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Synthesis of Corticosteroid Haptens Possessing the Bridge at the C-4 Position[1]

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In order to obtain specific antisera for use in radioimmunoassay and enzyme immunoassay, new haptens of cortisol, 11-deoxycortisol and cortisone were prepared. The synthesis of the 4-hemisuccinates of 4-hydroxycortisol and 4-hydroxy-11-deoxycortisol was accomplished by employing suitably protected 21-mono-*tert*-butyldimethylsilyl ethers of the 4-hydroxysteroids as key intermediates. As another type of hapten, the 4-carboxymethylthio derivatives of corticosteroids were prepared by base-catalyzed ring opening of the 4,5-epoxide with mercaptoacetic acid. In addition, the preparation of related haptens is described.

Keywords——cortisol haptens; 11-deoxycortisol haptens; cortisone haptens; 4,5-epoxide ring opening; *tert*-butyldimethylsilylation; 4-hydroxycortisol 4-hemisuccinate; 4-hydroxy-11-deoxycortisol 4-hemisuccinate; corticosteroid 4-carboxymethyl thioether; corticosteroid 4-carboxyethyl thioether

A number of attempts have been made to prepare antisera for use in the radioimmunoassay and enzyme immunoassay of steroid hormones. It is reasonably well substantiated that the site of conjugation of a protein carrier on the steroid molecule, the nature of the "bridge" and the stereochemistry of the hapten greatly influence the specificity of antisera. In enzyme immunoassay, the combination of antibody and enzyme-labeled steroid is an important factor determining the sensitivity. Therefore, the preparation of various hapten derivatives is required to develop these immunoassay methods. As for corticosteroids, few types of hapten are so far available, since the chemical modification of steroids is relatively difficult owing to the instability of the side chain at C-17. Anti-cortisol antisera raised against hapten-bovine serum albumin (BSA) conjugates linked through C-3,[3] C-6[4] and C-21[5] are not necessarily satisfactory with respect to specificity. Previously, we prepared anti-testosterone antisera by utilizing the C-4 position of the hapten molecule as the coupling site to BSA and found that the specificity in radioimmunoassay was comparable to that of antisera prepared by linkage through C-3, C-6 and C-7.[6] The present paper deals with the synthesis of new haptens of cortisol, 11-deoxycortisol and cortisone having different bridges at C-4.

Initial effort was directed to the preparation of 4-hydroxycortisol 4-hemisuccinate (**15**) and 4-hydroxy-11-deoxycortisol 4-hemisuccinate (**21**) *via* 4-hydroxycortisol (**11**) and 4-hydroxy-11-deoxycortisol (**16**), respectively. There are three general routes leading to the diosphenol:

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[1] Part CLV of "Studies on Steroids" by T. Nambara; Part CLIV: J. Goto, K. Suzaki, and T. Nambara, *Chem. Pharm. Bull.*, **28**, 1258 (1980). The following trivial names are used in this paper: cortisol=11β,17,21-trihydroxy-4-pregnene-3,20-dione, 11-deoxycortisol=17,21-dihydroxy-4-pregnene-3,20-dione, cortisone=17,21-dihydroxy-4-pregnene-3,11,20-trione, testosterone=17β-hydroxy-4-androsten-3-one.

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[3] D. Fahmy, G.F. Read, and S.G. Hillier, *Steroids*, **26**, 267 (1975); R.J. Dash, B.G. England, A.R. Midgley, Jr., and G.D. Niswender, *ibid.*, **26**, 647 (1975).

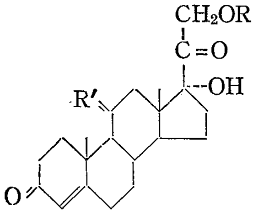
[4] T. Nishina, A. Tsuji, and D.K. Fukushima, *Steroids*, **24**, 861 (1974); H. Sone and M. Kojima, *Chem. Pharm. Bull.*, **26**, 2819 (1978).

[5] G.E. Abraham, J.E. Buster, and R.C. Teller, *Anal. Lett.*, **5**, 757 (1972); H.J. Ruder, R.L. Guy, and M.B. Lipsett, *J. Clin. Endocrinol. Metab.*, **35**, 219 (1972).

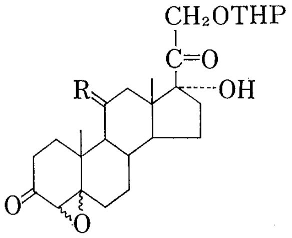
[6] H. Hosoda, K. Tadano, S. Miyairi, and T. Nambara, *J. Steroid Biochem.*, **10**, 513 (1979).

one of these utilizes the acid-catalyzed ring opening of a 4,5-epoxide[6,7] and the remaining two involve oxidation of a 3-keto-5β-steroid with molecular oxygen in the presence of potassium *tert*-butoxide[8] and rearrangement of an oxime derivative.[9] In the present work the former method was employed since the latter two require transformation into the bismethylenedioxy derivative to protect the side chain, during which the formation of by-products is inevitable.[10]

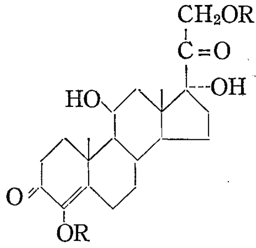
For the preparation of the 4,5-epoxides from cortisol and 11-deoxycortisol, protection of the alkali-sensitive ketol side chain is a prerequisite.[11] Recently, a convenient method for derivatizing the hydroxyl group into the tetrahydropyranyl ether has been developed by Miyashita *et al.*[12] Treatment of cortisol (**1**) with 2,3-dihydropyran in methylene chloride in the presence of pyridinium *p*-toluenesulfonate as a catalyst afforded the tetrahydropyranyl



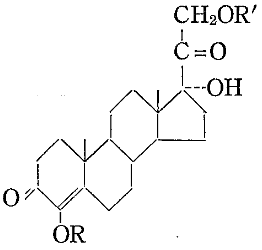
1: R=H, $R'={<}^{OH}_{H}$
2: R=H, $R'=H_2$
3: R=H, R'=O
4: R=THP, $R'={<}^{OH}_{H}$
5: R=THP, $R'=H_2$
6: R=THP, R'=O



7: $R={<}^{OH}_{H}$
8: $R=H_2$
9: R=O



10: $R=R'=Si(CH_3)_2\text{-}Bu^t$
11: R=R'=H
12: R=H, $R'=Si(CH_3)_2\text{-}Bu^t$
13: $R=Si(CH_3)_2\text{-}Bu^t$, R'=H
14: $R=COCH_2CH_2COOH$, $R'=Si(CH_3)_2\text{-}Bu^t$
15: $R=COCH_2CH_2COOH$, R'=H



16: R=R'=H
17: R=H, $R'=Si(CH_3)_2\text{-}Bu^t$
18: $R=R'=Si(CH_3)_2\text{-}Bu^t$
19: $R=Si(CH_3)_2\text{-}Bu^t$, R'=H
20: $R=COCH_2CH_2COOH$, $R'=Si(CH_3)_2\text{-}Bu^t$
21: $R=COCH_2CH_2COOH$, R'=H

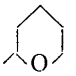
THP= 

Chart 1

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7) B. Camerino, B. Patelli, and A. Vercellone, *J. Am. Chem. Soc.*, **78**, 3540 (1956); R.H. Bible, Jr., C. C. Placek, and R.D. Muir, *J. Org. Chem.*, 22, 607 (1957); D.J. Collins, *J. Chem. Soc.*, **1959**, 3919; K. Heusler, J. Kalvoda, P. Wieland, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, **45**, 2575 (1962).

8) *a*) B. Camerino, B. Patelli, and R. Sciaky, *Tetrahedron Lett.*, **1961**, 554; *b*) *Idem*, *Gazz. Chim. Ital.*, **92**, 676 (1962).

9) M.V. Bhatt, C.G. Rao, and S. Rengaraju, *Chem. Commun.*, **1976**, 103.

10) D.K. Fukushima and S. Daum, *J. Org. Chem.*, **26**, 520 (1961); R.E. Beyler, F. Hoffman, R.M. Moriarty, and L.H. Sarett, *ibid.*, **26**, 2421 (1961).

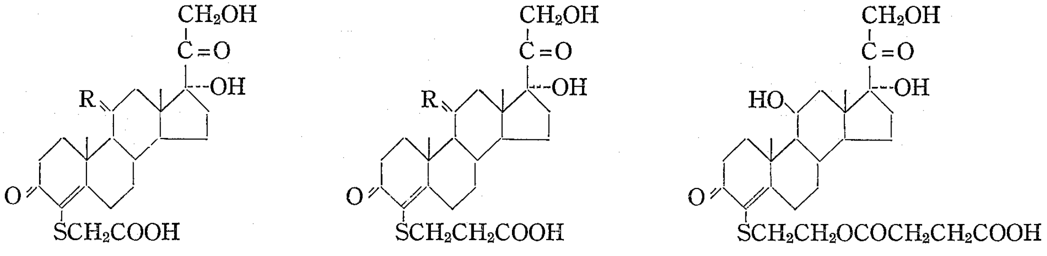
11) H.J. Ringold, E. Batres, O. Mancera, and G. Rosenkranz, *J. Org. Chem.*, **21**, 1432 (1956).

12) M. Miyashita, A. Yoshikoshi, and P.A. Grieco, *J. Org. Chem.*, **42**, 3772 (1977).

ether (**4**) in excellent yield. Epoxidation of **4** with alkaline hydrogen peroxide gave the epimeric 4,5-epoxides (**7**). On treatment with 50% sulfuric acid in methanol, both epimers were transformed into the diosphenol (**11**), which in turn was derivatized into the 4,21-disilyl ether (**10**). Here, selective protection of the hydroxyl group at C-21 was necessary. Previously, we observed that, in contrast to the alcoholic *tert*-butyldimethylsilyl ether, the phenolic ether is susceptible to basic conditions[13] and applied this finding to the synthesis of testosterone haptens.[6] However, selective hydrolysis of the silyloxyl group at C-4 in the disilyl ether (**10**) was not possible by treatment with methanolic potassium hydroxide. Instability of the silyloxyl group at C-21 under these conditions may be due to the nature of the ketol function of the side chain and/or to the participation of the hydroxyl group at C-17. Selective silylation of the diosphenol (**11**) was effected by the use of a limited anount of *tert*-butyldimethylsilyl chloride with imidazole in dimethylformamide-pyridine providing the desired 21-monosilyl ether (**12**) in a satisfactory yield. This monosilyl ether was distinguishable on thin-layer chromatography from the 4-monosilyl ether (**13**) prepared from **10** by hydrolysis with acetic acid. Treatment of **12** with succinic anhydride in pyridine followed by desilylation with sulfuric acid furnished the desired compound (**15**). Similarly, the 11-deoxycortisol hapten (**21**) was prepared through the sequence of reactions (**2**)→(**5**)→(**8**)→(**16**)→(**17**)→(**20**)→(**21**).

The synthesis of another type of hapten, the 4-mercapto derivatives (**22**—**27**) was then undertaken. The ring opening of the 4,5-epoxysteroids (**7**—**9**) with an appropriate nucleophilic reagent appeared to be useful for this purpose. Tomoeda *et al.*[14] reported that treatment of a 4,5-epoxide with 2-mercaptoethanol and mercaptoacetic acid under acidic conditions provides an oxathiane derivative and 4-hydroxy-Δ^4-3-ketone, respectively. The base-catalyzed reaction with an alkylmercaptan has also been investigated by Krämer *et al.*[15] In our previous work, a base-catalyzed cleavage of 4,5-epoxytestosterone with mercaptoacetic acid was found to proceed in the desired fashion.[16] When the 4,5-epoxide (**7**) was treated with mercaptoacetic acid in methanolic potassium hydroxide, ring opening took place to give the carboxymethyl thioether, which, on removal of the protecting group at C-21 with sulfuric acid, was converted to the desired compound (**22**) in good yield. The 2-carboxyethylthio derivative (**23**) was also prepared by reaction with mercaptopropionic acid as a nucleophile. The analogous haptens for 11-deoxycortisol and cortisone (**24**—**27**) could be obtained in a similar manner in reasonable yields.

Base-catalyzed ring opening was found to be convenient for the preparation of haptens having a longer bridge. For example, compound **28** was obtained from **7** by treatment with



22: R=$<^{\text{OH}}_{\text{H}}$
24: R=H_2
26: R=O

23: R=$<^{\text{OH}}_{\text{H}}$
25: R=H_2
27: R=O

28

Chart 2

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[13] H. Hosoda, K. Yamashita, H. Sagae, and T. Nambara, *Chem. Pharm. Bull.*, 23, 2118 (1975).

[14] M. Tomoeda, M. Ishizaki, H. Kobayashi, S. Kanatomo, T. Koga, M. Inuzuka, and T. Furuta, *Tetrahedron*, 21, 733 (1965).

[15] J.M. Krämer, K. Brückner, K. Irmscher, and K.-H. Bork, *Chem. Ber.*, **96**, 2803 (1963).

[16] H. Hosoda, Y. Sakai, H. Yoshida, S. Miyairi, K. Ishii, and T. Nambara, *Chem. Pharm. Bull.*, 27, 742 (1979).

2-mercaptoethanol and subsequent hemisuccinylation and removal of the protecting group at C-21. In the present study the 4,5-epoxide was prepared from a Δ^4-3-ketone, protecting the 21-hydroxyl group as the tetrahydropyranyl ether. Tetrahydropyranylation gave a mixture of diastereoisomers, and hence *tert*-butyldimethylsilylation might be rather conveniently applicable.[13,17]

To the best of our knowledge, hydroxylation at C-4 has not yet been reported in the metabolism of Δ^4-3-ketosteroids. This position is therefore suitable for the attachment of a carrier protein for use in the production of antibodies. The [C-4]-haptens obtained in the present study may help in clarifying the effect of a bridge at a certain position on the specificity of the antisera elicited. In addition, the availability of these derivatives should be useful for enzyme labeling in enzyme immunoassay employing a "bridge" heterologous combination as well as with a homologous system. Inspection of the specificity of the antisera raised against the conjugates of these haptens with BSA is now in progress and the details will be reported elsewhere in the near future.

Experimental[18]

General Procedure for the Preparation of Corticosteroid 21-Tetrahydropyranyl Ether——A mixture of corticosteroid (1—3) (8.3 mmol), 2,3-dihydropyran (32 mmol), and pyridinium *p*-toluenesulfonate (1.2 mmol) in CH_2Cl_2 (90 ml) was stirred at room temperature for 5 hr. The resulting solution was diluted with ether, washed twice with half-saturated brine, dried over anhydrous Na_2SO_4, and evaporated down. The product obtained was recrystallized from acetone–hexane.

Cortisol 21-Tetrahydropyranyl Ether (4)——Colorless plates. mp 180—183°. $[\alpha]_D^{25}$ +119.6° (c=1.65). *Anal.* Calcd for $C_{26}H_{38}O_6$: C, 69.93; H, 8.58. Found: C, 69.85; H, 8.69.

11-Deoxycortisol 21-Tetrahydropyranyl Ether (5)——Colorless plates. mp 155—157°. $[\alpha]_D^{25}$ +104.7° (c=1.11). *Anal.* Calcd for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.34; H, 9.15.

Cortisone 21-Tetrahydropyranyl Ether (6)——Colorless plates. mp 183—185°. $[\alpha]_D^{25}$ +166.9° (c=0.88). *Anal.* Calcd for $C_{26}H_{36}O_6$: C, 70.24; H, 8.16. Found: C, 70.20; H, 8.56.

General Procedure for the Preparation of Corticosteroid 4,5-Epoxide——A solution of the tetrahydropyranyl ether (4—6) (2.3 mmol), 10% NaOH (2.8 ml), and 30% H_2O_2 (3.0 ml) in MeOH (45 ml) was stirred at 0° for 4 hr. After careful neutralization with AcOH, the resulting solution was concentrated to one-third of its volume under reduced pressure. The residue was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The crude product obtained was chromatographed on silica gel (16 g). Elution with hexane–AcOEt (1: 1) gave the epimeric 4,5-epoxides (7—9).

4-Hydroxycortisol 4,21-Bis(*tert*-butyldimethylsilyl) Ether (10)——A solution of the 4,5-epoxide (7) (1.7 g) and 50% H_2SO_4 (6 ml) in MeOH (60 ml) was stirred at room temperature for 8 hr. After neutralization with 50% NaOH followed by removal of the methanol under reduced pressure, the resulting mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The residue was chromatographed on silica gel (30 g). Elution with hexane–AcOEt (1: 4) gave the crude diosphenol. Imidazole (1 g) and *tert*-butyldimethylsilyl chloride (500 mg) were added to a solution of this product in pyridine (0.5 ml)-dimethylformamide (1 ml), and the resulting solution was stirred overnight at room temperature. The reaction mixture was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The crude product was chromatographed on silica gel (16 g). Elution with hexane–AcOEt (5: 1) and recrystallization of the eluate from ether–hexane gave **10** (500 mg) as colorless needles. mp 144—145°. $[\alpha]_D^{25}$ +110.0° (c=0.21). NMR ($CDCl_3$) δ: 0.12 (6H, s, 21-$OSi(CH_3)_2$), 0.11 and 0.19 (each 3H, s, 4-$OSi(CH_3)_2$), 0.96 (21H, s, 18-CH_3, 4- and 21-OSi-*t*-Bu), 1.44 (3H, s, 19-CH_3), 3.10 (1H, m, 6ξ-H), 4.42 (1H, m, 11α-H), 4.42 and 4.56 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{33}H_{56}O_6Si_2 \cdot 3/4H_2O$: C, 63.88; H, 9.67. Found: C, 63.86; H, 9.65.

4-Hydroxycortisol (11)——A solution of **10** (450 mg) and 5 N H_2SO_4 (1.5 ml) in MeOH (15 ml) was stirred at room temperature for 1 hr. The resulting solution was diluted with AcOEt, washed with 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Recrystallization of the crude product from acetone–hexane gave **11** (280 mg) as colorless prisms. mp 208—211° (lit. mp 205—208°).[8b] $[\alpha]_D^{23}$ +149.0° (c=0.18). *Anal.* Calcd for $C_{21}H_{30}O_6 \cdot 1/4H_2O$: C, 65.86; H, 8.03. Found: C, 66.01; H, 7.89.

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17) E.J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, **94**, 6190 (1972).

18) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were determined in $CHCl_3$ unless otherwise specified. Nuclear magnetic resonance (NMR) spectra were measured with a JEOL PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviations used: s=singlet, d=doublet, and m=multiplet.

4-Hydroxycortisol 21-*tert*-Butyldimethylsilyl Ether (12)——A mixture of **11** (280 mg), imidazole (400 mg), and *tert*-butyldimethylsilyl chloride (200 mg) in pyridine (0.3 ml)–dimethylformamide (0.6 ml) was stirred at room temperature for 1 hr. The resulting solution was diluted with ether, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The crude product was chromatographed on silica gel (10 g). Elution with hexane–AcOEt (4: 3) and recrystallization of the resulting material from acetone–hexane gave **12** (295 mg) as colorless needles. mp 92—95°. $[\alpha]_D^{20}$ +77.2° (c=0.19). NMR ($CDCl_3$) δ: 0.12 (6H, s, 21-$OSi(CH_3)_2$), 0.94 (9H, s, 21-OSi-t-Bu), 0.96 (3H, s, 18-CH_3), 1.45 (3H, s, 19-CH_3), 3.03 (1H, m, 6ξ-H), 4.43 and 4.58 (each 1H, d, J=18 Hz, 21-H), 4.45 (1H, m, 11α-H). *Anal.* Calcd for $C_{27}H_{44}O_6Si \cdot 3/4H_2O$: C, 64.06; H, 9.06. Found: C, 64.12; H, 9.04.

4-Hydroxycortisol 4-*tert*-Butyldimethylsilyl Ether (13)——A solution of **10** (55 mg) and 50% AcOH (1 ml) in MeOH (4 ml) was allowed to stand at room temperature for 5 hr. The resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The residue obtained was chromatographed on silica gel (8 g). Elution with hexane–AcOEt (2: 3) and recrystallization of the resulting material from acetone gave **13** (27 mg) as colorless needles. mp 149°. $[\alpha]_D^{23}$ +112.1° (c=0.20). NMR ($CDCl_3$) δ: 0.11 and 0.18 (each 3H, s, 4-$OSi(CH_3)_2$), 0.96 (12H, s, 18-CH_3 and 4-OSi-t-Bu), 1.45 (3H, s, 19-CH_3), 3.10 (1H, m, 6ξ-H), 4.34 and 4.67 (each 1H, d, J=18 Hz, 21-H), 4.48 (1H, m, 11α-H). *Anal.* Calcd for $C_{27}H_{44}O_6Si$: C, 65.82; H, 9.00. Found: C, 65.60; H, 8.87.

4-Hemisuccinoyloxycortisol 21-*tert*-Butyldimethylsilyl Ether (14)——A mixture of **12** (200 mg) and succinic anhydride (400 mg) in pyridine (1 ml) was allowed to stand at 80° for 10 hr. After removal of the pyridine followed by addition of H_2O, the resulting mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. Purification of the crude product by preparative TLC using $CHCl_3$–MeOH–AcOH (20: 2: 0.1) as a developing solvent gave **14** (100 mg) as colorless semicrystals. NMR ($CDCl_3$) δ: 0.13 (6H, s, 21-$OSi(CH_3)_2$), 0.96 (12H, s, 18-CH_3 and 21-OSi-t-Bu), 2.86 (4H, m, $-COCH_2CH_2CO-$), 4.40 (1H, m, 11α-H), 4.49 and 4.65 (each 1H, d, J=18 Hz, 21-H).

4-Hydroxycortisol 4-Hemisuccinate (15)——A solution of **14** (200 mg) and 50% H_2SO_4 (0.2 ml) in EtOH (3 ml) was stirred at room temperature for 5 min. The resulting solution was diluted with AcOEt, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The crude product obtained was chromatographed on silica gel (16 g). Elution with AcOEt–AcOH (100: 0.1) and recrystallization of the resulting material from acetone–hexane gave **15** (33 mg) as colorless plates. mp 153—155°. $[\alpha]_D^{15}$ +130.1° (c=0.28, MeOH). NMR (CD_3OD) δ: 0.90 (3H, s, 18-CH_3), 1.53 (3H, s, 19-CH_3), 2.78 (4H, m, $-COCH_2CH_2CO-$), 4.32 and 4.64 (each 1H, d, J=18 Hz, 21-H), 4.44 (1H, m, 11α-H). *Anal.* Calcd for $C_{25}H_{34}O_9$: C, 62.75; H, 7.16. Found: C, 62.61; H, 7.23.

4-Hydroxy-11-deoxycortisol (16)——Treatment of the 4,5-epoxide (**8**) (6.5 g) with 50% H_2SO_4 was carried out in the manner described for **10**. Upon usual work-up, the residue obtained was rinsed with hexane and then MeOH. Recrystallization of the crude product from acetone–MeOH gave **16** (1.2 g) as colorless needles. mp 236—237°. (lit. mp 220—225°).[8b] $[\alpha]_D^{20}$ +104.9° (c=0.24, EtOH). NMR ($CDCl_3$–CD_3OD (4: 1)) δ: 0.68 (3H, s, 18-CH_3), 1.21 (3H, s, 19-CH_3), 4.33 and 4.63 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34: Found: C, 69.24; H, 8.47.

4-Hydroxy-11-deoxycortisol 21-*tert*-Butyldimethylsilyl Ether (17)——Silylation of **16** (180 mg) with *tert*-butyldimethylsilyl chloride was carried out in the manner described for **12**. After usual work-up, the crude product obtained was chromatographed on silica gel (8 g). Elution with hexane–AcOEt (6: 1) and recrystallization of the resulting material from ether–hexane gave **17** (200 mg) as colorless leaflets. mp 182—184°. $[\alpha]_D^{25}$ +72.0° (c=0.28). NMR ($CDCl_3$) δ: 0.12 (6H, s, 21-$OSi(CH_3)_2$), 0.72 (3H, s, 18-CH_3), 0.96 (9H, s, 21-OSi-t-Bu), 1.20 (3H, s, 19-CH_3), 3.06 (1H, m, 6ξ-H), 4.45 and 4.57 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{27}H_{44}O_5Si$: C, 68.03; H, 9.30. Found: C, 68.22; H, 9.66.

4-Hydroxy-11-deoxycortisol 4,21-Bis(*tert*-butyldimethylsilyl) Ether (18)——Silylation of **16** (135 mg) with excess *tert*-butyldimethylsilyl chloride was carried out in the manner described for **12**. After usual work-up, the crude product obtained was chromatographed on silica gel (8 g). Elution with hexane–AcOEt (6: 1) and recrystallization of the resulting material from MeOH gave **18** (165 mg) as colorless needles. mp 146—148°. $[\alpha]_D^{20}$ +72.4° (c=0.24). NMR ($CDCl_3$) δ: 0.11 (9H, s, 21-$OSi(CH_3)_2$ and one of 4-$OSi(CH_3)_2$), 0.17 (3H, s, one of 4-$OSi(CH_3)_2$), 0.71 (3H, s, 18-CH_3), 0.95 (18H, s, 4- and 21-OSi-t-Bu), 1.18 (3H, s, 19-CH_3), 3.41 (1H, m, 6ξ-H), 4.42 and 4.56 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{33}H_{58}O_5Si_2$: C, 67.07; H, 9.89. Found: C, 67.12; H, 9.82.

4-Hydroxy-11-deoxycortisol 4-*tert*-Butyldimethylsilyl Ether (19)——Hydrolysis of **18** (90 mg) with AcOH was carried out in the manner described for **13**. After usual work-up the crude product obtained was recrystallized from ether to give **19** (70 mg) as colorless needles. mp 178—180°. $[\alpha]_D^{25}$ +96.4° (c=0.42). NMR ($CDCl_3$) δ: 0.11 and 0.17 (each 3H, s, 4-$OSi(CH_3)_2$), 0.72 (3H, s, 18-CH_3), 0.96 (9H, s, 4-OSi-t-Bu), 1.20 (3H, s, 19-CH_3), 4.32 and 4.63 (each 1H, d, J=18 Hz, 21-H). *Anal.* Calcd for $C_{27}H_{44}O_5Si$: C, 68.03; H, 9.30. Found: C, 68.00; H, 9.47.

4-Hemisuccinoyloxy-11-deoxycortisol 21-*tert*-Butyldimethylsilyl Ether (20)——Treatment of **17** (200 mg) with succinic anhydride was carried out in the manner described for **14**. After usual work-up the crude product obtained was chromatographed on silica gel (3 g). Elution with AcOEt and recrystallization of the resulting material from ether-hexane gave **20** (150 mg) as colorless granules. mp 151—152° $[\alpha]_D^{20}$+70.5°

(*c*=0.23). NMR ($CDCl_3$) δ: 0.12 (6H, s, 21-$OSi(CH_3)_2$), 0.72 (3H, s, 18-CH_3), 0.96 (9H, s, 4-OSi-*t*-Bu), 1.26 (3H, s, 19-CH_3), 2.81 (4H, m, -$COCH_2CH_2CO$-), 4.44 and 4.59 (each 1H, d, *J*=18 Hz, 21-H). *Anal.* Calcd for $C_{31}H_{48}O_8Si$: C, 64.55; H, 8.39. Found: C, 64.57; H, 8.46.

4-Hydroxy-11-deoxycortisol 4-Hemisuccinate (21)——Hydrolysis of **20** (120 mg) with 50% H_2SO_4 was carried out in the manner described for **15**. After usual work-up, the crude product obtained was chromatographed on silica gel (6 g). Elution with hexane–AcOEt (1 : 10) and recrystallization of the resulting material from AcOEt gave **21** (80 mg) as colorless granules. mp 159—160°. $[\alpha]_D^{25}$ +107.1° (*c*=0.28, MeOH). NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 1.25 (3H, s, 19-CH_3), 2.81 (4H, m, -$COCH_2CH_2CO$-), 4.31 and 4.61 (each 1H, d, *J*=18 Hz, 21-H). *Anal.* Calcd for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.73; H, 7.62.

General Procedure for the Preparation of Corticosteroid 4-Thioether——A solution of the 4,5-epoxide (**7**—**9**) (1.7 mmol) in EtOH (5 ml) was added to mercaptoacetic acid or mercaptopropionic acid (2.8 mmol) in 25% KOH (0.8 ml), and the resulting solution was stirred at room temperature under an N_2 gas stream for 2 hr. After addition of H_2O followed by extraction with AcOEt, the aqueous layer was acidified with 1 N HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The residue was dissolved in 50% AcOH (8 ml) and allowed to stand at room temperature for 5 hr. Upon addition of H_2O the precipitate formed was collected by filtration and dried. Recrystallization of the crude product from aqueous acetone gave the 4-thioether (**22**—**27**).

4-(Carboxymethylthio)cortisol (22)——Colorless needles. mp 113—117°. $[\alpha]_D^{25}$ +154.3° (*c*=0.20, EtOH). NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.94 (3H, s, 18-CH_3), 1.52 (3H, s, 19-CH_3), 3.37 (2H, s, 4-SCH_2CO), 3.66 (1H, m, 6ξ-H), 4.30 and 4.66 (each 1H, d, *J*=18 Hz, 21-H), 4.47 (1H, m, 11α-H). *Anal.* Calcd for $C_{23}H_{32}O_7S\cdot 3/2H_2O$: C, 57.60; H, 7.36. Found: C, 57.78; H, 7.24.

4-(2-Carboxyethylthio)cortisol (23)——Colorless needles. mp 177—178°. $[\alpha]_D^{25}$ +162.7° (*c*=0.35, EtOH). NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.92 (3H, s, 18-CH_3), 1.50 (3H, s, 19-CH_3), 2.55 (2H, m, 4-$SCH_2C\underline{H}_2CO$), 2.92 (2H, m, 4-$SC\underline{H}_2CH_2CO$), 3.66 (1H, m, 6ξ-H), 4.30 and 4.65 (each 1H, d, *J*=18 Hz, 21-H), 4.43 (1H, m, 11α-H). *Anal.* Calcd for $C_{24}H_{34}O_7S\cdot 1/2H_2O$: C, 60.61; H, 7.42. Found: C, 60.62; H, 7.26.

4-(Carboxymethylthio)-11-deoxycortisol (24)——Colorless needles. mp 180—182°. $[\alpha]_D^{25}$ +144.4° (*c*=0.25, EtOH). NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.69 (3H, s, 18-CH_3), 1.25 (3H, s, 19-CH_3), 3.35 (2H, s, 4-SCH_2CO), 3.75 (1H, m, 6ξ-H), 4.30 and 4.65 (each 1H, d, *J*=18 Hz, 21-H). *Anal.* Calcd for $C_{23}H_{32}O_6S\cdot H_2O$: C, 60.77; H, 7.54. Found: C, 60.88; H, 7.74.

4-(2-Carboxyethylthio)-11-deoxycortisol (25)——Colorless needles. mp 109—111°. $[\alpha]_D^{25}$ +149.0° (*c*=0.20, EtOH). NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.71 (3H, s, 18-CH_3), 1.29 (3H, s, 19-CH_3), 2.53 (2H, m, 4-$SCH_2C\underline{H}_2CO$), 2.93 (2H, m, 4-$SC\underline{H}_2CH_2CO$), 3.76 (1H, m, 6ξ-H), 4.33 and 4.68 (each 1H, d, *J*=18 Hz, 21-H). *Anal.* Calcd for $C_{24}H_{34}O_6S\cdot 3/2H_2O$: C, 60.35; H, 7.81. Found: C, 60.72; H, 7.91.

4-(Carboxymethylthio)cortisone (26)——Colorless needles. mp 181—183°. $[\alpha]_D^{25}$ +204.8° (*c*=0.19, EtOH). NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.62 (3H, s, 18-CH_3), 1.45 (3H, s, 19-CH_3), 3.35 (2H, s, 4-SCH_2CO), 3.77 (1H, m, 6ξ-H), 4.25 and 4.61 (each 1H, d, *J*=18 Hz, 21-H). *Anal.* Calcd for $C_{23}H_{30}O_7S\cdot H_2O$: C, 58.95; H, 6.88. Found: C, 59.03; H, 7.04.

4-(2-Carboxyethylthio)cortisone (27)——Colorless needles. mp 162—164°. $[\alpha]_D^{25}$ +208.3° (*c*=0.25, EtOH). NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.65 (3H, s, 18-CH_3), 1.49 (3H, s, 19-CH_3), 2.52 (2H, m, 4-$SCH_2C\underline{H}_2CO$), 2.83 (2H, m, 4-$SC\underline{H}_2CH_2CO$), 3.78 (1H, m, 6ξ-H), 4.26 and 4.63 (each 1H, d, *J*=18 Hz, 21-H). *Anal.* Calcd for $C_{24}H_{32}O_7S\cdot 3/4H_2O$: C, 60.29; H, 7.06. Found: C, 60.54; H, 7.09.

4-(2-Hemisuccinoyloxyethylthio)cortisol (28)——Treatment of **7** (1.15 g) with 2-mercaptoethanol was carried out in the manner described above. After extraction of the reaction mixture with AcOEt followed by the usual work-up, the crude product obtained was chromatographed on silica gel (16 g). Elution with hexane–AcOEt (1 : 3) gave 4-(2-hydroxyethylthio)cortisol 21-tetrahydropyranyl ether (830 mg) as colorless semicrystals. A solution of this product in pyridine (2.5 ml) was treated with succinic anhydride (510 mg) and the resulting solution was allowed to stand overnight at 35°. After dilution with H_2O the resulting mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. The residue was dissolved in 50% AcOH (14 ml) and allowed to stand at room temperature for 5 hr. The reacton mixture was diluted with AcOEt, washed with H_2O, and dried over anhydrous Na_2SO_4 Upon removal of the solvent, **28** (580 mg) was obtained as hygroscopic semicrystals. NMR ($CDCl_3$–CD_3OD (4 : 1)) δ: 0.90 (3H, s, 18-CH_3), 1.48 (3H, s, 19-CH_3), 2.61 (4H, s, -$COCH_2CH_2CO$-), 2.87 (2H, m, 4-$SC\underline{H}_2CH_2O$), 3.64 (1H, m, 6ξ-H), 4.10 (2H, m, 4-$SCH_2C\underline{H}_2O$), 4.28 and 4.60 (each 1H, d, *J*=18 Hz, 21-H), 4.42 (1H, m, 11α-H).

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