PATENT SPECIFICATION

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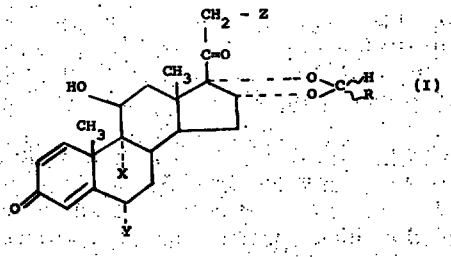
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(19)

(54) NEW STEROID ACETALS, PROCESSES FOR THEIR MANUFACTURE AND PREPARATIONS CONTAINING THEM

(71) We, AKTIEBOLAGET BOFORS, a Swedish Joint Stock Company of S-690 20 Bofors, Sweden, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

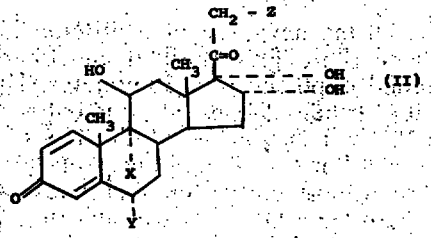
The present invention refers to new physiologically active steriods, to processes for their manufacture and to preparations containing such steroids. The new physiologically active steroids according to the invention have the general formula:



(I)

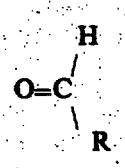
wherein Y is hydrogen fluorine, X is hydrogen or fluorine when Y is hydrogen and X is fluorine when Y is fluorine, Z is hydroxyl or esterified hydroxyl, R is a straight or branched alkyl radical having 2—10 and preferably 2—6 carbon atoms.

The above-mentioned steroids may be prepared by reacting a steroid having the general formula:



(II)

wherein X, Y and Z are as defined above with an aldehyde having the general formula



wherein R is as defined above, to form the desired steroid having formula (I) and when Z is hydroxyl, said hydroxyl is then esterified if desired. The esterification may be carried out with a fatty acid with a straight or branched hydrocarbon chain

having 1—12 carbon atoms, such fatty acid being for instance: acetic acid, propionic acid, butyric acid, valeric acid, isovaleric acid trimethylacetic acid, hexanoic acid, tert.-butylacetic acid or octanoic acid, or with a heterocyclic carboxylic acid, such as pyridine-3-carboxylic acid, pyridine-4-carboxylic acid, benzofuran-2-carboxylic acid or a menthoxy acetic acid. To prepare water-soluble steroid derivatives, the esterification is suitably carried out with a dicarboxylic acid, preferably having 2—12 carbon atoms, or with phosphoric or sulphuric acid.

The reaction between the starting compound II and the aldehyde is suitably carried out by adding the steroid II to a solution of the aldehyde together with an acid catalyst, for instance perchloric acid, p-toluene-sulphonic acid or hydrochloric acid, in dioxane or an equivalent solvent, the reaction mixture being then taken up in methylene chloride and neutralized and the acetal derivative formed being isolated and purified by gel filtration on a suitable material, for instance cross-linked dextran gels of the Sephadex LH-type or copolymers of vinyl acetate, such as Merckogel OR-PVA, in equilibrium with suitable solvents, for instance halogen hydrocarbons, ethers or esters, such as ethyl acetate, chloroform, methylene chloride, ethylene dichloride, tetrahydrofuran and dioxane.

To prepare the particularly useful 21-acyloxy derivatives the free acid, its halogenide or anhydride may be used in the esterification.

For the preparation of the 21-phosphate derivatives, phosphorus oxychloride in the presence of a tertiary base, for instance pyridine-3-ethylamine is used. The intermediate chloride formed is hydrolyzed with water in the presence of the same tertiary base. If it is desired, the 21-phosphate may be converted to an alkali metal salt thereof by treatment with an alkali metal hydroxide, such as sodium or potassium hydroxide, or an alkali metal carbonate, such as sodium or potassium carbonate.

The steroids of this invention may, if desired, be separated into their stereoisomers by gel filtration on a suitable material in conformity with patent Application No. 22195/73 (Serial No. 1,428,416) filed simultaneously herewith. The stereoisomerism is due to the space orientation about the 2-carbon atom of the dioxolane ring.

The invention will now be further illustrated by the following Examples. In these Examples there is used for the chromatography a column having a length of 85 cm, an inner diameter of 2.5 cm, the flow rate being 1 ml/min. The molecular weights are determined by mass spectroscopy. The retention volumes given in the Examples refer to the use of chloroform as an eluant. All the melting points are determined by means of a Reichert melting point microscope, and all derivatives melt under decomposition.

Example 1.

16α, 17α - propylidenedioxy - 9 - fluoropregna - 1,4 - diene - 11β, 21 - diol-3,20 - dione.

To a solution of 59.0 mg newly distilled propionaldehyde and 0.1 ml 72% perchloric acid in 30 ml thoroughly purified and dried dioxane 200.0 mg of triamcinolone were added in portions for 30 minutes. The reaction mixture was then allowed to stand for further 5.5 hours at room temperature under stirring and was then diluted with 200 ml methylene chloride. The solution was washed twice with a 15% potassium bicarbonate solution and then three times with water and dried. The solvents were evaporated in vacuum, the residue was taken up in ether and precipitated with petroleum ether. The dried crude product (220.0 mg) was chromatographed on a column packed with hydroxypropylated, cross-linked dextrane gel (Sephadex LH 20, molecular weight range 100—4000; Pharmacia Fine Chemicals, Uppsala, Sweden) using chloroform as an eluant, the retention volume being 840—990 ml. This resulted in 174.6 mg (79%) pure isomer mixture having the following characteristics: Melting point about 155—97°C; $[\alpha]_D^{25}$ = +97.5° (c = 0.2 in CH_2Cl_2); molecular weight = 434 (theor. 434.5).

Similar separation results were obtained by using a gel of copolymers of vinyl acetate (Merckogel OR—PVA 2000, molecular weight range up to 1000), as well as using, in addition to chloroform, methylene chloride, ethylene chloride, ethyl acetate, tetrahydrofuran and dioxane as eluants for both types of gel materials.

Examples 2—12.

In a manner analogous to that described in Example 1 the substances given in Tables 1—3 below were prepared, purified and chromatographed.

TABLE 1

Example No.	16α, 17α-derivative of triamcinolone with:	$[\alpha]_D^{25}$ (c=0.2 in CH_2Cl_2)°	Mp. °C	Molecular weight: found:	Molecular weight: calculated:	Retention volume mls
2	n-butyraldehyde	+ 94.0	130–45	448	448.5	822–984
3	n-valeric aldehyde	+ 86.4	96–108	462	462.6	780–924
4	n-caproic aldehyde	+ 82.3	94–100	476	476.6	702–828
5	n-decylaldehyde	+ 76.4	70–80	532	532.7	540–630

TABLE 2

Example No.	16α, 17α-derivative of fluocinolone with:	$[\alpha]_D^{25}$ (c=0.2 in CH_2Cl_2)°	Mp. °C	Molecular weight: found:	Molecular weight: calculated:	Retention volume mls
6	n-butyraldehyde	+ 88.3	155–65	476	476.5	1130–1320
7	n-caproic aldehyde	+ 83.6	150–7	494	494.6	870–1000
8	n-caprylic aldehyde	+ 73.1 [1)]	117–30	522	522.6	750–850

1) c = 0.1 in CH_2Cl_2

TABLE 3

Example No.	16α, 17α-derivative of 11β,16α,17α,21-tetrahydroxy-pregna-1,4-diene-3,20-dione with:	$[\alpha]_D^{25}$ (c=0.2 in CH_2Cl_2)°	Mp. °C	Molecular weight found:	Molecular weight calculated:	Retention volume mls
9	n-butyraldehyde	+ 96.9	215–22	430	430.5	456–540
10	n-caproic aldehyde	+ 87.3	190–4	458	458.6	414–498
11	n-caprylic aldehyde	+ 82.0	75–90	486	486.7	372–420
12	n-decylaldehyde	+ 79.1	65–80	514	514.7	336–390

Example 13.

16α, 17α - Butylidenedioxy - 11β - hydroxy - 21 - (benzofuran - 2 - carbonyloxy) - 9 - fluoropregna - 1,4 - diene - 3,20 - dione.

A solution of 50.0 mg of 16α, 17α-butylidenedioxy-9-fluoropregna-1,4-diene-11β,21-diol-3,20-dione in 2 ml dry pyridine was added to 56.4 mg benzofuran-2-carboxylic acid chloride dissolved in 1 ml of dry dioxane. The reaction mixture was allowed to stand under stirring at room temperature overnight, the main part of the solvents were evaporated in vacuum and the residue was poured into 20 ml of 3% ammonium chloride solution. The precipitate obtained was separated by centrifugation and dissolved in 75 ml of chloroform. The chloroform solution was washed once with 15% sodium bicarbonate solution, three times with water, dried over magnesium sulphate and evaporated in vacuum. The residue was dissolved in ether and precipitated with petroleum ether. The dried, solid crude product (54.5 mg) was chromatographed on Sephadex LH—20 using chloroform as eluant. The retention volume was 372—420 ml. This resulted in 48.5 mg (73%) 21-benzofuran-2-carboxylic ester of a pure isomeric mixture of the desired compound having the following characteristics: Melting point about 175—90°C; $[\alpha]_D^{25}$ = +150.2° (c = 0.2 in CH_2Cl_2); molecular weight = 592 (theor. 592.7).

Examples 14—36.

In a manner analogous to that described in Example 13 the 21-esters of the compounds prepared in Examples 1—12 and shown in Table 4 below were prepared, purified and chromatographed.

The acid chlorides used for the esterification are abbreviated in Table 4 in the following manner:

NAC = nicotinic acid chloride
IAC = isonicotinic acid chloride
AAC = acetyl chloride
VAC = valeric acid chloride
BAC = benzofuran-2-carboxylic acid chloride.

TABLE 4

Example No.	the 21-ester of the compound of Ex. No.	with	$[\alpha]_D^{25}$ (c=0.2 in CH_2Cl_2)°	Mp. °C	Molecular weight found:	Molecular weight calculated	Retention volume mls
14	2	NAC	+ 119.4	212–35	553	553.6	342–420
15	2	IAC	+ 115.5	198–201	553	553.6	320–380
16	2	AAC	+ 83.3	216–34	490	490.6	310–360
17	2	VAC	+ 88.2	224–43	532	532.7	270–315
18	4	VAC	+ 79.3	189–97	560	560.7	235–80
19	5	VAC	+ 71.8	162–63	616	616.8	234–58
20	6	BAC	+ 138.9	197–200	610	610.7	282–348
21	6	NAC	+ 108.3	187–99	571	571.6	282–348
22	6	AAC	+ 78.3	260–78	508	508.6	335–395
23	6	VAC	+ 81.2	238–45	550	550.7	270–306
24	7	AAC	+ 73.0	250–55	536	536.6	336–84
25	8	VAC	+ 69.2	192–98	606	606.8	245–75
26	9	BAC	+ 142.9	120–30	574	574.7	230–270
27	9	NAC	+ 120.6	110–25	535	535.6	275–340
28	9	AAC	+ 86.2	157–63	472	472.6	240–65
29	9	VAC	+ 93.9	166–78	514	514.7	220–50
30	10	VAC	+ 83.3	177–87	542	542.7	222–46
31	11	BAC	+ 112.2	103–12	658	658.8	204–28
32	11	AAC	+ 78.4	185–7	528	528.7	225–50
33	11	VAC	+ 77.4	153–4	570	570.8	216–46
34	12	BAC	+ 123.5	79–90	658	658.8	198–222
35	12	AAC	+ 75.2	142–5	556	556.7	215–35
36	12	VAC	+ 96.7	108–10	598	598.8	195–225

Example 37.

The 21 - (disodium phosphate) of 16α, 17α - butylidenedioxy - 9 - fluoropregna-1,4 - diene - 11β,21 - diol - 3,20 - dione.

To a solution of 0.016 ml newly distilled phosphorus oxychloride in 2.0 ml of dry pyridine a solution of 40.0 mg of 16α, 17α-butylidenedioxy-9-fluoropregna-1,4-diene-11β,21-diol-3,20-dione (II) in 2.0 ml of dry pyridine was added under stirring at –26°C. The temperature was allowed to rise

to −10°C, whereafter the reaction mixture was allowed to stand a few minutes in the cold. Water (0.16 ml) was added dropwise, the temperature not being allowed to exceed −10°C. After 13 minutes the pyridine was evaporated in vacuum; the residue was taken up in chloroform and the chloroform solution was extracted three times with water. The water phase was neutralized with 1.4 ml of 5% sodium carbonate solution to pH 7.0 and freeze-dried. The solid substance (95.3 mg) was taken up in dry methanol and the insoluble part was separated by centrifugation. The methanol solution was treated with excess of Dowex 50W—X8 (H-form), (this material is a cation exchange resin consisting of a copolymer of styrene and divinyl benzene (8%) and sold by Dow Chemical Company, USA), filtrated and transferred to a column packed with Sephadex LH—20 (length 80 cm, inner diameter 25 mm) and eluted (flow rate 1 ml/min.) with methanol of weak sulphuric acid acidity (Retention volume 255—330 ml). The methanol was evaporated in vacuum, the residue was taken up in water, neutralized to pH 7.0 with 5% sodium carbonate solution and freeze-dried.

The resulting solid substance was slurried in dry methanol. The methanol solution was centrifuged and evaporated to 1 ml and petroleum ether was then added. This resulted in 22.7 mg (45%) of 21-(disodium phosphate) of 16α,17α-butylidenedioxy-9-fluoropregna-1,4-diene-11β,21-diol-3,20-dione with R_f = 0.78 (thin layer chromatography on cellulose with isopropanol:ammonia:water 7:1:2).

Elementary analysis: Calculated for $C_{25}H_{32}O_9FPNa_2$ (572.488) C: 52.45; H: 5.63; P: 5.41. Obtained: C: 51.87; H: 5.41; P: 5.59.

Example 38.

The 21-(disodium phosphate) of 16α,17α-butylidenedioxy-6α,9-difluoropregna-1,4-diene-11β,21-diol-3,20-dione was obtained in a manner analogous to that described in Example 37 and showed the following data:

Retention volume: 250—305 ml

Rf = 0.69 (thin layer chromatography on cellulose with isopropanol:ammonia:water 7:1:2).

Elementary analysis: Calculated for $C_{25}H_{31}O_9F_2PNa_2$ (590,473). C: 50.85; H: 5.29; P: 5.25. Obtained C: 50.53; H: 5.12; P: 5.38.

The following Examples of pharmaceutical preparations were prepared in a conventional manner:

Example 39.

Ointment, fat, anhydrous,

Steroid	0.001—0.2	g
Cetanol	5	g
Liquid paraffin	20	g
Vaseline	*to* 100	g

Example 40.

Cream

Steroid	0.001—0.2	g
Monolein	2.5	g
Wool fat	5	g
Vaseline	42	g
Citric acid	0.3	g
Sodium citrate	0.9	g
Water	*to* 100	g

Example 41.

Liniment.

Steroid	0.001—0.2 g
Cetanol	3.2 g
Stearol	0.2 g
Polyoxyethylene sorbitan monolaurate	2 g
Sorbitan monopalmitate	0.5 g
Propylene glycol	4.8 g
Metagin	0.08 g
Propagin	0.02 g
Water	*to* 100 g

Example 42.

Tincture.

Steroid	3—500 mg
Ethanol 60%	*to* 100 ml

Example 43.

Suspension for injection.

Steroid	0.05—10 mg
Sodium carboxymethyl cellulose	7 mg
Sodium chloride	7 mg
Tween 80[a]	0.5 mg
Phenyl carbinol	8 mg
Water, sterile	*to* 1 ml

a) Polyoxyethylene(20)sorbitane monoleate.

Example 44.

Foam aerosol.

Steroid	0.001—0.2 g
Glycerol	4 g
N-cetylstearyl alcohol	0.2 g
Cetylstearyl alcohol	3 g
Isopropylmyristate	2 g
Metagin	0.1 g
Water	80 g
Tetrafluordichloroethane/ difluordichloromethane 40:60	*to* 100 g

All the steroids of this invention are physiologically active compounds, possessing antiinflammatory activity. The antiinflammatory activity of the substances according to the invention was investigated in granuloma test on rats subjected to adrenalectomy. The experimental procedure used corresponds largely to that described by G. Engelhardt: Arzneimittel-Forschung, 13, p. 588, 1963. According to this procedure the test substances are applied topically in the implanted cotton wads. It is thereby possible to study the local antiinflammatory effect in granulomas and also systemic effects in the form of retrogression of thymus and inhibition of bodyweight growth.

Young male rats of the Sprague-Dawley strain weighing about 110—130 g were subjected to adrenalectomy under ether narcosis. Two sterilized cotton wads of each about 6 mg were implanted simultaneously subcutaneously on the lateral side of the spinal. After awakening the animals were stored 5 per cage and were supplied with normal food and 1% sodium chloride solution as drinking water. On the eighth test day the animals were sacrificed by ether narcosis. The granulomas formed around the cotton wads were carefully recovered and thymus and body weights were measured. The two granulomas from each animal were dried over night at 80°C and weighed. After subtraction of the initial weight of the cotton wads the weight increase was used as a measure of the granuloma growth.

The test substances were used dissolved in ethyl acetate. Under aseptic conditions 0.05 ml of said solutions were injected into each of the cotton wads, whereafter the solvent was allowed to evaporate in a desiccator. Normally 3 concentrations of each test substance with the standard doses 3.3, 30 and 270 γ/animal were investigated. Each test group normally comprised 10 rats. The cotton wads of the control group were injected with only ethyl acetate, but were in other respects treated in the same way. When considering the effects of the test substances the average values of granuloma growth, thymus weight and body weight increase day 0—8 were measured in each group in absolute figures and in % of the corresponding figure of the control group. Dose-response curves were drafted and used for estimating the doses giving 50% reduction of granuloma growth and of thymus weight and 25% decrease of body weight increase.

The results of testing the steroids of this invention by the procedure described above are summarized in Table 5 below. From this Table it is clear that the investigated compounds of the invention are physiologically active substances showing significantly stronger antiinflammatory effect than steroids of the prior art. The dose required for obtaining 50% reduction of the granuloma growth is lower for the compounds of the present invention than the corresponding dose of the reference substances triamcinolone acetonide, fluocinolone acetonide and prednacinolone, which substances have wide-spread use.

Moreover it is clear from the experimental data presented that the new compounds possess a better relation between the local antiinflammatory activity (inhibition of granuloma growth) and systemic effects in the form of weight decrease of thymus and in the form of decreased body growth. When using the previously known substances, higher doses are required for obtaining 50% reduction of granuloma growth than for negatively effecting thymus and body weights. Contrary to this, for the new compounds of this invention a 50% antiinflammatoric activity was obtained at lower doses than the doses resulting in the systemic effects in question. Therefore, the new compounds of the invention show surprisingly a better relation between desirable main effect and non-desirable side effects.

The fact that the steroids of this invention possess this high level of anti-inflammatory activity and other desirable properties is most surprising having regard to the lack of such activities and properties in the immediately lower homologues, e.g. the triamcinolone derivative where R is methyl, and in close analogues e.g. those 16α,17α-dialkylmethylenedioxy compounds in Table 5 below.

TABLE 5

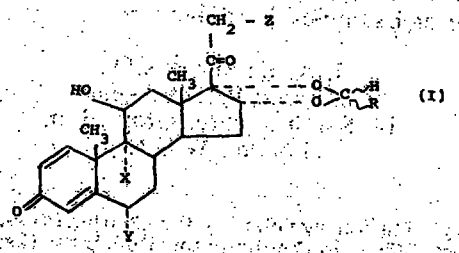
Table summarizing biological effects of investigated compounds

Compound according to Ex. No.	Required dose γ/animal to obtain: 50 % inhibition of		25 % inhibition of
	Granuloma growth	Thymus weight	Body weight increase
Triamcinolone acetonide	125	70	100
1	35	100	140
2	10	>30	>30
3	<3	70	170
4	17	130	>270
5	<30	>30	>30
Fluorocinolone acetonide	50	14	20
6	5	10	50
7	<3	25	30
8	<30	>30	>30
Prednacinolone	270	105	>270
9	7	200	60
10	10	175	90
11	<30	>270	30
12	<30	>270	270
13	<3	25	20
14	7	35	10
15	15	17	25
16	<3	30	20
17	<7	50	30
20	<3	10	10
21	10	30	20
22	<3	10	5
26	<3	60	40
27	3	90	70
28	<3	150	>800
29	<3	60	20

The words "Sephadex", "Dowex", "Vaseline" and "Tween" are Registered Trade Marks.

WHAT WE CLAIM IS:—

1. A steroid having the general formula:



(I)

wherein Y is hydrogen or fluorine, X is hydrogen or fluorine when Y is hydrogen, and X is fluorine when Y is fluorine, Z is hydroxyl or esterified hydroxyl, and R is a straight or branched alkyl radical having 2—10 carbon atoms.

2. A steroid according to claim 1, wherein R has 2—6 carbon atoms.

3. A steroid according to claim 1 or 2, wherein X is fluorine and Z is hydroxyl.

4. A steroid according to claim 3, wherein Y is fluorine.

5. A steroid according to any one of the preceding claims, wherein Z is hydroxyl esterified with acetic acid, propionic acid, butyric acid, valeric acid, isovaleric acid, trimethylacetic acid, hexanoic acid, tert.butylacetic acid or octanoic acid.

6. A steroid according to any one of claims 1—4, wherein Z is hydroxyl esterified with pyridine-4-carboxylic acid, benzofuran-2-carboxylic acid or menthoxyacetic acid.

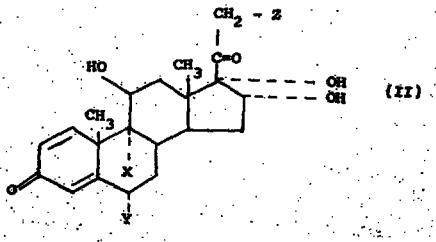
7. A steroid according to any one of claims 1—4, wherein Z is hydroxyl esterified with a dicarboxylic acid.

8. A steroid according to claim 7, wherein the dicarboxylic acid has 2—12 carbon atoms.

9. A steriod according to any one of claims 1—4, wherein Z is hydroxyl esterified with phosphoric or sulphuric acid.

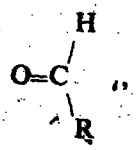
10. Each and every steroid according to claim 1 hereinbefore specifically mentioned.

11. A process for the preparation of a steroid as defined in claim 1, comprising reacting a steroid having the general formula



(II)

wherein symbols X, Y and Z are as defined in claim 1, with an aldehyde having the general formula



wherein R is as defined in claim 1.

12. A process according to claim 11, wherein a steriod is obtained in which Z is hydroxyl and the hydroxyl group is then esterified.

13. A process according to claim 11 or 12, wherein R has 2—6 carbon atoms.

14. A process according to any one of claims 11—13, wherein X is fluorine and Z is hydroxyl.

15. A process according to any one of claims 11—14, wherein Y is fluorine.

16. A process according to claim 11 or 12, substantially as hereinbefore described in any one of Examples 1 to 38.

17. A steriod obtained by a process according to any one of claims 11—16.

* C L M S *

18. A pharmaceutical preparation containing at least one steroid according to any one of claims 1—10 or 17 together with a pharmaceutically acceptable carrier.

19. A preparation according to claim 18, substantially as hereinbefore described in any one of Examples 39 to 44.

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* C L M S *