VITAMIN B_6 ANALOGS

VIII.* IR SPECTRA

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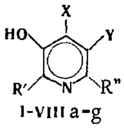
The IR spectra of 2- and 6-alkyl analogs of three forms of vitamins of the B_6 group, viz., pyridoxine, pyridoxamine, and pyridoxal, their 5-phosphates, and a number of derivatives, were investigated. Correlations for the characteristic frequencies of the spectra were made.

Anderson and Martell [2] have studied the IR spectra of pyridoxal (2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine, P) and its 5'-phosphate ester (PP) in deuterium oxide. In an investigation of the band of the valence vibrations of the carbonyl group at 1660 cm^{-1} as a function of the pH of the medium, they showed that P, particularly in acid media, exists predominantly as the hemiacetal, while the formyl group of PP is strongly hydrated. The IR spectra of Schiff bases of P with a number of amino acids [3] and the spectra of model compounds of 4-formylpyridine and 3-hydroxy-4-formylpyridine [4] were subsequently studied, and correlations were made for the fundamental bands, viz., for $\nu_{C=O}$ of the aldehyde group and for ν_{OH} of the phenolic hydroxyl group.

This communication is devoted to an investigation of the IR spectra of a number of analogs and derivatives of vitamins of the B_6 group (I-VIII):

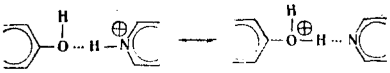


I-VIII a-g

I $X=Y=COOCH_3$;
II $X=Y=CH_2OH$;
III $X=CHO$, $Y=CH_2OH$;
IV $X=CH_2NH_2$, $Y=CH_2OH$;
V $X=CH=NOH$, $Y=CH_2OH$;
VI $X=CH=NC_6H_4OC_2H_5$, $Y=CH_2OH$;
VII $X=CHO$, $Y=CH_2OPO_3H_2$;
VIII $X=CH_2NH_2$, $Y=CH_2OPO_3H_2$
a $R'=R''=H$; b $R'=CH_3$, $R''=H$; c $R'=H$, $R''=CH_3$; d $R'=R''=CH_3$;
e $R'=i\text{-}C_3H_7$, $R''=H$; f $R'=n\text{-}C_4H_9$, $R''=H$; g $R'=H$, $R''=i\text{-}C_3H_7$

2000-4000 cm^{-1} REGION

The absorption in this region, which pertains to the valence vibrations of the hydroxyl group, can be divided into three basic types: extremely broad band (B-1) at 2500-2900 cm^{-1}, a broad peak (B-2) at 3400 cm^{-1}, and a rather narrow band of high intensity (B-3) at 3200-3300 cm^{-1}. Band B-1 pertains to vibrations of the phenolic hydroxyl group which is bonded by a hydrogen bond to the nitrogen atom of the pyridine ring:



for hydrochlorides

for bases

This assignment is confirmed by the following. Band B-1 is characteristic for hydrochlorides of diesters of 5-hydroxycinchomeronic acids (I). Protonation of the nitrogen of the pyridine ring lowers the basicity of

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*See [1] for communication VII.

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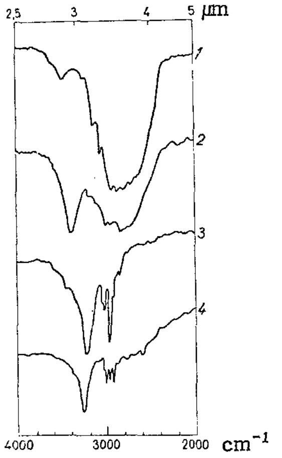


Fig. 1. Transmission at 2000-4000 cm^{-1}: 1) hydrochloride of dimethyl 5-hydroxycinchomeronate (Ia); 2) hydrochloride; and 3) base of dimethyl 2,6-dimethyl-5-hydroxycinchomeronate (Id); 4) 2-norpyridoxylidene-p-phenetidine (VIa).

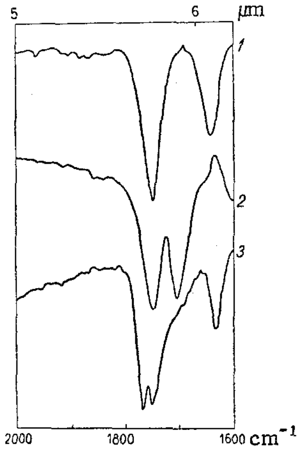


Fig. 2. Transmission at 1600-2000 cm^{-1}: 1) hydrochloride and 2) base of dimethyl 2-methyl-5-hydroxycinchomeronate (Ic); 3) hydrochloride of dimethyl 6-methyl-2-isopropyl-5-hydroxycinchomeronate (Ig).

the carbonyl group of the ester grouping to such an extent that the chelate intramolecular hydrogen bond cannot form. (This conclusion is confirmed by an examination of the absorption at 1600-2000 cm^{-1}, which will be discussed below.) In the case of hydrochlorides of I, the nitrogen atom in which is sterically hindered, viz., diesters of 2,6-dimethyl-5-hydroxycinchomeronic acid (Id) and 6-n-butyl-5-hydroxycinchomeronic acid (If), the intensity of B-1 decreases, but absorption appears at 3400 cm^{-1} (B-2), which we relate to the valence vibrations of the hydroxyl group bonded by a hydrogen bond with the oxygen atom (dimers or polymers). Finally, in compounds for which one should expect the presence of an intramolecular chelate hydrogen bond, both B-1 and B-2 are practically absent. Their position is occupied by an intense peak at 3200-3300 cm^{-1} (B-3). Band B-3 is characteristic for the spectra of Schiff bases VI. The chelate hydrogen bond is realized in free bases I and is reflected in the spectra of these compounds over the examined region. The correlations are illustrated in Fig. 1.

It should be noted that the $-O-\cdots O=C$ bond of the chelate for 3-hydroxy-4-formylpyridine lies precisely at 3250 cm^{-1}.

In addition to the B-1 band, the spectra of pyridoxine analogs (II) contain B-2, which is sometimes split into two or three bands which correspond to the individual hydroxyl groups.

The intense absorption at 3250-3300 cm^{-1} in the spectra of dihydrochlorides of amines IV should, in our opinion, be ascribed to the valence vibrations of the 5-hydroxyl group.

An intense band of the valence vibrations of the 5-hydroxyl group at 3480-3580 cm^{-1} is also characteristic for oximes V. The spectra of pyridoxal analogs (III) contain a band at ~3400 cm^{-1} in addition to B-1. Since, as will be shown below, these compounds exist as hemiacetals in the solid state, this band should be related to the vibrations of the 4'-hydroxyl group.

As for the other absorption bands, specifically the valence vibrations of the methyl and methylene groups and the C−H group of the pyridine ring, they are usually manifested as a shoulder on B-1 and are clearly separated from the valence vibrations of the hydroxyl group only in compounds with a chelate hydrogen bond. In addition, "ammonium bands" ($\overset{\oplus}{N}H_3$ and $C=\overset{\oplus}{N}H$), which are also covered by B-1, also lie in this region.

1600-2000 cm^{-1} REGION

This region gives the maximum amount of information since the characteristic frequencies of both the carbonyl group itself and its hetero analogs lie within it.

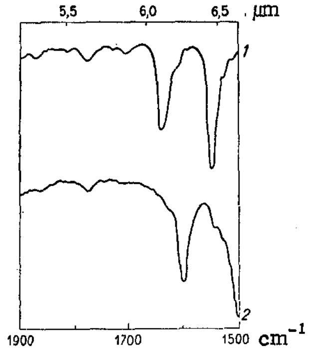


Fig. 3. Transmission at 1500-1900 cm^{-1}: 1) hydrochloride and 2) base of 3-hydroxy-4,5-bis(hydroxymethyl)pyridine (IIa).

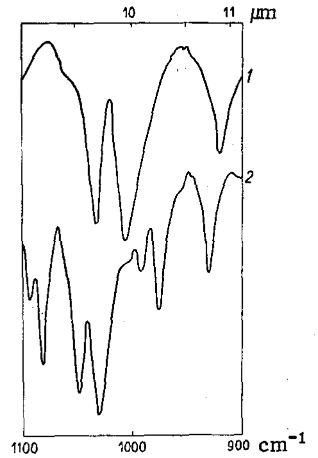


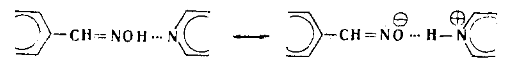
Fig. 4. Transmission at 900-1100 cm^{-1}: 1) hydrochloride of 3-hydroxy-4,5-bis-(hydroxymethyl)pyridine (IIa); 2) hydrochloride of 2-methyl-3-hydroxy-4-α-hydroxyethyl-5-hydroxymethylpyridine.

An examination of the spectra of I (Fig. 2) confirms the previously drawn conclusion that the chelate hydrogen bond arises only in free bases. Only one broad band of the valence vibrations of C = O at 1750-1752 cm^{-1} is present in the spectra of hydrochlorides of I. It is the result of the superimposition of the close frequencies of the 4- and 5-ester groupings. The carbonyl band is split into two bands at 1750 and 1703 cm^{-1} on passing to the free bases. The first band relates to $\nu_{C=O}$ of the 5-COOR group, while the second relates to the valence vibrations of the 4-ester grouping, shifted to lower wave numbers due to the formation of a hydrogen bond with the phenolic hydroxyl group. (The shift of 47 cm^{-1} corresponds to the formation of a hydrogen bond of average strength.) This fact is in good agreement with the appearance of an absorption which corresponds to the vibrations of the hydroxyl group which participates in the chelate hydrogen bond (3250 cm^{-1}).

In addition, two carbonyl bands at 1750 and 1770 cm^{-1} (Fig. 2) are also present in the spectrum of the hydrochloride of dimethyl 6-methyl-2-isopropyl-5-cinchomeronate (Ig). In our opinion, this is explained by the fact that the large substituent in the ortho position to the 5-carbomethoxy group partially removes it from conjugation with the pyridine ring, which leads to a shift in $\nu_{C=O}$ to higher frequencies by 20 cm^{-1}.

The position of the valence vibrations of the C = N bonds of Schiff bases VI makes it possible to assume the presence of a strong chelate hydrogen bond between the phenolic hydroxyl group and the nitrogen atom of the imine. In addition, $\nu_{C=N}$ in the spectra of oximes V lies at 1650-1660 cm^{-1} which, allowing for the character of the absorption at 2000-4000 cm^{-1}, makes it possible to assume the absence of a chelate hydrogen bond for V in the solid state.

It is interesting to note that a broad band of moderate intensity appears at 1700-1900 cm^{-1} in the spectra of V; this band is probably related to the vibrations of the hydroxyl group of the oxime bonded by a hydrogen bond to the nitrogen atom of the pyridine ring:



The absence of a carbonyl frequency in the spectra of the hydrochlorides of pyridoxal derivatives III indicates that the latter exist as hemiacetals in the solid state.

Pyridoxal-5'-phosphate analogs (VII) exist at least partially in the aldehyde form in the solid state, which is confirmed by the presence in their spectra of a carbonyl band at 1720 cm^{-1}. The high position of this band indicates the absence of a chelate hydrogen bond.

The spectra of all of the compounds studied by us contain one of two bands (1630-1660 cm^{-1} and 1592-1610 cm^{-1}, both of medium intensity), which are related to the vibrations of the pyridine ring. The band at 1630-1660 cm^{-1} is manifested only in the spectra of the hydrochlorides, while the band at 1592-1610 cm^{-1} appears only in the spectra of the bases (Fig. 3). This sort of behavior makes it possible to relate the first band to $C = \overset{\oplus}{N}H$ ("hydrochloride band") and the second to C = N ("base band") of the pyridine ring. Thus, the spectra of VII contain a "hydrochloride band" at 1650 cm^{-1}, which attests to the fact that the ring nitrogen atom is protonated, which makes the absence of a chelate hydrogen bond in these compounds understandable.

TABLE 1. IR Spectra of Hydrochlorides of Dimethyl Esters of Alkyl-Substituted 5-Hydroxycinchomeronic Acids (Ia-g)

Hydrochloride of compound	ν, cm^{-1}							ν, cm^{-1}						
	$-O-H \vdots HO-$	$-OH \cdots O=C$ chelate	$OH \cdots N \leqslant$	C–H pyridine	CH_3- $-CH_2-$	5-C=O 4-CO_2CH_3	4-C=O 6-CO_2CH_3	C=N ring	$C=\overset{+}{N}H$ ring	C=C ring	C=C ring	C=C ring	CH_3- $-CH_2-$	Overtone C=O
Ia	3480 w	—	2900— 2800 s	3060 m	2860 2940	1650 vs		—	1638 m	1556 m	1512 w	—	1450 s 1370 s	1250 s 1031 m
Ic	3490 w	—	3000 s	—	2870 2940	1658 vs		—	1642 w	(1580) 1560 m	1509 s	1422m	1460 m 1380 m	1258 vs 1056 s
Ic*	3450 m	3160 s	2780 m	3030 m	2870 2950	1650 vs	1603 vs	1593 m	—	1570 w	—	1410m	1450 s 1366 s	1250s 1050s
Id	3300 w	—	2710s	—	2860 2940	1646 vs		—	1648m	1559 m	—	1404 m	1444 s 1382 w	1265s 1048m
Id*	3470w	3240s	—	—	2890 2970	1650 vs	1603 vs	1600m	—	—	—	1420m	1447 s 1390 s	1247s 1056s
Ie	3490 w	—	260 s	—	2860 2940	1661 vs		—	1631 m	1542 m	—	1416 w	1470 s 1378 s	1241 s 1042m
If	3420 s	—	2750m	3030w	— 2950	1652 vs		—	1630m	1541 m	—	—	1460 s	1242m 1033s
Ig	3460 s	—	2570m	—	2860 2950	1672 vs	1650 vs	—	1638m	1564 s	—	—	1470 s 1387 m	1246 s 1052 m

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* Bases Ic and Id.

TABLE 2. IR Spectra of 2- and 6-Alkyl-Substituted 3-Hydroxy-4,5-bis(hydroxymethyl)pyridines [pyridoxine analogs (IIa-f)]

Compound	ν, cm^{-1}				ν, cm^{-1}						
	$-O-H \vdots H-O-$	$-OH \cdots N \leqslant$	CH_3- $-CH_2-$	C–H pyridine	$C=\overset{+}{N}H$ ring	C=C ring	C=C ring	C=C ring	C–O 5-CH_2OH	C–O 4-CH_2OH	CH_3- $-CH_2$
Pyridoxine (IIb), hydrochloride	3200	2850	—	—	1632 w	1554m	1493 m	1400 m	1023 s	998 m	1440 w 1337 w
IIa, hydrochloride	3300	3000	—	—	1645 m	1547s	1510 w	—	1021 s	1005 s	1433s
IIc, base	3200	2750	—	3020	1600 m*	—	1494 s	—		1020 vs	1432s 1381m
IId, base	3200	2730	2850 2930	—	1608 m*	—	1496 m	—	1035 s	1006 s	1450s 1387m
IIf, hydrochloride	3310	2900	—	3020	1631 w	1550w	1509 s	1421 w	1007 s	992 s	1468m 1390m
2-Methyl-3-hydroxy-4-α-hydroxyethyl-5-hydroxymethylpyridine, hydrochloride	3260	1740	—	—	1634 w	1557w	1500 m	1417 w	1021 s	1043 s	1460s 1389m

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*C=N "base band."

TABLE 3. IR Spectra of Hydrochlorides of 2- and 6-Alkyl-Substituted 3-Hydroxy-4-formyl-5-hydroxymethylpyridines (IIIa-f)

Hydrochlor. of compound	ν, cm^{-1}										
	$-O-H \cdots H-O-$	$-OH\cdots N\leqslant$	CH_3- $-CH_2-$	C–H pyridine	$\overset{+}{C=NH}$ ring	C=C ring	C=C ring	C=C ring	C–O–C hemiacetal	C–OH hemiacetal	C–O hemiacetal
III b	—	2620	2970 2880	3060	1662 w	1578s	—	1423 w	1261s 1062m	1027s	991 s
III a	3390	2760	2940 2870	3110	1635 m	1561s	1509m	1431 w	1260m 1067m	1014s	1003 s
III d	3340	2690	2970 2880	—	1659 w	1583s	—	1439 w	1257m 1052s	1022s	997 s
III e	3450 3380	2770	2970 2880	3080	1657 w	1570s	—	1420 w	1280s 1069m	1027s	998 m
III f	3290	2670	2950 2890	3070	1660 w	1573s	—	1420 w	1250m 1059m	1028s	991 m

TABLE 4. IR Spectra of Dihydrochlorides of 2- and 6-Alkyl-Substituted 3-Hydroxy-4-aminomethyl-5-hydroxymethylpyridines

Hydrochlor. of compound	ν, cm^{-1}											
	$-O-H \cdots H-O-$	$OH\cdots N\leqslant$	CH_3- $-CH_2-$	C–H pyridine	$\overset{+}{C=N}-$ ring	C=C ring	C=C ring	C=C ring	$\overset{+}{N}H_3$ δ_{as}	$\overset{+}{N}H_3$ δ_s	CH_3- $-CH_2-$	C=O 5-CH_2OH
IVb	3470 3230	2620	2860 2940	3030	1642 m	1596m	—	1400s	1548s	1508s	1450 m 1362 s	1023s
IVa	3370	2980	2940 2850	3080	1623 m	1581w	—	—	1540s	1488 s	1448 m	1031 s
IVc	3340	2890	— —	3030	1623 m	—	—	1406s	1563m	1513 s	1463 m 1352 m	1020 s
IVd	3220	2900	2930 —	—	1641 m	1572m	1510 w	1400s	1552 s	1490 m	— 1363 m	1019 m
IVf	3270	2740	2940 2860	3070	1636 m	—	—	—	1550 s	1510 m	—	—

1000-1600 cm^{-1} REGION

In addition to the bands noted above, another three bands related to the vibrations of the pyridine ring at 1570, 1500, and 1430 cm^{-1} appear in the spectra of the investigated compounds. The position of these bands changes slightly on passing from the bases to the hydrochlorides.

Deformation vibrations of the $\overset{\oplus}{N}H_3$ group, which appear in the spectra of pyridoxamine analogs (VIII) as two high intensity bands at 1550 cm^{-1} (δ_{as}) and 1500 cm^{-1} (δ_s), lie in this region.

The C–O valence vibrations of the 4- and 5-hydroxymethyl groups are found at 1000 and 1030 cm^{-1}, respectively. This is confirmed by the fact that the first of the bands disappears in the spectra of compounds which do not have a 4-hydroxymethyl group. In addition, the band at 1030 cm^{-1} for 2-methyl-3-hydroxy-4-α-hydroxymethyl-5-hydroxymethylpyridine does not change its position, while the band at 1000 cm^{-1} is shifted to higher frequencies by 40 cm^{-1}, which corresponds to transition from a primary to a secondary alcohol (Fig. 4).

The valence vibrations of the ester group (C–O–C) appear at 1250 and 1040 cm^{-1} in the spectra of I. Both bands are of high intensity. The cyclic hemiacetal is characterized by a group of intense bands in this region, viz., at 1250 and 1050 cm^{-1} (–C–O–C–), 1025 cm^{-1} (C–OH), and 1000 cm^{-1} (C–O).

The presence of a 5'-phosphate grouping in pyridoxal-5'-phosphate analogs (VII) and pyridoxamine-5'-phosphate analogs (VIII) leads to the appearance in their spectra of characteristic frequencies of the valence vibrations of the P=O bond (1270-1290 cm^{-1}), of the C–O bond (1062-1084 cm^{-1}), and of the P–O bond (1169-1185 cm^{-1}). The correlation of the characteristic bands in the spectra of the investigated compounds is presented in Tables 1-7.

TABLE 5. IR Spectra of Oximes of 2- and 6-Alkyl-Substituted 3-Hydroxy-4-formyl-5-hydroxymethylpyridines (Va-f)

Oxime of compound	ν, cm^{-1}												
	—O—H ⋮ —O—H	—OH···N≤	CH_3— —CH_2—	CH oxime	C=C ring	C=C ring	C=C ring	C=C ring	C=N oxime	CH_3— —CH_2	C—O 5-CH_2OH	N—O oxime	NOH···N≤
Vb	3470 s	2470	2980 2930 2880 2820	—	1580 s	1547 w	1500m	1413s	1640 m	1047m	1396 w	1020s	1780
Va	3440s 3400 m	2580	2970 2940 2870	3090	1571m	1536m	1502 m	1414m	1620 m	1047 s	1466 w	988s	1790
Vc	3510 s	2700	2970 2930 2860	3060	1580w	—	1513 s	1411s	1649 m	1024 s	1474 s 1370 m	1010s	1730
Vd	3500s	2680	2940 2880 2830	3060	1580m.	—	1512m	1422 s	1647m	1040 m	1480 m 1370 m	1012s	1750
Ve	3500 s	2670	2940 2870	—	1581 w	1540 w	—	—	1644m	1032 s	1480 m 1383m	1014 s	1790
Vf	3490s	2480	2970 2920 2870	3050	1578m	1531 w	1499s	1400 s	1654m	1031 s	1481 s 1370m	1008 s	1800

TABLE 6. IR Spectra of Schiff Bases of 2- and 6-Alkyl-Substituted 3-Hydroxy-4-formyl-5-hydroxymethylpyridines (Va-f) with p-Phenetidine

Schiff base of comp.	ν, cm^{-1}									
	OH···N= chelate	CH_3— —CH_2—	C=C ring	C=C ring	C=C ring	C=C ring	C=N imine	C—O— —OC_2H_5	C—O 5-CH_2OH	CH_3— —CH_2
VIb	3160 s	2990 2940 2890 2840	1592 m	1568w	1512 s	1420 s	1629m	1259 s 1058 m	1030 s	1486m
VIa	3280 s	2980 2940 2890	1591 m	1560 w	1514 s	1436 m	1626m	1252 s 1060 m	1012 m	1482m
VId	3160 s	2990 2940 2880	1596m	1562 w	1520m	1411 w	1629m	1259 s 1052 m	1020 s	1486 m 1360m
VIc	3180 s	2990 2940 2890 2850	1590m	1561 w	1520 m	1422 s	1626m	1258 s 1050 s	1021 s	1486m
VIe	3250 s	2990 2940 2870	1600m	1567 w	1522m	1420m	1638m	1261 s 1058m	1022 m	1480m 1392m
VIf	3270 s	2980 2940 2890 2860	1590m	1558w	1520m	1410 s	1629m	1259s 1050 s	1026 s	1487m 1370 m

TABLE 7. IR Spectra of 5'-Phosphate Esters

Compound*	v, cm^{-1}												
	$-O-H \cdots H-O-$	$OH \cdots N$	$-CH_3-$ $-CH_2$	C−H pyridine	C=O aldehyde	$\overset{\oplus}{C=N}-$ ring	C=C ring	C=C ring	C=C ring	$\overset{+}{N}H_3$	P=O	P−O	C−O phosphate
VIIb	3440	2550	2940 2870	3070	1720 s	1646 w	1581m	1520 w	1415 s	—	1278m	1182 m	1070 s
VIIa	3430	2920	2940 2880	3070	1720 m	1650 m	1581m	1524 w	1414 m	—	1279m	1183 m	1070 s
VIId	3410	2750	2940 2870	—	1720 w	1660 m	1560m	—	1409 m	—	1274m	1185 m	1061 s
VIIf	3420	2700	2940 2870	—	1720 m	1654 m	1568 m	—	—	—	1290m	1169 m	1070 s
VIIIa	3400	2650	2950 2880	3090	—	1653 m	—	—	1400 m	1529 s —	1294m	1174s	1062 s
VIIIc	3440	3100	2930 2860	3030	—	1643 m	—	—	1412 m	1547 m	1290 w	1142s	1084 s

*VII as the monohydrate; VIII as the dihydrate.

The authors sincerely thank Academician A. E. Braunshtein for his interest in this work.

MATERIALS AND METHODS

Commercial samples of pyridoxine (IIb), pyridoxal (IIIb), pyridoxamine (IIIb), and pyridoxal-5'-phosphate (VIIb) were used. The remaining compounds were synthesized by us [5-7]. The IR spectra of compounds in the solid state (KBr pellets) were obtained with a UR-10 spectrometer.

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