Notes

Interaction of Pyridoxal with Cyanide[1]

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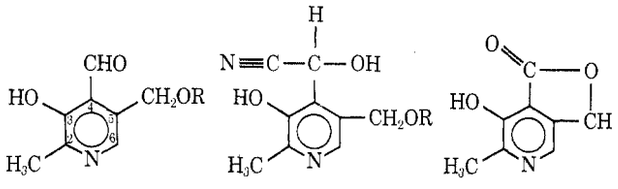
Pyridoxal (**1a**) and its 5′-phosphate (**1b**) are the most important forms of vitamin B_6. The 5′-phosphate serves as a cofactor for a number of enzymes, most of which are involved in the metabolism of amino acids; these enzymes include transaminases, decarboxylases, and racemases.[2]

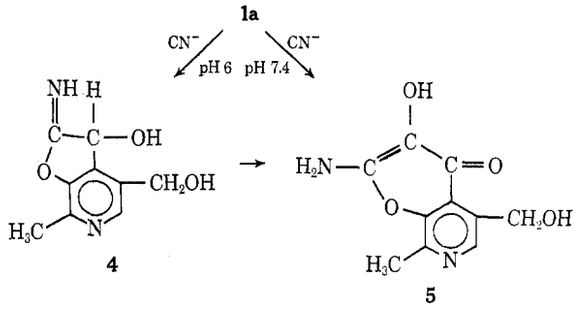


1 **2** **3**

a, R = H
b, R = PO_3^{2-}

We are interested in obtaining the cyanohydrin (**2a**) of pyridoxal as a convenient starting material for the synthesis of some potential antagonists for this vitamin. Indeed, Bonavita reported formation of the presumed cyanohydrin **2** by the interaction of **1a** or **1b** with KCN at pH 7.2, giving a fluorescent compound.[3] Subsequently this reaction became one of the standard fluorometric methods for the determination of **1b** in biological material.[4] More recently, however, Oishi and Fukui reexamined the reaction, and found pyridoxic acid lactone (**3**) to be the end product.[5] Thus the fluorescence observed is due to this lactone. About the same time, Takanishi, *et al.*, reported formation of the cyclic imine derivative **4**, with a five-membered ring, from a similar reaction, and determined the structure by X-ray crystallography.[6] However, they did not provide experimental details.

We have investigated the reaction in the hope of obtaining precursors of the lactone **3**. Tlc of a reaction mixture obtained on treating pyridoxal with cyanide at pH 7.4 indicated the formation of a mixture consisting of at least five products; on heating to 50°,



4 **5**

crystals precipitated, and the product, obtained in 47% yield, had two extra carbon atoms, as indicated by its mass spectrum and by elemental analyses. Other physical methods and degradation experiments (reported in the present paper) did not provide definitive information concerning the structure of this unexpected product, and X-ray crystallography[7] finally identified the substance as the azachromone derivative **5** (see Experimental Section). The new compound was found to be stable in acid, giving a yellow solution, but was readily degraded in 1 *N* NaOH to pyridoxic acid and HCN. It was further characterized as a crystalline triacetyl derivative and as the hydrobromide salt.

While X-ray crystallographic study was in progress, we carried out mild degradation experiments on **5** to learn what we could about its structure. Mild acid hydrolysis gave a mixture of products. When **5** was dissolved in 0.1 *N* HCl and treated with "nitrous fumes" generated from HNO_3 and As_2O_3,[8] a crystalline product was formed immediately. This product was condensed with phenylenediamine, and the resulting product in turn was acetylated. The structures of the degradation product and this derivative could not be established unequivocally, but the experiments leading to the two compounds are described (see Experimental Section).

Reaction of pyridoxal with cyanide in a potassium acetate buffer at pH 6 gave a product in 79% yield, and the composition of the product corresponded to that of the "normal" cyanohydrin **2**. Since the uv spectra of the new compound in acidic and neutral solutions are almost identical, we can assume that the phenolic hydroxyl is substituted. This assumption alone makes the structure **4** very probable, particularly since it is reasonable to postulate an intramolecular addition of the phenolic hydroxyl to the cyano group to form the five-membered ring. The compound is most likely the one described by Takanishi, *et al.*,[6] although the details reported by Takanishi's group are insufficient to establish this conclusion beyond any doubt. A structure of this type has been postulated as an intermediate in the interaction of salicylaldehyde with cyanide.[9] The structure of **4** has also been con-

(1) Chemistry and Biology of Vitamin B_6. 34. Previous paper in this series: W. Korytnyk, S. C. Srivastava, N. Angelino, P. G. G. Potti, and B. Paul, *J. Med. Chem.*, **16**, 1096 (1973).

(2) E. E. Snell in "Comprehensive Biochemistry," Vol. 11, M. Florkin and E. H. Stotz, Ed., Elsevier, Amsterdam, 1963, p 48.

(3) V. Bonavita, *Arch. Biochem. Biophys.*, **88**, 366 (1960).

(4) (a) C. A. Storvick and J. M. Peters, *Vitam. Horm. (New York)*, **22**, 833 (1964); (b) C. A. Storvick, E. M. Benson, M. A. Edwards, and M. J. Woodring, *Methods Biochem. Anal.*, **12**, 183 (1964); (c) Z. Tamura and S. Takanishi, *Methods Enzymol.*, **18A**, 471 (1970); (d) K. M. Grigor, D. von Redlich, and D. Glick, *Anal. Biochem.*, **50**, 28 (1972).

(5) S. Oishi and S. Fukui, *Arch. Biochem. Biophys.*, **128**, 606 (1968).

(6) S. Takanishi, Z. Tamura, A. Yoshino, and Y. Iidaka, *Chem. Pharm. Bull.*, **16**, 758 (1968).

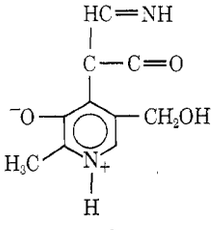
(7) G. Kartha, H. Ahrens, and W. Korytnyk, Abstracts, 23rd International Congress of Pure and Applied Chemistry, Boston, Mass., 1971, pp 134, 135.

(8) R. Möhlau, *Chem. Ber.*, **15**, 2472 (1882).

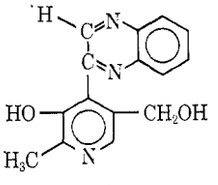
(9) K. Ladenburg, K. Folkers, and R. T. Major, *J. Amer. Chem. Soc.*, **58**, 1292 (1936).

firmed by the nmr and mass spectra. 5-Deoxypyridoxal also reacted with cyanide in a similar manner at pH 6, indicating that the 5′-OH group is not involved in the reaction. The compound **4** was found to be a powerful reducing agent, as indicated by immediate precipitation of silver on treatment with Tollens' reagent. It proved to be very unstable, decomposing in either acid or alkali to pyridoxic acid, even in chromatography on silica gel. Surprisingly, when a slightly alkaline solution of **4** was heated at 50° for 30 min, it was converted into **5** in about 50% yield.

These somewhat unexpected reactions can be rationalized by the assumption that the cyanohydrin **2** is too reactive to be capable of independent existence, and at pH 6 is converted into the cyclic imine **4**, with a five-membered ring. It is apparent that the formation of **5** from pyridoxal and cyanide involves two consecutive additions, since **5** has two extra carbon atoms. The formation of **5** from **4** probably involves a disproportionation reaction: part of **4** is degraded to pyridoxic acid, liberating CN^-, which in turn adds to an appropriate intermediate derived from **4**, forming **5**. Reaction of pyridoxal with CN^- at pH 7.4 was followed by tlc. Initially, an intermediate was detected as the main spot, but could not be isolated, presumably because of its instability. Since it was expected to have the α-ketoimine structure **6** (existing probably in the hemiketal form), the reaction mixture was treated with phenylenediamine, when the quinoxaline **7** precipi-



6

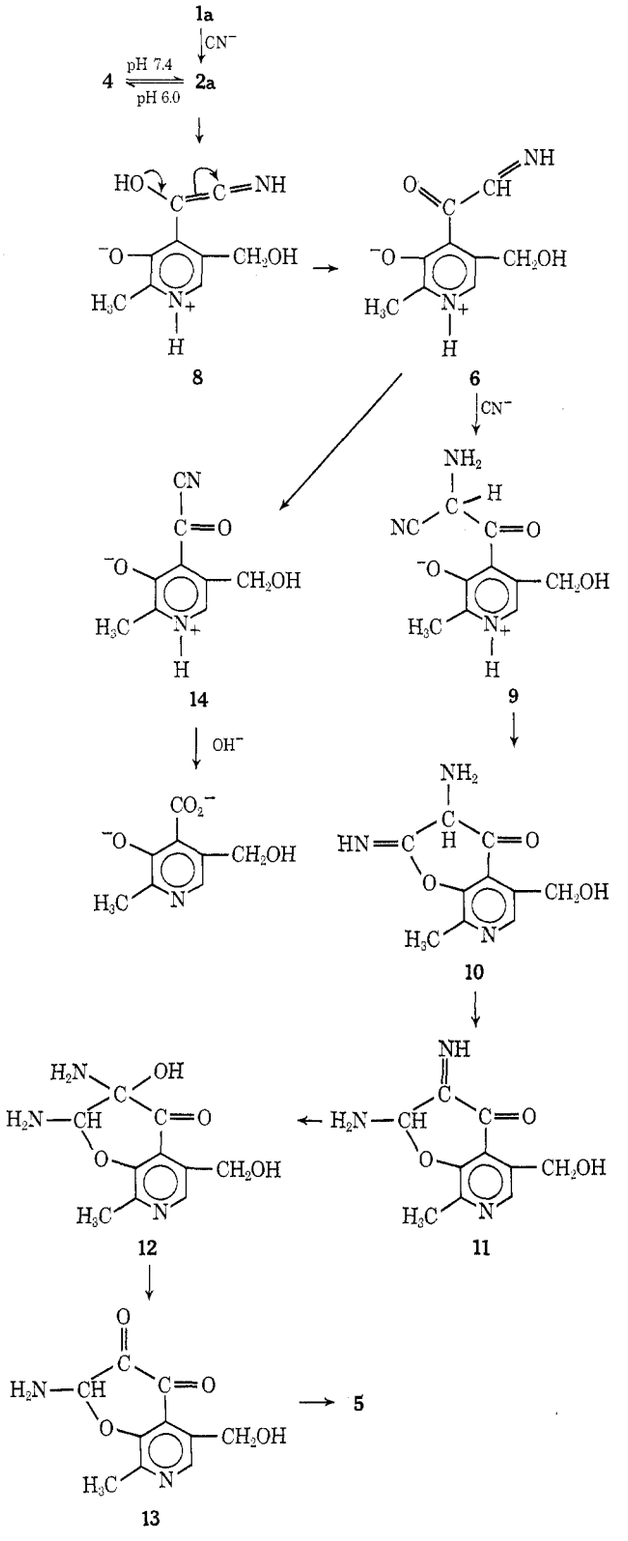


7

tated in 51% yield. The structure of **7** was proved by nmr spectroscopy and reaction of the phenolic OH with *p*-nitrobenzenesulfonyl chloride.

Formation of **6** from the initial cyanohydrin **2a** can be envisaged as occurring *via* the tautomeric ketoimine **8**,[10] which then undergoes an internal oxidation–reduction reaction to **6** (Scheme I). The imine **6** next reacts with CN^-, forming the α-aminonitrile **9**,[11] which then reacts intramolecularly with the phenolic hydroxyl, giving the amino imino intermediate **10**. Subsequently the latter tautomerizes to **11**, which adds H_2O to its imino group, giving **12**, which finally eliminates NH_3, giving **13**, a tautomer of **5**.[12] Conversion of **4** into **5** and pyridoxic acid can be assumed to proceed also by way of the cyanohydrin **2a**, forming the α-ketoimine **8**. It seems probable that the latter is readily oxidized by air to the acyl cyanide **14**, which hydrolyzes under mild alkaline conditions to pyridoxic acid and CN^-. While this process is under way, the liberated CN^- could at-

SCHEME I



tack the remaining **6**, forming **5** by the mechanism already discussed.

The suggested mechanism should be considered a rationalization based on analogies found in the literature and on observed facts. Although other interpretations are admissible, we would like to point out the participation of the nucleophilic phenolic hydroxyl group, in preference to the less nucleophilic 5′-hydroxyl group, as an interesting feature of these reactions.

(10) D. J. Cram and L. Gosser, *J. Amer. Chem. Soc.*, **86**, 2950 (1964), have postulated this type of ketenimine tautomer in the racemization of a nitrile by the so-called "conducted tour mechanism."

(11) There are a number of examples of additions of CN^- to imines giving aminonitriles: *e.g.*, G. E. P. Smith and F. W. Bergstrom, *J. Amer. Chem. Soc.*, **56**, 2095 (1934).

(12) Hydrolysis of a similar type of imines appears to be important in the Kiliani–Fischer cyanohydrin synthesis: R. Varma and D. F. French, *Carbohyd. Res.*, **25**, 71 (1972).

The latter group has been found to be important for the formation of the hemiacetal and for other reactions,[13] but apparently does not participate in any important manner in the reactions discussed here.

In connection with the "cyanohydrin reaction" as developed originally by Bonavita, it is significant that both **4** and **5** give rise to pyridoxic acid, the end product. This result suggests that a number of intermediates produced in the initial stages of the reaction, as indicated by our tlc studies, may be degraded to pyridoxic acid.[14] The synthesis of both **4** and **5** in a single step from the readily available pyridoxal provides readily available intermediates for synthesizing some unusual vitamin B_6 analogs.

Experimental Section

Ir spectra were determined with a Perkin-Elmer 457 spectrometer, uv spectra with a Perkin-Elmer 202 instrument, and nmr spectra with a Varian A-60A instrument. Some mass spectra were determined by Dr. D. C. DeJongh, of Wayne State University, with an Atlas CH4 mass spectrometer, the ionizing potential being 70 eV and the ionizing current 19 μA; others were determined at Roswell Park with a CEC 21-491 mass spectrometer under similar conditions. Tlc (silica gel) was used routinely, as described earlier.[15]

Reaction of Pyridoxal with KCN at pH 7.4. 2-Amino-3-hydroxy-5-(hydroxymethyl)-8-methyl-7-azachromone (5).—To a well-stirred solution of pyridoxal hydrochloride (1.05 g) in water (5 ml) a solution of potassium cyanide (650 mg) in water (5 ml) was added, the pH being carefully followed with a pH meter. Immediately after the addition of cyanide, the pH of the solution was adjusted to 7.4 with 6 *N* HCl and was kept at pH 7.8–7.4 by continued addition of 2 *N* HCl. After 10 min at room temperature, flurries of solid material began to appear. Tlc of the reaction mixture (1:1 MeOH–$CHCl_3$) gave six Gibbs-positive spots. The main spot (R_f 0.65) coincided with some unreacted pyridoxal, as indicated by a positive phenylhydrazine test. The reaction mixture was heated to 45–55° for 40 min to complete the formation of the crystalline precipitate, cooled in ice for 2 hr, and filtered; the precipitate was washed with ice–water. The yield was 530 mg (46%): mp 270–280°; R_f 0–0.1 in 1:1 MeOH–$CHCl_3$, and 0.38 in 0.1 *N* HCl. The Gibbs test gave a strong violet spot. The solution of the compound is strongly yellow at acid pH, but colorless at neutral or alkaline pH. In 1 *N* NaOH, the compound is rapidly converted into 4-pyridoxic acid: nmr (DMSO-d_6) 156 (CH_3), 296 (CH_2OH), 500 (pyridine α-H), 463 (broad singlet, OH), 300 (broad, OH); nmr (1 *N* DCl) 173 (CH_3), 314 (CH_2OH), 512 (α-pyridine H); ir 3400–2600 (very broad peak), 1650 cm^{-1} (C=O stretching); uv $\lambda_{max}^{0.1\ N\ HCl}$ 266 nm (ϵ 14,200), 404 (750), shoulder at 310; in phosphate buffer (pH 7.0), there is a change in spectrum, λ_{max} 252 and 318 nm being recorded after 45 min.

Anal. Calcd for $C_{10}H_{10}N_2O_4$: C, 54.06; H, 4.53; N, 12.61. Found: C, 53.91; H, 4.58; N, 12.66.

X-Ray Determination of the Structure of 5.—One very fine elongated yellow crystal, obtained from a dilute aqueous phosphoric acid solution, was used in the X-ray crystallographic determination of the structure of **5**. The crystal had an orthorhombic unit cell, with parameters a = 18.36, b = 7.12, c = 17.34 Å, and Z = 8. The three-dimensional X-ray diffraction data were collected to a Bragg angle of 75° on a General Electric automatic diffractometer, using Cu K_α radiation and the stationary crystal, stationary counter method. Of the 2688 reflections measured, 2154 (about 80%) were considered observed. Systematic absences indicated P_{bcn} as the most probable space group.

The chemical formula and the structure of the molecule being uncertain at the beginning of the investigation, initial attempts to establish the crystal structure by interpretation of the Patterson map on the basis of known molecular features were unsuccessful. Finally, the correct solution was obtained by utilizing the multisolution tangent refinement techniques that required minimal prior knowledge concerning the structure.[16] Using 151 reflections of E greater than 1.75, 32 phase sets were computed, and the E map with the most consistent set was calculated. This map revealed probable atomic positions for 14 atoms belonging to a planar molecule. Iterative cycles of calculation of structural factors, assigning carbon scattering factors to all atoms, followed by least-squares refinement and calculations of electron density, revealed two more atoms belonging to the molecule. In addition, two water molecules were located in the asymmetric unit of the cell. Detailed inspection of the intramolecular bond lengths and angles, as well as the peak heights in the electron density maps, enabled the correct atomic types (C, N, and O) to be established. Further least-squares refinement of the parameters, followed by difference electron density maps, gave the locations of all of the hydrogen atoms in the molecule. The reliability index R at this stage of refinement is 0.088, and the fractional atomic coordinates are given in Table I. The estimated standard deviations in the atomic positions

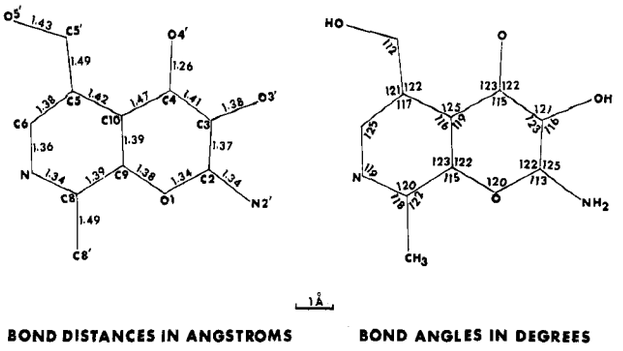


Figure 1.—2-Amino-3-hydroxy-5-(hydroxymethyl)-8-methyl-7-azachromone.

TABLE I

FRACTIONAL COORDINATES OF NONHYDROGEN ATOMS

Atom	X/a	Y/b	Z/c	ESD in position, Å
O_1	0.3113	0.0745	0.3952	0.003
$O_{4'}$	0.3141	0.2203	0.6236	0.003
$O_{5'}$	0.0869	0.2391	0.6450	0.004
$O_{3'}$	0.4437	0.1719	0.5412	0.003
W_1	0.9872	0.0910	0.3356	0.004
W_2	0.4169	0.0598	0.7301	0.004
N_7	0.1184	0.0964	0.4192	0.004
$N_{2'}$	0.4324	0.0727	0.3887	0.004
C_8	0.1843	0.0750	0.3878	0.004
C_9	0.2462	0.1002	0.4332	0.004
C_{10}	0.2435	0.1505	0.5104	0.004
C_5	0.1728	0.1696	0.5430	0.004
C_6	0.1137	0.1440	0.4947	0.004
$C_{5'}$	0.1620	0.2156	0.6263	0.004
$C_{8'}$	0.1884	0.0190	0.3049	0.005
C_4	0.3120	0.1769	0.5530	0.004
C_3	0.3757	0.1530	0.5089	0.004
C_2	0.3741	0.1010	0.4331	0.004

are also given in Table I. The thermal parameters for these atoms are given in Table II.

The analysis shows that the molecule is 2-amino-3-hydroxy-5-(hydroxymethyl)-8-methyl-7-azachromone. It is essentially a planar molecule, with none of the 16 nonhydrogen atoms being away from the least-squares plane by more than 0.03 Å. The intramolecular bond lengths and angles are shown in Figure 1. The C–O bonds obtained correspond to the value usually found in aromatic rings. The C_3–C_4 bond shows appreciably more sp^2 character, both from consideration of bond length and bond angles compared to the C_4–C_{10} bond. Bond length C_4=$O_{4'}$ is

(13) W. Korytnyk and H. Ahrens, *J. Med. Chem.*, **14**, 947 (1971).

(14) Certain aliphatic cyanohydrins have been found to be degraded to carboxylic acids by an intramolecular oxidation–reduction reaction; *cf.* V. Franzen and L. Fikentscher, *Justus Liebigs Ann. Chem.*, **623**, 68 (1959).

(15) H. Ahrens and W. Korytnyk, *Methods Enzymol.* **18A**, 489 (1970).

(16) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. B*, **26**, 276 (1970).

TABLE II

THERMAL PARAMETERS OF ATOMS[a]

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O_1	0.0207	0.0506	0.0249	0.0003	0.0000	−0.0010
$O_{4'}$	0.0308	0.0569	0.0254	0.0013	−0.0004	−0.0024
$O_{5'}$	0.0257	0.0605	0.0489	−0.0013	0.0014	0.0049
$O_{3'}$	0.0203	0.0689	0.0370	0.0011	−0.0006	−0.0041
W_1	0.0276	0.0649	0.0539	0.0008	−0.0008	−0.0013
W_2	0.0576	0.0526	0.0402	−0.0023	−0.0003	−0.0003
N_7	0.0247	0.0440	0.0328	0.0002	−0.0005	0.0000
$N_{2'}$	0.0245	0.0730	0.0338	0.0005	0.0006	−0.0029
C_8	0.0244	0.0362	0.0328	0.0000	−0.0004	−0.0006
C_9	0.0221	0.0315	0.0274	0.0007	0.0001	0.0005
C_{10}	0.0237	0.0278	0.0266	0.0001	0.0001	0.0008
C_5	0.0230	0.0288	0.0314	0.0000	0.0003	0.0007
C_6	0.0243	0.0412	0.0366	0.0004	−0.0003	−0.0011
$C_{5'}$	0.0245	0.0451	0.0364	−0.0001	0.0007	−0.0013
$C_{3'}$	0.0358	0.0594	0.0344	−0.0003	−0.0004	−0.0013
C_4	0.0237	0.0346	0.0278	0.0009	−0.0002	−0.0003
C_3	0.0214	0.0432	0.0279	0.0004	−0.0005	−0.0004
C_2	0.0205	0.0397	0.0312	0.0005	0.0003	−0.0008

[a] $T = \exp[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)]$.

the zwitterion type, presumably due to the hydrogen bonding between $O_{4'}$ and the water molecule. In addition to the intermolecular hydrogen bonding through the water molecules there is also a weak intramolecular hydrogen bond between $N_{2'}$ and $O_{3'}$.

The eight molecules in the unit cell are stacked together in the crystal in a parallel fashion in two layers at a stacking distance of 3.48 Å. A view of the crystal packing viewed down the b axis is shown in Figure 2.[17] The crystal is stabilized by many hydrogen bonds involving water molecules. Table III shows the relevant lengths and angles involving the intermolecular hydrogen bonds.

TABLE III

H BONDING DISTANCES (Å) AND BOND ANGLES (DEGREE) IN 2-AMINO-3-HYDROXY-5-(HYDROXYMETHYL)-8-METHYL-7-AZACHROMONE

X—H···Y	X—H	H···Y	X—Y	∠X-H-Y	∠H-X-Y
O_{w1}—H···N_7	0.99	2.20	2.81	145	23
$O_{3'}$—H···O_{w1}	1.00	1.90	2.84	148	21
$O_{5'}$—H···O_{w1}	1.08	1.67	2.74	176	3
O_{w2}—H···$O_{5'}$	1.00	1.74	2.72	170	7
O_{w2}—H···$O_{4'}$	0.99	1.99	2.88	142	25
$N_{2'}$—H···O_{w2}	0.99	1.94	2.91	163	7

Hydrobromide of 5.—Compound **5** (83 mg, free base) was added in small portions to an aqueous HBr solution (0.5 ml of 48% HBr and 0.5 ml of water) and the reaction mixture was kept at 4° for 1 hr. The resulting brown crystals were filtered, washed with ether, and dried, yielding 38 mg of material. Addition of ether precipitated an additional 37 mg of the hydrobromide. The combined fractions were dissolved in boiling ethanol (150 ml), and the solution was filtered. The filtrate was evaporated to 50 ml, when the compound crystallized. It decomposed over the range 240–245°. It gave the same tlc spot and uv spectrum (in 0.1 *N* HCl) as did the free base **5** and ir λ_{max}^{KBr} 3420, 2650 (broad, OH), 1650, 1620, 1590 (broad bands C=O).

Anal. Calcd for $C_{10}H_{11}N_2O_4Br$: C, 39.64; H, 3.66; N, 9.25; Br, 26.04. Found: C, 39.50; H, 3.66; N, 8.96; Br, 26.37.

Acetylation of 5.—Compound **5** (170 mg) was stirred with acetic anhydride (5 ml) in pyridine (dry, 7 ml) for 24 hr. In addition to the triacetyl derivative of **5** (R_f 0.30 in EtOAc), a spot of high R_f, 0.77, was formed; both spots were Gibbs positive after spraying with HCl and heating, but the triacetyl derivative appeared to predominate. After evaporation, the resulting oil was kept at 0.1 Torr for 2 hr to remove most of the acetylation reagents. The oil was then dissolved in EtOAc (1.0 ml), and 0.1 ml of the solution was subjected to preparative tlc. The two zones (R_f 0.15–0.35 and 0.40–0.65) were scraped off, and the scrapings were eluted with EtOAc. Both eluates show the same spot (R_f 0.30), indicating that the material of higher R_f has been converted into the triacetate (R_f 0.30). From these eluates, 14.4 mg of crystalline material, mp 179°, was isolated. The rest of the EtOAc solution was crystallized on the addition of petroleum ether. A total of 176.4 mg (66%) of crystalline triacetate was obtained (several recrystallizations from mixtures of chloroform with petroleum ether and of THF with petroleum ether gave the raised mp 180°); ir λ_{max}^{KBr} 1781, 1739, 1688, 1660, 1620 (split), 1590, 1554 (C=O), 3190 and 2325 cm^{-1} (broad, NH and C–H, respectively); uv λ_{max}^{EtOH} 233 mμ (ε 2.14 × 10^4), 247 (sh, 1.28 × 10^4), 316 (1.38 × 10^4); $\lambda_{max}^{0.1\ N\ HCl}$ 234 mμ (ε 1.94 × 10^4), 265 (sh, 6.7 × 10^3), 327 (9.7 × 10^3); nmr ($CDCl_3$), 131 and 141.5 (acetyl CH_3), 166 (8-CH_3), 347 (5-CH_2OH), 481 (NH, exchanged with D_2O), 510 (C_6H); mass spectrum 348 (molecular ion).

Anal. Calcd for $C_{16}H_{16}N_2O_7$: C, 55.17; H, 4.63; N, 8.04. Found: C, 55.34; H, 4.63; N, 8.30.

Degradation of 5. A. Treatment with 0.1 *N* HCl.—The hydrobromide of **5** was dissolved in 0.1 *N* HCl, and the solution was left standing at room temperature for 24 hr. The peak at 404 nm disappeared, and new peaks at 258 and 357 nm appeared. Tlc (1:1 MeOH–$CHCl_3$) gave three spots of low R_f. A well-defined product could not be isolated.

B. Treatment of 5 with "Nitrous Fumes" in 0.1 *N* HCl.—A stream of "nitrous fumes," generated from nitric acid and As_2O_3,[8] was passed through a solution of **5** (100 mg) in ice-cold 0.1 *N* HCl (4 ml). After a few minutes, tlc showed essentially one spot (R_f 0.4 in 1:1 MeOH–$CHCl_3$). After evaporation *in vacuo*, water was added, and the new compound crystallized. It was recrystallized from hot DMF and decomposed above 140°: uv $\lambda_{max}^{0.1\ N\ HCl}$ 292 and 340 nm (broad, weak band); ir λ_{max}^{KBr} 3455, 3338, 2150, 710 cm^{-1} (C=O); molecular ion *m/e* 238.

Anal. Calcd for $C_{10}H_{10}O_3N_2$: C, 50.41; H, 4.23; N, 11.76. Found: C, 50.72; H, 4.57; N, 11.69.

Condensation of the "Nitrous Acid" Degradation Product with *o*-Phenylenediamine.—To *o*-phenylenediamine (370 mg) in 10% acetic acid (2.5 ml) a solution of the preceding degradation product (110 mg) was gradually added, with stirring. After 3 hr, the resulting precipitate was filtered and washed with water, acetone, and ether; it was recrystallized twice from hot DMF: yield 55%; mp 293–300° dec; ir λ_{max}^{KBr} 1770 (C=O), 3200 cm^{-1} (broad); molecular ion peak at 295.

Anal. Calcd for $C_{16}H_{13}N_3O_3$: C, 65.15; H, 4.44; N, 14.24. Found: C, 65.12; H, 4.19; N, 14.39.

Acetylation of Phenylenediamine Condensation Product.—The phenylenediamine condensation product (86 mg) was heated with a mixture of pyridine (3 ml) and acetic anhydride (3 ml). Then the reagents were evaporated, and the residue was dissolved in $CHCl_3$. After being washed with H_2O, the $CHCl_3$ solution was dried ($MgSO_4$) and was evaporated to an oil. The oil was crystallized from hot dimethylformamide (DMF): mp 248–253°; ir λ_{max}^{KBr} 1742 and 1765 cm^{-1} (C=O).

(17) See paragraph at end of paper regarding supplementary material.

Anal. Calcd for $C_{18}H_{15}N_3O_4$: C, 64.15; H, 4.49; N, 12.47. Found: C, 63.93; H, 4.24; N, 12.54.

Reaction of Pyridoxal with KCN at pH 6.0. 2,3*H*-2-Imino-3-hydroxy-4-(hydroxymethyl)furo[2,3-*c*]pyridine (4).—To a solution of KOAc (1.0 g) and KCN (1.0 g) in water (10 ml), glacial acetic acid was added until pH 7.0 was reached (*ca.* 0.9 ml). Now pyridoxal HCl (0.5 g) was added to the solution, lowering the pH to about 6.0. The solution became darker and started depositing crystals. After cooling for 2 hr in ice, the resulting acetate salt of **4** was filtered, washed with acetone and ether, and dried. The yield was 0.49 g (79%): mp 149° dec (the compound could not be recrystallized, since it readily decomposes to 4-pyridoxic acid lactone); nmr (DMSO-d_6) 114 (CH_3, ionic acetate), 147 (2-CH_3), 298 (4-CH_2CH), 476 (C_7 H); ir λ_{max}^{KBr} 1650 (C=N), 1750 cm^{-1} (ionic acetate); uv λ_{max}^{DMSO} 315 nm (ϵ 5700); $\lambda_{max}^{0.1\ N\ HCl}$ 249 nm (ϵ 8150), 352 (18,900); $\lambda_{max}^{pH\ 7.0}$ 251 nm (ϵ 6800), 357 (8350).

Anal. Calcd for $C_9H_{11}N_2O_3{}^+CH_3COO^-$: C, 51.98; H, 5.55; N, 11.08. Found: C, 52.25; H, 5.56; N, 11.08.

Picrate of 4.—With picric acid, **4** forms a violet picrate, mp 176° dec.

Anal. Calcd for $C_{15}H_{13}N_5O_{10}$: C, 42.55; H, 3.10; N, 16.54. Found: C, 42.84; H, 3.27; N, 16.80.

Hydrobromide of 4.—To a solution of **4** (acetate salt, 220 mg) in water (2.0 ml), 48% HBr (1.0 ml) was added. After a short time, brown crystals precipitated. The reaction mixture was kept in a refrigerator for 2 hr and filtered, and the precipitate was recrystallized from a mixture of ethanol and petroleum ether: mp 165–170° dec.

Anal. Calcd for $C_9H_{11}BrN_2O_3$: C, 39.30; H, 4.03; N, 10.19. Found: C, 39.41; H, 3.96; N, 10.50.

Quinoxaline Derivative (7).—Initially the reaction was carried out as for **5**, using the same amounts of reagents. After the addition of KCN (pH 7.4), about 5 min was allowed to elapse before *o*-phenylenediamine (0.56 g, 5.15 mmol) was added. Then the pH was lowered to 5 with 6 *N* HCl, and the reaction mixture was left standing in the cold for 4 hr. Since no precipitate formed, the pH was raised to 7.5 with NaOH solution, and the reaction mixture was left standing in a refrigerator overnight. The next day, 0.58 g of product had precipitated. The product was then washed with ethanol and ether. Additional material was isolated from the mother liquors, increasing the yield to 0.72 g (51%): mp 228–230° dec; tlc R_f 0.3 (9:1 $CHCl_3$–CH_3OH); the Gibbs test gives a green spot, which changes to purple on standing; nmr (DMSO-d_6) 151 (2-CH_3), 265.5 (5-CH_2OH), 469–492 (multiplet of unresolved quinoxaline phenyl protons and of C_6 H of pyridoxine), 534 (quinoxaline diazine C–H); uv λ_{max}^{EtOH} 215, 243, 323, 390 nm; $\lambda_{max}^{0.1\ N\ HCl}$ 211, 240, 260, 305–325 nm; $\lambda_{max}^{0.1\ N\ NaOH}$ 222, 239.5, 312, 386 nm.

Anal. Calcd for $C_{15}H_{13}N_3O_2\cdot 0.5H_2O$: C, 65.20; H, 5.10; N, 15.20. Found: C, 65.44; H, 5.07; N, 14.94.

The *p*-nitrobenzenesulfonyl derivative of the preceding compound was prepared by treating the compound (90 mg, 0.33 mmol) in ice-cold pyridine (4 ml) with *p*-nitrobenzenesulfonyl chloride (73 mg, 0.37 mmol). After the reaction mixture had been left standing overnight, pyridine was evaporated *in vacuo*, the residue was dissolved in chloroform, and the solution was washed with a bicarbonate solution and water. After drying (Na_2SO_4) and evaporating off the $CHCl_3$, the oil was dissolved in ethanol. The yield was 24 mg (30%): mp 186–188°; tlc (in ethyl acetate, R_f 0.5) gave one spot, which was Gibbs-negative, indicating substitution on the phenolic hydroxyl. When the tlc plate was sprayed with an NaOH solution and heated, the Gibbs test was positive, indicating hydrolysis of the sulfonate ester. The nmr spectrum is consistent with the structure.

Anal. Calcd for $C_{21}H_{16}N_4O_6S$: C, 55.74; H, 3.56; N, 12.38. Found: C, 56.60; H, 3.70; N, 12.17.

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Registry No.—**1a**, 66-72-8; **4**, 41203-44-5; **4** picrate, 41203-45-6; **4** acetate salt, 41203-46-7; **4** HBr, 19839-38-4; **5**, 41203-47-8; **5** HBr, 41203-48-9; **5** triacetate, 41203-49-0; **7**, 41203-50-3; **7**, *p*-nitrobenzenesulfonyl derivative, 41296-58-6; KCN, 151-50-8; *p*-nitrobenzenesulfonyl chloride, 98-74-8.

Supplementary Material Available.—Figure 2 and a table of structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20 × reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JOC-73-3793.

6α- and 6β-Hydroxyestradiol. Circular Dichroism and Substantiation of Configurational Assignments

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Recently we reported[2] the stereospecific syntheses of 6α- and 6β-hydroxyestriol[3] (**1a** and **2a**) and made configurational assignments by a comparison of the C-6 pmr signals of the respective tetraacetates (**1b** and **2b**). A broad, poorly defined triplet (spacing 8 Hz) was assigned to the pseudoaxial proton at C-6 in **1b**, while a narrow doublet (spacing 2.5 Hz) was assigned to the pseudoequatorial proton at C-6 in **2b**. The CD spectra of **1a** and **2a** show a strong and configurationally specific effect of the 6-hydroxyl substituent on the ^1L_b and ^1L_a dichroic absorption bands associated with the aromatic chromophore. While estradiol[4] and estriol each display ^1L_b and ^1L_a dichroic absorption of opposite sign (negative and positive, respectively), both CD maxima are negative for **1a** ($[\theta]_{283}^{EtOH}$ −1700 and $[\theta]_{229}^{EtOH}$ −5200) and positive for **2a** ($[\theta]_{280}^{EtOH}$ +620 and $[\theta]_{227}^{EtOH}$ +20,000). In the light of these data, a report[5] that the ORD spectrum of 6β-hydroxyestradiol (**2c**) shows a weak negative Cotton effect centered at 272 nm and a strong positive Cotton effect centered at 221 nm is puzzling. Since the C-16 hydroxyl group is so remote as to have little effect on the CD absorption within either the ^1L_b or the ^1L_a band,[6] one would predict that **2c** should display two positive Cotton effects, in strict analogy to **2a**.

The synthesis of the two epimeric 6-hydroxyestradiols, mp 239–249 and 191–195°, was reported by Wintersteiner and Moore in 1959,[7] and the higher melting triol was tentatively assigned the thermodynamically more stable 6α configuration on the basis of its method of preparation. It was noted, however, that the observed order of elution of the two triacetates **1d** and **2d** from alumina was opposite to that expected

(1) Supported by NIH Grant HD-05797.
(2) E. P. Burrows, D. L. Di Pietro, and H. E. Smith, *J. Org. Chem.*, **37**, 4000 (1972).
(3) Estriol is the trivial name for 1,3,5(10)-estratriene-3,16α,17β-triol.
(4) Estradiol is the trivial name for 1,3,5(10)-estratriene-3,17β-diol.
(5) P. Crabbé and W. Klyne, *Tetrahedron*, **23**, 3449 (1967).
(6) G. Snatzke and P. C. Ho, *Tetrahedron*, **27**, 3645 (1971).
(7) O. Wintersteiner and M. Moore, *J. Amer. Chem. Soc.*, **81**, 442 (1959).