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(54) 【発明の名称】 21-ヒドロキシ-6, 19-オキシドプロゲステロン (21OH-6OP) の製造方法

(57) 【要約】

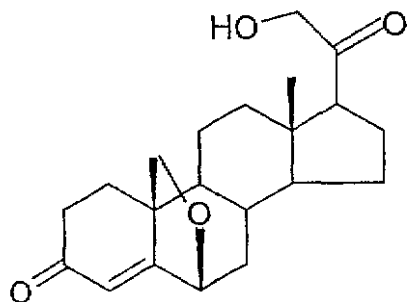
本発明は、21-ヒドロキシ-6, 19-オキシドプロゲステロン (21OH-6OP)、及び／又はその21-アセテート、21-プロピオネート、21-ヘミサクシネート、21-ホスフェート、21-オレエート誘導体の新しい製造方法に関する。21OH-6OP及びそのエステル誘導体は、グルココルチコイド過剰に関連した病気の治療又は予防、特にクッシング症候群、医原性高コルチゾール症、又はうつ病の治療のための抗グルココルチコイド物質である。

【特許請求の範囲】

【請求項 1】

以下の 21-ヒドロキシ-6, 19-オキシドプロゲステロン (1)、

【化 1】



(1)

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あるいはそのアシル又はホスフェート誘導体の製造方法であって、以下のステップ：

- a) 21-アセトキシプレグネノロンを準備し；
 - b) 21-アセトキシプレグネノロンの C-3 ヒドロキシ基を不安定なエステルの形成により保護し；
 - c) 上記エステル保護された 21-アセトキシプレグネノロンの C5-C6 の位置の二重結合への臭素及びヒドロキシ基の付加によりそのプロモヒドリン産物を得；
 - d) 照射下、「スアレス試薬」(ジアセトキシヨードベンゼン) 及びヨウ素を用いて C19 原子による分子内の環化を実施し、それにより骨格内に 6, 19 オキシド架橋を得、
 - e) 選択的な加水分解を実施し、続いて、酸化を実施して、プロモケトンを得、
 - f) ステップ e) から得られたプロモケトンの加水分解を実施して、21-ヒドロキシ-6, 19-オキシドプロゲステロン (1) を得；そして、場合により
 - g) 化合物 (1) をアシル化剤又はリン酸化剤と反応させる、
- を含む上記製造方法。

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【請求項 2】

21-アセテート誘導体が、ステップ f) の 21-ヒドロキシ-6, 19-オキシドプロゲステロン (1) のアセチル化により得られる、請求項 1 に記載の方法。

【請求項 3】

C1-18 アシル誘導体が、C1-18 の酸、無水物、活性エステル、又は塩化アシルを用いた 21-ヒドロキシ-6, 19-オキシドプロゲステロン (1) のエステル化により得られる、請求項 1 に記載の方法。

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【請求項 4】

21-プロピオネート、21-ヘミサクシネート、21-ホスフェート、21-オレエート誘導体が、プロピオン酸、コハク酸、オレイン酸、又はそれらの無水物、活性エステル、若しくは酸塩化物を用いたステップ f) の 21-ヒドロキシ-6, 19-オキシドプロゲステロン (1) のエステル化により得られる、請求項 1 に記載の方法。

【請求項 5】

前記 21-ホスフェート誘導体が、リン酸化剤を用いたステップ f) のオキシドプロゲステロン (1) の反応により得られる、請求項 1 に記載の方法。

【請求項 6】

ステップ b) に関して、ギ酸エステルを与える、請求項 1 ～ 5 にいずれか 1 項に記載の方

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法。

【請求項 7】

前記分子内の環化ステップ d) を、溶媒としてのジクロロメタン中で実施する、請求項 1～6 のいずれか 1 項に記載の方法。

【請求項 8】

前記ステップ e) における選択的な加水分解を、溶媒としての MeOH / ジクロロメタン中で HCl を用いて実施する、請求項 1～7 のいずれか 1 項に記載の方法。

【請求項 9】

前記ステップ e) における酸化を、塩化クロム酸ピリジニウム (PCC) を用いて実施する、請求項 1～8 のいずれか 1 項に記載の方法。

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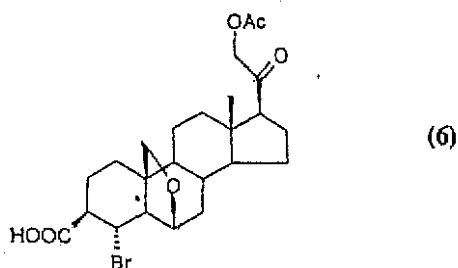
【請求項 10】

前記ステップ f) における加水分解を、溶媒としての MeOH / ジクロロメタン中で KOH を用いて実施する、請求項 1～9 のいずれか 1 項に記載の方法。

【請求項 11】

以下の (6) :

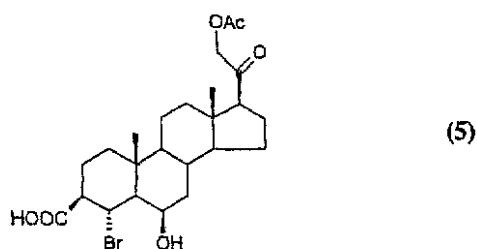
【化 2】



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で表される分子の製造方法であって、以下の分子 (5) :

【化 3】



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の 6-OH と C₂₁ の間の分子内の環化を実施するステップを含む上記製造方法。

【請求項 12】

前記の分子内の環化を、DIBAL 及び I₂ を用いた光化学反応により行う、請求項 11 に記載の方法。

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【請求項 13】

前記の分子内の環化を、CH₂Cl₂ 中で行う、請求項 11 又は 12 に記載の方法。

【発明の詳細な説明】

【0001】

本発明の分野：

本発明は、21-ヒドロキシ-6, 19-オキシドプロゲステロン (21OH-6OP) 及び／又はその 21-アセテート、21-プロピオネート、21-ヘミサクシネート、21-ホスフェート、及び 21-オレエート誘導体の新しい製造方法に関する。21OH-

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6 O P 及びそのエステルは、グルココルチコイド平衡失調に関係する病気の治療又は予防、特にクッシング症候群又はうつ病を治療するための抗グルココルチコイド物質である。

【0002】

本発明の背景：

コルチコステロイドは、構造上コレステロールに関連するステロイドホルモンである。これらのホルモンは、副腎皮質で合成され、グルココルチコイド（例えば、コルチゾール）、電解質コルチコイド（例えば、アルドステロン）、並びにわずかなアンドロゲン及びエストロゲンを含んでいる。甲状腺のそのような副腎の機能は、視床下部（H P T）及び脳下垂体（P I T）の制御下にある。コルチゾール（天然のグルココルチコイド）のレベルがセットポイント以下に下がった時、視床下部は、脳下垂体からの副腎皮質刺激ホルモン（A C T H）放出を刺激する C R H（コルチコトロピン放出ホルモン）を放出する。A C T H は、以下の

・コルチゾールの合成及び分泌（それは、アルドステロンの合成／分泌に対して最小限の影響しか与えない）、そして

・副腎の成長、

を刺激する刺激ホルモンである。コルチゾールがレベルが上昇した時、これは、C R H 及び A C T H の分泌を止める（図 1 を参照のこと）。

【0003】

コルチゾールは、生合成及びグルコースの代謝に関連するその性質、並びに非特異的並びに特異的免疫性に関連する性質により特徴づけられる。グルコース代謝へのそれらの効果のために、コルチゾール並びに天然又は合成のそれらのアナログが、通常グルココルチコイドと名付けられる。それらは、グルココルチコイド・レセプター（G R）に結合する。

【0004】

グルココルチコイド・レセプターは、リガンド活性化転写因子として機能する細胞内レセプターに密接に関連するタンパク質スーパーファミリーのメンバーである。このスーパーファミリーの他のメンバーは、電解質コルチコイド・レセプター（M R）及びプロゲステロン・レセプター（P R）である。M R と G R は高いホモロジーを示し、それ故に、天然及び合成ステロイドでさえ、これらのレセプターの間に交差反応を示した。P R に関して、その天然のリガンドであるプロゲステロンが同様に M R 及び G R と交差反応する。

【0005】

クッシング症候群は、コルチゾールの副腎皮質からの分泌の増加の結果として生じる不調である。副腎皮質の機能亢進は、A C T H に依存しているかもしれないし、又は A C T H 調節に非依存、例えば副腎皮質腺腫又は悪性腫瘍によるコルチゾールの産生であるかもしれない。外因性のコルチゾール又は関連する合成アナログの超生理学的な量の投与は、副腎皮質の機能を抑圧して、そして A C T H 非依存性グルココルチコイド機能亢進に似た症状を呈する。副腎皮質の A C T H 依存性機能亢進は、脳下垂体による A C T H の分泌過多、肺の小細胞癌（異所性 A C T H 症候群）のような非脳下垂体腫瘍による A C T H の分泌、又は外因性 A C T H の投与によるかもしれない。「クッシング症候群」という用語は、原因にかかわらず起こるコルチゾール過剰によりもたらされる臨床像に適用される一方、脳下垂体の A C T H 過剰から起こっている副腎皮質の機能亢進は、しばしばクッシング病と呼ばれ、特定の生理学的な異常を示す。クッシング病の患者は、脳下垂体の好塩基性腺腫か色素嫌性腺腫を持っているかもしれない。微小腺腫は、通常 C T か、好ましくはガドリニウムにより増強させられた高解像度技術を用いた M R I スキャンにより可視化されることができる。いくつかの微小腺腫は、これらの手法によってでさえ可視化することが困難である。いくつかの症例で、A C T H 過剰産生の明らかな証拠にもかかわらず、脳下垂体で組織学的異常は見つからなかった。

【0006】

医原性かもしれない、原因に関係しないコルチゾール過剰から起こっている臨床像を意味するように本明細書中で意図されているクッシング症候群への言及は、A C T H の注射薬又はコルチゾール、あるいはアレルギー性、喘息性、炎症性、又は免疫学的を含む様々な

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タイプの病気で広く使用されるプレドニゾン、プレドニゾロン、デキサメタゾン、又はその他のもののような合成アナログの直接的な投与によるいずれかの医原性のものでもありうる。クッシング症候群は、副腎腫瘍のコルチコイドの分泌に加え、異所性 A C T H 産生、及びクッシング病を含んでいる。

【0007】

臨床徴候は、膨れ上がった外観を持つ丸みのある「満月様」顔貌を含んでいる。目立った鎖骨上の及び背中の頸部の脂肪体（「バッファロー瘤」）を有する体幹性肥満症である；末梢の手足及び指は通常、非常にほっそりしている。筋肉消耗性であり、そして脱力感がある。皮膚は、薄く、そして創傷治癒に乏しく及び傷つきやすい萎縮性である。紫色の線条が、腹部に現われる。高血圧、腎臓結石、骨粗鬆症、グルコース不耐性、感染耐性の低下、及び精神障害であることが珍しくない。直線的な成長の停止が子供に独特である。女性

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【0008】

エストロゲン及びアンドロゲン・レセプターに関連する抗ホルモン薬剤の開発がうまくいっているが、選択的な抗コルチコイドの搜索は、より制限されている。

【0009】

様々なレベルでステロイドホルモンの合成を抑制する既知の薬剤（すなわち、ステロイドホルモンの合成の様々な段階を触媒する酵素の阻害剤）が J. Steroid Biochem., vol. 5, p. 501 (1974) 中で検討され、そして以下を含む：

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a) ジフェニルメタンの誘導体、例えばアンフェノン B (amphenon B) (11-ベーター、17-及び21-のヒドロキシラーゼの段階でのステロイドホルモンの合成を抑制する)；

b) ピリジンの誘導体 (SU-c シリーズ)、例えばメチラポン (metirapon) (11-ベーターのヒドロキシラーゼの段階で合成を抑制する)；

c) 置換されたアルファ、アルファ-グルタルアミド、例えばアミノグルテチミド (aminogluteimide) (20-アルファ-ヒドロキシラーゼと C₂₀、C₂₂-リアーゼの抑制によりコレステロールからプレグネノロンの合成を妨げる)；

d) ステロイド物質、例えばトリロスタン (3-ベーター置換されたステロイド-3-ベーターヒドロキシ-5-アンドロステン-17-オン)、3-ベーターデソキシステロイドヒドロゲナーゼ-5.4-イソメラーゼを抑制する (Steroids, vol. 32, p. 257)。

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【0010】

e) 素速く解離する抗電解質コルチコイドとして使用されるスピロノラクトン・ファミリーのステロイド (PNAS USA 71 (4) p. 1431-1435 (1974))。

【0011】

f) 腎臓 (Z. Naturforsch., 45b, p. 711-715 (1990)、及び海馬 I 型 MR (Life Science, 59, p. 511-21 (1996)) について特異的な結合特性を示し、II 型 GR については示さない抗電解質コルチコイドと説明される合成ステロイド、ZK 91587。従って、両方のレセプター・システムを含んでいる組織における MR の機能の調査の道具として都合よく役に立つかもしれない。

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【0012】

グルココルチコイドホルモンとホルモン・レセプターの相互作用を特異的に抑制する物質は以下のとおりである：

a) ミフェプリストン (11 β , 17 β)-11-[4-(ジメチルアミノ)フェニル]-17-ヒドロキシ-17-(1-プロピニル)エストラ-4, 9-ジエン-3-オン、グルココルチコイドホルモンのレセプターに働きかけ、グルココルチコイド効果をもたらす機構を開始できない複合体を形成する (Annals of New-York Ac

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ademy of Science, vol. 761, p. 5-28 (1995))。

【0013】

b) 非ステロイド系の物質 (J. Steroid Biochem., Vol. 31, p. 481-492 (1988)、例えば塩酸ドロタベリーナ (drotaverina hydrochloride) (イソキノリン-1-(3,4-ジエトキシベンジリデン)-6,7-ジエトキシ-1,2,3,4-テトラヒドロキノリンの誘導体)、又はアセチルサリチル酸 (Moskovskaya Meditsina, 1990, "Receptor mechanisms of the glucocorticoid effect" by V. P. Golikov)。

今まで、臨床条件で試みられた、抗グルココルチコイド物質に関する唯一の治療のための適用 (例えば、ミフェプリストン) は、非脳下垂体性クッシング症候群の手術不可能な症例の治療である。ミフェプリストン (抗プロゲステロン物質及び抗グルココルチコイド物質の両方) の場合に、高い服用量 (1日につき800 mgまで) が必要とされる。

【0014】

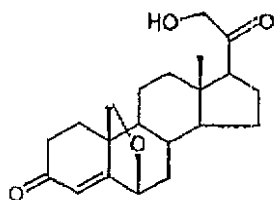
活性を増加させ、そして交差感受性と好ましくない副作用を減少させるための戦略の系統的な適用の利用により、特に抗エストロゲン及び抗アンドロゲンの分野でより大きな効力及び選択性を有する抗ホルモン物質の開発における進展が報告された。

【0015】

EP-903'146において、以下の式 (I) :

【0016】

【化4】



(I)

【0017】

により表される合成ステロイド21-ヒドロキシ-6,19-オキシドプロゲステロン (210H-6OP) が、実質的に子宮PR又は腎臓MRと交差反応しない選択的な抗グルココルチコイド物質として開示された。前記21-ヒドロキシ-6,19-オキシドプロゲステロン抗グルココルチコイド物質は、電解質コルチコイド又はグルココルチコイドの性質、並びにMR又はPRに対する親和性を事実上欠いている抗グルココルチコイド物質が望ましい、グルココルチコイド過剰に関係している病気の治療に使用されうることができた。

【0018】

21-ヒドロキシ-6,19-オキシドプロゲステロン (1)、及び21-アセテート (2) の合成は、21-ヒドロキシプロゲステロン・ジアセテートから開始し、19-ヒドロキシ-デソキシ-コルチコステロンの合成における中間体としてDeghenghiにより1966年に最初に達成された。

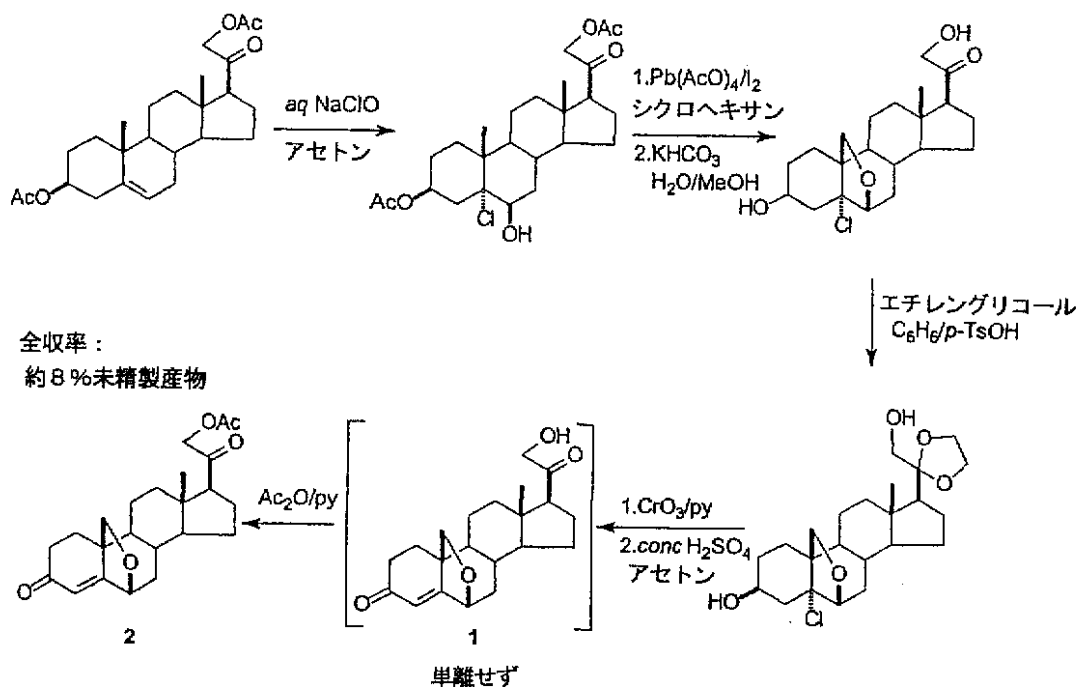
【0019】

スキーム1に要約した手順は、酢酸鉛 (Pb (AcO)₄) を用いた次亜ヨウ素酸型の反

応の使用を含む。化合物 1 は、単離せず、特徴づけられもしないが、その位置でアセテート (2) にアセチル化される。部分的に精製した (2) の全収率は、たった 8.3 % だった。低い収率以上に、この調製法は、特にクロロヒドリンの形成のために再生するのが困難であると一般に知られる。

【0020】

【化5】



スキーム 1 : Deghenghi による 21-ヒドロキシ-6, 19-オキシドプロゲステロン、

及びその 21-アセテート誘導体の合成

【0021】

さらに最近 19-ヒドロキシデオキシコルチコステロンの合成において、Kirk 及び Yeoh (J. Chem. Soc. Perkin Trans. 1, 2945 (1983)) が、中間体としてアセテート (2) を製造した。プレグネロン・アセテートから開始するこの手順をスキーム 2 に表す。最初の 3 ステップの完全な詳細がそれらの公開の実験項に記載されていなかったが、引用されていた文献によるこれらのステップの収率は、約 34 ~ 37 % と見積もられ、プレグネロン・アセテートから約 12 % のアセテート (2) の全収率を得た。

【0022】

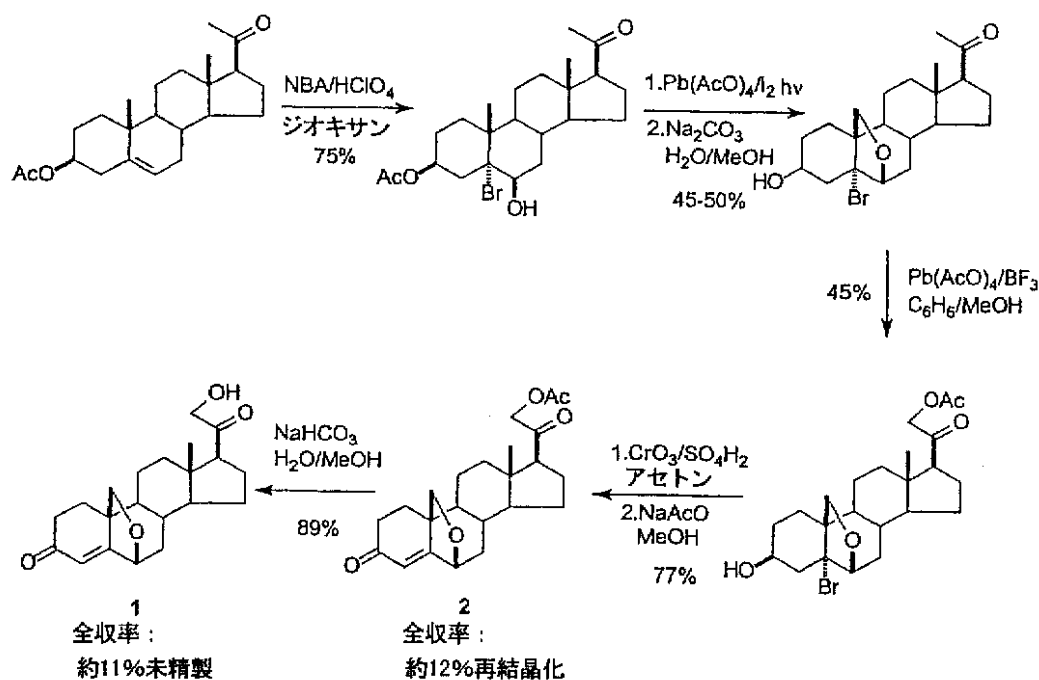
【化6】

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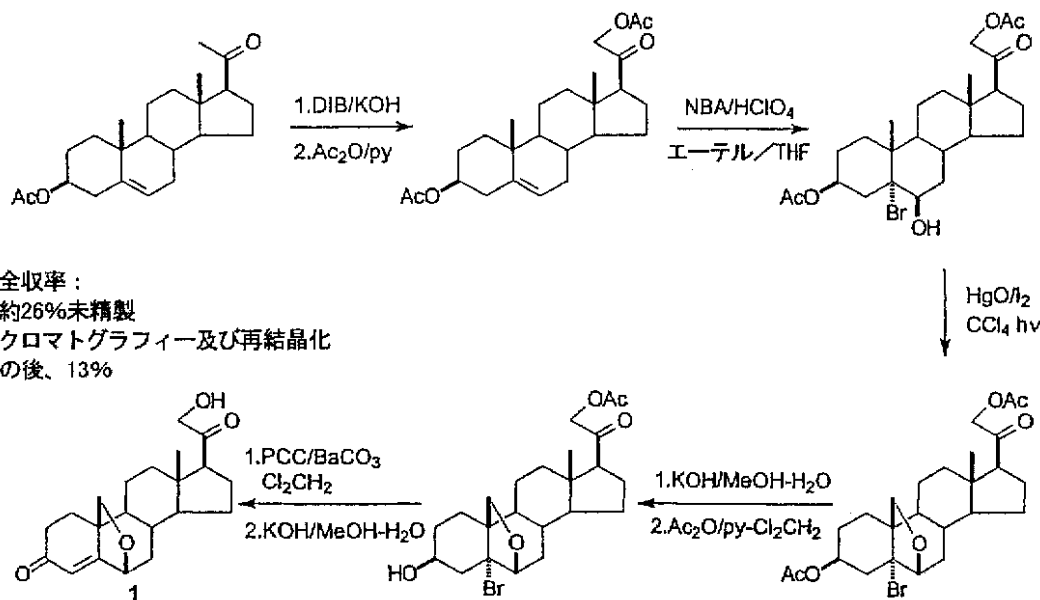
スキーム2：Kirkらによる21-ヒドロキシ-6, 19-オキシドプロゲステロン、
及びそのアセテートの合成

【0023】

スキーム3に表したさらなる方法により、 $Pb(AcO)_4$ 、及び熱的又は光化学的条件下のヨウ素によるわずかな官能化反応を、より再現性のある、そして光化学条件下の「より温和な」 HgO /ヨウ素系と交換し、21-ヒドロキシル化のステップを多価ヨウ素化合物を用いて実施される(A. S. Veleiro, M. V. Nevado, M. C. Monteserin and G. Burton, Steroids, 60, 268-272 (1995); M. Akhtar and D. H. R. Barton, J. Am. Chem. Soc., 86, 1528-1534 (1964); R. M. Moriarty, L. S. John and P. C. Du, J. Chem. Soc. Chem. Commun., 641-642 (1981)を参照のこと)。

【0024】

【化7】



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スキーム3：Veleiroらによる21-ヒドロキシ-6,19-オキシドプロゲステロンの合成

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【0025】

前述の手順も、プレグネノロン・アセテートから開始し、それは最初に21-ヒドロキシプロゲステロンジアセテートに変換される。最善の場合で、(1)の全収率は、約26%である；TLC及びNMRによると純粋なようだったが、しかし、この産物は結晶化することができず、このようにクロマトグラフィーによる追加の精製ステップを必要とした；純粋な結晶(1)の収率は13% (21-ヒドロキシプレグネノロン・ジアセテートから19%)であった。アセテート(2)は、この合成経路において中間体であるが、完全な精製は、(すなわち、化合物(1)の)脱アセチル化後にのみ達成されうる。さらに、この手順は、1996年に実施した最初の生物学的な試験のための少量の(1)の合成をもたらすが、この手順をスケールアップするための試みは失敗した。現在知られている方法は、21-ヒドロキシ-6,19-オキシドプロゲステロン(1)を提供するが、それらは、より不十分な収率、並びに取り除くのが困難な副産物を相変わらず生じる。

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【0026】

本発明の説明：

21-ヒドロキシ-6,19-オキシドプロゲステロン(21OH-6OP)及び／又はそのエステル、新しい製造方法を提供することが本発明の目的である。

【0027】

21-ヒドロキシ-6,19-オキシドプロゲステロン及び／又はそのエステルを提供することが本発明のさらなる目的である。

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【0028】

第1の側面において、本発明は、21-ヒドロキシ-6,19-オキシドプロゲステロン(1)又はそのエステル、例えばカルボキシレート、ホスフェート又はスルフェートの新しい製造方法を提供する。好ましい態様において、本発明は、21OH-6OP、及びその21-アセテート、21-プロピオネート、21-ヘミサクシネート、21-ホスフェート、及び21-オレエート誘導体の新しい製造方法を提供する。本発明の方法は、本質的に今まで知られている不都合の全てを回避する。本発明の方法は、許容される程度の純度を持つ21-ヒドロキシ-6,19-オキシドプロゲステロン(1)を優れた収率で製

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造し、そして試薬としての重金属を避ける。本発明の方法は、21-ヒドロキシ-6, 19-オキシドプロゲステロン(1)及びそのエステルの大規模な産業上の製造に好適である。

【0029】

本発明の目的は、メイン・クレームにより満たされる。好ましい態様は、それと一緒に組み込まれている従属クレームの範囲内で記載される。

【0030】

本発明による新しい合成手順は、以下の連続した基本的なステップを含むか又はそれから成る：

- a) 21-アセトキシプレグネノロン(3) (実際は市販品である)を準備し； 10
- b) 21-アセトキシプレグネノロンのC-3ヒドロキシ基を不安定なエステル、好ましくはホルメート・エステルに変換し；
- c) 保護された21-アセトキシプレグネノロンからプロモヒドリン産物を得、上述のプロモヒドリンは、C5-C6の位置の二重結合への臭素とヒドロキシ基の付加によりもたらされる；
- d) 照射下、「スアレス試薬」(ジアセトキシコードベンゼン；Annas et al., J. Chem. Soc. Perkin 1, 405 (1989)を参照のこと)及びヨウ素を用いてC19原子による分子内の環化を実施し、よって、骨格内に6, 19オキシド架橋を得、
- e) 好ましくはHCl/MeOH/ジクロロメタンにより選択的な加水分解を実施し、 20
続いて、好ましくは塩化クロム酸ピリジニウム(PCA)により酸化し、よって、プロモケトンを得、
- f) ステップe)から得られたプロモケトンの加水分解を実施して、21-ヒドロキシ-6, 19-オキシドプロゲステロン(1)を得、そして、場合により
- g) 21-ヒドロキシ-6, 19-オキシドプロゲステロン(1)をアシル化して、21-エステル(このアセテートを(2)と示す)を得る。

【0031】

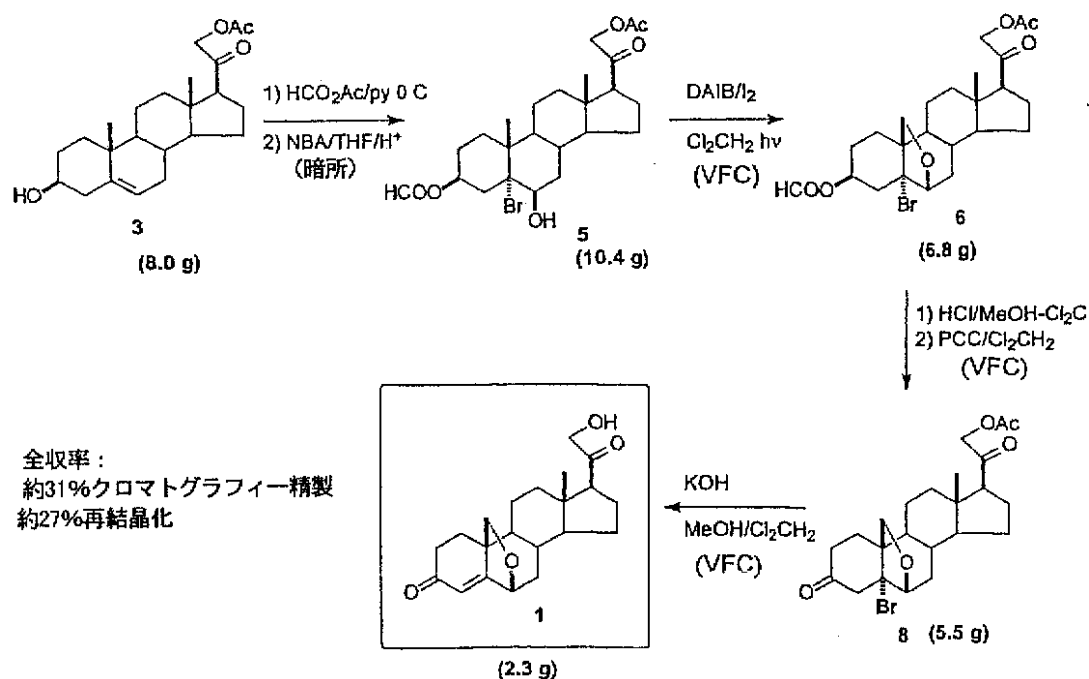
好ましいエステルは、(場合により、COOHにより、1又は2回置換された)C1-18アシル・エステル、及びホスフェート・エステルである。アシル・エステルは、カップリング剤(例えば、N, N'-ジシクロヘキシルカルボジイミド)の存在下、有機酸と、 30
又は活性化された有機エステル(例えば、ニトロフェノール・エステル)、又はハロゲン化アシル(例えば、塩化アシル)、又はアシル無水物と21-ヒドロキシ-6, 19-オキシドプロゲステロン(1)を反応させることにより得られる。ホスフェート・エステルは、リン酸化剤(例えば、リンオキシクロライド、塩基性加水分解が続く)と21-ヒドロキシ-6, 19-オキシドプロゲステロンを反応させることにより得られる。

【0032】

21-プロピオネート、又は21-ヘミサクシネート、及び21-オレエート、及び誘導体は、プロピオン酸、コハク又はオレイン酸、それらの無水物、活性化されたエステル、又は塩化アシルを用いたステップf)の21-ヒドロキシ-6, 19-オキシドプロゲステロン(1)をエステル化することにより得られる。本発明による好ましい合成手順を、 40
スキーム4に表す。

【0033】

【化8】



スキーム4：21-ヒドロキシ-6,19-オキシドプロゲステロンの新規合成

【0034】

スキーム4で説明した好ましい方法によると、ギ酸基は、3位の保護基として導入される。ギ酸は、第1級酢酸、そして α -アセトキシケトンすら安定な比較的温和な酸性の条件(HCl/MeOH -ジクロロメタン)の下でそれらは加水分解されるという利点がある。ギ酸部分の導入は、好ましくは混合した酢酸-ギ酸無水物(ギ酸と無水酢酸からその場で準備される)を用いて、非常に温和な条件下で好ましくは実施される。THF中、N-ブromoアセトアミドによりブromo-ヒドリン形成を実施し、80%の所望のブromoヒドリン(5)、及び約20%の $5\alpha\text{-HO}-6\beta\text{-Br}$ 異性体をもたらす。前記混合物は、結晶形成か又は薄層クロマトグラフィーにより分離されうる。 C_{-19} を伴った分子内の環化のために、好ましくは標準のタングステンランプによる一好適な照射の下で「スプレッド試薬」(ジアセト-オキシヨードベンゼン)(DAIB)及びヨウ素を、ジェネレーティング・システムとしての次亜ヨウ素酸として使用する。

【0035】

全く驚いたことに、ジクロロメタン(CH_2Cl_2)中の分子内の環化ステップを実施により、この反応は、迅速で(1時間未満で完全な変換を完了する)及び無菌だけでなく、優れた収率が得られることが判明した。

【0036】

水循環を伴うガラス・ジャケットを備えた反応器を使用することにより達成される環化ステップの温度調節(25°C)は、収率を増やし、過剰酸化副産物を減らす。さらに、エポキシド形成を解消し、異性体ブromoヒドリンは、前記試薬に不活性であり、そしてシリカゲルカラムを通す吸引ろ過(VFC)により容易に所望のブromoエーテル(6)から(ヨードベンゼン副産物と一緒に)分離することができる。

【0037】

好ましくは、ホルメート・エステルの選択的な加水分解は、 MeOH -ジクロロメタン中でHClにより実施され、続いて、好ましくはPCCにより酸化し、よって、ブromoケト

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ン（８）を得る。V F C（非常に短いシリカゲルカラム）精製に供した時、この産物は、部分的にH B rが除かれ、不飽和ケトン（２）を生じる、しかし、これは、不飽和産物が、以降のステップの所望の産物であるので問題を引き起こさない、ここで上記ステップは、塩基を用いた処理が２１位を脱アシル化し、そしてC－５臭素の除去を完了することにより所望の産物（１）を得る。

【００３８】

（１）の精製は、短いシリカゲルカラムを通す高速V F Cで達成され、３１％以上の収率でクロマトグラフィーによる精製産物を得る。これを無水エタノールから結晶化させて２７％の収率の結晶（１）を生じる。

【００３９】

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２１－アセテート（２）は、ピリジン中、無水酢酸による（１）の標準的なアセチル化により製造される。このアセテートを、無水エタノールから再結晶させる。

【００４０】

本発明による新しい合成には以下の利点がある：

a）それは、選択的なアセチル化のステップの必要性を取り除くか、又はC－２１とC－３のヒドロキシル基を区別するあらゆる必要を取り除く。

【００４１】

b）それは、重金属（鉛、銀、又は水銀ベースの試薬）を必要としないように、次亜ヨウ素酸反応を修飾し、そしてそれは、同質の条件下で進むので、数グラム、そして最終的にはキログラム量までのスケールアップを可能にする。

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【００４２】

c）それは、精製ステップを最小限にするために、さまざまな段階で形成された二次的な産物（特に５，６－エポキシド形成）を減らし、そしてプロモエーテル、化合物（１）及び（２）がこれらの条件下で本質的に不安定であるため、シリカゲルによるカラムクロマトグラフィーの必要性を特に回避するか又は最小限にする。

【００４３】

d）それは、全体にわたって反応量を減らし、スケールアップを可能にする。

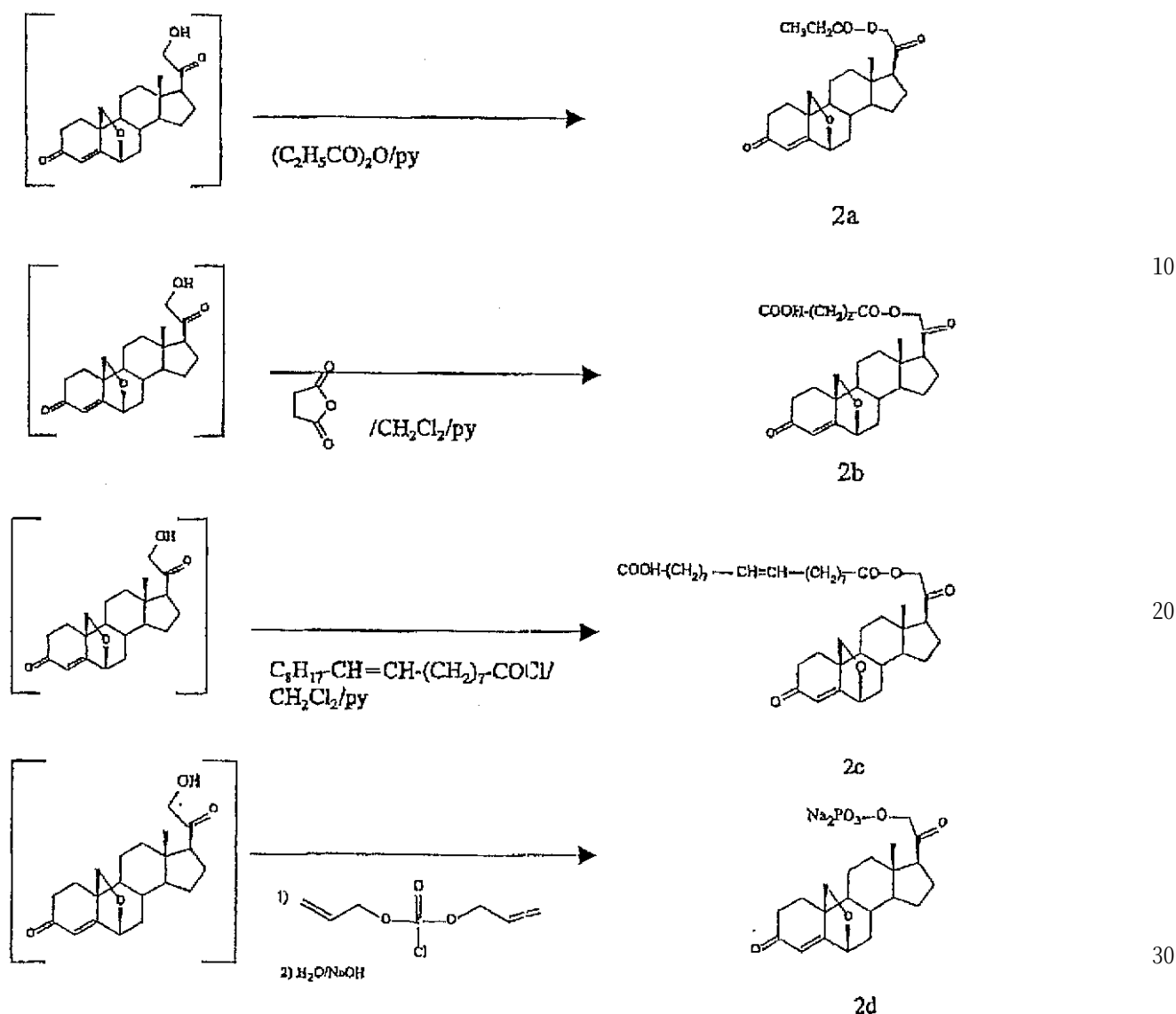
【００４４】

化合物（１）から始まる、アシル・エステル、例えば２１－プロピオネート（２ a）、及び２１－ヘミサクシネート（２ b）、及び２１－オレエート（２ c）－６，１９－オキシドプロゲステロン誘導体の合成は、スキーム５に説明されるように実施されうる。同様に、２１－ホスフェート誘導体（２ d）の製造がスキーム５に示される。

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【００４５】

【化９】



スキーム5：21-プロピオネート(2a)、21-ヘミサクシネート(2b)、21-オレ
エート(2c)、及び21-PO₄(2d) 6,19-オキシドプロゲステロン誘導体の合成

【0046】

本発明に従って合成された化合物は、単独で又は医薬として許容される担体及び／又は賦形剤との組み合わせで、薬剤として使用するためのものである。そのような薬剤は、グルココルチコイド過剰に関係している病気の治療又は予防、例えばクッシング症候群、医原性高コルチゾール症、又はうつ病の治療のための薬剤の製造に特に好適である。

【0047】

本発明を、説明のためだけに以下の実施例を参照してここで説明する：

実施例

材料及び方法

融点をフィッシャー・ジョン装置で測り、矯正していない。IRスペクトルを、Nicolet Magna IR 550 FT-IRスペクトロメーターによりKBrディスクを用いて薄膜に記録した。¹H及び¹³C NMRスペクトルを、(内部標準としてTMS

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を用いて) 重水素クロホルム中、B r u k e r A C - 2 0 0 又は A M - 5 0 0 N M R スペクトロメーターにより計測した。J 値を H z で与える。スペクトルを、D E P T、C O S Y 4 5、及び H E T C O S Y スペクトルの解析とプロゲステロンのそれとの比較により割り当てた。

【0048】

電子衝撃質量スペクトル (E I) を、直接導入により 7 0 e V で V G T r i o 2 質量分析計で計測した。F A B 質量スペクトル及び電子衝撃高解像度質量スペクトル (H R M S) を、V G Z A B B E Q Q 質量分析計で得た。使用した全ての溶媒が試薬等級だった。溶媒を、真空下、約 4 5 ° C で留去した。亜鉛粉末を、1 M の H C l 中への懸濁し、水、無水エタノール、及びジエチルエーテルにより洗浄し、そして 1 2 0 ° C で 2 時間乾燥させることにより活性化した。全ての化合物の均一性を、薄層クロマトグラフィーにより確認した。

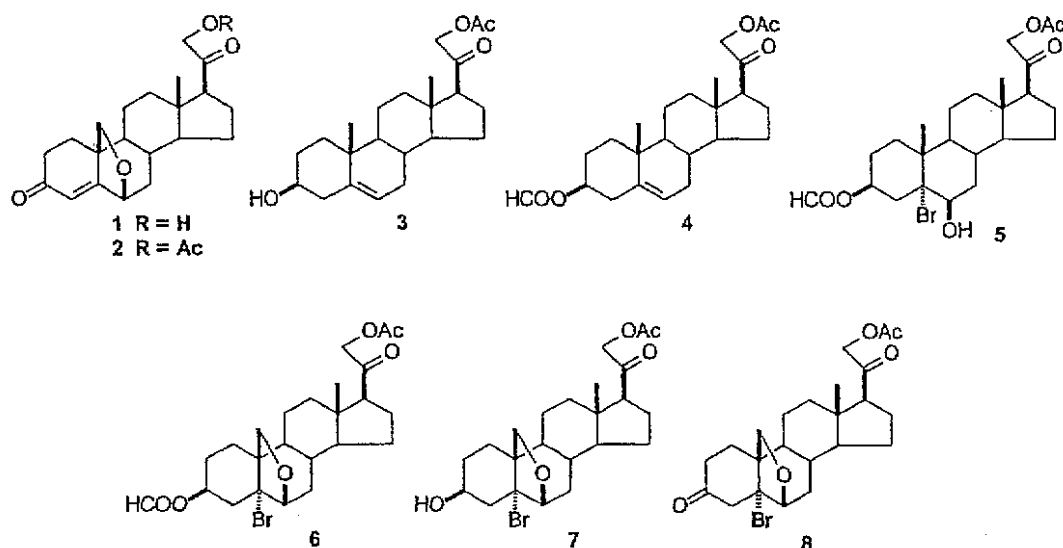
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【0049】

2 1 - ヒドロキシ - 6, 1 9 - オキシドプロゲステロン (1) の大規模合成
出発物質、中間体、及び最終産物の構造 (1) 及び (2) :

【0050】

【化10】



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【0051】

3 β - ホルミルオキシ - 2 1 - a アセチルオキシ - 5 - プレグネン - 2 0 - オン (4)
無水酢酸 (1 3 . 4 m l) を、0 ° C でギ酸 (6 . 6 m l) に滴下にて添加し、この溶液
を 5 0 ° C で 1 5 分間加温し、そして 0 ° C に急冷した。得られたアセトギ酸無水物溶液を、
0 ° C で、ピリジン (2 0 . 8 m l) 中、2 1 - アセトキシプレグネノロン (3、8 . 0
g) の攪拌された懸濁液に滴下にて添加し、攪拌をその温度で 2 時間続ける。前記反応産
物を、冷却した飽和重炭酸ナトリウム水性溶液に注ぎ、ろ過し、そしてその固体を飽和重
炭酸ナトリウム水性溶液、水、及び 1 N H C l により (中和するまで) 洗浄し、ホルメ
ート (4) を得た (8 . 0 g) ; ¹ H N M R (2 0 0 . 1 3 M H z) ^δ S H 0 . 7
0 (3 H, s, 1 3 - C H ₃), 1 . 0 2 (3 H, s, 1 0 - C H ₃), 2 . 1
6 (3 H, s, 2 1 - C H ₃ C O), 2 . 5 3 (1 H, t, J = 8 . 0 H z,
1 7 - H), 4 . 5 0 (1 H, d, J = 1 7 . 0 H z, 2 1 a - H), 4 . 7 0
(1 H, d, J = 1 7 . 0 H z, 2 1 b - H), 5 . 3 2 (1 H, m, 3 - H

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), 5.38 (1H, d, J = 3.0 Hz, 6-H), 8.02 (1H, s, HCOO)。

【0052】

3β-ホルミルオキシ-5α-ブromo-6β-ヒドロキシ-21-アセチルオキシプレグナン-20-オン (5)

ホルメート (4) (8.0 g) を、ジエチルエーテル (100 ml) 及び THF (37.2 ml) 中に溶解し、10℃に冷却する。10~15℃で攪拌している一遮光された一溶液に、7.5%の過塩素酸 (11.88 ml) を添加し、続いて8つの部分で25分間にわたりN-ブromoアセトアミド (4.75 g) を添加する。攪拌を、25℃で45分間続け、そして完全に脱色するまで10%のチオ硫酸ナトリウム水性溶液を添加することにより反応を止める。次に、反応混合物を、ジクロロメタン/メタノール10:1で抽出し、そして有機層を、水により洗浄し、無水硫酸ナトリウムにより乾燥させて、そして溶媒を留去して、ブromoヒドリ (5) (10.4 g、¹H NMRにより測定されたとおり20%の5α-ヒドロキシ-6β-ブromo異性体を含んでいる) を得る。 10

【0053】

3β-ホルミルオキシ-5α-ブromo-21-アセチルオキシ-6, 19-オキシドプレグナン-20-オン (6)

25℃の循環する水による外部冷却ジャケット及び攪拌機を備えた1リットルのガラス容器に含まれた新たに蒸留したジクロロメタン (723 ml) 中、ブromoヒドリ化合物 (5) (10.4 g、20%の5α-ヒドロキシ-6β-ブromo異性体を含んでいる) の溶液を通して窒素を5分間バブリングする。ジアセトキシヨードベンゼン (スアレス試薬、7.66 g) 及びヨウ素 (5.46 g) を、攪拌しながら連続して添加する。容器を2つの300ワット・タングステンランプ (各5000 lm) に晒し、激しい攪拌を25℃で1時間続ける。照射を止め、そして完全に脱色するまでチオ硫酸ナトリウムの飽和水性溶液を添加する。有機層を分離し、無水硫酸ナトリウムにより乾燥させて、溶媒を留去する。得られた固体をジクロロメタン (8 ml) で溶解し、ヘキサンで前もってフラッシュしたシリカゲル G-60 カラム (12 cmの直径×8 cmの高さ) に適用する; ヘキサン-酢酸エチル9:1 (1100 ml)、8:2 (700 ml)、7:3 (700 ml)、及び6:4 (600 ml) により (真空を出口に適用して) 連続して溶出し、31×100 mlの画分を得る。画分をTLCにより分析し、そしてブromoエーテル (6) を含んでいるそれらをプールし、乾燥するまで留去して (6) (6.8 g) を得る。¹H NMR (200.13 MHz) ^δ H 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, J = 8.8 Hz, 17-H), 3.73 (1H, d, J = 8.4 Hz, 19a-H), 3.94 (1H, d, J = 8.4 Hz, 19b-H), 4.08 (1H, d, J = 4.2 Hz, 6-H), 4.50 (1H, d, J = 16.8 Hz, 21a-H), 4.71 (1H, d, J = 16.8 Hz, 21b-H), 5.34 (1H, m, 3-H), 8.02 (1H, s, HCOO)。 20

【0054】

3β-ヒドロキシ-5α-ブromo-21-アセチルオキシ-6, 19-オキシドプレグナン-20-オン (7) 40

先に得られたブromoエーテル (6) (6.8 g) の攪拌した溶液を、ジクロロメタン (45.7 ml) とメタノール (154.7 ml) 中に溶解し、そして氷浴中で0℃に冷却し、そして水 (10.9 ml)、さらに濃HCl (23.0 ml) を添加する。0℃での激しい攪拌の約30分後 (TLCにより出発材料の消失が観察される)、この反応混合物を20%の水酸化ナトリウム水性溶液により中和し、そしてジクロロメタンで抽出する。有機層を無水硫酸ナトリウムにより乾燥させ、そして溶媒を留去し、アルコール化合物 (7) (6.5 g) を得る; ¹H NMR (200.13 MHz) ^δ H 0.69 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, J = 8.5 Hz, 17-H), 3.62 (1H, d, J = 9.0 Hz, 19a-H), 3.83 (1H, d, J = 9.0 Hz, 19b-H), 4.08 (1H, d, J = 4.2 Hz, 6-H), 4.50 (1H, d, J = 16.8 Hz, 21a-H), 4.71 (1H, d, J = 16.8 Hz, 21b-H), 5.34 (1H, m, 3-H), 8.02 (1H, s, HCOO)。 40

8.5 Hz, 19a-H), 3.92 (1H, d, $J = 8.5$ Hz, 19b-H), 4.07 (1H, d, $J = 4.0$ Hz, 6-H), 4.15 (1H, m, 3-H), 4.51 (1H, d, $J = 17.0$ Hz, 21a-H), 4.70 (1H, d, $J = 17.0$ Hz, 21b-H)。

【0055】

5 α -ブromo-21-アセチルオキシ-6, 19-オキシドプレグナン-3, 20-ジオン (8)

無水ジクロロメタン (480 ml) 中、塩化クロム酸ピリジニウム (12.1 g)、炭酸バリウム (5.0 g)、及び3 Åモレキュラーシーブ (9.60 g) の懸濁液を、窒素下で10分間攪拌する。得られたオレンジ色のスラリーに、無水ジクロロメタン (324 ml) 中の先に得られたブromoエーテル (7) (6.5 g) の溶液を添加し、そして出発材料 (TLC) が消えるまで、約90分間攪拌を続ける。反応混合物を短いシリカゲル G 60 カラム (12 cm の直径 $\times$ 8 cm の高さ) に透過させ、ジエチルエーテル (2 $\times$ 150 ml) 及びヘキサノール酢酸エチル 1:2 (3 $\times$ 150 ml) により洗浄した。産物を含んでいる画分をプールし、乾燥するまで留去して、5.5 g のケトン (8) を得る (約10% の Δ^4 -3-ケトン (2) を含む) ; $^1\text{H NMR}$ (200.13 MHz) $^\delta\text{H}$ 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.51 (1H, t, $J = 8.5$ Hz, 17-H), 2.85 (1H, d, $J = 16.0$ Hz, 4a-H), 3.40 (1H, d, $J = 16.0$ Hz, 4b-H), 3.90 (1H, d, $J = 9.0$ Hz, 19a-H), 4.07 (1H, d, $J = 4.0$ Hz, 6-H), 4.15 (1H, d, $J = 9.0$ Hz, 19b-H), 4.50 (1H, d, $J = 17.0$ Hz, 21a-H), 4.71 (1H, d, $J = 17.0$ Hz, 21b-H)。

【0056】

21-ヒドロキシ-6, 19-オキシド-4-プレグネン-3, 20-ジオン (1)

前ステップから得たケトン (8) (5.5 g) をメタノール (263.8 ml) 及びジクロロメタン (13.2 ml) 中に溶解する。攪拌しているこの溶液に、14% のメタノール中、KOH (53.4 ml) を添加し、そして出発材料 (TLC) が消えるまで、約15分間室温で攪拌を続ける。この反応混合物を1N HCl で中和し、ジクロロメタンにより抽出する。無水硫酸ナトリウムを用いて有機層を乾燥させて、そして溶媒を留去することで、未精製の21-ヒドロキシ-6, 19-オキシドプロゲステロン (1, 4.6 g) を得る。この固体をジクロロメタン (5 ml) 中に溶解し、そして前もってヘキサノール酢酸エチル 7:3 によりフラッシュしたシリカゲル G-60 カラム (8.5 cm の直径 $\times$ 5 cm の高さ) に通す; (真空を出口に適用して) ヘキサノール酢酸エチル 6:4 (1350 ml) 及び1:1 (900 ml) で連続して溶出し、30の画分を得る。TLCにより画分を分析し、(1) を含んでいるものをプールして、そして乾燥するまで留去して21-ヒドロキシ-6, 19-オキシドプロゲステロン (1, 2.3 g) 。 $^1\text{H NMR}$ (200.13 MHz) $^\delta\text{H}$ 0.74 (3H, s, 13-CH₃), 2.45 (1H, t, $J = 8.5$ Hz, 17-H), 3.51 (1H, d, $J = 8.8$ Hz, 19a-H), 4.18 (3H, s, 21-CH₃), 4.20 (1H, d, $J = 8.8$ Hz, 19b-H), 4.69 (1H, d, $J = 5.0$ Hz, 6-H), 5.82 (1H, s, 4-H)。

【0057】

無水エタノールからの再結晶形成は、1回目の回収の結晶 (1) (1.27 g) を与える mp 165~166 °C。母液を濃縮して2回目の回収の (1) (0.68 g) 得る。

【0058】

21-アセチルオキシ-6, 19-オキシド-4-プレグネン-3, 20-ジオン (2)

未精製の21-ヒドロキシ-6, 19-オキシドプロゲステロン (1, 2 g クロマトグラフィー精製前) を、無水ピリジン (15.6 ml) 中に溶解し、そして無水酢酸 (15.6 ml) を添加した。この溶液は25 °Cで90分間攪拌して、1MのHCl上に注

ぎ、そしてろ過した（あるいは、この固体をジクロロメタンにより抽出する）。この固体を（中性まで）水により洗浄し、乾燥させて、ジクロロメタン（2 ml）中に溶解し、そして前もってヘキサン-酢酸エチル 7 : 3 によりフラッシュしたシリカゲル G-60 カラム（7 cm の直径 × 5 cm の高さ）に適用し；ヘキサン-酢酸エチル 7 : 3（700 ml）及び 6 : 4（700 ml）を用いて（真空を出口に適用して）連続的に溶出して、20 の画分を得た。画分を TLC により分析し、そして（2）を含んでいるものをプールし、そして乾燥するまで留去し、21-アセチルオキシ-6, 19-オキシドプロゲステロン（2、1.12 g）を得た。（メタノールの滴下により）無水エタノールからの再結晶は、結晶（2）（0.72 g）を与えた、mp 190 ~ 191 °C。¹H NMR（200.13 MHz）^δ H 0.76（3H, s, 13-CH₃）, 2.17（3H, s, 21-CH₃CO）, 2.51（1H, t, J = 8.5 Hz, 17-H）, 3.51（1H, d, J = 8.2 Hz, 19a-H）, 4.20（1H, d, J = 8.2 Hz, 19b-H）, 4.50（1H, d, J = 16.7 Hz, 21a-H）, 4.69（1H, d, J = 5.0 Hz, 6-H）, 4.72（1H, d, J = 16.7 Hz, 21b-H）, 5.82（1H, s, 4-H）。

【0059】

21-プロパノイルオキシ-6, 19-オキシド-4-プレグネン-3, 20-ジオン（2a）

未精製の 21-ヒドロキシ-6, 19-オキシドプロゲステロン（1、0.2 g クロマトグラフィー精製前）を、無水ピリジン（0.28 ml）中に溶解し、そしてプロパン酸無水物（0.2 ml）を添加した。この溶液は 25 °C で 1 時間攪拌して、メタノールを添加し過剰な無水物を無効化し、真空中でこの溶液を濃縮した。残渣をジクロロメタンにより希釈し、1 M HCl 及び水により洗浄し、乾燥するまでに留去した（0.233 g）。前記と同様な精製で 21-プロパノイルオキシ-6, 19-オキシドプロゲステロン（2a、0.116 g）を得た。¹H NMR（200.13 MHz）^δ H 0.76（3H, s, 13-CH₃）, 1.18（3H, t, J = 7.6 Hz, 21-CH₃CH₂CO）, 2.46（2H, q, J = 7.6 Hz, 21-CH₃CH₂CO）, 2.51（1H, t, J = 8.0 Hz, 17-H）, 3.51（1H, d, J = 8.2 Hz, 19a-H）, 4.20（1H, d, J = 8.2 Hz, 19b-H）, 4.50（1H, d, J = 16.8 Hz, 21a-H）, 4.70（1H, d, J = 5.0 Hz, 6-H）, 4.73（1H, d, J = 16.8 Hz, 21b-H）, 5.82（1H, s, 4-H）。EI MS m/z 400（17）[M]⁺, 342（5）, 313（24）, 285（23）, 267（10）, 57（100）。

【0060】

21-サクシノイルオキシ-6, 19-オキシド-4-プレグネン-3, 20-ジオン（2b）

21-ヒドロキシ-6, 19-オキシドプロゲステロン（1、0.75 g）を、無水ジクロロメタン（37.5 ml）及びピリジン（1.9 ml）中に溶解し、そしてコハク酸無水物の第 2 部分（0.375 g）を添加し、そして 6 時間（出発材料が消失するまで）攪拌を続けた。反応混合物を濃縮し、そして残渣をジエチルエーテルにより抽出した。エーテル溶液を 1 M の HCl により洗浄し、そして 10 % の炭酸ナトリウム水溶液により抽出した。この水層を濃 HCl により pH = 3 まで酸性化し、そしてジクロロメタンにより抽出した。水により洗浄し、そして乾燥するまで留去して、未精製 21-サクシノイルオキシ-6, 19-オキシドプロゲステロン（2b、0.823 g）を得た。この固体をジクロロメタン（1 ml）中に溶解し、そしてヘキサン-酢酸エチル 2 : 8 により前もってフラッシュしたシリカゲル G-60 カラム（6 cm の直径 × 4 cm の高さ）に適用し；ヘキサン-酢酸エチル 2 : 8（200 ml）及び酢酸エチル（500 ml）を用い

て（真空を出口に適用して）連続的に溶出し、14の画分を得た。画分をTLCにより分析して、(2b)を含んでいるそれらをプールし、乾燥するまで留去して、21-サクシノイルオキシ-6, 19-オキシドプロゲステロン(2b、0.455 g)を得た。¹H NMR (200.13 MHz) δ H 0.75 (3H, s, 13-CH₃), 2.52 (1H, t, J = 8.0 Hz, 17-H), 2.75 (4H, m, 21-HCOOCH₂CH₂CO), 3.51 (1H, d, J = 8.2 Hz, 19a-H), 4.20 (1H, d, J = 8.2 Hz, 19b-H), 4.54 (1H, d, J = 16.9 Hz, 21a-H), 4.70 (1H, d, J = 5.0 Hz, 6-H), 4.75 (1H, d, J = 16.9 Hz, 21b-H), 5.82 (1H, s, 4-H)。EIMS m/z 444 (3) [M]⁺, 344 (24), 313 (55), 285 (44), 267 (17), 91 (60), 79 (54), 55 (100)。

【0061】

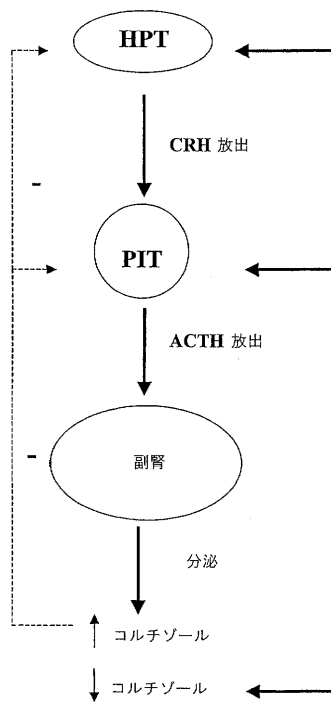
21-オレオイルオキシ-6, 19-オキシド-4-プレグネン-3, 20-ジオン(2c)
21-ヒドロキシ-6, 19-オキシドプロゲステロン(1、0.052 g)を無水ジクロロメタン(1 ml)中に溶解し、そしてピリジン(0.12 ml)及び塩化オレオイル(0.1 ml)を添加した。この溶液を25℃で24時間攪拌して、ジクロロメタンにより希釈し、1MのHCl及び水により洗浄し、乾燥するまで留去した。予備のTLCによる精製によって、21-オレオイルオキシ-6, 19-オキシドプロゲステロン(2c、0.075 g)を得た。¹H NMR (200.13 MHz) δ H 0.76 (3H, s, 13-CH₃), 0.88 (3H, t, J = 7.0 Hz, CH₃-CH-CO), 2.34 (2H, t, J = 7.7 Hz, CH-CH₂CO), 2.50 (1H, t, J = 8.5 Hz, 17-H), 3.51 (1H, d, J = 8.3 Hz, 19a-H), 4.20 (1H, d, J = 8.3 Hz, 19b-H), 4.49 (1H, d, J = 16.9 Hz, 21a-H), 4.70 (1H, d, J = 4.5 Hz, 6-H), 4.73 (1H, d, J = 16.9 Hz, 21b-H), 5.34 (2H, m, CH-CH=CH-CH-CO), 5.82 (1H, s, 4-H)。

【0062】

21-ホスフェート-6, 19-オキシド-4-プレグネン-3, 20-ジオン(2d)
21-ヒドロキシ-6, 19-オキシド-4-プレグネン-3, 20-ジオン(1、0.052 g)を、無水ジクロロメタン(1 ml)中に溶解し、そしてピリジン(0.12 ml)及びホスホクロライド酸ジアリル・エステル(0.024 g、1当量)を0℃で30分以上にわたって滴下により添加した。この混合物を室温まで加温し、そして一晩攪拌した。この混合物を5%のNaHCO₃により3回洗浄し、有機層を乾燥させて、そして加熱することなく真空内で留去した。得られたシロップに、水中(3 ml)、NaOH(2.2化学当量)の溶液を添加した。この溶液を、還流状態に徐々に至らせ、12時間緩やかに還流した。凍結乾燥により、エタノールから再結晶された未精製ホスフェート二ナトリウム塩を得た。あるいは、未精製二ナトリウム塩を水中に溶解し、そしてHClによりpHを調整して、遊離のホスフェートの沈殿を引き起こす。

【 図 1 】

Figure 1



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(54) Title: METHOD FOR THE PREPARATION OF 21-HYDROXY-6,19-OXIDOPROGESTERONE (21OH-6OP)

(57) Abstract: The present invention relates to a novel method for preparing 21-hydroxy-6,19-oxidoprogesterone (21OH-6OP) and/or its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate, 21-oleate derivatives. 21OH-6OP and its ester derivatives are antigluccorticoids for the treatment or prophylaxis of diseases associated to an excess of glucocorticoids, in particular for treating Cushing's syndrome, iatrogenic hypercortisolism or depression.

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Method for the preparation of 21-hydroxy-6,19-oxidoprogesterone
(21OH-6OP)

Field of the invention:

- 5 The present invention relates to a novel method of preparing 21-hydroxy-6,19-oxidopro-gesterone (21OH-6OP) and/or its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate and 21-oleate derivatives. 21OH-6OP and its esters are antiglucocorticoids for the treatment or prophylaxis of diseases associated with a glucocorticoid imbalance, in particular for treating Cushing's syndrome or
10 depression.

Background of the invention:

- Corticosteroides are steroid hormones related structurally to cholesterol. These hormones are synthesized in the adrenal cortex and include the glucocorticoids (e.g.
15 cortisol), the mineralocorticoids (e.g aldosterone) as well as weak androgens and estrogens. The adrenal function, like that of the thyroid gland, is under the control of the hypothalamus (HPT) and the pituitary (PIT). When cortisol (the naturally-occurring glucocorticoid) levels drop below a setpoint, the hypothalamus releases CRH (corticotropin releasing hormone) which stimulates adrenocorticotropic
20 hormone (ACTH) release from the pituitary. ACTH is a tropic hormone which stimulates

- the synthesis and secretion of cortisol (it has minimal effects on aldosterone synthesis/secretion), and
- the growth of the adrenal gland. When cortisol levels increase, this shuts off

- 25 CRH and ACTH secretion (cf. **Figure 1**).

Cortisol is characterized by its properties related to the biosynthesis and metabolism of glucose and propeties related to non-specific as well as specific

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immunity. Due to their effects on the glucose metabolism, cortisol and natural or synthetic analogues thereof are usually named glucocorticoids. They bind to the glucocorticoid receptor (GR).

- 5 The glucocorticoid receptor is a member of a protein super family of closely related intracellular receptors which function as ligand-activated transcription factors. Other members of this super family are the mineralocorticoid receptor (MR) and the progesterone receptor (PR). MR and GR have shown to be highly homologous, thus natural and even synthetic steroids exhibit cross-reaction between these
10 receptors. With respect to PR, its natural ligand progesterone also cross-reacts with MR and GR.

- Cushing's syndrome is a disorder resulting from increased adrenocortical secretion of cortisol. Hyperfunction of the adrenal cortex may be ACTH-dependent or it may be independent of ACTH regulation, e.g. production of cortisol by an
15 adrenocortical adenoma or carcinoma. The administration of supraphysiologic quantities of exogenous cortisol or related synthetic analogs suppresses adrenocortical function and mimics ACTH-independent glucocorticoid hyperfunction. ACTH-dependent hyperfunction of the adrenal cortex may be due to hypersecretion of ACTH by the pituitary, secretion of ACTH by a nonpituitary
20 tumor such as small cell carcinoma of the lung (the ectopic ACTH syndrome), or administration of exogenous ACTH. While the term "Cushing's syndrome" has been applied to the clinical picture resulting from cortisol excess regardless of the cause, hyperfunction of the adrenal cortex resulting from pituitary ACTH excess has frequently been referred to as Cushing's disease, implying a particular
25 physiologic abnormality. Patients with Cushing's disease may have a basophilic adenoma of the pituitary or a chromophobe adenoma. Microadenomas can usually be visualized by CT or, preferably, MRI scan, using a high-resolution technique

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augmented by gadolinium. Some micro-adenomas are difficult to visualize even with these modalities. In some cases, no histological abnormality is found in the pituitary despite clear evidence of ACTH overproduction.

Reference to Cushing's syndrome is herein intended to mean the clinical picture
5 resulting from cortisol excess regardless of the cause, which may be also iatrogenic, both by the injection of ACTH or by the direct administration of cortisol or synthetic analogs such as prednisone, prednisolone, dexamethasone or others that are widely used in various types of diseases including allergic, asthmatic, inflammatory or immunologic. Cushing's syndrome includes in addition adrenal
10 tumours secreting corticoids, ectopic ACTH production and Cushing's disease.

Clinical manifestations include rounded "moon" faces with a plethoric appearance. There is truncal obesity with prominent supraclavicular and dorsal cervical fat pads ("buffalo hump"); the distal extremities and fingers are usually quite slender. Muscle wasting and weakness are present. The skin is thin and atrophic, with poor
15 wound healing and easy bruising. Purple striae may appear on the abdomen. Hypertension, renal calculi, osteoporosis, glucose intolerance, reduced resistance to infection, and psychiatric disturbances are common. Cessation of linear growth is characteristic in children. Females usually have menstrual irregularities. An increased production of androgens, in addition to cortisol, may lead to
20 hypertichosis, temporal balding, and other signs of virilism in the female.

Although development of antihomonal agents related to the estrogen and androgen receptors has been successful, the search for selective anti-corticoids is more restricted.

Known agents suppressing the synthesis of steroid hormones at various levels
25 (i.e. inhibitors of enzymes which catalyze various stages of the synthesis of

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steroid hormones) are reviewed in *J.Steroid Biochem.*, vol.5, p.501 (1974) and include the following:

- 5 a) derivatives of diphenylmethane, e.g. amphenon B (which suppresses the synthesis of steroid hormones at stages 11- beta -, 17 - and 21 - of hydroxylase);
- b) derivatives of pyridine (SU-c series), e.g. metirapon (which suppresses synthesis at stage 11- beta of hydroxylase);
- 10 c) substituted alpha , alpha - glutaramides, e.g. aminoglutetamide (which impedes the synthesis of pregnenolone from cholesterol through suppression of 20- alpha -hydroxylase and C₂₀, C₂₂-liase;
- d) steroid substances e.g. trilostan (3 beta -substituted steroid- 3 beta -hydroxy-5-androsten-17-one), which suppresses 3 beta -desoxysteroidhydrogenase -5.4- isomerase (*Steroids*, vol.32, p.257).
- 15 e) steroids of the spironolactone family which are used as rapidly dissociating anti-Mineralocorticoids (*PNAS USA* 71(4) p.1431-1435 (1974).
- 20 f) a synthetic steroid described as an anti-Mineralocorticoids, ZK91587, showing specific binding properties for the kidney (*Z.Naturforsch.*, 45b, p.711-715 (1990)) and hippocampus type I MR (*Life Science*, 59, p.511-21 (1996)), but not for type II GR. It may therefore be conveniently useful as a tool in the investigation of MR function in tissues containing both receptor systems.

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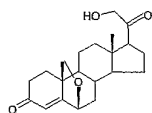
Agents that specifically suppress the interaction of glucocorticoid hormones with hormone receptors are:

- a) Mifepriston (11 β ,17 β)-11-[4-(Dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one, which acts on receptors of glucocorticoid hormones to form a complex incapable of initiating mechanisms leading to glucocorticoid effect (*Annals of New-York Academy of Science*, vol. 761, p.5-28 (1995)).
- b) non-steroid substances (*J.Steroid Biochem.*, vol. 31, p.481-492 (1988)) e.g. drotaverina hydrochloride (a derivative of isoquinoline-1-(3,4-dietoxibene zilidene)-6,7 - dietoxy-1,2,3,4- tetrahydroquinoline) or acetylsalicylic acid (*Moskovskaya Meditsina*, 1990, "Receptor mechanisms of the glucocorticoid effect" by V.P.Golikov).

To-date, the only therapeutical application for antiglucocorticoids (e.g. Mifepristone) that has been attempted in a clinical setting is to treat inoperable cases of nonpituitary Cushing's syndrome. In the case of Mifepristone (both an anti-progesterone and an anti-glucocorticoid), high doses (up to 800 mg per day) are required.

Employing a systematic application of strategies to increase activity and decrease cross-reactivity and undesirable side effects, progress has been reported in the development of antihormonal agents with greater potency and selectivity, especially in the antiestrogen and antiandrogen fields.

In EP-903'146 the synthetic steroid, 21-hydroxy-6,19-oxidoprogesterone (21OH-6OP) of formula I,



(I)

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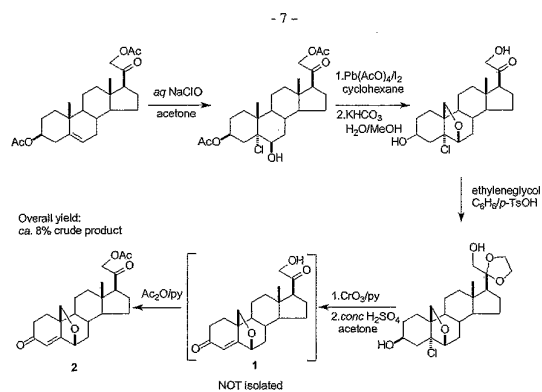
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is disclosed as a selective antiglucocorticoid which does not substantially cross-react with uterus- PR or kidney-MR. Said 21-hydroxy-6,19-oxidoprogesterone antiglucocorticoid could be used in the treatment of diseases associated with an excess of glucocorticoids, where an anti-glucocorticoid virtually lacking
5 mineralocorticoid or glucocorticoid properties as well as affinity for MR or PR is desirable.

The synthesis of 21-hydroxy-6,19-oxidoprogesterone (1) and its 21-acetate (2) was first accomplished by Deghenghi in 1966 as intermediate in a synthesis of 19-hydroxy-desoxy-corticosterone, starting from 21-hydroxypregnenolone diacetate.
10 The procedure summarized in **Scheme 1** involves the use of a "hypiodite type" reaction with lead tetraacetate ($\text{Pb}(\text{AcO})_4$). Compound 1 was neither isolated nor characterized, but acetylated *in situ* to acetate 2. Overall yield of partially purified 2 was only 8.3 %. Further to the low yield, this method of preparation is generally
15 perceived as being difficult to reproduce notably due to the formation of the chlorohydrin.

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Scheme 1: Synthesis of 21-hydroxy-6,19-oxidoprogesterone and its 21-acetate derivative by Deghenghi.

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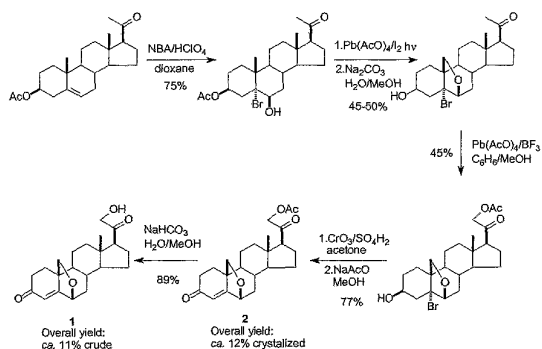
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In a more recent synthesis of 19-hydroxydeoxycorticosterone, Kirk and Yeoh (*J. Chem. Soc. Perkin Trans. I*, 2945 (1983)) prepared acetate **2** as an intermediate.

This procedure starting from pregnenolone acetate is depicted in **Scheme 2**.

Although full details of the first 3 steps are not given in the experimental section of their publication, according to the literature cited the yield for these steps may be estimated as *ca.* 34-37% giving an overall yield of acetate **2** of *ca.* 12% from pregnenolone acetate.



Scheme 2: Synthesis of 21-hydroxy-6,19-oxidoprogesterone and its acetate by Kirk *et al.*

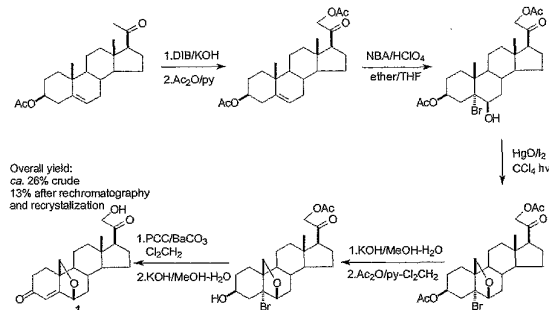
According to a further method, depicted in **Scheme 3**, the remote functionalization reaction with $\text{Pb}(\text{AcO})_4$ and iodine under thermal or photochemical conditions is replaced with the more reproducible and "milder" HgO/iodine system under

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photochemical conditions and the 21-hydroxylation step is carried out with a hypervalent iodine compound (see A. S. Veleiro, M. V. Nevado, M. C. Monteserín and G. Burton, *Steroids*, **60**, 268-272 (1995); M. Akhtar and D. H. R. Barton, *J. Am. Chem. Soc.*, **86**, 1528-1534 (1964); R. M. Moriarty, L. S. John and P. C. Du, *J. Chem. Soc. Chem. Commun.*, 641-642 (1981).



Scheme 3: Synthesis of 21-hydroxy-6,19-oxidoprogesterone by Veleiro *et al.*

The above procedure starts also from pregnenolone acetate which is first converted into 21-hydroxypregnenolone diacetate. In the best case, overall yield of **1** is ca. 26%; however this product, although apparently pure according to TLC and NMR, could not be crystallized, and thus required additional purification steps by chromatography; yield of pure crystalline **1** is about 13% (19% from 21-hydroxypregnenolone diacetate). Although the acetate **2** is an intermediate in this synthetic route, complete purification could only be achieved after deacetylation (i.e. on compound **1**). Furthermore, although this procedure allows the synthesis of small amounts of **1** for the initial biological tests carried out in 1996, attempts to

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scale up the procedure failed. Although, the currently known methods provide 21-hydroxy-6,19-oxidoprogesterone (1), they consistently give poorer yields as well as byproducts which are difficult to eliminate.

5 Description of the invention:

It is an object of the invention to provide a new method for preparing 21-hydroxy-6,19-oxidoprogesterone (21OH-6OP) and/or its esters.

It is a further object of the invention to provide 21-hydroxy-6,19-oxidoprogesterone and/or its esters.

- 10 In a first aspect, the present invention provides a new method of preparing 21-hydroxy-6,19-oxidoprogesterone (1) or its esters, for example carboxylate, phosphate or sulfate esters. In preferred embodiments, the invention provides a new method for preparing
- 21 OH-6OP and its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate
- 15 and 21-oleate derivatives. The method of the invention avoids essentially all of the hitherto known inconveniences. The method of the invention produces 21-hydroxy-6,19-oxidoprogesterone (1) in good yield, having an acceptable degree of purity, and avoids heavy metals as reagent. The method of the invention is suitable for large scale industrial preparation of 21-hydroxy-6,19-oxido-progesterone (1) and its
- 20 esters.

The objects of the invention are met according to the main claim. Preferred embodiments are set out within the dependent claims which are incorporated herewith.

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The novel synthetic procedure according to the present invention comprises or consists of the following consecutive basic steps :

- a) Providing 21-acetoxypregnenolone (3) (which is actually a commercial product);
- 5 b) Transforming the C-3 hydroxy group of 21-acetoxypregnenolone into a labile ester, preferably a formate ester;
- c) Obtaining the bromohydrin product from the protected 21-acetoxypregnenolone, said bromohydrin resulting from the addition of a bromine and a hydroxy group onto the double bond of position of C5-C6;
- 10 d) Performing an intramolecular cyclisation with the C19 atom with the "Suarez-reagent" (diacetoxyiodobenzene; see Armas et al., *J.Chem.Soc. Perkin I*, 405 (1989)) and iodine under irradiation, thus obtaining the 6,19 oxido-bridge within the scaffold;
- e) Performing a selective hydrolysis, preferably with
- 15 HCl/MeOH/dichloromethane, followed by an oxidation, preferably with pyridinium chlorochromate (PCC), thus obtaining a bromoketone;
- f) Performing a hydrolysis of the bromoketone resulting from step e) to obtain 21-hydroxy-6,19-oxidoprogesterone (1), and optionally
- g) Acylating 21-hydroxy-6,19-oxidoprogesterone (1), to afford the 21-ester
- 20 (the acetate is shown as 2);

Preferred esters are C1-18 acyl esters (optionally substituted with COOH, 1 or 2 times) and phosphate esters. Acyl esters may be obtained by reacting 21-hydroxy-6,19-oxidoprogesterone (1) with an organic acid, in the presence of a coupling agent (for example N, N'-dicyclohexylcarbodiimide), or with an activated organic

25 ester (for example, a nitrophenol ester), or with an acyl halide (for example, an acyl

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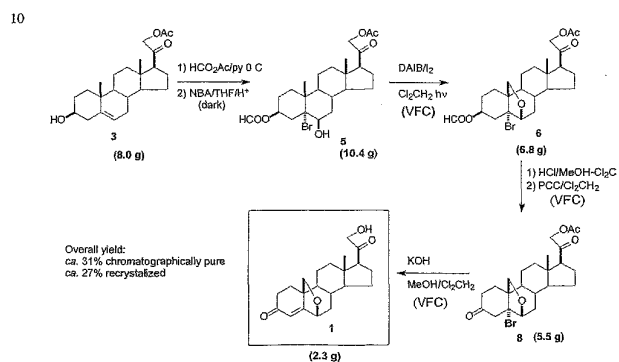
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chloride), or with an acyl anhydride. Phosphate esters may be obtained by reacting 21-hydroxy-6,19-oxidoprogesterone with a phosphorylating agent (for example phosphorus oxychloride, followed by basic hydrolysis).

The 21-propionate or 21-hemisuccinate, and 21-oleate and derivatives are obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone (1) of step f) with propionic acid, succinic or oleic acid, their anhydrides, activated esters or acyl chlorides.

A preferred synthetic procedure according to the present invention is depicted in Scheme 4.



Scheme 4: Novel Synthesis of 21-hydroxy-6,19-oxidoprogesterone

15

According to the preferred method illustrated in Scheme 4, a formate group is introduced as protecting group for position 3. Formates have the advantage that

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they may be hydrolyzed under relatively mild acid conditions (HCl/MeOH-dichloromethane) in which primary acetates and even α -acetoxy-ketones are stable. The introduction of a formate moiety is preferably carried out under very mild conditions using preferably mixed acetic-formic anhydride (prepared *in situ* from formic acid and acetic anhydride). The bromo-hydrin formation, is carried out with N-bromoacetamide in THF yielding 80% of the desired bromohydrin **5** and about 20% of the 5 α -HO-6 β -Br isomer. The mixture may be separated by crystallization or Thin Layer Chromatography. For the intramolecular cyclisation involving C-19, the "Suarez reagent" (diacet-oxyiodobenzene (DAIB)) and iodine is employed as the "hypoiodite" as generating system under suitable irradiation – preferably with a standard tungsten lamp.

Quite surprisingly, it turns out that upon performing the intramolecular cyclisation step in di-chloromethane (CH₂Cl₂), the reaction is not only rapid (complete conversion in less than 1 hour) and clean, but also excellent yields could be obtained.

Temperature control (25 °C) of the cyclisation step, achieved by using a glass jacketed reactor with water circulation, increases the yield and diminishes excessive oxidation by-products. Furthermore, epoxide formation is eliminated, the isomeric bromohydrin is inert to the reagent and could be easily separated (together with the iodobenzene side product) from the desired bromoether **6** by vacuum filtration through a silica gel column (VFC).

The selective hydrolysis of the formate ester, is preferably performed with HCl in MeOH-dichloromethane, followed by an oxidation, preferably with PCC, thus affording the bromoketone **8**. This product partially eliminates HBr to give the unsaturated ketone (**2**) when subjected to VFC (very short silica gel column) purification, however this poses no problem as the unsaturated product is the

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desired product of the following step, in which the treatment with a base deacetylates position 21 and completes the elimination of the C-5 bromine thus giving the desired pro-duct (1).

Purification of 1 may be achieved with a fast VFC through a short silica gel column to afford chromatographically pure product in over 31% yield. This may be crystallized from absolute ethanol to yield crystalline 1 in 27% yield.

The 21-acetate (2) is prepared by standard acetylation of 1 with acetic anhydride in pyri-dine. The acetate is recrystallized from absolute ethanol.

The novel synthesis according to the present invention has the following advantages :

- a) It eliminates the need of the selective acetylation step or any need to differentiate the hydroxyl groups at C-21 and C-3.
- b) It modifies the hypiodite reaction so that no heavy metals are required (lead, silver or mercury based reagents) and it proceeds under homogeneous conditions, thus allowing a scale up to multigram and eventually kilogram amounts.
- c) It reduces the secondary products formed at different stages (specially 5,6-epoxide for-mation) so as to minimize the purification steps, and specially to avoid or minimize the need of column chromatography on silica gel, as bromoethers, compound 1 and 2 are intrinsically unstable under these conditions.
- d) It reduces reaction volumes throughout to allow scale up.

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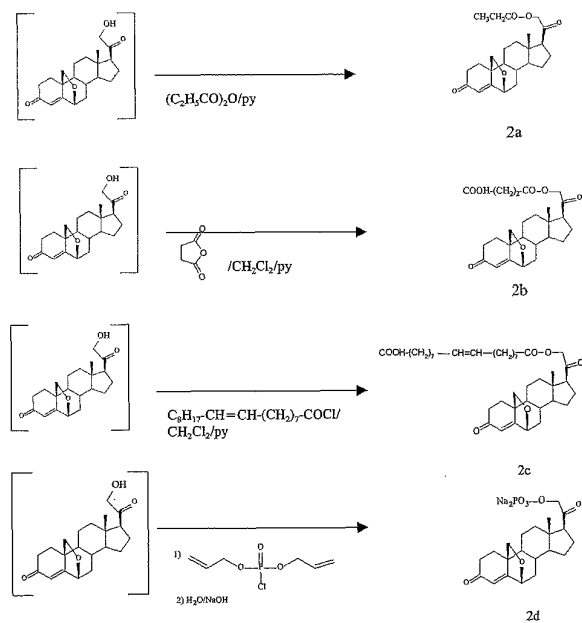
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Starting from compound 1, synthesis of acyl esters, such as 21-propionate (2a), and 21-hemisuccinate (2b) and 21-oleate (2c)-6,19-oxidoprogesterone derivatives can be performed as illustrated in Scheme 5.

Also shown in Scheme 5 is the preparation of the 21-phosphate derivative (2d).

5



Scheme 5: Synthesis of 21-propionate (2a), 21-hemisuccinate (2b), 21-oleate (2c)- and 21- PO_4 (2d) 6,19-oxidoprogesterone derivatives.

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The compounds synthesised according to the present invention are for use as a medicament, alone or in combination with pharmaceutically acceptable carriers and/or excipients. Such medicament is notably suitable in the manufacture of a
5 medicament for the treatment or prophylaxis of diseases associated with an excess of glucocorticoids, e.g. for the treatment of Cushing's syndrome, iatrogenic hypercortisolism or depression.

The invention will now be described, by way of illustration only, with reference to
10 the following examples :

EXAMPLES

15 MATERIALS AND METHODS

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna IR 550 FT-IR spectrometer. ¹H and ¹³C NMR spectra were measured in Bruker AC-200 or AM-500 NMR spectrometers in deu-teriochloroform (using TMS as internal
20 standard). The *J* values are given in Hz. Spectra were assigned by analysis of the DEPT, COSY 45 and HETCOSY spectra and by com-parison with those of progesterone.

The electron impact mass spectra (EI) were measured in a VG Trio 2 mass spectrometer at 70 eV by direct inlet. FAB mass spectra and electron impact high
25 resolution mass spectra (HRMS) were obtained in a VG ZAB BEQQ mass spectrometer. All solvents used were reagent grade. Solvents were evaporated at ca. 45°C under vacuum. Zinc dust was activated by suspending in 1M HCl, washing

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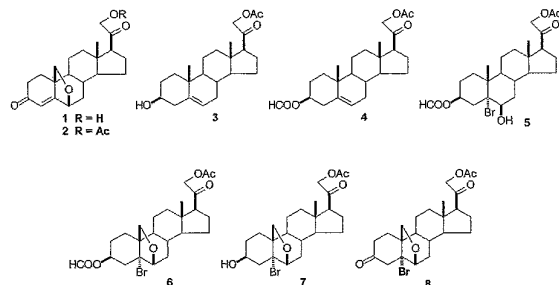
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with water, absolute ethanol and diethyl ether and drying 2 h at 120°C. The homogeneity of all compounds was confirmed by thin layer chromatography.

Large scale synthesis of 21-hydroxy-6,19-oxidoprogesterone (1)

- 5 Structures of starting material, intermediates and final products (1) and (2):



- 10 **3 β -Formyloxy-21-acetyloxy-5-pregnen-20-one (4)**

Acetic anhydride (13.4 ml) is added dropwise to formic acid (6.6 ml) at 0°C, the solution is heated at 50°C for 15 min and cooled rapidly to 0°C. The resulting acetoformic anhydride solution is added dropwise to a stirred suspension of 21-acetoxypregnenolone (3, 8.0 g) in dry pyridine (20.8 ml) at 0°C, and stirring is continued at that temperature for 2 h. The reaction product is poured over cold saturated aqueous sodium bicarbonate solution, filtered and the solid is washed with saturated aqueous sodium bicarbonate solution, water and 1N HCl and water (until neutral) rendering formate 4 (8.0 g); ¹H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 1.02 (3H, s, 10-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.53 (1H, t, J = 8.0 Hz,

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17-H), 4.50 (1H, d, $J = 17.0$ Hz, 21a-H), 4.70 (1H, d, $J = 17.0$ Hz, 21b-H), 5.32 (1H, m, 3-H), 5.38 (1H, d, $J = 3.0$ Hz, 6-H), 8.02 (1H, s, HCOO).

3 β -Formyloxy-5 α -bromo-6 β -hydroxy-21-acetyloxypregnan-20-one (5)

Formate **4** (8.0 g), is dissolved in diethyl ether (100 ml) and THF (37.2 ml) and
5 cooled to 10°C. To the stirred solution at 10-15°C - which protected from light -
7.5% perchloric acid (11.88 ml) is added, followed by N-bromoacetamide (4.75 g)
in 8 portions over a 25 min period. Stirring is continued for 45 min at 25°C and the
reaction is stopped by addition of 10% aqueous sodium thiosulfate solution until
complete decoloration. The reaction mixture is then extracted with
10 dichloromethane/methanol 10:1 and the organic layer, is washed with water, dried
with anhydrous sodium sulfate and the solvent is evaporated to afford bromo-
hydrin **5** (10.4 g, containing about 20% of the 5 α -hydroxy-6 β -bromo isomer as
determined by ¹H NMR).

3 β -Formyloxy-5 α -bromo-21-acetyloxy-6,19-oxidopregnan-20-one (6)

15 Nitrogen is bubbled for 5 min through a solution of bromohydrin compound **5** (10.4
g, containing about 20% of the 5 α -hydroxy-6 β -bromo isomer) in freshly distilled
dichloro-methane (723 ml) contained in a 1 liter glass vessel fitted with an external
cooling jacket with circulating water at 25°C and magnetic stirrer.
Diacetoxiodobenzene (Suarez reagent, 7.66 g) and iodine (5.46 g) are successively
20 added with stirring. The vessel is exposed to two 300 Watt tungsten lamps (5000
lm each) and vigorous stirring is continued for 1 h at 25°C. Irradiation is turned off
and a saturated aqueous solution of sodium thiosulfate is added until complete
decoloration. The organic layer is separated, dried with anhydrous sodium sulfate
and the solvent evaporated. The resulting solid is dissolved in dichloro-methane (8
25 ml) and applied to a silicagel G-60 column (12 cm diameter x 8 cm height)
previously flushed with hexane; successive elution (applying vacuum to the outlet)

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with hexane-ethyl acetate 9:1 (1100 ml), 8:2 (700 ml), 7:3 (700 ml) and 6:4 (600 ml) affords 31 x 100 ml fractions. Fractions are analyzed by TLC and those containing bromoether **6** are pooled and evaporated to dryness to afford **6** (6.8 g).

¹H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, J = 8.8 Hz, 17-H), 3.73 (1H, d, J = 8.4 Hz, 19a-H), 3.94 (1H, d, J = 8.4 Hz, 19b-H), 4.08 (1H, d, J = 4.2 Hz, 6-H), 4.50 (1H, d, J = 16.8 Hz, 21a-H), 4.71 (1H, d, J = 16.8 Hz, 21b-H), 5.34 (1H, m, 3-H), 8.02 (1H, s, HCOO).

3 β -Hydroxy-5 α -bromo-21-acetyloxy-6,19-oxidopregnan-20-one (7)

A stirred solution of the bromoether **6** (6.8 g) obtained above, is dissolved in dichloro-methane (45.7 ml) and methanol (154.7 ml) and is cooled to 0°C in an ice bath and water (10.9 ml) while conc. HCl (23.0 ml) is added. After about 30 min of vigorous stirring at 0°C (disappearance of the starting material is monitored by TLC) the reaction mixture is neutralized with 20% aqueous sodium hydroxide and extracted with dichloromethane. The organic layer is dried with anhydrous sodium sulfate and the solvent evaporated to afford the alcohol compound **7** (6.5 g); ¹H NMR (200.13 MHz) δ_{H} 0.69 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, J = 8.5 Hz, 17-H), 3.62 (1H, d, J = 8.5 Hz, 19a-H), 3.92 (1H, d, J = 8.5 Hz, 19b-H), 4.07 (1H, d, J = 4.0 Hz, 6-H), 4.15 (1H, m, 3-H), 4.51 (1H, d, J = 17.0 Hz, 21a-H), 4.70 (1H, d, J = 17.0 Hz, 21b-H).

5 α -Bromo-21-acetyloxy-6,19-oxidopregnane-3,20-dione (8)

A suspension of pyridinium chlorochromate (12.1 g), barium carbonate (5.0 g) and 3Å molecular sieves (9.60 g), in dry dichloromethane (480 ml) is stirred under nitrogen for 10 min. To the resultant orange slurry a solution of bromoether **7** (6.5 g) obtained above in dry dichloromethane (324 ml) is added and stirring is continued for about 90 min, until the starting material (TLC) has disappeared. The reaction mixture is percolated through a short silicagel G 60 column (12 cm

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diameter x 8 cm height) washed with diethyl ether (2 x 150 ml) and hexane-ethyl acetate 1:2 (3 x 150 ml). Fractions containing the product are pooled and evaporated to dryness affording 5.5 g of ketone 8 (containing about 10 % of Δ^4 -3-ketone (2); ¹H NMR (200.13 MHz) δ_H 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.51 (1H, t, J = 8.5 Hz, 17-H), 2.85 (1H, d, J = 16.0 Hz, 4a-H), 3.40 (1H, d, J = 16.0 Hz, 4b-H), 3.90 (1H, d, J = 9.0 Hz, 19a-H), 4.07 (1H, d, J = 4.0 Hz, 6-H), 4.15 (1H, d, J = 9.0 Hz, 19b-H), 4.50 (1H, d, J = 17.0 Hz, 21a-H), 4.71 (1H, d, J = 17.0 Hz, 21b-H).

21-Hydroxy-6,19-oxido-4-pregnene-3,20-dione (1)

10 The ketone 8 (5.5 g) obtained from the preceding step is dissolved in methanol (263.8 ml) and dichloromethane (13.2 ml). To the stirred solution 14% methanolic KOH (53.4 ml) is added and stirring is continued at room temperature for about 15 min, until the starting material (TLC) has disappeared. The reaction mixture is neutralized with 1N HCl and extracted with dichloromethane. The organic layer is
 15 dried with anhydrous sodium sulfate and the solvent is evaporated thus yielding crude 21-hydroxy-6,19-oxidoprogesterone (1, 4.6 g). The solid is dissolved in dichloromethane (5 ml) and sent through to a silicagel G-60 column (8.5 cm diameter x 5 cm height) previously flushed with hexane-ethyl acetate 7:3; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 6:4
 20 (1350 ml) and 1:1 (900 ml) affords 30 fractions. The fractions are analyzed by TLC and those containing 1 are pooled and evaporated to dryness to afford 21-hydroxy-6,19-oxidoprogesterone (1, 2.3 g). ¹H NMR (200.13 MHz) δ_H 0.74 (3H, s, 13-CH₃), 2.45 (1H, t, J = 8.5 Hz, 17-H), 3.51 (1H, d, J = 8.8 Hz, 19a-H), 4.18 (3H, s, 21-CH₃), 4.20 (1H, d, J = 8.8 Hz, 19b-H), 4.69 (1H, d, J = 5.0 Hz, 6-H), 5.82 (1H,
 25 s, 4-H).

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Re-crystallization from absolute ethanol affords a first crop of crystalline 1 (1.27 g), mp 165-166°C. The mother liquor is concentrated to yield a second crop of 1 (0.68 g).

5 *21-Acetyloxy-6,19-oxido-4-pregnene-3,20-dione (2)*

Crude 21-hydroxy-6,19-oxidoprogesterone (1, 2 g before chromatographic purification) was dissolved in dry pyridine (15.6 ml) and acetic anhydride (15.6 ml) added. The solution was stirred for 90 min at 25°C, poured over 1M HCl and filtered (alternatively the solid may be extracted with dichloromethane). The solid
10 was washed with water (until neutral), dried, dissolved in dichloromethane (2 ml) and applied to a silicagel G-60 column (7 cm diameter x 5 cm height) previously flushed with hexane-ethyl acetate 7:3; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 7:3 (700 ml) and 6:4 (700 ml) afforded 20 fractions. Fractions were analyzed by TLC and those containing 2 pooled and 15 evaporated to dryness to afford 21-acetyloxy-6,19-oxidoprogesterone (2, 1.12 g). Recrystallization from absolute ethanol (with drops of methanol) afforded crystalline 2 (0.72 g), mp 190-191°C. ¹H NMR (200.13 MHz) δ_H 0.76 (3H, s, 13-CH₃), 2.17 (3H, s, 21-CH₃CO), 2.51 (1H, t, *J* = 8.5 Hz, 17-H), 3.51 (1H, d, *J* = 8.2 Hz, 19a-H), 4.20 (1H, d, *J* = 8.2 Hz, 19b-H), 4.50 (1H, d, *J* = 16.7 Hz, 21a-H), 4.69
20 (1H, d, *J* = 5.0 Hz, 6-H), 4.72 (1H, d, *J* = 16.7 Hz, 21b-H), 5.82 (1H, s, 4-H).

21-Propanoyloxy-6,19-oxido-4-pregnene-3,20-dione (2a)

Crude 21-hydroxy-6,19-oxidoprogesterone (1, 0.2 g before chromatographic purification) was dissolved in dry pyridine (0.28 ml) and propanoic anhydride (0.2 ml) added. The solution was stirred for 1 h at 25°C, methanol was added to
25 destroy excess anhydride and the solution concentrated *in vacuo*. The residue was diluted with dichloromethane, washed with 1M HCl and water, and evaporated to dryness (0.233 g). Purification as above afforded 21-propanoyloxy-6,19-

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oxidoprogesterone (**2a**, 0.116 g). ¹H NMR (200.13 MHz) δ_{H} 0.76 (3H, s, 13-CH₃), 1.18 (3H, t, $J = 7.6$ Hz, 21-CH₃CH₂CO), 2.46 (2H, q, $J = 7.6$ Hz, 21-CH₃CH₂CO), 2.51 (1H, t, $J = 8.0$ Hz, 17-H), 3.51 (1H, d, $J = 8.2$ Hz, 19a-H), 4.20 (1H, d, $J = 8.2$ Hz, 19b-H), 4.50 (1H, d, $J = 16.8$ Hz, 21a-H), 4.70 (1H, d, $J = 5.0$ Hz, 6-H), 4.73 (1H, d, $J = 16.8$ Hz, 21b-H), 5.82 (1H, s, 4-H). EIMS m/z 400 (17) [M]⁺, 342 (5), 313 (24), 285 (23), 267 (10), 57 (100).

21-Succinoyloxy-6,19-oxido-4-pregnene-3,20-dione (2b)

21-hydroxy-6,19-oxidoprogesterone (**1**, 0.75 g) was dissolved in dry dichloromethane (37.5 ml) and pyridine (1.9 ml) and succinic anhydride (0.75 g) added. The solution was stirred for 4 h at 25°C, a second portion of succinic anhydride (0.375 g) added and stirring continued for 6 h (until disappearance of starting material). The reaction mixture was concentrated, and the residue extracted with diethyl ether. The ethereal solution was washed with 1M HCl and extracted with aqueous 10% sodium carbonate. The aqueous layer was acidified with conc. HCl to pH = 3 and extracted with dichloromethane. Washing with water and evaporation to dryness afforded crude 21-succinoyloxy-6,19-oxidoprogesterone (**2b**, 0.823 g). The solid was dissolved in dichloromethane (1 ml) and applied to a silicagel G-60 column (6 cm diameter x 4 cm height) previously flushed with hexane-ethyl acetate 2:8; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 2:8 (200 ml) and ethyl acetate (500 ml) afforded 14 fractions. Fractions were analyzed by TLC and those containing **2b** pooled and evaporated to dryness to afford 21-succinoyloxy-6,19-oxidoprogesterone (**2b**, 0.455 g). ¹H NMR (200.13 MHz) δ_{H} 0.75 (3H, s, 13-CH₃), 2.52 (1H, t, $J = 8.0$ Hz, 17-H), 2.75 (4H, m, 21-HCOOCH₂CH₂CO), 3.51 (1H, d, $J = 8.2$ Hz, 19a-H), 4.20 (1H, d, $J = 8.2$ Hz, 19b-H), 4.54 (1H,

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d, $J = 16.9$ Hz, 21a-H), 4.70 (1H, d, $J = 5.0$ Hz, 6-H), 4.75 (1H, d, $J = 16.9$ Hz, 21b-H), 5.82 (1H, s, 4-H). EIMS m/z 444 (3) $[M]^+$, 344 (24), 313 (55), 285 (44), 267 (17), 91 (60), 79 (54), 55 (100).

5 *21-Oleoyloxy-6,19-oxido-4-pregnene-3,20-dione (2c)*

21-hydroxy-6,19-oxidoprogesterone (1, 0.052 g) was dissolved in dry dichloromethane (1 ml) and pyridine (0.12 ml) and oleoyl chloride (0.1 ml) added. The solution was stirred for 24 h at 25°C, diluted with dichloromethane, washed with 1M HCl and water, and evaporated to dryness. Purification by
 10 preparative TLC afforded 21-oleoyloxy-6,19-oxidoprogesterone (2c, 0.075 g). 1H NMR (200.13 MHz) δ_H 0.76 (3H, s, 13-CH₃), 0.88 (3H, t, $J = 7.0$ Hz, CH₃-CH-CO), 2.34 (2H, t, $J = 7.7$ Hz, CH-CH₂CO), 2.50 (1H, t, $J = 8.5$ Hz, 17-H), 3.51 (1H, d, $J = 8.3$ Hz, 19a-H), 4.20 (1H, d, $J = 8.3$ Hz, 19b-H), 4.49 (1H, d, $J = 16.9$ Hz, 21a-H), 4.70 (1H, d, $J = 4.5$ Hz, 6-H), 4.73 (1H, d, $J =$
 15 16.9 Hz, 21b-H), 5.34 (2H, m, CH-CH=CH-CH-CO), 5.82 (1H, s, 4-H).

21-Phosphate-6,19-oxido-4-pregnene-3,20-dione (2d)

21-hydroxy-6,19-oxido-4-pregnene-3,20-dione (1, 0.052g) was dissolved in dry dichloromethane (1 ml) and pyridine (0.12 ml), and phosphochloridic acid diallyl
 20 ester (0.024g, 1 eq.) was added dropwise at 0°C, over 30 minutes. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was washed three times with 5% NaHCO₃, the organic layer was dried and evaporated in vacuo without heating. To the resulting syrup was added a solution of NaOH (2.2 equivalents) in water (3 ml). The solution was brought slowly to
 25 reflux, and gently refluxed for 12 hours. Lyophilization yielded the crude

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phosphate disodium salt, which was recrystallized from ethanol. Alternatively, the crude disodium salt may be dissolved in water, and the pH adjusted with HCl, to cause precipitation of the free phosphate.

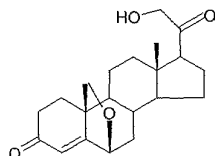
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Claims

1. A method of preparing 21-hydroxy-6,19-oxidoprogesterone (1) or an acyl or
 5 phosphate derivative thereof.



(1)

the method comprising the steps

- a) providing 21-acetoxypregnenolone;
- 10 b) protecting the C-3 hydroxy group of 21-acetoxypregnenolone through
 formation of a labile ester;
- c) obtaining the bromohydrin product thereof, by addition of a bromine and a
 hydroxy group onto the double bond of position of C5-C6 of the ester
 protected 21-acetoxy-pregnenolone;
- 15 d) performing an intramolecular cyclization with the C19 atom with the
 "Suarez-reagent" (diacetoxyiodobenzene) and iodine under irradiation, thus
 obtaining the 6,19 oxido-bridge within the scaffold;
- e) performing a selective hydrolysis, followed by an oxidation, to obtain a
 bromo-ketone;

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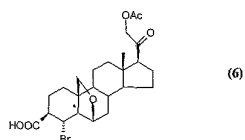
- f) performing a hydrolysis of the bromoketone resulting from step e) to obtain 21-hydroxy-6,19-oxidoprogesterone (1); and optionally
- g) reacting compound (1) with an acylating agent or phosphorylating agent.
2. The method according to claim 1, wherein the 21-acetate derivative is obtained
5 by acetylating 21-hydroxy-6,19-oxidoprogesterone (1) of step f).
3. The method according to claim 1, wherein a C1-18 acyl derivative is obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone with a C1-18 acid, anhydride, active ester or acyl chloride.
4. The method according to claim 1, wherein a 21-propionate, 21-hemisuccinate,
10 21-phosphate, 21-oleate derivative is obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone (1) of step f) with propionic acid, succinic acid, oleic acid or their anhydrides or active esters or acid chlorides.
5. The method according to claim 1, wherein the 21-phosphate derivative is
15 obtained by reacting oxidoprogesterone (1) of step (f) with a phosphorylating agent.
6. The method according to any of the preceding claims wherein for step b) a formate ester is afforded.
7. The method according to any of the preceding claims, wherein the intramolecular cyclisation step d) is performed in dichloromethane as solvent.
- 20 8. The method according to any of the preceding claims, wherein the selective hydrolysis of step e) is performed with HCl in MeOH/dichloromethane as solvent.

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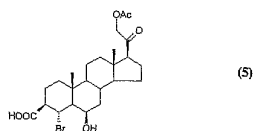
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9. The method according to any of the preceding claims, wherein the oxidation in step e) is performed with pyridinium chlorochromate (PCC).
10. The method according to any of the preceding claims, wherein the hydrolysis in step f) is performed with KOH in MeOH/dichloromethane as solvent.
- 5 11. A method for preparing (6)



comprising the step of performing an intramolecular cyclisation between the 6-OH and the C₂₁ of the molecule (5)



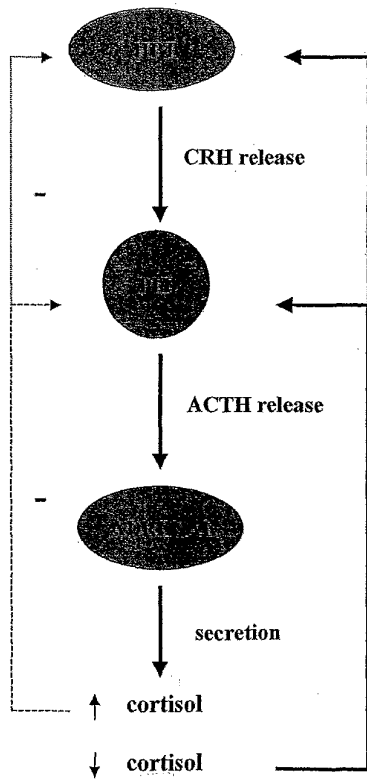
12. A method according to claim 11, wherein the intramolecular cyclisation is carried out by photoreaction with DIAB and I₂.
- 10
13. A method according to claim 11 or 12, wherein the intramolecular cyclisation is carried out in CH₂Cl₂.

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Figure 1



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(54) Title: METHOD FOR THE PREPARATION OF 21-HYDROXY-6,19-OXIDOPROGESTERONE (21OH-6OP)

(57) Abstract: The present invention relates to a novel method for preparing 21-hydroxy-6,19-oxidopro-gesterone (21OH-6OP) and/or its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate, 21-oleate derivatives. 21OH-6OP and its ester derivatives are antigluccorticoids for the treatment or prophylaxis of diseases associated to an excess of glucocorticoids, in particular for treating Cushing's syndrome, iatrogenic hypercortisolism or depression.

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(54) Title: METHOD FOR THE PREPARATION OF 21-HYDROXY-6,19-OXIDOPROGESTERONE (21OH-6OP)

(57) Abstract: The present invention relates to a novel method for preparing 21-hydroxy-6,19-oxidopro-gesterone (21OH-6OP) and/or its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate, 21-olate derivatives. 21OH-6OP and its ester derivatives are antigluccorticoids for the treatment or prophylaxis of diseases associated to an excess of glucocorticoids, in particular for treating Cushing's syndrome, iatrogenic hypercortisolism or depression.

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Method for the preparation of 21-hydroxy-6,19-oxidoprogesterone
(21OH-6OP)

Field of the invention:

- 5 The present invention relates to a novel method of preparing 21-hydroxy-6,19-oxidopro-gesterone (21OH-6OP) and/or its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate and 21-oleate derivatives. 21OH-6OP and its esters are antigluccorticoids for the treatment or prophylaxis of diseases associated with a glucocorticoid imbalance, in particular for treating Cushing's syndrome or
10 depression.

Background of the invention:

- Corticosteroides are steroid hormones related structurally to cholesterol. These hormones are synthesized in the adrenal cortex and include the glucocorticoids (e.g.
15 cortisol), the mineralocorticoids (e.g aldosterone) as well as weak androgens and estrogens. The adrenal function, like that of the thyroid gland, is under the control of the hypothalamus (HPT) and the pituitary (PTT). When cortisol (the naturally-occurring glucocorticoid) levels drop below a setpoint, the hypothalamus releases CRH (corticotropin releasing hormone) which stimulates adrenocorticotropic
20 hormone (ACTH) release from the pituitary. ACTH is a tropic hormone which stimulates
- the synthesis and secretion of cortisol (it has minimal effects on aldosterone synthesis/secretion), and
 - the growth of the adrenal gland. When cortisol levels increase, this shuts off
25 CRH and ACTH secretion (cf. **Figure 1**).

Cortisol is characterized by its properties related to the biosynthesis and metabolism of glucose and propeties related to non-specific as well as specific

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immunity. Due to their effects on the glucose metabolism, cortisol and natural or synthetic analogues thereof are usually named glucocorticoids. They bind to the glucocorticoid receptor (GR).

- 5 The glucocorticoid receptor is a member of a protein super family of closely related intracellular receptors which function as ligand-activated transcription factors. Other members of this super family are the mineralocorticoid receptor (MR) and the progesterone receptor (PR). MR and GR have shown to be highly homologous, thus natural and even synthetic steroids exhibit cross-reaction between these
10 receptors. With respect to PR, its natural ligand progesterone also cross-reacts with MR and GR.

- Cushing's syndrome is a disorder resulting from increased adrenocortical secretion of cortisol. Hyperfunction of the adrenal cortex may be ACTH-dependent or it may be independent of ACTH regulation, e.g. production of cortisol by an
15 adrenocortical adenoma or carcinoma. The administration of supraphysiologic quantities of exogenous cortisol or related synthetic analogs suppresses adrenocortical function and mimics ACTH-independent glucocorticoid hyperfunction. ACTH-dependent hyperfunction of the adrenal cortex may be due to hypersecretion of ACTH by the pituitary, secretion of ACTH by a nonpituitary
20 tumor such as small cell carcinoma of the lung (the ectopic ACTH syndrome), or administration of exogenous ACTH. While the term "Cushing's syndrome" has been applied to the clinical picture resulting from cortisol excess regardless of the cause, hyperfunction of the adrenal cortex resulting from pituitary ACTH excess has frequently been referred to as Cushing's disease, implying a particular
25 physiologic abnormality. Patients with Cushing's disease may have a basophilic adenoma of the pituitary or a chromophobe adenoma. Microadenomas can usually be visualized by CT or, preferably, MRI scan, using a high-resolution technique

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augmented by gadolinium. Some micro-adenomas are difficult to visualize even with these modalities. In some cases, no histological abnormality is found in the pituitary despite clear evidence of ACTH overproduction.

Reference to Cushing's syndrome is herein intended to mean the clinical picture resulting from cortisol excess regardless of the cause, which may be also iatrogenic, both by the injection of ACTH or by the direct administration of cortisol or synthetic analogs such as prednisone, prednisolone, dexamethasone or others that are widely used in various types of diseases including allergic, asthmatic, inflammatory or immunologic. Cushing's syndrome includes in addition adrenal tumours secreting corticoids, ectopic ACTH production and Cushing's disease.

Clinical manifestations include rounded "moon" faces with a plethoric appearance. There is truncal obesity with prominent supraclavicular and dorsal cervical fat pads ("buffalo hump"); the distal extremities and fingers are usually quite slender. Muscle wasting and weakness are present. The skin is thin and atrophic, with poor wound healing and easy bruising. Purple striae may appear on the abdomen. Hypertension, renal calculi, osteoporosis, glucose intolerance, reduced resistance to infection, and psychiatric disturbances are common. Cessation of linear growth is characteristic in children. Females usually have menstrual irregularities. An increased production of androgens, in addition to cortisol, may lead to hypertichosis, temporal balding, and other signs of virilism in the female.

Although development of antihormonal agents related to the estrogen and androgen receptors has been successful, the search for selective anti-corticoids is more restricted.

Known agents suppressing the synthesis of steroid hormones at various levels (i.e. inhibitors of enzymes which catalyze various stages of the synthesis of

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steroid hormones) are reviewed in *J.Steroid Biochem.*, **vol.5**, p.501 (1974) and include the following:

- 5 a) derivatives of diphenylmethane, e.g. amphenon B (which suppresses the synthesis of steroid hormones at stages 11- beta -, 17 - and 21 - of hydroxylase);
- b) derivatives of pyridine (SU-c series), e.g. metirapon (which suppresses synthesis at stage 11- beta of hydroxylase);
- 10 c) substituted alpha , alpha - glutaramides, e.g. aminoglutetimide (which impedes the synthesis of pregnenolone from cholesterol through suppression of 20- alpha -hydroxylase and C₂₀, C₂₂-liase;
- d) steroid substances e.g. trilostan (3 beta -substituted steroid- 3 beta -hydroxy-5-androsten-17-one), which suppresses 3 beta -desoxysteroidhydrogenase -5.4- isomerase (*Steroids*, **vol.32**, p.257).
- 15 e) steroids of the spironolactone family which are used as rapidly dissociating anti-Mineralocorticoids (*PNAS USA* 71(4) p.1431-1435 (1974).
- 20 f) a synthetic steroid described as an anti-Mineralocorticoids, ZK91587, showing specific binding properties for the kidney (*Z.Naturforsch.*, **45b**, p.711-715 (1990)) and hippocampus type I MR (*Life Science*, **59**, p.511-21 (1996)), but not for type II GR. It may therefore be conveniently useful as a tool in the investigation of MR function in tissues containing both receptor systems.

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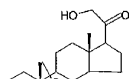
Agents that specifically suppress the interaction of glucocorticoid hormones with hormone receptors are:

- a) Mifepristone (11 β ,17 β)-11-[4-(Dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one, which acts on receptors of glucocorticoid hormones to form a complex incapable of initiating mechanisms leading to glucocorticoid effect (*Annals of New-York Academy of Science*, vol. 761, p.5-28 (1995)).
- b) non-steroid substances (*J.Steroid Biochem.*, vol. 31, p.481-492 (1988)) e.g. drotaverina hydrochloride (a derivative of isoquinoline-1-(3,4-dietoxibene zilidene)-6,7 - dietoxy-1,2,3,4- tetrahydrizoquinoline) or acetylsalicylic acid (*Moskovskaya Meditsina*, 1990, "Receptor mechanisms of the glucocorticoid effect" by V.P.Golikov).

To-date, the only therapeutical application for antiglucocorticoids (e.g. Mifepristone) that has been attempted in a clinical setting is to treat inoperable cases of nonpituitary Cushing's syndrome. In the case of Mifepristone (both an anti-progesterone and an anti-glucocorticoid), high doses (up to 800 mg per day) are required.

Employing a systematic application of strategies to increase activity and decrease cross-reactivity and undesirable side effects, progress has been reported in the development of antihormonal agents with greater potency and selectivity, especially in the antiestrogen and antiandrogen fields.

In EP-903'146 the synthetic steroid, 21-hydroxy-6,19-oxidoprogesterone (21OH-6OP) of formula I,



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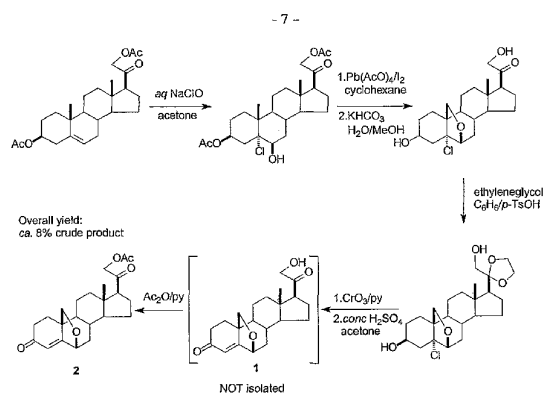
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is disclosed as a selective antiglucocorticoid which does not substantially cross-react with uterus- PR or kidney-MR. Said 21-hydroxy-6,19-oxidoprogesterone antiglucocorticoid could be used in the treatment of diseases associated with an excess of glucocorticoids, where an anti-glucocorticoid virtually lacking
5 mineralocorticoid or glucocorticoid properties as well as affinity for MR or PR is desirable.

The synthesis of 21-hydroxy-6,19-oxidoprogesterone (1) and its 21-acetate (2) was first accomplished by Deghenghi in 1966 as intermediate in a synthesis of 19-hydroxy-desoxy-corticosterone, starting from 21-hydroxypregnenolone diacetate.
10 The procedure summarized in **Scheme 1** involves the use of a "hypoiodite type" reaction with lead tetraacetate ($\text{Pb}(\text{AcO})_4$). Compound 1 was neither isolated nor characterized, but acetylated *in situ* to acetate 2. Overall yield of partially purified 2 was only 8.3 %. Further to the low yield, this method of preparation is generally perceived as being difficult to reproduce notably due to the formation of the
15 chlorohydrin.

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Scheme 1: Synthesis of 21-hydroxy-6,19-oxidoprogesterone and its 21-acetate derivative by Deghenghi.

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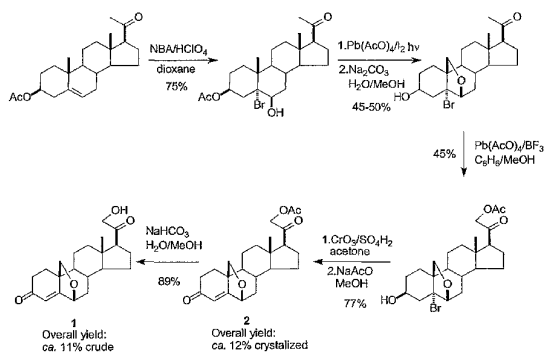
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In a more recent synthesis of 19-hydroxydeoxycorticosterone, Kirk and Yeoh (*J. Chem. Soc. Perkin Trans. I*, 2945 (1983)) prepared acetate **2** as an intermediate.

This procedure starting from pregnenolone acetate is depicted in **Scheme 2**.

Although full details of the first 3 steps are not given in the experimental section of their publication, according to the literature cited the yield for these steps may be estimated as ca. 34-37% giving an overall yield of acetate **2** of ca. 12% from pregnenolone acetate.



Scheme 2: Synthesis of 21-hydroxy-6,19-oxidoprogesterone and its acetate by Kirk *et al.*

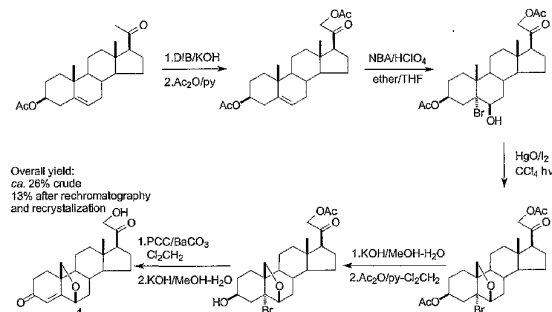
According to a further method, depicted in **Scheme 3**, the remote functionalization reaction with $\text{Pb}(\text{AcO})_4$ and iodine under thermal or photochemical conditions is replaced with the more reproducible and "milder" HgO /iodine system under

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photochemical conditions and the 21-hydroxylation step is carried out with a hypervalent iodine compound (see A. S. Veleiro, M. V. Nevado, M. C. Monteserín and G. Burton, *Steroids*, **60**, 268-272 (1995); M. Akhtar and D. H. R. Barton, *J. Am. Chem. Soc.*, **86**, 1528-1534 (1964); R. M. Moriarty, L. S. John and P. C. Du, *J. Chem. Soc. Chem. Commun.*, 641-642 (1981).



Scheme 3: Synthesis of 21-hydroxy-6,19-oxidoprogesterone by Veleiro *et al.*

The above procedure starts also from pregnenolone acetate which is first converted into 21-hydroxypregnenolone diacetate. In the best case, overall yield of **1** is ca. 26%; however this product, although apparently pure according to TLC and NMR, could not be crystallized, and thus required additional purification steps by chromatography; yield of pure crystalline **1** is about 13% (19% from 21-hydroxypregnenolone diacetate). Although the acetate **2** is an intermediate in this synthetic route, complete purification could only be achieved after deacetylation (i.e. on compound **1**). Furthermore, although this procedure allows the synthesis of small amounts of **1** for the initial biological tests carried out in 1996, attempts to

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scale up the procedure failed. Although, the currently known methods provide 21-hydroxy-6,19-oxidoprogesterone (1), they consistently give poorer yields as well as byproducts which are difficult to eliminate.

5 Description of the invention:

It is an object of the invention to provide a new method for preparing 21-hydroxy-6,19-oxidoprogesterone (21OH-6OP) and/or its esters.

It is a further object of the invention to provide 21-hydroxy-6,19-oxidoprogesterone and/or its esters.

- 10 In a first aspect, the present invention provides a new method of preparing 21-hydroxy-6,19-oxidoprogesterone (1) or its esters, for example carboxylate, phosphate or sulfate esters. In preferred embodiments, the invention provides a new method for preparing
- 21 OH-6OP and its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate
- 15 and 21-oleate derivatives. The method of the invention avoids essentially all of the hitherto known inconveniences. The method of the invention produces 21-hydroxy-6,19-oxidoprogesterone (1) in good yield, having an acceptable degree of purity, and avoids heavy metals as reagent. The method of the invention is suitable for large scale industrial preparation of 21-hydroxy-6,19-oxido-progesterone (1) and its
- 20 esters.

The objects of the invention are met according to the main claim. Preferred embodiments are set out within the dependent claims which are incorporated herewith.

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The novel synthetic procedure according to the present invention comprises or consists of the following consecutive basic steps :

- a) Providing 21-acetoxypregnenolone (3) (which is actually a commercial product);
- 5 b) Transforming the C-3 hydroxy group of 21-acetoxypregnenolone into a labile ester, preferably a formate ester;
- c) Obtaining the bromohydrin product from the protected 21-acetoxypregnenolone, said bromohydrin resulting from the addition of a bromine and a hydroxy group onto the double bond of position of C5-C6;
- 10 d) Performing an intramolecular cyclisation with the C19 atom with the "Suarez-reagent" (diacetoxyiodobenzene; see Armas et al., *J.Chem.Soc. Perkin I*, 405 (1989)) and iodine under irradiation, thus obtaining the 6,19 oxido-bridge within the scaffold;
- e) Performing a selective hydrolysis, preferably with
- 15 HCl/MeOH/dichloromethane, followed by an oxidation, preferably with pyridinium chlorochromate (PCC), thus obtaining a bromoketone;
- f) Performing a hydrolysis of the bromoketone resulting from step e) to obtain 21-hydroxy-6,19-oxidoprogesterone (1), and optionally
- g) Acylating 21-hydroxy-6,19-oxidoprogesterone (1), to afford the 21-ester
- 20 (the acetate is shown as 2);

Preferred esters are C1-18 acyl esters (optionally substituted with COOH, 1 or 2 times) and phosphate esters. Acyl esters may be obtained by reacting 21-hydroxy-6,19-oxidoprogesterone (1) with an organic acid, in the presence of a coupling agent (for example N, N'-dicyclohexylcarbodiimide), or with an activated organic

25 ester (for example, a nitrophenol ester), or with an acyl halide (for example, an acyl

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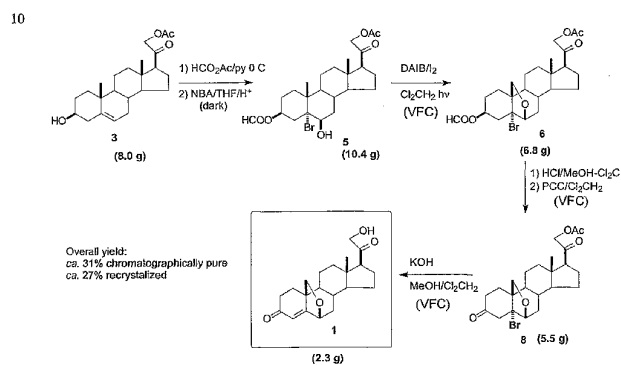
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chloride), or with an acyl anhydride. Phosphate esters may be obtained by reacting 21-hydroxy-6,19-oxidoprogesterone with a phosphorylating agent (for example phosphorus oxychloride, followed by basic hydrolysis).

The 21-propionate or 21-hemisuccinate, and 21-oleate and derivatives are obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone (**1**) of step f) with propionic acid, succinic or oleic acid, their anhydrides, activated esters or acyl chlorides.

A preferred synthetic procedure according to the present invention is depicted in Scheme 4.



15

According to the preferred method illustrated in Scheme 4, a formate group is introduced as protecting group for position 3. Formates have the advantage that

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they may be hydrolyzed under relatively mild acid conditions (HCl/MeOH-dichloromethane) in which primary acetates and even α -acetoxy-ketones are stable. The introduction of a formate moiety is preferably carried out under very mild conditions using preferably mixed acetic-formic anhydride (prepared *in situ* from formic acid and acetic anhydride). The bromo-hydrin formation, is carried out with N-bromoacetamide in THF yielding 80% of the desired bromohydrin **5** and about 20% of the 5 α -HO-6 β -Br isomer. The mixture may be separated by crystallization or Thin Layer Chromatography. For the intramolecular cyclisation involving C-19, the "Suarez reagent" (diacet-oxyiodobenzene (DAIB)) and iodine is employed as the "hypoiodite" as generating system under suitable irradiation – preferably with a standard tungsten lamp.

Quite surprisingly, it turns out that upon performing the intramolecular cyclisation step in di-chloromethane (CH₂Cl₂), the reaction is not only rapid (complete conversion in less than 1 hour) and clean, but also excellent yields could be obtained.

Temperature control (25 °C) of the cyclisation step, achieved by using a glass jacketed reactor with water circulation, increases the yield and diminishes excessive oxidation by-products. Furthermore, epoxide formation is eliminated, the isomeric bromohydrin is inert to the reagent and could be easily separated (together with the iodobenzene side product) from the desired bromoether **6** by vacuum filtration through a silica gel column (VFC).

The selective hydrolysis of the formate ester, is preferably performed with HCl in MeOH-dichloromethane, followed by an oxidation, preferably with PCC, thus affording the bromoketone **8**. This product partially eliminates HBr to give the unsaturated ketone (**2**) when subjected to VFC (very short silica gel column) purification, however this poses no problem as the unsaturated product is the

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desired product of the following step, in which the treatment with a base deacetylates position 21 and completes the elimination of the C-5 bromine thus giving the desired pro-duct (1).

Purification of 1 may be achieved with a fast VFC through a short silica gel column to afford chromatographically pure product in over 31% yield. This may be crystallized from absolute ethanol to yield crystalline 1 in 27% yield.

The 21-acetate (2) is prepared by standard acetylation of 1 with acetic anhydride in pyri-dine. The acetate is recrystallized from absolute ethanol.

The novel synthesis according to the present invention has the following advantages :

- a) It eliminates the need of the selective acetylation step or any need to differentiate the hydroxyl groups at C-21 and C-3.
- b) It modifies the hypoiodite reaction so that no heavy metals are required (lead, silver or mercury based reagents) and it proceeds under homogeneous conditions, thus allowing a scale up to multigram and eventually kilogram amounts.
- c) It reduces the secondary products formed at different stages (specially 5,6-epoxide for-mation) so as to minimize the purification steps, and specially to avoid or minimize the need of column chromatography on silica gel, as bromoethers, compound 1 and 2 are intrinsically unstable under these conditions.
- d) It reduces reaction volumes throughout to allow scale up.

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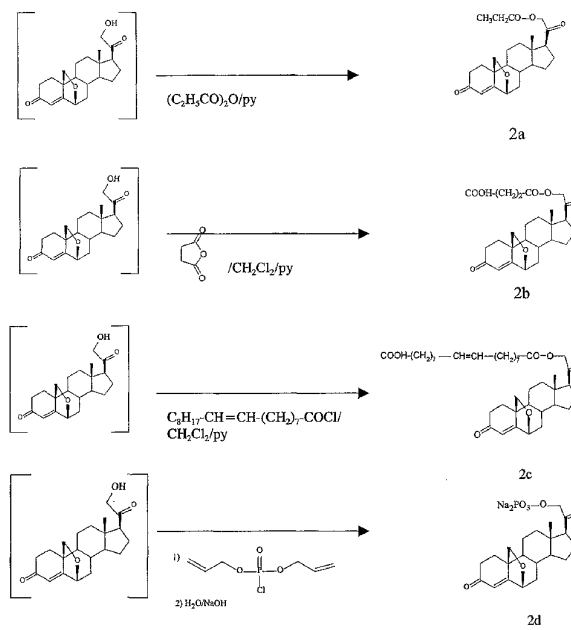
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Starting from compound **1**, synthesis of acyl esters, such as 21-propionate (**2a**), and 21-hemisuccinate (**2b**) and 21-oleate (**2c**) -6,19-oxidoprogesterone derivatives can be performed as illustrated in Scheme 5.

Also shown in Scheme 5 is the preparation of the 21-phosphate derivative (**2d**).

5



Scheme 5: Synthesis of 21-propionate (**2a**), 21-hemisuccinate (**2b**), 21-oleate (**2c**), and 21-phosphate (**2d**) -6,19-oxidoprogesterone derivatives

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The compounds synthesised according to the present invention are for use as a medicament, alone or in combination with pharmaceutically acceptable carriers and/or excipients. Such medicament is notably suitable in the manufacture of a
5 medicament for the treatment or prophylaxis of diseases associated with an excess of glucocorticoids, e.g. for the treatment of Cushing's syndrome, iatrogenic hypercortisolism or depression.

The invention will now be described, by way of illustration only, with reference to
10 the following examples :

EXAMPLES

15 MATERIALS AND METHODS

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna IR 550 FT-IR spectrometer. ^1H and ^{13}C NMR spectra were measured in Bruker AC-200 or AM-500 NMR spectrometers in deu-teriochloroform (using TMS as internal
20 standard). The J values are given in Hz. Spectra were assigned by analysis of the DEPT, COSY 45 and HETCOSY spectra and by com-parison with those of progesterone.

The electron impact mass spectra (EI) were measured in a VG Trio 2 mass spectrometer at 70 eV by direct inlet. FAB mass spectra and electron impact high
25 resolution mass spectra (HRMS) were obtained in a VG ZAB BEQQ mass spectrometer. All solvents used were reagent grade. Solvents were evaporated at *ca.* 45°C under vacuum. Zinc dust was activated by suspending in 1M HCl, washing

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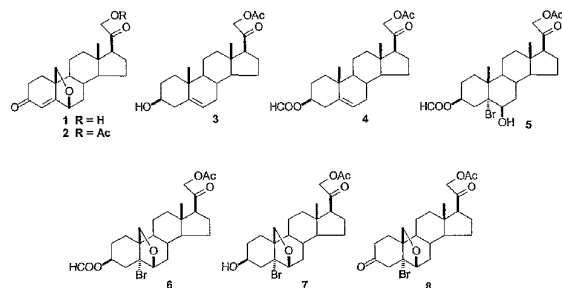
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with water, absolute ethanol and diethyl ether and drying 2 h at 120°C. The homogeneity of all compounds was confirmed by thin layer chromatography.

Large scale synthesis of 21-hydroxy-6,19-oxidoprogesterone (1)

- 5 Structures of starting material, intermediates and final products (1) and (2):



- 10 *3 β -Formyloxy-21-acetyloxy-5-pregnen-20-one (4)*

Acetic anhydride (13.4 ml) is added dropwise to formic acid (6.6 ml) at 0°C, the solution is heated at 50°C for 15 min and cooled rapidly to 0°C. The resulting acetoformic anhydride solution is added dropwise to a stirred suspension of 21-acetoxypregnenolone (3, 8.0 g) in dry pyridine (20.8 ml) at 0°C, and stirring is continued at that temperature for 2 h. The reaction product is poured over cold saturated aqueous sodium bicarbonate solution, filtered and the solid is washed with saturated aqueous sodium bicarbonate solution, water and 1N HCl and water (until neutral) rendering formate 4 (8.0 g); ¹H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 1.02 (3H, s, 10-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.53 (1H, t, $J = 8.0$ Hz,

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17-H), 4.50 (1H, d, $J=17.0$ Hz, 21a-H), 4.70 (1H, d, $J=17.0$ Hz, 21b-H), 5.32 (1H, m, 3-H), 5.38 (1H, d, $J=3.0$ Hz, 6-H), 8.02 (1H, s, HCOO).

3 β -Formyloxy-5 α -bromo-6 β -hydroxy-21-acetyloxy-pregnan-20-one (5)

- Formate 4 (8.0 g), is dissolved in diethyl ether (100 ml) and THF (37.2 ml) and cooled to 10°C. To the stirred solution at 10-15°C - which protected from light - 7.5% perchloric acid (11.88 ml) is added, followed by N-bromoacetamide (4.75 g) in 8 portions over a 25 min period. Stirring is continued for 45 min at 25°C and the reaction is stopped by addition of 10% aqueous sodium thiosulfate solution until complete decoloration. The reaction mixture is then extracted with dichloromethane/methanol 10:1 and the organic layer, is washed with water, dried with anhydrous sodium sulfate and the solvent is evaporated to afford bromohydrin 5 (10.4 g, containing about 20% of the 5 α -hydroxy-6 β -bromo isomer as determined by ^1H NMR).

3 β -Formyloxy-5 α -bromo-21-acetyloxy-6,19-oxidopregnan-20-one (6)

- Nitrogen is bubbled for 5 min through a solution of bromohydrin compound 5 (10.4 g, containing about 20% of the 5 α -hydroxy-6 β -bromo isomer) in freshly distilled dichloro-methane (723 ml) contained in a 1 liter glass vessel fitted with an external cooling jacket with circulating water at 25°C and magnetic stirrer. Diacetoxyiodobenzene (Suarez reagent, 7.66 g) and iodine (5.46 g) are successively added with stirring. The vessel is exposed to two 300 Watt tungsten lamps (5000 lm each) and vigorous stirring is continued for 1 h at 25°C. Irradiation is turned off and a saturated aqueous solution of sodium thiosulfate is added until complete decoloration. The organic layer is separated, dried with anhydrous sodium sulfate and the solvent evaporated. The resulting solid is dissolved in dichloro-methane (8 ml) and applied to a silicagel G-60 column (12 cm diameter x 8 cm height) previously flushed with hexane; successive elution (applying vacuum to the outlet)

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with hexane-ethyl acetate 9:1 (1100 ml), 8:2 (700 ml), 7:3 (700 ml) and 6:4 (600 ml) affords 31 x 100 ml fractions. Fractions are analyzed by TLC and those containing bromoether 6 are pooled and evaporated to dryness to afford 6 (6.8 g).

¹H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, *J* = 8.8 Hz, 17-H), 3.73 (1H, d, *J* = 8.4 Hz, 19a-H), 3.94 (1H, d, *J* = 8.4 Hz, 19b-H), 4.08 (1H, d, *J* = 4.2 Hz, 6-H), 4.50 (1H, d, *J* = 16.8 Hz, 21a-H), 4.71 (1H, d, *J* = 16.8 Hz, 21b-H), 5.34 (1H, m, 3-H), 8.02 (1H, s, HCOO).

3 β -Hydroxy-5 α -bromo-21-acetyloxy-6,19-oxidopregnan-20-one (7)

A stirred solution of the bromoether 6 (6.8 g) obtained above, is dissolved in dichloro-methane (45.7 ml) and methanol (154.7 ml) and is cooled to 0°C in an ice bath and water (10.9 ml) while conc. HCl (23.0 ml) is added. After about 30 min of vigorous stirring at 0°C (disappearance of the starting material is monitored by TLC) the reaction mixture is neutralized with 20% aqueous sodium hydroxide and extracted with dichloromethane. The organic layer is dried with anhydrous sodium sulfate and the solvent evaporated to afford the alcohol compound 7 (6.5 g); ¹H NMR (200.13 MHz) δ_{H} 0.69 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, *J* = 8.5 Hz, 17-H), 3.62 (1H, d, *J* = 8.5 Hz, 19a-H), 3.92 (1H, d, *J* = 8.5 Hz, 19b-H), 4.07 (1H, d, *J* = 4.0 Hz, 6-H), 4.15 (1H, m, 3-H), 4.51 (1H, d, *J* = 17.0 Hz, 21a-H), 4.70 (1H, d, *J* = 17.0 Hz, 21b-H).

5 α -Bromo-21-acetyloxy-6,19-oxidopregnane-3,20-dione (8)

A suspension of pyridinium chlorochromate (12.1 g), barium carbonate (5.0 g) and 3Å molecular sieves (9.60 g), in dry dichloromethane (480 ml) is stirred under nitrogen for 10 min. To the resultant orange slurry a solution of bromoether 7 (6.5 g) obtained above in dry dichloromethane (324 ml) is added and stirring is continued for about 90 min, until the starting material (TLC) has disappeared. The reaction mixture is percolated through a short silicagel G 60 column (12 cm

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diameter x 8 cm height) washed with diethyl ether (2 x 150 ml) and hexane-ethyl acetate 1:2 (3 x 150 ml). Fractions containing the product are pooled and evaporated to dryness affording 5.5 g of ketone **8** (containing about 10 % of Δ^4 -3-ketone (**2**); ^1H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.51 (1H, t, J = 8.5 Hz, 17-H), 2.85 (1H, d, J = 16.0 Hz, 4a-H), 3.40 (1H, d, J = 16.0 Hz, 4b-H), 3.90 (1H, d, J = 9.0 Hz, 19a-H), 4.07 (1H, d, J = 4.0 Hz, 6-H), 4.15 (1H, d, J = 9.0 Hz, 19b-H), 4.50 (1H, d, J = 17.0 Hz, 21a-H), 4.71 (1H, d, J = 17.0 Hz, 21b-H).

21-Hydroxy-6,19-oxido-4-pregnene-3,20-dione (1)

10 The ketone **8** (5.5 g) obtained from the preceding step is dissolved in methanol (263.8 ml) and dichloromethane (13.2 ml). To the stirred solution 14% methanolic KOH (53.4 ml) is added and stirring is continued at room temperature for about 15 min, until the starting material (TLC) has disappeared. The reaction mixture is neutralized with 1N HCl and extracted with dichloromethane. The organic layer is
15 dried with anhydrous sodium sulfate and the solvent is evaporated thus yielding crude 21-hydroxy-6,19-oxidoprogesterone (**1**, 4.6 g). The solid is dissolved in dichloromethane (5 ml) and sent through to a silicagel G-60 column (8.5 cm diameter x 5 cm height) previously flushed with hexane-ethyl acetate 7:3; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 6:4
20 (1350 ml) and 1:1 (900 ml) affords 30 fractions. The fractions are analyzed by TLC and those containing **1** are pooled and evaporated to dryness to afford 21-hydroxy-6,19-oxidoprogesterone (**1**, 2.3 g). ^1H NMR (200.13 MHz) δ_{H} 0.74 (3H, s, 13-CH₃), 2.45 (1H, t, J = 8.5 Hz, 17-H), 3.51 (1H, d, J = 8.8 Hz, 19a-H), 4.18 (3H, s, 21-CH₃), 4.20 (1H, d, J = 8.8 Hz, 19b-H), 4.69 (1H, d, J = 5.0 Hz, 6-H), 5.82 (1H,
25 s, 4-H).

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Re-crystallization from absolute ethanol affords a first crop of crystalline **1** (1.27 g), mp 165-166°C. The mother liquor is concentrated to yield a second crop of **1** (0.68 g).

5 *21-Acetyloxy-6,19-oxido-4-pregnene-3,20-dione (2)*

Crude 21-hydroxy-6,19-oxidoprogesterone (**1**, 2 g before chromatographic purification) was dissolved in dry pyridine (15.6 ml) and acetic anhydride (15.6 ml) added. The solution was stirred for 90 min at 25°C, poured over 1M HCl and filtered (alternatively the solid may be extracted with dichloromethane). The solid
10 was washed with water (until neutral), dried, dissolved in dichloromethane (2 ml) and applied to a silicagel G-60 column (7 cm diameter x 5 cm height) previously flushed with hexane-ethyl acetate 7:3; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 7:3 (700 ml) and 6:4 (700 ml) afforded 20 fractions. Fractions were analyzed by TLC and those containing **2** pooled and
15 evaporated to dryness to afford 21-acetyloxy-6,19-oxidoprogesterone (**2**, 1.12 g). Recrystallization from absolute ethanol (with drops of methanol) afforded crystalline **2** (0.72 g), mp 190-191°C. ¹H NMR (200.13 MHz) δ_H 0.76 (3H, s, 13-CH₃), 2.17 (3H, s, 21-CH₃CO), 2.51 (1H, t, *J* = 8.5 Hz, 17-H), 3.51 (1H, d, *J* = 8.2 Hz, 19a-H), 4.20 (1H, d, *J* = 8.2 Hz, 19b-H), 4.50 (1H, d, *J* = 16.7 Hz, 21a-H), 4.69
20 (1H, d, *J* = 5.0 Hz, 6-H), 4.72 (1H, d, *J* = 16.7 Hz, 21b-H), 5.82 (1H, s, 4-H).

21-Propanoyloxy-6,19-oxido-4-pregnene-3,20-dione (2a)

Crude 21-hydroxy-6,19-oxidoprogesterone (**1**, 0.2 g before chromatographic purification) was dissolved in dry pyridine (0.28 ml) and propanoic anhydride (0.2 ml) added. The solution was stirred for 1 h at 25°C, methanol was added to
25 destroy excess anhydride and the solution concentrated *in vacuo*. The residue was diluted with dichloromethane, washed with 1M HCl and water, and evaporated to dryness (0.233 g). Purification as above afforded 21-propanoyloxy-6,19-

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oxidoprogesterone (2a, 0.116 g). ¹H NMR (200.13 MHz) δ_H 0.76 (3H, s, 13-CH₃), 1.18 (3H, t, *J* = 7.6 Hz, 21-CH₃CH₂CO), 2.46 (2H, q, *J* = 7.6 Hz, 21-CH₃CH₂CO), 2.51 (1H, t, *J* = 8.0 Hz, 17-H), 3.51 (1H, d, *J* = 8.2 Hz, 19a-H), 4.20 (1H, d, *J* = 8.2 Hz, 19b-H), 4.50 (1H, d, *J* = 16.8 Hz, 21a-H), 4.70 (1H, d, *J* = 5.0 Hz, 6-H), 4.73 (1H, d, *J* = 16.8 Hz, 21b-H), 5.82 (1H, s, 4-H). EIMS *m/z* 400 (17) [M]⁺, 342 (5), 313 (24), 285 (23), 267 (10), 57 (100).

21-Succinoyloxy-6,19-oxido-4-pregnene-3,20-dione (2b)

21-hydroxy-6,19-oxidoprogesterone (1, 0.75 g) was dissolved in dry dichloromethane (37.5 ml) and pyridine (1.9 ml) and succinic anhydride (0.75 g) added. The solution was stirred for 4 h at 25°C, a second portion of succinic anhydride (0.375 g) added and stirring continued for 6 h (until disappearance of starting material). The reaction mixture was concentrated, and the residue extracted with diethyl ether. The ethereal solution was washed with 1M HCl and extracted with aqueous 10% sodium carbonate. The aqueous layer was acidified with conc. HCl to pH = 3 and extracted with dichloromethane. Washing with water and evaporation to dryness afforded crude 21-succinoyloxy-6,19-oxidoprogesterone (2b, 0.823 g). The solid was dissolved in dichloromethane (1 ml) and applied to a silicagel G-60 column (6 cm diameter x 4 cm height) previously flushed with hexane-ethyl acetate 2:8; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 2:8 (200 ml) and ethyl acetate (500 ml) afforded 14 fractions. Fractions were analyzed by TLC and those containing 2b pooled and evaporated to dryness to afford 21-succinoyloxy-6,19-oxidoprogesterone (2b, 0.455 g). ¹H NMR (200.13 MHz) δ_H 0.75 (3H, s, 13-CH₃), 2.52 (1H, t, *J* = 8.0 Hz, 17-H), 2.75 (4H, m, 21-HCOOCH₂CH₂CO), 3.51 (1H, d, *J* = 8.2 Hz, 19a-H), 4.20 (1H, d, *J* = 8.2 Hz, 19b-H), 4.54 (1H,

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d, $J = 16.9$ Hz, 21a-H), 4.70 (1H, d, $J = 5.0$ Hz, 6-H), 4.75 (1H, d, $J = 16.9$ Hz, 21b-H), 5.82 (1H, s, 4-H). EIMS m/z 444 (3) [M]⁺, 344 (24), 313 (55), 285 (44), 267 (17), 91 (60), 79 (54), 55 (100).

5 21-Oleoyloxy-6,19-oxido-4-pregnene-3,20-dione (2c)

21-hydroxy-6,19-oxidoprogesterone (1, 0.052 g) was dissolved in dry dichloromethane (1 ml) and pyridine (0.12 ml) and oleoyl chloride (0.1 ml) added. The solution was stirred for 24 h at 25°C, diluted with dichloromethane, washed with 1M HCl and water, and evaporated to dryness. Purification by preparative TLC afforded 21-oleoyloxy-6,19-oxidoprogesterone (2c, 0.075 g). ¹H NMR (200.13 MHz) δ _H 0.76 (3H, s, 13-CH₃), 0.88 (3H, t, $J = 7.0$ Hz, CH₃-CH-CO), 2.34 (2H, t, $J = 7.7$ Hz, CH-CH₂CO), 2.50 (1H, t, $J = 8.5$ Hz, 17-H), 3.51 (1H, d, $J = 8.3$ Hz, 19a-H), 4.20 (1H, d, $J = 8.3$ Hz, 19b-H), 4.49 (1H, d, $J = 16.9$ Hz, 21a-H), 4.70 (1H, d, $J = 4.5$ Hz, 6-H), 4.73 (1H, d, $J = 16.9$ Hz, 21b-H), 5.34 (2H, m, CH-CH=CH-CH-CO), 5.82 (1H, s, 4-H).

21-Phosphate-6,19-oxido-4-pregnene-3,20-dione (2d)

21-hydroxy-6,19-oxido-4-pregnene-3,20-dione (1, 0.052g) was dissolved in dry dichloromethane (1 ml) and pyridine (0.12 ml), and phosphochloridic acid diallyl ester (0.024g, 1 eq.) was added dropwise at 0°C, over 30 minutes. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was washed three times with 5% NaHCO₃, the organic layer was dried and evaporated in vacuo without heating. To the resulting syrup was added a solution of NaOH (2.2 equivalents) in water (3 ml). The solution was brought slowly to reflux, and gently refluxed for 12 hours. Lyophilization yielded the crude

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phosphate disodium salt, which was recrystallized from ethanol. Alternatively, the crude disodium salt may be dissolved in water, and the pH adjusted with HCl, to cause precipitation of the free phosphate.

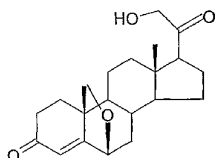
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Claims

1. A method of preparing 21-hydroxy-6,19-oxidoprogesterone (1) or an acyl or
 5 phosphate derivative thereof.



(1)

the method comprising the steps

- a) providing 21-acetoxypregnenolone;
- 10 b) protecting the C-3 hydroxy group of 21-acetoxypregnenolone through formation of a labile ester;
- c) obtaining the bromohydrin product thereof, by addition of a bromine and a hydroxy group onto the double bond of position of C5-C6 of the ester protected 21-acetoxy-pregnenolone;
- 15 d) performing an intramolecular cyclization with the C19 atom with the "Suarez-reagent" (diacetoxyiodobenzene) and iodine under irradiation, thus obtaining the 6,19 oxido-bridge within the scaffold;
- e) performing a selective hydrolysis, followed by an oxidation, to obtain a bromo-ketone;

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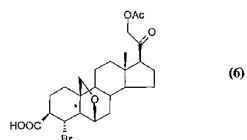
- f) performing a hydrolysis of the bromoketone resulting from step e) to obtain 21-hydroxy-6,19-oxidoprogesterone (1); and optionally
- g) reacting compound (1) with an acylating agent or phosphorylating agent.
2. The method according to claim 1, wherein the 21-acetate derivative is obtained
5 by acetylating 21-hydroxy-6,19-oxidoprogesterone (1) of step f).
3. The method according to claim 1, wherein a C1-18 acyl derivative is obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone with a C1-18 acid, anhydride, active ester or acyl chloride.
4. The method according to claim 1, wherein a 21-propionate, 21-hemisuccinate,
10 21-phosphate, 21-oleate derivative is obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone (1) of step f) with propionic acid, succinic acid, oleic acid or their anhydrides or active esters or acid chlorides.
5. The method according to claim 1, wherein the 21-phosphate derivative is obtained by reacting oxidoprogesterone (1) of step (f) with a phosphorylating
15 agent.
6. The method according to any of the preceding claims wherein for step b) a formate ester is afforded.
7. The method according to any of the preceding claims, wherein the intramolecular cyclisation step d) is performed in dichloromethane as solvent.
- 20 8. The method according to any of the preceding claims, wherein the selective hydrolysis of step e) is performed with HCl in MeOH/dichloromethane as solvent.

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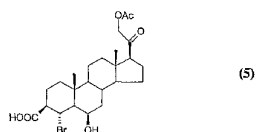
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9. The method according to any of the preceding claims, wherein the oxidation in step e) is performed with pyridinium chlorochromate (PCC).
10. The method according to any of the preceding claims, wherein the hydrolysis in step f) is performed with KOH in MeOH/dichloromethane as solvent.
- 5 11. A method for preparing (6)



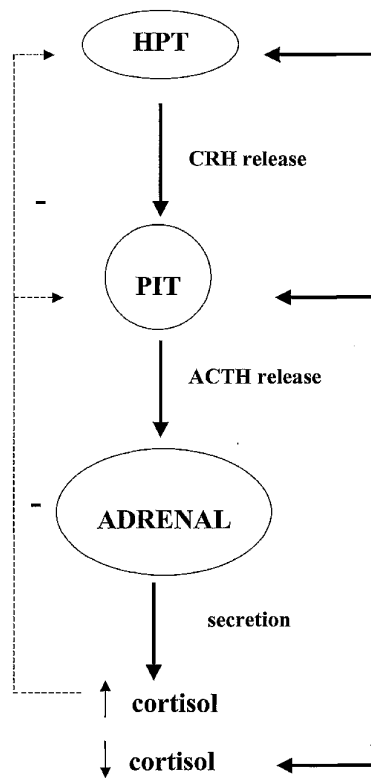
comprising the step of performing an intramolecular cyclisation between the 6-OH and the C₂₁ of the molecule (5)



12. A method according to claim 11, wherein the intramolecular cyclisation is carried out by photoreaction with DIAB and I₂.
- 10 13. A method according to claim 11 or 12, wherein the intramolecular cyclisation is carried out in CH₂Cl₂.

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Figure 1



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(54) Title: METHOD FOR THE PREPARATION OF 21-HYDROXY-6,19-OXIDOPROGESTERONE (21OH-6OP)

(57) Abstract: The present invention relates to a novel method for preparing 21-hydroxy-6,19-oxidopro-gesterone (21OH-6OP) and/or its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate, 21-oleate derivatives. 21OH-6OP and its ester derivatives are antigluccorticoids for the treatment or prophylaxis of diseases associated to an excess of glucocorticoids, in particular for treating Cushing's syndrome, iatrogenic hypercortisolism or depression.

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Method for the preparation of 21-hydroxy-6,19-oxidoprogesterone
(21OH-6OP)

Field of the invention:

- 5 The present invention relates to a novel method of preparing 21-hydroxy-6,19-oxidopro-gesterone (21OH-6OP) and/or its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate and 21-oleate derivatives. 21OH-6OP and its esters are antiglucocorticoids for the treatment or prophylaxis of diseases associated with a glucocorticoid imbalance, in particular for treating Cushing's syndrome or
10 depression.

Background of the invention:

- Corticosteroides are steroid hormones related structurally to cholesterol. These hormones are synthesized in the adrenal cortex and include the glucocorticoids (e.g.
15 cortisol), the mineralocorticoids (e.g aldosterone) as well as weak androgens and estrogens. The adrenal function, like that of the thyroid gland, is under the control of the hypothalamus (HPT) and the pituitary (PIT). When cortisol (the naturally-occurring glucocorticoid) levels drop below a setpoint, the hypothalamus releases CRH (corticotropin releasing hormone) which stimulates adrenocorticotropic
20 hormone (ACTH) release from the pituitary. ACTH is a tropic hormone which stimulates

- the synthesis and secretion of cortisol (it has minimal effects on aldosterone synthesis/secretion), and
- the growth of the adrenal gland. When cortisol levels increase, this shuts off
25 CRH and ACTH secretion (cf. **Figure 1**).

Cortisol is characterized by its properties related to the biosynthesis and metabolism of glucose and propeties related to non-specific as well as specific

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immunity. Due to their effects on the glucose metabolism, cortisol and natural or synthetic analogues thereof are usually named glucocorticoids. They bind to the glucocorticoid receptor (GR).

- 5 The glucocorticoid receptor is a member of a protein super family of closely related intracellular receptors which function as ligand-activated transcription factors. Other members of this super family are the mineralocorticoid receptor (MR) and the progesterone receptor (PR). MR and GR have shown to be highly homologous, thus natural and even synthetic steroids exhibit cross-reaction between these
- 10 receptors. With respect to PR, its natural ligand progesterone also cross-reacts with MR and GR.

- Cushing's syndrome is a disorder resulting from increased adrenocortical secretion of cortisol. Hyperfunction of the adrenal cortex may be ACTH-dependent or it may be independent of ACTH regulation, e.g. production of cortisol by an
- 15 adrenocortical adenoma or carcinoma. The administration of supraphysiologic quantities of exogenous cortisol or related synthetic analogs suppresses adrenocortical function and mimics ACTH-independent glucocorticoid hyperfunction. ACTH-dependent hyperfunction of the adrenal cortex may be due to hypersecretion of ACTH by the pituitary, secretion of ACTH by a nonpituitary
- 20 tumor such as small cell carcinoma of the lung (the ectopic ACTH syndrome), or administration of exogenous ACTH. While the term "Cushing's syndrome" has been applied to the clinical picture resulting from cortisol excess regardless of the cause, hyperfunction of the adrenal cortex resulting from pituitary ACTH excess has frequently been referred to as Cushing's disease, implying a particular
- 25 physiologic abnormality. Patients with Cushing's disease may have a basophilic adenoma of the pituitary or a chromophobe adenoma. Microadenomas can usually be visualized by CT or, preferably, MRI scan, using a high-resolution technique

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augmented by gadolinium. Some micro-adenomas are difficult to visualize even with these modalities. In some cases, no histological abnormality is found in the pituitary despite clear evidence of ACTH overproduction.

Reference to Cushing's syndrome is herein intended to mean the clinical picture
5 resulting from cortisol excess regardless of the cause, which may be also iatrogenic, both by the injection of ACTH or by the direct administration of cortisol or synthetic analogs such as prednisone, prednisolone, dexamethasone or others that are widely used in various types of diseases including allergic, asthmatic, inflammatory or immunologic. Cushing's syndrome includes in addition adrenal
10 tumours secreting corticoids, ectopic ACTH production and Cushing's disease.

Clinical manifestations include rounded "moon" faces with a plethoric appearance. There is truncal obesity with prominent supraclavicular and dorsal cervical fat pads ("buffalo hump"); the distal extremities and fingers are usually quite slender. Muscle wasting and weakness are present. The skin is thin and atrophic, with poor
15 wound healing and easy bruising. Purple striae may appear on the abdomen. Hypertension, renal calculi, osteoporosis, glucose intolerance, reduced resistance to infection, and psychiatric disturbances are common. Cessation of linear growth is characteristic in children. Females usually have menstrual irregularities. An increased production of androgens, in addition to cortisol, may lead to
20 hypertichosis, temporal balding, and other signs of virilism in the female.

Although development of antihormonal agents related to the estrogen and androgen receptors has been successful, the search for selective anti-corticoids is more restricted.

Known agents suppressing the synthesis of steroid hormones at various levels
25 (i.e. inhibitors of enzymes which catalyze various stages of the synthesis of

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steroid hormones) are reviewed in *J.Steroid Biochem.*, **vol.5**, p.501 (1974) and include the following:

- 5 a) derivatives of diphenylmethane, e.g. amphenon B (which suppresses the synthesis of steroid hormones at stages 11- beta -, 17 - and 21 - of hydroxylase);
- b) derivatives of pyridine (SU-c series), e.g. metirapon (which suppresses synthesis at stage 11- beta of hydroxylase);
- 10 c) substituted alpha , alpha - glutaramides, e.g. aminoglutetimide (which impedes the synthesis of pregnenolone from cholesterol through suppression of 20- alpha -hydroxylase and C₂₀, C₂₂-liase;
- d) steroid substances e.g. trilostan (3 beta -substituted steroid- 3 beta - hydroxy-5-androsten-17-one), which suppresses 3 beta - desoxysteroidhydrogenase -5.4- isomerase (*Steroids*, **vol.32**, p.257).
- 15 e) steroids of the spironolactone family which are used as rapidly dissociating anti-Mineralocorticoids (*PNAS USA* 71(4) p.1431-1435 (1974).
- f) a synthetic steroid described as an anti-Mineralocorticoids, ZK91587, showing specific binding properties for the kidney (*Z.Naturforsch.*, **45b**, p.711-715 (1990)) and hippocampus type I MR (*Life Science*, **59**, p.511-20 21 (1996)), but not for type II GR. It may therefore be conveniently useful as a tool in the investigation of MR function in tissues containing both receptor systems.

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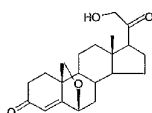
Agents that specifically suppress the interaction of glucocorticoid hormones with hormone receptors are:

- a) Mifepriston (11 β , 17 β)-11-[4-(Dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one, which acts on receptors of glucocorticoid hormones to form a complex incapable of initiating mechanisms leading to glucocorticoid effect (*Annals of New-York Academy of Science*, vol. 761, p.5-28 (1995)).
- b) non-steroid substances (*J. Steroid Biochem.*, vol. 31, p.481-492 (1988)) e.g. drotaverina hydrochloride (a derivative of isoquinoline-1-(3,4-dietoxibene zilidene)-6,7 - dietoxy-1,2,3,4- tetrahydrizoquinoline) or acetylsalicylic acid (*Moskovskaya Meditsina*, 1990, "Receptor mechanisms of the glucocorticoid effect" by V.P.Golikov).

To-date, the only therapeutical application for antiglucocorticoids (e.g. Mifepristone) that has been attempted in a clinical setting is to treat inoperable cases of nonpituitary Cushing's syndrome. In the case of Mifepristone (both an anti-progesterone and an anti-glucocorticoid), high doses (up to 800 mg per day) are required.

Employing a systematic application of strategies to increase activity and decrease cross-reactivity and undesirable side effects, progress has been reported in the development of antihormonal agents with greater potency and selectivity, especially in the antiestrogen and antiandrogen fields.

In EP-903'146 the synthetic steroid, 21-hydroxy-6,19-oxidoprogesterone (21OH-6OP) of formula I,



(I)

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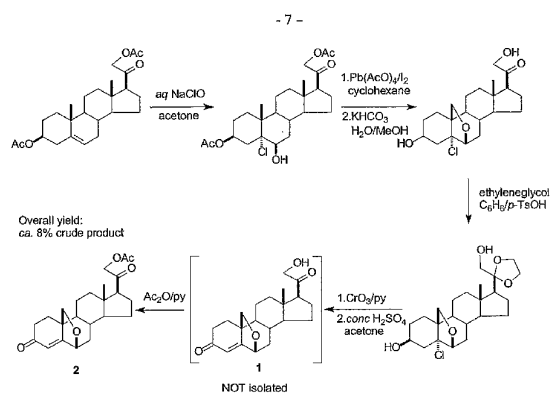
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is disclosed as a selective antiglucocorticoid which does not substantially cross-react with uterus- PR or kidney-MR. Said 21-hydroxy-6,19-oxidoprogesterone antiglucocorticoid could be used in the treatment of diseases associated with an excess of glucocorticoids, where an anti-glucocorticoid virtually lacking
5 mineralocorticoid or glucocorticoid properties as well as affinity for MR or PR is desirable.

The synthesis of 21-hydroxy-6,19-oxidoprogesterone (1) and its 21-acetate (2) was first accomplished by Deghenghi in 1966 as intermediate in a synthesis of 19-hydroxy-desoxy-corticosterone, starting from 21-hydroxypregnenolone diacetate.
10 The procedure summarized in **Scheme 1** involves the use of a "hypoiodite type" reaction with lead tetraacetate ($\text{Pb}(\text{AcO})_4$). Compound 1 was neither isolated nor characterized, but acetylated *in situ* to acetate 2. Overall yield of partially purified 2 was only 8.3 %. Further to the low yield, this method of preparation is generally perceived as being difficult to reproduce notably due to the formation of the
15 chlorohydrin.

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Scheme 1: Synthesis of 21-hydroxy-6,19-oxidoprogesterone and its 21-acetate derivative by Deghenghi.

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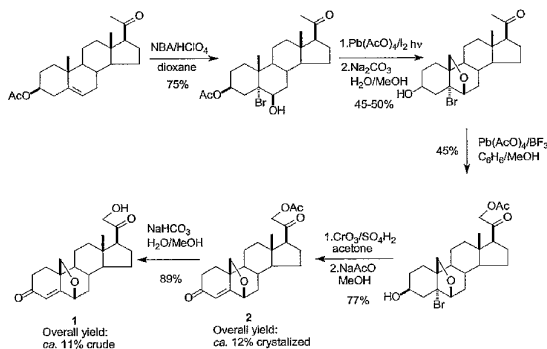
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In a more recent synthesis of 19-hydroxydeoxycorticosterone, Kirk and Yeoh (*J. Chem. Soc. Perkin Trans. I*, 2945 (1983)) prepared acetate **2** as an intermediate.

This procedure starting from pregnenolone acetate is depicted in **Scheme 2**.

Although full details of the first 3 steps are not given in the experimental section of their publication, according to the literature cited the yield for these steps may be estimated as *ca.* 34-37% giving an overall yield of acetate **2** of *ca.* 12% from pregnenolone acetate.



Scheme 2: Synthesis of 21-hydroxy-6,19-oxidoprogesterone and its acetate by Kirk *et al.*

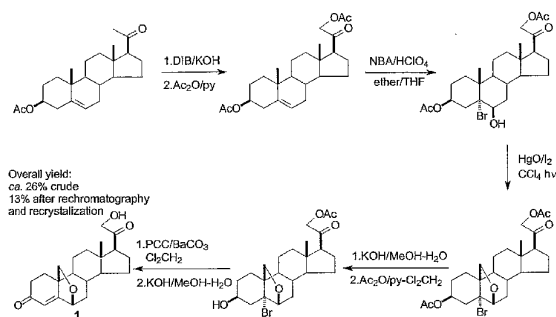
According to a further method, depicted in **Scheme 3**, the remote functionalization reaction with $\text{Pb}(\text{AcO})_4$ and iodine under thermal or photochemical conditions is replaced with the more reproducible and "milder" HgO /iodine system under

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- photochemical conditions and the 21-hydroxylation step is carried out with a hypervalent iodine compound (see A. S. Veleiro, M. V. Nevado, M. C. Monteserín and G. Burton, *Steroids*, **60**, 268-272 (1995); M. Akhtar and D. H. R. Barton, *J. Am. Chem. Soc.*, **86**, 1528-1534 (1964); R. M. Moriarty, L. S. John and P. C. Du, *J. Chem. Soc. Chem. Commun.*, 641-642 (1981).



Scheme 3: Synthesis of 21-hydroxy-6,19-oxidoprogesterone by Veleiro *et al.*

- The above procedure starts also from pregnenolone acetate which is first converted into 21-hydroxypregnenolone diacetate. In the best case, overall yield of **1** is ca. 26%; however this product, although apparently pure according to TLC and NMR, could not be crystallized, and thus required additional purification steps by chromatography; yield of pure crystalline **1** is about 13% (19% from 21-hydroxypregnenolone diacetate). Although the acetate **2** is an intermediate in this synthetic route, complete purification could only be achieved after deacetylation (i.e. on compound **1**). Furthermore, although this procedure allows the synthesis of small amounts of **1** for the initial biological tests carried out in 1996, attempts to

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scale up the procedure failed. Although, the currently known methods provide 21-hydroxy-6,19-oxidoprogesterone (1), they consistently give poorer yields as well as byproducts which are difficult to eliminate.

5 Description of the invention:

It is an object of the invention to provide a new method for preparing 21-hydroxy-6,19-oxidoprogesterone (21OH-6OP) and/or its esters.

It is a further object of the invention to provide 21-hydroxy-6,19-oxidoprogesterone and/or its esters.

- 10 In a first aspect, the present invention provides a new method of preparing 21-hydroxy-6,19-oxidoprogesterone (1) or its esters, for example carboxylate, phosphate or sulfate esters. In preferred embodiments, the invention provides a new method for preparing
- 21 OH-6OP and its 21-acetate, 21-propionate, 21-hemisuccinate, 21-phosphate
- 15 and 21-oleate derivatives. The method of the invention avoids essentially all of the hitherto known inconveniences. The method of the invention produces 21-hydroxy-6,19-oxidoprogesterone (1) in good yield, having an acceptable degree of purity, and avoids heavy metals as reagent. The method of the invention is suitable for large scale industrial preparation of 21-hydroxy-6,19-oxido-progesterone (1) and its
- 20 esters.

The objects of the invention are met according to the main claim. Preferred embodiments are set out within the dependent claims which are incorporated herewith.

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The novel synthetic procedure according to the present invention comprises or consists of the following consecutive basic steps :

- a) Providing 21-acetoxypregnenolone (3) (which is actually a commercial product);
- 5 b) Transforming the C-3 hydroxy group of 21-acetoxypregnenolone into a labile ester, preferably a formate ester;
- c) Obtaining the bromohydrin product from the protected 21-acetoxypregnenolone, said bromohydrin resulting from the addition of a bromine and a hydroxy group onto the double bond of position of C5-C6;
- 10 d) Performing an intramolecular cyclisation with the C19 atom with the "Suarez-reagent" (diacetoxyiodobenzene; see Armas et al., *J.Chem.Soc. Perkin I*, 405 (1989)) and iodine under irradiation, thus obtaining the 6,19 oxido-bridge within the scaffold;
- e) Performing a selective hydrolysis, preferably with
- 15 HCl/MeOH/dichloromethane, followed by an oxidation, preferably with pyridinium chlorochromate (PCC), thus obtaining a bromoketone;
- f) Performing a hydrolysis of the bromoketone resulting from step e) to obtain 21-hydroxy-6,19-oxidoprogesterone (1), and optionally
- g) Acylating 21-hydroxy-6,19-oxidoprogesterone (1), to afford the 21-ester
- 20 (the acetate is shown as 2);

Preferred esters are C1-18 acyl esters (optionally substituted with COOH, 1 or 2 times) and phosphate esters. Acyl esters may be obtained by reacting 21-hydroxy-6,19-oxidoprogesterone (1) with an organic acid, in the presence of a coupling agent (for example N, N'-dicyclohexylcarbodiimide), or with an activated organic ester (for example, a nitrophenol ester), or with an acyl halide (for example, an acyl

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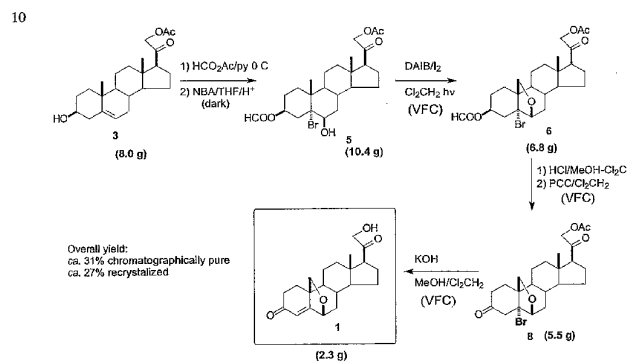
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chloride), or with an acyl anhydride. Phosphate esters may be obtained by reacting 21-hydroxy-6,19-oxidoprogesterone with a phosphorylating agent (for example phosphorus oxychloride, followed by basic hydrolysis).

The 21-propionate or 21-hemisuccinate, and 21-oleate and derivatives are obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone (1) of step f) with propionic acid, succinic or oleic acid, their anhydrides, activated esters or acyl chlorides.

A preferred synthetic procedure according to the present invention is depicted in Scheme 4.



Scheme 4: Novel Synthesis of 21-hydroxy-6,19-oxidoprogesterone

15

According to the preferred method illustrated in **Scheme 4**, a formate group is introduced as protecting group for position 3. Formates have the advantage that

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they may be hydrolyzed under relatively mild acid conditions (HCl/MeOH-dichloromethane) in which primary acetates and even α -acetoxy-ketones are stable. The introduction of a formate moiety is preferably carried out under very mild conditions using preferably mixed acetic-formic anhydride (prepared *in situ* from formic acid and acetic anhydride). The bromo-hydrin formation, is carried out with N-bromoacetamide in THF yielding 80% of the desired bromohydrin **5** and about 20% of the 5 α -HO-6 β -Br isomer. The mixture may be separated by crystallization or Thin Layer Chromatography. For the intramolecular cyclisation involving C-19, the "Suarez reagent" (diacet-oxyiodobenzene (DAIB)) and iodine is employed as the "hypoiodite" as generating system under suitable irradiation – preferably with a standard tungsten lamp.

Quite surprisingly, it turns out that upon performing the intramolecular cyclisation step in di-chloromethane (CH₂Cl₂), the reaction is not only rapid (complete conversion in less than 1 hour) and clean, but also excellent yields could be obtained.

Temperature control (25 °C) of the cyclisation step, achieved by using a glass jacketed reactor with water circulation, increases the yield and diminishes excessive oxidation by-products. Furthermore, epoxide formation is eliminated, the isomeric bromohydrin is inert to the reagent and could be easily separated (together with the iodobenzene side product) from the desired bromoether **6** by vacuum filtration through a silica gel column (VFC).

The selective hydrolysis of the formate ester, is preferably performed with HCl in MeOH-dichloromethane, followed by an oxidation, preferably with PCC, thus affording the bromoketone **8**. This product partially eliminates HBr to give the unsaturated ketone (**2**) when subjected to VFC (very short silica gel column) purification, however this poses no problem as the unsaturated product is the

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desired product of the following step, in which the treatment with a base deacetylates position 21 and completes the elimination of the C-5 bromine thus giving the desired product (1).

Purification of 1 may be achieved with a fast VFC through a short silica gel column to afford chromatographically pure product in over 31% yield. This may be crystallized from absolute ethanol to yield crystalline 1 in 27% yield.

The 21-acetate (2) is prepared by standard acetylation of 1 with acetic anhydride in pyridine. The acetate is recrystallized from absolute ethanol.

The novel synthesis according to the present invention has the following advantages :

- a) It eliminates the need of the selective acetylation step or any need to differentiate the hydroxyl groups at C-21 and C-3.
- b) It modifies the hypoiodite reaction so that no heavy metals are required (lead, silver or mercury based reagents) and it proceeds under homogeneous conditions, thus allowing a scale up to multigram and eventually kilogram amounts.
- c) It reduces the secondary products formed at different stages (specially 5,6-epoxide formation) so as to minimize the purification steps, and specially to avoid or minimize the need of column chromatography on silica gel, as bromoethers, compound 1 and 2 are intrinsically unstable under these conditions.
- d) It reduces reaction volumes throughout to allow scale up.

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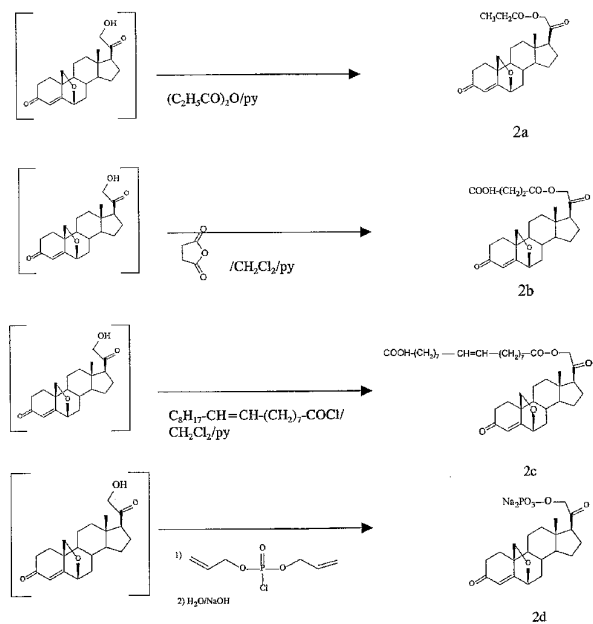
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Starting from compound **1**, synthesis of acyl esters, such as 21-propionate (**2a**), and 21-hemisuccinate (**2b**) and 21-oleate (**2c**) -6,19-oxidoprogesterone derivatives can be performed as illustrated in Scheme 5.

Also shown in Scheme 5 is the preparation of the 21-phosphate derivative (**2d**).

5



Scheme 5: Synthesis of 21-propionate (**2a**), 21-hemisuccinate (**2b**), 21-oleate (**2c**) and 21- PO_4 (**2d**) 6,19-oxidoprogesterone derivatives.

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The compounds synthesised according to the present invention are for use as a medicament, alone or in combination with pharmaceutically acceptable carriers and/or excipients. Such medicament is notably suitable in the manufacture of a
5 medicament for the treatment or prophylaxis of diseases associated with an excess of glucocorticoids, e.g. for the treatment of Cushing's syndrome, iatrogenic hypercortisolism or depression.

The invention will now be described, by way of illustration only, with reference to
10 the following examples :

EXAMPLES

15 MATERIALS AND METHODS

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna IR 550 FT-IR spectrometer. ¹H and ¹³C NMR spectra were measured in Bruker AC-200 or AM-500 NMR spectrometers in deu-teriochloroform (using TMS as internal
20 standard). The *J* values are given in Hz. Spectra were assigned by analysis of the DEPT, COSY 45 and HETCOSY spectra and by com-parison with those of progesterone.

The electron impact mass spectra (EI) were measured in a VG Trio 2 mass spectrometer at 70 eV by direct inlet. FAB mass spectra and electron impact high
25 resolution mass spectra (HRMS) were obtained in a VG ZAB BEQQ mass spectrometer. All solvents used were reagent grade. Solvents were evaporated at *ca.* 45°C under vacuum. Zinc dust was activated by suspending in 1M HCl, washing

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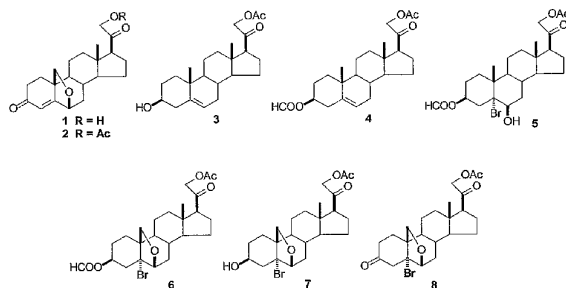
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with water, absolute ethanol and diethyl ether and drying 2 h at 120°C. The homogeneity of all compounds was confirmed by thin layer chromatography.

Large scale synthesis of 21-hydroxy-6,19-oxidoprogesterone (1)

- 5 Structures of starting material, intermediates and final products (1) and (2):



- 10 **3 β -Formyloxy-21-acetyloxy-5-pregnen-20-one (4)**

Acetic anhydride (13.4 ml) is added dropwise to formic acid (6.6 ml) at 0°C, the solution is heated at 50°C for 15 min and cooled rapidly to 0°C. The resulting acetoformic anhydride solution is added dropwise to a stirred suspension of 21-acetoxypregnenolone (3, 8.0 g) in dry pyridine (20.8 ml) at 0°C, and stirring is continued at that temperature for 2 h. The reaction product is poured over cold saturated aqueous sodium bicarbonate solution, filtered and the solid is washed with saturated aqueous sodium bicarbonate solution, water and 1N HCl and water (until neutral) rendering formate 4 (8.0 g); ¹H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 1.02 (3H, s, 10-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.53 (1H, t, J = 8.0 Hz,

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17-H), 4.50 (1H, d, $J = 17.0$ Hz, 21a-H), 4.70 (1H, d, $J = 17.0$ Hz, 21b-H), 5.32 (1H, m, 3-H), 5.38 (1H, d, $J = 3.0$ Hz, 6-H), 8.02 (1H, s, HCOO).

3 β -Formyloxy-5 α -bromo-6 β -hydroxy-21-acetyloxypregnan-20-one (5)

Formate **4** (8.0 g), is dissolved in diethyl ether (100 ml) and THF (37.2 ml) and
 5 cooled to 10°C. To the stirred solution at 10-15°C - which protected from light -
 7.5% perchloric acid (11.88 ml) is added, followed by N-bromoacetamide (4.75 g)
 in 8 portions over a 25 min period. Stirring is continued for 45 min at 25°C and the
 reaction is stopped by addition of 10% aqueous sodium thiosulfate solution until
 complete decoloration. The reaction mixture is then extracted with
 10 dichloromethane/methanol 10:1 and the organic layer, is washed with water, dried
 with anhydrous sodium sulfate and the solvent is evaporated to afford bromo-
 hydrin **5** (10.4 g, containing about 20% of the 5 α -hydroxy-6 β -bromo isomer as
 determined by ^1H NMR).

3 β -Formyloxy-5 α -bromo-21-acetyloxy-6,19-oxidopregnan-20-one (6)

15 Nitrogen is bubbled for 5 min through a solution of bromohydrin compound **5** (10.4
 g, containing about 20% of the 5 α -hydroxy-6 β -bromo isomer) in freshly distilled
 dichloro-methane (723 ml) contained in a 1 liter glass vessel fitted with an external
 cooling jacket with circulating water at 25°C and magnetic stirrer.
 Diacetoxyiodobenzene (Suarez reagent, 7.66 g) and iodine (5.46 g) are successively
 20 added with stirring. The vessel is exposed to two 300 Watt tungsten lamps (5000
 lm each) and vigorous stirring is continued for 1 h at 25°C. Irradiation is turned off
 and a saturated aqueous solution of sodium thiosulfate is added until complete
 decoloration. The organic layer is separated, dried with anhydrous sodium sulfate
 and the solvent evaporated. The resulting solid is dissolved in dichloro-methane (8
 25 ml) and applied to a silicagel G-60 column (12 cm diameter x 8 cm height)
 previously flushed with hexane; successive elution (applying vacuum to the outlet)

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with hexane-ethyl acetate 9:1 (1100 ml), 8:2 (700 ml), 7:3 (700 ml) and 6:4 (600 ml) affords 31 x 100 ml fractions. Fractions are analyzed by TLC and those containing bromoether **6** are pooled and evaporated to dryness to afford **6** (6.8 g).

¹H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, J = 8.8 Hz, 17-H), 3.73 (1H, d, J = 8.4 Hz, 19a-H), 3.94 (1H, d, J = 8.4 Hz, 19b-H), 4.08 (1H, d, J = 4.2 Hz, 6-H), 4.50 (1H, d, J = 16.8 Hz, 21a-H), 4.71 (1H, d, J = 16.8 Hz, 21b-H), 5.34 (1H, m, 3-H), 8.02 (1H, s, HCOO).

3 β -Hydroxy-5 α -bromo-21-acetyloxy-6,19-oxidopregnan-20-one (7)

A stirred solution of the bromoether **6** (6.8 g) obtained above, is dissolved in dichloro-methane (45.7 ml) and methanol (154.7 ml) and is cooled to 0°C in an ice bath and water (10.9 ml) while conc. HCl (23.0 ml) is added. After about 30 min of vigorous stirring at 0°C (disappearance of the starting material is monitored by TLC) the reaction mixture is neutralized with 20% aqueous sodium hydroxide and extracted with dichloromethane. The organic layer is dried with anhydrous sodium sulfate and the solvent evaporated to afford the alcohol compound **7** (6.5 g); ¹H NMR (200.13 MHz) δ_{H} 0.69 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.52 (1H, t, J = 8.5 Hz, 17-H), 3.62 (1H, d, J = 8.5 Hz, 19a-H), 3.92 (1H, d, J = 8.5 Hz, 19b-H), 4.07 (1H, d, J = 4.0 Hz, 6-H), 4.15 (1H, m, 3-H), 4.51 (1H, d, J = 17.0 Hz, 21a-H), 4.70 (1H, d, J = 17.0 Hz, 21b-H).

5 α -Bromo-21-acetyloxy-6,19-oxidopregnane-3,20-dione (8)

A suspension of pyridinium chlorochromate (12.1 g), barium carbonate (5.0 g) and 3 Å molecular sieves (9.60 g), in dry dichloromethane (480 ml) is stirred under nitrogen for 10 min. To the resultant orange slurry a solution of bromoether **7** (6.5 g) obtained above in dry dichloromethane (324 ml) is added and stirring is continued for about 90 min, until the starting material (TLC) has disappeared. The reaction mixture is percolated through a short silicagel G 60 column (12 cm

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diameter x 8 cm height) washed with diethyl ether (2 x 150 ml) and hexane-ethyl acetate 1:2 (3 x 150 ml). Fractions containing the product are pooled and evaporated to dryness affording 5.5 g of ketone 8 (containing about 10 % of Δ^4 -3-ketone (2); ^1H NMR (200.13 MHz) δ_{H} 0.70 (3H, s, 13-CH₃), 2.16 (3H, s, 21-CH₃CO), 2.51 (1H, t, J = 8.5 Hz, 17-H), 2.85 (1H, d, J = 16.0 Hz, 4a-H), 3.40 (1H, d, J = 16.0 Hz, 4b-H), 3.90 (1H, d, J = 9.0 Hz, 19a-H), 4.07 (1H, d, J = 4.0 Hz, 6-H), 4.15 (1H, d, J = 9.0 Hz, 19b-H), 4.50 (1H, d, J = 17.0 Hz, 21a-H), 4.71 (1H, d, J = 17.0 Hz, 21b-H).

21-Hydroxy-6,19-oxido-4-pregnene-3,20-dione (1)

10 The ketone 8 (5.5 g) obtained from the preceding step is dissolved in methanol (263.8 ml) and dichloromethane (13.2 ml). To the stirred solution 14% methanolic KOH (53.4 ml) is added and stirring is continued at room temperature for about 15 min, until the starting material (TLC) has disappeared. The reaction mixture is neutralized with 1N HCl and extracted with dichloromethane. The organic layer is
15 dried with anhydrous sodium sulfate and the solvent is evaporated thus yielding crude 21-hydroxy-6,19-oxidoprogesterone (1, 4,6 g). The solid is dissolved in dichloromethane (5 ml) and sent through to a silicagel G-60 column (8.5 cm diameter x 5 cm height) previously flushed with hexane-ethyl acetate 7:3; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 6:4
20 (1350 ml) and 1:1 (900 ml) affords 30 fractions. The fractions are analyzed by TLC and those containing 1 are pooled and evaporated to dryness to afford 21-hydroxy-6,19-oxidoproges-terone (1, 2.3 g). ^1H NMR (200.13 MHz) δ_{H} 0.74 (3H, s, 13-CH₃), 2.45 (1H, t, J = 8.5 Hz, 17-H), 3.51 (1H, d, J = 8.8 Hz, 19a-H), 4.18 (3H, s, 21-CH₃), 4.20 (1H, d, J = 8.8 Hz, 19b-H), 4.69 (1H, d, J = 5.0 Hz, 6-H), 5.82 (1H,
25 s, 4-H).

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Re-crystallization from absolute ethanol affords a first crop of crystalline 1 (1.27 g), mp 165-166°C. The mother liquor is concentrated to yield a second crop of 1 (0.68 g).

- 5 *21-Acetyloxy-6,19-oxido-4-pregnene-3,20-dione (2)*
Crude 21-hydroxy-6,19-oxidoprogesterone (1, 2 g before chromatographic purification) was dissolved in dry pyridine (15.6 ml) and acetic anhydride (15.6 ml) added. The solution was stirred for 90 min at 25°C, poured over 1M HCl and filtered (alternatively the solid may be extracted with dichloromethane). The solid
10 was washed with water (until neutral), dried, dissolved in dichloromethane (2 ml) and applied to a silicagel G-60 column (7 cm diameter x 5 cm height) previously flushed with hexane-ethyl acetate 7:3; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 7:3 (700 ml) and 6:4 (700 ml) afforded 20 fractions. Fractions were analyzed by TLC and those containing 2 pooled and
15 evaporated to dryness to afford 21-acetyloxy-6,19-oxidoprogesterone (2, 1.12 g). Recrystallization from absolute ethanol (with drops of methanol) afforded crystalline 2 (0.72 g), mp 190-191°C. ¹H NMR (200.13 MHz) δ_H 0.76 (3H, s, 13-CH₃), 2.17 (3H, s, 21-CH₃CO), 2.51 (1H, t, *J* = 8.5 Hz, 17-H), 3.51 (1H, d, *J* = 8.2 Hz, 19a-H), 4.20 (1H, d, *J* = 8.2 Hz, 19b-H), 4.50 (1H, d, *J* = 16.7 Hz, 21a-H), 4.69
20 (1H, d, *J* = 5.0 Hz, 6-H), 4.72 (1H, d, *J* = 16.7 Hz, 21b-H), 5.82 (1H, s, 4-H).

21-Propanoyloxy-6,19-oxido-4-pregnene-3,20-dione (2a)

- Crude 21-hydroxy-6,19-oxidoprogesterone (1, 0.2 g before chromatographic purification) was dissolved in dry pyridine (0.28 ml) and propanoic anhydride (0.2 ml) added. The solution was stirred for 1 h at 25°C, methanol was added to
25 destroy excess anhydride and the solution concentrated *in vacuo*. The residue was diluted with dichloromethane, washed with 1M HCl and water, and evaporated to dryness (0.233 g). Purification as above afforded 21-propanoyloxy-6,19-

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oxidoprogesterone (**2a**, 0.116 g). ¹H NMR (200.13 MHz) δ_H 0.76 (3H, s, 13-CH₃), 1.18 (3H, t, *J* = 7.6 Hz, 21-CH₂CH₂CO), 2.46 (2H, q, *J* = 7.6 Hz, 21-CH₂CH₂CO), 2.51 (1H, t, *J* = 8.0 Hz, 17-H), 3.51 (1H, d, *J* = 8.2 Hz, 19a-H), 4.20 (1H, d, *J* = 8.2 Hz, 19b-H), 4.50 (1H, d, *J* = 16.8 Hz, 21a-H), 4.70 (1H, d, *J* = 5.0 Hz, 6-H), 4.73 (1H, d, *J* = 16.8 Hz, 21b-H), 5.82 (1H, s, 4-H). EIMS *m/z* 400 (17) [M]⁺, 342 (5), 313 (24), 285 (23), 267 (10), 57 (100).

21-Succinoyloxy-6,19-oxido-4-pregnene-3,20-dione (2b)

21-hydroxy-6,19-oxidoprogesterone (**1**, 0.75 g) was dissolved in dry dichloromethane (37.5 ml) and pyridine (1.9 ml) and succinic anhydride (0.75 g) added. The solution was stirred for 4 h at 25°C, a second portion of succinic anhydride (0.375 g) added and stirring continued for 6 h (until disappearance of starting material). The reaction mixture was concentrated, and the residue extracted with diethyl ether. The ethereal solution was washed with 1M HCl and extracted with aqueous 10% sodium carbonate. The aqueous layer was acidified with conc. HCl to pH = 3 and extracted with dichloromethane. Washing with water and evaporation to dryness afforded crude 21-succinoyloxy-6,19-oxidoprogesterone (**2b**, 0.823 g). The solid was dissolved in dichloromethane (1 ml) and applied to a silicagel G-60 column (6 cm diameter x 4 cm height) previously flushed with hexane-ethyl acetate 2:8; successive elution (applying vacuum to the outlet) with hexane-ethyl acetate 2:8 (200 ml) and ethyl acetate (500 ml) afforded 14 fractions. Fractions were analyzed by TLC and those containing **2b** pooled and evaporated to dryness to afford 21-succinoyloxy-6,19-oxidoprogesterone (**2b**, 0.455 g). ¹H NMR (200.13 MHz) δ_H 0.75 (3H, s, 13-CH₃), 2.52 (1H, t, *J* = 8.0 Hz, 17-H), 2.75 (4H, m, 21-HCOOCH₂CH₂CO), 3.51 (1H, d, *J* = 8.2 Hz, 19a-H), 4.20 (1H, d, *J* = 8.2 Hz, 19b-H), 4.54 (1H,

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d, $J = 16.9$ Hz, 21a-H), 4.70 (1H, d, $J = 5.0$ Hz, 6-H), 4.75 (1H, d, $J = 16.9$ Hz, 21b-H), 5.82 (1H, s, 4-H). EIMS m/z 444 (3) $[M]^+$, 344 (24), 313 (55), 285 (44), 267 (17), 91 (60), 79 (54), 55 (100).

5 21-Oleoyloxy-6,19-oxido-4-pregnene-3,20-dione (2c)

21-hydroxy-6,19-oxidoprogesterone (1, 0.052 g) was dissolved in dry dichloromethane (1 ml) and pyridine (0.12 ml) and oleoyl chloride (0.1 ml) added. The solution was stirred for 24 h at 25°C, diluted with dichloromethane, washed with 1M HCl and water, and evaporated to dryness. Purification by preparative TLC afforded 21-oleoyloxy-6,19-oxidoprogesterone (2c, 0.075 g). ¹H NMR (200.13 MHz) δ_H 0.76 (3H, s, 13-CH₃), 0.88 (3H, t, $J = 7.0$ Hz, CH₃-CH-CO), 2.34 (2H, t, $J = 7.7$ Hz, CH-CH₂CO), 2.50 (1H, t, $J = 8.5$ Hz, 17-H), 3.51 (1H, d, $J = 8.3$ Hz, 19a-H), 4.20 (1H, d, $J = 8.3$ Hz, 19b-H), 4.49 (1H, d, $J = 16.9$ Hz, 21a-H), 4.70 (1H, d, $J = 4.5$ Hz, 6-H), 4.73 (1H, d, $J = 16.9$ Hz, 21b-H), 5.34 (2H, m, CH-CH=CH-CH-CO), 5.82 (1H, s, 4-H).

21-Phosphate-6,19-oxido-4-pregnene-3,20-dione (2d)

21-hydroxy-6,19-oxido-4-pregnene-3,20-dione (1, 0.052g) was dissolved in dry dichloromethane (1 ml) and pyridine (0.12 ml), and phosphochloridic acid diallyl ester (0.024g, 1 eq.) was added dropwise at 0°C, over 30 minutes. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was washed three times with 5% NaHCO₃, the organic layer was dried and evaporated in vacuo without heating. To the resulting syrup was added a solution of NaOH (2.2 equivalents) in water (3 ml). The solution was brought slowly to reflux, and gently refluxed for 12 hours. Lyophilization yielded the crude

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phosphate disodium salt, which was recrystallized from ethanol. Alternatively, the crude disodium salt may be dissolved in water, and the pH adjusted with HCl, to cause precipitation of the free phosphate.

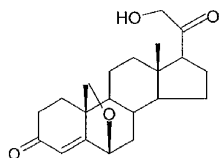
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Claims

1. A method of preparing 21-hydroxy-6,19-oxidoprogesterone (1) or an acyl or
 5 phosphate derivative thereof.



(1)

the method comprising the steps

- a) providing 21-acetoxypregnenolone;
- 10 b) protecting the C-3 hydroxy group of 21-acetoxypregnenolone through
 formation of a labile ester;
- c) obtaining the bromohydrin product thereof, by addition of a bromine and a
 hydroxy group onto the double bond of position of C5-C6 of the ester
 protected 21-acetoxy-pregnenolone;
- 15 d) performing an intramolecular cyclization with the C19 atom with the
 "Suarez-reagent" (diacetoxyiodobenzene) and iodine under irradiation, thus
 obtaining the 6,19 oxido-bridge within the scaffold;
- e) performing a selective hydrolysis, followed by an oxidation, to obtain a
 bromo-ketone;

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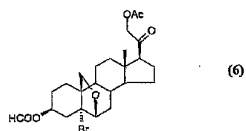
- f) performing a hydrolysis of the bromoketone resulting from step e) to obtain 21-hydroxy-6,19-oxidoprogesterone (1); and optionally
- g) reacting compound (1) with an acylating agent or phosphorylating agent.
2. The method according to claim 1, wherein the 21-acetate derivative is obtained by acetylating 21-hydroxy-6,19-oxidoprogesterone (1) of step f).
3. The method according to claim 1, wherein a C1-18 acyl derivative is obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone with a C1-18 acid, anhydride, active ester or acyl chloride.
4. The method according to claim 1, wherein a 21-propionate, 21-hemisuccinate, 21-phosphate, 21-oleate derivative is obtained by esterifying 21-hydroxy-6,19-oxidoprogesterone (1) of step f) with propionic acid, succinic acid, oleic acid or their anhydrides or active esters or acid chlorides.
5. The method according to claim 1, wherein the 21-phosphate derivative is obtained by reacting oxidoprogesterone (1) of step (f) with a phosphorylating agent.
6. The method according to any of the preceding claims wherein for step b) a formate ester is afforded.
7. The method according to any of the preceding claims, wherein the intramolecular cyclisation step d) is performed in dichloromethane as solvent.
8. The method according to any of the preceding claims, wherein the selective hydrolysis of step e) is performed with HCl in MeOH/dichloromethane as solvent.

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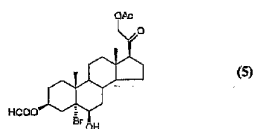
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9. The method according to any of the preceding claims, wherein the oxidation in step e) is performed with pyridinium chlorochromate (PCC).
10. The method according to any of the preceding claims, wherein the hydrolysis in step f) is performed with KOH in MeOH/dichloromethane as solvent.
- 5 11. A method for preparing (6)

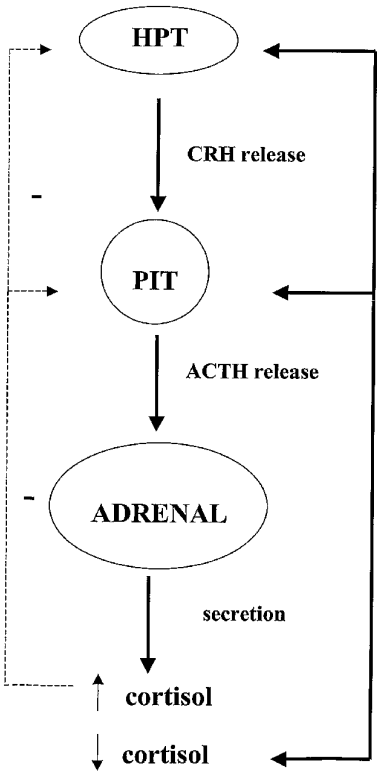


comprising the step of performing an intramolecular cyclisation between the 6-OH and the C₁₉ of the molecule (5)



12. A method according to claim 11, wherein the intramolecular cyclisation is carried out by photoreaction with DIAB and I₂.
- 10 13. A method according to claim 11 or 12, wherein the intramolecular cyclisation is carried out in CH₂Cl₂.

Figure 1



【国際調査報告】

INTERNATIONAL SEARCH REPORT

Int. l. Application No.
PCT/EP 01/10734

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07J1/00		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07J		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) BIOSIS, EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data, PAJ		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VELEIRO ADRIANA S ET AL: "Syntheses of d21-hydroxy-11,19-oxidopregn-4-ene-3,20-dione and 21-hydroxy-6,19-oxidopregn-4-ene-3,20-dione." STEROIDS, vol. 60, no. 3, 1995, pages 268-271, XP002163146 ISSN: 0039-128X	11,13
A	page 271; figure 2 --- -/--	1-10
☒ Further documents are listed in the continuation of box C. ☐ Patent family members are listed in annex.		
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *Z* document member of the same patent family		
Date of the actual completion of the international search 5 March 2002		Date of mailing of the international search report 13/03/2002
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2260 HW Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo NL Fax. (+31-70) 340-3010		Authorized officer Watchorn, P

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INTERNATIONAL SEARCH REPORT

Int. Application No.
PCT/EP 01/10734

C/(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COSTA S C P ET AL: "Ultrasound assisted remote functionalization of non-activated carbon atoms: efficient in situ formation of tetrahydrofurans by sonolysis of bromohydrins with (diacetoxyiodo)benzene/I ₂ " TETRAHEDRON LETTERS, NL, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 40, no. 49, 3 December 1999 (1999-12-03), pages 8711-8714, XP004184879 ISSN: 0040-4039	11,13
A	page 8712, paragraph 2 page 8712, paragraph 2 page 8713; table 1	1-10
A	DE ARMAS, PEDRO ET AL: "Intramolecular hydrogen abstraction. Hypervalent organoiodine compounds, convenient reagents for alkoxy radical generation" J. CHEM. SOC., PERKIN TRANS. 1 (1989), (3), 405-11, XP002163147 cited in the application page 405, column 1, paragraphs 1-3 page 406; table 1	1-10
A	CONCEPCION, JOSE I. ET AL: "Intramolecular hydrogen abstraction. Iodobenzene diacetate, an efficient and convenient reagent for alkoxy radical generation" TETRAHEDRON LETT. (1984), 25(18), 1953-6, XP002163148 page 1955; table 1	1-10

Form PCT/ISA210 (continuation of second sheet) (July 1992)

フロントページの続き

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