Spectroscopic investigation of the tautomeric equilibria in the guanine derivatives of acyclovir

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The effect of solvent polarity on the keto–enol tautomerism of *N*- and *O*-acetylated acyclovir derivatives has been studied. Results of different spectroscopic methods (^{1}H NMR, UV/VIS, IR, Raman) have been interpreted in combination with the results of semi-empirical calculations and by comparison with two derivatives of deoxyacyclovir. From the UV/VIS spectra it was found that the keto–enol equilibrium in acyclovir is strongly dependent on the solvent polarity; in methylene chloride the enol form was observed, whereas in water the keto form dominates. However, acetylation on the N terminal function stabilises the keto tautomer in all tested solvents, due to the formation of an intramolecular hydrogen bond between the acetyl CO function and H(^{1}N). Substitution on the OH group in the side function does not influence the tautomeric equilibrium but changes the polarity of the substance and makes it almost insoluble in apolar solvents such as methylene chloride.

Introduction

Guanidine derivatives are well known for their antiviral activity. This makes this class of compounds interesting for the development of further effective compounds having the guanine moiety and different substituents. In particular, acyclovir or 9-(2-hydroxyethoxymethyl)guanine, has been established as an efficient drug in the treatment of herpes.[1,2] For pharmaceutical purposes the octanol–water partition coefficients are generally regarded as important parameters for the estimation of the distribution and transport properties of drugs in biological systems. The prediction of partition coefficients from the structure of the compounds is, therefore, an important task in drug discovery, especially with regard to their use in quantitative structure–activity relationships, QSARs.[3] Kristl *et al.* have studied a number of properties and behaviour of guanine derivatives in solution.[4–6] In particular, they characterised the solid properties of the guanine derivatives, acyclovir (ACV), deoxyacyclovir (DCV) and their *O*-acetyl (OAcACV, OAcDCV), *N*-acetyl (NAcACV) and *N*,*O*-diacetyl (diAcACV) derivatives. The solubility and partitioning behaviour of the substances were investigated by the conventional shake-flask method as well as by RP-HPLC using a C18 column as the stationary phase and an aqueous methanol mobile phase.[7] When the log *P* values were calculated according to the Rekker method[8,9] very strong deviations from the experimental values were shown in the case of OAcACV and diAcACV; these compounds were more hydrophilic than calculated. In the case of ionic amino acids such deviations can be reconciled by the conformational flexibility of the substances which can be taken into account by the use of various correction factors.[10] However, the flexibility of the guanine moiety is limited by the rigidity of the conjugated π-electron system. Thus, the question arises as to how the deviation between calculated and experimental values of various properties can be explained. One possibility is that of the tautomeric equilibrium in acyclovir derivatives as shown in Fig. 1.

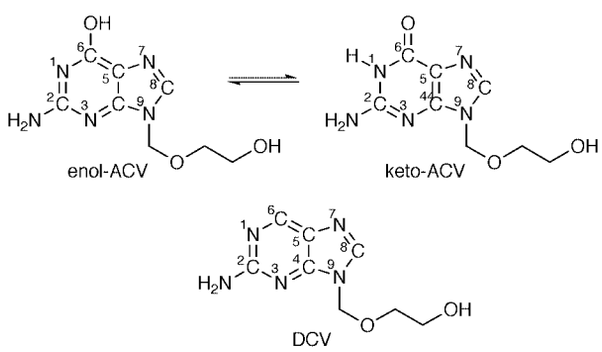

Fig. 1 Structures of acyclovir ACV (enol and keto tautomer) and deoxyacyclovir DCV derivatives.

From the arrangement of the functional groups in the *N*-acetylated ACV derivatives intramolecular hydrogen bond formation between the amide function and the H^{1}N function in the guanine system can occur. Also conformational effects in the flexible side chain need to be taken into account.

In order to investigate the hydrogen bonding properties of the guanine derivatives, IR, Raman and UV/VIS and NMR spectroscopic studies were carried out. Additionally, semi-empirical AM1 calculations were performed in order to help to explain the hydrophilic anomalies of the ACV and DCV derivatives.

Results and discussion

AM1 calculation

Based on its enormous importance as a DNA and RNA base the isolated guanine moiety has been the subject of numerous quantum mechanical studies.[11,12] In guanine itself two different kinds of tautomers have to be considered. Firstly, there exists an oxo-amino form of the H^{9}N and H^{7}N tautomer. Secondly, an

equilibrium of the oxo-amino and the hydroxo-imido tautomers may occur. In the compounds studied in this work, the substitution on the H^9N positions reduces the possibilities and only oxo-amino and hydroxy-imido tautomers can occur. These are named keto and enol tautomers throughout this paper.

As Kristl and Pecar reported previously, the folded conformation of ACV is favoured over the extended conformation only by *ca.* 1 kcal mol^{-1}.[7] Thus, the arrangement of the flexible side chain seems to be less important for the stability of the derivatives if no intramolecular hydrogen bonds of the OH or acetyl function can be formed. For the semi-empirical calculations a number of starting conformations of the keto as well as of the enol form were created which would also allow intramolecular hydrogen bonding of the OH group to acceptor functions as the *N*-acetyl function or the π-electron system of the purine ring. However, in no case do these conformations correspond to the global minimum of potential energy. Thus, in the remainder of the AM1 calculations we focussed on the purine function and left the side chain in the folded arrangement of an all-*gauche* conformation of the aliphatic group. This approach was substantiated by the NOESY results.

Generally, the outcome of these calculations showed that the keto forms of the ACV derivatives are thermodynamically favoured. The differences in the total energies of the keto and the enol form were found to be *ca.* 5 kcal mol^{-1} for ACV and OAcACV. *Ab initio* quantum mechanical calculations performed for 9-methylguanine with 6-31G basis set at the MBPT2 level with zero point energy gave a stabilisation of the keto tautomer by just −5.1 kJ mol^{-1}.[13] The energy differences calculated by the AM1 method seem rather large but can be explained by the AM1 parameterisation which obviously overestimates the energetic differences between the tautomers.

In the case of the *N*-acetylated derivatives a further stabilisation occurs due to inclusion of the amide function into the conjugated π-electron system. In the resulting planar system the formation of an intramolecular hydrogen bond was found, as the $O\cdots H(^1N)$ distance of 2.3 Å shows. Thus, the keto tautomer in these compounds is stabilised by *ca.* −10.5 kcal mol^{-1}. The properties in solution or in the crystal lattice are, however, different from those of the isolated molecule of the semi-empirical calculations. Thus, if the energy barrier can be surmounted both isomers may exist in equilibrium.

The solubility of a compound reflects the result of solute–solute interactions in the solid, and solute–solvent interaction in solution. Both of these interactions will depend, in part, on the overall dipole moment. The vector sum over the bond moments of the AM1 calculation in the backfolded conformers shows that the dipole moment for the DCV is only half the magnitude of the keto form of the ACV derivatives. However, the dipole moment of the enol ACV derivatives was found to be of the same order as the DCV derivatives. As a consequence of different solute–solvent interactions the solubility of the keto tautomer should be promoted by polar solvents whereas the solubility of the enol form should be favoured in less polar media.

The keto–enol tautomerism also affects the electron density distribution in the π-electron system of the purine ring. Although the partial atomic charges depend strongly on the parameterisation of the semi-empirical approach the relative comparison should indicate differences in the electron distribution. Generally, the net charges on the purine atoms in ACV and DCV are different. With insertion of an OH group on ^6C its immediate environment experiences an electronic distortion which diminishes with the distance to ^6C. Consequently, the net charge on the ^9N is similar for ACV and DCV but on ^1N the deviation is quite strong. Substitutions in the side chain of the DCV and ACV derivatives do not cause major disturbance. Secondly, as a consequence of the keto–enol tautomerism the net charges on the atoms in the purine ring are changed drastically. Thus, the net charges in the enol-ACV are more similar to those of DCV than to keto-ACV.

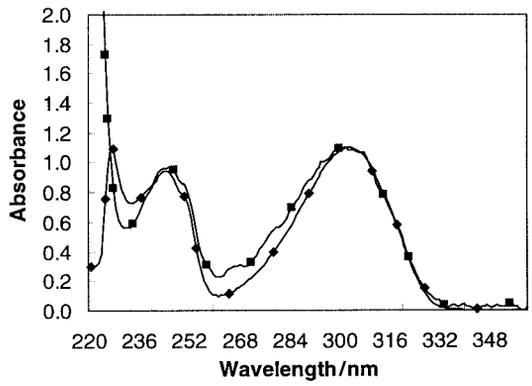

Fig. 2 UV spectra of ACV (■) and DCV (◆) dissolved in methylene chloride.

O-Acetylation in the side chain does not contribute to the change of the purine system. The amide formation on the NH_2 group and, therefore, the extension of the conjugated π system has mainly a positive effect on the ^2C net charge but generally the other atoms are similar in their partial charges.

The change of the electronic properties accompanied by tautomerism can be demonstrated experimentally by means of UV/VIS spectroscopy.

UV/VIS investigation

The conjugated π-electron system of purine acts as an appropriate chromophore for the excitation of electronic transitions. The high sensitivity of this spectroscopic method enables the observation of intense signals even in concentrations as low as 10^{-6} mol l^{-1}. Fig. 2 gives the UV spectra between 190 and 350 nm for DCV and ACV dissolved in methylene chloride. Despite the fact that the signal/noise ratio for the ACV substance was low due to the low solubility of the sample in apolar solvents it can be clearly seen that all signals found for DCV were also found in the spectra of ACV. It might be surprising that the insertion of an OH group does not affect the electronic transitions. However, similar observations were described by Albinsson and Norden[14] for differently substituted purine compounds; the position of the inserted methyl group did not influence the absorption signals.

Based on a comparison with UV studies of purine bases the signals of DCV at 300 nm can be assigned to an isolated n→π* transition. Generally, π→π* transitions occur approximately at 245 nm exhibiting a shoulder at the red shifted region.[14,15] Using methylene chloride as a solvent the region below 230 nm is overlapped by solvent absorptions and, thus, the two in-plane polarised π→π* transitions expected at 200 nm cannot be observed.

Table 1 gives the positions of the absorption maxima and their intensity ratios with respect to the most intense band. For DCV and its derivative, OAcDCV, there was almost no solvent effect found. Only the splitting of the bands at 250 and 245 nm becomes less resolved in H_2O.

However, as Fig. 3 shows, the number and shape of the bands in the ACV spectra changes when another solvent was used. Thus, in acetonitrile we observe a signal at 276 nm whereas the signal at 252 nm is almost unchanged and the band at 301 nm disappears. In the aqueous solutions of ACV five signals were found with absorption maxima characteristic for the polar acetonitrile and the less polar methylene chloride. Thus, it may be deduced that there exists two different species of ACV, namely, just the tautomers in an equilibrium. Based on the similarities of ACV and DCV spectra in methylene chloride and the results of the theoretical investigations mentioned

Table 1 Positions of UV/VIS maxima and their intensity ratios in different solvents

Substance	CH_2Cl_2	Acetonitrile	H_2O
DCV		219 (1.0)	218 (1.0)
	245 (0.859)	245 (0.270)	243 (0.249)
	250 (shoulder, 0.706)	251 (shoulder, 0.208)	
	303 (1.0)	305 (0.308)	304 (0.265)
OAcDCV		219 (1.0)	218 (1.0)
	245 (0.846)	245 (0.282)	243 (0.242)
	250 (shoulder, 0.702)	251 (shoulder, 0.225)	
	303 (1.0)	305 (0.308)	305 (0.257)
ACV		191 (1.0)	200 (shoulder, 1.0)
			216 (0.693)
	245 (0.884)	252 (0.471)	248 (0.664)
	251 (0.759)	276 (0.280)	273 (0.417)
	302 (1.0)		301 (0.156)
OAcACV	—	192 (1.0)	192 (1.0)
	255 (1.0)	255 (0.543)	253 (0.569)
	277 (0.777)	272 (shoulder, 0.375)	276 (0.373)
NAcACV	—	199 (1.0)	200 (1.0)
	253 (1.0)	253 (0.487)	
	260 (shoulder, 0.972)	259 (shoulder, 0.481)	259 (0.620)
	283 (0.736)	282 (0.372)	282 (0.414)
diAcACV		199 (1.0)	200 (1.0)
	253 (1.0)	253 (0.471)	
	260 (shoulder, 0.969)	259 (shoulder, 0.463)	259 (0.606)
	283 (0.743)	282 (0.355)	280 (0.419)

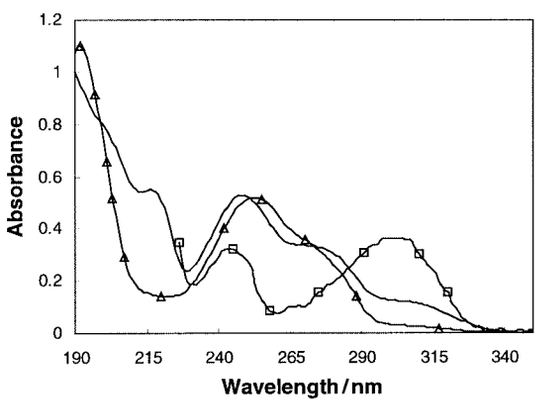

Fig. 3 UV spectra of ACV dissolved in different solvents: methylene chloride (□), acetonitrile (△) and water.

above we would assign the species occurring in methylene chloride as the enol-ACV whereas due to its higher dipole moment the keto species should be preferred in acetonitrile. Since the extinction coefficients of the signals are not known it is difficult to form any conclusions about the concentration of the enol and keto species in water. In this regard the intensity ratio of the proton signals in the ^{1}H NMR spectra is more informative.

The extremely low solubility of OAcACV does not allow any statement of its tautomerism in methylene chloride. However, in acetonitrile the same bands for the *O*-acetylated ACV were observed as discussed for ACV. Based on the observation of a missing solvent effect on the *N*-acetyl derivatives, if there should be any shift of the signals observed in aqueous solution, for the *O*-acetylated species these should correspond to the keto-OAcACV. Surprisingly, OAcACV behaves differently from ACV. From the theoretical results the side group does not change the purine system. Thus, we would conclude that the dipole moment determines the solution properties of the compound in the various solvents.

Both *N*-acetylated derivatives, NAcACV and diAcACV gave almost identical spectra in the three solvents. The shift of the band maxima was within the range of a few nanometres. Compared to ACV dissolved in acetonitrile the bands are bathochromically shifted, which reflects the enlargement of the conjugated π system due to the substitution on the NH_2 function. As the semi-empirical calculations have shown, and as is well-known in organic chemistry, this enlargement stabilises the molecules. Furthermore, as seen above, the O · · · H distance between the ester carbonyl function and the NH group of the keto tautomer allows the formation of an intramolecular hydrogen bond.

From the change of the electronic properties of the purine system due to the tautomerism there should be a consequent effect on the shielding effects of the aromatic protons. Thus, ^{1}H NMR spectroscopy might be able to confirm our interpretation. However, we note that the lower sensitivity of this method and the problems of the solubility of the samples make it necessary to work in saturated solutions.

^{1}H NMR investigation

The use of D_2O as a solvent forces the acidic protons in the NH_2/NH and OH function to exchange rapidly. Thus, signals of these functions are not observed in the ^{1}H NMR spectra. Based on the comparison of the derivatives and the NOESY spectra an assignment of the signals can be carried out (Table 2).

From the comparison of the ^{1}H NMR spectra we can unambiguously assign the proton chemical shifts. However, in ACV we have found an abnormal behaviour: the ^{8}C–H proton in the five membered ring gives two signals. The intensity of both signals changes with the concentration, which might be an effect of aggregate formation at higher concentration, which forces the substances to transform into the enol compound. In saturated D_2O the intensity ratio was found to be equal to 1 : 1. Two singlets were also found for the CH_2 group on the ^{9}N position; again each signal representing approximately half of the expected intensity. Comparing the positions of the low field shifted band of ACV with that of DCV and OAcDCV we found almost the same shielding effects. From the UV/VIS and AM1 results we would assign the low field signal to the enol arrangement exhibiting a widely spread conjugated π-electron system and the high field signal to the keto form of the ACV.

The proton resonances of OAcACV corresponded to the high field signals of ACV. Here the shielding effect due to the ring current is lower which is probably due to the keto arrangement. Again only one tautomer is detected for OAcACV dissolved in water.

Table 2 ^{1}H NMR signals of the ACV and DCV derivatives (in ppm) in solution in D_2O

DCV	OAcDCV	ACV	OAcACV	NAcACV	diAcACV	Assignment
	1.816 (singlet)		1.847 (singlet)		1.793 (singlet)	CH_3CO (*O*-ac)
				2.183 (singlet)	2.186 (singlet)	CH_3CO (*N*-ac)
3.569 (singlet)		3.569 (singlet)		3.577 (singlet)		CH_2OH
	3.751 (multiplet)		3.746 (multiplet)		3.772 (multiplet)	CH_2O
	4.054 (multiplet)		4.056 (multiplet)		4.064 (multiplet)	$CH_2OC(O)$
		5.403 (singlet)	5.409 (singlet)			NCH_2O
5.542 (singlet)	5.543 (singlet)	5.506 (singlet)		5.516 (singlet)	5.514 (singlet)	
		7.817 (singlet)	7.827 (singlet)			^8CH
8.192 (singlet)	8.197 (singlet)	8.119 (singlet)		8.035 (singlet)	8.039 (singlet)	
8.625 (singlet)	8.641 (singlet)					^6CH

The substitution on the NH_2 function influences the properties in the purine ring, as the AM1 calculations and the UV/VIS spectra have shown. In contrast to the ACV spectra there was only one set of signals found but the chemical shifts of ^8CH and $^9NCH_2$ are close to the signals found for DCV and the enol-ACV derivative. However, the low field shift of the ^8CH and the $^9NCH_2$ signals is probably caused by the insertion of the amide function in the π-electron system and not by the presence of enol tautomers.

O-Acetylation of the DCV and ACV derivatives caused a coupling of the ethoxy signals which was not found for the derivatives having an alcohol function in the side chain. However, two signals for the ethoxy functions were found in the ^{13}C spectra and, thus, it happens more or less by chance that the ^1H chemical shift of both CH_2 functions is identical in the alcohol compounds. This effect disappears with a change in solvent.

The absence of cross peaks in the NOESY experiments of diAcACV dissolved in DMSO-d_6 indicate that the distance between the amide NH and the ester carbonyl function in the side chain is too large for the formation of an intramolecular hydrogen bond. However, the correlation signals observed for the ^8CH and the protons of the ethoxy function on diAcACV and NAcACV confirm the quantum mechanical result of a stable conformation exhibiting a backfolded side chain by a correlation signal of ^8CH and the side function.

IR and Raman investigation

Although IR spectroscopy is a well-acknowledged technique for studying hydrogen bonds, in our case the low solubility of the substances in apolar solvents and the presence of crystal water complicates the analysis of the NH stretching region. But as already reported the tautomeric equilibrium reflects on the aromatic system. Gould *et al.* have studied guanine tautomers by means of matrix isolation technique and have assigned typical signals based on theoretical calculations.[16] Comprehensive IR and Raman studies on alkyl guanine derivatives were also performed by Szczepaniak *et al.* in order to describe the effect of hydrogen bonding and tautomerism in solids.[17–21] Hence, we studied the spectra of different solutions in the regions of the aromatic modes and the carbonyl stretching vibration. The results are given in Table 3.

It is reasonable to suppose that none of the bands in the region between 1800 and 1400 cm^{-1} corresponds to a pure group vibration. Thus, all bands may be considered as ring vibrations in which some group motions contribute more or less strongly. However, the comparison of the typical signals for the DCV derivatives found allowed the assignment of the bands at *ca.* 1630, 1580 and 1480 cm^{-1} to in-plane ring vibrations of the aromatic system. The experimental data in combination with theoretical calculations show crucially that the signal at 1480 cm^{-1} is only found in the enol tautomer of guanine. Based on this band any enol tautomers can be identified.

For ACV this signal was found in the solid phase (1479 cm^{-1}) as well as in methylene chloride (1484 cm^{-1}). In acetonitrile there was also found a signal at 1490 cm^{-1} and in D_2O a shoulder at 1488 cm^{-1}. Thus, these findings seem to be contradictory to the former observation in the UV/VIS spectra. However, the recording of IR spectra was generally difficult since the substances under investigation were poorly soluble in the series of solvents.

Despite these discrepancies we will discuss any further IR data based on the assignment by Gould and the similarity correlation between guanine derivatives and our compounds.

In the spectra of OAcACV the characteristic ester carbonyl signal at 1755 cm^{-1} with a shoulder at the low frequency side and signals of the ring vibrations at 1605 and 1543 cm^{-1} were observed. The band at 1480 cm^{-1} was not observed. The non-occurrence of this signal might indicate that there is only one OAcACV tautomer found in apolar solutions which might be explained by the higher polarity *e.g.* dipole moment of the enol-OAcACV in comparison to ACV and, thus, the lower solubility of this tautomer. However, this does not mean that the tendency to form both tautomers is less in the OAcACV compound but only that the solubility properties of the tautomers are different and, therefore, the enol-OAcACV was not observed in solution.

The amide I band of the *N*-acetyl group cannot be distinguished from the signal of the amide I band of the ring system. Thus, the spectra of NAcACV, diAcACV and OAcACV look very similar in D_2O solution. Due to the intermolecular solvent–solute interactions the signals are generally very broad and, thus, the guanine ring vibrations were not found and the amide I signals were shifted to lower wavenumbers. Additionally, the *O*-acetyl protected derivatives exhibit a signal for the ester carbonyl stretching vibration.

Conclusion

Our spectroscopic and quantum mechanical studies have shown that the tautomerism in acyclovir is substantially different to that in its *N*-acetyl derivatives. Thus, the tautomeric equilibrium in ACV is strongly dependent on the solvent. Based on a comparison of ACV with DCV, the UV/VIS spectra of methylene chloride solution indicate the presence of mainly the enol form whereas in acetonitrile solution only the keto form of ACV is found. In water, both tautomers are present, and based on the ^1H NMR results the enol/keto ratio is almost unity in the saturated solution. According to Gould the signal at 1480 cm^{-1} can be assigned to a ring mode. It was found in all cases where we had evidence of the existence of keto-tautomers. It was also observed in the spectra of solid ACV. The strong influence of solvent on the ACV tautomeric equilibrium was not found for any of the acetyl derivatives. Net charges calculated for the backfolded conformer of OAcACV show that the conjugated π-electron system is comparable with that of ACV, and the acetyl group seems not to influence the system. However, our spectrocopic studies suggest that only the keto form of OAcACV is present in methylene chloride, acetonitrile and water, in marked contrast to ACV itself. This might be an

Table 3 CO stretching vibrations and phenyl in-plane modes of ACV and DCV and their derivatives given in cm^{-1}

Compound	KBr	Raman	Methylene chloride	Toluene	Acetonitrile	DMF	D_2O
DCV	1632 s	1613 m	1627 (shoulder)	not soluble			
			1613 s		1616 s		1624 s
	1584 s	1581 w	1603 (shoulder)		1579 m		1584 s
	1521 w	1520 m	1581 s		—		1532 m
			1512 w		—		1489 w
	1477 m	1451 w			—		1438 w
OAcDCV	1737 (shoulder)	1720 w	1737 s	1745 w	1740 s	1740 s	
	1720 s		1722 (shoulder)	1723 w			1720 (broad)
	1631 s		1613 s				
	1610 w	1617 s	1603 (shoulder)		1616 s	1610 s	1624 s
	1581 s	1577 w	1581 s	1579	1579 m	1570 w	1584 s
	1520 w	1520 s	1511 w		—		
	1481 w				—		
ACV	1696 s	1693 w	1725 w	1727 s	1705 w	1723 w	1665 s
	1663 w		1635 w	1629 s	1631 s		1624 w
	1629 s	1627 w	1620 w		1527 w		1581 s
	1602 w		1609 s		1515 w		1570 (shoulder)
	1574 m	1574 s	1542 w		1490 m		1533 w
	1539 m	1483 s	1484 w				1488 (broad)
	1479 m	1455 w					1438 w
OAcACV	1755 s	1735 w	1757 s	1756 w	1755 w	1740 w	1718 (broad)
	1737 (shoulder)	1694 w	1737 (shoulder)	1729 w	1634 s	1723	1674 s
	1721 s		1727 s	1635 w			1578 (shoulder)
	1637 s		1667 w		1541 w		1561 m
	1605 s		1635 s		1520 w		1526 m
	1572 w	1572 s	1605 s		1489 w		1467 w
	1543 w		1543 m				
	1491 m	1489 s	1490 m				
	1452 w						
NAcACV	1714 s		1716 (broad)	not soluble	1723 (shoulder)	1721 (shoulder)	
	1706 (shoulder)	1676 m	1667 (broad)		1634 s	—	1676 s
	1676 s						
	1617 m		1609 m		1613 s		1557 m
	1557 m	1559 s	1557 w (broad)				1527 m
	1539 m						1466 w
	1492 w	1492 m					
diAcACV	1737 (shoulder)		1737 (shoulder)	not soluble	1740 s	1740 s	1718 (shoulder)
	1730 s				1712 s		
	1707 s		1716 s			—	
	1694 (shoulder)	1695 m	1703 s		1697 m		
	1667 (shoulder)	1665 m					1676 s
	1609 s	1562 s			1613 s	1610 s	1559 m
	1559 m		1609 s		1558 m		1526 m
	1535 m		1557 w				1467 w
	1476 m	1475 m	1539 w				

effect of the low solubility of the keto tautomer in polar as well as in apolar solvents.[22]

In the case of the *N*-acetyl derivative, NAcACV, our calculations show that the keto form is energetically considerably more favoured than the enol form. Very probably, the stabilisation of the keto form is helped by the intramolecular hydrogen bonding between the NH group in the ring at position 1, and the C=O function of the *N*-acetyl group. In any event, it appears that NAcACV occurs generally in the keto form, as is the case also for the di-acetylated derivative, diAcACV.

Our ^1H NMR correlation experiment showed that the coupling pattern of the ethoxy group in D_2O was not observed for DCV, ACV and NAcACV, indicating that the OH group can rotate freely and is not restricted by intramolecular interactions. NOESY results show that the distance between the acetyl group and the imidazolinic proton is less than 5 Å, a distance that can be realised in a backfolded arrangement as proposed by Kristl.[7]

We started these investigations because of discrepancies between observed and calculated water–solvent partition coefficients for ACV and its acetyl derivatives. From our studies, it appears that ACV itself can exist as different tautomeric forms in different solvents, so that attempts to calculate water–solvent partitions for ACV will be very difficult. Furthermore, it will obviously be difficult to use partition data on ACV in order to predict partitions for acetyl derivatives, because partition coefficients for ACV will refer to some mixture of tautomers whereas partition coefficients for acetyl derivatives will refer to the keto form only.

Experimental

Materials

ACV (9-(2-hydroxyethoxymethyl)guanine), NAcACV (N^2-acetylacyclovir), OAcACV (*O*-acetylacyclovir), diAcACV (N^2,*O*-diacetylacyclovir), DCV (2-amino-9-(2-hydroxyethoxymethyl)-9*H*-purine) and OAcDCV (*O*-acetyldeoxyacyclovir) were synthesised at the National Institute of Chemistry (Ljubljana, Slovenia) as described.[23] Throughout the paper the abbreviations given in Table 4 were used. The positions of the substituents R′ and R″ are indicated in Fig. 1.

Semi-empirical calculations

In order to get a first impression concerning the energetic differences of the tautomers, semi-empirical calculations using the AM1 method of the HYPERCHEM packet (Version 4.5) were performed. The standard parameters of the program were used for the geometry optimisation. The general structures were

Table 4 Nomenclature of the compounds used in this investigation

Abbreviation	R′	R″
DCV	H	H
OAcDCV	H	CH_3CO
ACV	H	H
OAcACV	H	CH_3CO
NAcACV	CH_3CO	H
diAcACV	CH_3CO	CH_3CO

based on previous results[7] of a force field calculation, which found the backfolded conformation to be the most stable. A number of starting conformations were created recognising the possible occurrence of intramolecular hydrogen bond formation. The differences of the total energies and the net charges of the atoms were compared and used in the discussion of the NMR spectra.

UV/VIS measurements

A double beam UV/VIS spectrometer Lambda 16 (Perkin Elmer) was used for the recording of the UV/VIS spectra in the region between 500 and 190 nm. A slit width of 2 nm and recording speed of 120 nm min^{-1} were used as experimental parameters. Solutions of the compounds in H_2O, methylene chloride and acetonitrile solutions were measured in a 1 cm quartz cell using the pure solvents as reference probes. The concentrations were approximately 10^{-6} mol l^{-1}.

NMR-measurements

^1H NMR spectra of the saturated D_2O solutions were recorded on a 500 MHz NMR spectrometer (Varian). 32 scans were accumulated for the ^1H NMR spectra at a temperature of 25 °C. In selected cases $^1H/^{13}C$ correlation experiments (COSY and NOESY) of the DMSO-d_6 solutions were performed to support the assignment of the signals.

IR and Raman measurements

All studies were performed on a FTIR spectrometer IFS66 (Bruker) with a resolution of 2 cm^{-1}. 32 scans were accumulated for the IR spectrum to enhance the signal to noise ratio. Due to the low solubility of the substances, NaCl cells of 3 mm thickness were used. The temperature was kept constant at 25 °C. The concentration of the solutions in methylene chloride, toluene, acetonitrile, dimethylformamide and deuteriated water were less than 10^{-3} mol l^{-1}. For the D_2O solutions a 0.03 mm CaF_2 cell was used.

For the Raman spectra of the solid compound 2000 scans were recorded on the IFS66/FRA106 FTIR/Raman spectrometer using a resolution of 2 cm^{-1}.

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