Experimental

All mass spectra were determined with a CEC mass spectrometer, Model 21–103 C, using a recently described[16] inlet system for the direct insertion of samples near the ion source. The ionization energy was maintained at 70 e.v. and the ionization current at 50 μa.

(16) J. F. Lynch, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *Experientia*, **19**, 211 (1963).

After the preparation of this manuscript, a communication appeared by Martell, *et al.*,[17] in which it is mentioned briefly that attempted measurement of the mass spectra of the aporphines bulbocapnine (III) and glaucine, using a heated metal inlet system, resulted in dehydrogenation to afford fully aromatic structures. Our results thus represent another instance where the superiority of the direct inlet procedure is demonstrated.

(17) M. J. Martell, T. O. Soine, and L. B. Kier, *J. Am. Chem. Soc.*, **85**, 1022 (1963).

[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF., AND VARIAN ASSOCIATES, PALO ALTO, CALIF.]

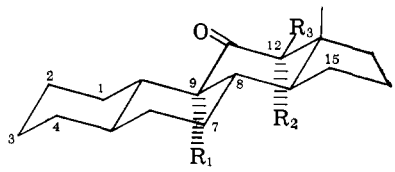
Unusual Chemical Shifts in the Nuclear Magnetic Resonance Spectra of 7- and 11-Keto Steroids

BY D. H. WILLIAMS, N. S. BHACCA, AND CARL DJERASSI

RECEIVED MAY 13, 1963

By a study of the n.m.r. spectra of 5α-androstan-11-one (Ia) and several deuterated analogs it has been established that the carbonyl group induces a considerable diamagnetic shift on the equatorial proton at C-1. A similar effect is observed in the deshielding of the equatorial proton at C-15 in a 7-keto steroid.

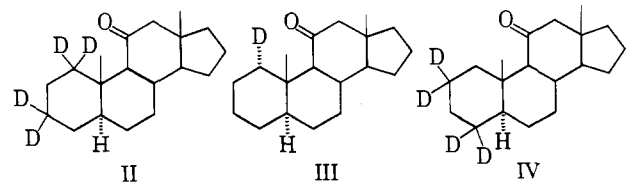
During the course of our investigation of the mass spectrometric fragmentation of 5α-androstan-11-one (Ia) *via* a study of its deuterated analogs,[1] we established that two enolizable hydrogens could readily be replaced by deuterium on treatment of the 11-ketone with base to give 9α,12α-d_2-5α-androstan-11-one (Ib), while a third deuterium atom could only be introduced with difficulty to give 9α,12,12-d_3-5α-androstan-11-one (Ic). The 9α,12α-d_2-structure was assigned to Ib on the basis of the known preference for axial as opposed to equatorial ketonization of enols[2] and mass spectrometric evidence.[1] In order to confirm our assignment we obtained the 100-Mc. n.m.r. spectra of Ia, Ib, and Ic, since the 60-Mc. spectra were not clearly resolved in the relevant downfield region. The spectrum (Fig. 1) of Ia showed downfield signals representing three protons, the tall peak at δ = 2.27 p.p.m. corresponding to two protons and a pair of smeared triplets at 2.45 p.p.m. accounting for the other proton. Initially they were assigned, very reasonably, to the protons at C–12 and C–9, respectively. The large splitting in the resonance pattern was assumed to arise



Ia, $R_1 = R_2 = R_3 = H$
b, $R_1 = R_2 = D, R_3 = H$
c, $R_1 = R_2 = R_3 = D$
d, $R_1 = R_2 = H, R_3 = D$
e, $R_1 = D, R_2 = R_3 = H$

via coupling of the C–9 proton with the single axial hydrogen at C-8 and the small splitting attributed to the hydrogens at C-7. However, very surprisingly, the spectrum (Fig. 2) of the 9α,12α-d_2-derivative Ib showed signals due to two downfield protons, the singlet at δ = 2.27 p.p.m. now containing one proton and the resonance at δ = 2.45 p.p.m. remaining unchanged. In the spectrum (Fig. 3) of the 9α,12,12-d_3-analog Ic the peak at δ = 2.27 p.p.m. had completely disappeared, but the signals at δ = 2.45 p.p.m. remained. Consistent with the assignment of the two-proton signal as being due to the protons at C-12, the singlet was reduced to half intensity in the spectrum (Fig. 4) of the 12β-d_1-ketone Id. *It must therefore be concluded that some proton in the molecule which is not adjacent to the carbonyl function must be giving rise to the most downfield signals at δ = 2.45 p.p.m., i.e.*, some proton is being deshielded much more than would normally be anticipated, and furthermore that the resonance due to the proton at C-9 does not occur in the expected downfield region. These deductions were confirmed by the spectrum (Fig. 5) of 9α-d_1-5α-androstan-11-one (Ie), which still showed three downfield protons.

In an effort to rationalize the above observations, we obtained the spectra of several other deuterated 5α-androstan-11-ones.[1] The spectrum of 8β-d_1-5α-androstan-11-one still exhibited the same downfield pattern as the undeuterated steroid, thus excluding the C-8 proton as giving rise to the resonance at δ = 2.45 p.p.m., despite the fact that it lies roughly in the plane of the carbonyl group and could therefore theoretically be deshielded. However, in the spectrum (Fig. 6) of 1,1,3,3-d_4-5α-androstan-11-one (II), the signals at δ = 2.45 p.p.m. did not occur. This result strongly suggested that the proton whose resonance occurred at δ = 2.45 p.p.m. was that oriented equatorially at C-1, in view of its proximity to the carbonyl function. That this was indeed the case was confirmed by the spectrum (Fig. 7) of 1α-d_1-5α-androstan-11-one (III)



which exhibited a broad single resonance at δ = 2.45 p.p.m.; the large coupling ($J \sim 12$ c.p.s.) in the spectrum (Fig. 1) of the parent ketone therefore arises due to geminal coupling of the two C-1 protons. The smeared triplet pattern (see Fig. 1) is due to further coupling with protons at C-2, a conclusion which is confirmed by the spectrum (Fig. 8) of 2,2,4,4-d_4-5α-androstan-11-one (IV). Now the resonance at δ = 2.45 p.p.m. appears as a broad doublet, since spin coupling with the C-2 protons has now been eradicated; the broadening of the doublet is due to the small coupling of the 1β-proton with deuterium nuclei at C-2. Even more interesting is the sharpening of the one proton

(1) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2091 (1963).

(2) E. J. Corey and R. A. Sneen, *ibid.*, **78**, 6269 (1956).

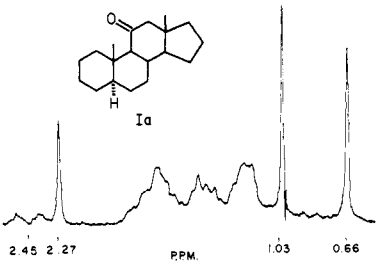


Fig. 1.—100-Mc. n.m.r. spectrum of 5α androstan-11-one (Ia).

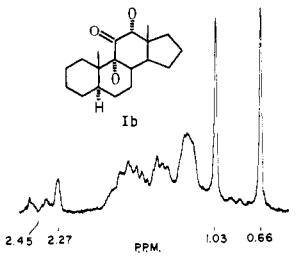


Fig. 2.—100-Mc. n.m.r. spectrum of 9α,12α-d_2-5α-androstan-11-one (Ib).

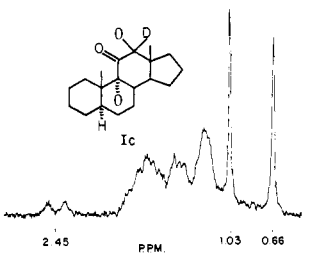


Fig. 3.—100-Mc. n.m.r. spectrum of 9α,12,12-d_3-5α-androstan-11-one (Ic).

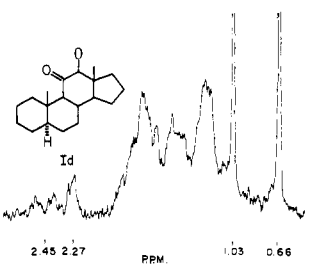


Fig. 4.—100 Mc. n.m.r. spectrum of 12β-d_1-5α-androstan-11-one (Id).

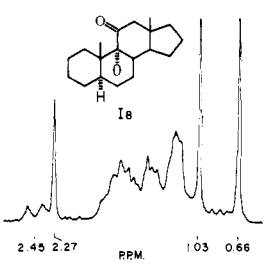


Fig. 5.—100-Mc. n.m.r. spectrum of 9α-d_1-5α-androstan-11-one (Ie).

resonance at δ = 0.78 p.p.m., which therefore is probably due to the C-1 axial proton. This was confirmed by performing a double resonance experiment[3]; double irradiation at the frequency of the C-1 equatorial proton caused the resonance at δ = 0.78 p.p.m. to become a singlet (Fig. 9). Incidentally, the 1α-d_1-11-ketone III, prepared *via* catalytic deuteration of Δ^1-5α-androsten-3,11-dione, is now established as being stereochemically pure; its freedom from the 1β-d_1-derivative was previously open to question.[1]

It has previously been concluded[4] that protons lying in conical regions extending above and below the plane of the trigonal carbon atom of a carbonyl group (V) will be shielded by this function, while those lying

(3) N. S. Bhacca, M. E. Wolff, and R. Kwok, *J. Am. Chem. Soc.*, **84**, 4976 (1962).

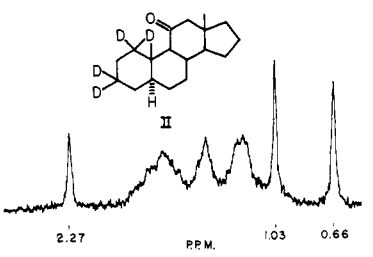


Fig. 6.—100-Mc. n.m.r. spectrum of 1,1,3,3-d_4-5α-androstan-11-one (II).

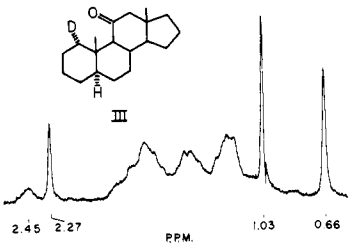


Fig. 7.—100-Mc. n.m.r. spectrum of 1α-d_1-5α-androstan-11-one (III).

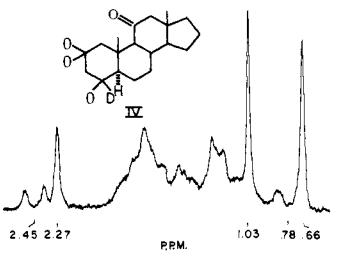


Fig. 8.—100-Mc. n.m.r. spectrum of 2,2,4,4-d_4-5α-androstan-11-one (IV).

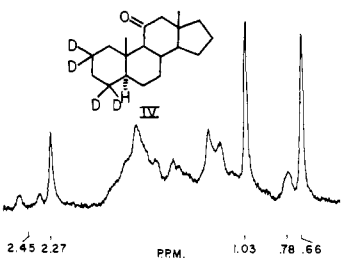


Fig. 9.—100-Mc. n.m.r. spectrum of 2,2,4,4-d_4-5α-androstan-11-one (IV); spin-decoupled by irradiation at 245 c.p.s.

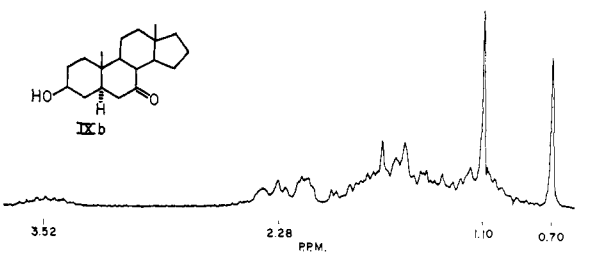


Fig. 10.—100-Mc. n.m.r. spectrum of 5α-androstan-3β-ol-7-one (IXb).

(4) L. M. Jackman, "Applications of N.M.R. Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, pp. 122–124.

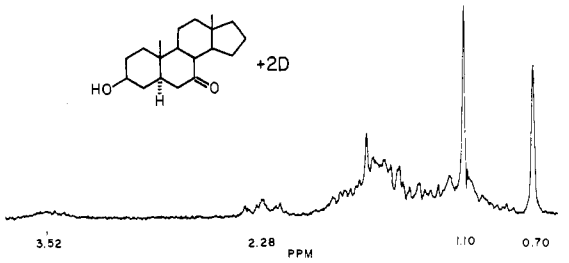


Fig. 11.—100-Mc. n.m.r. spectrum of d_2-5α-androstan-3β-ol-7-one.

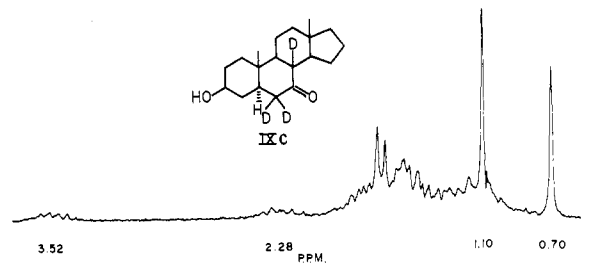
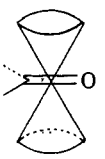
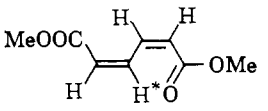


Fig. 12.—100-Mc. n.m.r. spectrum of 6,6,8β-d_3-5α-androstan-3β-ol-7-one (IXc).

elsewhere, and particularly those in the plane of the trigonal atom, will be deshielded. In support of this view, large paramagnetic shifts of γ-protons which are held in the plane of the carbonyl group have been observed[4,5] (*e.g.*, see H* in VI). The C-1 equatorial



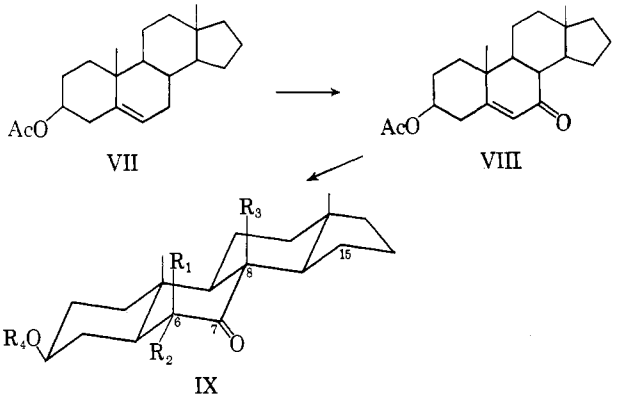
V



VI

proton is similarly a γ-proton held in the plane of the carbonyl group in 5α-androstan-11-one (Ia). The C-1 axial proton is abnormally shielded and therefore presumably lies in the shielded cone of the carbonyl group. The latter is a surprising result, as are indeed the findings that the axial and equatorial protons at C-12 have the same chemical shift and that the C-9 proton is shielded relative to those at C-12. Further experiments will be undertaken on other rigid six-membered ketones to try to rationalize these findings.

As a logical extension of the above observations, an analogous interaction was sought between the equatorial hydrogen atom at C-15 and a 7-keto group. (Further interactions will be studied in the less readily available 1- and-15-keto steroids.) Allylic oxidation of Δ^5-androsten-3β-ol acetate (VII) with sodium dichromate in acetic acid[6] gave Δ^5-androsten-7-one-3β-ol acetate (VIII), m.p. 179–180°, λ_{max} 234 mμ, ϵ 13,000, purer than material hitherto obtained (m.p. 173.5–174.5°, λ_{max} 234 mμ, ϵ 11,200[7]). Reduction of VIII with hydrogen over palladium–charcoal in cyclohexane solution gave 5α-androstan-7-one-3β-ol acetate (IXa), m.p. 130–132°, $[\alpha]_D$ −62°.[8] Hydrolysis of IXa with ethanolic



VII VIII IX

a, $R_1 = R_2 = R_3 = H$, R_4 = Ac
b, $R_1 = R_2 = R_3 = R_4 = H$
c, $R_1 = R_2 = R_3 = D$, $R_4 = H$

potassium hydroxide solution gave 5α-androstan-7-one-3β-ol (IXb).[7]

When the hydrolysis was performed in deuteriomethanol containing 30% deuterium oxide and dissolved sodium, there was obtained after 30 min. reflux, d_2-5α-androstan-7-one-3β-ol of 72% isotopic purity with smaller quantities of d_1- and d_3-contaminants. However, when the hydrolysis was followed by a long period of reflux (3 days), there was obtained 6,6,8β-d_3-5α-androstan-7-one-3β-ol (IXc) of 93% isotopic purity. The predominantly dideuterated ketol may theoretically be 6β,8β- or 6,6-d_2-5α-androstan-7-one-3β-ol. If the preference for axial over equatorial enolization of the Δ^6-enol is very great and the enolization to C-8 only slightly less facile than to C-6, then the 6β,8β-d_2-derivative would be obtained. However, if the preference for axial over equatorial enolization of the Δ^6-enol is not very great and enolization to C-8 considerably more difficult than to C-6, then the 6,6-d_2-derivative would result. Since the relative rates of these processes in aqueous methanol have not been established (Jones[9] has demonstrated the preference of enolization of a 7-ketone to C-6 and Corey[2] the preference for axial ketonization of the Δ^6-enol in some solvents), further experiments are being undertaken to establish the structure of the dideuterated ketol. However, the precise location of the two deuterium atoms need not be known for our present arguments.

The n.m.r. spectrum of the nondeuterated 7-ketone IXb (Fig. 10) showed a multiplet three-proton absorption in the region δ = 2.0–2.7 p.p.m. Consistent with expectations, the spectrum of d_2-5α-androstan-7-one-3β-ol (Fig. 11) showed a two-proton multiplet resonance around δ = 2.28 p.p.m., indicating that a proton which is not adjacent to the carbonyl function was absorbing in the downfield region. Confirmation of this state of affa rs was provided by the spectrum (Fig. 12) of the 6,6,8-d_3-ketone IXc which exhibited only a one-proton multiplet resonance around δ = 2.28 p.p.m., whose broad pattern was consistent with coupling with four nonequivalent protons, as would be the case for the equatorial hydrogen at C-15.

The present work emphasizes the caution which must be exercised in the interpretation of ketone spectra, in view of the pronounced chemical shifts which may be observed with hydrogens bearing special spatial relationships to the carbonyl group.

Experimental[10]

The preparation of all deuterated 5α-androstan-11-ones utilized in this paper has previously been reported.[1]

(5) L. M. Jackman and R. H. Wiley, *J. Chem. Soc.*, 2887 (1960).
(6) N. W. Atwater, *J. Am. Chem. Soc.*, **83**, 3071 (1961).
(7) R. D. H. Heard and A. F. McKay, *J. Biol. Chem.*, **165**, 677 (1946).
(8) This material has previously been described in the literature as having m.p. 110–113°,[6] but no other parameters were recorded. In view of the discrepancy between our melting point and the recorded value, we have fully characterized the compound (see Experimental).
(9) E. R. H. Jones and D. J. Wluka, *J. Chem. Soc.*, 907 (1959).
(10) Melting points are corrected and were determined in capillaries. Rotations were measured in chloroform and ultraviolet absorption spectra in 95% ethanol. The microanalyses were performed by Messrs. E. Meier and J. Consul (Stanford University, Microanalytical Laboratory). The n.m.r. spectra were obtained on a Varian HR-100 spectrometer. The samples were run as $CDCl_3$ solutions with a trace of tetramethylsilane added to act as internal reference.

Δ^5-Androsten-7-one-3β-ol Acetate (VIII).—Δ^5-Androsten-3β-ol acetate (VII, 10 g.) in acetic acid (80 ml.) and acetic anhydride (70 cc.) were warmed to 40° and sodium dichromate tetrahydrate (13.5 g.) was added portionwise during 5 min. The mixture was stirred until homogeneous and then kept at 40° for 2 days. The solution was then poured into water (300 cc.) and the crude crystalline product (6.4 g.) isolated by filtration and washed with water. An ethereal solution of this material was washed with 10% sodium bicarbonate solution and twice with water, dried, and evaporated. The residue was crystallized from methanol–methylene chloride giving VIII (2.92 g.), m.p. 179–180°, λ_{max} 234 mμ (ϵ 13,000).

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 76.32; H, 9.15. Found: C, 76.07; H, 9.06.

5α-Androstan-7-one-3β-ol Acetate (IXa).—A solution of VIII (2.0 g.) in cyclohexane (55 cc.) and 30% palladium-on-charcoal (500 mg.) was stirred under a hydrogen atmosphere for 1 hr., during which time one mole of hydrogen was absorbed. Filtration and evaporation of the filtrate gave a crystalline residue (1.97 g.) which was recrystallized from aqueous methanol giving IXa (1.56 g.), m.p. 130–132°, $[\alpha]D$ −62° (*c* 2.3), λ_{max}^{Nujol} 5.75 and 8.03 μ (acetate) and 5.85 μ (six-membered ketone).

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 75.86; H, 9.70. Found: C, 75.62; H, 9.72.

5α-Androstan-7-one-3β-ol (IXb).—A solution of IXa (112 mg.) and potassium hydroxide (200 mg.) in ethanol (5 cc.) was kept at room temperature for 3 hr. The product (100 mg.), isolated in the usual manner, was crystallized from acetone–hexane giving IXb (62 mg.), m.p. 128–129.5°.

d_2-5α-Androstan-7-one-3β-ol.—A solution of IXa (22 mg.) in deuteriomethanol (2.8 cc.) and deuterium oxide (1.2 cc.) containing dissolved sodium (50 mg.) was heated under reflux for 30 min. and then poured into ether (15 cc.). The ether phase was washed three times with water, dried, and evaporated, giving a crystalline residue (20 mg.), which was recrystallized from acetone–hexane giving 14.7 mg. of the deuterated ketone (d_1, 6%; d_2, 72%; d_3, 17%; d_4, 5%), m.p. 128–129°.

6,6,8β-d_3-5α-Androstan-7-one-3β-ol (IXc).—A solution of IXa (50 mg.) in deuteriomethanol (4 cc.) and deuterium oxide (2.9 cc.) containing dissolved sodium (100 mg.) was heated under reflux for 3 days and then poured into ether (10 cc.). The ether phase was washed with water, dried, and evaporated. Crystallization of the residue from ether–hexane gave IXc (42 mg.), m.p. 141–142.5° (d_2, 6%; d_3, 93%; d_4, 1%). This material showed the same behavior on thin-layer chromatography as material of melting point 128–129° and is merely another crystalline modification of the ketol.

Acknowledgment.—The work at Stanford University was supported in part by grant No. CRTY-5061 from the National Institutes of Health of the U. S. Public Health Service.

[CONTRIBUTION FROM THE DEPARTMENT OF EXPERIMENTAL THERAPEUTICS, ROSWELL PARK MEMORIAL INSTITUTE, BUFFALO 3, N. Y.]

Proton Magnetic Resonance Spectra of Compounds in the Vitamin B_6 Group[1]

BY W. KORYTNYK AND R. P. SINGH

RECEIVED APRIL 27, 1963

The p.m.r. spectra of compounds in the vitamin B_6 group have been determined in D_2O solution, and the proton peaks have been assigned on the basis of comparison with several analogs of pyridoxol. Considerable changes in p.m.r. spectra have been observed in acid, neutral, and alkaline solutions and have been rationalized on the basis of the electronic properties of the various ionic forms. A facile base-catalyzed deuterium exchange has been observed in pyridoxol derivatives in which the heterocyclic nitrogen is quaternized. The nature of the aldehyde group in pyridoxal and in pyridoxal phosphate has been elucidated.

Introduction

The vitamin B_6 group of compounds includes pyridoxol,[2] pyridoxal, pyridoxamine, and their phosphate derivatives. Ultraviolet spectroscopy has played an important role, not only as a tool in the initial structural studies, but also in elucidation of the biological functions of pyridoxal phosphate. Metzler and Snell[3] have made a detailed study of the ultraviolet spectra of the vitamin B_6 group, especially with respect to the ionic forms in solution and the nature of the aldehyde function in pyridoxal and pyridoxal phosphate.

Proton magnetic resonance spectroscopy has been extensively applied to molecules of biological interest[4] and has provided valuable information on such questions as tautomeric equilibria, hydrogen bonding, electron densities, conformation, and complex formation.

Electron distribution may be an important property in determining the catalyst or antimetabolite action of pyridoxol and its analogs, and Katritzky and Lagowski[5] have shown that proton resonance shifts in substituted pyridines could be correlated with the electron densities in different positions on the ring. In this connection it should be noted that Pullman and his co-workers[6] recently calculated the electronic properties of pyridoxal and some transition forms involved in reactions catalyzed by pyridoxal phosphate.

Thus a study of a series of closely related compounds of this type should provide an experimental indication of the electron densities in these derivatives. It was hoped to resolve at the same time some questions regarding the structures of the compounds. Our main purpose in undertaking this study, however, was to determine the value of p.m.r. spectroscopy as a tool in establishing the structures of synthetic analogs being prepared in our laboratory.[7] Heavy water was the solvent of choice because these compounds are generally soluble in water, and a study of their behavior in heavy water may contribute to our understanding of their biological functions.

Experimental

All spectra were obtained at 60 Mc., using a Varian A-60 instrument, which was calibrated by standard techniques.[8] The reproducibility was better than 1 c.p.s., and the accuracy was within 0.5 c.p.s. Most compounds used in this study were commercial products of highest purity. 3-Deoxypyridoxol was kindly provided by Dr. S. A. Harris of Merck and Co., Inc. Whenever possible, a compound was used in the form of a 10% solution in D_2O. A few compounds were not sufficiently soluble and hence were used in the form of saturated solutions. The exact concentration was not a critical factor in determining the positions of the peaks. The internal standard was 1,4-dioxane, as described by Jones, *et al.*[9] The pH was determined with a standard pH meter, and the true pD was computed by adding 0.40 to the pH reading.[10]

(1) Pyridoxine Chemistry IV; for preceding paper in this series, see ref. 7.

(2) This compound is generally known as pyridoxine. Nevertheless, according to the I.U.P.A.C. "Definitive Rules for Nomenclature of the Vitamins" [*J. Am. Chem. Soc.*, **82**, 5545 (1960)], the name *pyridoxine* has been extended to designate all naturally occurring pyridine derivatives with vitamin B_6 activity.

(3) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **77**, 2431 (1955).

(4) O. Jardetzky and C. D. Jardetzky, "Methods of Biochemical Analysis," Vol. IX, Interscience Publishers, Inc., New York, N. Y., 1962, p. 235.

(5) A. R. Katritzky and J. M. Lagowski, *J. Chem. Soc.*, 43 (1961).

(6) B. Pullman, C. Spanjaard, and C. Valdemoro, *Compt. rend.*, **248**, 2413 (1959); A.-M. Perault, B. Pullman, and C. Valdemoro, *Biochim. Biophys. Acta*, **46**, 555 (1961).

(7) W. Korytnyk, *J. Org. Chem.*, **27**, 3724 (1962), and previous papers.

(8) Varian Associates Publication No. 87-100-119.

(9) R. A. Y. Jones, A. R. Katritzky, J. N. Murrel, and N. Sheppard, *J. Chem. Soc.*, 2576 (1962).

(10) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).