Chem. Pharm. Bull. 28(9)2809—2812(1980)

Synthesis of Optically Active 2-(N-Benzyl-N-methylamino)ethyl Methyl 2,6-Dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Nicardipine[1])

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(Received March 6, 1980)

(+)- and (−)-2-(N-benzyl-N-methylamino)ethyl methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**6**) (nicardipine) were synthesized from (−)- and (+)- 1-ethoxymethyl-5-methoxycarbonyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (**3**), respectively. Resolution of **3** was carried out using cinchonidine and cinchonine as resolving agents. The vertebral vasodilating activity of (+)-YC-93 (the hydrochloride of (+)-(**6**)) was about 3 times that of (−)-YC-93.

Keywords——1,4-dihydropyridine; vasodilating activity; vasodilator; optical resolution; esterification; hydrolysis; nicardipine

In a previous paper, 2-(N-benzyl-N-methylamino)ethyl methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride (YC-93) was reported to be a very promising vasodilator among a large number of water-soluble 1,4-dihydropyridine derivatives tested.[3] YC-93 is a racemic compound because of the asymmetric center at the C-4 position of dihydropyridine ring. Therefore, it is of interest to characterize the optical isomers as regards their vasodilating activity and bioavailability.

Initially, we focused our efforts on the resolution of the racemic free base **6** of YC-93 itself, using various resolving agents (D-camphor-10-sulfonic acid, *etc.*), but unfortunately this failed because the diastereomeric derivatives could not be crystallized.

However, we have now succeeded in preparing optically pure (+)- and (−)-**6** *via* the resolution of racemic 1-ethoxymethyl-5-methoxycarbonyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine)-3-carboxylic acid (**3**). The methods for the preparation of (+)- and (−)-**6** are described in detail below.

Dimethyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**1**) was readily prepared from *m*-nitrobenzaldehyde, methyl acetoacetate and methyl 3-amino crotonate by the Hantzsch method.[4] Treatment of **1** with chloromethyl ethyl ether in the presence of sodium hydride provided dimethyl 1-ethoxymethyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate[5] (**2**), which was hydrolyzed with 1-dimethylamino-2-propanol-H_2O and sodium to give the key intermediate[3] **3**.

Subsequently, the resolution of racemic **3** obtained above was accomplished by recrystallization of its cinchonidine salt to constant rotation of the resolved **3**. The compound ((−)-(**3**)), which was obtained from its cinchonidine salt, was hydrolyzed with 1N hydrochloric

1) Prop. INN: WHO Chronicle, 33, Supplement (List 42), 13, Sept., 1979. The code designation of the hydrochloride of nicardipine is YC-93.
2) Location: *Azusawa 1-1-8, Itabashi-ku, Tokyo 174, Japan.*
3) M. Iwanami, T. Shibanuma, M. Fujimoto, R. Kawai, K. Tamazawa, T. Takenaka, K. Takahashi, and M. Murakami, *Chem. Pharm. Bull.*, **27**, 1426 (1979).
4) A. Hantzsch, *Ann. Chim.*, **215**, 1 (1882).
5) M. Murakami, K. Takahashi, T. Ozasa, K. Tamazawa, R. Kawai, and T. Takenaka, Japan Patent (Kokai) 85574 (1973).

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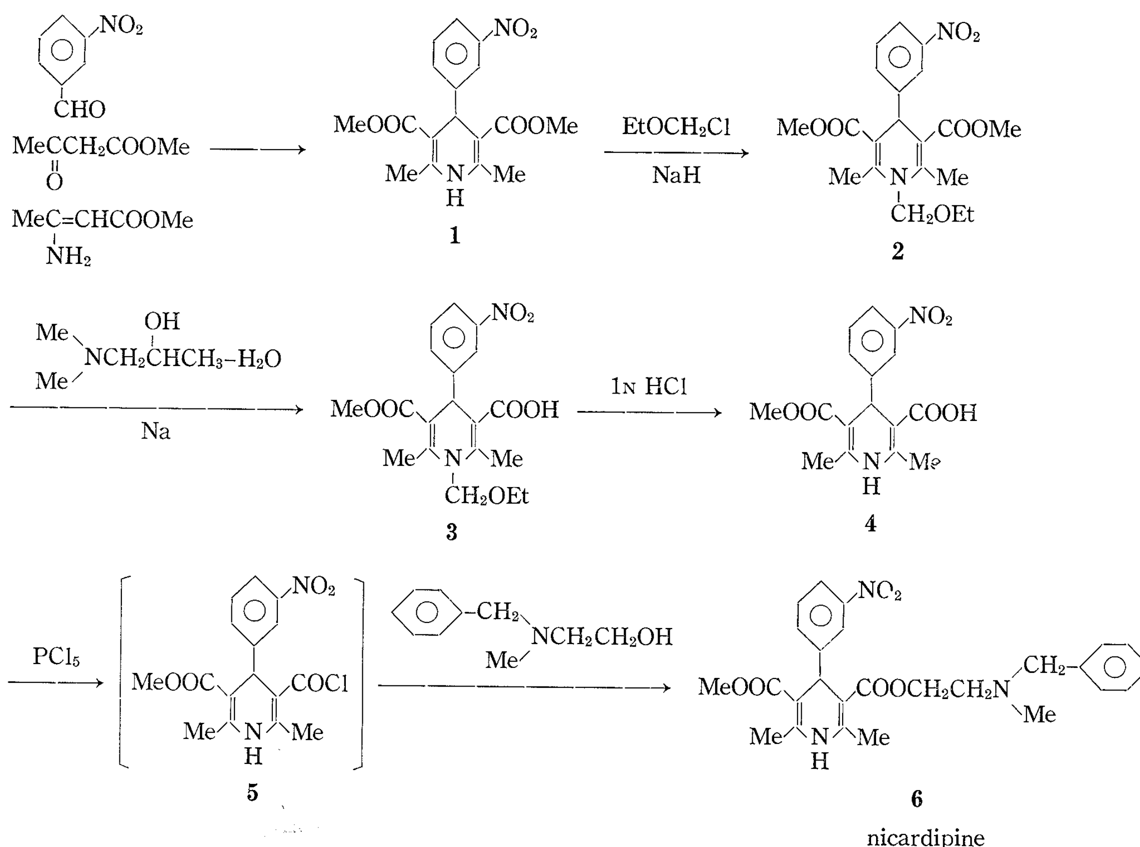

Chart 1

acid to afford (—)-5-methoxycarbonyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid ((—)-(**4**)). The monocarboxylic acid ((—)-(**4**)) was converted by treatment with phosphorus pentachloride into the corresponding acid chloride, which, without isolation, was treated with 2-(N-benzyl-N-methylamino)-ethanol to yield the desired (+)-**6**, $[\alpha]_D^{20}$ +26.8 (c=5.00, MeOH).

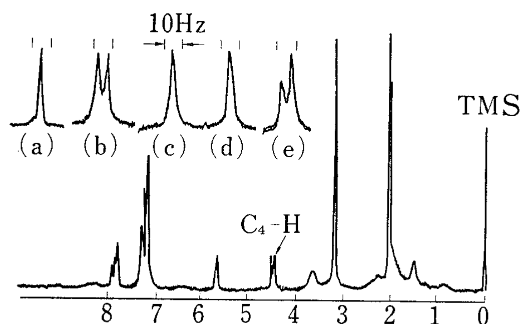

Fig. 1. Spectrum of (±)-**6** (0.2M) in $CDCl_3$ in the Presence of the Chiral Shift Reagent, Pr–TFMC (0.06M)

Expanded portions show the signal of the methine proton at the C_4-carbon. (a) (±)-**6**, (b) (±)-**6** (0.2M) +Pr–TFMC (0.06M), (c) (+)-**6** (0.2M)+Pr–TFMC (0.06M), (d) (—)-**6** (0.2M)+Pr–TFMC (0.06M) (e) (+)-**6** (0.07M)+(—)-**6** (0.13M)+Pr–TFMC (0.06M).

On the other hand, **3** recovered from the mother liquor after the original crystallization of cinchonidine salt of **3** was resolved through its cinchonine salt to permit the preparation of pure (+)-**3**. By the same procedure, (—)-**6**, $[\alpha]_D^{20}$ —26.7 (c=5.00, MeOH) was obtained from (+)-**3**.

The nuclear magnetic resonance (NMR) spectra of (+)-, (—)- and (±)-**6** in the presence of the chiral shift reagent are shown in Figure 1. The methine proton at the C_4- carbon of (±)-**6** was observed at both 4.48 ppm due to (—)-**6** and at 4.53 ppm due to (+)-**6** in $CDCl_3$ containing the chiral shift reagent, tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]praseodymium (III) (Pr–TFMC). However, (+)- and (—)-**6** each exhibited only a single peak at the expected position. These results confirmed that (+)-and (—)-**6** were optically pure.

The cardiovascular activity of (+)- and (—)-YC-93 (the hydrochlorides of (+)- and (—)-(**6**)), after intravenous administration, was determined in pentobarbital anesthetized dogs.[6]

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(+)-YC-93 in a dose range of 0.3 to 10 μg/kg and (−)-YC-93 in a dose range of 1 to 30 μg/kg produced a dose-dependent increase in vertebral blood flow with an associated reduction of arterial blood pressure. The vertebral vasodilating activity of (+)-YC-93 was approximately 3 times that of (−)-YC-93. Furthermore, the effective duration of action of (+)-YC-93 was longer than that of (−)-YC-93. This is also the first example of the synthesis of an optically active dihydropyridine with different ester groups at the 3- and 5- positions. The present method should be suitable for the preparation of various such dihydropyridines.

Experimental

All melting points are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL FX 100 (PFT-NMR) spectrometer (100 MHz) using Me_4Si as an internal standard. The following abbreviations are used; s=singlet, d=doublet, t=triplet, m=multiplet. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. For column chromatography, silica gel (Wakogel C-200) was used. All evaporation procedures were carried out *in vacuo*.

1-Ethoxymethyl-5-methoxycarbonyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3-carboxylic Acid (3)——Na (13.3 g) was added to 1-dimethylamino-2-propanol (150 ml). After stirring for 1 hr, a solution of H_2O (3.8 ml) in 1-dimethylamino-2-propanol (40 ml) was added dropwise to the mixture and the Na was completely dissolved with warming. Dimethyl-1-ethoxymethyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate[5] (2) (38 g) in benzene (190 ml) was added to this solution under cooling in an ice bath and the reaction mixture was stirred at room temperature for 3 hr. The solvent was then evaporated off and the residue was acidified to pH 2 by careful addition of 3 N HCl under cooling in a dry ice-acetone bath. The aqueous solution was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel with benzene–AcOEt (4:1) to give the monocarboxylic acid (3) (18 g) as crystals, mp 192—194°. NMR (d_6-DMSO) δ: 1.09 (3H, t, $-CH_2$-$C\underline{H}_3$), 2.46 (6H, s, $C_{2,6}$–CH_3), 3.38 (2H, q, $-C\underline{H}_2CH_3$), 3.64 (3H, s, $-COOCH_3$), 4.95 (2H, s, $-C\underline{H}_2OEt$), 5.12 (1H, s, C_4–H).

Resolution of Racemic 1-Ethoxymethyl-5-methoxycarbonyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3-carboxylic Acid (3)——Cinchonidine (9.357 g) was dissolved in a solution of racemic **3** (12.413 g) in MeOH (200 ml) with warming and the solvent was removed. The salt formed was dissolved in EtOH (150 ml) and kept overnight at room temperature. The resulting crystals were collected by filtration, and recrystallized twice from EtOH to afford the (−)-3-cinchonidine salt (6.4 g), mp 197—199°. The (−)-3-cinchonidine salt was treated with 0.1 N HCl and the aqueous layer was extracted with $CHCl_3$. The extract was washed with water and dried over $MgSO_4$. Removal of the solvent gave (−)-**3** (3.2 g), mp 134—135°, $[\alpha]_D^{22}$ −16.00 (*c*=1.78, acetone). The mother liquor from the original crystallization was concentrated and worked up in the same way for the preparation of (−)-**3** from the (−)-3-cinchonidine salt. The recovered **3** (8.393 g), which contained excess (+)-**3**, was dissolved in a solution of cinchonine (6.33 g) in MeOH (200 ml). After removal of MeOH, the residue was recrystallized three times from EtOH to give the (+)-3-cinchonine salt (6.8 g), mp 209—212°. (+)-**3** (3.85 g), mp 133—134°, $[\alpha]_D^{22}$ +15.3 (*c*=1.90, acetone) was obtained from its cinchonine salt by the procedure described above.

(−)-5-Methoxycarbonyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3-carboxylic Acid ((−)-(4))——1 N HCl (20 ml) was added to a solution of (−)-**3** (3.28 g) in acetone (100 ml) and the solution was stirred at room temperature for 1 hr. After removal of the acetone, water (20 ml) was added to the residue and the aqueous solution was extracted with AcOEt. The AcOEt extract was washed with water, dried over $MgSO_4$ and concentrated. The residual crystals were washed with ether to yield (−)-**4** (1.65 g), mp 196—197°, $[\alpha]_D^{22}$ −19.6 (*c*=0.542, acetone). NMR (d_6-DMSO) δ: 2.31 (6H, s, $C_{2,6}$–CH_3), 3.58 (3H, s, –COO-CH_3), 5.04 (1H, s, C_4–H), 8.94 (1H, s, >NH). *Anal.* Calcd for $C_{16}H_{16}N_2O_6$: C, 57.83; H, 4.85; N, 8.43. Found: C, 57.82; H, 4.84; N, 8.45.

(+)-5-Methoxycarbonyl-2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3-carboxylic Acid ((+)-(4))——Hydrolysis of (+)-**3** (4.0 g) with 1 N HCl in a manner similar to that used for the preparation of (−)-**4** gave crystals of (+)-**4**, (2.35 g), mp 194—195°, $[\alpha]_D^{22}$ +19.1 (*c*=0.556, acetone). The NMR spectrum of this sample was identical with that of (−)-**4**. *Anal.* Calcd for $C_{16}H_{16}N_2O_6$: C, 57.83; H, 4.85; N, 8.43. Found: C, 57.83; H, 4.93; N, 8.42.

(+)-2-(N-Benzyl-N-methylamino)ethyl Methyl 2,6-Dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate ((+)-(6))——Phosphorus pentachloride (1.13 g) was added in small portions to a stirred suspension of (−)-**4** in dry CH_2Cl_2 (22 ml) at 3—6°. After being stirred at the same temperature for 1 hr, the reaction mixture was cooled to −30°, then a solution of 2-(N-benzyl-N-methylamino)ethanol (7.5 g) in dry CH_2Cl_2 (7.5 ml) was added dropwise. After being stirred for 2 hr under cooling in an ice bath, the reaction

6) T. Takenaka, S. Usuda, T. Nomura, H. Maeno, and T. Sado, *Arzneim.-Forsch.*, **26**, 2172 (1976).

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mixture was washed with ice-cooled 5% aqueous Na_2CO_3 and water, then dried over $MgSO_4$. The solvent was evaporated off and the residue was chromatographed on a silica gel column with benzene–AcOEt (4:1). After concentration of the eluate, the addition of ether–petr. ether (1:2) afforded crystals of (+)-**6**, (1.28 g) mp 107—108°, $[\alpha]_D^{20}$ +26.8 (c=5.00, MeOH). NMR ($CDCl_3$) δ: 2.19 (3H, s, >N–CH_3), 2.33 (6H, s, $C_{2,6}$–CH_3), 2.61 (2H, t, –CH_2CH_2N<), 3.48 (2H, s, –CH_2–C_6H_5), 3.63 (3H, s, –$COOCH_3$), 4.16 (2H, t, –$COOCH_2$-CH_2–), 5.10 (1H, s, C_4–H), 5.82 (1H, s, >NH). *Anal.* Calcd for $C_{26}H_{29}N_3O_6$: C, 65.12; H, 6.10; N, 8.76. Found: C, 65.05; H, 6.12; N, 8.66.

(−)-2-(N-Benzyl-N-methylamino)ethyl Methyl 2,6-Dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate ((−)-(6))——Treatment of (+)-**4** (1.1 g) as described for the preparation of (+)-**6** gave (−)-**6** (0.91 g) as crystals, mp 106—107°, $[\alpha]_D^{20}$ −26.7 (c=5.00, MeOH). The NMR spectrum of this sample was identical with that of (+)-**6**. *Anal.* Calcd for $C_{26}H_{29}N_3O_6$: C, 65.12; H, 6.10; N, 8.76. Found: C, 65.10; H, 6.21; N, 8.76.

Chem. Pharm. Bull. 28(9)2812—2816(1980)

Determination of Fluorescein and Fluorescein Monoglucuronide excreted in Urine

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(Received March 25, 1980)

A simple method for the fluorometric determination of fluorescein and fluorescein monoglucuronide in urine was developed. Free fluorescein was determined by measuring its native fluorescence at 494 nm excitation and 516 nm emission. The conjugate was determined as fluorescein after hydrolysis at 37° for 20 min with β-glucuronidase. This method was applied to investigate the glucuronidation function of Wistar rats with liver injury in comparison with that of normal rats.

Keywords——fluorescein; fluorescin; fluorescein monoglucuronide; β-glucuronidase; fluorometry; glucuronidation; conjugation function; liver

As reported previously,[2] fluorescein (F) and fluorescein monoglucuronide (FG) are excreted in the urine of animals administered F. For metabolic studies of F, a precise method for the determination of both F and FG is required.

Although F had been determined by measuring its absorbance,[3] Menaker and Parker[4] devised a fluorometric method for the determination of urinary and biliary F in order to eliminate the interference of the bile pigments and urochromogens. However, they neglected the interference of fluorescent FG with the determination of F. Later, Webb and his coworkers[5] confirmed the presence of FG and estimated the total fluorescein (TF) in urine photometrically after hydrolysis with sodium hydroxide, despite the decomposition of F by the alkali.[6,7] Thus, no precise method has been reported for the simultaneous determination of F, FG and TF in urine.

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1) Location: *7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan.*
2) S.C. Chen, H. Nakamura, and Z. Tamura, *Chem. Pharm. Bull.*, **28**, 1403 (1980).
3) J.M. Crismon and F.A. Fuhrman, *J. Clin. Invest.*, **26**, 259 (1947).
4) G.J. Menaker and M.L. Parker, *Surgery*, **27**, 41 (1950).
5) J.M. Webb, M. Fonda, and E.A. Brouwer, *J. Pharmacol. and Exptl. Therap.*, **137**, 141 (1961).
6) S.C. Chen, H. Nakamura, and Z. Tamura, *Chem. Pharm. Bull.*, **27**, 475 (1979).
7) R.C. Elderfield (ed.), "Heterocyclic Compounds," Vol. 2, John Wiley and Sons Inc., New York, 1951, p. 512 and 519.

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