C_4 plants has been reported[8,16-18,21,22]. These differences between C_3 and C_4 plants were mainly attributed to preferential utilization of ^{12}C and partial exclusion of ^{13}C during the initial carboxylation step in photosynthesis of these plants[25,26]. Less discrimination against $^{13}CO_2$ by PEP carboxylase than by RuBP carboxylase is believed to result in the differences in $\delta^{13}C$ values observed between C_3 and C_4 plants[25]. Since PEP carboxylase performs the initial carboxylation step of photosynthesis in C_4 plants, the $\delta^{13}C$ values reported for these plants are far less negative than those of typical C_3 plants which exhibit initial carboxylation step by RuBP carboxylase.

Earlier studies have shown that the PEP carboxylase activity in leaf extracts of *A. ficoides*[13] and *P. hysterophorus*[14,27] was slightly higher than in typical C_3 plants. C_3-like $\delta^{13}C$ values of the two recently identified C_3–C_4 intermediate species, *A. ficoides* and *P. hysterophorus*, reported here have clearly indicated that slightly higher levels of PEP carboxylase reported earlier may contribute less in reducing photorespiration as well as overall growth in these intermediate species. Further, C_3-like $\delta^{13}C$ values obtained for *A. ficoides* and *P. hysterophorus* in the present study clearly indicate that the contribution of C_4 type of photosynthetic carbon assimilation in reducing photorespiratory CO_2 loss is very unlikely in these C_3–C_4 intermediate species.

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Crystal structure of putrescine aspartic acid complex

S. Ramaswamy and M. R. N. Murthy
Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

Polyamines, putrescine, spermidine and spermine are ubiquitous biogenic cations believed to be important for a variety of cellular processes. In order to obtain structural information on the interaction of these amines with other biomolecules, the structure of a complex of putrescine with aspartic acid was determined using single crystal X-ray diffraction methods. The crystals belong to monoclinic space group C2 with $a=21.504$ Å, $b=4.779$ Å, $c=8.350$ Å and $\beta=97.63°$. The structure was refined to an R factor of 8.4% for 664 reflections. The asymmetric unit contains one aspartic acid and half putrescine molecule. The conformation of aspartic acid corresponds to its most favourable extended structure. The putrescine molecule, although on a 2-fold special position, lacks 2-fold symmetry. The putrescine backbone has a *trans-gauche* conformation. The energy required for distorting the putrescine molecule from its most favourable zigzag structure is presumably derived from both hydrogen bonding and electrostatic interactions.

PUTRESCINE, spermine and spermidine are ubiquitous biogenic amines believed to be important for a variety of cellular functions. It has been suggested that they are important for the structural integrity of certain biomolecular assemblies[1,2], protein synthesis[3], cell differentiation[4], flowering in plants[5] and many other biochemical phenomena. Spermidine is known to enhance the efficiency of *in vitro* translation[6]. Usually, inorganic cations either fail to fulfill the role of polyamines, or, are required at much higher concentrations[1]. In order to understand the functional role of polyamines, it is necessary to determine the structure of a large number of complexes of these amines with other ubiquitous biomolecules. These studies will provide information on the conformational flexibility of the polyamine backbone and on their preferred bonding patterns. We have earlier reported the structure of

putrescine glutamic acid[7] complex where the hydrogen bonding requirements of putrescine are satisfied by changes in the conformations of the two crystallographically independent glutamic acid molecules. Here, we present the structure of 1:2 complex of putrescine with aspartic acid.

Crystals of 2:1 complex of L-aspartic acid and putrescine were obtained from an aqueous solution by slow diffusion of propanol. The needle-shaped crystals were very hygroscopic and dissolved when exposed to air. For X-ray examination, the crystals were mounted in a glass capillary. The excess solvent was removed using a thin filter paper and the capillary was sealed with low melting wax. X-ray examination revealed that the crystals belong to monoclinic space group C2 with $a=21.504$ Å, $b=4.779$ Å, $c=8.350$ Å and $\beta=97.63°$. The unit cell volume was found to be compatible with 2 putrescine and 4 aspartic acid molecules for which the calculated density was 1.38 g cm^{-3}.

X-ray diffraction data were collected on an Enraf–Nonius 4 circle diffractometer to a Bragg angle of 65° using $w/2\theta$ scan. A microfocus sealed tube with a copper anode ($\lambda=1.5418$ Å) provided the X-ray beam. A total of 1440 reflections from the $K\geqslant 0$ hemisphere were collected. The reflections were corrected for Lorentz and polarization factors and the symmetry-related observations were averaged to yield 816 unique measurements. During the data collection the crystals did not exhibit significant radiation damage as revealed by periodically monitored standard reflections.

The structure was solved by using the direct methods program MULTAN[8] and refined using a block diagonal structure factor least squares program to an R value of 8.4% for 664 reflections with $F_0\geqslant 4\sigma(F_0)$. During the initial refinement with C, N and O atoms, the central carbon atoms of putrescine were found to have unusually high-temperature factors. Examination of the difference fourier after omitting these atoms revealed a density that was spread considerably along the b direction in comparison with a or c. Also, when these atoms were refined anisotropically, the B_{22} factors were about 5 times larger than B_{11} or B_{33}. This suggested that these atoms were either disordered or occupied two alternative positions separated along the b-axis with partial occupancy. However, dynamic disorder of these central atoms of putrescine is unlikely in view of the fact that the terminal atoms are well ordered. Hence two possible positions separated by about 0.8 Å within the unresolved streak of electron density in the difference fourier map were selected as partially occupied positions for the carbon atom. The occupancy, position and temperature parameters were refined and were found to behave normally. The independently refined occupancies of the two positions were equal and added to unity. The hydrogen atoms of the aspartic acid and amino groups of putrescine could be located in a difference fourier map. These hydrogen positions were refined isotropically. The other hydrogens were not included in the refinement. The inability to locate these hydrogen atoms is partially due to the fractional occupancy of the putrescine central carbon atoms and also probably due to somewhat poorer quality of the intensity data.

The final positional parameters and equivalent temperature factors are given in Table 1. Tables 2–5 give the bond-lengths and bond-angles of putrescine and aspartic acid in the structure. Table 6 gives a comparison of the conformational angles of putrescine in the present case with those of putrescine chloride[9]

Table 1. Positional parameters (× 10000) and equivalent temperature factors of non-hydrogen atoms. The estimated standard deviations (for parameters refined) are given in parentheses.

Atom	X	Y	Z	Equivalent B
N1	2523(3)	4059	2534(8)	2.8(2)
O1	1205(3)	5301(18)	−464(7)	3.9(2)
O2	2030(3)	8006(16)	380(7)	3.3(2)
C1	1693(4)	5982(24)	509(9)	2.9(2)
C2	1838(4)	4064(23)	2010(9)	2.7(2)
C3	1488(4)	5332(23)	3356(9)	2.9(2)
C4	1561(4)	3499(24)	4864(10)	3.1(3)
O5	2037(3)	3966(17)	5881(7)	3.8(2)
O6	1166(3)	1720(21)	4964(7)	5.1(2)
N11	−844(3)	363(22)	2062(8)	3.5(2)
C12	−153(5)	267(36)	2272(12)	5.1(4)
1C13	124(9)	1055(62)	835(21)	4.2(7)
2C13	125(9)	−595(65)	767(23)	4.3(7)

Table 2. Bond lengths (in Å) of putrescine.

N11–C12	1.47(1)
C12–1C13	1.46(2)
C12–2C13	1.52(2)
1C13–1C13′	1.43(2)
2C13–2C13′	1.32(2)
1C13–2C13	0.79(4)
IC13–2C13′	1.58(3)
2C13–1C13′	1.58(3)

Table 3. Bond angles (in degrees) of putrescine.

N11–C12–1C13	114.2(1.2)
N11–C12–2C13	114.3(1.2)
C12–1C13–1C13′	131.5(1.9)
C12–2C13–2C13′	130.3(2.0)
C12–1C13–2C13′	115.8(1.7)
C12–2C13–1C13′	115.9(1.7)

Table 4. Bond lengths of (in Å) aspartic acid.

N1–C2	1.48(1)
O1–C1	1.28(1)
O2–C1	1.23(1)
C1–C2	1.55(1)
C2–C3	1.56(1)
C3–C4	1.53(1)
C4–O5	1.26(1)
C4–O6	1.21(1)

Table 5. Bond angles (in degrees) of aspartic acid.

O1–C1–O2	126.3(0.8)
O1–C1–C2	114.9(0.8)
O2–C1–C2	118.7(0.8)
N1–C2–C1	109.0(0.7)
N1–C2–C3	110.2(0.7)
C1–C2–C3	106.8(0.7)
C2–C3–C4	111.5(0.8)
C3–C4–O5	116.1(0.8)
C3–C4–O6	117.6(0.8)
O5–C4–O6	126.3(0.9)

Table 6. Torsion angles of putrescine in different forms.

Angle	Putrescine aspartate	Putrescine chloride	Putrescine phosphate
N11–C12–1C13–2C13′	50.7	180	60.9
C12–1C13–2C13′–C12′	178.7	180	180.0
1C13–2C13′–C12′–N11′	−53.7	180	60.0

and putrescine phosphate[10]. Table 7 gives a similar comparison of aspartic acid with free aspartic acid[11] and histidine aspartic acid complex[12]. The crystal packing as it appears when viewed along the b-axis is shown in Figure 1.

The asymmetric unit of the crystal consists of one aspartic acid and half putrescine molecule. In contrast, the asymmetric unit in the crystal structure of putrescine–glutamic acid, consists of one putrescine and 2 glutamic acids in slightly different conformations.

The charged carboxylates of aspartate are most separated when the side chain carboxyl group is *trans* to the main chain carboxyl. This conformation appears to be the most stable as it is observed in all aspartate structures except in its complex with histidine and metal ions. The stability of the *trans* conformation arises both due to electrostatic repulsion between the carboxyl anions and steric factors. The conformation observed in the present case agrees with that of the most favourable *trans* geometry. The orientation of the terminal carboxyl groups is found to vary in different crystal structures. Presumably, the carboxyl oxygens orient so as to maximize hydrogen bonding interactions. In the present structure, one of the oxygens of each carboxyl group accepts two hydrogen bonds while the other oxygen forms only one bond. The asymmetry in hydrogen bonding leads to slight but significant

Table 7. Torsion angles of aspartic acid in different forms.

Angle	Putrescine aspartate	Aspartic acid	Histidine aspartate
N1–C2–C1–O1	−2.4	−37.7	−9.7
N1–C2–C1–O2	174.3	144.8	166.5
N1–C2–C3–C4	−75.2	−61.9	52.8
C2–C3–C4–O5	−38.5	131.4	1.8
C2–C3–C4–O6	142.1	−51.3	−173.2
C1–C2–C3–C4	176.2	178.2	−72.8
O1–C1–C2–C3	−90.0	83.2	117.2
O2–C1–C2–C3	87.7	−94.2	−66.5

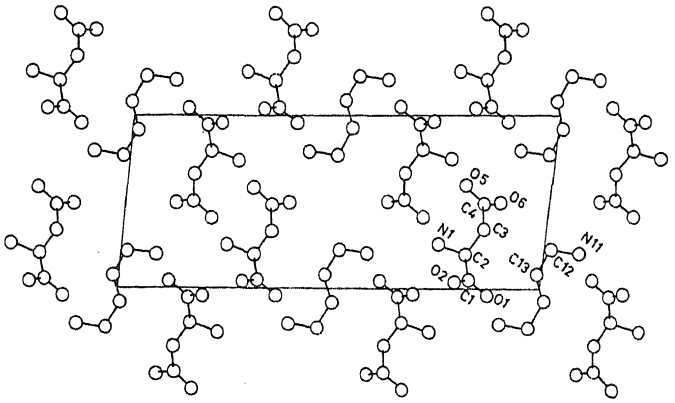

Figure 1. Packing diagram of putrescine–aspartic acid complex as viewed down the b-axis.

increase (0.05 Å) in the bond length for the oxygen involved in 2 hydrogen bonds.

The crystal asymmetric unit consists of half putrescine molecule. Hence, the molecule is situated on the 2-fold special position in the crystal structure. Two positions in the asymmetric unit designated as 1C13 and 2C13 were assigned to this central carbon atom of putrescine. The positional, temperature and occupancy parameters associated with these positions were refined independently. The occupancy converged to 0.5. These positions are separated by 0.79 Å along the b-axis. Interestingly, the bonding pattern N11–C12–1C13–1C13′–C12′–N11′ for which the molecule will have exact 2-fold symmetry is not compatible with the observed ranges of bond lengths and angles of putrescine backbone. The distance 1C13–1C13′ is only 1.32 Å and the angle C12–1C13–1C13′ is 130.3°. In contrast, the structure of the molecule N11–C12–1C13–2C13′–C12′–N11′ has bond-lengths and angles much closer to normal values (Tables 2–4). The backbone of putrescine has *trans* and *gauche* conformation for the bonding pattern N11–C12–1C13–2C13′–C12′–N11′. In contrast, the other symmetrical alternative N11–C12–1C13–1C13′–C12′–N11′ has a *syn* C12–1C13–1C13′–C12′ angle which is energetically much less favourable. Thus, the conformation of the putrescine molecule lacks a centre of symmetry leading to the unusual formation of a crystallographic 2-fold axis of symmetry due to half occupancy of two unsymmetrical structures. The most favourable structure for putrescine is the fully extended zigzag *trans* configuration. In the zigzag structure, the positively charged amino groups are maximally separated and the torsion angles lead to least steric hindrance. However, here as well as in putrescine phosphate, the molecule adopts a *trans-gauche* conformation. The energy required for this distortion is provided by the hydrogen bonding and electrostatic interactions.

Examination of the crystal packing diagram shows that the crystal structure derives its stability from electrostatic, hydrogen bonding and nonpolar vander-

Waals interactions. Each of the positively charged amino groups of putrescine is surrounded by carboxyl groups from both the main and side chain of aspartic acid molecules. Within a radius of 5 Å from the amino group, a total of 10 anionic groups and 3 cationic groups are found. Similarly, the side chain carboxyl groups of aspartate are surrounded by 6 amino groups within a radius of 5 Å. This pattern of charge clustering is due to the electrostatic component of complex formation. The nonpolar atoms of the aspartic acid side chain and putrescine backbone form a layer of the structure separated by polar layers formed by carboxylates and amino groups. Putrescine amino groups form all possible hydrogen bonds in both of its complexes with aspartic and glutamic acids, whereas the carboxylates form only part of the possible hydrogen bonds. This is apparently due to the inherent flexibility in the backbone of polyamines which allows them to adopt structures suitable for optimizing stabilizing interactions with other biomolecules. These hydrogen bonds, therefore, must form part of any structural model describing polyamine interactions.

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A simple method for derivatization of a complex organic compound for biological purposes

A. Bhattacharyya
Institute of Microbial Technology, Sector 39-A, Chandigarh 160 036, India

Derivatization of an inherently complex organic compound for biological purposes often faces serious problems from the viewpoint of physical and chemical nature of the compound. The present study takes a complex molecule rifamycin B, which is in itself complicated by the presence of several functional groups, its chemical lability and extremely low solubility. The C_{21}/C_{23} functionalization has been achieved leaving aside the aromatic region. Of the two varieties of derivatives prepared, the citrate has been found to be soluble in aqueous medium, and hence more suitable for biological purposes. Immunization is also reported. This simple methodology of preparation of the water soluble citrate derivative should find general application in biochemistry provided the organic compound under consideration contains an NH_2 or OH group.

For various purposes like affinity design, antibody production, drug delivery, etc. it is often necessary to derivatize an organic compound under mild condition at a suitable position[1-7]. Keeping in view the complexities of many biologically active compounds it is often necessary to deal with every compound separately by complicated synthetic routes[1,3,8-10]. In addition to the chemical complexities, many compounds are insoluble in water adding further problems in chemical conversions as well as difficulties in biochemical experiments which are mostly carried out in aqueous medium. The physical problem of insolubility in water makes it extremely difficult to carry out further conjugation reaction of the derivative with macromolecules like proteins. Even in case the reaction can be carried out, the product becomes insoluble and hence unusable for biological experiments.

It is thus desirable to have a way out to tackle this multidimensional problem of physicochemical nature. Ideally, we should have a methodology that is i) simple so that it can be easily carried out in a biochemical laboratory, and ii) of general applicability which can be widely used for large number of compounds. In the present study we have chosen a sparingly soluble, chemically labile and biologically important compound rifamycin B (Ciba-Geigy, USA) which is also complicated in itself by the presence of several functional groups[11]. The compound has successfully been converted to functional derivatives at desired position, and it has been found that the derivative which is soluble in water has more potential for biological application like