Studies on Topical Antiinflammatory Agents. IV.[1,2] 21-(Alkylthio)acetates and (Methylthio)methoxides of Corticosteroids

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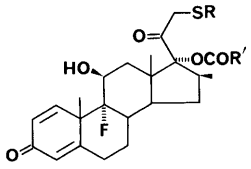
A series of 21-(alkylthio)acetates and 21-(methylthio)methoxides of corticosteroids were synthesized and examined for vasoconstrictive activities. The activities of seven compounds were equal to or greater than that of 9α-fluoro-11β,21-dihydroxy-16β-methyl-17α-valeryloxy-1,4-pregnadiene-3,20-dione (betamethasone 17-valerate, BV). Among them, betamethasone 21-(methylthio)acetate 17-propanoate (2Ca) was found to have the most potent activity, which is superior to that of BV. A structure–activity relationship study revealed that substitution of the 21-hydroxy group of corticosteroids with the (methylthio)acetate function is a useful approach for obtaining potent activity.

Keywords corticosteroid; antiinflammatory agent; vasoconstrictive activity; 9α-fluoro-11β-hydroxy-16β-methyl-21-(methylthio)acetoxy-17α-propanoyloxy-1,4-pregnadiene-3,20-dione; structure–activity relationship

Various topical antiinflammatory agents contain a 20-keto-21-hydroxy function as an essential moiety for their biological activity in a corticosteroid skeleton. In a previous paper,[1] we described the synthesis and the topical antiinflammatory activity of corticosteroid 21-thio substituted derivatives (I). In particular, the 21-methylthio compound (Ia) (Chart 1) had potent activity. In the course of our studies on the development of topical antiinflammatory agents, we have been attempting to elucidate the possible usefulness of other functional groups bearing a sulfur atom instead of a methylthio group at the 21-position and to establish structure–activity relationships for these compounds. In order to determine whether the sulfur atom introduced into the 21-hydroxy group influences the activity or not, in the present work, we describe the preparation and biological activities of a new series of the 21-(alkylthio)acetates (**2**) and 21-(methylthio)methoxides (**3**) of corticosteroids.



I
Ia: R=Me, R'=Et

Chart 1

Chemistry

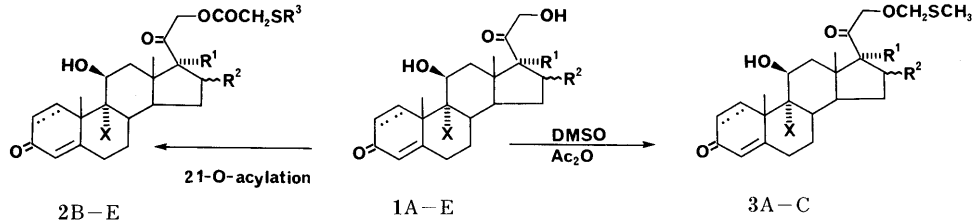
The synthetic scheme is outlined in Chart 2. The 21-acyl derivatives (**2**Ba—Ea) listed in Table I were synthesized from the corresponding corticosteroids (**1**B—E) by two methods (methods A and B). Compounds **2**Ba—Cc were obtained by acylation of the 17-ester compounds (**1**B, C) with the corresponding carboxylic acid and thionyl chloride in the presence of pyridine (method A).

In order to complete the structure–activity relationship studies, the 16α,17α-ketals (**2**Da, Ea) were prepared. Compounds **1**D and **1**E were esterified with (methylthio)acetic acid and 2-chloro-1-methylpyridinium iodide in the presence of tributylamine according to the method of Mukaiyama *et al.*[3] (method B). The 21-(methylthio)methoxides (**3**A—C) (Table II) were prepared from the 17-esters (**1**A—C) by Pummerer reaction with dimethyl sulfoxide (DMSO) and acetic anhydride, but the yields were not satisfactory. Physical data for compounds **2** and **3** are given in Tables I and II, respectively.

Safety of the Compounds Tested Before application to volunteers, the safety of all the compounds was checked by the method reported previously.[2]

Biological Results and Discussion

Primary Skin-Irritating Activity All the compounds were evaluated at 1, 2, 3 and 7 d by the Draize method.[4] As



2B—E 1A—E 3A—C

	A	B	C	D	E
C1–C2	–	–	Δ	Δ	Δ
X	H	H	F	H	H
R1	OCOEt	OCOPr	OCOEt		
R2	H	H	β-Me		

Chart 2

TABLE I. Corticosteroid 21-(Alkylthio)acetate Derivatives (**2**)

Compd. No.	R^3	Yield[a] (%) (Method)	mp (°C) (Solvent[b])	Formula (SIMS *m/z*: MH^+)	Analysis (%) Calcd (Found) C	H	F	S
2Ba	Me	33	68—70	$C_{28}H_{40}O_7S$[c]	63.49	7.80		6.05
		(A)	(E–W)	(521)	(63.77	7.66		6.31)
2Bb	Et	49	63—65	$C_{29}H_{42}O_7S$[d]	64.60	7.94		5.95
		(A)	(E–W)	(535)	(64.49	7.91		5.99)
2Bc	Pr	78	60—62	$C_{30}H_{44}O_7S$	65.66	8.08		5.84
		(A)	(E–W)	(549)	(65.61	8.18		5.91)
2Ca	Me	53	198—201	$C_{28}H_{37}FO_7S$	62.67	6.95	3.54	5.97
		(A)	(E)	(537)	(62.39	7.01	3.48	6.27)
2Cb	Et	82	93—95	$C_{29}H_{39}FO_7S$[c]	62.23	7.20	3.39	5.73
		(A)	(E–W)	(551)	(62.33	7.16	3.46	5.67)
2Cc	Pr	84	82—85	$C_{30}H_{41}FO_7S$[d]	63.30	7.35	3.34	5.63
		(A)	(E–W)	(565)	(63.37	7.33	3.37	5.67)
2Da	Me	64	170—171	$C_{27}H_{36}O_7S$	64.26	7.19		6.35
		(B)	(A–H)	(505)	(64.24	7.20		6.50)
2Ea	Me	86	149—151	$C_{28}H_{38}O_7S$	64.84	7.39		6.18
		(B)	(A–H)	(519)	(64.74	7.41		6.08)

a) Yields are based on the preceding isolated intermediates. *b*) Recrystallization solvents: A, AcOEt; H, hexane; E, EtOH; W, H_2O. *c*) 1/2 H_2O. *d*) 1/4 H_2O.

TABLE II. Corticosteroid 21-(Methylthio)methoxide Derivatives (**3**)

Compd. No.	Yield[a] (%)	mp (°C) (Solvent[b])	Formula (SIMS *m/z*: MH^+)	Analysis (%) Calcd (Found) C	H	F	S
3A	22	155—157	$C_{26}H_{38}O_6S$	65.24	8.00		6.70
		(E–H)	(479)	(65.28	8.13		6.94)
3B	18	106—108	$C_{27}H_{40}O_6S$	65.82	8.18		6.51
		(E–H)	(493)	(65.84	8.34		6.73)
3C	25	215—218	$C_{27}H_{37}FO_6S$	63.76	7.33	3.73	6.30
		(A–H)	(509)	(63.96	7.44	3.59	6.26)

a, *b*) See footnotes *a* and *b* in Table I.

a result, it was considered that none of the compounds exhibits primary skin irritation.

Mutagenicity All the compounds tested were negative in the Ames' spot test.[5]

Thus, no significant toxic signs were observed in the primary skin irritation and bacterial reverse mutation tests of all the compounds.

Vasoconstrictive Activities A number of methods for evaluating topical antiinflammatory activity of corticosteroids have been described. However, it is well known that corticosteroids that are predicted to be potent on the basis of animal studies may be much less potent than expected in humans. Only the vasoconstriction activity test is considered to be reliable for predicting the antiinflammatory potency of topical corticosteroids,[6] because a remarkably good correlation has been found between the results of this test and the topical efficacy in the clinic.[7] Using this method, for instance, clobetasol propionate[8] and betamethasone 17-valerate[9] (BV) were selected and are now widely used in the clinic. Evaluation by this method is recommended as a preclinical study for topically applied corticosteroids.[6]

The compounds prepared in this study were tested for vasoconstrictive activities in twenty healthy male volunteers by the method reported previously.[2] The vasoconstrictive activities of the compounds tested were compared with that of BV, which was used as a positive control for the activity. Statistical analysis was performed by Wilcoxon's signed-ranks test.[10] The results are summarized in Table III.

TABLE III. Vasoconstrictive Activity Ratios of Corticosteroid 21-(Alkylthio)acetate and 21-(Methylthio)methoxide Derivatives (**2** and **3**)

Compd. No.	After 2 h	After 4 h	Compd. No.	After 2 h	After 4 h
2Ba	114	97	**3A**	56[b]	59[c]
2Bb	82	80[a]	**3B**	119	103
2Bc	89	87	**3C**	63[c]	70[c]
2Ca	142[b]	133[b]			
2Cb	119	128[a]			
2Cc	112	100			
2Da	97	93			
2Ea	114	107			

Vaseline ointment (0.01%) was used. Each compound was tested on 20 volunteers. The potency is expressed as the ratio of vasoconstrictive activity to that of BV taken as 100. *a*) $p<0.1$. *b*) $p<0.02$. *c*) $p<0.01$ for BV, using Wilcoxon's signed-ranks test.[10]

The activities of seven compounds, **2Ba**, **2Ca**—**2Cc**, **2Da**, **2Ea** and **3B**, at 2 h were equal to or greater ($p<0.1$) than that of BV. In particular, the activities of six compounds, **2Ba**, **2Ca**—**2Cc**, **2Ea** and **3B**, were equal to or greater

($p<0.1$) than that of BV at both 2 and 4 h. With regard to the terminal substituents at the 21-position, the compounds with a methyl group (**2**Ba, Ca, Ea) showed potent activities. Conversion of the terminal methyl group into larger alkyl groups such as ethyl (**2**Bb, Cb) and propyl groups (**2**Bc, Cc) tended to decrease the activity. These results in the present series of compounds are in accordance with our previous findings on the 17-succinyl esters.[2b] Generally, the activities of betamethasone 21-(alkylthio)acetates (**2**Ca—Cc) were more potent than those of the corresponding hydrocortisone 21-acetate derivatives (**2**Ba—Bc). The structure–activity studies were extended to the 16,17-ketal series to include **2**Da and **2**Ea. These compounds were as potent as or more potent than BV.

On the other hand, in the case of the 21-(methylthio)-methoxides, the 17-butanoate (**3B**) had the most potent activity, which was equivalent to that of BV, but the activities of the 17-propanoates (**3**A, C) were markedly reduced as compared with that of **3**B. Variation of the 17-ester chain length resulted in a remarkable change of the activity.

However, introduction of the (methylthio)acetate group into the 21-position of the corticosteroids resulted in significant enhancement of the activity. Among the compounds tested in this study, betamethasone 21-(methylthio)acetate 17-propanoate (**2**Ca) had the most potent activity, which was significantly ($p<0.02$) greater than that of BV. Accordingly, it is concluded that substitution of the 21-hydroxy group of corticosteroids with a (methylthio)acetate function is a potentially useful approach for obtaining higher activity.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO DS-301 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian XL-200 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. The chemical shifts are given in δ (ppm). The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The unit (Hz) of coupling constants (J) is omitted. The secondary ionization mass spectra (SIMS) were taken with a Hitachi M-80A spectrometer. The extracted organic solutions were dried over $MgSO_4$. Column chromatography was carried out on Wakogel C-200.

Typical examples are given to illustrate the general procedure.

Method A 17α-Butanoyloxy-11β-hydroxy-21-(methylthio)acetoxy-4-pregnene-3,20-dione (**2**Ba): A solution of (methylthio)acetic acid (1.1 g), benzene (10 ml) and thionyl chloride (2.2 ml) was refluxed for 2 h. After removal of the solvent, the residue was dissolved in chloroform (3 ml). This solution was added to a solution of hydrocortisone 17-butanoate[11] (**2**B, 1.50 g) in pyridine (12 ml) with stirring under ice-cooling and the whole was stirred for 40 min. After completion of the reaction, the mixture was neutralized with 10% HCl, and extracted with ethyl acetate (AcOEt). The AcOEt extract was washed with 10% Na_2CO_3 and brine, then dried and concentrated *in vacuo*. The residue was purified by column chromatography (AcOEt : hexane = 1 : 3) followed by recrystallization from EtOH–H_2O, affording **2**Ba (0.60 g, 33%) as a colorless powder. IR: 3400, 2920, 1720, 1650 cm^{-1}. NMR: 0.96 (3H, t, $J=7$), 1.03 (3H, s), 1.45 (3H, s), 2.26 (3H, s), 2.33 (2H, t, $J=7$), 3.34 (2H, s), 4.50 (1H, m), 4.72, 4.98 (2H, each d, $J=12$), 5.71 (1H, br s).

The following compounds were similarly prepared. The yields and physical properties are listed in Table I. Spectral data for compounds (**2**Bb—Cc) are as follows.

17α-Butanoyloxy-21-(ethylthio)acetoxy-11β-hydroxy-4-pregnene-3,20-dione (**2**Bb): IR: 3400, 2920, 1720, 1650 cm^{-1}. NMR: 0.95 (3H, t, $J=6$), 1.01 (3H, s), 1.28 (3H, t, $J=7$), 1.45 (3H, s), 2.32 (2H, t, $J=6$), 2.71 (2H, q, $J=7$), 3.37 (2H, s), 4.50 (1H, m), 4.71, 4.98 (2H, each d, $J=12$), 5.71 (1H, br s).

17α-Butanoyloxy-11β-hydroxy-21-(propylthio)acetoxy-4-pregnene-3,20-dione (**2**Bc): IR: 3410, 2920, 1720, 1650 cm^{-1}. NMR: 0.95 (3H, t, $J=7$), 0.99 (3H, t, $J=7$), 1.02 (3H, s), 1.45 (3H, s), 2.33 (2H, t, $J=7$), 2.67 (2H, t, $J=7$), 3.37 (2H, s), 4.50 (1H, m), 4.70, 4.98 (2H, each d, $J=12$), 5.72 (1H, br s).

9α-Fluoro-11β-hydroxy-16β-methyl-21-(methylthio)acetoxy-17α-propanoyloxy-1,4-pregnadiene-3,20-dione (**2**Ca): IR: 3310, 1742, 1726, 1718, 1655 cm^{-1}. NMR: 1.00 (3H, s), 1.16 (3H, t, $J=7$), 1.35 (3H, d, $J=7$), 1.56 (3H, s), 2.24 (3H, s), 2.39 (2H, q, $J=7$), 3.34 (2H, s), 4.36, 4.91 (2H, each d, $J=12$), 4.44 (1H, m), 6.15 (1H, br s), 6.37 (1H, dd, $J=10$, 2), 7.23 (1H, d, $J=10$).

21-(Ethylthio)acetoxy-9α-fluoro-11β-hydroxy-16β-methyl-17α-propanoyloxy-1,4-pregnadiene-3,20-dione (**2**Cb): IR: 3400, 2920, 1730, 1660 cm^{-1}. NMR: 0.99 (3H, s), 1.15 (3H, t, $J=8$), 1.29 (3H, t, $J=8$), 1.35 (3H, d, $J=8$), 1.56 (3H, s), 2.39 (2H, q, $J=8$), 2.70 (2H, q, $J=8$), 3.19 (2H, s), 4.35, 4.91 (2H, each d, $J=12$), 4.43 (1H, m), 5.15 (1H, br s), 6.37 (1H, dd, $J=10$, 2), 7.23 (1H, d, $J=10$).

9α-Fluoro-11β-hydroxy-16β-methyl-17α-propanoyloxy-21-(propylthio)-acetoxy-1,4-pregnadiene-3,20-dione (**2**Cc): IR: 3390, 2920, 1728, 1658 cm^{-1}. NMR: 0.99 (3H, s), 1.00 (3H, t, $J=7$), 1.15 (3H, t, $J=7$), 1.35 (3H, d, $J=8$), 1.55 (3H, s), 2.38 (2H, q, $J=7$), 2.64 (2H, t, $J=7$), 3.36 (2H, s), 4.36, 4.90 (2H, each d, $J=16$), 4.43 (1H, m), 6.15 (1H, br s), 6.36 (1H, dd, $J=11$, 2), 7.23 (1H, d, $J=11$).

Method B 11β-Hydroxy-16α,17α-isopropylidenedioxy-21-(methylthio)-acetoxy-1,4-pregnadiene-3,20-dione (**2**Da): A solution of 2-chloro-1-methylpyridium iodide (319 mg) in CH_2Cl_2 (2 ml) was added to a solution of desonide[12] (**1**D, 400 mg), (methylthio)acetic acid (112 mg) and tributylamine (0.59 ml) in CH_2Cl_2 (6 ml). The resulting mixture was refluxed for 4 h and concentrated *in vacuo*. The residue was directly chromatographed with AcOEt–hexane (2 : 3) and the product was recrystallized from AcOEt–hexane, affording **2**Da (310 mg, 64%) as colorless prisms. IR: 3360, 2920, 1740, 1720, 1645 cm^{-1}. NMR: 0.95 (3H, s), 1.22 (3H, s), 1.44 (3H, s), 1.46 (3H, s), 2.26 (3H, s), 3.35 (2H, s), 4.53 (1H, m), 4.90, 5.05 (2H, each d, $J=18$), 4.99 (1H, d, $J=4$), 6.05 (1H, br s), 6.29 (1H, dd, $J=10$, 2), 7.28 (1H, d, $J=10$).

The following compound was similarly prepared. The yield and physical properties are listed in Table I. Spectral data for **2**Ea are as follows.

16α,17α-Butylidenedioxy-11β-hydroxy-21-(methylthio)acetoxy-1,4-pregnadiene-3,20-dione (**2**Ea): IR: 3340, 2900, 1740, 1720, 1650 cm^{-1}. NMR: 0.93 (3H, t, $J=7$), 0.97 (3H, s), 1.46 (3H, s), 2.26 (3H, s), 3.34 (2H, s), 4.53 (1H, m), 4.64 (1H, t, $J=5$), 4.85, 4.94 (2H, each d, $J=18$), 4.86 (1H, d, $J=4$), 6.05 (1H, br s), 6.29 (1H, dd, $J=10$, 2), 7.27 (1H, d, $J=10$).

9α-Fluoro-11β-hydroxy-16β-methyl-21-(methylthio)methoxy-17α-propanoyloxy-1,4-pregnadiene-3,20-dione (**3**C): Acetic anhydride (4.21 ml), acetic acid (1.27 ml) and DMSO (3.16 ml) were added to a solution of **1**C (1.00 g) in acetonitrile (50 ml). The resulting solution was refluxed for 4 h. The reaction mixture was neutralized with 10% Na_2CO_3 and the precipitate was filtered and directly chromatographed with acetone–$CHCl_3$–hexane (3 : 3 : 16). The product was recrystallized from AcOEt–hexane, yielding **3**C (280 mg, 25%) as colorless plates. IR: 3460, 1720, 1660 cm^{-1}. NMR: 1.00 (3H, s), 1.16 (3H, t, $J=8$), 1.41 (3H, d, $J=7$), 1.57 (3H, s), 2.15 (3H, s), 2.39 (2H, q, $J=8$), 4.08, 4.20 (2H, each d, $J=17$), 4.46 (1H, m), 4.69, 4.78 (2H, each d, $J=12$), 6.16 (1H, s), 6.37 (1H, dd, $J=9$, 1), 7.22 (1H, d, $J=9$).

The following compounds were similarly prepared. The yields and physical properties are listed in Table II. Spectral data for **3**A, B are as follows.

11β-Hydroxy-21-(methylthio)methoxy-17α-propanoyloxy-4-pregnene-3,20-dione (**3**A): IR: 3370, 1720, 1645 cm^{-1}. NMR: 0.96 (3H, s), 1.14 (3H, t, $J=7$), 1.44 (3H, s), 2.15 (3H, s), 4.36 (2H, s), 4.51 (1H, m), 4.74 (2H, s), 5.71 (1H, s).

17α-Butanoyloxy-11β-hydroxy-21-(methylthio)methoxy-4-pregnene-3,20-dione (**3**B): IR: 3400, 1720, 1650 cm^{-1}. NMR: 0.96 (3H, t, $J=7$), 0.98 (3H, s), 1.45 (3H, s), 2.15 (3H, s), 4.38 (2H, s), 4.52 (1H, m), 4.75 (2H, s), 5.72 (1H, s).

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