Synthesis of (−)-2-Chloroshikimic Acid

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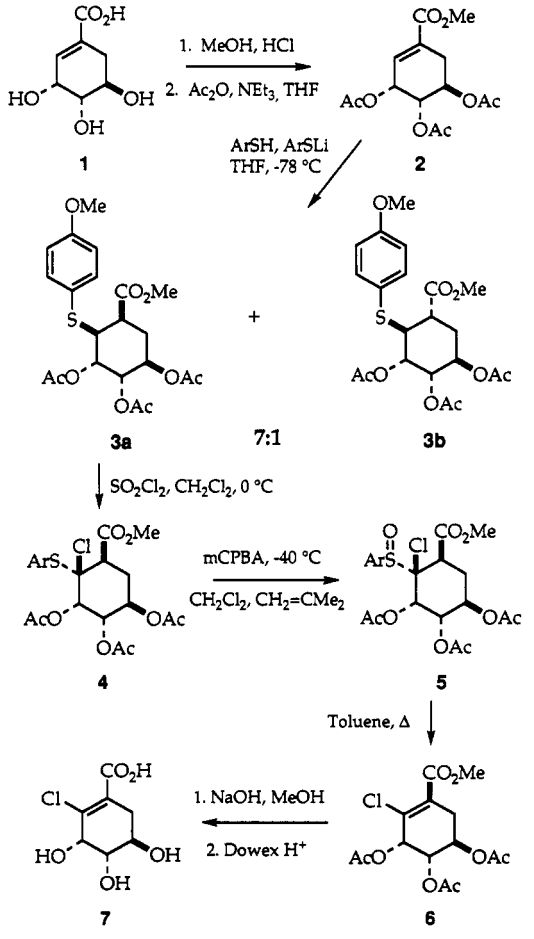
Halogenated analogs of natural substrates are useful as inhibitors, suicide substrates, or mechanistic probes of enzymatic reactions. In the shikimic acid pathway, for example, fluorinated and brominated analogs of substrates and intermediates have served these roles in the reactions catalyzed by DAHP synthase and DHQ synthase,[1] dehydroquinase,[2] EPSP synthase,[3] and chorismate synthase.[4] We now describe a convenient synthesis of the 2-chloro analog of shikimic acid (**7**) which is of potential interest as an alternative substrate of the enzymes that act on shikimic acid itself and, in derivatized form, of the enzymes that operate earlier and later in the pathway.

Methyl esterification and acetylation of (−)-shikimic acid provide the known tetraester **2**.[5] Michael addition of *p*-methoxybenzenethiol in the presence of a catalytic amount of the lithium thiolate affords the 2β-sulfides as a 7:1 mixture of *cis* and *trans* isomers **3a** and **3b**. The configuration of the major isomer was assigned from the vicinal coupling constants, and it was shown to exist in the chair conformation with the sulfide moiety in an axial position.[6]



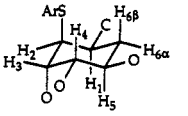
1 **2** **3a** **3b** **4** **5** **6** **7**

The 2β-chloro 2α-sulfide **4** is isolated in 78% yield from reaction of the mixture of sulfide diastereomers with sulfuryl chloride.[7] A route involving addition of thiophenol, to give the unsubstituted analog of **3**, was explored initially, but a higher yield is obtained in the chlorination step with the more electron-rich *p*-methoxy derivative.[8] A single chloro sulfide diastereomer was detected by NMR, suggesting that the product from oxidation of the minor isomer **3b** is lost on purification. Oxidation of chloro sulfide **4** was optimized with *m*-CPBA in methylene chloride at −40 °C to give sulfoxide **5** in 62% yield (1.6:1 mixture of sulfoxide diastereomers); isobutylene is introduced into the reaction mixture before workup to minimize over-oxidation. Both sulfoxide diastereomers **5** undergo elimination readily in refluxing toluene, which provided evidence for the indicated *trans* relationship between the carbomethoxy and aryl sulfide moieties in **4**. The desired vinyl chloride **6** is obtained in 32–66% yield; alkaline hydrolysis of this material proceeds in excellent yield to give (−)-2-chloroshikimic acid (**7**) after ion-exchange chromatography.

Experimental Section[9]

Methyl 3(*S*),4(*S*),5(*R*)-Triacetoxy-2(*S*)-[(4-methoxyphenyl)thio]-1(*R*)-cyclohexanecarboxylate (3a). To a stirred solution of 4-methoxybenzenethiol (4.11 g, 29.32 mmol) in THF (75 mL) at −78 °C was added *n*-butyllithium in hexanes (375 μL of 2.30 M, 0.86 mmol), followed by a solution of the tetraester[5] **2** (2.71 g, 8.62 mmol) in THF (100 mL). The solution was stirred for 50 min and then partitioned between saturated $NaHCO_3$ (250 mL) and ether (250 mL). The oily residue obtained on workup was chromatographed (35% EtOAc/hexane) to give adduct **3** (3.83 g, 98%) as a foamy white mixture of diastereomers (7:1). For the major diastereomer: ^{1}H NMR δ 7.45 (d, 2, J = 8.8), 6.84 (d, 2, J = 8.9), 5.63 (dd, 1, J = 3.1, 10.4), 5.44 (dd, 1, J = 2.1, 3.1), 5.12 (m, 1), 3.77 (s, 3), 3.69 (s, 3), 3.52 (dd, 1, J = 2.1, 4.0), 3.20 (td, 1, J = 4.0, 13.2), 2.35 (ddd, 1, J = 4.0, 5.1, 14.1), 2.040 (s, 3), 2.035 (s, 3), 1.99 (s, 3), 1.88 (td, 1, J = 13.2, 14.1); ^{13}C NMR δ 171.4, 170.3, 170.0, 169.4, 160.2, 135.8, 114.8, 114.5, 71.4, 69.7,

(1) Pilch, P.; Somerville, R. L. *Biochemistry* **1976**, *15*, 5315–5319.
(2) Harris, J.; Manthey, M. K.; Kleanthous, C.; Coggins, J. R.; Hawkins, A. R.; Abell, C. *J. Chem. Soc., Chem. Commun.*, in press.
(3) (a) Alberg, D. G.; Lauhon, C. T.; Nyfeler, R.; Fässler, A.; Bartlett, P. A. *J. Am. Chem. Soc.* **1992**, *114*, 3535–3546. (b) Walker, M. C.; Jones, C. R.; Somerville, R. L.; Sikorski, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 7601–7603.
(4) (a) Balasubramanian, S.; Davies, G. M.; Coggins, J. R.; Abell, C. *J. Am. Chem. Soc.* **1991**, *113*, 8945–8946. (b) Bartlett, P. A.; McLaren, K. L.; Alberg, D. G.; Fässler, A.; Nyfeler, R.; Lauhon, C. T.; Grissom, C. B. in *Prospects for Amino Acid Biosynthesis Inhibitors in Crop Protection and Pharmaceutical Chemistry*; Copping, L. G., Ed.; Society of Chemical Industry, 1989; pp 155–170.
(5) Delfourne, E.; Despeyroux, P.; Gorrichon, L.; Veronique, J. *J. Chem. Res. (S)* **1991**, 56–57.
(6) ^{1}H NMR 1D homonuclear decoupling experiments revealed the following coupling constants for the major adduct **3a**:



$J_{1,2}$ = 4.0	$J_{4,5}$ = 10.4	$J_{5,6\alpha}$ = 5.1
$J_{2,3}$ = 2.1	$J_{1,6\alpha}$ = 4.0	$J_{5,6\beta}$ = 13.2
$J_{3,4}$ = 3.1	$J_{1,6\beta}$ = 13.2	$J_{6\alpha,6\beta}$ = 14.1

(all coupling constants in Hertz)

(7) cf: Hancock, J. R.; Hardstaff, W. R.; Johns, P. A.; Langler, R. R.; Mantle, W. S. *Can. J. Chem.* **1983**, *61*, 1472–1480.
(8) cf: Robins, M. J.; Mullah, K. B.; Wnuk, S. F.; Dalley, N. K. *J. Org. Chem.* **1992**, *57*, 2357–2364.
(9) Unless otherwise specified, reaction workups culminated in washing the organic layer with saturated $NaHCO_3$ and brine, drying over Na_2SO_4, filtration, and evaporation at reduced pressure. J values are reported in hertz.

0022-3263/94/1959-0693$04.50/0

69.1, 55.2, 52.1, 51.5, 40.2, 27.5, 21.0, 20.9, 20.7. Anal. Calcd for $C_{21}H_{26}O_9S$: C, 55.49; H, 5.77. Found: C, 55.48; H, 5.57.

Methyl 3(*S*),4(*S*),5(*R*)-Triacetoxy-2(*S*)-chloro-2-[(4-methoxyphenyl)thio]-1(*R*)-cyclohexanecarboxylate (4). To a rapidly stirred solution of sulfide **3** (3.3 g, 7.26 mmol) in CH_2Cl_2 (200 mL) at 0 °C was added a 1.0 M solution of sulfuryl chloride in CH_2Cl_2 (36.3 mL, 5.0 equiv) and stirring was continued for 1 h. The reaction mixture was worked up and the residue was chromatographed (30% EtOAc/hexane) to give chloro sulfide **4** (2.77 g, 78%) as a white solid: mp 145–146 °C; ^{1}H NMR δ 7.37 (d, 2, J = 8.9), 6.83 (d, 2, J = 8.9), 5.42 (dd, 1, J = 2.9, 10.3), 5.15 (d, 1, J = 2.9), 5.05 (m, 1), 3.79 (s, 3), 3.77 (s, 3), 3.36 (m, 1), 2.27 (m, 1), 2.20 (s, 3), 2.01 (m, 1), 2.00 (s, 3), 1.90 (s, 3); ^{13}C NMR δ 170.4, 169.7, 169.2, 168.9, 161.5, 139.3, 118.0, 114.4, 78.6, 72.6, 70.3, 68.2, 55.3, 52.2, 46.2, 29.2, 21.0, 20.9, 20.5. Anal. Calcd for $C_{21}H_{25}ClO_9S$: C, 51.58; H, 5.15. Found: C, 51.33; H, 5.15.

Methyl 3(*S*),4(*S*),5(*R*)-Triacetoxy-2(*S*)-chloro-2-[(4-methoxyphenyl)sulfinyl]-1(*R*)-cyclohexanecarboxylate (5). To a stirred solution of chloro sulfide **4** (2.77 g, 5.66 mmol) in CH_2Cl_2 (125 mL) at −41 °C was added 85% *m*-chloroperoxybenzoic acid (4.89 g, 22.66 mmol, 4.0 equiv) and the reaction mixture was stirred for 1.5 h. The reaction was quenched by bubbling isobutylene into the mixture at −41 °C for 5 min. The flask was allowed to warm to room temperature for the gases to evolve, and the mixture was diluted with CH_2Cl_2 (25 mL). The solution was worked up, and the product was chromatographed (40% EtOAc/hexane) to give chloro sulfoxide **5** (1.78 g, 62%), 1.6:1 ratio of diastereomers, as a colorless solid: major isomer: ^{1}H NMR δ 7.51 (d, 2, J = 8.9), 6.97 (d, 2, J = 9.0), 5.33 (dd, 1, J = 2.9, 10.3), 5.09 (m, 1), 5.06 (d, 1, J = 2.9), 3.84 (s, 3), 3.8 (m, 1), 3.75 (s, 3), 2.36 (m, 1), 2.14 (s, 3), 2.00 (s, 3), 2.0 (m, 1), 1.88 (s, 3) (minor isomer: δ 7.71 (d, 2, J = 8.8), 6.99 (d, 2, J = 8.8), 3.81 (s, 3)); ^{13}C NMR δ 170.3, 169.6, 169.0, 168.4, 163.5, 129.0, 126.9, 114.5, 86.6, 72.1, 70.5, 67.9, 55.5, 52.7, 43.4, 29.7, 21.0, 20.9, 20.5 (minor isomer: δ 128.5, 114.9); FAB-MS (NBA): m/z = 505.3 (100%, M − H$^+$), 469.3 (25%, MH$^+$ − HCl); HRMS calcd for $C_{21}H_{26}ClO_{10}S$: m/z = 505.0935, found 505.0945.

Methyl 3(*S*),4(*S*),5(*R*)-Triacetoxy-2-chloro-1-cyclohexenecarboxylate (6). Chloro sulfoxide **5** (980 mg, 1.94 mmol) was heated in refluxing toluene (125 mL) for 12.5 h. The solution was allowed to cool and was evaporated under reduced pressure. Chromatography (35% EtOAc/hexane) led to the isolation of major product **6** (214 mg, 32%) as a colorless oil (on a smaller scale (141 mg), this reaction has provided up to 66% yield of purified product): ^{1}H NMR δ 5.80 (m, 1), 5.23 (m, 2), 3.79 (s, 3), 3.07 (m, 1), 2.45 (m, 1), 2.12 (s, 3), 2.06 (s, 3), 2.02 (s, 3); ^{13}C NMR δ 169.6, 169.4, 169.3, 164.8, 130.4, 128.5, 69.6, 68.5, 65.2, 52.1, 31.2, 20.6, 20.24, 20.18; FAB-MS (LiCl): m/z = 355.0 (54, M + Li), 289.0 (35, M − OAc); HRMS calcd for $C_{14}H_{18}ClO_8$: m/z = 349.0690, found 349.0698.

2-Chloroshikimic Acid (7). A solution of the tetraester **6** (23.0 mg, 0.06 mmol) and 1 N aqueous NaOH (1.32 mL, 20.0 equiv) in methanol (1 mL) was stirred at room temperature for 15 min. The solution was applied directly to an ion-exchange column (Dowex 50X2-400 resin (H$^+$ form), prewashed, with methanol and water) and eluted with water. The product was contained in the first fractions, which were lyophilized to give 18.4 mg (80% yield) of 2-chloroshikimic acid as a white solid: mp 154-155 °C; $[\alpha]^{23}_D$ = −115.3° (c = 0.17 in H_2O); ϵ_{193} = 7200; ^{1}H NMR (D_2O) δ 4.23 (d, 1, J = 4.2), 3.84 (ddd, 1, J = 9.6, 8.2, 5.6), 3.66 (dd, 1, J = 9.7, 4.4), 2.73 (dd, 1, J = 17.6, 5.6), 2.21 (dd, 1, J = 17.6, 8.2); ^{13}C NMR (CD_3OD) δ 169.5, 134.4, 129.5, 74.1, 73.0, 66.9, 35.5; FAB-MS (NBA·NaCl) 231 (70, MNa$^+$), 209 (20, MH$^+$); HRMS calcd for $C_7H_9ClO_5Na$: m/z = 231.0036, found 231.0031. Anal. Calcd for $C_7H_9ClO_5$: C, 40.3, H, 4.35. Found: C, 38.24; H, 4.38 (corresponding to 8·0.6 H_2O).

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