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INFRARED STUDY OF THE PROTON ACCEPTOR ABILITY OF METYRAPONE

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The hydrogen bond complexes between metyrapone [methyl-1,2-di(3-pyridyl)propan-1-one] and hydroxylic proton donors (phenols, water) were investigated by infrared spectroscopy. The thermodynamic and spectroscopic data determined in carbon tetrachloride suggest that the nitrogen atom of the pyridine ring A [bonded to the $C(CH^3)_2$ group] is the main hydrogen bond interaction site. The data are compared with di-2-pyridylglyoxal complexes where the hydrogen bonds are formed on the oxygen atom of the carbonyl function. In the solid adduct of metyrapone with HCl, protonation takes place on the two nitrogen atoms of the pyridine rings. The data from this work are compared with those from chemical oxidation, which leads predominantly to the formation of mono-*N*-oxide A and di-*N*-oxide.

INTRODUCTION

Metyrapone [2-methyl-1,2-di(3-pyridylpropan-1-one] is a powerful inhibitor of certain cytochromes P-450.[1,2] The availability of nucleophilic functional groups is a feature common to most direct inhibitors of cytochrome P-450 enzymes and is manifested in metyrapone by the presence of basic atoms. These factors may be necessary for the interaction with the protein.

The structure of metyrapone determined by x-ray diffraction is characterized by a twist of the molecule about the central two-carbon link (C_7—C_{10}) connecting the two pyridine rings. The torsion angle C_1—C_7—C_{10}—C_{12} is 57°. The nitrogen atoms N_5 and N_{10} are on the same side of the molecule but opposite the carbonyl oxygen O_{31} (Figure 1). Metyrapone exhibits no hydrogen bonding in the crystal structure. Approximate molecular orbital techniques show that excess negative charge resides on the carbonyl oxygen and pyridyl nitrogens[3] and, as a consequence, these three sites can act as hydrogen bond acceptors.

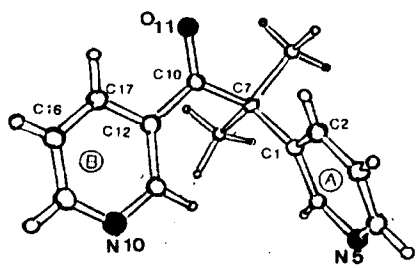

Figure 1. Molecular structure of metyrapone[3]

Despite the biological importance of metyrapone, there are no data in the literature on its proton acceptor ability. In this work, the interaction between this base and hydroxylic proton donors was investigated by infrared spectrometry in carbon tetrachloride solution. The thermodynamic and spectroscopic data allow one to determine the interaction site(s). The infrared spectrum of protonated metyrapone is also reported.

EXPERIMENTAL

The IR spectra were recorded on Perkin-Elmer Model 580 B and on Brucker Model 88 Fourier transform IR spectrometers at a resolution of 1 cm^{-1}. The equilibrium constants (K) were measured from the absorbance of the ν_{OH} stretching vibration of the phenols. The concentration of the phenols ranged between 0·007 and 0·008 mol dm^{-3} in order to avoid self-association and the concentration of the base was in excess (0·01–0·03 mol dm^{-3}). The K values calculated on the assumption of complexes of 1:1 stoichiometry, did not show any variation with the concentration and resulted from ten separate determinations. The enthalpies of complex formation were calculated from the K values determined at 298 and 323 K.

Metyrapone from Aldrich (98%) was crystallized from ethanol and phenols from Aldrich or Fluka were crystallized from ether petroleum–CCl_4. The solvents were carefully dried by the standard methods and kept on molecular sieves (4 Å).

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The metyrapone–HCl adduct was prepared by passing gaseous HCl through a chloroform solution of metyrapone. The spectrum of the resulting precipitate was taken in a KBr pellet.

RESULTS AND DISCUSSION

Complexes between metyrapone and hydroxylic proton donors

Metyrapone has mainly three basic sites available for hydrogen bond formation, the two nitrogen atoms of the heteroaromatic ring and the oxygen atom of the carbonyl function. The most probable interaction site can be found by comparing the thermodynamic and spectroscopic properties of the complexes with the data on similar molecules.

The thermodynamic parameters for the interaction between some phenol derivatives and metyrapone are given in Table 1, which lists the formation constants, the enthalpies ($-\Delta H$) and entropies ($-\Delta S°$) of complex formation along with the frequency shifts ($\Delta\nu_{OH}$) of the ν_{OH} stretching vibration. One example of an IR spectrum in the ν_{OH} stretching region is reproduced in Figure 2.

The logarithms of the K values are linearly related to the pK_a values of the proton donors:

$$\log K^{298} = 5{\cdot}65 - 0{\cdot}40\ pK_a \qquad (r = 0{\cdot}994) \qquad (1)$$

$$\log K^{323} = 4{\cdot}81 - 0{\cdot}35\ pK_a \qquad (r = 0{\cdot}994) \qquad (2)$$

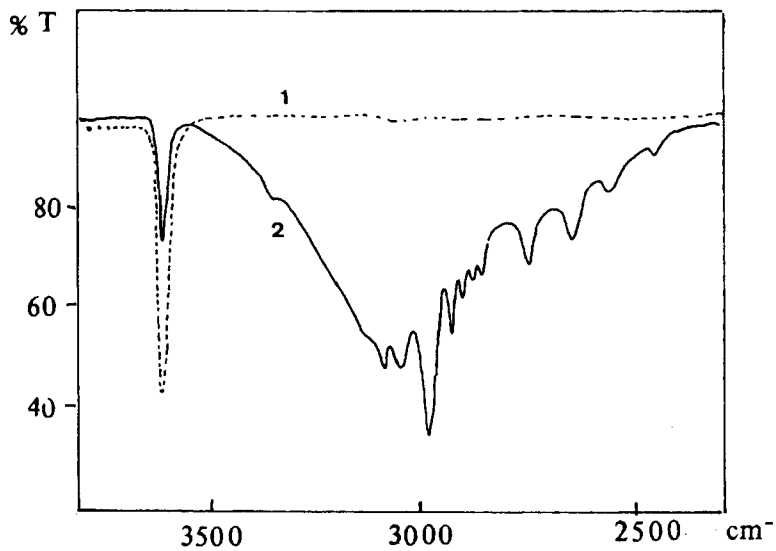

Figure 2. IR spectra (3600–3000 cm^{-1}) of (1) 3,4-cichlorophenol ($c = 0{\cdot}020$ mol dm^{-3}) and metyraone ($c = 0{\cdot}025$ mol dm^{-3}). Cell thickness, 0·1 cm; solvent, carbon tetrachloride

Table 1. Thermodynamic data for the interaction between metyrapone and phenols with carbon tetrachloride as solvent

Phenol derivative[a]	K^{298} [b] ($dm^3\ mol^{-1}$)	K^{298} [b] ($dm^3\ mol^{-1}$)	$-\Delta H$[c] ($kJ\ mol^{-1}$)	$-\Delta S°$ ($J\ K^{-1}\ mol^{-1}$)	$\Delta\nu_{OH}$ (cm^{-1})
Phenol (9·93)	43	21	23	46	455
4-Br (9·34)	96	43	26	48	485
3-Br (9·03)	106	45	27	53	505
3,4-Cl_2 (8·58)	179	72	29	54	525
3,5-Cl_2 (8·18)	286	108	30	55	555
3,4,5-Cl_3 (7·75)	433	158	32	57	570
3-CF_3-4-NO_2 (6·07)	1530	465	38	67	610

[a] The pK_a values in water are given in parentheses.
[b] Error on $K = \pm 5\%$.
[c] Error on $-\Delta H = \pm 1{\cdot}5$ kJ mol^{-1}.
[d] Error on $\Delta\nu_{OH} = \pm 10$ cm^{-1}.

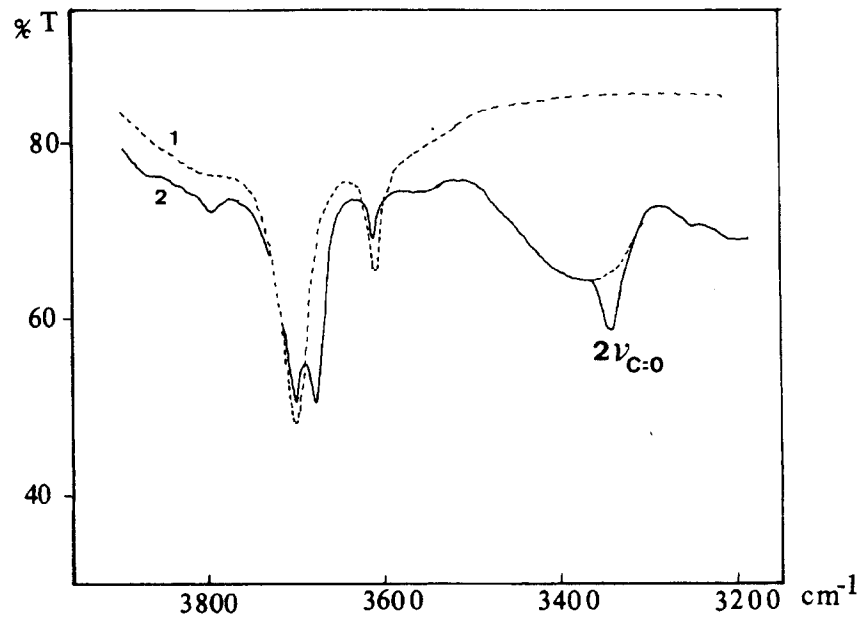

Figure 3. IR spectra (3900–3200 cm^{-1}) of (1) H_2O ($c = 0{\cdot}007$ mol dm^{-3}) and (2) H_2O ($c = 0{\cdot}007$ mol dm^{-3}) and metyrapone ($c = 0{\cdot}04$ mol dm^{-3}). Cell thickness, 1 cm; solvent, carbon tetrachloride

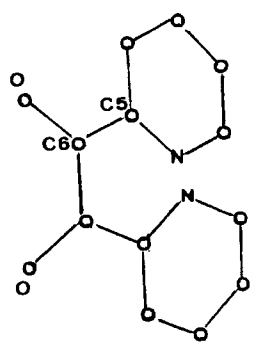

Figure 4. Molecular structure of di-2-pyridylglyoxal[19]

In this limited ΔH–$\Delta\nu_{OH}$ domain, the correlation of Badger–Bauer is valuable and can be written as

$$-\Delta H \text{ (kJ mol}^{-1}) = -17{\cdot}7 + 0{\cdot}089\,\Delta\nu_{OH} \text{ (cm}^{-1})\,(r = 0{\cdot}977) \quad (3)$$

The thermodynamic data and the ν_{OH} values do not differ greatly from the data reported for phenol–3-methylpyridine complexes in the same solvent.[4–6]. For the complex between phenol and this base, these values are $K^{298} = 60$ dm^3 mol^{-1}, $-\Delta H = 28{\cdot}4$ kJ mol^{-1}, $-\Delta S° = 660$ J K^{-1} mol^{-1} and $\Delta\nu_{OH} = 475$ cm^{-1}. Further, the slope and intercept of equation (1) are very similar to those calculated from Refs 4–6 for the pyridine complexes:

$$\log K^{298} = 5{\cdot}881 - 0{\cdot}42\,pK_a \quad (4)$$

In the di-2-pyridylglyoxal complexes, where complex formation undoubtedly occurs at the oxygen atom of the carbonyl function, the thermodynamic constants and $\Delta\nu_{OH}$ values are much weaker, and for the interaction with unsubstituted phenol re $K^{298} = 9$ dm^3 mol^{-1}, $-\Delta H = 17$ kJ Mol^{-1}, $-\Delta S° = 37$ J K^{-1} mol^{-1} and $\Delta\nu_{OH} = 145$ cm^{-1}.

These features strongly suggest that in the present case the hydrogen bond complexes are formed on one of the pyridine nitrogens. This conclusion is strengthened by the following spectroscopic observations:

(1) The ν_{OH} complex band is broad and characterized by several submaxima originating from Fermi resonance interaction with the internal modes of the proton donor. Similar band shapes were observed for the complexes involving phenols and heterocyclic nitrogen bases.[8,9]

(2) The $\nu_{C=O}$ vibration is shifted to 2–4 cm^{-1} to higher wavenumbers. This upward shift explained by the decreased delocalization in the carbonyl bond.

(3) Some vibrations of the pyridine ring are perturbed by complex formation. The ν_{8a} vibration observed at 1584 cm^{-1} in free metyrapone is observed at 1600 cm^{-1} in the 3,4,5-trichlorophenol complex, the ν_{19b} vibration

shifts from 1416 to 1425 cm^{-1} and the ν_1 vibration from 1023 to 1025 cm^{-1}. Owing to the overlapping with the phenol vibrations, only a few perturbations could be observed but very similar shifts were observed for the complexes involving pyridine and methanol[10] or diazines with phenols.[11]

The nitrogen atoms in each of the two pyridine rings have different proton acceptor ability. Pyridines with 3-alkyl substituents are more basic ($pK_a \approx 5{\cdot}5$)[12] than 3-acetylpyridine ($pK_a = 3{\cdot}23$).[13] This suggests that the nitrogen atom of the A ring is preferred over the nitrogen atom of the B ring. As previously noted,

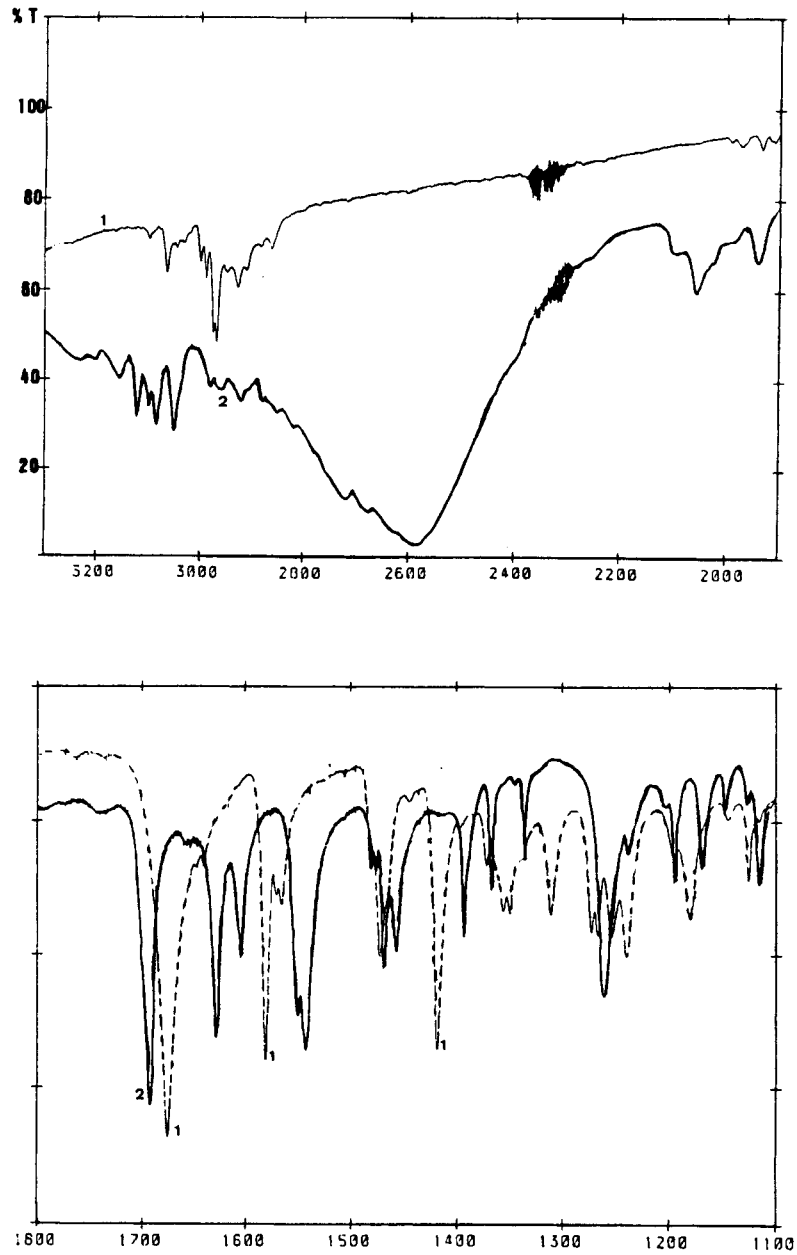

Figure 5. IR spectra (3300–550 cm^{-1}) of (1) metyrapone and (2) metyrapone–HCl adduct

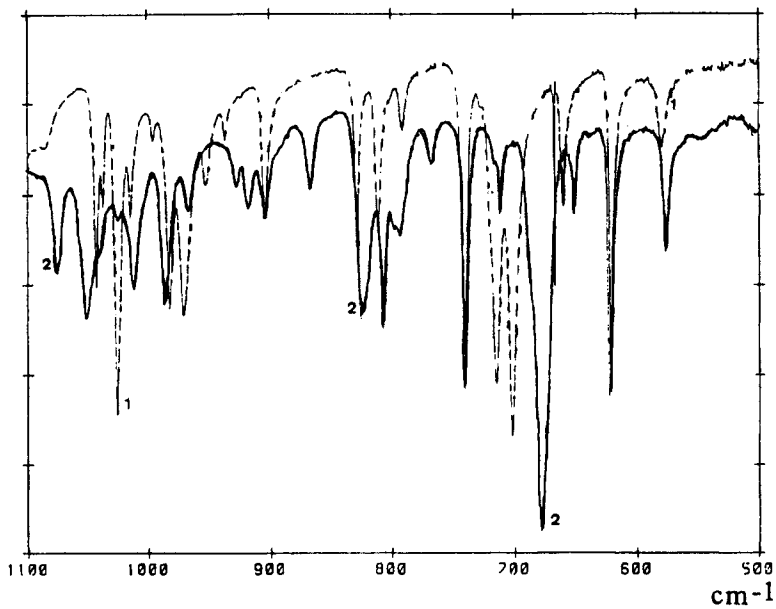

Figure 5. *Continued*

the thermodynamic data on metyrapone and 3-alkylpyridine complexes are very similar. Further, in the 4-acetylpyridine–phenol complex (no data are available for 3-acetylpyridine) where 95% of the hydrogen bond interaction takes place on the nitrogen atom of the heterocyclic ring, the thermodynamic data in carbon tetrachloride are[14] $K^{298} = 25{\cdot}1$ dm^{3}A mol^{-1}, $-\Delta H = 17{\cdot}2$ kJ mol^{-1} and $-\Delta S° = 31{\cdot}4$ J K^{-1} mol^{-1}. A small amount of complexes formed on the nitrogen atom of the B ring is not excluded, the metyrapone complexes being slightly weaker than those involving the same proton donor and 3-alkylpyridines. It is worth noting that the nitrogens in each of the two pyridyl groups of metyrapone are different in their susceptibility to chemical *N*-oxidation. 3-Chloroperoxybenzoic acid leads predominantly to the formation of the mono-*N*-oxide A and the di-*N*-oxide whereas the mono-*N*-oxide B is formed only in small amounts.[2]

Similar conclusions can be drawn for the interaction between metyrapone and water. As shown in Figure 3, the ν^{as} and ν^{s} stretching vibration of water in carbon tetrachloride are observed at 3708 and 3615 cm^{-1}. In the metyrapone complex, these vibrations are observed at 3696 cm^{-1} ($\Delta\nu^{as} = 12$ cm^{-1}) and 3385 cm^{-1} ($\Delta\nu^{s} = 232$ cm^{-1}). The values are very similar to those observed for the 3-methylpyridine–water complex ($\Delta\nu^{as} = 15$ cm^{-1}, $\Delta\nu^{s} = 232$ cm^{-1}).[15] The experimental shifts for water–carbonyl bases are much smaller.[16–18]

The difference between the hydrogen bond acceptor sites in di-2-pyridylglyoxal and metyrapone can be discussed as a function of the different structures of the two molecules. As shown in Figure 4, the angle between the two pyridine planes of di-2-pyridylglyoxal is 83°, the nitrogen and oxygen atoms being in a *trans* relationship around the C_5—C_6 bond.[19,20] In this structure, the accessibility of the lone pairs on the carbonyl oxygen is obviously greater than that of the pyridine nitrogens and, as stated previously, the hydrogen bonds involving a hydroxylic proton donor are formed on one of the carbonyl oxygens. Owing to the skew structure of di-2-pyridylglyoxal, a bifide hydrogen bond involving the two oxygen atoms seems very unlikely.[7]

As depicted in Figure 1, in metyrapone the nitrogen atoms are *anti* to the exocyclic oxygen, making the accessibility of the basic nitrogens greater. The lower availability of the carbonyl oxygen also clearly appears when considering the molecular volumes calculated from the Van der Waals radii.[1] It has also been shown that the availability of nucleophilic functional groups is a feature common to most direct inhibitors of cytochrome P-450 enzymes.[13] The availability in hydrogen bond formation processes has been discussed for other systems of biological interest.[21]

IR spectrum of the metyrapone–HCl adduct in the solid state

The IR spectra of metyrapone and its adduct with HCl, taken in a KBr suspension, are reported in Figure 5. The

experimental wavenumbers are listed in Table 2. Table 2 also indicates the probable assignment of the vibrations. This assignment was made by comparison of literature data for pyridine[22–24] and protonated pyridine,[25–27] which are also reported in Table 2.

As discussed previously, the two heteroaromatic rings of metyrapone are not equivalent. However, as shown by the data in Table 2, splitting of the pyridine (ring or CH) vibrations is observed only for a few vibrational modes of free metyrapone, namely the ν_{8b}, ν_{12}, ν_1 and ν_{11} vibrations, the splitting of the other vibrations being to small to be observed.

Protonation of pyridine derivatives brings about a spectacular increase in the frequencies of some vibrations, more particularly of the ν_{8a}, ν_{8b} and ν_{19b} ring modes. The perturbation of the vibrations are very similar in metyrapone. No double perturbation (arising from the free and protonated molecule) is observed and this strongly suggests that both nitrogen atoms are involved in the protonation. This double perturbation is observed when pyridinium ions form NH^+–N homoconjugated bonds with neutral pyridine molecules.[25] Only one very broad $\nu_{NH^+-Cl^-}$ and culminating at 2500 cm^{-1} is observed. The splitting of the δ_{NH^+}, γ_{NH^+} and ν_{19b} vibrations probably originates from the fact that the two pyridine moeities are not equivalent. For the other vibrational modes of protonated metyrapone, no splitting could be observed. New bands (called by Cook[28] Σ bands) are observed at 2095, 2065 and 1940 cm^{-1}. These absorptions are very often observed in the IR spectra of protonated nitrogen or oxygen bases.

An increase in the frequency of the $\nu_{C=N}$ vibration has also be observed when imines are protonated by strong acids.[29]

The weak frequency shifts of the skeletal C—C—C or rocking vibration of the methyl groups probably reflect small variations of the C—C—C or

Table 2. Spectroscopic data (cm^{-1}) and tentative assignment of the vibrations in metyrapone and its adducts with HCl and comparison with pyridine and pyridine–HCl

Metyrapone	Metyrapone–HCl	Assignment[a]	Pyridine[b]	Pyridine–HCl[c]
3058	3078	2 (ν_{CH})	3054	3081
3035	3045	7b (ν_{CH})	3036	3041
—	2590	$\nu_{NH^+-Cl^-}$	—	2500
	2095			
	2060	Σ bands[d]	—	—
	1940			
1675	1692	$\nu_{C=O}$	—	—
1581	1628	8l (R)	1583	1639
1566[e]	1604	8b (R)	1572	1605
1470	1473	19a (R)	1482	1483
1419	1540d	19b (R)	1439	1532
1480, 1476	1459	δ_{CH_3}	—	—
1375	1380	14 (R)	1375	1379
1351	1340	δ_{CH_3}	—	—
	1273, 1261	δ_{NH^+}	—	1213
1261	1313	3 (δ_{CH})	1288	1321
1181	1172	9a (δ_{CH})	1218	1196
1113	1128	r_{CH_3}	—	—
1043[e]	1013	12 (R)	1030	1027
1025[e]	1031	1 (R)	993	1003
981	986	Skel. C–C–C	—	—
971	976			
	928, 918	γ_{NH^+}	—	963
907	903	10a (γ_{CH})	891	883
821	817	Skel. C–C–C	—	—
812	807	Skel. C–C–C		
740[e]	683	11 (ν_{CH})	703	685
658	548	6b (δR)	652	637
619	619	6a (δR)	603	607

[a] ν = Stretching vibration; δ and γ in-plane and out-of-plane vibration, respectively; R = ring vibration; r = rocking vibration; skel = skeleton vibration.
[b] Refs 22–24.
[c] Refs 25–27.
[d] Ref. 28.
[e] Doublets. The wavenumber of the first component is indicated.

CH_3 valence angles brought about by protonation. This is difficult to discuss, however, because the structure of biprotonated metyrapone has not yet been determined.

CONCLUSIONS

It can be concluded that the hydrogen bonds formed between metyrapone and hydroxylic proton donors are predominantly formed on the nitrogen atoms of the heterocyclic ring A. Interaction with a strong acid leads to protonation of both nitrogen atoms. Similar conclusions have been reported for the *N*-oxidation with peroxyacids.

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