Molecular and Crystal Structure of the Linear Tricatechol Siderophore, Agrobactin

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Abstract: Agrobactin, or *N*-[3-(2,3-dihydroxybenzamido)propyl]-4-[4-(2,3-dihydroxybenzamido)butyl]-2-(2,3-dihydroxyphenyl)-*trans*-5-methyl-2-oxazoline-4-carboxamide, is the new linear tricatechol-type siderophore of *Agrobacterium tumefaciens*. The ligand was crystallized as the ethyl acetate solvate ($C_{32}H_{36}O_{10}N_4 \cdot 2C_4H_8O_2$, 812.9 g/fw) in the orthorhombic space group $P2_12_12_1$ ($Z = 4$), with unit cell dimensions $a = 17.846$ (18) Å, $b = 28.634$ (19) Å, and $c = 7.835$ (3) Å at $V = 4003.7$ Å^3 and at −135 (2) °C. The structure was refined by least-squares methods to an R factor of 0.052 (3749 observed data) and 0.071 for all 4627 unique reflections. The molecule assumes a conformation which is relatively flat (3.842-Å thickness) and is efficiently stabilized by five relatively strong intramolecular hydrogen bonds. The formation of the oxazoline ring by the carbonyl group of one of the 2,3-dihydroxybenzoic acid moieties and the C^α–C^β-N^α atoms of L-threonine is accompanied by inversion of the C^β carbon atom. Three amide bonds link the ligating functional groups to the spermidine backbone. The results show that a large conformational change must occur on chelation with trivalent iron.

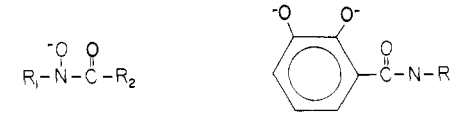
Introduction

Siderophores are low molecular weight (500–1500 daltons) Fe-(III) specific chelating agents syntheized by eucaryotic and procaryotic aerobic and faculative anaerobic microorganisms to facilitate growth.[1-3] These potent ligands are thought to mediate the high-affinity transport system for ferric ion across the cell membrane by functioning as either free or membrane-bound ionophores or as nontransportable sequestering agents.[4] Seven families of cyclic and linear siderophores have been characterized, which utilize two general classes of ligating groups, the hydroxamates (A), and the catecholates (B). Ferrichrome,[5] a cyclic hexapeptide containing three residues each of glycine and N^δ-acetyl-N^δ-hydroxy-L-ornithine,[6] and enterobactin (enterochelin), a cyclic triester of 2,3-dihydroxy-*N*-benzoyl-L-serine,[7,8] are prototypes of the two classes and are the most widely characterized to date. Both types of siderophores give rise to 5-membered chelate rings and form very stable[9,10] ($K_f = 10^{29.1}$ and 10^{52}, respectively) octahedral, high-spin[11] ferric chelates.

Enterobactin, the siderophore produced by *Escherichia coli*,[7] *Salmonella typhimurium*,[8] and *Aerobacter aerogenes* (now *Klebsiella pneumoniae*),[7,12] is the sole cyclic tricatechol-type siderophore known; linear monomeric and dimeric species, 2,3-dihydroxybenzoylglycine and bis(2,3-dihydroxybenzoylserine), have been isolated from cultures of *Bacillus subtilis*[13] and *Azotobacter vinelandi*,[14] respectively. Recently, the chemical structure of the linear tricatechol originally identified[15] as N^1,N^8-bis(2,3-dihydroxybenzoyl)-N^4-(*N*-salicylthreonyl)spermidine from *Micrococcus* (now *Paracoccus*) *denitrificans* was reexamined, revised[16] to *N*-[3-(2,3-dihydroxybenzamido)propyl]-*N*-[4-(2,3-dihydroxybenzamido)butyl]-2-(2-hydroxyphenyl)-5-methyloxazoline-4-carboxamide, and called parabactin.

The chemical structure of a second linear tricatechol type siderophore was elucidated[17] as *N*-[3-(2,3-dihydroxybenzamido)propyl]-*N*-[4-(2,3-dihydroxybenzamido)butyl]-2-(2,3-dihydroxyphenyl)-*trans*-5-methyl-2-oxazoline-4-carboxamide, with the trivial name agrobactin. It is a close structural analogue of parabactin and was recently isolated from "low-iron" cultures of the pathogen, *Agrobacterium tumefaciens*, an organism known to induce crown-gall in higher plants.[18]

Chart I



A B

The molecular structure and absolute configuration of agrobactin and siderophores in general (both the metal-free ligands and the ferric chelates) are of particular interest for a number of reasons. First, significant conformational changes must occur in the ligand upon coordination of ferric ion[19,20] which are intimately related to the ability of the chelate to be actively assimilated. Second, there is increasing evidence suggesting stereospecific requirements of the chelate for transport,[21] that is, a "hand-in-glove" relationship between the chelate and its specific outer membrane receptor. This is further exemplified by the observation that the ferric enterobactin cannot be utilized by *P. denitrificans*, while neither ferric agrobactin nor ferric parabactin are actively assimilated by *E. coli*.[22] There is another interesting difference between enterobactin, agrobactin, and parabactin. In enterobactin, the ferric chelate is formed by coordination to the three catechol moieties, forming a Δ-cis configuration,[23] while in agrobactin and parabactin the iron coordination sphere is Λ-cis and is suspected to involve two catechol rings and the imine N of the oxazoline ring and the *o*-hydroxy group of the third and neighboring catechol.[22]

To date no single-crystal X-ray diffraction study has been made of any tricatechol-type siderophores or their ferric chelates nor, for that matter, of any natural siderophore (free ligand). The crystal structures of several model tris catecholato complexes of Fe^{3+} and Cr^{3+} have been reported.[24] Also, no X-ray diffraction

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Table I. Crystal Data and Intensity Data Collection

mol formula: $C_{32}H_{36}O_{10}N_4 \cdot 2C_2H_5OC(O)CH_3$
fw: 812.87 g
cryst system: orthorhombic
space group: $P2_12_12_1$, $Z = 4$
unit cell dimens [−135 (2) °C]: a = 17.846 (18) Å, b = 28.634 (19) Å, c = 7.835 (3) Å, V = 4003.7 Å^3
ρ_{calcd} = 1.348 g/cm^3
crystallizatn: ethyl acetate–ethanol equilibrated with hexane under N_2 at 0 °C
cryst size: 0.045 × 0.087 × 0.587 mm
diffractometer: Enraf Nonius CAD-4; PDP8/e controlled
radiatn: Cu Kα (λ = 1.541 78 Å); 40 KV, 26 ma
temp: −135 (2) °C
2θ limit: 0–150°
scan technique: θ–2θ
scan angle: $\theta = (1.0 + 0.14 \tan \theta)°$
scan time: 60 s
receiving aperature: width, variable, (5.5 + 0.86 tan θ) mm; height, 6 mm; distance, 173 mm
no. of unique reflctns: 4627
no. of obsd reflctns $I > 2\sigma(I)$: 3806
intens monitors: 3 reflctns every 75 m of X-ray exposure
orientatn control: 3 reflctns every 200 measurements

study and description of the oxazoline ring has been reported, although it does appear in the structures of the membrane-bound siderophore, mycobactin P,[25] and the steroidal glycoside, phytolaccagenin.[26]

Recently, a NMR study has been made on the structure and behavior of spermidine siderophores, suggesting the existence of two conformational isomers in solution.[27] We report here the molecular and crystal structure of agrobactin, as determined by single-crystal X-ray diffraction, and define for the first time the structure of the oxazoline group. Also, the stereochemistry involved in oxazoline ring closure and the potential conformational changes in the ligand accompanying iron coordination are discussed.

Experimental Section

Crystallization and Intensity Data Collection. A 10-mg sample of the title compound was obtained from Professor J. B. Neilands, Department of Biochemistry, University of California, Berkeley. Clusters of thin colorless rectangular plates were obtained by repeated crystallization from solutions of ethyl acetate and a minimal amount of ethanol (OMNISOLV, MCB Manufacturing Chemists, Inc.) equilibrated with hexane under N_2 at 0 °C. The latter precautions (inert atmosphere, low temperature) were implemented in an effort to reduce the chances of oxidation of the catechol moiety. At room temperature (22 °C), the crystals were moderately unstable and developed internal fractures from volatilization of solvate molecules; however, the crystals were stable at low temperature (−135 °C).

Single crystals were selected, mounted, and quickly transferred from the mother liquor to the low-temperature nitrogen gas stream. The unit cell dimensions (Table I) were determined by a least-squares fit to the 2θ values of 48 reflections. On the basis of observed systematic absences ($h00$, $h = 2n + 1$; $0k0$, $k = 2n + 1$; $00l$, $l = 2n + 1$), the noncentrosymmetric orthorhombic space group $P2_12_12_1$ was uniquely determined. No meaningful room-temperature cell constants and density measurements could be obtained due to the instability of the crystals at that temperature.

The intensities of all 4627 unique reflections with $2\theta \leq 150°$ were measured by using Cu Kᾱ radiation on an Enraf-Nonius CAD-4 diffractometer equipped with a low-temperature nitrogen stream cooling device, having a working temperature of −135 (2) °C. The method of data collection has been described.[28] Specific parameters are given in Table I. The data were corrected for Lorentz and polarization factors but were not corrected for absorption effects.

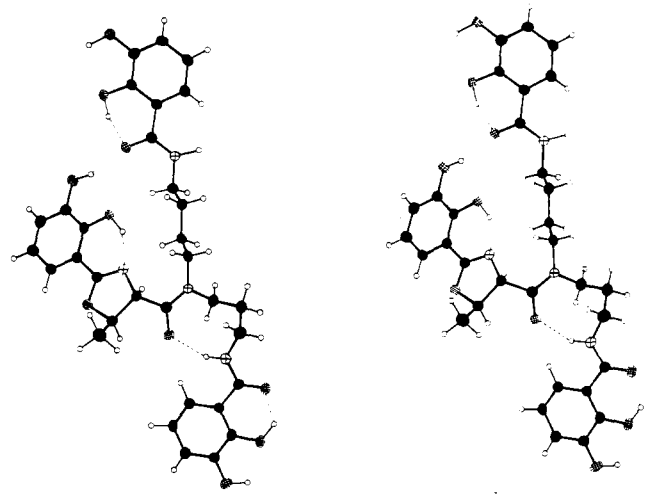

Figure 1. Stereoscopic presentation of a single molecule of agrobactin, without ethyl acetate solvate molecules, viewed down the c axis. C, N, O, and H atoms are presented as shaded, crossed, lined, and open, respectively.

Structure Solution and Refinement. The structure of agrobactin was solved by direct methods, using the program MULTAN 78[29] and successive difference Fourier syntheses. The phases of 325 reflections with the highest E values ($E \geq 1.66$) were generated by tangent formulas and refined. The largest peaks in the E map revealed 44 of the 46 nonhydrogen atoms of the molecule in two chemically recognizable fragments. The positions of these atoms were refined isotropically to an R factor ($R = [\sum(|kF_o| - |F_c|)]/\sum|kF_o|]$) of 0.169 for the observed data. A difference Fourier map was computed, which yielded 14 significant peaks (e/Å^3 = 2.1 − 4.7). Two of these peaks were at positions corresponding to the missing methyl [C(43)] and methylene [C(4)] carbon atoms of the postulated chemical structure while the remaining 12 peaks were assigned as two molecules of ethyl acetate solvate. All atomic parameters were refined isotropically to an R value of 0.111 and then anisotropically to 0.095 for all data. The atomic positions of 46 of the 52 independent hydrogen atoms were obtained from two successive difference Fourier syntheses. The remaining 6 hydrogen atoms associated with the terminal methyl carbons of one ethyl acetate molecule appeared disordered. The positions of these 104 atoms were refined, the nonhydrogen atoms anisotropically and the hydrogen atoms isotropically, until the maximum parameter shifts were less than 0.4 of their corresponding standard deviation. The final R factor was 0.052 for 3749 observed reflections and 0.071 for all 4627 data, while the final difference Fourier map was featureless.

All structure factor refinements were carried out with a block-diagonal least-squares program[30] in which the quantity $\sum W_F(|kF_o| - |F_c|)^2$ was minimized. The weight W_F for each structure amplitude was defined as $W_F = 1/\sigma_F^2$, where σ_F^2 was obtained from counting statistics.[28] The scattering factors for C, O, and N atoms were taken from ref 31 and those for hydrogen from Stewart, Davidson and Simpson.[32]

Crystallographic Results and Discussion

General Description of the Structure. The final atomic coordinates of the nonhydrogen atoms and selected hydrogen atoms are given in Table II. A complete listing of the thermal parameters for the nonhydrogen atoms, positional parameters, and isotropic temperature factors for all hydrogen atoms are provided with the supplementary materials.

A stereoscopic view of a single molecule of agrobactin is given in Figure 1; the atom numbering scheme is presented in Figure 2. The molecular structure of the title compound, as determined

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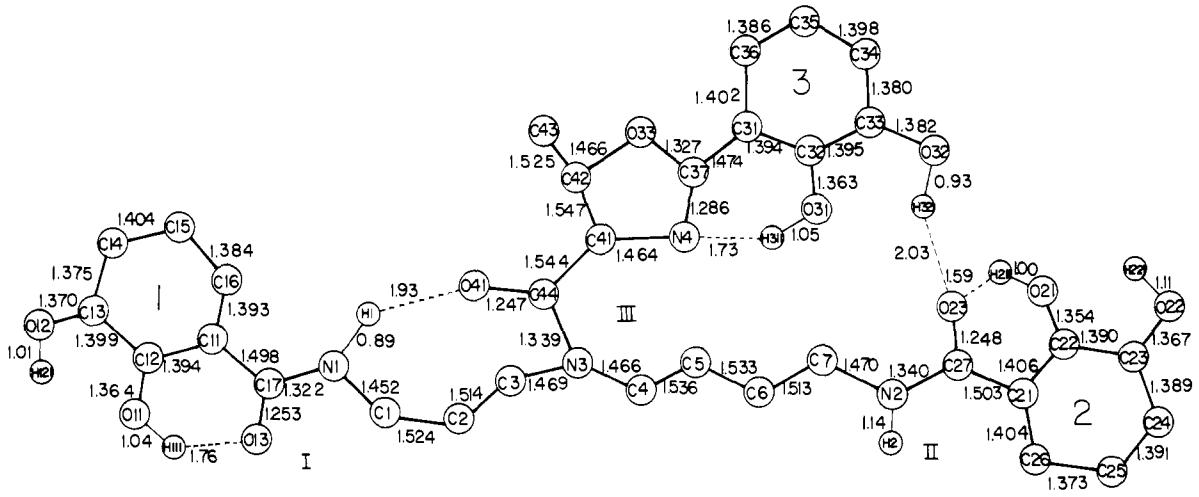

Figure 2. Atom numbering scheme and bond distances (in Å) for all C, N, O, and selected hydrogen atoms in agrobactin. Calculated standard deviations are in the range 0.004–0.006 Å.

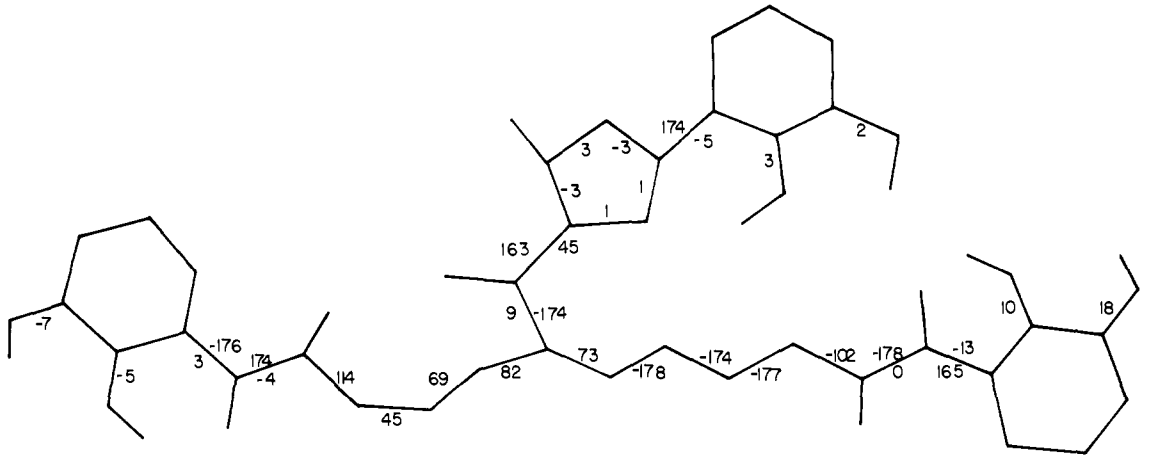

Figure 3. Torsion angles in agrobactin (in degrees). Standard deviations are between 0.4 and 0.6° for angles involving C, N, and O atoms. The standard deviations are 4–5° if the angle involves an H atom. A complete listing of the conformation angles for amide groups I, II, and III are given in Table IV.

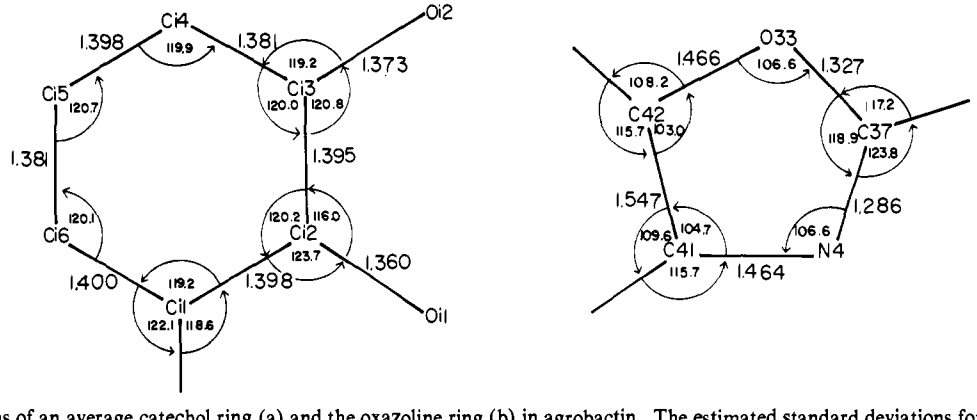

Figure 4. Dimensions of an average catechol ring (a) and the oxazoline ring (b) in agrobactin. The estimated standard deviations for the bond distances and angles are 0.004 Å and 0.6° in a and 0.005 Å and 0.3° in b.

by single-crystal X-ray diffraction, confirms the chemical structure postulated by Ong, Peterson, and Neilands.[17] It is described as an L-threonyl peptide of spermidine, linked at its secondary amine nitrogen (amide group III). Two residues of 2,3-dihydroxybenzoic acid (2,3-DHB 1 and 2) are linked to the terminal primary amine nitrogens of spermidine (amide groups I and II). The carbonyl group of a third 2,3-DHB(3) moiety, C(37) and O(33), and the N-C$^{\alpha}$-C$^{\beta}$ atoms of L-threonine, N(4), C(41), and C(42), participate in the formation of a 5-methyl-*trans*-2-oxazoline ring.

Bond Distances and Angles. The bond lengths for the carbon, oxygen, nitrogen, and selected hydrogen atoms are given in Figure 2. Bond and torsion angles are presented in Table III and Figure 3, respectively.

The bond distances and angles in the three catechol groups are closely the same. The largest individual differences are 0.014 Å and 2.1°. An average catechol group is presented in Figure 4a. It is interesting to note that the *m*-C–O distance is longer than the *o*-C–O length and that the C(*i*3)–C(*i*4) and C(*i*5)–C(*i*6) distances are shorter than the other four aromatic C–C bond lenghts. Also, the pattern of the O–C–C angles should be noted, in which the C(*i*1)–C(*i*2)–O(*i*1) angles are significantly larger than the C(*i*3)–C(*i*2)–O(*i*1) angles. This, however, is not a result

Table II. Positional Parameters for Oxygen, Nitrogen, Carbon, and Selected Hydrogen Atoms in Agrobactin[a]

atom	10^5x	10^5y	10^4z
O(11)	4 078 (17)	7 738 (10)	4700 (5)
O(12)	−459 (18)	15 635 (12)	3265 (6)
O(13)	14 720 (16)	2 897 (10)	6010 (4)
O(21)	99 404 (15)	−493 (8)	5754 (4)
O(22)	111 006 (15)	−6 438 (10)	6309 (4)
O(23)	85 472 (15)	769 (9)	5407 (4)
O(31)	72 637 (15)	8 939 (9)	5756 (4)
O(32)	86 215 (16)	10 440 (9)	4279 (4)
O(33)	56 368 (15)	19 511 (9)	5790 (4)
O(41)	39 257 (15)	11 175 (9)	5788 (4)
N(1)	26 542 (19)	5 728 (12)	6166 (5)
N(2)	77 591 (18)	−5 359 (11)	5052 (4)
N(3)	46 582 (17)	4 771 (11)	6013 (4)
N(4)	58 959 (18)	11 984 (11)	6372 (4)
C(1)	29 893 (24)	2 035 (16)	7182 (6)
C(2)	35 556 (24)	−817 (15)	6166 (6)
C(3)	41 004 (23)	1 971 (14)	5090 (6)
C(4)	53 282 (22)	2 294 (13)	6607 (5)
C(5)	58 730 (21)	987 (13)	5166 (5)
C(6)	65 488 (21)	−1 740 (14)	5848 (5)
C(7)	71 232 (21)	−2 529 (14)	4458 (6)
C(11)	17 083 (23)	10 380 (13)	4785 (6)
C(12)	9 538 (23)	10 951 (14)	4376 (7)
C(13)	7 009 (24)	15 073 (15)	3611 (7)
C(14)	11 984 (25)	18 583 (15)	3222 (7)
C(15)	19 636 (24)	17 966 (14)	3573 (7)
C(16)	22 104 (22)	13 905 (13)	4347 (6)
C(17)	19 440 (22)	6 020 (14)	5696 (6)
C(21)	90 401 (21)	−6 795 (13)	6001 (5)
C(22)	97 752 (22)	−5 033 (12)	6054 (5)
C(23)	103 765 (21)	−7 984 (14)	6386 (5)
C(24)	102 434 (22)	−12 620 (14)	6812 (5)
C(25)	95 132 (23)	−14 320 (13)	6855 (6)
C(26)	89 190 (22)	−11 483 (13)	6448 (5)
C(27)	84 232 (22)	−3 517 (13)	5479 (5)
C(31)	68 464 (22)	16 716 (13)	4961 (5)
C(32)	73 828 (21)	13 179 (13)	5012 (5)
C(33)	80 787 (22)	13 879 (14)	4243 (6)
C(34)	82 369 (23)	18 066 (15)	3448 (6)
C(35)	77 049 (24)	21 649 (14)	3424 (6)
C(36)	70 070 (22)	20 982 (13)	4161 (6)
C(37)	61 060 (21)	15 917 (13)	5741 (5)
C(41)	51 278 (21)	12 642 (13)	6983 (5)
C(42)	49 363 (22)	17 766 (13)	6532 (5)
C(43)	47 294 (26)	20 892 (15)	8036 (7)
C(44)	45 346 (22)	9 357 (14)	6198 (5)
Ethyl Acetate Molecules			
O(51)	78 701 (18)	14 676 (10)	9285 (4)
O(52)	81 873 (18)	21 701 (10)	8280 (5)
O(61)	16 953 (26)	19 911 (13)	8495 (6)
O(62)	13 691 (20)	12 649 (11)	9160 (5)
C(51)	69 437 (27)	20 755 (16)	9138 (7)
C(52)	77 043 (26)	18 650 (14)	8933 (6)
C(53)	89 481 (27)	20 057 (16)	8008 (7)
C(54)	93 821 (33)	23 866 (19)	7132 (9)
C(61)	26 541 (32)	14 521 (22)	9395 (9)
C(62)	18 663 (33)	16 036 (16)	8955 (7)
C(63)	5 936 (31)	14 058 (18)	8863 (8)
C(64)	1 180 (34)	9 808 (19)	9184 (10)
Hydrogen Atoms			
	10^4x	10^4y	10^4z
H(1)	2964 (25)	815 (15)	6009 (65)
H(2)	7562 (33)	−915 (19)	5090 (84)
H(111)	594 (31)	459 (19)	5204 (84)
H(121)	−315 (30)	1261 (18)	3507 (78)
H(211)	9428 (28)	89 (17)	5744 (79)
H(221)	11139 (33)	−256 (21)	6380 (87)
H(311)	6711 (27)	877 (16)	6213 (68)
H(321)	8480 (29)	767 (18)	4809 (77)

[a] Calculated standard deviations for the least significant digit are given in parentheses.

Table III. Bond Angles (Deg) in Agrobactin[a]

angle	i = 1	i = 2	i = 3
C(i1)-C(i2)-C(i3)	120.6 (4)	120.5 (3)	119.6 (4)
C(i2)-C(i3)-C(i4)	120.2 (4)	119.6 (4)	120.1 (4)
C(i3)-C(i4)-C(i5)	119.5 (4)	120.0 (4)	120.3 (4)
C(i4)-C(i5)-C(i6)	120.1 (4)	120.7 (4)	120.2 (4)
C(i5)-C(i6)-C(i1)	120.8 (4)	120.3 (4)	119.3 (4)
C(i6)-C(i1)-C(i2)	118.7 (4)	118.6 (3)	120.3 (4)
C(i2)-C(i1)-C(i7)	118.6 (4)	117.9 (3)	119.4 (4)
C(i6)-C(i1)-C(i7)	122.7 (4)	123.5 (3)	120.2 (4)
C(i1)-C(i2)-O(i1)	124.6 (4)	122.9 (3)	123.5 (3)
C(i3)-C(i2)-O(i1)	114.7 (4)	116.6 (3)	116.8 (3)
C(i2)-C(i3)-O(i2)	119.9 (4)	121.6 (3)	120.9 (4)
C(i4)-C(i3)-O(i2)	119.9 (4)	118.8 (3)	119.0 (4)
C(i1)-C(i7)-O(i3)	120.0 (4)	119.8 (3)	117.2 (3)
N(i)-C(i7)-O(i3)	123.0 (4)	122.2 (4)	
C(i1)-C(i7)-N(i)	117.0 (4)	118.0 (3)	

angle		angle	
C(17)-N(1)-C(1)	126.4 (4)	N(4)-C(37)-O(33)	118.9 (3)
N(1)-C(1)-C(2)	112.2 (4)	C(37)-N(4)-C(41)	106.6 (3)
C(1)-C(2)-C(3)	115.7 (4)	N(4)-C(41)-C(42)	104.7 (3)
C(2)-C(3)-N(3)	116.7 (4)	C(41)-C(42)-O(33)	103.0 (3)
C(3)-N(3)-C(4)	116.4 (3)	C(42)-O(33)-C(37)	106.6 (3)
C(3)-N(3)-C(44)	118.5 (3)	C(41)-C(42)-C(43)	115.7 (3)
C(4)-N(3)-C(44)	125.1 (3)	O(33)-C(42)-C(43)	108.2 (3)
N(3)-C(4)-C(5)	113.7 (3)	N(4)-C(41)-C(44)	115.7 (3)
C(4)-C(5)-C(6)	111.5 (3)	C(42)-C(41)-C(44)	109.6 (3)
C(5)-C(6)-C(7)	111.0 (3)	C(41)-C(44)-N(3)	121.8 (3)
C(6)-C(7)-N(2)	112.2 (3)	C(41)-C(44)-O(41)	116.5 (3)
C(7)-N(2)-C(27)	123.0 (3)	N(3)-C(44)-O(41)	121.7 (4)
C(31)-C(37)-N(4)	123.8 (4)		
Ethyl Acetate Molecules			
C(51)-C(52)-O(52)	111.4 (4)	C(61)-C(62)-O(62)	112.7 (5)
C(51)-C(52)-O(51)	125.3 (4)	C(61)-C(62)-O(61)	124.6 (5)
C(52)-O(52)-C(53)	116.7 (4)	C(62)-O(62)-C(63)	114.4 (4)
O(52)-C(53)-C(54)	108.3 (4)	O(62)-C(63)-C(64)	106.5 (5)

[a] Standard deviation of the last significant digit is given in parentheses.

of the exocyclic substitution on C(i1), because the same pattern is observed in free catechol.[33,34] The H atoms of the hydroxyl groups are approximately coplanar with the catechol groups to which they are attached, as can be seen from the torsion angles given in Figure 3, while in all of the three catechol moieties the OH groups have the same relative direction as is indicated in Figures 2 and 3.

The 5-methyl-*trans*-2-oxazoline ring[35] results from the yet undescribed enzymatic coupling of the carboxy group of 2,3-DHB 3 with the atoms N(4)–C$^{\alpha}$(41)–C$^{\beta}$(42) of threonine. Hitherto, the dimensional parameters defining an oxazoline moiety have not been described, despite the fact that this group has appeared in the crystal structures of several natural compounds including the siderophore, mycobactin P,[25] and the steroidal glycoside, phytolaccagenin.[26] The bond distance and angles for the oxazoline ring are summarized in Figure 4b and reflect certain similarities with other 5-membered heterocycles containing O and N atoms such as isoxazoline[36,37] and oxazolo[38,39] compounds. The C(37)–O(33) length (1.327 Å) is significantly shorter than the C(42)–O(33) bond distance (1.466 Å), while the same is true for

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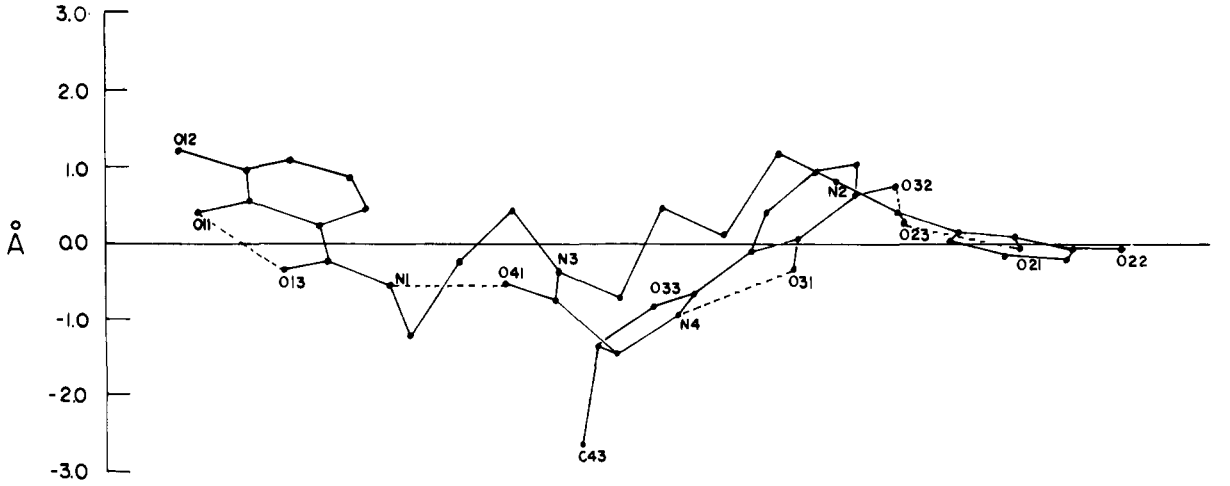
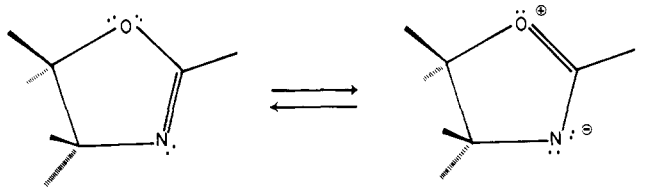


Figure 5. Deviations of all nonhydrogen atoms in agrobactin from the least-squares plane through these atoms (in Å).

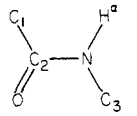
Scheme I



the C(37)–N(4) length (1.286 Å) with respect to the C(41)–N(4) bond distance (1.464 Å). This indicates similar double-bond character in both O(33)–C(37) and C(37)–N(4), as reflected in the resonance structures in Chart I. Due to the charged nature of the second resonance structure, one may expect N(4) to be a strong hydrogen bond acceptor and O(33) to be a poor acceptor. This is born out by the hydrogen bonding scheme (vide infra), which shows O(33) to be the only oxygen atom not involved in any hydrogen bond.

The configuration of the exocyclic atoms, C(43) and C(44), about the two adjacent asymmetric carbon atoms [C^{α}(41) and C^{β}(42)] of the oxazoline ring is trans, as proposed by Ong, Peterson, and Neilands.[17] The absolute configuration of agrobactin has not been proven in the present study, but the L configuration for C^{α}(41) has been assumed and is shown in all figures. The relative configuration of the threonine moiety, which can be recognized in the oxazoline ring, is L-(*R*)-allothreonine, in contrast to the chromatographic, after acid hydrolysis, finding of L(*S*)-threonine in agrobactin.[17] With the assumption that L(*S*)-threonine, the biologically active stereoisomer of threonine,[40,41a,b] is the precursor in agrobactin biosynthesis, it is apparent that on oxazoline ring formation in *A. tumefaciens*, the L configuration at C^{α} is retained. If this is indeed the case, there is inversion (*S* → *R*) at C^{β} to obtain the relative configuration observed. Two reasonable mechanisms might be advanced. If L(*S*)-threonine is the precursor, ring closure and inversion at C^{β} would occur simultaneously, if preceded by acylation of the α-amino group to form an N^{α}-benzoylthreonyl intermediate followed by imine formation. Some credence to this mechanism is found in the chemical studies of Attenburrow, Elliott, and Penny[42] with D,L-threonine. A second mechanism for oxazoline ring formation might involve the coupling of the 2,3-dihydroxybenzoic acid ester of serine with its α-amino group, as proposed by Martin and Parcell,[43] followed by methylation at C(42) to generate the appropriate asymmetric center.

Table IV. Conformational Angles Defining the Three Amide Bonds in Agrobactin



conformational angle	definition[b]	I	II	III
ω_1	C(1)–C(2)–N–C(3)	174.5 (4)	−177.6 (3)	−173.5 (4)
ω_2	O–C(2)–N–H	−174.0 (37)	−178.1 (43)	−174.1 (4)
ω_3	O–C(2)–N–C(3)	−4.1 (7)	0.3 (6)	8.5 (6)
ω_4	C(1)–C(2)–N–H	4.5 (38)	4.0 (43)	3.9 (6)
τ	$\tau = {}^1/_2(\omega_1 + \omega_2)$ $[\lvert\omega_1 - \omega_2\rvert < \pi]$	180.2 (37)	182.1 (43)	186.2 (6)
χ_C	$\omega_1 - \omega_3 + \pi$	−1.4 (8)	2.1 (7)	−2.0 (9)
χ_N	$\omega_2 - \omega_3 + \pi$	10.1 (38)	1.6 (44)	−2.6 (9)

[a] Atom C(4) in amide III. [b] See ref 50. [c] Estimated standard deviations for τ', χ_C, and χ_N, as $\sigma = (\sigma_1^2 + \sigma_2^2)^{1/2}$.

The bond distances and bond angles in the spermidine backbone of the molecule are normal and are in close agreement with those values reported for spermidine trihydrochloride.[44] The C–N distances, C(3)–N(3) and C(4)–N(3), average 1.467 Å, are the same as those observed in spermidine trihydrochloride and spermine tetrahydrochloride,[45] and are quite expected for tertiary amides.[46a–c] Several observations concerning the bond angles of the spermidine moiety in agrobactin deserve comment. The angles about the atoms C(4), C(5), C(6), and C(7), average value 111.6°, are normal and close to the tetrahedral angle of 109.5°. The butyl arm of spermidine is nearly planar (torsion angles, −178, −173, and −177°) and possesses the all anti conformation about these atoms. In contrast, the angles around atoms C(1), C(2), and C(3) of the propyl arm are significantly different (average 116.2°) from the expected 109.5° in spermidine. The opening of these angles is attributed to the steric effect of the gauche conformation about these atoms.

Amide Bonds. The two amide groups, I and II, are both trans and can be likened to peptide linkages. The bond distances in these groups are closely the same to those observed in cyclic[47a,b] and linear[48] peptides, with the possible exception of the C=O

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Table V. Hydrogen Bonding Interactions in Agrobactin

bond[a]	donor D	acceptor A	D-H, Å	H···A, Å	D-H···A, Å	D-H···A, deg
		Intramolecular				
i	O(32)-H(321)	O(23)[i]	0.93	2.03	2.910	156
ii	N(1)-H(1)	O(41)[i]	0.89	1.93	2.769	155
iii	O(31)-H(311)	N(4)[i]	1.05	1.73	2.637	142
iv	O(11)-H(111)	O(13)[i]	1.04	1.76	2.566	131
v	O(21)-H(211)[b]	O(23)[i]	1.00	1.59	2.527	154
		Intermolecular				
vi	O(12)-H(121)	O(32)[ii]	1.01	2.09	2.915	138
vii	O(22)-H(221)	O(13)[iii]	1.11	1.70	2.764	159
viii	O(11)-H(111)[b]	O(21)[ii]	1.04	1.92	2.633	123
ix	N(2)-H(2)	O(51)[iv]	1.14	1.87	2.956	157

[a] Bonds: i, x, y, z; ii, $x-1, y, z$; iii, $x+1, y, z$; iv, $1^1/_2 - x, \bar{y}, z - {}^1/_2$. [b] Bifurcated.

bonds, which together with the same bond in amide III appear significantly longer by 0.015, 0.022, and 0.014 Å. This may be caused by the strong intramolecular H bonds formed by these groups (vide infra).

The degree of nonplanarity of the three amide linkages can be described by the parameters τ', χ_N, and χ_C, defined by Winkler and Dunitz,[49] which describe respectively the rotation around the C–N bond, and the nonplanarity of the three bonds around the N and C atoms. From the results shown in Table IV, it can be seen that amide groups I and II are planar while the tertiary amide (III) experiences a small rotation around the N(3)–C(44) bond.

Planarity. The conformer of agrobactin crystallized from ethyl acetate–ethanol solution is remarkably flat, with a maximum thickness at any point of 3.842 Å. The deviation of each atom from the least-squares plane of the molecule is given in Figure 5.

The three aromatic rings of the catechol groups are planar. The *o*- and *m*-hydroxyl O atoms lie alternately above and below the planes of the rings by 0.025 and –0.011 Å, –0.104 and 0.075 Å, and –0.003 and 0.013 Å, for rings 1, 2, and 3, respectively. The corresponding hydroxyl protons, all of which are involved in intra- and intermolecular contacts, lie 0.09 and 0.11 Å, –0.27 and –0.25 Å, and –0.06 and –0.00 Å from these planes. Also, the oxazoline ring is planar and approximately coplanar with the attached 2,3-DHB group (torsion angle about C(31)–C(37) is –5°), which permits electron delocalization over the two rings, as is reflected in the short C(31)–C(37) bond length of 1.474 Å.

Hydrogen Bonding. The intramolecular and intermolecular contacts in agrobactin are extensive and efficiently stabilize the molecular structure in the crystalline state by utilizing all 8 active hydrogen atoms. The parameters defining these hydrogen bonds are tabulated in Table V.

There are five intramolecular hydrogen bonds in the molecular structure. Of the five, three [O(11)–H(111)···O(13), O(21)–H(211)···O(23), and O(31)–H(311)···N(4)] are quite strong and make 6-membered rings, all of which are essentially coplanar with the 2,3-DHB rings. The first of these contacts is bifurcated with a strong intermolecular hydrogen bond between O(11)–H(111)···O(21) (Table V). The N(1)···O(41) (2.769 Å) and the O(32)···O(23) (2.910 Å) intramolecular contacts are moderately strong. Both anchor the *N*-(3-(2,3-dihydroxybenzamido)propyl) and *N*-(4-(2,3-dihydroxybenzamido)butyl) groups about the tertiary amide group with respect to the 2,3-dihydrophenyl-*trans*-5-methyl-2-oxazoline-4-carboxamide group. The N(1)···O(41) contact requires the gauche conformation of the propyl arm of the spermidine backbone.

Crystal Structure. Agrobactin crystallizes as the ethyl acetate solvate in the orthorhombic space group $P2_12_12_1$ and possesses one relatively short crystal axis (c = 7.835 Å). The overall crystal-packing scheme can be described as stacks of molecules lying on top of one another. The two planar ethyl acetate solvate molecules are sandwiched between the stacked rings of the 2,3-DHB rings 1 and 2 at an average distance of 3.917 Å from the parallel planes of these rings. Both molecules are oriented along the C(i1)–C(i4) axis of the catechol rings. It is apparent that their purpose is merely for crystal packing, although there is one moderately strong (2.956 Å) intermolecular hydrogen bond between the carboxyl oxygen, O(51), by the symmetry transformation $1^1/_2 - x, y, {}^1/_2 + z$ (Table V) and N(2)–H(2). There are three additional intermolecular hydrogen bond interactions (see Table V). Of these, two [O(22)–H(211)···O(13) and O(11)–H(111)···O(21)] are unexpectedly strong, with the latter bifurcated with a strong intramolecular contact as described above.

Solution Conformations and Ferric Ion Chelation. Inspection of molecular models of agrobactin indicates that the structure reported here appears to represent the most stable, energetically favorable conformation of the molecule. It utilizes five intramolecular hydrogen bonds of which several are quite strong. It has been suggested[17] that the unusual α, β, and γ resonances of the ^{1}H NMR spectrum of the threonyl (oxazoline) group of agrobactin implicated the existence of a second conformation of the linear tricatehol in solution. This conclusion was supported by more recent findings[27] that in Me_2SO, the ratio of these two isomeric forms of agrobactin was unity and that these resonances coalesced to a more normal spectrum for threonine by warming. Speculatively, it was suggested[17] that the two solution conformers arose from alternate hydrogen bonding between the atoms O(31) and N(4) and O(31) and O(33). This is unlikely for two reasons. First, it implies rotation (~180°) about the C(31)–C(37) bond, which would require the mounting of a presumably large internal rotation energy barrier and the breaking of two strong intramolecular hydrogen bonds. Second, due to electron delocalization in the oxazoline ring, the ether O atom [O(33)] takes on a partial positive charge, rendering it unsuitable as a hydrogen acceptor. A nearly 180° rotation about the C(44)–C(41) bond would no doubt be more energetically favorable, with the breaking of only one internal hydrogen bond [O(23)–O(32)] and a small steric interaction of C(44) with the spermidine backbone.

An alternative ligand conformation for agrobactin in solution, recently proposed,[27] arises from rotation about the N(3)–C(44) tertiary amide bond resulting in cis–trans isomerization. However, for the most energetically favorable conformation to be obtained from such a rotation, additional rotations of the (2,3-dihydroxybenzamido)propyl and (2,3-dihydroxybenzamido)butyl groups are required with a concommitant change in the conformation of the propyl and butyl groups (gauche to staggered and anti to gauche, respectively). This would allow a maximum of five intramolecular contacts but requires a large energy of activation, i.e., the rotational barrier around N(3)–C(44) plus the breaking of two internal H bonds.

With regard to the chelation of ferric ion by agrobactin (and parabactin), the proposed model is based on two pieces of spectroscopic and chemical evidence.[22] First, in solution, the absolute configuration of the iron coordination sphere is Λ-cis, as determined by circular dichroism. And second, the chelate behaves as a dianion at neutral pH, with a proton count of five per mole. On the basis of these considerations, four of the six octahedral coordination sites of ferric ion are occupied by O(11), O(12), O(21), and O(22), in 5-membered chelate rings. The remaining

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positions allow coordination by O(31) and N(4), forming a 6-membered ring. Such a coordination model is not without precedence, having been observed in the crystal structure of ferrimycobactin P,[25] a linear dihydroxamate siderophore containing a single residue of *o*-hydroxyphenyloxazoline.

Upon examination of this coordination model,[22] it is apparent that a number of conformational changes occur in the ligand upon chelation. These include rotations (~180°) about bonds adjoining the tertiary amide carbonyl, N(3)–C(44) and C(44)–C(41), and about the bonds C(11)–C(17) and C(21)–C(27) to relieve the steric problems associated with close approach of O(13) and O(23) with the spermidine backbone. The butyl group undergoes a change from anti, anti, anti to gauche, anti, gauche conformations about the C(4)–C(5), C(5)–C(6), and C(6)–C(7) bonds, while the propyl arm remains gauche. Amide bonds I and II remain coplanar with 2,3-DHB groups 1 and 2; coplanarity of the oxazoline ring and 2,3-DHB 3 is retained, and the chelate is stabilized by at least one intramolecular hydrogen bond [O(41)–N(2)]. An alternative model[50] for metal coordination by agrobactin is the tricatecholate mode of chelation, made possible by the deprotonation of the meta catechol oxygen, O(32), and the transfer of the proton H(031) from the ortho oxygen, O(31), to the oxazoline nitrogen, N(4). The resulting ferric tricatecholate chelate remains dianionic at neutral pH with a proton count of five. This coordination model may possess less conformational strain in the spermidine backbone, with retention of coplanarity of the functionalities mentioned above. This mode of coordination is intriguing, although is not applicable to ferric chelation by the related linear ligand, parabactin.[22] Certainly, the coordination of ferric ion by agrobactin (and parabactin), the conformational changes occurring upon chelation, absolute configuration, and intramolecular contacts of these sequestering agents must be confirmed with solid-state studies, and experiments to this end have been initiated.

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Supplementary Material Available: Listings of hydrogen atom coordinates and isotropic temperature factors, anisotropic thermal parameters of the nonhydrogen atoms, and structure amplitudes (30 pages). Ordering information is given on any current masthead page.

A Nuclear Magnetic Resonance Kinetic and Product Study of the Ring Opening of Propylene Oxide. Nucleophilic and General Catalysis by Phosphate[1]

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Abstract: Systematic kinetic and product studies have been performed in an examination of the ring opening of propylene oxide over the entire pH region. The NMR kinetic method, which involves the integration of reactant and product resonances as a function of time, provided the rate data. The plots of log ($area_t - area_\infty$) vs. time were linear to better than three half-life times of reaction. Reproducibility error for rate measurements run under identical conditions was less than 5%. Products were identified by ^1H and ^{13}C NMR spectroscopy and with gated decoupler techniques. The latter was used to quantitatively determine the product composition. The validity of this method of product analysis was carefully established with a series of control experiments. The error in these determinations was shown to be less than 4%. Emphasis was placed on the search for general and nucleophilic mechanisms of catalysis. Kinetic and product analyses were performed on reaction solutions in both aqueous formate and aqueous phosphate buffers. The glycol monoformate esters proved to be labile under kinetic conditions. By contrast the glycol monophosphate esters permitted a complete dissection of the buffer catalytic components into nucleophilic and general modes. Thus careful analysis of the reaction species present in the buffer matrix shows that glycol monophosphate esters are always produced in amounts less than the buffer contribution to the overall rate and furthermore arise almost exclusively from HPO_4^{2-} attack upon neutral epoxide. This nucleophilic catalysis accounts for 80 ± 6% of $k_{HPO_4^{2-}}$ and leads to almost equal amounts of 1,2-propanediol-1-phosphate and 1,2-propanediol-2-phosphate. The remaining 20 ± 6% of $k_{HPO_4^{2-}}$ and the total $k_{H_2PO_4^-}$ represent general catalysis. These contributions arise mechanistically through hydrogen bonding which operates to increase the nucleophilic capacity of rear side water molecules or to enhance the electrophilic character of the epoxide ring, respectively.

During the past decade the properties of the epoxide linkage have been the subject of increasing interest in the realm of chemical and biochemical investigation. Special attention has focused upon two important investigative areas: the synthetic

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