Studies on Nilvadipine. IV.[1] Synthesis of Deuteriated and Optically Active Isopropyl 2-Cyano-3-methoxycarbonyl-4-(3-nitrophenyl)-6-methyl-1,4-dihydropyridine-5-carboxylate (Nilvadipine)

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Nilvadipine, I, has already entered clinical use for the treatment of hypertension. In the process of the developing nilvadipine, we prepared the deuteriated analogue of I as an internal standard for the determination of I in human plasma by capillary column gas chromatography-negative-ion chemical-ionization mass spectrometer.

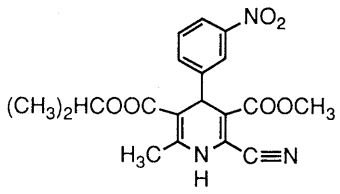
Nilvadipine has an asymmetric center at the C-4 position of the dihydropyridine ring, and characterization of the optical isomers with regard to their activity and bioavailability is of interest. Thus, we synthesized both the enantiomers of I by optical resolution *via* the 5-carboxy derivative (3), which was previously prepared as one of the metabolites of I.

Keywords nilvadipine; deuteration; optical resolution; (+)-nilvadipine; (−)-nilvadipine

In previous papers,[2,3] among many derivatives with a variety of functional groups at the 2-position of the 1,4-dihydropyridine nucleus, 2-hydroxymethyl- and 2-cyano-1,4-dihydropyridines were reported to possess very potent hypotensive activity in normotensive rats and coronary blood flow-increasing activity in pentobarbital-anesthetized dogs. Among a number of 2-hydroxymethyl- and 2-cyano-1,4-dihydropyridines which we prepared during optimization research, isopropyl 2-cyano-3-methoxycarbonyl-4-(3-nitrophenyl)-6-methyl-1,4-dihydropyridine-5-carboxylate (nilvadipine, I) was selected for further evaluation[4] and has entered clinical use for the treatment of hypertension.

In the process of developing nilvadipine, we prepared the deuteriated analogue of I for use as an internal standard for the determination of I in human plasma by capillary column gas chromatography-negative-ion chemical-ionization mass spectrometry, the method of which has already been reported by Tokuma.[5]

Nilvadipine has a unique 1,4-dihydropyridine structure whose substituents at all five positions of the nucleus differ from one another. It has an asymmetric center at the C-4 position of the dihydropyridine ring and characterization of both enantiomers with regard to activity[6] and bioavailability[7] is of interest.



(I) nilvadipine

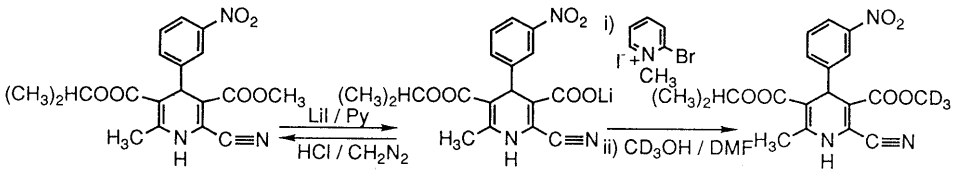
Fig. 1

This paper describes methods for the preparation of the deuteriated analogue and the optical resolution of nilvadipine, I, in detail *via* the 3-carboxy derivative (**1**) and the 5-carboxy derivative (**3**) respectively, which were prepared previously as metabolites of I.[8]

Synthesis of the Deuteriated Analogue of Nilvadipine It was thought at the outset that the 3-trideuteriomethyl analogue of I could be prepared easily by the reaction of the 3-carboxylic acid derivative with deuteriated methanol, which is commercially available. But, as described in a previous paper,[7] the 3-carboxylic acid analogue of I could not be isolated by cleavage of the *tert*-butyl ester at the 3-position with formic acid in the usual manner owing to its instability under acidic conditions. However, it could be isolated as a lithium salt under basic conditions (LiI/Py), which was confirmed by treatment with diazomethane immediately after neutralization with dilute hydrochloric acid to afford the starting material I accompanied with the 2-carbamoyl derivative and many other by-products.

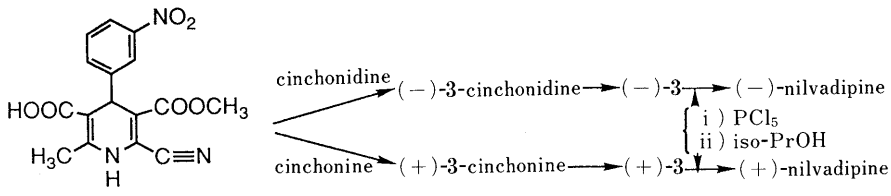
The obtained lithium salt (**1**) was reacted with Mukaiyama's reagent[9] in basic medium to give an activated ester, which was found to react easily with deuterated methanol coexisting in the reaction vessel to afford the desired deuteriated analogue (**2**) of nilvadipine (I), as shown in Chart 1.

Synthesis of the Optical Isomers of Nilvadipine The 5-carboxylic acid derivative (**3**), prepared by the treatment of *tert*-butyl 2-cyano-3-methoxycarbonyl-6-methyl-



I 1 2

Chart 1



3

Chart 2

4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate with formic acid,[7] was resolved by the formaion of the (−)-(**3**)·cinchonidine salt and recrystallization of the salt to constant rotation. The free acid, (−)-(**3**) was obtained by treating the salt with 2 N HCl, $[\alpha]_D^{20}$ −230.7° ($c=1.0$, MeOH). The 5-carboxylic acid ((−)-**3**) was converted to the corresponding acid chloride by treatment with phosphorus pentachloride followed by isopropyl alcohol to yield the desired (−)-nilvadipine, $[\alpha]_D^{20}$ −219.6° ($c=1.0$, MeOH).

On the other hand, the acid **3**, recovered from the first mother liquor of the cinchonidine salt after decomposition, was resolved *via* cinchonine salt formation to permit the preparation of pure (+)-**3**, $[\alpha]_D^{20}$ +234.1° ($c=1.0$, MeOH). By using the same procedure, (+)-nilvadipine, $[\alpha]_D^{20}$ +222.4° ($c=1.0$, MeOH), was obtained from (+)-**3**.

The optical purity of the isomers obtained above was checked by high-performance liquid chromatography (HPLC) with a chiral stationary-phase column (Chiral pak OT(+)) and each of them showed a single peak.

The absolute configuration of the isomers was determined by an X-ray analysis of (+)-**3**·cinchonine salt, the precursor of (+)-nilvadipine. The configuration at the C-4 carbon of the (+)-monocarboxylic acid ((+)-3) was determined to be *R*, based on the known configuration of cinchonine. It is thought that (+)-nilvadipine is synthesized from (+)-**3** without inversion, as shown in Chart 2, and (+)-nilvadipine was determined to be the *S*-conformer and (−)-nilvadipine the *R*-conformer.[10]

The biological evaluations of the optical isomers of nilvadipine for activity to increase of coronary blood flow in pentobarbital-anesthetized dogs and for hypotensive effect in spontaneously hypertensive rats will be reported elsewhere[5] and the results of pharmacodynamic and pharmacokinetic studies have already been reported.[6]

Experimental

Melting points were determined using a Thomas-Hoover capillary melting point apparatus, without correction. NMR spectra were recorded on a JNN-PMR (60 MHz) spectrometer using tetramethylsilane as an internal standard. IR spectra were taken on either a Hitachi 260-10 spectrophotometer or a Shimadzu IR-420 spectrophotometer. Mass spectra were taken on a Hitachi mass spectrometer M-1000H (LC-MS system). Column chromatography was performed on silica gel (Merck Kieselgel 60, 230—400 mesh).

Trideuteriomethyl 2-Cyano-5-isopropoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (2) A mixture of nilvadipine (I, 16.1 g, 41.8 mmol) and LiI (12.98 g, 97 mmol) in pyridine (80 ml) was stirred at 110 °C under a nitrogen atmosphere for 8 h. After cooling to ambient temperature, the reaction mixture was concentrated *in vacuo*. The residue was taken up in ethyl acetate and undissolved substances were filtered off. The filtrate was concentrated *in vacuo* to give a residue, to which was added H_2O (60 ml). The aqueous solution was washed with $(iso\text{-}Pr)_2O$ four times (50 ml × 4), and extracted with *n*-BuOH seven times (70 ml × 7). The extracts were combined, washed with a small amount of H_2O once and evaporated *in vacuo*. The residue was dissolved in a small amount of AcOEt, to which was added $(iso\text{-}Pr)_2O$, and crystallization occurred on standing. Collection by filtration, washing with $(iso\text{-}Pr)_2O$ and drying afforded the desired lithium salt, 12.0 g (76.1%), which was used in the following reaction without further purification.

A mixture of the lithium salt obtained above (11.43 g, 30.3 mmol), 1-methyl-2-bromopyridinium iodide (10.18 g, 33.9 mmol), *N*,*N*-dimethylaniline (4.92 g, 40.3 mmol) and CD_3OD (2 ml) in *N*,*N*-dimethylformamide (DMF, 30 ml) was stirred for 7.5 h at ambient temperature. The reaction mixture was poured into H_2O and extracted with AcOEt. The extract was washed with 5% HCl aqueous solution, H_2O and brine successively, and dried over $MgSO_4$. Removal of the solvent gave a residue, which was chromatographed on silica gel using a mixture of toluene and EtOAc (10 : 1—5 : 1) as the eluent. The fractions containing the desired compound were combined and the solvent was removed *in vacuo* to afford a residue as a viscous oil, which was dissolved in $(iso\text{-}Pr)_2O$ under warming and which crystallized out when the solution was left to stand at ambient temperature. Collection of the crystals by filtration, washing with $(iso\text{-}Pr)_2O$ and drying afforded the desired deuteriated analogue, **2** of I, mp 164—165 °C. Yield. 6.74 g (57.3%). *Anal.* Calcd for $C_{19}H_{16}D_3N_3O_6$[11]: C, 59.22; H, 4.97; N, 10.90. Found: C, 59.27; H, 4.93; N, 10.92. IR (Nujol) cm^{-1}: 3330 (NH), 3090 (Ar), 2260, 2180, 2110, 2080 (CD_3), 1700, 1680 (COOR), 1645, 1620 (C=CR), 1520, 1350 (NO_2), 1225, 1110, 1085 (C–O–C), 790, 703 (*m*-substituted Ar). ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, d, $J=6$ Hz, $CH(C\underline{H}_3)_2$), 1.26 (3H, d, $J=6$ Hz, $CH(C\underline{H}_3)_2$), 2.41 (3H, s, C_6–CH_3), 4.96 (1H, septet, $C\underline{H}(CH_3)_2$), 5.17 (1H, s, C_4-H), 6.67 —6.87 ((1H, br m, NH), 7.35—8.21 (4H, m, aromatic protons). LC-MS *m/z*: 389 $(M+1)^+$.

(−)-2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic Acid ((−)-3) A mixture of (±)-**3** (16.7 g) and cinchonidine (14.4 g) in MeOH (100 ml) was refluxed for 15 min and then allowed to stand at ambient temperature. The resulting precipitates were collected by filtration, washed with MeOH and air-dried to give the salt((−)-**3**·cinchonidine, 11.74 g), which was recrystallized twice from MeOH to give the pure salt (9.36 g, yield; 30.0%), mp 159—160 °C. $[\alpha]_D^{20}$ −198.9° ($c=1.0$, MeOH).

A suspension of the pure salt (9.05 g) in EtOAc (50 ml) was treated with 2 N HCl (20 ml) under stirring and cooling in an ice-bath for a period of 10 min and then the aqueous layer was removed. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give (−)-**3** (5.11 g), mp 205 °C (dec.). *Anal.* Calcd for $C_{16}H_{13}N_3O_6$: C, 55.98; H, 3.82; N, 12.24. Found: C, 55.87; H, 3.94; N, 12.19. $[\alpha]_D^{20}$ −230.7° ($c=1.0$, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 2.34 (3H, s, C_6-CH_3), 3.71 (3H, s, $COOCH_3$), 5.13 (1H, s, C_4-H), 7.56—8.23 (4H, m, aromatic protons), 10.25 (1H, br s, NH).

(+)-2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylic Acid ((+)-3) The mother liquor obtained after removal of the (−)-**3** cinchonidine salt by filtration was evaporated *in vacuo*. The crystalline residue was washed with a mixture of EtOAc and $(iso\text{-}Pr)_2O$ and extracted with EtOAc after neutralization with 2 N HCl under ice-bath cooling. The extract was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give the acid, **3** (11.15 g). The acid and cinchonine (9.54 g) were dissolved in EtOAc under heating and the solution was allowed to stand at ambient temperature. The resulting precipitates were collected by filtration, washed with EtOAc and recrystallized twice from EtOH to afford the pure cinchonine salt (7.62 g, yield; 25.0%), mp 164°C, $[\alpha]_D^{20}$ +243.2° ($c=1.0$, MeOH).

A 2 N HCl solution (20 ml) was added to a suspension of the above-obtained salt (7.41 g) in EtOAc (50 ml) with stirring and cooling in

an ice-bath over a period of 10 min, and then the aqueous layer was removed. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to afford (+)-**3** (3.67 g), mp 203 °C. *Anal.* Calcd for $C_{16}H_{13}N_3O_6$: C, 55.98; H, 3.82; N, 12.24. Found: C, 55.86; H, 3.90; N, 12.21. $[\alpha]_D^{20}$ +234.1° (c=1.0, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 2.34 (3H, s, C_6-CH_3), 3.71 (3H, s, $COOCH_3$), 5.13 (1H, s, C_4-H), 7.56—8.25 (4H, m, aromatic protons), 10.25 (1H, br s, NH).

Isopropyl (−)-2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate ((−)-I) A suspension of (−)-**3** (4.47 g) in CH_2Cl_2 (45 ml) was treated with PCl_5 (3.62 g) under cooling in an ice-bath and the mixture was stirred for another 30 min. Then a solution of iso-PrOH (2.5 g) in CH_2Cl_2 (7.5 ml) was added dropwise under the same conditions over a period of 10 min. Stirring was continued for 20 min, then 5% aqueous Na_2CO_3 solution (30 ml) was added to the reaction mixture and the whole was stirred at ambient temperature for 1 h. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography on slica gel (80 g) with a mixture of C_6H_6 and EtOAc (10 : 1) as the eluent. The fractions containing the desired compound were combined and evaporated *in vacuo* to give a residue, which was crystallized from (iso-Pr)$_2$O to afford (−)-I (4.9 g, 98.0%), mp 120—122 °C. *Anal.* Calcd for $C_{19}H_{19}N_3O_6$: C, 59.22; H, 4.97; N, 10.90. Found: C, 59.17; H, 4.92; N, 10.91. $[\alpha]_D^{20}$ −219.6° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$), 1.25 (3H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$), 2.39 (3H, s, C_6-CH_3), 3.78 (3H, s, $COOCH_3$), 4.98 (1H, septet, J=6.5 Hz, $C\underline{H}(CH_3)_2$), 5.19 (1H, s, C_4-H), 7.0 (1H, br s, NH), 7.25—8.21 (4H, m, aromatic protons). LC-MS m/z: 385 $(M+1)^+$.

Isopropyl (+)-2-Cyano-3-methoxycarbonyl-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-5-carboxylate ((+)-I) (+)-I (3.34 g, 95.0%) was obtained from (+)-**3** (3.12 g) by using a similar procedure to that employed for the synthesis of (−)-I, mp 120—122 °C. *Anal.* Calcd for $C_{19}H_{19}N_3O_6$: C, 59.22; H, 4.97; N, 10.90. Found: C, 59.38; H, 5.08; N, 10.98. $[\alpha]_D^{20}$ +222.4° (c=1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$), 1.26 (3H, d, J=6.5 Hz, $CH(C\underline{H}_3)_2$), 2.40 (3H, s, C_6-CH_3), 3.76 (3H, s, $COOCH_3$), 4.97 (1H, septet, J=6.5 Hz, $C\underline{H}(CH_3)_2$), 5.17 (1H, s, C_4-H), 6.96 (1H, br s, NH), 7.21—8.21 (4H, m, aromatic protons). LC-MS m/z: 386 $(M+1)^+$.

The optical purity of (+)- and (−)-I was checked by HPLC analysis under the following conditions: column, Chiral pak OT (+) (4.6 mm × 250 mm)/Japan Spectroscopic Corp.); column temterature, 0 °C; eluent, MeOH–H_2O (9 : 1, v/v); flow rate, 0.6 ml/min; detection, UV 280, 260 nm (Waters instrument).

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