Synthesis and Antihypertensive Activities of New 1,4-Dihydropyridine Derivatives Containing Nitrooxyalkylester Moieties at the 3- and 5-Positions[1)]

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We have synthesized new 1,4-dihydropyridine derivatives having nitrooxyalkylester moieties at the 3- and 5-positions in order to develop potent and long-lasting vasodilators. The antihypertensive activities of these compounds were compared with that of nifedipine. One of them, 2-nitrooxypropyl 3-nitrooxypropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (CD-349) was selected for further development. The structure–activity relationship is discussed.

Keywords 1,4-dihydropyridine; calcium antagonist; antihypertensive activity; nitrate; structure–activity relationship

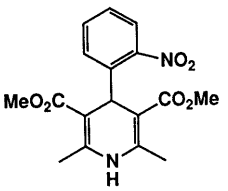
Organic nitrate compounds such as nitroglycerine and nicorandil increase cyclic guanosine 5′-monophosphate (GMP) production in various vascular smooth muscle tissues and promote relaxation.[2)] So the combination of nitro-like and calcium-blocking action in a single molecule was expected to have a potential vasodilating activity superior to that of known 1,4-dihydropyridines. We have previously[3)] reported that 1,4-dihydropyridines having a nitrooxyalkylester moiety at the 3-position have potent antihypertensive activities similar to those of nifedipine[4)] and nicardipine.[5)]

These results encouraged us to prepare 1,4-dihydropyridine derivatives having two nitrooxyalkylester moieties at the 3- and 5-positions in the expectation of increased activity. The structure–activity relationships are also described in this paper.

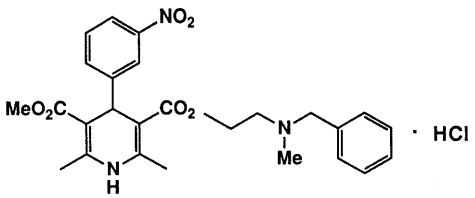
Chemistry

The compounds listed in Tables I, II, and III were synthesized *via* either the Hantzsch reaction[6)] (method A) and its modification (method B) or the esterification of 1,4-dihydropyridine monocarboxylic acid (VII)[3)] with alcohols or alkyl bromides.

2-Nitrooxypropanol and 3-nitrooxypropanol, employed in the preparation of the ester side chains, were prepared from the corresponding methyl 2-hydroxypropionate or ethylene cyanohydrin according to the reported method.[7)] 3-Nitooxy-2-propanol (X) was also prepared from bromoacetone (VIII) by using silver nitrate, followed by reduction of the obtained intermediate (IX) with sodium borohydride.



nifedipine



nicardipine

Fig. 1

4-Nitrooxybutyl bromide (XII), employed in the esterification of monocarboxylic acids (VII), was prepared by nitration of 4-bromobutyl alcohol (XI) with a mixture of concentrated nitric acid/sulfuric acid. The intermediate 4-bromobutyl alcohol (XI) of XII was obtained by the hydrolysis of 4-bromobutyloxy-trimethylsilane by a known method.[8)]

Acetoacetates (III), required for the preparation of 1,4-dihydropyridines by method A or B, were prepared by condensation of the corresponding alcohols with diketene in the presence of triethylamine. 4-Nitrooxybutyl acetoacetate (IIIb) was also obtained from 4-bromobutyl acetoacetate (XIII) by using silver nitrate in acetonitrile. These acetoacetates (IIIa, b) were treated with ammonia in tetrahydrofuran (THF) to give 3-aminocrotonate (IVa, b).

An alternative route to I involves esterification of the mono-carboxylic acid (VII) prepared by removal of the cyanoethyl group of VI (method C). Treatment of VII with acetic anhydride gave the mixed anhydride, which reacted with the nitrooxyalkyl alcohol in the presence of a catalytic amount of acetyl chloride to give I. Compounds I were also obtained from VII by esterification of nitrooxyalkyl bromide in dimethylformamide (DMF) in the presence of potassium carbonate.

Results and Discussion

Twenty-three compounds were tested for antihypertensive activities and duration of action. The screening test for vasodilating activity was carried out by measurement of femoral and vertebral blood flow in anesthetized dogs. The test drugs were administered into the artery. The potency was represented as the amplitude of the maximum increase in blood flow and its duration was represented as the half duration of the peak effect. The antihypertensive activities of the compounds prepared in this work were expressed as the ratio of the potency to that of nifedipine. The results are summarized in Tables I, II, and III.

The activities of compounds **2**, **3**, **4**, **5**, **6**, **9**, and **10** were

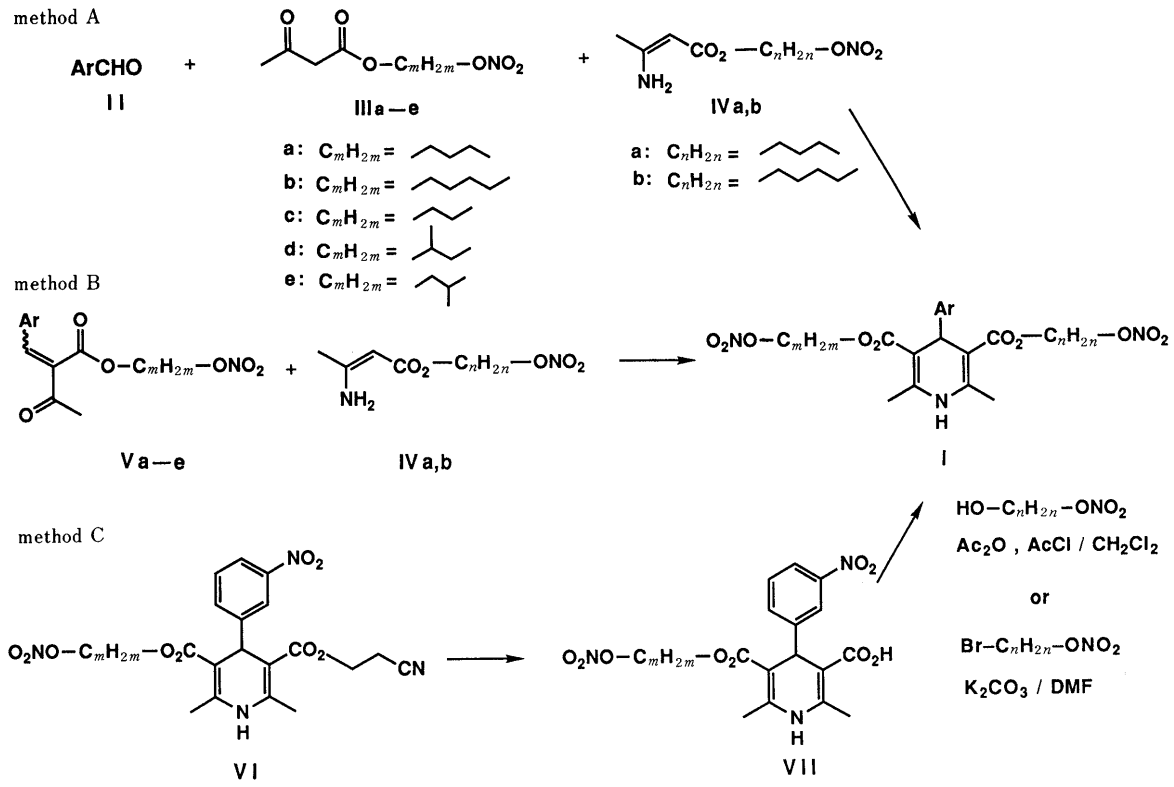


Chart 1

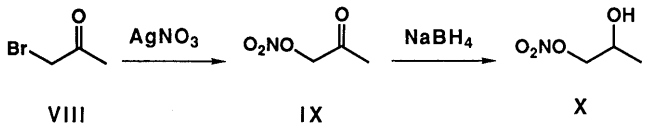


Chart 2

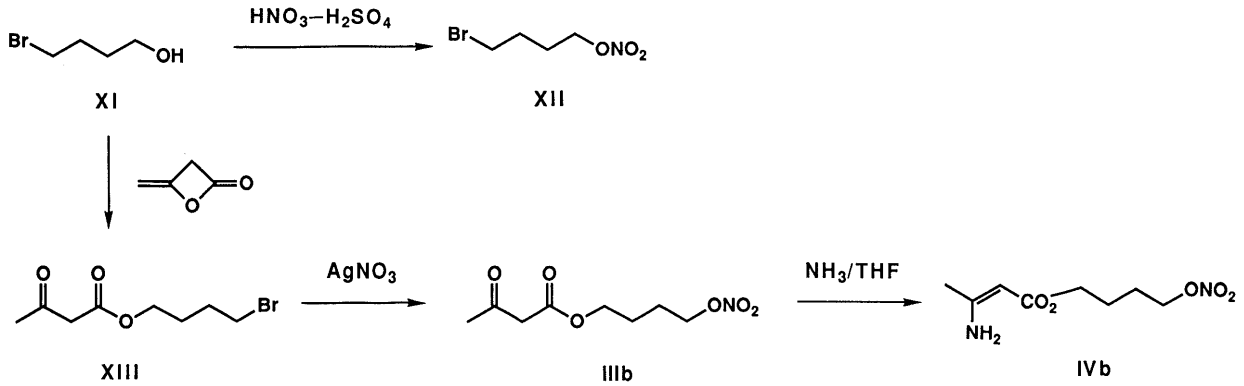


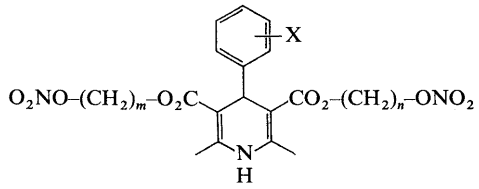
Chart 3

equal to or greater than that of nifedipine in terms of both potency and duration. Some of the effective 1,4-dihydropyridines (I) were selected from those listed in Table I and their antihypertensive activity was examined in unanesthetized spontaneously hypertensive rats (SHR). In particular, the activity of **4** was equal to that of nifedipine. All other compounds were either weakly effective (compounds **3**, **7**, and **10**) or inactive (compound **1**).

Attempts to increase the potency of **4** and **7** by replacing the phenyl group with heterocyclic compounds such as pyridine and thiophene rings met with little success (Table II).

Furthermore, the effect on the activity of introducing a methyl group at the α or β-position of the nitrooxyalkylester moiety at C-3 of compound **4** was examined (Table III). Compounds **19** and **20** having a methyl group at the β-position of the nitrooxyalkylester moiety were considerably less active than **4**. On the other hand, interestingly, when a methyl group was introduced into the α-position of the nitrooxyalkylester moiety, the activity was similar to that of the parent compound **4**. In particular, the activity of compound **23** (CD-349) was more potent and/or long-

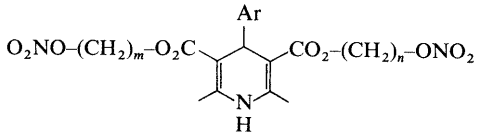
TABLE I. Physical and Biological Properties of 1,4-Dihydropyridines (I)[a]

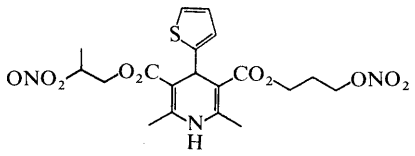


Compd. No.	X	m	n	Method	Yield (%)	mp (°C)	Recrystn. solvent[b]	Formula[c]	Vasodilating FBF	Activity[d] VBF	SHR[e]
1	2-NO_2	2	2	C	30.8	140—141	C–IPE	$C_{19}H_{20}N_4O_{12}$	0.8 (1.9)	1.4 (3.5)	–
2	3-NO_2	2	2	C	46.8	143—145	D–IPE	$C_{19}H_{20}N_4O_{12}$	1.4 (1.6)	1.0 (1.8)	
3	2-NO_2	2	3	B	53.8	123—124	D–IPE	$C_{20}H_{22}N_4O_{12}$	1.6 (2.9)	1.3 (2.3)	++
4	3-NO_2	2	3	C	54.9	135—137	A–H	$C_{20}H_{22}N_4O_{12}$	1.6 (1.9)	1.3 (2.3)	+++
5	2-NO_2	2	4	B	42.5	Oil		$C_{21}H_{24}N_4O_{12}$	1.0 (3.0)	1.0 (1.0)	
6	3-NO_2	2	4	C	53.4	84—85	D–IPE	$C_{21}H_{24}N_4O_{12}$	1.0 (1.5)	1.0 (1.5)	++
7	2-NO_2	3	3	B	47.3	78—80	D–IPE	$C_{21}H_{24}N_4O_{12}$	0.4 (1.9)	1.1 (3.1)	
8	3-NO_2	3	3	C	85.5	134—135	D–IPE	$C_{21}H_{24}N_4O_{12}$	0.3 (0.7)	0.7 (1.0)	
9	2-NO_2	3	4	B	40.5	Oil		$C_{22}H_{26}N_4O_{12}$	1.5 (1.5)	1.5 (1.5)	
10	3-NO_2	3	4	A	55.8	73—74	D–IPE	$C_{22}H_{26}N_4O_{12}$	2.0 (2.0)	1.0 (1.0)	++
11	2-NO_2	4	4	B	46.9	Oil		$C_{23}H_{28}N_4O_{12}$	1.2 (2.0)	0.6 (1.0)	
12	3-NO_2	4	4	A	32.0	58—59	D–IPE	$C_{23}H_{28}N_4O_{12}$	0.7 (1.0)	0.4 (1.0)	

a) Structures of all compounds were confirmed by IR, mass and NMR spectra. For typical examples, see Experimental. *b*) Solvents for recrystallization: A, acetone; D, dichloromethane; IPE, diisopropyl ether; E, ether; EA, ethyl acetate; H, *n*-hexane; M, methanol. *c*) All compounds were analyzed for C, H, and N. Analytical results obtained for these elements were within 0.4% of the calculated values for the formulae shown. *d*) For the biological methods, see Experimental. The figures represent the ratio of the increase in blood flow; the degree of the increase induced by nifedipine was taken as 1.0. The figures shown in brackets are half-maximum duration in minutes of the increased blood flow. Half-maximum duration means the period for which the increase of blood flow remained above half of the maximum increase in blood flow. Abbreviations: FBF, femoral arterial blood flow; VBF, vertebral arterial blood flow. *e*) SHR: conscious spontaneously hypertensive rats.

TABLE II. Physical and Biological Properties of 1,4-Dihydropyridines (I)[a]



Compd. No.	Y	m	n	Method	Yield (%)	mp (°C)	Recrystn. solvent[b]	Formula[c]	Vasodilating FBF	Activity[d] VBF	SHR[e]
13	2-Py[f]	3	2	A	28.2	92—94	D–IPE	$C_{19}H_{22}N_4O_{10}$	0.9 (0.3)	0.2 (1.1)	–
14	2-Py	3	3	A	30.5	99—100	D–IPE	$C_{20}H_{24}N_4O_{10}$	0.2 (0.1)	0.6 (0.2)	
15	3-Py	3	2	A	51.1	108—110	D–Et	$C_{19}H_{22}N_4O_{10}$	0.4 (0.3)	0.4 (0.3)	++
16	4-Py	3	2	A	48.8	78—80	D–Et	$C_{19}H_{22}N_4O_{10}$	0.2 (1.9)	0.3 (2.3)	++
17	2-Th[g]	3	2	A	57.4	Oil		$C_{18}H_{21}SN_3O_{10}$	0.5 (0.4)	0.2 (0.1)	
18				A	40.5	66—69	D–IPE	$C_{19}H_{23}SN_3O_{10}$	0.4 (0.2)	0.4 (0.1)	

a—*e*) See footnotes *a*—*e* in Table I. *f*) 2-Py; 2-pyridyl. *g*) 2-Th; 2-thienyl.

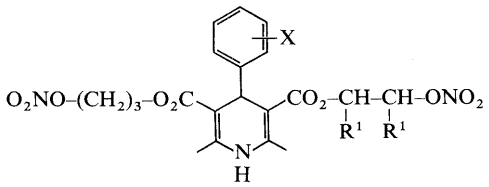
lasting than that of nifedipine. CD-349 also showed a potent effect compared to nifedipine in SHR.

In conscious, unrestrained dogs, CD-349 at oral doses of 0.3, 0.6, and 1 mg/kg lowered blood pressure dose-dependently. In a comparative study, CD-349 at 1 μg/kg orally was more effective and had a longer duration of action than either nifedipine or nicardipine. Reflex tachycardia was less pronounced with CD-349 than with nifedipine. Coronary and cerebral blood flows were increased more markedly with CD-349 than with nicardipine or nimodipine[9] in dogs.[10] In surviving rats, the decrease in blood pressure, and histopathological improvement were more pronounced with CD-349 than with nicardipine or nimodipine in stroke-prone spontaneously hypertensive rats. CD-349 increased cyclic GMP levels in blood vessels, being similar to nitrovasodilators in that respect. This drug inhibited the contraction induced by norepinephrine in Ca^{2+}-free solution in rat aorta or the contration induced by phorbol ester in rabbit aorta. Such an inhibition was not observed with other calcium antagonists such as nifedipine. This is considered to be due to the increase in cyclic GMP levels caused by CD-349.[11] CD-349 is currently undergoing clinical evaluation.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO DS-301 spectrometer. NMR spectra were recorded on Varian XL-200 (200 MHz) spectrometer using tetramethylsilane as an internal standard.

TABLE III. Physical and Biological Properties of 1,4-Dihydropyridines (I)[a]



Compd. No.	X	R^1	R^2	Method	Yield (%)	mp (°C)	Recrystn. solvent[b]	Formula[c]	Vasodilating FBF	Activity[d] VBF	SHR[e]
19	2-NO_2	Me	H	B	55.3	Oil		$C_{21}H_{24}N_4O_{12}$	1.4 (2.7)	0.6 (1.5)	
20	3-NO_2	Me	H	C	43.9	132—135	D–Et	$C_{21}H_{24}N_4O_{12}$	1.1 (3.3)	0.8 (2.3)	++
21	3-NO_2	H	Me	C	43.1	93—95	D–IPE	$C_{20}H_{22}N_4O_{12}$	1.0 (2.2)	1.0 (1.8)	
22	2-NO_2	H	Me	B	48.7	Oil		$C_{21}H_{24}N_4O_{12}$	1.7 (1.9)	1.5 (2.3)	+++
23 (CD-349)	3-NO_2	H	Me	ABC	93.1[f]	97—99	D–IPE	$C_{21}H_{24}N_4O_{12}$	1.5 (3.3)	1.3 (1.8)	++++
Nifedipine									1.0 (1.0)	1.0 (1.0)	+++

a—e) See footnotes *a—e* in Table I. *f*) Yield by method C.

Chemical shifts are given in ppm. The following abbreviations are used: s = singlet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra (MS) were measured on a Shimadzu LKB 9000 spectrometer. Column chromatography was performed on 70—230 mesh silica gel from Merck.

3-Nitrooxy-2-propanol (X) Silver nitrate (254.81 g, 1.5 mol) was added portionwise to a solution of bromoacetone (VIII) (136.98 g, 1 mol) in MeCN (700 ml) under ice cooling, and then the mixture was stirred for 10 h at room temperature. The precipitate was filtered off and washed with MeCN. The filtrate was evaporated to dryness to give nitrooxyacetone (IX) as a pale yellow oil (82.0 g, 68.9%). Nitrooxyacetone (IX) thus formed was used in the next reaction without further purification or after brief purification by column chromatography (hexane : AcOEt = 1 : 1) on silica gel. MS (electron impact (EI)) *m/z*: 76 ($M^+ - ONO_2$). IR (neat) cm^{-1}: 1729 (C=O), 1653, 1288 (ONO_2). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.09 (3H, s), 4.95 (2H, s). $NaBH_4$ (7.57 g, 0.2 mol) was added portionwise to a solution of IX (59.54 g, 0.5 mol) and $NaHCO_3$ (8.40 g, 0.1 mol) in EtOH (50 ml) under ice cooling, and then the mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated, diluted with water, and extracted with AcOEt. The extract was washed with water, and dried (Na_2SO_4). The solvent was evaporated off to give 2-nitrooxypropanol (X) as a pale yellow oil, which was purified by distillation; Yield 27.42 g (45.3%); bp 48—51 °C (0.5 mmHg). MS (EI) *m/z*: 76 ($M^+ - NO_2$). IR (neat) cm^{-1}: 3392 (OH), 1635, 1281 (ONO_2). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.37 (3H, d, J = 7 Hz), 2.39 (1H, br s), 4.15 (1H, m), 4.40 (2H, m). *Anal.* Calcd for $C_3H_7NO_4$: C, 29.76; H, 5.83; N, 11.57. Found: C, 29.38; H, 5.61; N, 11.77.

4-Bromobutyl Nitrate (XII) HNO_3 (90%, 28.0 g, 0.4 mol) was added dropwise to an ice-cold solution of H_2SO_4 (43.59 g, 0.4 mol) with stirring. The solution was stirred for 1 h at 0 °C, then 4-bromobutanol (XI) (30.60 g, 0.2 mol) was added dropwise at the same temperature and the mixture was stirred for 3 h. It was then poured into ice-water and extracted with Et_2O. The extract was washed with saturated $NaHCO_3$ and water, and dried (Na_2SO_4). The solvent was evaporated off to give XIII as a yellow oil (31.22 g, 84.8%). MS (EI) *m/z*: 153 ($M^+ - NO_2$). IR (neat) cm^{-1}: 1631, 1281 (ONO_2). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.00 (4H, m), 3.43 (2H, m), 4.50 (2H, t, J = 5 Hz).

2-Nitrooxypropyl Acetoacetate (IIIe) Diketene (100.82 g, 1.2 mol) was added dropwise to stirred, preheated (50—60 °C) 2-nitrooxypropyl alcohol (121.09 g, 1 mol) in the presence of triethylamine (10.12 g, 0.1 mol), and the mixture was stirred for 1—3 h at 50—60 °C. Then saturated $NaHCO_3$ was added, and the resulting mixture was extracted with CH_2Cl_2. The extract was washed with water and drid (Na_2SO_4). The solvent was evaporated off to give the acetoacetate (IIIe) as a pale yellow oil (173.0 g, 84.3%). MS (EI) *m/z*: 205 (M^+). IR (neat) cm^{-1}: 1751, 1719 (C=O), 1635 (ONO_2). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.41 (3H, d, J = 6 Hz), 2.29 (3H, s), 3.51 (2H, s), 4.19 (1H, dd, J = 11, 7 Hz), 4.40 (1H, dd, J = 11, 3 Hz), 5.33 (1H, m). *Anal.* Calcd for $C_7H_{11}NO_6$: C, 40.98; H, 5.40; N, 6.83. Found: C, 40.69; H, 5.35; N, 6.77.

The acetoacetate (IIIe) thus formed was used in the next reaction without further purification or after rapid purification by column chromatoraphy on silica gel. The other acetoacetate (IIId) was prepared similarly.

1-Methyl-2-nitrooxyethyl Acetoacetate (IIId): yield 78.0%. MS (EI) *m/z*: 205 (M^+). IR (neat) cm^{-1}: 1747, 1719 (C=O), 1641 (ONO_2). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.34 (3H, d, J = 5 Hz), 2.27 (3H, s), 3.48 (2H, s), 4.42 (1H, dd, J = 10, 5 Hz), 4.60 (1H, dd, J = 10, 3 Hz), 5.25 (1H, m). *Anal.* Calcd for $C_7H_{11}NO_6$: C, 40.98; H, 5.40; N, 6.83. Found: C, 50.21; H, 5.25; N, 6.96.

4-Nitrooxybutyl Acetoacetate (IIIb) Diketene (100.82 g, 1.2 mol) was added dropwise to stirred, preheated (50—60 °C) 4-bromobutyl alcohol (153.02 g, 1 mol) (XI) in the presence of triethylamine (10.12 g, 0.1 mol), and the mixture was stirred for 1—3 h at 50—60 °C. Then saturated $NaHCO_3$ was added, and the resulting mixture was extracted with CH_2Cl_2. The extract was washed with water, and dried (Na_2SO_4). The solvent was evaporated off and the remaining crude product was distilled to provide the pure 4-bromobutyl acetoacetate (XIII); yield; 130.0 g (54.8%); bp 130—132 °C (0.7 mmHg). MS (EI) *m/z*: 238 ($M^+ + 1$). IR (neat) cm^{-1}: 1708 (C=O). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.89 (4H, m), 2.29 (3H, s), 3.45 (2H, t, J = 5 Hz), 3.49 (2H, s), 4.19 (2H, t, J = 5 Hz). *Anal.* Calcd for $C_8H_{13}BrO_3$: C, 40.52; H, 5.53. Found: C, 40.31; H, 5.48. Silver nitrate (101.62 g, 0.6 mol) was added portionwise to a solution of the acetoacetate (XIII) (118.55 g, 0.5 mol) in MeCN (500 ml) at room temperature, and the mixture was stirred for 10 h at the same temperature. The precipitate was filtered off, and washed with MeCN. The filtrate and washing were combined and evaporated under reduced pressure. The residue was subjected to chromatography (hexane : AcOEt = 1 : 1) on silica gel to give 4-nitrooxybutyl acetoacetate (IIIb) as a pale yellow oil (87.0 g, 79.4%). MS (EI) *m/z*: 219 (M^+). IR (next) cm^{-1}: 1748, 1719 (C=O), 1631, 1282 (ONO_2). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.83 (4H, m), 2.29 (3H, s), 3.48 (2H, s), 4.20 (2H, t, J = 6 Hz), 4.51 (2H, t, J = 6 Hz). *Anal.* Calcd for: $C_8H_{13}NO_6$: C, 43.83; H, 5.98; N, 6.39. Found: C, 43.85; H, 5.77; N, 6.38.

4-Nitrooxybutyl 3-Aminocrotonate (IVb) Ammonia was bubbled for 3 h into a solution of 4-nitrooxybutyl acetoacetate (IIIb) (0.5 mol) in THF (300 ml) at 0 °C. The reaction flask was tightly stoppered and the content was stirred at 0 °C for 24 h. The solvent and excess NH_3 were evaporated off to give the crude 3-aminocrotonate derivative (IVb), which was used for the next step. MS (EI) *m/z*: 218 (M^+). IR (neat) cm^{-1}: 3459 (NH), 1666 (C=O). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.81 (4H, m), 1.91 (3H, s), 4.10 (2H, t, J = 5 Hz), 4.50 (2H, t, J = 5 Hz), 4.53 (1H, s), 7.70 (1H, br s).

4-Nitrooxybutyl 2-Cyanoethyl 2,6-Dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (VIb) A solution of 3-nitrobenzaldehyde (II) (15.11 g, 0.1 mol), 4-nitrooxybutyl acetoacetate (IIIb) (21.92 g, 0.1 mol), and cyanoethyl 3-aminocrotonate (15.55 g, 0.1 mol) in 2-propanol (300 ml) was refluxed for 3 h with stirring. The solvent was removed and the residue was purified by chromatography on silica gel with hexane–AcOEt (1 : 1, v/v) to give VIb (26.50 g, 54.3%) as yellow crystals, mp 118–120 °C (from CH_2Cl_2–iso-Pr_2O). MS *m/z*: 488 (M^+). IR (KBr) cm^{-1}: 3351 (NH), 1703 (C=O). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.72 (4H, m), 2.39 (6H, s), 2.68 (2H, t, J = 5 Hz), 4.09 (2H, m), 4.28 (2H, t, J = 5 Hz), 4.45 (2H, m), 5.10 (1H, s), 5.99 (1H, br s), 7.35—8.15 (4H, m). *Anal.* Calcd for $C_{22}H_{24}N_4O_9$: C, 54.09; H, 4.95; N, 11.47. Found: C, 53.91; H, 4.90; N, 11.27.

Hydrogen 4-Nitrooxybutyl 2,6-Dimethyl-4-(3-nitrophenyl)-1,4-dihydro-

3,5-pyridinedicarboxylate (VIIb) A suspension of 4-nitrooxybutyl 2-cyanoethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (VIb) (48.84 g, 0.1 mol) in acetone (200 ml) and 5 N NaOH (200 ml) was stirred at room temperature for 2 h. The reaction mixture was diluted with water, and extracted with CH_2Cl_2. The aqueous layer was acidified with phosphoric acid (24.50 g, 0.25 mol) under ice cooling. The precipitated product was collected by filtration, washed with water, and then dried *in vacuo*. Recrystallization from methanol–ether gave VII (34.0 g, 83.5%) as yellow crystals, mp 171—172 °C. MS (EI) *m/z*: 392 ($M^+ - ONO_2$). IR (KBr) cm^{-1}: 3373 (NH), 1704, 1675 (C=O). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.61 (4H, m), 2.28 (3H, s), 2.32 (3H, s), 4.00 (2H, m), 4.48 (2H, t, J=5 Hz), 5.00 (1H, s), 7.47—8.08 (4H, m), 8.98 (1H, br s). Signals due to CO_2H are masked by other signals. *Anal.* Calcd for $C_{19}H_{21}N_3O_9$: C, 52.41; H, 4.86; N, 9.65. Found: C, 52.10; H, 4.51; N, 9.63.

2-Nitrooxypropyl 3-Nitrooxypropyl 2,6-Dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (23) (CD-349) Method A: A solution of 3-nitrobenzaldehyde (II) (1.51 g, 10 mmol), 2-nitrooxypropyl acetoacetate (IIIe) (2.05 g, 10 mmol), and 3-nitrooxypropyl 3-aminocrotonate (IVa) (2.04 g, 10 mmol) in 2-propanol (30 ml) was refluxed for 3 h with stirring. The solvent was removed and the residue was purified by chromatography on silica gel with hexane–AcOEt (1 : 1, v/v) to give **23** (CD-349) (1.86 g, 35.5%) as yellow crystals, 97—99 °C (from CH_2Cl_2–iso-Pr_2O). MS (EI) *m/z*: 524 (M^+). *Anal.* Calcd for $C_{21}H_{24}N_4O_{12}$: C, 48.09; H, 4.61; N, 10.68. Found: C, 48.12; H, 4.58; N, 10.49.

Method B: A solution of 3-nitrobenzaldehyde (II) (30.22 g, 0.2 mol), 2-nitrooxypropyl acetoacetate (IIIe) (41.03 g, 0.2 mol), AcOH (3.0 g, 50 mmol), and piperidine (4.26 g, 50 mmol) in benzene (500 ml) was refluxed for 2 h with continuous removal of water by a Dean–Stark apparatus. The benzene layer was washed with water, dried (Na_2SO_4) and concentrated to give 2-nitrooxypropyl 2-(3-nitrobenzylidene) acetoacetate (Ve) as a yellow oil (54.12 g, 80.0%). The ratio of isomers was 2 : 1 as judged from the NMR spectrum. MS (EI) *m/z*: 338 (M^+). IR (neat) cm^{-1}: 1737, 1703, 1672 (C=O).

A solution of Ve (3.38 g, 10 mmol) and 3-nitrooxypropyl 3-aminocrotonate (IVa) (2.04 g, 10 mmol) in 2-propanol (30 ml) was refluxed for 3 h with stirring. The solvent was removed and the residue was purified by chromatography on silica gel with hexane–AcOEt (1 : 1, v/v) to give **23** (CD-349) (3.21 g, 61.3%) as yellow crystals, mp 97—99 °C (from CH_2Cl_2–iso-Pr_2O).

Method C: Acetic anhydride (3.06 g, 30 mmol) was added to a suspension of hydrogen 3-nitrooxypropyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate[12] (VIIa) (4.21 g, 10 mmol) in CH_2Cl_2 (30 ml) at room temperature. A solution of 2-nitrooxypropanol (1.33 g, 12 mmol) in CH_2Cl_2 (10 ml) containing a catalytic amount of acetyl chloride was added to the reaction mixture at the same temperature, and the mixture was stirred for 5 h. The reaction mixture was diluted with CH_2Cl_2, washed with 1 N NaOH and brine, and dried (Na_2SO_4). The solvent was removed, and the residue was recrystallized from CH_2Cl_2–iso-Pr_2O to give **23** (CD-349) as yellow crystals (4.88 g, 93.1%).

TABLE IV. Spectral Data for 1,4-Dihydropyridines (I) Listed in Table I

Compd. No.	IR (KBr) cm^{-1} (NH)	IR (KBr) cm^{-1} (CO)	^{1}H-NMR ($CDCl_3$) δ (ppm)
1	3347	1700	2.37 (6H, s), 4.30 (4H, m), 4.61 (4H, t, J=5 Hz), 5.79 (2H, s), 7.20—7.74 (4H, m)
2	3330	1705, 1686	2.41 (6H, s), 4.34 (4H, m), 4.67 (4H, t, J=5 Hz), 5.06 (1H, s), 5.90 (1H, s), 7.35—8.17 (4H, m)
3	3416	1704	2.02 (2H, m), 2.36 (3H, s), 2.38 (3H, s), 4.13 (2H, m), 4.25 (2H, m), 4.37 (2H, t, J=5 Hz), 4.63 (2H, t, J=5 Hz), 5.77 (1H, s), 5.83 (1H, br s), 7.22—7.75 (4H, m)
4	3376	1700	2.04 (2H, m), 2.35 (3H, s), 2.38 (3H, s), 4.16 (2H, t, J=6 Hz), 4.35 (2H, m), 4.40 (2H, t, J=6 Hz), 4.65 (2H, t, J=6 Hz), 5.06 (1H, s), 6.08 (1H, br s), 7.33—8.15 (4H, m)
5	3351	1730, 1698	1.68 (4H, m), 2.37 (3H, s), 2.38 (3H, s), 4.06 (2H, m), 4.29 (2H, m), 4.40 (2H, t, J=6 Hz), 4.62 (2H, t, J=5 Hz), 5.79 (1H, s), 5.80 (1H, br s), 7.22—7.77 (4H, m)
6	3333	1698	1.72 (4H, m), 2.37 (3H, s), 2.40 (3H, s), 4.09 (2H, m), 4.34 (2H, m), 4.45 (2H, m), 4.65 (2H, m), 5.07 (1H, s), 5.84 (1H, br s), 7.34—8.15 (4H, m)
7	3386	1698, 1677	2.04 (4H, m), 2.36 (6H, s), 4.14 (4H, m), 4.38 (4H, t, J=6 Hz), 5.76 (1H, s), 5.80 (1H, br s), 7.23—7.76 (4H, m)
8	3384	1698	2.06 (4H, m), 2.39 (6H, s), 4.17 (4H, m), 4.40 (4H, t, J=6 Hz), 5.07 (1H, s), 5.94 (1H, br s), 7.36—8.12 (4H, m)
9	3350	1728	1.68 (3H, s), 2.03 (3H, m), 2.37 (3H, s), 2.39 (3H, s), 4.00 (2H, m), 4.12 (2H, m), 4.36 (2H, t, J=6 Hz), 4.42 (2H, t, J=6 Hz), 5.76 (1H, br s), 5.78 (1H, s), 7.22—7.76 (4H, m)
10	3339	1695	1.72 (4H, m), 2.06 (2H, m), 2.38 (3H, s), 2.39 (3H, s), 4.10 (2H, m), 4.15 (2H, m), 4.41 (2H, t, J=5 Hz), 4.45 (2H, t, J=5 Hz), 5.07 (1H, s), 5.83 (1H, br s), 7.37—8.12 (4H, m)
11	3338	1696	1.68 (8H, m), 2.36 (6H, s), 4.06 (4H, m), 5.75 (1H, br s), 5.77 (1H, s), 7.20—7.76 (4H, m)
12	3328	1698	1.72 (8H, m), 2.39 (6H, s), 4.11 (3H, s), 4.43 (4H, t, J=5 Hz), 5.08 (1H, s), 5.75 (1H, br s), 7.34—8.15 (4H, m)
13	3420	1702, 1680	2.01 (2H, m), 2.28 (3H, s), 2.30 (3H, s), 4.14 (2H, m), 4.33 (2H, m), 4.39 (2H, t, J=6 Hz), 4.62 (2H, m), 5.18 (1H, s), 7.15—7.74 (3H, m), 8.03 (1H, br s), 8.49—8.60 (1H, m)
14	3435	1703	2.02 (4H, m), 2.27 (6H, s), 4.16 (4H, m), 4.42 (4H, t, J=7 Hz), 5.17 (1H, s), 7.12—7.71 (3H, m), 8.34 (1H, br s), 8.47—8.56 (1H, m)
15	3383	1694	2.03 (2H, m), 2.36 (3H, s), 2.39 (3H, s), 4.16 (2H, m), 4.33 (2H, m), 4.40 (2H, d, J=6 Hz), 4.64 (2H, m), 4.95 (1H, s), 6.70 (1H, br s), 7.15—8.59 (4H, m)
16	3420	1699	2.04 (2H, m), 2.35 (3H, s), 2.40 (3H, s), 4.18 (2H, m), 4.35 (2H, m), 4.42 (2H, t, J=6 Hz), 4.65 (2H, m), 4.98 (1H, s), 6.12 (1H, br s), 7.21 (2H, dd, J=6, 1.5 Hz), 8.48 (2H, dd, J=6, 1.5 Hz)
17	3343	1697	2.06 (2H, m), 2.34 (3H, s), 2.39 (3H, s), 4.22 (2H, m), 4.41 (2H, t, J=6 Hz), 4.43 (2H, m), 4.72 (2H, m), 5.28 (1H, s), 5.91 (1H, br s), 6.74—7.21 (3H, m)
18	3346	1698	1.35, 1.38 (3H, each d, J=3 Hz), 2.11 (2H, m), 2.34 (3H, s), 2.36 (3H, s), 4.21 (2H, m), 4.40 (2H, m), 4.66 (2H, t, J=6 Hz), 5.28 (1H, s), 5.35 (1H, s), 6.16 (1H, br s), 6.73—7.12 (3H, m)
19	3344	1698	1.23, 1.32 (3H, each d, J=8 Hz), 2.02 (2H, m), 2.31, 2.33, 2.36, 2.39 (6H, each s), 4.03 (2H, m), 4.24 (2H, m), 4.41 (2H, m), 5.16 (1H, m), 5.81 (1H, s), 5.87 (1H, br s), 7.20—7.77 (4H, m)
20	3381	1697	1.17, 1.35 (3H, each d, J=7 Hz), 2.05 (2H, m), 2.36 (3H, s), 2.41 (3H, s), 2.41 (3H, s), 4.16 (2H, m), 4.37 (2H, m), 4.43 (2H, t, J=6 Hz), 5.06 (1H, s), 5.18 (1H, m), 5.92 (1H, br s), 7.31—8.20 (4H, m)
21	3346	1700	1.29, 1.34 (3H, each d, J=7 Hz), 2.37 (3H, s), 2.38 (3H, s), 4.18 (2H, m), 4.34 (2H, m), 4.66 (2H, t, J=5 Hz), 5.04 (1H, s), 5.30 (1H, m), 6.03 (1H, br s), 7.34—8.14 (4H, m)
22	3344	1699	1.25, 1.29 (3H, each d, J=6 Hz), 2.04 (2H, m), 2.36 (3H, s), 2.38 (3H, s), 4.06 (2H, m), 4.18 (2H, m), 4.38 (2H, m), 5.28 (1H, m), 5.73, 5.75 (1H, each s), 5.91 (1H, br s), 7.21—7.74 (4H, m)
23	3361	1699	1.28, 1.34 (3H, each d, J=6 Hz), 2.06 (2H, m), 2.38 (3H, s), 2.40 (3H, s), 4.10 (1H, dd, J=12, 3 Hz), 4.15 (2H, m), 4.29 (1H, dd, J=12, 3 Hz), 4.43 (2H, t, J=6 Hz), 5.07 (1H, s), 5.30 (1H, m), 6.06 (1H, s), 7.33—8.12 (4H, m), 7.22—7.44 (4H, m)

Bis(nitrooxybutyl) 2,6-Dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (12) A solution of hydrogen 4-nitrooxybutyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate (VIIb) (4.35 g, 10 mmol), 4-bromobutyl nitrate (XII) (2.02 g, 12 mmol), and potassium carbonate (1.66 g, 12 mmol) in DMF (30 ml) was stirred for 12 h at room temprature. The mixture was extracted with CH_2Cl_2 (100 ml). The CH_2Cl_2 layer was washed with water and brine, and dried (Na_2SO_4), then the solvent was removed, and the residue was purified by chromatography on silica gel with hexane–AcOEt (1 : 1, v/v) to give the product **12** (1.77 g, 32.0%). MS (EI) *m/z* 552 (M^+). *Anal.* Calcd for $C_{23}H_{28}N_4O_{12}$: C, 50.00; H, 5.11; N, 10.14. Found: C, 49.98; H, 4.92; N, 10.11.

Other 1,4-dihydropyridines (I) were similarly prepared by methods A, B, and C and their spectral data are listed in Table IV.

Biological Evaluation Procedures Male and female mongrel dogs weighing 8—15 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v. supplemented with additional doses as necessary). Femoral arterial blood pressure was measured with a pressure transducer (Nihon Kohden MPV-0.5, Tokyo, Japan) connected to a rigid polyethylene tube introduced to the femoral artery. Heart rate was measured by using a heart rate counter (Nihon Kohden T-600G, Japan) driven by the waves of arterial pulse pressure. An extracorporeal path was constructed in the femoral or vertebral arteries. The heparinized blood was perfused from the proximal sites of the femoral and vertebral arteries to the distal sites of their arteries through the extracorporeal path. Each test compound or nifedipine (Bayer, Germany) was dissolved in 50% ethenol solution at a concentration of 100—500 μg/ml. The dose of 0.1, 0.3 or 1 μg was administered intra-arterially *via* the extracorporeal path. The flow meter probe was interposed in the extracorporeal path. The femoral and vertebral blood flow was measured by means of an electromagnetic blood flow meter (Nihon Kohden MF-27 and MFV-1200, Japan). The maximum increase in blood flow induced by each compound was represented as a ratio with respect to the maximum increase in blood flow induced by nifedipine. Half-maximum duration of increased blood flow induced by each compound was represented in minutes; half-maximum duration means the period for which the increase of blood flow was maintained at over half the maximum increase in blood flow.

References

1) This work was presented at the 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1987.
2) S. A. Waldman and F. Murad, *J. Cardiovasc. Pharmacol.*, **12**, (Supple. 5): S 115, (1988); S. Holzmann, *J. Cardiovasc. Res.*, **5**, 346 (1983).
3) T. Ogawa, A. Nakazato, K. Tsuchida, and K. Hatayama, *Chem. Pharm. Bull.*, **41**, 108 (1993).
4) H. S. Mueller and R. A. Chahine, *Am. J. Cardiol.*, **71**, 645 (1981).
5) T. Takenaka and J. Handa, *Inter. J. Clin. Pharm. Biopharm.*, **17**, 1 (1979); *Chem. Abstr.*, **90**, 32975 (1979).
6) A. Hantzsh, *Justus Liebigs Ann. Chem.*, **1**, 215 (1982); F. Bossert, H. Meyer, and E. Wehinger, *Angew. Chem. Int. Ed. Engl.*, **20**, 762 (1981).
7) T. Ogawa, A. Nakazato, M. Sato, and K. Hatayama, *Synthesis*, **1990**, 459.
8) U. Kruerke, *Chem. Ber.*, **95**, 174 (1962).
9) R. Towart, E. Wehinger, H. Meyer, and S. Kazda, *Arzneim.-Forsch.*, **38**, 1662 (1988).
10) K. Tsuchida, M. Muramatsu, K. Kaneko, and H. Aihara, *Cardiovasc. Drug Rev.*, **8**, 45 (1990).
11) N. Miyata, K. Tsuchida, and S. Otomo, *J. Cardiovasc. Pharmacol.*, **17**, 786 (1991).
12) T. Ogawa, K. Matsumoto, T. Yokoo, K. Hatayama, and K. Kitamura, *J. Chem. Soc., Perkin Trans. 1*, **1993**, 525.