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# Experimental and *ab initio* calculated structures of 2-aminoindane-2-phosphonic acid, a potent inhibitor of phenylalanine ammonia-lyase, and theoretical studies of its binding to the model enzyme structure†

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The structure of 2-aminoindane-2-phosphonic acid (AIP) was studied using X-ray crystallography, NMR spectroscopy, molecular modelling, IR spectroscopy, and potentiometric titration. In the solid state, a thermodynamically stable conformer of AIP is one in which the phosphonic group occupies the equatorial position and the amino group the axial position. The NMR data suggest that fast equilibrium in solution between the two conformers of AIP is significantly shifted toward the equatorial conformer (EC). Both solid state studies, that is X-ray analysis and IR spectroscopy of AIP, revealed the presence of hydrogen-bonded water. *Ab initio* calculations in the gas phase indicate only a small barrier between the two possible conformations of AIP. Binding studies of both conformers, in various protonation states, to the model of the phenylalanine ammonia-lyase structure suggest that only the axial phosphonic group conformer is docked specifically. Indications from modelling are that phenylalanine ammonia-lyase binds AIP's conformer with higher specificity and that the molecular reorganisation required can be responsible for the experimentally observed time-dependent inhibition.

## Introduction

Phenylalanine ammonia-lyase (PAL), an important enzyme of plant secondary metabolism, catalyses the elimination of ammonia from (*S*)-phenylalanine to give (*E*)-cinnamic acid (Fig. 1).[1] The enzyme opens the door to many diverse metabolites in the phenylpropanoid pathway with a wide range of functions, including lignins, flavonoids, coumarins, benzoic acid derivatives, *etc.*[1,2] The mechanism of action of PAL is still not completely understood, despite many investigations.[3,4] The three-dimensional structure of PAL has also not been solved, so the design of inhibitors based on the enzyme's structure is not possible. Nevertheless, a few potent inhibitors of PAL have been found: (±)-2-aminooxy-3-phenylpropanoic acid,[5] (*R*)-(−)-1-amino-2-phenylethylphosphonic acid,[6,7] 2-aminoindane-2-phosphonic acid‡ (AIP, Fig. 1),[8] and (+)-1-amino-3′,4′-dichlorobenzylphosphonic acid.[9] Of the known inhibitors, 2-aminoindane-2-phosphonic acid has the highest potency and potential as a specific inhibitor, both *in vitro* and *in vivo*.[8] AIP inhibits pure recombinant phenylalanine ammonia-lyase from parsley competitively in a time-dependent manner, resulting in the formation of an enzyme–inhibitor complex.[10] AIP has been used in numerous investigations of PAL[11–14] but the precise molecular mode of inhibition of the enzyme is not known. In this project, we have studied solid, solution and *ab initio* structures of 2-aminoindane-2-phosphonic acid. We have also done binding analyses for two possible conformers and various protonation states of the inhibitor in the active site model of phenylalanine ammonia-lyase, which was obtained by the homology approach.[4]

## Experimental

### Syntheses

**2-Aminoindane-2-phosphonic acid monohydrate (1).** 2-Aminoindane-2-phosphonic acid as the monohydrate (**1**) was obtained from 2-indanone according to the literature procedure.[8] Crystals were obtained by evaporating a water solution of 2-aminoindane-2-phosphonic acid under atmospheric pressure until the first crystals precipitated out. The solution was

† Electronic supplementary information (ESI) available: bond lengths and angles for **1** and **2**, selected infrared frequencies and their assignment for **1** and **1D$_4$**, geometries of different conformations of **1** in various protonation states, optimized at different levels of theory. See http://www.rsc.org/suppdata/nj/b3/b307533h/

‡ We have updated the name of 2-aminoindan-2-phosphonic acid to 2-aminoindane-2-phosphonic acid, in accordance with the recommendation in *Guide to IUPAC Nomenclature of Organic Compounds (Recommendations 1993)*.

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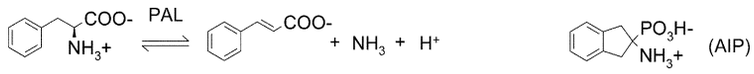


**Fig. 1** Scheme of reaction catalysed by phenylalanine ammonia-lyase and structure of 2-aminoindane-2-phosphonic acid (AIP)—the potent *in vivo* and *in vitro* inhibitor of the enzyme.

allowed to stand at 20 °C, depositing small needless that were filtered off and air dried. Melting point 257–260 °C (literature[8] mp 257–260 °C). $^1$H NMR ($D_2O$–DCl, pD = 1.04, $T$ = 293 K): $\delta$ 6.57 ppm (m, 4H, aromatic ring), 2.88 (dd, $^3J_{PH}$ = 12.5 12.5 Hz, $^2J_{HH}$ = 17.3 Hz, 2H, $CH_2$), 2.51 (dd, $^3J_{PH}$ = 4.2 Hz, $^2J_{HH}$ = 17.3 Hz, 2H, $CH_2$).$^{31}$P{$^1$H} NMR: $\delta$ 17.84 (s). $^{13}$C NMR: $\delta$ 137.48, 137.45, 127.31, 124.56 (aromatic ring), 59.68 (d, $^1J_{PC}$ = 160.0 Hz), 39.12 ($CH_2$).$^1$H NMR ($T$ = 340 K): $\delta$ 7.35 (m, 4H, aromatic ring), 3.69 (dd, $^3J_{PH}$ = 12.6 Hz, $^2J_{HH}$ = 17.4 Hz, 2H, $CH_2$), 3.33 (dd, $^3J_{PH}$ = 5.8 Hz, $^2J_{HH}$ = 17.5 Hz, 2H, $CH_2$).$^1$H NMR ($D_2O$–NaOD, pD = 8.93, $T$ = 293 K): $\delta$ 7.14 (m, 4H, aromatic ring), 3.46 (dd, $^3J_{PH}$ = 9.3 Hz, $^2J_{HH}$ = 17.5 Hz, 2H, $CH_2$), 2.93 (dd, $^3J_{PH}$ > 1 Hz, $^2J_{HH}$ = 17.5 Hz, 2H, $CH_2$).$^{31}$P{$^1$H} NMR: $\delta$ 13.96 (s). $^{13}$C NMR: $\delta$ 139.68, 139.57, 127.24, 125.06 (aromatic ring), 62.84 (d, $^1J_{PC}$ = 143.0 Hz), 40.69 ($CH_2$). $^1$H NMR ($T$ = 340 K): $\delta$ 7.72 (m, 4H, aromatic ring), 3.90 (dd, $^3J_{PH}$ = 9.8 Hz, $^2J_{HH}$ = 16.5 Hz, 2H, $CH_2$), 3.14 (dd, $^3J_{PH}$ = 1.9 Hz, $^2J_{HH}$ = 16.5 Hz, 2H, $CH_2$).

**2-Aminoindane-2-phosphonic acid trihydrate (2).** Crystals of 2-aminoindane-2-phosphonic acid trihydrate (**2**) were obtained by slow evaporation of a water solution of 2-aminoindane-2-phosphonic acid at room temperature. The large crystals obtained in this way were unstable at room temperature in open air (they turned into an amorphous white powder). This procedure sometimes yielded crystals with higher water contents.

**Tetradeutero-2-aminoindane-2-phosphonic acid (1D$_4$).** Tetradeutero-2-aminoindane-2-phosphonic acid, probably as the mono(deuterium oxide), was obtained from **1** by dissolving it in deuterium oxide, followed by evaporation to dryness under reduced pressure, and repeating this procedure two more times. The residual crystalline **1D$_4$** was air dried.

### Crystallography

The diffraction data for both crystals of **1** and **2** were measured at 293 K on a KUMA KM4 computer-controlled four-circle diffractometer with graphite-monochromated Cu-K$_\alpha$ ($\lambda$ = 1.5418 Å) and Mo-K$_\alpha$ ($\lambda$ = 0.71069 Å) radiation, respectively. In the case of 2-aminoindane-2-phosphonic acid trihydrate (**2**), only those reflections for which the pre-scan procedure gave an intensity greater than zero were measured. Crystal data and details of the measurements are summarized in Table 1.§ Both structures were solved by direct methods (SHELXS97[15]) and refined by full-matrix least-squares on $F^2$ using SHELXL97.[15] All non-H atoms were refined with anisotropic thermal parameters. All positions of the hydrogen atoms were found from difference Fourier maps and refined with anisotropic thermal parameters. Molecular structures were drawn with XP in SHELXTL.[16]

### NMR spectroscopy

$^1$H, $^{13}$C and $^{31}$P NMR spectra of 2-aminoindane-2-phosphonic acid monohydrate (**1**) were recorded on Bruker Avance DRX 300 MHz instrument operating at 300.13 MHz ($^1$H). Measurements were made in $D_2O$ adjusted in pD with DCl (20%) or NaOD (40%). The pD values of the solutions were determined with the use of a Mettler Toledo MP225 pH-meter and were not corrected. The NMR spectra were recorded for a solution containing 20 mg of sample **1** in 0.7 ml of solvent at 293 and 340 K with TMS as external standard ($^1$H and $^{13}$C NMR) and with 20% $H_3PO_4$ as external standard ($^{31}$P NMR). Proton decoupling was achieved by power gated decoupling using the WALTZ-16 sequence and relative concentrations of components were measured by integration of signals. However, as the observed signals did not have complete baseline resolution, the coupling constants were estimated directly from the spectra.

### IR spectroscopy

Infrared absorption spectra of compounds **1** and **1D$_4$** were measured on a Perkin–Elmer FT-IR 1600 spectrophotometer as KBr discs and on a Perkin–Elmer system FT-IR 1200 spectrophotometer (for the range 600–100 cm$^{-1}$ as nujol mulls on polyethylene plates).

### Potentiometric titration

Solutions (1.5–2.0 ml) of 1 mM 2-aminoindane-2-phosphonic acid monohydrate (**1**) in 0.1 M $KNO_3$ were titrated with 0.1 M NaOH at 25 °C using a Molspin automatic titrator (Molspin Ltd, Newcastle-upon-Tyne, UK) within the pH range 2–11.5. Changes in pH were monitored with a combined glass–Ag/AgCl electrode (ATI Russell pH Ltd, Fife, Scotland). Results from the titrations were analysed by SUPERQUAD, giving the corresponding p$K_{1-3}$ values.[17]

### Molecular modelling

**Gas phase.** *Ab initio* ground state geometries and energies of two conformations of 2-aminoindane-2-phosphonic acid and the barrier between them were calculated using Hartree–Fock (HF) level, 6-31G(d) basis set. The results were additionally tested using density functional theory [using the hybrid B3LYP exchange-correlation functional, 6-31G(d) basis set] and at the level of the Møeller–Plesset (MP2) method,

**Table 1** Crystal data and measurement details for compounds **1** and **2**.

| | **1** | **2** |
|---|---|---|
| Chemical formula | $C_9H_{12}NO_3P{\cdot}H_2O$ | $C_9H_{12}NO_3P{\cdot}3H_2O$ |
| Formula weight | 231.18 | 267.21 |
| Temperature/K | 293(2) | 293(2) |
| Crystal system | Monoclinic | Monoclinic |
| Space group | $P2_1/n$ | $C2/c$ |
| $a$/Å | 13.261(2) | 25.237(6) |
| $b$/Å | 6.604(2) | 6.167(2) |
| $c$/Å | 13.588(2) | 20.349(6) |
| $\beta$/° | 119.09(2) | 122.95(3) |
| $U$/Å$^3$ | 1039.9(4) | 2657.6(2) |
| $Z$ | 4 | 8 |
| Total reflections | 2487 | 3039 |
| Indep. reflections | 1871 | 2470 |
| $R_{int}$ | 0.024 | 0.023 |
| $\mu$/mm$^{-1}$ | 2.342 | 0.220 |
| $R$ [$I \geq 2\sigma(I)$] | 0.0398 | 0.045 |
| $wR$ [$I \geq 2\sigma(I)$] | 0.1097 | 0.110 |

§ CCDC reference numbers 213957–213958. See http://www.rsc.org/suppdata/nj/b3/b307533h/ for crystallographic data in .cif or other electronic format.

TSBI>6-31G(d) basis set. All *ab initio* calculations were done using the Gaussian package[18] on an SGI Origin 3200 work station. Transition state (TS) geometries were located using the standard optimisation algorithm implemented in the Gaussian package at the HF/6-31G(d) level. The TS geometry was identified by a single imaginary frequency. Convergence criteria used in the geometry optimisation were: 0.000450 (maximum force), 0.000300 (RMS force), 0.001800 (maximum displacement) and 0.001200 (RMS displacement) for the AC and EC conformers. In the case of the transition state, tighter criteria were used: 0.000015 (maximum force), 0.000010 (RMS force), 0.000060 (maximum displacement) and 0.000040 (RMS displacement). All values are in atomic units.

### Binding model

Modelling of the docking of 2-aminoindane-2-phosphonic acid was done with the AutoDock package.[19] The structure of the active site of phenylalanine ammonia-lyase from *Petroselinum crispum* was modelled using a homology approach based on the available X-ray structure of histidine ammonia-lyase.[4] This structure contains a spherical part of the enzyme around the active site (25 Å). The original structure was supplemented by polar hydrogen atoms. In some amino acids, such as histidine, only one possible protonation state was chosen. Partial charges were set for every atom using the Charmm27 force field in the Insightii package (Accelrys Inc., San Diego, CA, USA). In the next step, solvation parameters were set using the Addsol programme in the AutoDock package. Partial charges for the inhibitor were calculated using the CHELPG method at the Hartree–Fock level in the 6-311G(d) basis set.[20]

In the first part of the docking procedure, grids describing the interaction of different atom types with the protein were created.[21] This was done by placing a probe atom on a grid point followed by interaction calculations. A grid of electrostatic potential was calculated in a similar way using a charged probe. During the docking, the energy or potential for a given position was calculated by interpolation between eight points of the above-mentioned grids. 2-Aminoindane-2-phosphonic acid was docked using the Lamarckian genetic algorithm.[19] The AIP molecule was allowed to move on a grid of $60 \times 60 \times 60$ points separated by 0.375 Å and centred on the active site. The whole procedure was repeated fifty times for each of twelve structures (from AC-a, EC-a to AC-f, EC-f) of the inhibitor, every time starting from a randomly chosen population of fifty different conformations generated by the algorithm. A group of conformations with RMS deviations less than 1.0 Å was classified as a cluster of conformations (see Fig. 8 below). The limiting number of energy evaluations was set to 1,000,000 and the limiting number of generations was set to 27,000. The whole enzyme structure was treated as rigid. Amino and carboxylic groups present in the active site were ionised. Water molecules were not considered in the docking. The carbon skeleton of the inhibitor was also rigid whereas the attached phosphonic and amino groups could rotate during docking. For evaluation of the binding energy, an empirical scoring function provided in the AutoDock package was applied.[19]

## Results and discussion

2-Aminoindane-2-phosphonic acid is expected to exist in two conformations, either with the phosphonic group equatorial (designated EC) or axial (designated AC). Additionally, AIP can exist in six protonation states (a–f, see Table 5 below).

### Crystallography

The structures of 2-aminoindane-2-phosphonic acid monohydrate and trihydrate are shown in Fig. 2. As can be seen, both

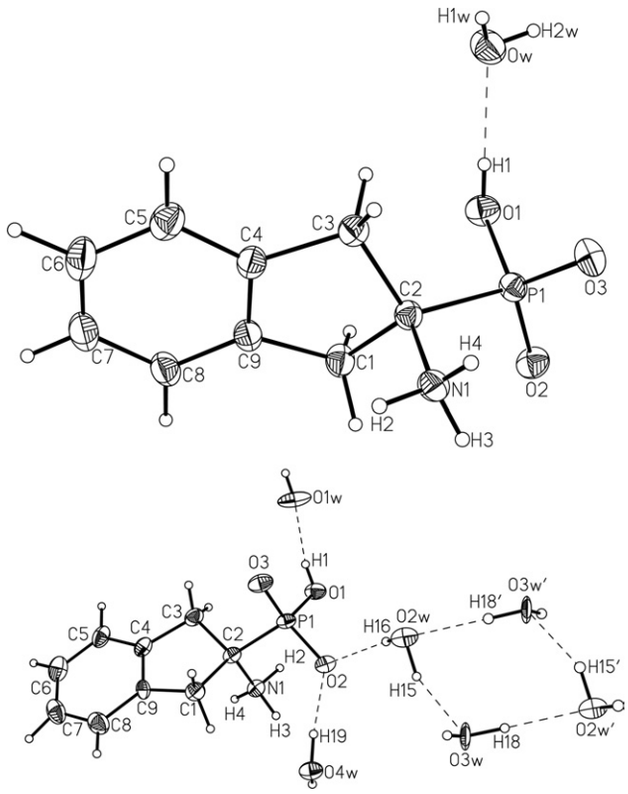


**Fig. 2** The molecular structures, with numbering scheme, of 2-aminoindane-2-phosphonic acid monohydrate (**1**, top) and 2-aminoindane-2-phosphonic acid trihydrate (**2**, bottom). Displacement ellipsoids are drawn at the 40% probability level. Occupancy factors are 0.5 for O3w and O4w. Primed atoms are at equivalent positions: $-x+1, -y+2, -z$.

exist as zwitterions. Bond distances and angles are given in the Electronic supplementary information (ESI). As expected, the angular disposition of the bonds around the phosphorus atom in both phosphonic groups deviates significantly from that of a regular tetrahedron. The bond lengths and angles are very similar in both structures and are in good agreement with those found in other 1-aminoalkylphosphonic acids.[22] The geometry of the indane portion of the molecules does not differ from those of other indane derivatives.[23,24] The indane moiety as a whole is non-planar [the dihedral angle between the best plane of the phenyl and the plane defined by C3–C4–C9–C1 is 3.6(2)° and 2.0(4)° for **1** and **2**, respectively]. The five-membered rings in both structures adopt an envelope conformation. Carbon atom C2 is −0.409(3) and −0.447(2) Å out of the least-squares plane of C3–C4–C9–C1 with the amino group being axially oriented [the nitrogen N1 and phosphorus P1 atoms deviate from this plane by −1.910(3) and 0.286(3) Å for **1**, and −1.948(2) and 0.226(1) Å for **2**]. The orientations of the amino and phosphonic groups with respect to the C1–C9 and C3–C4 bonds in the indane system are defined by the torsion angles N1–C2–C(3,1)–C(4,9) (Table 2). These fragments adopt synclinal and antiperiplanar conformations, respectively. It is interesting to note that, in both structures, the indane portion of AIP has an identical conformation but the molecules differ drastically in the conformation around the P1–C2 bond. This difference is visible in the orientation of the hydroxyl group relative to the amino group, described by the O1–P1–C2–N1 torsion angle (Table 2).

There is extensive hydrogen bonding in the crystal structures of compounds **1** and **2**, with all potential donor and acceptor atoms participating (Figs. 3 and 4). A collection of hydrogen bonds parameters in both crystals is presented in Table 3. In the monohydrate the first level of molecular organisation can

**Table 2** Selected torsion angles (°) for compounds **1** and **2**

| | **1** | **2** |
|---|---|---|
| O1–P1–C2–N1 | −168.4(2) | 57.9(5) |
| O2–P1–C2–N1 | 79.9(2) | −54.4(6) |
| O3–P1–C2–N1 | −49.9(2) | 178.0(5) |
| N1–C2–C1–C9 | 89.5(2) | −88.0(5) |
| N1–C2–C3–C4 | −90.3(2) | 88.1(5) |
| P1–C2–C3–C9 | −151.4(2) | 151.1(4) |
| P1–C2–C3–C4 | 149.0(2) | −150.7(4) |
| C1–C9–C4–C3 | 1.0(2) | 1.3(4) |
| C2–C3–C4–C9 | −16.4(2) | 16.3(4) |
| C3–C2–C1–C9 | −24.5(2) | 27.3(5) |
| C4–C9–C1–C2 | 15.0(2) | −18.2(5) |
| C1–C2–C3–C4 | 24.8(2) | −26.7(5) |

be perceived as formation of a centrosymmetric dimer realised by the two hydrogen bond interactions O1–H1···Ow [2.592(3) Å] and Ow–H2w···O3 [2.767(2) Å]. On the other hand, adjacent molecules are held together by pairs of N1–H2···O1 [3.083(2) Å] and N1–H3···O3 [2.733(3) Å] hydrogen bonds related by a centre of symmetry in a centrosymmetric cyclic dimer. All dimers in the crystal are connected to each other *via* Ow–H1w···O2 [2.749(2) Å] and N1–H4···O2 [2.760(2) Å] hydrogen bonds along a crystallographic axis. Consequently, compound **1** forms a channel-like structure in which water molecules reside, surrounded by phosphonic groups, separated by a network of indane rings (Fig. 3). The complex hydrogen bonding pattern in **2** (Fig. 4), in which each water molecule participates in two, three and four hydrogen bonds to as many different acid molecules, provides five distinct types of intermolecular hydrogen bonds (Table 3).

## NMR spectroscopy

AIP was studied in acidic and basic solutions and at variable temperatures. The geometry was analysed based on coupling constants and chemical shift as has previously been described.[25] AIP is a conformationally restricted isostere of phenylalanine[8] but the cyclopentane ring can, in principle, adopt two different conformers with the phosphonic group in either equatorial or pseudo-axial positions. Since phenylalanine ammonia-lyase is highly stereoselective,[7] it is likely that only one conformer fits into the active site of the enzyme. Thus, the stability of a particular conformer as well as the interconversion barrier between conformers is expected to be important.

Significant differences can be observed in the coupling constants ($^3J_{HP}$) between the phosphorus atom and the two diastereotopic methylene protons ($H_A$ and $H_B$), indicating a difference in the HCCP dihedral. For the two conformers with the phosphonic group either axial or equatorial, the calculated dihedral angles and predicted coupling constants are collected in Table 4.[25]

Analysis of the NMR coupling constant data leads to the conclusion that, in solution, the two conformers of AIP are in fast equilibrium, which significantly shifts toward the EC conformer in basic media, in accordance with literature data.[26] At pD 8.93 **1** exists mostly in the EC conformation ($^3J_{PHA} = 9.3$, $^3J_{PHB} = 1.0$ Hz) while at pD 1.03 the equilibrium is slightly shifted to the AC one ($^3J_{PHA} = 12.5$, $^3J_{PHB} = 4.2$ Hz). The last value is considerably different from that predicted for AC ($^3J_{PHB} = 15$–$17$ Hz, Table 4), but it should be noted that a 20° change in $PCCH_B$ results in a reduction of $^3J_{PHB}$ to 5 Hz. In acidic media the amine group is protonated, so it is hydrophilic and hence hydrated. Thus, it prefers to occupy the equatorial position, which has more space in comparison with the axial one. The reverse holds for basic media.

Raising the temperature does not change the shape of the spectra dramatically, with the exception of a sharpening of multiplets. Also, since we did not observe any doubling of the signals, it seems likely that the 2-aminoindane-2-phosphonic acid exist predominantly in one conformation state (EC) in all studied media.

## IR spectroscopy

Band assignments in 2-aminoindane-2-phosphonic acid monohydrate (**1**) were achieved by spectral comparison with similar

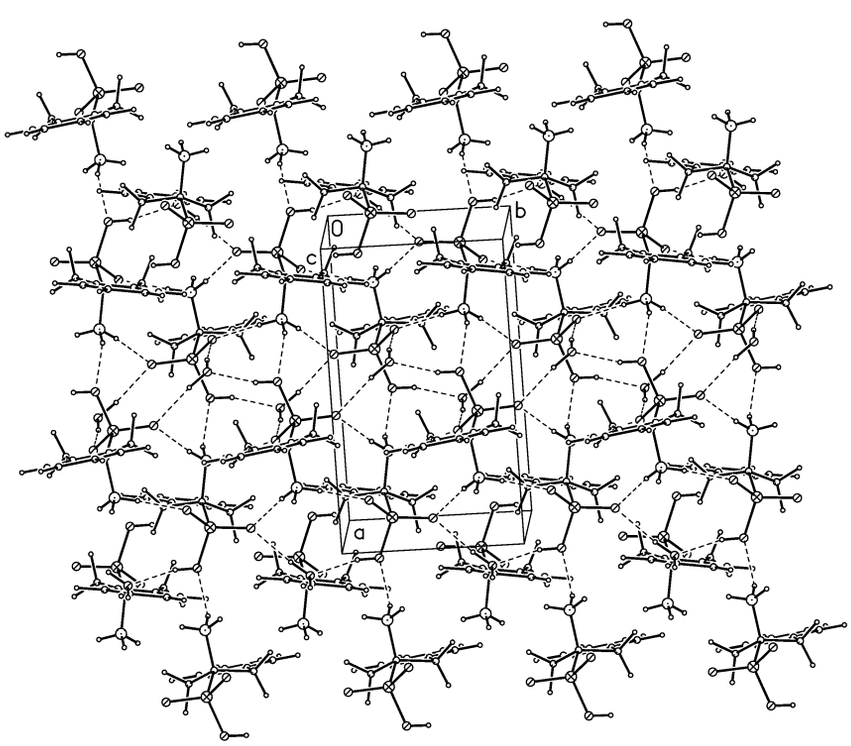


**Fig. 3** Crystal packing arrangement of compound **1**. Hydrogen bonds are shown as dashed lines.

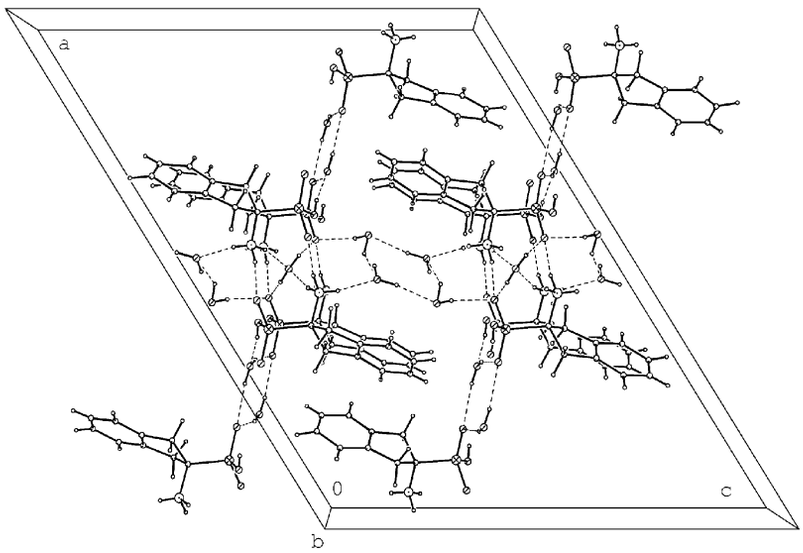


**Fig. 4** Crystal packing arrangement of compound **2**. Hydrogen bonds are shown as dashed lines.



compounds[27–29] and the deuterated[28] compound (**1D$_4$**). The data are tabulated in the ESI. Both the zwitterionic character of the functional groups (1640, 1620 and 1535 cm$^{-1}$) and the presence of the water molecule involved in strong hydrogen bonding (3259, 3169, and 2739 cm$^{-1}$) in the crystal of compound **1** are evident.

## Potentiometric titration

p$K_{1-3}$ values for 2-aminoindane-2-phosphonic acid monohydrate (**1**) were calculated from the dissociation constants and they are 2.50, 5.62 and 9.37 (internal confidence ±0.04). p$K_2$ and p$K_3$ are similar to that reported for 1-aminoalkylphosphonic acids, whereas p$K_1$ is higher (usually 1.50).[30]

**Table 3** Hydrogen bond parameters for crystals **1** and **2**

| D–H···A | D–H/Å | H···A/Å | D···A/Å | D–H···A/° |
|---|---|---|---|---|
| Compound **1**[a] | | | | |
| O1–H1···Ow | 0.80(5) | 1.81(5) | 2.592(3) | 166(4) |
| Ow–H1w···O2$^{i}$ | 0.86(4) | 1.91(4) | 2.749(2) | 166(3) |
| Ow–H2w···O3$^{ii}$ | 0.83(4) | 1.94(4) | 2.767(2) | 170(4) |
| N1–H2···O1$^{iii}$ | 0.89(3) | 2.25(3) | 3.083(2) | 157(2) |
| N1–H3···O3$^{iv}$ | 0.89(3) | 1.85(3) | 2.733(3) | 172(3) |
| N1–H4···O2$^{v}$ | 0.86(3) | 1.90(3) | 2.760(2) | 173(3) |
| C3–H31···O3$^{v}$ | 0.98(2) | 2.63(3) | 3.610(3) | 172(2) |
| C3–H32···O1 | 1.00(2) | 2.65(3) | 3.146(3) | 111(2) |
| Compound **2**[b] | | | | |
| O1–H1···O1w | 0.76(3) | 1.80(3) | 2.545(3) | 169(3) |
| N1–H2···O4w$^{i}$ | 1.03(3) | 1.88(3) | 2.879(3) | 164(2) |
| N1–H3···O2$^{ii}$ | 0.95(3) | 1.89(3) | 2.827(2) | 170(3) |
| N1–H4···O3w$^{iii}$ | 0.85(3) | 1.90(3) | 2.741(3) | 170(3) |
| O1w–H13···O3$^{iv}$ | 0.77(4) | 1.99(4) | 2.738(3) | 163(4) |
| O1w–H14···O3$^{i}$ | 0.83(3) | 1.91(3) | 2.715(3) | 164(3) |
| O2w–H15···O3w | 0.94(5) | 2.07(5) | 2.867(5) | 141(4) |
| O2w–H16···O2 | 0.90(4) | 2.03(5) | 2.852(3) | 156(4) |
| O3w–H18···O2w$^{v}$ | 0.90(5) | 1.97(5) | 2.834(6) | 163(4) |
| O4w–H19···O2 | 0.87(3) | 1.98(3) | 2.789(2) | 154(3) |

[a] Symmetry codes for **1**: (i) $x, y+1, z$; (ii) $-x+1, -y+1, -z+1$; (iii) $x-0.5, -y+0.5, z-0.5$; (iv) $-x+0.5, y-0.5, -z+0.5$; (v) $-x+0.5, y+0.5, -z+0.5$. [b] Symmetry codes for **2**: (i) $x-1, y, z$; (ii) $-x+1, y, -z+0.5$; (iii) $-x+1, y-1, -z+0.5$; (iv) $-x+0.5, -y+0.5, -z$; (v) $-x+1, -y+2, -z$.

## Molecular modelling

**Gas phase calculations.** We calculated the energy of the AC and EC conformations and barriers between them. In addition, AIP can exist in six protonation states (Table 5). The state (a) requires relatively strong acidic conditions, whereas the state (f) occurs under relatively basic conditions. For each structure, total energies and geometrical parameters (see ESI) were calculated. Geometries of the AC-d, TS-d and EC-d structures of AIP are shown in Fig. 5.

At the HF/6-31G(d) level we were able to find all the structures of AIP, expect AC-e and AC-f, which converge during optimisation to EC-d, EC-e or EC-f. In all cases, EC was lower in energy than AC with a relatively low activation barrier (Table 5). It is possible that, after including zero-point energy (ZPE) and thermal corrections to the energy, the higher minimum vanishes completely. It seems that the lower minimum is flat and the structure of the cyclopentane ring in AIP is flexible, because the imaginary frequency calculated for the transition state is relatively small [−47.35 cm$^{-1}$ in (a), −47.79 cm$^{-1}$ in (b), −43.86 cm$^{-1}$ in (c) and −40.70 cm$^{-1}$ in (d)]. In this way AIP can exist in both conformations. Table 6 summarises the results of the calculations performed at HF and for comparison at higher levels of theory (DFT/B3LYP, MP2) with the 6-31G(d) basis set.

In the DFT calculations we could not locate the minima for most of the zwitterionic structures, even when a finer grid was used. These structures usually converge in the gas phase to the appropriate neutral state, but can be found again at the MP2 level using the same 6-31G(d) basis set. If diffuse functions are used [6-311++G(d,p) basis set] at the MP2 level, the structure converges to the neutral state, as in the case of the DFT optimisation. For the AC forms the Hartree–Fock results are overestimated and for the TS are similar (TS-c) or slightly underestimated (TS-b, TS-d) compared to the MP2 level. DFT underestimates all these values (Table 6).

**Table 4** Some calculated and predicted NMR data for 2-aminoindane-2-phosphonic acid.

| | Calculated dihedral angle/° | | Predicted coupling constants/Hz | |
|---|---|---|---|---|
| Conformer | PCCH$_A$ | PCCH$_B$ | $^3J_{PHA}$ | $^3J_{PHB}$ |
| AC | −19 to −26 | −139 to −140 | 14 to 17 | 15 to 17 |
| EC | 35 to 45 | 72 to 84 | 10 to 11 | 1 to 3 |

**Table 5** Energies and barriers for different conformations and protonation states of 2-aminoindane-2-phosphonic acid

| | Total energy/Hartrees [rel. energy/kcal mol$^{-1}$] | | |
|---|---|---|---|
| Protonation state | Axial (AC)[a] | Transition state (TS) | Equatorial (EC)[b] |
| (a) $NH_3^+$, $PO_3H_2$ | −968.009401916 [4.41] | −968.008694564 [4.86] | −968.016432115 [0.00] |
| (b) $NH_2$, $PO_3H_2$ | −967.641380942 [2.70] | −967.640345708 [3.35] | −967.645688475 [0.00] |
| (c) $NH_3^+$, $PO_3H^-$ | −967.615067714 [4.24] | −967.614391503 [4.66] | −967.621824495 [0.00] |
| (d) $NH_2$, $PO_3H^-$ | −967.096475868 [1.72] | −967.096039564 [2.00] | −967.099222029 [0.00] |
| (e) $NH_3^+$, $PO_3^{-2}$ | _[c] | _[c] | −967.036525123 |
| (f) $NH_2$, $PO_3^{-2}$ | _[c] | _[c] | −966.359549058 |

[a] Phosphonic group in the axial position. [b] Phosphonic group in the equatorial position. [c] Not found.

**Binding model.** Gas phase calculations and NMR studies showed that AIP can exist in two conformers and several protonation states. Docking experiments were done for each possible conformation and all protonation states. All structures of AIP except AC-e and AC-f were prepared for docking by optimisation at the HF/6-311G(d) level. The same method was used for calculation of the CHELPG charges on atoms used in the docking. All data are presented in Table 7.

Structures AC-e and AC-f do not exist in the gas phase, but can still exist in the enzyme active site. Therefore, in order to dock these forms we have done only single-point HF/6-311G(d) calculations (without optimisation) of a molecule created by deleting hydrogen atoms in the AC-c structure. The AC-e structure was created by deletion of the hydrogen atom in the phosphonic group only and the AC-f structure was created by deleting one hydrogen atom in the phosphonic group and one hydrogen atom in the amino group.

In our docking calculations only the AC conformer of AIP, for each protonation state (a–f), binds in proximity to the MIO group and Arg354 in the PAL active site model (like AC-d on Fig. 6). EC structures bind in various places (like EC-d on

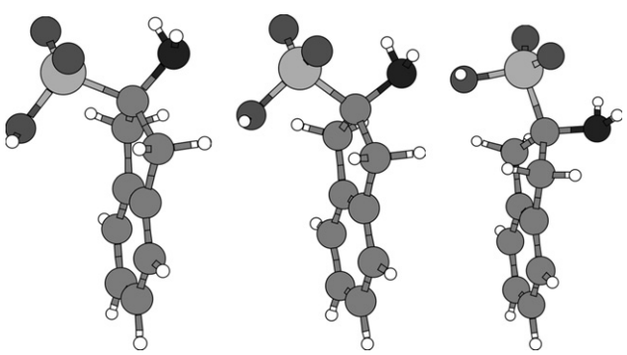

**Fig. 5** Geometries of AC-d (left), TS-d (middle) and EC-d (right) of 2-aminoindane-2-phosphonic acid calculated at the HF/6-31G(d) level.

**Table 6** Relative energies of selected forms 2-aminoindane-2-phosphonic acid compared on different theory levels

| | Relative energy/kcal mol$^{-1}$ | | |
|---|---|---|---|
| Protonation state | Axial (AC) | Transition state (TS) | Equatorial (EC) |
| (b) $NH_2$, $PO_3H_2$ | | | |
| HF | 2.70 | 3.35 | 0.00 |
| DFT/B3LYP | 2.16 | 3.12 | 0.00 |
| MP2 | 2.32 | 4.57 | 0.00 |
| (c) $NH_3^+$, $PO_3H^-$ | | | |
| HF | 4.24 | 4.66 | 0.00 |
| DFT/B3LYP | _[a] | _[a] | _[a] |
| MP2 | 2.22 | 4.62 | 0.00 |
| (d) $NH_2$, $PO_3H^-$ | | | |
| HF | 1.72 | 2.00 | 0.00 |
| DFT/B3LYP | 0.87 | 1.46 | 0.00 |
| MP2 | 1.26 | 2.62 | 0.00 |

[a] Not found.

**Table 7** Binding energies estimated by AutoDock (in kcal mol$^{-1}$).

| Structure | Number of clusters | Size of the largest cluster | Lowest energy | Lowest energy of the biggest cluster | Minimum energy of the biggest cluster |
|---|---|---|---|---|---|
| AC-a | 1 | 50 | −14.40 | −14.40 | −14.37 |
| EC-a | 10 | 23 | −9.27 | −9.27 | −9.19 |
| AC-b | 2 | 49 | −9.41 | −9.41 | −9.33 |
| EC-b | 10 | 20 | −9.51 | −9.51 | −9.29 |
| AC-c | 4 | 46 | −9.45 | −9.45 | −9.31 |
| EC-c | 19 | 8 | −9.79 | −9.09 | −9.05 |
| AC-d | 2 | 47 | −4.88 | −4.88 | −4.75 |
| EC-d | 12 | 13 | −4.18 | −4.12 | −3.98 |
| AC-e | 3 | 48 | −5.12 | −5.12 | −5.04 |
| EC-e | 4 | 32 | −3.80 | −3.63 | −3.62 |
| AC-f | 1 | 50 | −0.57 | −0.57 | −0.54 |
| EC-f | 6 | 17 | +0.30 | +0.69 | +0.71 |

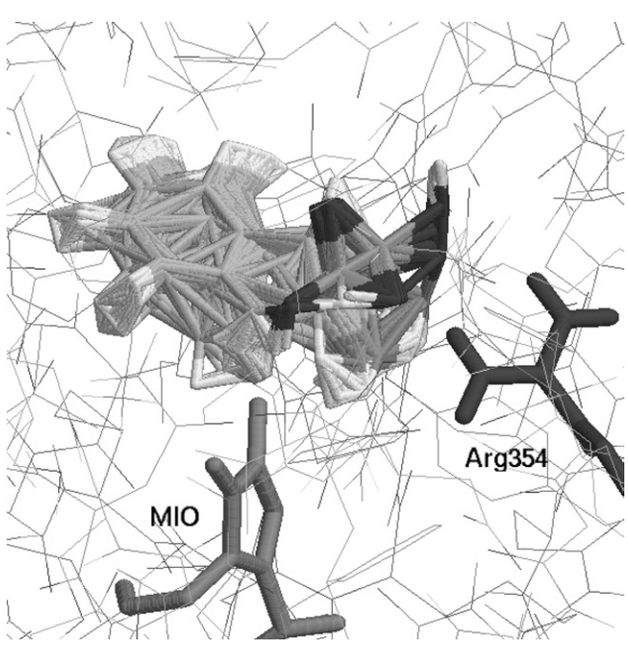


**Fig. 6** Fifty axial conformations of 2-aminoindane-2-phosphonic acid (AC-d) docked in the PAL model.

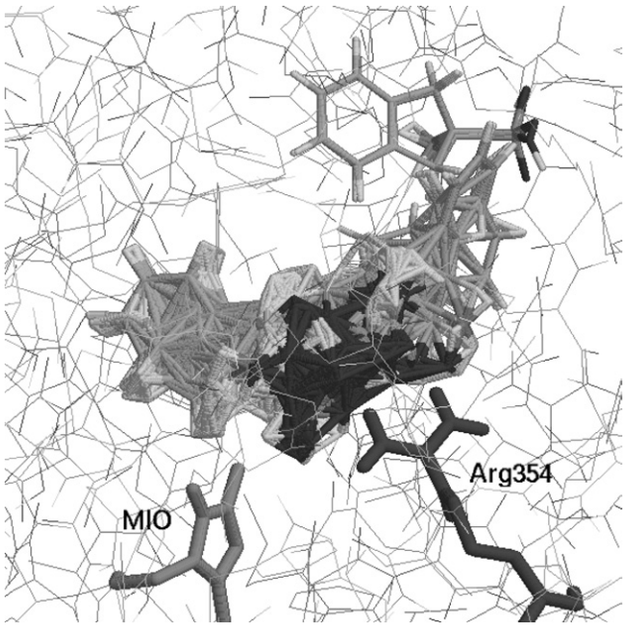


**Fig. 7** Fifty equatorial conformations of 2-aminoindane-2-phosphonic acid (EC-d) docked in the PAL model.

Fig. 7), often outside of the active site of the PAL model. In contrast to the AC forms, the calculated structures of AIP having equatorial phosphonic group (EC) do not form narrow clusters after multiple docking trials (compare AC-a–f *versus* EC-a–f in Fig. 8). This suggests that the AC conformer of AIP is preferred to the EC one in binding to the active site model of PAL (Fig. 8 AC-a–f and Fig. 6 *vs.* Fig. 8 EC-a–f and Fig. 7). It is also worth noting that the protonation state of AIP seems to have an impact only on the value of the scoring function, which estimates the enthalpy of binding (the strength of binding), and not on the specificity of binding.

## Conclusions

2-Aminoindane-2-phosphonic acid (**1**) in the solid, solution and gas states exists in the conformation with an equatorial phosphonic group and consequently with an axial amino group. Depending on the crystallisation conditions, 2-aminoindane-2-phosphonic acid contains one or more molecules of water. The calculated barrier between the two conformers of 2-aminoindane-2-phosphonic acid is low in the gas phase (2–5 kcal $mol^{-1}$, depending on the protonation state). Docking analyses indicate that only 2-aminoindane-2-phosphonic acid with the phosphonic group in the axial position, and hence with the equatorial amino group, binds more specifically to the model of the active site of phenylalanine ammonia-lyase.

The presented data are in accordance with our earlier results[10] stating that the inhibition of phenylalanine ammonia-lyase by 2-aminoindane-2-phosphonic acid is time-dependent. Such inhibition, as postulated by Schloss,[31] can involve reversible alteration of the enzyme or inhibitor. Data presented in this paper confirm this suggestions. However, we think that the largest influence is due to the change of conformation of the inhibitor when passing from solution to the active site of phenylalanine ammonia-lyase. This last conclusion is based on the fact that the non-cyclic (*R*)-1-amino-2-(4′-fluorophenyl)ethylphosphonic acid[10] does not inhibit phenylalanine ammonia-lyase in a time-dependent manner. We do not exclude, however, that observed inhibition of phenylalanine ammonia-lyase by 2-aminoindane-2-phosphonic acid[10] is due to additional changes of protonation or hydration states of the enzyme or the inhibitor.

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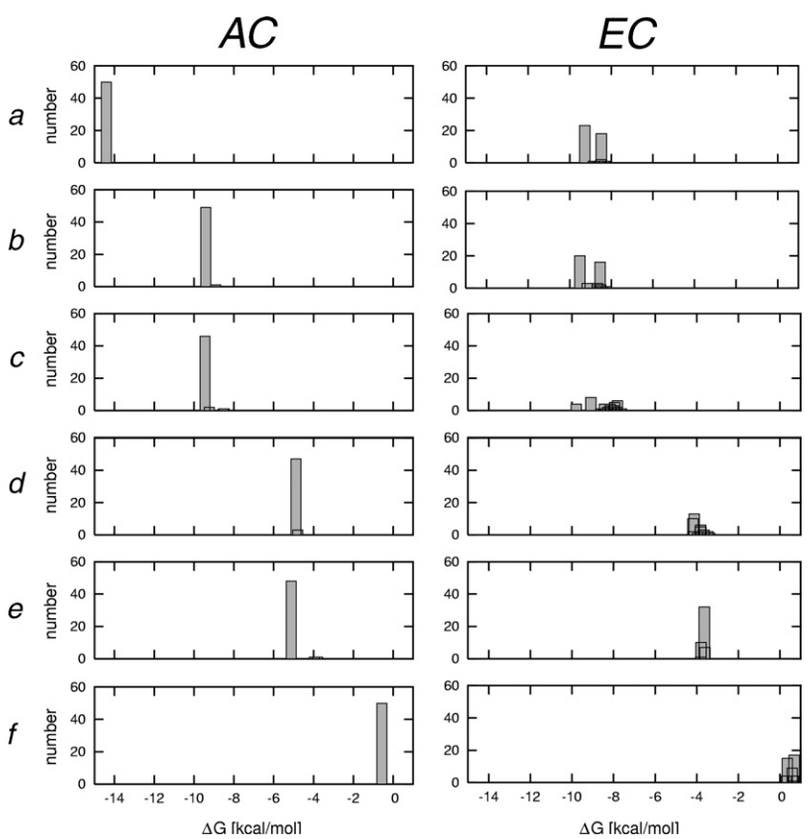


**Fig. 8** Number and size of clusters from the docking procedure of 2-aminoindane-2-phosphonic acid in the PAL model.

site homology model of phenylalanine ammonia-lyase. Calculations have been carried out in the Wrocław (WCSS), Poznań (PSC) and Warsaw (ICM) Supercomputer Centers. This work was supported in part by grants from the Center of Biomonitoring, Biotechnology and Preservation of Ecosystems of Lower Silesia (Wrocław, Poland) and Wrocław University of Technology (Wrocław, Poland).

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