



A Publication
of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

The procedures in *Organic Syntheses* are intended for use only by persons with proper training in experimental organic chemistry. All hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

In some articles in *Organic Syntheses*, chemical-specific hazards are highlighted in red "Caution Notes" within a procedure. It is important to recognize that the absence of a caution note does not imply that no significant hazards are associated with the chemicals involved in that procedure. Prior to performing a reaction, a thorough risk assessment should be carried out that includes a review of the potential hazards associated with each chemical and experimental operation on the scale that is planned for the procedure. Guidelines for carrying out a risk assessment and for analyzing the hazards associated with chemicals can be found in Chapter 4 of Prudent Practices.

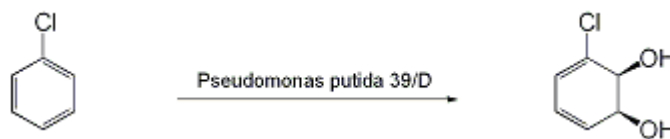
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September 2014: The paragraphs above replace the section "Handling and Disposal of Hazardous Chemicals" in the originally published version of this article. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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1-CHLORO-(2S,3S)-DIHYDROXYCYCLOHEXA-4,6-DIENE

[3,5-Cyclohexadiene-1,2-diol, 3-chloro-, (1S-cis)-]



Submitted by Tomas Hudlicky¹, Michele R. Stabile¹, David T. Gibson², and Gregory M. Whited³.

Checked by Michel Chartrain, Norihiro Ikemoto, and Ichiro Shinkai.

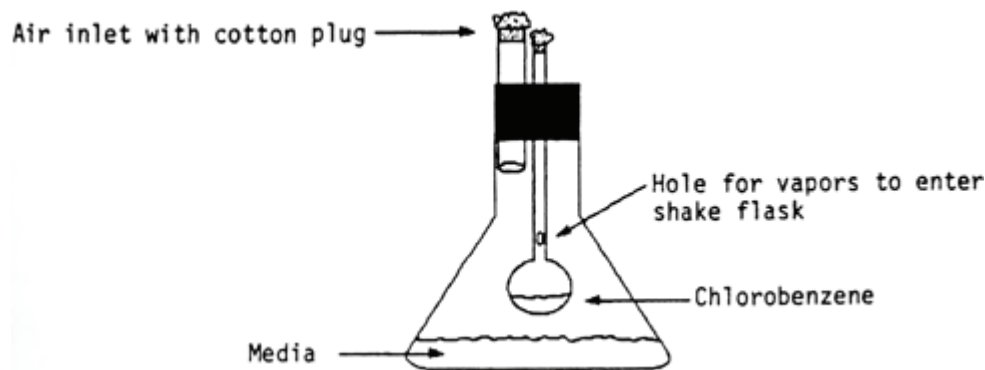
1. Procedure

Caution! Chlorobenzene is an irritant and possible carcinogen.

A. Preculture preparation. A 250-mL Erlenmeyer flask containing 50 mL of mineral salt broth (MSB) (Note 1) with 0.2% L-arginine hydrochloride (L-arginine-HCl) and fitted with a cotton plug is sterilized (Note 2). In a laminar flow hood, a single colony of *Pseudomonas putida* 39/D (*Pp* 39/D) (Note 3) is selected from a fully grown plate (Note 4), (Note 5), (Note 6) and transferred to the solution with a sterile loop taking care not to place the hand over the plate or flask. The flask is then placed in a benchtop orbital incubator shaker at 30°C and 200 rpm for 24 hr.

B. Shake flask. A 2800-mL Fernbach flask fitted with a vapor bulb and an air inlet (see Figure 1) containing 500 mL of MSB solution with 0.2% L-arginine-HCl is sterilized (Note 2). After cooling, the 50-mL preculture is added via aseptic transfer (Note 7). The vapor bulb is filled with 10 mL of chlorobenzene, and the flask is carefully placed in an orbital incubator shaker at 150 rpm and 30°C for 48 hr. The excess chlorobenzene is removed from the central chamber with a pipette, and the pH of the solution is adjusted, if necessary, to approximately 8 or 9. The suspension is poured into two large centrifuge tubes, and the cells are removed by centrifugation for 30 min at 10°C and 8000 rpm. The supernatant solution is saturated with salt and extracted with ethyl acetate (4 × 100 mL). The organic layers are combined, dried with magnesium or sodium sulfate, and filtered (Note 8). The solvents are evaporated under reduced pressure to afford a tan-colored solid (190 mg). The diene diol is further purified by recrystallization from methylene chloride/hexanes to yield an off-white solid (160 mg) (Note 9). The material has a reasonable shelf life (Note 10) when kept acid-free to inhibit decomposition to chlorophenols.

Figure 1. Shake Flask Apparatus



2. Notes

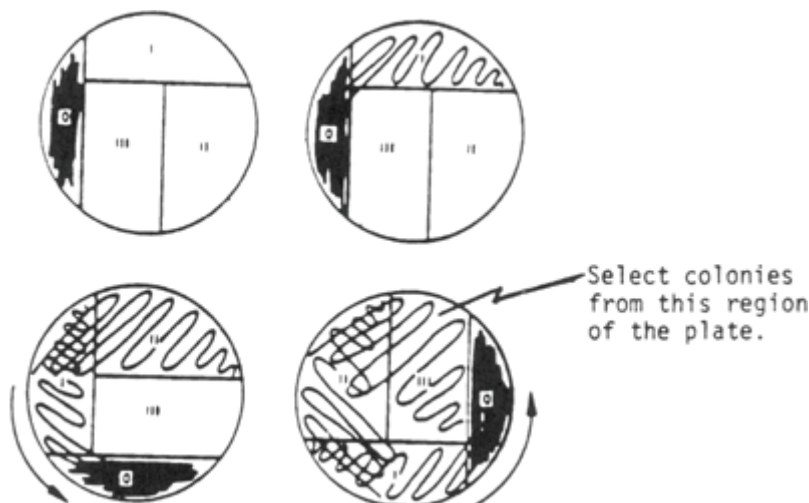
1. MSB components⁴ are as follows: 4% of solution A, 2% of solution B, 1.5% of solution C. Mix solutions A, B, C and add distilled water to 90% volume. Adjust pH to 7.2 with 10 M KOH, and adjust to final volume with distilled water. Solution A (for 1 L): 1 M KH_2PO_4 (136 g/L) and 1 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (268.1 g/L). Adjust the solution to pH 7.2 with 10 M KOH. Solution B (Hunter's Base – Vitamin-free, for 1 L): **Nitrilotriacetic acid (NTA)**, 10 g/L; KOH, 7.5 g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 29.6 g/L; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3.3 g/L; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 9.3 mg/L; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 99 mg/L; Metals 44 solution 50 mL/L. [note: 10.0 g of **nitrilotriacetic acid (NTA)** is dissolved with stirring in 150 mL of distilled H_2O containing 7.5 g of KOH. Next, 29.6 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ is dissolved separately in 150 mL of distilled H_2O . A solution of 3.3 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 150 mL of distilled water is prepared. The MgSO_4 solution is then added to the NTA solution; add *slowly*, to avoid any clouding of the mixture. Once the solutions are mixed, the CaCl_2 solution is *gradually* added with stirring. Again, do NOT allow the mixture to become cloudy as this will result in the formation of insoluble precipitates. Prepare a solution from 9.3 mg of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ and 99 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 150 mL of distilled water. Add this solution with stirring to the mixture already prepared. A pale yellow color should appear when the solutions have been mixed. Add the Metals 44 solution and bring the total volume to 1.0 L with distilled H_2O . The pH of the solution should be adjusted to 6.8 CAREFULLY with 10 N NaOH – preferably in 0.2-mL aliquots - otherwise, insoluble precipitates will form. Store refrigerated at 4-10°C]. Metals 44 solution (for 100 mL): **ethylenediaminetetraacetic acid (EDTA)**, 250 mg ; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.095 g ; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 500 mg ; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 154 mg ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 39.2 mg ; $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 24.8 mg ; $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 17.7 mg . [The solids are dissolved one at a time in 100 mL of distilled H_2O and ~1 drop of **sulfuric acid** (1 M) is added to retard precipitation. The solution should be aquamarine blue.] Solution C (for 1 L): 200 mg/L of $(\text{NH}_4)_2\text{SO}_4$ in distilled water.

2. All flasks, solutions and other accessories that come into contact with the media solutions must be sterilized or purchased pre-sterilized. For sterilization, a high pressure steam autoclave (AMSCO 3021) was used with a liquid cycle that holds the contents at a temperature of 121°C/14 psi for 25 min.

3. The organism has been deposited with DSM, the German Collection of Microorganisms and Cell Cultures, as # 6414 and also with ATCC, the American Type Culture Collection, as strain number ATCC 700008.

4. Agar plate preparation. Dilute 20 mL of solution A, 10 mL of solution B, 7.5 mL of solution C and 2.5 g of **L-arginine** to 250 mL in a 1-L flask equipped with a stir bar. In a separate 1-L flask, mix 10 g of Bacto-Agar in 250 mL of distilled H_2O . Sterilize the solutions. A precipitate present in the MSB solution at this point will dissolve with stirring and cooling of the solution. When the MSB and agar solutions are at ~45-50°C, quickly combine, stir and pour the mixed solution into 100-mm diameter plastic Petri dishes (Fisher Scientific Company) (approximately 20 mL of solution per plate). In general, the method used for obtaining single colonies is called "streaking for isolation," shown in [Figure 2](#). Each direction of streaking is to be done with a flame-sterilized wire loop or a new disposable plastic loop, four loops for the entire procedure. All procedures are performed under sterile conditions in a laminar flow hood.

Figure 2. Summary of Steps Used to Streak for Isolation



5. Storage of cells: A culture of cells was grown in sterile Lauria broth (10 g/L of tryptone, 5 g/L of yeast extract, 5 g/L of NaCl) that had been inoculated (transfer of cells as described in part A) with a single colony from an agar plate. These organisms were grown for 24 hr. The above solution (0.8 mL) was transferred to a sterile cryovial, combined with sterile glycerol solution (0.8 mL) and frozen at -78°C . (Glycerol solution: 60 mL of glycerol, 2.5 g of tryptone, 1.25 g of yeast extract, 2.5 g of NaCl, 182 mL of distd water.) The cells are periodically checked for activity (Note 6).

6. Indigo test: Two plates of *Pseudomonas* agar (MSB + 0.2% arginine + 2% Bacto-Agar) were streaked and allowed to grow in the presence of chlorobenzene. In the lid of one dish was placed a vial containing toluene (for use as control) and plugged with cotton. In the lid of the other plate was placed a vial containing chlorobenzene. The plates were placed in an incubator (30°C) overnight. After the plate was grown (~24 hr), it was removed from the incubator and the vial containing the substrate was removed and replaced with several crystals of indole. After the plate was left at room temperature for 30 min, the cells were checked for the presence of a blue color, indicative of indigo formation. (Color should be very apparent in 30 min for toluene-induced cells.) In this case, a blue color was apparent for both plates thus proving that chlorobenzene would induce the production of toluene dioxygenase and that the cells were viable. The same technique can be used for substrates other than chlorobenzene; if a blue color is not observed, the substrate will not induce production of the dioxygenase enzyme during growth of the organism.

7. An aseptic transfer involves first passing the lip of the preculture flask through a flame and repeating the flaming procedure with the lip of the Fernbach flask. The preculture is then poured into the larger flask carefully, without allowing the flasks to come into contact.

8. To facilitate breaking up any emulsion, the checkers centrifuged (20 min, 7000 rpm) the combined organic layers.

9. The spectral/physical data of 1-chloro-(2S,3S)-dihydroxycyclohexa-4,6-diene are as follows: $R_f = 0.32$ (1:1 hexane/ethyl acetate) mp $82-84^{\circ}\text{C}$; $[\alpha]_D^{25} +54^{\circ}$ (CHCl_3 , c 0.59); $^1\text{H NMR}$ (CDCl_3) δ : 2.63 (d, 1 H, $J = 8.4$), 2.74 (d, 1 H, $J = 7.3$), 4.19 (t, 1 H, $J = 7.3$), 4.48 (m, 1 H), 5.87 (m, 2 H), 6.12 (m, 1 H); $^{13}\text{C NMR}$ (CDCl_3) δ 69.1 (CH), 71.4 (CH), 122.7 (CH), 123.4 (CH), 128.0 (CH), 134.9 (C).

10. The half-life of 1-chloro-(2S,3S)-dihydroxycyclohexa-4,6-diene ($t_{1/2}$) is 4 days in CDCl_3 at room temperature. The diol can be stored at -20°C or -80°C for several months without decomposition.

Waste Disposal Information

All toxic materials were disposed of in accordance with "Prudent Practices in the Laboratory"; National Academy Press; Washington, DC, 1995. The bacterial cell mass from fermentation is, after sterilization, judged suitable for disposal to municipal sewers.

3. Discussion

The pioneering work of Gibson^{5, 6} on the isolation and mutation of *Pseudomonas* strains that oxidatively degrade aromatic compounds has led, 25 years later, to the application of cyclohexadiene cis-diols in asymmetric synthesis. The first applications of these types of compounds to synthesis were the use of meso-diol derived from benzene for production of polyphenylene⁷ by ICI and in the synthesis of racemic pinitol by Ley in 1987.⁸ The first use of the title compound in synthesis was in 1990 in the preparation of both enantiomers of erythrose.⁹ This field increased greatly in the late 1980s and there are now numerous reports on the use of the title compound as a versatile chiral synthon. Several reviews have appeared.^{10 11 12 13 14 15 16 17 18 19 20 21}

Over 300 diol metabolites are known.^{22 23 24} Several diols and some secondary synthons derived from them have recently become commercially available: Eastman Fine Chemicals, Genencor: (1S-cis)-3-Chloro-3,5-cyclohexadiene-1,2-diol, (1S-cis)-3-bromo-3,5-cyclohexadiene-1,2-diol, (1S-cis)-3-iodo-3,5-cyclohexadiene-1,2-diol, (5S-cis)-5,6-dihydroxy-1,3-cyclohexadiene-1-carbonitrile, cis-2R,3S-2,3-dihydroxy-2,3-dihydrobenzimidazole acetone, (1R-cis)-1,2-dihydro-1,2-naphthalenediol, (1R-cis)-1,2,3,4-tetrahydro-1,2-naphthalenediol, (4S-trans)-4,5-dihydroxy-3-oxo-1-cyclohexene-1-carboxylic acid, furo[3,4-d]-1,3-dioxol-4(3aH)-1-dihydro-6-hydroxy-2,2-dimethyl-[3aR-(3aa,6aa)].

References and Notes

1. Department of Chemistry, Virginia Polytechnic Institute and State University *Address correspondence to this author at: Department of Chemistry, University of Florida, Gainesville, FL 32611;
2. Department of Microbiology, University of Iowa;
3. Genencor International Incorporated, Kimball Way, South San Francisco, CA.
4. Modified from Cohen-Bazire, G.; Sistrom, W. R.; Stanier, J. *Cell. Comp. Phys.* **1957**, *49*, 25; See also, Hudlicky, T.; Boros, C. H.; Boros, E. E. *Synthesis*, **1992**, 174.
5. Gibson, D. T.; Hensley, M.; Yoshioka, H.; Mabry, T. J. *Biochemistry* **1970**, *9*, 1626;
6. Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, *7*, 2653.
7. Ballard, D. G. H.; Courtis, A.; Shirley, I. M.; Taylor, S. C. *Macromolecules* **1988**, *21*, 294.
8. Ley, S. V.; Sternfeld, F.; Taylor, S. *Tetrahedron Lett.* **1987**, *28*, 225.
9. Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *J. Org. Chem.* **1990**, *55*, 4683.
10. Reviews: (a) Boyd, D. R. *Nat. Prod. Reports* **1998**, 309;
11. Grund, A. D. *SIM News* **1995**, *45*, 59;
12. Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, *96*, 1195;
13. Hudlicky, T. In "Green Chemistry: Designing Chemistry for the Environment"; Anastas, P. T.; Williamson, T., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 1996; Vol. 626, p. 180;
14. Sheldrake, G. N. In "Chirality and Industry"; Collins, A. N.; Sheldrake, G. N.; Crosby, J., Eds.; John Wiley and Sons Ltd., 1992; p. 127;
15. Hudlicky, T.; Thorpe, A. J. *J. Chem. Soc., Chem. Commun.* **1996**, 1993;
16. Hudlicky, T. *Chem. Rev.* **1996**, *96*, 3;
17. Hudlicky, T.; Reed, J. W. *Adv. Asymmetric Synth.* **1995**, *1*, 271;
18. Illman, D. L. *Chemical and Engineering News*, September 5, 1994, p. 22;
19. Brown, S. M.; Hudlicky, T. *Org. Synth.: Theory Appl.* **1993**, *2*, 113;
20. Carless, H. A. H. *Tetrahedron: Asymm.* **1992**, *3*, 795;
21. Widdowson, D. A.; Ribbons, D. W.; Thomas, S. D. *Janssen Chim. Acta* **1990**, *8*, 3.
22. Stabile, Michele R., Ph.D thesis, Virginia Polytechnic Institute and State University, 1995;
23. McMordie, R. A. S. Ph.D Thesis, School of Chemistry, The Queen's University of Belfast, Belfast BT9 5AG, U. K., 1991;
24. Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1998** .

Appendix
Chemical Abstracts Nomenclature (Collective Index Number);
(Registry Number)

1-Chloro-(2S,3S)-dihydroxycyclohexa-4,6-diene:
 3,5-Cyclohexadiene-1,2-diol, 3-chloro-, (1S-cis)- (10); (65986-73-4)

Potassium dihydrogen phosphate:
 Phosphoric acid, monopotassium salt (9); (7778-77-0)

Disodium hydrogen phosphate:
 Phosphoric acid, disodium salt (8,9); (7558-79-4)
 Nitrotriacetic acid: CANCER SUSPECT AGENT:
 Acetic acid, nitrotri- (8);
 Glycine, N,N-bis(carboxymethyl)- (9); (139-13-9)

Ammonium molybdate(VI) tetrahydrate:
 Molybdic acid, hexaammonium salt, tetrahydrate (8,9); (12027-67-7)

Ethylenediaminetetraacetic acid, tetrasodium salt: CANCER SUSPECT AGENT:

Glycine, N,N'-1,2-ethanediylbis[N-(carboxymethyl)-, tetrasodium salt, trihydrate (9); (67401-50-7)

Cobalt nitrate hexahydrate:
Nitric acid, cobalt (2+ salt), hexahydrate (9); (10026-22-9)

Sodium tetraborate decahydrate:
Borax (8,9); (1303-96-4)

L-Arginine hydrochloride:
L-Arginine, monohydrochloride (9); (1119-34-2)

Chlorobenzene:
Benzene, chloro- (8,9); (108-90-7)

Agar (8,9); (9002-18-0)

Tryptones (Bacteriological): *Chem. Abstr.* See: Peptones, Bacteriological (10); (73049-73-7)

Indole (8);
1H-Indole (9); (120-72-9)