Absolute Configuration of (+)-[Fluoro(hydroxyphenylphosphinyl)methyl]-phosphonic Acid, a Specific Inhibitor of Na^+-Gradient-Dependent Na^+–Phosphate Cotransport across Renal Brush Border Membrane, by X-ray Crystallographic Analysis of Its (−)-Quinine Salt

Robert Bau,* Phuong-Truc T. Pham, Gregory D. Duncan, and Charles E. McKenna*

Department of Chemistry, University of Southern California, Los Angeles, California 90089-0744

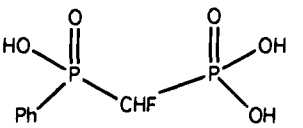
Received June 6, 1994[⊗]

Racemic [fluoro(hydroxyphenylphosphinyl)methyl]phosphonic acid (**1**) and its individual enantiomers [(+), 98% ee; (−), 67% ee] were previously shown to inhibit Na^+-gradient-dependent Na^+–phosphate cotransport across renal brush border membrane, without measurable stereospecificity. Resolution of **1** was effected by fractional recrystallization of its (−)-quinine salts. The more levorotatory, diquinine product **2**, corresponding to (+)-**1**, has now been analyzed by X-ray crystallography and found to be composed of the *S* enantiomer of **1**. This result confirms the absence of stereochemical preference in inhibition of the cotransporter by the enantiomers of **1** and provides the first absolute configuration assignment of an asymmetrical α-halomethylene pyrophosphate analogue.

Introduction

Biophosphate analogues containing a halomethylene function in place of the bridging oxygen of pyrophosphate were first exemplified by achiral methanediphosphonates.[1–3] α-Halogenated pyrophosphate analogues possessing a chiral α-carbon, such as α-monohalo- or mixed α,α-dihalophosphonoacetates, have previously been synthesized and evaluated for antiviral or other biological activities as racemates.[2,4–6] The possible influence of stereoisomerism on the interactions of these types of compounds with enzymes or other chiral receptor sites has not received much attention. In fact, examples of resolved enantiomers of such inhibitors have not been previously described.[5–7]

(Phosphinylmethyl)phosphonates, differing from methanediphosphonates only in the replacement of a P-OH by a P-R group (where R = aryl or alkyl), have a prochiral α-carbon atom. Recently, the pyridinium salt of racemic [fluoro(hydroxyphenylphosphinyl)methyl]-phosphonic acid (**1**) was shown to be a competitive inhibitor of Na^+–phosphate (Na^+–P_i) cotransport in rat renal brush border membrane.[8,9] In the nonstereospecific synthesis of α-halogenated [(hydroxyphenylphosphinyl)methyl]phosphonates,[9] it was noted that the triethyl esters of monohalo compounds such as **1** were obtained as mixtures of diastereomers (due to the presence of a second chiral center, the phenylphosphinyl P atom) which could be distinguished spectroscopically by ^{13}C, ^{31}P, and (where applicable) ^{19}F NMR. This led to attempted resolution of racemic **1** by classical fractional crystallization of its diastereomeric (−)-quinine salts, with stereochemical product analysis by ^{31}P and ^{19}F NMR in a nondissociating solvent ($CDCl_3$).[9] The head crop, a monoquinine salt by elemental analysis, manifested only partial resolution by NMR. However the tail crop, by analysis a diquinine salt, **2**, was 98% diastereomerically pure by both ^{31}P and ^{19}F NMR analysis.



1

We have now determined the X-ray structure of a single crystal of **2**. The results confirm its composition as a 2:1 (−)-quinine:**1** salt, verify its diastereomeric purity to be consistent with the NMR analysis, and reveal that the stereoisomeric form of **1** present is the *S* enantiomer.

Results and Discussion

In a previous study of seven α,α-dihalo- and racemic α-monohalo[(phenylphosphinyl)methyl]phosphonates as inhibitors of Na^+–P_i cotransport in rat renal BBM, a small range of activity variation with α-halogen (22–44% at 1 mM inhibitor, 0.1 mM P_i) was found.[9] The most active compound was racemic **1**, which is a competitive inhibitor of the cotransporter with a K_i (0.36 mM) slightly larger than the reference inhibitor PFA (K_i = 0.27 mM, both K_i determined at I = 1 mM).[9] Fractional crystallization of (−)-quinine salts of **1** produced a tail crop which was recrystallized to constant $[\alpha]^{25}_D$, corresponding to **2**. ^{31}P and ^{19}F NMR analysis revealed only a single fluoromethylene phosphonophenylphosphinate species in $CDCl_3$, whereas the head crop, with a different mp and $[\alpha]^{25}_D$, showed two species in a ratio of 2:1. Elemental analysis indicated that the former, more levorotatory salt contained two molecules of quinine, whereas the latter salt contained only one quinine. The data were interpreted in terms of diastereomeric quininium salts of the individual (+)/(−)-**1** enantiomers, one being completely resolved (diquinine salt) and the other partially resolved (monoquinine salt) on the basis of NMR integration.

Our initial efforts to verify the structure of **2** crystals by X-ray crystallography were unsuccessful. However,

[⊗] Abstract published in *Advance ACS Abstracts*, April 1, 1995.

0022-2623/95/1838-1575$09.00/0

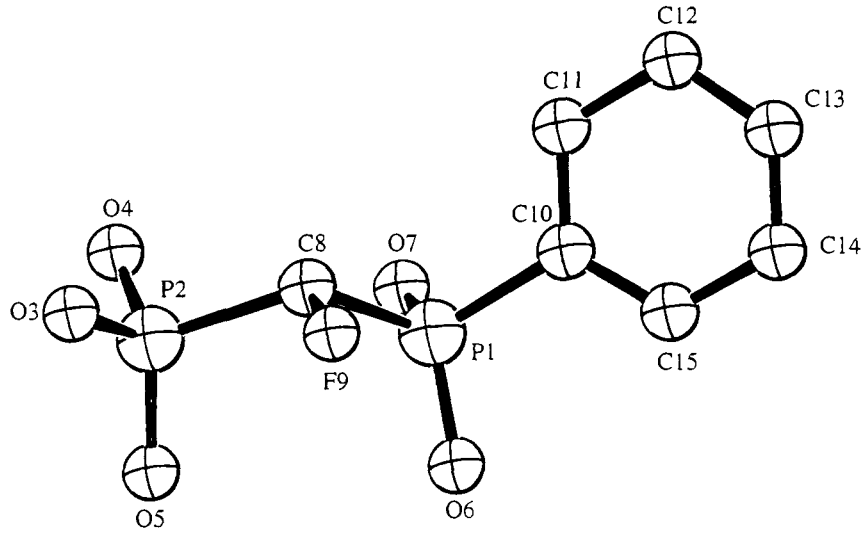
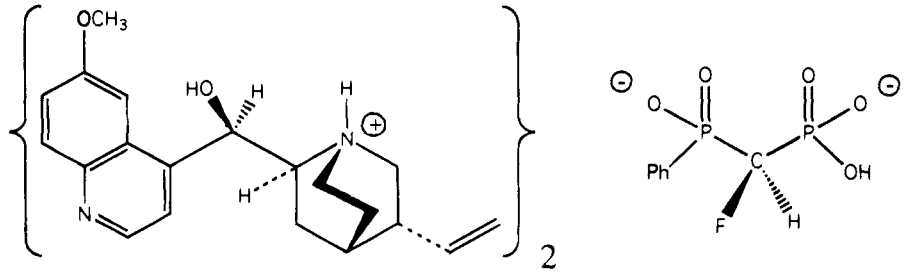


Figure 1. Molecular plot of the [fluoro(hydroxyphenylphosphinyl)methyl]phosphonate anion in compound **2**, showing the *S* absolute configuration about the α-carbon atom [C(8)]. Selected distances are as follows: P(1)–C(8) = 1.827(11) Å, P(2)–C(8) = 1.860(11) Å, P(1)–C(10) = 1.808(11) Å, C(8)–F(9) = 1.425(13) Å. The P(2)–O(3) distance [1.592(8) Å] is significantly longer than the other four P–O distances [P(2)–O(4) = 1.473(9) Å, P(2)–O(5) = 1.475(8) Å, P(1)–O(6) = 1.503(9) Å, P(1)–O(7) = 1.502(9) Å], suggesting that atom O(3) is probably protonated. Negative charges are thus assumed to be delocalized over the O(4)–P(2)–O(5) and O(6)–P(1)–O(7) moieties. A full list of distances and angles for **2** is given in the supplementary material.



2

when a high-intensity X-ray source recently became available to us, it proved possible to obtain the crystallographic structure of **2** with reasonable refinement. Several alternate solvate formulations giving similar *R* values were obtained, of which only one ($R = 7.5\%$) was consistent with a 500 MHz ^{1}H NMR analysis of the residual sample of **2** crystals (Experimental Section). As revealed in Figure 1, only a single enantiomer of 1 is present in the crystal, confirming the structural conclusion reached from the ^{31}P and ^{19}F NMR work. The overall salt structure also contains two molecules of quinine (one is illustrated in Figure 2; for details, see supplemental material), validating the original, combustion analysis-based formulation.

This quinine bis-salt composition of **2** is further supported by consideration of P–O bond lengths in its anion moiety (Figure 1). One (phosphonic acid) P–O bond is 0.09–0.12 Å longer than the other four, consistent with ionization of two out of the three OH protons in the **1** enantiomer to form two conjugate base (quinine) cations. It was shown previously, that pK_1 and pK_2 for **1** are 1.5–2.0 and 2.7, whereas pK_3 is 7.1 (the pK_1 and pK_2 of quinine are 5.1 and 9.7, respectively, at 18 °C).[10a] The absolute configuration of the [fluoro(hydroxyphenylphosphinyl)methyl]phosphonate anion in **2** was readily assigned as *S* (Figure 1) using its (–)-quinine counterions as known[10] stereochemical references (Figure 2).

The salt **2** and its partly resolved diastereomer were converted to the corresponding [(+) and (–)] sodium salts by adjustment of the pH to 11 with dilute NaOH, in order to test for stereospecificity in inhibition by **1** enantiomers.[9] The two enantiomers were not significantly different in inhibitory potency with an SE of 7–10% for their average inhibition activities,[9] indicating a maximal stereospecificity in interaction with the BBM cotransporter of <10% when the 2:1 *R*:*S* ratio in the partially resolved enantiomer is taken into account.[11] This finding is consistent with the result that inhibition of Na^+–P_i transport by racemic **1** differs little from that of the α,α-difluoro analogue. The absolute **1** stereoisomer structure reported here is nevertheless of interest, both to confirm the NMR analysis and in view of the potential value of chiral fluorine compounds as NMR probes of asymmetric binding sites in enzymes or biological receptors.

Experimental Section

Crystallization. The mother liquor from repeated MeOH/acetone precipitation of the salt from racemic **1**[9] and quinine was left standing partially covered in a fume hood for 4 days at room temperature, resulting in evaporation of about one-half of the solvent and separation of crude **2**. This was

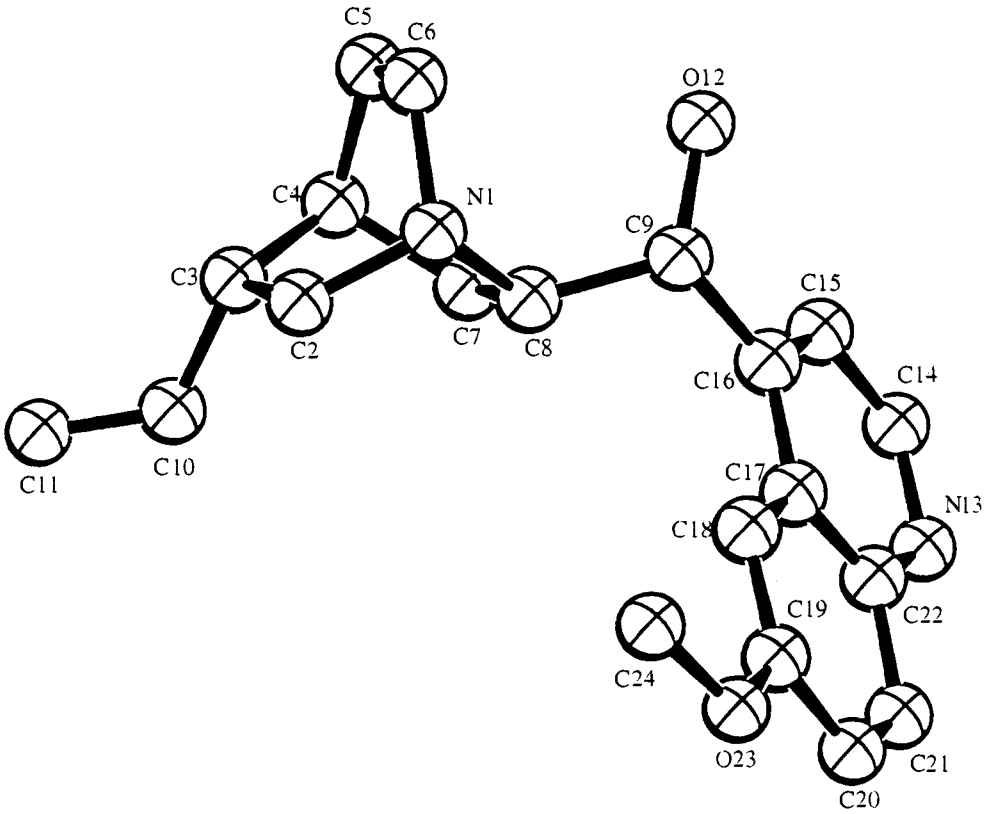


Figure 2. Molecular plot of one of the two quinine cations in compound **2**, showing an absolute configuration consistent with that reported in the literature.[10]

dissolved in a small amount of hot MeOH. After 2-fold concentration and treatment with about 8 volumes of hot acetone, the resulting solution was allowed to stand for 2 days in a chamber kept saturated with acetone vapor, whereupon crystals of **2** separated. These crystals were recrystallized to a constant $[\alpha]^{25}_{D}$.[9]

X-ray Data. The diquinine salt of [fluoro(hydroxyphenylphosphinyl)methyl]phosphonic acid (**2**) crystallizes as colorless prisms in the orthorhombic space group $P2_12_12$ (No. 18), with unit cell parameters a = 18.703(6) Å, b = 25.152(8) Å, c = 11.006(3) Å, V = 5177(3) Å^3, and Z = 4. Data were collected at room temperature on a Siemens P4/RA diffractometer with Cu Kα radiation at 12 kW. The structure was solved using direct methods[13] and refined to final agreement factors of R = 7.5% and $R(w)$ = 7.6% for 2528 nonzero reflections [$F > 4\sigma(F)$].[14]

NMR Analysis. We originally formulated the salt sample **2** with an alternative solvate composition on the basis of elemental analysis, specifically 2 MeOH.[9] This result fits the X-ray data, however, with a slightly higher crystallographic R factor than three alternative solutions differing only in the interpretation of the solvent peaks (acetone, H_2O, or MeOH). Of these, only one (1:1.5 acetone:H_2O) satisfies the previously obtained elemental (C,H) analysis, giving however agreement (C, +0.40%; H, −0.29%) less good than 2MeOH (C, +0.31%; H, −0.03%).[9] To resolve this detail, we confirmed the absence of MeOH and the presence of H_2O and acetone in the crystals used in this study by ^{1}H NMR (500.1 MHz) analysis. The remaining sample of **2** from X-ray crystallographic experiments (ca. 4 mg) was analyzed by 500.1 MHz ^{1}H NMR analysis (Bruker AMX 500) in $CDCl_3$ dried over powdered, activated 4 Å molecular sieves (99.8 atom % D; no H_2O peak detectable). Peaks at δ 2.15 and 1.85 (referenced to solvent $CHCl_3$) were identified as acetone and H_2O, respectively, by authentic compound spiking. Approximate integration ratios of 1:1 were measured for both these peaks (prespiking) relative to the quininium ion methoxy group (δ 3.84), taken as reference (1 acetone:1.5 H_2O:2 quinines gives a predicted integration ratio of 1:0.5:1). A peak at δ 3.43 (MeOH) was not observed. The presence of H_2O in this crystal may be attributed to the lengthy evaporation−crystallization procedure using hydrophilic solvent mixtures and possibly also to protracted (>2 years) crystal storage with intermittent exposure to air.

Acknowledgment. This research was supported by NIH Grant AI-25697 to C.E.McK. and Research Corporation Grant R-171 to R.B. G.D.D. was a U.S. Department of Education National Needs Fellow (1991−1994). We thank Mr. Allan Kershaw for assistance in obtaining 500 MHz NMR spectra and Ms. Joyce Laquindanum for assistance in the early stages of the X-ray analysis. Funds for the purchase of the rotating anode X-ray diffractometer were provided in part from NSF Instrument Grant CHE-92-16167.

Supplementary Material Available: Atomic coordinates, bond lengths and angles, and thermal parameters for diquininium [fluoro(hydroxyphenylphosphinyl)methyl]phosphonate (acetone/1.5 water solvate) (**2**) in tabular form: Structure Determination Summary (Table 1), Atomic Coordinates and Temperature Factors (Table 2), Bond Distances (Table 3), Bond Angles (Table 4), Anisotropic Displacement Coefficients (Table 5), and Hydrogen Atom Coordinates (Table 6) (10 pages); a table of observed and calculated structure factors (Table 7) (11 pages). Ordering information is given on any current masthead page.

References

(1) Blackburn, G. M.; England, D. A.; Kolkman, F. Monofluoro- and Difluoromethylenebisphosphonic Acids: Isopolar Analogues of Pyrophosphoric Acid. *J. Chem. Soc. Chem. Commun.* **1981**, 930−932.
(2) McKenna, C. E.; Khawli, L. A. Synthesis of α-Fluorinated Phosphonoacetates. *Phosphorus Sulfur Relat. Elem.* **1983**, *18*, 483.
(3) Hutchinson, D. W.; Cload, P. A. Haugh, M. C. Organophosphorus Compounds as Antiviral Agents. *Phosphorus Sulfur Relat. Elem.* **1983**, *14*, 285−293.

(4) McKenna, C. E.; Khawli, L. A. Synthesis of Halogenated Phosphonoacetate Esters. *J. Org. Chem.* **1986**, *51*, 5467–5471.
(5) McKenna, C. E.; Khawli, L. A.; Bapat, A.; Harutunian, V.; Cheng, Y. C. Inhibition of Herpesvirus and Human DNA Polymerases by α-Halogenated Phosphonoacetates. *Biochem. Pharmacol.* **1987**, *36*, 3103–3106.
(6) Hoppe, A.; McKenna, C. E.; Harutunian, V.; Levy, J. N.; Dousa, T. P. α-Cl-α-Br-Phosphonoacetate is a Potent and Selective Inhibitor of Na^+/Phosphate Cotransport Across Renal Cortical Brush Border Membrane. *Biochem. Biophys. Res. Commun.* **1988**, *153*, 1152–1158.
(7) For chiral α-fluoromethyl(mono)phosphonates, see: Hall, C. R.; Inch, T. D.; Williams, N. E. The Preparation and Properties of some Chiral Fluoromethylphosphonates, Phosphonothioates, and Phosphonamidothioates. *J. Chem. Soc., Perkin Trans. I* **1985**, 233–237.
(8) McKenna, C. E.; Pham, P.; Hoppe, A.; Rassier, M. E.; Dousa, T. P. α-Halophenyl(phosphonylmethyl)phosphinates as Specific Inhibitors of Na^+-P_i Cotransport across Renal BBM. *Kidney Int.* **1990**, *37*, 460.
(9) McKenna, C. E.; Pham, P.-T. T.; Rassier, M. E.; Dousa, T. P. α-Halo [(phenylphosphinyl)methyl]phosphonates as Specific Inhibitors of Na^+-Gradient Dependent Na^+-P_i Cotransport Across Renal Brush Border Membrane. *J. Med. Chem.* **1992**, *35*, 4885–4892. We wish to draw the reader's attention to the following typographical errors on p 4891 in this paper: paragraph 1, line 6, read $^2J_{FP}$ and $^2J_{FH}$ not $^2J_{PF}$ and $^2J_{PH}$; paragraph 2, lines 16/17 and also 19/20, the symbols **19** and **19′** were reversed.
(10) (a) Klyne, W.; Buckingham, J. *Atlas of Stereochemistry*, 2nd ed.; Oxford University Press: London, 1978; Vol. 1, p 145. (b) Windholz, M., Ed. *The Merck Index*, 10th ed.; Merck and Co.: Rahway, NJ, 1983.
(11) Although both enantiomeric sodium salts exhibited (opposite) optical activity, the possibility of racemization in the conversion of **1** bis-quinine salts to sodium salts was nevertheless investigated by repeating the conversion procedure in deuterium oxide.[9] After pH adjustment to 11 (NaOH), concentration, and isolation of the sodium salt, ^1H NMR showed no evidence for deuterium exchange at the fluoromethylene carbon.[9] Racemization of **1** would proceed via removal of the proton from this carbon, generating an intermediate carbanion. The pK value for the α-proton is shown by the above result to be $\gg pK_3$ in **1** (= 7; see text). Given that it must be ionized from a trianion, the fluoromethylene proton in **1** should be several orders of magnitude less acidic[12] than the α-proton in the neutral, triethyl ester of **1**, which will react with 1–2 equiv of a strong base like KO-*t*-Bu.[9] In the BBM Na^+–P_i transport assays, the pH was buffered to 7.5 with 5 mM Tris-HEPES. We conclude that **1** did not racemize during its conversion to a sodium salt and is very unlikely to have racemized during cotransport assays.
(12) Atkins, P. W. *Physical Chemistry*, 3rd ed.; W. H. Freeman and Co.: New York, 1986; p 277.
(13) Sheldrick, G. M. *SHELX-86*; University of Göttingen, Germany, 1986.
(14) Sheldrick, G. M. *SHELX-76*; University of Cambridge: U.K., 1976.

JM940361N