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Research Article

A short synthesis of [^{14}C]-labelled levamisole and its major metabolite

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Summary

Levamisole (**I**) is the *levo* isomer of tetramisole. It is a broad spectrum anthelmintic which is used extensively as a veterinary drug for food producing animals. Metabolism and environmental studies necessitated the synthesis of ^{14}C-labelled levamisole (**6**) and of its major metabolite (**12**).

^{14}C-Tetramisole was obtained in two steps from ^{14}C-thiourea. Resolution via salt formation and crystallization afforded ^{14}C-levamisole and ^{14}C-dexamisole. Racemisation followed again by resolution made it possible to improve the over-all radiochemical yield of ^{14}C-levamisole to 51.0%. The compound had a specific activity of 73.6 MBq/mmol, a HPLC purity of 99.8% and an enantiomeric excess of 99.4%.

The ^{14}C-labelled metabolite of levamisole (**II**) was obtained from ^{14}C-potassium cyanate in 4 consecutive steps. Resolution via chiral HPLC afforded the desired compound in a 16.2% overall radiochemical yield. It had a specific activity of 895 MBq/mmol, a HPLC purity of 98.7% and an enantiomeric excess of 100%.

Key Words: carbon-14; synthesis; levamisole; levamisole metabolite; resolution

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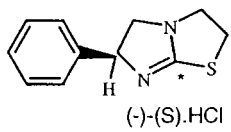
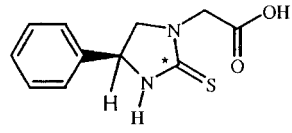
Introduction

Levamisole (**I**) is the *levo* isomer and the most active form of the broad spectrum anthelmintic D,L-tetramisole.[1–4] It is used extensively as a veterinary drug for food producing animals.[5] For research reasons, specifically tritium-labelled levamisole at high specific activity has been synthesized according to a procedure already described in this Journal.[6] Later, for additional metabolism studies, the need arose to obtain levamisole with a carbon-14 label located in the imidazo[2,1-*b*]thiazolidine ring system. Because of extensive use of levamisole-containing drug, environmental safety studies on its major metabolite (II) in ruminants were indicated. Of this compound one



(-)-(S).HCl

I, Levamisole.HCl

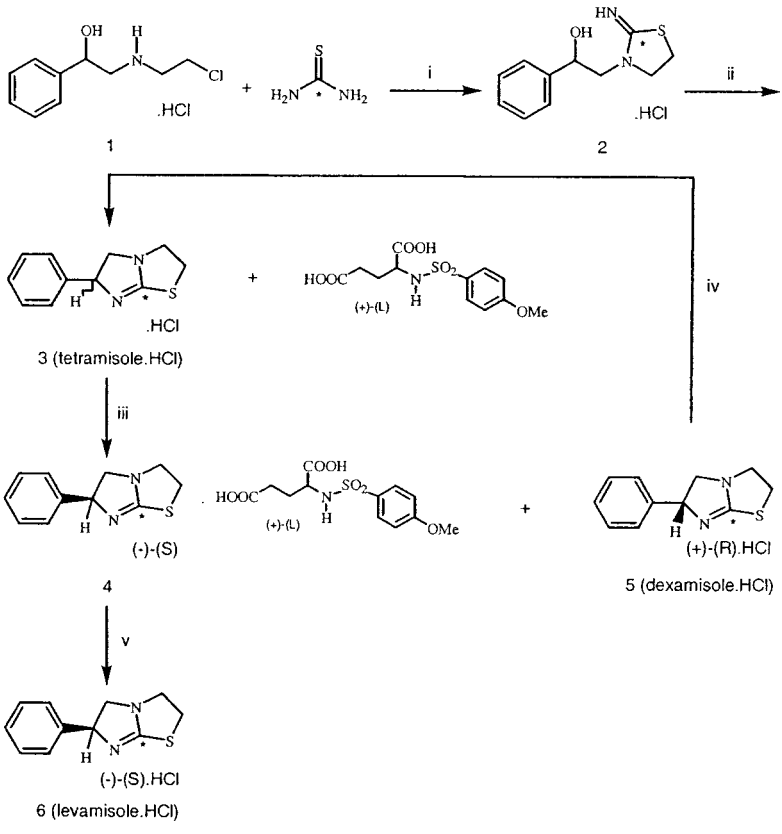


II, Levamisole metabolite

desired a synthetic approach which was preferably aimed towards the introduction of the carbon-14 label at the same position as had the metabolite formed from the parent drug; the synthesis of which is described herewith.

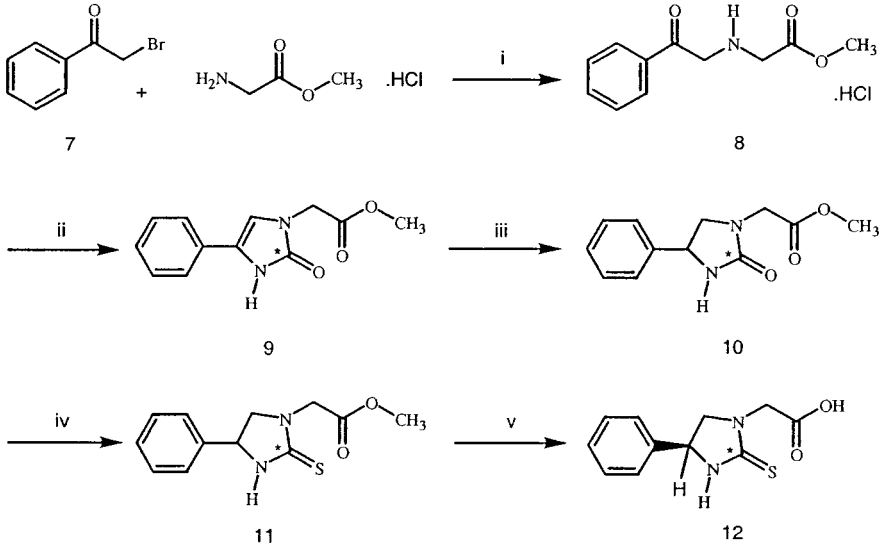
Results and discussion

Levamisole is a potent anthelmintic which is already marketed since 1968. Although the use of levamisole-racemate (tetramisole) carrying the ^{14}C-label on the 7α-position[7] or on the 6-position[8] has been mentioned, only one preparative procedure with a ^{14}C-label uniformly distributed in the phenyl group was revealed in the literature.[9] Our choice to introduce a carbon-14 label situated on the 7α-position proved to be most fruitful in providing a short two-step synthetic approach towards tetramisole. Resolution with (+)-(*L*)-2-[[(4-methoxyphenyl)sulfonyl]amino]-pentane-dioic acid gave an almost quantitative isolation of the desired levamisole at an 99.4% enantiomeric excess. The undesired dexamisole was easily racemized, offering the possibility to improve the over-all radiochemical yield to 51.0% by repeating the racemization–resolution cycle up to four times (Scheme 1).



Scheme 1. Synthesis of [^{14}C]-labelled levamisole. Reagents and conditions: (i) HCl 0.1 N, reflux, 12 j; (ii) conc. H_2SO_4, 0°C, 5 min; HPLC-purification; (iii) Sodium salt formation of optically pure acid, then 3, unlabelled 6, careful acidification with HCl; (iv) Base liberation with NaOH 50%, then toluene, DMF, KO-*t*-Bu, 70°C, 11/2 h. Isolation of 3-base in toluene, then HCl; (v) NaOH 10 N, isolation of base, then toluene and HCl

In vivo, the metabolite in question is formed via hydroxylation of the thiazolidine ring system, followed by oxidation and hydrolysis of the tautomeric thiolactone form of the thiohydantoic acid metabolite.[7] The synthetic approach comprised four radiochemical steps, which were followed by HPLC purification and resolution to offer the optically pure compound in an overall radiochemical yield of 16.2%. Identification of the correct isomer was performed by co-chromatography of the isolated enantiomers with a radioactive sample of the desired metabolite, stemming from in-vivo samples of a ^{14}C-levamisole metabolism study (Scheme 2).



Scheme 2. Synthesis of [^{14}C]-labelled levamisole. Reagents and conditions: (i) Base liberation: Na_2CO_3, 4-methyl-2-pentanone, 18 h, RT, then + 7, 4 h, RT, isolation, then HCl; (ii) KO*CN, MeOH-H_2O (70-30, v/v), 1 h RT, 1 h 100°C, 18 h RT, 1 h 0°C; (iii) H_2, Pd-C, 10%, MeOH, 18 h, 50°C; (iv) Lawesson's reagent, toluene; (v) NaOH 1 N, H_2O, resolution

Experimental

Analytical HPLC was performed on an apparatus consisting of a Gilson model 305 (master) pump and a Gilson model 306 (slave) pump, each equipped with a 10 SC pumphead. The samples were injected by a Rheodyne 7125 injector. Chemical and radiochemical purity were determined by on-line UV detection on a Gilson 218 detector and radioactivity detection on a Berthold Radioactivity monitor LB 506 C system with a flow-through cell of 0.500 ml. The eluate was mixed with Pico-Fluor TM30 (Packard, used as a scintillation cocktail) in an FMI ultrograd mixing unit. The normalized areas of the radioactivity peaks were computed and visualized by a Compaq Deskpro computer unit. Preparative HPLC and resolution were performed with only on-line UV detection. Conditions of analytical and preparative HPLC are described in the text. Radioactivity of known aliquots was measured by liquid scintillation counting (Packard Tri-Carb 4530), using Ultima Gold (Packard) as scintillation cocktail. The specific activity was determined by measuring the UV absorbance (on HPLC) relative to the absorbance

of known amounts of injected unlabelled standards and the radioactivity contents in the HPLC-eluate by means of liquid scintillation counting. ^{1}H-NMR spectra were obtained on a 400 MHz spectrometer (Brüker, AMX 400). Chemical shifts are reported in ppm with tetramethylsilane as internal reference.

2-Imino-α-phenyl-3-[2-^{14}C]thiazolidineethanol hydrochloride (2)

A mixture of α-[2-chloroethyl)aminomethyl]benzenemethanol hydrochloride (**1**, 1.65 g, 7.00 mmol), [^{14}C]-thiourea (0.532 g, 7.00 mmol, 14.1 GBq)[10] and hydrochloric acid 0.1 N (10.5 ml) was stirred at reflux for 12 h. The mixture was then cooled to 80°C and sodium chloride (700 mg) was introduced. The mixture was brought to reflux and was then allowed to slowly cool to room temperature. After 5 h, the precipitate was filtered, washed with acetone (18 ml) and dried to the air to leave **2** (1.40 g, 10.9 GBq, yield 77.4%). ^{1}H-NMR (DMSO): 3.78 (3H, s), 4.06 (2H, s), 4.83 (2H, s), 7.59–8.03 (5H, m), 9.88 (1H, br).

(±)-2,3,5,6-Tetrahydro-6-phenyl[7α-^{14}C]imidazo[2,1-b]thiazole hydrochloride (3, tetramisole hydrochloride)

To nitrogen-covered, ice cooled and stirred concentrated sulphuric acid (5.0 ml), **2** (1.40 g, 5.42 mmol) was slowly added. After addition, the solution was stirred for 5 min more at ice bath temperature and was then allowed to slowly come to room temperature. Crushed ice was added and the mixture was made strongly alkaline with ammonium hydroxide (conc.), while maintaining the temperature below 25°C. The mixture was extracted with chloroform (50 ml) and the organic layers were washed with brine (10 ml), dried on magnesium sulphate and filtered. HCl-saturated 2-propanol was added to the filtrate, which was evaporated to yield impure tetramisole hydrochloride. The solid was dissolved in water (4.0 ml)-methanol (0.3 ml) and purified via preparative HPLC (Hypersil ODS (5 μm), column 7.1 mm ID × 300 mm; flow rate: 3.6 ml/min; eluate: acetonitrile–water–diisopropylamine (32–68–0.2, v/v/v)). The purified fractions were combined, evaporated, dissolved in methanol and acidified with HCl-saturated 2-propanol to pH 1, evaporated and dried for 18 h at 60°C under vacuum to yield HPLC-pure **3** (1.16 g, 9.73 GBq, yield 89.2%).

Resolution of 3 into levamisole hydrochloride (6) and dexamisole hydrochloride (5) and racemization of dexamisole to tetramisole

A mixture of (+)-(L)-2-[[(4-methoxyphenyl)sulfonyl]amino]-pentanedioic acid (3.19 g, 10.1 mmol) and sodium hydroxide 2 N (10.3 ml, 20.6 mmol) was stirred for 10 min at 60°C. A mixture of **3** (1.16 g, 4.82 mmol, 9.73 GBq), unlabelled tetramisole hydrochloride (1.16 g, 4.82 mmol) and unlabelled levamisole hydrochloride (1.25 g, 5.18 mmol) was introduced. After 5 more minutes, sodium citrate $2H_2O$ (60 mg, 0.26 mmol) was added. The solution was removed from its heat source and slowly acidified at 40°C with concentrated hydrochloric acid to pH 4.5 and then with hydrochloric acid 1 N to pH 4.2. Stirring was continued for 2 h more after which the precipitate was filtered, washed with water (3×5.0 ml) and dried to the air to give **4** (4.90 g, 94%).

The filtrate was, under nitrogen atmosphere, strongly alkalinized with 50% sodium hydroxide, stirred for 5 min and extracted with chloroform (12 ml). The chloroform layers were dried on magnesium sulphate and evaporated under nitrogen to give a residue of dexamisole (**5**-base, 0.93 g). The residue was dissolved in toluene (10.0 ml) and *N,N*-dimethyl-formamide (0.23 ml). Potassium-*tert*-butoxide (0.16 g) was added under nitrogen at 70°C. The mixture was stirred for 90 min at 70°C after which it was cooled to 50°C. The reaction mixture was washed twice with water and was separated after 10 min. The toluene layer was dried, filtered, to the filtrate was slowly added a solution of HCl-saturated 2-propanol (2 ml) and the whole was evaporated under aspirator pressure at 40°C to give tetramisole hydrochloride (**3**, 1.07 g, 4.40 mmol). With this material the above described resolution cycle was repeated five times. Combination of all the diastereomeric salts (**4**, 18.2 g) and crystallization from nearly boiling water (180 ml) gave pure **4** (16.9 g, 32.4 mmol) with a radioactivity of approx. 7.2 GBq.

The obtained **4** was suspended in water (25 ml) and, under nitrogen atmosphere, strongly alkalinized with sodium hydroxide 10 N. The mixture was extracted with chloroform (50 ml), the chloroform layers were washed once with brine, dried on magnesium sulphate, filtered and evaporated to give levamisole **6**-base (6.51 g, 3.19 mmol). Unlabelled levamisole hydrochloride (16.1 g, 67.0 mmol) was dissolved in water (25 ml), alkalinized and isolated as above to give unlabelled levamisole (13.5 g, 66.1 mmol). Both the labelled and the unlabelled levamisole were dissolved in toluene and combined (200 ml). The solution was acidified with HCl-saturated 2-propanol and the mixture was stirred

for 20 h. The precipitate was filtered and dried for 8 h at 75°C under vacuum to supply levamisole hydrochloride **6** (23.5 g) with a total radioactivity of 7.20 GBq (radiochemical yield: 51.0%). The material had a specific activity of 73.6 MBq/mmol. The radiochemical purity, as determined via HPLC (Hypersil ODS (5 μm), 4.6 mm ID × 300 mm; flow rate: 1.5 ml/min; eluate: acetonitrile-water-diisopropylamine (35-65-0.2, v/v/v)) was 99.8% with an enantiomeric excess (Chiralcel OJ (10 μm), 4.6 mm ID × 300 mm; flow rate: 0.7 ml/min; eluate: hexane–ethanol (80–20, v/v)) of 99.4%. The material was HPLC identical to unlabelled material.

Glycine, N-(2-oxo-2-phenylethyl) methyl ester hydrochloride (8)

Glycine methyl ester hydrochloride (1.24 g, 10.0 mmol) was dissolved in 4-methyl-2-pentanone (20.0 ml) and liberated from its salt by stirring it for 18 h at room temperature with sodium carbonate (3.52 g, 30 mmol). To the stirred mixture was dropped a solution of α-bromo acetophenone (**7**, 1.79 g, 9.0 mmol) in 4-methyl-2-pentanone (10.0 ml). After 1 h, the mixture was filtered and the solids were washed with hot 4-methyl-2-pentanone (14.0 ml). To the filtrate was added a 6 N solution of hydrochloric acid in 2-propanol (1.6 ml), causing the product to precipitate as the HCl salt. It was filtered, washed with 4-methyl-2-pentanone and dried to the air. Crystallisation from acetonitrile (60 ml) afforded **8** (812 mg, 37.2%).

Methyl[12-^{14}C]-2-oxo-4-phenyl-4,5-didehydroimidazolidineacetate (9)

To a solution of ^{14}C-labelled potassium cyanate (161 mg, 2.0 mmol, containing 1.68 GBq of radioactivity)[†] in a mixture of methanol–water (70–30, v/v; 1.75 ml) was dropped a solution of **8** (487 mg, 2.0 mmol) in the same solvent mixture (2.25 ml). The solution was stirred for 1 h at room temperature, causing a precipitate to form. The mixture was brought to reflux temperature and was stirred for 1 h. It was then stirred for 18 h at room temperature and then for 1 h at 0°C. The precipitate was filtered and washed with ice-cooled water to yield **9** (330 mg, 71%). The material was used as such in the next reaction step.

[†]The material was purchased from NEN, Brussels, Belgium.

Methyl (±)-[12-^{14}C]-2-oxo-4-phenyl-1-imidazolidineacetate (10)

A mixture of **9** (330 mg, 1.42 mmol), palladium on carbon (10%, 50 mg) and methanol (15 ml) was hydrogenated at 50°C for 18 h. It was filtered hot over celite, and the celite was rinsed several times with small portions of hot methanol. The filtrate was concentrated at aspirator pressure and the residue was recrystallized from 2-propanol (5.0 ml) to yield **10** (323 mg, 98%, containing 1.05 GBq of radioactivity). It was 92.7% HPLC pure (Kromasil KR100-10 (5 μm), 4.6 mm ID × 300 mm; flow rate: 1.0 ml/min; programmed run consisting of an isocratic run for 20 min with aqueous 0.1 M ammonium acetate solution brought to pH 9.0–methanol–acetonitrile (64–18–18, v/v/v), followed by a 5 min linear gradient run to solvent composition (10–45–45, v/v/v) and again a 10 min isocratic run at the latter composition).

Methyl (±)-[12-^{14}C]-2-thiooxo-4-phenyl-1-imidazolidineacetate (11)

A suspension of **10** (323 mg, 1.38 mmol) and Lawesson's reagent (279 mg, 0.69 mmol) in toluene (7.0 ml) was stirred for 10 h at 90°C. The obtained light yellow solution was allowed to cool to room temperature, causing the product to crystallize. The crystals were filtered, washed with toluene (1.0 ml) and diisopropyl ether (4.0 ml) and dried to the air to give **11** (235 mg). The HPLC radiochemical purity (Kromasil KR100-10 (5 μm), 4.6 mm ID × 300 mm; flow rate: 1.0 ml/min; programmed isocratic run for 10 min with aqueous 0.1 M ammonium acetate solution (pH 9.0)–methanol–acetonitrile (48–26–26, v/v/v), followed by a 5 min linear gradient run to solvent composition (20–40–40, v/v/v) and again a 10 min isocratic run at the latter solvent composition) was 92.6%.

(S)-[2-^{14}C]-2-thiooxo-4-phenyl-1-imidazolidineacetic acid (12)

The obtained **11** (293 mg) was stirred with sodium hydroxide **1** N (1.25 ml) for 1.5 h at 80°C. The solution was then acidified with conc. HCl (0.12 ml), causing the product to precipitate. The mixture was stirred for 30 min and the precipitate was then filtered, washed with water and diisopropyl ether and dried to the air to give the racemate of **12** (183 mg). Part of this material (130 mg) was dissolved in methanol (1.2 ml) and purified via preparative HPLC by injection of 50 μl portions with an extra added 200 μl of

water (Kromasil RP18 100-10, 7.1 mm ID × 300 mm; flow rate: 4.0 ml/min; UV detection at 235 nm, eluate: water-acetonitrile-formic acid (72-28-0.2; v/v/v)). The combined product peaks were concentrated at 40°C (aspirator pressure), dissolved in acetonitrile (20.0 ml) and again thoroughly concentrated at 30°C (aspirator pressure). The residue was dissolved in ethanol (10.0 ml) and resolved by means of chiral HPLC by multiple 30 µl injections (Chiralpak AD (10 µm), 4.6 mm ID × 50 min; flow rate 3.0 ml/min; UV detection at 250 nm, eluate: hexane–ethanol–trifluoroacetic acid (76–24–0.03; v/v/v)). The desired material was the first eluting peak, as verified with material obtained from *in vivo* samples. Those fractions were combined and concentrated at 30°C (aspirator pressure), dissolved in acetonitrile (20.0 ml) and again thoroughly concentrated at 30°C (aspirator pressure). The residue was dissolved in acetonitrile (25.0 ml) and was stored as such. It contained the desired metabolite at a purity of 98.7% (Kromasil RP18 100-5, 4.6 mm ID × 300 mm; flow rate: 1.0 ml/min; programmed run consisting of an isocratic run for 20 min with aqueous 0.1 M ammonium acetate solution (pH 9.0)–methanol-acetonitrile (80–10–10, v/v/v), followed by a 5 min linear gradient run to solvent composition (10–45–45, v/v/v) and again a 15 min isocratic run at the latter solvent composition) and with an enantiomeric excess of 100% (Chiralpak AD (10 µm), 4.6 mm ID × 50 mm; flow rate: 0.7 ml/min; eluate: hexane–ethanol–trifluoroacetic acid (80–20–0.03, v/v/v)). It contained 194 MBq of radioactivity, overall radiochemical yield: 16.2%. The specific activity was 895 MBq/mmol. The material was HPLC and NMR identical to unlabelled material.

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