242. The Structure of Diastereomeric Oxaprotiline Metabolites. An NMR Study

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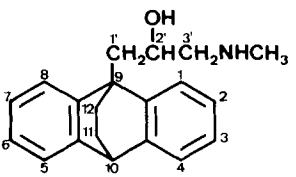
Summary

The structures of phenolic metabolites of oxaprotiline, obtained from a study with rats and dogs, are assigned with NMR-spectroscopic methods in combination with MS data and an X-ray structure analysis of synthetic (9*R**,2'*S**)-2-chlorooxaprotiline. The metabolites are shown to be (9*R**,2'*R**)-2-hydroxy-, 3-hydroxy-, and 2-hydroxy-3-methoxyoxaprotiline, and (9*R**,2'*S**)-2-hydroxy-, 3-hydroxy-, and 3-hydroxy-2-methoxyoxaprotiline, as well as 2,6-dihydroxyoxaprotiline.

Introduction. – Oxaprotiline (**1**, *α-[(methylamino)methyl]-9,10-ethanoanthracene-9(10H)-ethanol)*, a potent and highly selective inhibitor of noradrenaline uptake [1], is the 2'-hydroxy derivative of the antidepressant drug maprotiline **2** (*Ludiomil®*). In the



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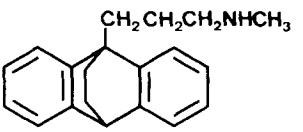

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course of an investigation of the biotransformation of oxaprotiline [2], phenolic metabolites were obtained. Due to the chiral center C(2') in the side chain, the two aromatic rings in **1** are diastereotopic. Metabolic hydroxylation, therefore, leads to diastereomers, which are indeed observed. For a structure elucidation of these diastereomers, the position of the OH-group at the aromatic rings and the spatial position of the hydroxylated ring with respect to the chiral center C(2') had to be deduced. This was carried out, as shown in this paper, by assigning the chemical shifts of the protons in the two aromatic rings of the parent compound **1** by various NMR methods, and correlating these chemical shifts with the chiral center by a comparison with the aromatic proton shifts of the synthetic (9*R**,2'*S**)-2-chloro-derivative of **1**, the configuration of which was established by X-ray structure analysis. Use of substituent effect data led to the structure of the individual metabolites.

Chemical Shift Assignment of the Aromatic Protons of 1. – The chemical shifts are collected in the *Table*. CD_3OD was used as solvent, as it generally dissolves also the non-derivatized conjugates (*e.g.* glucuronides). The assignment of the protons was achieved in the following manner: the proton at lowest field (7.38 ppm) shows only one *ortho*-coupling and is, therefore, either H–C(1), H–C(4), H–C(5) or H–C(8). It is labelled H–C(1) here for convenience and is used as starting point of the chemical shift assignment. The protons H–C(1), H–C(4), H–C(5) and H–C(8), as a group, can be distinguished from the protons H–C(2), H–C(3), H–C(6) and H–C(7) by the number of *ortho*-couplings. The protons H–C(1) and H–C(8) can be differentiated against H–C(4) and H–C(5) through the vicinal coupling between H–C(10) and C(4) or C(5) in a proton-coupled ^{13}C-NMR spectrum, whereby the ^{1}H chemical shifts are connected with the ^{13}C chemical shifts by a heterocorrelated 2-D NMR spectrum. The two four-spin systems of the aromatic rings are separated by a COSY experiment. The assignment of H–C(2) *vs.* H–C(3), finally, is established by irradiating H–C(1), and that of H–C(6) *vs.* H–C(7) by irradiating H–C(5). Irradiation of H–C(10) corroborated the assignment of H–C(1) and H–C(8) *vs.* H–C(4) and H–C(5), since the extended 'zig-zag' coupling $^5J(1,10)$ is larger (*ca.* 0.6 Hz) than the *ortho*-benzylic coupling $^4J(4,10)$ (*ca.* 0.3 Hz) [3].

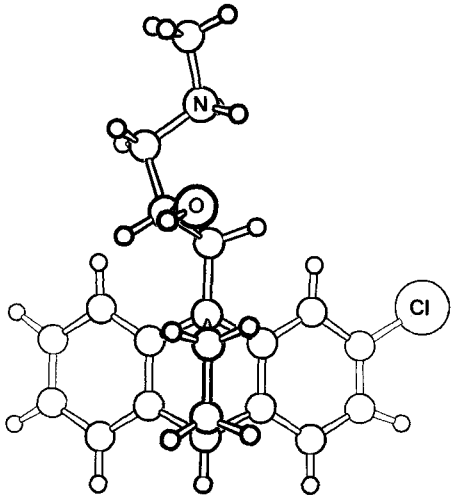

Figure. *Perspective view of the (9S,2'R)-enantiomer of the cation of* **3**·HCl (Plot program SCHAKAL [6]). The crystal is racemic.

With the aromatic proton shift data of **1** ascertained, the chemical shifts of the two diastereomeric 2-chloro derivatives of **1** (**3** and **5**, preparation and separation *cf. Exper. Part*) were easily assigned *(Table)* allowing for the (small) substituent effect of the Cl-atom [5]. In **3**, the low-field signal H–C(1) is *ortho* to the Cl-substituent, as shown by the absence of the *ortho*-coupling $^3J(1,2)$, whereas in **5**, the analogous low-field signal is very similar to that of **1**, since the ring containing H–C(1) is not substituted. The remaining shifts and splitting patterns are also in agreement with the structural assignment given in the *Table*. The diastereomer **3** is the (9*R**,2'*S**)-2-chloro-derivative

Table. *Aromatic Proton Chemical Shifts of Oxaprotiline Derivatives in CD_3OD*

Compound	Chemical shift [ppm]							
	H–C(1)	H–C(2)	H–C(3)	H–C(4)	H–C(5)	H–C(6)	H–C(7)	H–C(8)
Oxaprotiline (**1**)[a])	7.38	7.09	7.04	7.23	7.26	7.05	7.08	7.14
	(122.07)	(126.26)	(126.22*)	(124.09)	(124.35)	(126.24*)	(126.16)	(121.95)
Maprotiline (**2**)[b])	7.25	7.09	7.04	7.23	7.23	7.04	7.09	7.25
	(122.08)	(126.14)	(126.11)	(124.14)	(124.14)	(126.11)	(126.14)	(122.08)
(9R*,2'S*)-*2-Chlorooxaprotiline* (**3**)	7.40	–	7.05	7.21	7.26	7.08	7.11	7.15
(9R*,2'S*)-*2-Hydroxyoxaprotiline* (**4**)	6.83	–	6.49	7.04	7.24	7.05	to	7.11
(9R*,2'R*)-*2-Chlorooxaprotiline* (**5**)[c])	7.40	7.12	7.08	7.25	7.23	7.06	–	7.16
(9R*,2'R*)-*2-Hydroxyoxaprotiline* (**6**)[c])	7.34	7.09	7.05	7.22	7.07	6.50	–	6.57
(9R*,2'S*)-*3-Hydroxyoxaprotiline* (**7**)	7.15	6.51	–	6.74	7.25	7.03	to	7.12
(9R*,2'R*)-*3-Hydroxyoxaprotiline* (**8**)[d])	7.33	7.10	7.05	7.24	6.76	–	6.51	6.91
3,6-Dihydroxyoxaprotiline (**9**)	7.12	6.50	–	6.72	6.74	–	6.50	6.88
(9R*,2'S*)-*3-Hydroxy-2-methoxyoxaprotiline* (**10**)	7.03	–	–	6.76	7.23	7.05	to	7.10
(9R*,2'R*)-*2-Hydroxy-3-methoxyoxaprotiline* (**11**)[c])[d])	7.32	7.08	7.05	7.22	6.94	–	–	6.61

[a]) Other proton resonances: 4.29 (H–C(2')); 4.26 (H–C(10)); 2.94 (J(3',3') = 12.5, J(2',3') = 3.2, H–C(3')); 2.85 (J(3',3') = 12.5, J(2',3') = 9.0, H–C(3')); 2.67 (J(1',1') = 15.0, J(1',2') = 3.0, H–C(1')); 2.56 (J(1',1') = 15.0, J(1',2') = 6.0, H–C(1')); 2.49 (NCH_3); 1.80, 1.75 and 1.63 (H–C(11), H–C(12)). Chemical shifts of the corresponding aromatic C-atoms in brackets, other C-resonances: 146.7, 146.5, 146.4, 146.1 (non-protonated aromatic C-atoms); 68.1 (C(2')), 60.4 (C(3')); 45.8 (C(9)); 45.6 (C(10)); 38.5 (C(1')); 36.1 (NCH_3); 31.1 (C(12)); 28.9 (C(11)). For the ^{13}C-NMR data of 9,10-dihydro-9,10-ethanoanthracene see [4].

[b]) Other proton resonances: 4.24 (H–C(10)); 2.85 (H–C(3')); 2.48 (H–C(1')); 2.46 (NCH_3); 1.98 (H–C(2')); 1.78 (H–C(11)); 1.54 (H–C(12)). Chemical shifts of the corresponding aromatic C-atoms in brackets, other C-resonances: 146.5, 146.2 (non-protonated aromatic C-atoms); 53.6 (C(3')); 45.8 (C(9)); 45.7 (C(10)); 36.2 (NCH_3); 30.8 (C(12)); 29.8 (C(1')); 28.7 (C(11)); 25.7 (C(2')).

[c]) To facilitate comparisons, the substituent is given the 7-position in the data representation.

[d]) To facilitate comparisons, the substituent is given the 6-position in the data representation.

of **1** as shown by an X-ray structure analysis of its hydrochloride (see *Fig.*). This result defines the spatial position of H–C(1) with respect to the configuration of C(2') so that a substitution in the aromatic ring containing H–C(1) leads to the (9*R**,2'*S**)-diastereomer.

The Structures of the Phenolic Metabolites of 1. – With the ^{1}H-shift data of **1** correlated with the chiral center C(2'), the structures of the diastereomeric phenolic metabolites, obtained in a study with rats and dogs [7] can be assigned *(Table)*. The molecular weight of the metabolites and, consequently, the nature of the metabolic modification was determined by mass spectrometry *(Exper. Part)*.

The positions of the OH-group were deduced with the aid of the substituent effects of the OH-group in phenol [5]. The results were corroborated by calculating the shifts of **4** and **6–8** with OH-substituent effect data obtained from an analysis of the NMR spectra of maprotiline **2** *(Table)* and its phenolic metabolites [8], *i.e.* using the OH-group substituent effects of the same polycyclic aromatic system. The calculated values obtained are within 0.02 ppm of the observed ones with only one exception (0.05 ppm). Two hydroxy, methoxy metabolites were also isolated [7], and the substituent positions were assigned by a comparison with the aromatic proton shifts of 2-methoxy phenol and the 3-hydroxy-2-methoxy derivative of **2** [8]. Interestingly, only the 3-hydroxy-2-methoxy derivative of the (9*R**,2'*S**)-diastereomer and the 2-hydroxy-3-methoxy derivative of the (9*R**,2'*R**)-diastereomer were found. The results are also in agreement with the observation of the long-range coupling ^5J in H–C(1) or H–C(8) even though it can only be observed as a slight broadening of the corresponding signals in the spectra of the metabolites.

In conclusion, the chemical-shift difference of 0.24 ppm between the diastereotopic protons H–C(1) and H–C(8) introduced by the OH-substitution in 2'-position in oxaprotiline proved to be sufficient for the structure elucidation of the diastereomeric metabolites.

Our thanks are due to Mr. *B. Weber* for his skilful experimental work.

Experimental Part

Origin and Preparation of Compounds. The preparation of **1–3** and **5** is given in [9]. Compounds **3** and **5** were separated by semiprep. HPLC (steel column, 10 × 250 mm) on silica gel *LiChrosorb Si 60*, 10 μm *(Merck)* using cyclohexane/i-PrOH/MeOH/conc.aq. NH_3 80:17:3:0.5 as mobile phase, and crystallized as hydrochlorides. Hydrochloride of **3**, m.p. 238–240°. Hydrochloride of **5**, m.p. 251–253°. The separation and isolation of **4**, **6–11** are described in a detailed investigation of the biotransformation of oxaprotiline in rat and dog [7].

NMR Spectra. The spectra were recorded on *Bruker HX 360* and *WM 400* spectrometers. The two-dimensional NMR experiments were performed on the *Bruker WM 400*. COSY: Pulse sequence $(\pi/2) - (t_1) - (\pi/2) - (\text{FID}, t_2)$; spectral with F1: 90 Hz, 128 data points with zero-filling (128 increments), F2: 180 Hz, 512 data points; filter: sine bell for F1 and F2. ^{1}H-^{13}C shift correlation: Pulse sequence $(\pi/2)_H - (t_1/2) - (\pi)_C - (t_1/2) - (\tau_1) - (\pi/2)_{H,C} - (\tau_2) - (BB_H, \text{FID}, t_2)$, $\tau_1 = 0.0036$s, $\tau_2 = 0.0018$s; spectral width F1: 230 Hz, 256 increments, F2: 2732 Hz, 2048 data points; filter: Gauss for F1 and F2.

Mass Spectra. The mass spectra (*m/z*, (%)) were recorded with a *Varian MAT CH5-DF* spectrometer equipped with a combined EI/FI/FD ion source (70 eV ionizing energy, 180° ion-source temperature, direct sample insertion). Base-peak normalization in the metabolite spectra was carried out above *m/z* 50. The peak assignment is given only for the first of each pair of isomers.

1: 293 (16, *M*), 218 (1, $M - C_3H_9NO$), 203 (1, $C_{16}H_{11}$), 191 (6, $C_{15}H_{11}$), 75 (6, C_3H_9NO), 44 (100, CH_2NHCH_3). **2**: 277 (52, *M*), 218 (12, $M - C_3H_9N$), 205 (16, $M - C_4H_{10}N$), 191 (24, $M - C_3H_8N/C_2H_4$), 178 (18, $M - C_4H_9N/C_2H_4$), 71 (54, C_4H_9N), 59 (64, C_3H_9N), 44 (100, CH_2NHCH_3). **3**: 327 (4, *M*(1 Cl)), 225 (3, $M - HOCHCH_2NHCH_3$), 189 (4, 225 – HCl), 75 (4, $H_2OCHCH_2NHCH_3$), 74 (4, $HOCHCH_2NHCH_3$), 44 (100, CH_2NHCH_3). **4**: 309 (100, *M*), 264 (4, $M - C_2H_7N$), 236 (18, 264 – C_2H_4), 234 (19, 236 – 2H), 207 (77, $M - OHCHCH_2NHCH_3/C_2H_4$), 194 (15, $M - C_4H_9NO/C_2H_4$), 178 (21, 207 – H/CO), 165 (14, 194 – H/CO), 75 (24, $H_2OCHCH_2NHCH_3$), 74 (25, $HOCHCH_2NHCH_3$). **5**: 327 (6), 225 (3), 189 (4), 75 (4), 74 (4), 44 (100). **6**: 309 (100), 264 (20), 236 (22), 234 (25), 207 (72), 194 (28), 178 (28), 165 (26), 75 (62), 74 (80). **7**: 309 (52, *M*), 281 (16, $M - C_2H_4$), 236 (26, 281 – C_2H_7N), 234 (25, 236 – 2H), 207 (72, $M - HOCHCH_2NHCH_3/C_2H_4$), 194 (29, $M - C_4H_9NO/C_2H_4$), 178 (29, 207 – H/CO), 165 (3, 194 – H/CO), 75 (100, $H_2OCHCH_2NHCH_3$), 74 (97, $HOCHCH_2NHCH_3$). **8**: 309 (34), 281 (9), 236 (22), 234 (24), 207 (66), 194 (29), 178 (29), 165 (25), 75 (100), 74 (90). **9**: 325 (52, *M*), 297 (66, $M - C_2H_4$), 279 (20, $M - H_2O/C_2H_4$), 252 (13, $M - C_3H_7NO$), 224 (100, 252 – C_2H_4), 223 (66, 252 – H/C_2H_4), 210 (26, $C_{14}H_{10}O_2$), 74 (92, $HOCHCH_2NHCH_3$). **10**: 339 (36, *M*), 311 (20, $M - C_2H_4$), 266 (12, $M - C_3H_7NO$), 264 (18, $M - C_3H_9NO$), 238 (100, 311 – $HOCH{=}CHNHCH_3$), 237 (46, 311 – $HOCHCH_2NHCH_3$), 224 (20, 311 – $CH_2{=}C(OH)CH_2NHCH_3$), 194 (23, 224 – CH_2O), 165 (20, 194 – H/CO), 74 (88, $HOCHCH_2NHCH_3$). **11**: 339 (62), 311 (11), 266 (31), 264 (55), 238 (100), 237 (71), 224 (30), 194 (45), 165 (42), 74 (82).

X-Ray Structure Analysis of the Hydrochloride of **3**. $C_{20}H_{23}OClN^{\oplus}Cl^{\ominus}$. Crystals are monoclinic, space group $P_{2_1/c}$, $a = 7.810$ Å, $b = 8.293$ Å, $c = 29.221$ Å, $\beta = 98.38°$, $Z = 4$. On a *Philips PW1100* diffractometer 4023 independent reflections were measured of which 3087 were considered observed ($I > 2\,\sigma\,(I)$). The structure was solved by direct methods using the program system MULTAN 77 [10]. All the H-atoms could be located in difference maps and included in the refinement with isotropic temperature factors. For all the other atoms anisotropic temperature factors were introduced. The refinement converged to a final value of $R = 0.064$.

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