

- (21) Application No. 25187/75 (22) Filed 12 June 1975  
 (23) Complete Specification filed 13 Sept. 1976  
 (44) Complete Specification published 16 April 1980  
 (51) INT CL<sup>3</sup> C07J 41/00; A61K 31/57; C07J 43/00  
 (52) Index at acceptance  
 C2U 2 3 4A1A 4A1B 4B2B 4C11 4C4X 4N6A 4N6Y 5 6B  
 8A1 8B  
 (72) Inventor NICOLAS GUERITEE

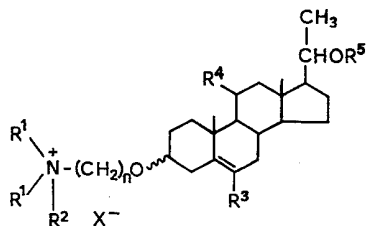


(54) NEW STEROID AMINOETHERS

(71) We, THERAMEX S.A., a French Company, of 11 Boulevard Lannes, Paris 16<sup>e</sup>, France, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to new steroid derivatives, their preparation and use in human or veterinary therapeutics by the oral, parenteral or local (cutaneous or mucous) routes, as anti-bacterial, anti-fungal, anti-parasitic or anti-viral products.

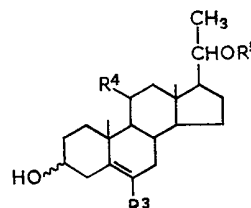
The new derivatives according to the invention have the following general formula



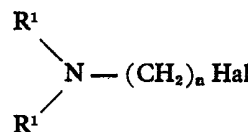
in which

- $n$  is an integer from 2 to 6;  
 $R^1$  is an alkyl group, e.g.  $-\text{CH}_3$ ,  $-\text{C}_2\text{H}_5$ , or  $-\text{C}_6\text{H}_{13}$ , or the groups  $R^1$  together with the intervening nitrogen atom form a 1-piperidinyl - 1 - pyrrolidinyl, 4 - methyl - 1-piperazinyl or 4 - morpholinyl group;  
 $R^2$  is a hydrogen atom or a methyl or ethyl group;  
 $R^3$  is a hydrogen atom or a methyl group;  
 $R^4$  is a hydrogen atom or a hydroxyl group;  
 $R^5$  is a hydrogen atom, an alkyl group, or a tetrahydropyran-2-yl group (provided that  $R^2$  and  $R^5$  are not both hydrogen); and  
 $X^-$  is an anion of a mineral or organic acid such as hydrochloric, hydrobromic, hydriodic, sulphuric, tartaric, succinic, methanesulphonic, toluenesulphonic, benzenesulphonic, fluorosulphonic or *p*-nitrobenzenesulphonic acid.

The new compounds according to the invention may be prepared by reacting a 3-hydroxy compound of the formula:



at various temperatures firstly with potassium tert-butyrate or *n*-butyl-lithium in an appropriate solvent (such as THF) and then with an aminoalkyl halide of the formula



(in which  $n$  and  $R^1$  have the meanings defined above and Hal is a halogen atom). The resultant 3-substituted derivative is then converted to the corresponding ammonium salt or quaternary ammonium derivative by reaction with an appropriate acid or quaternising agent.

The derivatives with  $3\alpha$ -stereochemistry can also be obtained by the substitution of the corresponding alcohols, or by alcoholysis of the  $\Delta^5$   $3\beta$ -tosyloxy derivatives by the desired amino-alcohols, with inversion of configuration at  $C_{(3)}$ , according to the method described by D. D. Evans and J. Hussey (J. Chem. Soc., 1969, 2504).

EXAMPLE 1.

$3\beta$  - [2 - (Diethylamino)ethoxy] -  $20\beta$ -tetrahydropyran - 2 - yloxy] pregn - 5-ene hydrochloride (IB002).

$3\beta$  - Acetoxy -  $20\beta$  - hydroxy - pregn - 5-ene (12g) described by Hirschman et al. (J. Biol. Chem. 1951, 192, 115) is transformed into its tetrahydropyran - 2 - yl ether by reaction in 360 ml anhydrous benzene containing 30 ml freshly distilled dihydropyran and 0.5g paratoluene sulphonic acid. After allowing to settle for 10h at room temperature, the reaction mixture is washed with a

sodium bicarbonate solution and dried over sodium sulphate.

The product obtained crystallises from methanol (yield: 85%). M.P. 153°—155°C.

5 The 3 $\beta$ -ester group is then hydrolysed by treating with N methanolic potassium and refluxing for 1½ hours.

10 A quantitative yield of 3 $\beta$  - hydroxy - 20 $\beta$ - (tetrahydropyran- 2 - yloxy) - pregn - 5 - ene is obtained. M.P. 202°C.

15 10g of this alcohol are placed in a 500 ml Woulfe's bottle attached to a magnetic stirrer and placed in an oil bath. 150 ml toluene are added to the alcohol in the Woulf's bottle and 22 ml n-butyl lithium solution in hexane are then added. The reaction mixture is brought to reflux temperature and refluxed for 4 hours. Diethylaminoethyl chloride solution, 20 ml in 80 ml benzene, is then added little by little to the reaction mixture. Refluxing is continued for 30 hours after adding the reagent.

20 The reaction mixture is then concentrated and the product, 3 $\beta$  - [2 - (diethylamino)-ethoxy] - 20 $\beta$  - (tetrahydropyran - 2 - yloxy-pregn - 5 - ene, crystallises from acetone. M.P.: 76°C (yield: 60%).

25 A hydrochloride is easily obtained from this derivative by treating with a solution of hydrogen chloride in ether. M.P.: 180°—30 182°C (IB 002).

#### EXAMPLE 2.

3 $\beta$  - [2 - (Diethylamino)ethoxy] - 20 $\beta$ -hydroxypregn - 5 - ene methyl *p*-toluene-sulphonate (TX 047).

35 2g of 3 $\beta$  - [2 - (diethylamino)ethoxy]-

20 $\beta$  - (tetrahydropyran - 2 - yloxy) - pregn-5 - ene are dissolved in 50 ml of ethanol containing 5 ml water and 5 ml concentrated hydrochloric acid. After settling for 3 hours at room temperature, the reaction mixture 40 alkalised with dilute sodium hydroxide solution and extracted with ether to give 3 $\beta$  - [2-(diethylamino)ethoxy] - 20 $\beta$  - hydroxypregn-5 - ene (M.P. 78°C. (IB 003).

45 2g of this latter compound, 40 ml anhydrous acetone and 2g methyl paratoluene-sulphonate are placed in a 100 ml flask provided with a magnetic stirrer and a condenser. The reaction mixture is refluxed for 5 hours and allowed to settle overnight at room 50 temperature. After distilling off the solvent, the residue is triturated several times with anhydrous ether and crystallised from a hexane-acetone mixture (3:1).

55 White crystals of the title produce are obtained. M.P.: 170—172°C (yield 98%).

60 From a biological point of view, the derivatives according to the invention are characterised by anti-bacterial, anti-fungal, anti-protozoal and anti-viral properties which are linked to the amino-ether structure of the basic steroid and not to the secondary modifications such as the salification of the amino group.

65 Table I summarises the *in vitro* minimum inhibiting concentrations (MIC) of two derivatives according to the invention with respect to several micro-organisms of a varied nature: 70 banal germs, yeasts, dermatophytes, a protozoon and a virus. As can be seen, the anti-infectious activities of the two derivatives (IB 002 and TX 047) are closely analogous.

TABLE  
Minimum Inhibiting Concentrations ( $\mu\text{g/ml}$ )<sup>o</sup> In Vitro

	IB 002	TX 047
Diplococcus pneumoniae	10	
Enterococcus	40	50 ; 100
Streptococcus pyogenes A (Pasteur Institute)	10	10 ; 25 ; 50
Streptococcus viridans G (Pasteur Institute)	20	12.5 ; 25 ; 33
Staphylococcus path. A & B (Pasteur Institute)	40	10 ; 12.5 ; 33
Staphylococcus path. N° 9 (Broussais Hospital)	10	50
Brucella abortus	100	
Bacillus carbonis II (Pasteur Institute) <sup>oo</sup>	50 <sup>oo</sup> ; 40	10 ; 33 <sup>oo</sup> ; 50 <sup>oo</sup>
Erysipelotrix insidiosa	20	33
Corynebacterium vaginalis <sup>oo</sup>	20 ; 50 <sup>oo</sup>	10 ; 25 ; 25 <sup>oo</sup> ; 50
Candida albicans I or 844 (Pasteur Institute)		10 ; 5 ; 20 ; 33
Trichophyton mentagrophytes n° 44 (Pasteur Institute) <sup>oo</sup>		10 <sup>oo</sup> ; 20 <sup>oo</sup> ; 40
Trichophyton rubrum		10 ; 20 ; 80
Trichomonas vaginalis		100
100 CTD <sub>50</sub> /calf kidney cell of animal Herpes	200	

<sup>o</sup> When there are several figures mentioned, they correspond to different experiments.  
<sup>oo</sup> The tests were performed on different strains.

Table II shows the bactericidal and fungicidal effects of compound TX 047 according to its concentration and duration of effect.

TABLE II

Strain	MIC ( $\mu\text{g}/\text{ml}$ )	after a contact of ... minutes
Bacillus carbonis 2nd Pasteur strain	200 50 25	100 270 1140
Bacillus carbonis Sterne strain	200 100 50	60 210 1140
Corynebacterium (an aerobic strain)	200 100	100 210
Pathogenic Staphylococcus (Pasteur V 12-8)	200 100	270 1140
Candida albicans Pasteur 844 strain	200 100 50 25	30 100 270 1140

## IN VITRO BACTERICIDAL EFFECT OF TX 047

5 Whether the anti-iral activity of the com-  
pounds of the invention is linked to the amino-  
ether structure of the steroid and not to the  
quaternisation of nitrogen, the proof is the  
viro-static effect of derivative IB 002 (hydro-  
chloride) on animal herpetic virus in a culture  
10 of calf kidney cells, at the minimum inhibiting  
dose of 200  $\mu\text{g}/\text{ml}$ .

More detailed tests were performed with  
compound TX 047 on human herpetic viruses  
I and II. The virostatic effect of this com-  
15 pound on 1000 CTD<sub>50</sub> of virus I and II is  
revealed at a concentration of 100  $\mu\text{g}/\text{ml}$  in  
10 days.

Compound TX 047, in a culture of cat  
kidney cells, in 10 days, also inhibited:

- 20 — 100 CTD<sub>50</sub> infectious rhinotracheitis  
virus, strain RIC 870  
(Pasteur Institute) at a  
concentration of 25  $\mu\text{g}/\text{ml}$ .  
and  
25 — 100 CTD<sub>50</sub> coryza virus, strain 306  
(Pasteur Institute) at a  
concentration of 100  $\mu\text{g}/\text{ml}$ .

30 Lastly, the virucidal effect of compound  
TX 047 on  $1 \times 10^6$  CTD<sub>50</sub> (cytotoxic doses

of virus) was revealed in 30 minutes at a  
dose of 200  $\mu\text{g}$ .

Salification of the basic aminoethers has, in  
particular, a practical interest, such as better  
adaptation to certain therapeutic uses (e.g.  
when better solubility is required).

However, quaternisation of the nitrogen of  
the aminoether is likely to modify consider-  
ably the penetration of a compound through  
epithelial tissue: skin and mucosa. The con-  
40 sequence is, for instance, an important decrease  
in toxicity in the case of external or local  
treatment, or by the digestive route: the com-  
pound can then be used at relatively high  
concentrations to obtain optimum local effec-  
45 tiveness and without risking toxic effects due  
to an important systemic passage.

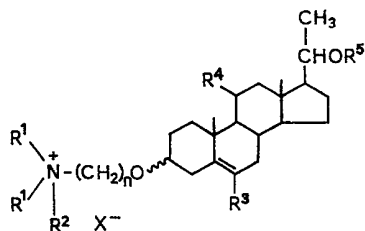
As an example, with respect to compound  
IB 003, the oral LD<sub>50</sub> is only 5 times greater  
than intraperitoneal LD<sub>50</sub>:

850 mg/kg  $\pm$  55.8 versus  $145 \pm 12.2$  re-  
spectively, whilst it is approximately 100 times  
greater with compound TX 047 (which is the  
methyl p-toluenesulphonate of IB 003):

LD<sub>50</sub>: 67 mg/kg by the intra-peritoneal  
55 route, versus >5000 mg/kg orally.

## WHAT WE CLAIM IS:—

1. Compounds of the general formula:

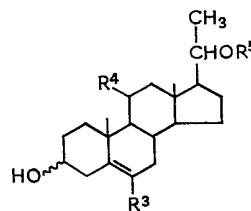


in which

- 5  $n$  is an integer from 2 to 6;  
 $R^1$  is an alkyl group or the groups  $R^1$  together with the intervening nitrogen atom form a 1-piperidinyl, 1-pyrrolidinyl, 4-methyl-1-piperazinyl or 4-morpholinyl group;  
 10  $R^2$  is a hydrogen atom or a methyl or ethyl group;  
 $R^3$  is a hydrogen atom or methyl group;  
 $R^4$  is a hydrogen atom or a hydroxyl group;  
 15  $R^5$  is a hydrogen atom or alkyl group or a tetrahydropyran-2-yl group ( $R^2$  and  $R^5$  not both being hydrogen); and  
 $X^-$  is an anion of a mineral or organic acid.  
 20 2.  $3\beta$  - [2 - (Diethylamino)ethoxy] -  $20\beta$  - (tetrahydropyran - 2 - yloxy)pregn - 5 - ene hydrochloride.  
 25 3.  $3\beta$  - [2 - (Diethylamino)ethyl] -  $20\beta$  - hydroxy - pregn - 5 - ene methyl paratoluene-sulphonate.  
 4. A process for the preparation of a compound as claimed in claim 1 which comprises

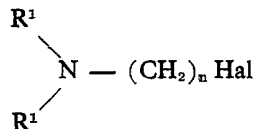
reacting a 3-hydroxy compound of the formula:

30



(in which  $R^3$ ,  $R^4$  and  $R^5$  have the meanings defined in claim 1) firstly with potassium tert. butylate or  $n$ -butyl-lithium in a solvent and then with an aminoalkyl halide of the formula:

35



(in which  $n$  and  $R^1$  have the meaning defined in claim 1), and then reacting the product with an appropriate acid or quaternising agent to convert it into the desired ammonium salt or quaternary ammonium derivative.

40

5. A pharmaceutical composition comprising a compound as claimed in any one of claims 1—3 in association with a pharmaceutical carrier or diluent.

45

MARKS & CLERK,  
 Chartered Patent Agents,  
 57—60 Lincoln's Inn Fields,  
 London, WC2A 3LS.  
 Agents for the Applicants.