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ABSTRACT

A process for preparing and purifying alfaxalone (3α-hydroxy-5α-pregnane) from a crude mixture of 3α- and 3β-hydroxy-5α-pregnane; and subsequent purification of the 17α- and 17β-enantiomers to obtain the 17β-enantiomer (alfaxalone).

ALFAXALONE SYNTHESIS

CROSS REFERENCE

This application is a divisional of Australian Application No. 2025283610 filed 19 December 2025, which is a divisional of Australian Application No. 2024227565 filed 23 October 2024, and claims priority to U.S. Provisional Application No. 63/593,334 filed 26 October 2023, the disclosure of each of which is incorporated by reference in its entirety herein for all purposes.

TECHNICAL FIELD

This invention relates to a novel process for preparing and purifying the steroid 3α-hydroxy-5α-pregnane (alfaxalone) from a crude mixture of 3α- and 3β-hydroxy-5α-pregnane to achieve >95% of pure alfaxalone.

BACKGROUND OF THE INVENTION

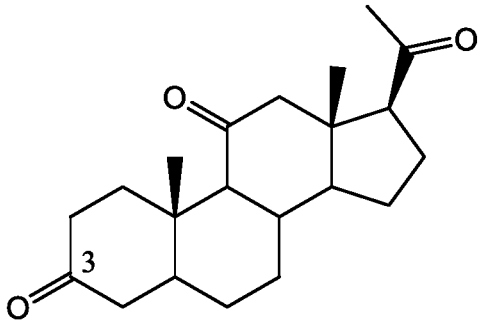
It is known that steroids give rise to profound depression of the central nervous system and act pharmacologically as anaesthetics or hypnotics. Such compounds have been the subject of considerable study to find viable alternatives to known anaesthetics.

Anaesthetic steroids are generally relatively simple pregnane derivatives, often hydroxylated in the 3-position. In the past, 3β-hydroxy steroids were studied in preference to 3α-steroid compounds. Many 3α-hydroxy steroids have now been found to possess useful properties, in particular, 5α-pregnane steroids having a 3α-hydroxy group, hereinafter referred to as 3α-hydroxy-5α-steroids, have been found to possess desirable anaesthetic properties and are included in pharmaceutical preparations as the active ingredient. For pharmaceutical use, it is desirable to obtain the 3α-hydroxy stereoisomer of the 5α-pregnane steroid in as pure a form as possible. The endeavor to produce high purity product continues to be a difficult task to achieve through catalysis and different purification techniques.

The 3α-hydroxy-5α-steroids may be formed by biotransformation or chemical synthesis. A number of different synthetic routes have been proposed in the past to prepare 3α-hydroxy-5α-steroids. One method involves the reduction of the corresponding 3-oxo compound using an iridium catalyst reduction system. The yield and purity of the desired 3α-hydroxy product, however, was unsatisfactory due to, for example, isomerisation at the 17-position of the 20-oxo pregnanes and the formation of the 3β-hydroxy isomer. In a modified version of this process, a 3-oxo-5α-steroid is reduced in the presence of an organic base using an iridium catalyst system prepared from a trivalent phosphorous compound, an iridium compound and water. It was

[Text continues on page 2]

found that the ratio of the desired 3α-hydroxy steroid to the 3β-hydroxy steroid can be improved and the 17-position epimerization controlled if the reduction was carried out in the presence of sufficient added organic base to bring the pH substantially to neutrality. Alternatively, the catalyzed reduction of the C3 ketone (C=O; shown below) can be performed using a ruthenium catalyst (US Patent No. 11,472,833B2) to prepare the C3-hydroxy (C-OH) steroid with a



carbonate salt in an organic solvent like isopropyl alcohol. C3 refers to the 3rd numbered carbon of the organic molecule. Use of the ruthenium catalyst provides for about an 80:20 stereoselectivity of the 3α-hydroxy:3-β-hydroxy compounds of Formula (1a) and Formula (1b), respectively. However, the endeavor to produce high purity product of Formula (1a) continues to be a difficult task to achieve through catalysis and different purification techniques. However, the present inventors have found that the purity of the desired 3α-hydroxy-5α-steroid is still unacceptable, especially for commercial pharmaceutical manufacturing purposes.

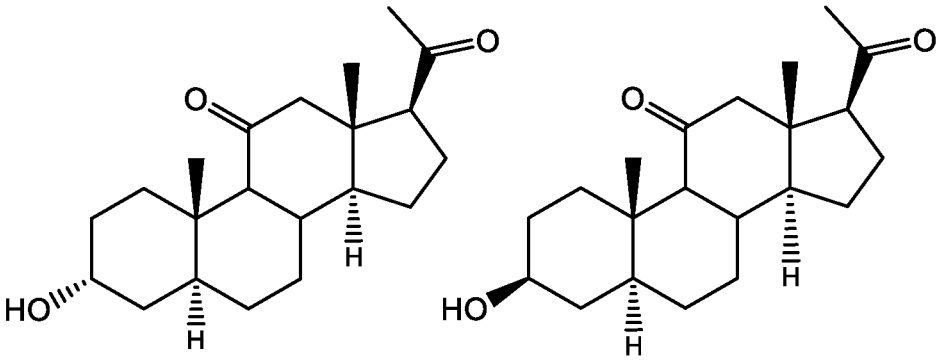
There is clearly a need for a process of preparing the 3α-hydroxy stereoisomer of 5α-pregnane steroids in a substantially high purity especially for a process that is easily performed and inexpensive. The inventors have recently discovered a means to further control and improve the purity of the desired 3α-hydroxy-5α-pregnane steroid, through improvements to the hydrolysis reaction and purification stages.

SUMMARY OF THE INVENTION

Described herein is a new process for purifying a crude mixture of 3α- and 3β-hydroxy-5α-pregnane, as defined below by Formula (1a; alfaxalone)

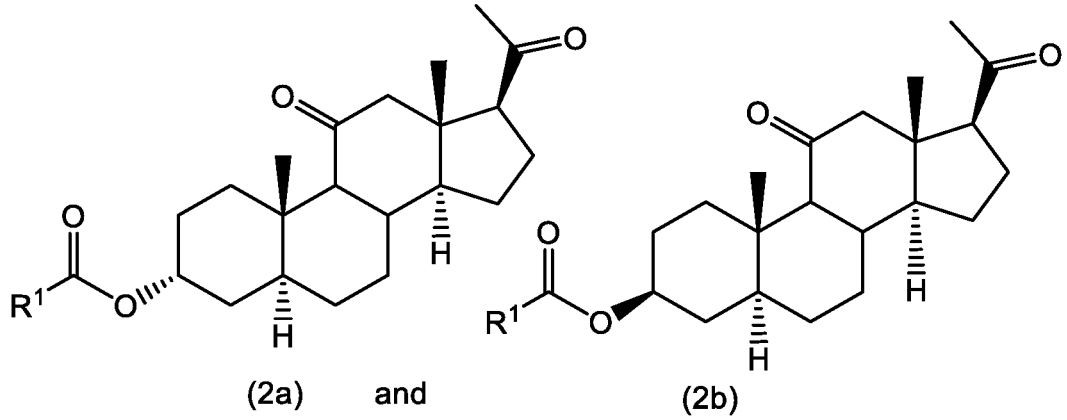
and (1b), to provide the individual 3α- and 3β-hydroxy stereoisomers, and particularly, to provide the 3α-hydroxy stereoisomer in substantially high purity. The present inventors have surprisingly found that this can be achieved by converting both the 3α- and 3β-hydroxy groups on the 5α-pregnane steroid to the corresponding 3-acetoxy isomers (2a and 2b) which are surprisingly easier to separate from one another when compared to the corresponding 3-hydroxy isomers. Each individual or desired 3-acetoxy isomer is then subject to hydrolysis to convert the acetate back to the hydroxy group. This new process addresses the previously technical difficulty of synthetically providing the 3α-hydroxy-5α-pregnane steroid as a substantially single isomer with relative ease.

In one aspect of the invention, is a process for purifying a crude mixture of the Formula (1a) and (1b) compounds into individual stereoisomers to substantially obtain the single stereoisomer of Formula (1a), comprising



(1a) and (1b)

a) treating the mixture of Formula (1a) and (1b) compounds with an acylating agent in at least one non-protic solvent to form a mixture of compounds according to Formula (2a) and (2b)



(2a) and (2b)

wherein

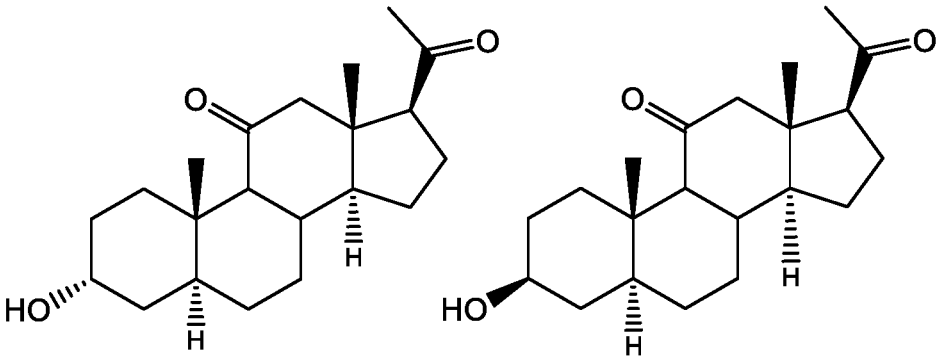
R^1 is a substituted or unsubstituted alkyl, and wherein the alkyl can be substituted with at least one or more substituents selected from hydroxy, chloro and oxo;

b) separating the Formula (2a) and (2b) compounds to obtain substantially single stereoisomers of (2a) and (2b);

c) hydrolysing the acetylated compound of Formula (2a) compound under basic conditions in one or more solvents to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and optionally,

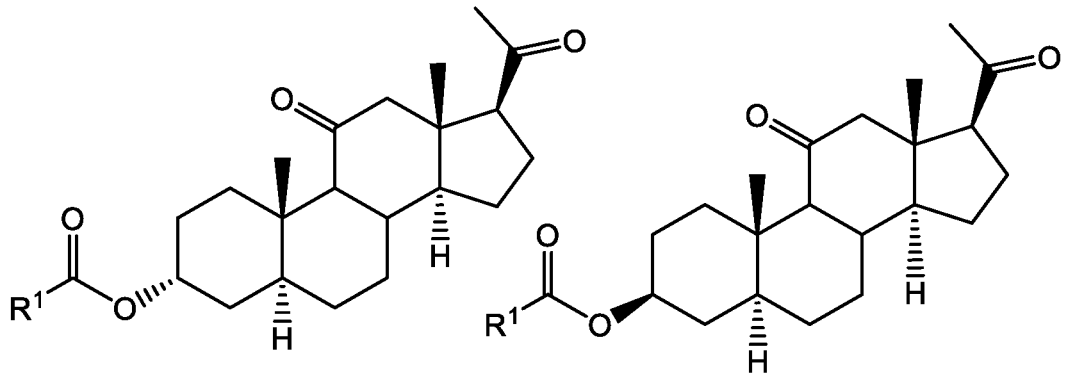
d) enantio-enrichment of the enantiomers to obtain >95% of the 17β-enantiomer.

In another aspect, is a process for purifying a crude mixture of the Formula (1a) and (1b) compounds into individual stereoisomers to substantially obtain the single stereoisomer of Formula (1a), comprising



(1a) and (1b)

a) treating the mixture of Formula (1a) and (1b) compounds with an acylating agent in at least one non-protic solvent to form a mixture of compounds according to Formula (2a) and (2b)



(2a) and (2b)

wherein

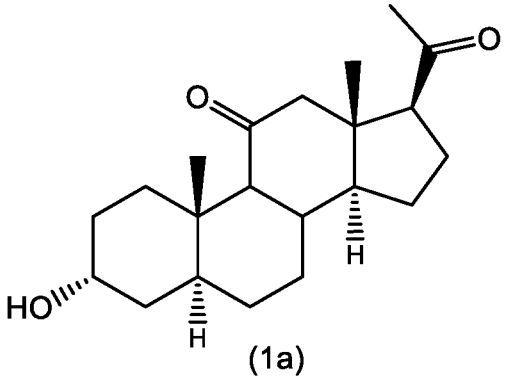
R^1 is a substituted or unsubstituted alkyl, and wherein the alkyl can be substituted with at least one or more substituents selected from hydroxy, chloro and oxo;

b) separating the Formula (2a) and (2b) compounds to obtain substantially single stereoisomers of (2a) and (2b);

c) hydrolysing the acetylated compound of Formula (2a) compound under basic conditions in one or more solvents to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and optionally,

d) enantio-enrichment of the enantiomer mixture comprising the epimerization of the 17α-enantiomer in an aqueous basic solution comprising 1-2 mole equivalents of sodium hydroxide or potassium hydroxide in methanol and water or ethanol and water and then recrystallizing the resulting product from a mixture of acetone and hexane, methanol and water or ethanol and water to obtain >95% of the 17β-enantiomer.

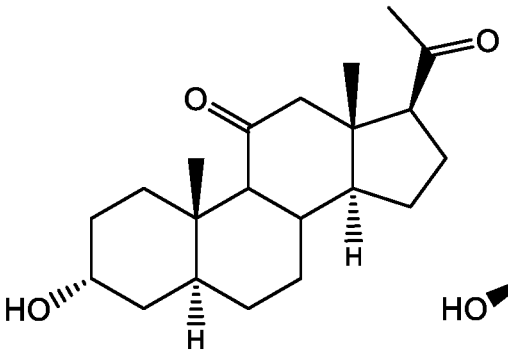
In another aspect, is a process for the preparation of a substantially single stereoisomer of Formula (1a),



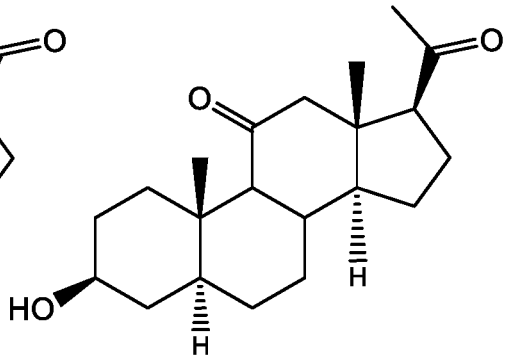
(1a)

comprising the steps of:

a) treating the mixture of Formula (1a) and (1b) compounds

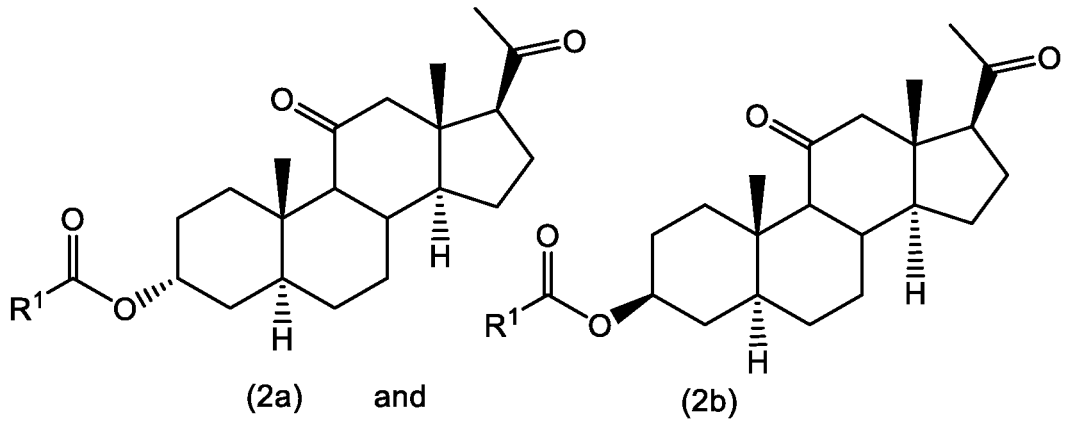


(1a) and



(1b)

with an acylating agent selected from the group consisting of acetic anhydride, acetyl chloride, propionic anhydride and proprionyl chloride; in at least one non-protic solvent selected from the group consisting of tetrahydrofuran, pyridine, ether, dimethyl formamide, dioxane and triethylamine, or mixture thereof; and optionally in the presence of a catalyst; at a temperature of about 15-30°C for about 5 to 40 hours; to form a mixture of compounds according to Formula (2a) and (2b)



(2a) and (2b)

wherein

R^1 is a substituted or unsubstituted alkyl, and wherein the alkyl can be substituted with at least one or more of substituents selected from hydroxy, chloro and oxo;

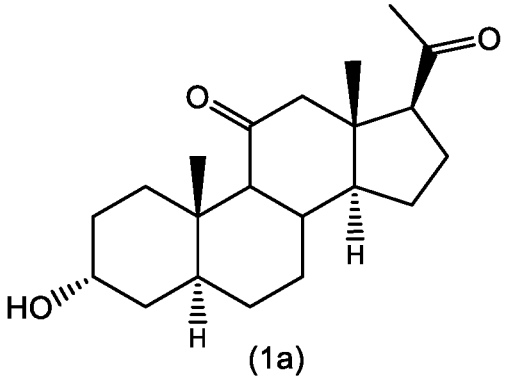
b) separating the Formula (2a) and (2b) compounds to obtain substantially single stereoisomers of Formula (2a) by recrystallization of the mixture in a solvent/anti-solvent wherein the solvent is selected from acetone and ethyl acetate and the anti-solvent is selected from hexane, pentane and heptane

c) hydrolysing the Formula (2a) compound under basic conditions using an aqueous solution of sodium hydroxide, potassium hydroxide or potassium carbonate in an alcoholic solvent selected from methanol, ethanol, propanol and isopropanol, and water to prepare the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and optionally,

d) enantio-enrichment of the enantiomer mixture comprising the epimerization of the 17α-enantiomer in an aqueous basic solution comprising 1-2 mole equivalents of sodium hydroxide or potassium hydroxide in methanol and water or ethanol and water and then recrystallizing the resulting product from a mixture of acetone and hexane, methanol and water or ethanol and water to obtain >95% of the 17β-enantiomer.

In another aspect, the preferred R^1 substituent is methyl.

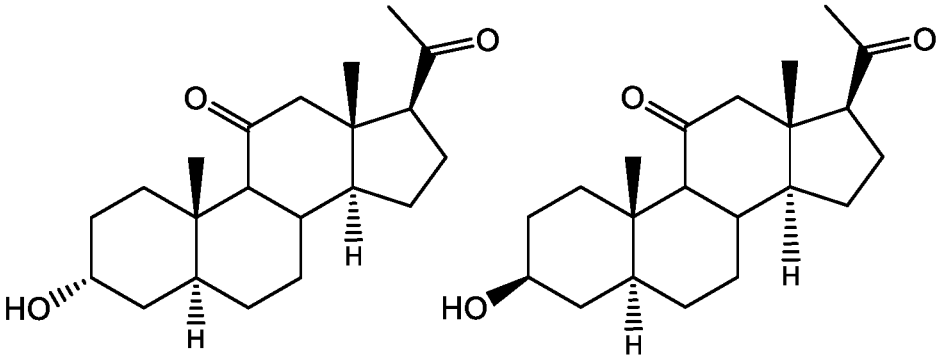
In another aspect, is a process for the preparation of a substantially single stereoisomer of Formula (1a),



(1a)

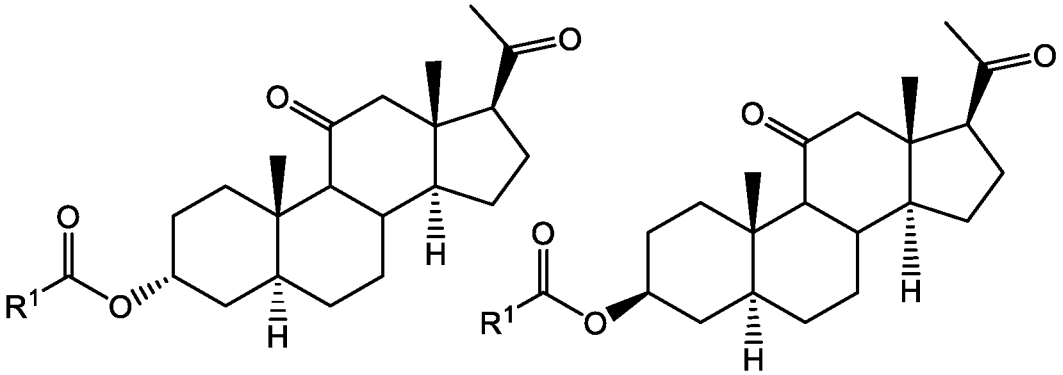
comprising the steps of:

a) treating the mixture of Formula (1a) and (1b) compounds with an acylating agent selected from acetic anhydride or acetyl chloride; in a non-



(1a) and (1b)

protic solvent selected from tetrahydrofuran, pyridine or mixture thereof; in the presence of a catalyst selected from the group consisting of 4-dimethylaminopyridine, 1-hydroxy-7-azabenzotriazole or 1-hydroxy-7-azabenzotriazole; at a temperature of about 15-30ºC for about 5 to 40 hours; to form a mixture of 3-acetoxy compounds of Formula (2a') and (2b')



(2a) and (2b)

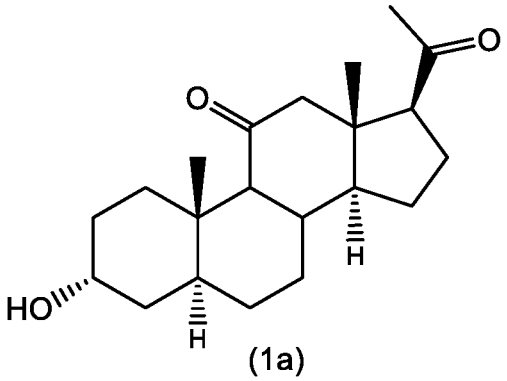
;

b) separating the Formula (2a') and (2b') compounds to obtain substantially the single stereoisomer of (2a') by fractional crystallization in a solvent mixture selected from the group consisting of acetone and hexane, ethyl acetate and hexane, methanol and water or ethanol and water;

c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of sodium hydroxide in an alcoholic solvent selected from methanol, ethanol, propanol and isopropanol, and water to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and optionally,

d) enantio-enrichment of the enantiomer mixture comprising the epimerization of the 17α-enantiomer in an aqueous basic solution comprising 1-2 mole equivalents of sodium hydroxide or potassium hydroxide in methanol and water or ethanol and water and then recrystallizing the resulting product from a mixture of acetone and hexane, methanol and water or ethanol and water to obtain >95% of the 17β-enantiomer.

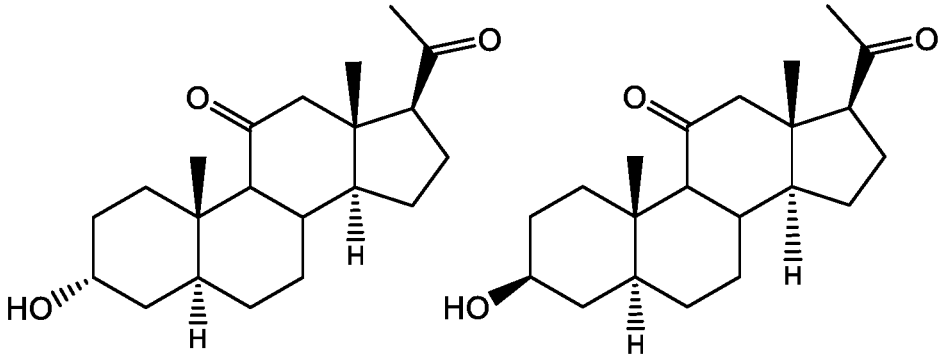
In another aspect, is a process for the preparation of a substantially single stereoisomer of Formula (1a),



(1a)

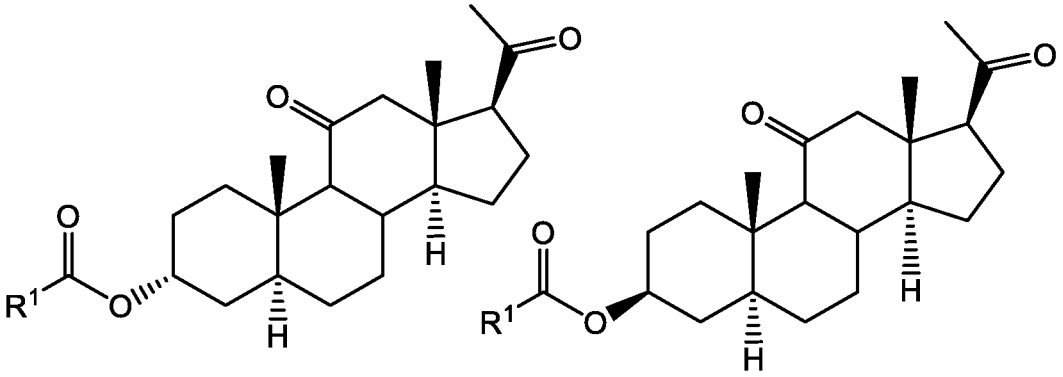
comprising the steps of:

a) treating the mixture of Formula (1a) and (1b) compounds with acetic



(1a) and (1b)

anhydride in a mixture of tetrahydrofuran and pyridine; in the presence of a catalyst that is 4-dimethylaminopyridine; at a temperature of about 15-30ºC for about 5 to 40 hours; to form a mixture of 3-acetoxy compounds of Formula (2a') and (2b')



(2a) and (2b)

;

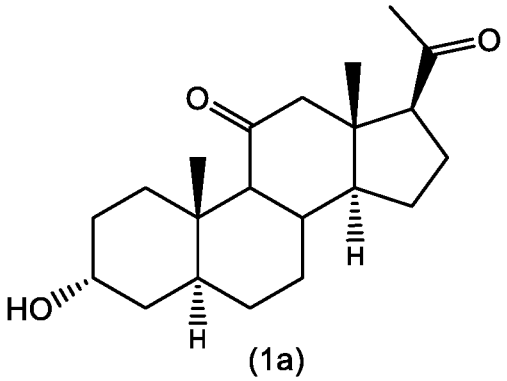
b) separating the Formula (2a') and Formula (2b') compounds to obtain substantially single stereoisomers by fractional crystallization in a mixture of acetone and hexane in a ratio of about 1:2 to about 1:10;

c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of sodium hydroxide in methanol and water or ethanol and water to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and optionally,

d) enantio-enrichment of the enantiomer mixture comprising the epimerization of the 17α-enantiomer in an aqueous basic solution comprising 1-2 mole equivalents of sodium hydroxide or potassium hydroxide in methanol

and water or ethanol and water and then recrystallizing the resulting product from a mixture of acetone and hexane, methanol and water or ethanol and water to obtain >95% of the 17β-enantiomer.

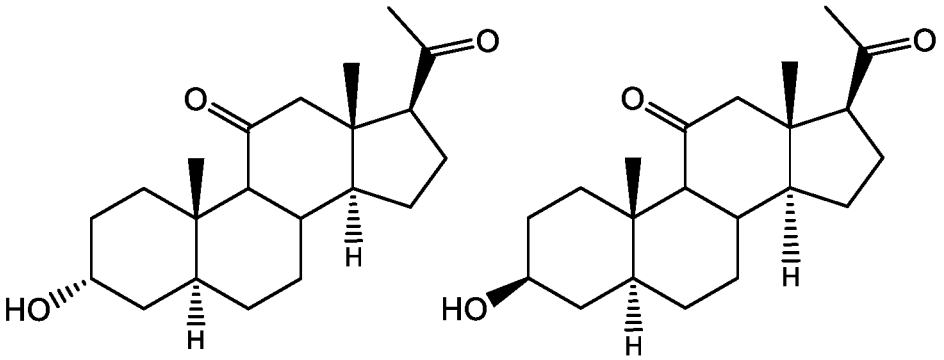
In another aspect, is a process for the preparation of a substantially single stereoisomer of Formula (1a),



(1a)

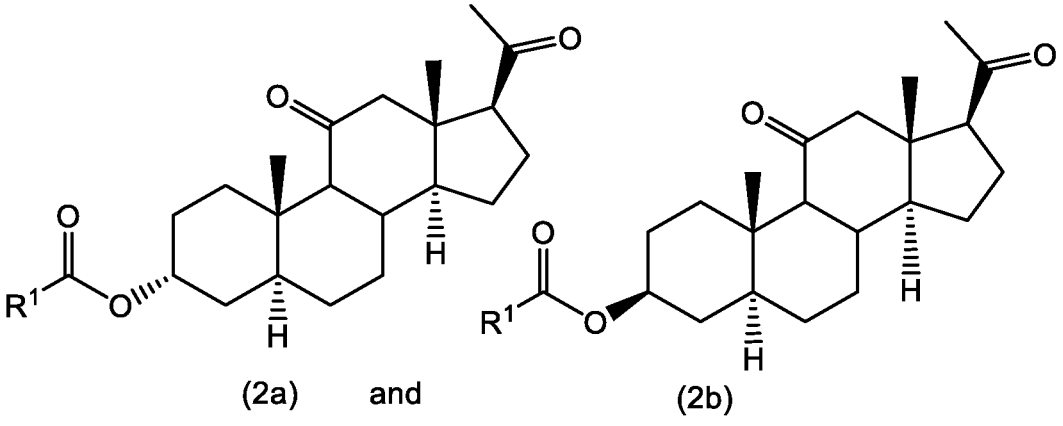
comprising the steps of:

a) treating the mixture of Formula (1a) and (1b) compounds with acetic



(1a) and (1b)

anhydride in a mixture of tetrahydrofuran and pyridine in a ratio of about 4:1; in the presence of a catalyst that is 4-dimethylaminopyridine; at a temperature of about 15-30ºC for about 5 to 40 hours; to form a mixture of 3-acetoxy compounds of Formula (2a') and (2b')



(2a) and (2b)

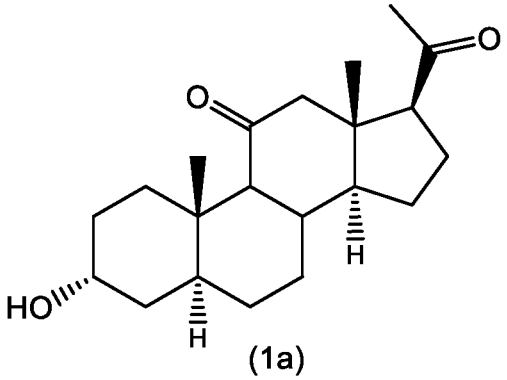
;

b) separating the Formula (2a') and (2b') compounds to obtain substantially the single stereoisomer of Formula (2a') by fractional crystallization in a mixture of acetone and hexane in a ratio of about 1:2 to 1:10;

c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of sodium hydroxide at 1 to 2 mole equivalents to Formula (2a') in methanol and water or ethanol and water to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and optionally,

d) enantio-enrichment of the enantiomer mixture comprising the epimerization of the 17α-enantiomer in an aqueous basic solution comprising 1-2 mole equivalents of sodium hydroxide or potassium hydroxide in methanol and water or ethanol and water and then recrystallizing the resulting product from a mixture of acetone and hexane in a ratio of about 1:4 or 2:5, methanol and water in a ratio of about 1:1 to 1:2 or ethanol and water in a ratio of about 1:1 to 1:2 to obtain >95% of the 17β-enantiomer.

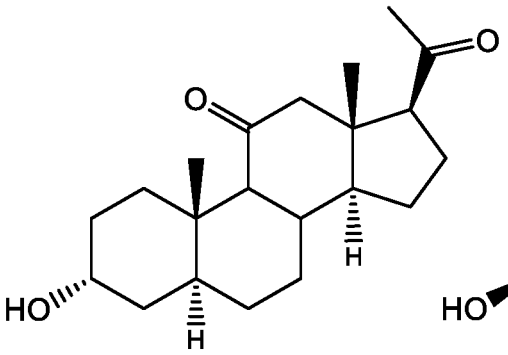
In another aspect, is a process for the preparation of a substantially single stereoisomer of Formula (1a),



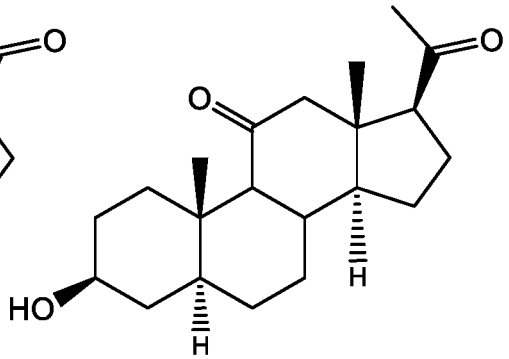
(1a)

comprising the steps of:

a) treating the mixture of Formula (1a) and (1b) compounds with acetic

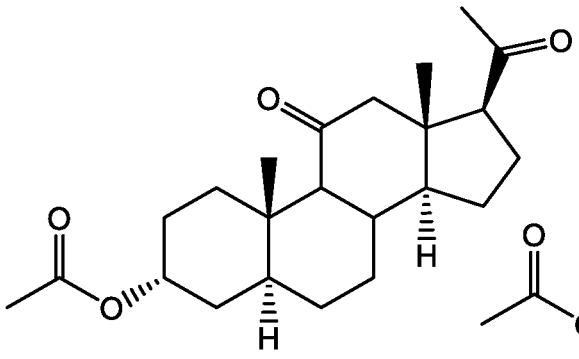


(1a) and

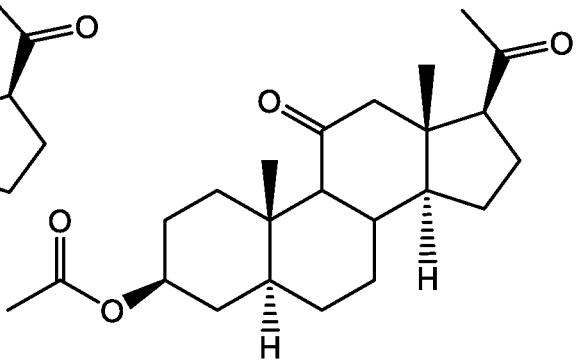


(1b)

anhydride in a mixture of tetrahydrofuran and pyridine in a ratio of about 4:1; in the presence of a catalyst that is 4-dimethylaminopyridine; at a temperature of about 15-30ºC for about 5 to 40 hours; to form a mixture of 3-acetoxy compounds of Formula (2a') and (2b')



(2a') and



(2b')

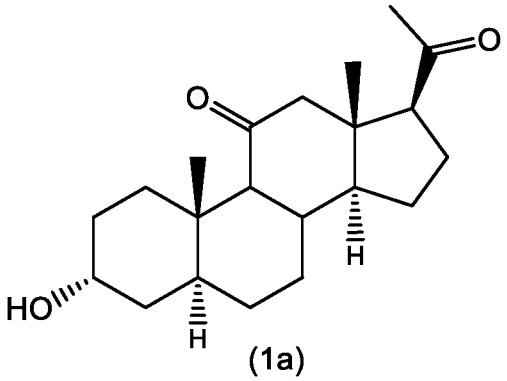
;

b) separating the Formula (2a') and (2b') compounds to obtain substantially the single stereoisomer of Formula (2a') by fractional crystallization in a mixture of acetone and hexane in a ratio of about 1:2 to 1:10;

c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of sodium hydroxide at 1 mole equivalent to Formula (2a') in methanol and water or ethanol and water to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and

d) enantio-enrichment of the enantiomer mixture comprising the epimerization of the 17α-enantiomer in an aqueous basic solution comprising 1-2 mole equivalents of sodium hydroxide or potassium hydroxide in methanol and water or ethanol and water and then recrystallizing the resulting product from a mixture of acetone and hexane in a ratio of about 1:4 or 2:5, methanol and water in a ratio of about 1:1 to 1:2 or ethanol and water in a ratio of about 1:1 to 1:2 to obtain >95% of the 17β-enantiomer.

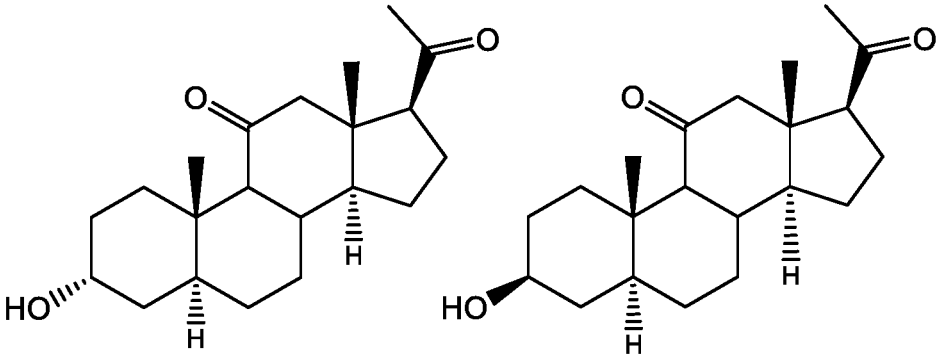
In another aspect, is a process for the preparation of a substantially single stereoisomer of Formula (1a),



(1a)

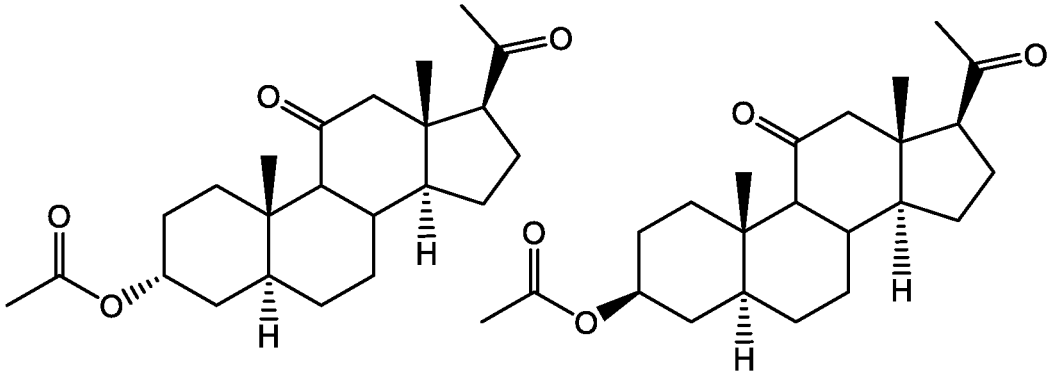
comprising the steps of:

a) treating the mixture of Formula (1a) and (1b) compounds with acetic



(1a) and (1b)

anhydride in a mixture of tetrahydrofuran and pyridine in a ratio of about 4:1; in the presence of a catalyst that is 4-dimethylaminopyridine; at a temperature of about 15-30ºC for about 5 to 40 hours; to form a mixture of 3-acetoxy compounds of Formula (2a') and (2b')



(2a') and (2b')

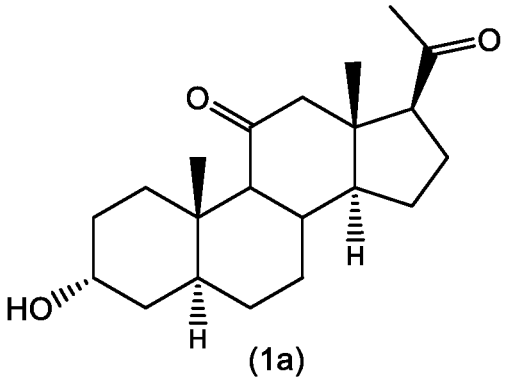
;

b) separating the Formula (2a') and (2b') compounds to obtain substantially the single stereoisomer of Formula (2a') by fractional crystallization in a mixture of acetone and hexane in a ratio of about 1:2 to 1:10;

c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of sodium hydroxide at 2 mole equivalents to Formula (2a') in methanol and water or ethanol and water to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers and wherein the mixture contains >95% of the 17β-enantiomer; and

d) enantio-enrichment of the enantiomers by recrystallizing the mixture in methanol and water in a ratio of about 1:1 to 1:2, or ethanol and water in a ratio of about 1:1 to 1:2 to obtain the 17β-enantiomer.

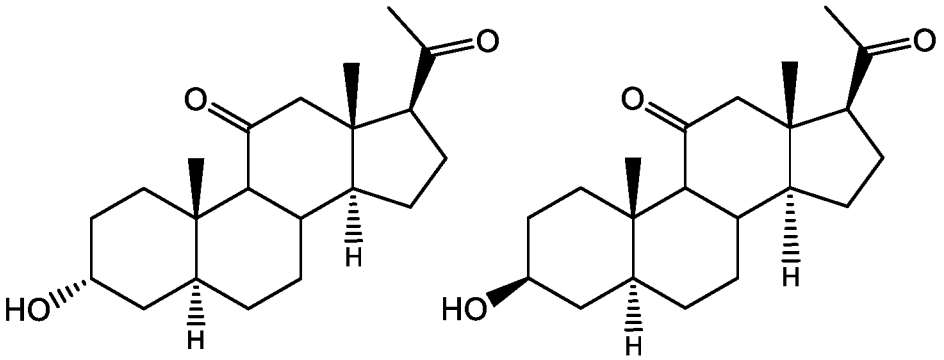
In another aspect, is a process for the preparation of a substantially single stereoisomer of Formula (1a),



(1a)

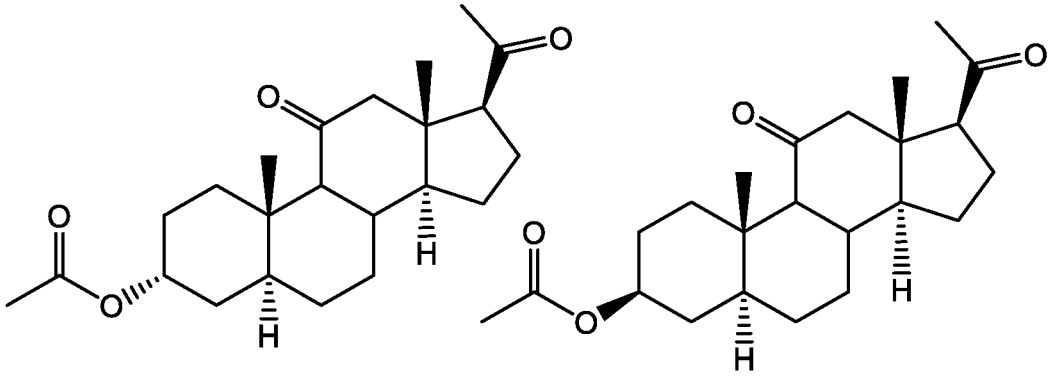
comprising the steps of:

a) treating the mixture of Formula (1a) and (1b) compounds with acetic



(1a) and (1b)

anhydride in a mixture of tetrahydrofuran and pyridine in a ratio of about 4:1; in the presence of a catalyst that is 4-dimethylaminopyridine; at a temperature of about 15-30ºC for about 5 to 40 hours; to form a mixture of 3-acetoxy compounds of Formula (2a') and (2b')



(2a') and (2b');

b) separating the Formula (2a') and (2b') compounds to obtain substantially the single stereoisomer of Formula (2a') by fractional crystallization in a mixture of acetone and hexane in a ratio of about 1:2 to 1:10;

c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of sodium hydroxide at 2 mole equivalents to Formula (2a') in methanol and water to form the compound of Formula (1a) as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers and wherein the mixture contains >98% of the 17β-enantiomer, and

d) enantio-enrichment of the enantiomers by recrystallizing the mixture in methanol and water in a ratio of about 1:1 to 1:2, or ethanol and water in a ratio of about 1:1 to 1:2 to obtain the 17β-enantiomer.

The purity of the alfaxalone obtained by the present invention is about 95-100% relative to the British Pharmacopoeia 2019 standard. The more preferred range is about 97-100%. Generally, the percentage of other impurities, such as the 3β-hydroxy isomer and the 17α-enantiomer, is < about 2%.

Preferably, the mixture of 3α- and 3β-hydroxy compounds according to Formula (1a) and (1b) are formed from Henbest reduction of allopregnatrione. Even more preferably, the allopregnatrione is synthesised via a series of steps starting with the readily available 11-hydroxyprogesterone or 11-ketoprogesterone as indicated in Scheme 1.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the

exclusion of any other element, integer or step, or group of elements, integers or steps, or including but not limited to.

In the description of this specification reference may be made to subject matter which is not within the scope of the appended claims. That subject matter should be readily identifiable by a person skilled in the art and may assist in putting into practice the invention as defined in the appended claims.

DETAILED DESCRIPTION

The present invention is directed to a process for manufacturing alfaxalone. Alfaxalone is a neuroactive steroid and general anaesthetic which is used currently in veterinary practice as an injectable anaesthetic agent for the induction and maintenance of anaesthesia. Alfaxalone is a preferred anaesthetic due to minimal depressive effects on the cardiovascular system. The most common side effect seen in current veterinary practice is respiratory depression when alfaxalone is administered alone or concurrently with other sedative and anaesthetic drugs. Alfaxalone works as a positive allosteric modulator on gamma-aminobutyric acid A (GABA-A) receptors and, at high concentrations, as a direct agonist of the GABA-A receptor. The GABA-A receptor is an ionotropic receptor and ligand-gated ion channel. Its endogenous ligand is γ-aminobutyric acid (GABA), type A; the major inhibitory neurotransmitter receptors responsible for fast inhibition in the basal ganglia. Alfaxalone is cleared quickly by the liver, giving it a relatively short terminal half-life and preventing it from accumulating in the body, lowering the chance of overdose.

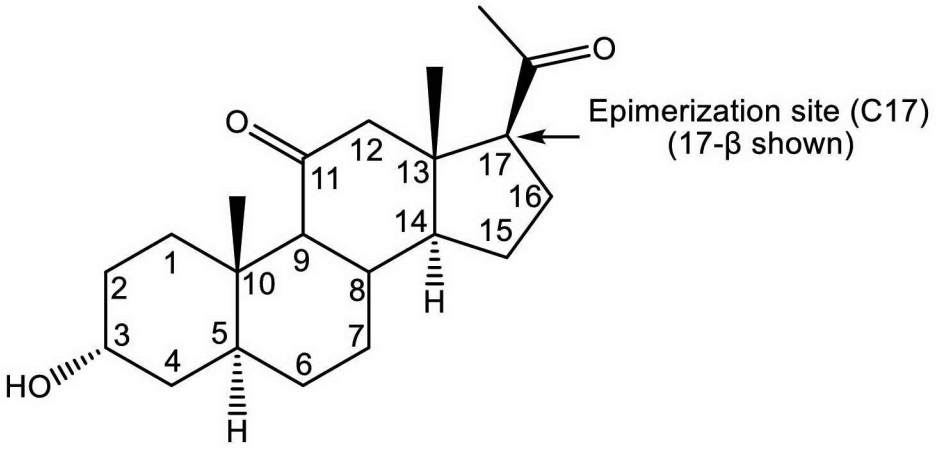
Alfaxalone is used as an injectable anaesthetic agent for the induction and maintenance of anaesthesia or as a sedative in animals. While it is commonly used in cats and dogs, it has also been successfully used in other animals like rabbits, horses, sheep, pigs, and exotics (turtles, iguanas, marmosets, bears and fish). As an induction agent, alfaxalone causes the animal to relax enough to be intubated, which then allows the administration of inhalational anaesthesia. Pre-medication reduces the dose requirement of

alfaxalone as an induction agent. Alfaxalone can be used instead of gas anaesthetics in surgeries that are under 30 minutes, where it is given intravenously via constant rate infusion; this is especially useful in procedures such as bronchoscopies or repairing tracheal tears, as there is no requirement for inhalation anaesthesia, and therefore, no endotracheal tube in the way. Once the administration of alfaxalone is ceased, there is minimal intervention required for animal recovery from anaesthesia. Alfaxalone can also be used as a sedative when given intramuscularly. Based on the commercial use of alfaxalone by veterinarians, there is a need for better manufacturing processes to reduce time, cost of goods and increase yield. The processes described herein, provide a new and more efficient and cost- effective process for manufacturing alfaxalone.

As described herein, the term enantio-enrichment, refers to a) the process of epimerization of the 17α-enantiomer under basic conditions to achieve greater amounts of the 17β-enantiomer (alfaxalone) and/or b) to the recrystallization of any mixture of the 17α- and 17β-enantiomers in a solvent/anti-solvent system (*e.g.*, acetone/hexane, methanol/water and ethanol/water) to obtain purer 17β-alfaxalone.

Alfaxalone, Formula (1a), is also described herein as 3α-hydroxy-5α-pregnane, 3α-hydroxy-17β-alfaxalone and 17β-alfaxalone.

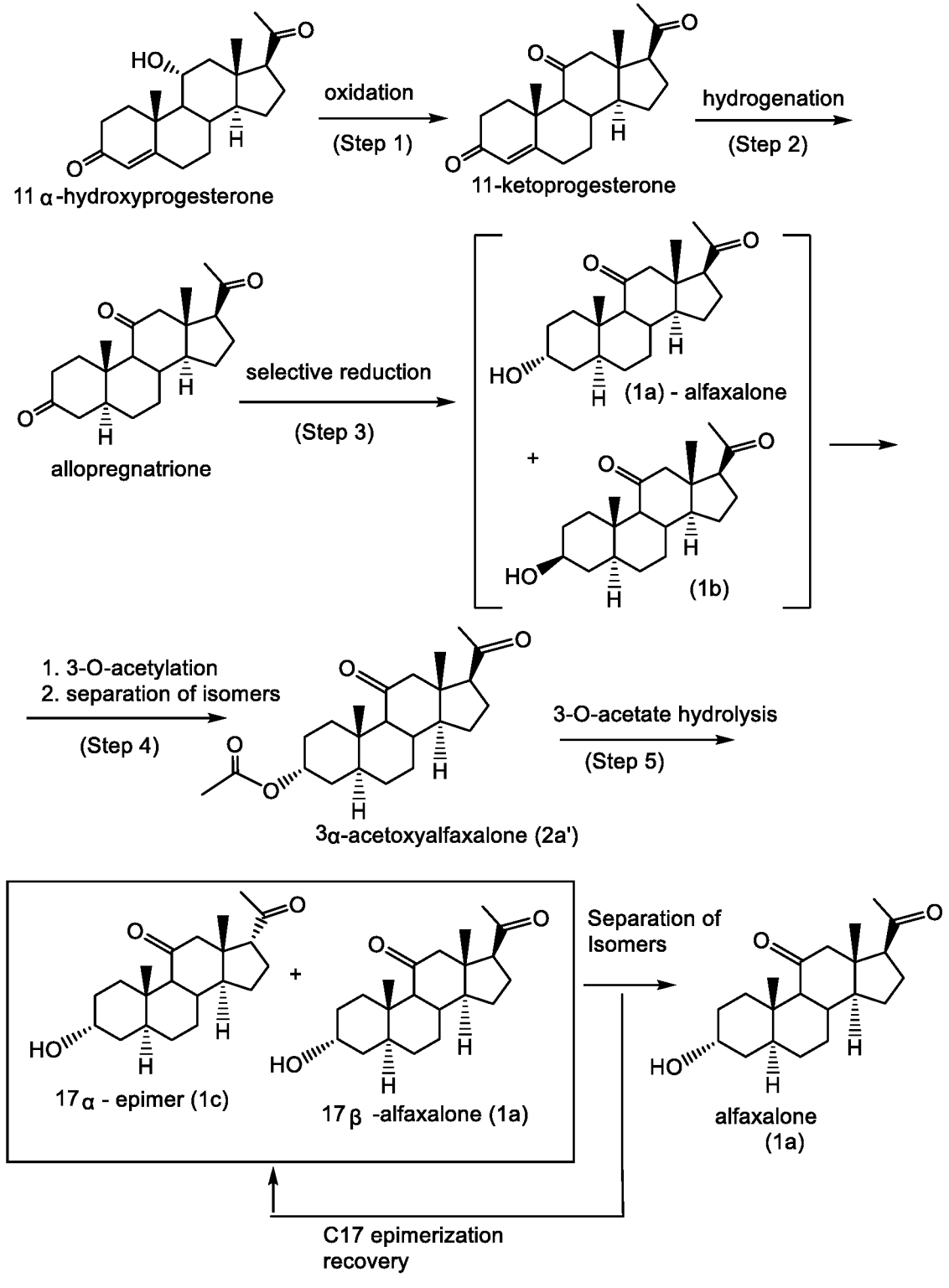
The structural numbering for alfaxalone is shown below:



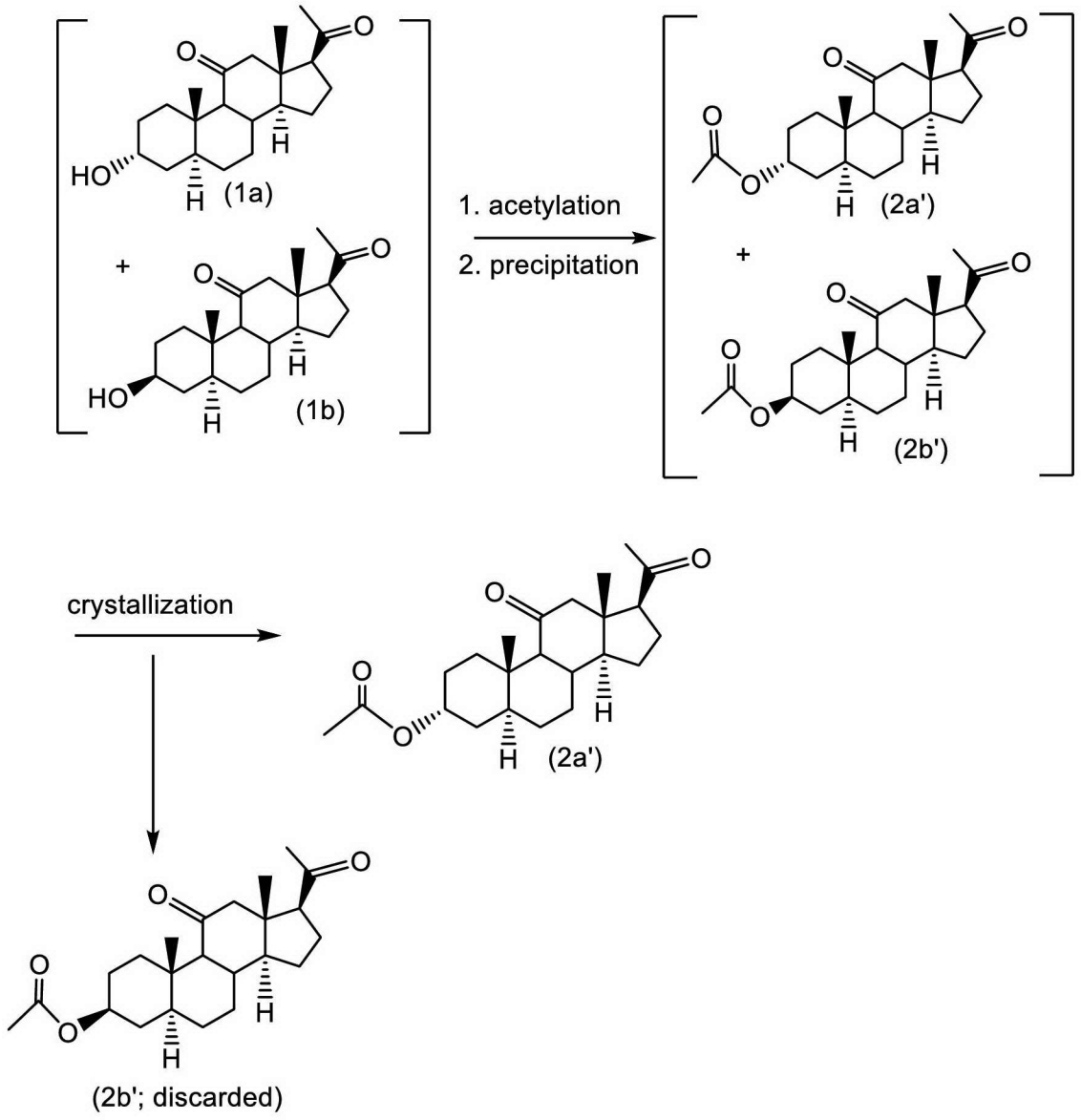
Overall, progesterone and 11-ketoprogesterone are examples of 3-keto steroids that are useful starting materials for 3-hydroxy pregnane steroids. In

particular, 11-ketoprogesterone is a useful starting material for the synthesis of some corticosteroids like alfaxalone and hydrocortisone. Synthesis of these corticosteroids requires separation of the 3α- and 3β-hydroxy isomers in a simple high yielding process to isolate an essentially pure 3α-hydroxy isomer. To achieve greater and purer yields of the 3α-hydroxy isomer, Applicant transformed the mixture of 3α- and 3β-hydroxy isomers to a mixture of 3α- and 3β-acetoxy isomers which can be selectively crystallized to essentially pure 3α-acetoxy from a solvent/antisolvent system. Depending on the hydrolysis conditions of the acetoxy isomers, further epimerization of C17 can be controlled. To overcome this epimerization at C17 mixture and to essentially acquire >97% 17β-alfaxalone, the hydrolytic conditions used to deprotect the 3-position acetate is to use a minimum of 2 mole equivalents of sodium hydroxide in a small amount of water at a lower temperature to allow equilibration to the preferred thermodynamic 17β-alfaxalone. Recrystallization from a solvent/anti-solvent system provides greater yields of the 17β enantiomer, that is alfaxalone, with about a 40% reduction in manufacturing time and less solvent usage. Alfaxalone synthesis is shown in Scheme 1.

SCHEME 1. Preparation of Alfaxalone



SCHEME 2. Preparation of 3α-acetoxyalfaxalone (2a') (Step 4 of Scheme 1)



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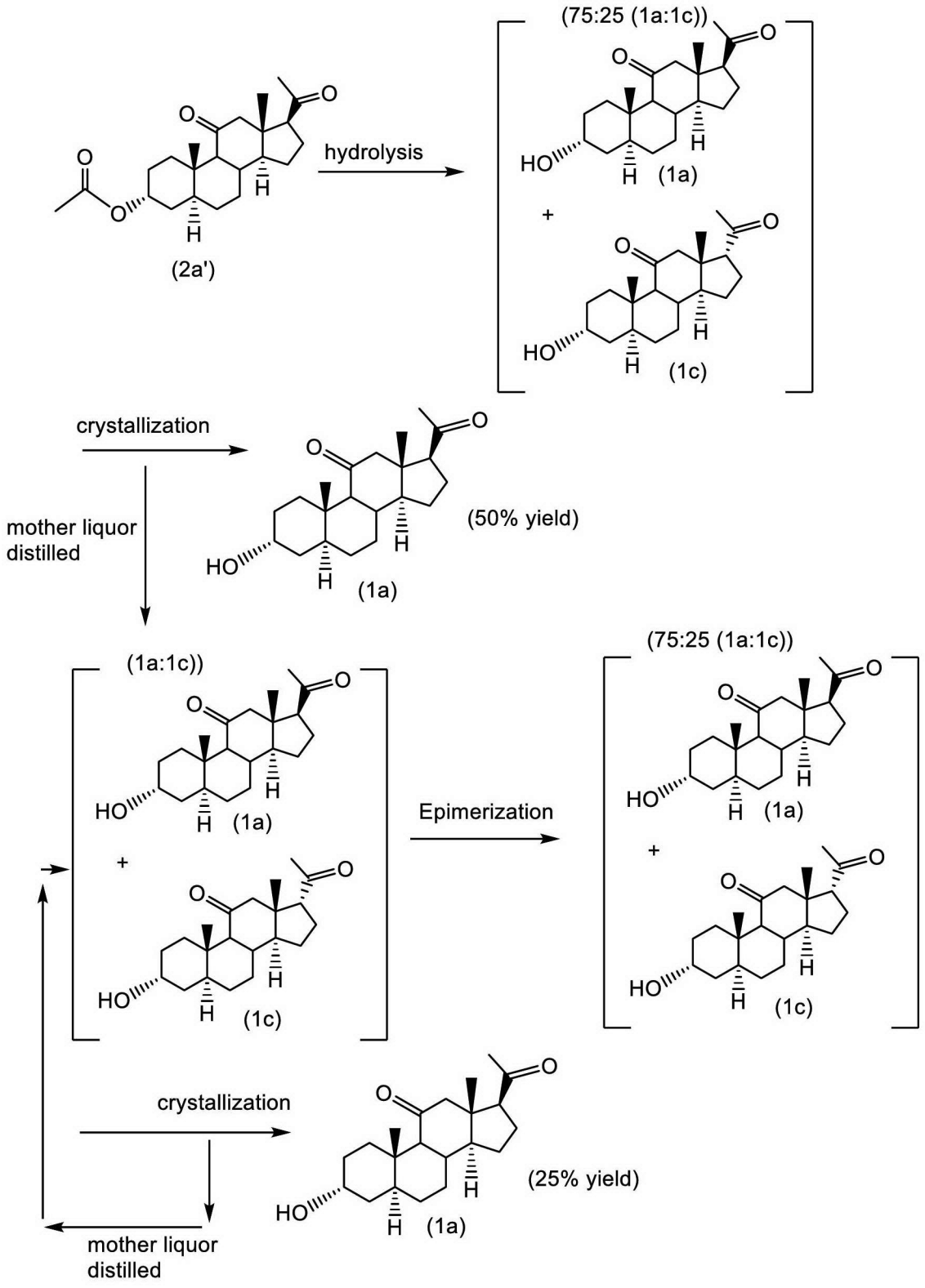
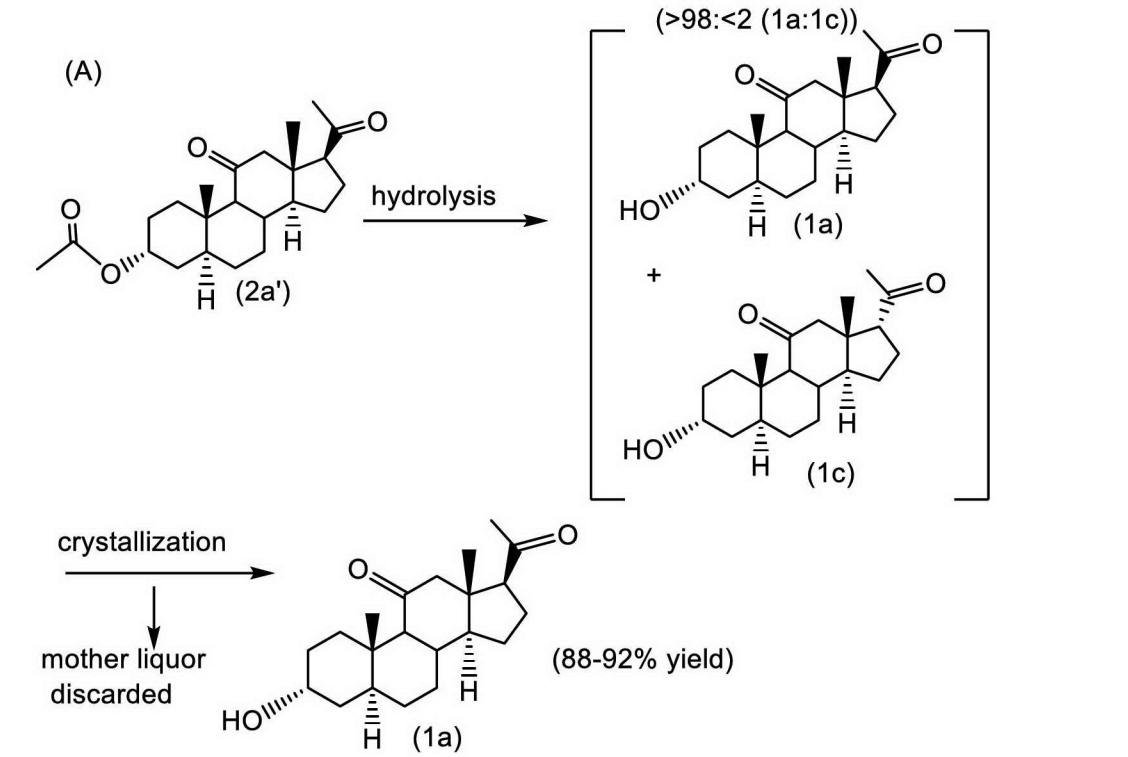
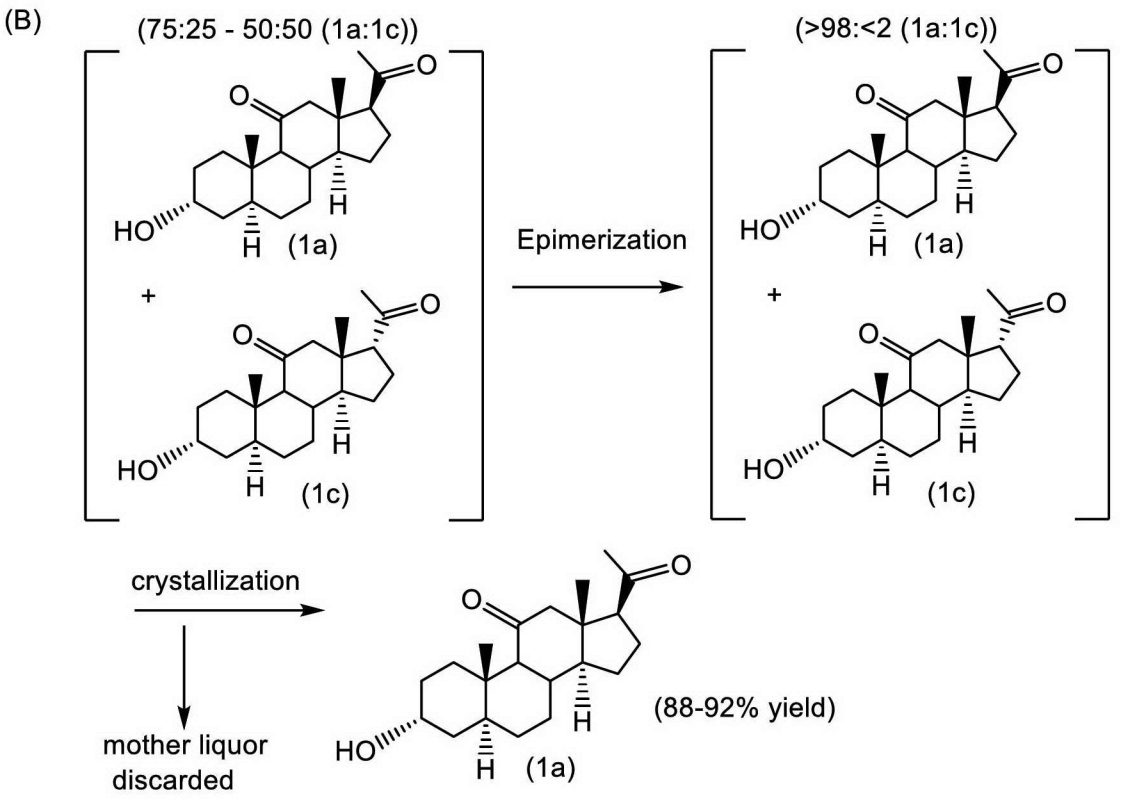
SCHEME 3. Hydrolysis of 3α-acetoxyalfaxalone and Preparation of Alfaxalone (1a) - (Step 5 of Scheme 1)



SCHEME 4. Hydrolysis of 3α-acetoxyalfaxalone (2a') and Hydrolysis of Crude 17α:17β-enantiomers (1c:1a) to Prepare Alfaxalone (1a) - (Step 5 of Scheme 1)





Overall, Step 1 describes the oxidation of 11α-hydroxyprogesterone to 11-ketoprogesterone (80-85% yield) with chromium trioxide in an aqueous acid (*e.g*. sulfuric acid, acetic acid and the like) with or without solvents (*e.g*., acetone and isopropyl alcohol). The reaction is very smooth and produces few by-products. The product is isolated by precipitation, involving dilution with water and filtration.

In Step 2, catalytic (Pd/C) hydrogenation of 11-ketoprogesterone in ethanol and/or ethyl acetate gives a 4:1 mixture of 5α-and 5β-pregnatrione (allopregnatrione). The desired 5α product is readily crystallized from a solution of ethyl acetate/ethanol with a yield of about 55-60%. The unwanted 5β-isomer stays in solution. Prior separation was conducted with column chromatography. The 5α:5β-isomer ratio has been found to depend on the pH of the reaction medium; the higher the pH the more unwanted 5β-isomer is produced. Preferably, the pH for this reaction medium is neutral. Overall, the hydrogenation is simple and has been described previously. Purification is by fractional crystallisation and yield is dictated by 5-α/β ratio formed in the reaction.

In Step 3, the 3-keto group of the pregnatrione is selectively reduced by transfer hydrogenation using a catalyst (iridium) formed in-situ using Henbest reaction conditions which selectively reduces the 3-keto group of the pregnatrione to give an 8.5:1.5 to 9.4:0.6 mixture of the 3α-hydroxy (1a) and 3β-hydroxy (1b) stereoisomers, respectively. Use of hydrogen hexa-chloroiridate (iv) hydrate, isopropyl alcohol, triethylamine trimethylphosphite and water aid in the C3 reduction reaction. These stereoisomers have similar physical properties and crystallize together from various different solvent systems. It can be extremely difficult to separate these stereoisomers by fractional crystallization. The reaction was studied extensively by varying the reaction conditions, nature of the reagents, and the pH of the medium as a means of preventing or reducing the formation of the unwanted 3β-isomer and other undesired by-products. In acidic pH (<6) conditions, the reaction is slow and results in higher impurities. An ideal pH range of 6-7 produces optimal

yield and the fewest impurities, without impacting the 3α:3β ratio. At higher pH, reaction rates are faster with higher amounts of impurities including the formation of the unwanted 3β isomer. Use of trimethylphosphite at pH 6-7 gave an approximately 8.5:1.5 to 9.4:0.6 ratio of 3α:3β-isomers in 90-95% (crude) yield. There was no significant variation of the 3α:3β ratio by changing phosphite reagent to tri-n-butylphosphite, triisopropylphosphite or triethylphosphite. Use of tris(2-chloroethyl) phosphite improved the 3α:3β ratio to 12:1. However, there was a higher percentage of impurities. Surprisingly, the 3α:3β-isomers can be separated by derivatization of the 3-hydroxy group using a three-step chemical process involving (a) acetylation of the 3-hydroxy group; (b) separation of the stereoisomers by fractional crystallization and (c) hydrolysis of the acetyl group. It will be appreciated that the acylation, separation and hydrolysis can be carried out using known methodologies.

In Step 4 (Scheme 2), the 3-hydroxy isomers are derivatized to acetate analogs which can be completed using any number of non-limiting acylating agents, for example, acetic anhydride, acetyl chloride, propionic anhydride, proprionyl chloride and the like. Preferred acetylating agents are acetic anhydride and acetyl chloride. More preferably, the acetylating agent is acetic anhydride. It will be appreciated that the acylation step will be carried out in one or more appropriate solvent(s). Preferably, the acylation step is carried out in a non-protic solvent in the presence of a base, wherein the base is also a solvent. The base may act as solvent and/or cosolvent. Non-exclusive examples of suitable solvents include tetrahydrofuran (THF), pyridine, ether, dimethyl formamide (DMF), dioxane, methanol, hexane, acetone, triethylamine (TEA), or mixtures thereof. Preferably, the acylation step is carried out in THF and pyridine. It will be appreciated that one or more catalysts may be used in the acylation reaction. The role of the catalyst is to increase the efficiency of the reaction. Examples of suitable catalysts include but are not limited to 4-dimethylaminopyridine (DMAP); 1-Hydroxy-7-azabenzotriazole (HOAt) and 1-Hydroxy-7-azabenzotriazole (HOBt). The preferred catalyst is DMAP.

It will be appreciated that the acylation reaction can be carried out under conditions and for a period of time to allow the required conversion of the alcohol to the acetate to take place. The reaction may take place at a temperature in the range of about 0-30ºC, preferably, about 15-30ºC. The time required for the acylation step will depend on a number of factors including but not limited to the percentage conversion of acylation achieved, the reaction temperature, the solvents used and whether or not a catalyst is present. For instance, the acylation reaction may take place over a period of about 5 to 40 hours. Preferably at least about 98% conversion, more preferably >98%, from the 3-hydroxy to the 3-acetoxy is achieved. It will be appreciated that the extent of the reaction may be followed using Thin Layer Chromatography (TLC) or HPLC analysis. Preferably, the acylating agent is combined with a catalyst and mixed with Formula (1a) and (1b) combined with one or more solvents. In a preferred embodiment, Formula (1a) and (1b) are dissolved in THF/pyridine (4:1) and mixed with acetic anhydride/DMAP while stirring at room temperature (about 20-25ºC) for about 20 hours to prepare the 3α- and 3β-acetoxy compounds. The reaction is very smooth and produces no other by-products or impurities. The 3α- and 3β-acetoxy compounds can be precipitated out of solution by the addition of a large amount of methanol and then hot water followed by cooling to room temperature. Yields of the 3α-isomer and 3β-isomer range from about 95-85% and 5-15%, respectively.

It will be appreciated that the separation of 3α- and 3β-acetoxy compounds can be carried out using known methodologies including but not limited to recrystallisation and chromatography. Preferably, separation of the 3α and 3β enantiomers is achieved using recrystallization, more preferably fractional crystallization. Recrystallisation may be carried out using solvents in which the crude 3α-acetoxy is preferably sparingly soluble at lower temperatures (about room temperature or below, preferably ≤25˚C and soluble at higher temperatures (temperatures at or near the boiling point of the solvent). Preferably, the 3α-acetoxy is separated from the 3β-acetoxy isomers by fractional crystallisation. Preferably, fractional crystallisation is achieved

using a solvent and an anti-solvent mixture system in which both compounds are soluble at a high temperature that is or is close to the boiling point of the solvent such that the target compound crystallizes out at a lower temperature (room temperature or below). Examples of suitable solvent systems include but are not limited to acetone and ethyl acetate and non-exclusive examples of suitable anti-solvents include hexane, pentane and heptane, including the respective isomers. Hexane isomers include n-hexane, 2-methylpentane, 3-methylpentane, cyclopentane, 2,3-dimethylbutane, and 2,2-dimethylbutane. Heptane isomers include n-heptane, 2-methylhexane, 3-methylhexane, 2,2-dimethylpentane, 2,3-dimethylpentane, 2,4-dimethylpentane, 3,3-dimethylpentane, 3-ethylpentane and 2,2,3-trimethylbutane. Pentane isomers include n-pentane, isopentane and neopentane. Preferably, the mixture of 3α- and 3β-acetoxy is dissolved in hot acetone and then hot hexane is added as an anti-solvent and the mixture is cooled to allow selective crystallization of the 3α-acetoxy. The 3α-acetoxy crystals may be collected by standard filtration methodology. The filtrate may be distilled to remove the acetone fraction followed by cooling to precipitate additional crystals of 3α-acetoxy. Preferably, the solvent system is a mixture of acetone and hexane in about a 1:2 to 1:10 ratio. The recrystallization from the mixture of acetone and hexane gives about a 70% yield of 3α-acetoxy alfaxalone as a white crystalline solid with a purity of >98%. The 3β-acetoxy isomer stays in the hexane solution.

In Step 5 (Scheme 3), the hydrolysis process is preferably carried out under basic conditions. Examples of aqueous solutions that may be used in the hydrolysis reaction include but are not limited to aqueous alkali salts including: sodium hydroxide, potassium hydroxide, lithium hydroxide, potassium carbonate, sodium carbonate and calcium carbonate. A preferred aqueous alkali salt is sodium hydroxide or potassium hydroxide. The more preferred aqueous alkali salt is sodium hydroxide. Preferably, the hydrolysis reaction further includes a solvent, more preferably a solvent in which the 3α- and/or 3β-acetoxy is soluble. Examples of suitable solvents include but are not limited to alcohols, such as methanol, ethanol, isopropanol, n-propanol or

mixtures thereof. The preferred hydrolysis solvent is methanol or ethanol. It will be appreciated that the hydrolysis reaction may be carried out at 10°C to reflux and that the extent of the hydrolysis reaction may be followed by standard methodologies, *e.g.*, TLC or HPLC.

In one aspect, the 3α- and/or 3β-acetoxy is hydrolysed with an aqueous solution of sodium hydroxide, more preferably the 3α- and 3β-acetoxy is dissolved in methanol or ethanol and heated under reflux in the presence of aqueous sodium hydroxide until hydrolysis is complete. Hydrolysis of the 3α-acetoxy alfaxalone with aqueous sodium hydroxide (1 mole equivalent) yields alfaxalone in two enantiomeric forms, the 17β-isomer (*i.e.*, Formula (1a)) and the undesired 17α-isomer (Formula (1c)) due to the base catalyzed isomerization at the C17-position of the molecule. This leads to an enantiomeric ratio that is about 75:25 of the 17β:17α-enantiomers that requires further purification and selective crystallization of the precipitant to increase the 17β-alfaxalone yield. Selective crystallization of the precipitated 75:25 mixture can be carried out to purify mixtures of the 3α-hydroxy-17α- and 3α-hydroxy-17β-compounds that form in the hydrolysis reaction to obtain the 17β-alfaxalone. This selective recrystallization process yields about 50% of the wanted 3α-hydroxy-17β-compound (*i.e.*, alfaxalone). It will be appreciated that a variety of solvents (a solvent to dissolve and an anti-solvent to recrystallize) may be used in the recrystallization steps. Non-limiting examples of suitable solvents include: acetone, ethyl acetate, ethanol and methanol; and non-limiting examples of anti-solvents include: water, pentane, heptane and hexane. A preferred solvent/anti-solvent mixture for a 17β:17α enantiomeric ratio of 75:25 is acetone and hexane. The desired 17β-isomer readily crystallizes from the mixture of acetone and hexane (1:4 or 2:5) to give pure alfaxalone which can be separated by basic filtration techniques. As described, the 1 mole equivalent of sodium hydroxide produces the 17β:17α-enantiomers in a ratio of 75:25 with an overall yield of about 50%. Once the 17β-alfaxalone crystals have been collected after crystallization, the mother liquor still containing 17α- and 17β-isomers (1:1) can be re-treated, after distillation of the

solvents, and then by the further addition of the hydrolysis reagents (*i.e.*, 1 mole aqueous sodium hydroxide in methanol or ethanol and water to promote further epimerization of the 17α-enantiomer to 17β-alfaxalone. Each successive cropping produces the 17β:17α-enantiomers in the 75:25 ratio with subsequent yields each being about 1:1 of the two isomers. Multiple croppings can be conducted using these conditions to increase the yield of 17β-alfaxalone to >97%. Further, at any cropping stage, the 17α- and 17β-isomeric precipitated mixture can be epimerized in a mixture of methanol, water and 2 mole equivalents of sodium hydroxide to yield a mixture of the 17α- and 17β-isomers in a ratio of <2:>98, respectively; which can then be recrystallized from a solvent/anti-solvent to yield 17β-alfaxalone with a yield of about 88-92%, thereby eliminating the need for multiple cropping steps (Scheme 4B).

Alternatively, to eliminate the cropping procedure as described above, if the hydrolysis reactants, particularly the mole equivalent of sodium hydroxide is increased to 2 mole equivalents, the hydrolysis reaction of the 3α-acetoxyalfalone produces the 17β-isomer and the 17α-isomer in a ratio of >98:<2, respectively (Scheme 4A). This mixture is then recrystallized from a solvent/antisolvent system to prepare 17β-alfaxalone (1a) in a yield of about 88-92%. The precipitated 17β- and 17α-enantiomers can be recrystallized from the mixture of acetone and hexane (1:4 or 2:5), or preferably ethanol and water (1:1 to 1:2) or methanol and water (1:1 to 1:2) to give pure alfaxalone which can be separated by basic filtration techniques. The preferred hydrolysis reaction of the 3-acetoxyalfaxalone is to dissolve the 3α-acetoxyalfaxalone in methanol or ethanol, preferably methanol, in aqueous sodium hydroxide (2 mole equivalents) to prepare the 17β-alfaxalone and 17α-enantiomer in a ratio of >98:<2, respectively, and then recrystallizing this enantiomeric mixture from ethanol and water to yield 3α-17β-alfaxalone. This material meets the British Pharmacopeial (2019) requirement for alfaxalone.

Experimental

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

All reactions were performed under normal laboratory condition and the progress of the reactions were monitored by thin layer chromatography (TLC) on pre coated silica gel TLC plates or pre coated reverse phase C_{18} bonded TLC plates or by HPLC. The products were isolated by precipitation with adding water as an anti-solvent and by filtration. All products were purified by recrystallization. Melting points were determined with an electrothermal digital melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectrums were recorded at the Department of Chemistry at University of New South Wales using a Bruker AC300F spectrometer operating at 300 MHz and 75 MHz respectively; or at the Department of Chemistry at the University of Newcastle using Bruker NMR spectrometer operating at 300 MHz and 75 MHz respectively. The purities were determined by HPLC analysis and recorded related to the British Pharmacopeial (2019) quality reference standard. Technical grade reagents and solvents were used unless specified.

Example

Step 1. Oxidation of 11-hydroxyprogesterone:

To a solution of 11α-hydroxyprogesterone (11-α-hydroxy-4-pregnene-3,20-dione) (25 kg) in 95 L of 90% acetic acid was added a solution of chromium trioxide (12.5 kg) in 25 L of water very slowly with cooling. The initial reaction was exothermic and therefore, care was taken to control the rate of addition of chromium trioxide solution to maintain the reaction temperature below 70°C. The mixture was stirred at room temperature overnight. Water (420 L) was added and allowed to crystallize the product. This was stored in the cold room for two days and filtered using basket centrifuge. The product

was washed with water several times to remove residual chromium oxide impurities. Drying in open air gave 11-ketoprogesterone as a white powder. Typical yield ~ 80-82 %. The crude product was sufficiently pure and further purification was not necessary.

Step 2. Preparation of 5-α-pregnane-3,11,20-trione (allopregnatrione) by selective reduction:

To a solution of 1.65 kg 11-ketoprogesterone in 27 L of ethyl acetate and 13 L of 96% ethanol was added 8.0 g of 10% palladium on activated carbon and hydrogenated. The reaction mixture was degassed using an aspirator for 30 minutes and then connected to a hydrogen supply line, and the hydrogen pressure was adjusted slightly higher than atmospheric pressure. Stirring was carried out vigorously until all the 11-ketoprogersterone hydrogenated (~1 day). The reaction mixture was warmed to dissolve the precipitated product and filtered using a 30cm disc filter and an S-10 packed filter pad. The filtrate was allowed to cool to room temperature and then stored in the cold room (at 4°C) overnight. The allopregnatrione crystallized out as a white crystal. The crystals were collected by filtration and washed with cold ethyl acetate/ethanol (30%) (the solvent recovered from fractional distillation was used for washing). The product was allowed to dry in a ventilated area or in a drying oven. The filtrate (mother liquor) was concentrated to one third of its volume by distillation and stored in the cold room for several days and filtered. This gave a second crop of allopregnatrione. Typical yield was about 55-60%. The procedure was repeated several times to convert all of the 11α-hydroxyprogesterone to the allopregnatrione.

Step 3: Preparation of Crude Mixture of 3α- and 3β-hydroxy-5α-pregnane-11,20-dione

Chloroiridic acid (12 g) and trimethylphosphite (4.85 L) in 28 L of isopropanol and 4.7 L of water were heated under reflux for 20 hours. The solution was cooled to about 50 to 60°C and the pH of the solution was

adjusted to pH ~6.5-7 by adding triethylamine (5.17 L). Allopregnatrione (4.6 kg) was added and the reaction was heated to reflux for 24 hours. The progress of the reaction was monitored by TLC and HPLC. About 15L of the solvent was removed by distillation and then the concentrated reaction mixture was transferred to a 100L stainless steel container. Water (60L) was added slowly with stirring. The mixture was allowed to cool to room temperature and then stored in the cold room overnight. The white crystalline solid was filtered using a basket centrifuge, washed with water several times to remove residual solvents and dried in a hot air drying oven. Typical yield was about 95%. HPLC analysis showed 9:1 mixture of 3α and 3β isomers. A small sample was recrystallized from the mixture of acetone and hexane (1:4) to give needle like crystals with a melting point of 165-166°C.

Step 4. Preparation of 3α-acetoxy-5α-pregnane-11,20-dione (3α-acetoxyalfaxalone (2a')):

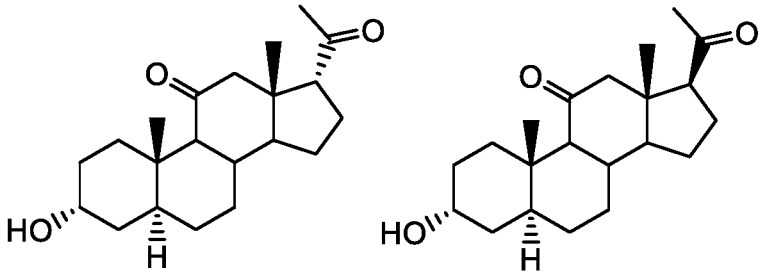
In a very dry 50 L glass reaction vessel, 3-α/β-hydroxy-5α-pregnane-11,20-dione (5.7 kg; a 9:1 mixture of 3α and 3β isomers) was dissolved in 12L of pre-cooled tetrahydrofuran and 2.76 L of pre-cooled pyridine. To this was added 2.5 L of acetic anhydride and 18.08g of 4-dimethylaminopyridine (DMAP) and the mixture was stirred at room temperature for 20 hours. The progress of the reaction was monitored using TLC. 2.7 L of methanol was added and stirred at room temperature for an hour. The reaction mixture was added to a 145 L of hot water with vigorous stirring. The mixture was allowed to cool to room temperature and then stored in the cold room for 1-2 days. The white oily precipitate formed was collected by filtration and washed with water several times. The crude acetoxy mixture was allowed to dry in the fume cupboard and then dried in a hot air drying oven. The product was purified by fractional crystallization as described below.

The dried crude product was dissolved in 8 L of hot acetone and then 54 L of hot hexane was added with stirring. The hot solution was allowed to cool to room temperature slowly and then stored in the cold room for two days.

The crystals were collected by filtration. The filtrate was distilled to remove the acetone fraction (~20 L) and allowed to cool to room temperature and then stored in the cold room. The second crop of product was obtained by filtration. Combination of first and second crops gave 5.1 kg of 3α-acetoxy compound. The product was analyzed by reverse phase TLC, ^{1}H NMR and HPLC and revealed that the ratio of 3α:3β was >99:1. The purity of 3α-acetoxyalfaxalone was calculated as >98%.

Step 5. Hydrolysis of 3α-acetoxyalfaxalone (2a'):

To a suspension of the 3α-acetoxyalfaxalone (4.4 kg) in 44 L of methanol was added 5.0L of 10% sodium hydroxide (1.0 mole equivalent) and heated at reflux for 3-4 hours (until all the acetate hydrolyzed). The progress of the reaction was tested by TLC and then 20L of methanol was distilled off. The reaction mixture was transferred to a larger container and stirred at room temperature overnight. 80 L of reverse osmosis (RO) water was then added with stirring and allowed to cool in the cold room for two days. The precipitate was filtered using a basket centrifuge and washed with water several times. The product was collected and dried in a hot air drying oven. TLC analysis of the product showed a mixture of the 17α and the 17β optical isomers.



17α-enantiomer (1c) 17β-alfaxalone (1a)

The isolated precipitate mixture of 17α- and 17β-enantiomers can be further enriched to >95% 17β-alfaxalone by dissolving the precipitate in methanol or ethanol and aqueous sodium hydroxide (2.0 mole equivalents) as a 10% w/w solution at room temperature. An example being, alfaxalone precipitate (2.0 kg, in any 17α and 17β ratio) is dissolved in methanol (7.2 kg)

and treated with an aqueous 10% sodium hydroxide solution (0.47 kg sodium hydroxide in 7.4 kg RO water), the reaction mixture is heated to reflux for 1 hour and then permitted to cool to <20°C for an adequate amount of time to allow it to equilibrate (*e.g.*, overnight). The cooled reaction slurry is charged with RO water at <30°C and aged with agitation for 1 hour, before filtration through a Buchner funnel or centrifuge. The filter cake is washed thoroughly with water and then dried in an oven at 60°C. Typically, yield recovery is >97% for 17β-alfaxalone. The same yield outcome is obtained when methanol is replaced with ethanol. Where 1.0 mole equivalent of sodium hydroxide is used (0.24 kg) with the same mass inputs for alfaxalone precipitate (17β-alfaxalone and 17α-enantiomer) and solvents and the same process, the ratio of 17β-alfaxalone to 17α-enantiomer is 75:25.

Crystallization steps for alfaxalone, as described herein, were conducted in the following manner. The crude product (17β-alfaxalone and 17α-enantiomer (75:25)) was dissolved in a minimum amount of hot acetone (approximately 20 L) and the hot solution was filtered through 10S disc filter pad. The filter pad was washed with 2-3 L of acetone. The solution was concentrated to ~ 15L and 60 L of hot hexane was added and the solution was allowed to cool to room temperature and then stored in the cold room overnight. Gentle agitation has been found to reduce the co-crystallisation of the unwanted isomer (17α-enantiomer) with alfaxalone. Fine needle like crystals were collected by filtration. The mother liquor containing a mixture of 3α-hydroxy-17α (unwanted) and 3α-hydroxy-17β alfaxalone (wanted) was evaporated and the residue was dissolved in 8.8 kg methanol and stirred with 400 mL of 10% sodium hydroxide solution for 24 hours. The alfaxalone was precipitated by adding water and collected by filtration. Drying and recrystallization as above gave pure alfaxalone. This was combined with first crop. Similarly, the mother liquor was evaporated and the residual was dissolved in methanol, 10% sodium hydroxide was added and the mixture was stirred overnight. Water was then added and the solution filtered and the product dried. The product was purified by recrystallization as above to collect

a third crop. This procedure was repeated with the mother liquor for two more times to collect fourth and fifth crop (*i.e.*, cropping). The purity of the alfaxalone was analyzed using HPLC according to the British Pharmacopoeia (veterinary) standard methods. The purity of the alfaxalone was ~96.4%. The amount of the 17α-isomer was <0.1%. The product may optionally be recrystallized again from acetone and hexane. Alternatively, the mother liquor (of any crop) can be reduced by 70% of the initial volume, cooled to <30°C, optionally, anti-solvent added, and aged for 3 hours before isolation by filtration and dried.

Alternatively, hydrolysis of the 3α-acetoxyalfaxalone in Step 5 can be conducted using different hydrolysis conditions to produce the desired 17β-alfaxalone drug product as the major product and to minimize the 17α-enantiomer and thereby limit the crystallization and cropping steps, thereby reducing the need for additional solvent use/waste and providing a more cost effective route for making the 17β-alfaxalone drug product.

Rather than using the procedure described above, that results in a 75:25 (17β:17α) ratio with a yield of about 50% for the first crop; the method can be altered using a short chain alcohol (such as methanol or ethanol) with increased sodium hydroxide (2 mole eq.) and water and then heated to >55°C for >10 minutes before cooling to <25°C and aging the mixture at this temperature for an adequate amount of time to allow it to equilibrate before precipitation with water. The hold time is critical to the ratio of 17-α/β-enantiomers, to allow the equilibration to prefer the 17β-enantiomer (alfaxalone). Employing the optimised reaction conditions provides >97% of the 17β-alfaxalone after the precipitation step, without the need for further recrystallisation and cropping. Where the product is enantiomerically enriched an alternate high yielding and safer crystallising solvent using an alcohol (methanol or ethanol) and water versus acetone and hexane can be used to recrystallise and further purify the product. This optimised method can be used to prepare alfaxalone in a higher yield with less solvent/anti-solvent usage which also has a time savings of around 40%.

An example of the optimised conditions, using methanol or ethanol, to a suspension of the 3α-acetoxyalfaxalone (660 g) in 1.5 L of methanol was heated to >60°C/reflux and charged with 20% aqueous sodium hydroxide (sodium hydroxide (150 g) in RO water (760 g)). Mixture was held at temperature for 20 minutes and then cooled to 20°C. As the reaction cools, the 17β-enantiomer begins to precipitate out of solution in preference to the 17α-enantiomer. The slurry mixture was held at this temperature for a minimum of 12 hours, before RO water (12 kg) was added to complete precipitation and substantially provide the desired 17β-enantiomer. The precipitate was isolated by filtration, washed with RO water and dried. The material analysed was found to have >98% 17β-alfaxalone (1a) by HPLC analysis. The alfaxalone precipitate was recrystallised from hot ethanol (3 mass equivalents) and hot water (3 to 4.5 mass equivalence) and the mixture, then, permitted to stir slowly and cool to (10°C) overnight. Product was isolated by any filtration means, washed with cold ethanol:water and then dried. The purity of the alfaxalone was analyzed using HPLC to afford >99% and abides to the British Pharmacopeial 2019 quality. The overall yield (over two isolatable steps) of this optimised process is 88-92%. Additional material can be isolated from the filtrate, if desired, by reducing the mother liquor volume with distillation (atmospheric or under vacuum) to cause (1a) and (1b) to precipitate out. The precipitate can be isolated by filtration, dried and further enriched to Formula (1a) using the enrichment process described above. The product was characterized by ^{1}HNMR, ^{13}CNMR and MS spectra. Formula (1a) has a melting point of 172-173°C with a specific rotation $[\alpha]_D$ of 110.11 at 18ºC.

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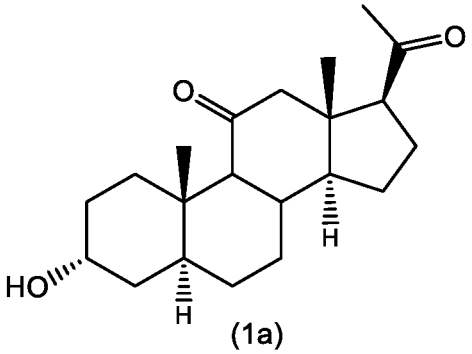
We Claim:

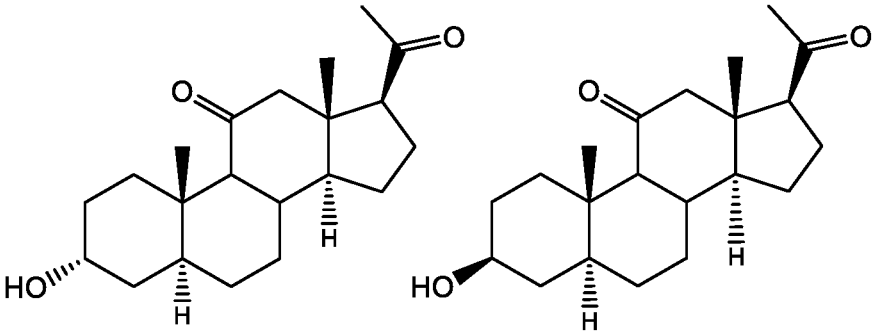
1. A process for preparing the compound of Formula (1a), alfaxalone,



(1a)

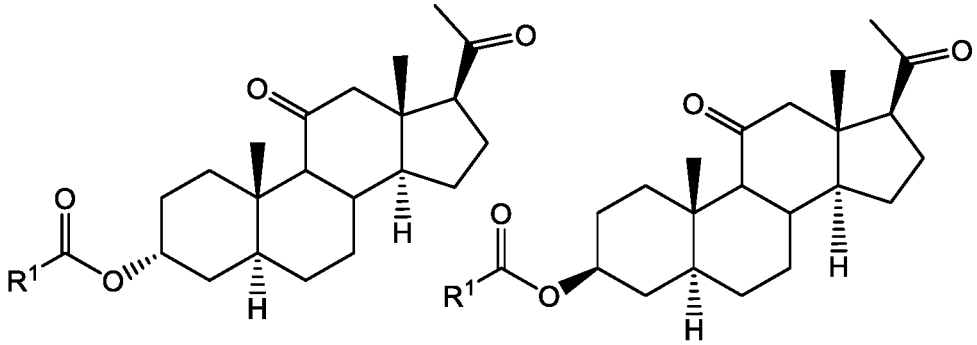
comprising:

a) treating the mixture of the 3α-hydroxy Formula (1a) and 3β-hydroxy Formula (1b) compounds



(1a) and (1b)

with an acylating agent in the presence of a catalyst in at least one non-protic solvent to form a mixture of acetylated 3α Formula (2a) and 3β Formula (2b) compounds



(2a) and (2b)

wherein R^1 is a substituted or unsubstituted alkyl, wherein the alkyl can be substituted with at least one or more of substituents selected from hydroxy, chloro and oxo;

b) separating the Formula (2a) and Formula (2b) compounds to obtain substantially single stereoisomers thereof;

c) hydrolysing the acetylated compound of Formula (2a) under basic conditions in one or more solvents to form the Formula (1a) compound as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers, and optionally,

d) enantio-enrichment of the enantiomer mixture comprising the epimerization of the 17α-enantiomer in an aqueous basic solution comprising 1-2 mole equivalents of sodium hydroxide or potassium hydroxide in methanol and water or ethanol and water and then recrystallizing the resulting product from a mixture of acetone and hexane, methanol and water or ethanol and water to obtain >95% of the 17β-enantiomer.

2. The process of claim 1, wherein the acetylation in Step (a) is conducted using an acetylation agent selected from the group consisting of acetic anhydride, acetyl chloride, propionic anhydride and proprionyl chloride; and wherein the non-protic solvent in Step (a) is selected from the group consisting of tetrahydrofuran, pyridine, ether, dimethyl formamide, dioxane, triethylamine or mixtures thereof; and wherein the catalyst is selected from the group consisting of 4-dimethylaminopyridine, 1-hydroxy-7-azabenzotriazole and 1-hydroxy-7-azabenzotriazole.

3. The process of claim 2, wherein the acetylation agent is acetic anhydride or acetyl chloride and the non-protic solvent is a mixture of tetrahydrofuran and pyridine.

4. The process of claim 3, wherein the acetylation agent is acetic anhydride and the ratio of tetrahydrofuran to pyridine is about 4:1.

5. The process of claim 4, wherein the catalyst is 4-dimethylaminopyridine.

6. The process of claim 1, Step (b), wherein Formula (2a) is separated from Formula (2b) by dissolving the compounds with ethyl acetate or acetone and then recrystallizing the compounds from a solvent and anti-solvent mixture, wherein the solvent is acetone and the anti-solvent is hexane in an amount with a ratio of about 1:2 to 1:10, respectively, and then filtering the solvent/anti-solvent mixture to obtain the Formula 2(a) compound.

7. The process of claim 1, wherein the hydrolysis in Step (c) is completed with the use of an alkali salt selected from the group consisting of potassium hydroxide, sodium hydroxide, lithium hydroxide, sodium carbonate, potassium carbonate and calcium carbonate and the solvent is selected from acetone, methanol or ethanol.

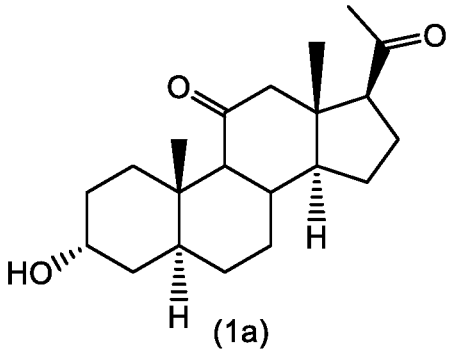
8. The process of claim 7, wherein the alkali salt is sodium hydroxide and wherein the mole equivalent of sodium hydroxide is at least 1 to 2 moles to the amount of Formula (1a).

9. The process of claim 1, wherein the process further comprises Step (d), and wherein the enantio-enrichment to the 17β-enantiomer is completed by the epimerization of the 17α-enantiomer in a 1 or 2 mole equivalent solution of sodium hydroxide in methanol and water and wherein the 17β product is recrystallized from a mixture of acetone and hexane, methanol and water or ethanol and water.

10. The process of claim 9, wherein the mixture of acetone and hexane is in an amount with a ratio of about 1:4 or about 2:5; the mixture of methanol and water or ethanol and water, are each in an amount with a ratio of about 1:1 to 1:2, respectively.

11. The process of claim 10, wherein the 17β product is recrystallized from a mixture of methanol and water or ethanol and water, each in an amount with a ratio of about 1:1 to 1:2, respectively, and preferably 1:1.5.

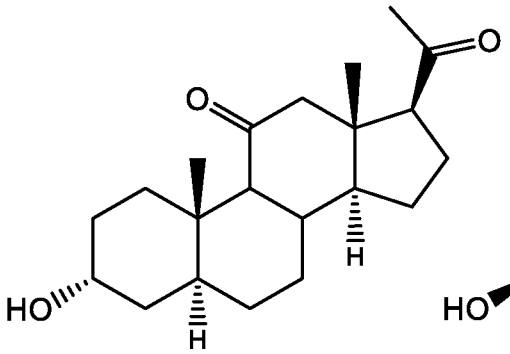
12. A process for preparing the compound of Formula (1a), alfaxalone,



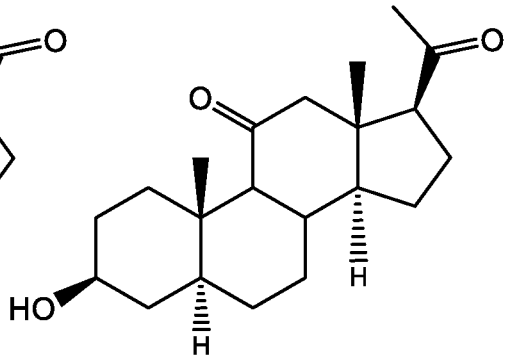
(1a)

comprising:

a) treating the mixture of the 3α-hydroxy Formula (1a) and 3β-hydroxy Formula (1b) compounds

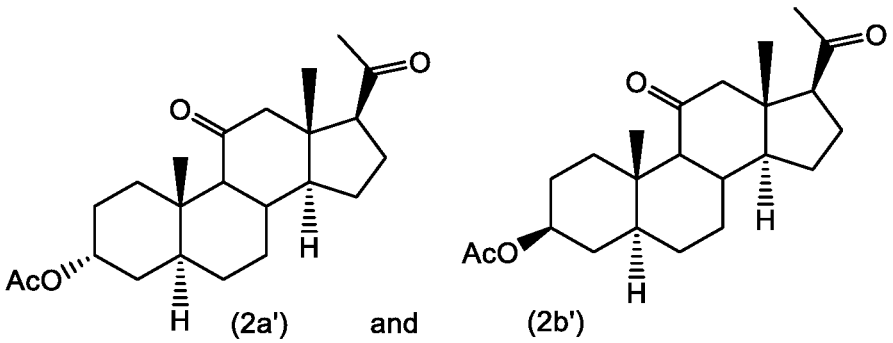


(1a) and



(1b)

with acetic anhydride in a solvent mixture of tetrahydrofuran and pyridine, in the presence of a catalyst that is 4-dimethylaminopyridine; at a temperature of about 15-30°C for about 5 to 40 hours; to form a mixture of 3-acetoxyalfaxalone compounds of Formula (2a') and Formula (2b')



(2a') and (2b')

b) separating the Formula (2a') and Formula (2b') compounds to obtain substantially single stereoisomers by fractional crystallization in a mixture of acetone and hexane in a ratio of about 1:2 to about 1:10;

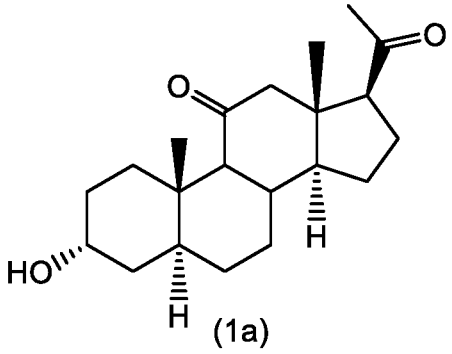
c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of a 1 mole equivalent of sodium hydroxide to the amount of Formula (2a') in methanol and water or ethanol and water, to prepare the Formula (1a) compound as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and optionally,

d) enantio-enrichment of the enantiomers in an aqueous solution comprising 1 or 2 mole equivalents of sodium hydroxide in methanol and water or ethanol and water and recrystallizing the resulting product from a mixture of acetone and hexane, methanol and water or ethanol and water, to obtain >95% of the 17β-enantiomer.

13. The process of claim 12, further comprising Step (d), and wherein the sodium hydroxide in Step (d) is in the amount of about 1 mole equivalent and the 17β product is recrystallized from a mixture of acetone and hexane in an amount with a ratio of about 1:4 or 2:5.

14. The process of claim 13, wherein the sodium hydroxide in Step (d) is 2 mole equivalents and the product is recrystallized from a mixture of methanol and water or ethanol and water; each in an amount with a ratio of about 1:1 to 1:2, and preferably about 1:1.5.

15. A process for preparing the compound of Formula (1a), alfaxalone,



(1a)

comprising:

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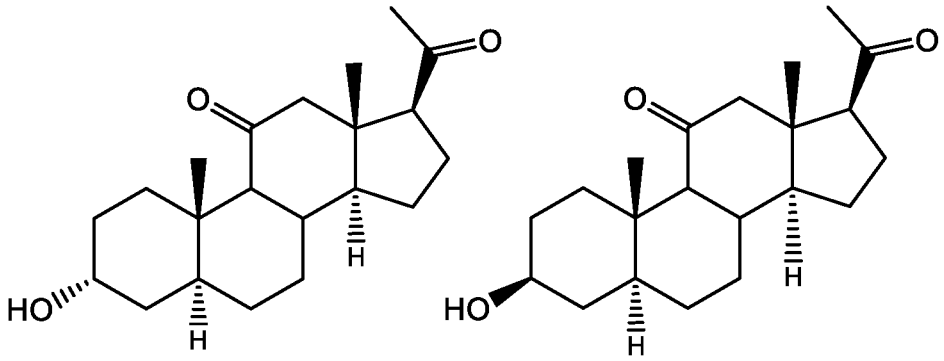
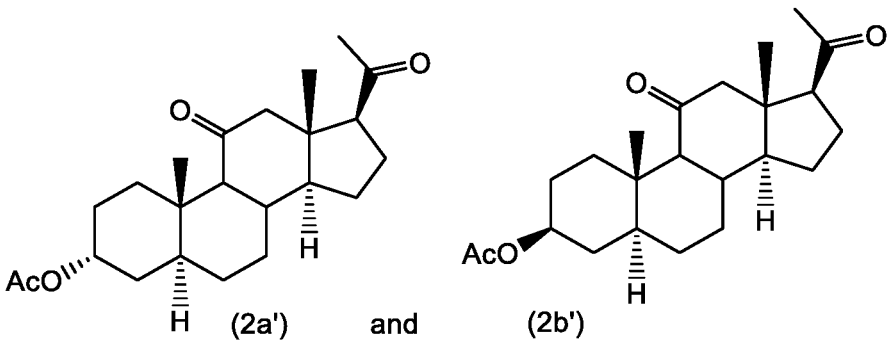


(1a) and (1b)

with acetic anhydride in a solvent mixture of tetrahydrofuran and pyridine in a ratio of about 1:4, in the presence of a catalyst that is 4-dimethylaminopyridine; at a temperature of about 15-30ºC for about 5 to 40 hours; to form a mixture of 3-acetoxyalfaxalone compounds of Formula (2a') and Formula (2b')



(2a') and (2b')

b) separating the Formula (2a') and Formula (2b') compounds to obtain substantially single stereoisomers by fractional crystallization in a mixture of acetone and hexane in a ratio of about 1:2 to about 1:10;

c) hydrolysing the Formula (2a') compound under basic conditions using an aqueous solution of sodium hydroxide in 2 mole equivalents to the Formula (2a') compound in methanol and water to form the Formula (1a) compound as a substantially single stereoisomer which comprises a mixture of 17α- and 17β-enantiomers; and

d) enantio-enrichment of the enantiomers by recrystallizing the 17β product in a mixture of methanol and water or ethanol and water, each in an amount with a ratio of about 1:1 to 1:2, respectively, and preferably 1:1:1.5.