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Synthesis and Anti-inflammatory Activity of Steroidal 17-Yl-α-Oxothiocarboxamides and Related Compounds

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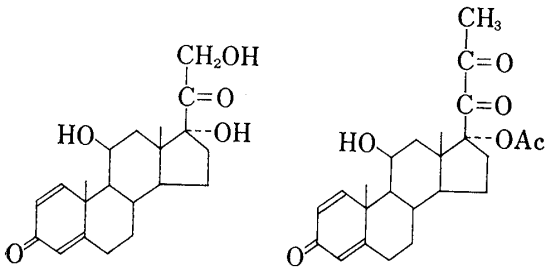
The Willgerodt–Kindler reaction of 21-chloro-20-ketosteroids (**5**) or sodium 21-thiosulfate derivatives of 20-ketosteroids (**6**) with sulfur in secondary amines gave the corresponding steroidal α-oxothiocarboxamides (**3**) in good yields. Among these compounds (**3**), 11β,17α-dihydroxy-21-morpholinopregna-1,4-diene-3,20-dione-21-thione (**3a**) and 11β,17α-dihydroxy-21-morpholinopregn-4-ene-3,20-dione-21-thione (**3b**) showed potent anti-inflammatory activities comparable to or a little less than those of the parent compounds, prednisolone and hydrocortisone, respectively, in the carrageenin edema test in rats. **3a** also showed potent activity in the granuloma pouch test in rats but much less activity in the cotton pellet test in rats. These results suggest that **3a** might be more active against acute or subacute inflammation than against chronic inflammation.

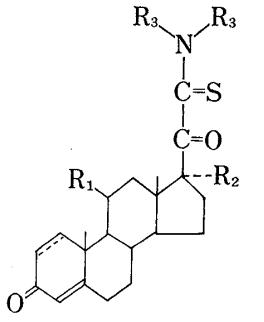
Keywords——21-chloro-20-ketosteroid; α-oxothiocarboxamide; steroidal 21-yl-disulfide; Willgerodt–Kindler reaction; anti-inflammatory activity

The significance of the 21-hydroxyl group of glucocorticoids in relation to anti-inflammatory activity is of considerable interest. It has been reported that the 21-hydroxyl group of 11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione (prednisolone) (**1**) can be replaced by N-methylpiperidine without any loss of activity of the parent compound[2] and that desoxygenation at C-21 does not totally abolish the activity of anti-inflammatory corticosteroids.[3] The present authors have also reported[4] the synthesis of a series of 17α-acyloxy-21-methyl-20, 21-diketosteroids, among which 11β,17α-dihydroxy-21-methylpregna-1,4-diene-3,20,21-trione 17-acetate (TSC-5) (**2**) showed significant anti-inflammatory activity of approximately the same order of magnitude as prednisolone.[5] These findings suggest that the 21-hydroxyl group of corticosteroids may not be essential for their biological activities. As an extension



prednisolone **1** TSC-5 **2**

Chart 1



3

R_1, R_2: H or OH
R_3: alkyl residue of secondary amine

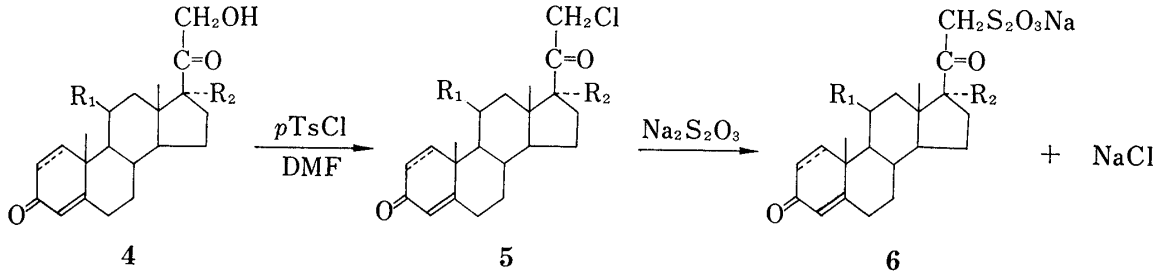
Chart 2

1) Location: *Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.*
2) J. Toth and Z. Tuba, *Nature* (London), **191**, 607 (1961).
3) E.R. Boland, *Am. J. Med.*, **31**, 581 (1961).
4) *a*) S. Noguchi and K. Morita, *Chem. Pharm. Bull.* (Tokyo), **11**, 235 (1963); *b*) S. Noguchi, F. Nakayama, and K. Morita, *ibid.*, **12**, 1180 (1964); *c*) S. Noguchi, H. Otsuka, M. Obayashi, M. Imanishi, and K. Takahashi, *Steroids*, **12**, 9 (1968).
5) K. Kawai, The 32nd Kinki Meeting of the Pharmacological Society of Japan, Tokyo, April, 1967.

of our studies to elucidate the possible role of the functional group at C-21 of corticosteroids, we have reported[6] briefly the preparation of steroidal 17-yl-α-oxothiocarboxamides (**3**), which are similar in chemical structure to TSC-5 (**2**). In this paper, we describe the preparation in more detail, and report details of the anti-inflammatory activities of the steroidal 17-yl-α-oxothiocarboxamides (**3**) and related compounds.

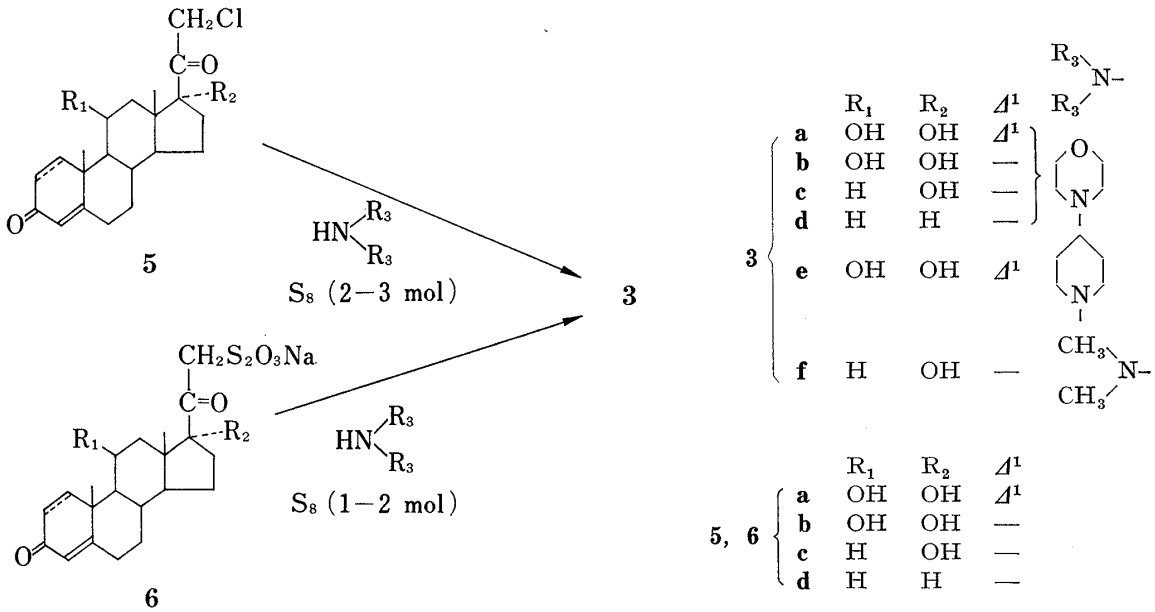
Chemistry

The Willgerodt–Kindler reaction has been conveniently applied to obtain carboxylic acids from various types of compounds, such as aromatic or aliphatic ketones, aromatic aldehydes, unsaturated compounds and enamines.[7] In the present study, 21-chloro-20-ketosteroids (**5**) and sodium 20-ketosteroid 21-thiosulfates (**6**) were employed as starting materials because of their ready availability. Thus, **5** was obtained in excellent yield by treating the corresponding 21-hydroxyl compound (**4**) with *p*-toluenesulfonyl chloride in dimethylformamide;[8] **6** was obtained as an equimolar mixture with sodium chloride by treating **5** with equimolar sodium thiosulfate.



		R_1	R_2	Δ^1
4, 5, 6	**a**	OH	OH	Δ^1
	b	OH	OH	—
	c	H	OH	—
	d	H	H	—

Chart 3



		R_1	R_2	Δ^1	
3	**a**	OH	OH	Δ^1	R_3N–R_3
	b	OH	OH	—	O, N
	c	H	OH	—	O, N
	d	H	H	—	O, N
	e	OH	OH	Δ^1	N
	f	H	OH	—	CH_3N–CH_3

		R_1	R_2	Δ^1
5, 6	**a**	OH	OH	Δ^1
	b	OH	OH	—
	c	H	OH	—
	d	H	H	—

Chart 4

6) M. Obayashi and S. Noguchi, *Takeda Kenkyusho Ho*, **30**, 1 (1971).
7) W. Walter and K.D. Bode, *Angew. Chem.*, **78**, 517 (1966).
8) S. Noguchi, *Yakugaku Zasshi*, **81**, 374 (1961).

The 21-chloro derivative (**5**) thus obtained was treated with 2—3 mol of sulfur in a large excess of secondary amine to afford the desired α-oxothiocarboxamide (**3**) in excellent yield. Thus, a mixture of 21-chloro-11β,17α-dihydroxypregna-1,4-diene-3,20-dione (**5a**) and 3 mol of sulfur was stirred in morpholine at room temperature for 30 min to give 11β,17α-dihydroxy-21-morpholinopregna-1,4-diene-3,20-dione-21-thione (**3a**) in 92.5% yield. Similarly, the 21-morpholino (**3b**, **c**, **d**), the 21-piperidino (**3e**) and the 21-dimethylamino (**3f**) derivatives were obtained in good yields from the corresponding 21-chloro derivatives (**5a—d**), 2—3 mol of sulfur and secondary amine.

Stirring of the sodium 21-thiosulfate (**6a—d**) and sulfur (1—2 mol) in secondary amines for a few minutes also gave **3** in good yields.

The chemical structure of **3** was confirmed by elemental analysis and the spectral properties. In the infrared (IR) spectra, strong absorption bands characteristic of a thiocarboxamide group were observed at 1460—1550 cm^{-1}, and nuclear magnetic resonance (NMR) spectra showed the presence of signals due to the alkyl residue of the secondary amine and the disappearance of the signals due to 21-CH_2 of the starting material.

The reaction was conducted with various amounts of sulfur and was monitored by silica gel thin-layer chromatography (TLC). First, **5a** was allowed to react with 1 mol, instead of 2—3 mol, of sulfur in morpholine. The spot of **5a** disappeared within 5 min and two new spots appeared on the plate: one was **3a** and the other was 11β,17α-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl disulfide (**7a**). Chromatographic separation of the reaction mixture gave **3a** and **7a** in 29% and 38% yields, respectively. Similarly, when **5c**, 1 mol of sulfur and dimethylamine were stirred for 5 min, **3f** and **7c** were obtained in 37% and 32% yields, respectively.

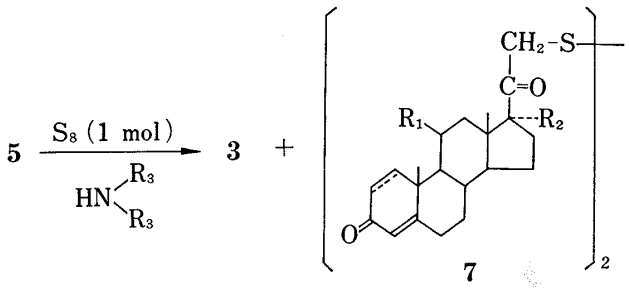


Chart 5

The chemical structure of the 21-disulfide (**7**) was determined by elemental analysis and from spectral data,

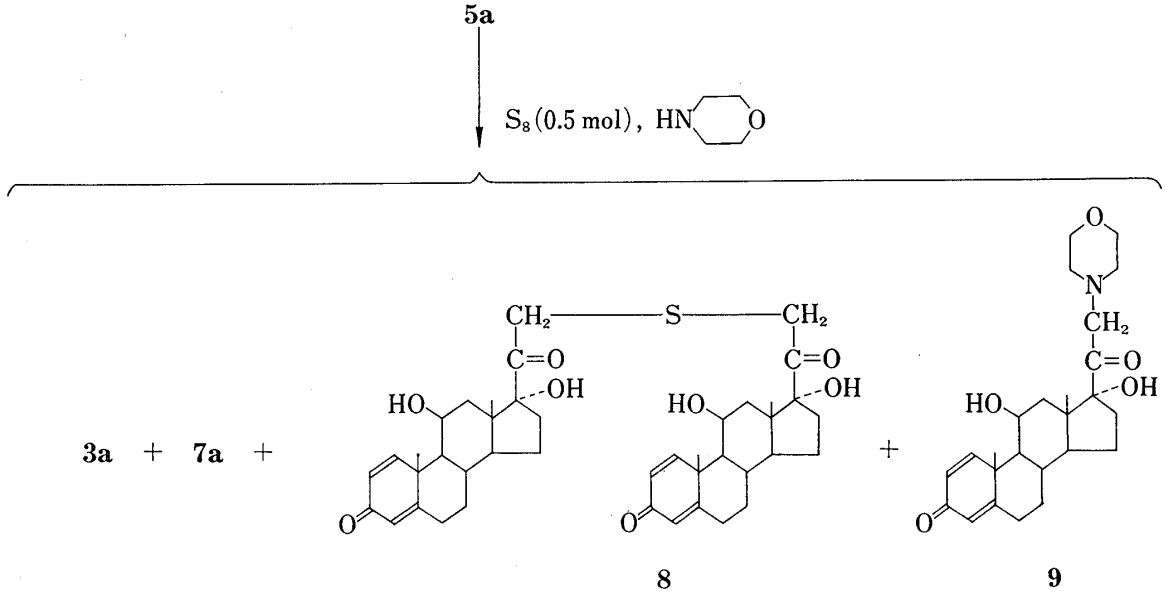


Chart 6

and was confirmed by direct comparison with a sample prepared by the quantitative reaction of **5** and sodium disulfide.

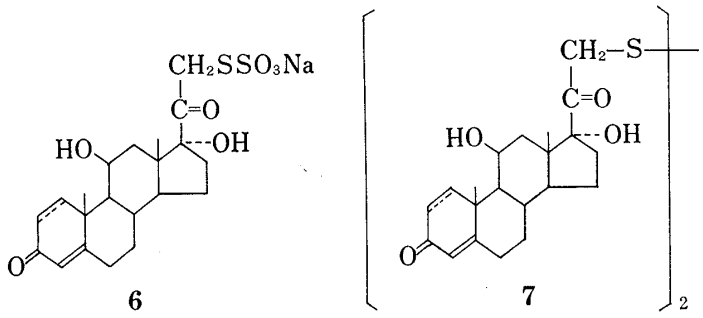
Secondly, a mixture of **5a** and 0.5 mol of sulfur was stirred in morpholine at room temperature. After 15 min, the spot of **5a** disappeared on the TLC plate and 4 spots appeared. Chromatographic separation of the reaction mixture gave α-oxothiocarboxamide (**3a**) (13.3%), 21-disulfide (**7a**) (16.4%), 21-monosulfide (**8**) (10.9%) and 21-morpholinoprednisolone (**9**) (14.0%).

The chemical structure of **8** was determined by elemental analysis and from the spectral data, and it was confirmed to be identical with a sample obtained from the quantitative reaction of **5a** with 0.5 mol of sodium sulfide. The chemical structure of **9** was confirmed by comparing the melting point (191.5—193°), with the reported value[2] (191—193°), as well as by the spectral data and direct comparison with a sample prepared by the reaction of **5a** with morpholine.

In order to check whether the 21-disulfide (**7a**), 21-monosulfide (**8**) and 21-morpholino derivative (**9**) are intermediates of the reaction, each compound was allowed to react with 1 mol of sulfur in morpholine and the reaction was again monitored by TLC. It was found that the 21-morpholino derivative (**9**) did not give **3a**, even after 5 hr and even when the reaction temperature was elevated to 70—80°. On the other hand, the disulfide (**7a**) gave **3a** quantitatively after 5 min and the reaction proceeded even in the absence of sulfur, although the reaction was very slow (completion of the reaction took about 24 hr). The monosulfide (**8**) also gave **3a** but the reaction was very slow, and in the absence of sulfur, the reaction (**8**→**3a**) did not take place. The findings suggest that the disulfide (**7a**) and the monosulfide (**8**) are intermediates in the present Willgerodt-Kindler reaction of **5a**.

Although the sodium 21-thiosulfate (**6**) afforded **3** very rapidly in the presence of 1 mol of sulfur, the formation of disulfide (**7**) was found to be predominant when sulfur was not

Table I. Anti-inflammatory Activities of the Sodium 21-Thiosulfate Derivative of the 20-Ketosteroid (**6**) and 20-Ketosteroid-21-yl-disulfide (**7**) in the Carrageenin Edema Test in Rats



6, 7 { **a**: Δ^1 **b**: — }

Compound	Δ^1	Oral dose mg/kg	% inhibition	Significance
6a	Δ^1	26.5*	0.2	N.S.
6b	—	26.5*	5.3	N.S.
7a	Δ^1	10.4	−22.9	N.S.
7b	—	20.8	5.2	N.S.
		62.4	17.8	N.S.
Prednisolone		2.5	44.6	0.01
Hydrocortisone		20.0	45.6	0.01

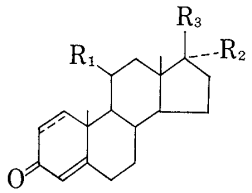
N.S.: not significant.
* Equimolar mixture of **6** and NaCl.

present. Thus, when an equimolar mixture of **6c** with sodium chloride was treated with dimethylamine for 5 min, the α-oxothiocarboxamide (**3f**) and the disulfide (**7c**) were obtained in 5% and 70% yields, respectively.

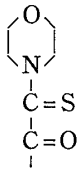
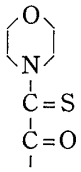
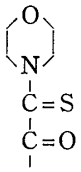
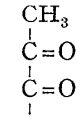
Anti-inflammatory Activity

Compounds **3**, **6** and **7** thus obtained were tested for anti-inflammatory activities using the carrageenin edema test, granuloma pouch test and cotton pellet test in rats. As shown in Table I, steroidal 21-thiosulfates (**6**) and 21-disulfides (**7**) did not show any significant anti-inflammatory activity in the carrageenin edema test in rats. It is known[9] that an –S–S– bond is easily cleaved after intestinal absorption to give –SH groups and therefore, compound

Table II. Anti-inflammatory Activity of the Steroidal 17-α-Oxothiocarboxamide (**3**) in the Carrageenin Edema Test in Rats



3

	Compound				Oral dose mg/kg	% Inhibition	Significance
	R_1	R_2	R_3	Δ^1			
3a	OH	OH		Δ^1	3.18 10.0	34.8 57.3	<0.05 <0.01
3b	OH	OH		—	12.7	29.2	<0.01
3c	H	H		—	20.0	−4.2	N.S.
TSC-5	OH	OAc		Δ^1	2.5 5.0	39.8 52.1	<0.01 <0.01
Prednisolone	OH	OH		Δ^1	2.5 5.0	44.6 58.2	<0.01 <0.01
Hydrocortisone	OH	OH		—	5.0 10.0	32.1 54.1	<0.01 <0.01

N.S.: not significant.

9) *a*) H. Hirano, Z. Suzuoki, and K. Murakami, *Bitamin*, **34**, 364 (1966); *b*) M. Hamada, T. Hayakawa, T. Yamaguchi, and M. Koike, *ibid.*, **35**, 474 (1967); *c*) F. Honda, *ibid.*, **36**, 452 (1967); *d*) F. Honda, *ibid.*, **36**, 459 (1967); *e*) I. Utsumi, K. Kohno, Y. Kakie, and M. Mizobe, *ibid.*, **37**, 264 (1968).

7 is considered to be biologically equivalent to 21-thiol derivatives. The present results indicate that replacement of the 21-hydroxyl group by –SH results in loss of activity.

On the other hand, as shown in Table II, 21-morpholino-21-thiocarbonyl derivatives (**3a**) and (**3b**) showed significant anti-inflammatory activity, comparable to or a little less than that of the parent compounds, prednisolone and hydrocortisone, respectively, as evaluated in the carrageenin edema test. The results suggest that the 21-hydroxy group is not essential for biological activities of corticosteroids; in addition, it is interesting that the steroidal 17-yl-α-oxothiocarboxamide (**3**), which is similar in structure to a steroidal α-diketone (TSC-5), also showed significant anti-inflammatory activity.

3a was further tested for anti-inflammatory activity in the granuloma pouch test and the cotton pellet test in rats. **3a** showed significant activity in the granuloma pouch test (Table III), but essentially no activity in the cotton pellet test (Table IV), in contrast to TSC-5 and known anti-inflammatory corticosteroids such as prednisolone, which show significant anti-inflammatory activities in the carrageenin edema, granuloma pouch and cotton pellet tests.

Table III. Effects of 11β,17α-Dihydroxy-21-morpholinopregna-1,4-diene-3,20-dione-21-thione (**3a**), TSC-5 and Prednisolone on the Exudate induced by Croton Oil in Rats

Compound	Oral dose mg/kg/day	% inhibition	Thymus mg	Adrenal mg	Spleen mg
Control	—	—	444.4 ±23.2	24.2 ±0.36	667.8±34.3
3a	1.27	7.8	444.4 ±22.6	24.77±1.1	646.7±39.3
	5.09	38.4*	316.7 ±15.6**	24.3 ±0.75	568.9±39.2
TSC-5	2.0	32.2*	321.25±23.02**	20.23±1.16	450.0±20.96**
	4.0	45.7**	226.3 ±16.14**	19.2 ±0.64**	465.0±26.52*
Prednisolone	1.0	27.2	302.2 ±20.1**	24.6 ±0.96	587.8±32.6
	4.0	73.5**	157.8 ±14.1**	21.64±1.43	498.9±28.7**

*: $p<0.05$, **: $p<0.01$.

Table IV. Effects of 11β,17α-Dihydroxy-21-morpholinopregna-1,4-diene-3,20-dione-21-thione (**3a**) and Prednisolone on Granuloma induced by Cotton Pellets in Rats

Compound	Oral dose mg/kg/day	% inhibition	Thymus mg	Adrenal mg	Spleen mg
Control	—	—	610.0±36.3	16.8 ±0.68	737.5±17.4
3a	3.18	3.4 N.S.	481.3±29.5*	13.93±0.64**	723.8±88.0
Prednisolone	2.5	19.3**	345.9±24.4**	14.0 ±0.92*	561.3±34.9**

*: $p<0.05$, **: $p<0.01$, N.S.: not significant.

This finding suggests that **3a** might be active against acute and subacute inflammation but inactive against chronic inflammation. It was also found that **3a** significantly reduced the adrenal and thymus weights in both granuloma pouch and cotton pellet tests. These features of **3a** suggest that, although **3a** is different from TSC-5 or anti-inflammatory corticosteroids as regards the mode of anti-inflammatory activity, **3a** still has a corticosteroid-like effect on the organs.

Recently, a Schering group[10] has reported that *n*-butyl 6α-fluoro-11β-hydroxy-16α-methylpregna-1,4-diene-3,20-dione-21-oate (flucortin butyl ester) has anti-inflammatory effect upon

10) J.F. Kapp, H. Koch, M. Toepert, H.J. Kessler, and E. Gerhards, *Arzneim.-Forsch.*, **27**, 2191 (1977).

topical application. This compound bears an α-keto acid moiety at the 17 position, like **3a** and **3b**. It is interesting that, though flucortin butyl ester is active only on topical administration, **3a** is active on systemic administration.

Experimental[11]

Synthesis of α-Oxothiocarboxamide (3) from 21-Chloro-20-ketosteroid (5)

a) Reaction of 21-Chloro-20-ketosteroid (5) with 3 Mol Equivalents of Sulfur in a Secondary Amine——11β,17α-Dihydroxy-21-morpholinopregna-1,4-diene-3,20-dione-21-thione (**3a**): A solution of 2.3 g of 21-chloro-11β,17α-dihydroxypregna-1,4-diene-3,20-dione (**5a**) and 0.58 g of sulfur in 20 ml of morpholine was stirred at room temperature for an hour then poured into ice-water. The resulting crystals were collected by filtration, and washed with H_2O to give 2.6 g (92.5%) of **3a**. After drying, the crystals were recrystallized from MeOH–CH_2Cl_2 to give 2.3 g, mp 253°. $[\alpha]_D^{25}$ +77.4° (c=1, dioxane). *Anal.* Calcd. for $C_{25}H_{33}NO_5S$: C, 65.34; H, 7.33; N, 3.05; S, 6.96. Found: C, 65.03; H, 7.33; N, 3.08; S, 6.70. IR ν_{max}^{KBr} cm^{-1}: 3400, 1695, 1660, 1605, 1505. UV λ_{max}^{EtOH} nm (ε): 249.5 (22900).

The following compounds were synthesized similarly.

11β,17α-Dihydroxy-21-morpholinopregn-4-ene-3,20-dione-21-thione (**3b**): Yield 86%. mp 248—250° (recrystallized from MeOH). $[\alpha]_D^{25}$ +108.8° (c=1, dioxane). *Anal.* Calcd. for $C_{25}H_{35}NO_5S$: C, 65.05; H, 7.64; N, 3.04; S, 6.93. Found: C, 64.85; H, 7.81; N, 3.04; S, 6.63. IR ν_{max}^{KBr} cm^{-1}: 3486, 1692, 1667, 1626, 1516. UV λ_{max}^{EtOH} nm (ε): 247 (22800).

17α-Hydroxy-21-morpholinopregn-4-ene-3,20-dione-21-thione (**3c**): Yield 95%. mp 262—264° (recrystallized from MeOH–CH_2Cl_2). $[\alpha]_D^{25}$ +87.8° (c=1, dioxane). *Anal.* Calcd. for $C_{25}H_{35}NO_4S$: C, 67.39; H, 7.92; N, 3.14; S, 7.18. Found: C, 67.61; H, 7.91; N, 3.30; S, 6.97. IR ν_{max}^{KBr} cm^{-1}: 3550, 1698, 1673, 1620. UV λ_{max}^{EtOH} nm (ε): 245 (23000).

21-Morpholinopregn-4-ene-3,20-dione-21-thione (**3d**): Yield 94%. mp 189—190° (recrystallized from MeOH). $[\alpha]_D^{25}$ +84.5° (c=1, dioxane). *Anal.* Calcd. for $C_{25}H_{35}NO_3S$: C, 69.09; H, 8.21; N, 3.26; S, 7.66. Found: C, 68.84; H, 8.33; N, 3.36; S, 7.22. IR ν_{max}^{KBr} cm^{-1}: 1686, 1642, 1620, 1507. UV λ_{max}^{EtOH} nm (ε): 243 (21700).

11β,17α-Dihydroxy-21-piperidinopregna-1,4-diene-3,20-dione-21-thione (**3e**): Yield 85%. mp 242—245° (recrystallized from acetone). $[\alpha]_D^{25}$ +84.5° (c=1, dioxane). *Anal.* Calcd. for $C_{26}H_{35}NO_4S$: C, 68.25; H, 7.71; N, 3.06; S, 6.99. Found: C, 67.96; H, 8.01; N, 2.95; S, 6.23. UV λ_{max}^{EtOH} nm (ε): 249 (20800).

b) Reaction of 5 with One Mol Equivalent of Sulfur in a Secondary Amine——**3c** and 17α-Hydroxy-3,20-dioxopregn-4-en-21-yl Disulfide (**7c**): A solution of 910 mg of 21-chloro-17α-hydroxypregn-4-ene-3,20-dione (**5b**) and 80 mg of sulfur in 10 ml of morpholine was stirred under heating for 5 min. After cooling, the solution was poured onto ice and extracted with ethyl acetate. The ethyl acetate layer was washed with H_2O, dried with Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was separated by chromatography on silica gel using acetone–$CHCl_3$=1:9 to afford 320 mg (29%) of **3c** and 360 mg (38%) of **7c**.

7c, mp 254—256°. $[\alpha]_D^{25}$ +110° (c=1, pyridine). *Anal.* Calcd. for $C_{42}H_{58}O_6S_2$: C, 69.77; H, 8.09; S, 8.87. Found: C, 69.52; H, 8.10; S, 8.47. IR ν_{max}^{KBr} cm^{-1}: 3370, 1710, 1660, 1612. UV λ_{max}^{EtOH} nm (ε): 241 (31000).

17α-Hydroxy-21-dimethylaminopregn-4-ene-3,20-dione-21-thione (**3f**): Similarly, **3f** in 37% yield and **7c** in 32% yield were obtained from **6c**, sulfur and dimethylamine. **3f**: mp 211—212° (recrystallized from MeOH). $[\alpha]_D^{25}$ +62° (c=1, dioxane). *Anal.* Calcd. for $C_{23}H_{33}NO_3S$: C, 68.46; H, 8.24; N, 3.42; S, 7.93. Found: C, 68.48; H, 8.20; N, 3.48; S, 7.91. IR ν_{max}^{KBr} cm^{-1}: 3410, 1704, 1647, 1619, 1540. UV λ_{max}^{EtOH} nm (ε): 245 (24800).

Synthesis of 3 from Sodium 20-Ketosteroid 21-Thiosulfate (Bunte Salt) (6)

a) Synthesis of 6——Sodium 11β,17α-Dihydroxypregna-1,4-diene-3,20-dione 21-Thiosulfate (**6a**): To a solution of **5a** in 25 ml of EtOH, 3.5 ml of a 10% solution of $Na_2S_2O_3$ was added. The mixture was heated with stirring at 80° for 30 min and evaporated to dryness *in vacuo* to give an equimolar mixture of **6a** and NaCl, which was used for the subsequent reaction without separation.

Sodium 11β,17α-Dihydroxypregn-4-ene-3,20-dione 21-Thiosulfate (**6b**), Sodium 17α-Hydroxypregn-4-ene-3,20-dione 21-Thiosulfate (**6c**) and Sodium Pregn-4-ene-3,20-dione 21-Thiosulfate (**6d**): Similarly, **6b**, **6c** and **6d** were obtained from the corresponding 21-chloro-20-ketosteroids **5b**, **5c** and **5d**.

b) Reaction of 6a with Sulfur in Secondary Amine——Sulfur (100 mg) was added to 300 mg of an equimolar mixture of **6a** and NaCl in 10 ml of morpholine and the resulting solution was stirred at room temperature. After 30 min, the solution was poured onto ice and the resulting crystals were obtained by filtration and dried to give 220 mg (90%) of **3a**.

11) Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded on a Hitachi EPI-S2 spectrophotometer. NMR spectra were recorded on a Varian A-60 spectrometer. Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane (TMS) as an internal standard.

Similarly, **3b**, **3c** and **3d** were obtained from the corresponding sodium 21-thiosulfates (**6b**, **6c** and **6d**). Yields, 88%, 92% and 87%, respectively.

c) **Reaction of 6b with Dimethylamine**——An equimolar mixture (300 mg) of **6b** and NaCl was dissolved in 10 ml of H_2O with heating, then 20 ml of 40% solution of dimethylamine in H_2O was added. After 5 min, the mixture was cooled, extracted with AcOEt and washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel (silica gel 60 g, $CHCl_3$–AcOEt=9: 1) to give 20 mg of **3f** and 108 mg of **7c**.

Isolation of Intermediates of the Reaction of 5a with Sulfur in Morpholine

A mixture of 1.13 g of **5a** and 48 mg (0.5 equivalent) of sulfur in 10 ml of morpholine was stirred overnight at room temperature and poured onto ice. The precipitated oil was extracted with AcOEt, washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica gel column (silica gel 300 g, acetone–$CHCl_3$=1: 4) to give 230 mg of **3a**, 185 mg of 11β,17α-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl disulfide (**7a**), 118 mg of 11β,17α-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl sulfide (**8**) and 180 mg of 11β,17α-dihydroxy-21-morpholinopregna-1,4-diene-3,20-dione (**9**).

7a——mp 244° (recrystallized from MeOH). $[\alpha]_D^{25}$ +94.4° (c=1.0, pyridine). *Anal.* Calcd. for $C_{42}H_{54}O_8S_2$: C, 67.16; H, 7.26; S, 8.54. Found: C, 67.32; H, 7.15; S, 8.30. IR ν_{max}^{KBr} cm^{-1}: 3400, 1708, 1659, 1580, 890. UV λ_{max}^{EtOH} nm (ε): 242 (34300). **8**: mp 168—169° (recrystallized from MeOH). *Anal.* Calcd. for $C_{42}H_{54}O_4S$: C, 77.01; H, 8.33; S, 4.90. Found: C, 77.12; H, 8.28; S, 5.10. IR ν_{max}^{KBr} cm^{-1}: 3400, 1710, 1650, 1615, 1600. UV λ_{max}^{EtOH} nm (ε): 244 (32000). **9**: mp 191.5—193° (recrystallized from MeOH). Reported[2] mp 191—193°.

7a, 8 and 9 as Possible Intermediates of the Reaction

Each compound, **7a**, **8** or **9**, was stirred at room temperature with or without an equivalent amount of sulfur in morpholine and the reactions were followed by TLC (silica gel, $CHCl_3$–acetone–EtOH=80: 20: 3).

Alternative Syntheses of 7, 8 and 9

7a——A solution of 0.64 g of sulfur and 4.8 g of $Na_2S\cdot 9H_2O$ in 30 ml of MeOH was heated under reflux for 30 min. After cooling, 7.0 g of **5a** was added to the solution and the mixture was stirred at room temperature for 30 min, then poured onto ice. The resulting crystals were collected by filtration, washed with H_2O and dried to give 7.2 g of crude **7a**.

7b and 7c——**7b** and **7c** were obtained similarly from **5b** and **5c**, respectively.

11β,17α-Dihydroxy-3,20-dioxopregn-4-en-21-yl Disulfide (7b)——mp 251° (dec.) (recrystallized from MeOH–CH_2Cl_2), $[\alpha]_D^{25}$ +119° (c=1, pyridine). *Anal.* Calcd. for $C_{42}H_{58}O_8S_2$: C, 66.81; H, 7.74; S, 8.49. Found: C, 66.92; H, 7.80; S, 8.50. IR ν_{max}^{KBr} cm^{-1}: 3380, 1697, 1638, 1620. UV λ_{max}^{EtOH} nm (ε): 242 (32000).

8——A solution of 0.9 g of **5a** and 1 g of $Na_2S\cdot 9H_2O$ in 30 ml of MeOH was stirred at room temperature for 1 hr then poured onto ice. The resulting crystals were collected by filtration, washed with H_2O and dried to give 0.88 g of crude **8**.

9——A solution of 500 mg of **5a** in 10 ml of morpholine was stirred at room temperature for 3 hr then poured onto ice. The resulting crystals were collected by filtration, washed with H_2O and dried to give 500 mg of **9**.

Anti-inflammatory Tests

Carrageenin Edema Test——Following the method of Winter *et al.*,[12] the unilateral hind paw volume of male rats weighing 180—200 g was measured and test agents were orally administered in various doses. One hour after the administration of drugs, 0.05 ml of 1% carrageenin suspension in physiological saline was injected subcutaneously into the plantar side of the hind paw, and 3 hr later the edema thus developed was estimated by measuring the volume of the paw. The edema in the treated rats was compared with that in the control animals.

Granuloma Pouch Test——Following the method of Robert and Nezamis,[13] an air pouch was formed subcutaneously on the back of female rats, and 0.5 ml of 1% croton oil dissolved in arachis oil was injected into the pouch. Two days later the air in the pouch was withdrawn to promote exudation. Test agents were orally administered daily for 5 days from the day of pouch formation. On the day after final administration the volume of exudate in the pouch was measured.

Cotton Pellet Test——Following the method of Winter *et al.*,[14] two sterilized cotton pellets weighing 30±1 mg were aseptically embedded subcutaneously in the lateral abdomen of male rats. The test agents were orally administered daily for 7 days. On the next day after final administration, the pellets were removed, dried and weighed to determine the weight of the granuloma formed around the pellets.

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