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Note

The ionisation properties of acyclovir and deoxyacyclovir

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Summary

The ionisation constant pK_a for N_2-acetylacyclovir was determined by a spectrophotometric method. The value obtained for pK_a of N_2-acetylacyclovir and previously reported pK_a values (Kozjek et al., Acta Pharm. Jugosl., 38 (1988) 61–69; Kristl et al., Int. J. Pharm., 57 (1989) 229–234.) for acyclovir and deoxyacyclovir were compared with the aim of locating basic and acidic moieties in the molecules.

Acyclovir (ACV) is one of the most effective and selective agents against viruses of the herpes group. Because of its low bioavailability (only 15–30% of the dose is absorbed from the gastrointestinal tract (Laskin, 1983; Dorsky and Crumpacker, 1985; Fletcher and Bean, 1985)) different analogues were synthesised. One of them is deoxyacyclovir (DCV) which is readily absorbed (about 75% of the dose) after oral administration (Petty et al., 1987). The problem of poor acyclovir absorption from the gastrointestinal tract was also approached by the synthesis of different esters and *N*-substituded derivatives of ACV (Colla et al., 1983; Boryski et al., 1988; Bundgaard et al., 1991).

There are only a few articles dealing with the physico-chemical properties of the above-mentioned guanine derivatives (Kozjek et al., 1988; Kristl et al., 1989; Bundgaard et al., 1991). In this work, we wished to elucidate the ionisation properties of these derivatives and discuss the location of the basic and acidic moieties in the molecules, regarding some recent interpretations (Bundgaard et al., 1991), claiming that the imidazole moiety in acyclovir possesses weakly basic properties, whereas the 2-NH moiety is weakly acidic. The ionisation constant of N_2-acetylacyclovir (N_2AcACV) (9-(2-hydroxyethoxymethyl)-N_2-acetyl)guanine) was determined by a spectrophotometric method.

Reagents and solutions: N_2AcACV was synthesised at Krka, Pharmaceuticals (Novo mesto, Slovenia) (Štimac and Kobe, 1990). The buffers used for spectrophotometric determination of K_a were the following: citrate (pH 1.1–5.0), phosphate (pH 5–8) and borate (pH 8–10.5). The ionic strength of all buffers was adjusted to 0.25 with NaCl.

Ionisation constant: the ionisation constant (K_a) of N_2AcACV was determined by a spec-

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trophotometric method (Kozjek et al, 1988). N_2AcACV was dissolved in water and the resulting solutions were suitably diluted with appropriate buffers. The pK_a was calculated using eqn 1:

$$pK_a = pH + \log(A_I - A)/(A - A_N) \quad (1)$$

where A is the absorbance at various pH values, A_N the absorbance of the nonionized form and A_I the absorbance of the anionic form.

The values for pK_a of N_2AcACV were calculated by linear regression method, where pH was taken as a dependent variable and $\log(A_I - A)/(A - A_N)$ as independent variable. The absorbances were measured with a Perkin Elmer 554 spectrophotometer at different wavelengths, close to the maximum of the absorption: λ = 259.6, 259.8, 260, 260.2, and 260.4 nm. pH values were measured on an Iskra MA 5705 apparatus.

The ionisation constants for ACV and DCV were determined previously by two different methods. The values obtained by spectrophotometry (Kozjek et al., 1988) ($pK_{a_1} = 2.19$; $pK_{a_2} = 9.35$ for ACV and $pK_a = 3.63$ for DCV) are in good agreement with those calculated by the partition method (Kristl et al., 1989) ($pK_{a_1} = 2.40$; $pK_{a_2} = 9.20$ for ACV and $pK_a = 3.63$ for DCV).

In a preliminary experiment it was established that in the pH range from 7.0 to 9.5 the absorbance of N_2AcACV increased with growing pH values and remained constant at lower and higher pH values (Fig. 1). Accurate determination of the pK_a thus required an experiment within the pH range 7.0–10.0 (Table 1). The calculated values of N_2AcACV were the following: 8.58, 8.55, 8.54, 8.52 and 8.50 and the mean value including standard deviation was $pK_a = 8.54 \pm 0.03$.

Following the molecules of growing complexity from purine to guanine, it is evident that the introduction of groups markedly changes the pK_a values of the molecule (Lister et al., 1971). Purine has two constants of ionisation, weakly basic, $pK_{a_1} = 2.4$ and weakly acidic, $pK_{a_2} = 8.9$. The predicted site of protonation is N1, while ionisation to the anion occurs at N9. The basic strength of 2- and 6-hydroxypurines is less than, and that of 8-hydroxypurine slightly greater than, that of

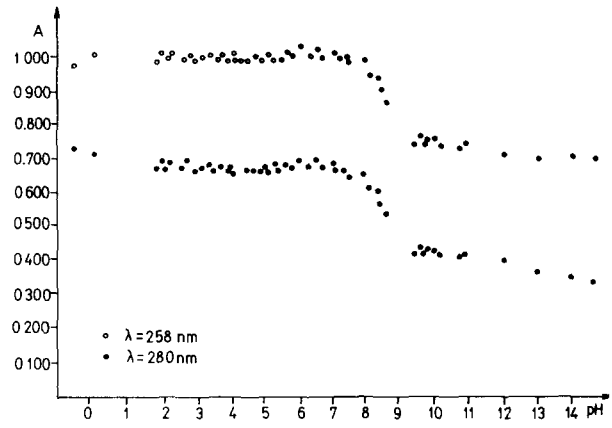


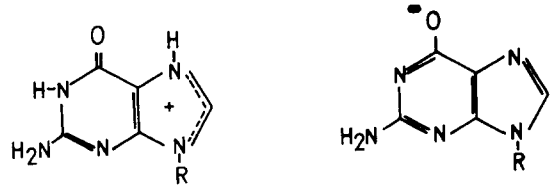
Fig. 1. Absorbances of N_2AcACV in buffer solutions at various pH, in 0.5 and 1 M HCl and in 0.5 and 1 M NaOH at two different wavelengths.

purine. As expected, an amino substituent exerts a generally base-strengthening action. Therefore, the values for the ionisation constants of adenine are $pK_{a_1} = 4.2$ and $pK_{a_2} = 9.8$ with predicted sites of protonation and anion ionisation at N1 and N9, respectively. The acido-basic character of guanine is expressed by the values of the ionisation constants being intermediate between those of purine and adenine: $pK_{a_1} = 3.3$ and $pK_{a_2} = 9.2$ with different site of protonation –N7, whereas

TABLE 1

Absorbances of N_2AcACV solutions in buffers ranging from pH 7.27 to 9.81 and in 1 M HCl and 1 M NaOH at different wavelengths

pH	$A_{259.6}$	$A_{259.8}$	$A_{260.0}$	$A_{260.2}$	$A_{260.4}$
7.27	1.007	1.005	1.002	0.999	0.997
7.48	0.988	0.986	0.984	0.980	0.977
7.88	0.994	0.992	0.990	0.987	0.985
8.09	0.961	0.959	0.958	0.954	0.953
8.28	0.946	0.944	0.943	0.942	0.939
8.43	0.909	0.908	0.905	0.904	0.903
8.59	0.861	0.861	0.861	0.860	0.860
8.66	0.882	0.881	0.880	0.878	0.877
9.42	0.753	0.753	0.752	0.753	0.753
9.60	0.786	0.786	0.787	0.786	0.786
9.68	0.748	0.750	0.751	0.750	0.749
9.74	0.780	0.780	0.782	0.781	0.781
9.81	0.781	0.781	0.782	0.782	0.782
1 M HCl	1.040	1.043	1.044	1.046	1.049
1 M NaOH	0.730	0.730	0.730	0.729	0.729



a b

Fig. 2. The predominant structures of protonated (a) and deprotonated (b) guanosine. R = D-ribofuranose.

the anion ionisation takes place at the same site – N9, as for purine and adenine (Lister et al., 1971). Considering the alkyl substitution at N9, i.e., ACV derivatives, the situation is very similar to that of guanosine. For guanosine, protonation of the imidazole ring is well established, the predominant structure of the cation being that presented in Fig. 2a. Acidic dissociation of guanosine is indicated to yield anions with the negative charge principally on the oxygen atom as shown in Fig. 2b.

The results obtained for the ionisation constants for ACV indicate that introducing a 2-hydroxyethoxymethyl group at position 9 of guanine lowers the basic strength of the molecule, whereas the value for the acidic ionisation constant remains unchanged; in fact, the pK_a values of ACV are close to those of guanosine, which are $pK_{a_1} = 2.1–2.2$ and $pK_{a_2} = 9.2–9.5$ (Shapiro, 1968). Regarding the molecule of DCV the basic strength is increased and the acidic character is lost, a single pK_a value of 3.63 being observed. In contrast, for the molecule of N_2AcACV only acidic properties are present. This can be explained by the presence of two amide moieties in the molecule. The negative charge of the anion can be more delocalised and the consequence is increased acidity (Fig. 3). Therefore, the value obtained for the ionisation constant ($pK_a = 8.54$) is very close to the pK_a values for imides (pK_a for phthalimide: 8.3 (Winholz, 1983)), where delocalisation of the negative charge is also the case. It has thus been shown that the introduction

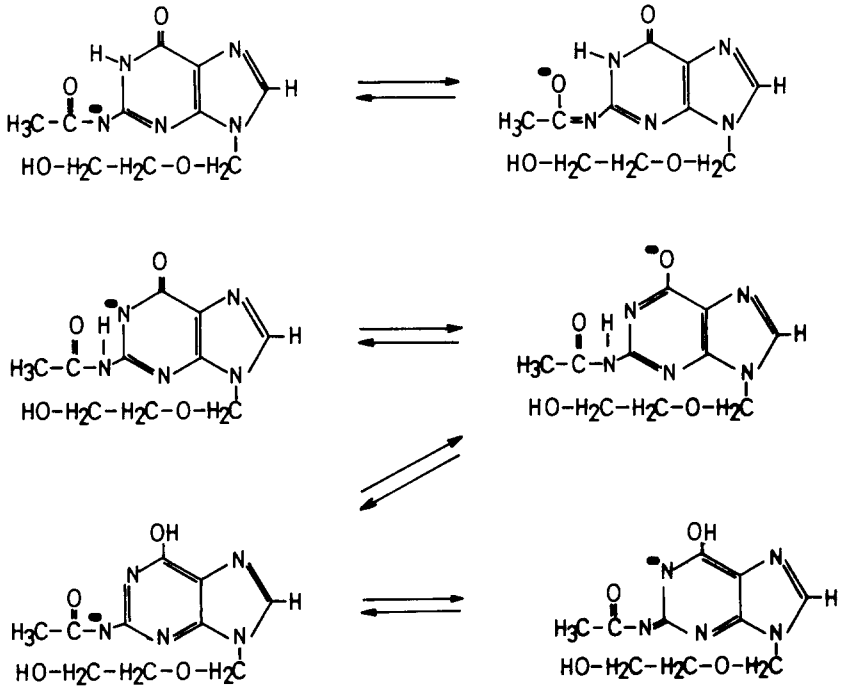


Fig. 3. Possible tautomers of N_2AcACV.

of an acetyl group into the ACV molecule at the 2-NH_2 position hinders the basic character of ACV. Therefore, the basic nature of treated guanine derivatives to a great extent depends on the 2-NH_2 group.

Overall, we assume that the weak basic properties of this series are attributed to the imidazole moiety with the contribution of the 2-NH_2 group, whereas the acidic moiety is at the oxygen atom (6-enol group, with the connection to theN1 moiety), which can be deprotonated.

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