# 1H Nuclear Magnetic Resonance Study of the Deprotonation of the Catecholamines in Solution: the Microconstants for the De-deuteronation of L-Dopa in D2O

By Reginald F. Jameson,* Geoffrey Hunter, and Tamas Kiss

(Department of Chemistry, The University, Dundee DD1 4HN, Scotland)

Summary The use of pulsed Fourier transform 1H n.m.r. spectroscopy has been shown to give detailed information concerning the protonation sites of the catecholamines; in particular, a method for the assignment of the microconstants for the deprotonation of L-dopa and the determination of their values has been rigorously established.

THE order of deprotonation of the available sites in the catecholamines, i.e., the determination of the microconstants and their assignment, has recently been questioned.[1,2] We now present data obtained from pulsed Fourier transform n.m.r. spectroscopy which unambiguously answers this question, and at the same time promises to be a most powerful tool for studies involving protonation, conformation, and metal complex formation when multicentre ligands are involved.

Provided that oxygen is rigorously excluded, the spectrum of L-dopa in dilute deuterium oxide solution consists of two, effectively separate, ABC multiplets, one arising from the three closely coupled aryl protons and the other from the three closely coupled alkyl protons of the side-chain (see 1).

(1)
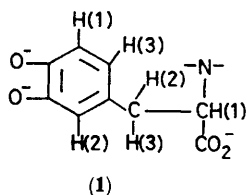
We have now obtained high quality spectra arising from solutions of varying pD (pH) and have analysed them via the use of the program LAOCOON 3,[3] obtaining accurate chemical shifts and spin–spin coupling constants {typical values obtained at 60 MHz and pD = 10·21 are as follows: (a) alkyl protons: $\Delta\nu_{1,2} = 39\cdot1$, $\Delta\nu_{2,3} = 13\cdot7$, $\Delta\nu_{1,3} = 52\cdot8$, $J_{1,2} = 4\cdot9$, $J_{2,3} = -14\cdot0$, $J_{1,3} = 7\cdot8$ Hz; (b) aryl protons: $\Delta\nu_{1,2} = 4\cdot3$, $\Delta\nu_{2,3} = 7\cdot7$, $\Delta\nu_{1,3} = 12\cdot0$, $J_{1,2} = -0\cdot4$; $J_{2,3} = 2\cdot6$, $J_{1,3} = 8\cdot1$ Hz; the solutions were at 0·100 M ionic strength (K[NO3]) and at ambient temperature}.
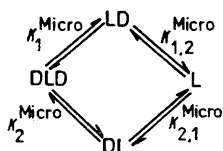
where $K_1^{Micro} = \dfrac{[LD][D^+]}{[DLD]}$, etc.

SCHEME

Data were collected over the pD range 8–12. Over this range only two sites for deuteronation (protonation) need be considered,[1,2] namely one of the phenolic –OD sites and the –ND3+ site. Thus we may write DLD to represent this

state of affairs with the –OD deuteron written to the left and the –ND3+ deuteron to the right of L, where L represents the dopa anion (omitting the charges and the other two sites for clarity). The equilibria are, thus, as shown in the Scheme and the chemical shifts can be used to obtain the mole ratios of non-deuteronated forms of the –OD site to total dopa (aryl shifts) and of non-deuteronated forms of the –ND3+ site to total dopa (alkyl shifts).
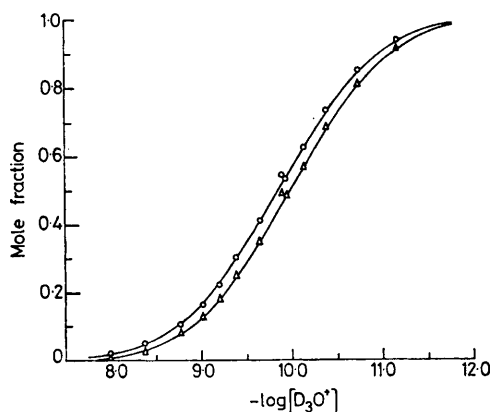

FIGURE. Mole fractions of L-dopa present in dilute D2O {0·100 M ionic strength (K[NO3]); ambient temperature} as species dedeuteronated at the –OD site (–○–) and at the –ND3+ site (–△–). The curves have been computed from data in the Table.

The Figure shows the mole fractions obtained from the 1H n.m.r. spectra as experimental points with curves calculated on the basis of the de-deuteronation (K_n) values listed in the Table, and illustrates the excellent agreement obtained. The overall acid dissociation constants were calculated from the microconstants and compared with those obtained from a direct pD titration method. Again the agreement is excellent (see Table).

TABLE. pK values for the de-deuteronation of L-dopa in D2O at ambient temperature and ionic strength = 0·100 M (K[NO3])

N.m.r. titration			pD Titration[a]
Microconstants		Macroconstants	Macroconstants
pK_1^{micro}　9·68	}	pK_1　9·42	pK_1　9·44
pK_2^{micro}　9·76			
$pK_{1,2}^{micro}$　10·16	}	pK_2　10·42	pK_2　10·40
$pK_{2,1}^{micro}$　10·08			

[a] At 25·00 ± 0·02 °C

From these results it is seen that the –OD deuteron is lost before the –ND3+ deuteron in dilute deuterium oxide solution, but that the difference is extremely small (only 0·08 log units). This would almost certainly confirm the views of Boggess and Martin[2] with respect to deprotonation in dilute aqueous solution and to rule out the ideas put forward by Jameson,[1] particularly in the assumption that

$K_1^{micro} = K_{2,1}^{micro}$ and $K_2^{micro} = K_{1,2}^{micro}$. However, as it is almost impossible to prevent some oxidation, the closeness of the pK values for $-ND_3^+$ and $-OD$ almost certainly precludes the use of optical spectroscopic techniques for this type of work.

[1] R. F. Jameson, *J.C.S. Dalton*, 1978, 43.
[2] R. K. Boggess and R. B. Martin, *J. Amer. Chem. Soc.*, 1975, **97**, 3076.
[3] A. A. Bothner-by and S. Castellano, Program 111, Quantum Chemistry Program Exchange, Indiana University.