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ABSOLUTE CONFIGURATION OF AN ALLENIC ENZYME INACTIVATOR

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Abstract: X-Ray crystallographic analysis of the 4-(4'-bromophenyl)phenacyl ester of (+)-2,3-decadienoic acid has shown that the allenic inhibitor of β-hydroxydecanoylthioester dehydrase has the S configuration.

The application of mechanism-based, irreversible enzyme inactivators[1] ("suicide substrates") has become a standard method for studying enzyme mechanisms and regulating metabolic pathways. In the pioneering demonstration of this technique,[2] Bloch and his coworkers showed that the *E. coli* enzyme β-hydroxydecanoylthioester dehydrase is irreversibly inactivated by the *N*-acetyl-2-mercaptoethylamine (*N*-acetylcysteamine; NAC) thioester of 3-decynoic acid. The latter is rearranged by dehydrase to 2,3-decadienoyl NAC, a potent electrophile that alkylates an active site histidine residue.[3]

Prior investigations have shown that dextrorotatory 2,3-decadienoyl NAC[4] and 2,3-decadienoic acid[5] are substantially more inhibitory toward dehydrase than are the corresponding racemates; however the absolute configurations of these allenes were unknown. Using x-ray crystallography, we have now shown that the allenic inhibitors possess the *S* configuration.

(±)-2,3-Decadienoic acid was made[6] from 1,2-propadiene by a sequence of lithiation (*n*-BuLi), alkylation (*n*-C_6H_{13}Br), lithiation (*n*-BuLi), and carboxylation (CO_2). To this racemic acid was added 0.5 equivalents of cinchonidine, and the resulting salt was fractionally crystallized from acetone four times, until the optical rotation of the salt had become constant ($[\alpha]_D^{27} = +14.1^\circ$, acetone). Acidification gave the allenic acid, $[\alpha]_D^{29} = +141^\circ$ (acetone; the published[5] rotation, measured under unspecified conditions, was +145°).

In our hands, the cinchonidine salt of (+)-2,3-decadienoic acid would not crystallize in a form suitable for crystallographic analysis. Accordingly, a heavy atom derivative of the acid was required, enabling determination of the absolute configuration via the anomalous scattering technique. Synthesis of an appropriate derivative was less straightforward than had been hoped; while the melting point of the 4-bromophenacyl ester[7] of racemic 2,3-decadienoic acid was 43 °C, the corresponding ester of the dextrorotatory acid melted at only 34 °C. A satisfactory solution was provided through the synthesis and application of a modified derivatizing reagent, 2-bromo-1-(4'-bromo[1,1'-biphenyl]-4-yl)ethanone (4-(4'-bromophenyl)phenacyl bromide).[8] This compound was made from 4-bromobiphenyl in two steps: Friedel-Crafts acetylation[9] gave the acetophenone[9c] (77%), which was brominated[10] using bromine in glacial acetic acid (87%). Using the crown ether method,[7] (+)-2,3-decadienoic acid was converted to its 4-(4'-bromophenyl)phenacyl ester, $[\alpha]_D^{23}$ = +87.3°, mp 78-80 °C.[11]

The heavy atom derivative, 4-(4'-bromophenyl)phenacyl (+)-2,3-decadienoate, crystallized (from diethyl ether) in the monoclinic space group $P2_1$ with two molecules of composition $C_{24}H_{30}O_3Br$ forming the asymmetric unit. Accurate cell constants of $a = 8.620(1)$, $b = 15.575(3)$, $c = 16.757(2)$ Å, and $\beta = 102.00(1)^\circ$ were determined by a least-squares fit of fifteen moderate angle 2Θ-values. All unique diffraction maxima with $2\Theta \leq 114^\circ$ were collected on a computer controlled four-circle diffractometer using graphite monochromated Cu $K\bar{\alpha}$ radiation (1.54178 Å) and variable speed 1° ω-scans. After correction for Lorentz, polarization and background effects, 2786 (90%) of the 3105 unique reflections were judged observed ($|F_o| \geq 3\sigma(F_o)$). The structure was solved routinely using heavy atom and weighted Fourier techniques.[12] Hydrogen atoms were located in a difference electron density synthesis after partial least-squares refinement of the heavy atoms. The absolute configuration was determined by utilizing the anomalous scattering of the bromine atoms ($\Delta f'' = 1.5$) in two different ways. In the first method both the structure and its enantiomer were refined. In the second method 26 reflections with large differences in the Friedel pairs were repeatedly measured. Local scaling was done by using nearby reflections in reciprocal space that did not have Friedel differences. Both methods agreed and the final x-ray model is shown in Figure 1. As can be seen, the chiral allene has the <u>S</u> configuration. The final conventional crystallographic residual is 0.059 for the observed reflections. Additional crystallographic details have been deposited with the Cambridge Crystallographic Data Centre.[13]

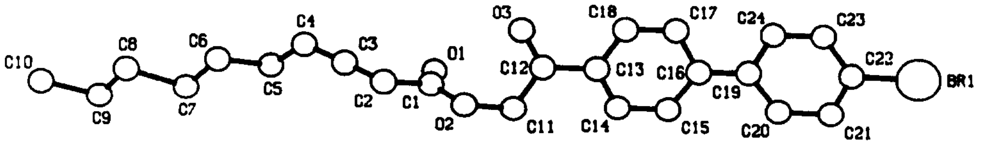

Figure 1. A computer generated perspective drawing of the final x-ray model of 4-(4'-bromophenyl)phenacyl (+)-2,3-decadienoate. Hydrogens are omitted for clarity and the absolute configuration was determined using anomalous dispersion effects of the bromine.

Assignments of absolute configurations to chiral allenes are often based on indirect evidence.[14] While the configuration of (+)-2,3-decadienoic acid was predicted to be S, based on the empirical rules formulated by Lowe[15] and Brewster,[16] the experiments described herein provide unambiguous proof.

We had hoped to correlate the configuration of the allenic acid with that of the inhibitory NAC thioester. Unfortunately, however, thioesterification[17] of the dextrorotatory acid affords 2,3-decadienoyl NAC with a negligible optical rotation. Nevertheless, the Lowe[15] and Brewster[16] rules predict that (+)-2,3-decadienoyl NAC (the inhibitory enantiomer) also has the S configuration. Even more compelling, however, is the fact that the S configuration is predicted based upon a) our recent determination of the steric course of the "normal" dehydrase-catalyzed isomerization of E-2-decenoyl NAC to Z-3-decenoyl NAC,[17] and b) the assumption that "suicide" inactivation of dehydrase by 3-decynoyl NAC is truly mechanism-based.

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8. Recrystallized from abs. EtOH as a white powder, mp 138-140 °C. NMR: 4.47 (s, 2 H, CH_2Br); 7.4-7.75 (m, 6 H, Ar-H); 8.07 (d, *J* = 8.5 Hz, 2 H, C-3',5' H). IR (KBr): 2950, 1690 cm^{-1}. Anal. Calcd. for $C_{14}H_{10}Br_2O$: C, 47.5; H, 2.85; Br, 45.14. Found: C, 47.29; H, 2.85; Br, 44.87.

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11. 4-(4'-Bromophenyl)phenacyl esters of *E*-2-octenoic acid, oleic acid, and geranic acid melt 14, 16, and 15 degrees higher, respectively, than the corresponding 4-bromophenacyl esters. 4-(4'-Bromophenyl)phenacyl esters should be useful for derivatization of very nonpolar carboxylic acids.

12. All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 78 and 80, systems of computer programs for the automatic solution of crystal structures from x-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq and M. M. Woolfson, University of York, England, 1978; BLS78A, an anisotropic block diagonal least squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; CRYSTALS, a crystallographic system written by D. J. Watkin and J. R. Carruthers, Chemical Crystallography Laboratory, University of Oxford, 1981; PLUTO78, a crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.

13. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, England CB2 1EW and are available from them.

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