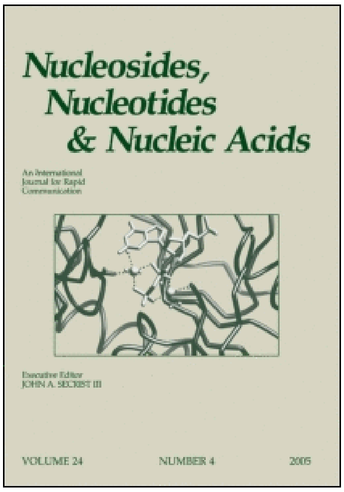

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# $^{31}P$-$^{15}N$ ONE-BOND COUPLINGS OF DIASTEREOISOMERIC ADENOSINE CYCLIC 3',5'-PHOSPHORAMIDATES

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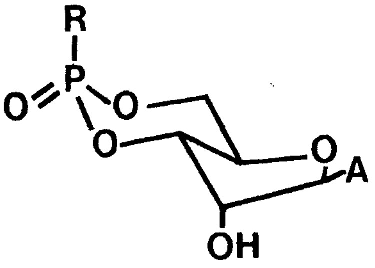
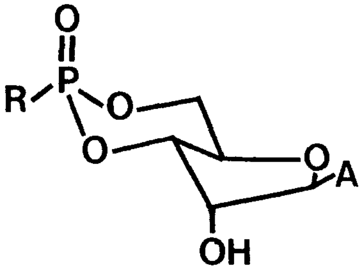
Abstract. $^1J(^{31}P^{15}N)$ coupling constants of $\underline{R}_P$ and $\underline{S}_P$ adenosine cyclic 3',5'-phosphoramidates (1), -N-methylphosphoramidates (2) and -N,N-dimethylphosphoramidates (3) increase in the order of 1<2<3 and obey the Stec rule ($J(\underline{R}_P)<J(\underline{S}_P)$). A possible interpretation of coupling constant differences based on differences in substituent electronegativities and variation in hybridization at nitrogen atom, is suggested.

Phosphoramidate derivatives, epimeric at phosphorus of adenosine cyclic 3',5'-monophosphate, $\underline{R}_P$ and $\underline{S}_P$ adenosine cyclic 3',5'-phosphoramidates (1), -N-methylphosphoramidates (2) and N,N-dimethylphosphoramidates (3) were prepared according to the methods developed in our laboratory, recently.[1-3] The configuration at phosphorus of compounds 1-3



$\underline{R}_P$



$\underline{S}_P$

A = adenin-9-yl

| | R |
|---|---|
| 1 | $NH_2$ |
| 2 | $NHCH_3$ |
| 3 | $N(CH_3)_2$ |

was assigned on the basis of the $^{31}P$ chemical shift criterion established for 2-substituted-2-oxo-1,3,2-dioxaphosphorinanes.[4] According to this empirical rule the $\underline{R}_P$ diastereoisomer is expected to absorb several ppm at higher field, since this isomer has the amino (or substituted amino) group in the axial position, i.e. locked into gauche conformations relative to the endocyclic ester bonds. The $^{31}P$ shielding for the gauche conformation may be interpreted as a manifestation of the $\gamma$-effect often observed in $^{13}C$ chemical shifts either on the basis of steric interactions[5] or on stereoelectronic grounds.[6]

The $^{31}P$ chemical shift criterion tacitly involves the supposition that the dioxaphosphorinane ring of both diastereoisomers exists in the same chair conformation. If this criterion is not fulfilled, discrepancies from the rule may occur, since the amino substituent at phosphorus is oriented axially also in the $\underline{S}_P$ diastereoisomer that has the dioxaphosphorinane ring in a twist-boat conformation. To the best of our knowledge such a discrepancy has not yet been found for the diastereomeric 2-amino(or substituted amino)-2-oxo-1,3,2-dioxaphosphorinanes, but has been observed for the 2-p-nitrophenoxy -2-oxo-5,6-tetramethylene-1,3,2-oxazaphosphorinanes of similar structure.[7] On the other hand, the amino groups at phosphorus are equatorially (or pseudo-equatorially) oriented in both diastereoisomers, when the $\underline{R}_P$ isomer has a twist-boat-shaped dioxaphosphorinane ring, as in the case of thymidine cyclic 3',5'-N,N-dimethylphosphoramidate.[8] Thymidine cyclic 3',5'-N,N-dimethylphosphoramidate diastereoisomers, however, obey the $^{31}P$ chemical shift criterion, though the difference between the shifts is an unusually small value (0.62 ppm).[9]

Another and more reliable approach for the configurational assignment at phosphorus in compounds 1-3 would be a comparison of the $^{1}J(^{31}P^{15}N)$ spin-spin coupling constants. Stec and his coworkers have found that of the two diastereoisomers of a given 2-amino(or substituted amino)-2-oxo-1,3,2-dioxaphosphorinane, the $\underline{S}_P$ diastereoisomer with an equatorially oriented amino group at phosphorus has a $^{1}J(^{31}P^{15}N)$ value of 10-15 Hz higher than the $\underline{R}_P$ diastereoisomer having the amino group in the axial position.[9-13] This Stec rule for exocyclic spin 1/2 nuclei has been explained on the basis of the shorter equatorial P-N bond containing a nitrogen atom of more $sp^2$ character compared to the axial P-N linkage that has a nitrogen atom of more $sp^3$ character.[13] Although the Stec rule is strictly valid for chair-shaped dioxaphosphorinane rings, it is still less sensitive to conformational changes than the $^{31}P$ chemical shift criterion. For example, the difference of 11 Hz existing between the $^{1}J(^{31}P^{15}N)$ values of thymidine cyclic 3',5'-N,N-dimethylphosphoramidate diastereoisomers[9], can be found in the expected range in contrast that the $\underline{R}_P$ isomer populates predominantly a twist-boat conformation.[8]

To test the correctness of the earlier configurational assignment at phosphorus based on the $^{31}P$ chemical shift criterion, the $^{1}J(^{31}P^{15}N)$ coupling constants of diastereoisomeric phosphoramidates $\underline{1}$-$\underline{3}$ were determined and compared. Results of these $^{15}N$ and $^{31}P$ NMR investigations are presented in this paper.

## RESULTS AND DISCUSSION

$^{1}J(^{31}P^{15}N)$ coupling constants, $^{15}N$ and $^{31}P$ chemical shift values as well as $^{15}N$-induced isotope effects on the $^{31}P$ chemical shifts for compounds $\underline{1}$-$\underline{3}$ are displayed in TABLE 1.

In accordance with the Stec rule, the one-bond couplings of the $\underline{S}_P$ diastereoisomers are always higher than those of the respective $\underline{R}_P$ diastereoisomers. The difference between the coupling constants of the two diastereoisomers of a given phosphoramidate has the same value (13.7 Hz) for methylated phosphoramidates $\underline{2}$ and $\underline{3}$ and an approximately 1 Hz larger value (14.6 Hz) for the unsubstituted derivative $\underline{1}$. Coupling constants increase with increasing degree of methylation ($\underline{1}<\underline{2}<\underline{3}$) in the two series of diastereoisomers.

TABLE 1

$^{15}N$ and $^{31}P$ NMR data of diastereoisomeric adenosine cyclic 3',5'-phosphoramidates 1-3 in DMSO-$d_6$.

| Compound | | $^1J(^{31}P^{15}N)$ (Hz) | $\Delta^1J(^{31}P^{15}N)$ (Hz) $S_P$-$R_P$ | $\Delta^1J(^{31}P^{15}N)$ (Hz) 2-1 (3-2) | $\delta^{15}N$ (ppm) | $\delta^{31}P$ (ppm) | $\Delta^{31}P^a$ (ppm) |
|---|---|---|---|---|---|---|---|
| 1 | $R_P$ | 32.36 | 14.57 | | -340.27 | 8.02 | -0.007 |
| | $S_P$ | 46.93 | | | -339.58 | 11.57 | -0.011 |
| 2 | $R_P$ | 36.40 | 13.72 | 4.04 | -349.57 | 7.69 | - |
| | $S_P$ | 50.12 | | 4.19 | -348.83 | 9.33 | - |
| 3 | $R_P$ | 38.73[b] | 13.73 | (2.33) | -352.26 | 7.94 | -0.009 |
| | $S_P$ | 52.46[b] | | (2.34) | -353.57 | 9.27 | -0.011 |

[a] $\Delta^{31}P = \delta^{31}P(^{15}N) - \delta^{31}P(^{14}N)$[14]

[b] $^1J(^{31}P^{15}N)$ values of 45.0 Hz ($R_P$) and 55.0 Hz ($S_P$) in $CHCl_3$ for $N^6,N^6,O^{2'}$-tribenzoyl-adenosine cyclic 3',5'-N,N-dimethylphosphoramidates have been reported.[15]

Differences of 4.0-4.2 and 2.3 Hz were found between the individual diastereoisomers of 2 and 1 and of 3 and 2, respectively. These differences may be interpreted as being a result of methyl substitution and variation in hybridization at nitrogen atom.

The magnitude of one-bond couplings increases with increasing electronegativity of the substituent.[16] As a result of the greater electronegativity of carbon compared to hydrogen, in the two series of diastereoisomers, an increase of $^1J(^{31}P^{15}N)$ values in the order of 1<2<3 is anticipated. Exactly the same differences (2.3 Hz) were found between the coupling constants of $R_P$-2 and $R_P$-3 and of $S_P$-2 and $S_P$-3. This may be ascribed to substitution of H by $CH_3$, and may suggest similar hybridization at nitrogen atom for diastereoisomers of the same configuration of 2 and 3. The larger coupling constant differences between the diastereoisomers of 1 and 2 (4.0 Hz for $R_P$ and 4.2 Hz for $S_P$) may, however, only partly be attributed to substitution of H by $CH_3$. Variations in hybridization at nitrogen atom may also be considered for the interpretation of these differences.

In compounds 1 and 2, the $^{15}$N nuclei in the $R_P$ diastereoisomers are more shielded than in the $S_P$ diastereoisomers. A reverse correlation can be found for $R_P$-3 and $S_P$-3. In the case of 2-phenylamino-2-oxo(-thio or -seleno)-4-methyl-1,3,2-dioxaphosphorinane diastereoisomers a similar relationship found here for 1 and 2, exists.[13] Adenosine cyclic 3',5'-phosphoranilidate diastereoisomers show, however, the reverse correlation as to that observed for $R_P$-3 and $S_P$-3.[13] This reverse correlation was considered a result of the deshielding influence of the adenine ring on the axially oriented $^{15}$N nucleus.[13] In the light of our results this interpretation seems to be very unlikely.

The sign and magnitude of one-bond $^{15}$N-induced isotope effects of the $^{31}$P chemical shifts, are in good agreement with the literature data on analogous compounds.[13]

Amide nitrogen atom of pyramidal geometry has recently been assumed for $R_P$-1 and $S_P$-1.[17] The above NMR results do not exclude the correctness of this assumption.

## EXPERIMENTAL

Compounds 1 and 3 labelled with $^{15}$N and unlabelled 2 were synthesized according to literature procedures.[2,3] For the preparation of 1 and 3, $(^{15}NH_4)_2CO_3$ and $(CH_3)_2{}^{15}NH$ (30% aqueous solution, 94.8% $^{15}$N, VEB Berlin-Chemie, Berlin-Adlershof, G.D.R.) were used.

A Varian Associates model XL-400 instrument was used to obtain the $^{31}$P (161.9 MHz) and $^{15}$N (40.5 MHz) NMR spectra. The measurements were made at ambient temperature, under broad-band $^{1}$H decoupling on 0.05-0.3 M solutions of the compounds in DMSO-$d_6$. Chemical shifts are referenced to external $H_3PO_4$ and $NH_4{}^{15}NO_3$. Digital resolution of the transformed spectra was 0.1 Hz ($^{31}P$) and 0.25 Hz ($^{15}N$) which set the estimated accuracy of frequency determinations at ± 0.2 Hz and ± 0.5 Hz, respectively. $^1J(^{31}P^{15}N)$ values were obtained from the splittings of the $^{15}N$ and $^{31}P$ resonances, or, for 2, from the $^{15}N$-satellite frequencies of the $^{31}P$ signal. Results are summarized in TABLE 1. The $^1J(^{31}P^{15}N)$ values in TABLE 1 are taken from the more accurate frequency lists of the phosphorus spectra.

Preparation of $(^{15}NH_4)_2CO_3$

$(^{15}NH_4)_2SO_4$ (2.0 g, 94.5% $^{15}N$, VEB Berlin-Chemie, Berlin-Adlershof, G.D.R.) was dissolved in a solution of KOH (5.0 g) in deionized $H_2O$ (30 mL) in a 100 mL two-necked, round-bottomed flask. The solution was boiled, and a slow stream of dry nitrogen gas was passed in. The departing gas was transferred through a reflux condenser and a drying tube filled with KOH pellets and finaly through a gas washing bottle containing dry DMF (270 mL) that had been cooled to (-)40 - (-)50°C with the exclusion of atmospheric moisture. After 3 h, dry $CO_2$ gas was bubbled gently into the DMF solution for 30 min. The suspension thus obtained was mixed with anhydrous pyridine (30 mL) at room temperature and used directly for the synthesis of 1.

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