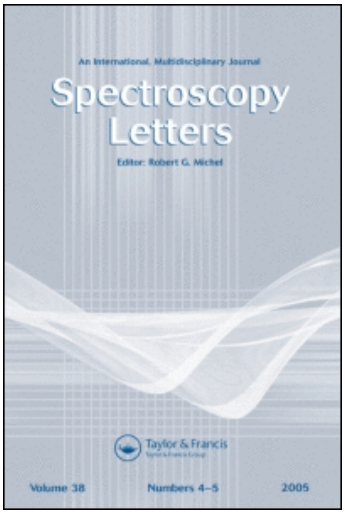

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IR Evidences for *N*-9 and *N*-7 Regioisomers of the Acyclic Purine Nucleoside Analogues

Silvana Raí[a]; Mario Pongraĉić[b]; Mladen Mintas[a]
[a] Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia [b] Pliva Research Institute, Zagreb, Croatia

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IR EVIDENCES FOR *N*-9 AND *N*-7 REGIOISOMERS OF THE ACYCLIC PURINE NUCLEOSIDE ANALOGUES

Key words: Acyclic Nucleoside Analogues, Purine, N-9 and N-7 Regioisomers, IR Spectroscopy

Silvana Raić[a], Mario Pongračić[b] and Mladen Mintas[a]*

[a]*Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Croatia*

[b]*Pliva Research Institute, Prilaz Baruna Filipovića 25, 10000 Zagreb,Croatia*

ABSTRACT

The IR spectra have been recorded in the solid state for the parent molecule, 6-(*N*-pyrrolyl)purine (**1**) and its *N*-9- and *N*-7-substituted derivatives: 9- and 7-(2-hydroxyprop-1-yl)-6-(*N*-pyrrol-1-yl)purine (**2** and **3**), 9- and 7-(2-acetoxyprop-1-yl)-6-(*N*-pyrrol-1-yl)purine (**4** and **5**), 9- and 7-(2,3-dihydroxyprop-1-yl)-6-(*N*-pyrrol-1-yl)purine (**6** and **7**) as well as 9- and 7-(2,3-diacetoxyprop-1-yl)-6-(*N*-pyrrol-1-yl)purine (**8** and **9**). Analysis of the characteristic bands has proved to be useful in differentiating between *N*-9 and *N*-7 regioisomers.

INTRODUCTION

The biological importance of the nucleic acid bases is widely recognized.[1] Furthermore, a number of acyclic nucleoside analogues have been found to posses potent antiviral activities against HIV (Human Immunodeficiency Virus).[2-4]

Compared with numerous experimental and theoretical infrared studies on *N*-9- and *N*-7-H tautomers of nucleic acid bases and adenine in particular,[1,5-8] relatively few such reports on the infrared spectra of *N*-9-[1,8,9,13] and *N*-7-substituted adenine derivatives[8,13] are available. We have recently reported on simultaneous formation of *N*-9 and *N*-7 regioisomers in the alkylation reactions of 6-(*N*-pyrrolyl)purine as established by means of ^{1}H and ^{13}C NMR spectroscopy.[10,11]

In this paper we present the IR spectra in the solid state of the parent molecule 6-(*N*-pyrrolyl)purine (**1**) and its *N*-9-(**2**, **4**, **6** and **8**) and *N*-7-(**3**, **5**, **7**, and **9**) substituted acyclic derivatives (Scheme), the assignments of the characteristic absorption bands and their use for differentiation between *N*-9 and *N*-7 regioisomers.

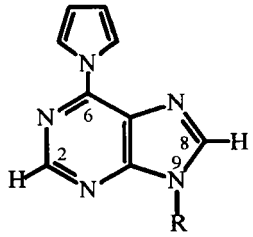
EXPERIMENTAL

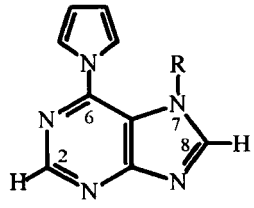
The IR spectra were recorded on a Perkin Elmer M 782 spectrophotometer with a Data station 3600 operated in the double-beam mode. The measurements were made by a potassium bromide pellet technique in the 4000-400 cm^{-1} frequency range. The preparation of compounds **1-9** has been described in our previous paper.[10]

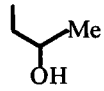
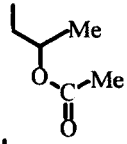
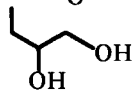
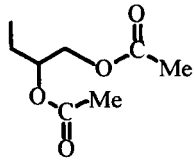
RESULTS AND DISCUSSION

The IR spectra of all compounds **1-9** are well resolved and provide useful experimental data for elucidating the structure of *N*-9- and *N*-7 regioisomers. This can be seen from the FIG.1 showing the IR spectra of **1** and its *N*-9- and *N*-7-substituted derivatives **6** and **7**, respectively.

Analysis of the recorded spectra may be profitably performed by comparison with those of purine and pyrrole moieties as well as model molecules containing the same groups. The most characteristic absorption bands observed in the spectra of compounds **1-9** and their assignments are given in TABLE 1. These assignments are in good agreement with those reported for pyrrole and 1-substituted pyrrole,[12,17] purine,[6-8,13-16] adenine[1,5-8,13-16] and adenosine.[14,15] In addition to the





R	*N*-9	*N*-7
H	**1**	–
	**2**	**3**
	**4**	**5**
	**6**	**7**
	**8**	**9**

Scheme

frequencies given in TABLE 1, two strong and broad absorption bands in the 3000-2500 cm^{-1} range are seen in the IR spectrum of 6-(*N*-pyrrolyl)purine (**1**, FIG. 1), centred at 2975 and 2815 cm^{-1}. According to Tsuboi and Kyogoku[14] "a broad absorption band in this region consisting of a number of absorption peaks located with nearly equal spacing" is caused by the *N*-9-H vibration of purine. This assignment has been confirmed in the recent literature: 2790 cm^{-1}, strong, for polycrystalline adenine,[16] 2798 cm^{-1}, strong, for adenine in KBr matrix[5] and 2980

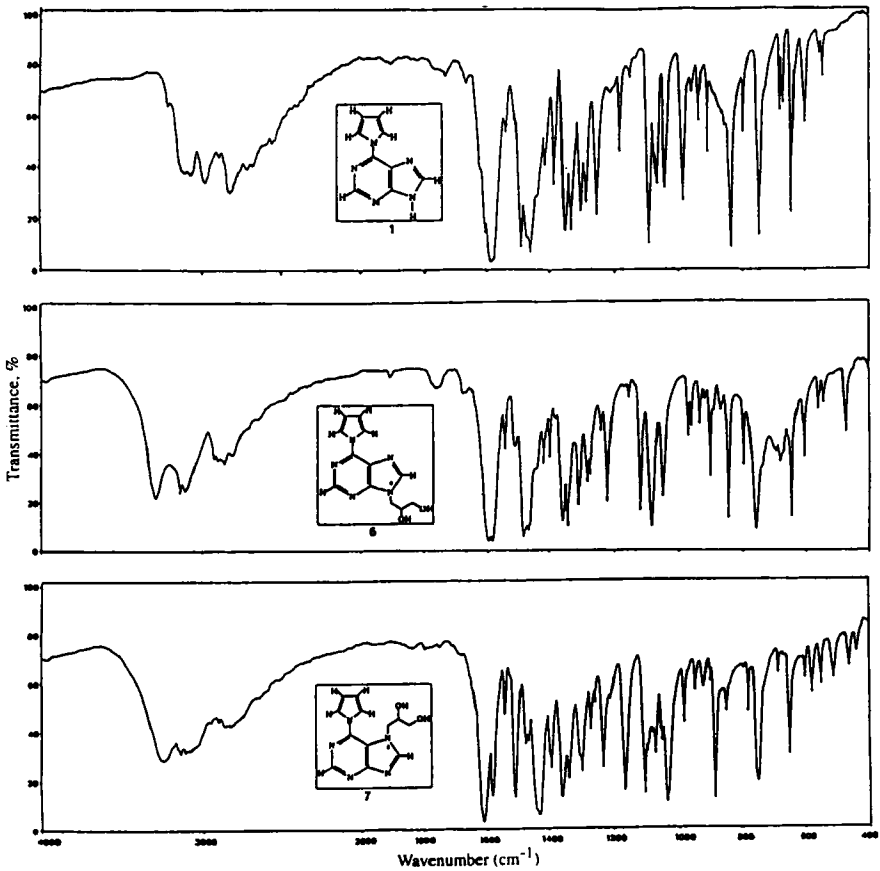


FIG. 1. The IR spectra of 1 and its *N*-9 and *N*-7 substituted derivatives 6 and 7, respectively.

TABLE 1. Asignation at characteristic IR bands (υ cm^{-1}) of the parent compound **1** and its *N*-9- and *N*-7-substituted derivatives **2, 4, 6, 8** and **3, 5, 7, 9** respectively.

Pyrrole C-H stretching		*Purine Ring stretching (2)*		*Methyl C-H sym. bending*		*Acetate C-O streching*	
1 3120		**1** 1490, 1470, 1460, 1438, 1413					
2 3150	**3** 3155 3140	**2** 1475 1445 1411	**3** 1505 1478 1433	**2** 1365	**3** 1375	**2** -	**3** -
4 3165 3150	**5** 3165 3150	**4** 1482 1438 1418	**5** 1509 1480 1432	**4** 1373	**5** 1367	**4** 1238 1060	**5** 1230 1100
6 3140	**7** 3160 3140	**6** 1482 1440 1417	**7** 1506 1430	**6** -	**7** -	**6** -	**7** -
8 3155	**9** 3135 3120	**8** 1480 1435	**9** 1472 1432* 1506	**8** 1374	**9** 1365	**8** 1240 1085 1050	**9** 1232 1117 1048*

Pyrrole Out- of-plane C-H wagging		*Purine C-8-H deformation*		*Alcohol O-H stretching*	
1 750		**1** 839			
2 740	**3** 745 736	**2** 840	**3** 844	**2** 3360	**3** 3460
4 736	**5** 738*	**4** 838	**5** 851	**4** -	**5** -
6 760	**7** 752(b)	**6** 844	**7** 851	**6** 3300	**7** 3260
8 749	**9** 753 743	**8** 845 834	**9** 849	**8** -	**9** -

Pyrrole Out-of-phase C=C stretching		*Purine C-2-H deformation*		*Alcohol C-O stretching*	
1 1538		**1** 800			
2 1537	**3** 1540*	**2** 798	**3** 785	**2** 1076	**3** 1112
4 1538	**5** 1545	**4** 799	**5** 784	**4** -	**5** -
6 1540	**7** 1543*	**6** 798	**7** 784	**6** 1120 1050	**7** 1108 1038
8 1538	**9** 1540*	**8** 799	**9** 784	**8** -	**9** -

Purine Ring stretching(1)		*Methyl C-H asym. stretching*		*Acetate C=O stretching*	
1 1608,1588, 1575(sh)					
2 1600 1580	**3** 1620(b) 1577	**2** 2975	**3** 2965*	**2** -	**3** -
4 1599 1579	**5** 1618 1575*	**4** 2980	**5** 2975	**4** 1741	**5** 1745*
6 1597 1585	**7** 1611(b) 1581*	**6** -	**7** -	**6** -	**7** -
8 1597 1576	**9** 1626(b) 1573*	**8** 2955 2880	**9** 2975 2860	**8** 1737 1718	**9** 1752 1741

*The differences not be considered statistically significant.
b=broad; sh=shoulder.

cm^{-1} for solid adenine.[15] The fact that both of these strong bands disappear in the spectra of *N*-9 and *N*-7 substituted derivatives **2-9** additionally supports this assignment. Furthermore, the strong and sharp band at 1252 cm^{-1}, also lacking in the spectra of **2-9**, may be ascribed to *N*-9-H deformation mode, as was found for adenine in KBr matrix at exactly the same frequency.[5]

Pyrrole Moiety

The bands characteristic for this moiety are found in the spectra of **1-9** at approximately the same frequencies to those given in the literature[12,17]: C-H stretching band at 3180-3090 cm^{-1}, strong and somewhat broad band at 770-715 cm^{-1} caused by the in-phase out-of-plane *cis* C-H wagging and a band of medium intensity at 1560-1540 cm^{-1} due to out-of-phase C=C stretching in 1-monosubstituted pyrroles. Other bands characteristic for 1-monosubstituted pyrroles[12,17] at 1510-1490, 1390-1380, 1095-1080 and 1065-1055 cm^{-1} cannot be assigned with certainty because of the appearance of numerous bands in nearby the same regions arising from ring stretching modes of the purine skeleton combined with C-8-H and C-2-H bending modes.[1,5,7,8,16] The only exception is the in-plane C-H deformation vibration (1095-1080 cm^{-1})[12,17] that is clearly seen in the spectrum of **1** at 1090 cm^{-1} as a very strong band. Comparison of the spectra of *N*-9 and *N*-7 regioisomers shows that out-of-phase C=C stretching vibrations of the pyrrole moiety appear at higher frequencies (2-7 cm^{-1}) in *N*-7 than in the corresponding *N*-9 substituted derivatives.

Purine Moiety

(i) *Ring Stretching Bands*

Stretching vibrations of the C=N and C=C double bonds of purine and adenine are generally found in the region at *ca* 1600 cm^{-1}.[1,5-8,13-16] Purine shows two medium-intensity bands at 1610 and 1570 cm^{-1}.[14] The bands at 1618 and 1598 cm^{-1} in the spectrum of adenine (in argon matrix) are assigned to ring streching vibration of the pyrimidine ring.[1] In adenine, the very strong band at 1608-1604

cm^{-1} may overlap with another one which is barely apparent as a shoulder at 1580-1570 cm^{-1}; these bands correspond to whole ring vibrations, the latter being of pyrimidic character[8,16] (the proper frequency of pyrimidine is 1583 cm^{-1}) [8]. In the spectra of purine derivatives **2-9** one observes two very strong and well separated bands at 1626-1597 and 1585-1573 cm^{-1}, instead of three closely spaced very strong bands at 1608-1575 cm^{-1} found for the parent molecule **1**. In the spectra of *N*-9 regioisomers, these two bands of nearly equal intensity are found at almost constant positions (1600-1597 and 1585-1576 cm^{-1} , respectively), the spacing between them being *cca* 20 cm^{-1}(with exception of 12 cm^{-1} for **6**). For *N*-7 regioisomers, however, the first band is shifted to higher frequencies (1626-1611 cm^{-1}) and is somewhat broadened, whereas the other one remains in the same region (1581-1573 cm^{-1}) but with significantly decreased intensity. This shifting causes bigger spacing between the two bands (30-53 cm^{-1}).

In the spectrum of adenine (in argon matrix) the band at 1481 cm^{-1} is predicted to be associated with ring stretching vibrations of the imidazole ring, with a contribution from C-8-H bending mode.[1] This assignment is supported by the presence of a band at 1484 cm^{-1} in the spectrum of imidazole (in argon matrix).[8] Therefore, the frequencies at 1490-1470 cm^{-1} in the spectrum of adenine (in argon matrix) correspond to the vibrations localised on the imidazole fragment.[8] Mathlouthi[15] also assigned the band at 1453 cm^{-1} in the spectrum of solid adenine to ring stretching vibration of imidazole moiety, since this band is absent in the spectra of the acyclic derivatives substituted at *N-9*.

Analysis of the spectra of compounds **2-9** in this region is somewhat complicated, because some vibrations other than ring stretching of imidazole moiety give rise to prominent bands: CH_3 asymmetrical bending at 1470-1430 cm^{-1}, medium to strong)[18], CH_2 scissoring at 1475-1445 cm^{-1} (usually at 1465 cm^{-1}, medium to strong)[18] and in phase C=C stretching of 1-substituted pyrrole at 1510-1490 cm^{-1}(medium).[12] In the spectrum of the parent compound **1**, one observes a group of five strong bands crowded at 1490-1413 cm^{-1} region. The spectra of *N-9* substituted derivatives show a very strong, somewhat broad band at

1482-1475 cm^{-1} and two medium bands at 1445-1435 cm^{-1} and 1418-1411 cm^{-1}. Another strong band at *cca* 1465 cm^{-1} is probably due to CH_3 asymmetrical bending and CH_2 scissoring modes, whereas a band of medium intensity at 1508-1495 cm^{-1} could be assigned to in-phase C=C stretching of the pyrrole moiety. For *N*-7 regioisomers, however, a strong band is observed at 1509-1506 cm^{-1}, the band at 1480-1472 cm^{-1} becomes weak to medium, whereas the band at 1433-1430 cm^{-1} becomes strong and the band at 1418-1411 cm^{-1} disappears. The strong band at 1448-1440 cm^{-1} is probably due to CH_3 asymmetrical bending and CH_2 scissoring modes.

(ii) *C-H Stretching Bands*

C-8-H and C-2-H stretching bands are found in the spectra of purine and adenine at 3098 and 3023 cm^{-1}, respectively as very weak bands usually hidden under the broad, strong bands of the NH groups.[13,14,16] These bands are not observed in the IR spectra of adenine and 9-methyladenine due to their low intensity.[1,5,8] The calculated frequencies for adenine tautomers and for 9-methyladenine are in accord with the presence of bands at 3126 cm^{-1} in the spectrum of imidazole (in argon matrix) and 3020 cm^{-1} in the spectrum of pyrimidine.[1,8] In the spectra of purine derivatives **1-9** several weak absorption bands are observable in the 3100-3080 and 3060-3020 cm^{-1} regions, but due to their very low intensity they cannot be assigned with certainty.

(iii) *C-H Deformation Bands*

Bands of medium intensity are found at 845 and 847 cm^{-1} as well as 796 and 797 cm^{-1} in the spectrum of adenine in KBr matrix[5] and argon matrix,[7,16] respectively, they are ascribed to C-8-H and C-2-H deformation vibrations, respectively. A sharp band at 800 cm^{-1} in the spectrum of adenine is due to C-2-H deformation mode, as established by deuteration experiments.[13,14] A very strong band at 839 cm^{-1} and a medium sharp band at 800 cm^{-1}, corresponding to C-8-H and C-2-H vibrations, respectively, are observable in the spectrum of parent

compound **1**. Both bands remain at nearly constant positions (845-838 and 799-798 cm^{-1}) in the spectra of *N*-9 substituted derivatives, the first one decreasing somewhat in intensity (medium to strong), and, for compound **8**, being split into two bands of medium intensity. In the spectra of *N*-7 regioisomers, both bands are shifted: the first one (C-8-H) to higher frequencies (4-13 cm^{-1}) with significantly decreased intensity (weak to medium), and the second one (C-2-H) to lower frequencies (13-15 cm^{-1}), remaining of medium intensity.

Methyl Group

Weak to medium bands 2980-2955 cm^{-1} due to C-H asymmetrical stretching vibration and medium to strong bands at 1375-1365 cm^{-1} arising from C-H symmetrical bending mode, as the most characteristic for methyl group[18] are observable in the spectra of compounds **2-5, 8** and **9.** Bands due to C-H asymmetrical bending usually found at *cca* 1460 cm^{-1} [18], cannot be assigned with certainty, because of the presence of few ring stretching bands of the purine moiety in this region. Thus, the bands at *cca* 1465 cm^{-1} for *N*-9 isomers and at 1448-1440 cm^{-1} for *N*-7 isomers are probably due to C-H asymmetrical bending mode of methyl groups, as well as to the scissoring vibration of methylene groups.

Alcohol

The characteristic bands observed in the spectra of *N*-9 and *N*-7 regioisomeric pairs: 9- and 7-(2-hydroxyprop-1-yl)-6-(*N*-pyrrolyl)purine (**2** and **3**) and 9- and 7-(2,3-dihydroxyprop-1-yl)-6-(*N*-pyrrolyl)purine (**6** and **7**) result from O-H stretching and C-O stretching vibrations. The presence of hydrogen bonds can be verified not only by the lowering of the O-H stretching band frequency, but also by the increase in the band width and intensity.[19] Thus compounds **2**, **3**, **6** and **7** exhibit a broad and strong band at 3460-3260 cm^{-1} which is assigned to O-H stretching vibration. The out of phase C-O stretching vibrations in primary and secondary alcohols produce a strong band in the 1150-1000 cm^{-1} region.[19] The compounds **2**, **3**, **6** and **7** show sharp and strong bands at 1069-1172 cm^{-1}

attributable to the C-C-O stretching vibrations. This assignment is in agreement to the corresponding one given in the literature.[19]

In conclusion, bands arising from C-O stretching vibrations in *N*-9 regioisomers are progressively shifted to lower frequencies (for 38-49 cm^{-1}) in relation to the corresponding *N*-7 regioisomers.

Acetate

Alkyl acetates exhibit generally, a strong band arising from C=O stretching vibration in the 1750-1735 cm^{-1} region.[20] Such carbonyl band is found at 1752-1737 cm^{-1} in acetylated derivatives **4**, **5**, **8** and **9.** In addition to that the compounds **4**, **5**, **8** and **9** show a strong C-O stretching band at 1240-1230 cm^{-1} and two strong C-O stretching bands for acetates of secondary and primary alcohol groups at 1117-1060 and 1050-1048 cm^{-1}, respectively. Assignment of these bands is consistent with the corresponding one given in the literature.[20] *N*-7 substituted derivatives display strong C=O stretching band shifted (for 4-23 cm^{-1}) to higher frequencies in relation to the corresponding *N*-9 derivatives.

Furthermore, comparison of the spectral data in TABLE 1 shows the following distinctive feature for two regioisomers: for *N*-7 substituted derivative **9,** additional bands of medium intensity appear at 818, 862 and 871 cm^{-1}, and one can observe an additional band at exactly 890 cm^{-1} of medium to strong intensity, for all *N*-7 regioisomers, not observable in the spectra of **1** and corresponding *N*-9 substituted regioisomers.

CONCLUSION

The most pronounced difference between spectra of two regioisomers is in the appearance of a strong and sharp absorption band at 890 cm^{-1} found in all *N*-7 substituted derivatives, whereas such band was not observed in the corresponding *N*-9 derivatives. Presumably this band arises from interaction of the aliphatic side chain on the purine ring with the pyrrole moiety. This is consistent with our computational and NMR studies of 7-(2-hydroxypropyl)- and 7-(2,3-

dihydroxypropyl)-6-(*N*-pyrrolyl)purines, **3** and **7** respectively[21], which show that such interactions exist in *N*-7 regioisomers.

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