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Spectral and chromatographic properties of 2-methoxyphenylmetyrapone and its potential metabolites

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Abstract

In the search for new metyrapone derivatives as radioligands for the functional diagnosis of adrenal pathology, 2-methoxyphenylmetyrapone [2-MPMP,1-(2-methoxyphenyl)-2-methyl-2-(3-pyridyl)-1-propanone] (**1**), and related 2-substituted phenylmetyrapone derivatives, have been separated as potent inhibitors of adrenal 11β-hydroxylase, with high affinity for adrenal mitochondrial binding sites. Surprisingly, 2-[^{11}C]MPMP showed a rapid loss of the radioactive label, which prompted investigation of its metabolism. Synthetic 2-MPMP (**1**) and its seven potential metabolites (**2–8**) have been identified spectroscopically (^{1}H- and ^{13}C-NMR and mass spectrometry) and further characterised by chromatography (TLC and gradient reversed-phase HPLC). Chromatographic and mass analysis of urinary extracts from rats dosed with 2-MPMP have confirmed the major metabolites as 2-hydroxyphenylmetyrapone (2-OHPMP), **2**) and its *N*-oxide (2-OHPMP–NO, **6**), which are present predominantly as conjugates.

Keywords: Adrenal pathology; Metabolites; 2-Methoxyphenylmetyrapone; Spectral and chromatographic properties

1. Introduction

Metyrapone [2-methyl-1,2-(di-3-pyridyl)-1-propanone] (Table 1) is a potent competitive inhibitor of the adrenal cytochrome P-450 11β-hydroxylase enzyme system (P-$450_{11\beta}$) [1,2], and of other steroid hydroxylases [3], thus resulting in the inhibition of adrenocortical steroidogenesis. Although metyrapone also inhibits cytochrome P-450 enzymes in hepatic and extrahepatic sites, its specificity for these P-450s is substantially less than for P-$450_{11\beta}$ in the adrenal cortex [4]. Based on these observations, radiolabelled metyrapone and its analogues were studied as candidates for use as potential adrenal cortical imaging agents [5–8].

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Replacement of one of the pyridyl rings of metyrapone (ring A) by a phenyl ring lead to the identification of 2-methoxyphenylmetyrapone [2-MPMP,1-(2-methoxyphenyl)-2-methyl-2-(3-pyridyl)-1-propanone] (**1**) and related derivatives (**2**, **9**) as potent inhibitors of adrenal 11β-hydroxylase (see Table 1) [9]. 2-Hydroxyphenylmetyrapone (**2**) is a precursor for labelling with carbon-11, a positron emitter [10,11]. Since 2-[^{11}C]MPMP showed a rapid loss of the radioactive label in preliminary biodistribution studies, more detailed metabolic studies have been carried out.

The aim of this paper was to synthesise a novel series of 2-substituted phenylmetyrapones considered to be potential metabolites of 2-MPMP (Table 1). For example, based on metabolism studies of metyrapone [12,13], 2-MPMP (**1**) would be expected to metabolise to 2-OHPMP (**2**) via a simple *O*-demethylation, 2-MPMPOL (**3**) and 2-OHPMPOL (**4**) via keto reduction of 2-MPMP and 2-OHPMP respectively, and *N*-oxides of these four compounds via *N*-oxidation at the pyridyl nitrogen.

In this paper, the spectral and chromatographic properties of 2-MPMP and the 2-substituted phenylmetyrapone derivatives are described in detail. The availability of the synthetic reference samples allowed the identification of the major urinary metabolites in rats dosed with 2-MPMP (**1**) by chromatography and mass analysis.

2. Experimental

2.1. Materials

The parent compounds (**1**, **2**, and **9**) were kindly supplied by Dr. I. Zolle (Department of Nuclear Medicine, University of Vienna, Austria). All solvents used in chromatographic analyses were of HPLC grade (BDH, Poole, UK and Merck, Darmstadt, Germany). Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). β-Glucuronidase/arylsulfatase (*Helix pomatia*, 100 000 Fishman units ml^{-1}) was obtained from Boehringer Mannheim (Mannheim, Germany). Animals (male Sprague–Dawley rats) were supplied by the Animal House, The Chinese University of Hong Kong.

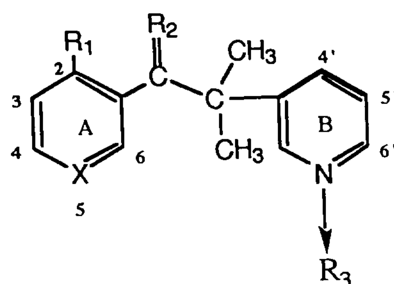
2.2. Synthesis of potential metabolites

Metabolites of (**1**) and (**2**) were synthesised according to methods described previously [7,14]. The "metyrapols" of (**1**) and (**2**) were obtained by reduction of the keto functional group with sodium borohydride to afford 2-methoxyphenylmetyrapol (2-MPMPOL, **3**) and 2-hydroxyphenylmetyrapol (2-OHPMPOL, **4**) respectively. Oxidation of the parent compounds (**1**) and (**2**) with *m*-chloroperoxybenzoic acid produced the corresponding *N*-oxides, 2-methoxyphenylmetyrapone-*N*-oxide (2-MPMP-NO, **5**) and 2-hydroxyphenylmetyrapone-*N*-oxide (2OHPMP-NO, **6**) respectively. Selective keto reduction of compounds (**5**) and (**6**) yielded the metyrapol-*N*-oxides, 2-methoxyphenylmetyrapol-*N*-oxide (2-MPMPOL-NO, **7**) and 2-hydroxyphenylmetyrapol-*N*-oxide (2-MPMPOL-NO, **8**) respectively. 2-Bromophenylmetyrapone (2-BrPMP, **9**) was used as an internal standard for the HPLC assay.

2.3 Collection of urine samples

Male Sprague–Dawley rats ($n = 4$, 250–300 g) were dosed with 2-MPMP (i.v. 50 mg kg^{-1}) as the HCl salt in normal saline. Faeces-free urine samples were collected over periods of 0–24 and 24–48 h and stored at −20°C until analysis. Bulked urine samples (50 ml) were combined and acetate buffer (pH 6, 20 ml) containing β-glucuronidase/arylsulfatase (1 ml) was added. The sample was then maintained at 37°C for 16 h in a water bath to hydrolyse any drug- or drug metabolite conjugates. Identical urine samples in the absence of β-glucuronidase/arylsulfatase were used to assess the presence of "free" drug and drug metabolites. The aqueous phases were alkalinised (pH 10) and then extracted with dichloromethane (3 × 200 ml), evaporated to dryness and reconstituted in methanol (1 ml). Analysis of 2-MPMP and its potential metabolites was by silica TLC and reversed-phase HPLC methods with gradient elution.

Table 1
Metyrapone and its 2-substituted phenyl derivatives: structure and chromatographic properties



X = N, R_1 = H, R_2 = O, R_3 = nothing	Metyrapone
X = CH, R_1 = H, R_2 = O, R_3 = nothing	Phenylmetyrapone
X = CH, R_1 = OCH_3, OH, Halogen, etc., R_2 = O, R_3 = nothing	2-Substituted phenylmetyrapone

Compound	R_1	R_2	R_3	TLC[a] (R_f)	HPLC[b] (t_R, min)
1 2-MPMP	OCH_3	O	–	0.65	12.64
2 2-OHPMP	OH	O	–	0.73	15.00
3 2-MPMPOL	OCH_3	H,OH	–	0.35	9.58
4 2-OHPMPOL	OH	H,OH	–	0.17	5.17
5 2-MPMP-NO	OCH_3	O	O	0.25	7.87
6 2-OHPMP-NO	OH	O	O	0.35	8.60
7 2-MPMPOL-NO	OCH_3	H,OH	O	0.35	7.33
8 2-OHPMPOL-NO	OH	H,OH	O	0.00	4.29
9 2-BrPMP	Br	O	–	0.71	16.49

[a] TLC solvent system: $CHCl_3$–MeOH–NH_3 (sp.gr. 0.88) (97.5:2.4:0.1 v/v/v).
[b] HPLC conditions as in text.

2.4 TLC

Pre-coated glass plates (20 × 20 cm^2, silica gel $60F_{254}$, E. Merck, Darmstadt, Germany) were used both qualitatively and preparatively to check the purity of authentic standards, and to identify and separate urinary metabolites. The plates were cleaned prior to use by running in methanol, followed by toluene, and reactivated in an oven at 100°C for 1 h [12]. Concentrated methanolic solutions of the synthetic reference compounds and/or urinary extracts were applied as spots to the TLC plates at 1–1.5 cm intervals or as bands (for preparative TLC). The plates were developed using chloroform–methanol–ammonia (sp. gr. 0.88) (97.5:2.4:0.1, v/v/v) as the solvent system. The recorded R_f values are shown in Table 1.

Separated bands (preparative-TLC) were carefully removed and the metabolites eluted with methanol containing 1% triethylamine. Qualitative identification of the eluates involved TLC, HPLC and mass analyses, with synthetic compounds as references.

2.5. HPLC

HPLC analyses were carried out on a Hewlett-Packard 1050 chromatograph using a Spherisorb ODS-2 column (25 × 0.46 cm^2). Gradient elution was employed with solvent system A, acetonitrile–water–triethylamine–acetic acid (27.3:69.1:0.9:2.7, v/v/v) and solvent system B, methanol. The gradient program used was as follows: initial and 0–4 min, A:B = 74:26; 4–10 min change linearly to A:B = 50:50 and maintain for 10–16 minutes; and at 16 min return to initial

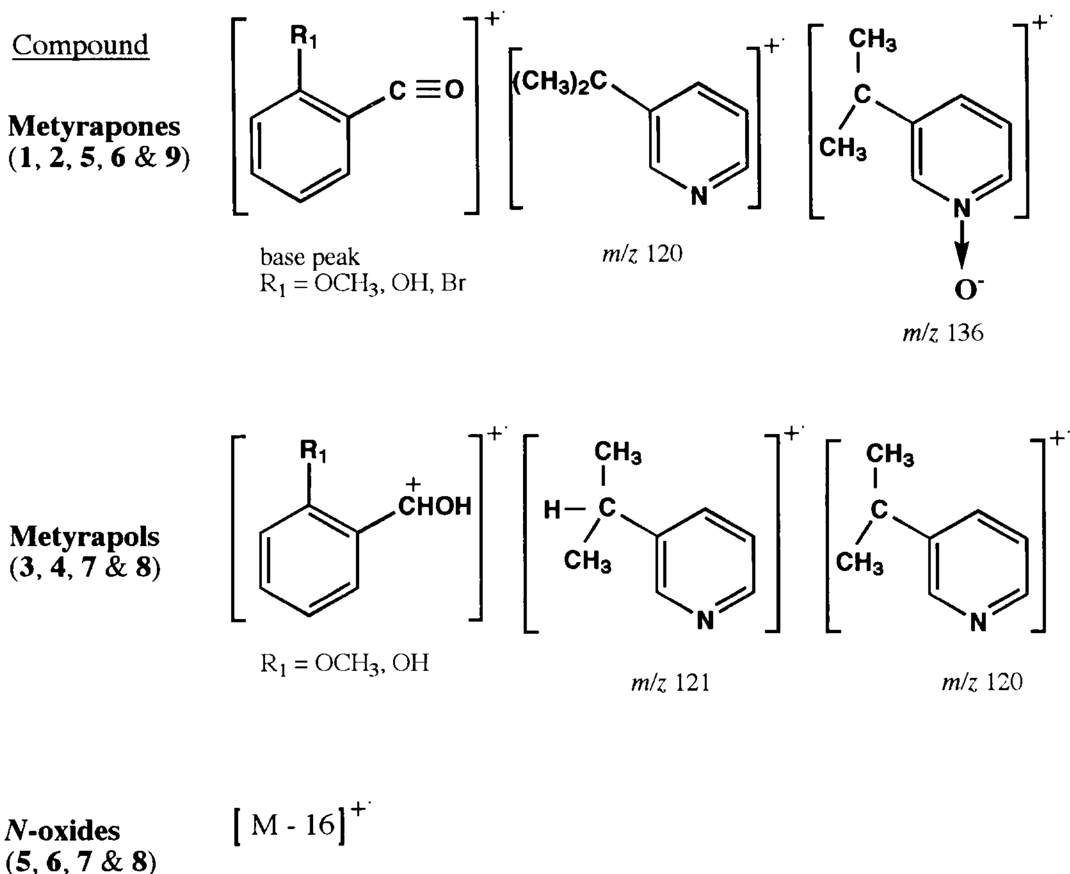


Fig. 1. Major fragment ions from mass analysis.

conditions (A:B = 74:26). The flow rate was kept constant at 1.25 ml min^{-1} and detection was by UV absorbance at 260 nm. The retention times (t_R) of the compounds are given in Table 1.

2.6. Mass spectrometry

High resolution mass spectra were recorded on a VG 70–70 high resolution mass spectrometer and low resolution mass spectra on a fourier transform (FT) ion cyclotron resonance spectrometer. In general, mass spectra were obtained by electron impact (EI) mode with an accelerating voltage of 70 eV. Samples were dissolved in HPLC-grade methanol prior to evaporation on the direct insertion probe filament. Data were generated with 64 scans over the mass range 43–1000 u. Major mass fragment ions are presented in Fig. 1.

2.7. ^{1}H and ^{13}C-NMR spectrometry

^{1}H- and ^{13}C-NMR spectra were performed on an ARX-500 superconducting high resolution FT-NMR spectrometer (500 MHz) running Bruker UX-NMR version 1.0 software. Pulse widths and relaxation delays of 1 μs and 1 s respectively for ^{1}H-NMR and 12.4 μs and 2 s for ^{13}C-NMR

Table 2
^{1}H-NMR chemical shift assignments (δ ppm)[a]

Proton no.	Compound								
	1	2	3	4	5	6	7	8	9
3	6.72, d (7.20)	6.97, d (8.20)	6.75, d (8.30)	6.78, m ([b])	6.81, d (8.35)	6.99, d (7.67)	6.80, d (8.25)	6.76, m ([b])	7.55, d (7.94)
4	7.28, pt[c] (7.54)	7.29, m ([b])	7.20, m ([b])	7.15, m –	7.20, pt (7.22)	7.23, pt (7.12)	7.03, m ([b])	7.07, m –	7.08, pt (7.62)
5	6.77, m ([b])	6.52, pt (7.77)	6.87, pt (7.56)	6.78, m ([b])	6.90, m ([b])	6.61, pt (7.89)	6.90, d (7.42)	6.76, m ([b])	6.97, pt (7.78)
6	7.65, d (7.95)	7.53, d (7.92)	7.00, d (7.33)	7.22, m –	7.35, d (8.65)	7.12, d (8.37)	7.12, d (6.48)	7.10, d (8.22)	7.76, d (8.00)
2′	8.61, bs	8.62, bs	8.55, bs	8.53, bs	8.27, bs	8.32, bs	8.23, bs	8.25, bs	8.68, bs
4′	7.22, d (8.38)	7.06, d (8.28)	7.57, d (8.30)	7.64, d (7.97)	7.26, d (7.43)	7.05, d (7.91)	7.24, m –	7.30, d (7.60)	7.31, m –
5′	6.77, m ([b])	7.29, m ([b])	7.20, m ([b])	6.78, m ([b])	6.90, m ([b])	7.36, pt (7.84)	7.03, m ([b])	6.76, m ([b])	6.35, m –
6′	8.47, d (4.30)	8.53, d (4.80)	8.43, d (3.42)	8.30, d (4.88)	8.10, d (6.19)	8.13, d (6.26)	8.08, d (7.39)	8.11, bs –	8.57, bs –
($C\underline{H}_3$)	1.65, s	1.69, s	1.37, s	1.38, s	1.65, s	1.67, s	1.37, s	1.35, s	1.68, s
$OC\underline{H}_3$	3.66, s	–	3.54, s	–	3.66, s	–	3.65, s	–	–
2-O$\underline{H}$	–	12.48, bs	–	8.63, bs	–	12.31, bs	–	nv[d]	–
CHO$\underline{H}$	–	–	2.19, bs	2.01, bs	–	–	2.70, bs	nv	–
C$\underline{H}$OH	–	–	5.01, s	4.91, s	–	–	5.00, s	4.90, s	–

[a] Values in parentheses represent the observed coupling constants in the 1-D ^{1}H-NMR spectra.
[b] Multiplet unresolved, but integrates to correct number of hydrogens.
[c] Pseudo-triplet; signal is a doublet of doublets but appears as a triplet due to equal coupling constants.
[d] Hydroxyl peak not visible.

spectra were used. Residual $CHCl_3$ in $CDCl_3$ was used as internal standard (assigned as 7.27 ppm and 77.00 ppm downfield from TMS respectively). ^{13}C-NMR distortionless enhancement by polarization transfer (DEPT) (135 and 90) experiments were also carried out. Spectral assignments are summarised in Table 2 for ^{1}H-NMR and Table 3 for ^{13}C-NMR. Data are reported as follows: chemical shift (δ/ppm), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet).

3. Results and discussion

3. TLC and HPLC

2-Methoxyphenylmetyrapone and its derivatives (**1**–**9**) were unequivocally identified by a

Table 3
^{13}C-NMR (DEPT) chemical shift assignments (δ ppm)

Carbon no.	Compound								
	1	2	3	4	5	6	7	8	9
1	129.90	116.60	128.36	132.48	129.29	116.23	127.98	132.49	138.12
2	155.46	164.02	156.68	167.79	155.43	164.11	156.63	167.76	118.67
3	110.85	118.06	110.37	117.35	110.97	118.41	110.48	118.52	130.39
4	134.46	135.61	129.34	129.70	131.51	136.12	129.20	130.88	134.64
5	120.21	119.15	120.15	118.68	120.59	119.46	120.36	120.56	126.43
6	127.35	131.93	128.70	128.82	127.61	131.36	129.04	128.82	126.60
2′	148.30	148.36	148.27	148.42	138.11	137.68	139.16	140.07	148.07
3′	139.38	141.59	141.80	141.16	144.12	145.83	146.23	146.30	141.36
4′	130.88	133.24	135.85	135.11	125.19	126.21	126.14	124.28	133.32
5′	122.90	123.81	121.80	123.04	124.97	123.93	124.33	123.33	123.46
6′	147.78	146.90	146.01	146.95	137.03	136.60	136.45	134.81	148.24
$(\underline{C}H_3)_2$	25.78	28.29	25.25	25.72	25.79	27.91	25.19	23.77	25.46
$\underline{C}(CH_3)_2$	51.12	50.77	42.91	43.56	50.96	50.53	43.08	42.70	51.00
$O\underline{C}H_3$	55.11	–	54.93	–	55.17	–	55.07	–	–
$\underline{C}HOH$	–	–	77.69	83.51	–	–	76.92	76.80	–
$\underline{C}=O$	208.55	208.42	–	–	207.30	206.55	–	–	206.62

combination of chromatography, ^{1}H and ^{13}C-NMR, and mass spectroscopic analyses. The synthesised reference compounds were ascertained to be pure spectroscopically (NMR) and by TLC and HPLC. The R_f and t_R values obtained are listed in Table 1. As expected, the highly polar 2-hydroxyphenylmetyrapol-*N*-oxide (**8**) remained at the baseline in TLC. However, it was found that 2-methoxyphenylmetyrapone (**1**) ($R_f = 0.65$) migrated slower than its more polar 2-hydroxy analogue (**2**) ($R_f = 0.73$). Similarly, 2-MPMP-NO (**5**) ($R_f = 0.25$) migrated slower than the more polar 2-OHPMP-NO (**6**) ($R_f = 0.35$). This trend was also observed during HPLC analysis. An explanation for this was readily evident when NMR spectra of these compounds were carefully studied (see later).

All nine compounds were resolved under reversed-phase HPLC conditions using gradient elution. In general, the elution order was in accordance with the polarity of the compound, with the most polar 2-OHPMPOL-NO (**8**) (t_R = 4.29 min) being eluted first and 2-BrPMP (**9**) (t_R = 16.49 min) last. 2-BrPMP was studied as this would seem to be the most ideal internal standard for future quantitative studies. As with TLC analysis, the elution times for 2-MPMP (**1**) (t_R = 12.64 min) and its *N*-oxide (**5**) (t_R = 7.87 min) were shorter than those for their more polar 2-hydroxy analogues: 2-OHPMP (**2**) (t_R = 15 min) and 2-OH-PMP-NO (**6**) (t_R = 8.60 min). This can be explained by the formation of an intramolecular hydrogen bond between the 2-hydroxyl group on the phenyl ring and the carbonyl oxygen, rendering the 2-hydroxy compounds (**2** and **6**) more lipophilic in character than the 2-methoxy analogues (**1** and **5**). The presence of H-bonding was further confirmed by ^{1}H-NMR spectral data (see later).

3.2. Mass spectrometry

The results from mass analysis (Fig. 1) agree well in general with the data obtained from an earlier study with metyrapone and its metabolites [12]. Direct EI low resolution mass analysis of the 2-substituted phenylmetyrapones within this novel series of compounds, together with their *N*-oxides (**1**, **2**, **5**, **6** and **9**), produced a base ion peak, the aromatic acylium ion ($ArC{\equiv}O^{+\cdot}$), with *m/z* consistent with cleavage of the bond β to the phenyl ring A (Table 1).

For each of the 2-substituted phenylmetyrapols (**3**, **4**, **7** and **8**), no molecular ion was observed under EI conditions. However, in all four compounds, an ion peak at *m/z* 121 was evident,

indicating the presence of the $[C_8H_{11}N]^{+\cdot}$ peak, which concurs with the data obtained from the earlier metyrapone study [12]. This peak originates from the mobile hydrogen of the C-1 hydroxyl group becoming attached to the adjacent quarternary C-2 atom.

With the *N*-oxides, only 2-MPMP-NO (**5**) and 2-OHPMP-NO (**6**) displayed molecular ions under EI conditions with intensities of 4% and 16% respectively. A weak to reasonably intense $[M-16]^+$ ion peak due to the loss of the *N*-oxide oxygen atom was also observed with intensities varying from 1% for 2-OHPMPOL-NO (**8**) to 29% for 2-MPMP-NO (**5**). Other ion peaks present in all four compounds were those at *m/z* 136 and 120, fragments resulting from cleavage of the bond β to the two aromatic rings and the subsequent loss of oxygen atom respectively (see Fig. 1).

The mass spectrum for 2-bromophenylmetyrapone (**9**) displayed the typical isotopic effect of ^{79}Br and ^{81}Br in the ratio of approximately 1:1 abundance. The fragmentation for this compound was analogous to that of 2-MPMP (**1**).

3.3. NMR

Expected ^1H- and ^{13}C-NMR chemical shifts were estimated by extrapolations from established empirical rules and data tables using sensible structural analogues, and assuming that substituent effects are additive. Proton assignments were based on relative chemical shifts and coupling constants, and ^{13}C DEPT (135 and 90) experiments enabled the straightforward assignments of methyl, methine and quaternary carbons. Spectral assignments for compounds (**1–9**) are summarized in Tables 2 and 3.

The presence of intramolecular hydrogen bonds in 2-OHPMP (**2**) and 2-OHPMP-NO (**6**) was confirmed by the observed downfield chemical shifts for the 2-hydroxyl group at δ_H 12.48 and 12.31 ppm respectively. A non-hydrogen-bonded phenoxy hydrogen would normally resonate at δ_H 4.5–10 ppm [15]. As a consequence of such hydrogen bonding, a shielding effect at the C-1 atom of both compounds **2** (δ_C 116.60) and **6** (δ_C 116.23) was also observed, an effect clearly absent in other structural analogues.

The ^{13}C spectrum for 2-BrPMP (**9**) exhibited a large upfield shift at the C-2 atom (δ_C 118.67 ppm), which demonstrated the "heavy halogen" effect caused by the bromine substituent [16]. This is in contrast to the typical downfield shifts normally associated with substituents with similarly high electronegativity values. For example, the observed chemical shifts for C-2 with either the methoxy (**1**) or hydroxyl (**2**) substituent were 155.46 and 164.02 ppm respectively. It is worth noting that strong upfield shifts at the C-3 atom (ranging from 10–18 ppm from benzene at δ_C 128.5) and to a lesser extent at C-5 ($\approx$10 ppm) were observed for all compounds (**1–8**), with the exception of the 2-bromo analogue, as a result of the powerful positive mesomeric effect exerted at the ortho and para positions by the methoxy- and hydroxyl-substituents.

The inductive and mesomeric electron-withdrawing properties of the ring nitrogen in the pyridyl ring result in a partial positive charge at the α positions and, to a lesser extent, the γ position. This is reflected in the downfield shifts of both the carbon and proton at the **2′** and **6′** (α) positions of the pyridyl ring, relative to equivalent positions on a phenyl ring.

Not surprisingly, the presence of the oxygen at the pyridyl nitrogen in compounds (**5**, **6**, **7** and **8**) caused an upfield shift in both the proton ($\approx$0.3 ppm) and carbon ($\approx$10 ppm) spectra for positions 2′ and 6′ when compared with the metyrapone (**1** and **2**) and metyrapol (**3** and **4**) parent compounds. The mesomeric release by the oxygen, which is analogous to a phenoxide oxygen, increases the electron density at positions 2′ and 6′ (α) and position 4′ (γ), although the upfield shifts for the proton and carbon at this latter position were found to be smaller.

3.4. Analysis of urine

TLC and HPLC analyses of the deconjugated urines from rats dosed with 2-MPMP (**1**) resulted in the isolation and identification of two major metabolites in urine, 2-OHPMP (**2**) and 2-OHPMP-NO (**6**), which correspond well with the authentic compounds (**2**) ($R_f = 0.79$, $t_R = 14.85$ min) and (**6**) ($R_f = 0.43$, $t_R = 8.54$ min). The same

metabolites were also present in the "free" (non-conjugated) form, but in very small amounts judging by the intensity of TLC spots under UV light, or by peak areas when analysed by HPLC.

The identities of the major metabolites were subsequently confirmed by high resolution mass spectrometry: 2-OHPMP (**2**), calcd. 241.110, found 241.117; and 2-OHPMP-NO (**6**), calcd. 257.105, found 257.112. The nature of the intact conjugates (i.e. glucuronides and/or sulfates) has not yet been confirmed, but is the subject of ongoing studies.

The majority of the drug/metabolites were excreted within 24 h, indicating a rapid metabolism and excretion of 2-MPMP. Although only two of the authentic compounds for which spectral data are reported herein were eventually found as urinary metabolites in the rat, the other compounds may be present as metabolites in other species. Species and sex differences in the metabolism of metyrapone have previously been reported [17].

Studies are ongoing to identify, synthesise and chemically characterise other 2-substituted phenyl derivatives of metyrapone as metabolically stable candidates for use as adrenal cortical imaging agents.

Acknowledgments

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References

[1] G.W. Liddle, D. Island, E.M. Lance and A.P. Harris, J. Clin. Endocrinol. Metab., 18 (1958) 906–912.
[2] T.E. Temple and G.W. Liddle, Annu. Rev. Pharmacol., 10 (1970) 199–218.
[3] N.P. Sanzari and F.G. Peron, Steroids, 8 (1966) 929–945.
[4] A.G. Hildebrandt, Biochem. J., 125 (1971) 69.
[5] J.L. Napoli and R.E. Counsell, J. Med. Chem., 20 (1977) 762–766.
[6] I. Zolle, W. Robien, H. Bergmann and R. Höfer, in R. Höfer and H. Bergmann (Eds), Radioaktive Isotope in Klinik and Forschung, Band 15 (2), Verlag H. Egermann, Vienna, 1982, pp. 589–595.
[7] W. Robien and I. Zolle, Int. J. Appl. Radiat. Isot., 34 (1983) 907–914.
[8] S.J. Hays, M.C. Tobes, D.L. Gildersleeve, D.M. Wieland and W.H. Beierwaltes, J. Med. Chem., 27 (1984) 15–19.
[9] I. Zolle, J. Yu, W. Robien, W. Woloszczuk and R. Höfer, J. Labelled Compd. Radiopharm., 30 (1991) 420–422.
[10] I. Zolle, C. Halldin, J. Yu and C.G. Swahn, J. Labelled Compd. Radiopharm., 32 (1993) 547–549.
[11] J. Yu, I. Zolle, J. Mertens and F. Rakias, Nucl. Med. Biol., 22 (1995) 257–262.
[12] L.A. Damani, P.A. Crooks and D.A. Cowan, Biomed. Mass. Spectrum., 8 (1981) 270–277.
[13] J.I. Usansky and L.A. Damani, Drug Metab. Dispos., 20 (1992) 64–69.
[14] P.A. Crooks, L.A. Damani and D.A. Cowan, J. Pharm. Pharmacol., 33 (1981) 309–312.
[15] D.H. Williams and I. Fleming, in Spectroscopic Methods in Organic Chemistry, 4th edn., McGraw-Hill, London, 1989.
[16] R.M. Silverstein, G.C. Bassler and T.C. Morrill, in Spectometric Identification of Organic Compounds, 5th edn., John Wiley & Sons, Chichester, UK, 1991.
[17] P.A. Dixon, S.E. Okereke and M.C. Enwelum, Biochem. Physiol. C., 81 (1985) 241–243.