Anal. Calcd for $C_8H_{11}ClO_2$: C, 55.02; H, 6.32. Found: C, 54.87; H, 6.39.

8-Bromo-8-methylbicyclo[4.2.0]oct-2-en-7-one (V). The reagents were reduced by $^1/_{10}$th that described in the general procedure. Consequently, the yield of cycloadduct was estimated by vpc. The isomers were separated by vpc: ir, both isomers, 1800 (C=O) and 1607 cm^{-1} (C=C); nmr (CCl_4) *exo*-methyl isomer, δ 1.92 (s, 3 H), 1.6–2.6 (m, 4 H), 3.6 (m, 1 H), 4.05 (m, 1 H), 5.95 (m, 2 H); *endo*-methyl isomer revealed a singlet at δ 1.59.

Anal. Calcd for $C_9H_{11}BrO$: C, 50.23; H, 5.12. Found: C, 50.34; H, 5.25.

8-Bromo-8-methyl-2-oxabicyclo[4.2.0]octan-7-one (VI). Distillation of the concentrated reaction mixture yielded the crude product at 75–88° (1.0 mm). Successive fractionations of this isomer mixture yielded a fraction at 76–77° (1.0 mm) which had an *endo*:*exo*-methyl isomer distribution of >10 and a fraction at 86–88° (1.0 mm) with an endo:exo distribution of <0.2: ir, both isomers, 1800 cm^{-1} (C=O); nmr (CCl_4) *endo*-methyl isomer, δ 1.60 (s, 3 H), 1.6 (m, 4 H), 3.5 (m, 2 H), 3.9 (m, 1 H), 4.35 (d, 1 H); *exo*-methyl isomer, δ 1.85 (s, 3 H), 1.6 (m, 4 H), 3.5 (m, 2 H), 3.7 (m, 1 H), 4.03 (d, 1 H).

Anal. Calcd for $C_8H_{11}BrO_2$: C, 43.84; H, 5.02. Found: C, 43.71; H, 5.14.

2-Bromo-2-methyl-3-ethoxycyclobutanone (VII). The mixture of isomers distilled at 39–45° (0.4 mm) with some decomposition occurring during distillation. An analysis of the fractions from successive fractionations indicated that the adducts were unstable to heat as one of the decomposition products was found to be the starting vinyl ether. A rapid vacuum distillation afforded a fraction at 39–40° (0.4 mm) with an *endo*:*exo*-methyl isomer ratio of 0.9. The starting ethyl vinyl ether, which appeared as a decomposition product in this fraction, was removed by rotoevaporation prior to elemental analysis: ir, both isomers, 1800 cm^{-1} (C=O); nmr (CCl_4) both isomers, δ 1.25 (t, 3 H), 1.73 (s, 1.5 H), 1.80 (s, 1.5 H) 3.2 (m, 2 H), 3.6 (q, 2 H), 4.3 (m, 1 H).

Anal. Calcd for $C_7H_{11}BrO_2$: C, 40.51; H, 5.32. Found: C, 40.37; H, 5.34.

7-Iodo-7-methylbicyclo[3.2.0]hept-2-en-6-one. An 80% yield of cycloadduct was obtained which distilled at 77–80° (1.6 mm): ir, both isomers, 1780 (C=O) and 1640 cm^{-1} (C=C); nmr (CCl_4) both isomers, δ 1.77 (s, 1 H), 2.14 (s, 2 H), 2.6 (m, 2 H), 4.2 (m, 2 H), 5.9 (m, 2 H).

Anal. Calcd for C_8H_9IO: C, 38.75; H, 3.63. Found: C, 39.06; H, 3.93.

Acknowledgment. Support for this investigation by the Robert A. Welch Foundation, the National Science Foundation (GP-14016), and the North Texas State University Faculty Research Fund is gratefully acknowledged.

The 4-Dechlorination of 4,6-Disubstituted Steroids

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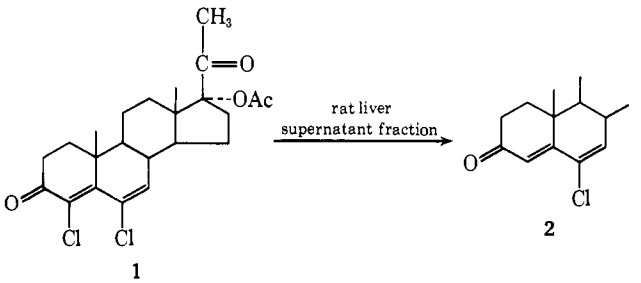
Contribution from the Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received August 18, 1970*

Abstract: Treatment of a steroid incorporating the 4,6-dichloro-4,6-dien-3-one system with various mercaptans results in 4-dechlorination. The 4-chloro-6-methyl and 4-monochloro analogs are also dechlorinated but to a lesser extent. Possible mechanisms for these transformations are discussed.

Earlier work from these laboratories[1] has shown that the major *in vitro* metabolite of the 4,6-dichloro steroid **1**[2] using 105,000 *g* rat liver supernatant fraction is the 6-chloro compound **2**.[3] It was also demonstrated that conditions which usually suffice to denature an enzyme (boiling, acidification) did not completely inhibit the dechlorination reaction.[1] This led us to suspect that the *in vitro* dechlorination of **1** is not necessarily an enzymatic process.



1 2

Hydride transfer was initially considered as a possible mode of 4-dechlorination, but no dependence on the pyridine nucleotides NADPH or NADH could be demonstrated. Sulfhydryl compounds, functioning in an oxidation–reduction reaction, were then considered as 4-dechlorinating agents. We first investigated the heat-stable, ubiquitous tripeptide, glutathione (GSH, **3**),[4] which is the most abundant sulfhydryl compound in most mammalian tissues. Incubation of **1** with 100 mol equiv of GSH at 37° in pH 7.4 buffer afforded an 80% yield of **2**. The dechlorination could also be carried out on a preparative scale at 25° using 2.5 mol equiv of GSH in methanol containing dilute sodium hydroxide. The product was identified by spectral data and by mixture melting point with authentic **2**.[3] No dechlorination of **1** was observed using oxidized GSH. The GSH-mediated 4-dechlorination in buffer was pH dependent and gave better yields at pH's higher than 7.4. Complete inhibition by a 10% molar excess of the known sulfhydryl group inhibitors, *N*-ethyl maleimide and iodoacetamide,[5] demonstrated that the sulfhydryl group of GSH is essential for the dechlorination.

(1) D. E. Maynard, O. Gurny, M. Carson, R. A. LeMahieu, M. A. Schwartz, M. K. Taylor, and R. W. Kierstead, *Biochemistry*, **10**, 355 (1971). A preliminary account of portions of this work was presented at the VIth International Symposium on the Chemistry of Natural Products (Steroids and Terpenes), Mexico City, Mexico, April 21–25, 1969.

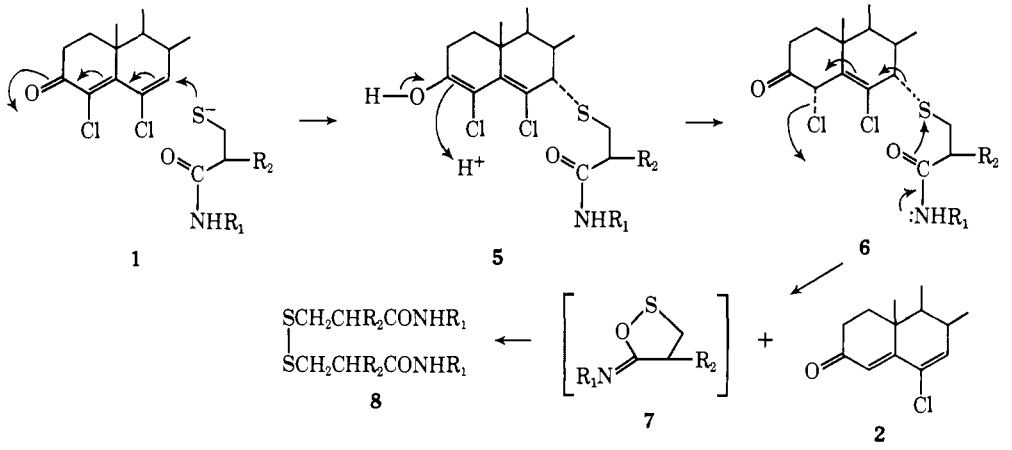
(2) R. W. Kierstead, J. Blount, K. E. Fahrenholtz, A. Faraone, R. A. LeMahieu, and P. Rosen, *J. Org. Chem.*, **35**, 4141 (1970).

(3) H. J. Ringold, E. Batres, A. Bowers, J. Edwards, and J. Zderic, *J. Amer. Chem. Soc.*, **81**, 3485 (1959).

(4) S. P. Colowick, *et al.*, Ed., "Glutathione," Academic Press, New York, N. Y., 1954, Chapters 1–3.

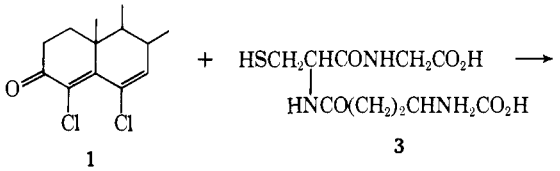
(5) J. L. Webb in "Enzyme and Metabolic Inhibitors," Vol. 3, Academic Press, New York, N. Y., 1965, Chapters 1 and 3.

Scheme I



With glutathione: $R_1 = CH_2CO_2H$; $R_2 = NHCO(CH_2)_2CHNH_2CO_2H$
With β-mercaptopropionamide: $R_1 = R_2 = H$

Other naturally occurring sulfhydryl compounds, such as *dl*-α-dihydrolipoic acid, cysteine, and coenzyme A (Table I), also converted **1** to **2** in phosphate buffer,



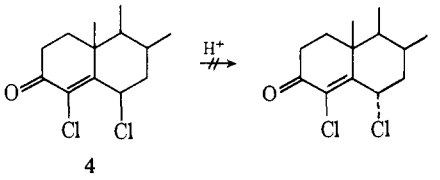
but not nearly as well as GSH. These compounds could also be involved in the *in vitro* rat liver dechlorination but probably make only minor contributions since their concentration in rat liver supernatant fractions is much lower than that of GSH. β-Mercaptopropionamide was investigated as a model for GSH, since it has the same spatial arrangement of sulfhydryl and amide groups (see Scheme I). It is only slightly less efficient in the dechlorination than glutathione. Other sulfhydryl compounds which were examined in the dechlorination reaction in phosphate buffer are shown in Table I.

Table I. Conversion of **1** to **2** with Mercaptans in Phosphate Buffer

Mercaptan[a]	% yield Recovered	**1**	**2**
Cysteine	10		26
Coenzyme A	8		33
dl-α-Dihydrolipoic acid	0		55
β-Mercaptopropionamide	7		59
γ-Mercaptobutyramide	0		26
1,3-Propanedithiol	24		58
1,2-Ethanedithiol	0		84
2-Mercaptoethanol	0		34
Ethyl mercaptan	42		3

[a] Each mercaptan was used in 100-fold molar excess.

Conjugate addition of mercaptans to steroidal $\Delta^{4,6}$-dien-3-ones yielding 7α-alkylthio-Δ^4-3-ones has been reported using basic conditions.[6] A mechanism for the dechlorination involving conjugate addition of the sulfhydryl compound to C-7 can be proposed (Scheme I). Earlier work from these laboratories[2] had shown that in a 4,6β-dichloro-Δ^4-3-keto steroid (**4**) the 6β-chloro substituent could not be isomerized to the 6α-chloro isomer under acidic conditions. In compounds

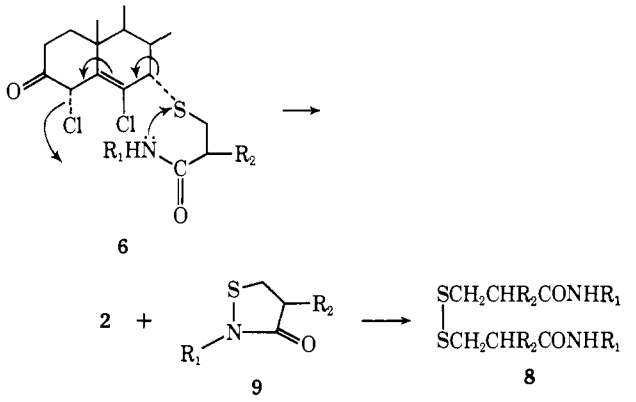


such as **4**, the steric interaction between the 6β chlorine and the 1,3-diaxial angular methyl is apparently less severe than that between the 6α chlorine and the C-4 chlorine. The enol **5** formed by the addition of mercaptide at C-7 could then protonate in an axial manner at C-4 or C-6.

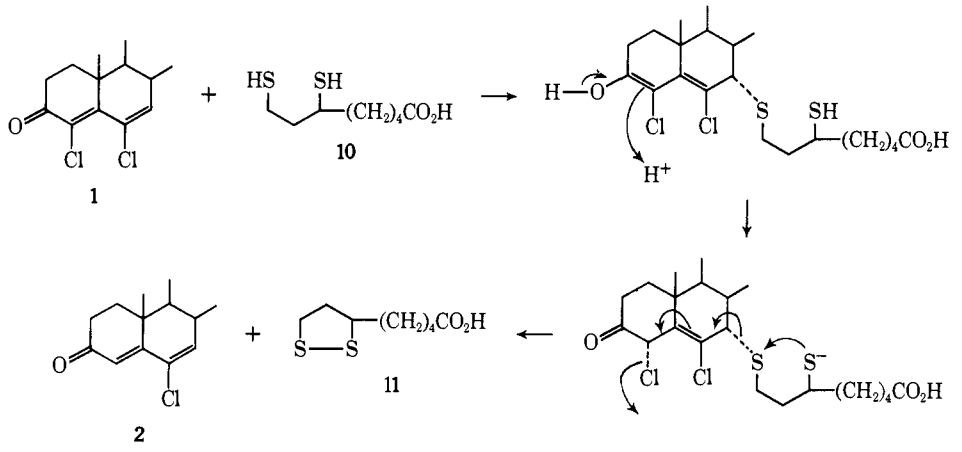
Axial protonation of the enol **5** at C-4 leading to a chair conformation in ring A or axial protonation at C-6 would introduce an unfavorable steric interaction between the two chlorine atoms. The product from protonation at C-4 can, however, flip into the ring A boat conformation[7] to relieve the chlorine–chlorine interaction. Intramolecular decomposition of the Michael adduct **6** by nucleophilic attack of the amide carbonyl[8] on sulfur would give **7** and the dechlorinated product **2**. Since 1,4 elimination reactions are preferentially cis,[9] **6** is well oriented sterically for this type of elimination. Hydrolysis of the unstable intermediate **7** and disproportionation of the resulting sulfenic acid,[10] or further reaction of **7** with external mercaptide ion by attack on sulfur would lead to disulfide **8**. Such

(6) H. Kaneko, K. Nakamura, Y. Yamato, and M. Kurokowa, *Chem. Pharm. Bull. (Tokyo)*, **17**, 11 (1969).
(7) For a related compound which exists in a ring A boat form see ref 2.
(8) L. A. Cohen and B. Witkop in "Molecular Rearrangements," Part 2, P. de Mayo, Ed., Interscience, New York, N. Y., 1964, p 1004.
(9) S. J. Cristol, W. Barasch, and C. H. Tieman, *J. Amer. Chem. Soc.*, 77, 583 (1955).
(10) A. Lüttringhaus and R. Schneider, *Justus Liebigs Ann. Chem.*, **679**, 123 (1964).

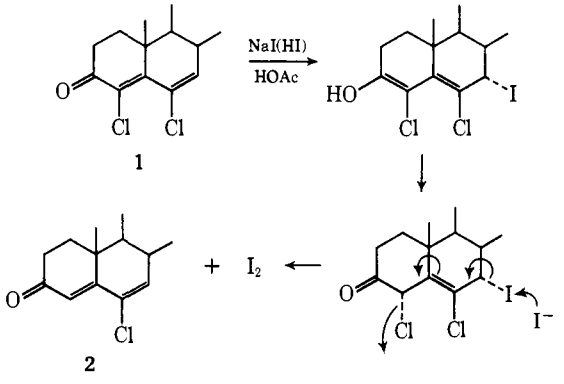
Scheme II



Scheme III



Scheme IV



disulfides have been isolated as by-products from this reaction when γ-mercaptobutyramide or *dl*-α-dihydrolipoic acid (*vide infra*) were utilized as dechlorinating agents.

An alternate mode of intramolecular decomposition of the adduct **6** may involve nucleophilic attack of the amide nitrogen on sulfur to yield an isothiazolidin-3-one by-product **9** (Scheme II). The latter system is known to undergo facile ring opening in aqueous solution to yield disulfides.[10]

Intermolecular decomposition of the initial adduct (see ethyl mercaptan in Table I) by attack of a second mercaptan molecule on sulfur is apparently a much less likely process than intramolecular decomposition. Mercaptans with an internal sulfhydryl group such as *dl*-α-dihydrolipoic acid (**10**), 1,3-propanedithiol, and 1,2-ethanedithiol give rise to facile dechlorination of **1** (Table I). In these cases, cyclic disulfide may be formed directly *via* internal nucleophilic displacement in the initial adduct (Scheme III). When *dl*-α-dihydrolipoic acid (**10**)[11] was utilized as the dechlorinating agent, *dl*-α-lipoic acid (**11**)[11] was identified as the by-product by comparison of its nmr and mass spectra and glpc retention time (silyl ester) with that of authentic silyl ester.

Dechlorination of **1** can also be accomplished in poor yield by refluxing for an extended period with sodium iodide and hydriodic acid in acetic acid. A mechanism involving conjugate addition of iodide ion can be proposed for this reaction (Scheme IV).

It was of interest to determine the effect of various C-6 substituents on the 4-dechlorination reaction. Therefore, the reactions of GSH with the 4-chloro-6-methyl compound **12** and the 6-unsubstituted compound **16**[12] were examined. Treatment of **12** with a 100-fold molar excess of GSH in phosphate buffer gave a 20% yield of **15** as well as 53% unreacted **12**. The 6-unsubstituted compound **16** under the same conditions afforded a 2% yield of dechlorinated product **20** along with 46% unreacted **16**. Identification of the dechlorinated products in both cases was accomplished by comparison of ultraviolet and mass spectra as well as glpc retention time with the authentic compounds.

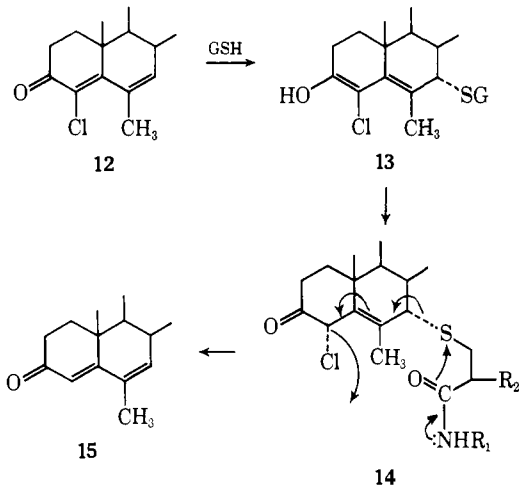
The electron-releasing 6-methyl group of **12** would be expected to retard nucleophilic addition to C-7 when compared with **1** and thus reduce the yield of dechlorination product. Protonation of the enol **13** (Scheme V) at C-4 (rather than at C-6) to give the ring A boat conformation would be favored for the steric reasons discussed earlier. Dechlorination can then proceed by intramolecular attack of the amide carbonyl on sulfur **14** as discussed before to yield **15**.

The greatly reduced yield of dechlorinated product from **16** compared to that from **1** is probably due to several factors. Due to the absence of the electron-withdrawing 6-chloro substituent in **16**, C-7 should be somewhat more electronegative than in **1** and consequently less susceptible to nucleophilic attack by GSH. However, some attack at C-7 would be expected to take place. In this case protonation of the enol **17** (Scheme VI) at C-6 to give **18** is possible since this does not introduce an unfavorable steric interaction with the C-4

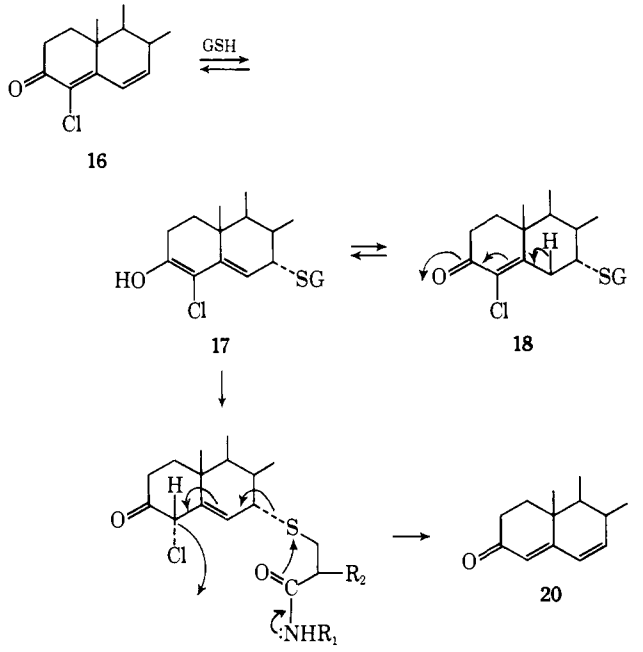
(11) U. Schmidt, P. Grafen, and H. W. Goedde, *Angew. Chem., Int. Ed. Engl.*, 4, 846 (1965).

(12) K. Brückner, B. Hampel, and U. Johnsen, *Chem. Ber.*, 94, 1225 (1961).

Scheme V



Scheme VI

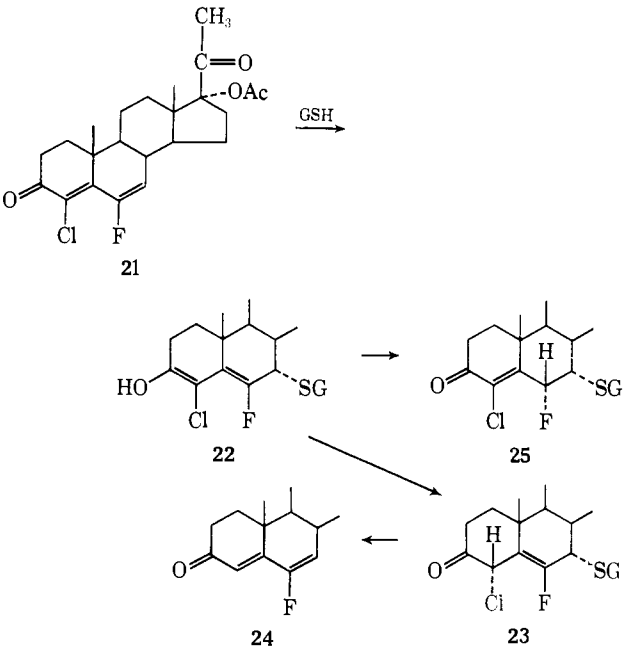


19, $R_1 = CH_2CO_2H$; $R_2 = NHCO(CH_2)_2CHNH_2CO_2H$

chlorine atom. Furthermore, protonation at C-6 is probably favored over C-4[13] due to the higher electron density on the former. Enolization of **18** would give rise to its precursor **17**, which on further reversal would give back starting material **16**. The lower total recovery in this case is due to loss of some material as a water-soluble adduct (possibly **18**).

However, some protonation of the enol **17** at C-4 could be expected to take place. The resulting product **19** could then undergo intramolecular decomposition as depicted to yield the dechlorinated product **20**.

Incubation of the 4-chloro-6-fluoro compound **21** with a 100-fold molar excess of GSH in phosphate buffer resulted in complete consumption of **21**. However, the 4-dechlorinated product **24** was formed in only 27% yield. Protonation of the enol **22** at C-4 to give **23** followed by 1,4 elimination yields **24**. Protonation of **22** at C-6 is not precluded because of the smaller size of fluorine relative to chlorine. The product of C-6 protonation, **25**, would then be lost in the aqueous phase due to the incorporation of the GSH moiety.



Experimental Section

General. All melting points were taken in glass capillaries and are corrected. The infrared spectra were determined using a Beckman IR-9 spectrophotometer. The nuclear magnetic resonance spectra were determined using a Varian A-60 spectrometer with tetramethylsilane as the internal standard. A Cary 14 spectrophotometer was used to obtain the ultraviolet spectra. The high-resolution mass spectra were obtained with a Consolidated Electrodynamics Corporation 21-110 mass spectrometer. The gas–liquid chromatographs were obtained with a Hewlett-Packard Model 402 instrument equipped with dual glass U columns and flame ionization detectors. For the steroid separations a 4 ft × 4 mm column packed with 3.8% UC-W98 (methyl silicone) on 80–100 mesh Diatoport S was used at a column temperature of 240°.

General Procedure for Incubations with Mercaptans. To 100 μmol of the mercaptan in 7.0 ml of pH 7.4 phosphate buffer (0.1 *M*) was added 1 μmol of steroid dissolved in 0.2 ml of propylene glycol–acetone (3:1). The solution was incubated under nitrogen at 37° on a Dubnoff metabolic shaker for 1 hr and then extracted twice by shaking for 5 min with 10-ml portions of ethyl acetate. The extract was concentrated at 50° in a stream of nitrogen and the extract was applied to a silica gel tlc plate (20 × 20 cm) along with a standard mixture of starting material and expected product. The plate was developed once in 2% methanol–benzene and then in 5% methanol–benzene. Examination of the dried plate under ultraviolet light revealed the product and unreacted substrate which were eluted separately with 15 ml of ethanol–chloroform (1:1). The silica gel was removed by filtration and the filtrates were concentrated to dryness in a stream of nitrogen at 50°. The residues were dissolved in appropriate amounts of ethanol and quantitation was accomplished by measurement of the uv absorption against a blank prepared by extraction of an equivalent area of the silica gel plate. Identification of the products was completed by comparison of the mass spectra and glpc retention time with the authentic compounds. When inhibitors were utilized, the mercaptan and inhibitors were preincubated for 5 min before addition of the substrate.

Dechlorination of 1 with GSH in Methanol. To 50 mg (0.114 mmol) of **1** dissolved in 50 ml of methanol was added 88 mg (0.285 mmol) of glutathione dissolved in the minimum amount of water. The solution was made alkaline with 2 drops of 6 *N* NaOH and then stirred under nitrogen for 1 hr. The solution was then acidified with acetic acid and concentrated to dryness at reduced pressure. Water was added and the product was extracted with ethyl acetate. The extract was dried ($MgSO_4$) and concentrated to dryness at reduced pressure. Crystallization of the product from methylene chloride–ether gave 22 mg (50% yield) of **2**: mp 211–

(13) S. K. Malhotra and H. J. Ringold, *J. Amer. Chem. Soc.*, **87**, 3228 (1965).

213°; λ_{max}^{EtOH} 285 mμ (ε 23,290). The ir and nmr spectra were identical with those of authentic **2**.[3] The mixture melting point with authentic **2** exhibited no depression.

Dechlorination of 1 with *dl*-α-Dihydrolipoic Acid. Nitrogen was bubbled through a solution of 45.4 mg (0.1 mmol) of **1** in 45 ml of methanol for 10 min. A solution of 20.2 mg (0.1 mmol) of *dl*-α-dihydrolipoic acid[14] in 5 ml of methanol was then added followed by 4 drops of 6 *N* NaOH. The solution was stirred under nitrogen for 80 min, acidified with 6 *N* HCl, and concentrated to dryness at reduced pressure. Ethyl acetate (20 ml) and water (3 ml) were added and the organic layer was extracted with two 10-ml portions of 5% $NaHCO_3$ solution to remove the acidic products. The bicarbonate extract was cooled and acidified with 6 *N* HCl to pH 2, saturated with NaCl, and extracted with ethyl acetate. Concentration of the dried ($MgSO_4$) extract gave a semisolid. The nmr and mass spectra of this material were identical with those of authentic *dl*-α-lipoic acid[11] except for several very weak peaks. In addition to the molecular peak at *m/e* 206, the mass spectrum showed a minor peak at *m/e* 208 indicating the presence of some *dl*-α-dihydrolipoic acid. The acidic product (0.7 mg) and 0.1 ml of *N,O*-bis(trimethylsilyl)acetamide were combined and left in a stoppered vial at room temperature for 16 hr to form the silyl ester. Analysis by glpc using a 1% OV-17 on Gas-Chrom Q column at 148° revealed two peaks: one at 16.5 min (86% of the mixture) and the second at 48 min (14% of the mixture). These retention times are identical with those of the silyl esters which were prepared from the authentic *dl*-α-lipoic and *dl*-α-dihydrolipoic acid, respectively. The initial ethyl acetate extract containing the neutral product was dried ($MgSO_4$) and concentrated to dryness at reduced pressure. Analysis of the crude product by glpc showed a single peak with the same relative retention time as authentic **2**. The crude product was dissolved in benzene and passed through a column of 0.25 g of neutral alumina. Elution with 40 ml of benzene and concentration of the eluent gave a colorless crystalline solid which was recrystallized from methylene chloride–ether to give 18.1 mg (45% yield) of **2**, mp 207–209°. The mixture melting point with authentic **2** showed no depression.

Dechlorination of 1 with Sodium Iodide. A solution of 0.5015 g of **1**, 1.53 g of sodium iodide, and 2 drops of 55–58% hydriodic acid in 20 ml of acetic acid was refluxed for 32 hr. Sodium iodide (1.50 g) and 5 drops of hydriodic acid were added and reflux was continued for 7 hr. The deep red reaction mixture was cooled and poured into 300 ml of cold 1 *N* NaOH. The product was extracted with ether–methylene chloride ((3:1) and the extract was washed with 1 *N* NaOH and H_2O, dried ($MgSO_4$), and concentrated to dryness at reduced pressure. The crude product was dissolved in benzene and passed through a column of 2.5 g of neutral alumina. The eluent contained only **1** and **2** by tlc. Crystallization from methylene chloride–ether and then from ethyl acetate gave 0.1370 g of material which contained 22% **1** and 78% **2** by glpc. Preparative tlc of 60 mg of this mixture on silica gel served to separate the two compounds. Crystallization of the purified fraction from methylene chloride–ether gave 9.8 mg of **2**, mp 207.5–211.5°. The mixture melting point with authentic **2** showed no depression.

4-Chloro-6-methyl-17α-hydroxypregna-4,6-diene-3,20-dione Acetate (12). To a solution of 3.00 g (7.9 mmol) of **15**[15] in 90 ml of chloroform cooled to 0° was added 9.9 ml (8.1 mmol) of a 0.82 *M* solution of chlorine in carbon tetrachloride. After 3 hr at 0°, 2.0 ml of 0.82 *M* chlorine in carbon tetrachloride was added. After 17 hr at 0°, the solvent was removed at reduced pressure and 25 ml of pyridine was added. The solution was left at room temperature for 6 hr and then concentrated to dryness at ~1 mm. The residual oil was taken up in methylene chloride, washed with 1 *N* HCl, 5% $NaHCO_3$ solution, and H_2O, dried ($MgSO_4$), and concentrated to dryness at reduced pressure. Two crystallizations from methylene chloride–ether gave 0.74 g of **12**: mp 215–217°; λ_{max}^{EtOH} 301 mμ (ε 19,750); $[\alpha]^{25}D$ +223.9° (*c* 1.03, $CHCl_3$). The analytical sample, mp 217.5–218.5°, was obtained from the same solvent pair.

Anal. Calcd for $C_{24}H_{31}ClO_4$: C, 68.80; H, 7.46; Cl, 8.46. Found: C, 68.50; H, 7.68; Cl, 8.57.

4-Chloro-6-fluoro-17α-hydroxypregna-4,6-diene-3,20-dione Acetate (21). To 2.185 g (5.62 mmol) of **24**[16] in 50 ml of chloroform cooled in an ice bath was added 7.6 ml (6.38 mmol) of 0.84 *M* chlorine in carbon tetrachloride. The solution was kept at 3° for 2.5 hr and then concentrated to dryness *in vacuo*. Pyridine (15 ml) was added and the solution was left at 25° for 19 hr. The pyridine was removed at ~1 mm, methylene chloride was added, and the extract was washed with 1 *N* hydrochloric acid and 5% sodium bicarbonate solution, dried ($MgSO_4$), and concentrated to dryness *in vacuo*. The resultant oil was chromatographed on 80 g of silica gel. Elution with 5% ethyl acetate–benzene gave several fractions containing pure **21** (by tlc analysis). These fractions were combined and crystallized from methylene chloride–ether to yield 1.031 g (43%) of **21**: mp 237–240°; λ_{max}^{EtOH} 298 mμ (ε 19,250); $[\alpha]^{25}D$ +37.1° (*c* 0.48, $CHCl_3$).

Anal. Calcd for $C_{23}H_{28}ClFO_4$: C, 65.32; H, 6.67; Cl, 8.38; F, 4.49. Found: C, 65.17; H, 6.75; Cl, 8.07; F, 4.38.

Acknowledgment. We wish to thank the following members of our physical chemistry department (Dr. P. Bommer, director), Dr. W. Benz, Dr. V. Toome, Mr. S. Traiman, and Dr. T. Williams, for the mass, ultraviolet, infrared, and nmr spectra, respectively. Thanks are also due to Dr. F. Scheidl for the microanalyses.

(14) Sigma Chemical Co., St. Louis, Mo. 63118.

(15) H. J. Ringold, J. P. Ruelas, E. Batres, and C. Djerassi, *J. Amer. Chem. Soc.*, **81**, 3712 (1959).

(16) A. Bowers, L. C. Ibáñez, and H. J. Ringold, *ibid.*, **81**, 5991 (1959).