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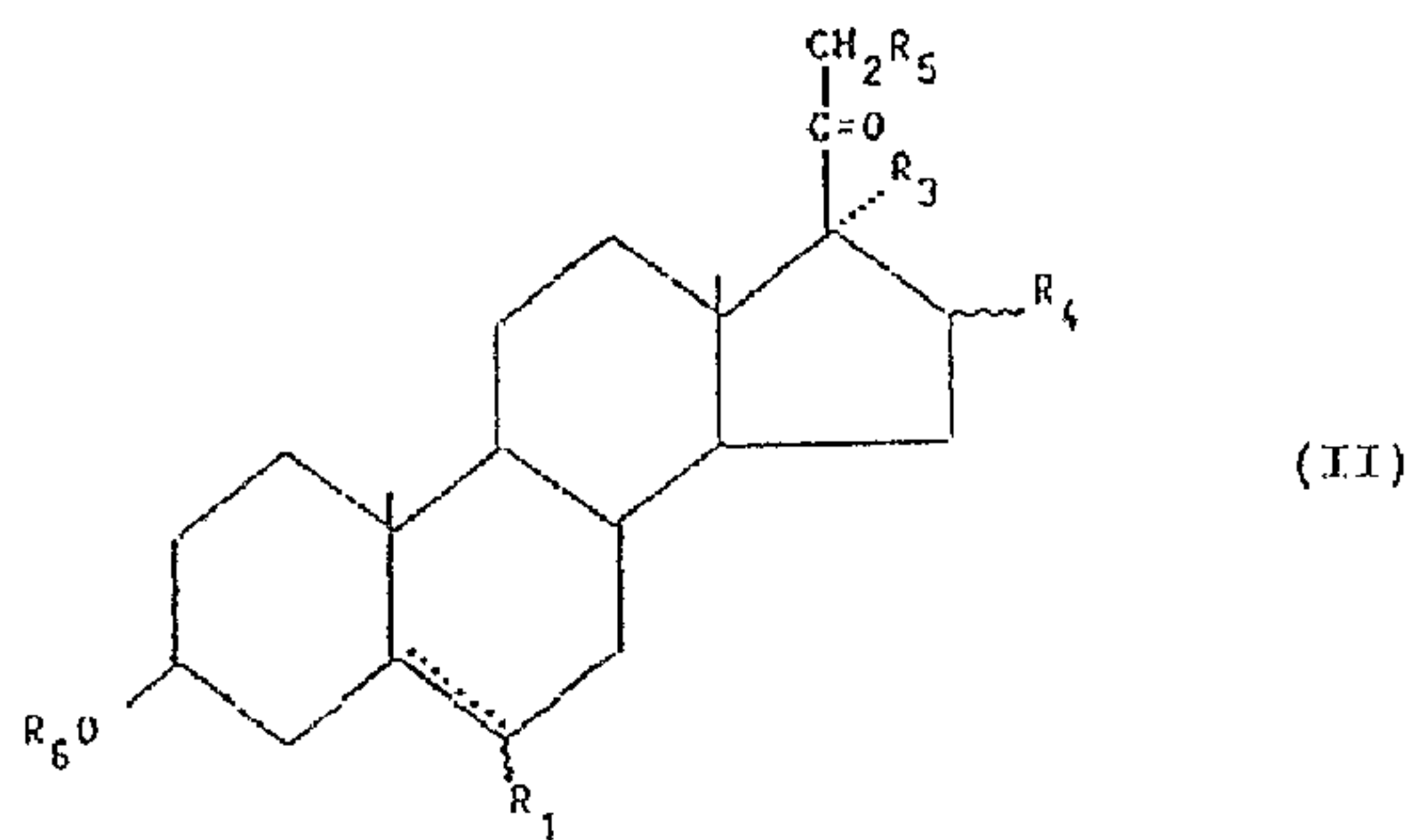
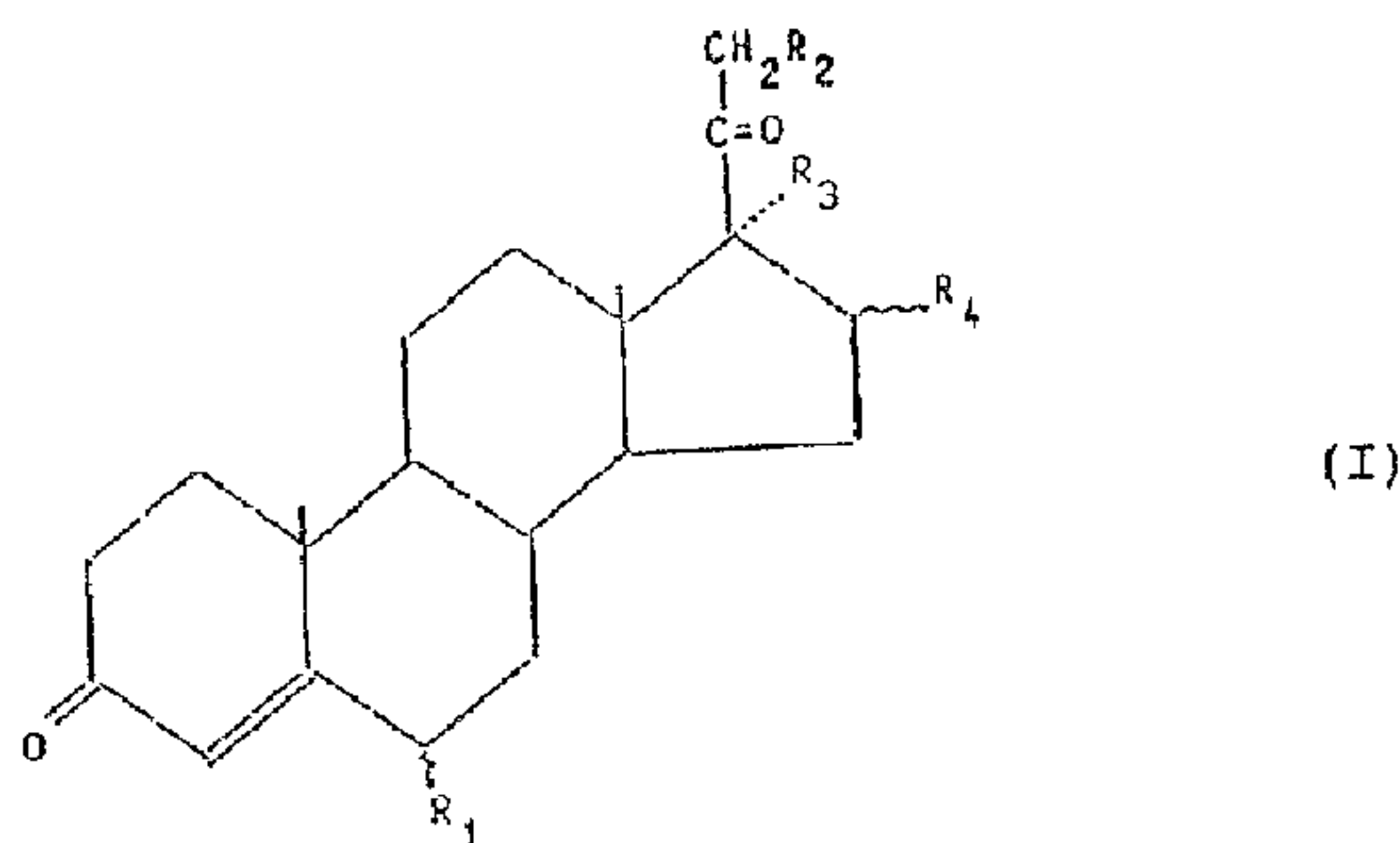
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(54) **PROCEDE POUR LA PRODUCTION DE 4-PREGNENE-3,20-
DIONE ET DE SES DERIVES**

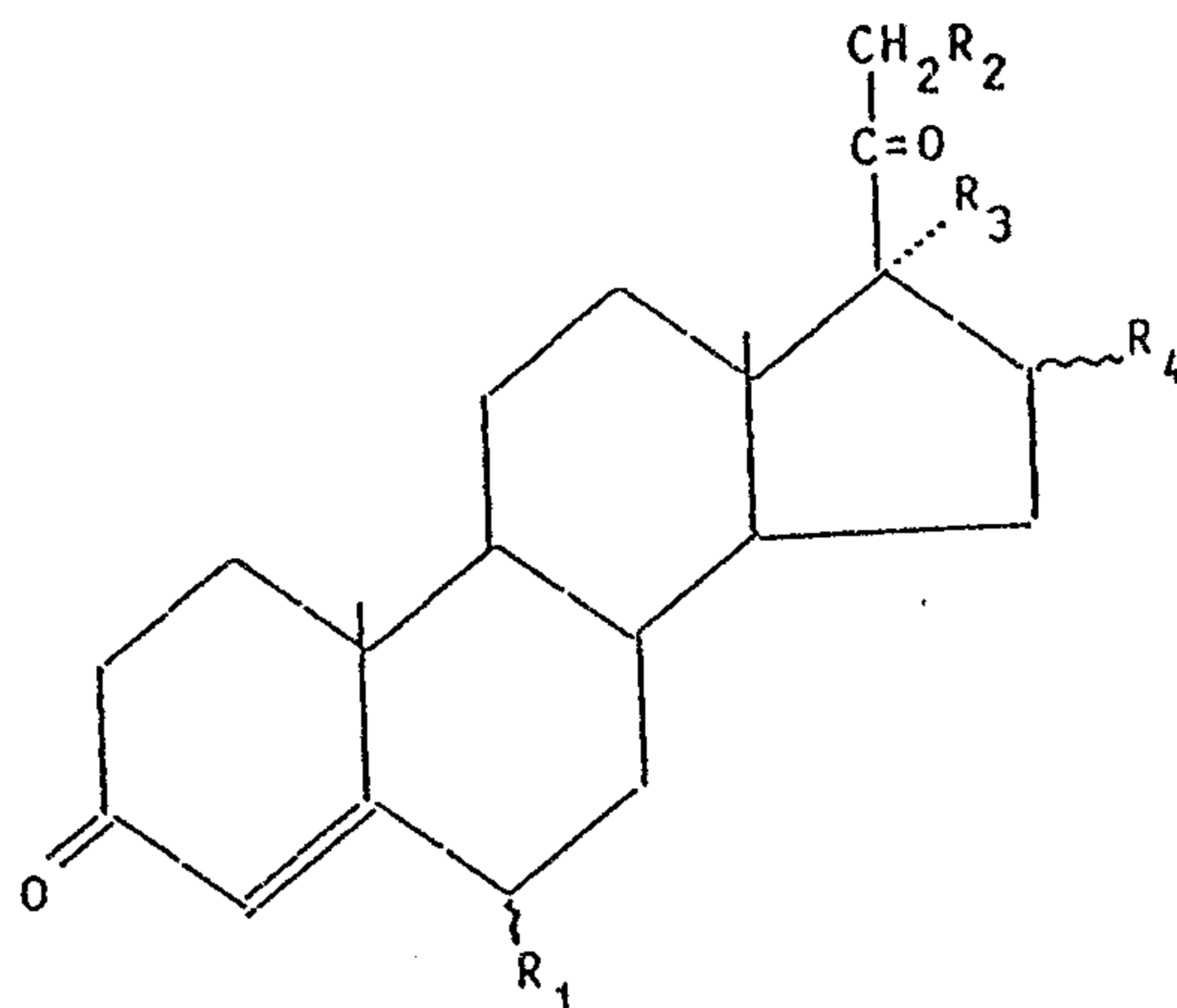
(54) **PROCESS FOR THE PRODUCTION OF 4-PREGNENE-3,20-
DIONE AND ITS DERIVATIVES**



(57) A process for the production of 4-pregnene-3,20-dione and its derivatives of general formula I (see formula I), in which R₁ means a hydrogen atom, a fluorine atom or a methyl group, R₂ represents a hydrogen atom or a hydroxy group, and R₃ and R₄ together symbolize a carbon-carbon bond or R₃ represents a hydrogen atom, a hydroxy group or an alkanoyloxy group with up to 6 carbon atoms and R₄ means a hydrogen atom or a methyl group, is described, which is characterized in that a pregnane derivative of general formula II (see formula II), in which R₁, R₃ and R₄ have the above-mentioned meaning, symbolizes a single bond or a double bond, R₅ represents a hydrogen atom, a hydroxy group or an alkanoyloxy group with at most 6 carbon atoms and R₆ means a hydrogen atom or an alkanoyl group with at most 6 carbon atoms, is fermented with a bacterial culture of species *Mycobacterium spec.* NRRL B-3805.

Abstract

A process for the production of 4-pregnene-3,20-dione and its derivatives of general formula I



(I),

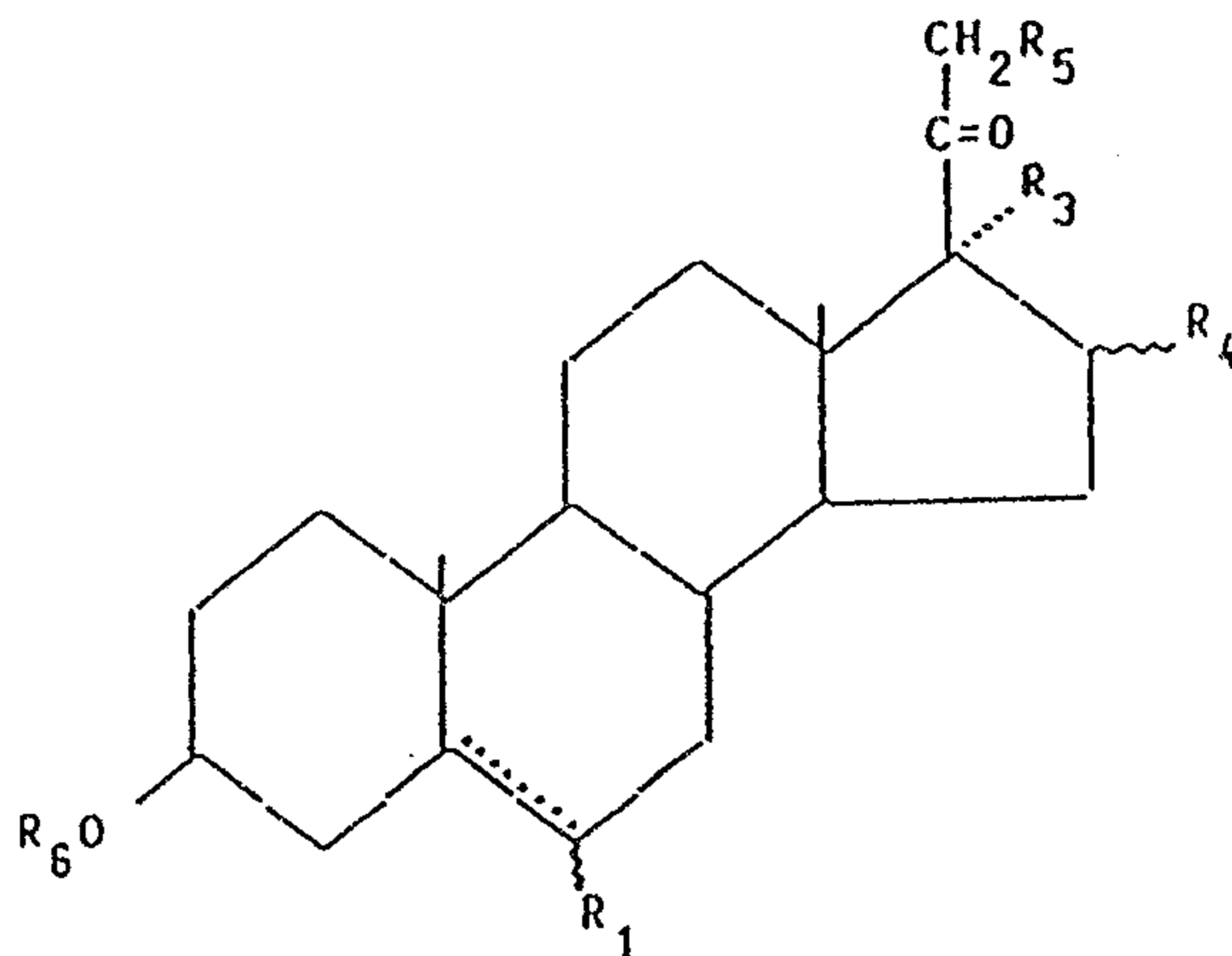
in which

R_1 means a hydrogen atom, a fluorine atom or a methyl group,
 R_2 represents a hydrogen atom or a hydroxy group, and
 R_3 and R_4 together symbolize a carbon-carbon bond or
 R_3 represents a hydrogen atom, a hydroxy group or an
 alkanoyloxy group with up to 6 carbon atoms and

R_4 means a hydrogen atom or a methyl group, is described,
 which is characterized in that

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a pregnane derivative of general formula II



(II),

in which R_1 , R_3 and R_4 have the above-mentioned meaning,

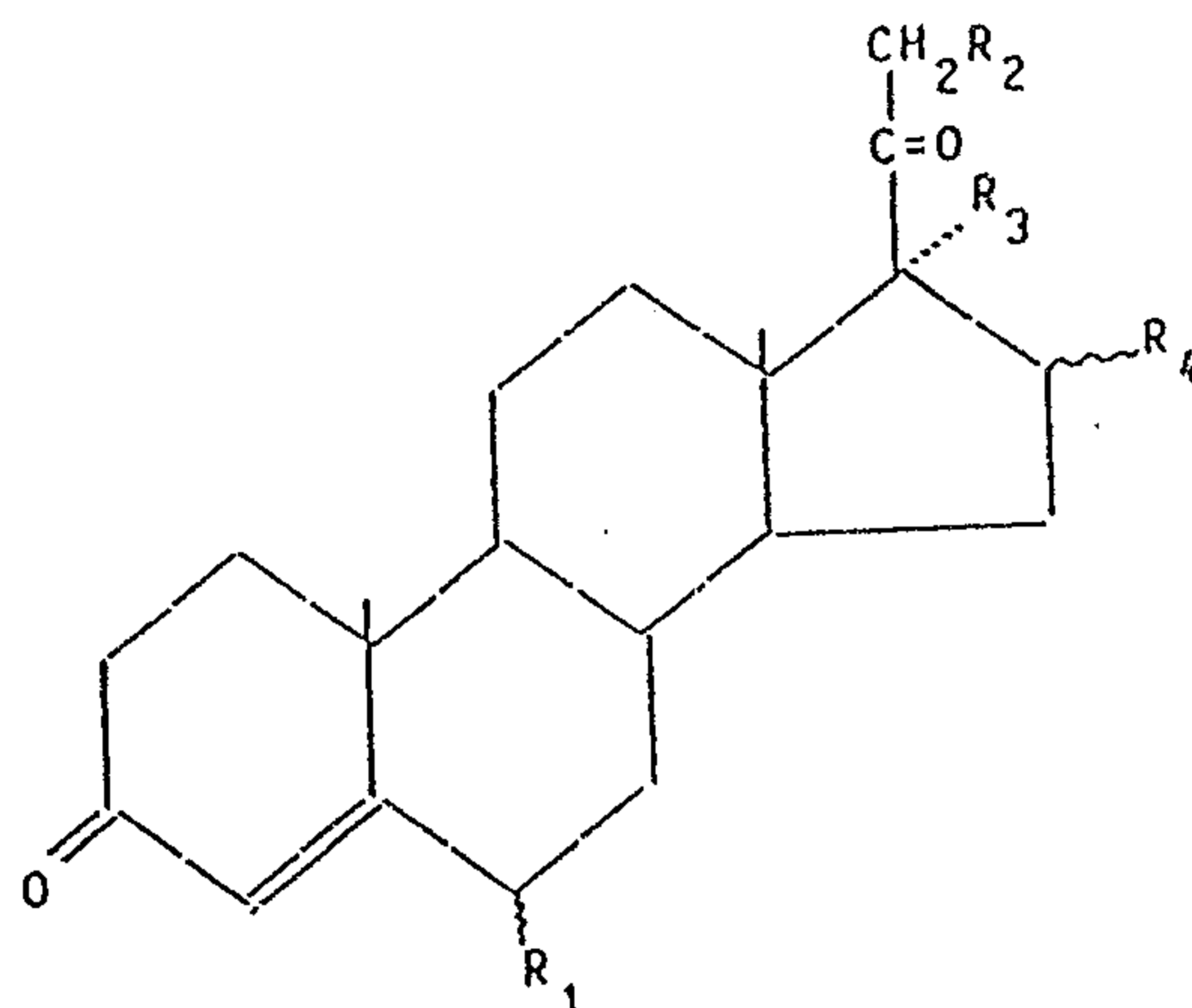
..... symbolizes a single bond or a double bond,

R_5 represents a hydrogen atom, a hydroxy group or an alkanoyloxy group with at most 6 carbon atoms and

R_6 means a hydrogen atom or an alkanoyl group with at most 6 carbon atoms, is fermented with a bacterial culture of species *Mycobacterium spec.* NRRL B-3805.

Process for the Production of 4-Pregnene-3,20-dione
and its Derivatives

The invention relates to a process for the production of 4-pregnene-3,20-dione and its derivatives of general formula I



(I),

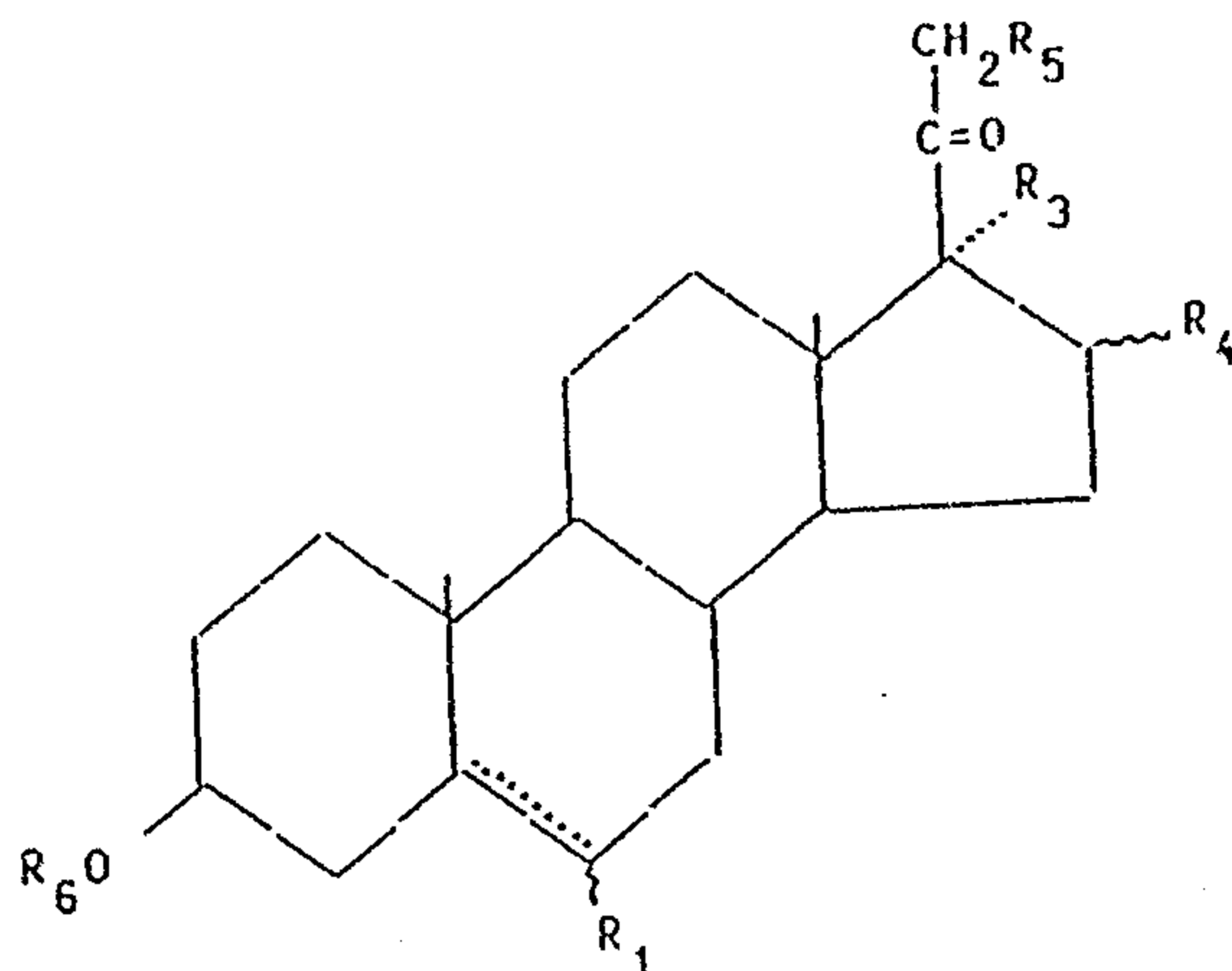
in which

R_1 means a hydrogen atom, a fluorine atom or a methyl group,
 R_2 represents a hydrogen atom or a hydroxy group, and
 R_3 and R_4 together symbolize a carbon-carbon bond or
 R_3 represents a hydrogen atom, a hydroxy group or an
alkanoyloxy group with up to 6 carbon atoms and

R_4 means a hydrogen atom or a methyl group,
characterized in that

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a pregnane derivative of general formula II



(II),

in which R_1 , R_3 and R_4 have the above-mentioned meaning,

..... symbolizes a single bond or a double bond,

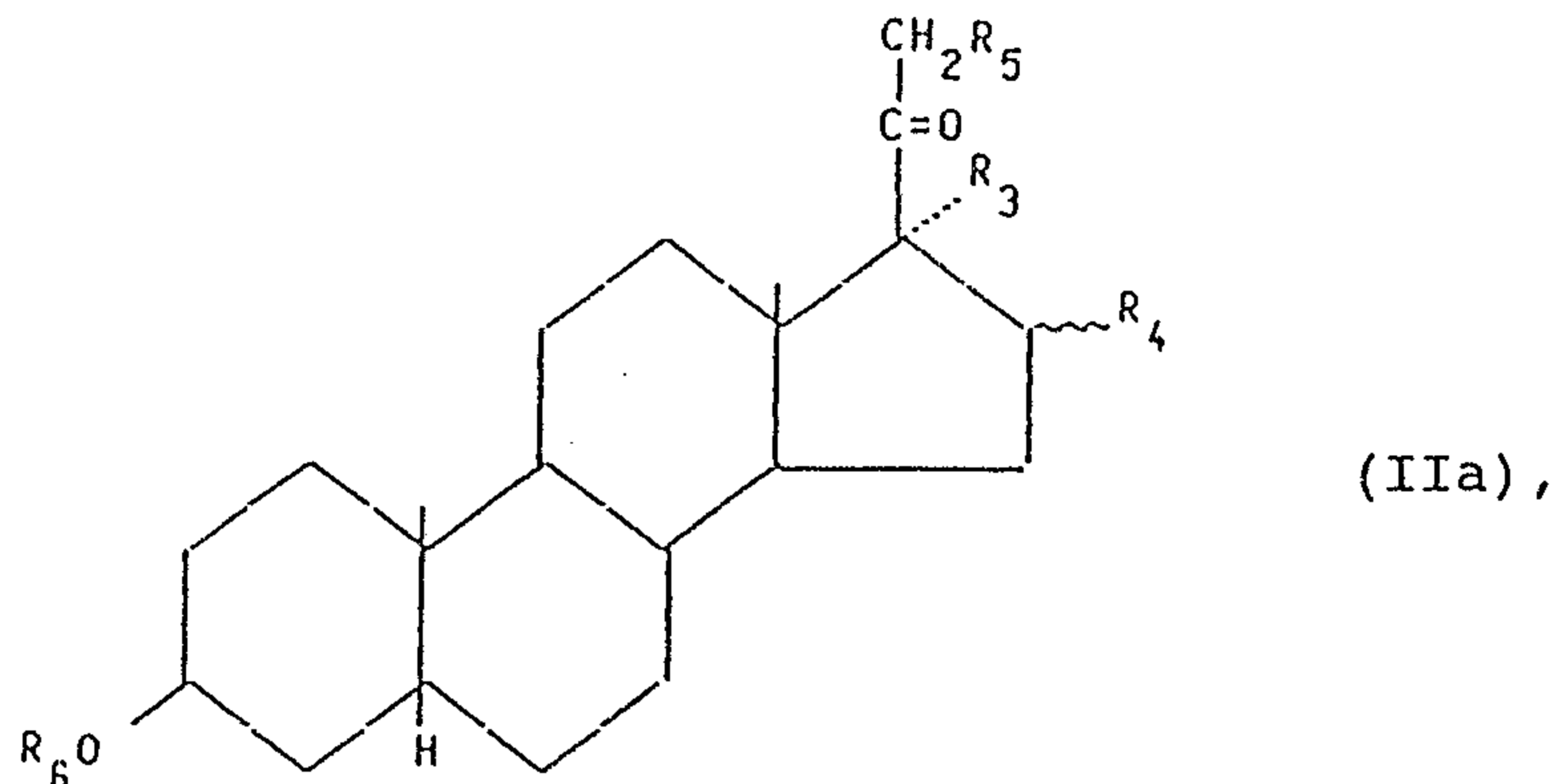
R_5 represents a hydrogen atom, a hydroxy group or an alkanoyloxy group with at most 6 carbon atoms and

R_6 means a hydrogen atom or an alkanoyl group with at most 6 carbon atoms, is fermented with a bacterial culture of species *Mycobacterium spec.* NRRL B-3805.

This invention is of special importance for the partial synthesis of pharmacologically effective pregnane derivatives from the steroid sapogenins smilagenin and sarsasapogenin widely occurring in nature. It has been known for a long time that these sapogenins can be catabolized relatively simply to 3 β -hydroxy-5 β -3 β -hydroxy-5 β -pregn-16-en-20-one or its 3-acetate (US-A 3,475,464 and Canadian Journ. of Chem., 46, 1968, 733 f). In these compounds, a methyl group in 16-position and/or a methyl group in 17 α - and/or 21-position and/or a hydroxy group or acyloxy group in 17 α - and/or 21-position can be introduced by

methods known in the art, or the 16-double bond of these substances can be hydrogenated. (John Fried and John A. Edwards "Organic Reactions in Steroid Chemistry"; van Nostrand Reinhold Comp. New York, etc. Vol. 1 1972, p. 125 ff, Vol. 2, 1972, p. 075 f and Vol. 2, 1972, p. 162 f and 176 f).

The thus represented pregnane derivatives of general formula IIa



in which R_3 , R_4 and R_5 and R_6 have the already mentioned meaning, are converted to the corresponding 3-oxo- Δ^4 steroids (US-A 3,475,464) according to the known prior art in a multistage chemical process which is performed by agents that are harmful to the environment.

In contrast, the process according to the invention makes it possible to convert these compounds to the corresponding 3-oxo- Δ^4 steroids in a one-stage process with good yields being achieved. That this is possible is very surprising to one skilled in the art, since it is known that the microorganism used in this process usually catabolizes the side chains of steroids to the corresponding 17-oxosteroids (GB-A 1,329,287 and US-A 4,179,336).

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The process according to the invention is performed under the same fermentation conditions which are also used with these bacterial cultures in the known microbiological conversions of substrates.

Under the culture conditions usually used for these microorganisms, submerged cultures are cultivated in a suitable nutrient medium with aeration. Then, the substrate (dissolved in a suitable solvent or in emulsified form) is added to the cultures and fermented, until a maximum substrate conversion is achieved.

Suitable substrate solvents are, for example, methanol, ethanol, glycol monomethyl ether, dimethylformamide or dimethylsulfoxide. The emulsification of the substrate can be brought about, for example, by the latter being sprayed in micronized form or dissolved in a water-miscible solvent (such as methanol, ethanol, acetone, glycol monomethyl ether, dimethylformamide or dimethylsulfoxide) under strong turbulence in (preferably decalcified) water, which contains the usual emulsifying aids. Suitable emulsifying aids are nonionogenic emulsifiers, such as, for example, ethylenoxy adducts or fatty acid esters of polyglycols. As suitable emulsifiers, the commercially available wetting agents Tegin^(R), Tween^(R) and Span^(R) can be mentioned as examples.

The optimum substrate concentration, substrate addition time and fermentation period depend on the type of substrate and microorganism used and the fermentation conditions. These values, as is generally necessary in microbiological steroid

conversions, have to be determined in the individual case by preliminary tests, as they are familiar to one skilled in the art.

The process according to the invention can also be performed by using other pregnane derivatives of general formula I than the process of formula Ia, but this brings hardly any advantages according to present knowledge relative to the known microbiological processes.

The following embodiments are used to explain the process according to the invention in more detail.

Example 1

a) A 2 l Erlenmeyer with 500 ml of sterile nutrient medium containing

1 % yeast extract

0.45 % Na_2HPO_4

0.34 % KH_2PO_4

0.2 % Tween 80

-- adjusted to pH 6.7 --

is inoculated with an elutriation of a dry culture of *Mycobacterium spec.* NRRL B-3805 and shaken for 3 days with 180 revolutions per minute at 30°C.

b) 10 g of 3 β -acetoxy-5 β -16-pregnen-20-one (Canad. J. of Chem., 46, 1968, 734 ff) is ground in a ball mill (PE 075, Netzsch Co., DE-Selb/Bavaria) with corundum balls of a particle size of about 1 μ and adjusted with distilled water to an end volume of 500 ml.

c) 50 Erlenmeyers (100 ml) with 20 ml of sterile nutrient medium each containing

2.5 % Cornsteep liquor

0.25 % soybean flour

0.3 % $(\text{NH}_4)_2\text{HPO}_4$

0.25 % Tween 80

-- adjusted to pH 6.5 --

are inoculated with 1 ml of the *Mycobacterium-spec.-growing* culture each. Then, 3 ml each of the ground suspension produced under b) is added, which corresponds to 0.06 g of 3 β -acetoxy-5 β -

16-pregnen-20-one and is fermented for 120 hours at 30°C on a rotary shaker with 220 revolutions per minute.

The combined cultures are extracted with methyl isobutyl ketone, mixed with 100 g of activated carbon and filtered on a folded filter. The filtrate is then concentrated by evaporation under vacuum at a maximum of 50°C in a rotary evaporator and chromatographed on aluminum oxide.

0.7 g of 4,16-pregnadiene-3,20-dione, which is identical with an authentic sample according to HPLC, is thus obtained.

Example 2

a) 10 g of 3 β -acetoxy-5 β -16-pregnen-20-one (Canad. J. of Chem., 46, 1968, 734 ff) is ground as described in example 1b and adjusted to 500 ml with distilled water.

b) Under the conditions of example 1c, 3 ml each of the above-named suspension is added in 50 Erlenmeyer flasks with 100 ml of fermentation culture each, fermented and worked up.

0.75 g of 4-pregnene-3,20-dione, which is identical with an authentic sample according to HPLC, is thus obtained.

Example 3

a) 10 g of 3 β -hydroxy-5 β -pregnan-20-one (Canad. J. of Chem., 46, 1968, 734 ff) is ground as described in example 1b and adjusted to 500 ml with 500 ml of distilled water.

b) Under the conditions of example 1c, 3 ml each of the above-named suspension is added in 50 Erlenmeyer flasks with 100 ml of fermentation culture each, fermented and worked up.

1.0 g of 4-pregnene-3,20-dione, which is identical with an authentic sample according to HPLC, is thus obtained.

Example 4

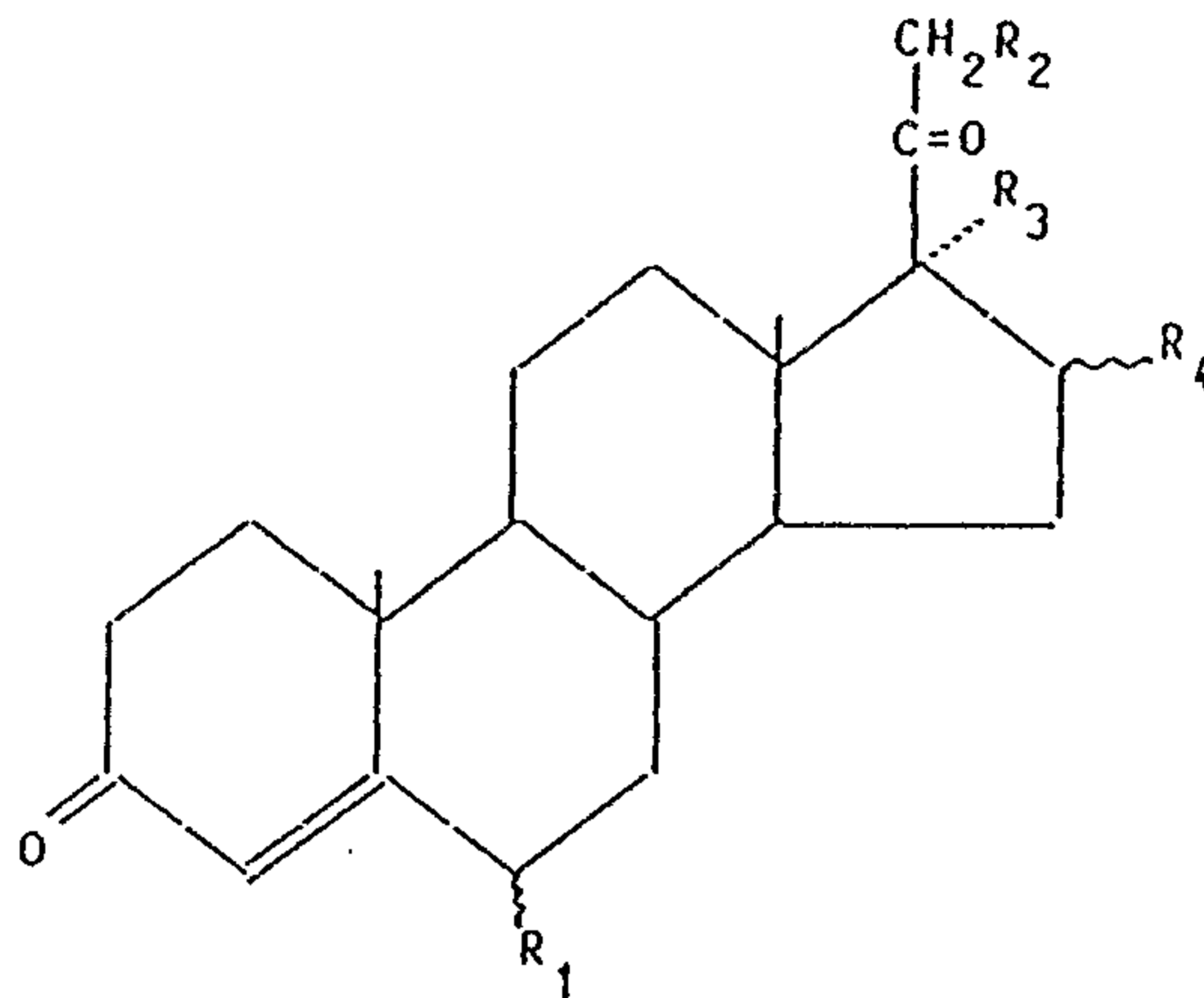
a) 10 g of 21-acetoxy-3 β -hydroxy-methyl-5 β -pregnan-20-one (DE-B 22 57 132) is ground, as described in example 1b and adjusted to 500 ml with distilled water.

b) Under the conditions of example 1c, 3 ml each of the above-named suspension is added in 50 Erlenmeyer flasks with 100 ml of fermentation culture each, fermented and worked up.

0.2 g of 21-hydroxy-16 α -methyl-4-pregnene-3,20-dione, which is identical with an authentic sample according to HPLC, is thus obtained.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE
PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

Process for the production of 4-pregnene-3,20-dione and its
derivatives of general formula I



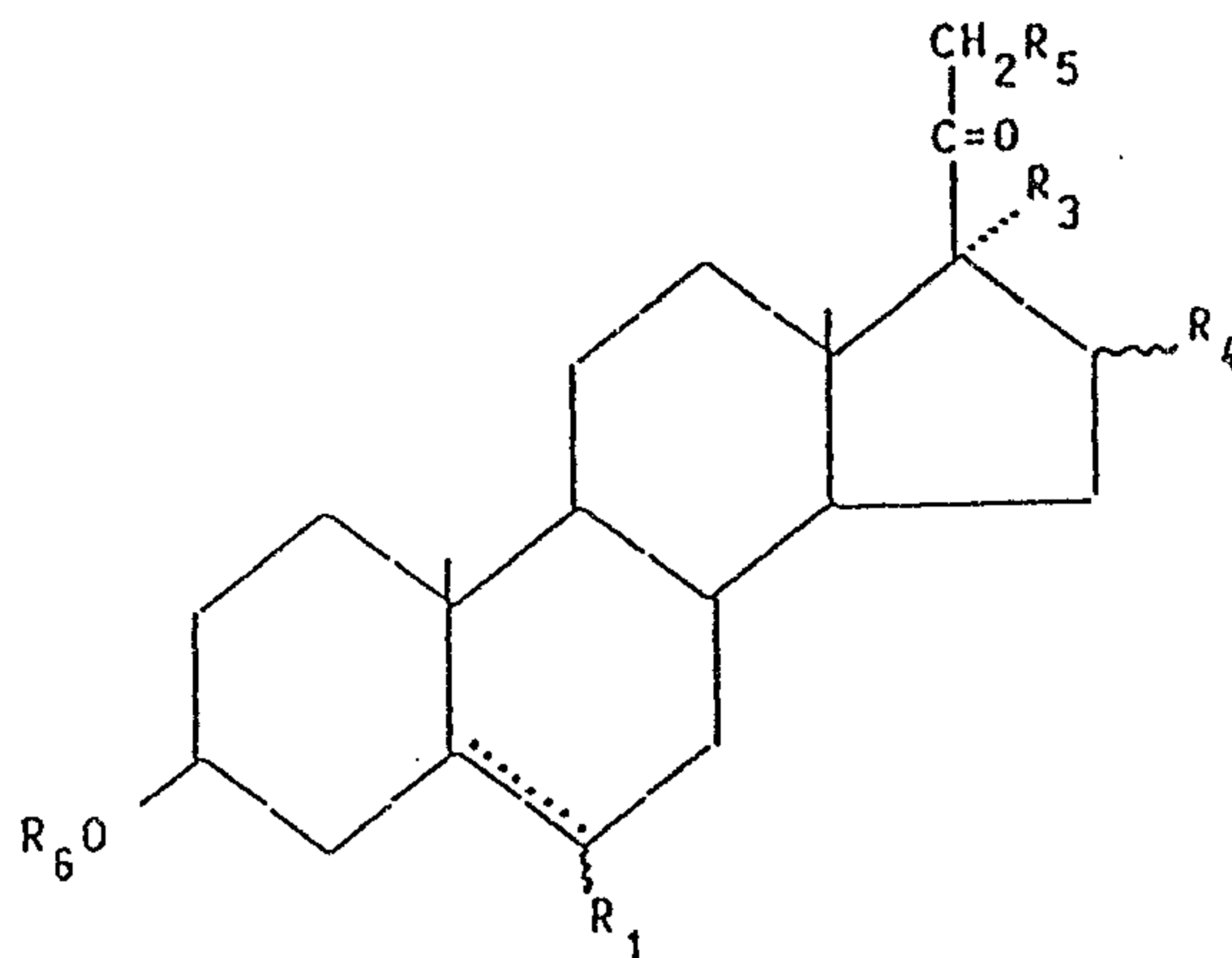
(I),

in which

R₁ means a hydrogen atom, a fluorine atom or a methyl group,
R₂ represents a hydrogen atom or a hydroxy group, and
R₃ and R₄ together symbolize a carbon-carbon bond or
R₃ represents a hydrogen atom, a hydroxy group or an
alkanoyloxy group with up to 6 carbon atoms and

R₄ means a hydrogen atom or a methyl group,
characterized in that

a pregnane derivative of general formula II



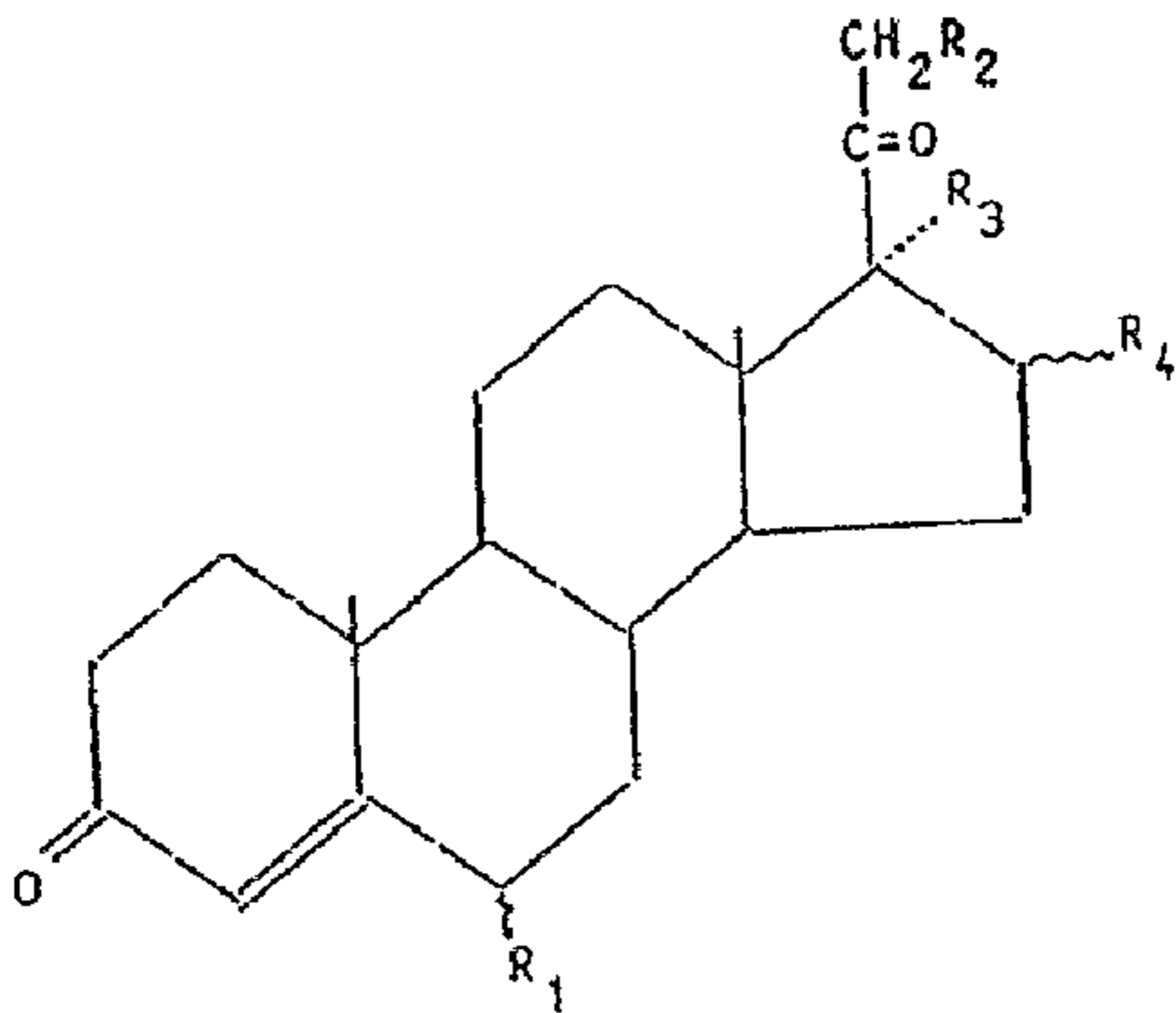
(II),

in which R₁, R₃ and R₄ have the above-mentioned meaning,

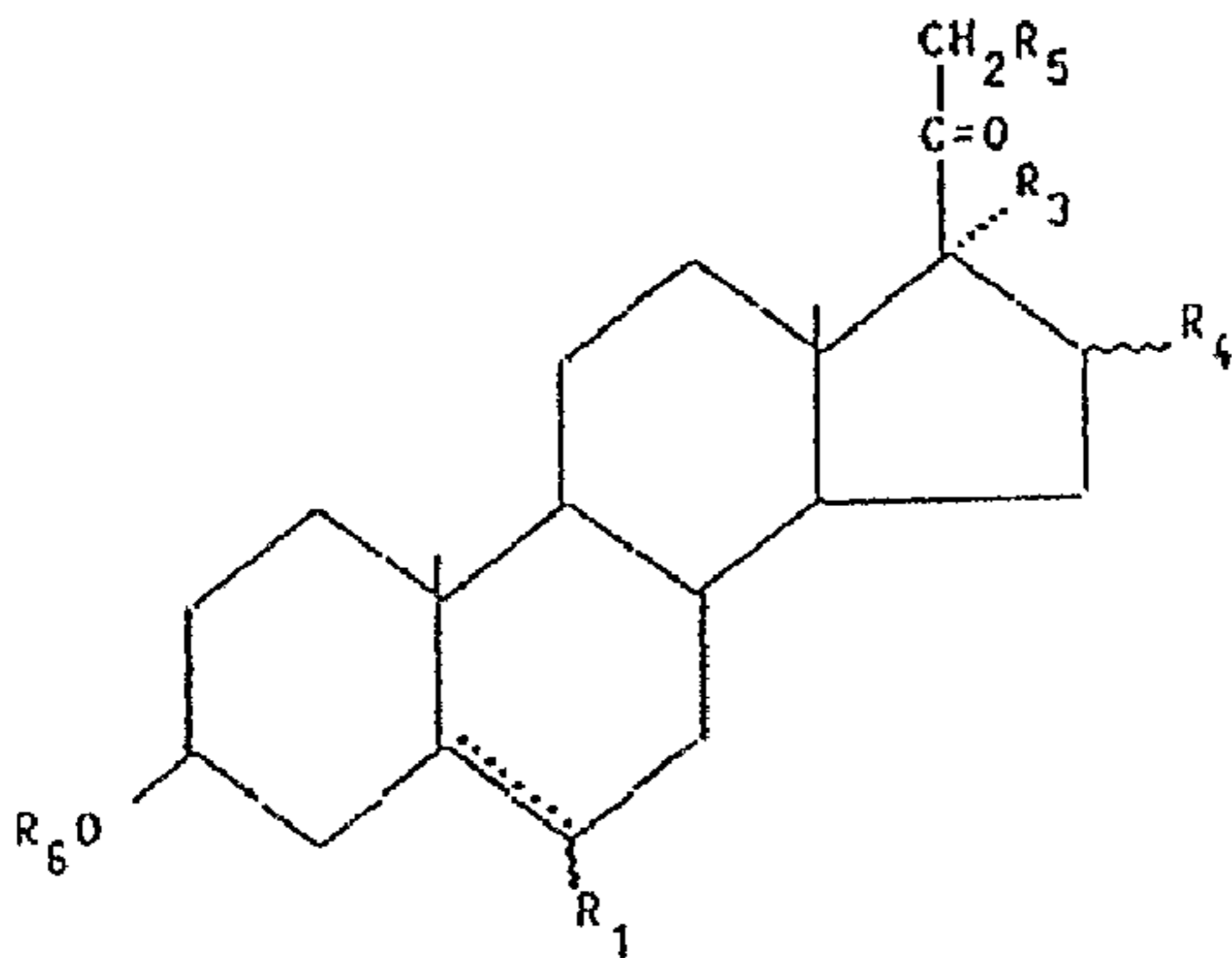
..... symbolizes a single bond or a double bond,

R₅ represents a hydrogen atom, a hydroxy group or an alkanoyloxy group with at most 6 carbon atoms and

R₆ means a hydrogen atom or an alkanoyl group with at most 6 carbon atoms, is fermented with a bacterial culture of species *Mycobacterium spec.* NRRL B-3805.



(I)



(II)