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BASICITY OF 3- AMINOPROPIONAMIDINE DERIVATIVES IN WATER AND DIMETHYL SULPHOXIDE. IMPLICATION FOR A PIVOTAL STEP IN THE SYNTHESIS OF DISTAMYCIN A ANALOGUES

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The acid–base properties of eight 3-aminopropionamidine derivatives $R_1R_2N(CH_2)_2C(=NH)NR_3R_4$ (1, $R_1=R_2=R_3=R_4=H$; 2, $R_1=R_3=R_4=H$, $R_2=Me$; 3, $R_1=R_2=R_4=H$, $R_3=Me$; 4, $R_1=R_2=H$, $R_3=R_4=Me$; 5, $R_1=Tos$, $R_2=R_3=R_4=H$; 6, $R_1=Tos$, $R_2=Me$ $R_3=R_4=H$; 7, $R_1=Tos$, $R_2=R_4=H$, $R_3=Me$; 8, $R_1=Tos$, $R_2=H$, $R_3=R_4=Me$; Tos = 4-toluenesulphonyl) related to the antiviral natural product distamycin A were investigated in water and dimethyl sulphoxide (DMSO). The measured pK_a values for the ammonium function in 1–4 in water ranged between 7·48 and 7·73, whereas the corresponding values in DMSO were $9·4 \pm 0·3$. The amidinium moiety of these compounds displayed pK_a values in the range 11·4–12·0 and 13·4–13·6 in water and DMSO, respectively. The tosylamide group in compounds 5–8 was deprotonated in the expected pH region and exhibited pK_a values between 9·49 and 10·02 in water, but was considerably less acidic in DMSO ($14·5 \leq pK_a \leq 15·7$). The behaviour of the amidinium cation of 5–8 in water and DMSO resembled that of 1–4. The measured pK_a values are discussed and the solvent-induced pK_a shifts are explained in terms of solvent and substituent effects. The observed pK_a differences between the ammonium and the amidinium functions in 1–4 render these compounds suitable intermediates in an alternative synthesis of distamycin A.

INTRODUCTION

Distamycin A (DA) (Figure 1) is a basic polyamide with a wide range of antibiotic properties.[1] The chemistry of this naturally occurring compound has received considerable interest since its discovery three decades ago. As a consequence, several synthetic routes to the parent compound and to a huge number of analogues have been devised. Some years ago, an alternative synthetic strategy to DA in which the preformed unprotected aliphatic amidine side-chain was attached to a trimeric pyrrolecarboxylic acid precursor as the final step was designed.[2] The yield in this coupling ranged between 40 and 80%, but was later increased to 80–90% by careful monitoring of the reaction.[3,4] In practice, this coupling was achieved by treatment of the corresponding succinimidyl ester with a considerable excess of 3-aminopropionamidine dihydrobromide in aqueous dioxane under essentially neutral conditions ($pH \approx 6–7$).

In order to simplify the procedure and to optimize the reaction conditions further, we explored miscellaneous alternative condensation agents, such as dicyclohexylcarbodiimide (DCC) and carbonyldiimidazole in this respect. Although various reaction conditions were tried, the crude reaction product always contained side products and only a modest quantity of DA could be isolated after a laborious workup. We have recently found that the yield of the desired product improved significantly when 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was used as a dehydrating agent in an aprotic polar solvent under anhydrous conditions.[5] This direct approach also exploits the fact that the amidine function generally is significantly more basic than the amino group, thus allowing selective acylation of the amine in the presence of an unprotected amidine moiety. However, it is well known that acylation of the amidine nitrogens, and also hydrolysis of the amidine function, might occur under the influence of strong alkali.[6,7] With the aim of

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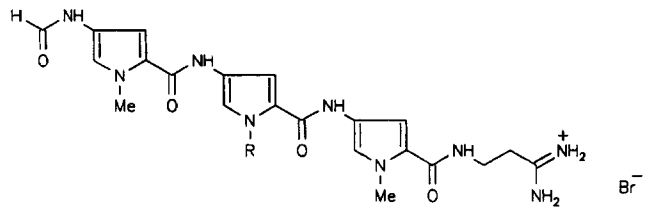

Figure 1. Distamycin A (hydrobromide), R = Me; compound **9** (hydrobromide), R = H (see Table 1)

elucidating this crucial step in the synthesis of DA and to gain further insight into the properties of these versatile precursors, we have now determined the pK_a values in water and DMSO of a small series of relevant 3-aminopropionamidine derivatives.

DA exerts its principal action by binding preferentially to AT-rich regions of the minor groove in helical DNA.[8,9] It is reasonable to assume that several properties of DA, such as its acid–base behaviour and its ability to interact via hydrogen bonds, contribute to its binding efficiency to DNA. The protolysis of DA is primarily governed by its amidine functions. Therefore, the goal of this study was to measure the acid–base properties (pK_a) of DA and the related 3-aminopropionamidine derivatives in order to gain further insight into the mechanism of action of DA.

RESULTS AND DISCUSSION

The pK_a values of a wide range of substituted amidine analogues have been amply reviewed recently.[10] Continued interest in this class of substances is further reflected in miscellaneous contemporary reports dealing with related aspects of their acid–base chemistry.[11,12]

The results of our pK_a measurements (in aqueous solution and in DMSO) of selected salts of 3-aminopropionamidine derivatives $R_1R_2N(CH_2)_2C(=NH)NR_3R_4$ related to DA are compiled in Table 1. All of the compounds **1–8** have three non-equivalent nitrogen atoms which may serve as sites for protonation or deprotonation. The R_1R_2N group behaves as a normal amine function and is attached to a $(CH_2)_2C$ fragment. Depending on the pH, it can act either as a basicity centre (for protonation on N) or as an acidity centre (for deprotonation of the R_1R_2N moiety of R_1 and/or $R_2 = H$). The imino (=NH) group and the remaining NR_3R_4 function can also display this dualistic behaviour, depending on the nature of the substituents and the pH of the surrounding medium. For the uncharged amidine molecule, the strong basicity is primarily governed by protonation of the =NH site to yield t:he very stable amidinium cation.[10] With few exceptions, the acidity of the =NH group is low (the pK_a in DMSO is usually in the range 16–31 pK_a units[13–15]).

The NR_3R_4 group behaves as an amine function, the basicity of which is generally lower than that of the =NH group.[10,15] Unless R_1–R_4 are not strongly electronegative substituents (e.g. CF_3SO_2, NO_2, CN and

Table 1. Acidity of $R_1R_2N(CH_2)_2C(=NH)NR_3R_4 \cdot a$HX in water and DMSO

No.	R_1	R_2	R_3	R_4	*a*HX	$R_1R_2N^c$	$C(=NH)NR_3R_4^c$	$R_1R_2N^c$	$C(=NH)NR_3R_4^c$
1	H	H	H	H	2HBr	7·50	11·6	9·7	13·6
2	H	ME	H	H	2HBr	7·73	11·4	9·2	13·6
3	H	H	Me	H	2HBr	7·53	11·9	9·4	13·4
4	H	H	Me	Me	2HBr	7·48	12·0	9·7	13·4
5	Tos[d]	H	H	H	HCl	9·49[e]	10·8	15·7[e]	13·3
6	Tos	Me	H	H	HCl	—	11·5	—	13·3
7	Tos	H	Me	H	HCl	10·02[e]	11·9	14·5[e]	13·3
8	Tos	H	Me	Me	HCl	9·84[e]	11·4	14·7[e]	13·0
9	—[g]	H	H	H	HBr	—[f]	11·2	—[f]	12·8

[a] ±0·03 pK_a units for pK_{a_1}, ±0·2 pK_a units for pK_{a_2}.
[b] ±0·2 pK_a units.
[c] Protonation or deprotonation centre.
[d] 4-Toluenesulphonyl.
[e] Refers to neutral tosylamide.
[f] Not determined.
[g] Tripyrrolecarboxamide residue lacking a methyl substituent on the second pyrrole fragment (see Figure 1).

$PhSO_2$), the NH acidity of the R_1R_2N and NR_3R_4 groups is extremely low.[13-17] This is evidently not the case for **5–8**, which contain the TosNH fragment. By analogy with $PhSO_2NH_2$[$pK_a(H_2O) = 10{\cdot}3$,[15] pK_a (DMSO) = 16·0[13,16]] or $PhSO_2NHMe$ [pK_a (H_2O) = 11·6[15]], one might expect the acidity of **5–8** to be at least comparable to, or even higher than, those of these two sulphonamides.

In most of the potentiometric titration experiments with the salts given in Table 1, in DMSO two definite equivalence points were monitored corresponding to consecutive deprotonations of those compounds. In water, **1–4** gave one well defined equivalence point. For **5**, **7** and **8** the equivalence point was very weak, almost non-existent. Compound **6** did not give an equivalence point at all. The reason for the absence of the second (or even the first) equivalence point on the titration curves is definitely the high values of the corresponding pK_a.

The DA analogue **9** (see Figure 1) has four carboxamide moieties, one pyrrole NH fragment and one protonated amidine group. The potentiometric titration curve for this compound also shows only one stoichiometric point in DMSO (none in water), which, by analogy with **6**, refers to protonation of the amidine function.

Assignment of the protonation centres for **1–4** seems to be straightforward. In these compounds, the $R_1R_2N(CH_2)_2$ fragment can be considered to be an amine function attached to the electron-withdrawing $C(=NH_2^+)NR_3R_4$ group. The pK_a (H_2O) for $EtNH_3^+$ is 10·7,[15] whereas in DMSO the corresponding value is in the range 10·6 – 10·9.[13,18] The replacement of an H in the methyl group of $EtNH_3^+$ by the positively charged $C(=NH_2^+)NR_3R_4$ group should theoretically reduce the basicity of the R_1R_2N fragment, as, is borne out in practice [$7{\cdot}5 \leq pK_a(H_2O) \leq 7{\cdot}8$; $9{\cdot}2 \leq pK_a$ (DMSO) $\leq 9{\cdot}7$] (see Table 1).

The second pK_a value for **1–4** evidently corresponds to the deprotonation of the amidinium fragment, thus closely resembling the pK_a pattern for simple amidines [pK_a (H_2O) is usually in the range 11–12[15] whereas pK_a (DMSO) is normally around 13–15[13,18-21]].

Compound **6**, owing to the absence of an NH bond in the R_1R_2N fragment, cannot be deprotonated at that site. In agreement with the literature[10,15,18] and the above discussion, the measured pK_a value corresponds to the deprotonation of the amidinium fragment.

The behaviour of the three remaining Tos-substituted 3-aminopropionamidines, **5**, **7** and **8**, is more complicated. These compounds could be considered as analogues of simple sulphonamides [pK_a (H_2O) for 4-$MePhSO_2NHMe$ is 11·7;[15] for further examples, see Refs 10–15] which carry the neutral or charged amidine function $C(=NH)NR_3R_4$. The latter should make these compounds (i.e. **5**, **7** and **8**) more acidic than the above-mentioned reference compound. Table 1 shows that this is indeed the case in aqueous solution, although this effect never exceeds 1 pK_a unit (see **7**). At the same time, the TosNH and TosNMe fragments decrease moderately the basicity of the amidine function in **5–8**.

However, the behaviour of compounds **5**, **7** and **8** in DMSO seems to be different from that in aqueous solution. The lower pK_a values (13·0–13·3) for these compounds seem to be associated with deprotonation of the amidine function (cf. also **6**), which is slightly affected by the remote neutral TosNH (or TosNMe) group. Consequently, the higher pK_a values for compounds **5**, **7** and **8** seem to reflect the deprotonation of the NH fragment of the sulphonamide function. Obviously, the acidifying effect [compared with the reference compound $PhSO_2NH_2$ (pK_a in DMSO = 16·0)[13,16]] of the deprotonated neutral amidine function [$C(\geq NH)NR_3R_4$] is not very significant. The solvent-induced reversal of the order of deprotonation of the TosNH group and the amidine function in **5**, **7** and **8** is evidently due to solvent effects of different intensity in this process when water is replaced by DMSO. As judged from Table 1, the solvent effects upon going from H_2O to DMSO on the R_1R_2N function in **1–4** or on the amidine function of all compounds studied amounts to as much as 2 pK_a units. At the same time, the solvent effects for **5**, **7** and **8**, associated with the acidic dissociation of the TosNH function, range from 4·9 (**8**) to 6·2 (**5**) pK_a units. These findings are further supported by literature data,[18] which show that the transfer of cationic acids from water to DMSO is relatively insensitive to solvent effects, whereas the acidity of neutral NH acids is much more solvent dependent.[17,22,23]

The acidifying effect of an adjacent CO group in **9** is, as expected, considerably less pronounced than that of the Tos function.[13-16] Under aqueous conditions, acidic dissociation of the neutral carboxamide moiety (CONH) does not occur, and the measured pK_a value in Table 1 in both solvents refers to deprotonation of the amidinium function, which, in turn, should exercise a moderate acidifying effect on the remote aliphatic 2-pyrrolecarboxamide fragment. Therefore, one can conclude that the 3-aminopropionamidine derivatives and **9** behave similarly.

CONCLUSION

The observed differences between the pK_a values of the amidinium and ammonium groups in **1–4** are sufficiently large to permit selective deprotonation of the latter, which, in turn, facilitates its acylation by a suitable DA carboxylic acid precursor. Such coupling reactions require meticulous monitoring of the pH in order to avoid excess base. In an aqueous environment it is important to suppress undesired hydrolysis of the amidine group, which often occurs under alkaline conditions. Our recent experience prompted us to focus on the use of anhydrous conditions to achieve this

crucial step and, owing mainly to its favorable solubility properties, DMF is the preferred solvent in this context. We measured the pK_a values in DMSO to facilitate meaningful comparison with data available previously.

The present results therefore provide a firm experimental basis for application of the 3-aminopropionamidine derivatives **1–4** for the final direct attachment of the unprotected aliphatic side-chain to the trimeric pyrrole-carboxylic acid. By choosing a suitable pH in aqueous solutions or by employing otherwise carefully controlled reaction conditions in anhydrous media in this crucial last step in our synthetic scheme, it is possible to acylate selectively the amino group in the presence of the unblocked protonated amidinium function in satisfactory yield with a minimum of side products.

EXPERIMENTAL

Compounds **1–8** were prepared by standard methods as described previously.[3] The DA analogue **9** originated from the same work.[3]

DMSO was purified as described earlier.[16–18]

The procedures for the determination of the pK_a values in water and DMSO were similar to those described earlier.[16–19,24] Potentiometric titration at 298 K with a glass electrode was used in both solvents; 0·1 M KOH was used as a titrant in water and a 0·01 M molar solution of Bu_4NOH in propan-2-ol–benzene (1 : 4, v/v) in DMSO. In water the glass electrode was calibrated using standard buffers, in DMSO benzoic acid served as reference compound.

The calculation method used was different from that used previously. Most of the acids studied in this work are diprotic and the two pK_a values are close to each other. Hence, the titration of the second acidic group begins before that of the first group has finished. This means that three different forms of the compound are present in the solution simultaneously: AH_2^{2+}, AH^+ and A in the case of **1–4** and AH_2^+, AH and A^- in the case of **5**, **7** and **8**. Therefore, a more complex data treatment was necessary. The calculation method used takes into account the presence of all three forms of a compound in solution and is described in Ref. 25.

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