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PATENT SPECIFICATION

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(54) ORGANIC ACID COMPOUND AND METHOD OF MAKING AND USING THE SAME FOR PHARMACEUTICAL PURPOSES

(71) We, PHILIP NICHOLAS SAWYER, of 7600 Ridge Boulevard, Brooklyn, State of New York, United States of America, and LEON DAVID FREEMAN, of 101 CasaBuena Drive, Corte Madera, State of California, United States of America, both citizens of the 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 compounds of organic acids and to methods of making and using the same.

U.S. Patent 3,722,504 describes a screen for testing pharmaceutical compounds based at least partly on their ability to increase the negative surface charge of the vascular system. U.S. Patent 3,722,504, by way of example, discloses a pharmaceutical compound which prevents thrombosis by modifying the intimal surface charge of the vascular system.

Para amino benzoic acid (PABA) increases the net negative surface charge of blood vessels and blood cells. It produces a marked increase in the current-induced occlusion time in rat mesentery vessels (see U.S. Pat. 3,722,504). It produces no measurable effect on blood coagulation studies. It has a limited effect on blood vessel wall pores as shown by electro-osmotic studies.

Para amino benzoic acid, further, has good antithrombotic characteristics but tends to have relatively little anticoagulant activity as shown by its lack of effect on the coagulation studies including partial thromboplastin time, thrombin time and recalcification time. It is therefore a very useful antithrombotic drug.

Para amino benzoic acid has various very significant properties as a drug:

1. It is inexpensive;
2. It is known to be nontoxic in humans;
3. It can be used for long periods of time without significant incidence of pathological manifestations;
4. The material can be taken orally in large dosage without significant toxicity.

In man, dosages have been administered, for example, as high as 12 to 24 grams a day without significant side effects. It has furthermore been used for a large number of diseases in man including the therapy of Ricketsial diseases before the development of antibiotics, for tuberculosis in very large dosage and in the treatment of certain protozoan diseases and other infestation. It has long been thought to be a mild anti-inflammatory agent.

In humans with arteriosclerotic peripheral vascular disease, para amino benzoic acid has been shown to increase blood flow in ischemic limbs as measured by Barcroft plethysmographic studies and doppler blood flow measurements. Studies which were carried out in approximately fifty patients resulted in an approximate doubling of blood flows in patients in which para amino benzoic acid salt was effective. U.S. Patent 3,722,504 has been issued on the effect of para amino benzoic acid on blood flow in man.

The long term effects of vitamin C on scurvy in man is a primary event of historical interest in medicine. The effects of vitamin C to prevent scurvy are

extraordinarily well documented throughout the world. Man, deprived of vitamin C for periods longer than approximately sixty days, starts to display evidence of capillary fragility and bleeding into all tissues and organs.

Vitamin C has the following characteristics:

1. It is a co-enzyme in the Kreb's cycle.
2. It is a reducing agent and electron donor in all known biological systems.

L-ascorbic acid or vitamin C has a variety of biological functions some of which are not completely understood (Ziten *et al* 1964). Large doses of L-ascorbic acid appear to have value in prevention and symptom reduction in the common cold and other viral diseases (Linus Pauling 1974). Recent experimental evidence indicates that L-ascorbic acid is effective in reducing serum chloresterol levels. C. Spittle (*The Lancet*, July 28, 1973; pp. 199—201 and December 11, 1971; pp. 1280—1281) suggested that dosages of 1 to 2 grams of vitamin C per day can be beneficial in the prevention of deep vein thrombosis as well as in reducing the incidence of atherosclerotic complications in man. Recent research indicates that ascorbic acid may reduce the incidence of myocardial infarction (Knox, E. G., *The Lancet*, pp. 1465, June 30, 1973). L-ascorbic acid, further, appears to offset the thrombogenic effects of oral contraceptives as demonstrated by prolongation of occlusion times in the mesenteric vessels.

Good results were obtained in a pilot study using seven volunteer subjects to determine the effects of ascorbic acid on (1) plasma coagulation characteristics (2) serum cholesterol levels (3) platelet aggregability and (4) surface charge characteristics of red cells and platelets. Vitamin C in these subjects was shown to decrease platelet aggregability and increase the electrophoretic mobility of all the tested cells.

There has been little evidence to indicate that vitamin C effects measured blood coagulability as demonstrated by partial thromboplastin time, thrombin times and thrombin recalcification times.

Spittle *et al* have tested vitamin C in a randomized group of patients with thrombophlebitis. The protective effect of vitamin C against thrombosis has been shown by a randomized double-blind trial using patients who were shown to be prone to deep venous thrombosis. In a total of fifty-three patients, it was observed that the incidence of deep venous thrombosis was 33% in patients dosed with L-ascorbic acid compared to 60% in the placebo groups (Spittle, C. R., *The Lancet* pp. 199, July 28, 1973). The dose given was 1 gram per day. This correlation between the intake of vitamin C and reduction in the number of thrombotic episodes has been confirmed by other sources. In burn patients, where it is customary to use large doses of vitamin C to speed healing, there has been an apparent demand for treatment of deep-vein thrombosis. This information is based on the experience of one hospital over a five and one-half year period during which time 159 patients over forty years of age were treated with large doses of vitamin C (Spittle, C. R., *The Action of Vitamin C on Blood Vessels, Amer. Heart J.* 88:387, 1974).

By practice of the present invention, there may be provided one or more of the following advantages:

(i) a synergistic combination of para amino benzoic acid and L-ascorbic acid.

(ii) an improved pharmaceutical compound having improved utility for extended periods following administration.

(iii) methods for the treatment of non-human hosts and the prevention of vascular conditions.

(iv) the promotion of the development of useful compounds based on organic acids.

(v) new methods for the development of pharmaceutical compounds.

According to the present invention there is provided a compound derived from L-ascorbic acid and para amino benzoic acid.

According to the present invention there is also provided a method of reducing thrombotic tendencies in a non-human host comprising administering to the host a compound derived from L-ascorbic acid and para amino benzoic acid.

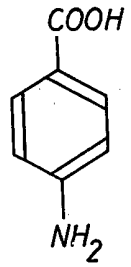
The compound which is derived may be an ester, salt or amide and this compound may be administered in a dosage of 1—100 mg/kg of body weight. The dosage is preferably administered orally, although it can be administered parenterally.

The compound may be formed by mixing solutions of L-ascorbic acid and para amino benzoic acid and evaporating the resulting mixture and recovering the

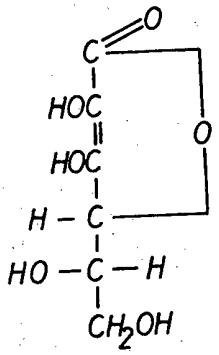
resulting solid. The acids are preferably used in a ratio of 1:1 on a molar basis. The solid may be recovered in crystal form or may be recovered as a cake.

The present invention further includes, as one aspect thereof, compounds prepared according to the above method or specifically salts, esters or amides of L-ascorbic acid and para amino benzoic acid.

Hereinabove, reference had been made to para amino benzoic acid. The formula for this organic acid is as follows:

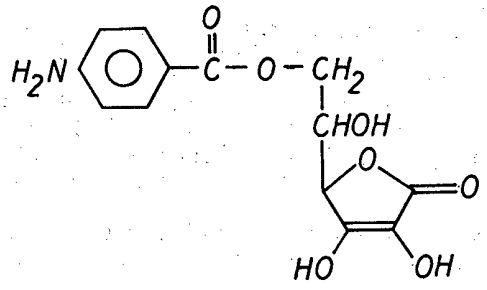


Reference has also been made to L-ascorbic acid or vitamin C, the formula for which is:

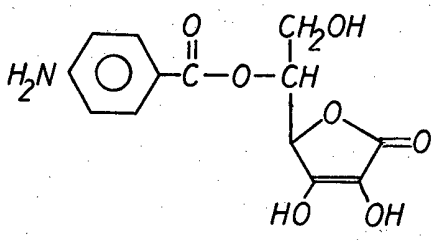


The present invention relates to compounds derived from these organic acids, namely, salts, esters and amides thereof, the preparation of such compounds and the applicability thereof to the treatment of the vascular system, notably in rats and dogs requiring such treatment.

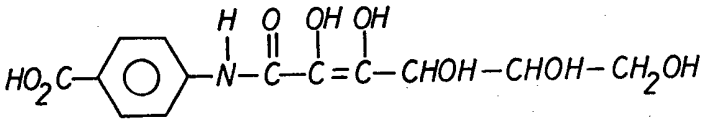
The formula for the 6 ester is as follows:—



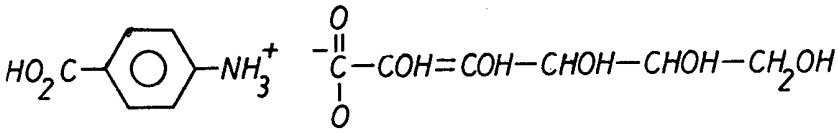
The formula for the 5 ester is as follows:—



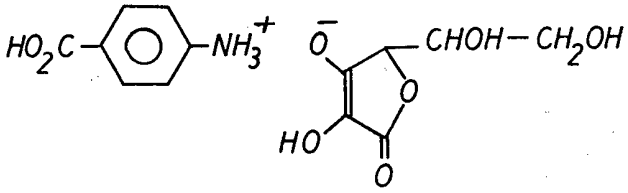
The formula for the amide is as follows:



The formula for a salt is as follows:



The formula for another salt is:



The original approach was to prepare a salt of ascorbic acid with para amino benzoic acid. Such a salt can be shown in two forms—one using the open form of ascorbic acid, the other using the enol form. In addition to the salts, it is possible to have two other kinds of derivatives—the amide and esters. Since there are two hydroxyl groups on ascorbic acid which could be readily esterified, the ester would probably be on either the 6 or 5 position. It is less likely, but also possible, to form an ester with one of the enolic hydroxyls (2 or 3 position) so that four esters are theoretically possible.

One manufacturing procedure used is described below. Because of the ease of oxidation of ascorbic acid, it is essential to perform all operations under nitrogen and free of water and air. In one case, substantial darkening of the product resulted. It was believed this was due to the presence of moisture.

EXAMPLE I

1. A solution of para amino benzoic acid in a mixture of 50% acetone and 50% methanol was prepared with gentle heating. The concentration was approximately 100 grams per liter.

2. A similar solution was prepared with L-ascorbic acid.

3. The two solutions were mixed. Water was excluded to the extent possible.

4. The mixture was evaporated under vacuum, with nitrogen being utilized to flush the system. As the material concentrated, crystals began to appear.

5. The mixture was evaporated until it was a thick slurry. It was then filtered through a coarse filter paper and the solid collected was allowed to drain as dry as possible. The surface was blanketed with nitrogen, with great care to exclude all filtered water.

6. The resultant solid was dried under high vacuum at room temperature. The ratio of L-ascorbic acid to para amino benzoic acid used was one to one on a molar basis.

EXAMPLE II

1. An alternative method of preparation used was to evaporate the solvent with careful exclusion of water and air to the point where a solid semi-dry cake was obtained in the container in which the evaporation was conducted.

2. This moist cake was then transfered to an appropriate container and dried under high vacuum. All operations are protected against exposure to air and moisture.

Other solvent systems will undoubtedly work. The above were used largely as a matter of convenience. An odor develops in the process which is removed with high vacuum drying, but must represent a by-product which is formed during the process. It must also be volatile, since it can be removed.

The first salt shown above, theoretically, only requires the removal of a molecule of water to form the amide. In general, this does not happen too readily. The distillation of the solvents may help pull off water.

It is also possible to form the esters shown above. This would probably require using the acyl chloride of para amino benzoic acid and reacting this with L-ascorbic acid.

Initial studies were directed toward determining the toxicity of the above indicated compound in mammalia. A very high dosage per kilogram of body weight in rats and dogs has been shown essentially non-toxic.

The new compound was next tested to determine its effect on rat-mesentery occlusion studies. Half lives of para amino benzoic acid have been shown to approximate one day. Half lives of vitamin C approximate 12 hours to a maximum of 1 day. The half lives of the new compound have been shown to approximate 4 to 5 days with a tail. A single dose lasts approximately 14 days. Mechanical mixing of para amino benzoic acid and L-ascorbic acid have been shown to have a maximum tail of approximately 5 to 6 days indicating by direct logic that the new compound is biologically different from the two components mixed together mechanically. Specifically, the compound derived appears to be more potent than the starting materials mixed together in a 50/50 ratio. If the agents are mixed together in the same ratio as used when making the new compound, the mechanical mixture is still not as potent as the compound derived.

The new compound has been evaluated in rat-mesentery studies. In the rat-mesentery, the new compound has been shown to prolong coagulation 3 to 4 times the normal rat-mesentery thrombosis time. The result is dramatic and prolonged since the effect of a single dose appears to extend out to 14 days before rat-mesentery occlusion times return to a normal level. This result is based on 26 rats.

Blood cells of male dogs fed with the new compound, display an increase in negative surface charge which increases sequentially from the third day to approximately the 14th day, so that at 14 days there is a doubling of electrophoretic mobility over the effects seen on the fifth, sixth and seventh day.

The available evidence suggests that, as with most normal pharmacologic agents, the new compound will not produce super normality. It will, however, return toward normal any grossly abnormal measurements concerning the electrokinetic characteristics of blood cells and blood vessels in dogs.

The available evidence indicates that the new compound derived from L-ascorbid acid and para amino benzoic acid is a rather potent anti-thrombotic agent. Its effect is cumulatively greater than the effect of either of its components even when they are mechanically mixed together and given orally to rats and/or dogs. The compound is an elegant example of an electron donor compound which is useful in treating the vascular system.

The following are some results of tests comparing the new compound with a mixture of the starting materials and with the starting materials individually:

TABLE

Dosages—Oral Route

Material	Range Mgs/kg/Day Minimum	Maximum	Duration Administration	Toxicity	Effect
PABA (X)	1	10	Indefinite	Low cutaneous manifestation.	Relatively limited.
L-Ascorbic acid (Y)	0.25 mgs	10	Indefinite	Low cutaneous manifestation. Gastritis. Some evidence disturbance gene pool in massive dosage. Catalytic oxidizer Krebs cycle essential vitamins.	Critical in maintenance of tissue integrity particularly vascular tree.
X+Y (mixture)	1	100 mg/kg rats	Unknown long term	Very low gastritis in one rat, in high dosage 1 mg/gm body weight dosage.	Prolonged rat mesentery occlusion time. Single dose run (day 1): 170±20 min. Tail—7 days
X–Y (compound)	2	100 mg/kg	May be given long term	Very low gastritis.	Prolonged. Longer tail than X+Y Tail—14 days. Occlusion time: 206=24 min.

X–Y tail—minimum 14 days in experimental animals.
X+Y tail—minimum 7 days in experimental animals.

The following additional data was obtained relative to the utility of the new compound:

Effect of New Compound on Electrically Induced Thrombosis in Rat Mesenteric Vessels

Single Loading Dose
20 mg/100 g B.W. Male
Melting Point—147°C

1 Day After Single Loading Dose
1 Rat The occlusion time was 240 minutes

3 Days After Single Loading Dose
1 Rat The occlusion time was 150 minutes
2 Rat The occlusion time was 195 minutes

The following data relates to rat-mesentery occlusion time:

Rat-Mesentery Occlusion Time

	Occlusion Time	Control
PABA		
35 mg p.o./d×3 d (F)	95 min (1F)	45+5 min (3F)
35 mg p.o./d×3 d (M)	53±13 min (7M)	
L-Ascorbic Acid		
10 mg/100 g body weight/ per day×3 days (F)	112±18 min (5F)	45±5 min (2F)
10 mg/100 g body weight/ per day×3 days (M)	135±14 min (2M)	38±10 min (3M)
PABA+L-Ascorbic Acid		
35 mg+10 mg/100 g body weight	108±23.0 min (6F) 128±18.0 min (2M)	45±5 min (2F) 38±10 min (3M)
10 mg+10 mg (3M+3F)	160 min (2F) 175 min (3M) AF+3M	50 minutes (1F) 55 minutes (1M)
PABA–L-Ascorbic Acid Salt (New Compound)		
20 mgs/100 grams	1 day after Single Loading Dose 240 min (1F) 3 days after Single Loading Dose 150 min (1M) 195 min (2F)	

The following data relates to the occlusion time tail in female rats:

(X–Y)–New Compound–Single Dose P.O.

100 mg/100 g Body Weight	Average Occluding Time
1 day	206±24.0 min (5F)
5 days	143± 9.5 min (5F)
9 days	137±12.0 min (5F)
11 days	127±34.0 min (4F)
14 days	67± 4.5 min (4F)
	Control—48± 1.1 min (5F)

Below are tabulated some physical characteristics of batches of the compound which were made:

	Melting Point	Solubility
Batch 1	157°C	Sparingly soluble; 1 gm/100 cc H_2O
Batch 2	157°C	Same
Batch 3	147°C	Same

The present invention will be further described with reference to the accompanying drawing which is a chart demonstrating the prolonged activity of dosages of the compound of the invention.

Referring to the drawing, it is seen that there is illustrated the effects of a single loading dose on a number of rats. A control is provided in the form of five animals and it is noted from the chart in the drawing that the control provides a rat-mesentery occlusion time relative to electrically-induced thrombosis which is, at the outset, less than a single loading dose of the new compound after 14 days.

More particularly, it will be noted that five animals were sacrificed after one day following administration of the new compound, five animals were sacrificed after five days, five more animals were sacrificed after nine days, four additional animals were sacrificed after 11 days and finally, four animals were sacrificed after 14 days. The occlusion time (in minutes) after the first day is markedly greater than that of the controls. After five days, the occlusion time is reduced but is still more than double that of the controls. Similarly, after nine days, the occlusion time is substantially greater than the controls and has reduced only very slightly from the

fifth day measurements. Similarly, after 11 days, there is very little reduction in occlusion time which is still at least twice as great as that of the controls.

After 14 days, measurement of four sacrificed animals still reveals an occlusion time which is greater than that of the controls.

The measurements in the drawing are based upon an administration of the compound in a dosage of 100 mg/100 g of body weight and single loading doses are employed for both the controls and the animals to which the new compound has been administered. This shows a substantial tail inures to the benefit of administration of the new compound and this is important with respect to the treatment of humans wherein oral administration of the new compound is expected to lead to a scheduled administration which provides for spaced-oral dosages over a period of days, such as, for example, one oral administration per week.

WHAT WE CLAIM IS:—

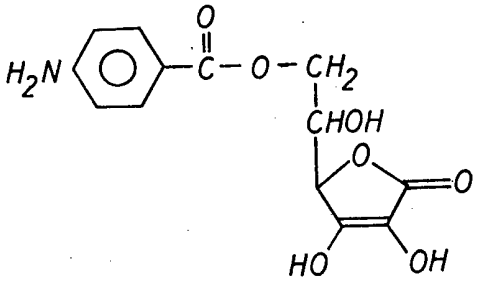
1. A compound derived from L-ascorbic acid and para amino benzoic acid.

2. A compound as claimed in Claim 1 which is a salt of L-ascorbic acid and para amino benzoic acid.

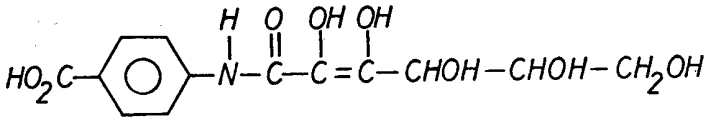
3. A compound as claimed in Claim 1 which is an ester of L-ascorbic acid and para amino benzoic acid.

4. A compound as claimed in Claim 1 which is an amide of L-ascorbic acid and para amino benzoic acid.

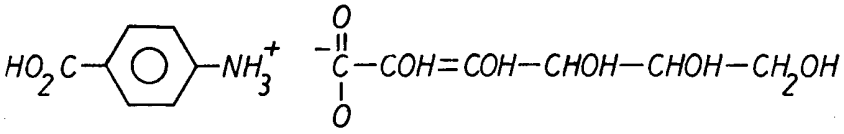
5. A compound as claimed in Claim 1 whose formula is:



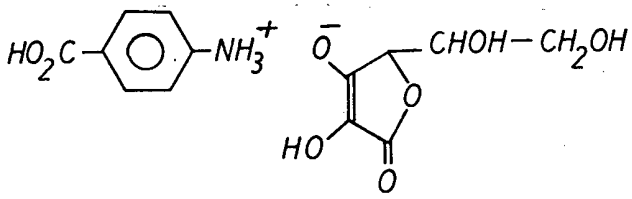
6. A compound as claimed in Claim 1 whose formula is:



7. A compound as claimed in Claim 1 whose formula is:



8. A compound as claimed in Claim 1 whose formula is:



9. A method of preparing a compound as claimed in Claim 1 comprising mixing solutions of L-ascorbic acid and para amino benzoic acid, evaporating the resultant mixture, and recovering the resultant solid.

10. A method as claimed in Claim 9 wherein the acids are used in a ratio of one to one on a molar basis.

11. A method of reducing thrombotic tendencies in a non-human host comprising administering to the host a compound as claimed in any of Claims 1 to 8.

12. A method as claimed in Claim 11 wherein the compound is administered in a dosage of 1—100 mg/kg of body weight.

13. A method as claimed in Claim 12 comprising using a dosage of 1—20 mg/kg of body weight.

14. A method as claimed in Claim 12 or 13 wherein the dosage is administered orally.

15. A method as claimed in Claim 12 or 13 wherein the dosage is administered interperitoneally.

16. A method as claimed in any one of Claims 11 to 15 wherein said compound is formed by mixing solutions of L-ascorbic acid and para amino benzoic acid, evaporating the resultant mixture, and recovering the resultant solid.

17. A method as claimed in Claim 16 wherein the acids are used in a ratio of one to one on a molar basis.

18. A method as claimed in Claim 16 or 17 wherein the solid is recovered in crystal form.

19. A method as claimed in Claim 16 or 17 wherein the solid is recovered in a cake form.

20. A compound as claimed in Claim 1 and substantially as hereinbefore described with reference to any one of the Examples.

21. A method as claimed in Claim 9 and substantially as hereinbefore described with reference to any one of the Examples.

22. A compound whenever prepared by a method as claimed in Claim 9 or 10.

23. A method as claimed in Claim 11 and substantially as hereinbefore described with reference to any one of the Examples.

W. P. THOMPSON & CO.,
Coopers Buildings,
Church Street,
Liverpool L1 3AB.
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1585887 COMPLETE SPECIFICATION

1 SHEET *This drawing is a reproduction of the Original on a reduced scale*