Structure of Amiprilose Hydrochloride, a Novel Anti-inflammatory Agent

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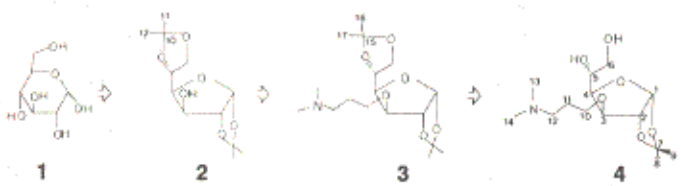
Abstract □ Amiprilose hydrochloride is a carbohydrate-derived, novel anti-inflammatory with potential application in the treatment of rheumatoid arthritis. A spectroscopy-based approach was undertaken to assign both the relative and absolute configuration of its five chiral centers. The fully assigned ^{13}C and ^{1}H NMR spectra of amiprilose hydrochloride was used to establish the relative stereochemistry of four of its five chiral centers held rigid in its furanose ring system. Parallel synthesis of the enantiomer of amiprilose hydrochloride from L-glucose was followed by CD spectropolarimetry to establish that no inversion of chiral centers had occurred in the synthesis. The hydrobromide salt of amiprilose and its enantiomer were prepared and, together with amiprilose hydrochloride, were crystallized. X-ray crystallographic analysis resulted in the assignment of the absolute configuration of all five chiral centers.

Amiprilose hydrochloride (4; Therafectin) is a novel anti-inflammatory drug derived synthetically from D-glucose, and is currently in advanced phase II/III clinical trials. In preclinical studies, amiprilose hydrochloride has been shown to affect inflammation[1] and to increase the killing activity of peritoneal-derived lymphocytes.[2,3] Additionally, the substance suppresses the appearance of Type II collagen-induced adjuvant arthritis in rats.[4] This drug is synthesized from D-glucose (1) by converting it to α-D-1,2:5,6-di-*O*-isopropylidene glucofuranose (2), thus leaving only the 3-hydroxyl group unblocked. Following 3-*O*-alkylation with 1,*N*,*N*-dimethylamino-3-chloropropane (3), the 5,6-*O*-isopropylidene blocking group is removed (Scheme I).

Amiprilose hydrochloride is one in a growing list of carbohydrate-based drugs, including polysaccharides,[5] glycosides,[6] nucleosides,[7] and derivatives of simple sugars,[8] containing a high level of chirality. In the past, chemists relied heavily on degradative analysis to characterize such carbohydrates.[9] Alternative approaches used to define chirality, such as X-ray crystallography or the parallel synthesis of an enantiomer of a molecule, are not always possible. Advances in modern NMR spectroscopy have made it possible to unequivocally assign the signals of all the protons and carbons present in a complex molecule such as a carbohydrate.[10] Nuclear magnetic resonance (NMR) spectroscopy can also be used to assign the relative stereochemistry of the rigid chiral centers located within the carbohydrate ring.

It was with the goal of developing such an integrated spectroscopic approach for the analysis of complex carbohydrate drugs that this project to assign the relative and absolute stereochemistry of amiprilose hydrochloride was undertaken.



Scheme I—Synthetic scheme for the preparation of amiprilose (4) from D-glucopyranose (1).

Experimental Section

Materials—Amiprilose hydrochloride (1,2-*O*-isopropylidene-3-*O*-3′-[*N*′,*N*′-dimethylamino-*n*-propyl]-α-D-glucofuranose hydrochloride; 4; lot #17267), its enantiomer (1,2-*O*-isopropylidene-3-*O*-3′-[*N*′,*N*′-dimethylamino-*n*-propyl]-α-L-glucofuranose hydrochloride), 1,2:5,6-di-*O*-isopropylidene-3-*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-α-D-glucofuranose hydrogen sulfate (3), its enantiomer (1,2:5,6-di-*O*-isopropylidene-3-*O*-3′(*N*′,*N*′-dimethylamino-*n*-propyl)-α-L-glucofuranose), and 1,2:5,6-di-*O*-isopropylidene-α-L-glucofuranose were obtained from Greenwich Pharmaceuticals, Chicago, IL. The 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2), D-glucopyranose (1), and L-glucopyranose were from Pfanstheil Laboratories, Waukegan, IL. The acetate form of anion exchange resin AG50W-X8, 100–200 mesh, was from Biorad, Richmond, CA. Deuteromethanol (99.5 atom% D), deuterochloroform (99.8 atom% D), and tetramethylsilane (99.9+%) were from Aldrich Chemical, Milwaukee, WI. All other chemicals and solvents were reagent grade.

Instrumentation—The NMR spectroscopy was performed at 80 MHz proton, 20.15 MHz carbon on an IBM NR80 spectrometer and at 360 MHz proton, 90.56 MHz carbon on a Bruker WM360 spectrometer. Low-resolution mass spectrometry was performed on a Ribermag 10C low resolution mass spectrometer. High-resolution mass spectrometry was performed on a VB ZAB-HF mass spectrometer with VG 11–250J data system and a PDP-11/73 computer. Fourier transform-IR was performed by diffuse reflectance on a Nicolet Fourier transform infrared spectrometer. X-ray crystallography was performed on an Enraf-Nonius CAD-4 diffractometer. Optical rotations were measured using a Perkin-Elmer 141 polarimeter. Circular dichroism (CD) spectropolarimetry was performed on a Jasco model J-500A spectropolarimeter having a 170 to 700-nm wavelength range. Melting points were performed on a Thomas Hoover capillary melting point apparatus.

Preparation of Derivatives—*Amiprilose*—Amiprilose hydrochloride (15 g) was dissolved in distilled water (100 mL) and the pH of the solution was adjusted from 6.0 to 11.5 using 5 M NaOH solution. The resulting solution was extracted three times with 100 mL of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated under reduced pressure affording a clear, colorless, viscous oil. After drying for 24 h under reduced pressure, ~12 g of amiprilose was obtained.

Amiprilose Hydrobromide—The acetate form of anion exchange resin (25 mL) was packed into a column (2 × 10 cm) pretreated with 100 mL of saturated potassium bromide solution and washed with 3 × 100 mL of distilled water. Amiprilose hydrochloride (or its enantiomer), 1 g in 5 mL of water, was then applied to the column. The column was washed with 10 mL of distilled water and the wash was collected, frozen, and freeze-dried, resulting in a clear oil which was solidified by triturating with a mixture of hexanes:diethyl ether. After drying under reduced pressure, 1 g of white powder was recovered.

Nuclear Magnetic Resonance (NMR) Analysis—The NMR was performed on the hydrochloride and hydrobromide salts of amiprilose in deuteromethanol at concentrations from 0.1 to 0.75 M using ~1%

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tetramethylsilane internal standard. The NMR spectrum of amiprilose was obtained in deuterochloroform at 3.0 M using ~1% tetramethylsilane as an internal standard. The delayed decoupling experiment was performed in amiprilose hydrochloride (0.75 M) in deuteromethanol at 360 MHz.[11] Two-dimensional (2D) decoupled ^1H homonuclear correlation spectroscopy (COSY) was performed on amiprilose (2.0 M in deuterochloroform) at 360 MHz.[11] The 2D ^1H,^{13}C heteronuclear COSY was performed on amiprilose hydrochloride (0.75 M in deuteromethanol) at 80 MHz ^1H, 20.15 MHz ^{13}C. Nuclear Overhauser (NOE) spectroscopy was performed on amiprilose hydrochloride (0.75 M in deuteromethanol) at 360 MHz.

Infrared (IR) Analysis—Fourier transform IR analysis was performed by diffuse reflectance[10] on amiprilose hydrochloride and its enantiomer (200 μg) in KBr (1 mg).

Mass Spectroscopy (MS) Analysis—Low resolution gas chromatography–mass spectrometry (GC–MS) was performed using a 5% phenyl methyl silicone GC column (0.22 mm × 25 m) mounted on a low-resolution mass spectrometer. High-resolution fast atom bombardment (FAB)–MS was performed on amiprilose hydrochloride and its enantiomer in the positive ion mode from a glycerol matrix on a stainless steel target using a standard VB-FAB source with Xe fast atoms at 8 Kev with 1 mamp current.

Circular Dichroism (CD) Analysis—The CD spectra of amiprilose hydrochloride (and its enantiomer) and intermediates in their synthesis were obtained in distilled water (pH 7.0) at 25 °C at a concentration of from 30 to 300 g/L.

Optical Rotations—Optical rotations were measured at the Na D-line in water at concentrations of 25–50 mg/mL using a 1-cm path length quartz cell.

Elemental Analysis—Elemental analysis (C,H,N,O, and Cl or Br) was performed by Gailbraith Laboratories, Knoxville, TN.

Crystallization of Amiprilose Hydrochloride, Amiprilose Hydrobromide, and Their Enantiomers—The hydrochloride salts could be crystallized from a variety of solvents including ethanol, isopropanol, 1-butanol, nitromethane, and acetonitrile. The best crystals for X-ray analysis were obtained by adding 500 mg of amiprilose hydrochloride (or its enantiomer) to 10 mL of isopropanol at 60 °C. (Amiprilose hydrochloride; mp 177–178 °C, $[\alpha]_D^{20} = -27.8°$. Amiprilose hydrochloride enantiomer; mp 176–178 °C, $[\alpha]_D^{20} = +23.5°$.) The resulting saturated solution was filtered hot through glass wool and allowed to crystallize at 25 °C. The hydrobromide salts could be crystallized from methanol, ethanol, isopropanol, nitromethane, and acetonitrile. The best crystals for X-ray analysis were obtained from ethanol by adding 500 mg of amiprilose hydrobromide (or its enantiomer) to 3.5 mL of absolute ethanol at 60 °C. The resulting saturated solution was filtered hot through glass wool and allowed to crystallize at 25 °C.

Crystallographic Methods—The diffraction data were obtained using monochromated Mo Kα-radiation, λ = 0.71073 A; 295 K data collection; scan, 0.6 + 0.35 tan (θ); background counts, 25% below and above range; peak counting time:background counting time = 2:1; and a scan speed of 1–4 deg/min based on intensity. Cell dimensions were obtained from at least 20 reflections in the 2 (θ) range of 35–40°. The remaining experimental parameters are summarized in Table I. X-ray crystal structures were obtained for amiprilose hydrochloride, amiprilose hydrobromide, and its enantiomer. The structure was initially solved by direct methods from diffraction data from the D-amiprilose hydrobromide. Although all but six hydrogen atoms were located from difference electron density maps, full matrix refinement was carried out with the hydrogen atoms in fixed positions. After least squares convergence, refinement was repeated based on a model with the opposite hand. The agreement indices, summarized in Table I, clearly fix the correct hand of the molecule. The refinement procedures were identical for the L-amiprilose hydrobromide, except that refinement began with starting parameters derived from the D-enantiomer. The data from the D-amiprilose hydrochloride permitted the refinement of the hydrogen atom positions (isotropically) and experimentally verified the calculated hydrogen atom positions assumed in the refinement of the bromide analogues. Again, both D- and L- form models were refined, and the results (in Table I) again indicate the correct choice of models to be the D form, in agreement with the bromide analogue. The small difference in agreement factors for the two enantiomeric forms in the chloride case might not be considered conclusive evidence of the correct handedness by itself. But, since it agrees with the unequivocal results obtained from the bromide analogue, this refinement lends support to the possibility of determining the correct handedness with a rather small anomalous scattering contribution.

The positional parameters and selected bond distances and angles for D-amiprilose hydrochloride are given in Tables II and III, and the

Table I—Crystallographic Parameters of Amiprilose Salts[a]

Parameter[b]	D-Amiprilose · HBr	L-Amiprilose · HBr	D-Amiprilose · HCl
a	10.745(2)	10.753(1)	10.572(4)
b	10.789(2)	10.737(2)	10.605(4)
c	15.609(3)	15.598(2)	15.487(6)
Vol.	1809.6	1800.1	1736.3
FW, *Z*	385.30, 4	385.30, 4	340.84, 4
d, g/mL	1.414	1.422	1.304
Data Collection			
Decay	10%	<2%	<1.3%
Lin. Absorp. (1/cm)	22.7	22.7	2.42
Absorp. Corr.			
Method	Templeton[c]	Emp. Absorp.	None
Min–Max	1.4–2.09	0.87–1.00	—
Equiv. Refl.			
Agree.F,F*F%	3.1, 2.7	1.9, 2.1	2.2, 2.7
Total Refl.	11935	11943	12225
Abs.	3438	2094	557
After Aver.	3198	3209	3082
$>3\sigma$	2145	2526	2467
Refinement			
Model	H atoms fixed	H atoms fixed	Isotropic, H
Parameters	199	199	312
SDOUW	1.08	1.11	1.12
D Enant. R_1, R_2	0.031, 0.051	0.055, 0.089	0.020, 0.025
L Enant. R_1, R_2	0.06, 0.09	0.020, 0.024	0.022, 0.027
Par. shift/esd.	0.16	0.22	0.01
Resid. el. dens.(el/A^3)	<0.6	—	<0.19
Weighting[d]	Q = 0.00, P = 0.035	Q = 0.00, P =0.01	Q = 0.00, P = 0.01

[a] Space group = $P2_12_12_1$. [b] For all salts: 2θ = 2–50; octants = all; size = 0.1–0.5 × 0.3–0.4 × 0.3–0.6 mm; boundary faces (0,0,0) x 1, −1, 0) x (1, 1, 0); refinement = anisotropic, non-H. [c] See ref 15. [d] See ref 16.

experimental details for all three structures are given in Table I. Positional and thermal parameters for all three structures have been submitted to the Cambridge Crystal Data Center.

Results and Discussion

Amiprilose hydrochloride had a melting point range of 177 to 178 °C and the following composition: C, 49.07%; H, 8.15%; N, 4.03%; O, 28.09%; Cl, 10.11% (theoretical: C, 49.19%; H, 8.26%; N, 4.10%; O, 28.08%; Cl, 10.37% for $C_{14}H_{28}NO_6Cl$). Low-resolution GC-MS of amiprilose showed a single GC peak with corresponding M + 1 peak in the MS of 306 mass units. High-resolution FAB–MS analysis gave a molecular ion of 306.192 (Δm = 0.1 mmu) corresponding to an M + 1 ion with a molecular formula for the free base of $C_{12}H_{28}O_6N$. The MS analysis is consistent with the calculated molecular mass of 341.834 for amiprilose hydrochloride. The enantiomer of amiprilose hydrochloride had an identical mass spectrum, with an M + 1 ion of 306.189 (Δm = 2.3 mmu).

Proton and carbon NMR of amiprilose hydrochloride in deuteromethanol at 0.75 M at 360 and 90.56 MHz showed signals corresponding to each of the 12 nonequivalent protons and 13 nonequivalent carbons, respectively (Table IV). Delayed decoupling spectroscopy[11] was used to determine the different types of carbon (i.e., methyl, methylene, methine, and quartenary) corresponding to each signal in the carbon spectrum (Table IV). The anomeric proton H1 was tentatively assigned to the peak at 5.87 ppm based on a reported shift of 5.85 ppm for H1 in the structurally similar 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2).[12] Adjacent protons exhibiting connectivity (H1-H6 and H10-H12) in amiprilose (4; the free base was over four times more soluble in organic solvents than the hydrochloride salt) were assigned

Table II—X-ray Crystallographic Coordinates for D-Amiprilose · HCl

Atom[a]	x	y	z	B(A2)
C1	0.66630(4)	0.12153(4)	0.36419(3)	4.155(8)
O1	0.60698(9)	0.4231(1)	0.77440(6)	3.33(2)
O2	0.4657(1)	0.3917(1)	0.88644(7)	3.80(2)
O3	0.5623(1)	0.2065(1)	0.91448(6)	3.25(2)
O4	0.70250(9)	0.1910(1)	0.70190(6)	3.07(2)
O5	0.9405(1)	0.3519(1)	0.77603(8)	4.74(3)
O6	0.8632(1)	0.5888(1)	0.84793(7)	4.84(3)
N1	0.9053(1)	0.1982(1)	0.46265(7)	2.96(2)
C1	0.5080(1)	0.3478(2)	0.80523(9)	3.27(3)
C2	0.5623(1)	0.2162(2)	0.82246(9)	2.93(3)
C3	0.6981(1)	0.2254(1)	0.79069(9)	2.73(3)
C4	0.7239(1)	0.3649(2)	0.80169(9)	2.85(3)
C5	0.8312(1)	0.4192(2)	0.74892(9)	3.32(3)
C6	0.8454(2)	0.5591(2)	0.7597(1)	3.85(3)
C7	0.4683(1)	0.2907(2)	0.9468(1)	3.31(3)
C8	0.3411(2)	0.2269(2)	0.9501(1)	5.65(5)
C9	0.5095(2)	0.3398(2)	1.0330(1)	4.48(4)
C10	0.7860(2)	0.0888(2)	0.68428(9)	3.25(3)
C11	0.8090(1)	0.0807(1)	0.58792(9)	3.15(3)
C12	0.8621(1)	0.2031(1)	0.55511(9)	2.88(3)
C13	1.0104(2)	0.1078(2)	0.4486(1)	4.28(4)
C14	0.9390(2)	0.3275(2)	0.4327(1)	4.31(4)
H1	0.439(1)	0.353(1)	0.7631(9)	1.0(3)
HN1	0.338(1)	0.330(1)	0.5692(9)	1.0(3)
H2	0.482(1)	0.646(1)	0.7022(8)	0.1(3)
H3	0.252(1)	0.326(1)	0.1757(9)	1.0
H4	0.762(1)	0.616(1)	0.3609(9)	1.0
H5	0.688(1)	0.597(1)	0.1904(9)	0.7(3)
HO5	0.503(2)	0.634(2)	0.246(1)	2.9(4)
HO6	0.645(2)	0.335(2)	0.354(1)	3.7(4)
H61	0.584(2)	0.409(2)	0.224(1)	2.6(4)
H62	0.766(2)	0.606(2)	0.739(1)	1.8(4)
H81	0.656(2)	0.655(2)	0.513(1)	3.9(5)
H82	0.280(2)	0.279(2)	0.970(1)	3.9(5)
H83	0.675(2)	0.688(2)	0.604(1)	4.2(5)
H91	0.593(2)	0.378(2)	0.026(1)	2.9(4)
H92	0.489(2)	0.767(2)	0.426(1)	3.5(5)
H93	0.451(2)	0.400(2)	0.053(1)	3.6(5)
H101	0.249(2)	0.487(1)	0.295(1)	1.4(3)
H102	0.366(1)	0.391(1)	0.2852(9)	1.3(3)
H111	0.227(1)	0.443(1)	0.4437(9)	0.7(3)
H112	0.369(1)	0.489(1)	0.4202(9)	1.0(3)
H121	0.296(1)	0.228(1)	0.4412(9)	1.0(3)
H122	0.433(1)	0.267(1)	0.4104(8)	0.2(3)
H131	0.481(2)	0.485(2)	0.536(1)	4.3(5)
H132	0.566(2)	0.382(2)	0.506(1)	3.2(4)
H133	0.544(2)	0.383(2)	0.608(1)	3.8(5)
H141	0.530(2)	0.678(2)	0.875(1)	3.5(4)
H142	0.638(2)	0.618(2)	0.933(1)	3.8(5)
H143	0.505(2)	0.139(2)	0.530(1)	3.0(4)

[a] All hydrogen atoms were refined isotropically; anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter which is defined as follows: $(4/3) \cdot [a^2 \cdot B(1,1) + b^2 \cdot B(2,2) + c^2 \cdot B(3,3) + ab(\cos\gamma) \cdot B(1,2) + ac(\cos\beta) \cdot B(1,3) + bc(\cos\alpha) \cdot B(2,3)]$ where a, b, and c are reciprocal lattice constants.

Table III—Interatomic Distances and Bond Angles for Amiprilose Hydrochloride

Atoms	Bond Distance, Å	Atoms	Bond Angle,°
C1–O1	1.401(2)	O1–C1–O2	110.6(1)
C1–O2	1.414(2)	O1–C1–C2	107.4(1)
C1–C2	1.533(2)	O2–C1–C2	105.3(1)
C2–O3	1.429(2)	C1–C2–C3	103.8(1)
C2–C3	1.520(2)	C1–C2–O3	103.8(1)
C3–O4	1.423(2)	C3–C2–O3	109.1(1)
C3–C4	1.514(2)	C2–C3–C4	101.3(1)
C4–O1	1.445(2)	C2–C3–O4	109.1(1)
C4–C5	1.513(2)	C4–C3–O4	110.7(1)
C5–O5	1.421(2)	C3–C4–C5	116.5(1)
C5–C6	1.500(2)	C3–C4–O1	103.3(1)
C6–O6	1.414(2)	C5–C4–O1	108.7(1)
C7–O2	1.422(2)	C4–C5–C6	113.0(1)
C7–O3	1.426(2)	C4–C5–O5	105.0(1)
C7–C8	1.506(2)	C6–C5–O5	112.5(1)
C7–C9	1.499(2)	C5–C6–O6	110.0(1)
C10–O4	1.425(2)	C1–O1–C4	107.2(1)
C10–C11	1.514(2)	C1–O2–C7	109.3(1)
C11–C12	1.503(2)	C2–O3–C7	107.8(1)
N1–C12	1.504(2)	C3–O4–C10	113.6(1)
N1–C13	1.483(2)	O2–C7–O3	104.8(1)
N1–C14	1.491(2)	O2–C7–C8	110.0(2)
		O2–C7–C9	109.2(2)
		O3–C7–C8	110.7(2)
		O3–C7–C9	109.1(1)
		C8–C7–C9	112.6(2)
		O4–C10–C11	109.3(1)
		C10–C11–C12	110.1(1)
		C11–C12–N1	113.9(1)
		C12–N1–C13	112.9(1)
		C12–N1–C14	109.7(1)
		C13–N1–C14	111.7(1)

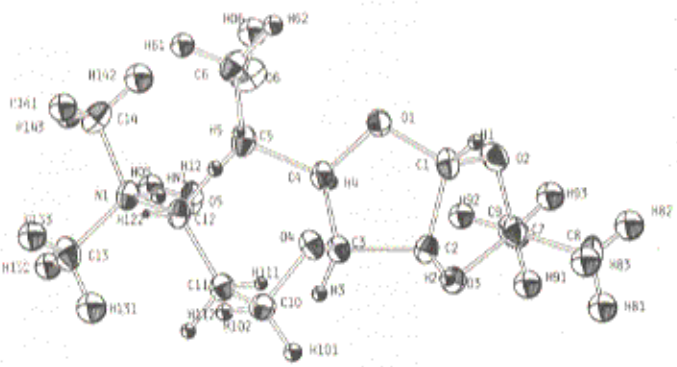

Figure 1—X-ray structure of amiprilose hydrochloride.

using 2D ^{1}H decoupled homonuclear COSY at 360 MHz.[11] Nuclear Overhauser effect (NOE) spectroscopy (360 MHz) with irradiation at 1.3 and 1.46 ppm and observation of the H-1, H-2, and H-4 signals permitted the assignment of the isopropylidene methyl groups.

Proton signals were correlated to their respective carbon signals using 2D ^{13}C,^{1}H heteronuclear COSY. The only signals in the carbon spectrum that could not be correlated were those at 70.22, 68.41, and 112.61 ppm. Delayed decoupling spectroscopy, however, had already given the carbon type for these three signals as methine, methylene, and quaternary, respectively, permitting their assignment to C-5, C-6, and C-7, and thus resulting in the complete, unequivocal assignment of the carbon spectrum. The assignment of the carbon spectrum of amiprilose hydrochloride closely parallels the published shifts and assignments for its precursor 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2).[13]

The complete assignment of carbon and proton signals in the spectra of amiprilose hydrochloride permitted the relative configuration of the chiral centers at C1, C2, C3, and C4 in the rigid glucofuranose ring to be established. The coupling constant, J (1,2), of 3.77 Hz demonstrates a *syn* relationship between H1 and H2. The J (2,3) coupling constant of <0.5 Hz demonstrates an *anti* relationship between H2 and H3. The coupling constants J (1,2) of 3.8 Hz and J (2,3) of 0 Hz are reported for 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2) which showed a similar *syn* (1,2) *anti* (2,3) relationship.[12] The J (3,4) coupling constant of 3.15 Hz demonstrates a *syn* relationship between H3 and H4. Thus, the assignment of the absolute configuration at any of these four centers would result in the assignment of all four centers.

The carbon and proton spectra of D-glucopyranose (1) and its enantiomer and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2) and its enantiomer were in each case identical and in each case in agreement with both their structure and the literature.[12,13] The 1,2:5,6-di-*O*-isopropylidene-3,*O*-3′-(*N*′,*N*′-dimethylamino-*n*-propyl)-α-D-glucofuranose (3) hydrogen sulfate gave a molecular ion of 346.221 (Δm = 2.3 mmu) corresponding to an M + 1 ion with a molecular formula for the free base of $C_{17}H_{32}O_6N$. Proton and carbon NMR at 360 and 90.56 MHz gave spectra consistent with the assigned structure (Table IV legend).

To complete this integrated spectral approach to assign the chirality of amiprilose hydrochloride two tasks still remained. The relative configuration of the flexible chiral center at C-5 had to be assigned and the absolute chirality of at least one of the chiral centers in the molecule needed to be established. Although NMR spectroscopy using chiral shift reagents might permit the assignment of the relative chirality of C-5 by analogy to a known compound, such as 1,2-isopropylidene glucofuranose, it would do nothing to resolve the larger question of absolute chirality.

The parallel chemical synthesis of amiprilose hydrochloride and its enantiomer from D-glucose and L-glucose was under-

Table IV—Nuclear Magnetic Resonance Spectral Assignments for Amiprilose Hydrochloride (0.75 M in CD_3OD)

Atom	Chemical Shift, ppm	Carbon Type	Atom	Chemical Shift, ppm[a]	Multiplicity	Coupling Constant, Hz
C1	106.27	Methine	H1	5.87	Doublet	J(1,2) 3.77
C2	82.80	Methine	H2	4.70	Doublet	J(2,1) 3.77 J(2,3) <0.5
C3	83.86	Methine	H3	3.95	Doublet	J(3,2) <0.5 J(3,4) 3.15
C4	80.81	Methine	H4	4.08	Doublet of doublets	J(4,3) 3.15 J(4,5) 8.20
C5	70.22	Methine	H5	3.87	Multiplet	—
C6	64.87	Methylene	H6a	3.77	Doublet of doublets	J(6a,5) 5.52 J(6b,5) 3.00 J(6a,6b) 11.52
			H6b	3.62	Doublet of doublets	J(6b,6a) 11.52
C7	112.61	Quaternary	H7	—	—	—
C8	27.04	Methyl	H8	1.31	Singlet	—
C9	26.47	Methyl	H9	1.45	Singlet	—
C10	68.41	Methylene	H10a	3.87	Multiplet	—
			H10b	3.62	Multiplet	—
C11	25.66	Methylene	H11	2.05	Multiplet	—
C12	57.41	Methylene	H12	3.30	Multiplet	—
C13	43.70	Methyl	H13	2.90	Singlet	—
C14	43.70	Methyl	H14	2.90	Singlet	—

[a] Deuteromethanol showed carbon signals from 48.27 to 49.68 ppm. The tentative assignments for 1,2:5,6-di-*O*-isopropylidene-3-*O*-3′-*N*′-*N*′-dimethylamino-*n*-propyl)-α-D-glucofuranose hydrogen sulfate carbon spectra are as follows: C1, 106.23; C2, 83.37; C3, 83.16; C4, 82.03; C5, 82.03; C6, 73.56; C7, 112.48; C8, 26.46; C9, 27.08; C10, 67.78; C11, 25.64; C12, 56.20; C13, 43.35; C14, 43.35; C15, 109.75; C16, 25.64; C17, 27.04; and for the proton spectra are as follows: H1, 5.87 (d, J(1,2) = 3.68); H2, 4.70 (d, J(2,3) = 0); H3, 3.89 (m); H4, 4.07 (m); H5, 3.30(m); H6a, 4.07 (m); H6b, 3.93 (m); H8, 1.44 (s); H9, 1.34 (s); H10a, 3.81 (m); H10b, 3.63 (m); H11, 2.05 (m); H12, 3.25 (m); H13, 2.90 (s); H14, 2.90 (s); H16, 1.39 (s); H17, 1.31 (s).

taken to examine if inversion of configuration took place during the chemical synthesis of amiprilose hydrochloride. The enantiomer of amiprilose hydrochloride had identical FT–IR, proton and carbon NMR, and FAB–MS spectral properties. Elemental analysis gave the following composition: C, 49.11; H, 8.26; N, 3.87; O, 28.29; and Cl, 10.62 (theoretical, C, 49.19; H, 8.26; N, 4.10; O, 28.08; and Cl, 10.37 for $C_{14}H_{28}NO_6Cl$). D-Glucopyranose and its enantiomer, L-glucopyranose, show characteristic, opposite, but identical CD curves, as previously reported.[9,14] The D-glucofuranose derivatives all show the expected negative CD curves at wavelengths <200 nm (in contrast to the positive CD curve for D-glucopyranose), while the L-glucofuranose derivatives show identical but positive curves suggesting that no inversion of configuration occurs during the synthesis of amiprilose hydrochloride.

Amiprilose hydrobromide had spectral properties similar to amiprilose hydrochloride. It had a mp of 146 to 148 °C, $[\alpha]_D^{20} = -21.8°$, and the following elemental analysis: C, 43.78; H, 7.19; N, 3.60; O, 24.78; and Br, 20.80 (theoretical, C, 43.53; H, 7.31; N, 3.63; O, 24.85; and Br, 20.69 for $C_{14}H_{28}O_6NBr$).

The crystal structure determination of the three salts unequivocally establishes the absolute configuration of the molecule. The table of bond distances and angles shows that all the bond distances and angles fall within the usual ranges for these values.

Conclusions

In conclusion, NMR spectroscopy was used to successfully assign the relative configuration of four of the five chiral centers in the carbohydrate-based drug amiprilose hydrochloride. Application of CD spectropolarimetry permitted the absolute assignment of all five chiral centers based on the pure D- and L-glucopyranose starting materials. Finally, X-ray crystallography of the hydrobromide salt of amiprilose and its enantiomer resulted in unequivocal assignment of the absolute configuration of amiprilose.

References and Notes

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