Synthesis and Pharmacological Evaluation of (Nitrooxy)alkyl Apovincaminates

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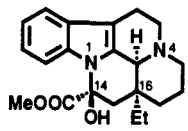
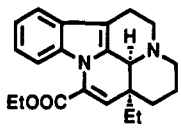
A series of (nitrooxy)alkyl apovincaminates has been synthesized and evaluated for their effects on vertebral and femoral blood flow. These derivatives were prepared from apovincaminic acid (4). In cerebral circulation, compound 5 (0.03–1.0 mg/kg iv) caused a dose-dependent increase in cerebral blood flow (CerBF) without affecting the blood pressure. It was more potent than vinpocetine (2). The structures of 2 and 5, determined by X-ray crystallography, showed differences in the electrostatic potential image and in the conformation of the ethyl group at the 16-position.

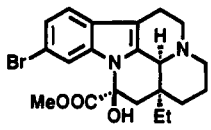
Vincamine (1),[1-3] an alkaloid of Vinca minor Linne, has a vasodilator effect.[4] It is known that ethyl vincaminate[5,6] (vinpocetine, 2) and 11-bromovincamine[7,8] (brovincamine, 3) have been used to treat cerebrovascular diseases. The structure of the eburunamenine skelton seemed to be of interest in view of its possible relationship with pharmacological activity. In the present investigation, (nitrooxy)alkyl apovincaminates were synthesized and evaluated for their effects on vertebral and femoral blood flow. Also, the structures of 5 and 2 were determined by X-ray crystallography.



vincamine (1)



vinpocetine (2)
(Eburunamenine-14-carboxylate)



brovincamine (3)

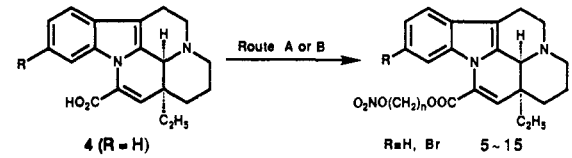
Chemistry

The compounds studied here were synthesized starting from apovincaminic acid (4),[5] as summarized in Scheme I. Esterification of 4 with (nitrooxy)alkyl bromides which were synthesized by nitration of bromoalkyl alcohols gave the corresponding (nitrooxy)alkyl apovincaminates under basic condition. Alternatively, chlorination of 4 with $SOCl_2$ gave an acid chloride, which was reacted with nitrooxyalkyl alcohols to give these (nitrooxy)alkyl compounds. (Nitrooxy)alkyl alcohols were obtained by reaction of bromoalkyl alcohols with $AgNO_3$–CH_3CN.[9] Physical and analytical data for 5–15 are recorded in Table I.

Results and Discussion

Pharmacological Results. Table I exhibits the results of the effect on vertebral blood flow (VBF) and femoral blood flow (FBF) after intraarterial administration in anesthetized dogs. The data (VBF and FBF) are given in terms of the potency ratio to the effect of vinpocetine. The activities of compounds 10–15 were less potent than those of vinpocetine. The activities of compounds 8 and 9 were roughly equivalent to vinpocetine, and compounds 5, 6, and 7 were more potent than vinpocetine. The

Scheme I



Route A; K_2CO_3/DMF, $O_2NO(CH_2)_nBr$[*1]

Route B; 1) $SOCl_2$/DMF, 2) $O_2NO(CH_2)_nOH$[*2]

$Br(CH_2)_nOH \xrightarrow[c.H_2SO_4]{HNO_3} Br(CH_2)_nONO_2$[*1]

$Br(CH_2)_nOH \xrightarrow[CH_3CN]{AgNO_3} O_2NO(CH_2)_nOH$[*2]

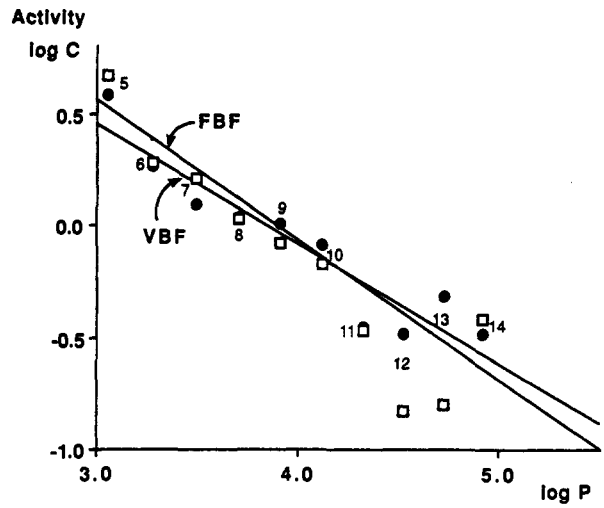


Figure 1. Relationship between biological activity (C: potency ratio to the effect of vinpocetine) and log P (calcd): (●) VBF, (□) FBF. The lines through the data were obtained from linear regression fit: VBF: log C = –0.53 log P + 2.05, r = 0.94. FBF: log C = –0.71 log P + 2.69, r = 0.93.

structure–activity relationships of (nitrooxy)alkyl apovincaminates (R = H) suggested that elongation of the methylene chain resulted in a decrease in VBF and FBF. Also, there seemed to be a relationship between the effect of blood flow and the log P, which was estimated by calculating the octanol/water partition coefficient.[10] This relationship is shown graphically in Figure 1 for compounds 5–15.

2-(Nitrooxy)ethyl apovincaminate (5), the most potent compound, was examined further. Note that introduction of a bromine atom at the 11-position of 5 (compound 15) decreased the effect on VBF and FBF.

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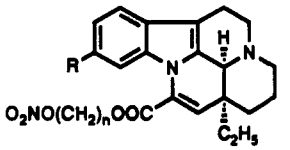
Table I. Physical and Pharmacological Properties of (Nitrooxy)alkyl Apovincaminates



compd no.	n	R	method	formula[a]	mp (°C)	yield (%)	recryst[b] solvent	activity[c] VBF[d]	activity[c] FBF[e]
5	2	H	B	$C_{22}H_{25}N_3O_5$	111–112	75.4	E-H	3.83	4.72
6	3	H	B	$C_{23}H_{27}N_3O_5$	88–90	56.4	E-H	1.85	1.91
7	4	H	A	$C_{24}H_{29}N_3O_5$	oil	46.9		1.25	1.63
8	5	H	A	$C_{25}H_{31}N_3O_5$·HCl	162–165	69.7	M-E	1.09	1.07
9	6	H	B	$C_{26}H_{33}N_3O_5$·HCl	145–147	25.6	M-E	0.99	0.84
10	7	H	A	$C_{27}H_{35}N_3O_5$	oil	43.0		0.82	0.67
11	8	H	A	$C_{28}H_{37}N_3O_5$	oil	30.6		0.35	0.34
12	9	H	A	$C_{29}H_{39}N_3O_5$	oil	34.5		0.33	0.15
13	10	H	A	$C_{30}H_{41}N_3O_5$	oil	44.8		0.48	0.16
14	11	H	B	$C_{31}H_{43}N_3O_5$·HCl	95–98	69.7	A-H	0.33	0.38
15	2	Br	A	$C_{22}H_{24}BrN_3O_5$·HCl	145–150	75.1	A-H	0.58	0.36
2 (vinpocetine)								1.00	1.00
3 (bromovinamine)								0.17	0.28

[a] Elemental analyses are within 0.4% of the theoretical values. [b] E, ether; H, *n*-hexane; M, methanol; A, acetone. [c] Potency ratio (vinpocetine = 1.0). [d] VBF, vertebral blood flow. [e] FBF, femoral blood flow.

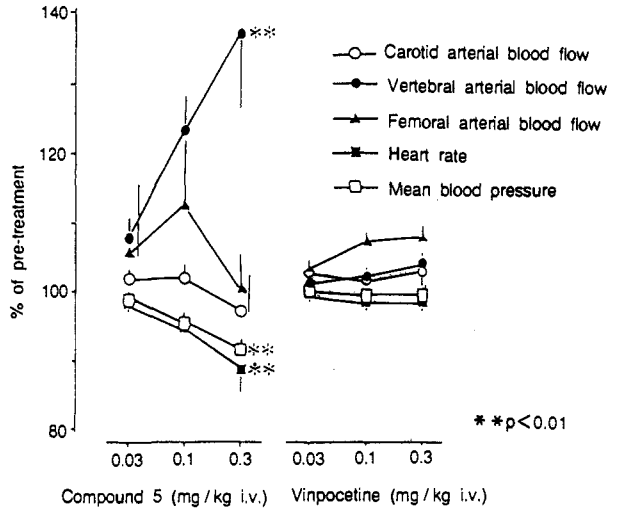


Figure 2. Effect of intravenously administered compound **5** and vinpocetine on peripheral circulation.

Evaluation of Compound 5. Effects of intravenously administered compound **5** and vinpocetine were examined on peripheral and cerebral circulation in anesthetized dogs (Figures 2 and 3). As shown in Figure 2, compound **5** (0.03–0.3 mg/kg iv) caused a dose-dependent increase in VBF without affecting the FBF and carotid blood flow (CBF). A dose of 0.3 mg/kg of compound **5** showed a significant decrease in blood pressure (BP) and heart rate (HR). In case of intravenous administration, vinpocetine (0.03–0.3 mg/kg iv) had no significant effects on VBF, FBF, VBF, BP, and HR.

In cerebral circulation, compound **5** (0.03–1.0 mg/kg iv) caused a dose-dependent increase in cerebral blood flow (CerBF) without affecting BP. At a dose of 1.0 mg/kg of compound **5**, a significant increase in CerBF was observed. Vinpocetine (0.03–1.0 mg/kg iv) also caused a dose-dependent increase in the CerBF without affecting BP, but this was not significant. Thus, compound **5** had a more potent effect than vinpocetine on CerBF.

X-ray Crystal Structure Analysis of Compound 5 and Vinpocetine. In order to consider the structure–activity relationships, we carried out X-ray structure analysis. Single crystals of **5** and vinpocetine were obtained

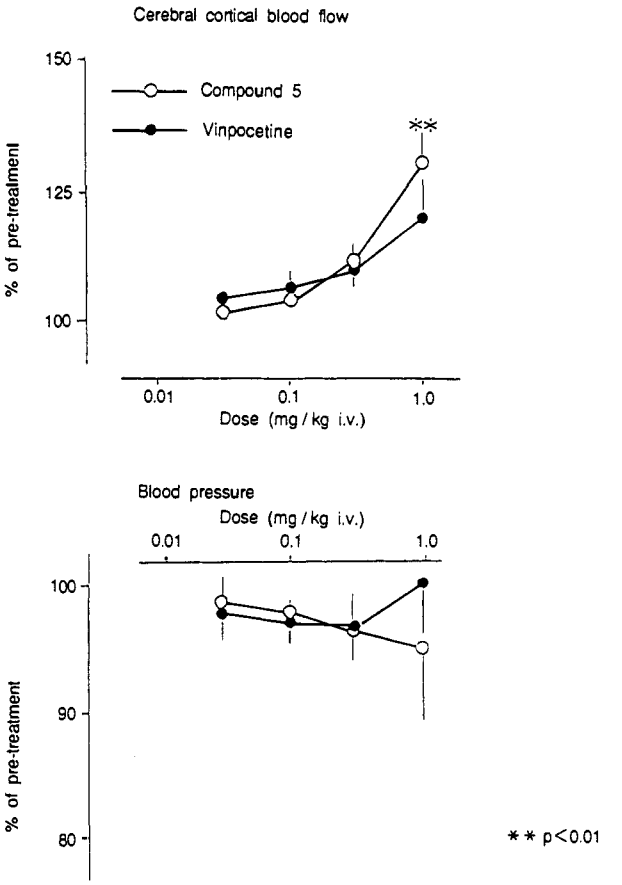


Figure 3. Effect of intravenously administered compound **5** and vinpocetine on central circulation.

by recrystallization from ethanol–methanol. The crystal data and the molecular structures are shown in Table II and Figure 4, respectively. As can be seen in the X-ray structures of **5** and vinpocetine, the conformations of the eburunamenine moiety are almost identical. The torsion angles of the C14–C15–C16–C17 bonds are −90.8° in **5** and −88.6° in vinpocetine. But the orientations of the ethyl group at C20–C21 are rather different. The torsion angles of the C15–C16–C20–C21 bonds are −60.3° in **5** and 66.38° in vinpocetine. Further, the electrostatic potentials of

Table II. Crystal and Refinement Data for 2-(Nitrooxy)ethyl Apovincaminate (5) and Vinpocetine

	5	vinpocetine
molecular weight	411.0	350.0
chemical formula	$C_{22}H_{25}N_3O_5$	$C_{22}H_{26}N_2O_2$
space group	$P2_12_12_1$	$P2_1$
crystal system	orthorhombic	monoclinic
a (Å)	24.67 (2)	11.267 (5)
b (Å)	9.85 (1)	9.518 (5)
c (Å)	8.251 (6)	8.883 (3)
V (Å^3)	2005.1 (5)	913.0 (4)
β (deg)		106.59 (3)
Z	4	2
D_c (g/cm^3)	1.36	1.27
μ (Cu Kα)	7.13	5.65
no. of reflections used	1932	1573
R value	0.100	0.043

these compounds were evaluated on the solvent-accessible surface using Cartesian coordinates and Mulliken net atomic charges obtained from a semiempirical molecular orbital calculation (CNDO method). The surfaces of electrostatic potentials (EP)[11] are shown in Figure 5, which reveal that the neutral region (yellow, −2 to 2 kcal/mol) appears around the surface of the eburunamenine moiety of vinpocetine. On the other hand, a large positive region (mauve, 3.0–9.0 kcal/mol) was observed on the surface of the eburunamenine moiety of **5**. The difference of the electrostatic potential image between the two compounds may result from the difference of charge densities of the (nitrooxy)ethyl group which has a negative potential region (blue, −15.0 to −9 kcal/mol) of compound **5**.

Thus, the differences of pharmacological activities between compound **5** and vinpocetine were thought to be mainly affected by source of (nitrooxy)ethyl ester group; furthermore, the differences of the conformation of ethyl group and electrostatic potential described above may also contribute to their pharmacological results.

Experimental Section

Melting points were determined on a Yanagimotokikai MP-S3 apparatus and were uncorrected. Infrared (IR) spectra were taken on a Perkin-Elmer 1760 spectrometer. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Varian VXR-200 spectrometer. Chemical shifts are given in ppm with tetramethylsilane as an internal standard, and the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), double doublet (dd), triplet (t), quartet (q), and multiplet (m). Mass spectra (MS) were taken on a JEOL JMS-SX102 or a Shimazu QP-1000 spectrometer.

2-(Nitrooxy)ethyl Apovincaminate (5). A mixture of **4** (25.0 g 77.5 mmol), $SOCl_2$ (22.8 g 192 mmol), *N*,*N*-dimethylformamide (DMF, 8.0 mL), and benzene (800 mL) was heated at reflux for 30 min, and concentrated in vacuo. Benzene (200 mL) was added to the residue, and the mixture was stirred for 10 min. To the resulting suspension was added, dropwise, a mixture of 2-(nitrooxy)ethanol (12.0 g 112 mmol), triethylamine (58.1 g 574 mmol), and benzene (80 mL). After stirring for 4 h, the reaction solution was poured into H_2O, extracted with ether, washed with H_2O, dried, and concentrated. The residual oil was purified by silica gel column chromatography (Wako-gel C-200, eluent; AcOEt/Hex = 1:1). The eluted solution was concentrated, and the residue was recrystallized from ether–hexane to give **5** as crystals: 23.9 g (75%); mp 111–112 °C; IR (KBr) 1733, 1636, 1436 cm^{-1}; MS *m*/*e* 411 (M$^+$); ^{1}H NMR (DMSO-d_6, 200 MHz) δ ppm 7.43 (1 H, m), 7.23 (1 H, m), 7.00–7.15 (2 H, m), 6.19 (1 H, s), 4.89 (2 H, m), 4.66 (2 H, m), 4.08 (1 H, s), 1.85 (2 H, q, J = 7 Hz), 0.95 (3 H, t, J = 7 Hz).

Compounds **6**, **9** and **14** were synthesized in the same manner. Data are listed in Table I.

2-(Nitrooxy)ethyl 11-Bromoapovincaminate Hydrochloride (15). A mixture of 11-bromoapovincaminic acid[12] (1.52 g 3.79 mmol), 2-(nitrooxy)ethyl bromide (770 mg 4.53 mmol), K_2CO_3 (1.0 g 7.24 mmol), and DMF (20 mL) was stirred at room temperature for 3 h, poured into H_2O, extracted with AcOEt, washed with H_2O, dried, and concentrated. The residual oil was converted into the hydrochloride and recrystallized from acetone–ether to give **15** as crystals: 1.5 g (75%); mp 145–150 °C; IR (KBr) 1732, 1636, 1464, 1385 cm^{-1}; MS *m*/*e* 489 (M$^+$), 491 (M$^+$ + 2); ^{1}H NMR (DMSO-d_6, 200 MHz) δ ppm 11.80 (1 H, br s), 7.66 (1 H, d, J = 1 Hz), 7.55 (1 H, d, J = 7 Hz), 7.33 (1 H, dd, J = 7, 1 Hz), 6.35 (1 H, s), 5.03 (1 H, br s), 4.92 (2 H, m), 4.67 (2 H, m), 4.67 (2 H, m), 0.99 (3 H, t, J = 6 Hz).

Compounds **10**–**13** were synthesized in the same manner. Data are listed in Table I.

6-(Nitrooxy)hexan-1-ol (20). A solution of 6-bromo-1-hexanol (30.0 g 166 mmol), $AgNO_3$ (42.0 g 247 mmol) and CH_3CN (300 mL) was stirred for 24 h at room temperature. The solution was concentrated and poured into H_2O. The precipitating AgBr was removed by filtration, and the filtrate was

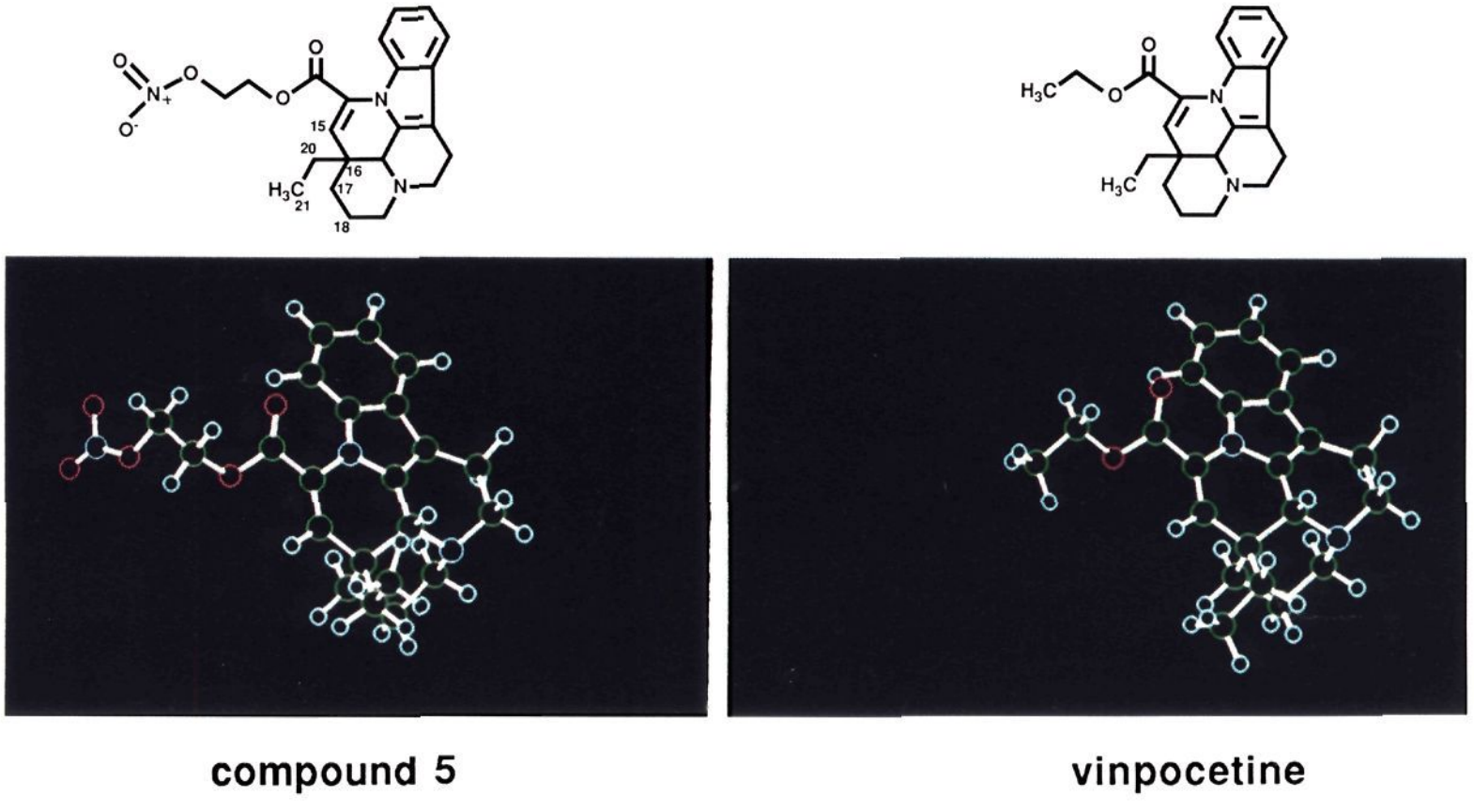


Figure 4. X-ray crystal structure of compound **5** and vinpocetine.

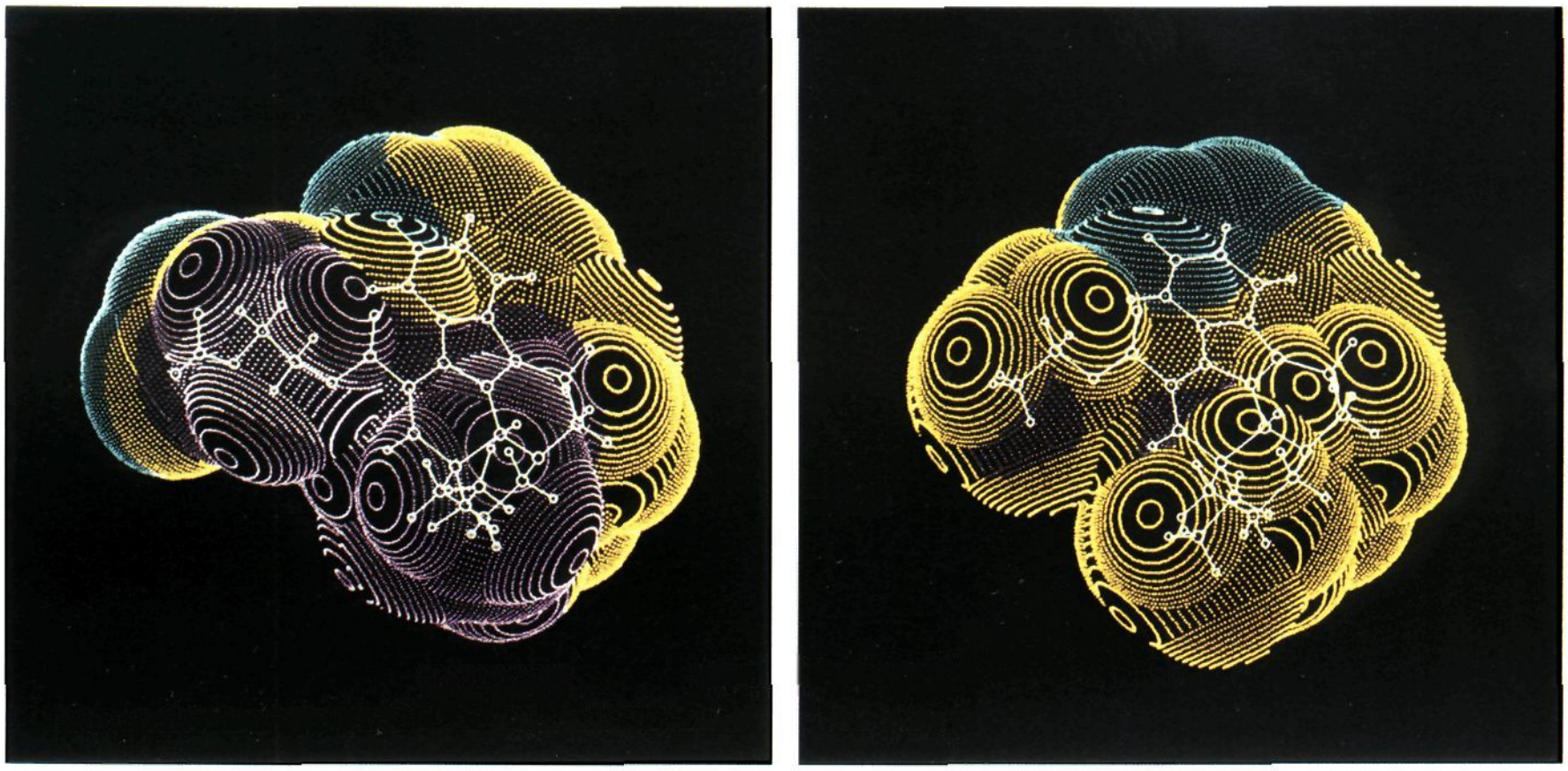


Figure 5. Electrostatic potentials (V) at surfaces of compound **5** and vinpocetine. Contour levels (kcal/mol): green, $V \leq -15.0$; blue, $-15.0 < V \leq -9.0$; light blue, $-9.0 < V \leq -3.0$; yellow, $-3.0 < V < 3.0$; mauve, $3.0 \leq V < 9.0$; red, $V \geq 9.0$.

extracted with ether, dried, and concentrated to give **20** as an oil: 25.0 g (92%); ^{1}H NMR ($CDCl_3$, 200 MHz) δ ppm 4.46 (2 H, t, J = 7 Hz), 3.65 (2 H, t, J = 7 Hz), 1.3–1.8 (8 H, m). Anal. ($C_6H_{13}NO_4$) C, H, N.

Other compounds of this series were prepared in the same manner (quantitative yield).

2-(Nitrooxy)ethanol (16):[13] oil. Anal. ($C_2H_5NO_4$) C, H, N.

3-(Nitrooxy)propanol (17): oil. Anal. ($C_3H_7NO_4$) C, H, N.

11-(Nitrooxy)undecan-1-ol (25): oil. Anal. ($C_{11}H_{23}NO_4$) C, H, N.

7-(Nitrooxy)heptyl Bromide (21). 7-Bromo-1-heptanol (2.0 g 10.3 mmol) was added dropwise to a solution of 70% HNO_3 (1.1 mL) and 95% H_2SO_4 (2.4 mL) at 0 °C and stirred at the same temperature for 1 h. The resulting suspension was poured into H_2O, extracted with CH_2Cl_2, dried, and concentrated to give **21** as an oil: 2.6 g (quantitative yield); ^{1}H NMR ($CDCl_3$, 200 MHz)

δ ppm, 4.45 (2 H, t, J = 7 Hz), 3.42 (2 H, t, J = 7 Hz), 1.87 (2 H, q, J = 7 Hz), 1.42 (2 H, q, J = 7 Hz), 1.30–1.60 (6 H, m). Anal. ($C_7H_{14}NO_3Br$) C, H, N.

Other compounds of this series were prepared in the same manner (quantitative yield).

4-(Nitrooxy)butyl bromide (18): oil. Anal. ($C_4H_8NO_3Br$) C, H, N.

5-(Nitrooxy)pentyl bromide (19): oil. Anal. ($C_5H_{10}NO_3Br$) C, H, N.

8-(Nitrooxy)octyl bromide (22): oil. Anal. ($C_8H_{16}NO_3Br$) C, H, N.

9-(Nitrooxy)nonyl bromide (23): oil. Anal. ($C_9H_{18}NO_3Br$) C, H, N.

10-(Nitrooxy)decyl bromide (24): oil. Anal. ($C_{10}H_{20}NO_3Br$) C, H, N.

Pharmacology. Effects on Peripheral Circulation. Male and female mongrel dogs were anesthetized with sodium pentobarbital, PB (30 mg/kg, iv), and artificially ventilated. Throughout the experiment, PB (5 mg/kg/h, iv) was continuously infused into keep anesthesia constant. Femoral arterial blood pressure was measured with a pressure transducer (TP-300T, Nihon-Kohden, Tokyo, Japan) connected to a rigid polyethylene tube introduced to a femoral artery. Heart rates were measured by a heart rate counter (AT-601G, Nihon-Kohden, Tokyo, Japan) driven by the waves of pulse pressure.

The carotid artery, vertebral artery, and femoral artery were exposed, and each blood flow was measured by a electromagnetic flowmeter (MFV-3200, MFV-1200 or MFV-1100; Nihon-Kohden, Tokyo, Japan). Compound **5** and vinpocetine dissolved in 10% ascorbic acid were administered into the femoral vein via a cannula inserted into the femoral vein. Dose–response curves were made on the basis of the peak values of the responses.

Effects on Cerebral Circulation. Male and female beagles, 5–12 months old, were anesthetized with sodium pentobarbital, PB (30 mg/kg, iv), and artificially ventilated. Throughout the experiment, PB (5 mg/kg/h, iv) was continuously infused to keep anesthesia constant. Femoral arterial blood pressure was measured with a pressure transducer (TP-300T, Nihon-Kohden, Tokyo, Japan) connected to a rigid polyethylene tube introduced to a femoral artery. The head skin was cut open to disclose the cranium, and a small hole was made on the cranium according to the brain atlas. The measurement of cerebral blood flow was performed using a laser-flometer (ALF-2100; Advance, Tokyo, Japan). The probe was inplace on the cerebral cortex. Compound and vinpocetin dissolved in 10% ascorbic acid were administered into femoral vein via a cannula inserted into the femoral vein. Dose–response curves were made on the basis of the peak values of the responses.

The data obtained (difference from the initial value) were presented as mean values and standard error for each group. Significance tests were done between the 10% ascorbic acid or saline group and each of the test compound or reference compounds according to the following method: the one-way analysis of variance (ANOVA) and Dunnett's test. The significance level was set at less than 5% ($p < 0.05$) in all the cases.

X-ray Crystal Structure Analysis. Single crystals of **5** and vinpocetine were obtained by recrystallization from a mixture of methanol/ethanol under concentration of the mother liquor by evaporation of the solvent at 293 K. These crystals were used for data collection on a Mac-Science mxc 18 diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.541 78 Å) at 288 K using the $\omega - 2\theta$ scan technique to a 2θ maximum 130°. Three standard reflections were measured every 100 reflections, and no crystal decay was detected. Cell constants were refined from 20 reflections in the range $55 \leq 2\theta \leq 60°$. Lorentz and polarization corrections were applied to the data; no corrections were made for absorption. The structures were solved by direct methods using SHELXS86[14] and refined with a full-matrix least-squares refinement. H atoms were located by difference Fourier synthesis. Anisotropic thermal parameters were used for all non-H atoms, while H atoms were refined isotropically. The atomic scattering factors were taken from ref 15.

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