

## SYNTHESIS OF $^3\text{H}$ -LABELLED LEVAMISOLE

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### SUMMARY

Levamisole, the levo isomer of tetramisole, is a potent anthelmintic also showing immunotropic properties. Renewed interest in the latter domain necessitated the synthesis of specifically  $^3\text{H}$ -labelled levamisole of high specific activity.

Racemic 1-(2-bromophenyl)-1,2-ethane diamine dihydrochloride (V) was obtained via bromination of 1-(2-bromophenyl)ethanone (I), followed by reaction with 2,5-pyrrolidinedione, Leuckart amination and hydrolysis. The racemate was then resolved by successive salt formation with (S)-(-) and (R)-(+)-tartaric acid. The liberated (S)-(+)-base of VI was dehalogenated with approximately 30 Ci of tritium gas and immediately ring-closed with carbon disulfide to VIII. The HPLC-purified material was finally cyclized with 1,2-dibromoethane to (S)-(-)-2,3,5,6-tetrahydro-6-[2-Tlphenyl-imidazo-(2,1-b)-thiazole IX and the isomerically ringclosed (S)-2,3,5,6-tetrahydro-5-[2-Tlphenyl-imidazo-(2,1-b)-thiazole X, of which levamisole IX was isolated via HPLC. The radiochemical yield over the cyclization steps was 28.2 %, the specific activity was 10.82 Ci/mmol, and the product was 99.9 % HPLC pure.

**Key-words:**  $^3\text{H}$ -levamisole, anthelmintic, immunotropic, optical resolution

### INTRODUCTION

Levamisole is the levo isomer and the more active form of the broad spectrum anthelmintic d,l-tetramisole.<sup>1-4</sup> The drug is active against most

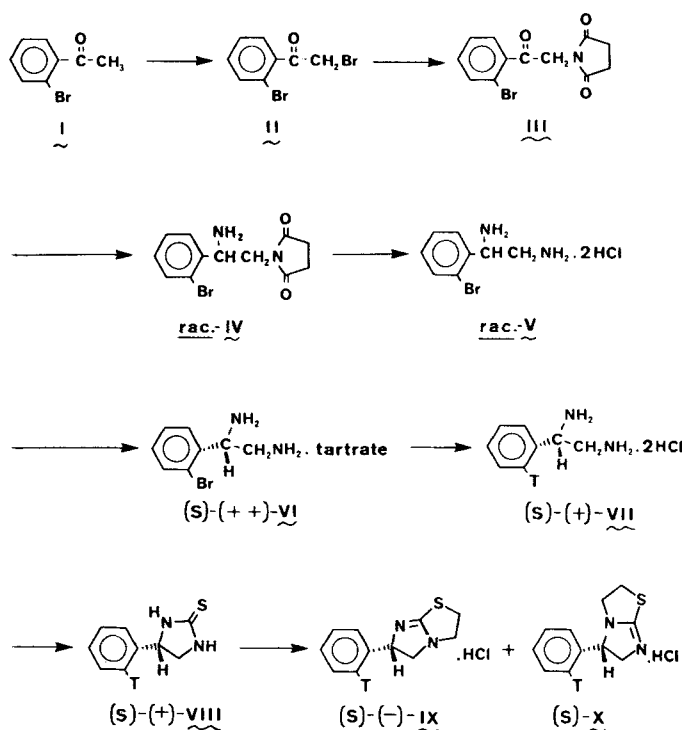
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nematodes of animals and man and has been extensively used since it was marketed in 1968.<sup>5</sup> The first evidence of immunotropic properties was obtained in 1971.<sup>6,7</sup> It exerts a marked influence on the immunological responses of animal and man, particularly in those individuals whose immune defences have for some reasons become impaired.<sup>8,9</sup> Notwithstanding the observed effects, the mechanism of action on the immuno response system has only recently been resolved.<sup>10,11,12</sup> As levamisole is still the subject of intensive research, we were urged to synthesize specifically <sup>3</sup>H-labelled levamisole with a high specific activity.

Because of possible metabolic 3- and 4-hydroxylation, the stable 2-position of the phenyl group was chosen as suitable for labelling. As dehalogenation of 2-bromo tetramisole led to complete destruction of the molecule, the obvious way of resolution and reductive dehalogenation with tritium of 2-bromo-

#### Scheme I

#### Synthesis of <sup>3</sup>H-Levamisole



levamisole was cut off. Earlier, the synthesis of d,l-tetramisole-<sup>3</sup>H with a low specific activity and its resolution by salt formation with (S)-(-)- and (R)-(+)-camphorsulphonic acid was carried out in our laboratories. Such a procedure however would be quite unfeasible for the preparation of the tritiated drugs at high specific activity. Therefore a more convenient method was worked out, based on the stereospecific synthesis of both optical isomers of tetramisole.<sup>2</sup> It comprised a resolution of non-radioactive 1-(2-bromophenyl)-1,2-diaminoethane, an introduction of the tritium label by catalytic dehalogenation followed by two consecutive ring closures. The latter approach is described in scheme I.

#### METHODS AND MATERIALS

Purification with normal-phase liquid chromatography (LC) was conducted at atmospheric pressure on columns, slurry packed with silica (Kieselgel 60, Merck art. 7734). The column sizes and solvent systems used are described in the text where appropriate. Purification by analytical reversed-phase high-performance liquid chromatography (HPLC) was conducted on Hypersil ODS (5 μm) bonded phase packed in a narrow-bore stainless steel column (4.6 x 300 mm), with a flow rate of 1 ml/min and U.V. detection at 250 nm. The required solvent compositions are described in the text. The apparatus used for radioactivity measurements and for analytical HPLC has been described earlier.<sup>13</sup>

The apparatus used for purification by means of HPLC consisted of a Gilson model 303 pump, a pumphead 25 S and a Varian U.V. detector. The samples were injected by a Rheodyne 7125 injector.

The specific activity was determined by measuring the U.V.-absorbance (on HPLC) relative to the absorbance of known levels of injected unlabelled standards and the radioactivity contents in the HPLC-eluate by means of liquid scintillation counting.

The specific rotation of VI and IX was measured in a Perkin-Elmer 141 Polarimeter.

Tritiation of VI to crude VII, including the removal of labile tritium was

carried out at the National Institute for Radio-Elements, B-6220-Fleurus, Belgium.

1-[2-(2-bromophenyl)-2-oxoethyl]-2,5-pyrrolidinedione III

A solution of bromine (120 g, 0.75 mol) in chloroform (200 ml) was dropped to a solution of 1-(2-bromophenyl)ethanone (I, 150 g, 0.75 mol) in chloroform (300 ml), stirred at room temperature. After stirring for 18 h, the mixture was washed with a 10 %  $\text{NaHCO}_3$  aqueous solution, the organic layer was dried over  $\text{MgSO}_4$  and evaporated to leave 205 g of crude II which was G.C. 72 % pure and was used as such. To a stirred solution of 2,5-pyrrolidinedione (65 g, 0.6 mol) in *N,N*-dimethylformamide (DMF, 300 ml) was added portionwise sodium hydride (50 % in mineral oil, 28.5 g, 0.6 mol). The mixture was then brought to 60° C and stirred until hydrogen evolution ceased. Crude II (205 g, containing 148 g of pure material, 0.53 mol), dissolved in DMF (50 ml) was dropped to the formed anion and the whole was stirred at 80° C for one hour. The reaction mixture was then poured into water (3000 ml), the precipitate was filtered off and crystallized from 4-methyl-2-pentanone yielding 120 g (59 %) of pure III, mp 161.5° C. Anal. Calcd. for  $\text{C}_{12}\text{H}_{10}\text{BrNO}_3$ : C, 48.67; H, 3.40; N, 4.73; Br, 26.98 Found: C, 48.58; H, 3.41; N, 4.66; Br, 26.90 %.

1-[2-amino-2-(2-bromophenyl)ethyl]-2,5-pyrrolidinedione IV

Ammonium formate (106 g, 1.67 mol) was heated for 20 minutes at 160° C and water was distilled off. At 60° C, III (120 g, 0.405 mol) was added and the whole was stirred at 180° C for 3.5 hours. The mixture was cooled to 110° C and boiling water (120 ml) was added. After stirring for 30 minutes the supernatant was decanted and the residue was refluxed for 2.5 h with 6 N hydrochloric acid (80 ml). The reaction was cooled to room temperature and filtered. The filtrate was alkalized and extracted with chloroform. The organic solution was dried over magnesium sulphate, concentrated and the oily residue was purified by column chromatography (column size: 300 mm x 100 mm i.d.) over silica gel using chloroform:methanol (98:2; by volume) as an eluate. Crystallization from 2-propanol yielded 17.9 g (14.8 %) of pure IV, mp 125.5° C.

1-(2-bromophenyl)-1,2-ethanediamine dihydrochloride (V)

The 2,5-pyrrolidinedione derivative IV (17.9 g, 0.060 mol) was hydrolyzed by refluxing for 12 hours with sodium hydroxide (18 g, 0.450 mmol) in water (85 ml). The reaction was cooled and extracted with chloroform. The organic layer was dried over magnesium sulphate, and the solvent was evaporated at aspirator pressure. The residue was dissolved in methanol and acidified with hydrogen chloride-saturated 2-propanol. The dihydrochloric acid crystallized from the solution yielding 13.6 g (71 %) of pure material, mp 300° C. Anal. Calcd. for C<sub>8</sub>H<sub>11</sub>BrN<sub>2</sub>·2HCl: C, 33.36; H, 4.55; N, 9.73 Found: C, 33.60; H, 4.71; N, 9.71 %.

Resolution of V

To the free base of V (10.2 g, 0.047 mol), dissolved in 60 ml of methanol was added a solution of (S)-(-)-tartaric acid (7.1 g, 0.047 mol) in methanol (55 ml). After two days the precipitate was filtered off and crystallized from a water:methanol (130 ml:60 ml) mixture. The crystals were once more crystallized from water (60 ml) to yield 3.6 g of (R)-(-)-1-(2-bromophenyl)-1,2-ethanediamine.(S)-(-)-tartrate (1:1), mp 231.5° C,  $[\alpha]_D^{20} = -7.25^\circ$  (c = 0.5 % in water). The combined filtrates were concentrated, the residue was taken up in brine and basified with a 10 N sodium hydroxide solution. The oily precipitate was extracted with chloroform, dried over MgSO<sub>4</sub> and the solvent was evaporated. The residue (7.15 g, 0.033 mol) was dissolved in methanol (60 ml) and a solution of (R)-(+)-tartaric acid (5.0 g, 0.033 mol) in methanol (40 ml) was added. The precipitate was filtered off and crystallized from water:methanol (90 ml:40 ml), yielding 5.4 g of (S)-(+)-1-(2-bromophenyl)-1,2-ethanediamine.(R)-(+)-tartrate (1:1) (VI), mp 231.5° C,  $[\alpha]_D^{20} = +7.67^\circ$  (c = 0.5 % in water). The optical rotation was checked by reductively dehalogenating the material with hydrogen over palladium on charcoal (10 %) to (S)-(+)-phenyl ethylenediamine; it proved to be identical with values from the literature.<sup>2</sup>

(S)-(+)-1-[2-T]phenyl-1,2-ethanediamine (VII)

A mixture of VI (53.6 mg, 0.147 mmol), Pd10 % on charcoal (100 mg), pow-

dered calcium oxide (200 mg) and a 4 % thiophene solution in diisopropyl ether (0.03 ml) was dehalogenated in tetrahydrofuran (5 ml) with approx. 30 Ci of tritium gas for 18 hours at room temperature. The excess of tritium gas was adsorbed on active charcoal and the reaction mixture was lyophilized. The residue was dissolved in tetrahydrofuran and filtered through a Millipore filter (MIREX LSWP 0.2500). After removal of labile tritium by lyophilisation with methanol, the product was crudely purified by column chromatography over silica (column size: 180 mm x 12 mm i.d., eluate: chloroform containing 10 % of methanol saturated with ammonia). The fractions containing the product were combined, acidified with hydrogen chloride-saturated 2-propanol and evaporated to give 20 mg of VII (70 % pure according to HPLC) with a total radioactivity of 641 mCi. The material was used as such in the next reaction step.

(S)-(+)-4-[2-T]phenyl-2-imidazodimethione (VIII)

A mixture of VII (10 mg, 0.048 mmol; 70 % HPLC-pure, 320 mCi), NaOH 1N (0.20 ml) and water (0.60 ml) was stirred for 15 minutes under nitrogen. Ethanol (0.80 ml) was added and the reaction vessel was closed with a rubber seal, maintaining the nitrogen atmosphere. Carbon disulphide was introduced in two portions via syringe (2 x 0.03 ml; 76 mg, 1.0 mmol) with an interspace of 10 minutes each and the reaction was stirred for 2 h at room temperature. The rubber seal was replaced by a condenser and stirring was continued for 13 h at 105° C. The mixture was acidified with 10 N HCl (0.02 ml) and kept for 45 minutes at 105° C. It was then cooled to room temperature and extracted with chloroform (4 x 2 ml). The chloroform solution was dried over  $MgSO_4$ , concentrated and the residue (dissolved in 0.25 ml of DMF) was purified by HPLC [Eluate: acetonitrile:water:diisopropylamine (14:86:0.2 v/v)]. Pure VIII (99.0 %), containing 187.5 mCi of radioactivity (83.7 %, based upon 224 mCi of pure VII), was obtained. The elution solvent was evaporated in vacuo and the residue was used as such in the next reaction step.

(S)-(-)-2,3,5,6-tetrahydro-6-[2-T]phenyl-imidazo[2,1-b]thiazole monohydrochloride (levamisole, IX)

A solution of VIII (186 mCi) in 2-propanol (2.5 ml) and 16.8 % aqueous potassium hydroxide (0.03 ml, 0.09 mmol) respectively were added to a mixture of 1,2-dibromoethane (113 mg, 0.60 mmol), sodium bicarbonate (7 mg, 0.083 mmol) and 2-propanol (0.50 ml) stirred at 80° C under nitrogen. The reaction was stirred for 16 h at 80° C, the solvents were evaporated and to the residue was added water (1.5 ml) and 10 N HCl (0.05 ml). The acidic aqueous solution was extracted with chloroform (3 x 1 ml), basified with 50 % NaOH solution (0.05 ml) and again extracted with chloroform (5 x 1 ml). The latter solution contained 138 mCi of radioactivity, divided over products IX and X (51 and 47 % respectively according to HPLC) [eluate: acetonitrile:water:diisopropylamine (30:70:0.2 v/v)]. The solvent was evaporated at aspirator pressure and the remaining oil was dissolved in methanol (5 ml) and acidified with hydrogen chloride-saturated 2-propanol, which was then concentrated under a gentle stream of nitrogen. The residue, dissolved in 0.6 ml of the eluate (acetonitrile:water:diisopropylamine; 25:75:0.2 by volume) was purified by HPLC. The eluate was evaporated and the residue was dissolved in ethanol which was then acidified with hydrogen chloride-saturated 2-propanol. The levamisole-monohydrochloride solution was again concentrated and dissolved in 1.50 ml of methanol to measure the optical rotation, after which the solution was further diluted with 60.0 ml of ethanol to a stock solution of 61.5 ml. It contained 99.9 % pure  $^3\text{H}$ -levamisole monohydrochloride (62.7 mCi, radiochemical yield 33.7 %) with a specific activity of 10.8 Ci/mmol (44.8 mCi/mg) and a  $[\alpha]_D^{20}$  of  $-138.8^\circ$  ( $c = 0.934$  % in methanol).<sup>2</sup>

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