Synthesis of the Optically Active *trans*-Isomers of Diltiazem and Their Cardiovascular Effects and Ca-Antagonistic Activity

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Optically active *trans*-isomers of diltiazem were synthesized and their cardiovascular effects were evaluated in anesthetized dogs and in isolated guinea pig hearts.

Both (+)-2 (2*R*,3*S*) and (−)-2 (2*S*,3*R*) were much less active than diltiazem (1, 2*S*,3*S*) with short duration of action. No substantial enantiomeric difference in activity was seen between them. Their Ca-antagonistic activities on Ca^{2+}-induced contractions in K^+-depolarized canine basilar arteries were also examined.

Absolute stereochemistry of (+)-2 was determined to be 2*R*,3*S* by X-ray crystallographic analysis.

Keywords diltiazem; diltiazem (+)-*trans* isomer ; diltiazem (−)-*trans* isomer; absolute stereochemistry; cardiovasculer effect; Ca-channel blocking activity

Diltiazem hydrochloride, (2*S*,3*S*)-3-acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one hydrochloride (**1**),[3] is a representative calcium channel blocker and has been widely used throughout the world as an effective antianginal and antihypertensive agent along with 1,4-dihydropyridine and phenylalkylamine Ca-antagonists.

Since diltiazem has two chiral centers at positions 2 and 3, four optical isomers are possible for its plane structure. In our previous studies[4] on the structure–activity relationships of the stereoisomers of diltiazem, the racemic *cis*-isomer (2*RS*,3*RS*) was found to have more potent and longer lasting coronary vasodilating activity than the corresponding *trans*-isomer [(±)-**2**, (2*RS*,3*SR*)] (Chart 1). Upon optical resolution, the activity of the *cis*-racemate proved to reside almost entirely in the *dextro*-isomer (2*S*,3*S*, diltiazem, **1**), but the toxicity of **1** was equal to that of the racemate. On the basis of these results, **1** was chosen as a candidate for clinical studies.

However, there has been no comparative study on the activity of the optically active *trans*-isomer.[5] In the present study, we synthesized the optically active isomers of (±)-**2** and their cardiovascular effect and Ca-channel blocking activity were examined in comparison with the corresponding *cis*-isomers.

The absolute stereochemistry of (+)-**2** (2*R*,3*S*) determined by X-ray crystallographic analysis is also presented.

Chemistry Optical resolution of the *trans*-lactam ((±)-**3**) was conveniently effected by converting it into the diastereoisomeric esters with an optically active acid. Acylation of (±)-**3**[6] with (*S*)-*N*-(2-naphthylsulfonyl)-2-pyrolidinecarbonyl chloride (NSPCl)[7] in pyridine gave a 1:1 mixture of the diastereoisomeric esters (**4** and **5**). After ready separation of the mixture by column chromatography, (+)-**3** and (−)-**3** were obtained by the hydrolysis of the less polar (**4**) and more polar diastereoisomer

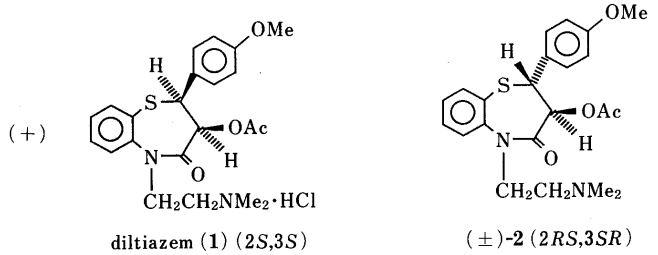

Chart 1

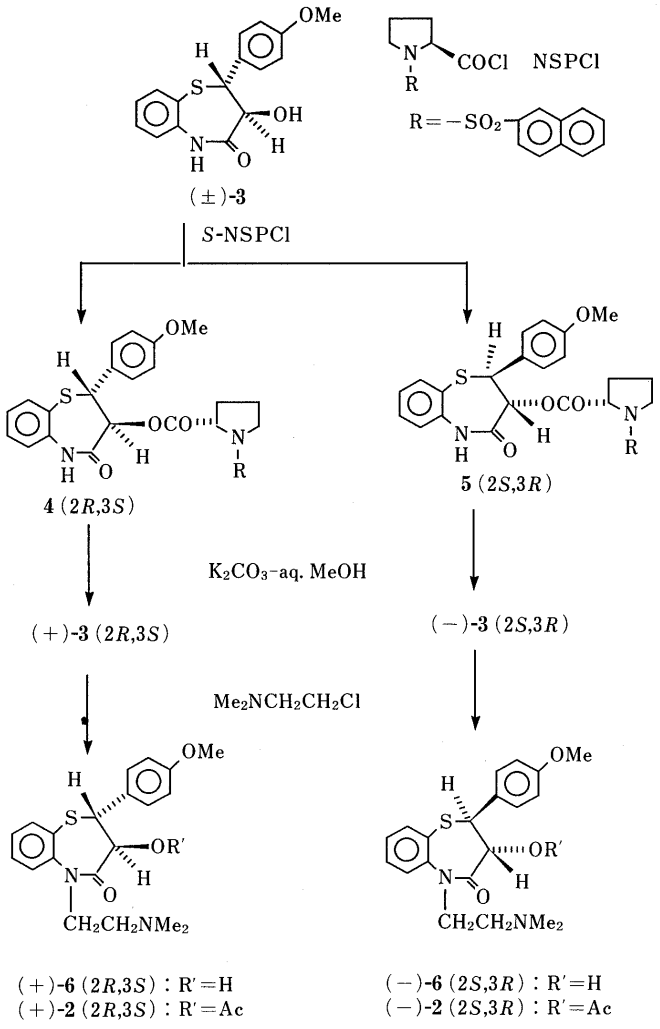

Chart 2

Dedicated to the memory of Professor Shigehiko Sugasawa.

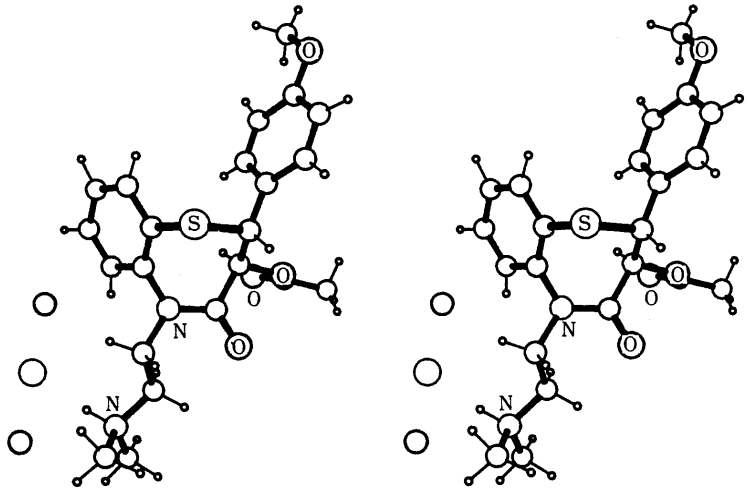

Fig. 1. Stereoscopic View of (+)-**2**·Hydrochloride (2*R*,3*S*)

TABLE I. Bijvoet Pair Ratio Table

h	*k*	*l*	F_o+/−	F_c+/−
4	1	1	1.134	1.082
13	1	1	1.151	1.183
19	1	1	1.125	1.122
2	2	1	0.788	0.749
11	2	1	1.113	1.058
12	2	1	1.068	1.050
13	2	1	1.146	1.105
4	3	1	1.142	1.126
5	3	1	1.083	1.108
9	3	1	0.920	0.935
12	3	1	0.868	0.865
12	4	1	1.080	1.076
13	4	1	0.936	0.941
1	5	1	0.939	0.933
3	5	1	0.898	0.914
4	5	1	1.056	1.064
5	5	1	0.884	0.896
8	5	1	1.263	1.067
10	5	1	1.137	1.144
11	5	1	0.885	0.935
10	1	2	1.139	1.084
2	2	2	0.857	0.829
13	2	2	1.116	1.087
15	2	2	1.136	1.129
16	2	2	0.916	0.895
1	3	2	1.090	1.072
9	3	2	1.067	1.075
11	3	2	0.890	0.817
14	3	2	1.069	1.061
2	4	2	1.078	1.087
3	4	2	0.916	0.904
9	4	2	1.064	1.069
6	5	2	0.929	0.948
8	5	2	0.935	0.933
12	5	2	1.058	1.102
9	1	3	1.126	1.068
14	1	3	1.107	1.060
4	2	3	1.135	1.069
5	2	3	1.069	1.079
2	3	3	0.928	0.893
5	3	3	0.911	0.921
3	4	3	1.114	1.132

42 Bijvoet pair observed

|*F*(obs.)|>50.00, |abs.(*d*+/−)|≧5.0%.

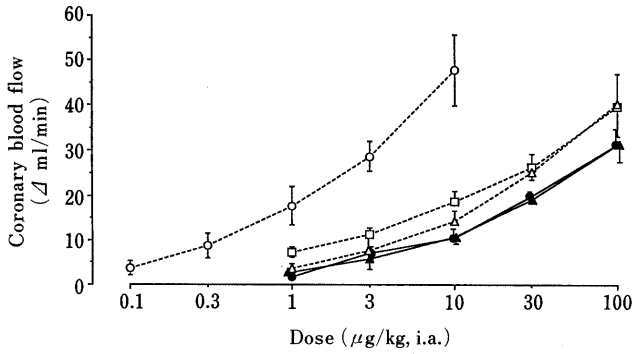

Fig. 2. Effects of Diltiazem and Its Stereoisomers on Coronary Blood Flow in Anesthetized Dogs

Mean±S.E.M. $n=3$. ---○---, **1** (diltiazem, 2*S*,3*S*); ---△---; (−)-isomer of **1** (2*R*,3*R*); —●—, (+)-**2** (2*R*,3*S*); —▲—, (−)-**2** (2*S*,3*R*); ---□---, papaverine.

TABLE II. Duration ($t_{1/2}$) of Coronary Vasodilating Effect in Anesthetized Dogs

	1 μg/kg	3 μg/kg	10 μg/kg	30 μg/kg	100 μg/kg
1 (Diltiazem, 2*S*,3*S*)	52.7±20.5	63.0±17.6	100.7±32.2	—	—
(−)-Isomer of **1** (2*R*,3*R*)	—	—	11.7± 2.3	15.3±2.7	20.3±0.3
(+)-**2** (2*R*,3*S*)	—	—	10.7± 2.4	13.0±2.5	16.7±1.3
(−)-**2** (2*S*,3*R*)	—	—	13.0± 3.4	19.0±3.9	21.3±0.3

(i.a., $n=3$) unit: s.

(**5**) as shown in Chart 2, respectively.

N-Alkylation of (+)-**3** (or (−)-**3**) with 2-(dimethylamino)ethyl chloride in the presence of K_2CO_3 in acetone, followed by *O*-acetylation gave (+)-**2** (or (−)-**2**). Their optical purity was determined by high performance liquid chromatography (HPLC) analysis using Opti-Pak XC (Nihon Millipore Ltd.) to be more than 99.95%, indicating that no isomerization took place during the sequence of reactions (**4**→**2**). The absolute stereochemistry of (+)-**2** was confirmed to be 2*R*,3*S* by X-ray crystallographic analysis as shown in Fig. 1.

Effects on Coronary Blood Flow in Anesthetized Dogs Figure 2 and Table II show the effect of the four stereoisomers of diltiazem on coronary blood flow in anesthetized dogs after intraarterial administration. Both of the optical isomers of the *trans*-isomer ((+)-**2** (2*R*,3*S*) and (−)-**2**

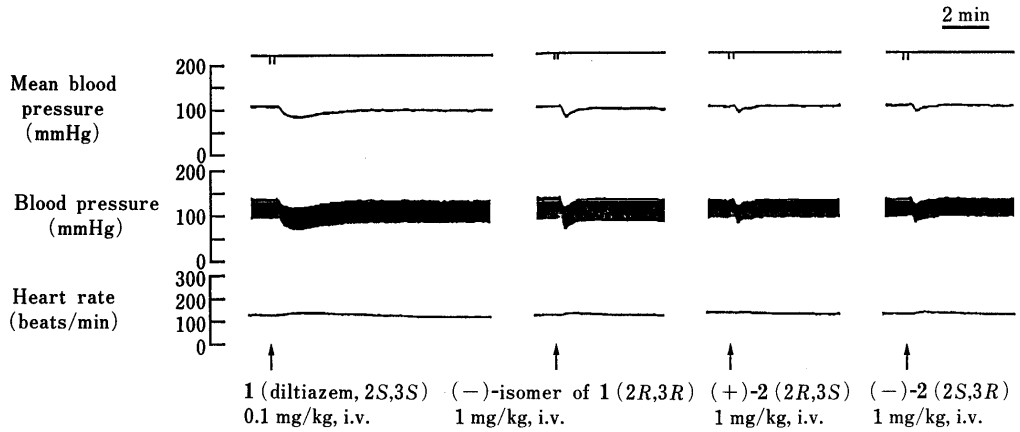

Fig. 3. Effects of Diltiazem and Its Stereoisomers on Blood Pressure and Heart Rate in an Anesthetized Dog (12 kg ♂)

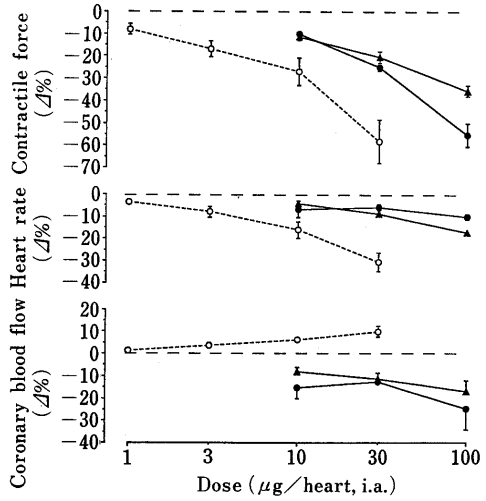

Fig. 4. Effects of Diltiazem and Its Stereoisomers on Myocardial Contractile Force, Heart Rate and Coronary Blood Flow in Isolated Guinea Pig Hearts

---○---, **1** (diltiazem, 2*S*,3*S*); —●—, (+)-**2** (2*R*,3*S*); —▲—, (−)-**2** (2*S*,3*R*).

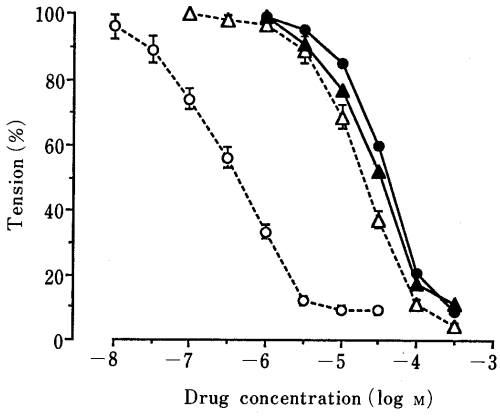

Fig. 5. Inhibitory Actions of Diltiazem and Its Stereoisomers on Calcium-Induced Contraction in Depolarized Basilar Arteries of Dogs

Mean±S.E.M. ---○---, **1** (diltiazem, 2*S*,3*S*) $n=10$; ---△---, (−)-isomer of **1** $n=6$; —●—, (+)-**2** (2*R*,3*S*) $n=9$; —▲—, (−)-**2** (2*S*,3*R*) $n=9$.

(2*S*,3*R*)) increased coronary blood flow dose-dependently. However, their potencies were almost the same and much less than that of diltiazem (**1**) (2*S*,3*S*) (about one-thirtyth). Like the (−)-*cis*-isomer (2*R*,3*R*), their duration of action was quite short. (−)-**2** (2*S*,3*R*) showed slightly longer duration of action than the (+)-isomer (2*R*,3*S*).

As a preliminary study, the effects of the stereoisomers of diltiazem on blood pressure and heart rate were examined in anesthetized dogs by intravenous administration (Fig. 3). Both (+)-**2** and (−)-**2** caused short lasting slight decreases in blood pressure. Their potencies were less than one-tenth of that of **1** (2*S*,3*S*) and less than that of the (−)-*cis*-isomer (2*R*,3*R*).

Effects on Myocardial Contractile Force, Coronary Blood Flow, and Heart Rate in Langendorff's Preparation of Isolated Guinea Pig Hearts As shown in Fig. 4, both (+)-**2** (2*R*,3*S*) and (−)-**2** (2*S*,3*R*) decreased contractile force and heart rate dose-dependently. However, they were from one-third to one-tenth as potent as diltiazem (**1**, 2*S*,3*S*). In contrast to the dose-dependent increasing effect of diltiazem (**1**) on coronary blood flow in isolated guinea pig hearts, both the *trans*-isomers ((+)-**2** and (−)-**2**) caused dose-dependent decreases in blood flow, that is a slight vasoconstrictor effect.[8]

TABLE III. IC_{50} Values of Diltiazem and Its Stereoisomers in Depolarized Basilar Arteries Contracted with Calcium

	IC_{50}[a] (M)	95% CL[b]	Potency ratio[c]
1 (Diltiazem, 2*S*,3*S*)	3.93×10^{-7}	(2.74—5.64)	1
(−)-Isomer of **1** (2*R*,3*R*)	1.88×10^{-5}	(1.38—2.57)	1/ 48
(+)-**2** (2*R*,3*S*)	3.99×10^{-5}	(2.99—5.32)	1/102
(−)-**2** (2*S*,3*R*)	3.38×10^{-5}	(2.54—4.51)	1/ 86

a) Concentration causing 50% relaxation of 1 mM calcium-induced tonic contraction. *b*) 95% confidence limits of IC_{50}. *c*) IC_{50} values of test compounds were divided by that of diltiazem.

Inhibitory Effect on Ca^{2+}-Induced Contraction in Depolarized Canine Basilar Arteries Diltiazem (**1**, 2*S*,3*S*) and the other stereoisomers caused concentration-dependent relaxation of Ca^{2+}-induced tonic contraction in K^+-depolarized canine basilar arteries at concentrations more than 10^{-8} and 10^{-6} mmol/l, respectively. Maximum relaxations induced by all the isomers were not different (about 100%) (Fig. 5). IC_{50} values of the inhibitory effect of these four isomers were shown in Table III. The order of the relaxation effects of these isomers was **1** (2*S*,3*S*)> (−)-*cis* (2*R*,3*R*)>(−)-*trans* (2*S*,3*R*)=(+)-*trans* (2*R*,3*S*). The relaxation effects of (+)-*cis*-(**1**) and (−)-*cis*-isomers were

about 100 and 50 times as potent as those of *trans*-isomers, respectively.

In summary, there is little difference between the effects of the optical isomers of the *trans*-isomer of diltiazem ((+)-**2** and (−)-**2**) on coronary vasodilation, heart rate, myocardial contractile force, and relaxation of Ca^{2+}-induced contraction in basilar arteries. The effect of both isomers were quite similar to the reported observation in the racemic *trans*-isomer ((±)-**2**).[4]

Experimental

All melting points (mp) were determined on a Yamato melting point apparatus Model MP-12 and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi IR-215 spectrometer. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were obtained on a Hitachi RH-90H. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (Me_4Si, 0.0) as an internal standard. Coupling constants (J) are reported in hertz (Hz), and s, d, m, and dd refer to singlet, doublet, multiplet, and double doublet, respectively. Mass spectra (MS) were measured using JEOL JMS-HX 100 mass spectrometer.

Synthesis of the Optically Active Isomers of *trans*-2,3-Dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (3) i) (*S*)-{*N*-(2-Naphthylsulfonyl)}-2-pyrrolidinecarbonyl chloride[7] (7.37 g, 22.8 mmol) was added to a suspension of (±)-**3**[6] (5.28 g, 17.5 mmol) in dry pyridine (10 ml) at 5 °C and the mixture was stirred at room temperature for 10 h. After dilution of the mixture with AcOEt and water, the organic layer was separated, washed with 10% HCl, water, aq. 5% $NaHCO_3$, and water successively, dried, and concentrated. The residual oil was separated by flash column chromatography (silica gel, eluted with AcOEt–toluene (3:7)). From the first fraction, **4** (2*R*,3*S*) (3.91 g, 38.0%) was obtained; mp 178.5—179.5 °C (from $CHCl_3$–EtOAc). IR ν_{max}^{Nujol} cm^{-1}: 1740, 1675. ^{1}H-NMR ($CDCl_3$): 1.2—1.9 (4H, m), 3.1—3.6 (2H, m), 3.77 (3H, s, OCH_3), 4.1—4.6 (1H, m), 4.67 (1H, d, J=11 Hz, C_2-H), 5.36 (1H, d, J=11 Hz, C_3-H), 6.83 (2H, d, J=9 Hz), 7.0—8.2 (12H, m), 8.39 (1H, s). MS m/z: 588 (M^+). $[\alpha]_D^{20}$ +8.4° (c=0.230, DMF), +228.2° (c=1.00, $CHCl_3$).

From the second fraction, **5** (2*S*,3*R*) (4.66 g, 45.2%) was obtained, mp 204.5—205.5 °C (from $CHCl_3$–EtOAc). IR ν_{max}^{Nujol} cm^{-1}: 1755, 1700. ^{1}H-NMR ($CDCl_3$): 1.5—2.5 (4H, m), 3.1—3.6 (2H, m), 3.80 (3H, s), 4.1—4.5 (1H, m), 4.58 (1H, d, J=11 Hz, C_2-H), 5.27 (1H, d, J=11 Hz, C_3-H), 6.87 (2H, d, J=9 Hz), 6.9—8.1 (12H, m), 8.32 (1H, s). MS m/z: 588 (M^+). $[\alpha]_D^{20}$ −0.8° (c=0.29, DMF), −376.8° (c=1.00, $CHCl_3$).

ii) **4** (2*R*,3*S*) (17.3 g, 29.4 mmol) was hydrolyzed by stirring in a mixture of MeOH (170 ml) and 40% aq. K_2CO_3 (85 ml) at room temperature overnight. The reaction mixture was diluted with water and the precipitated crystalls were collected and recrystallized from iso-PrOH to give (+)-**3** (2*R*,3*S*) (6.43 g, 77.6%), mp 197—198 °C. $[\alpha]_D^{20}$ +841° (c=0.50, DMF). The optical purity of this product was more than 99.8% on HPLC: column, Opti-Pak XC (3.9×300 mm, Nihon Millipore Ltd.); temperature, room temperature; eluant, hexane–EtOH (9:1); flow rate, 1.0 ml/min; pressure, 29 kg/cm^2; detector, 254 nm; retention time, 25.6 min for (+)-**3** and 32.1 min for (−)-**3**. IR ν_{max}^{Nujol} cm^{-1}: 1680. ^{1}H-NMR (DMSO-d_6): 3.72 (3H, s, OCH_3), 4.07 (1H, dd, J=7.9, 10 Hz, C_3-H), 4.36 (1H, d, J=10 Hz, C_2-H), 5.27 (1H, d, J=7.9 Hz, OH), 6.84 (2H, d, J=8.8 Hz), 7.15 (2H, d, J=8.8 Hz), 7.1—7.7 (4H, m).

(−)-**3** (2*S*,3*R*), mp 197—198.5 °C (iso-PrOH) was also obtained in 73.7% yield from **5** (2*S*,3*R*) in the same manner as described above, $[\alpha]_D^{20}$ −838° (c=0.29, DMF). IR and ^{1}H-NMR (DMSO-d_6) of (−)-**3** were superimposable over those of (+)-**3**.

(2*R*,3*S*)-5-[2-(Dimethylamino)ethyl]-2,3-dihydro-3-hydroxy-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one ((+)-6) A mixture of(+)-**3** (6.4 g, 21.3 mmol), 2-(dimethylamino)ethyl chloride hydrochloride (3.1 g, 22.4 mmol), K_2CO_3 (8.8 g, 63.9 mmol), acetone (250 ml), and water (2.5 ml) was stirred under reflux overnight. After cooling, the reaction mixture was diluted with $CHCl_3$, and inorganic compounds were removed by filtration and then the filtrate was concentrated. The residue was recrystallized from EtOAc–iso-Pr_2O to give (+)-**6** (2*R*,3*S*) (7.4 g, 98.7%), mp 131—133 °C, $[\alpha]_D^{20}$ +692° (c=1.00, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1675. ^{1}H-NMR (DMSO-d_6): 2.11 (6H, s, NCH_3), 3.71 (3H, s, OCH_3), 4.05 (1H, dd, J=10, 8.4 Hz, C_3-H), 4.32 (1H, d, J=10 Hz, C_2-H), 5.32 (1H, d, J=8.4 Hz, OH), 6.82 (2H, d, J=8.8 Hz), 7.05 (2H, d, J=8.8 Hz), 7.2—7.7 (3H, m).

Similarly, (−)-**6** (2*S*,3*R*) was obtained in 91.5% yield from (−)-**3** (2*S*,3*R*) in the same manner as described above, mp 130—132 °C, $[\alpha]_D^{20}$ −683° (c=0.30, MeOH). IR and ^{1}H-NMR spectra of (−)-**6** were superimposable over those of (+)-**6**.

(2*R*,3*S*)-3-Acetoxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one Hydrochloride ((+)-2·HCl) (+)-**6** (2*R*,3*S*) (7.85 g, 21.0 mmol) was acetylated by heating in Ac_2O (30 ml) and pyridine (1 ml) at 90 °C for 1.5 h. The mixture was concentrated under reduced pressure. The residual oil was converted to the hydrochloride and recrystallized from EtOH–Et_2O to give (+)-**2** (2*R*,3*S*)·HCl (7.65 g, 87.6%), mp 195—196.8 °C. Optical purity of this product was more than 99.95% on HPLC: column, Opti-Pak XC (3.9×300 mm); temperature, room temperature; eluant, hexane–iso-PrOH (30:1); flow rate, 0.7 ml/min; pressure, 18 kg/cm^2; detector, 250 nm; retention time, 34.8 min for (+)-**2** and 38.6 min for (−)-**2**. $[\alpha]_D^{20}$ +538° (c=0.30, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 1750, 1685. ^{1}H-NMR (D_2O): 1.99 (3H, s, $COCH_3$), 3.05 (6H, s, NCH_3), 3.80 (3H, s, OCH_3), 3.2—3.7 (2H, m), 3.9—4.8 (2H, m), 4.70 (1H, d, J=10.9 Hz), 5.22 (1H, d, J=10.9 Hz), 6.91 (2H, d, J=8.6 Hz), 7.11 (2H, d, J=8.6 Hz), 7.2—7.8 (3H, m). *Anal.* Calcd for $C_{22}H_{27}N_2O_4S$·HCl·1.6H_2O: C, 55.13; H, 6.35; Cl, 7.40; N, 5.84; S, 6.69. Found: C, 55.42; H, 6.32; Cl, 7.29; N, 5.98; S, 6.58.

Similarly, (−)-**2** (2*S*,3*R*) was obtained in 86.5% yield from (−)-**6** (2*S*,3*R*), mp 195—196.5 °C, $[\alpha]_D^{20}$ −533° (c=0.30, MeOH). *Anal.* Calcd for $C_{22}H_{27}N_2O_4S$·HCl·1.6H_2O: C, 55.13; H, 6.35; Cl, 7.40; N, 5.84; S, 6.69. Found: C, 55.22; H, 6.34; Cl, 7.35; N, 5.85; S, 6.59.

IR and ^{1}H-NMR spectra of (−)-**2** were superimposable over those of (+)-**2**.

X-Ray Crystallography of (+)-2 (2*R*,3*S*) Hydrochloride $C_{22}H_{27}ClN_2O_4S$·1.6H_2O M_r=479.81. Crystal data: orthorhombic, $P2_12_12_1$, a=34.621 (2), b=11.731 (1), c=6.071 (1) Å, V=2465.8 (2) Å^3, Z=4, D_X=1.29 g/cm^3, μ=24.42 cm^{-1}. The water molecules were disordered. The occupancies of these water molecules were converged to 1.0 and 0.6 during the refinement using the Full Matrix Least Square's method.

The crystal was obtained as a colorless transparent prism from the solution of 2-propanol with the dimensions of 0.60×0.60×0.40 mm. The four-circle diffractometer AFC-5 (Rigaku) was used with graphite-monochromated CuK_α radiation (λ=1.5418 Å). The unit cell parameters were determined from angular settings of 20 reflections in the range of 30°≦2θ≦60°. Three dimensional intensities were measured by the ω-2θ scan technique within the 2θ range of 120°. 2150 unique reflection data were measured, of which 1963 with $|F_O| \geqq 2.67\sigma(F_O)$ were considered as observed. No absorption correction was applied.

The structure was solved by direct method using MULTAN 80[9] and the difference Fourier method. The refinement of atomic parameters was carried out using the Full Matrix Least Square's method, with anisotropic temperature factors for non-hydrogen atoms. 20 hydrogen atoms were located on the difference Fourier maps and refined with isotropic temperature factors. The positions of the residual hydrogen atoms were assumed geometrically and fixed. Throughout the refinement, the function $\Sigma w(|F_O|-|F_C|)^2$ was minimized, and the weighting scheme of $\sqrt{w}=1/\sigma(F_O)$ was used during the final refinement stage.

The atomic scattering factors were taken from "International Tables for X-ray Crystallography."[10] The final R value was 0.061 (R_w=0.053). The maximum electron density on the final Fourier synthesis was 0.38 e/Å.

Absolute Configuration The absolute configuration was determined by Bijvoet pairs method.[11] The structure factors were calculated including anomalous scattering factor of Cl, S and O atoms for CuK_α radiation.[10] The intensity data of Bijvoet pairs (h, k, l and h, $-k$, l) were measured precisely in the right-handed set of coordinate axes. The results are shown in Table I.

The Effects on Coronary Blood Flow in Anesthetized Dogs Three mongrel dogs weighing about 20 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v., followed by a 3—5 mg/kg/h i.v. infusion). Animals were intubated with a tracheal cannula and ventilated with an artificial respirator (15 ml/kg/stroke×20 strokes/min).

The experiment for coronary blood flow was conducted in open chest dogs. Dogs were equipped with a flow probe of an electromagnetic flow meter (MF-27, Nihon Kohden, Tokyo) on the circumflex branch of the left coronary artery. In all experiments, arterial blood pressure was measured with a pressure transducer (MPU-0.5, Nihon Kohden) connected to a polyethylene cannula, which was inserted into the abdominal aorta *via* the femoral artery. Heart rate was also measured by a cardiotachometer triggered by arterial pressure pulse. Drugs were dissolved in 0.9% NaCl solution and administered intraarterially *via* a cannula inserted into the distal coronary artery to the probe. The volume of drug solution was set to be 0.01—0.03 ml/kg.

The Effects on Myocardial Contractile Force, Coronary Blood Flow, Heart Rate in Isolated Guinea Pig Hearts Hartley male guinea pigs (4 weeks of age, 280—320 g in body weight) were stunned and bled. Isolated hearts were perfused according to Langendorff's method with modified Locke–Ringer solution containing 2% defibrinated rabbit blood (40 cm H_2O, 29±1 °C) which had been oxygenated with a mixed gas of 95% O_2 and 5% CO_2. The drug dissolved in 0.9% NaCl solution (0.1 ml) was injected into the aortic cannula and the outflow of the perfusate was measured by means of a drop counter. Myocardial contractile force was measured by means of a strain gauge transducer (UL-50, Minebea, Tokyo) connected to the area of apex cardis with a thread, and heart rate was measured by a cardiotachometer triggered by the systolic pulse.

Calcium Antagonistic Activity in Depolarized Canine Basilar Arteries Basilar arteries of male mongrel dogs (8.6—15.0 kg) which were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) were exposed and cut into ring segments of 5 mm in length. The segments were suspended in a 10 ml organ bath containing physiological salt solution (PSS), which was aerated with 95% O_2 and 5% CO_2. The resting tension was adjusted to 1.0—1.3 g. The composition of PSS, prepared with deionized water, was as follows (mmol/l); NaCl 137, KCl 2.68, $MgCl_2$ 1.89, $CaCl_2$ 1.87, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5. After more than 60 min incubation in normal PSS, the segment was exposed to Ca^{2+} free, K^+-depolarizing PSS (containing 80 mmol/l KCl in equimolar replacement for NaCl). After Ca^{2+} (1 mmol/l)-induced contraction was ascertained to be constant and reproducible, diltiazem (10^{-8}—3×10^{-5} M) or (−)-*cis*-(10^{-7}—3×10^{-4} M), (+)-*trans*-(10^{-6}—3×10^{-4} M), or (−)-*trans*-isomers of diltiazem (10^{-6}—3×10^{-4} M) was cumulatively added to the organ bath during the sustained tonic contraction induced by 1 mmol/l Ca^{2+}. At the end of the experiment, 10^{-4} M papaverine was added to obtain the maximum relaxation. Results were expressed as percentages of papaverine-induced relaxation.

Acknowledgement The authors thank Drs. S. Saito, H. Nakajima, and S. Takeyama for their interest and encouragement. Thanks are also due to the staff of the analytical section of the Organic Research Laboratory for spectral and elemental analysis and Mr. H. Abe for HPLC analysis.

References and Notes

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3) *a*) H. Kugita, H. Inoue, M. Ikezaki, M. Konda, and S. Takeo, *Chem. Pharm. Bull.*, **19**, 595 (1971); *b*) H. Inoue, S. Takeo, M. Kawazu, and H. Kugita, *Yakugaku Zasshi*, **93**, 729 (1973); *c*) M. Sato, T. Nagao, I. Yamaguchi, H. Nakajima, and A. Kiyomoto, *Arzneim. Forsch.*, **21**, 1338 (1971); *d*) H. Nakajima, M. Hoshiyama, K. Yamashita, and A. Kiyomoto, *Jpn. J. Pharmacol.*, **26**, 571 (1976).
4) *a*) T. Nagao, M. Sato, H. Nakajima, and A. Kiyomoto, *Chem. Pharm. Bull.*, **21**, 92 (1973); *b*) T. Nagao, M. Sato, H. Nakajima, and A. Kiyomoto, *Jpn. J. Pharmacol.*, **22**, 1 (1972); *c*) T. Nagao, M. Sato, Y. Iwasawa, T. Takada, R. Ishida, H. Nakajima, and A. Kiyomoto, *Jpn. J. Pharmacol.*, **22**, 467 (1972).
5) After the completion of our study, M. Chiesi and his colleagues reported the synthesis of optically active *trans*-isomer (**2**) by the different method, optical resolution of intermediate *erythro*-2-hydroxy-3-(2-aminophenylthio)-3-(4-methoxyphenyl)propionic acid with cinchonidine, and their action on the mitochondrial Na–Ca exchange system and on sarcolemmal Ca-channels. However, the experimental detail of the synthesis and the absolute stereochemistry of the optical isomer of **2** and their *in vivo* cardiovascular activity have not been reported. M. Chiesi, H. Rogg, K. Eichenberger, P. Gazzotti, and E. Carafoli, *Biochem. Pharmacol.*, **36**, 2735 (1987).
6) H. Kugita, H. Inoue, M. Ikezaki, M. Konda, and S. Takeo, *Chem. Pharm. Bull.*, **18**, 2284 (1970).
7) H. Wada, K. Ishii, N. Tsumagari, M. Matsuo, and M. Matsumoto, Japan Kokai, 59-76051 (1984) [*Chem. Abstr.*, **101**, 151603*d* (1984)].
8) Similar vasoconstrictor effects of **2** in coronary arteries of anesthetized dogs (Fig. 2) and isolated basilar arteries of dogs (Fig. 5) were not observed. Moreover, no increase of contractile force in Langendorff's preparation (Fig. 4) was observed. Therefore, the decrease in coronary blood flow in Langendorff's preparation seems not to be attributable to the Ca-agonistic action of **2**.
9) P. Main, G. German, and M. M. Woolfson, MULTAN 80: A computer program for automatic analysis of phase problem, Univ. of York, England.
10) International Tables for X-Ray Crystallography Vol. IV, Kynoch Press, Birmingham, 1974.
11) J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature* (London), **168**, 271 (1951).