Structural Confirmation of the Nitration Product of the 1(2*H*)-Phthalazinone as the 2-Nitro-1(2*H*)-phthalazinone

Shigeru ITO,*[,a] Kentaro YAMAGUCHI,[b] and Yasuo KOMODA[a]

Institute for Medical and Dental Engineering, Tokyo Medical and Detal University,[a] 2–3–10 Surugadai, Kanda, Chiyoda-ku, Tokyo 101, Japan and School of Pharmaceutical Sciences, Showa University,[b] 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan. *Received May 20, 1992*

The structure of the dinitrated product from the reaction of the 1(2*H*)-phthalazinone (1a) with acetyl nitrate was confirmed definitively by X-ray crystallography. The structure (1d) proposed previously was revised as 2-nitro-1(2*H*)-phthalazinone (1c).

Keywords nitration; acetyl nitrate; 1(2*H*)-phthalazinone; 2-nitrophthalazinone; *N*-nitroamide; structural confirmation; ^{13}C-NMR; IR; X-ray crystallography; NO donor

Recently, the generation, metabolism, and the roles of the new vascular tone regulation factors such as endothelin[1] and endothelium derived relaxing factors (EDRFs)[2] have been extensively studied. Nitric oxide, which was one of the EDRFs and was generated from L-arginine in biochemical pathways,[3] activate soluble guanylate cyclase activity in cells and elevate the cyclic guanosine monophosphate (cGMP) level[4] causing not only smooth muscle relaxation[5] but also platelet aggregation inhibition.[6] Organic nitrates such as nitroglycerin (GTN) and isosorbide dinitrate (ISDN) which are applied therapeutically as antianginal drugs might be exogenous NO donors.[7] On the course of our synthetic studies on cardiovascular heterocyclic compounds, we previously[8] reported the conversion of the phthalazinone (**1a**)[9] to the corresponding mononitrate (**1b**) and dinitrate (**1d**) in order to enhance the anti-thrombotic activities and introduce the hypotensive effect on **1a**. Both resulting nitrates showed a potent hypotensive effect on anesthetized rabbit and efficient inhibitory activities against platelet aggregation induced by adenosine diphosphate, collagen, or arachidonic acid *in vitro*. Structural elucidation of **1d** described before[8] was based mainly on the ultraviolet (UV) and infrared (IR) absorption spectrum analyses, and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum analysis. The UV spectrum of **1d** was not similar to that of a typical pattern in most of 1(2*H*)-phthalazinone derivatives. On the other hand, an intense absorption supposedly due to carbonyl group at 1731 cm^{-1} and two signals of nitrate groups at 1647 and 1620 cm^{-1} were observed in the IR spectrum. In addition, two signals at δ 167.7, which was assigned as an ester carbonyl carbon at the 7-position, and δ 153.4, were observed on ^{13}C-NMR spectrum. Compared with lactam carbonyl carbons such as **1a** (δ 160.3), **1b** (δ 161.1), a corresponding methylsulfonyl derivative (**2**) (δ 159.1),[10] and the carbonyl carbon of the *N*-nitrocarboxamides (δ 167.0—173.2),[11] the later characteristic signal was markedly shifted toward upfield to be considered as a lactam carbonyl carbon. Moreover, ring carbons at 4-position of phthalazine and 4-acetoxy-1(2*H*)-phthalazinone (**3**)[12] were observed at δ 152.0[13] and 146.2 in the ^{13}C-NMR spectrum, respectively. From these data, we had assigned the signal at δ 153.4 to be a ring carbon directly attached to the nitroxy group of **1d**.

However, on the continuing studies on the structure and activity relationships of the analogous nitrates, we found that the IR spectrum of the simple dinitrophthalazinone (**4c**) obtained by the nitration of **4a**, which has no carbonyl group on the fused benzene ring, showed a carbonyl absorption at 1719 cm^{-1}, which extremely shifted to the high frequency side compared to that of starting material (**4a**) (1644 cm^{-1}), mononitrate (**4b**) (1692 cm^{-1}) and **2** (1731; 7-ester and 1698 cm^{-1}; lactam). This result suggested the

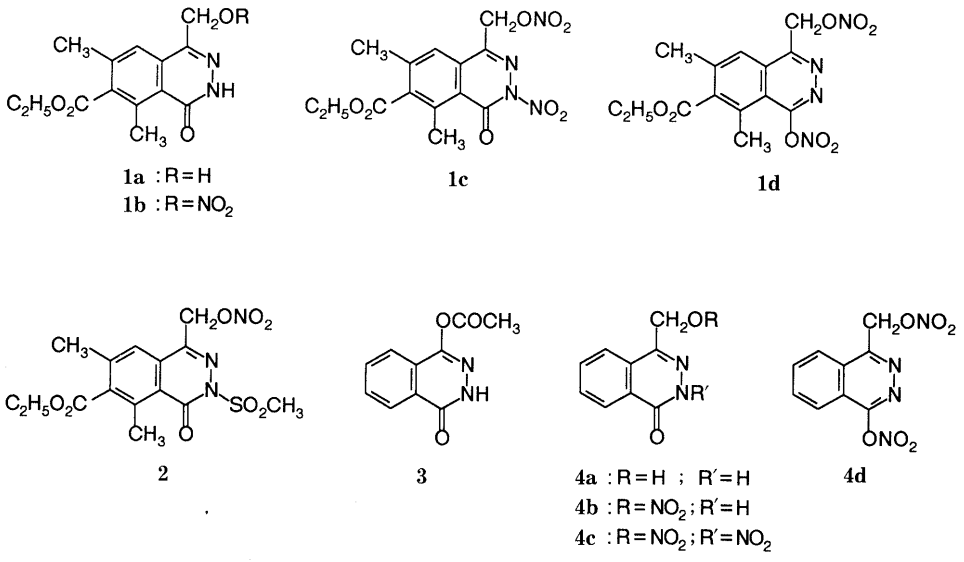

Fig. 1

structure of the dinitrated product of **4a** to be 2-nitrophthalazinone (**4c**) rather than 1-nitroxyphthalazine (**4d**). Furthermore, ^{13}C-NMR spectrum of **4c** showed a signal at δ 152.8, which is closely similar to that of the substituted dinitro derivative of **1a** described above, together with 7 signals of sp^2 carbon between the range of δ 125.3 to 135.5. Taking all these into consideration, it was suggested that the dinitro derivative of **1a** should also be 2-nitrophthalazinone (**1c**). Therefore, we carried out X-ray crystallographic analysis to confirm the structure of the dinitration product of **1a**.

The final structural feature of **1c** was shown in Fig. 2, and final atomic parameters were summarized in Tables I and II, respectively. As shown in Fig. 2, the internal rotation angles of C6–C5–C10–O26, C6–C5–C10–N9, and C5–C10–N9–N8 are 160.9°, −18.5°, and 24.8° respectively. This characteristic tortion on bond C5–C10 and C10–N9, mainly due to the steric hindrances of both the methyl group at *peri*-position and the 2-nitro group to the lactam carbonyl group, seems to reduce the conjugation of the lactam moiety with the fused benzene ring and was not observed on the conformation analyses on the basis of the semiempirical molecular orbital calculation using the AM1 method (MOPAC *ver.* 4.0) for the related compounds, **1a** (177.5°, −2.0°, and 0.4°) and **1b** (175.1°, −2.6°, and 1.8°), respectively. A comparatively long amide N9–N10 bond [1.421(4)Å] and a short C10–O26 bond [1.196(4)Å], whose distances are consistent with those found in *N*-nitrosoureas,[14] suggest a potent inductive effect by the nitro group introduced on the lactam moiety. All of these conformational features may contribute to both an upfield shift in ^{13}C-NMR spectrum and a high frequency side shift resulting in an overlap on the 7-ethoxycarbonyl absorption in IR spectrum of the lactam carbonyl group of **1c**.

We previously described that the nitro group on the

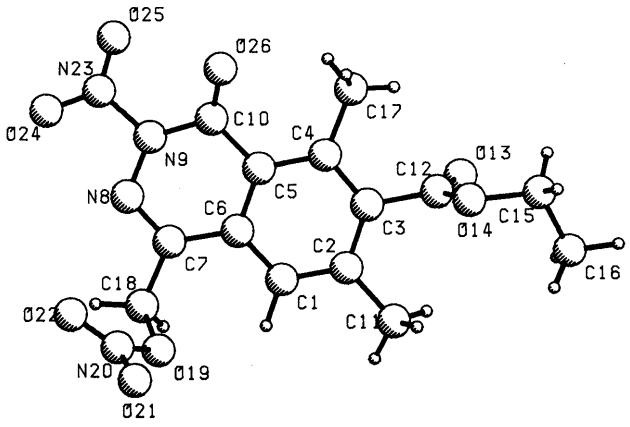

Fig. 2

TABLE I. Fractional Atomic Coordinates and Equivalent Isotropic Thermal Parameters of 2-Nitro-1(2*H*)-phthalazinone (**1c**)

	x	*y*	*z*	U_{eq} (Å^2)
C(1)	0.6154 (2)	0.4181 (1)	0.7608 (3)	0.041 (1)
C(2)	0.5095 (2)	0.3875 (1)	0.7325 (3)	0.040 (1)
C(3)	0.4603 (2)	0.3586 (1)	0.8585 (3)	0.039 (1)
C(4)	0.5120 (2)	0.3647 (1)	1.0099 (3)	0.039 (1)
C(5)	0.6196 (2)	0.3980 (1)	1.0365 (3)	0.038 (1)
C(6)	0.6721 (2)	0.4221 (1)	0.9118 (3)	0.037 (1)
C(7)	0.7863 (2)	0.4470 (1)	0.9432 (3)	0.040 (1)
N(8)	0.8447 (1)	0.4429 (1)	1.0784 (2)	0.045 (1)
N(9)	0.7932 (2)	0.4143 (1)	1.1952 (2)	0.046 (1)
C(10)	0.6791 (2)	0.4080 (1)	1.1945 (3)	0.043 (1)
C(11)	0.4491 (3)	0.3835 (2)	0.5699 (4)	0.054 (1)
C(12)	0.3513 (2)	0.3136 (2)	0.8229 (3)	0.045 (1)
O(13)	0.3402 (1)	0.2332 (1)	0.8094 (3)	0.069 (1)
O(14)	0.2694 (1)	0.3725 (1)	0.8076 (2)	0.055 (1)
C(15)	0.1592 (2)	0.3347 (3)	0.7620 (5)	0.064 (1)
C(16)	0.1367 (4)	0.3188 (4)	0.5941 (6)	0.091 (2)
C(17)	0.4541 (3)	0.3323 (2)	1.1392 (4)	0.054 (1)
C(18)	0.8486 (2)	0.4749 (2)	0.8169 (4)	0.046 (1)
O(19)	0.8004 (1)	0.5548 (1)	0.7357 (2)	0.056 (1)
N(20)	0.8339 (2)	0.6372 (2)	0.8077 (4)	0.068 (1)
O(21)	0.7871 (2)	0.7009 (2)	0.7453 (4)	0.103 (2)
O(22)	0.9046 (3)	0.6350 (2)	0.9176 (4)	0.098 (2)
N(23)	0.8673 (2)	0.4103 (2)	1.3395 (3)	0.060 (1)
O(24)	0.9511 (2)	0.4493 (2)	1.3447 (3)	0.113 (2)
O(25)	0.8393 (2)	0.3678 (2)	1.4396 (3)	0.104 (2)
O(26)	0.6415 (1)	0.4117 (1)	1.3120 (2)	0.062 (1)

$U_{eq} = 1/3\ \Sigma_i \Sigma_j U_{ij} a_i^* a_j^* \boldsymbol{a}_i . \boldsymbol{a}_j$.

TABLE II. Bond Distances (Å) and Angles (°) of **1c** or Non-hydrogen Atoms with Their Estimated Standard Deviations in Parentheses

Bond distance (Å)				Angle (°)			
C1–C2	1.377 (4)	C1–C6	1.400 (4)	C2–C1–C6	120.7 (2)	C1–C2–C3	118.5 (2)
C2–C3	1.408 (4)	C2–C11	1.504 (4)	C1–C2–C11	120.4 (3)	C3–C2–C11	121.1 (2)
C3–C4	1.384 (4)	C3–C12	1.497 (4)	C4–C3–C2	122.6 (2)	C4–C3–C12	119.9 (2)
C4–C5	1.409 (4)	C4–C17	1.511 (5)	C2–C3–C12	117.4 (2)	C3–C4–C5	117.9 (2)
C5–C6	1.403 (4)	C5–C10	1.471 (4)	C3–C4–C17	119.5 (2)	C5–C4–C17	122.5 (2)
C6–C7	1.451 (3)	C7–N8	1.291 (3)	C6–C5–C4	120.3 (2)	C6–C5–C10	118.7 (2)
C7–C18	1.503 (4)	N8–N9	1.356 (3)	C4–C5–C10	121.0 (2)	C1–C6–C5	119.8 (2)
N9–C10	1.421 (4)	N9–N23	1.445 (3)	C1–C6–C7	121.6 (2)	C5–C6–C7	118.5 (2)
C10–O26	1.196 (4)	C12–O13	1.197 (3)	N8–C7–C6	123.8 (2)	N8–C7–C18	114.1 (2)
C12–O14	1.328 (3)	O14–C15	1.475 (4)	C6–C7–C18	122.1 (2)	C7–N8–N9	116.3 (2)
C15–C16	1.473 (6)	C18–O19	1.455 (3)	N8–N9–C10	127.3 (2)	N8–N9–N23	111.1 (2)
O19–N20	1.402 (3)	N20–O21	1.190 (4)	C10–N9–N23	120.1 (2)	O26–C16–N9	121.2 (2)
N20–O22	1.198 (4)	N23–O25	1.174 (4)	O26–C16–C5	127.4 (2)	N9–C1–C5	111.5 (2)
N23–O24	1.185 (4)			O13–C13–O14	124.1 (2)	O13–C13–C3	123.1 (2)
				O14–C14–C3	112.8 (2)	C12–O12–C15	116.5 (2)
				C16–C16–O14	110.8 (3)	O19–C19–C7	111.1 (2)
				N20–O10–C18	114.2 (2)	O21–N21–O22	129.4 (3)
				O21–N21–O19	112.7 (3)	O22–N22–O19	117.9 (3)
				O25–N25–O24	126.3 (3)	O25–N25–N9	116.9 (2)
				O24–N24–N9	116.8 (2)		

lactam moiety was removed by the treatment of 1 N HCl at 80 °C to afford **1b**.[8] In addition, the structurally related compounds devoid of the nitro group on the lactam moiety, **2** and 2-acetyl-7-ethoxycarbonyl-6,8-dimethyl-1(2*H*)-phthalazinone (**5**) did not show any significant biological activities.[10] On the other hand, the extracts of the plasma incubate of **1a**, **1b** and **1c** on the same condition for the assessment of the antiaggregating activity, except absence of the aggregating inducers described above, were analyzed by means of HPLC. Although most of **1a** and **1b** remained intact, more than half the amount of **1c**, which showed remarkable inhibitory activity toward the platelet aggregation rather than **1a** and **1b**, was converted to the more polar metabolite.[15] All of these observations suggest that the *N*-nitroamide moiety of **1c** probably plays a potent exogenous NO donor like the nitrate groups of GTN and ISDN,[7] and nitrosothiol groups such as *S*-nitrosocisteine[16] and *S*-nitrosoglutathione.[16a,17] Pharmacological elucidation of **1c** is now in progress.

Experimental

Melting points were determined on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. NMR (^{1}H and ^{13}C) spectra were taken on a JEOL GX-270 spectrometer at 270 and 67.8 MHz, respectively. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard, and the following abbreviations are used: s = singlet, d = doublet, t = triplet. IR spectra were recorded with a JASCO PTL-396 spectrophotometer, and UV spectra were measured with a Hitachi U3200 spectrophotometer. Mass spectra (MS) were obtained with the aid of a JEOL D-300 spectrometer. Elemental analyses were determined with a Heraeus CHN-Rappid.

Nitration of 4a Nitration of **4a** and chromatographic purification were performed in the same manner as previously described[8] and recrystallization of the first fraction from diethyl ether/*n*-hexane afforded 23.5% of **4c** as colorless needles, mp 92.5—93.5 °C. *Anal.* Calcd for $C_9H_6N_4O_6$: C, 40.61; H, 2.27; N, 21.05. Found: C, 40.40; H, 2.39; N, 21.05. MS *m/z*: 266 (M^+), 221, 174, 90. UV λ_{max}^{EtOH} nm (log ε): 212 (4.38), 232 (4.30), 278 (3.65), 312 (3.55). IR (KBr): 1721, 1630, 1292, 1278, 1238 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.76 (2H, s, 4-CH_2O), 7.89 (1H, ddd, J = 7.6, 1.6, 0.7 Hz, 5-H), 7.93 (1H, dt, J = 7.6, 1.6 Hz, 7-H), 8.01 (1H, dt, J = 7.6, 1.6 Hz, 6-H), 8.56 (1H, ddd, J = 7.6, 1.6, 0.7 Hz, 8-H). ^{13}C-NMR δ: 70.0 (4-CH_2O), 125.3 (C-5), 128.1 (C-8a), 129.2 (C-8), 129.4 (C-4a), 133.4 (C-7), 135.5 (C-6), 136.8 (C-4), 152.8 (1-CO). Recrystallization of the second fraction from acetone gave 48.9% of **4b** as colorless fine needles, mp 194.5—195 °C. *Anal.* Calcd for $C_9H_7N_3O_4$: C, 48.87; H, 3.19; N, 19.00. Found: C, 48.84, H, 3.32; N, 19.15. MS *m/z*: 221 (M^+), 174. UV λ_{max}^{EtOH} nm (log ε): 208 (4.57), 244 (3.88), 253 (3.91), 284 (3.83). IR (KBr): 3454, 3180, 3116, 1692, 1620, 1294 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.90 (2H, s, 4-CH_2O), 7.91 (1H, dt, J = 7.6, 1.3 Hz, 7-H), 8.00 (1H, dt, J = 7.6, 1.3 Hz, 6-H), 8.10 (1H, ddd, J = 7.6, 1.3, 0.7 Hz, 5-H), 8.30 (1H, ddd, J = 7.6, 1.3, 0.7 Hz, 8-H). ^{13}C-NMR δ: 71.4 (4-CH_2O), 125.0 (C-5), 126.0 (C-8), 127.4 (C-8a), 128.3 (C-4a), 132.1 (C-7), 133.8 (C-6), 138.0 (C-4), 159.4 (1-CO). Assignment of the ^{1}H- and ^{13}C-NMR chemical shifts of both compounds were performed by the ^{1}H HOMO spin decoupling method and ^{13}C–^{1}H correlation spectroscopy (COSY) spectrum analyses, respectively.

X-Ray Crystallography Crystal Data: $C_{14}H_{14}N_4O_8$, M_r = 366.29. Monoclinic, $P2_1/a$, a = 12.434(1) Å, b = 14.735(1) Å, c = 8.761(3) Å, β = 98.98(2)°. Cell parameters from 20 reflections, θ = 27.5—30.5°, V = 1585.3(6) Å^3, Z = 4, D_x = 1.535 Mgm^{-3}, Cu$K\alpha_1$, λ = 1.5405 Å, μ = 10.58 mm^{-1}, $F(000)$ = 760, T = 297 K. Clear prism, 0.4 × 0.2 × 0.2 mm.

Data Collection: Rigaku AFC-5 diffractometer, $\omega/2\theta$ scan method, ω scan width $(1.3+0.14\tan\theta)°$ and scan speed 32° min^{-1}, no absorption correction, 2709 measured reflections, 2360 observed reflections [$F > \sigma(F)$], R_{int} = 0.018, θ_{max} = 60°, h = −13—13, k = 0—16, l = 0—9, 3 standard reflections, interval; 150 reflections, intensity variation; <3%.

Refinement: Refined on F^2 by the full-matrix least-squares method. Final R = 0.058, wR = 0.063 and S = 2.112 for 2021 reflections [$F > 3\sigma(F)$], 291 parameters, H-atom coordinates refined, $w = 1/[\sigma^2(F) + 0.014F^2]$, $(\Delta/\sigma)_{max}$ = 0.14, $\Delta\rho_{max}$ = 0.424 eÅ^{-3}, $\Delta\rho_{min}$ = −0.341 eÅ^{-3}. Source of atomic scattering factors; International Tables for X-Ray Crystallography (1974), Vol. IV, Table 2.2B).

Software: Data collection and cell refinement; AFD (Rigaku Corporation, 1985). Data reduction and structure refinement; RCRYSTAN (Rigaku Corporation, 1985). Program used to solve structure: SAPI85 (direct method). The final atomic coordinates are listed in Table I.

References and Notes

1) M. Yanagisawa, H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitui, Y. Yazaki, K. Goto, and T. Masaki, *Nature* (London), **332**, 411 (1988).
2) R. F. Furchgott and J. V. Zawadzki, *Nature* (London), **288**, 373 (1980).
3) *a*) R. M. J. Palmer, D. S. Ashton, and S. Moncada, *Nature* (London), **333**, 664 (1988); *b*) S. Moncada, E. A. Higgs, H. F. Hodson, R. G. Knowles, P. Loperz-Jaramillo, T. MaCall, R. M. J. Parmer, M. W. Radmski, D. D. Rees, and R. Shulz, *J. Cardiovasc. Pharmacol.*, **17** (Suppl. 3), s1 (1991); *c*) V. B. Schini and P. M. Vanhoutte, *ibid.*, s10 (1990).
4) *a*) S. Katuki, W. P. Arnold, C. Mittal, and F. Murad, *J. Cyclic Nucleotide Res.*, **3**, 23 (1977); *b*) L. J. Ignarro and P. J. Kadwitz, *Ann. Rev. Pharmacol. Toxicol.*, **25**, 171 (1985); *c*) P. M. Vanhoutte and H. Shimokawa, *Circulation*, **80**, 1 (1989); *d*) L. J. Ignarro, *Biochem. Pharmacol.*, **41**, 485 (1991).
5) *a*) C. A. Gruetter, B. K. Barry, D. B. McNamara, D. Y. Grutter, P. J. Kadwitz, and L. J. Ignarro, *J. Cyclic Nucleotide Res.*, **5**, 271 (1979); *b*) R. M. Palmar, A. G. Ferrige, and S. Moncada, *Nature* (London), **327**, 524 (1987).
6) *a*) B. T. Mellion, L. J. Ignarro, C. B. Myers, E. H. Ohlstein, B. A. Ballot, A. L. Hyman, and P. J. Kadwitz, *Mol. Pharmacol.*, **23**, 653 (1983); *b*) R. M. W. Radomski, R. M. J. Palmer, and S. Moncada, *Br. J. Pharmacol.*, **92**, 637 (1987).
7) *a*) M. Feelish, *J. Cardiovasc. Pharmacol.*, **17** (Suppl. 3), s25 (1991); *b*) J. E. MacKenzie and J. R. Parratt, *Br. J. Pharmacol.*, **60**, 155 (1977); *c*) K. L. Axelesson, J. E. Wikberg, and R. G. G. Andreson, *Life Sci.*, **24**, 1779 (1979).
8) S. Ito, Y. Komoda, S. Sekizaki, H. Azuma, and M. Ishikawa, *Chem. Pharm. Bull.*, **36**, 2669 (1988).
9) M. Ishikawa, Y. Eguchi, and A. Sugimoto, *Chem. Pharm. Bull.*, **28**, 2770 (1980).
10) S. Ito, H. Azuma, N. Funayama, T. Kubota, S. Sekizaki, and M. Ishikawa, *Reports Inst. Med. Dent. Eng., Tokyo Med. Dent. Univ.*, **24**, 41 (1990).
11) J. Garcia, J. Gonzalez, R. Segura, F. Urpi, and J. Vilarrasa, *J. Org. Chem.*, **49**, 3322 (1984); The IR spectra of the *N*-nitroamides show strong carbonyl absorptions at 1740—1710 cm^{-1}, agreed with our data, and N–NO_2 bonds at 1590—1560 cm^{-1}, deviated a little from our results.
12) H. D. K. Drew and H. H. Hatt, *J. Chem. Soc.*, **1937**, 16.
13) H. O. Kalinowski, S. Berger, and S. Braun, "Carbon-13 NMR Spectroscopy, Eng. ed.," John Wiley & Sons Ltd., Chichester, 1988, p. 392.
14) *a*) K. Yamaguchi, G. Matumura, M. Tanno, S. Sueyoshi, and N. Miyata, *Acta. Cryst.*, **C48**, 1051 (1992); *b*) M. Tanno and S. Sueyoshi, *Chem. Pharm. Bull.*, **35**, 1353 (1987); *c*) *Idem*, *ibid.*, **35**, 1360 (1987); *d*) *Idem*, *ibid.*, **38**, 49 (1990); *e*) *Idem*, *ibid.*, **38**, 2644 (1990).
15) Details will be reported in the article of the structure activity relationships in the future.
16) *a*) E. A. Ignarro and C. A. Gruetter, *Biochem. Biophys. Acta*, **631**, 221 (1980); *b*) P. R. Myers, R. L. Minor Jr., R. Guerra Jr., J. N. Bates and D. G. Harrison, *Nature* (London), **345**, 161 (1990).
17) T. W. Hart, *Tetrahedron Lett.*, **26**, 2013 (1985).