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"Twin" Phosphorous Atoms of Tetraethyl 2-Methyl-piperyd-1-ylmethylenebisphosphonates

Piotr Młynarz,[1] Michał Jewginski,[2] Sylwia Śliwińska,[1] Rafał Latajka,[1] Grzegorz Schroeder,[3] and Paweł Kafarski[1]

[1]*Department of Chemistry, Wroclaw University of Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Poland*

[2]*Institute of Chemistry, University of Opole, Oleska 48, 45-052 Opole, Poland*

[3]*Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznan, Poland*

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ABSTRACT: *Recently, bisaminophosphonates found applications as therapeutic agents for curing bone disorders. When trying to relate the structures of substituted piperid-1-ylmethylenebisphosphonic with their biological properties, non-typical findings that in ^{31}P NMR spectra of 2-methyl-piperid-1-ylmethylenebisphosphonic and 2-ethyl-piperid-1-ylmethylenebisphosphonic acids, two separate singlets from each of the phosphonic groups were observed, while their analogues bearing substituent in position 3 exhibit only one signal. Their presence was explained by freezing of the molecular motions by strong hydrogen bonding between NH and P=O atoms. In this work, synthesis as well as spectroscopic and theoretical investigations of the tetraethyl esters of 2-methyl-piperid-1-ylmethylenebisphosphonic in its racemic and enatiomerically pure forms are reported. Their ^{31}P NMR spectra revealed two sets of dublets, which indicate the presence of two nonequivalent phosphorous atoms. More detailed NMR and theoretical studies indicated that the nonequivalent phosphorous signals in ^{31}P NMR spectra may results from the absence of C_2 symmetry of the molecule along with the presence of large ester groups blocking the internal molecular motion around C—N bond, and thus blocking the interchange of ring conformation.* © 2007 Wiley Periodicals, Inc. Heteroatom Chem 18:774–781, 2007; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20349

Correspondence to: Piotr Młynarz; e-mail: piotr.mlynarz@pwr.wroc.pl.

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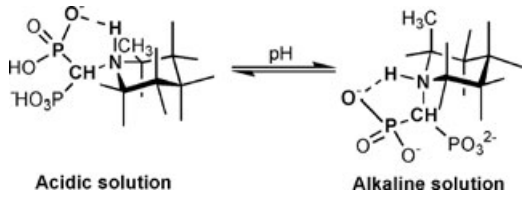
INTRODUCTION

Bisphosphonic acids are hydrolytically stable analogues of pyrophosphate characterized by a common P-C-P fragment, in which the carbon-to-phosphorous bonds replace oxygen-to-phosphorus bonds. They have been recognized as therapeutic agents for treatment of bone disorders, hypercalcemia of malignancy, and osteoporosis [1]. A subclass of bisphosphonates, derivatives of aminomethylenebisphosphonic acid exhibit promising antiparasitic [2] and herbicidal activities [3]. Because of these biological activities, their chemical properties, especially those considering their metal ion complexing abilities, have been deeply exploited in both solution and solid states [4]. Recent studies focusing on aminomethylenebisphosphonic acids bearing piperidine ring indicated that they might



SCHEME 1 The proposed pH switchable conformation [5].

appear in two stable conformations of different structures, one observed in acidic and second in basic aqueous solutions [5]. It was proposed that tertiary nitrogen is responsible for the formation of two "frozen" rotational structures that are stabilized by the formation of two different kinds of hydrogen bonds of N-H-O-P type (Scheme 1). First conformer is stabilized by hydrogen bond formed between protonated nitrogen and oxygen of phosphonic group and the second "locked structure" is stabilized by hydrogen bonds between protonated ring nitrogen and deprotonated oxygen of phosphonic group. Existence of these conformers was proposed for the explanation of the not so straightforward ^{31}P NMR spectra of 2-methyl-piperid-1-ylmethylenebisphosphonic and 2-ethyl-piperid-1-ylmethylenebisphosphonic acids. Interestingly, 3-methyl-piperid-1-ylmethylenebisphosphonic acid dose not give doublets in ^{31}P NMR spectra, although just as the 2-substuituted derivative, it is not of C_2 symmetry type. The one explanation might be based on the assumption that piperidine nitrogen atom substituted by bulky aminomethylenebisphosphonate moiety, which is most likely in the equatorial position (as shown previously [5] by interpretation of EXSY spectra), becomes the second chiral center along with the formation of mixtures of diastereomers (Fig. 1), and thus might result in splitting of phosphorus signal. This could be, of course, true only when nitrogen inversion is exceptionally slow in NMR time scale. The other explanation assumes the existence of barrier of rotation and only partial interchange of phosphonic groups within NMR time scale.

To understand this phenomenon in more detail, synthesis of diethyl 2-methyl-piperid-1-ylmethylenebisphosphonate (compound not able to form hydrogen bonds proposed as cause of "frozen" conformations in preceding paper) starting from racemic (Scheme 2) and enatiomerically pure (as isomer *S*) substrates was undertaken and the structures of the resulting products were analyzed by means of various NMR techniques.

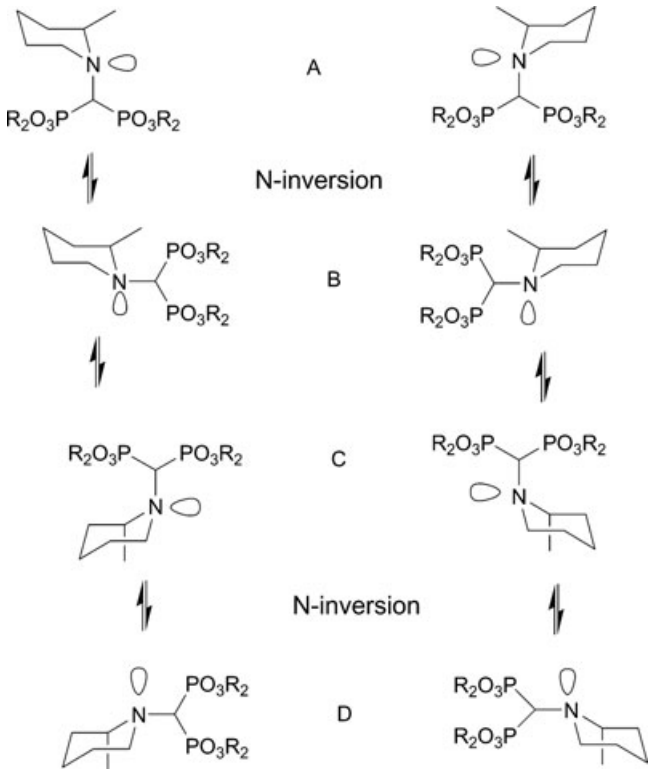
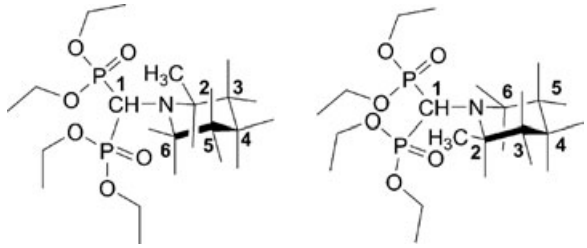


FIGURE 1 Ring and nitrogen inversions in tetraalkyl 2-methyl-piperyd-1-ylmethylene-bisphosphonate.



SCHEME 2 Structures of two enantiomers of 2-methyl-piperyd-1-ylmethylenebis-phosphonate *S* isomer is shown at right-hand side.

EXPERIMENTAL

Synthesis

Aminomethylene bisphosphonates were synthesized by reacting stoichiometric amounts of the corresponding amines, triethyl orthoformate, and diethyl phosphite as described earlier [6–8]. They were purified by column chromatography using ethyl acetate and methanol as eluents, starting from pure ethyl acetate and subsequently increasing the amount of methanol, finishing with pure methanol.

TABLE 1 Assignment of Peaks Found in ^{1}H and for ^{13}C Spectra (Phosphorous Signals Were Found at 21.13 (P1) and 17.99 ppm (P2))

Carbon Atom Number	*^{1}H*	*^{13}C*	*J_{C-P} (Hz)*
CH(1)	3.90	55.32	
CH(2)	2.94	55.46	14
CH_2(3)	1.60/1.20	36.20	2
CH_2(4)	1.68/1.33	24.87	
CH_2(5)	1.60/1.47	26.84	
CH_2(6)	3.59/2.76	50.56	6.4
CH_3(7)	1.07	20.54	
CH_2(8)	4.3–4.11	61.80	6.5
		62.02	7.1
		62.49	7.4
		64,18	7.0
CH_3 (9)	1.3–1.4	16.22–16.69	

NMR Measurements

NMR spectra were recorded on Bruker Avance DRX300 and Bruker AMX600 instruments in $CDCl_3$ using a variety of 1D and 2D (HMQC, COSY, TOCSY, H–P, C–P, P–P correlation spectra) techniques and chemical shifts are given in relation to TMS (^{1}H and ^{13}C spectra) or 85% phosphoric acid (phosphorous spectra). Structures of both enantiomeric forms of tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphononate along with the used enumeration of carbon atoms are given in Scheme 2. The observed ^{1}H, ^{13}C, and ^{31}P NMR spectra obtained for both racemic and enantiomeric forms revealed the same set and multiplicity of signals, differentiating only in chemical shift values (Table 1) within the experimental error.

Theoretical Studies

Structures of the studied compounds were optimized in the gas phase by using Gaussian 03 program at the HF/6-31g(d,p) level [9]. Optimization was carried out using default algorithm. To determine the energy of barrier of rotation, a scan of relaxed potential energy surface (PES) was applied. In this step, the Berny algorithm with the internal coordinates had been used [10]. During the experiment, the value of dihedral angle P-C1-N-C2 was increased stepwise. *Barrier of rotation* is defined as the difference between energy of the structure in local minimum and the highest energy obtained during the scan of relaxed PES [11].

RESULTS AND DISCUSSION

^{31}P NMR spectra of tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphonate were found to be quite complex and show two phosphorus signals of presumably AB type. The interpretation of this phenomenon is quite complex because piperidyl fragment of the molecule is highly flexible, whereas that of aminomethylenebisphosphonate fragment is quite rigid.

It is well known that in such a molecule as studied in this work, three types of motions can be considered, namely, ring inversion, nitrogen inversion, and rotation around C—N bond. All the possible conformers of both enantiomers of tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphonate are shown in Fig. 1.

Previous studies revealed that substituents located in piperidine ring are able to "lock" the ring inversions and set in a chair conformation [4]. It is also well known that the bulkiest substituent should be in energetically most favorable equatorial position, and therefore methylenebisphosphonic fragment of the molecule should appear in that position (structures B and D), which cause them to become diastereomeric (if considering one isomer of the substrate) or mixures of diastereomers (if synthesis was started from racemic substrate). Calculations have clearly shown that the presence of structures A and C are extremely unlikely since they tend to undergo spontaneous, rapid nitrogen inversion to structures B and D. Existence of conformation "frozen" by hydrogen bonding has to be rejected since 2-methyl-piperyd-1-ylmethylene-bisphosphonate is obtained in a nonprotonated form. In addition, the lack of hydrogen bonding was supported by the addition of phosphagene to the solution, a very strong Lewis base, which did not change the phosphorous signal pattern.

^{1}H Spectra

The assignment of ^{1}H and ^{13}C NMR spectra is shown in Table 1. The observed spectra confirm the structure of the studied compound yielding expected peak patterns.

EXSY spectra were not recorded for the system taking into account previous studies, which have shown that for far less complex system, conformational ring exchange does not occur owing to the presence of bulky substituents [4]. The most interesting proton H-C1 is split into doublet of doublets (for equivalent phosphorus atoms triplet is expected), with two coupling constants ^{2}J(C1H-P) = 29 and 22 Hz. The existence of doublet of doublets suggests the presence of two phosphorous atoms possessing different chemical surrounding, and thus appearing as two nonequivalent groups. The long-range proton–phosphorous coupling constants cannot be easily evaluated because of the complex multiplicity

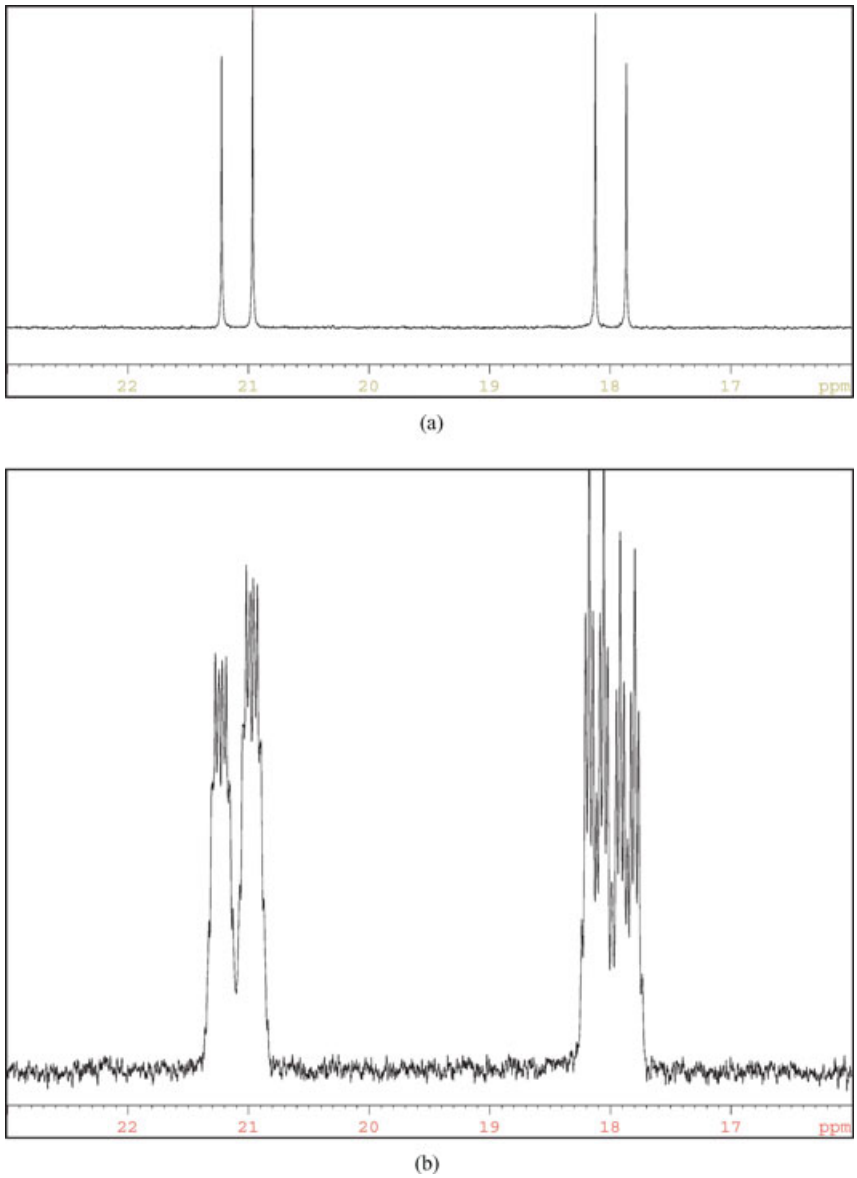


FIGURE 2 600 MHz ^{31}P NMR spectra recorded at 298 K: (a) with and (b) without proton decoupling.

of proton signals. It is also interesting that the presence of only one doublet was visible for methyl CH_3-protons in ^{1}H NMR spectra.

^{31}P Spectra

Phosphorous spectra are the most intriguing feature of tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphonate. One-dimensional phosphorous spectra, including proton decoupling ones, reveal pattern similar to AB system. If so, the P–P chemical coupling constant is equal to 61 Hz (Fig. 2). When switching off the decoupler set of two doubled multiplets is observable, for which coupling constants ^2J(H-C1-P1) = 22 Hz, ^2J(H-C1-P2) = 29 Hz and ^3J(-CH_2-O-P1) = 6 Hz and ^3J(-CH_2-O-P2) = 7 Hz could be assigned (Fig. 2). It is worth noticing that the pattern of each doubled multiplet is different, showing that each phosphorous atom is in different chemical environment. ^{31}P NMR measurements of temperature dependence in the range 300 to 360 K did not shown coalescence of both signals, while only a small shift of about 0.45 ppm was observed.

^{13}C Spectra

A fragment of ^{13}C NMR spectra of tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphonate is shown in Fig. 3. The difference between two diethyl phosphonate ester units is also very well visible in

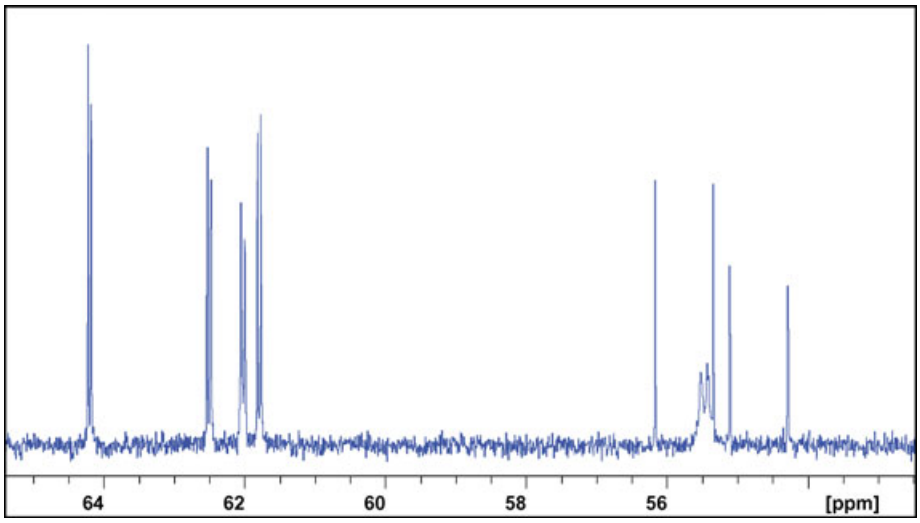


FIGURE 3 600 MHz ^{13}C NMR spectra recorded for tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphonate S isomer. Assignments from the left: 4xCH_2, C1 and C2 signals.

these spectra. The methylene protons of -OCH_2- groups have four different chemical shifts, with almost the same ^3J(C-P) coupling constants (6.5–7.4 Hz). One of these four groups is strongly deshielded. This effect is also visible for carbon atoms of methyl ester groups, where three of them have almost the same chemical shifts, while the fourth one is little deshielded (Table 1) because of the different chemical environment. The detailed analysis of C1 methylene signal showed that this peak is observed as a doublet of doublets, which clearly indicates the presence of two nonequivalent phosphonate groups and results in two coupling constants ^2J(P, C1) = 164 and 124 Hz (Fig. 3). It is obvious that in the case of two equivalent phosphorous atoms, C1 signal should arise as a triplet. The coupling between C—P atoms of the ^3J(P, C) type is also visible for C2 (14 Hz) and C6 (6.4 Hz) atoms. Surprisingly, there is also a small splitting observed for C3 signal with a coupling constant of 2 Hz.

The two phosphorous atoms split C2 and C6 atoms into two doublets instead of two doublet of doublets for each of them or into a triplet, which should be observed in the case of nonequivalence of two phosphorous atoms, or two equivalent phosphonate moieties resulting from fast rotating bisphosphonate group. This may be explained by the existence of different values of angles between 1P-N-2C and 2P-N-6C atoms due to diverse distribution of ester groups in the space.

2D P–P and P–C Correlation Spectra

P–P homo-correlation spectra reveal the cross signals between both phosphorous atoms. Interestingly, acidification of the solution with trifluoroacetic acid causes the depletion of multiplicity of these signals, and only the appearance of doublet is noticed. Moreover, P–P correlation spectra have shown that these two signals are not coupled. This finding seems to suggest that doubling of phosphorous signal results rather from the presence of chiral nitrogen, and thus from the existence of diastereomeric mixtures.

The results of 2D C–P correlation experiment (Fig. 4) are in a good agreement with the results of 1D ^{13}C NMR experiment (Table 1). In this spectrum, two sets of phosphorous signals corresponding to P1 and P2 atoms coupled to the respective carbon atoms are visible. The direct couplings of P1 with the following carbon atoms were found: ethyl ester carbon atoms (2x–CH_2-) and (2xCH_3-) as well as C1, C3, and C6 atoms of the piperidine ring. The P2 atom is coupled with carbons of ethyl ester (2x–CH_2-), (2xCH_3-), and methylene C1 and C2 of the heterocycle. Thus, each of the phosphorus atoms exhibits a little bit different coupling pattern. While interactions with C2 and C6 atoms could be easily explained on the basis of scalar coupling, those observed between P1 and C3, that is, through five single bonds, seems to be improbable. Therefore, this coupling most likely results from interaction through space, and might suggest the existence of weak hydrogen bond between one of the phosphorous oxygens of P1 group and hydrogen located at C3 atom.

THEORETICAL CALCULATIONS

The theoretical calculations were performed for conformers B and D characterized by equatorial

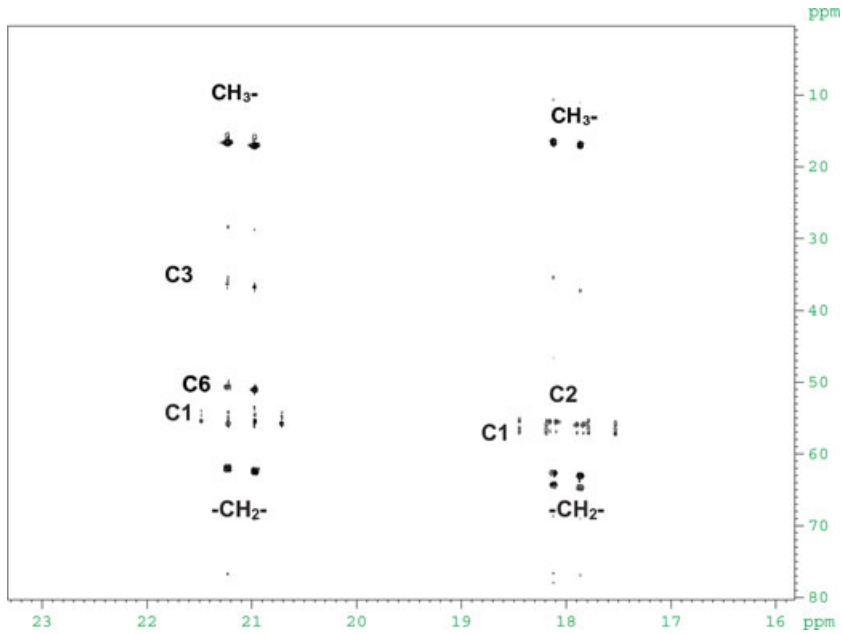


FIGURE 4 600 MHz ^{31}P-^{13}C correlation spectra of studied bisphosphonate. The numeration of ester group carbon atoms are omitted for clarity.Q

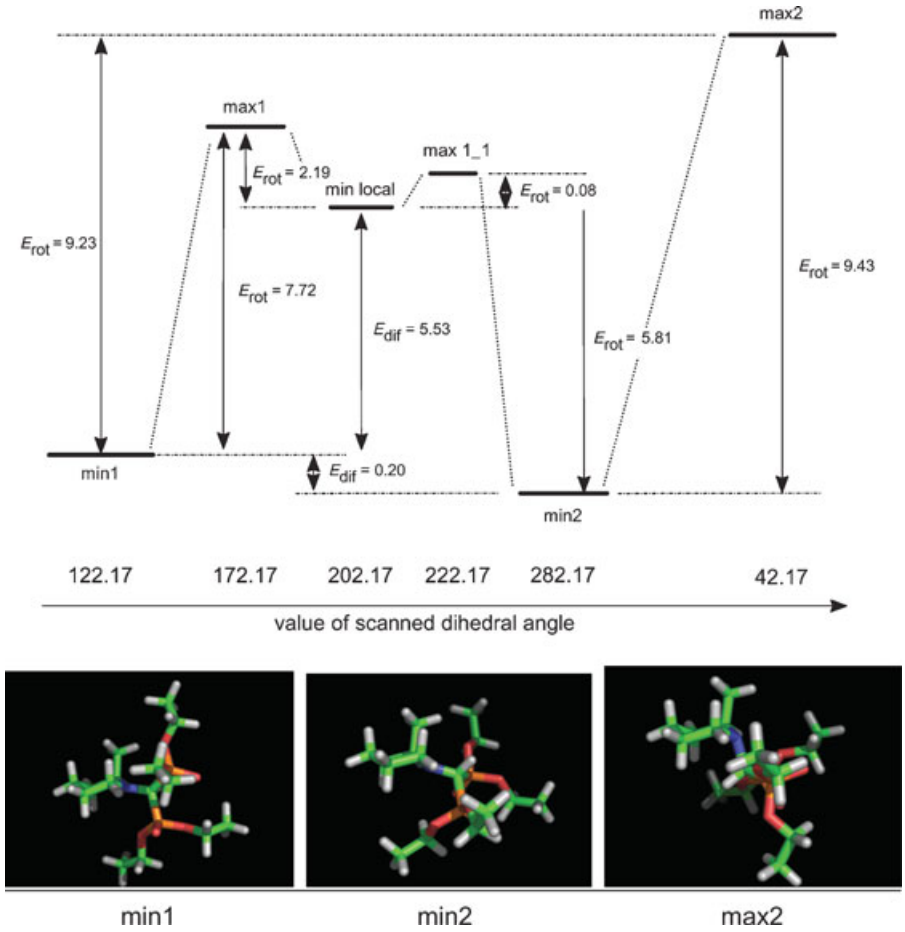


FIGURE 5 Graph of energy changes upon scanning of P-C-N-C angle of conformer D. Conformations of two minimal and maximal energies are shown below the graph.

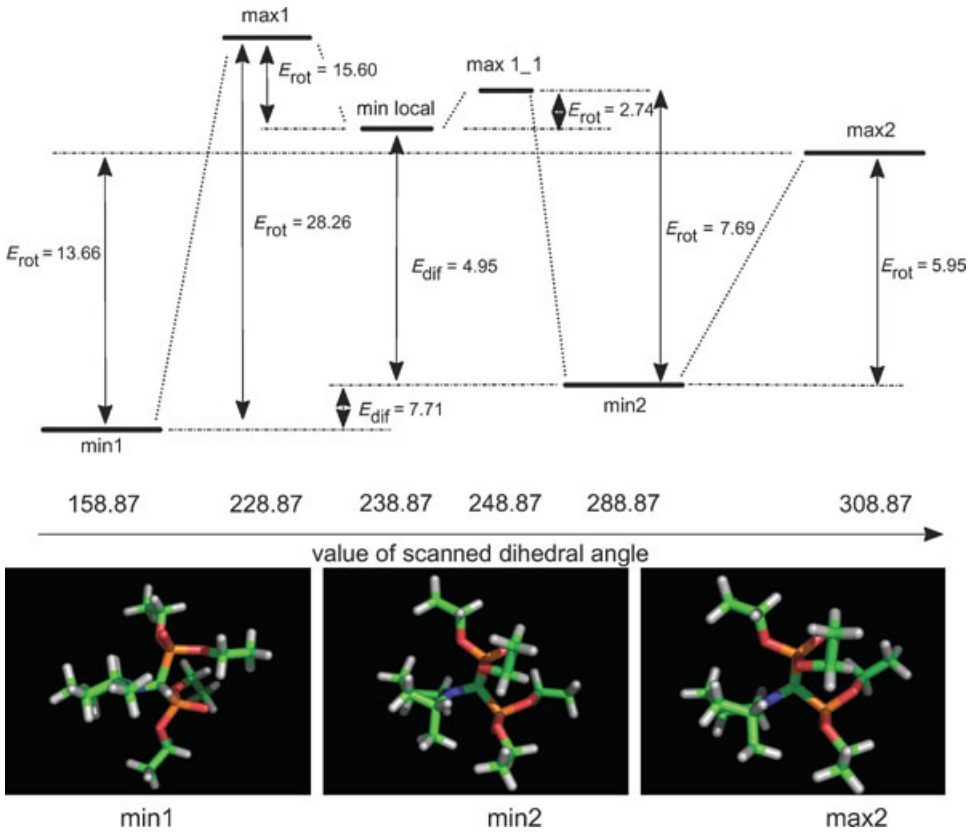


FIGURE 6 Graph of energy changes upon scanning of P-C-N-C angle of conformer B. Conformations of two minimal and maximal energies are shown below the graph.

placement of bulky aminomethylenebisphosphonate moiety. Placement of this group in the axial position results in rapid nitrogen inversion and formation of adequate conformers B and D. For both conformers, rotation energy around C1—N bond was calculated, and is presented in Figs. 5 and 6.

Maximal observed barrier of rotation is approximately 9 kcal mol^{-1}. This value is close to single hydrogen bond and suggests that each doubling of doublet in the ^{31}P NMR spectra might be a result of conformational barrier. Quite interestingly, the similar barrier calculated for free acid was close to zero (data not shown). This explains why there is only a doublet observed in the spectra of free acids.

Similarly, the calculations carried out for biequatorial conformer B gave quite unexpected results. In this case (Fig. 6), quite significant rotation barrier was observed. However, upon reaching a step shown with the arrow, the nitrogen inversion leading to conformer D was observed. This result shows that the rotational hindrance in this case is so high that inversion is an alternative route of releasing the resulting strain.

In conclusion, theoretical calculations indicate that methylenebisphosphonic fragment of tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphononate tends to appear in equatorial position, whereas the position of methyl group is less important. Calculations also showed that there exists significant barrier of rotation around N—C bond, which might be responsible for the observed patterns of phosphorus signals in NMR.

CONCLUSIONS

In this study, we have tried to explain the nontypical ^{31}P NMR spectra obtained for tetraethyl 2-methyl-piperid-1-ylmethylenebisphosphononate. In the case of 2-methyl-piperid-1-ylmethylenebisphosphononic acid, the existence of two energetically favorable populations, which predominate owing to the formation of P=O-H—N hydrogen bond was proposed as an explanation of this phenomenon [4]. The simplest elucidation of this effect is based on the lack of C_2 symmetry of the molecule. However, this does not explain why compounds substituted in position 3 do

not behave in the same manner (only one phosphorous signal was observed in this case). In addition, two phosphorus signals are observed for tetraethyl ester studied in this work, but the splitting of each signal into doublet is clearly visible. If considering that methylenebisphosphonate fragment of the molecule is preferentially placed at equatorial position of piperidyl ring, the compound preferentially exist in the solution in the form of conformers B and D. If the conformational interchange at NMR time scale is slow, they consequently exist in the form of diastereomeric mixtures, and hence the appearance of two signals is not surprising. Doubling of each signal observed in the case of tetraethyl 2-methylpiperid-1-ylmethylenebisphosphonate may be due to the steric hindrance around crowded nitrogen atom, resulting in restricted rotation of methylenebisphosphonate group. This assumption was supported by theoretical calculations, which showed the existence of quite significant rotational barriers in both conformers B and D. Obviously, all the observed effects are dependent on the dynamics of the system in NMR time scale, and therefore are not easy to interpret.

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