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Laboratory note

Synthesis of the *R* and *S* enantiomers of 1,2,3,4-tetrahydro-6,7-dihydroxy-1-methylisoquinoline-1-carboxylic acid

P Dostert, M Varasi, A Della Torre, C Monti, V Rizzo

Farmitalia Carlo Erba, Research and Development, Erbamont Group, Via C Imbonati 24, 20159 Milan, Italy

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Summary — 1,2,3,4-Tetrahydro-6,7-dihydroxy-1-methylisoquinoline-1-carboxylic acid (salsolinol-1-carboxylic acid) has been shown to be a bioprecursor of (*R*)-salsolinol in humans. We report here a synthesis and the determination of the absolute configurations of both enantiomers of salsolinol-1-carboxylic acid.

salsolinol-1-carboxylic acid / R and S enantiomers / mammalian alkaloids

Introduction

Non-enzymic bimolecular condensations between amino compounds and aldehydes or α-keto acids may be more common in mammalian tissues than has been assumed in the past. For example, various dopamine-derived alkaloids have long been shown to be present in animal species and man (for review see [1]). The possibility that changes in the levels of these alkaloids in fluids and tissues might cause, or contribute to, modifications in mental and/or neurophysiological states in various pathologies has been reviewed [2].

It has been suggested that the biosynthesis of tetrahydroisoquinoline alkaloids in mammals might originate from the condensation of β-phenylethylamines with aldehydes or α-keto acids [3]. There is increasing evidence that, at least in healthy subjects, the biosynthesis of salsolinol (1,2,3,4-tetrahydro-1-methyl-6,7-isoquinolinediol) occurs by condensation of dopamine with pyruvic acid [4] (fig 1). Salsolinol possesses an asymmetric center at C-1 and exists as R and S enantiomers. We have established that only the R enantiomer is present in the urine of healthy volunteers under normal dietary conditions [5]. The biosynthetic pathway for salsolinol formation in man is in line with Hahn's hypothesis [6], according to which the carbonyl group of α-keto acids might be the source of C-1 in tetrahydroisoquinoline alkaloids found in nature. This hypothesis was first validated by Kapadia *et al* [7] in the peyote Cactus.

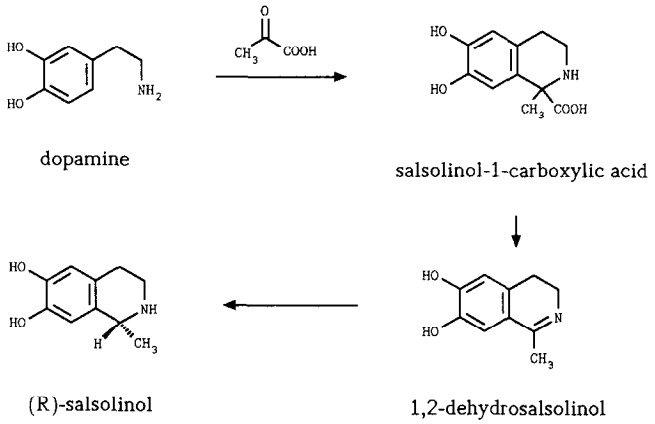


Fig 1. Biosynthetic pathway for (*R*)-salsolinol formation in healthy subjects.

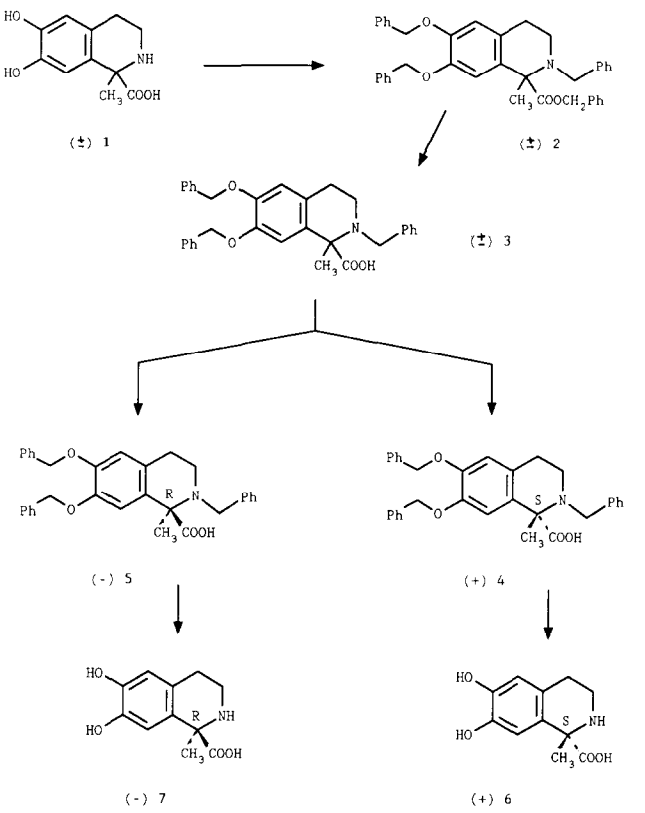
1,2,3,4-Tetrahydro-6,7-dihydroxy-1-methylisoquinoline-1-carboxylic acid (salsolinol-1-carboxylic acid), the first isoquinoline formed in the biosynthetic pathway leading from dopamine to (*R*)-salsolinol (fig 1), has been found in human urine and caudate nucleus [8, 9]. The stereochemistry of the endogenous product detected in these studies was not established. Decarboxylation of salsolinol-1-carboxylic acid to afford 1,2-dehydrosalsolinol (3,4-dihydro-1-methyl-6,7-isoquinolinediol; fig 1) has been shown to occur on prolonged aerial oxidation in basic media [10], by oxidative decarboxylation [11] or on incubation with

rat liver, or whole brain homogenates [12]. 1,2-Dehydrosalsolinol has recently been shown to be present in human urine [4].

The non-enzymic Pictet-Spengler reaction between amines and aldehydes, or α-keto acids, affords racemic tetrahydroisoquinolines. The absolute configuration of the two optical isomers of salsolinol has been determined as being *R* and *S* for the (+) and (–) isomers, respectively [13]. Here we report on a synthesis and the determination of the absolute configuration of the two enantiomers of salsolinol-1-carboxylic acid.

Synthesis and absolute configurations

Racemic salsolinol-1-carboxylic acid **1** was synthesized from dopamine hydrochloride and pyruvic acid according to a literature procedure [14]. Compound (±) **1** was transformed into the ester (±) **2**, which, when treated with potassium tert-butoxide and water under conditions known to hydrolyze very hindered esters [15], afforded the acid (±) **3** in excellent yield (scheme 1). Separation of (±) **3** into the enantiomers (+) **4** and (–) **5** was achieved by recrystallization of the salts obtained from *R*-(+)- and *S*-(–)-α-methylbenzylamine, respectively. Debenzylation of (+) **4** and (–) **5** using hydrogen in the presence of 10% Pd/C gave (+)- and (–)-1,2,3,4-tetrahydro-6,7-dihydroxy-1-methylisoquinoline-1-carboxylic acid **6** and **7**, respectively. The absolute configuration of the (+) and (–) enantiomers was established by comparison of the CD spectra of **4**, **5**, **6** and **7** with that reported [16] for *S*-(+)-methyl salsolidine-1-carboxylate **a**. For instance, the CD spectra of **4** and **a** both have three bands with an alternating positive-negative-positive sign at around 285, 240 and 215 nm. The wavelength of each maximum varies slightly in compounds **4** and **a**, and the corresponding intensities are also different. This is attributed to the influence of the substituents attached to the chromophore in the two compounds. Therefore, the (+) enantiomers **4** and **6** were found to have the *S* configuration, whereas the *R* configuration was assigned to the (–) enantiomers **5** and **7**.



Scheme 1. Chemical pathway followed for the synthesis of **6** and **7**.

Chemistry

Melting points were determined in open capillaries using a Büchi 512 apparatus and are uncorrected. Proton-NMR spectra were recorded on a Varian VXR-200. Chemical shifts are reported as δ values in parts per million relative to tetramethylsilane as an internal standard. EI-mass spectra were obtained on a CH7-Varian MAT mass spectrometer at an electron energy of 70 eV. Optical rotations were determined on a Perkin Elmer automatic polarimeter. CD spectra were recorded on a Mark V dichrograph, Jobin Yvon. Elemental analyses were performed by the analytical laboratory of Farmitalia Carlo Erba and agreed with theoretical values to within ± 0.4%. Common reagent-grade chemicals and starting materials were purchased from commercial sources and were used as received. Drying of solvents was performed by storage on 3 Å molecular sieves. Evaporations were made *in vacuo* (rotating evaporator) and were preceded by drying over sodium sulfate. Flash column chromatography was carried out with Carlo Erba RS silica gel (40–63 μm), and the solvent mixture reported within parentheses was used as eluant.

(±)-Benzyl 1,2,3,4-tetrahydro-6,7-dibenzyloxy-N-benzyl-1-methylisoquinoline-1-carboxylate (2)

To a stirred suspension of (±)-**1** (4.01 g, 18 mmol) and K_2CO_3 (12.42 g, 90 mmol) in 30 ml of anhydrous DMF, kept under N_2 at 0°C, a solution of benzyl bromide (15.36 g, 90 mmol) in 20 ml of anhydrous DMF was added dropwise. The mixture was then heated at 70°C for 3 h; after cooling at room temperature, water (80 ml) was added and the solution was extracted with ethyl acetate (70 ml, three times). The organic phases were combined, washed with water (100 ml), dried and evaporated. The residue was purified by flash chromatography (hexane:ethyl acetate 98:2) yielding 9.1 g (86%) of (±)-**2** as white crystals, mp = 110–112°C. ^{1}H-NMR ($CDCl_3$) δ 1.63 (s, 3H, CH_3); 2.5–3.20 (m, 4H, $PhCH_2CH_2N$); 3.45, 3.85 (two doublets, J = 13.2 H_z, 2H, NCH_2Ph); 4.95 (s, 2H, $COOCH_2Ph$); 5.05, 5.20 (two doublets, 2H, J = 11.9 H_z, OCH_2 Ph); 5.15 (s, 2H, OCH_2Ph); 6.65 (s, 1H, H-C (5)); 6.75 (s, 1H, H-C (8)); 7.1–7.5 (m, 20H, 4Ph). EI-MS: *m/z* 447 (30,

$[M\text{-}PhCH_2OCHO]^{+\cdot}$); 358 (20, $[M\text{-}PhCH_2OCHO\text{-}C_7H_7]^{+\cdot}$); 267 (6$[M\text{-}PhCH_2OCHO\text{-}2C_7H_7]^{+\cdot}$); 91 (100$[C_7H_7]^+$). Anal $C_{39}H_{37}NO_4$ (C, H, N).

*(±)-1,2,3,4-Tetrahydro-6,7-dibenzyloxy-N-benzyl-1-methylisoquinoline-1-carboxylic acid (**3**)*

Water (1.1 ml, 61 mmol) was added to a stirred, ice-cooled suspension of t-BuOK (26 g, 231 mmol) in 250 ml of diethyl ether. After stirring for 5 min, (±)-**2** (16 g, 27.4 mmol) was added to the slurry in a single portion. The mixture was allowed to warm to room temperature, stirred for 0.5 h and then refluxed for 10 h. After cooling, the reaction was quenched by adding water, acidified to pH 6 with 2 N HCl and extracted with ethyl acetate (150 ml, three times). The organic phases were combined, washed with water, dried and evaporated to give a pale yellow oil. The residue was purified by flash chromatography (CH_2Cl_2:MeOH 98:2); recrystallization from absolute ethanol afforded 11.8 g (88%) of (±)-**3**, mp = 115–120°C (dec). ^{1}H-NMR ($CDCl_3$) δ 1.65 (s, 3H, CH_3); 2.5–3.1 (m, 4H, NCH_2CH_2Ph); 3.45, 3.85 (two doublets, 2H, J = 13.2 H_z, NCH_2Ph); 5.06, 5.13 (two doublets, 2H, J = 11.9 H_z, OCH_2Ph); 5.07 (s, 2H, OCH_2Ph); 6.56 (s, 1H, H-C (5)); 7.12 (s, 1H, H-C (8)); 7.2–7.5 (m, 15H, 3Ph). EI-MS: *m/z* 448 (2, $[M\text{-}COOH]^+$); 434 (5, $[M\text{-}CH_3\text{-}CO_2]^+$); 357 (14, $[M\text{-}COOH\text{-}C_7H_7]^{+\cdot}$); 266 (5, $[M\text{-}COOH\text{-}2C_7H_7]^+$); 91 (100, $[C_7H_7]^+$). Anal $C_{33}H_{31}NO_4$ (C, H, N).

*S-(+)-1,2,3,4-Tetrahydro-6,7-dibenzyloxy-N-benzyl-1-methylisoquinoline-1-carboxylic acid (**4**)*

To a stirred solution of (±)-**3** (15 g, 30.3 mmol) in 400 ml of methanol, *R*-(+)-α-methylbenzylamine (3.67 g, 30.3 mmol) was added dropwise. After 15 min the solvent was evaporated and the solid obtained was dried under high vacuum at 30°C. Recrystallization from diethyl ether (1400 ml) afforded 5 g of a solid [$[\alpha]_D^{25}$ + 29.7 (c 0.47, MeOH)]. Two further recrystallizations from diethyl ether yielded 3.93 g (enantiomeric yield 42%) of the R-(+)-α-methylbenzylamine salt of **4**, mp = 151.5–154°C, $[\alpha]_D^{25}$ + 34.9° (c 0.44, MeOH). Anal $C_{40}H_{42}N_2O_4$ (C, H, N).

To a vigourously stirred solution of **4** *R*-(+)-α-methylbenzylamine salt (3.5 g, 5.69 mmol) in 200 ml of ethyl acetate, water (15 ml) and then 1 N HCl (6.25 ml, 6.25 mmol) was added. After 30 min the organic phase was separated and the aqueous phase was extracted with ethyl acetate (30 ml, three times). The combined organic phases were washed with water (50 ml), dried and evaporated. The residue was recrystallized from ethanol and gave 2.7 (96%) of **4**, mp = 120–125°C, $[\alpha]_D^{25}$ + 93.3° (c 0.58, MeOH). CD (c 0.0003M, 95% EtOH) $[\theta]_{215}$ + 110 000, $[\theta]_{240}$ – 27 000, $[\theta]_{285}$ + 4500. Anal $C_{32}H_{31}NO_4$ (C, H, N).

*R-(–)-1,2,3,4-Tetrahydro-6,7-dibenzyloxy-N-benzyl-1-methylisoquinoline-1-carboxylic acid (**5**)*

The mother liquors obtained during the preparation of the *R*-(+)-α-methylbenzylamine salt of **4** were mixed and evaporated to give 13.98 g (22.7 mmol) of a mixture of the *R*-(+)-α-methylbenzylamine salts of **4** and **5** enriched in **5**, which were dissolved in ethyl acetate (250 ml); after addition of 25 ml of water, 24.9 ml of 1 N HCl was added dropwise. The solution was kept under vigorous stirring for 30 min; the organic phase was separated and the aqueous phase was extracted with ethyl acetate (50 ml, twice). The combined organic phases were washed with water, dried and evaporated to afford a mixture of **4** and **5**, which proved to be slightly impure and was therefore purified by flash chromatography (CH_2Cl_2:MeOH 96:4) to give 10.1 g (20 mmol) of a mixture of **4** + **5**. This mixture was dissolved in methanol (300 ml) and *S*-(–)-α-methylbenzylamine (2.42 g, 20 mmol) was added dropwise. After 15 min the solvent was evaporated and the residue was dried under high vacuum at 30°C. Two recrystallizations from CH_3CN afforded 5.46 g of the *S*-(–)-α-methylbenzylamine salt of **5** (enantiomeric yield 58%), mp = 151.5–154°C, $[\alpha]_D^{25}$ – 34.85° (c 0.51, MeOH). Anal $C_{40}H_{42}N_2O_4$ (C, H, N).

4.90 g of this salt was treated as described above for the *S*-(+)-enantiomer **4** and afforded 3.90 g (99%) of **5**, mp = 122–126°C, $[\alpha]_D^{25}$ – 91.6° (c 0.59, MeOH). CD was a mirror image of **4** within experimental error (± 5%). $C_{32}H_{31}NO_4$ (C, H, N).

*S-(+)-1,2,3,4-Tetrahydro-6,7-dihydroxy-1-methylisoquinoline-1-carboxylic acid (**6**)*

A solution of 2.5 g (50.6 mmol) of **4** in 180 ml of ethanol and 80 ml of water was hydrogenated at room temperature and under 35 psi in the presence of 1.8 g of 10% Pd/C as a catalyst. After the absorption of hydrogen had ceased, the catalyst was filtered and washed with water. After evaporation to dryness, the residue was recrystallized from water to give 0.74 g (65%) of **6**, mp = 256–259°C, $[\alpha]_D^{25}$ + 71.9° (c 0.32, MeOH). ^{1}H-NMR [$(CD_3)_2SO$] δ 1.56 (s, 3H, CH_3); 2.5–2.9 (m, 2H, CH_2Ph); 3.0–3.4 (m, 2H, CH_2NH_2); 6.38 (s, 1H, H-C (5)); 7.18 (s, 1H, H-C (8)); 8.7 (bs, 4H, NH_2, OH, OH). EI-MS: *m/z* 178 (100, $[M\text{-}CO_2H]^+$); 164 (16, $[M\text{-}CH_3\text{-}CO_2]^+$). CD (c 0.0005, 95% EtOH) $[\theta]_{210}$ + 80 000, $[\theta]_{240}$ – 13 000, $[\theta]_{285}$ + 5000. Anal $C_{11}H_{13}NO_4$ (C, H, N).

*R-(+)-1,2,3,4-Tetrahydro-6,7-dihydroxy-1-methylisoquinoline-1-carboxylic acid (**7**)*

Starting from 2.5 g of **5** as described above for the preparation of **6**, 0.78 g (69%) of **7** was obtained after recrystallization from water; mp = 257–260°C, $[\alpha]_D^{25}$ – 71.6° (c 0.30, MeOH). CD was a mirror image of **6** within experimental error (± 5%). Anal $C_{11}H_{13}NO_4$ (C, H, N).

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