

PATENT SPECIFICATION

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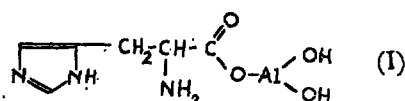
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(54) HISTIDINE SALT

(71) We, CARLO ERBA S.P.A., an Italian Body Corporate of Via Carlo Imbonati 24, Milan 20159, Italy, 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:—

The invention relates to the novel salt, dibasic aluminium histidinate which has the formula



The histidine can be in the natural L-form, or the D-form or a mixture of these forms, particularly the racemic (DL) form.

The invention also provides a process for preparing the salt comprising (a) reacting histidine with an aluminium alkoxide in the presence of water, or (b) reacting a histidine salt with an aluminium salt in an aqueous alcohol. The nature of the resulting salt is determined mainly by the molar ratio between the histidine and the aluminium alkoxide or salt; the use of 1 mole of histidine and 1 mole of aluminium alkoxide or 1/3 mole of aluminium salt produces the dibasic salt. The molar ratio of aluminium alkoxide, histidine and water is suitably 1:1:2.

The salt is a white amorphous powder, insoluble in water and organic solvents but, soluble in mineral acids (e.g. hydrochloric, sulphuric, and nitric acid); its molecular weight is 215. The salt is effective in the treatment of gastritis and of gastric and duodenal ulcers. The *in vivo* activity of dibasic aluminium histidinate was assessed in experimental animals by its ability to antagonize gastric ulcer formation induced by various methods, which has been examined, in order to choose those showing the best reproducibility associated with the best dose/effect relationship (regression); the most satisfactory results were obtained with ulcers obtained by action of phenylbutazone, glucose or caffeine.

Since dibasic aluminium histidinate is a

salt, it was interesting to assess which part of the activity was attributable to the aluminium and which to the histidine, and also assess whether the activity of the salt differed from that of an aluminium hydroxide/histidine mixture. Data obtained in all the tests showed that the activity of dibasic aluminium histidinate is greater than the sum of the activities of aluminium hydroxide and histidine given separately. Thus aluminium and histidine, when in the dibasic aluminium histidinate molecule, exert a synergistic effect in protecting gastric mucosa from ulcerogenic agents. This conclusion was checked, with the phenylbutazone-induced ulcers, by a direct comparison between the activity of dibasic aluminium histidinate and that of a mixture of aluminium hydroxide with histidine. Since this investigation allowed the characterisation of the new compound and its differentiation from a simple mixture of aluminium hydroxide with histidine, it will be described in more detail.

The method employed was in general that of Bonfils *et al.* (Compt. Rend. Soc. Biol., 1954, 148, 881). Rats, belonging to the CFE strain of our SPF breed, were installed two to a cage, and starved for 15 hours with only access to water. 80 Mg/kg of phenylbutazone were administered orally to the rats at zero time, and then the test preparation was administered. The same treatment was repeated after two hours. After 6 hr., the animals were sacrificed by cutting their throats under chloroform anaesthesia. The stomach, taken at an autopsy examination, was prepared according to Hanson and Brodie (J. Appl. Physiol., 1960, 15, 291). Gastric lesions were assessed immediately after withdrawal of the stomachs, according to an arbitrary scale plotted with values from 1 to 7 on the basis of models proposed by a number of authors (e.g. Osterloh *et al.*, *Arzneim. Forsch.*, 1966, 16, 901). The assessment of the lesions was performed by investigators unaware of the treatment given to the animals. Care was also taken to randomize the treatments so as to eliminate a polarization of the data; to achieve this, each animal was given a number, and the key only disclosed after the assessment. Dose levels of 100

- and 300 mg/kg for dibasic aluminium histidinate, and 300 and 900 mg/kg for an equimolecular mixture of aluminium hydroxide and histidine were investigated. The preparations were passed through a sieve (2900 holes/sq.cm.) and suspended in distilled water before use.
- Table I summarizes the results of the investigation.
- Table II relates to the variance analysis made according to a factorial design for biological assay, based on two parallel lines. Since the statistical comparison gave satisfactory results, the ratio of activity between aluminium histidinate and the mixture of aluminium hydroxide with histidine was calculated and found to be 5.61 with a fiducial limit of 3.91—8.03 for a probability (P) of 0.05.
- These results show that dibasic aluminium histidinate has an activity about 5 times that of the mixture of aluminium hydroxide and histidine.
- The invention also provides pharmaceutical compositions comprising a compound of general formula (I) and a pharmaceutically acceptable carrier or diluent. The compositions are preferably in a form suitable for oral administration, for example tablets, capsules, powder packets, liquid solutions, suspensions or elixirs.

TABLE I

Comparison between the activity of dibasic aluminium histidinate and an equimolecular mixture of aluminium hydroxide and histidine in inhibiting acute ulcers caused by phenyl butazone.

Ulcerogenic treatment	Protective treatment		Number of animals	Means $\pm$ standard error of the scores indicating the severity of the ulcerations
	Dose mg/kg given twice orally	Dose mg/kg given twice orally		
—	—	—	25	2.06 ± 0.228
Phenylbutazone	80	—	25	5.70 ± 0.299
"	80	Dibasic aluminium histidinate	25	4.34 ± 0.310
"	80	"	25	2.26 ± 0.260
"	80	Mixture of aluminium hydroxide + histidine	25	5.10 ± 0.252
"	80	"	25	3.56 ± 0.279

TABLE II
Variance analysis according to a 2 × 2 factorial design for biological assay on the data of TABLE I.

Source of variation	Degree of Freedom	Variance	F	Result	Probability
Days	4	7.7433	4.58	HS	<0.01
Control and Phenylbutazone versus treated animals	1	0.1408	<1	NS	>0.05
Drugs	1	26.5225	15.68	HS	<0.01
Regression	1	81.9025	48.43	HS	<0.01
Parallelism	1	1.8225	1.08	NS	>0.05
Groups x days	20	0.9533	<1	NS	<0.05
Error	140	1.6912	—	—	—

HS = Highly significant;

NS = Non-significant.

Activity ratio of $\frac{\text{Dibasic aluminium histidinate}}{\text{Mixture of aluminium hydroxide and histidine}} = 5.61$

(Fiducial limits : 3.91—8.03 for P = 0.05)

The invention is illustrated by the following Examples.

EXAMPLE 1

- 5 Freshly distilled aluminium isopropoxide (20.42 g.) and dry isopropanol (200 ml.) were heated in a round-bottomed, three-neck flask (500 ml.) having a mechanical stirrer, thermometer, and reflux condenser (fitted with a calcium chloride tube) at 40 to 45°C, then treated with L-histidine base (15.51 g.), agitated continuously at 50°C, and was left at 50°C for 10 minutes. Water (3.6 g.) was then added dropwise. The temperature rose spontaneously to 55 to 60°C. The voluminous white precipitate which formed was stirred for 2 hours at 55—60°C and then stirred for 1 hour at room temperature. The product was collected on a Buchner funnel and dried at 50°C and 30 mm Hg for 4 hours; the dry product was ground to a fine powder to give dibasic aluminium 1-histidinate (23 g.) (Water content 7.5%) [Found (corrected for the dry product): C, 33.04; N, 19.3; Al, 12.7%. $\text{C}_6\text{H}_{10}\text{N}_3\text{O}_4\text{Al}$ requires C, 33.49; N, 19.54; Al, 12.54%].

EXAMPLE 2

- 30 Aluminium isopropoxide (10.2 g.) was melted in a round-bottomed flask fitted with an efficient stirrer, cooled to 80 to 88°C, and treated with L-histidine base (7.76 g.) isopropanol (10 ml.), and then deionized water

(1.8 g.). Stirring was continued at 55 to 60°C for 5 hours; the mixture was dried in a crystallizer at 70°C under 30 mm Hg. The dried product was ground to a fine powder to give dibasic aluminium histidinate (10.7 g.; water content 5.11%) having the same physico-chemical characteristics as the product of Example 1. [Found (corrected for the dry product): C, 34.4; N, 19.4; Al, 12.1; histidine, 74%. $\text{C}_6\text{H}_{10}\text{N}_3\text{O}_4\text{Al}$ requires: C, 33.49; N, 19.54; Al, 12.54; histidine 71.65%].

EXAMPLE 3

45 Methanol (200 ml.) in a round bottomed flask (500 ml.) was treated with cooling with anhydrous aluminium chloride (6.6 g.), and then dropwise with stirring with L-histidine (23.2 g.), sodium hydroxide (6 g.), water (12.5 ml.), and methanol (50 ml.). The white precipitate which formed was stirred at 60°C for one hour, treated with water (50 ml.) and continuously agitated at 60°C for 4 hours. The cooled suspension was filtered; the product was dried at 50°C under reduced pressure to give dibasic aluminium histidinate (13 g.).

EXAMPLE 4

60 Proceeding as in Example 1, and using aluminium isopropoxide (20.42 g.), isopropanol (200 ml.), DL-histidine base (15.51 g.), and water (3.6 g.), dibasic aluminium DL-histidinate (23 g.) was obtained. The D-histidinate

can be obtained in the same way starting with D-histidine.

EXAMPLE 5

Proceeding as in Example 2, and using
5 molten aluminium isopropoxide (10.2 g.), DL-histidine (7.76 g.), isopropanol (10 ml.), and water (1.8 g.), dibasic aluminium DL-histidinate (10 g.), was obtained. The D-histidinate
10 can be obtained in the same way starting with D-histidine.

EXAMPLE 6

Proceeding as in Example 3 and using anhydrous aluminium chloride (6.6 g.) methanol (200 ml.), DL-histidine (23.2 g.), sodium hydroxide (6 g.), and water (12.5 g.) dibasic aluminium DL-histidinate (12 g.) was obtained. The D-histidinate can be obtained in the same way starting with D-histidine.

WHAT WE CLAIM IS:—

- 20 1. A salt which is dibasic aluminium histidinate.
2. A salt according to claim 1 wherein the histidine is in the L-, D- or DL-form.
25 3. A process for preparing a salt according to any one of the preceding claim 1 or 2, the process comprising (a) reacting histidine with an aluminium alkoxide in the presence of

water, or (b) reacting a histidine salt with an aluminium salt in an aqueous alcohol.

4. A process according to claim 3 wherein the aluminium alkoxide is dissolved in an alcohol or is molten. 30

5. A process according to claim 3 or 4 wherein the aluminium alkoxide is aluminium isopropoxide. 35

6. A process according to any one of claims 3 to 5 wherein the molar ratios of the aluminium alkoxide, histidine and water is 1:1:2.

7. A process according to claim 3 wherein the aluminium salt is anhydrous aluminium chloride. 40

8. A process according to claim 3 substantially as described in any one of the Examples.

9. A salt which is dibasic aluminium histidinate prepared by a process according to any one of claims 3 to 8. 45

10. A pharmaceutical composition comprising a salt according to any one of claims 1, 2 and 9, and a pharmaceutically acceptable carrier or diluent. 50

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can be obtained in the same way starting with D-histidine.

EXAMPLE 5

- Proceeding as in Example 2, and using
5 molten aluminium isopropoxide (10.2 g.), DL-histidine (7.76 g.), isopropanol (10 ml.), and water (1.8 g.), dibasic aluminium DL-histidinate (10 g.), was obtained. The D-histidinate
10 D-histidine.

EXAMPLE 6

- Proceeding as in Example 3 and using anhydrous aluminium chloride (6.6 g.) methanol (200 ml.), DL-histidine (23.2 g.), sodium hydroxide (6 g.), and water (12.5 g.) dibasic aluminium DL-histidinate (12 g.) was obtained. The D-histidinate can be obtained in the same way starting with D-histidine.

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4. A process according to claim 3 wherein the aluminium alkoxide is dissolved in an alcohol or is molten. 30

5. A process according to claim 3 or 4 wherein the aluminium alkoxide is aluminium isopropoxide. 35

6. A process according to any one of claims 3 to 5 wherein the molar ratios of the aluminium alkoxide, histidine and water is 1:1:2.

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10. A pharmaceutical composition comprising a salt according to any one of claims 1, 2 and 9, and a pharmaceutically acceptable carrier or diluent. 50

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