# ANALOGS OF PURINE NUCLEOSIDES AND PURINE MONO- AND POLYNUCLEOTIDES

## III.* INTERMOLECULAR AND INTRAMOLECULAR INTERACTIONS IN 6-SUBSTITUED 9-(α,ω-DIHYDROXYALKYL)PURINES

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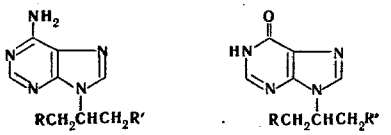
On the basis of a comparison of the protolysis constants and vibrational frequencies it is shown that intermolecular interactions are present in 6-substituted 9-(α,ω-dihydroxyalkyl)-purines and the corresponding mono- and diphosphates. In addition, in the case of the phosphates the existence of intramolecular interactions of electrostatic character between the phosphate group and the heteroring is also proposed. A comparison of the protolysis constants provides evidence for the different character of the interaction of the dihydroxyalkyl residue with the heteroring of the base in series of adenine and hypoxanthine derivatives.

The systematic synthesis and study of artificial analogs of nucleotides and nucleosides require a clear idea of the effect of the structure and configuration of the synthesized compounds on the intramolecular or intermolecular interaction of the most important reaction centers of the molecules under consideration. In the present research we have attempted to study this probelm in the case of synthetic analogs of adenosine and inosine using as physicochemical methods for the detection of the formation of hydrogen bonds the appropriate vibrational frequencies in the IR spectra and the protolysis constants, or, more precisely, the change in the indicated parameters on passing from the bases (adenine and hypoxanthine) to 6-substituted 9-(α,ω-dihydroxyalkyl)purines and, subsequently, to the corresponding mono- and diphosphates. A comparison of the results obtained with the data available in the literature, which pertains to natural nucleotides and nucleosides of adenine and hypoxanthine, makes it possible to expose definite differences between the natural nucleotides and their analogs.

In the present research we have studied 16 different derivatives of 9-(α,ω-dihydroxyalkyl)adenine (I) and hypoxanthine (II) and their corresponding mono-, di-, cyclophosphates, and which were synthesized by the methods in [1, 2]. (Tables 1 and 2).




I II

The protolysis constants (pK) obtained correspond to protonation or deprotonation of certain nitrogen atoms in molecules of I or their deprotonation in II. The evidence available in the literature that the protonation center in the adenine molecule is the N-1 nitrogen atom (the $pk^{+}_{NH}$ of adenine is 4.18) [3], whereas deprotonation occurs at the N-1 nitrogen atom in the case of hypoxanthine derivatives (the $pK^{+}_{NH}$ of hypo-

*See [1] for communication II.

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TABLE 1. Protolysis Constants, pK Values, and Some Vibrational Frequencies in the IR Spectra of Adenine (I) Diols and Phosphates

| R | R′ | $pK_{NH}^{+}$ | $pK_{PO_4^{2-}}$ | Vibrational frequencies in the IR spectra, $cm^{-1}$ | | | |
|---|---|---|---|---|---|---|---|
| | | | | $\nu_{OH}$ | $\nu_{NH_2}$ | $\delta_{NH_2}$ | $\nu_{P=O}$ |
| OH | OH | 3,97±0,02 | — | 3370 s | 3315 s 3255 m | 1697 s | |
| OH | $CH_2OH$ | 4,00±0,02 | — | 3435 s | 3345 s 3280 s | 1648 s | |
| $CH_2OH$ | $CH_2OH$ | 3,70±0,03 | — | 3450 s | 3340 s 3275 s | 1648 s | |
| OPO(OH)O* | | 4,19±0,03 | — | 3500 m | 3455 m 3330 m | 1695 s | 1230 m |
| $OPO(OH)_2$ | $OPO(OH)_2$ | 4,14±0,02 | 6,67±0,02† | 3370 m, br | 3120 m | 1695 s | 1230 m |
| $CH_2OPO(OH)_2$ | $OPO(OH)_2$ | 4,24±0,03 | 6,79±0,02† | 3370 m, br | 3125 m 3085 w | 1695 s | 1225 m |
| $CH_2OPO(OH)_2$ | $CH_2OPO(OH)_2$ | 4,01±0,03 | 6,56±0,02† | 3420 m, br | 3210 m 3170 w | 1690 s | 1160—1230 m, br |

* Cyclophosphate.

† Determined graphically from the titration curves with correction for the ionic strength of the solution.

TABLE 2. Protolysis Constants, pK Values, and Some Vibrational Frequencies in the IR Spectra of Hypoxanthine (II) Diols and Phosphates

| R | R′ | $pK_{NH}$ | $pK_{PO_4^{2-}}$ | Vibrational frequencies in the IR spectra, $cm^{-1}$ | | | |
|---|---|---|---|---|---|---|---|
| | | | | $\nu_{OH}$ | $\nu_{C=O}$ | $\delta_{NH}$ | $\nu_{P=O}$ |
| OH | OH | 9,38±0,02 | — | 3390 s, br | 1710 s | 1675 s | — |
| OH | $CH_2OH$ | 9,37±0,02 | — | 3450 s | 1710 m | 1670 m | — |
| $CH_2OH$ | $CH_2OH$ | 9,15±0,04 | — | 3330 m | 1680 s, br‡ | | — |
| OPO(OH)O* | | 9,23±0,02 | — | 3450 s, br | 1724 s, br | 1700 sh | 1210 m |
| OH | $OPO(OH)_2$ | 9,84±0,02 | 6,55±0,02 | 3400 s, br | 1710 s, br‡ | | 1180 m |
| OH | $CH_2OPO(OH)_2$ | 9,82±0,02 | 6,82±0,02 | 3375 m | 1725 s | 1690 s | 1175 s |
| $OPO(OH)_2$ | $OPO(OH)_2$ | 10,05±0,02 | 6,72±0,02† | 3440 m, br | 1715 s, br | 1700 sh | 1170—1190 s, br |
| $CH_2OPO(OH)_2$ | $OPO(OH)_2$ | 9,98±0,02 | 6,85±0,02† | 3440 m, br | 1715 s, br | 1700 sh | 1185 m |
| $CH_2OPO(OH)_2$ | $CH_2OPO(OH)_2$ | 9,28±0,03 | 6,57±0,02† | 3430 m, br | 1720 s, br | 1700 sh | 1210 s |

* Cyclophosphate.

† The pK values were determined graphically from the titration curves with correction for the ionic strength of the solution.

‡ The frequency corresponds to the overall band of $\nu_{C=O}$ and $\delta_{N-H}$ vibrations.

xanthine is 8.94) [4] was used for the assignment of these constants. This evidence was based on an interpretation of the results of IR and NMR spectroscopy, x-ray diffraction analysis, and quantum-chemical calculations [5-8].

A comparison of the protolysis constants of the investigated compounds with the pH values of the corresponding natural nucleotide bases – adenine and hypoxanthine – indicates the presence of interaction of the dihydroxyalkyl residues or phosphate groups with the purine base. The decreases in the pK values noted in the literature, respectively, from 4.18 to 3.52 and from 8.94 to 8.75 on passing from the base to the natural ribonucleosides of adenine and hypoxanthine have been explained in part by the –I inductive effect of the sugar residue [9]. The $pK_{NH}^{+}$ values of 9-($\alpha,\omega$-dihydroxyalkyl)-adenine derivatives do not decrease to the same extent as in natural nucleosides as compared with the adenine base, whereas in the case of inosine analogs the $pK_{NH}$ values, on the other hand, even increase (Tables 1 and 2). This evidently constitutes evidence that the effect of interaction of the hydroxyl groups of the dihydroxyalkyl residues with the nitrogen heteroatom of the purine ring or of the amino or carbonyl groups attached to the latter predominates over the –I effect. A comparison of the pK values and the vibrational frequencies in the IR spectra makes it possible to assume the formation of intermolecular hydrogen bonds (IHB) between the hydroxyl groups of the dihydroxyalkyl residues and the exocyclic amino groups in the case of adenosine analogs or between the carbonyl groups in the case of inosine analogs. The intermolecular character of the hydrogen bond is confirmed by the shift in the frequency of the coalesced total $\nu_{OH}$ vibrational bands as the solution concentration changes. In addition, the bands of deformation vibration of hydroxyl groups [$\beta$ (OH)] and the bands of stretching vibrations of amino groups [$\nu$ ($NH_2$)] are also shifted to higher frequencies but to a lesser extent as the concentration increases. Similar shifts of the vibrational band are also characteritic for 9-(1,4-dihydroxy-2-butyl)hypoxanthine. The characteristic values of the $\nu_{OH}$ vibrational frequencies at 3330-3450 $cm^{-1}$ (in the solid phase) also indicate the presence of IHB [10].

On the basis of a crystallographic investigation of 9-methyladenine [11], it can be assumed that an amino group enters into the IHB of the adenosines under investigation. In the case of 9-(1,5-dihydroxy-3-pentyl)adenine, the decrease in the $pK_{NH}^{+}$ value (3.70) as compared with the $pK_{NH}^{+}$ value of 1,3-dihydroxypropyl- and 1,4-dihydroxybutyl derivatives attests to a decrease in the electron density on the N-1 heteroatom, i.e., to the manifestation of electron-donor properties of the heteroring. This makes it possible to assume that, in addition to the IHB mentioned above, IHB are also formed between the hydroxyl groups of the dihydroxyalkyl residues and the nitrogen heteroatoms.

A strong IHB including a 6-amino group shows up distinctly in the case of 9-(1,3-dihydroxy-2-propyl)adenine (the anomalously high frequency of the $\delta_{NH_2}$ at 1697 $cm^{-1}$ and the low value of the $\nu_{OH}$ band at 3370 $cm^{-1}$). This is explained by the relatively favorable conditions for the formation of a hydrogen bond in the case of precisely 9-(1,3-dihydroxy-2-propyl)adenine. This is possibly also responsible for the anomalously low solubility of this compound ($2 \cdot 10^{-3}$ mole/liter).

In a number of the phosphates of analogs of adenosines and inosines an increase in the $pK_{NH}^{+}$ values is characteristic for adenine derivatives and an increase in the $pK_{NH}^{+}$ values is characteristic for hypoxanthine derivatives as compared with the corresponding protolysis constants of the nucleosides, just as in the case of natural nucleotides (the pK values of ribonucleoside 5'-phosphate – 3.88 for the adenine derivative and 9.62 for the hypoxanthine derivative, respectively – exceed the pK values of the corresponding ribonucleoside – 3.52 and 8.75 [3]).

In the case of natural adenosines the increase in the pK values of the nucleotides as compared with the pK values of the corresponding nucleosides was explained by intramolecular interaction of the phosphate residue with the protonated base [5, 12]. It is characteristic that the most distinct increase in the pK value is observed in the case of those compounds whose structures are most favorable for interaction of negatively charged phosphate groups with positively charged heterorings (for example, in the case of adenine ribonucleoside 5'-phosphate, $pK_{NH}^{+}$=3.88, which exceeds the corresponding protonation constants of adenine ribonucleoside 2'- and 3'-phosphates – 3.81 and 3.70).

A comparison of the pK values of the compounds that we investigated made it possible to establish that the $\Delta$pK values – the differences in the $pK_{NH}$ values for analogs of adenosines or the $pK_{NH}^{+}$ values for analogs of inosines between the corresponding diols and bases and between the diphosphates and diols – are definitely associated with the structural peculiarities of the molecules (Table 3).

It is characteristic that the $\Delta$pK values on passing from propyl to pentyl derivatives increase or decrease regularly. This justifies the assumption that there is a stronger interaction in the pentyl deriva-

TABLE 3. pK, ΔpK, and $\Delta\nu_{P=O}$* values of Adenine (I) and Hypoxanthine (II) Derivatives

| | Base | Derivatives | | |
|---|---|---|---|---|
| | | propyl | butyl | pentyl |
| Adenine derivatives | 4,18 | | | |
| pK values of the diols | | 3,97 | 4,00 | 3,70 |
| pK values of the diphosphates | | 4,14 | 4,24 | 4,01 |
| ΔpK (base – diol) values | | 0,21 | 0,18 | 0,48 |
| ΔpK (diphosphate – diol) values | | 0,17 | 0,24 | 0,31 |
| $\Delta\nu_{P=O}$, $cm^{-1}$ | | 20 | 25 | 20—90 |
| Hypoxanthine derivatives | 8,94 | | | |
| pK values of the diols | | 9,38 | 9,37 | 9,15 |
| pK values of the diphosphates | | 10,05 | 9,98 | 9,28 |
| ΔpK (diol – base) values | | 0,44 | 0,43 | 0,21 |
| ΔpK (diphosphate – diol) values | | 0,67 | 0,61 | 0,13 |
| $\Delta\nu_{P=O}$, $cm^{-1}$ | | 60—80 | 65 | 40 |

* The symbol $\Delta\nu_{P=O}$ is the difference in the frequencies of the bands of the associated (in the nucleotide) and free phosphate groups ($\nu_{P=O\ free}$ 1250 $cm^{-1}$ [10]).

tives both for diols and diphosphates in the adenine series and that there is a stronger interaction in the propyl derivatives in the hypoxanthine series. In addition, the ΔpK (diphosphate – diol) values correlate with the change in the frequency of the band of vibrations of the phosphate group ($\Delta\nu_{P=O}$).

In analogy with molecules of natural adenosine phosphates [5, 13] a zwitterion structure with a bent chain of the phosphate and dihydroxyalkyl residue is also proposed for the phosphates that we studied. A zwitterion structure is usually confirmed by the $\delta_{NH_2}$ vibrational band at 1690-1710 $cm^{-1}$ (in the solid phase). This band is actually characteristic for all of the natural adenosines with a protonated N-1 nitrogen atom [14] and also for adenosine 5'-mono-, di-, and triphosphates [5]. The bands of $\delta_{NH_2}$ vibrations at 1695 and 1690 $cm^{-1}$, the frequencies of which coincide with the above-indicated frequencies of the zwitterions bands that are observed in our case, attest to the presence of a zwitterion structure in 9-($\alpha,\omega$-dihydroxyalkyl)-adenine mono- and diphosphates.

In both the indicated natural and in the investigated 9-($\alpha,\omega$-dihydroxyalkyl)adenines the electrostatic interaction is expressed to the greatest extent at pH 1-4, where the residue of the phosphate group and the heteroring of the base have integral charges of opposite sign. It is clear that in the case of the investigated phosphates the interaction is greater, the closer the phosphate group is to the N-1 nitrogen atom, i.e., the longer the chain connecting the phosphate residue. The greatest change in the pK value should therefore correspond to the relatively long pentyl chain of the phosphate residue.

This is in agreement with the possible conformations of the molecules studied in the case of Stuart–Briegleb models. Thus, although the phosphate residue in the 9 position of adenine is not in contact with the N-1 atom, it nevertheless is at a distance sufficient for an electrostatic interaction.

It should be noted that a similar transannular interaction was observed in the case of $\alpha$-amino-$\beta$-(1-pyrimidyl)acids that have a zwitterion structure [15]. In both the case of the investigated synthetic nucleotides and in the case of the compounds mentioned above the presence of a transannular interaction was proved on the basis of a comparison of the pK values of the corresponding derivative.

A reaction of electrostatic character is also observed in the phosphates of inosine analogs. Inasmuch as here, as in the case of uracil phosphates [9], two charges of the same sign repulse one another, in view of the anionic destabilization of inosine a distinct increase in the $pK_{NH}$ values is observed in the phosphates. However, in view of the repulsion of the phosphate residue away from the ring of the base it might be expected that the chain of the phosphate and hydrocarbon residues can bend most of all (i.e., form the greatest distance from the phosphate residue to N-1) in the case of the pentyl derivative of the diphosphate. We therefore assumed that the most intimate interaction occurs in the case of the propyl derivative. This is not contradicted by the above-indicated regularities.

The effect of a phosphate group on the change in the $pK_{NH}$ values in analogs of inosines is confirmed by the absence of the indicated electrostatic interaction in the case of cyclophosphates. This is attested to by the relatively small $pK_{NH}$ value (9.23) in the case of 9-(1,3-dihydroxy-2-propyl) hypoxanthine 1',3'-cyclophosphate.

The diffuse form of the bands of the $\nu_{OH}$ stretching vibrations in the IR spectra of the phosphates of adenosine and inosine analogs makes it possible to assume the presence of IHB in these compounds along with the indicated interactions of electrostatic character. It is possible that the IHB are formed by those portions of the phosphate residues that do not participate in the electrostatic interactions.

## EXPERIMENTAL

The pK values were determined by potentiometric titration in aqueous solutions with an LPU-01 pH-meter at 20° (the solutions were stirred by means of argon). Except for compounds with low solubilities, the concentrations of the solutions were $10^{-2}$ mole/liter. The titrants were 0.1 N solutions of HCl and NaOH. In all cases except for the diphosphates, the pK values were calculated from a formula with the Debye–Hückel correction for the ionic strength [16].

The IR spectra of KBr pellets and DMSO solutions of the compounds were recorded with a UR-20 spectrometer.

The purity of the investigated compounds was monitored by chromatography.

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