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(54) A PROCESS FOR THE SEPARATION OF STEREOISOMERIC MIXTURES OF STEROID ACETALS INTO THEIR COMPONENTS AND COMPONENTS OBTAINED THEREBY

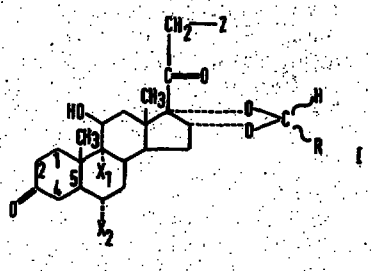
(71) We, AKTIEBOLAGET BOFORS, a Swedish Joint Stock Company of S—690 20 Bofors, Sweden, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to stereoisomeric components referred to herein as component A and component B of stereoisomeric mixtures of steroids, to the resolution of said stereoisomeric mixtures obtained by synthesis, to stereoisomeric components resolved thereby, and to pharmaceutical compositions comprising said stereoisomeric components.

It is well-known that mixtures of stereoisomers having the same molecular weight and possessing practically identical solubility characteristics may be extremely difficult to separate.

Gel filtration is normally used to separate lower molecular weight molecules from higher molecular weight molecules. It would therefore, be expected that stereoisomers, the molecules of which have the same molecular weight, could not be separated by this procedure since they would be expected to have the same retention volume in gel filtration. We have found, surprisingly that the stereoisomeric components A and B having the same molecular weight may be separated by gel filtration.

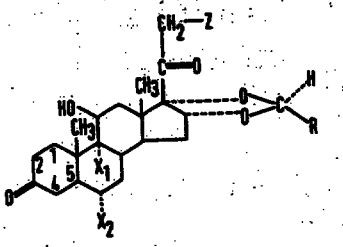
Accordingly, the present invention provides stereoisomeric components A and B of a stereoisomeric mixture (isomer pair) of a steroid of formula:—



I

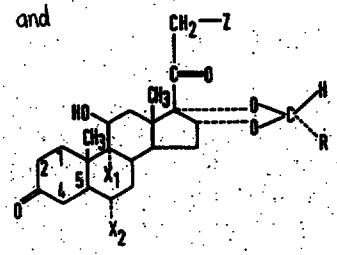
wherein the 1,2 and 4,5-positions are saturated or a double bond is present in at least one of said two positions, X_1 and X_2 each represent hydrogen or fluorine with the proviso that when X_2 is fluorine X_1 is fluorine, Z is hydroxyl or esterified hydroxyl, and R is a straight or branched alkyl group having 1—10 carbon atoms, preferably 1—6 carbon atoms, said component A being the isomer of the isomer pair showing the lower relative rotary power or having the smaller retention volume in gel filtration, said component B being the isomer of the isomer pair showing the greater relative rotary power or having the greater retention volume in gel filtration.

The individual stereoisomeric components A and B present in the mixture of a steroid of formula I may be represented in the following way:—



A

and



B

In the above formulae the stereoisomeric components differ from each other with regard to the space orientation about the 2'-carbon atom in the dioxolane ring.

The stereoisomeric mixture (isomer pair) of steroids of formula I may be resolved according to this invention into their stereoisomeric components A and B, which are distinguished with regard to the space orientation of the substituents on the 2'-carbon atom of the dioxolane ring, by subjecting the stereoisomeric mixture to gel filtration; the components being separated and being separately recoverable in view of their different retention volumes.

The separation process of this invention has made possible the investigation of the physiological characteristics of the separated stereoisomeric components A and B. It has been found surprisingly, that component B, has consistently better physiological characteristics than component A, and that it is better than the original mixture in this respect.

Thus it is possible by the new process to prepare, in pure form, new stereoisomeric components, which have certain advantages over the original synthetic stereoisomeric mixtures.

The gel filtration may be carried out on many different types of gel materials, for example cross-linked dextran gels, especially hydroxypropylated cross-linked dextran gels of the type Sephadex LH, for instance Sephadex LH 20 sold by Pharmacia Fine Chemicals, Uppsala, Sweden, resulting in a good fractionation within the molecular weight range 100—4000. Another useful gel type consists of copolymers of vinyl acetate having exclusion limits which render them useful in the molecular weight range up to about 1000. One such vinyl acetate gel is Merckogel, type OR PVA 2000 which is a copolymer of vinyl acetate swelling in organic solvents, sold by AG E. Merck, Darmstadt, Western Germany. The gel material is used in equilibrium with a suitable solvent. As an eluting agent acetonitrile or halogenated hydrocarbons, ethers, esters or mixtures thereof may be used, e.g. chloroform, methylene chloride, ethylene dichloride, tetrahydrofuran, dioxane and ethyl acetate. Thereby excellent separation of the stereoisomer A from the stereoisomer B results and in this way the stereoisomeric mixture A, B may be separated from by-products formed during the steroid synthesis.

In the steroids of formula I the hydroxyl group in the 21-position may be esterified with a fatty acid, which may have a straight or branched hydrocarbon chain preferably containing 1 to 12 carbon atoms. Examples of suitable acids are acetic, propionic, butyric, valeric, isovaleric, trimethylacetic, hexanoic, *tert*,-butylacetic and octanoic acid. The 21-hydroxyl group may also be esterified with a heterocyclic carboxylic acid, for instance pyridine-3-carboxylic acid, pyridine-4-carboxylic acid, or benzofuran-2-carboxylic acid Menthoxyacetic acid can also be used. For the preparation of water-soluble derivatives the esterification may be carried out with dicarboxylic acids preferably containing 2 to 12 carbon atoms, or with phosphoric or sulphuric acids.

The steroids of formula I in the form of cyclic acetals may be synthesized in a manner known *per se* from the 16α,17α-dihydroxy steroids and an aldehyde in the presence of an acid catalyst, for instance perchloric acid, *p*-toluene-sulphonic acid or hydrochloric acid, in dioxane or another suitable solvent. The reaction results in a mixture of stereoisomers, A and B, having the same molecular weight and

practically identical solubility characteristics, which makes the separation of the stereoisomers by conventional methods e.g. recrystallisation extremely difficult.

In the gel filtration on the column it has been found that it is easier to separate the by-products from the isomeric mixture in the crude product than to separate the isomers from each other, since the former show greater differences in retention volumes than the latter. It has also been found that the solubility of the crude product as well as the solubility of the purified isomeric mixture decreases, whereas the separation degree increases with decreasing polarity of the solvents used. This is particularly true for derivatives substituted with a shorter side chain on the 2'-carbon atom of the dioxolane ring. In the chromatographic separation described in the Examples it has been found that it is advantageous first to separate the isomeric mixture from the by-products of the crude product by a somewhat more polar solvent and then to separate the isomers from each other by a solvent of a lower polarity. This results is several advantages. First, the whole capacity of the column may be utilized and great amounts of crude product may be freed from by-products in each application. Second, a possible partial use of the column capacity resulting from limitations in the solubility of the isomeric mixture in the solvents showing a lower polarity may be compensated by making new test applications on the column at relatively short intervals day and night without awaiting the previous application to leave the column. The solvents which have been found to be highly active in the pre-separation, such as methylene chloride, ethylene dichloride, tetrahydrofuran and ethyl acetate, have also given a completely satisfactory isomeric separation, whereas chloroform and dioxane have given an even better result with regard to the isomers most difficult to separate.

The invention will now be illustrated by the following Examples in which a chromatography column was used having a length of 85 cm and an inner diameter of 2.5 cm; the flow rate was 1 ml/min. The retention volumes refer to chloroform as eluting agent.

The molecular weights were determined by mass spectroscopy, and in all NMR-investigations tetramethylsilane was used as an internal reference. All melting points were determined by means of a Reichert melting point microscope.

EXAMPLE 1.

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

To a solution of 112.0 mg newly distilled paraldehyde and 0.2 ml 72% perchloric acid in 75 ml well purified and dried dioxane, 500.0 mg of triamcinolone were added in portions for 40 minutes. The reaction mixture was stirred for a further 5.5 hours at room temperature, and then diluted with 200 ml methylene chloride. The solution was washed twice with a 15% sodium bicarbonate solution, three times with water and dried. The solvents were evaporated *in vacuo* and the residue was taken up in ether and precipitated with petroleum ether. The dried crude product (533.0 mg) was chromatographed on a column packed with hydroxypropylated, cross-linked dextran gel (Sephadex LH—20, molecular weight range 100—4000; Pharmacia Fine Chemicals, Uppsala, Sweden) using methylene chloride as eluting agent. This gave 431.7 mg (81%) pure isomeric mixture having the following characteristics: Melting point about 207—222°C; $[\alpha]_D^{25}$=+107.2° (c=0.3 in CH_2CL_2), molecular weight=420 (theor. 420.5).

The isomeric mixture (338.2 mg) was rechromatographed on a column packed with Sephadex LH 20 using chloroform as eluting agent. Two different isomers, A and B, of 16α,17α-ethylidenedioxy-9-fluoropregna-1,4-diene-11β,21-diol-3,20-dione were obtained in the following yields and having the following characteristics: A: 123.4 mg (37%); melting point 217—19°C: $[\alpha]_D^{25}$=+87.5° (c=0.3 in CH_2Cl_2); molecular weight=420 (theor. 420.5) (Retention volume 920—990 ml). B: 194.7 mg (58%); melting point 224—28°C; $[\alpha]_D^{25}$=+120.8° (c=0.3 in CH_2Cl_2); molecular weight=420 (theor. 420.5) (Retention volume 1020—1100 ml). The isomer purity (>98%) of A and B was determined by NMR spectroscopy by studying the signal for 18—CH_3 positioned at δ=1.00 ppm ($CDCl_3$) for A and at δ=0.92 ppm ($CDCl_3$) for B.

Similar separation results were obtained using a gel of copolymers of vinyl acetate (Merckogel OR—PVA 2000 molecular weight range up to 1000; AG E. Merck, Darmstadt, Western Germany), as well as by using, in addition to chloroform and methylene chloride, ethylene chloride, ethyl acetate, tetrahydrofuran and dioxane as eluting agents on both types of gel materials.

Examples 2—15.

Analogously to the process described in Example 1 different isomeric

mixtures were prepared, the isomers given in Table 1 below being obtained by means of separation according to Example 1. The NMR-investigations were carried out in $CDCl_3$ if not otherwise stated.

TABLE 1

Ex. No.	The 16α, 17α-derivative of	with	Isomer	$[\alpha]_D^{25}$ (c=0.2 in CH_2Cl_2)	Mp (°C)	Molecular weight found	Molecular weight calculated	18–CH_3 δ (ppm)	Retention volume (ml)
2	triamcinolone	propionaldehyde	A	+ 85.1°	204– 7	434	434.5	0.99	840–862
			B	+112.7°	189– 92	,,	,,	0.933	924–990
3	,,	n-butyraldehyde	A	+ 77.5°	150– 5	448	448.5	1.00	822–876
			B	+105.5°	147– 50	,,	,,	0.93	912–984
4	,,	n-valeric aldehyde	A	+ 73.7°	123– 7	462	462.6	0.99	780–801
			B	+ 93.6°	102– 6	,,	,,	0.93	864–924
5	,,	n-caproic aldehyde	A	+ 70.1°	172– 9	476	476.6	1.00	702–738
			B	+ 97.9°	180– 5	,,	,,	0.93	768–828
6	,,	n-decyl aldehyde	A	+ 63.6°	161– 4	532	532.7	0.99	540– 85
			B	+ 91.3°	147– 52	,,	,,	0.93	595–648
7	fluocinolone	acetaldehyde	A	+ 71.1°	232– 5	438	438.5	0.88(1)	1200–1250
			B	+110.8°	224– 7	,,	,,	0.83(1)	1260–1350
8	,,	n-butyraldehyde	A	+ 69.0°	196–200	466	466.5	0.98	1130–1190
			B	+ 94.5°	169– 72	,,	,,	0.93	1225–1320
9	,,	n-caproic aldehyde	A	+ 65.9°	143– 7	494	494.6	0.98	870–930
			B	+ 92.7°	167– 70	,,	,,	0.92	960–1015
10	,,	n-caprylic aldehyde	A	+ 61.0°	166– 9	522	522.6	0.98	735–765
			B	+ 88.2°	124– 7	,,	,,	0.93	790–850
11	11β, 16α, 17α, 21-tetra-hydroxy-4-pregnene-3,20-dione	acetaldehyde	A	+144.8°	177– 85	404	404.5	0.98	396–414
			B	+164.6°	202– 10	,,	,,	0.91	432–453
12	11β, 16α, 17α, 21-tetra-hydroxy pregna-1,4-diene-3,20-dione	n-butyraldehyde	A	+ 85.6°	225– 28	430	430.5	0.99	450– 68
			B	+105.3°	259– 60	,,	,,	0.93	510– 20

TABLE 1 (Continued)

Ex. No.	The 16α, 17α-derivative of	with	Isomer	$[\alpha]_D^{25}$ (c=0.2 in CH_2Cl_2)	Mp (°C)	Molecular weight found	Molecular weight calculated	18–CH_3 δ (ppm)	Retention volume (ml)
13	11β, 16α, 17α, 21-tetra-hydroxy pregna-1,4-diene-3,20-dione	n-caproic aldehyde	A	+ 72.9°	198–201	458	458.6	0.99	414– 32
			B	+104.5°	167– 71	,,	,,	0.93	462– 98
14	,,	n-caprylic aldehyde	A	+ 67.6°	169– 73	486	486.7	0.99	355–365
			B	+ 96.3°	143– 46	,,	,,	0.93	385–400
15	,,	n-decyl aldehyde	A	+ 66.0°	157– 66	514	514.7	0.99	330–350
			B	+ 93.2°	124– 7	,,	,,	0.93	365–385

(1) $DMSOd_6$

Example 16.

Resolution of 16α,17α-ethylidenedioxy-11β-hydroxy-21-(benzofuran-2-carbonyl-oxy)-9-fluoropregna-1,4-diene-3,20-dione into isomers.

A solution of 60 mg pure isomeric mixture of 16α,17α-ethylidenedioxy-9-fluoropregna-1,4-diene-11β,21-diol-3,20-dione in 2 ml of dry pyridine was added to 72.2 mg benzofuran-2-carboxylic acid chloride dissolved in 1 ml dry dioxane. The reaction mixture was allowed to stand under stirring at room temperature overnight. The major part of the solvents were evaporated *in vacuo* and the residue was poured into 30 ml of 3% ammonium chloride solution. The precipitate obtained was separated by centrifugation and dissolved in 100 ml of chloroform. The chloroform solution was washed once with 5% sodium carbonate solution, three times with water, dried over magnesium sulphate and evaporated *in vacuo*. The residue was chromatographed on Sephadex LH—20 using chloroform as an eluant. Two different isomers, A and B, of 16α,17α-ethylidenedioxy-11β-hydroxy-21-(benzofuran-2-carbonyloxy)-9-fluoropregna-1,4-diene-3,20-dione were obtained in the following yields and having the following characteristics:

A: 28.1 mg (35%); melting point 250—56°C; $[\alpha]_D^{25}$=+158.9° (c=0.2 in CH_2Cl_2); molecular weight=564 (theor. 564.6).

Retention volume 270—90 ml.

B: 24.2 mg (30%); melting point 247—50°C; $[\alpha]_D^{25}=+168.3°$ (c=0.2 in CH_2Cl_2); molecular weight=564 (theor. 564.6).
Retention volume 300—60 ml.

The isomer purity (>98%) of A and B was determined by NMR-spectroscopy by studying the signal for 18—CH_3 positioned at $\delta=1.06$ ppm ($CDCl_3$) for A and at $\delta=1.03$ ppm ($CDCl_3$) for B.

Similar separation results have been obtained using Merckogel OR—PVA 2000, as well as using, in addition to chloroform, methylene chloride, ethylene chloride, ethyl acetate, tetrahydrofuran and dioxane as eluting agents on both types of gel materials.

Examples 17—28.

Different 21-esters of the isomeric mixtures prepared according to Examples 1—15 were prepared purified and separated analogously to the process described in Example 16. The isomers given in Table 2 were thus obtained. The NMR-investigations were carried out in $CDCl_3$.

The acid chlorides used for the esterification are indicated in Table 2 in the following manner. NAC=nicotinic acid chloride, AAC=acetyl chloride, VAC=valeric acid chloride, BAC=benzofuran-2-carboxylic acid chloride.

TABLE 2

Ex. No.	the 21-ester of isomeric mixture according to Ex. No.	with	Isomer	$[\alpha]_D^{25}$ (c=0.2 in CH_2Cl_2)	Mp. °C	Molecular weight found:	Molecular weight calculated:	18–CH_3 δ (ppm)	Retention volume (mls)
17	1	NAC	A	+121.6°	268– 70	525	525.6	1.07	340– 65
			B	+135.0°	275– 7	,,	,,	1.03	375–410
18	1	VAC	A	+ 76.6°	248– 51	504	504.6	1.00	276–306
			B	+102.4°	261– 4	,,	,,	0.97	318–354
19	1	AAC	A	+ 87.2°	235– 8	462	462.5	0.96	325– 55
			B	+111.0°	259– 63	,,	,,	0.93	360– 90
20	3	BAC	A	+139.1°	135– 45	592	592.7	1.07	250– 75
			B	+157.8°	205– 12	,,	,,	1.03	280–320
21	3	NAC	A	+102.0°	207– 10	553	553.6	1.07	320– 45
			B	+132.9°	252– 4	,,	,,	1.04	350– 80
22	3	VAC	A	+ 67.0°	170– 1	532	532.7	1.00	255– 80
			B	+ 97.7°	254– 6	,,	,,	0.97	285–310
23	7	BAC	A	+143.6°	147– 52	582	582.6	1.06	280–320
			B	+164.4°	274– 8	,,	,,	1.02	325– 65
24	7	AAC	A	+ 79.7°	311– 4	480	480.5	0.99	366– 96
			B	+101.8°	322– 5	,,	,,	0.95	402– 38
25	8	BAC	A	+129.1°	129– 34	610	610.7	1.05	270– 94
			B	+147.8°	208– 10	,,	,,	1.03	300– 30
26	8	VAC	A	+ 66.8°	212– 16	550	550.7	0.98	258– 82
			B	+ 85.7°	261– 63	550	550.7	0.95	288–324
27	12	AAC	A	+ 86.2°	176– 9	472	472.6	0.99	235– 55
			B	+ 97.8°	183– 6	,,	,,	0.96	256– 75
28	12	BAC	A	+142.0°	118– 23	574	574.7	1.06	200– 25
			B	+153.6°	120– 30	,,	,,	1.03	230– 60

Examples of pharmaceutical preparations prepared in conventional manner are given below:

Example 29.
Ointment, fat anhydrous.

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

Example 30.
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 31.
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 32.
Tincture.

Steroid	3—500	mg
Ethanol 60% to	100	ml

Example 33.
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	mg

[a] Polyoxyethylene(20)sorbitan monooleate.

Example 34.
Foam aerosol.

Steroid	0.001—0.2	g.
Glycerol	4	g.
Na-cetyl stearyl alcohol	0.2	g.
Isopropylmyristate	2	g.
Metagin	0.1	g.
Water	80	g.
Tetrafluordichloroethane/ difluordichloromethane 40:60 to	100	g.

It is clear from the following presentation of experimental results that one of the stereoisomeric components, component B, has consistently superior physiological characteristics as compared to the other stereoisomeric component and the stereoisomeric mixture. The stereoisomeric component B which is herein after defined as the more active of the two components A and B in the stereoisomeric pair, may be defined as follows. It is the stereoisomeric component

showing the higher relative rotary power. In connection with gel filtration it is the stereoisomer, which shows the greater retention volume, i.e. it is the last to be eluted, and finally, it is the component which in NMR-measurements shows the lowest δ-value for 18—CH_3. As regards the steroids referred to in connection with the present invention the stereoisomeric mixture as well as the individual stereoisomeric components A and B have been investigated with regard to their antiinflammatory activity 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 each of about 6 mg were implanted simultaneously subcutaneously on the lateral side of the spinal cord. 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 killed by ether narcosis. The granuloma formed around the cotton wads were carefully recovered and thymus and body weights were measured. The two granuloma from each animal were dried overnight 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 the experiments carried out with the steroids in question are summarized in Table 3. From said Table it is immediately clear, that a difference in activity is found between the stereoisomeric components in each isomer pair, and that it is always the isomer last eluted from the gel column, which shows the highest activity. By means of the separation process of the present invention it has been possible to show that one of the stereoisomeric components, namely component B, clearly has stronger antiinflammatory effect than that obtained with the corresponding stereoisomeric mixture.

Irrespective of the interest to find steroid structures having high activity, there is a great demand for new compounds showing a better relation between antiinflammatory effect and the non-desired systemic effects, that may be observed after the resorption of the compounds. Thymus regression and inhibition of body growth may be seen as examples of such non-desired effects. From Table 3 it is clear that in order to obtain a good antiinflammatory effect (50% inhibition of granuloma growth) it is necessary with regard to the reference substances triamcinolone acetonide and fluocinolone acetonide to resort to doses of such a level (about 125 and 50 γ/animal, respectively) as to simultaneously result in a heavy decrease of thymus weight and body weight growth among the test animals. With regard to the compounds of the invention the doses needed for 50% inhibition of granuloma growth are, however, lower than or possibly of the same level as those giving a thymus regression or body growth inhibition.

Even if component A does not show an antiinflammatory effect as pronounced as that of corresponding component B it may in certain cases be more advantageous to use component A as such instead of the isomeric mixture with regard to better characteristics regarding non-desired systemic effects.

TABLE 3

Table summarizing biological effects of investigated compounds.

Compound prepared according to Example No.	Isomer	Dose (γ/animal) required to obtain the 50% inhibition of Granuloma growth	Thymus weight	25% inhibition of Body weight Increase
Triamcinolone acetonide	–	125	70	100
1	A+B	120	270	>270
1	A	270	115	60
1	B	30	50	30
2	A+B	35	100	140
2	A	25	130	90
2	B	10	60	100
3	A+B	10	>30	>30
3	A	25	>30	>30
3	B	3	17	100
4	A+B	<3	70	170
4	A	<3	100	170
4	B	<3	50	100
5	A+B	17	130	>270
5	A	30	90	>270
5	B	10	90	>270
Fluocinolone acetonide	–	50	14	20
7	A	15	12	20
7	B	10	6	15
8	A+B	5	10	50
8	A	6	13	50
8	B	4	10	50
Prednacinolone	–	270	105	>270
12	A+B	100	80	80
12	A	125	125	>270
12	B	40	70	50
13	A+B	10	175	90
13	A	8	>270	100

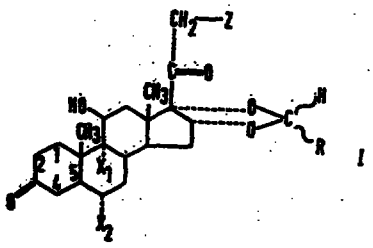
TABLE 3 (Continued)

Compound prepared according to Example No.	Isomer	Dose (γ/animal) required to obtain the 50% inhibition of Granuloma growth	Thymus weight	25% inhibition of Body weight Increase
13	B	5	210	>270
16	A	5	70	45
16	B	3	10	10
17	A	7	45	20
17	B	3	13	20
18	A	<3	70	33
18	B	<3	10	10
20	A+B	<3	25	20
20	A	<3	90	50
20	B	<3	20	5
21	A+B	7	35	10
21	A	<3	70	70
21	B	<3	10	5
22	A+B	10	50	30
22	A	<3	90	33
22	B	<3	35	5
25	A+B	<3	10	10
25	A	<3	15	20
25	B	<3	7	20
27	A+B	<3	60	40
27	A	<3	100	100
27	B	<3	60	35

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

WHAT WE CLAIM IS:—

1. A stereoisomeric component B of a stereoisomeric mixture (isomer pair) of a steroid of formula:—



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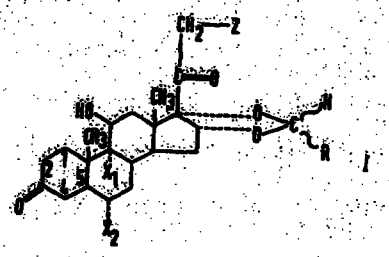
TABLE 3 (Continued)

Compound prepared according to Example No.	Isomer	Dose (γ/animal) required to obtain the 50% inhibition of Granuloma growth	Thymus weight	25% inhibition of Body weight increase
13	B	5	210	>270
16	A	5	70	>45
16	B	3	10	10
17	A	7	45	20
17	B	3	13	20
18	A	<3	70	33
18	B	<3	10	10
20	A+B	<3	25	20
20	A	<3	90	50
20	B	<3	20	5
21	A+B	7	35	10
21	A	<3	70	70
21	B	<3	10	5
22	A+B	10	50	30
22	A	<3	90	33
22	B	<3	35	5
25	A+B	<3	10	10
25	A	<3	15	20
25	B	<3	7	20
27	A+B	<3	60	40
27	A	<3	100	100
27	B	<3	60	35

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WHAT WE CLAIM IS:—

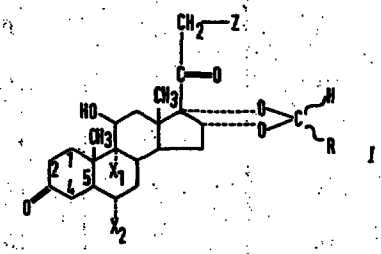
1. A stereoisomeric component B of a stereoisomeric mixture (isomer pair) of a steroid of formula:—



I

wherein the 1,2 and 4,5-positions are saturated or a double bond is present in at least one of said two positions, X_1 and X_2 each represent hydrogen or fluorine with the proviso that when X_2 is fluorine X_1 is fluorine, Z is hydroxyl or esterified hydroxyl, and R is a straight or branched alkyl group having 1—10 carbon atoms, said component B being the isomer of the isomer pair showing the greater relative rotary power or having the greater retention volume in gel filtration.

2. A stereoisomeric component A of a stereoisomeric mixture (isomer pair) of a steroid of formula:—



I

wherein the 1,2 and 4,5-positions are saturated or a double bond is present in at least one of said two positions, X_1 and X_2 each represent hydrogen or fluorine with the proviso that when X_2 is fluorine X_1 is fluorine, Z is hydroxyl or esterified hydroxyl, and R is a straight or branched alkyl group having 1—10 carbon atoms, said component A being the isomer of the isomer pair showing the lower relative rotary power or having the smaller retention volume in gel filtration.

3. A stereoisomeric component according to claim 1 or 2, wherein R is an alkyl group having 1—6 carbon atoms.

4. A stereoisomeric component according to any one of claims 1 to 3, 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.

5. A stereoisomeric component according to any one of claims 1 to 3, wherein Z is hydroxyl esterified with pyridine-3-carboxylic acid, pyridine-4-carboxylic acid, benzofuran-2-carboxylic acid or menthoxyacetic acid.

6. A stereoisomeric component according to any one of claims 1 to 3, wherein Z is hydroxyl esterified with a dicarboxylic acid.

7. A stereoisomeric component according to claim 6, wherein the acid has 2—12 carbon atoms.

8. A stereoisomeric component according to any one of claims 1 to 3, wherein Z is hydroxyl esterified with phosphoric or sulphuric acid.

9. A stereoisomeric component according to claim 1 or 2, hereinbefore specifically mentioned.

10. A stereoisomeric component B according to claim 1 which is 16α,17α-ethylidenedioxy-9-fluoropregna-1,4-diene-11β,21-diol-3,20-dione.

11. A stereoisomeric component B according to claim 1 which is 16α,17α-butylidenedioxy-9-fluoropregna-1,4-diene-11β,21-diol-3,20-dione.

12. A stereoisomeric component B according to claim 1 which is 16α,17α-ethylidenedioxy-6α,9-difluoropregna-1,4-diene-11β,21-diol-3,20-dione.

13. A stereoisomeric component B according to claim 1 which is 16α,17α-butylidenedioxy-6α,9-difluoropregna-1,4-diene-11β,21-diol-3,20-dione.

14. A stereoisomeric component B according to claim 1 which is 16α,17α-butylidenedioxypregna-1,4-diene-11β,21-diol-3,20-dione.

15. A process for the resolution of a stereoisomeric mixture (isomer pair) of a steroid as defined in claim 1, into its stereoisomeric components, which are distinguished with regard to the space orientation of the substituents on the 2'-carbon atom of the dioxolane ring, which comprises subjecting the stereoisomeric mixture to gel filtration, the components being separated and being separately recoverable in view of their different retention volumes.

16. A process according to claim 15, wherein the gel filtration is carried out on a cross-linked dextran gel.

17. A process according to claim 16, wherein the gel is a hydroxypropylated cross-linked dextran gel.

18. A process according to claim 15, wherein the gel filtration is carried out on a copolymer of vinyl acetate.

19. A process according to claim 18, wherein the copolymer of vinyl acetate is one swelling in organic solvents.

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20. A process according to any one of claims 15 to 19 wherein the gel filtration is carried out using an eluting agent which is acetonitrile or a halogenated hydrocarbon, ether or ester, of mixture thereof.

21. A process according to claim 20, wherein the eluting agent is ethyl acetate, methylene chloride or chloroform.

22. A process according to claim 15, substantially as hereinbefore described in any one of Examples 1 to 28.

23. A stereoisomeric component isolated by a process as claimed in any one of claims 15 to 22.

24. A pharmaceutical composition comprising a stereoisomeric component as claimed in any one of claims 1 to 14, and 23, together with a pharmaceutically acceptable carrier.

25. A composition according to claim 22, substantially as hereinbefore described in any one of Examples 29 to 34.

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