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SYNTHESIS OF (R)-(+)-^{3}H-ETOMIDATE

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SUMMARY

Etomidate, (R)-(+)-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate, is a short-acting hypnotic. A new synthesis, featuring optical resolution on a non-radioactive precursor and introduction of the tritium label by reductive dehalogenation, is described.
The title compound was obtained at a specific activity of 3.77 Ci/mmol and a 99.9 % HPLC purity.

Key words: ^{3}H-Etomidate, Hypnotic, Optical resolution, Reductive dehalogenation.

INTRODUCTION

Etomidate, (R)-(+)-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate (Hypnomidate®) is a short-acting hypnotic[1] in animals[2] and man.[3, 4] Only

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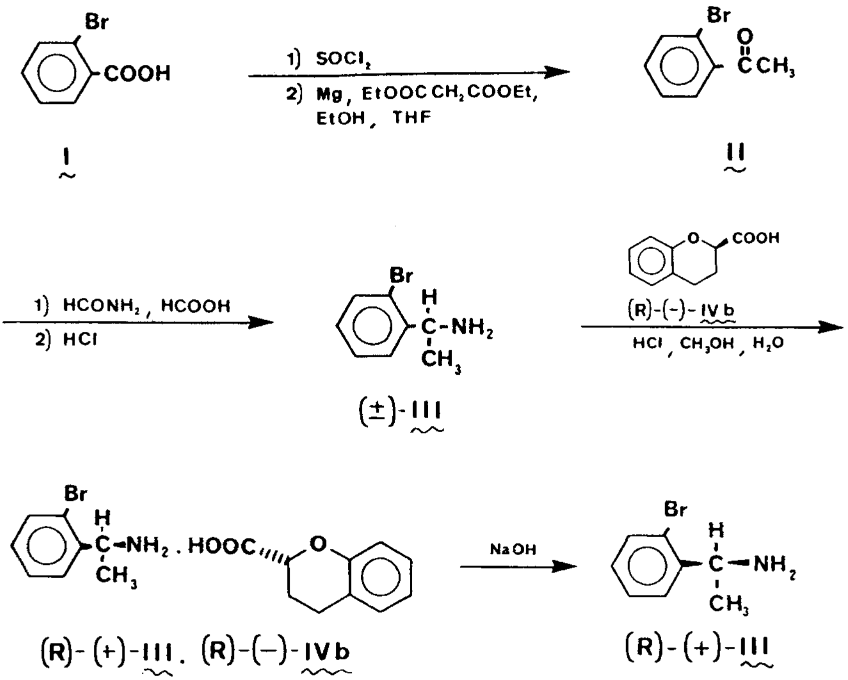
the (R)-(+)-isomer possesses hypnotic activity. Etomidate is a non-barbiturate hypnotic without analgesic properties, of which the principal advantages are its lack of cardiovascular and respiratory effects and its absence of histamine release. The drug possesses anticonvulsive properties and protects the brain tissue against cell damage by hypoxia.[5-8]

The synthesis of racemic etomidate has been described by Godefroi et al.[1] Formerly, the tritium labelled (R)-(+)-isomer had been obtained *via* resolution of the tritiated racemic metomidate,[9] a procedure requiring six "radioactive" steps and which, inherent to the resolution gave material with a relatively low specific activity. Because for research purposes a higher specific activity was needed, a new approach was developed. It essentially differed in the separation of optical isomers, which was carried out on a suitable non-radioactive precursor, and it allowed the tritiation to be performed in the last step. This new route minimalized the number of radioactive manipulations, it resulted in a higher yield and led to a 40-80 fold increase of the specific activity.

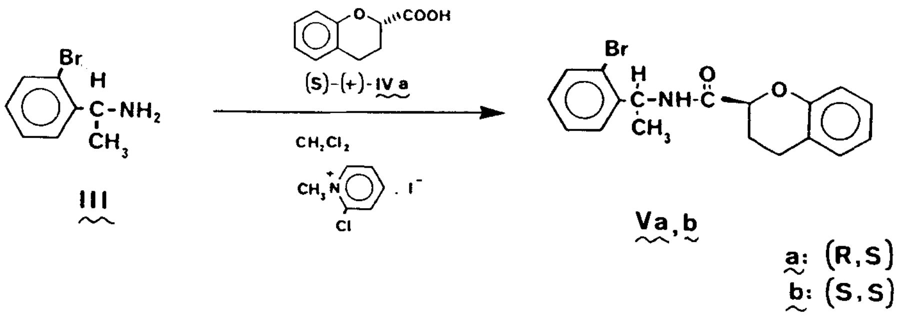
Conversion of 2-bromobenzoic acid I (Scheme I) with thionyl chloride gave 2-bromobenzoyl chloride (96 %), which was brought into reaction with freshly prepared diethyl ethoxymagnesiomalonate. The crude reaction mixture was hydrolized and decarboxylated to afford 94 % of 2-bromoacetophenone II. Leuckart amination gave racemic amino-1-(2-bromophenyl)ethane III (76 %). Several attempts were undertaken to obtain a resolution of amine III with optically pure acids by either amide formation-hydrolysis or by salt formation. (L)-(+)-Tartaric acid, (+)-(10)-camphorsulfonic acid and (L)-(+)-2-[(4-methylphenyl)sulfonylamino]pentanedioic acid gave a very poor yield of amides or salts with hardly any resolution. Use of (S)-(+)-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic acid IVb however turned out to be a very fortunate choice. Salt formation and recrystallization afforded 77.4 % of optically pure material which after alkalinization gave 72.7 % of (R)-(+)-III (99.9 % G.C. pure). Reductive dehalogenation of the resolved amine with hydrogen over Pd-10 % on charcoal and comparing the rotation with authentic (R)-(+)-α-methylbenzene methanamine proved the absolute configuration to be as indicated.

Scheme I



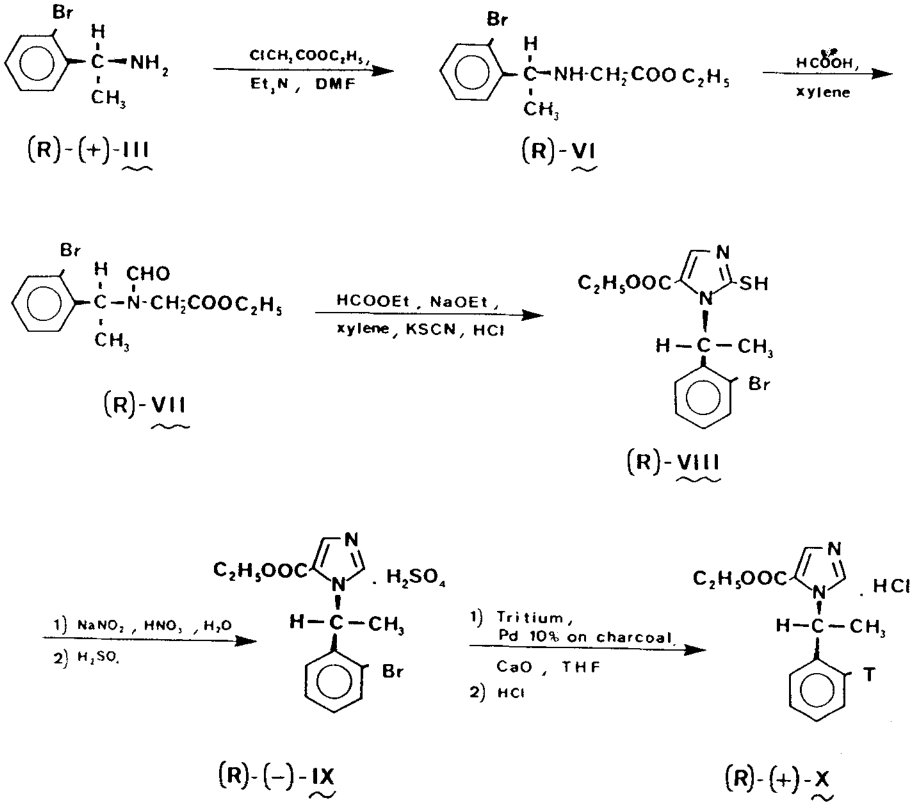
Amide formation of the racemate with the acid (or its enantiomer IVa), using a catalytic amount of N-methyl-2-chloro pyridinium iodide gave 100 % yield of both diastereomers which could be gas chromatographically separated, and hence formed a perfect tool to determine the optical purity of (R)-(+)-III (Scheme II).

Scheme II



The optically pure III was brought into reaction with ethyl chloroacetate to afford 88 % of glycine derivative (R)-VI (Scheme III). N-formylation to (R)-VII (95.3 %) and ringclosure with potassium isothiocyanate finally gave the etomidate skeleton with the proper stereochemistry ((R)-VIII, 96.3 %). Further modification by elimination of the mercapto group afforded TLC-pure (R)-(-)-IX-base (88 %), the sulfate (IX, 82 %) of which was used as the final precursor for the tritiation. The correct stereochemistry of the 2-bromoetomidate (IX) was proved by reductive dehalogenation with hydrogen, followed by salt formation with hydrochloric acid (69.4 %) to non-radioactive etomidate.HCl. The optical rotation, and hence the absolute configuration proved to be identical to the target molecule. Tritiation, performed in the same manner as the hydrogenation afforded (R)-(+)-^{3}H-X in a 32 % yield.

Scheme III



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 of the etomidate-base by preparative reversed-phase high performance liquid chromatography (HPLC) was conducted on Hypersil ODS (5 μm) bonded phase in a narrow-bore stainless steel column (7.1 x 300 mm), with a flow rate of 4 ml/min and U.V. detection at 250 nm. 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 apparatus used for radioactivity measurements and for analytical HPLC has been described earlier.[10]
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. Gas chromatography was performed on a Varian 3700 gas chromatograph using a 1 m OV-17 carbowax column with a linear temperature gradient run of 26 minutes from 50° C to 310° C.
The specific rotation of (R)-(+)-III.IVb, (R)-(+)-III, IX-base, IX-sulphate and non-labelled X was measured in a Perkin-Elmer 141 Polarimeter. Tritiation of IX to X, including the removal of labile tritium was carried out at the National Institute for Radio Elements, B-6220 Fleurus, Belgium.

2-Bromoacetophenone II

A mixture of magnesium curls (29.2 g, 1.2 mol) and diethyl malonate (10 g, 0.06 mol) in absolute ethanol (92 g, 2 mol) was brought to reflux. Tetrachloromethane (0.5 ml) was introduced to initiate the reaction. A solution of diethyl malonate (166.2 g, 1.04 mol) in 1300 ml of dry tetrahydrofuran was dropped to the reaction in such a rate that spontaneous reflux was maintained. Stirring at reflux temperature was continued for 1 h until all magnesium had disappeared. Freshly distilled 2-bromobenzoyl chloride (211.7 g, 0.96 mol, bp. 117-118° C (20 mm)), prepared from 2-bromobenzoic acid I (201 g, 1 mol) and thionyl chloride (216 ml, 3 mol), was dissolved in 200 ml of tetrahydrofuran and quickly introduced. After refluxing for 45 min, the solution was cooled on ice and hydrolized with 6.5 N sulfuric acid (240 ml). Tetrahydrofuran (1400 ml) was distilled off, 300 ml of water was added and the solution was extracted with ether (3 x 400 ml). The combined organic layers were dried on $MgSO_4$, filtered and evaporated at aspirator pressure. The oily residue (380 g) was refluxed for 18 h in a solvent mixture consisting of acetic acid (750 ml), sulfuric acid (50 ml) and water (250 ml). The reaction mixture was concentrated to half its volume and the remainder was slowly poured into 800 ml of 50 % sodium hydroxide, meanwhile keeping the temperature below 40° C by addition of ice. Water (500 ml) was added and the solution was extracted with diethyl ether, which was dried over $MgSO_4$, filtered and evaporized. Distillation afforded 181 g (94 % based upon **I**) of **II**, bp. 115-117° C (20 mm).

(±)-1-Amino-1-(2-bromophenyl)ethane **III**

A mixture of **II** (161.6 g, 0.81 mol), formamide (200.5 g, 4.45 mol) and formic acid (82.0 g, 1.78 mol) was stirred for 12 h at 190° C. The reaction was then brought to room temperature, poured into water (900 ml) and extracted with chloroform (2 x 250 ml). The chloroform layers were dried on $MgSO_4$, filtered and evaporated. The oily residue was refluxed with concentrated hydrochloric acid (270 ml) for 2 h. The cooled mixture was washed with diethyl ether and the water layer was cooled on ice and alkalinized with concentrated NH_4OH. The mixture was extracted with diethyl ether (3 x 250 ml), the combined organic solutions were washed with brine (1 x 250 ml), dried over $MgSO_4$, filtered and evaporated to leave 152 g of residue. Vacuum distillation afforded 121.7 g (75 %) of G.C. pure material, bp. 110° C (20 mm).

1(R)-1-(3,4-dihydro-2(S)*H*-1-benzopyran-2-carboxamido)-1-(2-bromophenyl)ethane **Va** and 1(S)-1-(3,4-dihydro-2(S)*H*-1-benzopyran-2-carboxamido)-1-(2-bromophenyl) ethane **Vb**

A nitrogen-covered solution of (S)-(+)-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic acid (0.89 g, 5 mmol, **IVa**) and triethylamine (0.99 g, 10 mmol) in dichloro methane (40 ml) was stirred for 30 min at room temperature. 2-Chloro-1-methyl pyridinium iodide (1.3 g, 5 mmol) was added, after 1.5 h followed by a solution of racemic **III** (1.0 g, 5 mmol) in 2 ml of dichloromethane. Stirring was continued for 20 h at room temperature. The reaction mixture was then washed with sodium hydroxide solution (0.1 N, 2 x 20 ml), aqueous hydrochloric acid (0.1 N, 2 x 20 ml) and water (2 x 20 ml), dried on $MgSO_4$ and concentrated to leave an oily residue (1.80 g, 96 %), consisting of gas chromatographically equal amounts of diastereomers **Va** and **b** (retention times of 1447 and 1479 seconds respectively).

Optical resolution of III

To a stirred solution of racemic III (70.8 g, 354 mmol) in 0.5 N hydrochloric acid (708 ml, 354 mmol), warmed to 85° C was dropped an at 85° C preheated solution of (R)-(-)-3,4-dihydro-2*H*-1-benzopyran-2-carboxylic acid (31.5 g, 177 mmol, IVb) in aqueous 10 % NaOH (70.8 ml, 177 mmol). After 4.5 h the formed precipitate was filtered and air-dried to afford crystalline material (63 g, 94 %), consisting for 96 % of the IVb salt of (R)-(+)-III and 4 % of the IVb salt of (S)-(-)-III, as controlled by above reaction to amide Va and b. The solids were recrystallized once from water (1250 ml) - methanol (1000 ml) to yield 52 g of 99.4 % pure (R)-(+)-III. IVb salt (77.7 %), $[\alpha]_{365}^{25}$ = - 10.805° (1 % in methanol). The crystals were suspended in water (400 ml) and the solution was strongly alkalinized with 50 % aqueous sodium hydroxide (20 ml). The amine was extracted with ether (3 x 150 ml), dried on $MgSO_4$, filtered and concentrated to give (R)-(+)-III (25.7 g, 94 %, overall-yield 72.7 %, 99.9 % G.C. pure), $[\alpha]_D^{25}$ = + 32.524 (1 % in methanol). Reductive dehalogenation of the material to α-methylbenzene methanamine and comparing it with authentic (R)-(+)-α-methylbenzene methanamine ($[\alpha]_D^{25}$= + 27.50° (1 % in methanol)) showed the product to be identical and hence to possess the same absolute configuration.

(R)-Ethyl-*N*-[1-(2-bromophenyl)ethyl]glycine VI

A solution of (R)-(+)-III (20.0 g, 0.1 mol) and triethylamine (10.2 g, 0.1 mol) in *N*,*N*-dimethylformamide (DMF, 40 ml) was stirred at 8° C. At this temperature ethyl chloroacetate (12.2 g, 0.1 mol) dissolved in DMF (10 ml) was slowly dropped to the reaction mixture. Stirring was continued at room temperature for 72 h, diethyl ether (150 ml) was introduced and the formed precipitate was filtered and washed with ether. The filtrate was washed with water (3 x 100 ml) and brine (100 ml) respectively, dried on $MgSO_4$, filtered and evaporated to leave 26.4 g of an oil. Filtration over silica (column: 100 mm x 100 mm i.d.; eluate: hexane - ethyl acetate 1:1 v/v) afforded 25.1 g (88 %) of TLC-pure VI.

(R)-Ethyl-*N*-[1-(2-bromophenyl)ethyl]-*N*-formyl glycine **VII**

A solution of **VI** (25.1 g, 88 mmol) in formic acid (9.66 g, 210 mmol) and xylene (70 ml) was stirred and refluxed for 5 h with continuous removal of water. The reaction mixture was cooled to room temperature and washed successively with water, aqueous sodium bicarbonate solution, water and brine. The organic layer was dried on $MgSO_4$, filtered and evaporated to leave a TLC-pure oily residue of 26.3 g (95.3 %) of **VII**.

(R)-Ethyl-1-[1-(2-bromophenyl)ethyl]-2-mercapto-1*H*-imidazole-5-carboxylate **VIII**

To a stirred, nitrogen-covered mixture of sodium ethanolate (10.1 g, 148 mmol) in xylene (100 ml) was dropped, at 15° C, a solution of **VII** (26.2 g, 83 mmol) and ethyl formate (21.9 g, 245 mmol) in xylene (20 ml). The reaction was stirred for 72 h at room temperature and then extracted with water (3 x 25 ml). The combined water layers were acidified below 30° C with concentrated hydrochloric acid (19.2 ml, 230 mmol) and potassium isothiocyanate (7.8 g, 80 mmol) dissolved in water (11 ml), was introduced in one portion. The mixture was refluxed for 6.5 h and left overnight at room temperature, slowly forming a precipitate. Water (100 ml) and chloroform (150 ml) were added and stirred until complete dissolution. The organic layer was washed with water (2 x 50 ml), dried on $MgSO_4$, filtered and thoroughly evaporated to afford 28.4 g of TLC-pure **VIII** (96.3 %), which was used as such in the next reaction step.

(R)-(-)-Ethyl-1-[1-(2-bromophenyl)ethyl]-1*H*-imidazole-5-carboxylate.sulphate **IX**

A stirred solution of sodium nitrite (250 mg, catalytically), nitric acid (26.5 ml, 369 mmol) and water (60 ml) was cooled to 10° C. A solution of **VIII** (28.4 g) in chloroform (50 ml) was slowly introduced and stirring was continued for 5 h. The organic phase was separated, stirred for 2 h with water (55 ml) and concentrated aqueous ammonium hydroxide (30 ml) and left for 18 h. The chloroform layer was separated and washed with brine-water (1:1 v/v), dried on $MgSO_4$, filtered and evaporated to furnish 24 g of a residue. Purification over silica (column size: 200 mm x 80 mm i.d.; eluate:

hexane-ethyl acetate (1:1 v/v) afforded 22.8 g of TLC-pure **IX**-base (88.3 %). Anal. Calcd. for $C_{14}H_{15}BrN_2O_2$: C, 52.03; H, 4.68; N, 8.67. Found: C, 51.79; H, 4.88; N, 8.60 %. $[\alpha]_D^{25}$ = - 5.045 (1 % in ethanol). The sulfate was obtained by dissolving **IX**-base (6.0 g) in refluxing ethanol (30 ml) - sulfuric acid (1.1 ml). The solution was evaporated thoroughly and stirred with methyl isobutyl ketone (100 ml). The precipitate was filtered and dried to the air to yield 6.4 g (82 %) of sulfate, mp 119° C. Anal. Calcd. for $C_{14}H_{15}BrN_2O_2.H_2SO_4$: C, 39.92; H, 4.07; N, 6.65. Found: C, 39.75; H, 4.19; N, 6.57 %. $[\alpha]_D^{25}$ = - 19.22° (1 % in ethanol).

(R)-(+)-ethyl-1-(1-phenylethyl)-1*H*-imidazole-5-carboxylate.hydrochloride (etomidate)

A mixture of **IX** (760 mg, 1.8 mmol), palladium 10 % on charcoal (700 mg), a 3 % solution of thiophene in diisopropyl ether (0.3 ml) and finely powdered calcium oxide (2.0 g) in tetrahydrofuran (100 ml) was hydrogenated for 18 h. The mixture was filtered over dicalite and evaporated. The residue was dissolved in chloroform (50 ml) and washed with water-brine (1:1 v/v), dried and concentrated. The oil (530 mg) was taken up in 2-propanol (5 ml) and acidified with hydrochloric acid-saturated 2-propanol. The solvent was evaporated and replaced by 4 ml of methyl isobutyl ketone (4 ml). The formed precipitate was filtered, washed with little methyl isobutyl ketone and dried under vacuum at 75° C, to furnish 350 mg (69.4 %) of etomidate, $[\alpha]_D^{25}$ = + 22.96 (1 % in ethanol), identical to authentic material.

(R)-(+)-ethyl-1-(1-[2T]phenylethyl)-1*H*-imidazole-5-carboxylate.hydrochloride **X** ((R)-(+)-^{3}H-etomidate)

A mixture of **IX** (74 mg, 0.176 mmol), palladium 10 % on charcoal (70 mg), a 3 % solution of thiophene in diisopropyl ether (0.03 ml) and finely powdered calcium oxide (200 mg) was dehalogenated in tetrahydrofuran (10 ml) with approx. 30 Ci of tritium gas for 18 h at room temperature. The excess of tritium gas was absorbed 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 lyophilization with methanol, the product was purified by means of HPLC (eluate: acetonitrile-water-diisopropyl amine 36:64:0.2 by volume); the eluate containing the product fraction was immediately acidified with hydrochloric acid saturated 2-propanol (0.5 ml). The product was 99.9 % pure and, according to HPLC, identical to unlabelled (R)-(+)-etomidate. It contained 214 mCi of radioactivity, had a specific activity of 3.77 Ci/mmol and a chemical and radiochemical yield of 32 % based upon IX.

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