABORATORIES
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RECEIVED JUNE 7, 1955

A CARCINOGENIC OXIDATION PRODUCT OF CHOLESTEROL

Sir:

The observation[1] that a crude progesterone preparation prepared[2] by permanganate oxidation of cholesterol dibromide and debromination produced tumors in 32% of the mice tested initiated an extended investigation in which various products of oxidation of cholesterol have been prepared in Cambridge and tested for carcinogenicity in Santa Barbara. Some of the compounds submitted for assay were suggested by specific hypotheses (an abnormal cholesteryl ester,[3] an epoxide derived from a 7,8,-9,11-diene,[4] Δ^5-cholestene-3-one[4]), others were empirically observed known or new[5] products of

(1) F. Bischoff and J. J. Rupp, *Cancer Research*, **6**, 403 (1946).
(2) M. A. Spielman and R. K. Meyer, THIS JOURNAL, **61**, 893 (1939).
(3) L. F. Fieser and W. P. Schneider, *ibid.*, **74**, 2254 (1952).
(4) L. F. Fieser, *Bull. soc. chim.*, **21**, 541 (1954); *Science*, **119**, 3099 (1954).
(5) L. F. Fieser, THIS JOURNAL, **75**, 4377, 4386, 4395 (1953).