Optical Activity of the Penicillin Nucleus Chromophores[1a]

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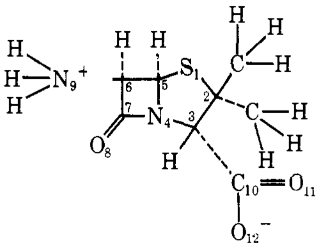
Abstract: Optical rotatory properties of 6-aminopenicillanic acid (6-APA) and related structures are calculated using siempirical LCAO-MO wave functions. Utilizing extended HÜCKEL MO's of model structures, the virtual orbital approximation, and full evaluation of all dipole velocity and angular momentum integrals, transition energies and rotatory strengths are found to predict circular dichroism (CD) spectra in remarkable agreement with experimental CD curves for 6-APA and various penicillin antibiotics. Transitions arising within the penam nucleus are found to be responsible for a strong, positive Cotton effect at ca. 235 nm (230–235 nm, experimental) and a negative Cotton effect at ca. 200 nm (204–211 nm, experimental). These bands may be characterized as principally sulfide to amide charge transfer and amide n → π^*, respectively. Results from SCF-Cl wave functions based on various deorthogonalized, neglect-of-differential-overlap MO's for 6-APA structures reveal that the excited states of the penam nucleus are especially difficult to describe by some of the current parameterization schemes.

As interesting and as widely used and studied as penicillins are, unequivocal signment of their circular dichroism (CD) spectra has been lacking. The difficulty lies in the fact that the penicillin nucleus, 6-aminopenicillanic acid[2] (6-APA) (1), consists of three chromophoric groups: sulfide, carboxyl, and β-lactam amide. All three groups absorb in the near-uv region, and the absorptions overlap to such a degree that the uv spectra of penicillins are devoid of characterizable bands. The groups are also close enough spatially that some mutual perturbation can occur. Strain in the four-membered ring will perturb to some extent the amide chromophore. Moreover, because the energies of the uv transitions of all three chromophores overlap in the 200–240-nm region, some charge transfer transitions may be expecte. All these factors confound the extrapolation or interpolation of spectral characteristics of related compounds in assigning CD spectra of penicillins. Consequently, we have approached the problem from the theoretical side and are able to present a predicted spectrum of 6-APA in excellent agreement with experiment.



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Experimental studies of the CD spectra of 6-APA and other penicillins have been sparked by recent debate. We shall not be interested here in chromophoric side chains of penicillins, such as various 6-acylamido groups. Instead we concentrate on the bicyclic penam nucleus, and hence 6-APA is the appropriate experimental compound to consider. The uv absorption of 6-APA consists of a structureless band peaking somewhere below 200 nm which tails out to about 270 nm.[3,4] More revealing, and therefore attracting more interest, is the CD spectrum. It displays a strong positive Cotton effect at ca. 233 nm and a pH sensitive negative or positive Cotton effect at ca. 204–211 nm.[4-9] Initially, the short wavelength (210 nm) band was assigned as the β-lactam n → π^* transition.[5] On the other hand, the characteristic disappearance of the long wavelength (233 nm) band upon hydrolytic opening of the β-lactam ring led others to suppose that this was the β-lactam n → π^* transition.[6] Siemion et al. analyzed the CD spectra of 6-APA by considering the three chromophores separately and proposed that the 233-nm band was due to the localized sulfide n → σ^* excitation.[7] Snatzke[8] countered by reassigning it to the β-lactam n → π^* transition, prompting Siemion et al. to again forward their sulfide n → σ^* assignment.[9] In the most recent chiroptical study of penicillins, Mitscher et al.[4] propose that the long wavelength Cotton effect is associated with some vicinal effect of the sulfide and β-lactam group. In view of published theoretical studies of penicillins (see below), they[4] surmise that this transition produces a strong CD signal because of penam's inherent asymmetry.

The history of theoretical studies of the electronic transitions of penams is brief. The first published calculational study employed extended Hückel (EH) molecular orbitals (MO's) and the virtual orbital approximation.[10] Despite apprehensions about the utility of such an expedient, the results turned out surprisingly well. Not only were plausible assignments based on excitation energies and oscillator strengths of penams possible, buthe calculations also gave excellent semiquantitative agreement with the uv absorptions of the related β-lactam antibiotics, Δ^3- and Δ^2-cephalosporins.[10] No empirical readjustments of the EH results were made in achieving this agreement. The EH calculations predicted the strongest long wavelength transition of a model structure of 6-APA at 245 nm with oscillator strength $f \approx 0.07$. This arose from excitation between the highest occupied MO, which had transannular π overlap of the S_1 and N_4 atoms, and the virtual orbital with amide π^* character. Thus, the interaction of the sulfide and amide chromophores is apparent in this transition. The β-lactam amide n → π^* transition was calculated at higher energy (ca. 199 nm).

The reader will notice that the association of the predicted 245-nm transition with the observed 233-nm positive CD band would mean that the excitation straddles the amide and sulfide chromophoric groups, i.e., the transition is not localized on either the amide or sulfide chromophores but rather involves mixing of the two in the charge transfer sense and in the perturbational sense.[11] The EH calculations indicate that the more nearly pure β-lactam n → π^* transition should be assigned to the Cotton effect at 204–211 nm. However, a comparison between theory[10,11] and experiment was hampered by the fact that only oscillator strengths were computed. To add credence to these assignments the rotatory strengths for transitions occurring in the penam nucleus are needed. The purpose of this paper is to present calculations of those rotatory strengths.

Experimental Section

In this section we describe our approach to the CD spectral calculations and details of our quantum mechanical computer experiments.

Methods of Calculation. Two types of all-valence-electron semiempirical MO methods were used to generate the wave functions. The first type employed neglect-of-differential-overlap (NDO) self-consistent-field (SCF) theory. At the complete neglect (CNDO) and intermediate neglect (INDO) levels of approximation, these MO's have proved to be useful in elucidating the chiroptical properties of numerous molecules.[12-20] Included among the molecules treated have been amides,[15,16,19] carboxyl groups,[14,18,20] and disulfides,[13,17] all of which have direct or indirect relevance to the chromophores of 6-APA. Initially, the CNDO/2 parameterization[21] with and without sulfur 3d orbitals was tried. Configuration interaction (CI) using single excitations between the five highest occupied (HO) and five lowest empty (LE) MO's in the virtual orbital approximation was employed to describe the excited, singlet state wave functions. After the CI but before the optical properties calculations, the NDO wave functions are deorthogonalized,[12-20,22] so that all contributions to the transition moment integrals could be and were rigorously included. The transition energies and rotatory strengths from these CNDO/2D-CI wave functions were less than satisfactory as described near the end of the paper. Consequently, alternate NDO parameterizations[23] were investigated, but none led to significantly improved results.

The second type of MO method employed was EH theory. Details of the method and parameters have been described previously.[10,11,24] The S 3d atomic orbitals (AO's) are included in the basis set. Transition energies from the EH calculations were expected to be satisfactory based on our previous experience,[10,11] but the quality to expect for the predicted chiroptical properties was uncertain in light of earlier rotatory strength calculations using EH wave functions.[25,26]

The spectral properties that we computefor each low energy transition include the transition energies in wavelengths λ_n, the reduced rotatory strengths $[R_n]$, dipole strengths D_n, dissymmetry factors g_n, and oscillator strengths f_n. The equations for calculating these quantities are well known.[12-16] Transition energies in the NDO schemes are taken as the difference between the total energy of an excited singlet state, multideterminant wave function and the total energy of the ground state, single determinant wave function. The NDO total energies are calculated using the appropriate parameters and two-electron integral approximations.[21] Transition energies in the EH scheme simplify to the difference in eigenvalues of the two MO's involved in the excitation.[10,11] The f_n value and D_n measure the electric dipole allowedness of a transition, whereas $[R_n]$ also reflects the magnetic dipole allowedness. These indices of absorption intensity or optical activity were calculated with all one-, two-, and three-center electric dipole and magnetic dipole integrals rigorously evaluated from overlap and dipole velocity integrals as before.[12-16] Of course, approximate dipole length transition moment integrals may be obtained by the usual transformation[12-16] which holds exactly for true dipole velocity transition moment integrals and true theoretically computed transition energies. Oscillator strengths which are thus generated from these approximate dipole length integrals will be compared later in the paper with f_n values obtained directly in the dipole length formalism used before.[10,11]

The transition energies and reduced rotatory strengths can be synthesized into a CD spectrum using the relation[27] below. Here $[\theta(\lambda)]$ is the molar ellipticity which can be related to the difference in extinction coefficients for left and right circularly polarized light.[27]

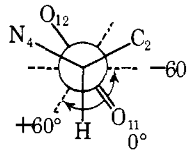
$$[\theta(\lambda)] = 75.15 \sum_{n=1}^{m} \frac{[R_n]\lambda_n}{\delta_n} \exp[-(\lambda - \lambda_n)^2/\delta_n{}^2]$$

Most of the published[4,7,9] CD spectra of penicillins have been in terms of molar ellipticities, so this is the more useful quantity for us to calculate. The 75.15 factor corresponds to $[\theta(\lambda)]$ being in units of degree square centimeter per decimole, $[R_n]$ in units of 100 Debye·Bohr magnetons per molecule, and λ_n and δ_n in units ofanometers.[27] As we will see later, only the eight lowest energy transitions ($m = 8$) need be included in the above summation when EH MO's are used because these are the transitions leading to absorption at wavelengths above 190 nm. These lower energy transitions are expected to be better treated in the virtual orbital approximation than the higher energy ones. Two values of the exponential half-width parameter δ_n were tested: a low value of 10 nm and a high value of 20 nm. These values are expected to bracket the half-widths observed in the solution spectra of large molecular systems. For convenience, all transitions are assigned identical δ_n parameters.

Structural Variables under Consideration. Several model systems of 6-APA, **1**, were chosen for the calculations so as to determine the spectral dependence on some of the conformational, rotational, and other structural variables, and to determine the contributions of some of the isolated chromophores. All the basic atomic coordinates and supplementary bond lengths, bond angles, and dihedral angles have been described before[10] and are used here unless specified otherwise. New structural features include the following:

(1) Besides the un-ionized (6-NH_2, 3-COOH) models of 6-APA treated previously,[10] the zwitterionic (6-NH_3^+, 3-COO^-) species are calculated here. The un-ionized forms are more suited for calculation, but the zwitterions are more relevant to experiment. The dissociation constants of 6-APA are such that the zwitterionic form comes into predominance at rather low pH's (3-4). The atomic coordinates for the zwitterions are obtained by removing the proton from COOH, adding one to NH_2 using tetrahedral hybridization and the same bond lengths as before,[10] and holding all other atomic coordinates fixed. The very low and high pH forms of 6-APA (6-NH_3^+, 3-COOH; 6-NH_2, 3-COO^-) were not included in our study because the reliability of EH MO's for ions is less than that for electrically neutral and nonpolar species and also because the two forms which were treated represent both the protonated and unprotonated states of the amino and crboxyl functionalities.

(2) The carboxyl group at the 3 position was rotated from its position observed in the solid state[28] (N_4-C_3-C_{10}-O_{11} dihedral angle ca. 216°) either +60 or −60° about the C_3-C_{10} axis.[29] A Newman projection of the carboxyl rotamers looking down the C_3-C_{10} axis is shown in **2**. We will refer to these as the carboxyl 0, +60, or −60° rotamers.



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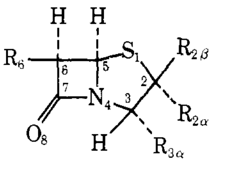
(3) The five-membered thiazolidine ring of the penam nucleus has a conformational degree of freedom: it may exist in a 2β-axial, 3α-axial conformation as found in crystalline 6-APA and penicillins V and G,[28] or it may exist in a 2β-equatorial, 3v5-equatorial conformation as observed in ampicillin and penicillin sulfoxides.[30] There is some as yet uncorroborated, preliminary evidence that the two penam conformers may coexist in solution with little or no barrier separating the two forms.[31] As will be apparent later in the paper, the total energies that we obtain show the two conformers to be of similar stability. In order to cut out extraneous variables and to make comparisons of the two conformers more valid, atomic coordinates for the 2β-equatorial, 3α-equatorial penam models were obtained by appropriate modification of Pitt's structure.[28] Whereas Pitt's data give a C_5-N_4-C_3-C_2 dihedral angle of ca. 33°, changing this to 352° places the molecule in the 2β-equatorial, 3α-equatorial conformation and leaves all bond lengths unchanged. Substituents on C_3 and the new C_2 can then be repositioned using the same bond lengths, bond angles, and certain dihedral angles as before.[10]

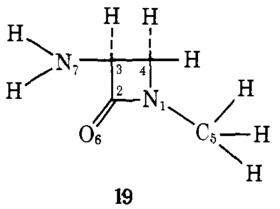
(4) The contributions of the carboxyl chromophore may be isolated by calculating a 3α-H model. Bond lengths and angles for locating the new proton are the standard values.[10]

(5) In most of the model structures that we computed, the *gem*-dimethyls on the 2 position of **1** are replaced by hydrogens in order to cut down on computer time. Some spectral calculations on diketopiperazines using INDO-MO's have been found to be quite sensitive to methyl substitution.[16] For the EH-MO's, the inclusion of the methyls is expected to make little contribution to the spectral properties of penam. However, we check this out by running calculations on structures having the *gem*-dimethyls. The CH_3 groups are positioned with standard[10,28] bond lengths and angles and with the methyl hydrogens staggered. So as to keep the number of valence AO basis

Table I. Structures Studied[a]



penam



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No.	Name
3	6-NH_3^+-3α-COO^-(0°, ax)penam
4	6-NH_3^+-3α-COO^-(+60°, ax)penam
5	6-NH_3^+-3α-COO^-(−60°, ax)penam
6	6-NH_3^+-3α-COO^-(0°, eq)penam
7	6-NH_3^+-3α-COO^-(+60°, eq)penam
8	6-NH_3^+-3α-COO^-(−60°, eq)penam
9	6-NH_2-3α-COOH(0°, ax)penam
10	6-NH_2-3α-COOH(+60°, ax)penam
11	6-NH_2-3α-COOH(−60°, ax)penam
12	6-NH_2-3α-COOH(0°, eq)penam
13	6-NH_2-3α-COOH(+60°, eq)penam
14	6-NH_2-3α-COOH(−60°, eq)penam
15	6-NH_2-3α-H(ax)penam
16	6-NH_2-3α-H(eq)penam
17	6-NH_2-3α-H(ax)-2-$(CH_3)_2$-penam
18	6-NH_2-3α-H(eq)-2-$(CH_3)_2$-penam
19	1-CH_3-3-NH_2-azetidin-2-one

[a] 0 or ±60° refers to the rotational position of the carboxyl group as explained in the text, and ax or eq refers to the 2β-axial, 3α-axial or 2β-equatorial, 3α-equatorial thiazolidine ring conformation.

functions as low as possible, the 2-$(CH_3)_2$-penam models are run without the carboxyl groups.

All of the model structures treated in this investigation are summarized in Table I. Note that structures **9**, **15**, and **19** (the free β-lactam) were also included in the earlier study.[10]

Results and Discussion

Before delving into an analysis of the spectral properties of each model, it will be helpful to begin this section by presenting the predicted CD spectra for the more appropriate penam models. The striking similarity between these curves and the experimental spectra of 6-APA encourages us to then proceed with the examination of the EH results for the other models. Later in this section the conformational energies of the penams will be discussed, the results of NDO calculations will be evaluated, and the question of d orbital participation will be considered.

Predicted CD Spectra and Assignments. The predicted CD spectrum of **3**, which is shown in Figure 1, is in close qualitative and even quantitative agreement with published CD spectra of 6-APA.[4,7] A long wavelength positive Cotton effect is computed at ca. 236 nm with a molar ellipticity of 42 000 or 91 000 depending on the band width parameter. The wider the assumed band widths, the smoother the spectral curve and the lower the molar ellipticities. Minor peaks and inflections, such as are present with our spectral curve with δ_n = 10 nm, cannot be discerned from published experimental CD curves. Experimentally, the first Cotton effect of 6-APA occurs at 230-235 nm with a molar ellipticity of 42 200-58 700 depending on experimental conditions.[4,7] The molar ellipticity that we compute with δnn = 20 nm is thus remarkably close to experiment.

The short wavelength CD extremum of **3** (Figure 1) is predicted to occur at 199 nm with a molar ellipticity of −40 000 to −83 000. Again the calculated λ_{max} is close to the experimental value for 6-APA (204-211 nm).[4,7] The sign of the second Cotton effect is quite sensitive to pH and apparently to other experimental conditions. Molar ellipticities of +17 900 and −20 000 have been reported in the literature for pH's near 6.5, and a value of −31 200 has been reported for pH 3.0.[4,7] The calculations appear to be consistent with the low pH, negative values for the second Cotton effect. The reported positive value corresponds to an inflection at 211 nm in the CD spectrum which was only recorded down to ca. 205 nm.[7]

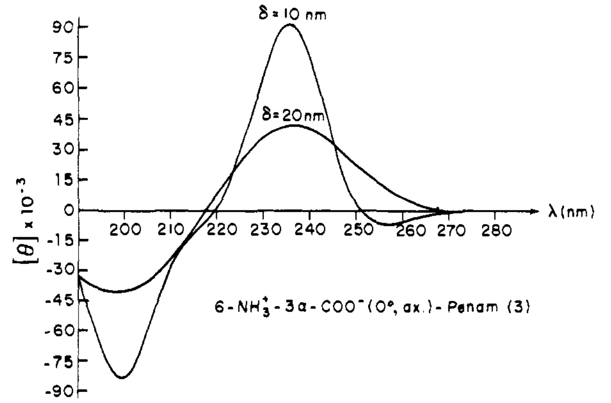

Figure 1. Predicted CD spectra of 6-NH_3^+-3α-COO^-(0°, ax)penam (**3**) based on EH-MO's. Two values of the exponential half-width δ parameter for the transitions are used in the calculations. Molar ellipticities are expressed in units of (deg cm^2)/dmol.

Considering next the chiroptical data which went into the calculation of Figure 1, we see in Table II the λ_{max} and [*R*] values for **3**. The MO's for this model are given in Table III. Note that the third and fourth transitions give rise to positive rotatory strengths in the 234-239-nm range. The MO's involved in these transitions may be designated S-N π → amide π* and S n → amide π* (Table III). Thus, the first excitation involves mixing of the chromophores and the second has distinct charge transfer character. Whereas the oscillator strengths of these transitions are very low, the inherent asymmetry of the penam nucleus results in these transitions being highly allowed magnetically. Thus, our calculations corroborate earlier expectations.[4] Many of the transitions (Table II) are predicted to lie rather close together, so the observed CD spectrum will be a composite of several strongly overlapping individual transitions. The net resultant of the longer wavelength transitions is the positive Cotton effect at ca. 236 nm as seen in Figure 1. From Tables II and III, we can see that it is best described as sulfide to amide charge transfer, although the orbitals involved also show some nonlocalized mixing of both chromophoric groups.

The rotatory strengths for the shorter wavelength transitions (Table II) are relatively small until one reaches the eighth. This transition occurring near 200 nm has a highly negativ rotatory strength. The oscillator strength is also very large. The MO's involved are the amide n and amide π* orbitals (Table III).

Table II. Spectral Properties of 6-NH_3^+-3α-COO^-(0°, ax)penam (**3**) from EH MO's[a]

Transition	λ	[*R*]	|*g*|	*D*	*f*
1 (29–28)	253.3	−7.22	0.0043	0.63	0.0116
2 (30–28)	248.0	−6.14	0.0020	1.13	0.0214
3 (29–27)	239.4	36.04	0.0269	0.50	0.0098
4 (30–27)	234.6	74.51	0.0100	2.76	0.0554
5 (29–26)	218.4	0.70	0.0005	0.50	0.0108
6 (30–26)	214.5	−16.43	0.0077	0.79	0.0173
7 (31–28)	209.4	14.90	0.0027	2.05	0.0461
8 (31–27)	199.8	−115.07	0.0179	2.39	0.0562
9 (31–26)	185.0	−13.70	0.0131	0.39	0.0099

[a] The MO's involved in each transition are given in the first column. Units are nm for λ, 100 · Debye · Bohr magnetons/molecule for [*R*], 10^{-36} esu^2 cm^2/molecule for *D*, and dimensionless for *g* and *f*.

Table III. EH Eigenvalues and MO's of 6-NH_3^+-3α-COO^-(0°,ax)-penam (3)

MO[a]	ε, eV	Large LCAO–MO Coefficients[b]	Mnemonic
26, NNLEMO	−6.13	$+0.40(S_1\ 3p_x) - 0.43(S_1\ 3d_{z2}) + 0.42(S_1\ 3d_{xz}) - 0.30(C_2\ 2p_z) - 0.33(C_{10}\ 2p_x)$	S 3d
27, NLEMO	−6.62	$-0.32(N_4\ 2p_x) + 0.82(C_7\ 2p_x) - 0.32(C_7\ 2p_y) - 0.46(O_8\ 2p_x)$	Amide π*
28, LEMO	−6.91	$+0.37(C_{10}\ 2p_z) + 0.76(C_{10}\ 2p_x) - 0.41(O_{11}\ 2p_x) - 0.37(O_{12}\ 2p_x)$	Carboxyl π*
29, HOMO	−11.80	$-0.59(S_1\ 3p_y) - 0.40(N_4\ 2p_y) + 0.28(C_5\ 2p_y)$	S–N π
30, NHOMO	−11.91	$+0.63(S_1\ 3p_y) - 0.30(N_4\ 2p_y) + 0.28(C_6\ 2p_z)$	S n
31, NNHOMO	−12.83	$-0.31(N_4\ 2p_x) + 0.36(C_6\ 2p_z) + 0.45(O_8\ 2p_z) - 0.33(O_8\ 2p_y) - 0.25(O_{11}\ 2p_y) - 0.28(O_{12}\ 2p_y)$	Amide n

[a] NNLEMO abbreviates next to the next lowest empty MO, NHOMO abbreviates next to the highest occupied MO, etc. [b] An arbitrary cut-off value of 0.25 was used for selecting coefficients for inclusion in this table. Each MO, ψ_i, is a linear combination of AO's, χ_j, which are designated, e.g., S_1 $3p_x$ for the $3p_x$ AO on S_1. See ref 10 for notation. The coordinate system has N_4 at the origin, C_5 on the +z axis, and C_2 in the xz plane; the α face of the molecule projects in the +y direction.

Table IV. Spectral Properties of 6-NH_2-3α-COOH(0°, ax)penam (9) from EH MO's

Transition	λ	[R]	f[a]	f[b]
1 (29–28)	259.5	−8.78	0.0144	0.0247
2 (30–28)	247.4	−7.60	0.0235	0.0233
3 (29–27)	244.8	42.09	0.0098	0.0677
4 (30–27)	234.1	53.90	0.0460	0.0223
5 (29–26)	222.9	−0.14	0.0158	0.0190
6 (30–26)	214.0	−12.53	0.0128	0.0012
7 (31–28)	208.5	22.57	0.0502	0.0585
8 (31–27)	198.9	−124.53	0.0946	0.1680
9 (31–26)	184.2	−18.73	0.0167	0.0064

[a] Oscillator strength using dipole velocity integrals. [b] Oscillator strength from ref 10 using dipole length integrals.

Thus, the second Cotton effect may be assigned as the β-lactam amide n → π* transition. The mixing of other chromophores into these two orbitals is small with the major contributing component coming from the carboxyl function.

Having seen the reasonableness of the EH predictions, let us next consider some structural variations in the model structures. In going from the zwitterionic model **3** to the corresponding un-ionized form **9** only some small changes occur. The calculated spectral properties of **9** are presented in Table IV. The MO's for this model are reported elsewhere,[10] and, in fact, have the same sequence and general energy and character as those given in Table III. The empty MO's of **3** and **9** are almost identical, whereas the filled MO's differ slightly in shape. Among the differences is a small mixing of amino nitrogen lone-pair character into the high-lying filled MO's; and, to a lesser extent, into the amide π* EHMO; this mixing occurs in all the models with unprotonated NH_2 groups and should be related to the pH sensitivity of the CD spectra. The transitions of **9** are quite similar in energy and rotatory strength to those for **3** (Table II). Shifts in λ_n are no greater than about 6 nm, and the third transition is the only transition with a large rotatory strength which shifts this much. There are some relatively large changes in the oscillator strengths, but since few of the *f* values are of large magnitude, the overall effect on a predicted uv spectrum would be minimal. In Table IV we also present *f* values obtained earlier[10] in the dipole length formalism. One can expect some change between the dipole length and dipole velocity formisms because of the approximate nature of the wave functions.[11] Changes in the *f* values in Table IV range from almost none to an order of magnitude difference. The amide n → π* transition (number 8) is the strongest in either formalism. There is one noticeable reversal of relative intensities involving the third and fourth transitions depending on the formalism used for the calculation. Both of these transitions involve interaction of the sulfide and β-lactam amide chromophores.

Table V. Prominent Spectral Characteristics and Relative Energies of Some Penam Models

Structure	ΔE,[a] kcal/mol	First CD band[b]		Second CD band[b]	
		λ_{max}, nm	[θ] × 10^{-3}	λ_{max}, nm	[θ] × 10^{-3}
3	0.18	237, 236	+42, +91	199	−40, −83
4	0.84	238, 236	+44, +94	201	−48, −89
5	0.0	236, 232	+41, +86	199, 200	−49, −99
6	2.07	236, 235	+51, +89	197, 199	−36, −77
7	3.05	237, 235	+52, +82	197, 198	−43, −89
8	2.15	236, 235	+54, +97	198, 199	−42, −80
9	22.06	238, 237	+35, +61	198	−41, −87
10	22.71	242, 239	+38, +68	201	−49, −91
11	21.87	239, 232	+32, +53	199	−50, −98
12	24.01	241, 242	+43, +73	197	−39, −80
13	24.97	241, 242	+46, +66	197, 198	−41, −81
14	24.05	241, 242	+42, +67	197, 198	−37, −76

[a] Relative energies from EH calculations using ½Σε as a measure of total energy; see ref 10. Structure **5** is most stable; **13** is the least stable. [b] Double entries correspond to the two δ_n parameters: the first value for δ_n = 20 nm, the second for δ_n = 10. Single entries are unchanged by the δ_n parameter. Molar ellipticities are in units of (deg cm^2)/dmol.

The predicted chiroptical properties for the other models of Table I are quite similar to those for **3** (or **9**). Consequently, data for the calculated spectral transitions for these models are given in tables as supplementary material. Likewise, predicted CD plots for models **4–14** show features similar to those in Figure 1. Plots for these other models are given as supplementary material. Salient features of the plots are summarized in Table V. The conformational and rotational variables, as well as the ionic form of the penam models, all have rather small effects on the λ_{max} and molar ellipticities of the two principal Cotton effects. For all cases in Table V, the first Cotton effect is predicted to be positive with λ_{max} close to 235 nm and the second Cotton effect is negative with λ_{max} hovering close to 200 nm. The choice of half-width parameter causes small shifts in λ_{max} and large changes in [θ]. The larger δ_n value appears to yield molar ellipticities closer to experiment for these penam models.

Some interesting trends can be discerned in Table V. For instance, calculated molar ellipticities of the first CD band tend to be somewhat smaller in magnitude in the carboxyl axial forms (**3–5**, **9–11**) than in the carboxyl equatorial forms. The opposite is true for the second CD band. Thus, increasing the proportion of carboxyl axial conformers would appear to strenghten the amplitude of the second band, due partially to an unmasking by the weakened first band. One can also observe from Table V that the first Cotton effect is calculated to be stronger in the zwitterionic model structures than in the un-ionized forms.

The relative energies of the penam structures are also reported in Table V. These are based on using[10,32] ½Σε obtained in the EH calculations. One notes that the structures are calculated to prefer a 2β-axial, 3α-axial conformation by about

2 kcal/mol. The zwitterionic forms are about 22 kcal/mol more stable than the un-ionized forms, but the reliability of EH theory in predicting the energy of ions puts the significance of this number in doubt. Rotation of the carboxyl group plane has a relatively small effect on the stability of the models because of the smallness of the rotational barrier about the C_3-C_{10} bond.[10] The structures with the carboxyl group rotated +60° are less stable than those with the carboxyl positioned with the x-ray determined dihedral angles or rotated −60° (see Experimental Section). In some cases, the 0° rotamer is of lowest energy, and, in other cases, the −60° rotamer is marginally more stable.

If a molecule in solution exists in an equilibrium between several different structures or conformations, then the observed spectrum will depend on the relative amount of each form present.[27] According to the data in Table V, **3** and **5** should be most representative of the molecule in solution. Moreover, the lack of high sensitivity of the predicted spectra to the structural variables means that even if the contributions of the other structures were taken into account, our resultant CD spectrum would not be very different from that in Figure 1. From another point of view, the lack of high sensitivity could indicate that experimental CD measurements would have to be of high resolution and deciphered in careful conjunction with theory in order to discern which form(s) of 6-APA predominate(s) in solution. Mitscher et al. have speculated that it may not be possible to use CD on penicillins to detect penam conformational changes and also noted that the spectra are even rather unaffected by the nature of the 6-acylamido side chain on penam.[4] We note that the experimentally observed second Cotton effect of 6-APA becomes more negative in going from neutral or basic pH to low pH. A question to consider is if this observation is related to simply protonation of the amino group or whether a conformational change in the penam nucleus is *also* involved. As brought out above, our calculations show that the carboxyl axial forms have a stronger second Cotton effect and a weaker first Cotton effect than the carboxyl equatorial forms. If this prediction is reliable and relevant, then one may conclude that CD is also revealing a greater proportion of carboxyl axial forms at low pH compared to high pH. On the other hand, based on electrostatic arguments and interatomic distances, there is little reason to expect that just changing the pH of the solution would be sufficient to induce a significant change in population of the conformers. Thus, the earlier view[4] may very well be borne out.

Thus far, we have seen that there are several minor and a few major transitions which combine to produce a CD spectrum of 6-APA with two prominent Cotton effects. Long wavelength transitions involving charge transfer between the sulfide orbitals and the β-lactam amide orbitals sult in the first Cotton effect, which is positive. These transitions are weak in the electric dipole sense, but the asymmetry of penam and their charge transfer character make them strongly allowed in the magnetic dipole sense. At shorter wavelengths, the strongly allowed β-lactam amide n → π^* transitions occurs. This fairly well localized transition results in the second Cotton effect. To better understand the nature of the transitions and the contributions and mixing of the chromophores of the penicillin nucleus, we next turn our attention to the rest of our structural models.

Spectral Dependence on Other Structural Variables. When the 3-carboxyl group of the model structures in Table V is replaced by a hydrogen, the main effects on the uv and CD are the elimination of the low-lying, empty carboxyl π^* orbital which this chromophoric group contributes (Table III) and the increased localization of the β-lactam amide n orbital. The other MO's which give rise to transitions above 190 nm remain similar in energy and shape to those given in Table III. Consequently, presentation of the MO's of one of the decarboxylated models (**15**) is confined to the supplementary material at the end of the paper. The calculated chiroptical data of **15** and **16** given in the supplementary material show S-N π → amide π^* and S n → amide π^* charge transfer transitions in the 230-245-nm region with low oscillator strengths and large positive rotatory strengths. The amide n → π^* transition at ca. 195 nm has a large oscillator strength and a very large negative rotatory strength. The similarity of this electronic description will be recognized as entirely similar to that given above for models **3**–**14**. Thus, we may conclude that the contributions of the carboxyl group to the basic features of the CD spectra of 6-APA and penicillins are rather small. This conclusion is entirely consistent with earlier deductions.[8,9,11]

Penam models with *gem*-dimethyl groups **17** and **18** display MO's and chiroptical data quite similar to those for **15** and **16**. Tabulations of the calculated data for **17** and **18** are included in the supplementary material. We may conclude that the methyl groups effect only small perturbations in the lower energy filled MO's according to the EH calculations. Replacing the methyl groups by hydrogens is thus a valid expedient in theoretical studies of the spectral properties of penam. Without a 3-carboxyl group, the penam ring system prefers the 2β-axial, 3α-axial conformation over the 2β-equatorial, 3α-equatorial one by about 2.5 kcal/mol.

The simplest model considered in this study is the monocyclic β-lactam ring structure **19**. From the MO and chiroptical data in the supplementary material one learns that the amide n → π^* transition at 205 nm again has a strong negative rotatory strength and a large oscillator strength. The only other transitions in the uv region are calculated at 192 and 215 nm and have fairly strong positive rotatory strengths. These excitations involve the amide π^* and filled MO's of nonlocalized character but with considerable amino nitrogen involvement.

Another structural variation of the penam models is the bonding of an oxygen to the sulfur corresponding to the well known penicillin sulfoxides. Partial CD spectra have been published for these compounds, and they appear to be surprisingly similar to those for the penicillins.[9] The sulfoxides display a strong, positive ($[\theta]$ = 23 500–34 000) Cotton effect at 227–228 nm, whereas the corresponding penicillins show a positive extremum of 24 100–36 000 (depending on solvent) at 233–237 nm.[9] The similarity of these Cotton effects is not hard to understand when one realizes that the relevant EH MO's of the sulfoxide are in the sequence: S 3d, amide π^*, carboxyl π^* (LEMO), HOMO, NHOMO, and amide n. This sequence holds whether the sulfoxide configuration is *R* (oxygen on α side in **1**) or *S* (oxygen on β side). The highest occupied MO (HOMO) is essentially a linear combination of the remaining sulfur lone pair lobe and coplanar 2p-shaped lobes on the sulfoxide oxygen. The next highest occupied MO (NHOMO) is mostly a lone pairon the amide N atom in the *R* sulfoxide, but this character mixes with the sulfur lone pair in the *S* sulfoxide to become an in-phase, transannular combination of the two lone pairs which we have been calling an S-N π-type MO. The NHOMO → amide π^* transition is calculated to occur in the range 230–238 nm, which is a few nanometers shorter than for the S-N π → amide π^* transition in the nonoxidized penams. In more recent calculations being published, we find that not only is the λ_{max} of the Cotton effect arising from this penam sulfoxide transition in reasonable agreement with experiment, but its sign and molar ellipticity are also satisfactorily predicted. Our assignment contrasts with that of Siemion et al.[9] They hypothesized that the long wavelength CD band of both penams and penam sulfoxides arises from localized sulfur n → σ^* type excitations. They did not consider transition mixing of the chromophores.

Results from Other MO Methods and the Participation of S 3d Orbitals. As mentioned in the Experimental Section, some

CNDO and INDO MO methods were also tried early in the course of our study. None of these methods proved as satisfactory for the penams as the EH method. The main problem with CNDO/2 and the other NDO parameterizations is that the localized sulfur excitations dominate the lower energy transitions and the CI moves these to very low energies. From a typical CNDO/2D-CI calculation, the two lowest energy transitions are mainly of the S n → S-C σ^* type and occur at unreasonably long wavelengths (274 and 341 nm). The third lowest energy transition is the amide n → π^* with only a small contribution from the 3-carboxyl group; this transition is at unreasonably short wavelengths (165 nm). Some calculated rotatory strengths seem to be too small or of unreasonable sign. The CNDO/2D-CI oscillator strengths for the transitions do not appear to have the right magnitudes relative to each other. Elimination of the S 3d atomic orbitals from the CNDO/2 basis set somewhat improves the situation by raising the energy of the S-C σ^* orbitals. However, the spectral predictions are still unsatisfactory. Other parameterizations[23] of the NDO-MO schemes were tried, but it appears very difficult to find one set of parameters which will work adequately for the penam ring system, as well as other less complex sulfides, amides, and carboxylic acids. The penam nucleus stands as a rigorous challenge to all present and future parameterizations of the NDO methods in regard to their ability to correctly predict excited state descriptions.

The reader may have noticed that the EH method assigns predominantly sulfur 3d character to one of the low-lying empty MO's of sulfides (see Table III, supplementary material, and ref 10). On the other hand, CNDO/2 places empty MO's which can be more nearly labeled as S-C σ^* orbitals below the more pure S 3d MO's. There is some d character in the CNDO/2 S-C σ^* orbitals, but their antibonding σ^* character is much more pronounced than in the EH MO's. This contrast between EH and CNDO/2 has been encountered previously.[10,13,33,34] Although the different approximations in the EH and CNDO/2 methods cannot help but give somewhat different descriptions of electronic structure, the question of the nature of the excited state orbitals of sulfides is partially a question of subjective semantics. In other words, how large do the LCAO-MO coefficients of the 3d orbitals have to be before an antibonding virtual orbital is labeled "empty S 3d" rather than "S-C σ^*"? Some recent ab initio[35] and semiempirical[36] MO treatments sulfides and a recent experimental study of the electronic absorption spectrum of dimethyl sulfide[37] have tended to minimize the role of the 3d type MO's. Eliminating the empty 3d type MO (NNLEMO of Table III) from consideration in the penam CD spectral assignments would not significantly alter our results because rotatory strengths involving this MO are all found to be very small. Likewise, it was prevously shown that the empty d-type MO's are involved in transitions of low oscillator strengths also.[10] However, the S 3d AO's remain important in the EH description as basis functions because of their small admixture into the other virtual orbitals. The above-mentioned ab initio[35] study of the optical activity of simple sulfides also conferred importance on the d orbitals in regard to quantitative, but not qualitative, predictions.

Conclusions

In summarizing this investigation of the chiroptical spectral properties of the penam nucleus of penicillins, the following points warrant recapitulation.

(1) The EH method, as least in the case of penam and some other β-lactam molecules, appears to yield a fortuitously satisfactory description of their excited state electronic structures. No doubt ab initio methods would yield more refined wave functions, and other minor electronic transitions in the 200–240-nm region would turn up. However, the EH MO's are adequate for predicting the gross features of the CD spectra of 6-APA, and they even give good quantitative agreement. The CNDO/INDO methods appear to need further parameterization before they will be able to do as well. Mention should also be made of the fact that the capability of the computer programs to evaluate rotatory strengths without integral neglect or approximation greatly enhanced our ability to reach these conclusions.

(2) The first Cotton effect in penams which occurs at ca. 235 nm with a large positive molar ellipticity is calculated quite well from the EH MO's. The band represents a superposition of several transitions, the main ones being of intramolecular charge transfer character with the sulfide group acting as the electron donor and the amide group as the electron acceptor. The mutual rturbations of the two chromophores are also evident in the transitions of this band. The strength of the first Cotton effect results from the inherent asymmetry of the penam nucleus. Upon standing in basic solutions, the β-lactam C-N bond of 6-APA and other penicillins is hydrolytically cleaved over a period of time. This reaction can be detected by the gradual disappearance of the first CD band. Although some earlier authors regarded this phenomenon as proof of a β-lactam amide n → π^* assignment, we see that, in fact, the rupture of the C-N bond destroys the acceptor part of the excited molecule.

(3) The second Cotton effect in penams, which some authors have thought to arise from a β-lactam n → π^* transition, is indeed predicted as such by our calculations. This band peaks close to 200 nm. The pH sensitivity of this band is explainable in terms of the small mixing of the amino group orbitals into the amide orbitals; the penam conformation could conceivably also be involved.

(4) In regard to the calculational models, we find that the penam nucleus itself is adequate for calculating the CD assignments of 6-APA and various penicillins. The contributions of the 3-carboxyl group to the calculated spectra are not large. Rotation of the carboxyl group has little consistent effect on the transitions. The conformation of the thiazolidine ring has a small effect on the predicted molar ellipticities: flipping the penam model from the 2β-equatorial, 3α-equatorial form to the 2β-axial, 3α-axial conformation causes the molar ellipticity of the first Cotton effect to decrease in magnitude and that of the second Cotton effect to increase in magnitude. The axial-type conformers are about 2 kcal/mol more stable according to the EH calculations and are nearly equivalent in stability by our CNDO/2 calculations. The coexistence of more than one structural form of 6-APA in solution seems likely.

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Supplementary Material Available: Tables VI–XXIII of spectral properties and Figures 2–12 of CD spectra (20 pages). Ordering information is given on any current masthead page.

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Structure and Force Fields of the Isomers of Dinitrogen Dioxide

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Abstract: The fully optimized geometries of the cis and trans isomers of dinitrogen dioxide, $(NO)_2$, have been calculated ab initio. No other minima in the potential surface corresponding to other possible stable isomers could be found. Vibrational force fields, compliance constants, and vibrational frequencies were computed. The localized orbital wave functions, structures, and force fields all show the importance of lone pair delocalization in weakening the N–N bond and of a weak O···O bond in stabilizing the cis form.

Although the nitric oxide dimer has been observed by many types of experiments,[2-10] its structure is not well known, the only two determinations being an x-ray study of the solid[10] and a gas phase infrared band shape analysis.[6] These two structure determinations differ greatly from each other and have large experimental uncertainties.[11,12] Two previous ab initio calculations using minimum basis sets[13,14] yielded results disagreeing both with each other and with experiment, the most important error being the failure to reproduce the uncommonly long N–N bond. We therefore decided to extend our preliminary studies which used a double ζ basis set[15] to a systematic calculation of the structures, force fields, and relative stability of the isomers of $(NO)_2$.

Method of Calculation

The SCF calculations employed the FORCE method of Pulay,[16] by which the forces acting on the atoms are calculated analytically as derivatives of the energy with respect to the coordinates. All calculations, unless otherwise stated in the discussion of the results, were done with a (7,3) basis set of Gaussian lobe functions contracted to $\langle 4,2 \rangle$.[17] The resulting canonical molecular orbitals were transformed into localized orbitals using the criterion of Boys.[18] The relative energies of the isomers found to be stable with this basis set were also calculated using (9,5), (9,5,1) and (10,6,1) basis sets[19] contracted to $\langle 4,2 \rangle$, $\langle 4,2,1 \rangle$, and $\langle 5,3,1 \rangle$.

Structures of the Stable Isomers

Only two isomers of $(NO)_2$ having minima in the energy surfaces were found, the planar cis and the planar trans species with energies of −257.988 379 6 and −257.986 168 8 au, respectively. Various other structures were calculated. A peroxide structure, Figure 1a, with O–O = 1.48 Å, N–O = 1.16 Å, and ∠OON = 107° was 87 kcal above the cis form. The cyclic structure of ref 14, shown in Figure 1b, was 48 kcal less stable than the cis form. A rectangular structure, Figure 1c, with N–O = 1.77 Å and N=O = 1.16 Å was 65 kcal less stable than the cis structure. All of these forms have very large forces