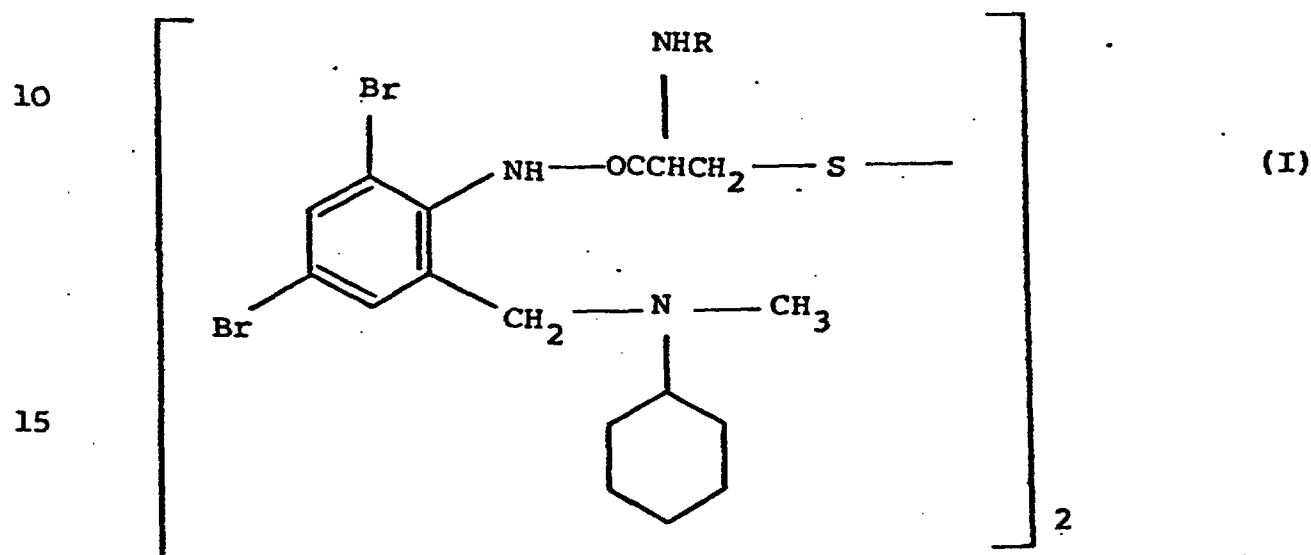


Cystine Derivatives

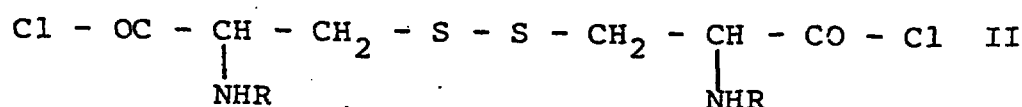
The invention relates to cystine derivatives and salts thereof, to methods for their preparation and to pharmaceutical compositions containing them.

The invention provides cystine derivatives having the general formula I



in which R represents a benzyloxycarbonyl or tosyl group and pharmaceutically acceptable acid addition salts thereof.

The invention further provides a method for the preparation of the cystine derivatives according to the invention, the method comprising reacting a cystyl dichloride of the general formula II



with 2,4-dibromo-5-(N-methyl-N-cyclohexyl-aminomethyl)-aniline (hereinafter "bromhexine").

The cystyl dichlorides of the general formula II may be obtained from the corresponding acids by any of the conventional methods for conversion of an acid into its chloride, for example by treatment with phosphorus pentachloride in a suitable solvent such as chloroform or diethyl ether. It is not necessary to isolate or purify the dichloride before proceeding with the method according to the invention. A suitable procedure is to precipitate the dichloride from the reaction mixture, for example by addition of ligroin (to diethyl ether solutions) or diethyl ether (to chloroform solutions), filter it off, and add to it a solution of bromhexine in a suitable solvent such as chloroform or ethyl acetate. The reaction with bromhexine proceeds at ambient temperature and may, if desired, be completed under reflux. The resultant cystine derivatives according to the invention may be isolated and purified, and optionally converted into a pharmaceutically acceptable acid addition salt by any of the conventional methods such as direct treatment with the chosen acid in a solvent. Salts of hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methansulphonic, formic benzoic, malonic, benzenesulphonic and naphthalene-2-sulphonic acids are preferred.

Cystine derivatives according to the invention and salts

thereof possess a good expectorant, antitussive and respiratory tract fluid fluidifying activity, whereas they show a low toxicity. The LD_{50} value, determined in the mouse and in the rat, both i.p., and per os, is ^{greater than} 3000 mg/kg for all the tested compounds.

The expectorant activity (ED_{50}), determined in the rabbit according to the Boyd method (Boyd and Sheppard, Arch. Int. Pharm. 1966, 163, 284) was 100 mg/kg (I, R=benzyloxy-carbonyl).

The same ED_{50} , determined in the mouse according to an improved Mavatari method (Graziani, Cazzulani, Il Farmaco, Ed. Prat., 1981, XXXVI, 3, 167), was respectively 1.2 mg/kg (I, R=benzyloxycarbonyl) and 3.7 mg/kg (I, R=tosyl).

Compound I (R=benzyloxycarbonyl) showed to be active i.p., as an antitussive agent, at a dose of 30 mg/kg (Method of Charlier, Prost et al., Arch. Int. Pharm., 1961, 134, 306), through inhalation of citric acid aerosol in the guinea-pig. The activity was determined testing depression of cough.

In order to evaluate the antitussive activity, another procedure was performed. The vagus nerve was stimulated and depression of cough was reported following the injection of the product (Pickering and Jones, Arzn. Forsch., 1979, 29, 287). The ED_{50} of the compounds I (R=benzyloxycarbonyl and tosyl) was respectively 88 and 74 mg/kg.

Accordingly, the invention also provides a pharmaceutical composition comprising a cystine derivative according to the invention or a salt thereof in admixture with a pharmaceutically acceptable diluent or carrier.

The following Examples illustrate the invention.

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EXAMPLE 1

N,N'-ditosyl-cystyl bis[2,4-dibromo-6-(N-methyl-N-cyclohexyl-aminomethyl)-anilide]₇

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To 17.20 g of N,N'-ditosyl-L-cystine dissolved in 185 ml of diethyl ether, 18.5 g of phosphorus pentachloride were slowly added at 20-25°C. The mixture was stirred for 30 minutes and then 650 ml of ligroin were added. The

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precipitate thus formed was filtered off and to it was added, at 20-25°C, a solution of 27.52 g of bromhexine in 74 ml of chloroform. The mixture was allowed to stand at the above temperature for 36 hours. The whole was extracted with chloroform and washed first with sodium carbonate, then with water. The organic layer was dried

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with calcium chloride. The solvent was evaporated off and the residue was purified on a silica column, using chloroform:ethyl acetate (3:2 by volume) as eluent.

The unreacted bromhexine was separated off. The product, as free base, was dried, dissolved in isopropanol and directly transformed into its hydrochloride by adding hydrogen chloride in isopropanol and then diethyl ether.

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The precipitate was crystallized from 95% ethanol and diethyl ether. Mp 191-192°C.

Elemental analysis for $C_{48}H_{60}Br_4N_6O_{16}S_4 \cdot 2HCl \cdot 2H_2O$

	C	H	N	Cl	S	Br	H ₂ O
Calculated (%):	41.96	4.66	6.11	5.16	9.33	23.26	2.76
Found (%) :	41.91	4.83	5.96	5.30	9.54	23.25	2.62

EXAMPLE 2

N,N'-dibenzoyloxycarbonyl-cystyl bis[2,4-dibromo-6-(N-methyl-N-cyclohexyl-aminomethyl)-anilide]

To 10.16 g of N,N'-dibenzoyloxycarbonyl-L-cystine
5 dissolved in 56 ml of anhydrous chloroform, 10 g of
phosphorus pentachloride were slowly added at from
-10°C to -5°C.

The reaction mixture was stirred for 10 minutes and then
60 ml of diethyl ether were added. The whole was cooled
10 for one hour. The precipitate thus formed was filtered
off and to it was added, at 22°C, a solution of 14.88 g of
bromhexine in 40 ml of ethyl acetate. The mixture was
stirred at the above temperature for one day and then
refluxed for 4 hours.

15 When the reaction was over, the mixture was extracted with
ethyl acetate, washed with sodium carbonate solution,
with water, then dried on anhydrous sodium sulphate. The
solvent was evaporated off and the residue was treated with
hexane. The product was filtered, collected, and
20 crystallized from ethanol. Mp 168°C.

Adding hydrogen chloride in ethanol, the corresponding
salt, melting at 195-197°C (with decomposition) was
obtained.

Elemental analysis for $C_{50}H_{60}Br_4N_6O_6S_2 \cdot 2HCl$

		C	H	N	Br	Cl	S
Calculated (%)	:	46.28	4.81	6.47	24.63	5.46	4.94
Found (%)	:	45.94	4.79	6.27	24.97	5.13	4.82

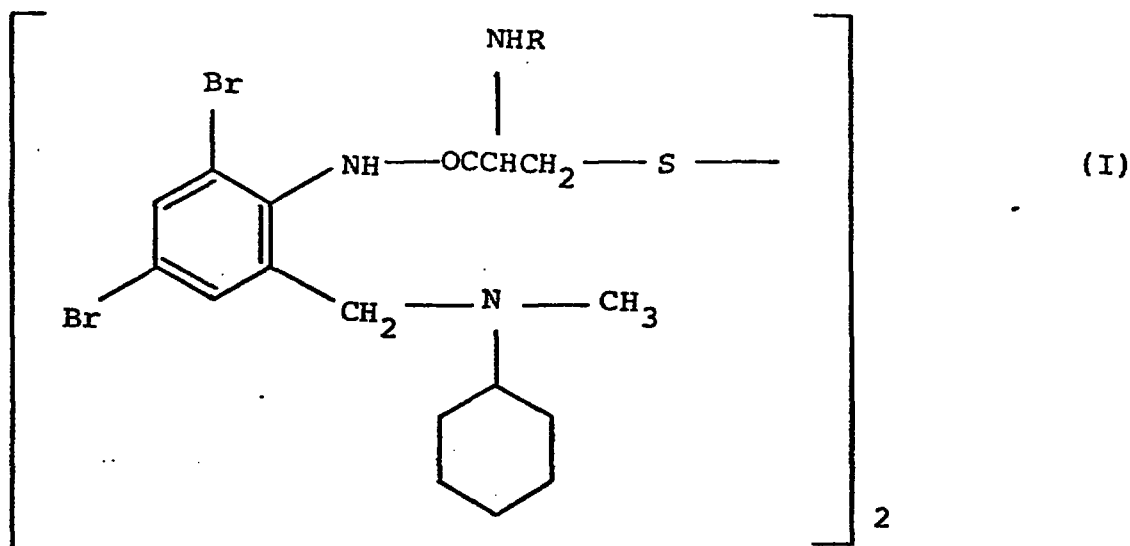
- 5 Following the same procedures, but varying the acid used for salt formation, the following salts were prepared.

	Hydrobromide	mp 190-195°C
	Formate	mp 167-169°C
	Mandelate	mp 148-149°C
10	Acetate	mp 169-171°C
	Tartrate	mp 131-134°C



CLAIMS

1. A cystine derivative having the general formula I

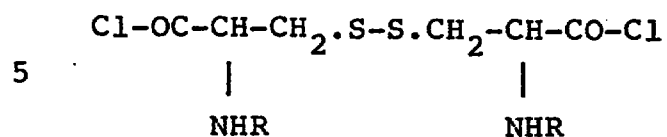


in which R represents a benzyloxycarbonyl or
5 tosyl group, or a pharmaceutically acceptable
acid addition salt of such a cystine derivative.

2. N,N'-ditosyl-cystyl bis [2,4-dibromo-6-(N-methyl-N-cyclohexyl-aminomethyl)-anilide].

10 3. N,N'-dibenzoyloxycarbonyl-cystyl bis
/2,4-dibromo-6-(N-methyl-N-cyclohexyl-aminomethyl)-
-anilide/.

4. A method for the preparation of a cystine derivative according to claim 1, the method comprising



II

with 2,4-dibromo-6-(N-methyl-N-cyclohexyl-aminomethyl)-
-aniline.

- 15 5. A method for the preparation of a cystine derivative
according to claim 1 the method being substantially as
described herein with reference to either of the Examples.
6. A pharmaceutical composition comprising a cystine
20 derivative according to any of claims 1 to 3 or a
pharmaceutically acceptable salt thereof in admixture
with a pharmaceutically acceptable diluent or carrier.

