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ERDEC-CR-216

COMPUTATIONAL MODELING OF THE FLUORESCENCE OF BACTERIAL SPORES

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February 1997

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U.S. Army Edgewood Research, Development and Engineering Center
Aberdeen Proving Ground, Maryland 21010-5423

ERRATUM SHEET

19 November 1997

REPORT NO.	ERDEC-CR-216
TITLE	COMPUTATIONAL MODELING OF THE FLUORESCENCE OF BACTERIAL SPORES
AUTHORS	Hendrik F. Hameka, James O. Jensen, Janet L. Jensen, Clifton N. Merrow, and Constantine P. Vlahacos
DATE	February 1997
CLASSIFICATION	UNCLASSIFIED

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE 1997 February	3. REPORT TYPE AND DATES COVERED Final, 94 Feb - 95 Aug

4. TITLE AND SUBTITLE

Computational Modeling of the Fluorescence of Bacterial Spores

5. FUNDING NUMBERS

PR-10162622A553C
PR-10161102A71A
C-DAAA15-92-D-0015

6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Pennsylvania, Philadelphia, PA 19104
DIR, ERDEC, ATTN: SCBRD-RTE, APG, MD 21010-5423
Science and Technology Corporation, Hampton, VA 23666-1340

8. PERFORMING ORGANIZATION REPORT NUMBER

ERDEC-CR-216

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

DIR, ERDEC, ATTN: SCBRD-RTE, APG, MD 21010-5423

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

COR: Dr. James O. Jensen, SCBRD-RTE, 410-671-5665

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

We interpret the fluorescence of bacterial spores by studying the fluorescence of dipicolinic acid (2,6-pyridinedicarboxylic acid) and its ion by performing calculations with the Gaussian 92 Program Package. We derived the optimized geometries, charge densities, energies, and vibrational frequencies for the ground state and first excited singlet state of dipicolinic acid and its ion by performing 6 in 6 complete active state MCSCF computations using a 6-31G basis set.

14. SUBJECT TERMS

Fluorescence
UV absorption
Dipicolinic acid

15. NUMBER OF PAGES

23

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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PREFACE

The work described in this report was authorized under Project No. 10162622A553C, CB Defense/General Investigation, Project No. 10161102A71A, Research in CW/CB Defense, and Contract No. DAAA15-92-D-0015. This work was started in February 1994 and completed in August 1995.

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1. INTRODUCTION

Dipicolinic acid (2,6-pyridinedicarboxylic acid, $C_5H_3N(COOH)_2$) is a major constituent of bacterial spores. In some cases, it constitutes up to 17% of the dry weight of the spores.[1-5] Dipicolinic acid (DPA) is also believed to be an important contributor to the resistance of spores with respect to both heat and UV radiation.[6-11] The material also appears to be important in spore stability and germination.[12-15] To some extent, the chemical structure of DPA is similar to quinolinate. It has been studied for its neurotoxicity and its neurotoxic effects on certain neurons[16-18] and as an activator and inhibitor of certain enzymes.[19-22] This acid also exhibits some additional biological functions.[23,24]

Udo[25] first discovered DPA in a biological system in 1936. The material was isolated from the viscous matter of Natto, a Japanese food made of steamed soybeans fermented with bacillus natto. The presence of DPA in all bacterial spores was reported by Powell[26] in 1953. This acid is also produced by some fungi.[27,28] Due to its inert nature and lack of reactivity, the molecule does not have much chemical significance and, therefore, did not attract much attention. However, because of its presence in bacterial spores, it has caught biologists' interests.

The DPA molecule is an important chelating agent.[29-37] This property has been used to study chiral-induced equilibrium shifts,[33-37] the so-called Pfeiffer effect.[38,39] The latter effect has been applied to probe the optical activity of dipicolinate ions chelated to various lanthanide metals. The chelated dipicolinate compounds have been made optically active by exposure to circular polarized light.

To a great extent, the detection and spectrometric analysis of bacterial spores is related to the spectrometric analysis of DPA and its ion (the ion is usually encountered as calcium dipicolinate). Therefore, the spectroscopic properties of DPA and its ion are of interest to bacteriologists and scientists interested in the properties of bacterial spores. The UV absorption spectra of various bacterial spores are all similar to the UV absorption spectrum of DPA and its ion.[40-43] The Raman spectra of spores[44] show strong evidence of calcium dipicolinate. We should also mention a combined study of the resonance Raman spectra of both bacterial spores and dipicolinate.[45] The infrared (IR) and Raman spectra of both DPA and its calcium salt were reported by Carmona in 1979.[46] We will use Carmona's data as a basis for comparison of our computed vibrational frequencies. Again, the IR spectra of DPA and its ion are in many ways similar to the corresponding spectra of bacterial spores.[47,48]

The crystal structure of calcium dipicolinate trihydrate was reported by Strahs and Dickerson in 1968.[49] Their results suggest a planar configuration of the DPA ion, a result which is consistent with the results of the computations reported herein.

We are primarily interested in the fluorescence of DPA and the dipicolinate ion. To interpret and predict the fluorescence spectra of both substances, we performed 6 in 6 complete active space multiconfiguration self consistent field (MCSCF) computations on the ground and first excited states of both molecules using the Gaussian 92 Program Package.[50] One of us, J.L. Jensen (personal communication), Research and Technology Directorate, U.S. Army Edgewood Research, Development and Engineering Center, measured the fluorescence of the acid in aqueous solution since we were unable to locate any experimental information in the literature. The experiment showed a fluorescence peak at 30,000 cm^{-1} and an excitation absorption peak around 36,000 cm^{-2}. We present a detailed description of our computations in Sections 2, 3, and 4 with the energies, geometries, and electronic structures presented in Section 2 and the vibrational frequencies and their interpretation in Sections 3 and 4.

2. GENERAL COMPUTATIONS

To predict and interpret the fluorescence of DPA and its ion, we performed 6 in 6 complete active space MCSCF (CASSCF) computations[50] on DPA and the dipicolinate ion for the ground state and the first excited singlet of each system using a 6-31G basis set. We use this particular approach because it enables us to compute the vibrational frequencies of the ground and excited states. This approach is very similar to our analysis of the fluorescence of toluene and p-cresol that we published recently.[51]

We show our numbering scheme for the atoms in the acid and the ion in the figure. This figure also represents the optimized geometries belonging to the lowest energies of the ground and lowest excited singlet states. We performed a variety of computations with different Z-matrices and starting geometries. All of the computations unambiguously predict a planar structure of the molecule and the ion in the ground and lowest excited singlet states. Recently published computations by Edgecombe, Weaver, and Smith,[52] based on the semiempirical AM1 (MOPAC) and ab initio HF/STO-3G, HF/3-21G, and HF/3-21+G methods, led to different geometry predictions for the DPA ground state.

The figure also represents the energy minimum of DPA as a function of the orientation of the COOH group in the plane of the molecule. We investigated all possible configurations, and we found that the configuration in the figure (C-O bond parallel to the molecular C-N axis and the O-H bond pointing away from the nitrogen atom) has the lowest energy in the molecular ground state. Obviously, C9-O11 and C10-O12 represent the C=O bonds. Therefore, we confined our computations to the configuration of the figure. In the case of the dipicolinate ion, there are no hydrogens in the COO- groups, and the two C-O bond lengths are smaller than the C-O and larger than the C=O bond lengths. In our optimization, we allowed the COO- group to rotate out of the molecular plane; but, we found that the optimized geometries correspond to planar configurations in all cases.

We attempt to interpret the nature of the excitation in DPA and the DPA ion from three sets of data: (a) the change in bond lengths, (b) the change in bond angles, and (c) the change in Mulliken atomic charges. We present the corresponding results of our computations in Tables 1 (bond lengths), 2 (bond angles), and 3 (atomic charges). Since we are primarily interested in a comparison between the ground and excited state data, we report more decimal places than are actually justified.

We present our computational results for the bond distances in DPA and its ion in Table 1. Strahs and Dickerson[49] report experimental data for the crystal structure of calcium dipicolinate trihydrate, and we present the experimental bond distances in the last column of the table. They should be compared with the computed bond distances of the ion's ground state. The data show that the differences between the experimental and theoretical bond distances are generally smaller than 0.02 Å. The theoretical bond angles of the four molecules are listed in Table 2 together with the experimental data[49] for the ground state of the ion. The agreement between the computed and experimental bond angles is poor. The differences are generally larger than the estimated standard deviation of 1° in the experimental bond angles.[49] However, the experimental bond angles of the carboxyl group are distorted because of the interaction with the calcium ions and the surrounding water molecules. This may explain the lack of symmetry in the experimental bond angles and the large discrepancies in the bond angles of the carboxyl groups. We present the Mulliken atomic charge densities of DPA and its ion in Table 3. There are no corresponding experimental data available.

In the first two columns of Table 1, comparison of bond distances of DPAs ground and excited states shows that the excitation is accompanied by an expanded pyridine ring. The ring diameter, corresponding to the distance N1-C2, increases by 0.1 Å, and all the other ring bond distances increase by corresponding amounts. The bond angles, presented in the first two columns of Table 2, remain practically unchanged upon excitation. This shows that the ring expands uniformly without accompanying distortions. We do not observe any significant changes in the bond distance and bond angles of the carboxyl groups. A comparison of the Mulliken atomic charge densities in the first two columns of Table 3 shows a small shift in electronic charge density from the ring to the carboxyl group in the C-C bond between the ring and the carboxyl carbon atom. We do not observe a significant rearrangement of the charge densities in either the ring or the carboxyl groups. All our theoretical data point to the same conclusion: the excitation corresponds to the excitation of an electron from a delocalized bonding orbital to a delocalized nonbonding orbital. The carboxyl groups are not involved in the excitation.

The computed bond distances in the dipicolinate ion are presented in the third and fourth columns of Table 1. The two bond distances C3-C5 and C4-C6 are increased by 0.06 Å, and the two bond distances C3-C9 and C4-C10 between the ring and the COO- groups are decreased by the same amount. In fact, the sum of the C3-C5 and C3-C9 bond distances remain almost unchanged upon excitation. The bond angles of the ground and excited states of the DPA ion are listed in the third and fourth columns of Table 2.

The largest bond angle changes are found in and around the carboxyl groups, but the changes are relatively small. The Mulliken atomic charge densities are presented in the third and fourth columns of Table 3, but our results raise some doubt about the accuracy and reliability of the data. In performing the computations, since we did not impose molecular symmetry requirements, the atomic charges for the excited state in the fourth column of Table 3 do not meet these requirements. Some of the discrepancies are significant. The charges on C9 and C10, which should be the same, are in fact 0.742 and 0.669 for the ground state and 0.721 and 0.616 for the excited state. We have repeated these computations a number of times, and we found similar discrepancies each time. Therefore, they are inherent to the program.

Our theoretical results for the DPA ion indicate that the excitation is accompanied by a change in conjugation. The increases in the C3-C5 and C4-C6 bond distances lead us to conclude that C3-C5 and C4-C6 have assumed single-bond character in the excited state and that the conjugation is now localized to the C5-C2-C6 and to the C9-C3-N1-C4-C10 regions of the molecule. This is confirmed by the shortening of the C3-C9 and C4-C10 bond distances due to conjugation. We conclude that the molecular excitation corresponds to the excitation of an electron from a delocalized orbital to an excited antibonding orbital localized in the C3-C5 and C4-C6 bonds.

The computed optimized energies of both the ground and excited states of DPA and its ion are listed in Table 4. We also present the energy of the ground state in the excited-state optimized geometry and the energy of the excited state in the ground-state optimized geometry. From the differences between these four computed energy values, we can derive the absorption peak of the excitation radiation and the peak of the fluorescence emission. We converted the latter two quantities from hartrees to cm^{-1}. We mentioned already that we had difficulty in finding experimental data in the literature. One of us, J.L. Jensen (personal communication), measured the fluorescence and excitation absorption of DPA in water solution. The experimental values are also listed in Table 4. We did not measure the fluorescence of the DPA ion.

Even though we report computational results for the vibrational frequencies of DPA and its ion in both ground and excited states, our information is not sufficient to derive accurate predictions of the effect of molecular vibrations on the excitation and fluorescence frequencies. Since this effect is not considered, the agreement between the computed and experimental excitation and fluorescence frequencies is about what we would expect, differences between 10 and 20%.

3. VIBRATIONAL FREQUENCIES OF DPA

We made use of the CASSCF procedure since this allowed us to compute the vibrational frequencies of the ground and excited states of the molecule. We present our theoretical results for DPA in Table 5. Our assignments are based on visual inspections of the detailed form of each vibrational mode. We obtained numerical values for the IR intensity of each frequency. Even though the exact numerical values of the intensities are not very accurate, we used them as guidelines to identify very strong lines (IR intensity larger than 500, three stars), strong lines (IR intensity larger than 100, two stars), and moderately strong lines (IR intensity larger than 20, one star). This classification is also helpful in matching the various IR lines with their experimental counterparts. The experimental IR and Raman spectra of DPA were reported by Carmona,[46] and we list the experimental frequencies in the third column of Table 5. We match the computed and the experimental frequencies by considering the frequency, the reported and computed intensities, and the reported and computed assignments. We list our computed frequencies of the electronic excited state in the second column of Table 5. We were unable to find corresponding experimental data.

The vibrational frequencies may be classified according to symmetry and to the functional group to which they belong. If we consider symmetry, then we differentiate between 31 in-plane vibrations, listed first, and 14 out-of-plane vibrations, listed last. Most of the vibrational modes are localized, and they may be assigned either to the carboxyl groups or to the pyridine ring.

The most intense experimental IR frequencies of the carboxyl group correspond to the O-H stretch mode around 3600 wave numbers, the C=O stretch mode around 1700 wave numbers, and the C-O stretch mode around 1140 wave numbers. In addition, we have C-O bend modes around 700 wave numbers, in-plane O-H bend modes at 1200 and 1400 wave numbers, and out-of-plane O-H bend modes around 600 wave numbers.

The pyridine ring is characterized by three C-H stretch modes around 3100 wave numbers, a number of C-C stretch frequencies between 1400 and 1600 wave numbers, a number of in-plane C-C bend modes, and a number of out-of plane C-C bend modes between 200 and 500 wave numbers. In addition, we have in-plane C-H bend modes between 1050 and 1250 wave numbers and out-of-plane C-H bend modes between 750 and 1000 wave numbers. Finally, there are a few vibrational modes (e.g., the C-COOH stretch modes around 450 wave numbers and the out-of-plane C-COOH bend modes around 750 wave numbers) involving both the ring and the carboxyl group.

We already mentioned that our computed ground state frequencies are presented in increasing order of magnitude in the first column of Table 5. The second column contains the corresponding excited state vibrational frequencies. Since they match the assignments of the ground state frequencies, they are not necessarily in increasing order. The third column contains the experimental vibrational frequencies of DPA,[46] matching the assignments of the computed ground state frequencies. In a recent publication,[51] we presented CASSCF calculations of the vibrational frequencies of toluene and p-cresol and reported a set of correction factors for the computed frequencies. We have used this same set of correction factors[51] to correct the computed DPA ground state frequencies. Those values are listed in the fourth column of Table 5. Since we did not have a correction factor for the C=O stretch frequency, we derived an empirical C=O stretch frequency correction factor by taking the average of the ratios between experimental and computed frequencies of the four C=O stretch frequencies of DPA and its ion. The result is 0.89737. We use this value to readjust our computed four C=O stretch frequencies.

The agreement between our corrected theoretical frequencies and the corresponding experimental quantities[46] is worse than in recently reported work on toluene and p-cresol,[51] and on other molecules.[52,53] It is possible that the CASSCF option does not constitute the most accurate approach to the calculation of vibrational frequencies. We performed various different frequency calculations of DPA by means of the CASSCF option with either slightly different starting points or different Z-matrices. The variations in our theoretical frequencies amounted to as much as 10 wave numbers in some cases. This led us to conclude that the CASSCF frequencies may be less accurate than those derived from other methods. However, the very large discrepancies for the O-H stretch frequencies should be attributed to environmental effects on the experimental data.

4. VIBRATIONAL FREQUENCIES OF THE DIPICOLINATE ION

The vibrational spectrum of the dipicolinate ion is of course similar to the spectrum of DPA. But, there are a few differences. The most obvious difference is that we now have two less atoms so that we obtain only 39 frequencies, 26 in-plane frequencies that we list first in Table 6, and 13 out-of-plane frequencies that we list at the bottom of this table.

We again identified the various vibrational modes and frequencies by visual inspection of the motion of the nuclei in the vibrational modes. However, we found the assignments more ambiguous than in the case of the acid. We have identified 3 C-H stretch modes and 6 C-H bend modes (3 in-plane and 3 out-of-plane). Even though the differences between C-O and C=O bonds are not as clear as the differences in the acid, we did identify two C=O and two C-O stretch modes. In addition, we identified two C-CO_2 stretch modes and four C-CO_2 bend modes (2 in-plane and 2 out-of-plane). The assignment of the five C-C ring stretch modes is also unambiguous; but, the assignment of the remaining modes is not quite as straightforward. Even identifying the in-plane and out-of-plane vibrations is not always clearcut since we make use of the nosym option in our computations. However, we have given careful consideration to the assignment of each ground state vibrational mode, and the result of our efforts is listed in Table 6; we use the same criteria as used in Table 5 to describe the intensities of the lines.

We also computed the vibrational modes of the first excited singlet state, and we made the same effort of labeling each vibrational mode. The results are listed in the second column of Table 6. It should again be noted that the order of assignment is sometimes different from the groundstate. The excited state frequencies are therefore not always listed in numerical order.

We again multiplied our computed frequencies by the correction factors that we derived in previous work,[51] supplemented by the correction factor of 0.89737 for the C=O stretch modes. The corrected frequencies are presented in the fourth and last columns of Table 6. These frequencies should be compared with the experimental frequencies[46] listed in the third column of Table 6. Again, the agreement between theory and experiment is reasonable but not as good as in other recent works.[53,54]

5. CONCLUSIONS

We hope that our theoretical results are useful for predicting and interpreting the fluorescence of dipicolinic acid (DPA) and its ion. By comparing the geometries and electron densities of the molecules in the ground and excited state, we conclude that the excitation of DPA is due to the promotion of an electron from a bonding delocalized orbital to an antibonding delocalized orbital. Similarly, the excitation of the DPA ion is due to the promotion of an electron from a bonding delocalized orbital to a localized antibonding orbital. The excitation of DPA is accompanied by a general expansion of the pyridine ring, while the excitation of the ion gives rise to a distortion of the pyridine ring accompanied by a decrease in conjugation.

We selected the 6 in 6 complete active space multiconfigurational self consistent field approach for our theoretical study because it enables us to compute the vibrational frequencies of the ground and excited states. We report the results in Table 5 for the acid and in Table 6 for the ion. The experimental frequencies for the ground state of both the acid and the ion have been reported,[46] and we also present those data in Tables 5 and 6. To evaluate the accuracy of our theoretical data, we multiply them by a set of previously derived empirical correction factors[51] and by a newly derived empirical correction factor for the C=O stretch frequency. Experimental vibrational frequencies of the excited states are not available. We do not report corrected frequencies for the excited states of the acid and the ion; however, they may easily be derived by using the same procedure that we used for the ground state.

Much of the experimental spectroscopic work on bacterial spores involves the spectra of DPA and calcium dipicolonate, and we hope that our present theoretical studies may be helpful in future spore studies.

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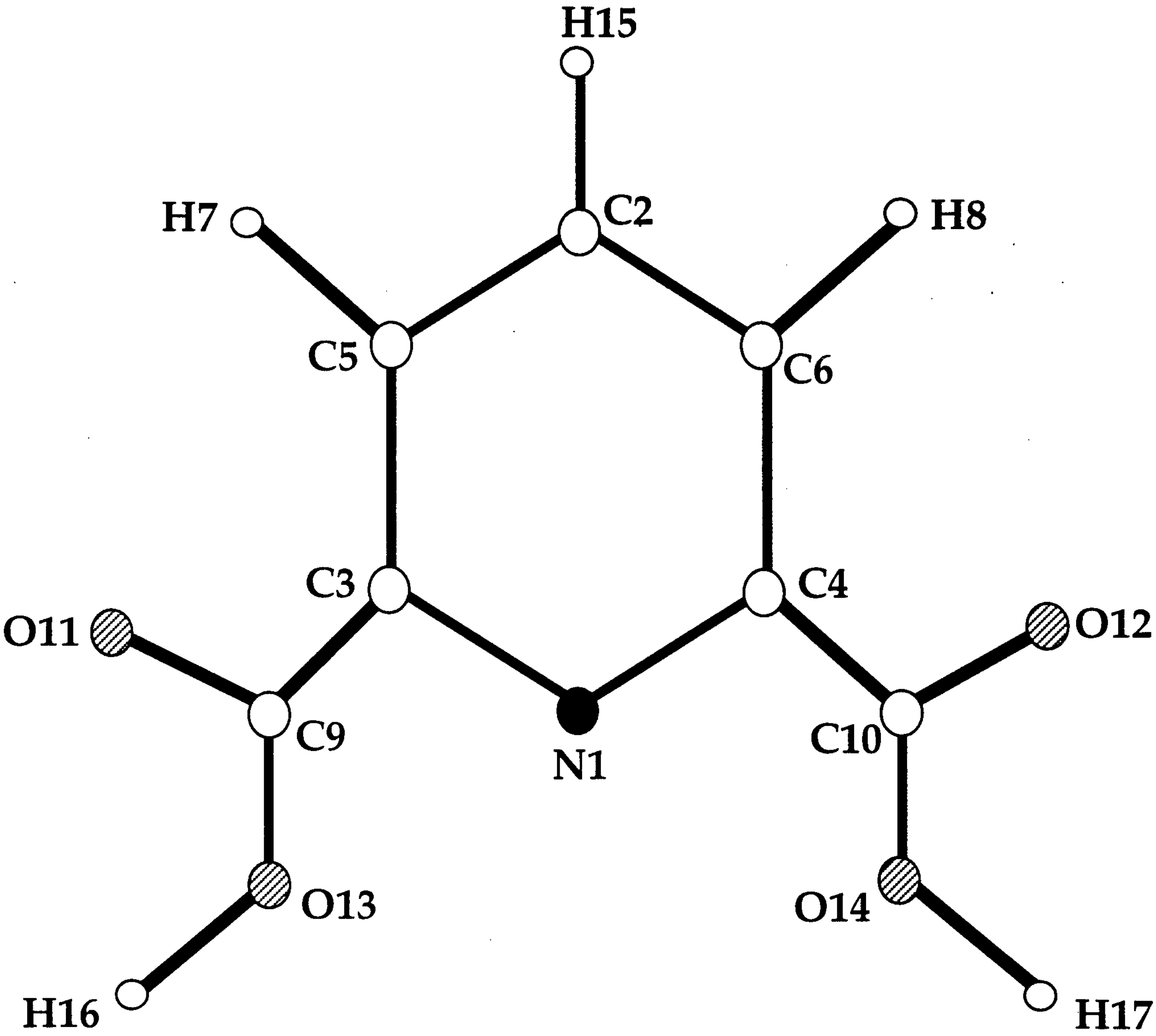

Figure. Structure and Atomic Numbering of Dipicolinic Acid

Table 1. Bond Distances of DPA and the DP Ion

	Dipic. Acid		Dipic. Ion		
Bond	Ground st.	Exc. st.	Ground st.	Exc. st.	Exp. gr. [49]
N1-C2	2.7861	2.8811	2.7605	2.7796	-
N1-C3	1.3377	1.3844	1.3382	1.3291	1.35
N1-C4	1.3377	1.3844	1.3382	1.3291	1.33
C2-C5	1.3950	1.4283	1.3883	1.3840	1.40
C2-C6	1.3950	1.4283	1.3883	1.3840	1.39
C2-H15	1.0712	1.0695	1.0779	1.0819	-
C3-C5	1.3983	1.4341	1.3956	1.4590	1.39
C3-C9	1.4837	1.4704	1.5353	1.4731	1.49
C4-C6	1.3983	1.4341	1.3956	1.4590	1.40
C4-C10	1.4837	1.4704	1.5353	1.4731	1.51
C5-H7	1.0697	1.0686	1.0689	1.0704	-
C6-H8	1.0697	1.0686	1.0689	1.0704	-
C9-O11	1.2157	1.2177	1.2757	1.3099	1.26
C9-O13	1.3361	1.3379	1.2517	1.2742	1.24
C10-O12	1.2157	1.2177	1.2757	1.3099	1.26
C10-O14	1.3361	1.3379	1.2517	1.2742	1.23
O13-H16	0.9545	0.9544	-	-	
O14-H17	0.9545	0.9544	-	-	
N1-H16	3.6497	3.6543	-	-	
N1-H17	3.6497	3.6543	-	-	
C3-H16	3.2291	3.2184	-	-	
C4-H17	3.2291	3.2184	-	-	

Table 2. Bond Angles of DPA and the DP Ion

	Dipic. Acid		Dipic. Ion		
Bond Angle	Ground st.	Exc. st.	Ground st.	Exc. st.	Exp [49]
C3-N1-C4	118.89	117.54	120.71	122.27	119.0
C5-C2-C6	118.93	118.53	119.12	121.19	120.2
C5-C2-H15	120.53	120.73	120.44	119.40	-
C6-C2-H15	120.53	120.73	120.44	119.40	-
N1-C3-C5	122.59	122.98	121.04	120.51	122.5
N1-C3-C9	118.73	118.37	121.22	120.17	115.1
C5-C3-C9	118.67	118.64	117.74	119.33	122.3
N1-C4-C6	122.59	122.98	121.04	120.51	122.6
N1-C4-C10	118.73	118.37	121.22	120.17	114.8
C6-C4-C10	118.67	118.64	117.74	119.33	122.6
C2-C5-C3	118.50	118.98	119.05	117.77	117.4
C2-C5-H7	121.93	122.00	123.02	122.65	-
C3-C5-H7	119.58	119.02	117.93	119.59	-
C2-C6-C4	118.50	118.98	119.05	117.77	118.3
C2-C6-H8	121.93	122.00	123.02	122.65	-
C4-C6-H8	119.58	119.02	117.93	119.59	-
C3-C9-O11	122.61	122.54	114.31	117.29	117.0
C3-C9-O13	115.21	115.31	119.03	123.29	117.8
O11-C9-O13	122.18	122.15	126.66	119.41	125.2
C4-C10-O12	122.61	122.54	114.31	117.29	115.7
C4-C10-O14	115.21	115.31	119.03	123.29	118.2
O12-C10-O14	122.18	122.15	126.66	119.41	126.0
C9-O13-H16	113.33	113.26	-	-	-
C10-O14-H17	113.33	113.26	-	-	-
N1-O13-H16	177.36	178.13	-	-	-
N1-O14-H17	177.36	178.13	-	-	-

Table 3. Mulliken Atomic Charges of DPA and Its Ion

	Dipic. Acid		Dipic. Ion	
Atom	Ground St.	Exc. St.	Ground St.	Exc.St.
N1	-0.481514	-0.476156	-0.543419	-0.519493
C2	-0.189603	-0.194199	-0.187294	-0.179782
C3	0.118711	0.142570	0.081785	-0.005529
C4	0.118628	0.142531	0.098858	0.043627
C5	-0.134320	-0.134946	-0.211242	-0.210010
C6	-0.134280	-0.134946	-0.198903	-0.269289
H7	0.270956	0.267121	0.210243	0.168893
H8	0.270956	0.267121	0.208261	0.113772
C9	0.774012	0.759550	0.742031	0.721075
C10	0.774238	0.759550	0.669441	0.615988
O11	-0.551764	-0.553551	-0.812412	-0.868368
O12	-0.552003	-0.553563	-0.794610	-0.449055
O13	-0.700229	-0.702111	-0.730205	-0.804302
O14	-0.700117	-0.702117	-0.658999	-0.443247
H15	0.227704	0.223384	0.126458	0.085721
H16	0.444322	0.444876	-	-
H17	0.444296	0.444876	-	-

Table 4. Computed Energies and Excitation Energies

	Dipic. Acid	Dipic. Ion
Ground St. En.(Gr. St. Geom.)	-621.716 4164	-620.457 1596
Ground St En.(Exc. St. Geom.)	-621.707 7061	-620.437 3189
Excited St En.(Gr. St. Geom.)	-621.535 5402	-620.270 5413
Excitation En	39,698 cm^{-1}	40,958 cm^{-1}
Excited St. En.(Exc. St. Geom.)	-621.544 0519	-620.286 1037
Fluorescence En.	35,918 cm^{-1}	33,188 cm^{-1}
Exp. Fluorescence [51]	30,000 cm^{-1}	-
Exp. Excitation abs. [51]	36,000 cm^{-1}	-

Table 5. Vibrational Frequencies of Dipicolinic Acid

Assignment	Gr. St.	Exc. St.	Exp. Gr. [46]	Corr
C-OH bend	146.3	143.7	107	-
C-OH bend	276.9	271.7	226	-
C-COOH stretch	387.2	369.8	330	353
C-C ring bend	419.2	403.9	365	383
C-COOH stretch	502.8	492.2	421	459
C-COOH stretch	612.9	604.7	-	559
C-C ring bend	697.2*	641.5*	647	657
C-O bend	706.0*	686.8*	647	665
C-O bend	786.5*	762.7*	705	741
C-H bend	960.8	896.1	890	882
C-C ring bend	1085.5	1010.3	987	991
C-H bend	1173.8**	1051.3*	1082	1077
C-O stretch	1214.4*	1140.3	1151	1133
C-H bend	1223.7	1293.0	-	1123
C-O stretch	1235.4**	1223.5**	1178	1153
C-H bend	1328.6	1444.2	1260	1220
O-H bend	1338.1*	1279.9	1275	1228
C-H bend	1340.0	1505.8	1275	1230
O-H bend	1529.6*	1320.2**	1341	1404
C-C ring stretch	1551.1**	1550.7*	1387	1415
C-C ring stretch	1582.7**	1556.8**	1422	1444
C-C ring str.	1632.0	1678.2	1470	1489
C-C ring str.	1722.8	1654.7	1571	1572
C-C ring str.	1742.2	1861.1**	1576	1590
C=O stretch	1892.9***	1886.7**	1700	1699
C=O stretch	1898.6**	1891.2*	1710	1704
C-H stretch	3391.8	3413.7	3070	3088
C-H stretch	3424.2	3436.9	3102	3117
C-H stretch	3428.0	3442.6	3115	3121
O-H stretch	3991.4**	3971.1**	3479	3647
O-H stretch	3991.6**	3973.8**	3520	3650
COOH wag	75.2	54.9	79	-
COOH wag	76.2	62.9	79	-
C-C ring bend	206.8	183.1	218	187
C-C ring bend	221.1	187.1	-	200
C-C ring bend	519.8	410.3	-	469
C-C ring bend	524.1	433.8	-	473
O-H bend	636.3**	660.7	583	584
O-H bend	669.0	671.9**	-	614
C-COOH bend	769.3**	801.1	692	700
C-H bend	840.2*	575.8*	751	747
C-COOH bend	871.0	819.7*	-	793
C-H bend	937.5	912.5	855	833
C-H bend	1067.6	758.0**	-	949
C-H bend	1128.4	858.2	-	1003

Table 6. Vibrational Frequencies of the Dipicolinate Ion

Assignment	Gr. St.	Exc. St.	Exp. Gr. [46]	Corr
O-C-O bend	161.5	116.0	167	152
O-C-O bend	299.5	244.2*	269	282
O-C-C bend	373.6	516.6	347	352
C-CO_2 stretch	429.9*	377.1	418	401
C-CO_2 stretch	516.3	403.3	485	482
C-C ring bend	600.1	551.5**	558	548
C-C ring bend	723.4	680.6	662	660
C-CO_2 bend	781.9*	778.8**	728	714
C-CO_2 bend	953.3*	836.1*	856	870
C-C ring bend	1098.5	723.1**	1014	1003
C-H bend	1155.5	930.2**	1084	1061
C-H bend	1248.8	1067.1**	1150	1137
C-C ring stretch	1282.9*	1137.7**	1186	1171
C-C ring stretch	1288.2	1190.1	1186	1175
C-H bend	1322.2	1460.2**	1200	1214
C-O stretch	1445.1***	1243.6**	1382	1348
C-O stretch	1464.8*	1418.2***	1382	1367
C-C ring stretch	1569.4*	1336.3***	1445	1432
C-H bend	1600.5	1539.9**	1466	1469
C-C ring stretch	1718.9**	1532.0**	1567	1568
C-C ring stretch	1741.9**	1680.7**	1598	1589
C=O stretch	1783.0**	1587.1*	1609	1600
C=O stretch	1827.1***	1640.6**	1623	1640
C-H stretch	3297.8*	3241.0**	3036	3002
C-H stretch	3425.7	3383.5*	3110	3118
C-H stretch	3429.9	3412.2*	3110	3122
CO_2 wag	67.6	82.9	64	67
CO_2 wag	99.9	96.6	104	98
C-C ring bend	192.5	150.2	183	175
C-C ring bend	220.0	186.8	213	200
C-C ring bend	512.1	425.4	485	462
CO_2 bend	564.0	473.1*	534	486
C-CO_2 bend	746.4*	599.7**	696	678
C-H bend	855.6	854.0*	763	760
C-CO_2 bend	856.0*	709.4*	763	772
C-CO_2 bend	872.5*	747.8*	823	787
C-H bend	963.8*	900.3	856	857
C-H bend	1094.3	1091.4*	-	973
C-H bend	1140.9	1108.9***	1014	1014

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