

PATENT SPECIFICATION

NO DRAWINGS

1.191.412

Inventors: PETER FREDERICK LESWELL and DONALD ROBERT MAXWELL

Date of filing Complete Specification: 15 Jan., 1969.

Date of Application (No. 2619/68): 17 Jan., 1968.

Complete Specification Published: 13 May, 1970.

Index at acceptance:—C2 C(3A19A3, 3A19B2, 3A19C3, 3A19D1); A5 B(38Y, 386, 42Y, 421, 422, 426, 48Y, 482, 483, 59Y, 595, 767)

International Classification:—A 61 k 28/00; C 07 c 49/28

COMPLETE SPECIFICATION

Aerosols and Compositions containing Prostaglandin Compounds

We, MAY & BAKER LIMITED, a British Company of Dagenham, Essex, England, 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 the use of prostaglandin E₁ and its esters and non-toxic salts in aerosols as bronchodilators.

Bronchodilators, i.e. substances which cause relaxation of the smooth muscle of the bronchial tree, are extensively used to relieve, i.e. remove or reduce, the state of bronchospasm, thereby facilitating breathing, in patients suffering from conditions such as bronchial asthma, bronchitis, bronchiectasis and emphysema. The most widely-used bronchodilators, which may be administered individually or in combination and in various pharmaceutical-presentation forms, such as injectable solutions and suspensions, tablets for oral administration, aerosols for inhalation and suppositories, are (a) xanthine derivatives, (b) corticosteroids, and (c) sympathomimetic agents. These bronchodilator agents have, however, drawbacks which limit their effectiveness.

Thus:—

(a) theophylline and aminophylline, the most frequently used xanthine derivative bronchodilators, produce undesirable side-effects upon the cardiovascular system and, when administered orally, suffer from the disadvantage of producing gastric irritation. In addition, the production of adequate and predictable blood concentrations is rendered difficult by variability of absorption;

(b) the corticosteroids, such as prednisolone, betamethasone, dexamethasone and hydrocortisone are very effective in relieving conditions of asthma and acute asthmatic

spasm (*status asthmaticus*) which have failed to respond to treatment with other bronchodilators, but, as is well known, the long-term administration of corticosteroids involves the risk of the appearance of a considerable number of very serious adverse side-effects such as peptic ulceration, osteoporosis with vertebral collapse, mental breakdown, fluid retention, systemic hypertension and diabetes;

(c) the most frequently-used sympathomimetic agents, which are adrenalinic (epinephrine), ephedrine and isoprenaline (also known as isoproterenol) are very powerful and effective agents for relieving the symptoms of acute asthmatic spasm, but suffer from the disadvantage of producing severe adverse side-effects including stimulation of the cardiovascular and central nervous systems and hyperglycemia. These side-effects are particularly to be expected, when over-dosage occurs, which happens not infrequently when the asthma sufferer is seeking rapid relief from the crippling effects of an acute attack. In addition, tolerance to the beneficial effects of these substances sometimes develops. It will be appreciated that these agents are commonly self-administered by the patient and are not under direct medical supervision, thereby increasing the chances of accidental or deliberate over-dosage. Isoprenaline is a particularly widely-used sympathomimetic bronchodilator administered by inhalation through the mouth as an aerosol. It is well known, however, that over-dosage with isoprenaline-containing aerosols can result in serious cardiac disturbances, and instances of fatality attributed to this cause are known, for example as described in 'Pressurized Aerosols: Asthma', Brit.med. J.(1967), i 756; ii 375. In addition, toler-

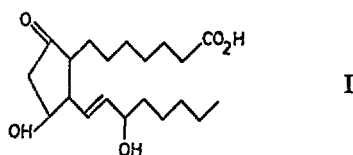
[Price 5s. 0d.]

ance to the beneficial bronchodilator effects of isoprenaline may develop. There is evidence to suggest that repeated administration of isoprenaline in man causes the accumulation in the lungs of 2-methoxyisoprenaline, a compound which itself blocks sympathetic β -receptors and thus inhibits further bronchodilator action by isoprenaline [Paterson, J.W. *et al* — Isoprenaline resistance and the use of pressurized aerosols — *Lancet*, ii, 426 (1968)].

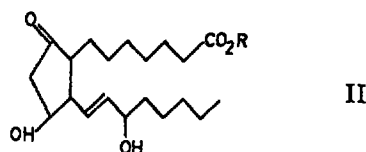
It will be readily appreciated from the limitations of currently-used bronchodilators described above, that it is a desirable objective to increase the range of available bronchodilators and more particularly to discover bronchodilators which are effective and well-tolerated by the body without serious adverse side-effects.

The prostaglandins are a family of naturally occurring unsaturated fatty acids which are known to have a wide range of pharmacological activities. More than 20 have been isolated to date from the tissues of warm-blooded animals including man.

Prostaglandin E_1 (hereinafter referred to as 'PGE₁') is a well-known member of this family, its isolation, identification, preparation by semi-synthetic methods and possession of a number of pharmacological properties being described in the literature. PGE₁ has the formula: —



As a result of research and experimentation, it has been found that the compounds of the general formula: —



wherein R represents a hydrogen atom (i.e. PGE₁) or a straight or branched alkyl group containing from 1 to 6 carbon atoms, and preferably from 1 to 4 carbon atoms, and, when R represents a hydrogen atom, non-toxic salts thereof, are highly potent as bronchodilators when administered orally by inhalation in the form of aerosols and may be used for the relief of bronchospasm. Accordingly, there is provided, as a feature of the present invention, aerosols comprising at least one prostaglandin compound of formula II or, when R represents a hydrogen atom, a non-toxic salt thereof, in association with a pharma-

ceutically-acceptable liquid medium. The compounds of formula II, and more especially PGE₁ and its non-toxic salts, are particularly valuable as bronchodilators by administration by oral inhalation in the form of aerosols, being more potent by this route of administration than isoprenaline, which is, as hereinbefore mentioned, widely-used as a bronchodilator in the form of aerosols, and devoid of the adverse side-effects associated with the use of isoprenaline-containing aerosols for bronchodilation. The higher bronchodilator potency of PGE₁ in comparison with isoprenaline is the more remarkable in that on intravenous administration PGE₁ is equipotent to, or less potent than, isoprenaline as a bronchodilator in the guinea-pig.

Examples of suitable values for R, when an alkyl group, include methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, *n*-pentyl, *n*-hexyl, 3-methylpentyl and 2,3-dimethylbutyl. The esters of general formula II, i.e. the compounds wherein R represents an alkyl group, may be prepared from PGE₁ by known methods for the esterification of carboxylic acids, for example by the treatment of a solution of PGE₁ in a suitable inert organic solvent such as diethyl ether, with the appropriate diazoalkane, e.g. diazomethane, diazoethane or 1-diazo-2-methylpentane. The ester may be isolated from the reaction mixture by distillation of the solvent and other components of the system which may be present, e.g. any unreacted diazoalkane. Thus, the methyl ester of PGE₁ can be prepared as follows: —

A solution of diazomethane in diethyl ether (1.5 ml. of solution containing 0.84 g. of diazomethane per litre) was added to a solution of PGE₁ (3 mg.) in diethyl ether (5 ml.). After standing for one hour at ambient temperature, the diethyl ether was removed under reduced pressure (20 mm.Hg) by warming gently on a steam bath, to give the methyl ester of PGE₁ in the form of a white solid.

By the term "known methods", as used in the specification, is meant methods heretofore used or described in the literature. By "non-toxic salts" is meant salts the cations of which are relatively innocuous to the animal organisms when used in therapeutic doses so that the beneficial pharmacological properties of the parent compound PGE₁ are not vitiated by side-effects ascribable to those cations. Preferably, the salts are water-soluble. Suitable salts include the alkali metal, e.g. sodium or potassium, and ammonium salts and pharmaceutically-acceptable, i.e. non-toxic, amine salts.

Amines suitable for forming such salts with carboxylic acids are well known and include, for example, amines derived in theory by the replacement of one or more of the hydrogen atoms of ammonia by groups, which may be the same or different when more than one

hydrogen atom is replaced, selected from alkyl groups containing from 1 to 6 carbon atoms, hydroxyalkyl groups containing from 1 to 3 carbon atoms, cycloalkyl groups containing from 3 to 6 carbon atoms, phenyl groups, phenylalkyl groups containing from 7 to 11 carbon atoms and phenylalkyl groups containing from 7 to 15 carbon atoms wherein the alkyl moieties are substituted by hydroxy groups. The alkyl moieties of such alkyl and phenylalkyl groups may be straight- or branched-chain and the phenyl group and phenyl moieties of phenylalkyl groups may be unsubstituted or substituted by one or two straight- or branched-chain alkyl groups containing from 1 to 6 carbon atoms. Suitable amines also include those derived in theory by the replacement of two of the hydrogen atoms of ammonia by a hydrocarbon chain, which may be interrupted by nitrogen, oxygen or sulphur atoms, to form, together with the nitrogen atom of ammonia to which its terminal groups are attached, a five- or six-membered heterocycle-containing heterocyclic ring, which heterocyclic ring may be unsubstituted or substituted by one of two straight- or branched-chain alkyl groups containing from 1 to 6 carbon atoms. Examples of suitable amine cations include mono-, di- and tri-methylammonium, mono-, di- and tri-ethylammonium, mono-, di- and tri-*n*-propylammonium, mono-, di- and tri-isopropylammonium, ethyldimethylammonium, mono-, di- and tri-2-hydroxyethylammonium, ethyl bis(2-hydroxyethyl)ammonium, *n*-butylmono-2-hydroxyethylammonium, tris(hydroxymethyl)methylammonium, cyclohexylammonium, benzylammonium, benzyl dimethylammonium, dibenzylammonium, phenyl-2-hydroxyethylammonium, piperidinium, morpholinium, pyrrolidinium, piperazinium, 1-methylpiperidinium, 4-ethylmorpholinium, 1-isopropylpyrrolidinium, 1,4-dimethylpiperazinium, 1-*n*-butylpiperidinium, 2-methylpiperidinium and 1-ethyl-2-methylpiperidinium.

The non-toxic salts may be prepared by reaction of stoichiometric quantities of PGE₁ and the appropriate base, e.g. an alkali metal hydroxide or carbonate, ammonium hydroxide, ammonia or an amine, in a suitable solvent which is preferably water in the case of the preparation of alkali metal salts and water or isopropanol in the case of amine salts. The salts may be isolated by lyophilisation of the solution or, if sufficiently insoluble in the reaction medium, by filtration, if necessary after removal of part of the solvent. If a pharmaceutically-acceptable solvent as hereinafter described is used in the preparation of the salt, the solution obtained may be used as such without isolation of the salt.

PGE₁ may be prepared by the incubation of di-homo- γ -linolenic acid with a lyophilised particulate fraction of sheep vesicular

gland in the presence of glutathione, as described by Nugteren, D.H., Beerthuis, R.K. and van Dorp, D.A., (1967) — 'Biosynthesis of prostaglandins' — *Prostaglandins — Nobel Symposium 2*, Ed. Bergström, S. and Samuelson, B., 45—50 — London: Interscience.

The compounds of general formula II and non-toxic salts thereof may, according to the present invention, be administered orally as bronchodilators by any method known *per se* for the administration by inhalation of drugs which are not themselves gaseous under normal conditions of administration. Thus, a solution of the active ingredient in a suitable pharmaceutically-acceptable solvent, for example water, can be nebulized by a mechanical nebulizer, for example a Wright Nebulizer, to give an aerosol of finely-divided liquid particles suitable for inhalation. Advantageously, the solution to be nebulized is dilute and preferably aqueous, solutions containing from 0.05 to 5.0 mg., and preferably 0.05 to 0.5 mg., of active ingredient per ml. of solution being particularly suitable. The solutions may contain stabilizing agents such as sodium bisulphite and buffering agents to give it an isotonic character, e.g. sodium chloride, sodium citrate and citric acid.

The active ingredients may also be administered orally by inhalation in the form of aerosols generated from self-propelling pharmaceutical compositions. Compositions suitable for this purpose may be obtained by dissolving or suspending in finely-divided form, preferably micronized to an average particle size of less than 5 microns, the active ingredients in pharmaceutically-acceptable solvents, e.g. ethanol, which are co-solvents assisting in dissolving the active ingredients in the volatile liquid propellants hereinafter described, or pharmaceutically-acceptable suspending or dispersing agents, for example aliphatic alcohols such as oleyl alcohol, and incorporating the solutions or suspensions obtained with pharmaceutically-acceptable volatile liquid propellants, in conventional pressurized packs which may be made of any suitable material, e.g. metal, plastics or glass, adequate to withstand the pressures generated by the volatile propellant in the pack. Pressurized pharmaceutically-acceptable gases, such as nitrogen, may also be used as propellants. The pressurized pack is preferably fitted with a metered valve which dispenses a controlled quantity of the self-propelling aerosol composition as a single dose.

Suitable volatile liquid propellants are known in the art and include fluorochlorinated alkanes containing from one to four, and preferably one or two, carbon atoms, for example dichlorodifluoromethane, dichlorotetrafluoroethane, trichloromonofluoromethane, dichloromonofluoromethane and monochlorotrifluoromethane. Preferably, the vapour pressure of the volatile liquid propellant is between about 25

and 65 pounds, and more especially between about 30 and 55 pounds, per square inch gauge at 70°F. As is well-known in the art, volatile liquid propellants of different vapour pressures may be mixed in varying proportions to give a propellant having a vapour pressure appropriate to the production of a satisfactory aerosol and suitable for the chosen container. For example dichlorodifluoromethane (vapour pressure 85 pounds per square inch gauge at 70°F) and dichlorotetrafluoroethane (vapour pressure 28 pounds per square inch gauge at 70°F) may be mixed in varying proportions to give propellants having vapour pressures intermediate between those of the two constituents, e.g. a mixture of dichlorodifluoromethane and dichlorotetrafluoroethane in the proportions 38.62 respectively by weight has a vapour pressure of 53 pounds per square inch gauge at 70°F.

The self-propelling pharmaceutical compositions may be prepared by dissolving the required quantity of active ingredient in the co-solvent or combining the required quantity of active ingredient with a measured quantity of suspending or dispersing agent. A measured quantity of this composition is then placed in an open container which is to be used as the pressurized pack. The container and its contents are then cooled to below the boiling temperature of the volatile propellant to be used. The required quantity of liquid propellant, cooled below its boiling temperature, is then added and the contents of the container mixed. The container is then sealed with the required valve fitting without allowing the temperature to rise above the boiling temperature of the propellant. The temperature of the sealed container is then allowed to rise to ambient with shaking to ensure complete homogeneity of the contents to give a pressurized pack suitable for generating aerosols for inhalation. Alternatively, the co-solvent solution of the active ingredient or combination of active ingredient and suspending or dispersing agent is placed in the open container, the container sealed with a valve, and the liquid propellant introduced under pressure.

Means for producing self-propelling compositions for generating aerosols for the administration of medicaments are, for example, described in detail in United States Patents No's 2,868,691 and 3,095,355.

Preferably, the self-propelling pharmaceutical compositions according to the present invention contain from 0.05 to 5.0 mg., and more particularly 0.05 to 0.5 mg., of active ingredient per ml. of solution or suspension. It is important that the pH of solution and suspensions used, according to the present invention, to generate aerosols should be kept within the range 3 to 8 and preferable that they should be stored at or below 4°C., to

avoid pharmacological deactivation of the active ingredient.

In carrying out the present invention, the means of producing an aerosol for inhalation should be selected in accordance with the physico-chemical properties of the active ingredient. For example, PGE₁ is sufficiently soluble in ethanol to give solutions which are readily miscible with fluorochlorinated alkane propellants to enable self-propelling compositions to be prepared readily. Alkali metal salts of PGE₁, although sufficiently soluble in water to give aqueous solutions suitable for mechanical nebulization to give aerosols, are, however, insufficiently soluble in the usual co-solvents used to assist solution in the usual volatile pharmaceutically-acceptable propellants to give suitable self-propelling compositions of this type but may be micronised, preferably to an average particle size of less than 5 microns, and suspended in the volatile propellants, if necessary utilizing a suspending or dispersing agent as hereinbefore mentioned, to form self-propelling compositions.

By the term "pharmaceutically-acceptable" as applied in this specification and claims to solvent, suspending or dispersing agents, propellants and gases is meant solvent, suspending or dispersing agents, propellants and gases which are non-toxic when used in aerosols suitable for inhalation therapy.

It is highly desirable that the aerosols should have a particle size less than about 10 microns and preferably less than 5 microns, for example between 0.5 and 3 microns, to ensure effective distribution to very narrow bronchioles. Preferably, administration is by means of devices enabling controlled quantities of the active ingredients to be administered, for example by means of the metered valves hereinbefore mentioned.

The dose of active ingredient to be used to relieve bronchospasm varies with the gravity of the symptoms exhibited by the patient and the individual characteristic response of the patient to the active ingredient. In general, however, doses of between 0.04 µg. and 0.8 µg. of active ingredient per kg. of body weight administered orally in aerosols give good symptomatic relief, more particularly with PGE₁. The treatment may be repeated as and when required by any return of the symptoms.

The following Examples illustrate the present invention.

EXAMPLE 1

An aerosol was generated from an aqueous solution of PGE₁ containing 0.1 mg. of active ingredient per ml. of solution by means of a nebulizer operating at a pressure of 20 pounds per square inch. The aerosol thus produced was suitable for use as a bronchodilator by inhalation.

EXAMPLE 2

Proceeding as in Example 1, but replacing the aqueous solution of PGE₁ by an aqueous ethanolic solution of the sodium salt of PGE₁ containing 0.1 mg. of PGE₁ per ml. of solution obtained by dissolving the PGE₁ in the minimum quantity of ethanol, adding the stoichiometric quantity of sodium carbonate and diluting with water, a similar aerosol suitable for use as a bronchodilator by inhalation was obtained.

EXAMPLE 3

A solution of PGE₁ (4.5 mg.) in absolute ethanol (0.45 ml.) was passed in a polyvinylchloride — coated glass bottle (20 ml. volume), the bottle cooled to a low temperature (−60°C.) and dichlorodifluoromethane (3.7 ml.; 4.9 g.) and dichlorotetrafluoroethane (4.85 ml.; 7.3 g.), cooled to the liquid state, added to give a self-propelling composition (9 ml.) containing 0.5 mg. of PGE₁/ml. of solution.

The bottle was sealed with a metered valve (with dip tube) delivering 55 μ l. doses and the contents rendered homogeneous by shaking, while allowing the temperature of the bottle and its contents to return to ambient. Each "puff" (generated from 55 μ l. of solution) of aerosol released from the pressurized pack thus obtained contained 27.5 μ g. of PGE₁.

EXAMPLE 4

Proceeding as in Example 3 but replacing the solution of PGE₁ (4.5 mg.) in absolute ethanol (0.45 ml.) by a solution ten times more dilute, i.e. PGE₁ (0.45 mg.) in absolute ethanol (0.45 ml.), a pressurized pack, containing 0.05 mg. of PGE₁/ml. of solution, was obtained, from which each "puff" (generated from 55 μ l. of solution) of aerosol released contained 2.75 μ g. of PGE₁.

EXAMPLE 5

Proceeding as in Example 3 but replacing the solution of PGE₁ (4.5 mg.) in absolute ethanol (0.45 ml.) by a solution one hundred times more dilute, i.e. PGE₁ (0.045 mg.) in absolute ethanol (0.45 ml.), a pressurized pack, containing 0.005 mg. of PGE₁/ml. of solution, was obtained, from which each "puff" (generated from 55 μ l. of solution) of aerosol released contained 0.275 μ g. of PGE₁.

EXAMPLE 6

PGE₁ (9 mg.) was added to a 0.4% w/v solution of triethanolamine in absolute ethanol (0.9 ml.) and a portion of the resulting solution of the triethanolamine salt of PGE₁ (0.25 ml.) was placed in a polyvinyl chloride-coated glass bottle (20 ml. volume). The bottle and contents were then cooled to −60°C. and dichlorodifluoromethane (1.93 ml.; 2.56 g.) and dichlorotetrafluoroethane (2.82 ml.; 4.16 g.), each at −60°C., were added to give a self-propelling composition (5 ml.) containing 0.5 mg. of PGE₁ (in terms of the free acid) per ml. of solution.

The bottle was sealed with a metered valve (with dip tube) delivering 55 μ l. doses and the

contents rendered homogeneous by shaking, while allowing the temperature of the bottle and its contents to return to ambient. Each "puff" (generated from 55 μ l. of solution) of aerosol released from the pressurized pack thus obtained contained 27.5 μ g. of PGE₁ (in terms of the free acid).

EXAMPLE 7

Self-propelling pressurized packs similar to those described in Examples 3 to 6 were prepared by the procedures described in the aforesaid examples but replacing the steps of cooling and introducing the dichlorodifluoromethane and dichlorotetrafluoroethane cooled to the liquid state and sealing the bottle with the metered valve by the steps of sealing the bottle with the metered valve and introducing these volatile liquid propellants under pressure through the valve.

The valuable bronchodilator activity of the compounds of general formula II and, when R represents a hydrogen atom, their non-toxic salts, is demonstrated by the following experiments. Throughout the experiments, isoprenaline was used in the form of its sulphate, but doses are quoted in terms of the free base. Unless otherwise indicated, PGE₁ was used in the form of its sodium salt, but doses are quoted in terms of the free acid.

Tests were carried out on the guinea-pig, which is generally considered to provide a reliable model for the human subject with good clinical correlation in the study of bronchodilators [Herxheimer, H. — The bronchoconstrictor action of propanolol aerosol in the guinea-pig — *J. Physiol., London*, 190 41—42P (1967)].

a) Albino guinea pigs of either sex, weighing 250—500 g., were anaesthetized with urethane (1.5—2.0 g./kg. animal body weight intraperitoneally), the depth of anaesthesia being designed to be sufficient to suppress voluntary respiration. The trachea of the animal was then cannulated and connected to a recording device operating upon the principles of the method of Konzett, H. and Rössler, R. *Arch. exp. Path. Pharmacol.*, 195, 71—74 (1940), whereby alterations in bronchial tone are reflected by changes in the volume of air entering lungs inflated at a constant pressure. The lungs were then artificially inflated to a constant pressure, using a respiration pump delivering a constant stroke volume. With each stroke of the respiration pump, that air which did not enter the lungs at the set pressure was diverted and its volume recorded using a piston recorder. Variations in the tone of the bronchiolar smooth muscle result in corresponding changes in the resistance of the lung to inflation, which are reflected by alterations in the volume of air recorded.

Aerosols were generated from aqueous solutions containing known concentrations of PGE₁

70

75

80

85

90

95

100

105

110

115

120

125

130

or from aqueous solutions containing known concentrations of isoprenaline, the latter compound being used as a standard. The dose administered was varied by altering the concentration of the solution from which the aerosol was generated. In each individual animal, standard severe, but sub-maximal, bronchoconstrictor responses were established by the intravenous administration of a bronchoconstrictor agonist, i.e. histamine, bradykinin, 5-hydroxytryptamine or acetylcholine. A bronchoconstrictor response to the same dose of agonist was then obtained by the administration of the agonist 15—20 seconds after completing the intratracheal administration of an aerosol of the substance under test by inhalation. Each aerosol was administered for 20 inflations of the lungs. The same volume of aerosol was administered in each instance. The standard bronchoconstrictor response to the agonist was then re-established and the procedure repeated with the same aerosol, with an aerosol generated from a solution containing a different concentration of the active ingredient from

that used to generate the first-tested aerosol, or with an aerosol containing the other compound under study. In each instance, aerosols generated from solutions of PGE₁ and aerosols generated from solutions of isoprenaline were administered to the same animal in order to obtain a direct comparison of the degree of antagonism of agonist-induced bronchoconstriction produced by PGE₁ or isoprenaline. Changes in resistance to inflation of the lungs were taken to be indicative of changes in bronchial tone and to provide a measure of bronchodilation, the results being expressed as a percentage reduction of the standard bronchoconstrictor response.

Bronchial tone was measured by recording the overflow from the lungs inflated at constant pressure. Bronchodilation was determined from the inhibition of agonist-induced bronchoconstriction.

The results obtained in a series of experiments of the type described in (a) above are set out in the following Table I (PGE₁ was used as free acid):—

TABLE I

Comparison of the bronchodilator potencies of PGE₁ and isoprenaline aerosols in the anaesthetised guinea-pig
(volume changes at constant pressure)

Experiment	Active Ingredient in Aerosol	Concentration of Active Ingredient in Aerosol— Generating Solution (μg./ml.)	Broncho- constrictor agonist (dose)	% reduction in Broncho- constriction	Approximate Bronchodilator Potency of PGE ₁ (Isoprenaline=1)
1	ISOPRENALINE	100	Histamine (0.75 μg.)	36	>10
	PGE ₁	10		50	
	PGE ₁	100		82	
2	ISOPRENALINE	100	Histamine (0.5 μg.)	25	>100
	ISOPRENALINE	1000		68	
	PGE ₁	10		78	
3	ISOPRENALINE	100	Histamine (1.0 μg.)	0	>20
	ISOPRENALINE	500		25	
	ISOPRENALINE	1000		52	
	PGE ₁	5		22	
	PGE ₁	10		38	
	PGE ₁	50		64	
4	PGE ₁	100	Bradykinin (0.5 μg.)	80	<100
	ISOPRENALINE	1000		50	
	PGE ₁	10		40	

TABLE I (Continued)

Experiment	Active Ingredient in Aerosol	Concentration of Active Ingredient in Aerosol— Generating Solution ($\mu\text{g./ml.}$)	Broncho- constrictor agonist (dose)	% reduction in Broncho- constriction	Approximate Bronchodilator Potency of PGE_1 (Isoprenaline=1)
5	ISOPRENALINE	1000		72	
	ISOPRENALINE	100		17	
	ISOPRENALINE	100	Histamine (0.7 $\mu\text{g.}$)	43	<10
	PGE_1	100		65	
6	ISOPRENALINE	1000		26	
	ISOPRENALINE	1000		25	
	ISOPRENALINE	1000		22	
	ISOPRENALINE	1000		30	>10
	ISOPRENALINE	1000	Histamine (0.5 $\mu\text{g.}$)	32	
	PGE_1	100		57	
7	ISOPRENALINE	100		55	
	PGE_1	10	Bradykinin (0.8 $\mu\text{g.}$)	75	>10
8	ISOPRENALINE	1000		25	
	ISOPRENALINE	1000		20	
	PGE_1	10	5-Hydroxy- tryptamine (1.5 $\mu\text{g.}$)	61	>100
	PGE_1	10		46	
	PGE_1	10		35	

TABLE I (Continued)

Experiment	Active Ingredient in Aerosol	Concentration of Active Ingredient in Aersol—Generating Solution ($\mu\text{g./ml.}$)	Bronchoconstrictor agonist (dose)	% reduction in Bronchoconstriction	Approximate Bronchodilator Potency of PGE_1 (Isoprenaline=1)
9	ISOPRENALINE	100	5-Hydroxy-tryptamine (1.0 $\mu\text{g.}$)	3	
	ISOPRENALINE	1000		59	
	PGE_1	10		61	100
	PGE_1	10		65	

b) Tests on urethane-anaesthetized guinea-pigs similar to those described in (a) above were carried out except that bronchodilator activity was measured by a recording device operating upon the principle of the method of Dixon, W.E. and Brodie, T.G., J. Physiol. (London), 29, 97—173 (1903) whereby in lungs inflated at constant volume, variations in bronchial tone produce corresponding changes in resistance to inflation of the lungs which are in turn reflected by alterations in intratracheal pressure. The lungs were artificially inflated at

constant volume using a respiration pump and variations in intratracheal pressure were recorded via a pressure transducer and pen recorder. Bronchial tone was measured by recording the intratracheal pressure changes during inflation of the lungs at constant volume. Bronchodilation was determined from reduction in the bronchoconstrictor responses to agonist.

The results obtained in a series of experiments described in (b) above are set out in the following Table II:—

15

20

25

5

10

TABLE II

Comparison of the bronchodilator potencies of PGE₁ and isoprenaline aerosols in the anaesthetised guinea-pig (pressure changes at constant volume.)

Experiment	Active Ingredient in Aerosol	Concentration of active ingredient in Aerosol—generating Solution (μg./ml.)	Broncho-constrictor agonist (dose)	% reduction in broncho-constriction	Approximate Broncho-dilator potency of PGE ₁ (Iso-prenaline = 1)
1	ISOPRENALINE PGE ₁	1000	Histamine (0.5 μg.)	74	>10
		100		84	
2	ISOPRENALINE PGE ₁	1000	Histamine (0.6 μg.)	56	10
		100		55	
3	ISOPRENALINE PGE ₁	1000	Histamine (1.0 μg.)	58	>10
		100		76	

c) Tests on urethane-anaesthetized guinea-pigs were carried out utilizing the experimental procedure described in (a) recording bronchodilation according to the principles of the method of Konzett and Rössler, except that the compounds under study for bronchodilator activity were administered

to the animals by rapid intravenous injection of an aqueous solution (0.05—0.2 ml.) instead of by inhalation of an aerosol.

The results obtained in a series of experiments described in (c) above are set out in the following Table III (PGE₁ was used as free acid): —

10

15

TABLE III

Comparison of the bronchodilator potencies of intravenous PGE₁ and isoprenaline in the anaesthetised guinea-pig (volume change at constant pressure).

Experiment	Compound under Test	Intra-venous Dose (μg.)	Broncho-constrictor agonist (dose)	% reduction of broncho-constriction	Approximate Broncho-dilator potency of PGE ₁ (Isoprenaline=1)
1	ISOPRENALINE	0.05	Histamine (0.5 μg.)	46	<0.5
	ISOPRENALINE	0.1		60	
	ISOPRENALINE	0.2		86	
	PGE ₁	0.05		20	
	PGE ₁	0.1		49	
	PGE ₁	0.2		54	
2	ISOPRENALINE	0.05	Acetylcholine (5 μg.)	37	0.4
	ISOPRENALINE	0.1		73	
	PGE ₁	0.1		21	
	PGE ₁	0.2		62	
	PGE ₁	0.3		76	
	ISOPRENALINE	0.125		30	
3	ISOPRENALINE	0.25	Acetylcholine (7.5 μg.)	43	0.5
	ISOPRENALINE	0.5		76	
	PGE ₁	0.25		34	
	PGE ₁	0.5		39	
	PGE ₁	0.75		44	
	ISOPRENALINE	0.125		30	

- d) A test on a urethane-anaesthetized guinea-pig was carried out utilizing the experimental procedure described in (b) above recording bronchial tone according to the principles of Dixon and Brodie, except that the compounds under study for
5
10
15
20
25
- bronchodilator activity were administered to the animals by rapid intravenous injection of an aqueous solution (0.05—0.2 ml.) instead of by inhalation of an aerosol. The results obtained are set out in the following Table IV:—

TABLE IV

Comparison of the bronchodilator potencies of intravenous PGE₁ and isoprenaline in the anaesthetised guinea-pig (pressure change at constant volume)

Compound under Test	Intravenous Dose (μg.)	Broncho-constrictor agonist (dose)	% reduction of bronchoconstriction	Approximate Bronchodilator Potency of PGE ₁ (Isoprenaline=1)
ISOPRENALINE	0.1	Histamine (1.0 μg.)	94	0.2
PGE ₁	0.5		97	

- The variations in bronchodilator potency of PGE₁ and isoprenaline found in the results shown in Tables I, II and III are usual in tests of this type for bronchodilator activity and probably result from the varying sensitivities of individual guinea-pigs to particular bronchodilator and bronchoconstrictor agents. These results, and those given in Table IV, show, however, that, in the anaesthetized guinea-pig, PGE₁ has at least 10 to 20 times the bronchodilator potency of isoprenaline when administered as an aerosol by inhalation but is less potent by intravenous administration.
e) In order to compare the bronchodilator activities of aerosols of PGE₁ free acid, PGE₁ sodium salt, and PGE₁ methyl ester (i.e. the compound of formula II in which R represents a methyl group), tests on urethane anaesthetized guinea-pigs similar to those described in (b) above were carried out, except that instead of administering each aerosol for 20 inflations of the lungs, the number of inflations was varied. The results are set out in the following Table V:—

TABLE V
Comparison of the bronchodilator potencies of PGE₁ free acid, PGE₁ sodium salt and PGE₁ methyl ester aerosols in the anaesthetized guinea-pig (pressure changes at constant volume).

Experiment	Active Ingredient in Aerosol	Concentration of active ingredient in Aerosol-Generating Solution (μg./ml.)	Number of inflations	Broncho-constrictor agonist (dose)	% reduction in broncho-constriction
1	PGE ₁ (Na salt)	50	25	Histamine (0.75 μg. i.v.)	72
	PGE ₁ (methyl ester)	100	25	Histamine (0.75 μg. i.v.)	60
2	PGE ₁ (methyl ester)	100	20	Histamine (0.5 μg. i.v.)	86
	PGE ₁ (free acid)	100	20	Histamine (0.5 μg. i.v.)	78
	PGE ₁ (free acid)	100	10	Histamine (1 μg. i.v.)	55
	PGE ₁ (methyl ester)	100	10	Histamine (1 μg. i.v.)	53

These results show that the bronchodilator activities of PGE₁ free acid, sodium salt, and methyl ester do not differ markedly when these substances are administered by aerosol to the anaesthetized guinea-pig.

In the experiments described above, the aerosols of PGE₁ and its sodium salt were generated from solutions similar to those hereinbefore described in Examples 1 and 2 with appropriate variations in the concentration of active ingredient. The aerosols of isoprenaline were generated from an aqueous solution of the sulphate of the indicated concentration in terms of the base. The aerosol of the methyl ester of PGE₁ was generated from a 100 μg./ml. solution obtained by dissolving the methyl ester of PGE₁ (3mg.) in ethanol (3 ml.) and diluting with a 1:1 by volume mixture of polyethylene glycol and water to a total volume of 30 ml.

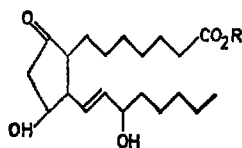
15

20

It is well-known that substantial species variations are to be expected in the study of bronchodilators although, as previously mentioned, the guinea-pig is generally considered to provide a reliable model for the human subject. In order to confirm that PGE₁, when administered orally by inhalation in an aerosol, does not produce bronchoconstriction in the healthy human subject, aerosols generated from pressurized packs of the type hereinbefore described in Example 7 were administered to healthy human subjects. The subjects received up to 2 "puffs" (each "puff" generated from 55 μ l. of solution) generated from a self-propelling composition containing 0.5 mg. of PGE₁/ml. of solution, i.e. a dose of 55 μ g. of PGE₁, and no bronchoconstriction or other significant adverse side-effect could be detected. It should be noted that, since the bronchial smooth muscle is normally fully relaxed in healthy subjects, it was not to be expected that bronchodilatation would be observed.

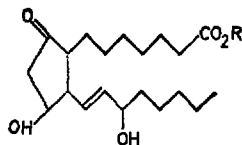
WHAT WE CLAIM IS:—

1. Aerosols suitable for inhalation by human subjects suffering from bronchospasm comprising, as a bronchodilator, at least one prostaglandin compound of the formula:



- wherein R represents a hydrogen atom or a straight or branched chain alkyl group containing from 1 to 6 carbon atoms, or, when R represents hydrogen, a non-toxic salt thereof in association with a pharmaceutically-acceptable liquid medium.

2. Compositions in liquid form capable of producing aerosols for administration to human subjects suffering from bronchospasm comprising a solution or suspension of at least one prostaglandin compound of the formula:



- wherein R represents a hydrogen atom or a straight or branched chain alkyl group containing from 1 to 6 carbon atoms, or, when R represents hydrogen, a non-toxic salt thereof, in a pharmaceutically-acceptable liquid medium and in association with a volatile liquid propellant.

3. Compositions according to claim 2 in which the pharmaceutically-acceptable liquid medium is water, an aliphatic alcohol or an aqueous aliphatic alcohol.

4. Compositions according to claim 3 in which the aliphatic alcohol is ethanol.

5. Compositions according to claim 2, 3 or 4 in which the volatile liquid propellant is a fluorinated alkane of from 1 to 4 carbon atoms.

6. Compositions according to claim 5 in which the volatile liquid propellant is at least one of dichlorodifluoromethane, dichlorotetrafluoroethane, trichloromonofluoromethane, dichloromonofluoromethane and monochlorotrifluoromethane.

7. Compositions according to any one of claims 2 to 6 in which the pH of the liquid compositions is within the range 3 to 8.

8. Compositions according to any one of claims 2 to 7 in which the prostaglandin compound content of the liquid solution or suspension is from 0.05 to 5 mg. per millilitre of liquid medium.

9. Compositions according to claim 8 in which the prostaglandin compound content of the liquid solution or suspension is from 0.05 to 0.5 mg. per millilitre of liquid medium.

10. Aerosols and compositions according to any one of claims 1 to 9 in which the prostaglandin in the aerosol or composition is the compound of the formula specified in claim 1 or 2 wherein R represents a hydrogen atom, or a non-toxic salt of said compound.

11. Aerosols and compositions according to claim 10 in which the non-toxic salt of the prostaglandin compound is an alkali metal, ammonium or amine salt.

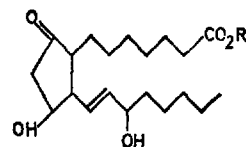
12. Aerosols and compositions according to claim 10 in which the prostaglandin in the aerosol or composition is the sodium or triethanolamine salt of the compound of the formula specified in claim 1 or 2 wherein R represents a hydrogen atom.

13. Aerosols and compositions according to any one of claims 1 to 9 in which the prostaglandin in the aerosol or composition is the compound of the formula specified in claim 1 or 2 wherein R represents the methyl group.

14. Compositions according to claim 2 substantially as hereinbefore described.

15. Compositions according to claim 2 substantially as described in any one of Examples 1 to 6.

16. Pressurized packs provided with a valve permitting emission of an aerosol containing a solution or dispersion of at least one prostaglandin compound of the formula:

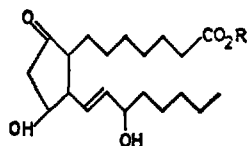


- wherein R represents a hydrogen atom or a straight or branched alkyl group containing from 1 to 6 carbon atoms, or, when R represents hydrogen, a non-toxic salt thereof, in a pharmaceutically-acceptable liquid medium under a gaseous pressure capable of converting

It is well-known that substantial species variations are to be expected in the study of bronchodilators although, as previously mentioned, the guinea-pig is generally considered to provide a reliable model for the human subject. In order to confirm that PGE₁, when administered orally by inhalation in an aerosol, does not produce bronchoconstriction in the healthy human subject, aerosols generated from pressurized packs of the type hereinbefore described in Example 7 were administered to healthy human subjects. The subjects received up to 2 "puffs" (each "puff" generated from 55 μ l. of solution) generated from a self-propelling composition containing 0.5 mg. of PGE₁/ml. of solution, i.e. a dose of 55 μ g. of PGE₁, and no bronchoconstriction or other significant adverse side-effect could be detected. It should be noted that, since the bronchial smooth muscle is normally fully relaxed in healthy subjects, it was not to be expected that bronchodilatation would be observed.

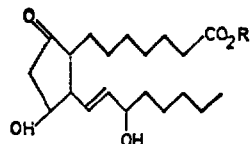
WHAT WE CLAIM IS:—

1. Aerosols suitable for inhalation by human subjects suffering from bronchospasm comprising, as a bronchodilator, at least one prostaglandin compound of the formula:



wherein R represents a hydrogen atom or a straight or branched chain alkyl group containing from 1 to 6 carbon atoms, or, when R represents hydrogen, a non-toxic salt thereof in association with a pharmaceutically-acceptable liquid medium.

2. Compositions in liquid form capable of producing aerosols for administration to human subjects suffering from bronchospasm comprising a solution or suspension of at least one prostaglandin compound of the formula:



wherein R represents a hydrogen atom or a straight or branched chain alkyl group containing from 1 to 6 carbon atoms, or, when R represents hydrogen, a non-toxic salt thereof, in a pharmaceutically-acceptable liquid medium and in association with a volatile liquid propellant.

3. Compositions according to claim 2 in which the pharmaceutically-acceptable liquid medium is water, an aliphatic alcohol or an aqueous aliphatic alcohol.

4. Compositions according to claim 3 in which the aliphatic alcohol is ethanol.

5. Compositions according to claim 2, 3 or 4 in which the volatile liquid propellant is a fluorinated alkane of from 1 to 4 carbon atoms.

6. Compositions according to claim 5 in which the volatile liquid propellant is at least one of dichlorodifluoromethane, dichlorotetrafluoroethane, trichloromonofluoromethane, dichloromonofluoromethane and monochlorotrifluoromethane.

7. Compositions according to any one of claims 2 to 6 in which the pH of the liquid compositions is within the range 3 to 8.

8. Compositions according to any one of claims 2 to 7 in which the prostaglandin compound content of the liquid solution or suspension is from 0.05 to 5 mg. per millilitre of liquid medium.

9. Compositions according to claim 8 in which the prostaglandin compound content of the liquid solution or suspension is from 0.05 to 0.5 mg. per millilitre of liquid medium.

10. Aerosols and compositions according to any one of claims 1 to 9 in which the prostaglandin in the aerosol or composition is the compound of the formula specified in claim 1 or 2 wherein R represents a hydrogen atom, or a non-toxic salt of said compound.

11. Aerosols and compositions according to claim 10 in which the non-toxic salt of the prostaglandin compound is an alkali metal, ammonium or amine salt.

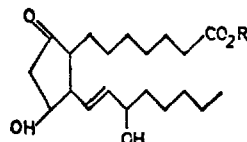
12. Aerosols and compositions according to claim 10 in which the prostaglandin in the aerosol or composition is the sodium or triethanolamine salt of the compound of the formula specified in claim 1 or 2 wherein R represents a hydrogen atom.

13. Aerosols and compositions according to any one of claims 1 to 9 in which the prostaglandin in the aerosol or composition is the compound of the formula specified in claim 1 or 2 wherein R represents the methyl group.

14. Compositions according to claim 2 substantially as hereinbefore described.

15. Compositions according to claim 2 substantially as described in any one of Examples 1 to 6.

16. Pressurized packs provided with a valve permitting emission of an aerosol containing a solution or dispersion of at least one prostaglandin compound of the formula:



wherein R represents a hydrogen atom or a straight or branched alkyl group containing from 1 to 6 carbon atoms, or, when R represents hydrogen, a non-toxic salt thereof, in a pharmaceutically-acceptable liquid medium under a gaseous pressure capable of converting



the prostaglandin solution or dispersion in the liquid form as claimed in any one of claims
container into an aerosol on release through the 2 to 15.
valve.

17. Pressurized packs according to claim 16
5 which contain a prostaglandin composition in

J. A. KEMP & CO.,
Chartered Patent Agents,
14, South Square,
Gray's Inn, London, W.C.1.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1970.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from
which copies may be obtained.

