N,N'-DIPHENYLACETAMIDINIUM CARBOXYLATES

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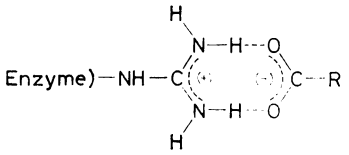
Received April 13, 1989
Accepted May 24, 1989

Various N,N'-diphenylacetamidinium carboxylates were prepared and the nature of the amidinium-carboxylate interactions was examined by ^1H and ^{13}C NMR spectra. Correlation between pK_a of the respective acids and ^1H NMR shifts in $CHCl_3$ is described. The compounds due to their solubility and other physico-chemical properties represent valuable models of lactate dehydrogenase active site.

In an effort to understand the elementary processes taking course in the active centers of certain enzymes great attention is given to the question of substrate fixation. The crucial role of carboxylic substrates fixation is played by arginine as was demonstrated for lactate dehydrogenase[1,2], carboxypeptidase[3], malate dehydrogenase[4] and other enzymes[5]. The mentioned fixation consists in the formation of two parallel hydrogen bonds between two nitrogens of the arginine guanidinium group and two oxygens of the carboxylate (Scheme 1).



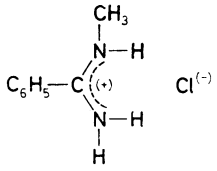
SCHEME 1

In our previous communications we have used[6] an MO approach to understand the basic principles of interaction between simple counter-ions of different proton affinities; we also tried to find mutual influence between the carboxylic and amidinium parts of more complex molecules for series of *p*-substituted benzamidinium carboxylates[7] and benzoates[8], respectively. We succeeded in establishing the nature of the amidinium-carboxylate interaction and our experimental work was supported by X-ray studies that resolved the structures of several our compounds[9–12] in the

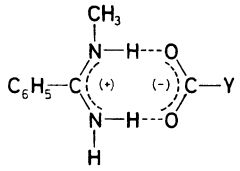
crystalline state. Our present effort is focused towards finding such a type of model compounds that will be suitable for modelling the lactate dehydrogenase.

Nitrogen substitued amidines were chosen for two reasons: (i) any lowering the amidine basicity increases the strength of hydrogen bonds between amidinium and carboxylate groups, respectively[7,8]. More pronounced mutual influence and therefore higher activation of the encaptured substrate can be observed; (ii) introducing substituents on the amidinium nitrogens increases the solubility of the compounds in media suitable for model reactions.

In the initial step carboxylates of the non symmetrical N-methylbenzamidinium were also examined. Starting N-methylbenzamidinium chloride (*Ia*) was prepared by reaction of ethyl benzenecarboximidate with methylammonium chloride[13]. N-Methylbenzamidinium formate (*Ib*) was obtained by the reaction of the chloride *Ia* with sodium formate. Respective trifluoroacetate *Ic* was prepared by ion exchange chromatography from the chloride *Ia*. Melting points and elemental analyses are summarized in Table I, ^{1}H NMR spectra can be found in Table II.

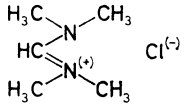


Ia



Ib, Y = H
Ic, Y = CF_3

In connection with this research tetramethylformamidinium chloride[14] (*II*) was prepared. The preparation of tetramethylformamidinium carboxylates which would not be able to form intramolecular hydrogen bonds N...H...O was not successful.



II

The main goal of this paper is the study of N,N'-diphenylacetamidinium carboxylates. Starting compound, N,N'-diphenylacetamidine (*IIIa*) was prepared according to ref.[15] from acetanilide and $POCl_3$. From the free base *IIIa* or its chloride *IIIb*, respectively, the N,N'-diphenylacetamidinium perchlorate *IIIc* and carboxylates *IIId*–*IIIl* were prepared. Their elemental analyses and melting points as well as those of the free base *IIIa*, its chloride *IIIb* and perchlorate *IIIc* are summarized in Table I. Two of the compounds sublime – chloride *IIIb* and trifloroacetate *IIIc*.

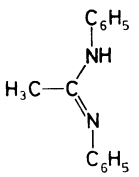
TABLE I

Melting points and elemental analyses for compounds *I* and *III*

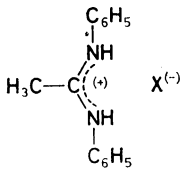
Compound	Formula (M.w.)	M.p., °C	Calculated/Found		
			%C	%H	%N
Ia	$C_8H_{11}N_2Cl$ (172·6)	221—222[a]	55·76 —[b]	6·42 —[b]	16·23 —[b]
Ib	$C_9H_{12}N_2O_2$ (180·2)	110—112	59·99 59·65	6·71 6·69	15·55 14·95
Ic	$C_{10}H_{11}N_2O_2F_3$[c] (248·2)	143—149	48·39 48·56	4·47 4·41	11·29 11·13
IIIa	$C_{14}H_{14}N_2$ (210·3)	131—132[d]	80·00 80·03	6·67 6·79	13·33 12·75
IIIb	$C_{14}H_{15}N_2Cl$[e] (246·7)	223—225	68·15 68·15	6·13 6·16	11·35 11·24
IIIc	$C_{14}H_{15}N_2O_4Cl$[f] (310·7)	134—135	54·11 54·15	4·87 5·03	9·02 9·04
IIId	$C_{16}H_{15}N_2O_2F_3$ (324·3)	179—180	59·26 58·83	4·66 4·82	17·57[g] 17·56[g]
IIIe	$C_{22}H_{20}N_2O_3$ (360·4)	136—137	73·32 73·00	5·59 5·67	7·77 7·59
IIIf	$C_{16}H_{16}N_2O_4$ (300·3)	139—140	63·99 63·53	5·37 5·26	9·33 8·89
IIIg	$C_{17}H_{18}N_2O_3$ (298·3)	102—103	68·44 68·30	6·08 6·37	9·39 9·47
IIIh	$C_{17}H_{20}N_2O_3$ (300·4)	109—110	67·98 68·02	6·71 6·57	9·33 8·91
IIIi	$C_{15}H_{16}N_2O_2$ (256·3)	115—120	70·29 69·88	6·29 6·08	10·93 10·90
IIIj	$C_{21}H_{20}N_2O_2$ (332·4)	119—120	75·88 75·92	6·06 6·15	8·43 8·29
IIIk	$C_{22}H_{22}N_2O_3$ (362·4)	131—132	72·91 72·72	6·12 6·13	7·73 7·82
IIIl	$C_{16}H_{18}N_2O_2$ (270·3)	65—66	71·09 71·09	6·71 6·70	10·36 10·29

[a] Ref.[13] m.p. 222—223°C; [b] compound identified by its m.p.; [c] %F: calculated. 22·96, found 23·20; [d] ref.[15] m.p. 132°C; [e] %Cl: calculated. 14·37, found 14·30; [f] %Cl: calculated. 11·41, found 11·03; [g] %F given.

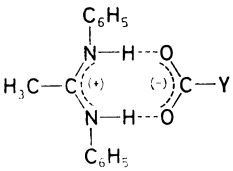
The values of the melting points are decreasing approximately in the same sense as the pK_a of the respective carboxylic acids is increasing.



IIIa



IIIb, X = Cl
IIIc, X = ClO_4



IIId, Y = CF_3
IIIe, Y = COC_6H_5
IIIf, Y = COOH
IIIg, Y = $COCH_3$
IIIh, Y = $CH(OH)CH_3$
IIIi, Y = H
IIIj, Y = C_6H_5
IIIk, Y = $CH(OH)C_6H_5$
IIIl, Y = CH

N,N'-Diphenylacetamidinium carboxylates *IIId–IIIl* are soluble not only in dimethylsulfoxide, water, methanol, ethanol and dimethylformamide as the similar compounds described previously[6–8] but also in solvents with lower polarity – chloroform, benzene, acetone, dioxane and acetonitrile. This enables utilization of chloroform as solvent for the NMR spectroscopical studies.

EXPERIMENTAL

Temperature data are not corrected. Melting points were determined on a Boetius apparatus. The spectra were measured on Bruker AM 400 in $CDCl_3$ solutions related to tetramethylsilane. Experimental parameters were: for ^1H NMR 400·13 MHz, 64 K data points, digital resolution 0·2 Hz/point, pulse width 4 μs, temperature (owing to the solubility) 27–80°C; for ^{13}C NMR 100·61 MHz, 64 K data points, digital resolution 1 Hz/point, used APT (Attached Proton Test),

TABLE II
^1H NMR spectra of the compounds *Ib*, *c* measured in $(CD_3)_2SO$

Compound	*o*-	*m*-	*p*-	CH_3	NH_2	Y
Ib	7·76	7·61	7·71	3·02	9·51	—
Ic	7·80	7·54	7·65	2·99	9·97	8·47

two-dimensional spectroscopic techniques COSY and HETCOR, pulse sequence, temperature 27—80°C. The solid state ^{13}C NMR spectra were measured on the Bruker MSL 200 apparatus, 50·32 MHz.

The potentiometric titration was carried out with an RTS-622 apparatus (Radiometer Copenhagen) as in ref.[16]. The initial acid concentration in chloroform was $5 \cdot 10^{-3}$ mol l^{-1}. Solution of tetrabutylammonium hydroxide in methanol was used as a titrant, the potentials of the cell glass electrode – calomel electrode (filled with the saturated solution of KCl in methanol) in half neutralization point are given in the Table III. Each titration was repeated 3—4 times. We have not calculated the values of pK_a, because there is no standard for calibration of the cell in chloroform but tabulated potentials are linearly dependent on values pK_a.

N-*Methylbenzamidinium chloride*[13] (Ia) and N,N,N',N'-*tetramethylformamidinium chloride*[14] (II) were prepared according to described procedures.

N-*Methylbenzamidinium formate* Ib was obtained by the reaction of equimolar amounts of chloride *Ia* and sodium formate in water–ethanol (9 : 1) mixture. N-*methylbenzamidinium trifluoracetate* (Ic) was prepared by the ion exchange chromatography described in our previous communication[6] using the Amberlite IRA-401 resin in trifluoracetate form.

N,N'-*Diphenylacetamidine* (IIIa) was prepared by modification of the described procedure[15]. The mixture of 20 g (0·15 mol) acetanilide, 100 ml of anhydrous benzene and 16 ml (26 g; 0·17 mol) phosphorus oxychloride was refluxed for 1·5 h. The residue after evaporation was dissolved in boiling water and washed twice with ether. The excess of 20% sodium hydroxide

Table III

^1H NMR spectra of the compounds *IIIa—IIIl* measured in $CDCl_3$ and pK_a of their respective acids

Compound	U^a	CH_3	*o*-	*m*-	*p*-	NH_2
IIIa	—	1·98	7·18	7·29	7·03	6·37
IIIb	−360	2·20	7·34	7·43	7·39	10·27
IIIc	−320	2·22	7·34	7·41	7·34	12·44
IIId	−60	2·26	7·27	7·45	7·37	13·63
IIIe[b]	0	2·19	7·30	7·43	7·34	14·35
IIIf	50	2·16	7·29	7·47	7·40	13·23
IIIg[c]	100	2·14	7·27	7·44	7·35	10·14
IIIh[d]	125	2·13	7·25	7·45	7·34	13·50
IIIi[e]	140	2·12	7·32	7·42	7·34	12·47
IIIj[f]	180	2·08	7·28	7·40	7·35	14·60
IIIk[g]	182	2·06	7·20	7·41	7·33	14·30
IIIl[h]	280	2·02	7·21	7·38	7·24	13·83

[a] The potential of the cell glass electrode vs SCE in the half neutralization point in $CHCl_3$. The following ^1H NMR signals for the substituent Y given: [b] phenyl *o*- 8·08, *m*- 7·43, *p*- 7·53; [c] methyl 2·39; [d] methyl 1·40, CH(OH) 4·14; [e] formate 8·52; [f] phenyl *o*- 8·07, *m*- 7·27, *p*- 7·37; [g] phenyl *o*- 7·52, *m*- 7·29, *p*- 7·21, CH(OH) 5·01, HO(CH) 4·65; [h] methyl 2·07.

was then added to the water layer and the free base was extracted with ether. After drying, evaporation of the solvent and five crystallizations from water–ethanol (1 : 1) was obtained 9·0g (51% of theoretic amount) N,N′-diphenylacetamidine (*IIIa*). ^{1}H NMR spectrum ($(CD_3)_2SO$): 1·90 s, 3 H (CH_3); 6·74 d, 2 H (*o*-, *E*); 6·92 t, 1 H (*p*-, *Z*); 6·94 t, 1 H (*p*-, *E*); 7·24 t, 2 H (*m*-, *Z*); 7·25 t, 2 H (*m*-, *E*); 7·78 d, 2 H (*o*-, *Z*); 8·89 s, 1 H (NH). ^{13}C NMR spectrum ($(CD_3)_2SO$): 17·67 (CH_3), 121·35 (*o*-, *E*), 121·23 (*p*-, *Z*), 121·35 (*p*-, *E*), 128·21 (*m*-, *Z*), 128·60 (*m*-, *E*), 118·86 (*o*-, *Z*), 152·90 (C_{amidin}), 141·20 (C-1, *E*), 151·12 (C-1, *Z*). ^{13}C NMR solid state spectrum: 18·47 (CH_3); 122·10, 127·40, 129·42, 130·46, 131·98 (all aromatics); 139·70, 141·76 (both C-1).

Salts of N,N′-*diphenylacetamidine* were prepared either by the reaction of equimolar amounts of base *IIIa* and respective acid (in the case of compounds *IIIb*, *IIIe*, *IIIf*, *IIIg* and *IIIl*) or by the reaction of *IIIb* with sodium salt of respective acid (in the case of compounds *IIIc*, *IIId*, *IIIh*—*IIIk*) in methanol–water solution. The crude amidinium salt was crystallized from methanol.

RESULTS AND DISCUSSION

^{1}H NMR spectra of the free base *IIIa*, its chloride *IIIb*, perchlorate *IIIc* and respective carboxylates *IIId*—*IIIl* are given in Table III. Certain trends follow from the Table III – depending on the value of the potential in mV of the cell glass electrode – modified calomel electrode in $CHCl_3$ in half neutralization point as value linearly dependent on pK_a of the respective acid changes in chemical shifts of various centers

TABLE IV

^{13}C NMR spectra of the compounds *IIIa*—*IIIl* measured in $CDCl_3$

Compd.	CH_3	*o*	*m*	*p*	C=N	C=O	C—I
IIIa	18·71	121·26	128·92	122·78	153·26	—	145·29
IIIb	15·81	125·89	130·03	129·16	166·52	—	134·36
IIIc	15·90	125·83	129·83	128·57	166·62	—	134·71
IIId[a]	15·93	125·87	129·91	128·46	165·72	163·87[b]	135·56
IIIe[c]	16·11	125·76	129·80	128·11	165·37	173·19	135·97
IIIf[d]	15·89	125·90	129·94	128·60	165·61	162·80	135·39
IIIg[e]	16·07	125·73	129·76	127·98	165·11	170·61	136·24
IIIh[f]	16·15	125·42	129·71	127·58	164·48	182·95	136·94
IIIi	16·30	125·63	129·78	128·32	—[g]	—[g]	135·14
IIIj[h]	16·35	124·97	129·47	126·61	162·97	174·17	135·62
IIIk[i]	16·01	125·67	129·73	127·90	164·98	179·86	136·34
IIIl[j]	16·55	124·30	129·41	126·05	161·61	178·91	139·37

The following ^{13}C NMR signals for the substituent Y given: [a] trifluormethyl group 116·38, ^1J(C, F) = 292 Hz; [b] observed ^2J(C, F) = 36 Hz; [c] CO 193·24, C-1 133·90, C-2 133·34, C-3 128·91, C-4 129·85; [d] COOH 186·14; [e] methyl 27·06, CO 201·06; [f] methyl 21·18, CH(OH) 68·88; [g] signals were not found; [h] C-1 138·55, C-2 130·88, C-3 127·75, C-4 129·58; [i] CH(OH) 74·15, C-1 141·82, C-2 126·61, C-3 128·05, C-4 127·12; [j] methyl 23·18.

may be observed. Fig. 1 shows this phenomenon schematically, observed differences are marked as positive for the case that chemical shift increases with decreasing pK_a of the acid, the value refers to maximum difference in the whole series of compounds. As follows either from Tables III and IV or from Fig. 1 also chemical shifts of the amidinium signals are substantially changed with respect to a given acid. Compared to similar survey made for benzamidinium carboxylates[7] the differences are 5 or 10 times bigger. This could be explained also by stronger hydrogen bonds in N-substituted amidinium carboxylates where basicity of the amidinium part is lower, therefore mutual interference between amidinium and carboxylate counter pairs is more pronounced. The most important response to the acid nature could be observed in *p*-position of the benzene ring.

The dependence of amidinium methyl ^{1}H NMR shifts on pK_a of the corresponding acids was treated statistically. For comparison also respective value for the free base was included. The plot equilibrated by the 2nd order polynome is depicted in Fig. 2.

^{13}C NMR solid state measurements were applied to free base *IIIa*, trifluoroacetate *IIId* and pyruvate *IIIg* and the results are summatized in Table V. One interesting point follows from this table – two different signals for carbonyl and methyl of the pyruvate *IIIg* could be detected. This may be explained by non symmetry of the compound *IIIg* in the solid state.

N,N'-Diphenylacetamidine (*IIIa*) deserves special attention although the free base, strictly speaking, does not represent desired lactate dehydrogenase active site

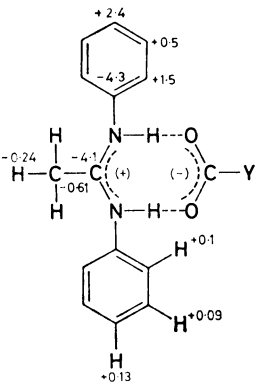


FIG. 1

The NMR chemical shift differencies (in ppm) of the amidinium parts of compounds *IIId*–*IIIl* in the dependence of the pK_a value of the corresponding carboxylic acid. The negative value means the decreasing chemical shift with the decreasing pK_a value

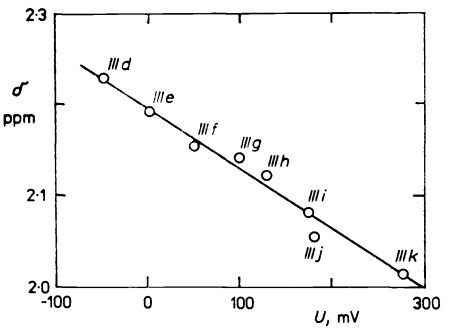
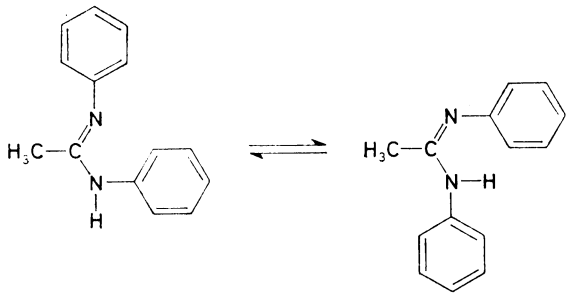


FIG. 2

A plot of the dependence of methyl ^{1}H NMR chemical shifts on the pK_a value of corresponding acid

model. Compound *IIIa* forms cyclic dimer, the attachment mediated by hydrogen bonds which can be observed in NMR spectra. This associate may be compared with similar interaction in amidinium carboxylates.

Several results follow from our observations: (i) NMR data are consistent with the presumption that in hexadeuterodimethylsulfoxide structures given in Scheme 2



E *Z*

SCHEME 2

TABLE V

^{13}C NMR solid state spectra of compounds *IIId* and *IIIg*

Signals	Compounds	
	IIId	*IIIg*
CH_3 (3 signals)	14·93	15·05
C=N (5–6 signals)	165·20	166·80
C=O	163·00	169·21
aromatic carbons	126·19	125·36
	129·34	127·35
	130·30	128·46
	134·02	130·55
	135·39	134·87
	136·20	135·80
Y	—[a]	27·47 (CH_3)
		28·18 (CH_3)
		200·00 (CO)
		201·09 (CO)

[a] Signals not distinct.

can be attributed to compound *IIIa*, no cyclic dimer is formed in this solvent. One benzene ring is oriented *cis* and the other *trans* in respect to the methyl group. Similar phenomenon is described for N,N'-dimethylamidines[17]; (ii) analogical behaviour may be observed by NMR in deuterochloroform solutions but only in diluted ones (less than 10%); (iii) although the possibility to isolate both tautomers was questioned earlier[18] when passing through HPLC column (Separon SGX) two compounds can be detected at 220 nm in molar ratio 1 : 1, probably tautomers *E* and *Z* (Scheme 2) of compound IIIa. However, we did not succeeded to prepare them in pure form; (iv) in concentrated deuterochloroform solutions as well as in the solid state compound *IIIa* exists in the form of dimer as follows from NMR measurements. All relevant data concerning the above mentioned observations are summarized in Tables III – V and in Experimental.

In conclusion it can be said that N,N'-diphenylacetamidine and its carboxylic salts are, thanks to their solubility and ability to mediate substitution effects, valuable tools for the lactate dehydrogenase active center study.

The authors thank Dr F. Pavlíková, Central Research Laboratories, Prague Institute of Chemical Technology for the ^{1}H *and* ^{13}C NMR *measurements and Dr B. Schneider, Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences for the solid state* NMR *experiments.*

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Translated by the author (J. Krechl).