Effective complexation of psychotropic phenethylammonium salts from a disodium dipyrazolate salt of macrocyclic structure†‡

Felipe Reviriego,[a] Pilar Navarro,*[a] Antonio Domènech*[b] and Enrique García-España[b]

[a] *Instituto de Química Médica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain. E-mail: iqmnt38@iqm.csic.es; Fax: +34 91 564 48 53; Tel: +34 91 5622900*
[b] *Facultad de Ciencias Químicas, Universidad de Valencia, 46100 Burjassot (Valencia), Spain*

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The equilibrium stability constants (K_s) of ammonium pyrazolate complexes $[L^{2-}]2RN(R')H_2^+$ (**3**, R′ = H and **4**, R′ = Me) formed from a macrocyclic disodium dipyrazolate salt **2**$[L^{2-}]$ $2Na^+$ and ammonium salts ($RNH_3^+X^-$ or $RN(Me)H_2^+X^-$) of psychotropic drugs and neurotransmitter catecholamines have been evaluated by electrochemical methods in DMSO solution. The resulting K_s values demonstrate that, except for (±)-amphetamine, the complexes formed by lipophilic primary [mescaline, (+)-amphetamine, (±)-*p*-methoxyamphetamine (PMA), (±)-3,4-methylenedioxyamphetamine (MDA)] and secondary [(±)-methamphetamine, (+)-methamphetamine and (±)-3,4-methylenedioxymethamphetamine (MDMA 'ecstasy')] phenethylamines are more stable than those formed from hydrophilic ones (dopamine and norepinephrine). A ^1H and ^{13}C NMR study on the formation of complexes of structure **3** and **4** formed from primary [mescaline, (+)-amphetamine] and secondary [(+)-methamphetamine] ammonium salts is given.

1 Introduction

An important class of psychotropic drugs is lipophilic phenethylamine derivatives which are usually commercialised as ammonium salts. Among them, amphetamine, dextroamphetamine, methamphetamine and dextromethamphetamine are psychostimulant drugs with subjective effects similar to those of cocaine. Other drugs are methoxy and/or methylenedioxy derivatives substituted on the aromatic ring of phenethylamine (*e.g.* mescaline), amphetamine (*e.g.* PMA, MDA), and methamphetamine (*e.g.* MDMA, 'ecstasy'), which act as psychedelic agents producing hallucinations, illusions and mental disorders such as paranoia (Fig. 1).[1,2]

For many years, the neurotoxic effects induced by such psychotropic drugs have been actively studied.[3] Thus, it is well known that MDMA produces a marked release of 5-hydroxytryptamine (5-HT) as well as a major release of dopamine which is likely to be responsible for sustained long term neurotoxic degeneration of both 5-hydroxytryptamine (5-HT) and dopamine nerve terminals.[4] Consequently, an abiotic receptor able to selectively complex a psychotropic drug (*e.g.* MDMA) and/or an undesirable excess of a neurotransmitter amine (*e.g.* dopamine) could also have useful applications.

We have previously reported on a 26 membered proton ionizable dioxatetraester crown containing two 3,5-disubstituted 1*H*-pyrazole units **1**$[LH_2]$ which after deprotonation in a basic medium leads to a disodium dipyrazolate salt of structure **2**$[L^{2-}]2Na^+$ (Fig. 2).[5] In DMSO-d_6 solution, an NMR study demonstrated the formation *in situ* of (1 : 2) complexes formed by the association of an L^{2-} host and two R–NH_3^+ ions derived from lipophilic and hydrophilic phenethylamines as guests.[6] The structure of these complexes was analysed by means of a variety of different experimental techniques like ^1H and ^{13}C NMR at variable temperature and measurements of intermolecular NOE effects (from NOESY and ROESY spectra), which demonstrated that hydrogen bonds between the R–NH_3^+ groups and the pyrazolate nitrogens stabilise the complexes. Furthermore, a theoretical study of their relative stability suggested that R–NH_3^+ ions of the lipophilic amines phenethylamine, and 3,4-dimethoxyphenethylamine (homoveratrylamine) may afford more stable complexes than those formed from the hydrophilic ones 3,4-dihydroxyphenethylamine (dopamine) and 1-(3,4-dihydroxyphenyl)-2-aminoethanol (norepinephrine).

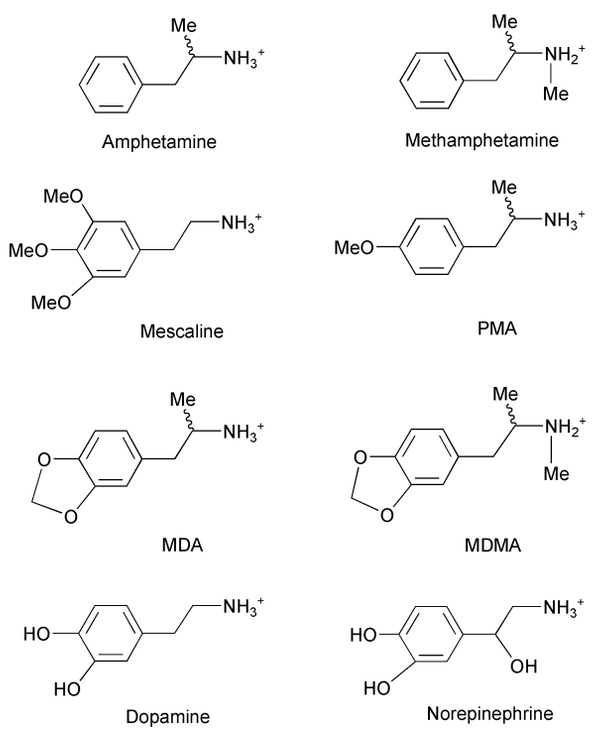

Fig. 1

In order to verify the above hypothesis, and taking as a model phenethylammonium chloride, an electrochemical procedure

† Electronic supplementary information (ESI) available: Table S1. See http://www.rsc.org/suppdata/p2/b2/b200607c/
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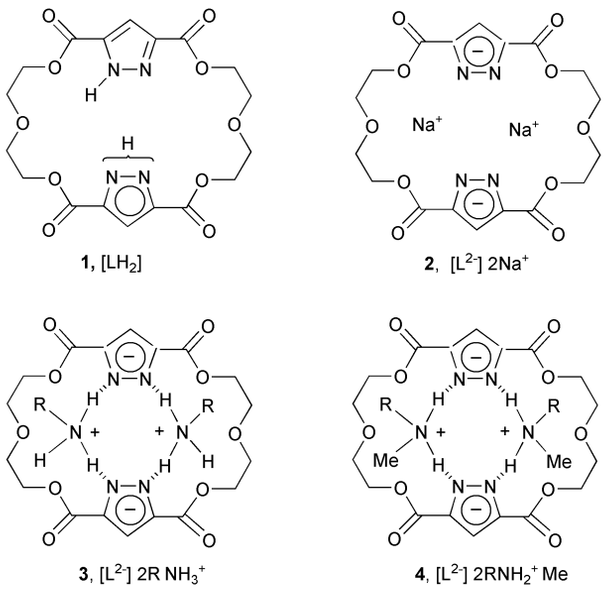

Fig. 2

was later developed based on cyclic, differential pulse and rotating-disc voltammetric experiments to elucidate the stoichiometry and stability constants of this interesting type of ammonium pyrazolate complexes.[7]

In this paper, we report for the first time the use of the disodium dipyrazolate salt **2**[L^{2-}]2Na^+ for the efficient and selective complexation of ammonium salts derived from psychostimulant and psychedelic drugs. With this purpose, using the electrochemical procedure mentioned above and DMSO as solvent, we have evaluated the stoichiometry and stability constants of complexes of structures **3** and **4** (Fig. 2) formed with primary or secondary ammonium salts of psychotropic [mescaline, (±)-amphetamine, (+)-amphetamine, (±)-4-methoxyamphetamine (PMA), (±)-3,4-methylenedioxyamphetamine (MDA), (±)-methamphetamine, (+)-methamphetamine, (±)-3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'] and neurotransmitter (dopamine and norepinephrine) amines. Furthermore, a ^{1}H and ^{13}C NMR study on the formation of complexes of structure **3** and **4** formed from primary [mescaline, (+)-amphetamine] and secondary [(+)-methamphetamine] ammonium salts is given.

2 Materials and methods

The sodium salt **2** [L^{2-}]2Na^+ was synthesized and characterized from the tetraester crown of the 1*H*-pyrazole **1** [3,6,9,16,19,22-hexaoxa-12,13,25,26-tetraazatricyclo[22.2.1.1^{11,14}]-octacosa-1(26),11,14(28),24(27)-tetraene-2,10,15,23-tetraone) as previously described.[6] Sulfates of (±)-amphetamine and (+)-amphetamine, and hydrochlorides of homoveratrylamine, mescaline, (±)-methamphetamine, (+)-methamphetamine, (±)-*p*-methoxyamphetamine (PMA), (±)-3,4-methylenedioxyamphetamine (MDA), (±)-3,4-methylenedioxymethamphetamine (MDMA), dopamine and norepinephrine were purchased from Sigma-Aldrich.

All electrochemical experiments were performed at 25 °C under atmosphere of dry argon in a thermostated three-electrode cell. Tetrabutylammonium hexafluorophosphate (Fluka) was used as a supporting electrolyte (0.10 M) in solutions in DMSO 0.10 M (1% water). Linear scan voltammograms were obtained by using a Metrohm E506 polarecord stand. A glassy carbon electrode (GCE; 0.12 cm^2 surface) was used as the working electrode, a platinum wire was used as the counter electrode, and an aqueous saturated calomel reference electrode (SCE) separated from the bulk solution by a salt bridge containing the solvent and supporting electrolyte only completed the standard three-electrode cell. Rotating disk voltammograms were obtained at v = 20 mV s^{-1} and ω = 2000 rpm by using a Metrohm 628–10 rotating-disk equipment. Prior to the series of experiments the working electrode was cleaned and activated. Electrochemical pretreatment was performed in blank solutions by applying +1.50 V *vs.* SCE for 10 min followed by −1.0 V for 1 min. Before each run the glassy carbon surface was polished with an aqueous suspension of alumina on a soft surface, dried and cleaned.

3 Results and discussion

Molar-ratio experiments were performed in solutions containing a constant concentration of macrocyclic ligand, c_L, and increasing concentrations of phenethylamine, c_N. Electrochemical data allowed calculation of the molar fraction of complexed ligand, L, from cyclic voltammetric peak currents and rotating disk limiting current measurements by means of the relationship:

$$L = (i^z - i_N^z)/(i_{LN}^z - i_N^z) \quad (1)$$

where i represents the actual limiting current for total concentrations c_N and c_L, and the limiting currents for zero complexation, i_N, obtained at a concentration c_N in the absence of macrocyclic ligand, and for complete complexation, i_{LN}, obtained with a sufficiently large excess of complexant. This equation was applied using z = 3/2 for rotating disk electrode voltammetry, and eventually using z = 2 for cyclic and differential pulse voltammetry at stationary electrodes, being similar to that used by Kodama and Kimura for polarography.[8] Three independent measurements of each limiting current were carried out for different concentrations of the ammonium salts, keeping constant the ligand concentration, allowing the direct estimate of a_L (mole fraction of free ligand) values for different c_N concentration of phenethylammonium salt at a constant c_L concentration of **2**[L^{2-}]2Na^+.

Then, for a general formation equilibrium:

$$mL + bN \rightleftharpoons L_mN_b \quad (2)$$

only the correct m, b, stoichiometric coefficients satisfy the relationship:

$$K^* = \frac{\alpha_L^{1/b}}{m^{1/b}(1-\alpha_L)^{m/b}(c_N - (b/m)\alpha_L c_L)} = \text{constant} \quad (3)$$

on inserting the a_L values calculated from electrochemical data. Accordingly, one can obtain the stoichiometry of the complex and its stability constant, K as $K = K^{*b} c_L^{(1-m)}$.

The application of this method to the L^{2-}–mescaline system shows that only the K^* values calculated for a 1 : 2 ligand : mescaline stoichiometry remain constant, whereas the K^* values monotonically increase or decrease for all the other tested stoichiometries (Supplementary material †). The mean value of K^* (2.33 × 10^3 M^{-1}) gives for the stability constant K a value of 5.4 × 10^6 M^{-2}.

It should be noted that the molar-ratio method strictly applies to the cases in which only one complex species is formed. However, when two or more complexes with different stoichiometries coexist, the method will produce constant K^* values only if one complex with a given stoichiometry largely prevails over the other ones. Therefore, although formation of 1 : 1 ligand : phenethylamine complexes cannot be dismissed, it appears that 1 : 2 complexes are much more stable and only values for the stability constants of such complexes are listed in Table 1.

The electrochemical experiments were performed in DMSO solution as indicated in the Materials and methods section. The

Table 1 Stability constants from differential pulse voltammetric data in DMSO (0.10 M Bu_4NPF_6). K_s values (M^{-2}) at 298 K

Primary ammonium salts		K_s/M^{-2}
[Homoveratrylamine]·HCl	**3a**	$(4.8 \pm 0.5) \times 10^5$
[Mescaline]·HCl	**3b**	$(5.8 \pm 0.4) \times 10^6$
[(±)-Amphetamine]$_2$SO$_4$	**3c**	$(4.6 \pm 0.4) \times 10^4$
[(+)-Amphetamine]$_2$SO$_4$	**3d**	$(2.4 \pm 0.2) \times 10^5$
(PMA)·HCl	**3e**	$(1.2 \pm 0.4) \times 10^7$
(MDA)·HCl	**3f**	$(1.1 \pm 0.3) \times 10^6$
[Dopamine]·HCl	**3g**	$(9.0 \pm 0.5) \times 10^4$
[Norepinephrine]·HCl	**3h**	$(5.3 \pm 0.4) \times 10^4$
Secondary ammonium salts		K_s/M^{-2}
[(±)-Methamphetamine]·HCl	**4a**	$(1.7 \pm 0.3) \times 10^5$
[(+)-Methamphetamine]·HCl	**4b**	$(2.1 \pm 0.3) \times 10^6$
(MDMA]·HCl	**4c**	$(2.3 \pm 0.5) \times 10^5$

phenethylammonium ions are electroactive and complex formation can be monitored from its electrochemical reduction.[9] This occurs by means of the following hydrogen-forming catalytic process: $R–NH_3^+ + e^- = R–NH_2 + ½\ H_2$. As shown in Fig. 3, a well defined cathodic peak at −0.6 V *vs.* SCE appears,

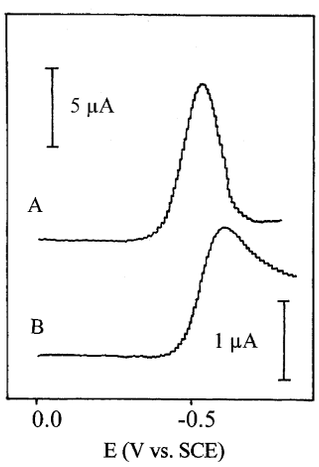

Fig. 3 Differential pulse voltammogram ($v = 20$ mV s^{-1}, $\Delta U = 80$ mV) (A) and linear scan voltammogram ($v = 20$ mV s^{-1}) (B) at a GCE for a solution of mescaline hydrochloride (0.5 mM) in DMSO (0.10 M Bu_4NPF_6)

for which the overall electron transfer process is diffusion-controlled, as judged by the proportionality between the concentration of phenethylammonium ion and the limiting rotating disk currents at a fixed potential scan rate.[10]

In the presence of the disodium dipyrazolate salt **2**[L^{2-}]2Na^+, the electrode process can be treated as the reduction of the uncomplexed RNH_3^+ (or $RN(Me)H_2^+$), and complexed phenethylammonium ions **3**[L^{2-}]2RNH_3^+ or **4**[L^{2-}]2RN(Me)H_2^+, which diffuse to the electrode surface with different diffusion coefficients, conforming to a case of codiffusion of different electroactive species.[11–13] As indicated in reference 7 the molar fraction of complexed phenethylammonium ions can be calculated from the limiting diffusion currents obtained from rotating disk electrode voltammetry. Three independent measurements of each limiting current were carried out for different concentrations of the ammonium salts, keeping constant the ligand concentration.

The experimental data gathered in Table 1 show that the new complexes studied are very stable (K_s 10^5–10^7 M^{-2}). Besides, except for (±)-amphetamine, the stability constants of all the ammonium pyrazolate complexes formed from psychostimulant drugs [dextroamphetamine (**3d**), methamphetamine (**4a**) and dextromethamphetamine (**4b**)] or psychedelic drugs with hallucinogenic effects [mescaline (**3b**), PMA (**3e**), MDA (**3f**) and MDMA, 'ecstasy' (**4c**) are slightly higher than that of dopamine (**3g**), and one or two orders of magnitude higher than that of norepinephrine (**3h**).

Consequently, although the theoretical stability order previously suggested for (1 : 2) complexes of structure **2** (homoveratrylamine > norepinephrine > dopamine)[6] is somewhat different to that experimentally found (homoveratrylamine > dopamine > norepinephrine), these results confirm that, in general, as the hydrophilic character of the phenethylammonium salts increases, the stability of the corresponding complexes is lower.

In addition to this general conclusion, other interesting observations can be pointed out in relation to the influence of the position, number, and nature of the phenethylamine substituents on the stability of ammonium dipyrazolate complexes of structure **3** or **4**. Firstly, as the number of methoxy groups on the aromatic ring of phenethylamine increases, the stability constant of the corresponding complexes becomes higher. Thus, the (1 : 2) complex **3b** formed from 3,4,5-trimethoxyphenethylammonium chloride (mescaline) is more stable than **3a** formed from 3,4-dimethoxyphenethylammonium chloride (homoveratrylamine) (Table 1).

In a similar way, a methoxy or methylenedioxy substituent on the aromatic nucleus of (±)-α-methylphenethylamine (amphetamine) strongly increases the stability of the corresponding complexes. Thus, the (±)-4-methoxyamphetamine (PMA) and (±)-3,4-methylenedioxyamphetamine (MDA) yield much more stable complexes (**3e** and **3f** respectively) than that of (±)-amphetamine (**3c**). On the other hand, it is well known that usually, the formation of ammonium–ligand hydrogen bonds plays an important role in stabilizing the corresponding complexes.[14] Taking this into account, it can be observed that contrary to expectations, the complexes **4a** and **4b** formed from secondary ammonium salts [(±)-methamphetamine and (+)-methamphetamine] are more stable than **3c** formed from a primary one [(±)-amphetamine)]. However, when the aromatic ring of (±)-methamphetamine is substituted by a 3,4-methylenedioxy group (MDMA) the above anomalous behaviour does not occur [the stability of **4a** is almost one order of magnitude lower than that of **3f** formed from (±)-3,4-methylenedioxyamphetamine (MDA)].

With the purpose of gaining additional insight into the complexation of psychotropic drugs, taking mescaline, (+)-amphetamine, and (+)-methamphetamine as reference of RNH_3^+ and $RN(Me)H_2^+$ salts, we have performed a ^{1}H and ^{13}C NMR study on the formation of complexes of structure **3** and **4** (Tables 2 and 3 respectively). In Tables 2A and 3A are compared the ^{1}H and ^{13}C NMR spectra of host samples with a 3-fold excess of ammonium salts, and in Tables 2B and 3B are compared the ^{1}H and ^{13}C NMR spectra of ammonium salts samples with a 3-fold excess of host.

The data gathered in Table 2 show that the ^{1}H chemical shift differences induced by complexation either in the free host **2** as in the respective free guests are in general very small. The most significant observation is that the ammonium groups of mescaline, (+)-amphetamine, and (+)-methamphetamine, which appear in the free bases as broad singlets, in the complexes **3b**, **3d** and **4b** change to very broad signals which have experienced upfield shifts of 3.3, 0.7 and 1.0 ppm respectively (Table 2B). However, the ^{13}C NMR data gathered in Table 3 afford relevant structural information. Thus, when the complexes are formed, the mescaline, (+)-amphetamine and (+)-methamphetamine carbons located in the β position to the complexation centre experience considerable downfield shifts of ~1.5–2.6 ppm. Furthermore, in the case of (+)-methamphetamine, the signal of the methyl carbon attached to the nitrogen atom shows a chemical shift difference of almost 2.5 ppm. On the other hand, the C(3,5) pyrazole carbons which in the disodium dipyrazolate salt **2**[L^{2-}]2Na^+ appear as a

Table 2 ^{1}H NMR (DMSO-d_6, δ, ppm) data for diammonium dipyrazolate complexes (**3b**, **3d** and **4b**) formed from mescaline hydrochloride (Mes), (+)-amphetamine sulfate [(+)-Am], and (+)-methamphetamine hydrochloride [(+)-Met] respectively. Chemical shifts induced by complexation in the free host **2** [ligand–amine, molar ratio 1 : 3] (A) and in the respective free guests [amine–ligand, molar ratio 2 : 3] (B)

	(A)	**2**	**3b**	**3d**	**4b**	
	HC-4	7.11 (s, 2H)	7.09 (s, 2H)	7.05 (s, 2H)	7.07 (s, 2H)	
	H_2C-7	4.34 (m, 8H)	4.37 (m(t), 8H)	4.39 [m(q), 8H]	4.37 (m, 8H)	
	H_2C-8	3.86 (m, 8H)	3.83 (m(t), 8H)	3.76 [m(t), 8H]	3.80 (m, 8H)	
(B)	(Mes)	**3b**	[(+)-Am]	**3d**	[(+)-Met]	**4b**
H_3N^+	8.13 (br s, 3H)	4.80 (br s, 6H)	5.97 (br s, 3H)	5.22 (br s, 6H)	9.2 (s, 2H)	8.2(v br s, 4H)
Me–N^+	—	—	—	—	2.55 (s, 3H)	2.37 (s, 3H)
H_2C-α	3.03 (m, 2H)	3.01 (t, 4H)	—	—	—	—
HC-α	—	—	3.22 (m, 1H)	3.20 (m, 2H)	3.35 (m, 1H)	2.91 (m, 1H)
Me-α	—	—	1.02 (d, 3H)	1.04 (d, 6H)	1.09 (d, 3H)	0.96 (d, 3H)
			J = 6.4 Hz	J = 6.4 Hz	J = 6.5 Hz	J = 6,0 Hz
H_2C-β	2.83 (t, 2H)	2.82 (t, 4H)	2.77 (dd, $1H_A$)	2.77 (dd, $2H_A$)	3.23 (dd,$1H_A$)	2.89 m, $1H_A$)
	J = 8.3 Hz		J = 13.0, 5.8 Hz	J = 13.0, 6.0 Hz	J = 13.0, 4.0 Hz	
			2.58 (dd, $1H_B$)	2.61 (dd, $2H_B$)	2.64 (dd, $1H_B$)	2.51 (m, $1H_B$)
			J = 13.2, 7.9 Hz	J = 13.0, 7.6 Hz	J = 13.0, 10.2 Hz	
HC-*o*	6.58 (s, 2H)	6.56 (s, 4H)	7.22 (m, 2H)	7.19 (m, 4H)	7.26 (m, 2H)	7.20 (m, 4H)
HC-*m*	—	—	7.31 (m, 2H)	7.27 (m, 4H)	7.34 (m, 2H)	7.29 (m, 4H)
HC-*p*	—	—	7.22 (m, 3H)	7.19 (m, 6H)	7.26 (m, 1H)	7.20 (m, 2H)
MeO-*m*	3.77 (s, 6H)	3.77 (s, 12H)	—	—	—	
MeO-*p*	3.62 (s, 3H)	3.63 (t, 6H)	—	—	—	

Table 3 ^{13}C NMR (DMSO-d_6, δ, ppm) data for diammonium dipyrazolate complexes (**3b**, **3d** and **4b**) formed *in situ* from mescaline hydrochloride (Mes), (+)-amphetamine sulfate [(+)-Am], and (+)-methamphetamine hydrochloride [(+)-Met] respectively. Chemical shift induced by complexation in the free host **2** [ligand–amine, molar ratio 1 : 3] (A) and in the free guests [amine–ligand, molar ratio 2 : 3] (B)

	(A)	**2**	**3b**	**3d**	**4b**	Δδ **3b**–**2**	Δδ **3d**–**2**	Δδ **4b**–**2**
	C-3,5	143.20[a]	142.01[b]	140.26[b]	139.08[b]	−1.19	−2.94	−4.12
	C-4	110.10	110.33	110.72	110.60	+0.23	+0.62	+0.50
	C-6	161.60[a]	161.24[c]	160.79	159.84	−0.36	−0.81	−1.76
	C-7	62.20	62.44	62.69	63.19	+0.24	+0.49	+0.99
	C-8	68.00	67.97	68.20	67.88	−0.02	+0.20	−0.12

(B)	(Mes)	**3b**	Δδ **3b**–(Mes)	[(+)-Am]	**3d**	Δδ **3d**–[(+)-Am]	[(+)-Met]	**4b**	Δδ **4b**–[(+)-Met]
Me–N^+	—		—	—	—	—	29.43	31.91	+2.48
C-α	39.67	40.41	+0.64	48.04	47.96	−0.08	55.17	55.71	+0.57
Me-α	—	—	—	19.86	21.37	+1.51	14.85	17.50	+2.65
C-β	33.11	34.38	+1.27	42.58	44.31	+1.73	38.16	40.78	+2.62
C-*i*	132.88	133.56	+0.68	138.25	138.94	+0.69	136.73	138.58	+1.85
C-*o*	105.83	105.98	+0.15	129.11	129.09	−0.02	129.19	129.16	−0.03
C-*m*	152.79	152.84	+0.05	128.32	128.20	−0.12	128.52	128.22	−0.30
C-*p*	152.79	152.84	+0.05	126.33	126.05	−0.28	126.70	126.09	−0.61
MeO-*m*	55.71	55.80	+0.10	—	—	—	—	—	—
MeO-*p*	59.81	59.89	+0.08	—	—	—	—	—	—

[a] Singlet. [b] Very broad signal. [c] Broad singlet.

singlet at 143.2 ppm, after complexation experience a variable chemical shift upfield of ~1 to 4 ppm, as well as a clear broadening, which indicates that in **3b**, **3d** and **4b** the protons belonging to RNH_3^+ or $RN(Me)H_2^+$ ions may be in fast equilibrium between the ammonium and the pyrazole nitrogens. In general, the above behaviour supports the previous suggestion[6] that electrostatic interactions with permanent charges should be the main force involved in the formation of such ammonium dipyrazolate complexes.

In conclusion, the authors believe that these results open a new area in the search for efficient and selective ligands for psychostimulant and psychedelic drugs.

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