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).

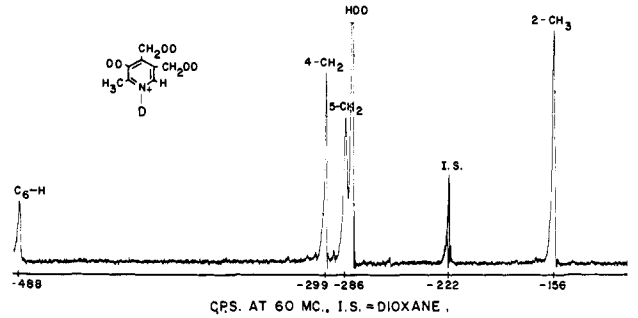


Fig. 1.—P.m.r. spectrum of pyridoxol cation in D_2O.

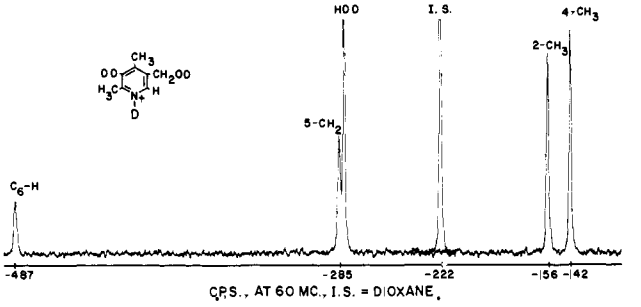


Fig. 2.—P.m.r. spectrum of 4-deoxypyridoxol cation in D_2O.

Solutions of cations were obtained either by dissolving the hydrochlorides in D_2O, the pD then being around 3.0, or by dissolving the bases in 1 *N* D_2SO_4. Both methods gave identical spectra. Anions were determined in 1 *N* NaOD solution, which was obtained by the addition of sodium to D_2O under a nitrogen atmosphere. Zwitterionic forms of bases were determined around pD 7.0, which was obtained on careful addition of 1 *N* NaOD to an acid solution of the compound.

Results

Spectra of Pyridoxol and Pyridoxol Analogs.—The p.m.r. spectrum of a solution of pyridoxol hydrochloride (I) at pD 3.0 has four sharp peaks (Fig. 1). The three-proton peak at −156 c.p.s. and the one-proton peak at −488 c.p.s. are obviously due to the methyl and C_6-protons. The closely spaced two-proton peaks at −286 and −299 c.p.s. are due to the non-exchangeable protons of the two hydroxymethyl side chains. The one phenolic and two alcoholic protons are exchanged immediately for deuterium.

The two-proton peaks in the side chain in pyridoxol were assigned by comparing the p.m.r. spectrum of pyridoxol with those of several of its analogs (Table I). In acid solution, all the pyridoxol analogs are in cationic form, and all have a two-proton peak between −285 and −287 c.p.s. In 4-deoxypyridoxol (II), the two-proton peak at −285 c.p.s. must be due to the 5-hydroxymethyl side chain (Fig. 2). In 4-methoxypyridoxol (III), the pair of the two-proton peaks are very close at −287 and −289 c.p.s., respectively, and hence no definite assignment could be made. Nevertheless, since the slight change in the 4-hydroxymethyl side chain is not expected to change greatly the position of the 5-hydroxymethyl side-chain peak, the latter probably appears at −287 c.p.s., as it is closer to the two-proton peak at −285 c.p.s. in 4-deoxypyridoxol.

For similar reasons, the −286 c.p.s. peak in pyridoxol is presumably due to the 5-hydroxymethyl side chain, and the −299 c.p.s. peak to the 4-hydroxymethyl side chain. The C_6-proton can be expected to be especially sensitive to any changes in the electronic nature of the substituents in the ring, and to a lesser extent this should also be reflected in the shielding of the methyl protons. Thus in pyridoxol, 4-deoxypyridoxol, and 4-methoxypyridoxol, the positions of these two peaks are very similar, as the changes in the

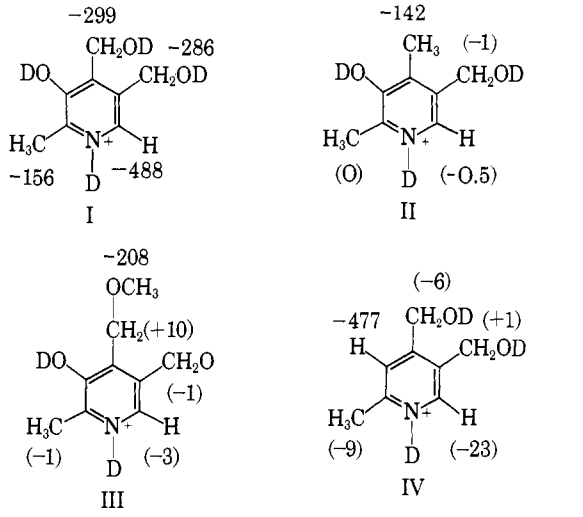


Fig. 3.—Comparison of p.m.r. spectra of pyridoxol and pyridoxol analog cations in D_2O. Positions of peaks are indicated in c.p.s. units. Numbers in parentheses represent displacement of the peaks in c.p.s. units with respect to pyridoxol cation I.

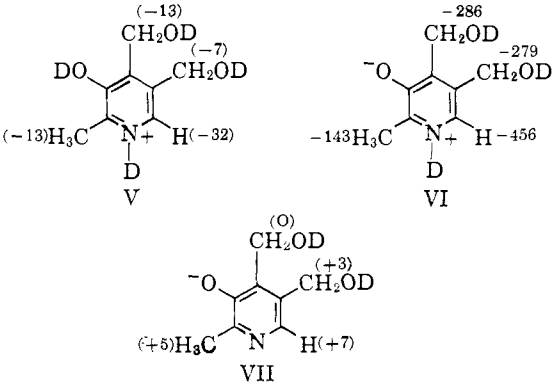


Fig. 4.—Comparison of p.m.r. spectra of the three ionic forms of pyridoxol in D_2O. Positions of peaks are indicated in c.p.s. units. Numbers in parentheses represent displacement of the peaks in c.p.s. units with respect to pyridoxol zwitterion VI.

electronic natures of the substituents are minor. 3-Deoxypyridoxol (IV) lacks the electron-donating phenolic group, however, and it is not surprising to find these two peaks considerably less shielded than in previous compounds. The assignment of the C_3-proton peak at −477 c.p.s. and the C_6-proton peak at −511 c.p.s. is based on the fact that in pyridine derivatives the α-protons are less shielded than the β-protons.[5] Lack of the phenolic group does not greatly affect the shielding of the hydroxymethyl groups on the 3-deoxypyridoxol cation. Changes in the shift of these cations with respect to pyridoxol are represented in Fig. 3.

Very marked shifts of peaks were observed in neutral and alkaline solutions. Here, again, peaks were assigned by comparing the spectra of several analogs (Table I).

It is now well established that pyridoxol, and those of its analogs which possess an unsubstituted phenolic group, exist predominantly in a zwitterionic form (VI) in neutral solution, and in an anionic form (VII) in alkaline solution. Dissociation of the phenolic group should increase the electron-donating properties and hence should result in increased shielding of protons. This is evident on comparing the cationic forms in acid solution with the zwitterionic forms in neutral solution (Fig. 4).

In the anionic forms, the removal of the positive charge on the heterocyclic nitrogen has resulted in a

TABLE I

P.M.R. SPECTRA OF PYRIDOXOL ANALOGS IN D_2O

Compound	2-CH_3 Acid	2-CH_3 Neutral	2-CH_3 Alkaline	4-CH_2OD Acid	4-CH_2OD Neutral	4-CH_2OD Alkaline	5-CH_2OD Acid	5-CH_2OD Neutral	5-CH_2OD Alkaline	C_6-H Acid	C_6-H Neutral	C_6-H Alkaline	Others Group	Others Acid	Others Neutral	Others Alkaline
Pyridoxol	−156	−143	−138	−299	−286	−286	−286	−279	−276	−488	−456	−449				
4-Deoxypyridoxol	−156	−138	−137				−285	−274	−271	−487.5	−452	−444	4-CH_3	−142	−142	−148
4-Methoxy-pyridoxol	−157	−139	−138	−287 or −289	[a]	−274 or −275	−287 or −289	−278	−274 or −275	−491	−454	−449	OCH_3	−208	−203	−200
3-Deoxypyridoxol	−165	−145	−145	−283	[a]	[a]	−285	−276	−276	−511	−494	−494	C_3-H	−477	−438	−438

[a] Peak obscured by HDO resonance line in the region between −279 and −284 c.p.s.

TABLE II

COMPARISON OF P.M.R. SPECTRA OF PYRIDOXOL WITH THOSE OF ITS METHYL DERIVATIVES

Compound	2-CH_3 Acid	2-CH_3 Neutral	2-CH_3 Alkaline	4-CH_2OD Acid	4-CH_2OD Neutral	4-CH_2OD Alkaline	5-CH_2OD Acid	5-CH_2OD Neutral	5-CH_2OD Alkaline	C_6-H Acid	C_6-H Neutral	C_6-H Alkaline	Others Group	Others Acid	Others Neutral	Others Alkaline
Pyridoxol	−156	−143	−138	−299	−286	−286	−286	−279	−276	−488	−456	−449				
N-Methylpyridoxol	−157	−148	−146	−299	−289	−284	−286	−280	−276	−483	−456	−453	$N-CH_3$	−251	−241	−239
3-O-Methylpyridoxol	−162	−144	−144	−294[a]	[b]	[b]	−291[a]	[b]	[b]	−509	−489	−489	$O-CH_3$	−235	−224	−224
3-O-Methylpyridoxol methiodide	−162	[c]	[d]	−293[a]	[c]	−288	−289[a]	[c]	[b]	−512	[c]	−504	$O-CH_3$	−232	[c]	−231
													$N-CH_3$	−253	[c]	−250

[a] Tentative assignment. [b] Peak obscured by HDO absorption. [c] Not determined. [d] Immediately exchanged for deuterium.

TABLE III

P.M.R. SPECTRA OF PYRIDOXAMINE AND PYRIDOXAMINE PHOSPHATE

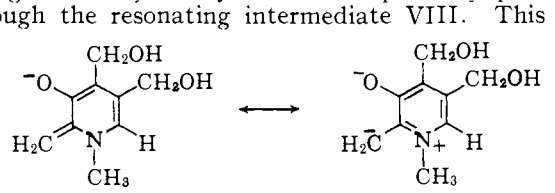
Compound	2-CH_3 Acid	2-CH_3 Neutral	2-CH_3 Alkaline	4-CH_2ND_2 Acid	4-CH_2ND_2 Neutral	4-CH_2ND_2 Alkaline	5-CH_2OR Acid	5-CH_2OR Neutral	5-CH_2OR Alkaline	C_6-H Acid	C_6-H Neutral	C_6-H Alkaline
Pyridoxamine	−161.5	[a]	−138	−265	[a]		−291	[a]	−272.5[b]	−494.5	[a]	447
Pyridoxamine phosphate	−163	−144.5	−139	−266	−256	−282	−305	−292	[c]	−499	−456	−453
							−313	[c]				

[a] Insufficiently soluble in neutral solution. [b] Tentative assignment. [c] Peak obscured by HDO resonance.

further increase in the shielding. The change in the shielding associated with the charge on the heterocyclic nitrogen is much more evident when the phenolic group is either absent, as in 3-deoxypyridoxol, or substituted, as in 3-O-methylpyridoxol (see below).

Interaction of pyridoxol with diazomethane yielded a mixture of the 3-O-methyl and N-methyl derivatives.[11] The latter has a fixed zwitterionic structure in neutral solution, unlike 3-O-methylpyridoxol, which cannot ionize. Comparison of the p.m.r. spectrum of pyridoxol with those of its methyl derivatives (Table II) indicates that in neutral solution the p.m.r. spectrum of pyridoxol is closely related to that of its N-methyl derivative. This is especially evident in the position of the C_6-proton resonance peak and provides independent evidence for the zwitterionic structure of pyridoxol in neutral solution. Similarly, at both extremities of the pD scale, the spectrum of pyridoxol is very similar to that of its N-methyl derivatives, and not to that of its O-methyl derivative. It is apparent that the ability of the phenolic group to be protonated or dissociated determines the shielding of the protons in its environment.

When the spectrum of N-methylpyridoxol was being determined, it was observed that the 2-methyl peak decreased in size and finally disappeared within half an hour. This indicated an exchange of protons for deuterons. The methyl peak was restored when the D_2O was evaporated and replaced with H_2O while alkaline conditions were maintained. Thus, the deuterium exchange is catalyzed by alkali and probably proceeds through the resonating intermediate VIII. This appears to be a general reaction. In the methiodide of O-methylpyridoxol,[11] deuterium exchange occurred immediately in a basic solution, but not in a neutral or acid one (Table II).



VIII

Spectra of Pyridoxamine and Pyridoxamine Phosphate.—In acid solutions, pyridoxamine and pyridoxamine phosphate have similar spectra (Fig. 5 and 6),

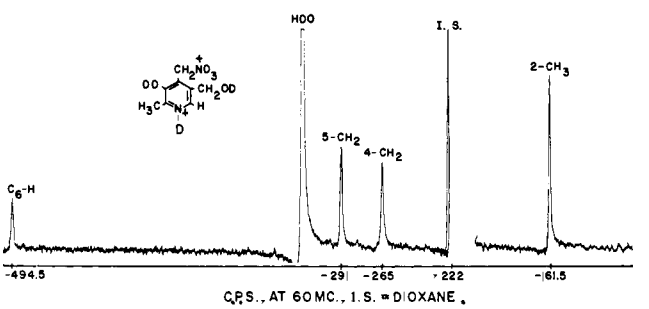


Fig. 5.—P.m.r. spectrum of pyridoxamine in 1 N D_2SO_4.

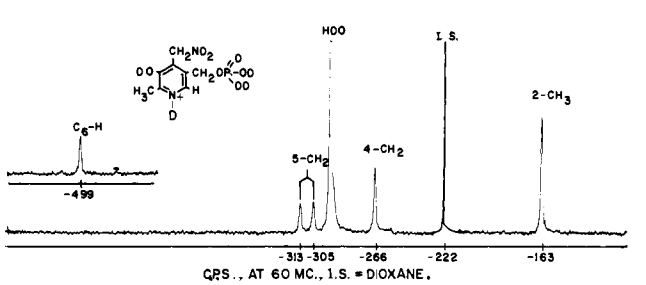


Fig. 6.—P.m.r. spectrum of pyridoxamine phosphate in 1 N D_2SO_4.

except that in pyridoxamine phosphate the 5-side chain two-proton peak is doubled and less shielded than in pyridoxamine (Table III). This doubling appears to be general with other phosphates in this series (see pyridoxal phosphate and 4-deoxypyridoxol phosphate below) and is probably due to phosphorus–proton coupling. A similar coupling has been observed in

(11) S. A. Harris, T. J. Webb, and K. Folkers, *J. Am. Chem. Soc.*, **62**, 1398 (1940).

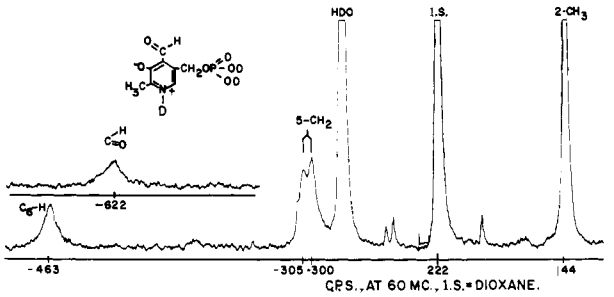


Fig. 7.—P.m.r. spectrum of pyridoxal phosphate at pH 7.8.

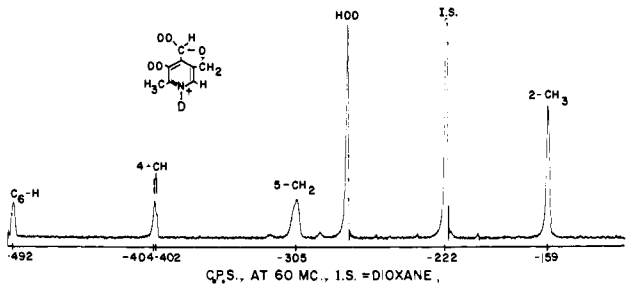


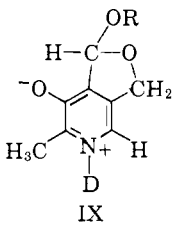
Fig. 8.—P.m.r. spectrum of pyridoxal cation in D_2O.

cyclic phosphates by Verkade and King.[12] By analogy with the latter work, the magnitude of the coupling (3–8 c.p.s.) may signify that the H–C–O–P bond is in a staggered configuration.

In neutral solution, only one part of the double peak of pyridoxamine phosphate can be seen at −292 c.p.s., and the other part is probably obscured by the HDO peak. In alkaline solution, apparently all 5-hydroxymethyl protons are obscured, and the −282 c.p.s. peak is probably due to the 4-aminomethyl side chain. The two two-proton peaks of pyridoxamine which appear at a similar pD could be assigned only tentatively.

Peaks due to the 2-methyl and C_6-protons appear at similar positions in the spectrum as compared to pyridoxol (Table I), except that in acid solution these peaks are somewhat less shielded, probably because the positively charged primary amino group acts as an electron sink.

Spectra of Pyridoxal, Pyridoxal Phosphate, and 4-Deoxypyridoxal Phosphate.—The nature of the aldehyde function in pyridoxal and pyridoxal phosphate has been a subject of extensive studies by ultraviolet spectroscopy.[3,13-15] Pyridine aldehydes have a pronounced tendency to form hydrates of the general structure $RCH(OH)_2$.[16] The situation in pyridoxal is complicated by the possibility of interaction between the aldehyde group and the 5-hydroxymethyl group to form a hemiacetal (IX, R = H). P.m.r. spectroscopy is uniquely suitable for following changes in the aldehyde group in solution, as the aldehyde proton generally



IX

appears in a very low field around −600 c.p.s. In pyridoxal phosphate, the aldehyde peak was found at −624 c.p.s. in neutral solution and at −622 c.p.s. in alkaline solution, whereas in acid solution no peak in this region could be detected (Table IV). Absence of the aldehyde peak in acid solution indicates that it is modified, most likely hydrated.

The ultraviolet spectrum of pyridoxal phosphate in acid solution has been interpreted in two different ways. Heyl, *et al.*,[13] have proposed that the aldehyde function remains unchanged, whereas Metzler and Snell[3] have maintained that it is a mixture of the free aldehyde and the hydrated form, the latter predominating.

(12) J. G. Verkade and R. W. King, *Inorg. Chem.*, **1**, 948 (1962).

(13) D. Heyl, E. Luz, S. A. Harris, and K. Folkers, *J. Am. Chem. Soc.*, **73**, 3430 (1951).

(14) A. K. Lunn and R. A. Morton, *Analyst*, **77**, 718 (1952).

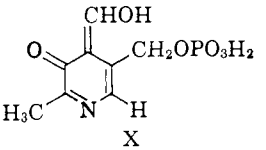
(15) V. R. Williams and J. B. Neilands, *Arch. Biochem. Biophys.*, **53**, 56 (1954).

(16) K. Nakamoto and A. E. Martell, *J. Am. Chem. Soc.*, **81**, 5857 (1959).

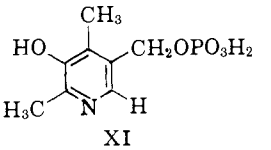
Although the p.m.r. spectrum shows conclusively that there are only insignificant amounts, if any, of the free aldehyde group in acid solution, the peak due to the proton of the hydrated form could not be located. A peak at −390 c.p.s. is observed, but its area is considerably less than one proton, and it is probably due to an impurity in the sample. It is likely that the proton of the hydrated form has been either exchanged for deuterium or is obscured by the HDO peak.

At pD 7.8, a broad aldehyde proton peak appears at −624 c.p.s. (Fig. 7). The ratio of its area to the area of the C_6-proton peak is 0.54. From this, the ratio of the hydrated form to the free aldehyde form has been calculated to be 0.86, which is in fair agreement with an estimate of 0.66 made from the ultraviolet spectrum of 5-deoxypyridoxal at pH 6.88.[3] The proportion of the free aldehyde form increases in alkaline solution. Thus, at pD 9.4 the ratio of the hydrated form to the free aldehyde form is only 0.06, which is again in agreement with the conclusions reached by Metzler and Snell.[3]

A quinonoid tautomeric form of pyridoxal phosphate (X) has been postulated[10] to account for the ultraviolet spectrum in alkaline solution. The p.m.r. spectrum



X



XI

of such a form could be expected to be quite different from that of 4-deoxypyridoxal phosphate (XI), which is incapable of forming a similar tautomeric form. The spectrum of 4-deoxypyridoxal phosphate is quite similar to that of pyridoxal phosphate (Table IV). There are no major differences in the shielding of the C_6-proton and the 2-methyl protons, which are very sensitive to any changes in the aromatic system. Thus the tautomeric form X is not a major contributor to the ultraviolet spectrum of pyridoxal phosphate in alkaline solution.

The p.m.r. spectra of pyridoxal (Fig. 8) in the pD range investigated (pD 1–11.1) did not show a free aldehyde proton peak, although its ultraviolet spectrum in alkaline solution has been interpreted[3] as being consistent with the existence of a certain fraction of the free aldehyde form. The p.m.r. spectra of pyridoxal and its ethylacetal (IX, R = C_2H_5) are similar in both acid and neutral solution (Table IV), indicating that pyridoxal exists exclusively in the hemiacetal form in these pD ranges. A characteristic feature of the p.m.r. spectrum of the hemiacetal form is the doubling or broadening of the proton resonances in the 5-side chain. The nonequivalence of these protons may be due to the puckering of the five-membered hemiacetal ring.

In alkaline solution, however, the spectrum of pyridoxal is not similar to that of its ethyl acetal, and its 5-side-chain protons appear as a sharp peak at −289

TABLE IV

P.M.R. SPECTRA OF PYRIDOXAL AND PYRIDOXAL PHOSPHATE

	2-CH_3			Aldehyde or hemiacetal H			5-CH_2OR			C_6-H			Others			
Compound	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline	Group	Acid	Neutral	Alkaline
Pyridoxal phosphate	−156	−144	−140.5	[a]	−624	−622	−303	−300	−297	−492	−463	−457				
							−306	−305	−303							
4-Deoxypyridoxol phosphate	−155	−143	−138				−296	−285	−283	−488	−457	−454	4-CH_3	−142	−131	−130
							−303	−291	−287							
Pyridoxal	−159	−145	−139	−402	−392[c]	−425	−315[b]	−304	−289	−492	−457	−442				
				−404				−307								
Pyridoxal ethylacetal	−159	−145	−140	−404	−378	−376	−314[b]	−304[b]	−301	−492	−455	−448				
				−405		−378			−305							

[a] A small peak at −390 c.p.s. is probably due to an impurity in the sample. [b] Broad peak. [c] Split by 1 c.p.s.

c.p.s., similar to that of other compounds in which the 5-hydroxymethyl side chain is unsubstituted (Table I). This would indicate that in alkaline solution the aldehyde group of pyridoxal is modified in a way which does not involve hemiacetal formation with the 5-hydroxymethyl side chain. The one-proton peak at −425 c.p.s. is probably associated with the modified aldehyde proton.

From the work described in this paper, it should be apparent that p.m.r. spectroscopy is potentially a valuable tool in such studies as the elucidation of reaction mechanisms catalyzed by pyridoxal phosphate, and the determination of the exact nature of the involvement of the aldehyde group in the binding of pyridoxal phosphate on various apoenzyme surfaces.[17]

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The Correlation of Biological Activity of Plant Growth Regulators and Chloromycetin Derivatives with Hammett Constants and Partition Coefficients

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An equation using two experimentally based variables, σ and π, has been developed for correlating the effect of a given substituent on the biological activity of a parent compound; σ is the Hammett substituent constant and π is an analogous constant representing the difference in the logarithms of the partition coefficients of the substituted and unsubstituted compounds ($\pi = \log P_X - \log P_H$). The value of this equation has been tested on two systems of biologically active molecules: the phenoxyacetic acids and chloromycetin analogs. Using π and σ it becomes possible to disentangle three of the most important parameters governing the biological activity of organic compounds: steric, electronic, and rate of penetration.

Since the classic paper by Koepfli, Thimann, and Went[2] pointing out that a variety of acids of quite different gross structure function as plant growth regulators in the cell elongation process, an enormous amount of work has been done on the chemical and/or physical properties responsible for the biological activity and common to the great assortment of compounds which will produce this effect. The theories which have been developed have been summarized and analyzed from various points of view.[3a,b]

In our "two point attachment" theory[3a] to rationalize chemical structure and biological activity, we have assumed that auxins react *via* two points, one on the side chain and one on the ring, with a plant substrate. The fact that a ring of considerable aromatic character seems essential for auxin activity[4] has caused us to focus our attention on the nature of the substituent effect. It was early apparent[5] that the electronegative groups such as nitro and halogen were more effective in increasing biological activity when substituted onto the ring than electron-releasing groups such as alkyl, OH, etc. However, our attempts to find any quantitative relationship between the biological activating ability of functional groups and their relative electronegativity were unsuccessful. The molecular orbital calculations of Fukui[6,7] and others[8,9] attempting to correlate activity with π-electron delocalizability at various points on the ring, while quite suggestive and of qualitative value, leave much to be desired. In setting up a more exact model to test our two-point reaction hypothesis, we have made the following assumptions: I. Consideration of three critical steps I, II, and III in the movement of auxin from solution to the site of action followed by a two point attachment to a plant substrate would be sufficient to rationalize growth rates caused by the different monosubstituted phenoxyacetic acids. Considering the vast number of molecules of such

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