

PATENT SPECIFICATION

771809



Date of Application and filing Complete Specification: May 11, 1954.

No. 13786/54.

Application made in Switzerland on Nov. 24, 1953.

Application made in Switzerland on Dec. 7, 1953.

Complete Specification Published: April 3, 1957.

Index at acceptance:—Class 2(3), C2B6(A2: F: G1B), C3A14A(3A: 5: 8C).

International Classification:—C07c, d.

COMPLETE SPECIFICATION

The Para-Amino Salicylic Acid Derivative of 2-Pyridyl-(4)-1:3:4-Oxadiazolone-(5) and processes of preparing same

We, ED. GEISTLICH SOHNE AG. FÜR CHEMISCHE INDUSTRIE, a joint stock company incorporated under the Laws of the Confederation of Switzerland, of Wolhusen, Switzerland, 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 is concerned with a new anti-tuberculosic compound effective in the treatment of human and animal tuberculosis.

In recent years a great deal of research has been carried out to discover chemotherapeutic agents having inhibiting action against the micro-organisms responsible for tuberculosis in humans and animals, i.e. M. tuberculosis. This research has revealed a number of compounds (called for convenience "tuberculostatics") which have found varying degrees of favour, some of the more important being streptomycin, *p*-amino salicylic acid and isonicotinic acid hydrazide and derivatives thereof.

Various drawbacks have however been found to be attendant upon the use of the tuberculostatics hitherto proposed for the treatment of tuberculosis. Thus, for example, streptomycin and *p*-amino salicylic acid often give rise to undesirable side effects; most of such tuberculostatics furthermore, including iso-nicotinic acid hydrazide, suffer, in varying degrees, from the serious defect that the tubercle bacillus develops a resistance against the tuberculostatic. This development of resistance may take place quite quickly after the commencement of therapy and may often produce a condition which is more difficult to treat than the original condition. Attempts have been made to overcome this last mentioned defect by the use of two or more different tuberculostatic compounds either together or consecutively, but with this procedure the treatment requires to be carefully controlled and frequent observations of the patient must be made.

[Price 3s. 6d.]

It may be said therefore that an ideal tuberculostatic should have, inter alia, the following characteristics:—

1. It should have a low toxicity and should give rise to substantially no side effects such as nausea, vomiting, dizziness, etc.

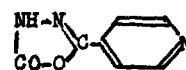
2. It should have high inhibiting action *in vivo* against the tubercle bacillus.

3. It should not easily induce the bacillus to form strains resistant to it, and should be active against strains of tubercle bacillus already resistant to known tuberculostatics.

4. It should be stable and capable of ready administration, preferably *per os*.

We have now found a new tuberculostatic compound which more nearly approaches the requirements of an ideal tuberculostatic than compounds hitherto used for this purpose.

Our new tuberculostatic compound is itself most conveniently regarded as a derivative of a parent compound of empirical formula $C_7H_5N_3O_2$; this parent substance which itself is a new compound though it does not form the subject of this application may be obtained by the reaction of isonicotinic acid hydrazide with phosgene, and is believed to be 2-pyridyl-(4)-1:3:4-oxadiazolone-(5), having the structural formula



This parent compound (which for convenience we shall hereinafter refer to as "the diazolone") is amphoteric in nature and thus forms salts or salt-like compounds with both acids and bases. It has a melting point of 274—6° C. (dec.). This melting point was determined for the purest material we have so far been able to obtain, and is of course liable to variation with purity.

As stated above, the diazolone is capable of forming salts or salt-like compounds with acids and bases. In particular, acid addition salts may be formed from, for example, hydro-

chloric, sulphuric, tartaric and citric acids. We use the term "salt or salt-like compounds" to designate the compounds which are obtained by salt-forming procedures applied to the diazalone; such compounds may not in fact be true salts but rather complex or addition compounds the nature of which is not entirely clear.

The present invention relates to one particular salt or salt-like derivative of the diazalone, which we have found to be particularly advantageous in the treatment of tuberculosis, namely the compound which may be obtained by reacting an acid addition salt of the above mentioned parent substance with *p*-amino salicylic acid (or by metathetically reacting an acid addition salt of the parent substance with an alkali metal, ammonium or alkali earth metal salt of *p*-amino salicylic acid). This derivative will, for convenience, be referred to as the *p*-amino salicylic acid (PAS) derivative; the said derivative does not appear to be a true salt of the diazalone, being coloured (whereas the diazalone and PAS themselves are colourless) and having a relatively low melting point viz. 165° C. (dec.).

According to the invention, therefore, there is provided, as a new compound, the para-amino salicylic acid derivative of 2-pyridyl-(4)-1:3:4-oxadiazalone-(5).

We shall now describe the physiological and like properties of our new compound.

Upon *in vitro* tests on *M. tuberculosis* our new compound in general shows rather less inhibitory action than isonicotinic acid hydrazide itself. Further *in vivo* tests carried out on man and upon animals show that the inhibitory effect of our new compound is slightly less than that of isonicotinic acid hydrazide, as judged by weight gain experiments, by pathological test on organs from animals treated by the new compound and by X-ray examination of treated humans.

However the toxicity of the new PAS-derivative of the diazalone is substantially less than that of isonicotinic acid hydrazide both on

oral administration and upon subcutaneous injection. Thus the LD₅₀ (subcutaneous) is approximately of the order of one tenth of that of isonicotinic acid hydrazide when determined in the mouse, and one third of that of isonicotinic acid hydrazide on oral administration in rabbits.

The new PAS-derivative of the diazalone shows no substantial side-effects on administration to humans such as are noticed with comparable dosages of, for example, streptomycin and *p*-amino salicylic acid.

The most important property of our new compound is however the fact that it has been shown to have a very much smaller tendency to give rise to resistant strains of *M. tuberculosis* than substantially all of the tuberculostatics hitherto in common use. Arising from this property in particular, we have obtained results in clinical trials upon the use of our new compound by itself which show striking improvement as against known tuberculostatics when the latter are used either singly, in admixture or consecutively.

The following is a description by way of example only of trials which we have carried out to demonstrate the last mentioned advantage of our new compound. The results of these trials will of course also illustrate the other advantages of the said new compound.

In vitro tests were first carried out upon *M. tuberculosis* (H.37 RV) to ascertain whether our new compound showed any tendency to produce resistant strains. Thus, the PAS-derivative was tested, by standard repeated subculturing techniques used for this purpose (see for example Barnet et al. Lancet, 1953, I, 314, and Barry et al. Lancet, 1953, I, 978), in comparison with isonicotinic acid hydrazide. Two separate experiments were conducted with each tuberculostatic (tests A and B) and the amount of each agent required to inhibit growth of each sub-culture was determined. The results are shown in the following table (I):—

TABLE I		Concn. of agent (μg/ml.) required to inhibit growth of organism											
		Orig. cult.	Sub-cultures										
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
Isonicotinic acid hydrazide	A	0.001	0.001	0.01	0.1	0.01	0.01	0.01	0.1	1.0	10.0	50.0	
	B	0.001	0.001	0.001	0.01	0.01	0.1	1.0	50.0				
PAS derivative of diazalone	A	0.1	0.1	1.0	1.0	0.1	0.1	0.1	1.0	1.0	1.0	1.0	1.0
	B	0.1	0.1	0.1	1.0	0.1	1.0	1.0	1.0				

These figures indicate that although *in vitro* the concentration of the new compound required to inhibit growth of this particular

strain of organism is with the initial culture as much as ten times greater than that of INH, with it —unlike INH—subsequent cultures

show almost no tendency to develop resistance, so much so that by the time the eleventh sub-culture is reached, while substantially the same amount of the PAS-derivative will inhibit growth as is required to inhibit growth of the original culture, with isonicotinic acid hydrazide it is seen that the amount of this substance required to inhibit growth after only ten sub-cultures is some 50,000 times that required to inhibit the original culture.

Tests were next carried out upon the *in vitro* action of the PAS-derivative of the diazalone against two strains of *M. tuberculosis* known to have a strong resistance against isonicotinic acid hydrazide. In these tests the action of the PAS-derivative is compared with the action of isonicotinic acid hydrazide (INH) at various dilutions. The results are shown in the following table (II):—

TABLE II Dilution of tuberculostatic compound	Strain "München"			Strain "Kornahl"	
	INH	PAS 'salt' of diazalone		INH	PAS 'salt'
		Test 1	Test 2		
1/ 10,000	+++	—	—	++	—
1/ 20,000	+++	—	—	++	—
1/ 40,000	+++	—	—	+++	—
1/ 80,000	+++	+	—	+++	—
1/ 160,000	+++	++	++	+++	—
1/ 320,000	+++	++	+++	+++	—
1/ 640,000	+++	+++	+++	+++	—
1/ 1,280,000	+++	+++	+++	+++	+
1/ 2,560,000	+++	+++	+++	+++	+++
1/ 5,120,000	+++	+++	+++	+++	+++
1/ 10,240,000	+++	+++	+++	+++	+++
1/ 20,480,000	+++	+++	+++	+++	+++
(Control) ∞		+++		+++	
In the above table:— +++ means strong growth ++ means moderate growth + means weak growth — means no growth					

These results show that the PAS-derivative of the diazalone is effective against these resistant strains at small dilutions at which isonicotinic acid hydrazide shows no effect.

These *in vitro* experiments have been confirmed by animal experiments and clinical trials in several countries. In these clinical trials it has been demonstrated that our new compound is highly effective *inter alia* against very resistant strains of *M. tuberculosis*. A high proportion of patients treated with the new compound have shown improvement, in

some cases markedly so, as a result of the treatment. This improvement has moreover occurred in cases which have failed to respond to all hitherto known treatments including treatment with isonicotinic acid hydrazide, p-amino salicylic acid, streptomycin and Terramycin (Registered Trade Mark). Thus for example, a group of 70 patients, including three moribund patients, who had already failed entirely to respond to high dosages of streptomycin, isonicotinic acid hydrazide, p-amino salicylic acid and Terramycin (Regis-

tered Trade Mark) showed in 70% of the cases marked improvement within 12 weeks of the commencement of treatment with the PAS-derivative of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5); the three moribund patients were able to leave their beds after a fortnight from commencement of treatment.

The dosage rate of the new compound is conveniently of the order of from 800—1,200 mg per day for a 70 Kg man. The compound is conveniently administered orally, for example in tablets or capsules, or by inhalation or insufflation of aqueous solutions.

The parent substance of the new tuberculo-static compound according to the invention may be prepared in any convenient manner, for example by the reaction of phosgene with isonicotinic acid hydrazide to form the diazolone. The diazolone may then be transformed as desired into the new salt or salt-like PAS-derivative.

According to a feature of the invention there is provided a process for the preparation of the *p*-amino salicylic acid derivative of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5) in which an acid addition salt of the diazolone is reacted with *p*-amino salicylic acid or an alkali metal, ammonium, or alkaline earth metal salt thereof.

It is preferred that an alkali metal salt, e.g. the sodium salt, of PAS and the hydrochloride of the oxadiazolone should be metathetically reacted in aqueous solution.

The preparation of the parent substance, i.e. the preparation of the diazolone used as starting material in the above process, is preferably effected by reacting phosgene with isonicotinic acid hydrazide (INH) or an acid addition salt thereof.

This reaction preferably takes place in a suitable solvent for the INH or its salts, according to which is being used, though it can be effected without a solvent under suitable conditions of temperature and pressure. In the case of INH salts, the reaction is conveniently carried out in an aqueous medium, whilst in the case of INH itself a non-aqueous inert organic solvent such as dioxan may be used, or dilute mineral acid is suitable. In the latter case water itself is also suitable as the reaction medium, since the introduction of phosgene into the aqueous medium will in fact give rise to the presence of hydrochloric acid. It is also possible to use an aqueous organic solvent.

The treatment with phosgene may be performed at any convenient temperature, for example between 0° C. and the boiling temperature of the mixture, preferably between room temperature and 50° C.

The equivalent amount or an excess of phosgene is introduced into the reaction mixture and the precipitated salt thus formed may then be isolated by filtration. It may be transformed in any convenient manner into the free compound e.g. by dissolving in aqueous alkali

solutions and precipitation of the compound by acidification to a pH of about 7.

In order that the invention may be well understood the following examples are now given by way of illustration only:—

It should be noted that the examples of Part A refer to the preparation of the PAS derivative claimed in this specification while Part B refers only to the preparation of starting materials.

PART A.

EXAMPLE 1.

Preparation of para-aminosalicylic acid "salt" of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5).

4.4 gms. of para-amino-salicylic acid are dissolved in 7.5 ml water and mixed with a solution of 4.1 gms. 2-pyridyl-(4)-1,3,4-oxadiazolone-(5), obtained according to Preparation 1 or 2 below, in 12.5 ml 2N hydrochloric acid at a temperature within the range of 20—40° C. The new orange-coloured compound precipitates immediately. Yield 6.2 gms., melting point 165° C. (with decomposition).

EXAMPLE 2.

Preparation of para-aminosalicylic acid "salt" of 2-pyridyl-(4)-1:3:4 oxadiazolone-(5).

2 gms. 2-pyridyl-(4)-1:3:4 oxadiazolone hydrochloride obtained according to Preparation 3 below, are dissolved in 25 mls of water at 60—70° C. and 2.1 gms. sodium para-aminosalicylate dissolved in 4 mls of water are added. The solution is cooled to room temperature immediately and the precipitated orange-yellow compound filtered and dried at room temperature *in vacuo*.

PART B.

PREPARATION 1.

Preparation of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5).

5 gms. isonicotinic acid hydrazide are dissolved in 300 ml of hot anhydrous dioxan. 80 gms. of gaseous phosgene are introduced at a temperature of 40° C. within 35 minutes. The compound precipitates in the form of its hydrochloride and is filtered with suction. Yield 8.0 gms.

14 gms. of this salt are dissolved in 100 ml aqueous caustic soda lye, treated with charcoal, filtered and then acidified with hydrochloric acid to pH 7. The precipitated substance is filtered with suction and dried. Yield 9 gms. It may be recrystallised from water or ethanol. Melting point 257° C. (with decomposition).

Analysis:

3.664 mg substance give 6.895 mg CO₂ and 0.960 mg H₂O
calculated for C₇H₅N₃O₂: C, 51.5%; H, 3.06%; N, 25.75%
found: C, 51.35%; H, 2.95%; N, 25.36%.

PREPARATION 2.

Preparation of 2-pyridyl-(4) 1:3:4-oxadiazolone-(5).

300 gms. isonicotinic acid hydrazide are dissolved in 600 mls water and 170 mls con-

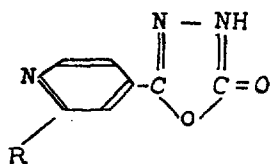
centrated hydrochloric acid and the solution cooled to room temperature. The solution is then placed in a 2000 ml flask equipped with an inlet tube of large diameter. In the course of 3 hours 1200 gms. of phosgene are fed into this solution while cooling and a precipitate is formed. The contents of the flask are then transferred into a 4000 ml beaker, and the pH brought to 6 with 25% ammonium hydroxide while cooling. After cooling for one hour at 5° C. the precipitate is filtered by suction, washed with water and dried. Yield: 340 gms. melting point 240° C. (with decomposition).

The product is purified by dissolving it in diluted ammonia and adding diluted nitric acid up to pH 6 when the product precipitates. It is also possible to recrystallise the product from water, alcohol or other suitable solvents. Melting point 265° C. (with decomposition).

PREPARATION 3.

Preparation of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5) using water as solvent.

138 gms. isonicotinic acid hydrazide are dissolved in 1 l. water at room temperature, and phosgene is led in slowly with good stirring. The temperature rises and a white precipitate of the oxydiazolone is formed, which dissolves as the hydrochloride in the course of the reaction. The reaction is complete after an approximately equal weight (140 gms.) of phosgene has been absorbed. On cooling the solution to 0° C., the hydrochloride crystallises out in a satisfactory manner. The free base may be obtained by neutralising the solution of the hydrochloride with 50% potassium carbonate solution until it is slightly alkaline. The oxydiazolone is precipitated as a fine white precipitate and is filtered and washed free of potassium salts with water. It may be crystallised from pyridine or alcohol, m.p. 274—276° (decomp.). In Patent No. 729,891, there are described and claimed new dihydro-oxadiazol derivatives of the general formula:



in which R is hydrogen or an alkyl radical.

What we claim is:—

1. As a new compound, the para-amino salicylic acid derivative of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5).

2. A process for the production of the new compound claimed in claim 1, which comprises reacting an acid addition salt of 2-pyridyl-(4)-

1,3,4-oxadiazolone-(5) with para-amino salicylic acid or an alkali metal, ammonium, or alkaline earth metal salt thereof.

3. A process as claimed in claim 2, in which the acid addition salt of the diazolone is its hydrochloride.

4. A process as claimed in either claim 2 or claim 3, in which the salt of para-amino salicylic acid is an alkali metal salt thereof.

5. A process as claimed in any of claims 2 to 4, in which the hydrochloride of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5) is reacted with sodium *p*-amino salicylate in an aqueous solution.

6. A process for the production of the new compound claimed in claim 1, substantially as herein described.

7. A process for the production of the new compound claimed in claim 1, substantially as herein described with reference to either of Examples 1 and 2.

8. A process as claimed in any of claims 2—7, wherein the 2-pyridyl-(4)-1,3,4-oxadiazolone-(5) or acid addition salt thereof used as starting material is prepared by reacting isonicotinic acid hydrazide or an acid addition salt thereof with phosgene to yield the hydrochloride of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5), followed, when desired, by neutralisation of said hydrochloride with an alkaline substance to give the free base.

9. A process as claimed in claim 8, wherein the reaction between isonicotinic acid hydrazide or an acid salt thereof and phosgene is effected without pressure in the presence of a solvent at a temperature less than the boiling temperature of the mixture.

10. A process as claimed in claim 9, in which isonicotinic acid hydrazide is reacted with phosgene in an inert organic solvent.

11. A process as claimed in claim 10, in which said solvent is dioxan.

12. A process as claimed in claim 9, in which isonicotinic acid hydrazide or an acid addition salt thereof is reacted with phosgene in water or an aqueous organic solvent.

13. A process as claimed in any of claims 8 to 12, in which the starting material is prepared substantially as herein described.

14. A process as claimed in any of claims 8 to 12, in which the starting material is prepared substantially as herein described with reference to any of Preparations 1 to 3.

For the Applicants,
FRANK B. DEHN & CO.,
Chartered Patent Agents,
Kingsway House, 103, Kingsway,
London, W.C.2.

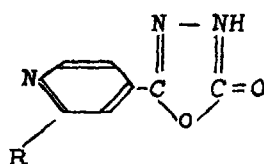
5 concentrated hydrochloric acid and the solution cooled to room temperature. The solution is then placed in a 2000 ml flask equipped with an inlet tube of large diameter. In the course of 3 hours 1200 gms. of phosgene are fed into this solution while cooling and a precipitate is formed. The contents of the flask are then transferred into a 4000 ml beaker, and the pH brought to 6 with 25% ammonium hydroxide while cooling. After cooling for one hour at 5° C. the precipitate is filtered by suction, washed with water and dried. Yield: 340 gms. melting point 240° C. (with decomposition).

10 The product is purified by dissolving it in diluted ammonia and adding diluted nitric acid up to pH 6 when the product precipitates. It is also possible to recrystallise the product from water, alcohol or other suitable solvents. Melting point 265° C. (with decomposition).

20 PREPARATION 3.

Preparation of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5) using water as solvent.

138 gms. isonicotinic acid hydrazide are dissolved in 1 l. water at room temperature, and phosgene is led in slowly with good stirring. The temperature rises and a white precipitate of the oxydiazolone is formed, which dissolves as the hydrochloride in the course of the reaction. The reaction is complete after an approximately equal weight (140 gms.) of phosgene has been absorbed. On cooling the solution to 0° C., the hydrochloride crystallises out in a satisfactory manner. The free base may be obtained by neutralising the solution of the hydrochloride with 50% potassium carbonate solution until it is slightly alkaline. The oxydiazolone is precipitated as a fine white precipitate and is filtered and washed free of potassium salts with water. It may be crystallised from pyridine or alcohol, m.p. 274—276° (decomp.). In Patent No. 729,891, there are described and claimed new dihydro-oxadiazol derivatives of the general formula:



45 in which R is hydrogen or an alkyl radical.

What we claim is:—

1. As a new compound, the para-amino salicylic acid derivative of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5).

50 2. A process for the production of the new compound claimed in claim 1, which comprises reacting an acid addition salt of 2-pyridyl-(4)-

1,3,4-oxadiazolone-(5) with para-amino salicylic acid or an alkali metal, ammonium, or alkaline earth metal salt thereof.

3. A process as claimed in claim 2, in which the acid addition salt of the diazolone is its hydrochloride.

4. A process as claimed in either claim 2 or claim 3, in which the salt of para-amino salicylic acid is an alkali metal salt thereof.

5. A process as claimed in any of claims 2 to 4, in which the hydrochloride of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5) is reacted with sodium *p*-amino salicylate in an aqueous solution.

6. A process for the production of the new compound claimed in claim 1, substantially as herein described.

7. A process for the production of the new compound claimed in claim 1, substantially as herein described with reference to either of Examples 1 and 2.

8. A process as claimed in any of claims 2—7, wherein the 2-pyridyl-(4)-1,3,4-oxadiazolone-(5) or acid addition salt thereof used as starting material is prepared by reacting isonicotinic acid hydrazide or an acid addition salt thereof with phosgene to yield the hydrochloride of 2-pyridyl-(4)-1,3,4-oxadiazolone-(5), followed, when desired, by neutralisation of said hydrochloride with an alkaline substance to give the free base.

9. A process as claimed in claim 8, wherein the reaction between isonicotinic acid hydrazide or an acid salt thereof and phosgene is effected without pressure in the presence of a solvent at a temperature less than the boiling temperature of the mixture.

10. A process as claimed in claim 9, in which isonicotinic acid hydrazide is reacted with phosgene in an inert organic solvent.

11. A process as claimed in claim 10, in which said solvent is dioxan.

12. A process as claimed in claim 9, in which isonicotinic acid hydrazide or an acid addition salt thereof is reacted with phosgene in water or an aqueous organic solvent.

13. A process as claimed in any of claims 8 to 12, in which the starting material is prepared substantially as herein described.

14. A process as claimed in any of claims 8 to 12, in which the starting material is prepared substantially as herein described with reference to any of Preparations 1 to 3.

For the Applicants,
FRANK B. DEHN & CO.,
Chartered Patent Agents,
Kingsway House, 103, Kingsway,
London, W.C.2.

