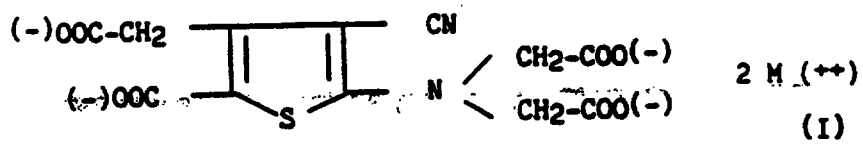
The present invention relates to novel divalent metal salts of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene, a process for the preparation thereof and pharmaceutical compositions containing them.

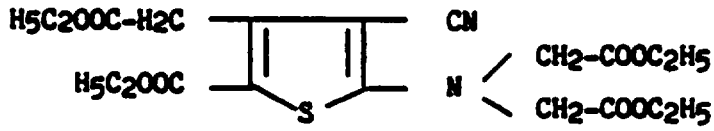
It relates to the divalent metal salts of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene of the general formula I:



(I)

wherein M represents a divalent metal, such as strontium, calcium or magnesium.

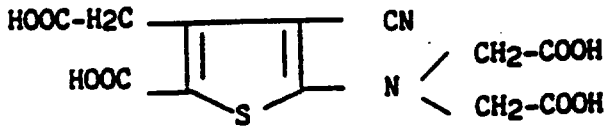
The present invention also relates to a process for the preparation of the salts of the general formula I, characterised in that there is used as starting material the tetraester of formula II:



(II)

which is :

a) either heated at reflux in the presence of sodium hydroxide in an aqueous alcoholic medium, then hydrolysed in an acidic medium, to yield the acid of formula III :



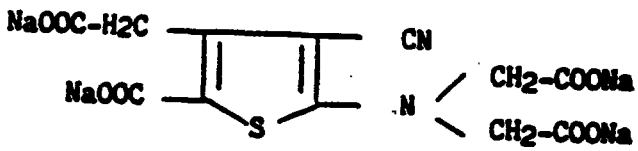
(III)

which is reacted, in aqueous medium, with a hydroxide of the general formula (IV) :

$$M(OH)_2 \qquad (IV)$$

wherein M is as defined above;

b) or heated at reflux in a 50/50 mixture by volume of a normal solution of sodium hydroxide and ethanol to yield, after distilling off the solvents, the tetrasodium salt of formula V



(V)

which is treated with an aqueous solution of a chloride of formula VI :

$$MCl_2 \qquad (VI)$$

wherein M is as defined above;

c) or heated at reflux, in an aqueous alcoholic medium, with a hydroxide of the general formula IV defined above.

Those three methods are, in fact, merely variants of one and the same process which comprises preparing salts of formula I from the tetraester of formula II either directly (case c), or with intermediate isolation of the tetracid of formula III (case a) or the tetrasodium salt of formula V (case b).

The starting material of formula II is described in

the literature (cf. M. Wierzbicki et al. Bull. Soc. Chim. (1975) pages 1786-92).

The acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene of formula III is a novel product which can be used as a starting material in the chemical and pharmaceutical industry, especially in the synthesis of divalent metal salts of formula I. It is thus included as such in the present invention.

The divalent metal salts of formula I have valuable therapeutic and pharmacological properties, especially remarkable anti-osteoporosis properties, as a result of which they can be used as medicaments especially in the treatment of bone disorders. They can also be used in the treatment of cutaneous and vascular ageing, hepatic disorders and dental disorders.

It is known from the prior art in this field that certain divalent metal salts can be used therapeutically especially in the treatment of bone disorders. For example, certain publications in the literature, notably Gastineau, Poc. Staff. Meetings Mayo Clinic 35, 105-11 (1960); Skoryna, Can. Med. Assoc. 125 (7), 702-12 (1981), Skoryna, Trace Subst. Environ. Health 18, 3-23 (1984) note the activity of the lactate, gluconate and carbonate of strontium in the treatment of osteoporosis.

The divalent metal salts of the present invention, apart from being novel compared with the salts mentioned above, have surprising advantages over the latter, notably an improved bioavailability which makes it possible to administer reduced doses of chemical in the treatment of osteoporosis.

The present invention also relates to pharmaceutical

compositions containing as active ingredient one of the salts of formula I in admixture or association with an appropriate pharmaceutical excipient, such as, for example, distilled water, glucose, lactose, starch, talc, ethyl cellulose, magnesium stearate or cocoa butter.

The pharmaceutical compositions so obtained are generally in dosage form and may contain from 200 to 300 mg of active ingredient. They may be formulated as tablets, dragées, gelatin capsules, drinkable solutions, injectable solutions or suppositories and, depending on the case in question, may be administered orally, rectally or parenterally at a dose of from 200 to 300 mg from 2 to 4 times per day.

The following Examples illustrate the invention.

Example 1

Synthesis of the distrontium salt of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene :

a) First method :

1 mol (454.5 g) of the tetraethyl ester of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene is heated at reflux for approximately 3 hours in a mixture of 4 litres of ethanol, 5 l of a normal sodium hydroxide solution and 4 litres of water.

The alcohol is then distilled off; the aqueous medium is cooled and added to 1.250 ml of 4N HCl solution and the whole is precipitated with 30 litres of acetone. The sodium chloride is filtered off, the acetone is distilled off and the aqueous solution is treated with a

sulphonic resin regenerated in a cycle to the ($H^{(+)}$ form until the sodium has disappeared from the solution. The whole is evaporated to dryness and the residue is recrystallised from ethyl ether then tetrahydrofuran or acetone. In this manner the pure acid is obtained, optionally crystallised with the solvent (26 % of solvent in the case of tetrahydrofuran and 11 % in the case of acetone) and corresponding to 240 g of pure acid (yield : 70 %).

The solvated equivalent of 34.2 g (0.1 mol) of the acid is added to 660 ml of water. The organic solvent is distilled off in vacuo at 20°C. 53.14 g of strontium hydroxide.$8H_20$ are added to the aqueous solution remaining. The whole is filtered, allowed to crystallise for 24 to 48 hours, then filtered.

In this manner, the octahydrate of the distrontium salt of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene is obtained which, dried under a stream of dry air, yields the heptahydrate.

After drying the latter under reduced pressure (10 mm) at 55°C, the corresponding tetrahydrate is obtained.

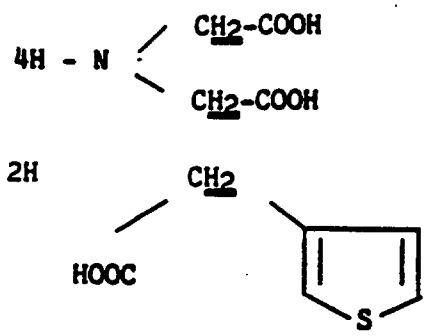
The free acid can also be recrystallised directly from ethyl ether. In that case it crystallises with 4 % of ether.

The physical characteristics of the products prepared in this manner are:

- Acid (ether):

IR ν (OH) : 2000 and 3700 cm^{-1}
ν (CN) : 2220 cm^{-1}
ν (CO) : 1680 and 1720 cm^{-1}

NMR



δ : 4.4 ppm

δ : 3.9 ppm

- octahydrate

IR ν (CN) : 2206 cm^{-1}

- heptahydrate :

IR ν (CN) : 2210 cm^{-1}
ν (COO-) : 1500-1700 cm^{-1}

-tetrahydrate :

IR ν (CN) : 2200 and 2220 cm^{-1}
ν (COO-) : centred at 1580 cm^{-1}

NMR 4H - δ : 4.4 ppm
2H - δ : 3.9 ppm

d) Second method

1 mol of the tetraethyl ester of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene is heated at reflux for approximately 4 hours in a mixture of 4 litres of normal sodium hydroxide

solution and 4 litres of ethanol.

The disappearance of the ester groups is verified by NMR. As soon as this is complete the ethanol and the majority of the water (up to a volume of 1 litre) are distilled off in vacuo in a water bath. The oil obtained is precipitated with 20 litres of ethanol. The sodium salt obtained is filtered and then dried in vacuo at 50°C.

One mol of the tetrasodium salt is dissolved in 4 litres of water. The filtered solution is added to a solution of 2 mols of strontium chloride in 4 litres of water. The whole is rapidly homogenized and then left to stand for 24 hours.

The salt formed, which is the distrontium salt, in the form of the octahydrate, of the acid 2-[N,N-di-(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxy-thiophene, is separated by filtration.

c) Third method

A mixture of 1 mol of the tetraethyl ester of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxy-methyl-5-carboxythiophene, two mols of strontium hydr-oxide, 4 litres of water and 4 litres of ethanol are refluxed for approximately one hour.

The ethanol is then distilled off, the aqueous solution is heated to 100°C, filtered hot, the residue is washed with several tens of ml of water and the octa-hydrate of the distrontium salt of the acid 2-[N,N-di-(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxy-thiophene so obtained is separated by filtration.

By proceeding as in the first method, the hepta-

hydrate and tetrahydrate of the distrontium salt of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene were prepared from the octahydrate obtained according to the above second and third methods.

Example 2

Pharmacological study

a) Anti-resorbent properties

Anti-resorbent bone properties were demonstrated on mice calvaria according to a model based on the method described by REYNOLDS and DINGLE - A sensitive in vitro method for studying the induction and inhibition of bone resorption, Calc. Tiss. Res., 4, 339-349 (1970).

In brief, the salting out of the Ca^{45} previously incorporated in the bone by subcutaneous injection into the animal is measured after culturing the calvaria for 48 hours in the presence or absence of the active ingredient.

The following results were obtained, using the distrontium heptahydrate salt of the acid 2-[N,N-di-(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene prepared in accordance with Example 1, in the evaluation of the bone resorption in mice calvaria:

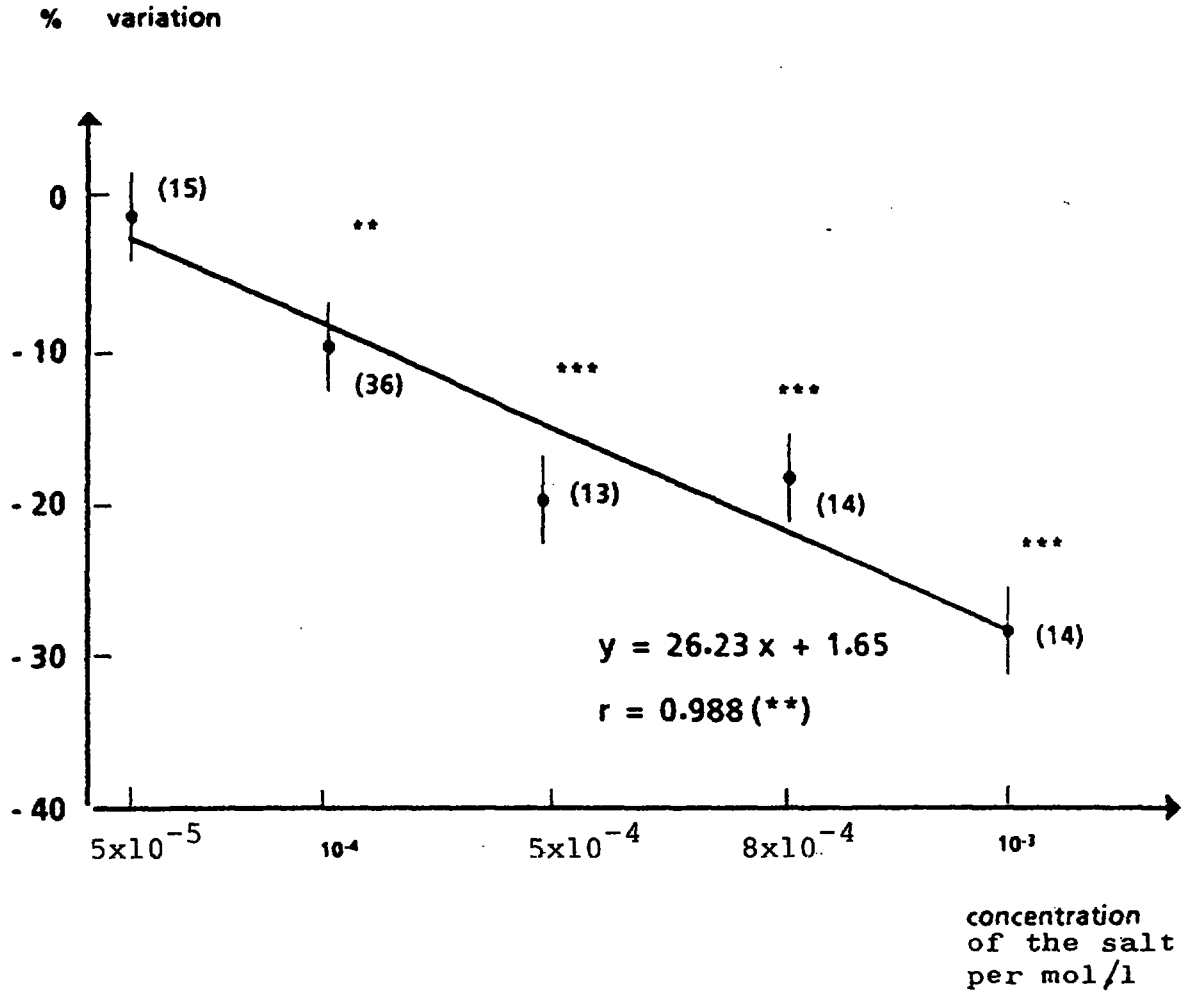
Concentration M		n	control	treated	statistical significance	% variation
salt	Sr					
5×10^{-5}	10^{-4}	15	11.22 ± 0.33	11.00 ± 0.26	NS	- 1.4 % ± 2.3
10^{-4}	2×10^{-4}	36	11.63 ± 0.26	10.96 ± 0.20	**	- 5.0 % ± 1.6
5×10^{-4}	10^{-3}	13	13.25 ± 0.26	10.95 ± 0.21	***	- 17.1 % ± 1.7
8×10^{-4}	1.6×10^{-3}	14	11.86 ± 0.44	9.35 ± 0.31	***	- 20.9 % ± 1.6
10^{-3}	2×10^{-3}	14	14.38 ± 0.39	10.30 ± 0.34	***	- 28.1 % ± 2.0

salt = distrontium heptahydrate salt of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene

n = number of calvaria/batch
average ± standard error
statistical comparison by t testing for paired series
NS : $p>0.05$; ** : $p<0.01$, *** : $p<0.001$

These results are expressed by the following graph:

ACTIVITY OF THE SAID SALT ON THE BASAL BONE RESORPTION IN VITRO (calvaria of mice)



( ) number of calvaria studied
average ± standard error
statistical comparison by t testing for paired series
NS : $p>0.01$; ** : $p<0.01$; *** : $p<0.001$.

b) Bioavailability

This is ascertained by a study of the serum kinetics of strontium after oral administration to rats of the distrontium heptahydrate salt of the the acid 2-[N,N-di-(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxy-

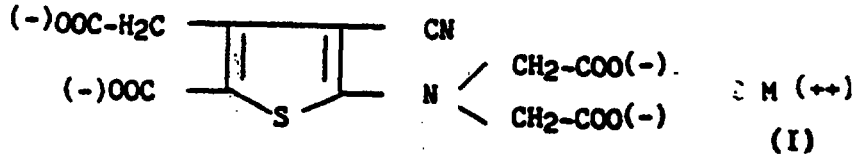
thiophene prepared according to Example 1.

After a single oral administration of 50 mg/kg (in equivalents of strontium) of the distrontium heptahydrate salt of the acid 2-[N,N-di(carboxymethyl)amino]-3-cyano-4-carboxymethyl-5-carboxythiophene, the strontium is absorbed with an absolute bioavailability of 36.3 %, the latter being calculated from that (taken as being 100 %) of the chloride of strontium by the i.v. route.

* C L M S *

CLAIMS for the Contracting States AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1- The divalent metal salts of the acid 2-(N,N-di(carboxymethyl)amino)-3-cyano-4-carboxymethyl-5-carboxythiophene of the general formula I :



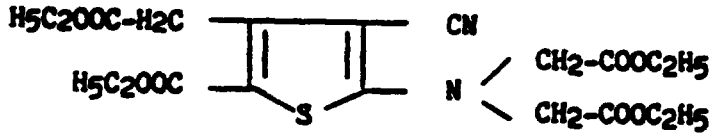
(I)

wherein M represents a divalent metal such as strontium, calcium or magnesium.

2. The distrontium salt of the acid 2-(N,N-di(carboxymethyl)amino)-3-cyano-4-carboxymethyl-5-carboxythiophene in the form of the octahydrate, heptahydrate or tetrahydrate.

3. The acid 2-(N,N-di(carboxymethyl)amino)-3-cyano-4-carboxymethyl-5-carboxythiophene.

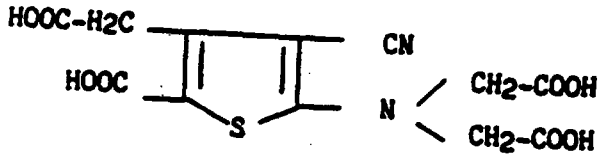
4. A process for the preparation of the divalent metal salts of claim 1, characterised in that there is used as starting material the tetraester of formula II :



(II)

which is :

a) either heated at reflux in the presence of sodium hydroxide in an aqueous alcoholic medium, then hydrolysed in an acidic medium, to yield the acid of formula III :



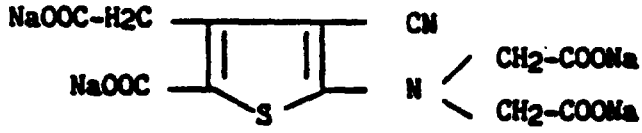
(III)

which is reacted, in aqueous medium, with a hydroxide of the general formula (IV) :

$$M(OH)_2 \qquad \text{(IV)}$$

wherein M is as defined in claim 1;

b) or heated at reflux in a 50/50 mixture by volume of a normal solution of sodium hydroxide and ethanol to yield, after distilling off the solvents, the tetrasodium salt of formula V



(V)

which is treated with an aqueous solution of a chloride of formula VI :

$$MCl_2 \qquad \text{(VI)}$$

wherein M is as defined in claim 1;

c) or heated at reflux, in an aqueous alcoholic medium, with a hydroxide of the general formula IV defined above.

5. Pharmaceutical compositions comprising as active ingredient a salt of claim 1 together with appropriate

* C L M S *

pharmaceutical excipients.

6. Pharmaceutical compositions comprising as active ingredient the salt of claim 2 together with appropriate pharmaceutical excipients.

7. Pharmaceutical compositions according to claims 5 and 6 presented in a form suitable especially for the treatment of bone disorders, cutaneous and vascular ageing, hepatic disorders and dental disorders.