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SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF 21-THIO DERIVATIVES OF CORTICOSTEROIDS

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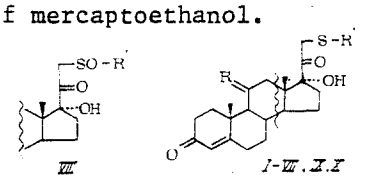
Among the derivatives of corticosteroids in which the primary alcoholic group at the 21-position is substituted by sulfur, the 21-thioaliphatic derivatives are known best [5-8, 10, 14]. Of these, tixocortol pivalate, which has a local anti-inflammatory activity equal to that of cortisol acetate, when there is no glucocorticoid or mineralocorticoid activity [11], is of considerable interest. The 21-sulfide derivatives of corticosteroids [9, 12, 13] have been much less studied.

In our search for new biologically active compounds, we synthesized 21-thio-derivatives of corticosteroids (I-X) that were not yet known, and studied their anti-inflammatory activity.

21-Thioalkanoic acids of corticosteroids I-V are obtained by the reaction of corticosteroid 21-tosylates with the disodium salts of mercaptoalkanoic acids, and acid VI from cortisol 21-tosylate and β-mercaptopropionic acid in the presence of triethylamine. The sodium salt IIIa is obtained from acid III and sodium methylate; the methyl ester VIII is obtained from cortisol 21-tosylate and the sodium salt of mercaptoacetic acid methyl ester, followed by oxidation to sulfoxide. Amide IX is obtained from acid III and diethylamine in the presence of dicyclohexylcarbodiimide. Alcohol X was synthesized by the reaction of cortexolone 21-tosylate with the sodium salt of mercaptoethanol.



III I-III, IX

I: $R = H_2$, $R^1 = CH_2COOH$; II: R = O, $R^1 = CH_2COOH$; III: R = H, β-OH, $R^1 = CH_2COOH$; IIIa: R = H, β-OH, $R^1 = CH_2COONa$; IV: $R = H_2$, $R^1 = CH_3(CH_2)COOH$; V: $R = H_2$; $R^1 = (CH_2)_2COOH$; VI: R = H, β-OH, $R^1 = (CH_2)_2COOH$; VII: R = H, β-OH, $R^1 = CH_2COOCH_3$; VIII: R = H, β-OH, $R^1 = CH_2COOCH_3$; IX: R = H, β-OH, $R^1 = CH_2CON(C_2H_5)_2$; X: $R = H_2$, $R^1 = (CH_2)_2OH$.

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The structure of 21-thio derivatives of corticosteroids I-X was confirmed by the data of elementary analysis, UV, IR and PMR spectra. In the IR spectra of compounds I-X, there are absorption bands in the regions of 1600-1620 cm^{-1} (4-C=C), 1630-1680 cm^{-1} (3-C=C), and 1690-1725 cm^{-1} (20-C=C). In the spectra of acids I-VI there are also absorption bands in the regions of 1710-1740 cm^{-1} (C=O of the carboxylic group) and 2400-3300 cm^{-1} (OH of the carboxylic group), while in the spectra of esters VII, VIII, at 1745 cm^{-1} (C=O of the ester group). In the IR spectrum of compound IX there is an absorption band of the amide carbonyl at 1660 cm^{-1}, and in the spectrum of alcohol X, a band at 3450 cm^{-1} (OH). In the PMR spectra of compounds I-X, proton signals of all the structural fragments were observed in the expected regions.

EXPERIMENTAL CHEMICAL

The melting points were determined on a Boetius stage (GDR). The specific rotation was determined on a Jasco J-20 spectropolarimeter (Japan). The UV spectra were obtained on a Specord UV-VIS spectrophotometer (GDR) in ethanol. The IR spectra were run on a UR-20 spectrophotometer (GDR), and the PMR spectra on a Bruker WM-360 spectrometer (GFR) in $CDCl_3$ solution, relative to TMS. The course of the reaction and the individual state of the compounds obtained was controlled by the TLC method, on Silufol UV-254 plates or on a LS 5/40 silica gel, using a 9.5:0.5 chloroform-methanol mixture as eluent.

Corticosteroid 21-tosylates were obtained by the method that we have already described in [1]. The reactions were carried out with stirring, in an argon atmosphere.

(17α-Hydroxy-4-pregnene-3,20-dion-21-yl-21-thio)acetic Acid (I). A freshly prepared disodium sulfidoacetate, obtained from 0.332 g (0.0035 mole) of mercaptoacetic acid and 0.378 g (0.007 mole) of sodium methylate in 14 ml of methanol, are added to a solution of 1.502 g (0.003 mole) of cortexolone 21-tosylate in 30 ml of acetone. After 15-20 min, 4 ml of acetic acid are added to the reaction mixture, which is evaporated to half of its volume, and water is added. The crystals formed are filtered. PMR spectrum, δ, ppm: 0.69 s (3H, 18-H_3), 1.21 s (3H, 19-H_3), 3.32 q (J 15.9 Hz, 2H, -S-CH_2-), 3.51 d (J 16.2 Hz, 1H, 21-H_B), 3.99 d (J 16.2 Hz, 1H, 21-H_A) 4.16 br. s (OH), 5.75 br. s (1H, 4-H).

(17α-Hydroxy-4-pregnene-3,11,20-trion-21-yl-21-thio)acetic Acid (II) is obtained from cortisone 21-tosylate and disodium sulfidoacetate in a methanol solution. The reaction mixture is acidified by 10% HCl, and partially evaporated, and the compound is precipitated by the addition of water. The precipitate is filtered, washed and dried. PMR spectrum, δ, ppm: 0.64 s (3H, 18-H_3), 1.39 s (3H, 19-H_3), 3.32 q (J 15.6 Hz, 2H, -S-CH_2-) 3.42 d (J 16.8 Hz, 1H, 21-H_B), 3.86 d (J 16.8 Hz, 1H, 21-H_A) 4.72 br. s (1H, 4-H).

Sodium (11β,17α-Dihydroxy-4-pregnene-3,20-dion-21-yl-21-thiol)acetic Acid (IIIa). In a similar way, from cortisol 21-toslyate, in an acetone solution and disodium sulfidoacete in methanol, (11β, 17α-dihydroxy-4-pregnene-3,20-dion-21-yl-21-thio)acetic acid (III) is obtained. This compound is dissolved in acetone, and sodium methylate in methanol is added. The salt is filtered, washed with acetone and dried. PMR spectrum of III, δ, ppm: 0.91 s (3H, 18-H_3), 1.48 s (3H, 19-H_3), 3.32 q (J 15.2 Hz, 2H, -S-CH_2-) 3.60 d (J 17.1 Hz, 1H, 21-H_B), 3.97 d (J 17.1 Hz, 1H, 21-H_A), 4.63 s (3H, OH), 5.68 s (1H, 4H).

α-(17α-Hydroxy-4-pregnene-3,20-dion-21-yl-21-thio)propionic Acid (IV) is obtained from cortexolone 21-tosylate and the disodium salt of α-mercaptopropionic acid. PMR spectrum, δ, ppm: 0.69 s (3H, 18H_3), 1.18 s (3H, 19-H_3), 1.46 d (J 7.2 Hz, 3H, CH_3-CH-), 3.52 q (J 7.2 Hz, 1H, CH_3-CH-), 3.52 d (J 16 Hz, 1H, 21-H_B), 4.02 d (J 16 Hz, 1H, 21-H_A), 5.77 br. s (1H, 4-H).

β-(17α-Hydroxy-4-pregnene-3,20-dion- 21-yl-21-thio)propionic Acid (V)is obtained from cortexolone 21-tosylate and the disodium salt of α-mercaptopropionic acid. PMR spectrum, δ, ppm 0.72 s (3H, 18-H_3), 1.18 s (3H, 19-H_3), 2.67 t (J 6.4 Hz, 2H, -S-CH_2), 2.83 t (J 6.4 Hz, 2H , -CH_2-COO-), 3.36 d (J 15 Hz, 1H, 21-H_B), 3.69 d (J, 15 Hz, 1H, 21-H_A), 5.76 br. s (1H, 4-H).

β-(11β,17α-Dihydroxy-4-pregnene-3,20-dion-21-yl-thio)propionic Acid (VI). A solution of 1.55 g (0.003 mole) of cortisol 21-tosylate, 0.37 g (0.0035 mole) of β-mercaptopropionic acid and 1.5 ml of triethylamine in 50 ml of acetone is stirred for 30 min. The reaction mixture is evaporated to 1/4 of the initial volume, diluted with water to 100 ml and acidified with 2% HCl to pH 5.0. The crystals are filtered, washed with water and are reprecipitated twice from ethanol. PMR spectrum δ, ppm: 0.91 s 3H, 18-H_3), 1.45 s (3H, 19-H_3), 2.60 t (J 6.1 Hz, 2H, -S-CH_2-), 2.83 t (J 6.1 Hz, 2H, -CH_2-COO-), 3.48 d (J 15.5 Hz, 1H, 21-H_B), 3.81 d (J 15.5 Hz, 1H, 21-H_A), 5.65 br, s (1H, 4-H).

TABLE 1. 21-Thio-Derivatives of Corticosteroids I-X

Compound	Yield, %	mp, °C	Found, %			Empirical formula	Calculated, %			$[\alpha]_D^{20}$ (c, acetone)	UV spectrum λ_{max}, nm (log ε)
			C	H	S (N)		C	H	S (N)		
I	91,3	179—182,5	65,65	7,71	7,53	$C_{28}H_{32}O_5S$	65,69	7,67	7,62	+108 (1)	242 (4,24)
II	77,6	181—2	63,61	7,25	7,35	$C_{28}H_{30}O_6S$	63,57	7,12	7,38	+144 (1)*	239 (4,19)
III	...	184,5—185,5	63,27	7,38	7,33	$C_{28}H_{32}O_6S$	63,27	7,39	7,34	+142 (1)*	243 (4,21)
IIIa	98,2	235—7	...	...	...	—	...	...	...	...	...
IV	80	183—5	66,31	8,06	7,17	$C_{24}H_{34}O_5S$	66,33	7,89	7,38	+84 (0,5)	243 (4,22)
V	78,2	144—7	66,40	7,91	7,28	$C_{24}H_{34}O_5S$	66,33	7,89	7,38	+126 (1)	243 (4,23)
VI	79,9	181—183,5	64,01	7,63	6,98	$C_{24}H_{34}O_6S$	63,97	7,61	7,12	+133 (0,33)	243 (4,20)
VII	94,6	159—61	63,98	7,60	7,09	$C_{24}H_{34}O_6S$	63,97	7,61	7,12	+132 (1)	243 (4,23)
VIII	71,4	154—8	61,69	7,41	6,77	$C_{24}H_{34}O_7S$	61,78	7,34	6,87	+132 (1)	242 (4,23)
IX	93,5	195—8	66,00	8,52	6,68	$C_{27}H_{41}NO_5S$	65,96	8,40	6,52 (2,85)	+129,5 (0,17)	243 (4,24)
X	96,6	137,5—140	68,01	8,37	7,70	$C_{28}H_{34}O_4S$	67,95	8,43	7,89	+116 (1)	242 (4,23)

*Determined in ethanol.

TABLE 2. Anti-inflammatory Activity and Toxicity of 21-Thio Derivatives of Corticosteroids

Compound	Anti-edematic action ED_{50}, mg/kg intraperineally	Relative activity	Toxicity LD_{50}, mg/kg	Latitude of therapeutic action LD_{50}/ED_{50}
I	23,3 (7,5—39,0)	0,81	447 (390—670)	19,6
II	51,5 (22,2—80,8)	0,37	815 (710—940)	16,0
IIIa	30,5 (6,7—27,3)	0,62	1030 (880—1180)	33,7
IV	23,0 (10,3—35,7)	0,82	410 (350—470)	18,0
V	21,0 (9,5—32,5)	0,9	420 (350—470)	20,0
VI	36,0 (19,0—53,0)	0,52	1030 (880—1180)	28,6
VII	84,6 (37,7—135,5)	0,22	2000	22,3
VIII	55,3 (23,3—87,0)	0,34	1400 (1200—1700)	25,1
IX	76,6 (38,6—114,6)	0,25	2000	26,1
X	40,0 (20,9—59,1)	0,47	708 (620—800)	17,7
Prednisolone	18,9 (13,4—24,4)	1	220	12,1

Methyl Ester of (11β,17α-Dihydroxy-4-pregnene-3,20-dion-21-yl-21-thio) (acetic Acid (VII) A 0.0027 mole portion of the sodium salt of a methyl ester of mercaptoacetic acid is added to a solution of 1.27 g (0.0025 mole) of cortisol 21-tosylate in 30 ml of acetone. The reaction mixture is treated in the usual way. PMR spectrum, δ, ppm: 0.95 s (3H, 18-H_3), 1.45 s (3H, 19-H_3), 3.27 s (2H, -S-CH_2-), 3.43 d (J 16 Hz, 1H, 21-H_B), 3.70 s (3H, OCH_3), 3.88 d (J 16 Hz, 1H, 21-H_A), 5.68 s (1H, 4H).

Methyl Ester of (11β,17α-Dihydroxy-4-pregnene-3,20-dion-21-yl-21-sulfenyl) acetic Acid (VIII). An aqueous-alcoholic soltuion of 0.077 g (0.0004 mole) of HIO_4 is added at 0-5°C to a solution of 0.15 g (0.00033 mole) of VII in 10 ml of ethanol, and the mixture is left to stand in the cold overnight. The reaction mixture is diluted with water and extracted by chloroform (3 × 25 ml). The extract is dried over $MgSO_4$, and evaporated, and the residue is crystallized from ethyl acetate. PMR spectrum, δ, ppm: 0.95 s (3H, 18-H_3), 1.45 s (3H, 19-H_3), 3.58 d (J 13.7 Hz, 1H, 21-H_B), 3.83 s (3H, OCH_3), 3.85 d (J 14.2 Hz, 1H, -SO- CH_B-), 4.09 d (J 14.2 Hz, 1H, -SO-CH_A-), 4.83 d (J 13.7 Hz, 1H, 21-H_A), 5.71 br s (1H, 4-H).

Diethylamide of (11β,17α-Dihydroxy-4-pregnene-3,20-dion-21-yl-21-thio)acetic Acid (IX). A 0.12 g portion (0.00058 mole) of dicyclohexylcarbodiimide is added to a solution of 0.22 g (0.0005 mole) of III in 10 ml of dioxane in the presence of 0.2 ml of diethylamine, and the mixture is left to stand overnight. The precipitate is filtered, and the filtrate is evaporated to dryness. The residue is dissolved in acetone and the solution is filtered. The compound is crystallized from methanol, from a mixture of ethyl acetate and methanol, and again from methanol. PMR spectrum, δ, ppm: 0.89 s (3H, 18-H_3), 1.13 t (J 7.2 Hz, 3H, =N-CH_2-CH_3), 1.25 t (J 7.2 Hz, 3H, =N-CH_2-CH_3), 1.46 s (3H, 19-H_3), 3.27-3.47 (7H, 21-H_B, -S-CH_2-, -N(CH_2-CH_3)$_2$), 4.08 d (J 18 Hz, 1H, 21-H_A), 4.38 br. s (2H, OH), 5.68 br. s (1H, 4-H).

2-(17α-Hydroxy-4-pregnene-3,20-dion-21-yl-21-thio)ethanol (X). A sodium salt of mercaptoethanol, obtained from 0.47 g (0.006 mole) of mercaptoethanol and 0.281 g (0.0052 mole) of sodium methylate in 2.85 ml of methanol is added to a solution of 2.503 g (0.005 mole) of cortexolone 21-tosylate in 40 ml of acetone. The reaction mixture is evaporated to 2/3 of initial volume, water is added, and the crystals are filtered. PMR spectrum δ, ppm: 0.72 s (3H, 18-H_3), 1.20 s (3H, 19-H_3), 2.78 t (2H, J 6.2 Hz, -S-CH_2-), 3.36 d (J 15.4 Hz, 1H, 21-H_B), 3.76 m (3H, 21-H_A and -CH_2-O-), 5.74 br. s (1H, 4H).

EXPERIMENTAL PHARMACOLOGICAL

The anti-exudative action of the compounds was evaluated on a model of a strong inflammation edema of a paw induced by carraghenin (0.1 ml of 1% solution) in nonpedigree rats weighing 200-220 g each [3]. The volume of the paw was measured oncometrically before and after the introduction of the phlogogenic agents and the compound studied. The anti-edematic activity of the compounds was evaluated from the decrease in the degree of limb edema, and was expressed in percent. The compounds were introduced with variation of the dose, as a single dose, intraperitoneally, 1 h before the introduction of carraghenin. An ED_{50}, which is dose causing a 50% decrease in the edema of the limb 1 h after the introduction of carraghenin [4] was determined. The activity of the compounds studied was compared with the activity of prednisolone, which was taken as unit value.

The toxicity of the compounds was studied in experiments on nonpedigree mice of both sexes, weighing 22-24 g each. The compounds studied were introduced with variation of the dose, as a single dose, intraperitoneally. The insoluble compounds were administered in the form of a suspension in 2% starch. The volume of the compounds introduced did not exceed 0.5 ml per 10 g of the body weight of the animal. The acute toxicity index (LD_{50}) was determined by a rapid method [2]. The latitude of the pharmacological action indexes (the LD_{50}/ED_{50} ratio) of the compounds studied and the pharmacological standard were calculated.

RESULTS AND DISCUSSION

The data obtained showed that like prednisolone, the 21-thio derivatives of corticosteroids inhibit the development of limb edema in rats, induced by the introduction of carraghenin. In their ED_{50} value, not one of the compounds tested has any advantages in comparison over the pharmacological standard (Table 2).

A comparison of the activity of the 21-thio-derivatives of corticosteroids with the activity of prednisolone taken as unit value showed that only the activities of compounds I, IV and V approach those of the standard preparation. Compounds IIIa, VI, and X have a moderate anti-inflammatory activity, reaching approximately 50% of the activity of prednisolone.

The anti-exudative activity of compounds II, VII, VIII and IX is 20-35% of the activity of prednisolone.

A comparison of the indexes characterizing the latitude of the pharmacological action of the compounds studied shows that in the most active compounds I, IV and V, this index does not appreciably differ from the latitude action index of prednisolone. In contrast, in compounds with a low anti-inflammatory activity, the latitude of the pharmacological action index is twice as high as that of prednisolone.

According to the value of mean lethal doses during a single intraperitoneal administration, the compounds studied can be classified as moderately toxic. Their LD_{50} for nonpedigree mice is 410-815 or 1030-2000 mg/kg. In the clinical pattern of the toxic action of the compounds, the depressing symptoms predominate in the animals. It was found that the compounds with a low anti-inflammatory activity are also slightly toxic.

Thus, our study showed that in their intensity of action, the 21-thio derivatives of corticosteroids are not inferior to prednisolone. Their activity is largely determined by the character of the initial structures used for the synthesis of the 21-thio derivatives. The overall toxicity, and probably the degree of expression of the undesirable effects goes in parallel with the decrease in the anti-edematous activity.

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