ethanol–water (40 ml) and hydrogenated in the presence of *ca.* 200 mg of 10% palladium on carbon catalyst for 15 min with the initial pressure of about 2 atm. The catalyst was filtered and washed with water. The combined filtrate and washings were evaporated to dryness, and then the residue was evaporated several times with ethanol until colorless microcrystals were obtained. After drying overnight at 78° *in vacuo*, 287 mg (92%) of product was obtained: mp 136° (sintered) 210–250° dec; $[\alpha]_D$ +44° (*c* 0.6, H_2O), nmr (D_2O) H-6, δ 7.77 (1 H, d), H-5, 6.03 (1 H, d), H-1′, 5.67 (1 H, d), NCH_3, 2.79 (3 H, s).

Anal. Calcd for $C_{16}H_{26}O_8N_6 \cdot H_2O$: C, 42.86; H, 6.29; N, 18.74. Found: C, 43.13; H, 6.36; N, 18.73.

1-[4-Deoxy-4-(sarcosyl-DL-seryl)amino-β-D-glucopyranosyl]-cytosine [mp 172–189° dec, $[\alpha]^{23}_D$ +11° (*c* 0.8, H_2O)] was synthesized from **1**, *N*-Cbz-DL-serine, and *N*-Cbz-sarcosin by the same procedure used for the preparation of **6a**.

Hydrolysis of Compound 6a and Isolation of D-Serine.—Compound **6a** (980 mg) was dissolved in 6 *N* HCl (50 ml) and refluxed gently for 24 hr. After concentration *in vacuo* the residue was taken up in water (50 ml) and passed through a column of Dowex-1 (OH^-) (2.6 × 9.6 cm). The column was washed with water (2 l.) to remove compound **1**. The amino acids were eluted from the column with 1 *N* HCl (100 ml). The acid eluate was evaporated to dryness and the residue was dissolved in a small amount of water and passed through a column of Amberlite IR-45 (OH^-) (2.6 × 10 cm) to remove hydrogen chloride. The column was washed with water (200 ml) and the effluent was evaporated to dryness. Crude D-serine (106 mg) was crystallized from the residue from methanol (5 ml). One recrystallization of the crude product from water–ethanol gave colorless needles: mp 216–217° dec; $[\alpha]^{23}_D$ +7° (*c* 1, H_2O); $[\alpha]^{23}_D$ −14° (*c* 0.9, 1 *N* HCl). The authentic D-serine, mp 214–215° dec, showed the identical optical rotations under the same conditions.

Both the methanolic and water–ethanol mother liquors of the crystallizations were combined and applied to two sheets of Whatman #1 paper (46 × 57 cm) and developed with 88% phenol. The serine bands were extracted with water and evaporated to dryness. The residue (34.7 mg) showed $[\alpha]^{23}_D$ −13° (*c* 0.9, 1 *N* HCl).

Degradation of Compound 6b and Characterization of D-Alanine.—Compound **6b** (890 mg) was hydrolyzed and D-alanine was obtained after essentially the same processing for the isolation of D-serine from compound **2a**. The crude residue from the Amberlite IR-45 column was taken up in 3 ml of water and applied to two sheets (46 × 57 cm) of Whatman #1 paper, developed with 88% phenol, and the alanine bands were eluted with water and evaporated to dryness. The residue (125 mg) had $[\alpha]_D$ −10° (*c* 1, 6 *N* HCl). One recrystallization of the crude sample gave pure D-alanine (65 mg), $[\alpha]_D$ −12° (*c* 1, 6 *N* HCl).

Registry No.—**3a**, 33780-67-5; **3b**, 33780-68-6; **3c**, 33780-69-7; **4a**, 33780-70-7; **4b**, 33780-71-1; **4c**, 33780-72-2; **5a**, 33780-73-3; **5b**, 33780-74-4; **5c**, 33780-75-5; **6a**, 31883-24-6; **6b**, 33780-77-7; **6c**, 33780-78-8; 1-[4-deoxy-4-(sarcosyl-DL-seryl]amino-β-D-glucopyranosyl]cytosine, 33780-79-9; D-serine, 312-84-5; D-alanine, 338-69-2.

Dioldithiol Analogs of the 1,2,4,5-Cyclohexanetetrols. Chemical and Nuclear Magnetic Resonance Studies[1,2]

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Reaction of 1,4-cyclohexadiene dioxide (cis–trans mixture) with sodium benzylmercaptide gave a mixture from which were isolated three of the four expected isomers of dibenzylmercaptocyclohexanediol. The two possible structures for each product were 4,6-dibenzylmercapto-1,3-cyclohexanediol (**2**) and 2,5-dibenzylmercapto-1,4-cyclohexanediol (**4**). For each structure, one meso and one DL diastereomer (**12** and **8**, or **9** and **10**) would be predicted. For the molecules of either meso isomer, only one (tetraequatorial) chair conformation (**14** or **16**) is predicted. The DL isomer molecules, however, should each be diaxial–diequatorial (**13a** or **15a**) and readily transformed by ring inversion into alternate chair conformations (**13b** or **15b**) indistinguishable from the original. This analysis permitted nmr spectral assignments based on (1) presence of time-averaging effects (DL isomers only); (2) equivalence between corresponding methylene protons at positions 3 and 6 (para isomers only). The assignments are para-DL (14/25), mp 92°; para-meso (15/24), mp 158°; meta-DL (14/36), mp 109°; meta-meso (13/46), unknown. The mp 109° isomer identity was confirmed by chemical correlation with the known *trans*-1,3-cyclohexanediol.

We wish to report nmr configurational proofs for the family of four trans–trans dioldithiols which are analogous to 1,2,4,5-cyclohexanetetrol and derived from 1,3- or 1,4-cyclohexanediol (see formulas **8**, **9**, **10**, and **12**, Scheme I). This family of structural and stereoisomers provides an interesting example of nmr configurational assignments based on conformational analysis and nmr spectroscopy. A similar study on the parent tetrols was previously reported by one of us.[5] Although only the di-*S*-benzyl derivatives were actually examined, the structures and configurations of the parent dioldithiols can now be easily established by simple chemical correlations.[6]

The synthetic and nmr studies here reported are part of a program for preparation of cyclitols and other carbohydrates[7] in which most or all of the oxygen functions will be replaced by sulfur functions (see Acknowledgment).[2b]

(1) Presented to the Division of Organic Chemistry at the 159th National Meeting of the American Chemical Society, Houston, Texas, Feb 1970.

(2) (a) Paper XXXVIII on Alicyclic Carbohydrates; for paper XXXVII, see N. Kurihara, Y. Sanemitsu, M. Nakajima, G. E. McCasland, and L. F. Johnson, *Agr. Biol. Chem.*, **35**, 71 (1971). For paper XXXVI, see G. E. McCasland, M. O. Naumann, and Lois J. Durham, *J. Org. Chem.*, **34**, 1382 (1969). (b) For preceding publication on thio carbohydrates, see G. E. McCasland and A. B. Zanlungo, *Carbohyd. Res.*, **17**, 475 (1971). (c) For preceding paper on (nonalicyclic) carbohydrates, see A. E. Lipska and G. E. McCasland, *J. Appl. Polym. Sci.*, **15**, 419 (1971).

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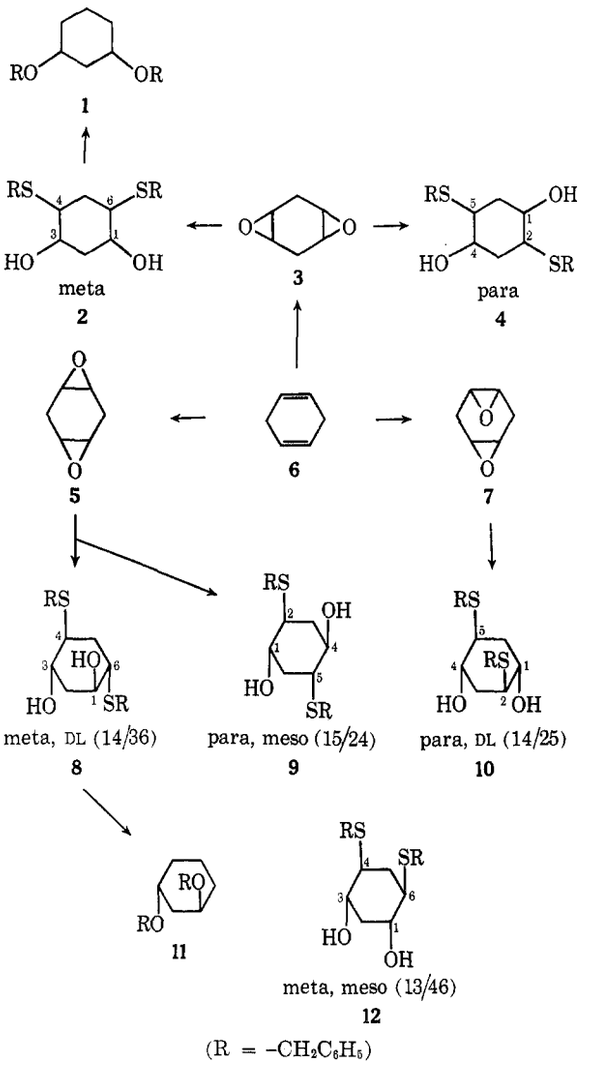
(4) Stanford University.

(5) For studies on the 1,2,4,5-cyclohexanetetrols, see G. E. McCasland, S. Furuta, L. F. Johnson, and J. N. Shoolery, *J. Org. Chem.*, **28**, 894 (1963).

(6) A benzylmercaptocyclohexane is easily converted to a mercaptocyclohexane, with retention of configuration, by reaction with sodium in liquid ammonia. See G. E. McCasland, S. Furuta, and A. Furst, *ibid.*, **29**, 724 (1964), for application of this reaction to mercaptodeoxyinositol derivatives.

(7) A report on sulfur analogs of D-iditol and D-mannitol was presented by G. E. McCasland and A. B. Zanlungo to the Division of Carbohydrate Chemistry at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970. See also G. E. McCasland and A. B. Zanlungo, *Carbohyd. Res.*, **17**, 475 (1971).

Scheme I



Reaction of 1,4-cyclohexadiene dioxide[8] (**3**) with sodium benzylmercaptide in methanol replaced each epoxy group with the pair of groups, –OH and $-SCH_2-C_6H_5$. The two hydroxyl groups necessarily are meta or para; likewise the two benzylmercapto groups (see formulas 2 and 4).

Since trans opening of epoxide rings by nucleophiles is assumed, the cis dioxide **7** should produce isomers **10** and **12**; the trans dioxide **5**, isomers **8** and **9**. For convenience, the dioxide[8] actually used was a cis–trans mixture[8] (64% trans). The three products actually isolated have mp 92, 109, and 158°.

The fourth predicted isomer, **12**, has not been obtained. For steric reasons, the yield of **12** may be very small. This diastereomer, once formed, should be quite stable in its all-equatorial conformation **16**. We believe, however, that in the nucleophilic attack on the *second* epoxy group of the dioxide **7** there would be strong steric repulsion between the benzylmercaptide ion attempting to enter at position 6 (formula **12**) and the benzylmercapto group already present at position **4**. In the transition state, these two large groups should have almost a 1,3-diaxial relationship. Similar steric effects have previously been noted by one of us in the formation of tetrol tetrabenzoates from 1,4-cyclohexadiene and silver benzoate (Prevost reaction).[9]

Structures, Configurations, and Conformations.—A large number of structural and stereoisomers are possible for any tetrasubstituted cyclohexane of the general type $C_6H_8A_2B_2$ or $C_6H_8A_2BC$. Since the compounds here reported are derived from 1,4-cyclohexadiene, the substituents are limited to ring positions 1, 2, 4, and 5.[10] Because of the reactions employed, only the "trans–trans" configurations need be considered for the products. In the case of the 1,2,4,5-cyclohexanetetrols[5] or tetrathiols,[11] only two trans–trans configurations are possible, *i.e.*, meso (15/24) and DL (14/25); compare the formulas **9** and **10**. The substitution type for the tetrols and tetrathiols is $C_6H_8A_4$. With four substituents in the substitution type $C_6H_8A_2B_2$, two trans–trans configurations are possible for the meta structure, and two different trans–trans configurations for the para structure (see formulas **12**, **8**, **9**, and **10**).

The isomer meta-meso (13/46), **12**, would be optically inactive, because its molecule in the conformation **16** has a plane of symmetry (see Scheme II). Since it has no axis of symmetry, the point group is C_s. The isomer para-meso (15/24), **9**, is inactive, because in the conformation **14** it has a center of symmetry but no plane or axis of symmetry (point group C_i).

For convenience, the stereoformulas in Schemes I and II depict only one enantiomer of each racemic product. The para (14/25) enantiomer depicted in formula **13a** has a nonsuperposable mirror image (not shown); on ring inversion the molecule is converted to **13b**, which is indistinguishable from the original. The formulas **15a** and **15b** for one of the meta (14/36) enantiomers have a similar relationship.

Nmr Configurational Proofs for the Four Dibenzylmercaptocyclohexanediols.—The spectrum of each isomer was first examined to see if the two protons in one ring methylene were respectively equivalent to those in the other. If so, the para structure **13a,b** or **14** must be present. If not, the meta structure **15a,b** or **16** was indicated. (In the meta isomer **15a,b**, the two protons within each ring methylene are geometrically equivalent only to each other; in the meta isomer **16**, there is no equivalence at all within the set of four methylene protons.)

The spectrum was next examined to see if the four O–C–H and S–C–H protons were strictly axial or a time average of axial and equatorial. This was apparent from the magnitudes of the chemical shifts and coupling constants. The presence of time averaging pointed to the DL configuration **13a,b** or **15a,b**; its absence to the meso configuration **14** or **16**.

These observations provided information necessary and sufficient to classify each isomer as (1) para and meso; (2) para and DL; (3) meta and meso; or (4) meta and DL.

Nmr Spectrum of the Mp 158° Isomer (Para-Meso).—Time averaging due to ring inversion was not observed; the four O–C–H and S–C–H protons were strictly axial (formula **14**).

(8) T. W. Craig, G. R. Harvey, and G. A. Berchtold, *J. Org. Chem.*, **32**, 3745 (1967).

(9) (a) Reference 5, p 897; (b) see also G. E. McCasland and E. C. Horswill, *J. Amer. Chem. Soc.*, **76**, 1654 (1954).

(10) Note that the position numbers for the meta isomers are 1, 3, 4, 6 (*Chemical Abstracts*) not 1, 2, 4, 5.

(11) G. E. McCasland, A. K. M. Anisuzzaman, S. R. Naik, and Lois J. Durham, unpublished results.

The O–C–H signal (3.84 ppm) was a triplet of doublets, due to couplings (large, large, small) with the two axial and one equatorial neighboring protons. The coupling pattern and coupling constant magnitudes of about 10–11 Hz show that the two O–C–H protons are axial and that there is little or no ring inversion.

The two equivalent S–C–H protons (maximum of eight lines expected) appeared actually as a seven-line multiplet centered at 2.92 ppm, due to coincidence of the two center lines. The spacings were 13, 10, and 4 Hz, due to couplings with the two axial and one equatorial neighboring protons. The two equivalent equatorial methylene protons produced a perturbed six-peak pattern (approximately a pair of triplets) centered at 2.64 ppm, with spacings of 4, 4, and 13 Hz. This pattern almost overlapped the S–C–H pattern at 2.92 ppm.

The two equivalent axial methylene protons produced a high-field six-line multiplet at approximately 1.86 ppm, with spacings of 13, 13, and 11 Hz, due to coupling with the one geminal and two neighboring axial protons.

The presence of the four equivalent sets of ring protons points strongly to the centrosymmetric molecule **14**. The coupling patterns are consistent with the para structure **9** and all-equatorial conformation.

Nmr Spectrum of the Mp 92° Isomer (Para-DL).—Time averaging of the signals, due to ring inversion, was observed. The pattern of the two-equivalent O–C–H protons appeared at 3.75 ppm, where it was partly obscured by a large peak at 3.74 ppm assigned to *S*-methylene.

Signals for the two equivalent S–C–H protons appeared as a 1:3:3:1 quartet with 6-Hz spacing at about 2.84 ppm suggesting averaging by ring inversion.

The ring methylene signals in the region 1.9–2.1 ppm consisted apparently of at least two overlapping multiplets. The complexity of these methylene multiplets results from the fact that, even with rapid ring inversion, the geminal protons within each methylene do not become equivalent. The result of averaging is rather to equate H_{3a} in conformation **13a** to H_{6a} in **13b**, and H_{3e} of **13a** to H_{6e} of **13b**, since the relative orientations of the flanking groups also change during ring inversion. For example, in **13a**, H_{3a} is flanked by equatorial OH and axial SR, but after ring inversion to **13b**, it becomes H_{3e}, which is flanked by axial OH and equatorial SR. The spectrum points to the para structure and the DL (14/25) configuration (formula **10** or **13a,b**).

Nmr Spectrum of the Mp 109° Isomer (Meta-DL).—The pattern of the two equivalent O–C–H protons appeared at 3.83 ppm, as a 1:3:3:1 quartet with splitting of 5–6 Hz, broadened by coupling to the OH signals. One quartet line was partly obscured by the *S*-methylene signal at 3.69 ppm.

Signals for the two (equivalent) S–C–H protons were observed at 2.80 ppm (1:3:3:1 quartet, splitting 6 Hz). The O–C–H and S–C–H regions were thus similar to those in the para-DL spectrum. The appearance of both the O–C–H and S–C–H signals as quartets was taken as evidence for averaging of the axial and equatorial protons in these positions by ring inversion.

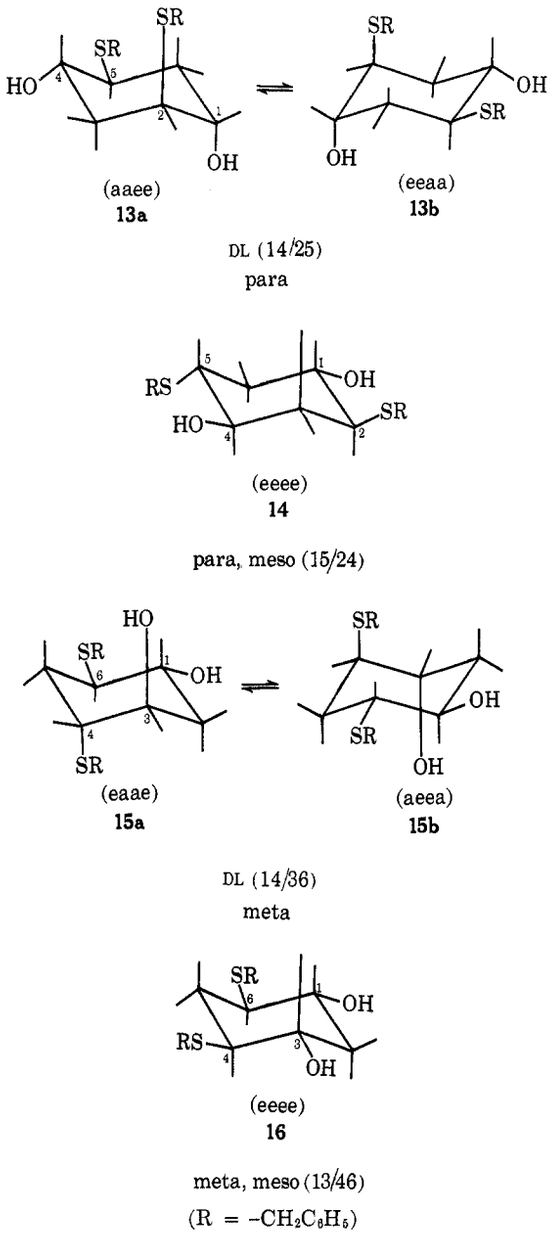
However, the ring methylene region was different from that of the other two isomers. This region consisted of two triplets centered at 1.93 and 2.04 ppm. At 100 MHz one line from each triplet coincided; at 60 MHz, two lines, giving an apparent quartet. The molecule thus must have two nonequivalent ring methylene groups, each of which averages to a triplet upon rapid ring inversion. This agrees with the meta structure, in which one methylene is flanked by axial and equatorial hydroxyl, the other by axial and equatorial –SR. Ring inversion averages the two methylene protons at each position between axial and equatorial, giving an average splitting.

The spectrum points to the meta structure and DL (14/36) configuration, formula **8** or **15a,b**.

Unknown Isomer (Meta-Meso).—By elimination, the remaining isomer obtainable from 1,4-cyclohexadiene dioxide (cis–trans mixture, or pure cis) should have the meta structure and meso (13/46) configuration, formula **12** or **16**.

Confirmation by Chemical Correlation.—The mp 109° isomer of dibenzylmercaptocyclohexanediol on treatment with Raney nickel catalyst in boiling ethanol

SCHEME II



was debenzylated and the sulfur removed, giving the previously known DL-*trans*-1,3-cyclohexanediol, characterized as the dibenzoate[12] **1** or **11** (R = $-COC_6H_5$), mp 124°. This chemical correlation confirms the structure and configuration, meta-DL (14/36), **8** or **15a,b**, based on nmr studies.

Other Features of the Nmr Spectra of the Dibenzylmercaptocyclohexanediols.—Signals from the ten aromatic protons in each compound were found at about 7.3 ppm.

In all three isomers, the two *S*-methylene groups were geometrically equivalent; however, the two protons *within* each *S*-methylene group were not geometrically equivalent. In the mp 158° isomer, the two protons within each *S*-methylene had distinctly different chemical shifts, resulting in an AB pattern, centered at 4.13 ppm (J = 13 Hz). In the mp 92° and mp 109° isomers, however, an apparent singlet was observed for *S*-methylene even at 100 MHz, due to accidental equality of the chemical shifts.

The hydroxyl protons in each isomer produced signals at about 2.15 ppm, using chloroform-*d* as solvent, which collapsed on addition of deuterium oxide (HDO peak appeared at 4.65 ppm). With pyridine, no OH signal was observed, due to exchange.

Experimental Section

All melting points have been corrected and were measured with a Nalge-Axelrod micro hot-stage. Microanalyses were performed by the Micro-Tech Laboratories, Skokie, Ill. The calculated[13] microanalyses, molecular weights, and per cent yields are taken from a computer printout. Found oxygen values are by difference. Infrared spectra were recorded on a Perkin-Elmer Model 337 spectrometer. Nmr spectra were recorded on a Varian A-60D spectrometer at the University of San Francisco or on a Varian HA-100 spectrometer at Stanford. Unless otherwise noted, chloroform-*d* was used as nmr solvent, and chemical shifts were reported in parts per million downfield from tetramethylsilane taken as internal reference. Product purity was confirmed where noted by thin layer chromatography on silica gel coated glass plates.[14a] Evaporations were conducted under reduced pressure with bath temperatures below 40°.

Meso (15/24) Diastereomer, Mp 158°, of (Para) 2,5-Dibenzylmercapto-1,4-cyclohexanediol (9).—Benzyl mercaptan (8.0 g) was added to a solution of 0.60 g of sodium metal in 20 ml of dry methanol under dry nitrogen. To this mixture was added a solution of 1.30 g of 1,4-cyclohexadiene[14b] dioxide (mixture about 64% trans, 36% cis)[8] in 60 ml of dry methanol. A higher yield could probably be obtained by using the pure cis dioxide as starting material.

The mixture was boiled under reflux for 20 hr (dry nitrogen), cooled, and mixed with 200 g of ice–water, and the resulting mixture was extracted with ether. The extract was washed with water, dried, and evaporated. The crystalline residue was purified by chromatography on a silica gel column. The column was eluted with chloroform to remove excess benzyl mercaptan. Elution with chloroform–acetone (15:1) then yielded the desired product: 200 mg (4.8%); mp 157–158°, after recrystallization from benzene; colorless crystals; infrared maximum (KBr) 3250, 3350 cm^{-1} (OH stretch). The nmr spectrum was recorded at 60 and 100 MHz (see discussion).

Anal. Calcd for $C_{20}H_{24}O_2S_2$ (360.538): C, 66.628; H, 6.710; O, 8.875; S, 17.787. Found: C, 66.56; H, 6.68; (O, 8.79); S, 17.97.

DL (14/25) Diastereomer, Mp 92°, of (Para) 2,5-Dibenzylmercapto-1,4-cyclohexanediol (10).—After elution of the para-meso (15/24) isomer (see above), the silica gel column was further eluted with chloroform–acetone (15:1). On evaporation of this eluate, there was obtained 3.0 g (72%) of an isomer melting at 91–92° after recrystallization from ether–hexane: colorless crystals; infrared maximum (KBr) 3400 cm^{-1} (OH stretch). The nmr spectrum was recorded at 60 and 100 MHz (see discussion).

Anal. Calcd for $C_{20}H_{24}O_2S_2$ (360.538): C, 66.628; H, 6.710; O, 8.875; S, 17.787. Found: C, 66.80; H, 6.66; (O, 8.83); S, 17.71.

DL (14/36) Diastereomer, Mp 109°, of (Meta) 4,6-Dibenzylmercapto-1,3-cyclohexanediol (8).—After elution of the para-DL (14/25) isomer (see above), the silica gel column was eluted further with chloroform–acetone (15:1). On evaporation of this eluate there was obtained 630 mg (15%) of an isomer melting at 108–109° after recrystallization from diethyl ether: infrared maximum (KBr) 3275 cm^{-1} (OH stretch). The nmr spectrum was recorded at 60 and 100 MHz (see discussion).

Anal. Calcd for $C_{20}H_{24}O_2S_2$ (360.538): C, 66.628; H, 6.710; O, 8.875; S, 17.787. Found: C, 66.92, H, 6.84; (O, 8.58); S, 17.66.

A higher yield of the mp 109° product could probably be obtained by using the pure trans dioxide in the reaction with sodium benzylmercaptide.

Chemical Correlation of the Dibenzylmercaptocyclohexanediol Isomer, Mp 109°, with DL-*trans*-1,3-Cyclohexanediol Dibenzoate (11).—A mixture of 102 mg of the meta-DL (14/36) isomer, mp 108–109°, with 20 ml of ethanol and about 2.0 g of Raney nickel catalyst was boiled under reflux for 5 hr. The catalyst was removed by filtration and the filtrate evaporated, giving 40 mg of a colorless syrup. This syrup was dissolved in 0.60 ml of pyridine, and 0.20 ml of benzoyl chloride was added with stirring. After 2 days, the mixture was stirred with 20 g of ice–water. The oil which separated was taken up in chloroform, and the extract was washed with sodium bicarbonate solution and with water. The dried extract on evaporation yielded a syrup, which on treatment with benzene–hexane gave 60 mg (65%) of colorless crystals, mp 123–124° after recrystallization from methanol (reported[12] mp 124°). A mixture melting point with an authentic sample[12] was not depressed, and the infrared spectra were identical.

Registry No.—DL (14/25), 33536-56-0; DL (14/36), 33536-57-1; meso (15/24), 33536-58-2.

Acknowledgment.—This research was made possible by a grant (AM-11433) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service. The 100-MHz nmr spectra were recorded and interpreted with the help of Dr. Malcolm R. Bramwell, Stanford University. We would like to thank Professor L. N. Owen (Imperial College of Science and Technology, University of London) for an authentic sample of *trans*-1,3-cyclohexanediol dibenzoate. Use of the IBM 360/67 computer system[13] at the Stanford University Campus Facility was supported by a Regional Network Grant from the National Science Foundation to the University of San Francisco (James N. Haag, Principal Investigator). We would like to thank Professor Haag and the Campus Facility consultants for helpful assistance. The Varian A-60D nmr spectrometer used was purchased with the help of a grant from the National Science Foundation to the University of San Francisco.

(12) M. F. Clarke and L. N. Owen, *J. Chem. Soc.*, 2105 (1950).

(13) One of us (G. E. M.) has written a computer program which is very convenient for printing out molecular formulas, molecular weights, and calculated elementary analyses for any elements over specified ranges. For example, for the present article it provided a 143-page table covering the ranges C, 6–36; H, 6–48; O, 0–12; and S, 0–9. By limiting the ranges, the execution time is kept to a reasonable value. Use of the ranges mentioned involved performing more than 150,000 calculations; the execution time was about 68 sec (IBM 360/67, WATFIV, Quick Partition).

(14) The reagents mentioned were products of (a) Mallinckrodt Chemical Works, New York, N. Y.; (b) Chemical Samples Co., Columbus, Ohio.