

COLCHICOSIDE ANALOGUES

Field of the invention

The present invention relates to colchicine derivatives, in particular 3-demethyl- and 3-demethylthio-colchicine derivatives with muscle relaxant, anti-inflammatory and anti-gout activity.

5 Technological background

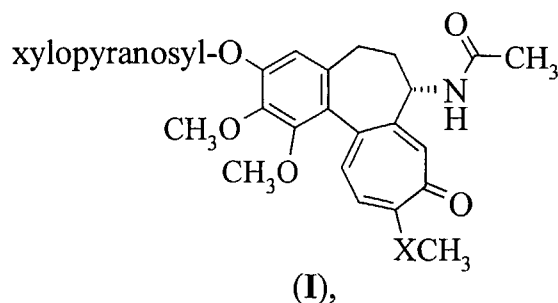
Relaxant drugs reduce muscle tone and are used in therapy for the treatment of contractures and muscle spasm. Muscle spasm is one of the main factors responsible for chronic pain; it characterises several pathologies of the locomotor apparatus as well as inflammatory-rheumatic and degenerative
10 orthopaedic pathologies; when it affects articulations, further to pain, it causes rigidity, which reduces joint mobility and flexibility in the affected part. For these reasons, the study of molecules endowed with muscle relaxant and antispasmodic properties still raises remarkable clinical interest.

As it is known, colchicine is a pseudoalcaloid that has been
15 widely used for some time for the treatment of gout. The use of 3-demethyl-thiocolchicine glucoside, thiocolchicoside, is also widespread in therapy for treating contractures and inflammatory conditions that affect the muscular system (Ortopedia e traumatologia Oggi XII, n. 4, 1992). It has been recently shown that the activity of thiocolchicoside is due to its ability to
20 interact with strychnine-sensitive glycine receptors; therefore, compounds having glycine-mimicking activity can be used in the rheumatologic-orthopaedic field, due to their muscle relaxant properties.

Disclosure of the invention

The present invention relates to colchicine derivatives of the general
25 formula (I):

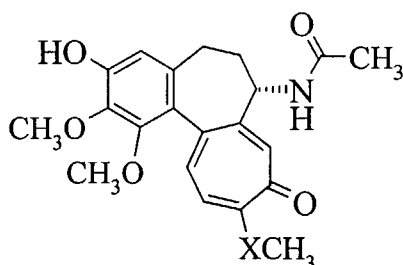
2



in which X is oxygen or sulfur.

For the purposes of the present description, the compound in which X is oxygen is referred to as (**Ia**), whereas the compound of formula (I) in which X is sulfur is referred to as (**Ib**). D and L isomers are comprised in the compounds of formula (I). The D and L isomers of compound (**Ib**), 3-O-β-D-xylopyranosyl-3-O-demethylthiocolchicine and 3-O-β-L-xylopyranosyl-3-O-demethylthiocolchicine are particularly preferred.

The compounds of the present invention are prepared by reaction of D- or L-xylopyranosyl-fluoride with 3-O-demethylcolchicine (**IIa**) and 3-O-demethylthiocolchicine (**IIb**)



IIa: X = O

IIb: X = S

according to the general method disclosed in EP 0 789 028.

In more detail, 3-O-demethylcolchicine (**IIa**) or 3-O-demethylthiocolchicine (**IIb**) are reacted with D- or L-xylopyranosyl-fluoride (**III**) or a protected form thereof, preferably peracetate. The reaction is carried out in polar aprotic solvents, preferably selected from acetonitrile and chlorinated solvents, at temperatures ranging from 0°C to the boiling temperature of the solvent, preferably at room temperature, and in the

presence of a base, preferably 1,1,3,3-tetramethylguanidine. The reaction is usually complete in a time ranging from 10 minutes to 2 hours. Hydrolysis of the protective groups can be carried out without recovery of the intermediates.

In particular, it has been observed that the β -D isomer of compound
5 (Ib) has a significant muscle relaxant activity, higher than that of the corresponding thiocolchicoside isomer, and is also endowed with a significant anti-inflammatory and anti-gout activity.

Muscle relaxant activity was evaluated with the rota-rod test. Swiss
male mice weighing 20-25 g were treated intraperitoneally with the β -D
10 isomer of compound (Ib) at doses of 1-3-10 mg/kg, thirty minutes before the test. Relaxant activity on striated muscles was evaluated by testing the resistance of the mice to the stimuli of a rotating plane revolving at increasing rate, from 2 to 50 r.p.m.. The results reported in the following table show that the compound of the present invention is more active than thiocolchicoside
15 used as the reference compound.

Table 1

Treatment	Dose (mg/Kg i.p.)	Resistance time (sec. M$\pm$S.E.)	DE₅₀ mg/Kg
Controls		400 $\pm$ 27	
Compound (Ib) isomer β -D	1	270 $\pm$ 19	
	3	175 $\pm$ 14	2.23 (1.84-2.82)
	10	80 $\pm$ 10	
Thiocolchicoside isomer β -D	1	345 $\pm$ 20	
	3	265 $\pm$ 17	4.47 (3.16-7.01)
	10	110 $\pm$ 12	

Moreover, the compound of the invention is significantly less toxic. In fact, its DL₅₀ is 80 (63-94) mg/kg i.p., whereas the DL₅₀ of thiocolchicoside is
20 20 mg/kg. These results show that the compound of the invention, further to

being more active, has a toxic/active dose ratio significantly more favourable than thiocolchicoside.

Table 2

Treatment	DE ₅₀ mg/kg i.p.	DL ₅₀ mg/kg i.p.	DL ₅₀ /DE ₅₀
Compound (Ib) isomer β -D	2.23	80	35.87
Thiocolchicoside isomer β -D	4.47	20	4.47

5 The compounds of the invention can be incorporated in pharmaceutical formulations intended to oral, intravenous, intramuscular, transdermal and topical administration with conventional excipients and methods, such as those reported in Remington's Pharmaceutical Sciences Handbook, XVII Ed., Mack Pub., N.Y., U.S.A.. Among the excipients useful for the preparation of
10 liposomal forms for the parenteral or topical administration, natural and synthetic phospholipids are particularly preferred. The doses can range from 5 to 50 mg a day depending on the disease and the administration route.

The invention will be now illustrated in greater detail by means of some examples.

15 **EXPERIMENTAL SECTION**

Melting points were measured with a Buchi 510 apparatus. NMR spectra were recorded with a Bruker AC 200.

Example 1 - 3-O-(2',3',4'-O-triacetyl- β -D-xylopyranosyl)-3-O-demethylthiocolchicine

20 3-O-Demethylthiocolchicine (IIb) (0.5 mmoles) and 2,3,4-O-triacetyl- α -D-xylopyranosyl fluoride (0.75 mmoles), prepared according to Hayashi et al. (*Chemistry Lett.* 1984, 1747), were suspended in dry acetonitrile at room temperature (10 ml), under nitrogen and with stirring. 1,1,3,3-Tetramethylguanidine (1.5 mmoles) was added and the suspension turned
25 clear red. Boron trifluoride etherate (4 mmoles) was added, thereafter the

solution turned colourless. The reaction was monitored by TLC (CH₂Cl₂:MeOH 9:1). After disappearance of the starting products (30 min), the reaction was quenched adding a saturated sodium bicarbonate solution (10 ml). The phases were separated and the aqueous one was extracted with ethyl acetate (3 x 10 ml). The combined organic phases were washed with a saturated potassium hydrogen sulfate solution (15 ml), brine (15 ml) and dried over magnesium sulfate. After evaporation of the solvent, the reaction products were separated by chromatography on silica gel. Alternatively, the crude was directly subjected to deprotection.

¹H-NMR (CDCl₃) - δ (ppm) 7.06 (NH, d, 7.4 Hz), 7.06 (H12, d, 10.3 Hz), 7.27 (H11, d, 10.3 Hz), 7.33 (H8, s), 6.71 (H4, s), 4.71-4.55 (H7, m), 2.60-1.90 (H5-H6, m), 3.90 (2-OMe, s), 3.66 (1-OMe, s), 2.44 (SMe, s), 2.00 (acetamide), 5.28-5.18, 5.08-4.98 (H1', H2', H3', H4', m), 4.30 (H5'a, ddd, 4.3, 7.0, 12.1 Hz), 3.58 (H5'b, ddd 4.3, 7.0, 12.1 Hz), 2.12 (OAc), 2.11 (OAc), 2.10 (OAc).

Example 2 - 3-O-(2',3',4'-O-triacetyl-β-L-xylopyranosyl)-3-O-demethylthiocolchicine

3-O-Demethylthiocolchicine (**IIb**) (0.5 mmoles) and 2,3,4-O-triacetyl-α-L-xylopyranosyl fluoride (0.75 mmoles), prepared according to Takanashi et al. (*Liebigs Ann. Chem.* 1997, 1081), were suspended in dry acetonitrile at room temperature (10 ml), under nitrogen and with stirring. 1,1,3,3-Tetramethylguanidine (1.5 mmoles) was then added and the suspension turned clear red. After addition of boron trifluoride etherate (4 mmoles) the solution turned colourless. The reaction was monitored by TLC (CH₂Cl₂:MeOH 9:1). After disappearance of the starting material (2 hours), the reaction was quenched by addition of a saturated sodium bicarbonate solution (10 ml). The phases were separated and the aqueous one was extracted with ethyl acetate (3 x 10 ml). The combined organic phases were washed with a potassium

hydrogen sulfate saturated solution (15 ml), brine (15 ml) and dried over magnesium sulfate. After evaporation of the solvent, the reaction products were separated by chromatography on silica gel. Alternatively, the crude was directly subjected to deprotection.

- 5 ¹H-NMR (CDCl₃) - δ (ppm) 7.34 (NH, d, 7.9 Hz), 7.07 (H12, d, 10.7 Hz), 7.30 (H11, d, 10.7 Hz), 7.37 (H8, s), 6.71 (H4, s), 4.71-4.55 (H7, m), 2.60-1.80 (H5-H6, m), 3.88 (2-OMe, s), 3.64 (1-OMe, s), 2.44 (SMe, s), 2.00 (acetamide), 5.28-5.18 e 5.10-4.90 (H1', H2', H3', H4' m), 4.25 (H5'a, ddd, 4.3, 4.4, 12.1 Hz), 3.58 (H5'b, ddd 4.3, 4.4, 12.1 Hz), 2.14 (OAc), 2.11 (OAc),
10 2.10 (OAc).

Example 3 - General method for deprotection in ethanol

The crude product (0.5 theoretical mmoles) from example 1 or 2 was dissolved in ethanol (4 ml) and 1N NaOH (2 ml) at room temperature. The reaction was checked by TLC. After disappearance of the starting product the
15 solvent was evaporated off and the residue was subjected to silica gel chromatography. The product can be further crystallized from methanol/isopropanol.

Example 4 - General method for deprotection in acetone

The crude product from example 1 or 2 (1 theoretical mmol) was
20 suspended with potassium carbonate in acetone (30 ml) and water (10 ml). The mixture was refluxed until disappearance of the starting product. The solvent was evaporated off and the product recovered by chromatography. The product can be further crystallized from methanol and diisopropyl ether.

Example 5 - 3-O-β-D-xylopyranosyl-3-O-demethylthiocolchicine

25 The product was obtained according to the deprotection method of example 3 or 4 with 45% yield, after chromatography on silica gel eluting with a CH₂Cl₂/MeOH gradient.

m.p. 193°C; [α]_D²² -201 (c 1, MeOH);

¹H-NMR (CDCl₃): ppm 8.64 (NH, d, 7.6 Hz), 7.15 (H12, d, 10.6 Hz), 7.28 (H11, d, 10.6 Hz), 7.03 (H8, s), 6.85 (H4, s), 4.37-4.25 (H7, m), 2.60-1.80 (H5-H6, m), 3.84 (2-OMe, s), 3.55 (1-OMe, s), 2.42 (SMe, s), 1.86 (acetamide), 4.97 (H1', 6.6 Hz), 3.20-3.90 (H2', H3', H4', H5', m), 4.40-5.60 (OH).

5 **Example 6 - 3-O-β-L-xylopyranosyl-3-O-demethylthiocolchicine**

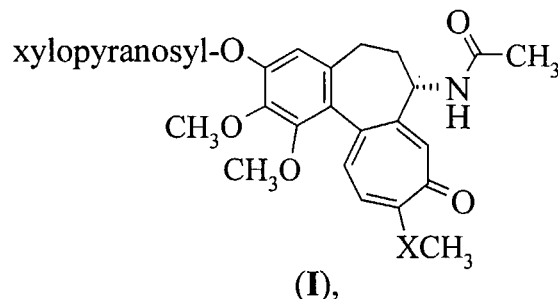
The product was obtained according to the deprotection method of example 3 or 4 with 45% yield, after chromatography on silica gel eluting with a CH₂Cl₂/MeOH gradient.

m.p. 220°C; [α]_D²² -176 (c 1, MeOH);

10 ¹H-NMR (CDCl₃): ppm 8.64 (NH, d, 7.3 Hz), 7.17 (H12, d, 10.2 Hz), 7.29 (H11, d, 10.2 Hz), 7.03 (H8, s), 6.87 (H4, s), 4.23-4.41 (H7, m), 2.70-1.90 (H5-H6, m), 3.84 (2-OMe, s), 3.55 (1-OMe, s), 2.42 (SMe, s), 1.86 (acetamide), 5.02 (H1', 6.9 Hz), 3.20-3.90 (H2', H3', H4', H5', m), 4.90-5.60 (OH).

CLAIMS

1. Compounds of the general formula (I)



- 5 in which X is oxygen or sulfur.
2. A compound as claimed in claim 1 wherein X is oxygen.
3. A compound as claimed in claim 1 wherein X is sulfur.
4. A compound selected from:
- 3-*O*- β -D-xylopyranosyl-3-*O*-demethylthiocolchicine and
- 10 3-*O*- β -L-xylopyranosyl-3-*O*-demethylthiocolchicine.
5. A compound of any one of claims 1 - 4 as a medicament.
6. Use of a compound of any one of claims 1 - 4 for the preparation of muscle relaxant medicaments.
7. Use of a compound of any one of claims 1 - 4 for the preparation of
- 15 anti-inflammatory medicaments.
8. Use of a compound of any one of claims 1 - 4 for the preparation of anti-gout medicaments.
9. Pharmaceutical compositions containing a compound of any one of claims 1 - 4 in admixture with suitable excipients and/or carriers.
- 20 10. Pharmaceutical compositions as claimed in claim 9 for topical use.
11. Pharmaceutical compositions as claimed in claim 9 for parenteral use.
12. Pharmaceutical compositions as claimed in claim 10 or 11 in which the excipients are selected from natural and synthetic phospholipids.