

1,097,157



PATENT SPECIFICATION

NO DRAWINGS

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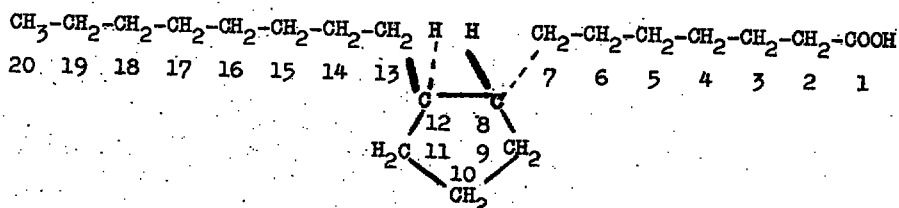
Int. Cl.:—C 07 c 61/36

COMPLETE SPECIFICATION

Improvements in or relating to Prostanic Acid Derivatives and the manufacture thereof

We, THE UPJOHN COMPANY, a corporation organised and existing under the laws of the State of Delaware, United States of America, of 301 Henrietta Street, Kalamazoo, State of Michigan, United States of America, 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:—

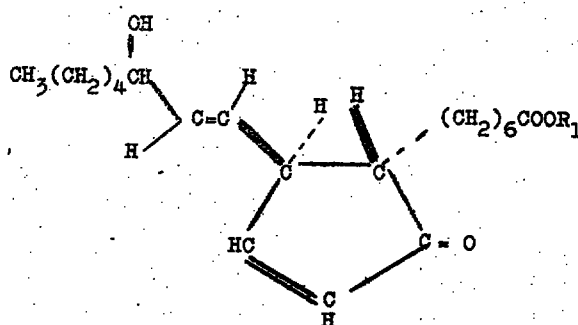
This invention relates to novel compositions of matter and to methods for producing them. In particular, this invention relates to certain derivatives of prostanic acid:



1

The hydrogen atoms attached to C-8 and C-12 in formula I are in trans configuration. See Bergström et al., J. Biol. Chem., 238, 3555—3564 (1963) and Horton, Experientia, 21, 113 (1965).

The compounds of this invention include novel compounds of the formula:



11

wherein R₁ is hydrogen or a pharmacologically acceptable cation.

Pharmacologically acceptable cations within the scope of R₁ in formula II can be the cationic form of a metal, ammonia, or an amine, or can be quaternary ammonium ions. Especially preferred metal cations are those derived from the alkali metals, e.g., lithium, sodium, and potassium, and from the alkaline earth metals, e.g., magnesium,

[Price 4s. 6d.]

calcium, strontium, and barium, although the cationic form of other metals, e.g., aluminum, zinc, iron, and silver are within the scope of this invention.

Pharmacologically acceptable amine cations within the scope of this invention can be derived from primary, secondary, or tertiary amines. Examples of suitable amines are methylamine, dimethylamine, trimethylamine, ethylamine, dibutylamine, triisopropylamine, N - methylhexylamine, decylamine, allylamine, crotylamine, cyclopentylamine, dicyclohexylamine, benzylamine, dibenzylamine, α - phenylethylamine, β - phenylethylamine, ethylenediamine, diethylenetriamine, and like lower - aliphatic, lower-cycloaliphatic, and lower - araliphatic amines containing up to and including 18 carbon atoms, as well as heterocyclic amines such as piperidine, morpholine, pyrrolidine, piperazine, and lower - alkyl derivatives thereof, such as 1 - methylpiperidine, 4 - ethylmorpholine, 1 - isopropylpyrrolidine, 2 - methylpyrrolidine, 1,4 - dimethylpiperazine, and 2 - methylpiperidine, as well as amines containing water - solubilizing or hydrophilic groups such as mono-, di- and triethanolamines, ethyldiethanolamine, N - butylethanolamine, 2 - amino - 1 - butanol, 2 - amino - 2 - ethyl - 1,3 - propanediol, 2 - amino - 2 - methyl - 1 - propanol, tris(hydroxymethyl)aminomethane, N - phenylethanolamine, N - (*p* - tert - amylphenyl)diethanolamine, galactamine, N - methylglucamine, N - methylglucosamine, ephedrine, phenylephrine, epinephrine, and procaine.

Examples of suitable pharmacologically acceptable quaternary ammonium cations within the scope of this invention are tetramethylammonium, tetraethylammonium, benzyltrimethylammonium and phenyltriethylammonium.

Prostanoic acid derivatives of formula II exhibit vaso-depressor activity in the normotensive state when assayed in dogs prepared according to the technique of Lee et al., *Circulation Res.* 13, 359 (1963). These dogs are anesthetized, vagotomized, and pentolinium-treated (AVPT dogs). The material to be assayed is administered in ethyl alcohol which is diluted 1 to 10 with physiological saline or 5 percent dextrose for intravenous injection.

Because of the above-described activity, prostanoic acid derivatives of formula II are valuable therapeutic agents for the treatment of hypertension, in normalizing serum lipids and thus reducing the danger of ischaemic heart disease, and in treating central nervous system disorders in mammals, including man. These compounds can be administered by intravenous infusion of sterile isotonic saline solutions at the rate of about 0.01 to about 10, preferably, about 0.1 to about 0.2 micrograms per kilogram of animal weight per minute.

It is known that other prostanoic acid derivatives lower systemic arterial blood pressure when injected intravenously, especially those substances known as the prostaglandins, e.g., PGE₁, PGE₂, and PGE₃. See Horton, *ibid.* The latter substances, however, also have a strong stimulatory effect on smooth muscle, for example mammalian intestinal smooth muscle, and antagonize epinephrine-induced mobilization of free fatty acids. It was quite surprising and unexpected, therefore, that the novel formula II compounds of this invention have far less smooth muscle stimulatory effect, as shown, for example, by tests on strips on guinea-pig and rabbit smooth intestinal muscle, than, for example, PGE₁, and far less antagonism of epinephrine-induced mobilization of free fatty acids than, for example, PGE₁. Therefore, the novel formula II compounds of this invention are especially useful for the above-described purposes because they are substantially more specific in their action and result in substantially lesser side effects than, for example, PGE₁.

These novel formula II compounds are also useful because they can be administered to laboratory animals, preferably rats, to produce animals containing high levels of the compounds. Such animals can then serve as test animals in the search for and study of compounds which are antagonists of the administered compounds and which, for that reason, would be useful in reversing the effects of inadvertent overdoses of the extremely potent novel formula II compounds, and in the treatment of allergic conditions. For that purpose, the formula II compound is advantageously administered to the test animal by continuous intravenous infusion in sterile saline solution at the rate of about 0.01 to about 10, preferably 0.05 to 0.2, micrograms per kilogram of animal weight per minute until the desired level of compound has been reached or until the desired response in the animal has been obtained. Infusion can then be continued or stopped depending on the particular use to be made of the test animal.

For any of the above purposes, there can be used either the free acid form or the pharmacologically acceptable salt form of formula II compounds.

In addition to the above uses, the essentially pure formula II compound wherein R₁ is hydrogen can be used as an assay standard in the study of animal tissues, plant

tissues, or biochemical conversion reaction mixtures for the possible presence and content of this novel compound.

Accordingly therefore the present invention also provides a therapeutic composition comprising as the active ingredient, a compound of Formula II together with a pharmaceutically acceptable carrier.

It is known that prostanoid acid derivatives, especially the prostaglandins, are present in numerous types of animal tissues. See Horton, *ibid.*, and Samuelsson, *Angew. Chem. Intern. Ed. Eng.* 4, 410 (1965). It is also known that various unsaturated acyclic carboxylic acids can be transformed to prostaglandins by interaction with the enzyme system present in certain animal tissues, for example, the tissues of sheep vesicular glands and guinea pig lungs. See references cited in Samuelsson, *ibid.*, and Klenberg et al., *Acta Chem. Scand.* 19, 534 (1965); Samuelsson, *J. Am. Chem. Soc.* 87, 3011 (1965); Rhyage et al., *Biochem. Biophys. Res. Commun.* 19, 279 (1965); Nugteren et al., *Biochim. Biophys. Acta*, 98, 654 (1965), and Wallach, *Life Sciences* 4, 361 (1965). Thus, all - cis - 8,11,14 - eicosatrienoic acid is transformed to PGE_1 , all - cis - eicosa-5,8,11,14 - tetraenoic acid to PGE_2 , and all - cis - eicosa - 5,8,11,14,17 - pentaenoic acid to PGE_3 . In those enzymatic transformations, oxygenase and oxidase enzymes play an essential role. See Samuelsson, *J. Am. Chem. Soc.* 87, 3011 (1965). Whole tissue homogenates, centrifuged tissue homogenates, and acetone powders have been used as sources of the necessary enzyme system.

We have now made the surprising discovery that essentially pure 15 - hydroxy - 9 - oxoprost - 10,trans - 13 - dienoic acid or a salt thereof can be separated from the mixture obtained by aerobic incubation of all cis - 8,11,14 - eicosatrienoic acid (homo - γ -linolenic acid) or a salt thereof with a substantially aqueous medium containing the oxygenase and oxidase enzymes present in animal tissue which also contains endogenous PGE_1 .

PGE_1 is a symbol for prostaglandin E_1 , also known as 11 α ,15 - dihydroxy - 9 - oxoprost - trans - 13 - enoic acid. See Horton, *ibid.* Animal tissue which contains endogenous PGE_1 is defined as animal tissue in which the PGE_1 is produced while that tissue is part of the intact animal, and does not include tissue to which the PGE_1 might be transported after it is produced, unless the latter tissue also produces or is capable of producing PGE_1 when part of the intact animal. Essentially pure 15 - hydroxy - 9 - oxo - prost - 10,trans - 13 - dienoic acid is defined as that acid essentially free of antigens, pyrogens, tissue debris, water and other ordinary diluents, and essentially free of PGE_1 , and other substances which stimulate mammalian intestinal smooth muscle and which antagonize epinephrine-induced mobilization of free fatty acids in vitro and in experimental animals. See, for example, Horton, *ibid.*, for references to test procedures.

As mentioned above, PGE_1 is known to be formed by aerobic incubation of all-cis-8,11,14-eicosatrienoic acid or a salt thereof with a substantially aqueous medium containing the oxygenase and oxidase enzymes present in animal tissue which also contains endogenous PGE_1 . It is uncertain whether the 15 - hydroxy - 9 - oxoprost - 10,trans - 13 - dienoic acid is formed directly from all - cis - 8,11,14 - eicosatrienoic acid, whether that dienoic acid is formed by an enzymatic or non-enzymatic dehydration of PGE_1 either during the incubation or during one or more of the isolation steps, or whether it is formed by some other reaction path. However, the novel aspect of the novel process of this invention does not reside in how 15 - hydroxy - 9 - oxoprost-10,trans - 13 - dienoic acid is formed. Rather, the novelty is in the unexpected and surprising observation that this useful dienoic acid can be isolated in essentially pure form from the mixture obtained by the above-described aerobic incubation. Any of the various methods known in the art, as exemplified by the above-cited references, or their obvious equivalent, for enzymatic production and isolation of PGE_1 from all - cis - 8,11-14 - eicosatrienoic acid or a salt thereof, can be used to produce mixtures from which essentially pure 15 - hydroxy - 9 - oxoprost - 10,trans - 13, - dienoic acid can be separated.

Sources of the oxygenase and oxidase enzymes necessary to produce mixture for the novel process of this invention can be minced tissues, whole tissue homogenates, centrifuged homogenates, and residues obtained by high-speed centrifugation, all as described by the above-cited prior art. Alternatively, the enzymes can be isolated from tissues in various stages of purity, for example, as acetone powders, by methods known in the art. See, for example, Neilands et al., "Outlines of Enzyme Chemistry," 2d. Ed., Wiley & Sons, Inc., New York, pp. 42-65 (1958); Schwimmer et al. in Nord, "Advances in Enzymology," Vol. 14, Interscience Publishers, Inc.,

New York, pp. 375—409 (1953); and Umbreit et al., "Manometric Techniques," 4th Ed., Burgess Publishing Co., pp. 114—176 (1964). The isolated enzymes can then be used in a substantially aqueous medium in place of tissue homogenates, for example, as described by Wallach, *ibid.*, for example.

As described in the above-cited prior art, the interaction of the oxygenate and oxidase enzymes and the all - cis - 8,11,14 - eicosatrienoic acid or salt thereof is preferably carried out in an aqueous buffered medium, preferably at a slightly basic pH, i.e., pH above 7 and up to about 8.5. Since molecular oxygen is necessary for the desired transformation, the reaction should also be carried out under aerobic conditions, using any of the known expedients for supplying oxygen to the reaction mixture.

All - cis - 8,11,14 - eicosatrienoic acid is known in the art. See, for example, Korn, J. Biol. Chem. 238, 3584 (1963). The free acid or a suitable salt thereof, for example, the sodium, potassium, calcium, or ammonium salt can be used. The amount of reactant acid to be used will depend upon the amount of oxygenase and oxidase enzymes used. The proportions described by the above-cited prior art are suitable.

The preferred reaction temperature is about normal mammalian body temperature, i.e., in the range about 35° to about 40° C., although moderately higher or lower temperatures are operative. The reaction time will depend upon such factors as the source and/or purity of the enzymes, the proportions of the enzymes and acid reactant, the reaction temperature, and the availability of oxygen. Times in the range about 30 minutes to about 24 hours usually give satisfactory results. The course of the reaction can be followed by taking small aliquots of the reaction mixture and estimating the amounts of PGE₁ and 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid. The latter compound is less polar than PGE₁ and can be separated from it and from the reactant acid by thin layer chromatography, for example, on silica gel with chloroform-methanol-acetic acid, 90:5:5 by volume. The PGE₁ and 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid can readily be detected on the thin layer chromatogram by spraying the dried plate with a vanillin - phosphoric acid mixture, and heating the plate at 110°C. for 10 minutes. Dark spots appear at the sites of the compounds.

For the above testing purpose as well as for final isolation, the PGE₁ and 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid can be separated from the enzymes and aqueous incubation medium as described for the isolation of PGE₁ in the above-cited prior art. Since the incubation reaction mixture is preferably kept slightly basic by buffers, a substantial proportion of the PGE₁ and 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid will be present in the final reaction mixture as anions. Preferably, the reaction mixture is acidified as described in said prior art to convert those anionic forms substantially completely to the free carboxylic acid forms before isolation. Indeed, the desired 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid may actually be formed by dehydration of PGE₁ during the acidification.

Conventional methods can be used to separate the PGE₁ and the desired 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid, and to further purify the latter to the essentially pure form, as defined above. For example, preparative thin layer chromatography on silica gel with the above-described solvent system or as described for various prostaglandins by Gr  n et al., J. Lipid Research 5, 117 (1964), or by partition chromatography or reversed phase partition chromatography, for example, as described for various prostaglandins by Samuelsson, J. Biol. Chem. 238, 3229 (1963). In its chromatographic behavior, the novel free acid of this invention is similar although not identical to the known prostaglandins, especially PGE₁, PGE₂, and PGE₃, and techniques useful for isolating, purifying, and characterizing those are generally useful for this novel compound.

It is especially advantageous to separate PGE₁ and the novel 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid of this invention by fractional crystallization from a suitable solvent or mixture of solvents. PGE₁ is readily separated in crystalline form from a mixture of PGE₁ and the novel formula II acid of this invention. The latter substance tends to remain as an oil at room temperature, and hence tends to be more soluble in the crystallization solvents and thus accumulate in the recrystallization mother liquors. The oily 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid is thus obtained substantially free of PGE₁, and can thereafter be obtained in essentially pure form, as defined above. It is especially preferred to use a combination of fractional crystallization, chromatography, and solvent extractions to obtain essentially pure 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid, as described hereinafter.

The novel essentially pure 15 - hydroxy - 9 - oxoprostano - 10,trans - 13 - dienoic acid of this invention, i.e., the formula II compound wherein R₁ is hydrogen, can be transformed to essentially pure pharmacologically acceptable salts, i.e., compounds of

formula II wherein R_1 is a pharmacologically acceptable cation, essentially free of the corresponding salt of PGE_1 , by neutralization with appropriate amounts of the corresponding inorganic or organic base, examples of which correspond to the cations listed above. These transformations can be carried out by a variety of procedures known in the art to be generally useful for the preparation of inorganic, i.e., metal or ammonium, salts, amine acid addition salts, and quaternary ammonium salts. The choice of procedure will depend in part upon the solubility characteristics of the particular salt to be prepared. In the case of the inorganic salts, it is usually suitable to dissolve the prostanoic acid derivative in water containing the stoichiometric amount of a hydroxide, carbonate, or bicarbonate corresponding to the inorganic salt desired. For example, such use of sodium hydroxide, sodium carbonate, or sodium bicarbonate gives a solution of the sodium salt of the formula II prostanoic acid derivative. Evaporation of the water or addition of a water-miscible solvent of moderate polarity, for example, a lower alcohol or a lower alkanone gives the solid inorganic salt if that form is desired.

To produce an amine salt, the prostanoic acid derivative can be dissolved in a suitable solvent of either moderate or low polarity. Examples of the former are ethanol, acetone, and ethyl acetate. Examples of the latter are diethyl ether and benzene. At least a stoichiometric amount of the amine corresponding to the desired cation is then added to that solution. If the resulting salt does not precipitate, it can usually be obtained in solid form by addition of a miscible diluent of low polarity or by evaporation. If the amine is relatively volatile, any excess can easily be removed by evaporation. It is preferred to use stoichiometric amounts of the less volatile amines.

Salts wherein the cation is quaternary ammonium are produced by mixing the prostanoic acid derivative with the stoichiometric amount of the corresponding quaternary ammonium hydroxide in water solution, followed by evaporation of the water.

The invention can be more fully understood by the following examples.

EXAMPLE 1

Essentially Pure 15 - Hydroxy - 9 - oxoprostanoic - 10,trans - 13 - dienoic Acid
Frozen sheep (young buck) seminal vesicles (5 kg.) were ground in a meat grinder. The resulting tissue mass was then homogenized in 500-gram batches in a one-gallon stainless steel Waring blender for 3 minutes with a total of 5 liters of 0.1 N aqueous ammonium chloride buffer, pH 8.5.

A solution of all - cis - 8,11,14 - eicosatrienoic acid (5.0 g.) in 50 ml. of ethanol was added slowly to 5 l. of the same 0.1 N aqueous ammonium chloride buffer with vigorous stirring. The resulting emulsion was then mixed with the above total gland homogenate, and the mixture was brought to pH 8.0 by addition of 6 N ammonium hydroxide solution, and was maintained at 37.5° C. for one hour with constant stirring to ensure aeration.

At the end of the incubation period, the pH of the mixture was brought to 7.5 by addition of 6 N hydrochloric acid. Cold acetone (45 l.) was then added with stirring, and that mixture was allowed to stand one hour at about 0° C. Suction filtration gave a first filtrate and a filter cake. The latter was suspended in 4 l. of a mixture of acetone and water, 3:1 by volume, and filtered. The first and second filtrates were combined and extracted twice with 27.5 l.-portions of hexane.

The extracted acetone filtrates were then concentrated by evaporation at reduced pressure and below 40° C. to a 4100-ml. volume, and the concentrate was brought to pH 2.8 with hydrochloric acid. The resulting acid solution was extracted successively with two 2-l. portions and two 1-l. portions of dichloromethane. The combined dichloromethane extracts were evaporated under reduced pressure to 2.5 l., and the concentrate was extracted successively with one 2-l. portion and two 1-l. portions of 0.2 M. sodium phosphate buffer, pH 8.0.

The combined buffer extracts were washed with 400 ml. of dichloromethane, and were then brought to pH 2.9 by addition of concentrated hydrochloric acid, and extracted successively with one 1-l. portion and three 500-ml. portions of dichloromethane. The combined dichloromethane extracts were washed successively with 450-ml., 300-ml., and 500-ml. portions of water. The dichloromethane solution was then evaporated under reduced pressure to give 2.84 g. of an oil. That oil was dissolved in 10 ml. of dichloromethane. Crystalline PGE_1 gradually separated and was filtered. The filtrate was evaporated, and the oily residue was dissolved in about 3 ml. of dichloromethane. Hexane was then added until the solution was slightly cloudy. Additional PGE_1 separated and was filtered.

The above-described dichloromethane-hexane mother liquor was combined with another mother liquor obtained in the same manner, starting with 5 kg. of sheep semi-

nal vesicles and 5.0 g. of all - cis - 8,11,14 - eicosatrienoic acid. To those two mother liquors was also added an ethyl acetate-hexane mother liquor obtained as follows: the four crops of crystalline PGE₁, obtained as described above were combined and dissolved in 10 ml. of dichloromethane; the crystals which separated were filtered and the filtrate was evaporated to dryness; the residue was dissolved in ethyl acetate, and that solution was made cloudy with hexane; the crystals which separated were filtered, giving said ethyl acetate-hexane mother liquor.

Those three combined mother liquors were evaporated, and the residue was dissolved in benzene containing 1% ethyl acetate. That solution was added to a 27 x 350 mm. column of acid-washed silica gel. The column was eluted successively with 500 ml. of benzene containing 1% ethyl acetate, 2 l. of benzene containing 20% ethyl acetate, and 2 l. of benzene containing 40% ethyl acetate. The eluate from the 40% ethyl acetate in benzene elution was evaporated to dryness to give 0.26 g. of residue.

A portion of the above residue (0.22 g.) was dissolved in hexane and added to a 14 x 360 mm. column of acid-washed silica gel. The column was eluted successively with 500 ml. of hexane, 500 ml. of hexane containing 1% ethyl acetate, 500 ml. of hexane containing 5% ethyl acetate, 500 ml. of hexane containing 10% ethyl acetate, 6000 ml. of hexane containing 20% ethyl acetate, and 12,000 ml. of hexane containing 30% ethyl acetate. The column was then eluted with an additional 3000 ml. of hexane containing 30% ethyl acetate, after which the silica gel in the column was removed, slurried with hexane containing 60% ethyl acetate, and reformed into the same size column. The resulting column was eluted with 2000 ml. of hexane containing 60% ethyl acetate.

The final 3000 ml. of 30% ethyl acetate in hexane eluate and the 2000 ml. of 60% ethyl acetate in hexane eluate were combined, filtered, and evaporated under reduced pressure to give 0.067 g. of residue. That residue was leached four times with 5-ml. portion of heptane. The heptane-insoluble residue was dissolved in absolute ethanol, filtered, and evaporated to give a residue which was leached successively with 5-ml., 4-ml., and 3-ml. portions of carbon tetrachloride. The carbon tetrachloride solutions were combined and evaporated to give 0.031 g. of residue. That residue was dissolved in about 1 ml. of dichloromethane and spotted on a 20 x 20 cm. thin layer silica gel chromatography plate. The plate was developed in an ethyl acetate - methanol - water solvent system, 8:2:5. The developed plate was air-dried, and the middle section (5 to 9 cm. from spot origin) was scraped from the plate and eluted with dichloromethane. That eluate was evaporated to give 0.026 g. of residue.

The above 26 mg. of residue behaved as a single compound on silica gel thin layer chromatography, using ethyl acetate - methanol - water, 8:2:5 or CHCl₃ - methanol - acetic acid, 90:5:5. The residue was further purified by dissolving in 30 ml. of dichloromethane and extracting the solution with three successive 10-ml. portions of water. The combined yellow water extracts (pH 6.5) were acidified to pH 3.5 with dilute hydrochloric acid, and were then extracted three times with dichloromethane. The combined dichloromethane solutions were dried and evaporated to dryness to give a residue which was dissolved in 15 ml. of warm absolute ethanol. The solution was cooled at -20°C. for about 16 hours. A small amount of residue was separated by filtration and washed on the filter with five 3-ml. portions of absolute ethanol. The filtrate and filtered washings were combined, and evaporated and dried to give 6.6 mg. of essentially pure 15 - hydroxy - 9 - oxoprostanoic acid in the form of an oil. This substance exhibited about 0.01 of the smooth muscle stimulatory effect of PGE₁, and about 0.005 of the antagonism of epinephrine-induced mobilization of free fatty acids shown by PGE₁.

I.R. (principal bands; dichloromethane solution) 3400, 2640, 1700, 1580, 1180, 970 cm⁻¹.

U.V. (ethanol solution) maximum at 220 mμ.

After heating 2.5 ml of the ethanol solution (39 μg. of acid) with 2.5 ml. of 1 N NaOH in ethanol, the U.V. spectrum maximum was 280 mμ.

Thin Layer Chromatography

A single spot with R_F 0.7 using acetic acid-water, 9:1.

A single spot with R_F 0.75 using chloroform-methanol-acetic acid, 90:5:5.

N.M.R.

The spectrum shows two doublets centered at 6.17 and 7.52δ; complex multiplet centered at 5-6δ; two multiples centered at 4.18 and 3.25δ. Spectrum taken with a Varian A-60 spectrophotometer on deuteriochloroform solution with tetramethylsilane as interval standard.

EXAMPLE 2

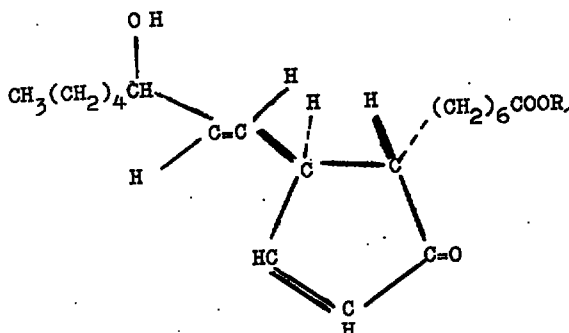
Sodium 15-hydroxy-9-oxoprost-10,trans-13-dienoate.

Essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid (2 mg.) was dissolved in 3 ml. of water-ethanol, 1:1. The solution was cooled to about 10°C. and was neutralized with an equivalent amount of 0.1 N aqueous sodium hydroxide solution. Evaporation to dryness gave essentially pure sodium 15-hydroxy-9-oxoprost-10,trans-13-dienoate.

Following the procedure of example 2, but using potassium hydroxide, calcium hydroxide, tetramethylammonium hydroxide, and benzyltrimethyl ammonium hydroxide, each in separate experiments in place of the sodium hydroxide, there are obtained the corresponding salts of essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid.

WHAT WE CLAIM IS:—

1. A compound of the formula:



wherein R_1 is hydrogen or a pharmacologically acceptable cation.

2. An essentially pure compound according to claim 1.
3. 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid.
4. Essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid.
5. A process for producing essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid which comprises separating said acid or a salt thereof in essentially pure form from the mixture obtained by aerobic incubation of all-cis-8,11,14-eicosatrienoic acid or a salt thereof with a substantially aqueous medium containing the oxygenase and oxidase enzymes present in animal tissue which also contains endogenous PGE_1 .
6. The process of claim 5 wherein a substantially aqueous solution of the prostanoic acid derivatives or salts present in said mixture is acidified and wherein said 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid in the resulting acid solution is subsequently separated from any other prostanoic acids in said acidified solution.
7. The process of claim 6 wherein said animal tissue is mammalian vesicular gland tissue.
8. The process of claim 7 wherein said animal is the sheep.
9. The process of claim 7 wherein said animal is the bull.
10. A process for producing essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid which comprises separating said acid or a salt thereof in essentially pure form from the mixture obtained by aerobic incubation of all-cis-8,11,14-eicosatrienoic acid or a salt thereof with a substantially aqueous medium containing comminuted animal tissue which also contains endogenous PGE_1 .
11. The process of claim 10 wherein a substantially aqueous solution of the prostanoic acid derivatives or salts present in said mixture is acidified and wherein said 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid in the resulting acid solution is subsequently separated from any other prostanoic acids in said acidified solution.
12. The process of claim 11 wherein the animal tissue is sheep vesicular gland tissue.
13. A process for the preparation of a compound as claimed in any of claims 1 to 4 substantially as herein described with reference to the Examples.
14. A compound as claimed in any of claims 1 to 4 when prepared by a process as claimed in claims 5 to 13.

EXAMPLE 2

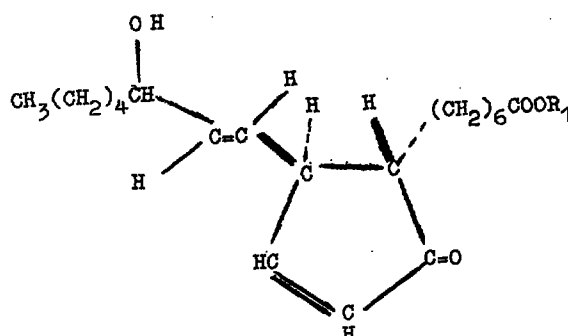
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Following the procedure of example 2, but using potassium hydroxide, calcium hydroxide, tetramethylammonium hydroxide, and benzyltrimethyl ammonium hydroxide, each in separate experiments in place of the sodium hydroxide, there are obtained the corresponding salts of essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid.

WHAT WE CLAIM IS:—

1. A compound of the formula:



wherein R_1 is hydrogen or a pharmacologically acceptable cation.

2. An essentially pure compound according to claim 1.
3. 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid.
4. Essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid.
5. A process for producing essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid which comprises separating said acid or a salt thereof in essentially pure form from the mixture obtained by aerobic incubation of all-cis-8,11,14-eicosatrienoic acid or a salt thereof with a substantially aqueous medium containing the oxygenase and oxidase enzymes present in animal tissue which also contains endogenous PGE_1 .
6. The process of claim 5 wherein a substantially aqueous solution of the prostanoic acid derivatives or salts present in said mixture is acidified and wherein said 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid in the resulting acid solution is subsequently separated from any other prostanoic acids in said acidified solution.
7. The process of claim 6 wherein said animal tissue is mammalian vesicular gland tissue.
8. The process of claim 7 wherein said animal is the sheep.
9. The process of claim 7 wherein said animal is the bull.
10. A process for producing essentially pure 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid which comprises separating said acid or a salt thereof in essentially pure form from the mixture obtained by aerobic incubation of all-cis-8,11,14-eicosatrienoic acid or a salt thereof with a substantially aqueous medium containing comminuted animal tissue which also contains endogenous PGE_1 .
11. The process of claim 10 wherein a substantially aqueous solution of the prostanoic acid derivatives or salts present in said mixture is acidified and wherein said 15-hydroxy-9-oxoprost-10,trans-13-dienoic acid in the resulting acid solution is subsequently separated from any other prostanoic acids in said acidified solution.
12. The process of claim 11 wherein the animal tissue is sheep vesicular gland tissue.
13. A process for the preparation of a compound as claimed in any of claims 1 to 4 substantially as herein described with reference to the Examples.
14. A compound as claimed in any of claims 1 to 4 when prepared by a process as claimed in claims 5 to 13.

15. A therapeutic composition comprising as the active ingredient a compound as claimed in any of claims 1 to 4 or 14 together with a pharmaceutically acceptable carrier.

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