# Newly Discovered Photodegradation Products of Nifedipine in Hospital Prescriptions

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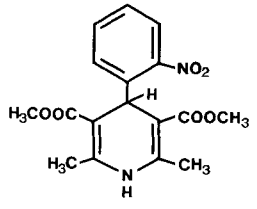
**Abstract** □ New photodegradation products of nifedipine (1) have been isolated. They were found in tablets dispensed in the pulverized form by hospitals. 1 decomposed concurrently into six components after storage of 30 days under exposure to normal room light. The main photoproduct was a nitroso derivative (2) and others were minor. Preparative thin-layer chromatography has been used to isolate the six photodegradation products. The chemical structures of these isolated compounds were identified or estimated by comparison with authentic samples and/or using UV, IR, $^1$H NMR, mass spectroscopy, melting point determination, and elementary analysis. From these analyses, it was found that 1 was converted into a *cis*-azoxy derivative (4), a *trans*-azoxy derivative (5), a *N,N'*-dioxide derivative (6) and a lactam derivative (7) in addition to 2 and a nitro derivative (3). Furthermore, it is proposed that 2 is mainly responsible for the formation of these new products (4–7) by photochemical condensation.

Nifedipine (1, dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate) is an important calcium-channel blocker, which causes vasodilation and lowering of peripheral circulatory resistance. 1 is widely used in the therapy of heart disease and hypertension. This compound, like most 1,4-dihydro-4-(2-nitrophenyl)pyridine derivatives is highly light-sensitive.[1] On exposure to visible light, 1 is converted to the 4-(2-nitrosophenyl)pyridine homologue[2,3] (nitroso derivative) (2), whereas on exposure to UV light, it changes to the 4-(2-nitrophenyl)pyridine homologue[4,5] (nitro derivative) (3).

1 is absorbed rapidly and almost completely after oral administration[6] so that the tablets are usually prescribed and prepared for patients as a dosage form. It is not unusual, however, when there are hospital patients, such as children or aged patients, who cannot swallow the tablets, for nifedipine tablets to be pulverized so as to ease administration.[7,8] In this case, there is a problem because pulverized tablets of 1 undergo decomposition into 2 and/or 3 under the artificial light (i.e. room light) of normal storage conditions. The photostability under fluorescent lamp or daylight irradiation conditions of several solid dosages forms of 1 have been evaluated by some investigators for quality assurance.[9,10] The photolytic degradation of 1 in solid-state follows pseudo-first-order kinetics.[7,9] However, most of the investigations have been interested in changes of 1 and the two photodegradation products of 1, i.e. 2 and 3.

In previous studies, Goerlitzer and Buss photochemically synthesized the dimer product of the nitrosopyridine homologue from its monomer,[11] and Matsuda and co-workers[9] isolated one of the photodegradation products of 1, an azoxy derivative, 2,2'-bis[(2,6-dimethyl-3,5-bis(methoxycarbonyl)pyridine-4-yl)azoxy]-benzene, from 1 under intense irradiation. But due to limited characterization data, the structures and formation mechanisms of these synthesized and isolated compounds have not been fully realized. In a previous study,[7] we too found several unknown photoproducts of 1, which increased in quantity during exposure to room light under storage conditions.

This study deals with isolation, structural elucidation and formation mechanism of the newly discovered photodegradation products of 1 in pulverized tablets.

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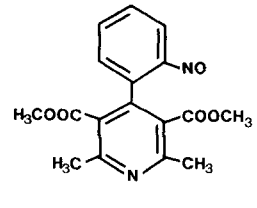


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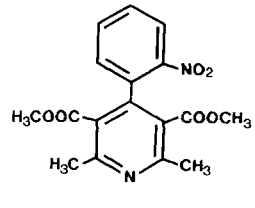


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## Experimental Section

**Equipment**—Melting points (mp) were determined by a model MP-21 melting point apparatus (Yamato Science, Ltd., Tokyo, Japan), and the values were uncorrected. The ultraviolet (UV) spectra were measured in analytical grade methanol in 1-cm cells with use of a model 200-10 spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The fluorescence spectra were also measured in nonfluorescent methanol (Dojindo Laboratories, Kumamoto, Japan) in 1-cm cells with use of a model 204-R fluorescence spectrophotometer (Hitachi, Ltd.). The infrared (IR) spectra were measured via KBr disks with use of a model 260-30 infrared spectrophotometer (Hitachi, Ltd.). Low- and high-resolution mass spectra, EI-MS and HRMS, respectively, were obtained with an electron ionization (70 eV) method using a model M-80 mass spectrometer (Hitachi, Ltd.). The mass spectrometry combined with liquid chromatography (LCMS) was also performed under atmospheric pressure ionization conditions with use of a model M-1000 LC/MS spectrometer (Hitachi, Ltd.). Proton nuclear magnetic resonance spectra ($^1$H NMR, $\delta$ in ppm) were recorded by a model JNM-FX 100 NMR spectrometer (JEOL, Tokyo, Japan) with tetramethylsilane as an internal reference ($\delta$ 0.00) in deuterated chloroform ($CDCl_3$) at 100 MHz. The signal multiplicities are expressed by both s (abbreviation of singlet) and m (abbreviation of multiplet).

**Materials**—Nifedipine (1) was purchased from Sigma Chemical Company (St. Louis, MO), and its photodegradation products, nitroso and nitro derivatives (2 and 3), were kindly donated by Bayer Yakuhin, Ltd., (Osaka, Japan). Four forms of nifedipine, tablets (Adalat-L; Bayer Yakuhin, Ltd., Emaberin; Takata Pharmaceutical Company, Tokyo, Japan), granule (Emaberin; Takata Pharmaceutical Company), and fine granule (Sepamit; Kanebo Yakuhin, Tokyo, Japan), all of which are on the Japanese market, were subjected to a photolabile test.

Methanol used was of a HPLC grade from Wako Pure Chemical Industries Ltd. (Tokyo, Japan), and water was distilled, deionized, and then filtered through a 0.45-$\mu$m membrane filter (Nihon Millipore Ltd., Tokyo, Japan) before use. All other reagents used were of an analytical grade and were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan), unless otherwise indicated. The preparative thin-layer

® Abstract published in *Advance ACS Abstracts*, January 15, 1994.



0022-3549/94/1200-532$04.50/0

**Table 1—Microanalytical Data[a]**

| Compound | Molecular Formula | C, % | H, % | N, % |
|---|---|---|---|---|
| 4 | $C_{34}H_{32}O_9N_4$ | 63.73 | 5.04 | 8.75 |
| | | 63.47 | 4.61 | 8.32 |
| 5 | $C_{34}H_{32}O_9N_4$ | 63.73 | 5.04 | 8.75 |
| | | 63.75 | 4.79 | 8.59 |
| 6 | $C_{34}H_{32}O_{10}N_4$ | 62.18 | 4.92 | 8.54 |
| | | 62.36 | 4.90 | 8.48 |
| 7 | $C_{16}H_{14}O_3N_2$ | 68.06 | 5.00 | 9.93 |
| | | 68.23 | 5.08 | 9.49 |

[a] For each element, the first value is the calculated percentage, and the second value is the experimentally determined percentage. Satisfactory analytical data (±0.5% for C, H, and N) were reported for all new compounds listed in the table.

chromatography (PLC) plate was a Merck silica gel 60 $F_{254s}$ with a concentrating zone of 20 × 20 cm and a thickness of 1 mm (Art. 13792).

**Preparation of Samples**—In a mortar and pestle, intact nifedipine tablets were ground to a fine powder and then mixed with dried powder of corn starch and lactose (1:2, w/w) to achieve 20 mg of 1 per gram. This mixture was divided in half, and each of half was packed in drug sealing paper. Pure 1 was also prepared and packed in the same way as a control sample. Besides these samples, commercial preparations of granule and fine-granule were repacked in drug sealing paper.

**Photoirradiation Procedure**—The sample preparations packed with a sealed paper was exposed to normal room light (mixture of fluorescent lamps and daylight) under the conditions of 750 lux (average irradation) and 35% relative humidity at room temperature for 30 days. The temperature and humidity were environmentally controlled.

**High-Performance Liquid Chromatography (HPLC)**—A 250-mg sample from the preparation package was subjected to extraction by methanol, and 1 and its photodegradation products were analyzed by HPLC.

The HPLC system consisted of a solvent pump (model L-6000), a solvent programmer (model L-5000), a variable wavelength detector (model L-4200), a chromatointegrator (model D-2500; Hitachi, Ltd., Tokyo, Japan), and a manual injector (Rheodyne 7125; California). The extracted sample solutions (20 μL injection size) were chromatographed at 30 °C on a 150 × 4.6 mm 1.d. column packed with 5-μm LiChrospher RP-18 (Kanto Chemical Co., Inc., Tokyo, Japan). The mobile phase consisted of methanol/water (55:45, v/v), and was degassed ultrasonically and used at a flow rate of 1.0 mL $min^{-1}$. The column effluent was monitored at 238 nm and at 0.08 absorbance units of full scale. The conditions for liquid chromatography of LCMS were the same as those of HPLC analysis described above, except for a separation column [#3056 (5 μm) 150 × 4.0 mm (Hitachi, Ltd.)].

**Separation of the Photodegradation Products**—All of the irradiated samples (approximately 45 g) which were collected from the preparations of pulverized Adalat-L tablets (100 packages from 100 tablets) were transferred into a funnel (1 L). The pooled samples were extracted three times with 500 mL of a methanol/chloroform (50:50, v/v) mixture and vigourous shaking. The extracts were filtered and transferred to an evaporating flask, and were then evaporated to dryness in vacuo. The residue was reextracted with 100 mL of acetone, filtered, and evaporated to a dark brownish syrup (approximately 1 g) in vacuo. The syrup was dissolved in 10 mL of acetone and then aliquots of the solution were chromatographed on PLC using petroleum ether/chloroform/acetone (50:30:20, v/v) as a developing solvent. The development was performed 3–4 times on a plate to separate photodegradation products. Each separated band was scraped from 10 plates and collected in six fractions (fraction A–F). These fractions were checked for their correspondences to the peaks analyzed by HPLC.

**Isolations and Characterizations of Photodegradation Products**—Each fraction (fraction A–F) obtained by PLC was extracted with 50 mL of chloroform. Each chloroformic extract of the adsorbent was filtered and evaporated nearly to dryness. These fractions afforded six crystalline compounds, 2–7.

*Compound 2*—The residue of fraction A was dissolved in about 10 mL of methanol by heating and left to stand in a refrigerator overnight. Pale greenish needles precipitated. The crystals were collected and recrystallized several times from methanol, yielding 300 mg of the needles. The chromatographical purity was 96.9% by area normalization. This compound showed a mp of 93 °C; UV (nm in methanol) $\lambda_{max}$ 280, 310 and $\lambda_{min}$ 255, 300; IR (KBr) $\nu$ 3450, 3000, 2950, 2850, 1725, 1555, 1495, 1435, 1420, 1290, 1240, 1155, 1110, 1042, 960, 945, 850, 820, 780, 770, and 690 $cm^{-1}$; EI-MS $m/z$ (relative intensity) 328 (27, $M^+$), 311 (2, $M^+$ – OH), 298 (7, $M^+$ – NO), 284 (22), 269 (100, $M^+ - COOCH_3$), 253 (32), 252 (24), 251 (7), 209 (12), 193 (20), 152 (21), 139 (17), 115 (10), 63 (10), 59 (27), and 15 (17); LCMS $m/z$ (relative intensity) 329 [74, $(M + H)^+$], 299 (14), 284 (100), 268 (46), 254 (11), 239 (7), 226 (5) and 210 (3); $^1H$ NMR ($CDCl_3$) $\delta$ 2.66 (s, 6H, $CH_3$), 3.37 (s, 6H, $OCH_3$), and 6.48–7.68 (m, 4H, aromatic-H).

*Compound 3*—The residue of fraction B was treated with methanol in the same manner as that of fraction A to yield 30 mg of yellow powdery material. The chromatographical purity was 97.0% by area normalization. This compound showed a mp of 106 °C; UV (nm in methanol) $\lambda_{max}$ 260 (shoulder); IR (KBr) $\nu$ 3450, 3000, 2950, 2850, 1725, 1610, 1560, 1525, 1435, 1400, 1350, 1290, 1235, 1110, 1042, 960, 940, 860, 840, 810, 788, 760, 750, 700, 685, and 665 $cm^{-1}$; EI-MS $m/z$ (relative intensity) 344 (5, $M^+$), 313 (7, $M^+ - OCH_3$), 298 (100, $M^+ - NO_2$), 267 (4, $M^+ - NO_2 - OCH_3$), 252 (5), 224 (4), 180 (3), 152 (3), 127 (5), 115 (4), 77 (3), 59 (6), and 15 (5); LCMS $m/z$ (relative intensity) 345 [100, $(M + H)^+$], 327 (6), 284 (60), 268 (48), 256 (18), 245 (10), 224 (12), and 211 (14); $^1H$ NMR ($CDCl_3$) $\delta$ 2.65 (s, 6H, $CH_3$), 3.49 (s, 6H, $OCH_3$), and 7.12–8.24 (m, 4H, aromatic-H).

*Compound 4*—The trace amount of residue of fraction C was minimally soluble in methanol. This was dissolved in about 2 mL of the mixture of methanol/ethanol (50:50, v/v) by heating for a short period of time and filtered. After standing in a refrigerator for 2 days, the precipitate formed was separated, washed with cold methanol without recrystallization, and dried under reduced pressure, giving 10 mg of brownish needles. The chromatographical purity was 96.6% by area normalization. This compound showed a mp of 197–202 °C; UV (nm in methanol) $\lambda_{max}$ (log $\epsilon$) 335 (4.13) and $\lambda_{min}$ 293; IR (KBr) $\nu$ 3450, 3000, 2950, 2850, 1725, 1558, 1510, 1435, 1370, 1295, 1240, 1210, 1110, 1040, 945, 860, 810, 770, 738, 665, 580, and 540 $cm^{-1}$; EI-MS $m/z$ (relative intensity) 640 (100, $M^+$), 625 (2, $M^+ - CH_3$), 624 (6, $M^+ - O$), 609 (5, $M^+ - OCH_3$ or $M^+ - NO$), 581 (18, $M^+ - COOCH_3$), 342 (7, $M^+ - C_{17}H_{16}O_4N_1$), 314 (72, $M^+ - C_{17}H_{16}O_4N_3$), 298 (97, $M^+ - C_{17}H_{16}O_5N_3$), 284 (44), 268 (31), 252 (19), 224 (17), 196 (12), 152 (11), 127 (9), 115 (7), 77 (3), 59 (15), and 28 (11); LCMS $m/z$ (relative intensity) 663 (6), 641 [100, $(M + H)^+$], 613 (12), 577 (8), 557 (8), and 513; $^1H$ NMR ($CDCl_3$) $\delta$ 2.63 (s, 6H, $CH_3$), 2.65 (s, 6H, $CH_3$), 3.39 (s, 6H, $OCH_3$), 3.43 (s, 6H, $OCH_3$), and 6.72–7.60 (m, 8H, aromatic H); HRMS spectrum $C_{34}H_{32}O_9N_4$ ($M^+$) requires 640.2171, found 640.2151.

*Anal.*—Calc. for $C_{34}H_{32}O_9N_4$: C, 63.73; H, 5.04; N, 8.75. Found: C, 63.47; H, 4.61; N, 8.32.

*Compound 5*—The residue of fraction D was dissolved in about 10 mL of ethanol by heating and allowed to stand in a refrigerator to give yellowish crystals. The crystals were collected and recrystallized several times from ethanol, yielding 200 mg of pale yellowish crystals. The chromatographical purity was 96.0% by area normalization. This compound showed a mp of 163–165 °C; UV (nm in methanol) $\lambda_{max}$ (log $\epsilon$) 325 (4.01) and $\lambda_{min}$ 295; IR (KBr) $\nu$ 3450, 3000, 2950, 2850, 1725, 1558, 1490, 1450, 1435, 1405, 1370, 1320, 1295, 1240, 1210, 1155, 1110, 1040, 965, 945, 918, 850, 805, 765, 758, 690, 660, 580, 530, and 495 $cm^{-1}$; EI-MS $m/z$ (relative intensity) 640 (62, $M^+$), 625 (3, $M^+ - CH_3$), 624 (2, $M^+ - O$), 609 (5, $M^+ - OCH_3$ or $M^+ - NO$), 581 (16, $M^+ - COOCH_3$), 565 (6), 539 (4), 521 (13), 342 (3, $M^+ - C_{17}H_{16}O_4N_1$), 314 (82, $M^+ - C_{17}H_{16}O_4N_3$), 298 (100, $M^+ - C_{17}H_{16}O_5N_3$), 284 (18), 268 (15), 252 (19), 224 (17), 196 (12), 152 (17), 127 (9), 115 (7), 77 (3), 59 (23), and 15 (8); LCMS $m/z$ (relative intensity) = 641 [100, $(M + H)^+$], 565 (10), 284 (8), 253 (7), and 226 (6); $^1H$ NMR ($CDCl_3$) $\delta$ 2.55 (s, 6H, $CH_3$), 2.59 (s, 6H, $CH_3$), 3.36 (s, 6H, $OCH_3$), 3.37 (s, 6H, $OCH_3$), and 6.80–8.05 (m, 8H, aromatic H); HRMS spectrum $C_{34}H_{32}O_9N_4$($M^+$) requires 640.2171, found 640.2160.

*Anal.*—Calc. for $C_{34}H_{32}O_9N_4$: C, 63.73; H, 5.04; N, 8.75. Found: C, 63.75; H, 4.79; N, 8.59.

*Compound 6*—Trace amounts of the residue of fraction E was dissolved in about 5 mL of ethanol and treated in the same manner as that of fraction D, giving 30 mg of dark brownish crystals. The chromatographical purity was 96.1% by area normalization. This compound showed a mp of 155–157 °C; UV (nm in methanol) $\lambda_{max}$ 325 and $\lambda_{min}$ 295; IR (KBr) $\nu$ 3450, 3000, 2950, 2850, 1725, 1670, 1558, 1490, 1450, 1435, 1400, 1370, 1330, 1295, 1240, 1210, 1155, 1130, 1110, 1040, 965, 945, 918, 850, 805, 765, 758, 690, 660, 580, 530, and 495 $cm^{-1}$; EI-MS $m/z$ (relative intensity) 656 (17, $M^+$), 640 (57, $M^+ - O$), 625 (3, $M^+ - O - CH_3$), 624 (3, $M^+ - O - O$), 609 (5, $M^+ - O - OCH_3$ or $M^+ - O - NO$), 581 (16, $M^+ - O - COOCH_3$), 565 (7), 539 (4), 521 (13), 342 (3, $M^+ - O - C_{17}H_{16}O_4N_1$), 314 (82, $M^+ - O - C_{17}H_{16}O_4N_3$), 298 (100, $M^+ - O - C_{17}H_{16}O_5N_3$), 282

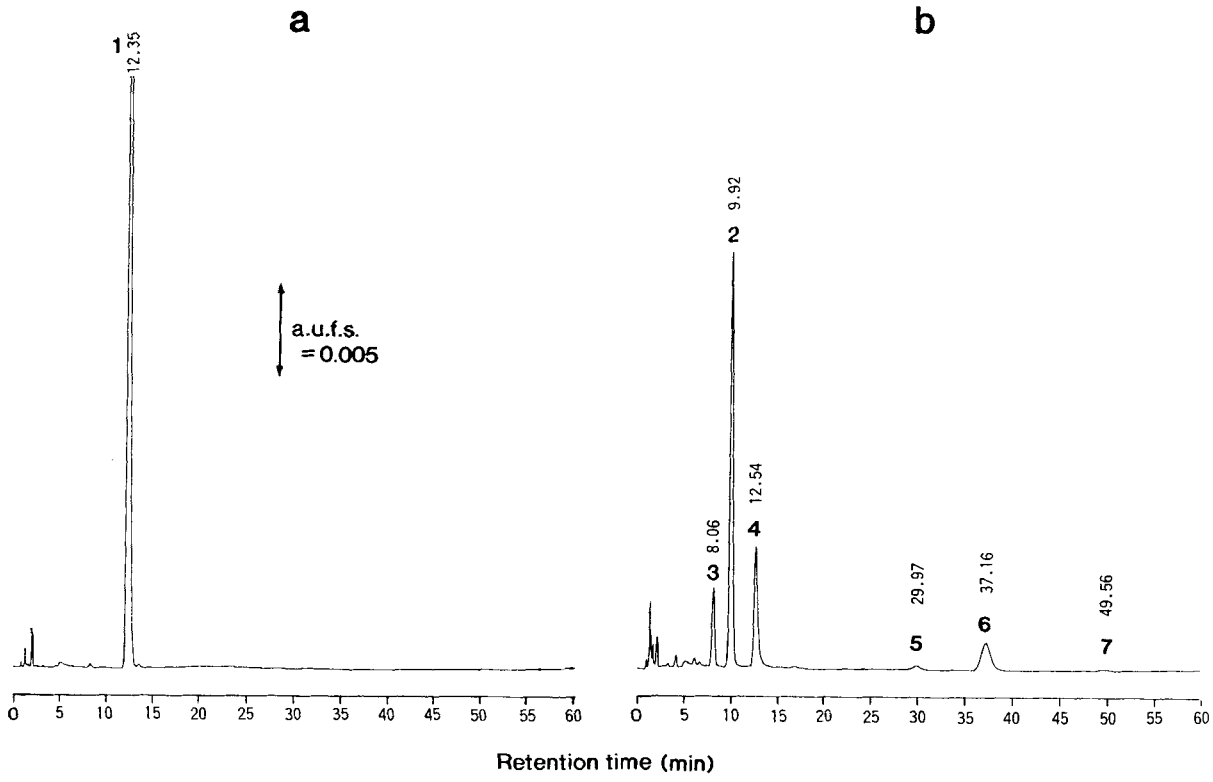



**Figure 1**—HPLC chromatograms of nifedipine in pulverized tablets (a) before and (b) after exposure to normal room light for 30 days. The HPLC conditions are seen in the text. Key: (1) nifedipine; (2) nitroso derivative; (3) nitro derivative; (4–7) unknown photodegradation products.

(82), 268 (33), 251 (53), 223 (23), 196 (15), 152 (23), 127 (15), 115 (9), 77 (5), 59 (28), and 15 (14); LCMS *m/z* (relative intensity) 699 (4), 677 (14), 655 [100, $(M - H)^+$], 609 (8), 579 (8), 328 (14), 311 (18), 296 (22), 282 (10), 267 (5), and 225 (9); $^1H$ NMR ($CDCl_3$) $\delta$ 2.55 (s, 6H, $CH_3$), 2.59 (s, 6H, $CH_3$), 3.36 (s, 6H, $OCH_3$), 3.40 (s, 6H, $OCH_3$), and 6.64–8.04 (m, 8H, aromatic H); HRMS spectrum $C_{34}H_{32}O_{10}N_4$ ($M^+$) requires 656.2120, found 656.2109.

*Anal.*—Calc. for $C_{34}H_{32}O_{10}N_4$: C, 62.18; H, 4.92; N, 8.54. Found: C, 62.36; H, 4.90; N, 8.48.

*Compound 7*—The residue of fraction F was slightly soluble in alcohol and soluble in acetone or chloroform. The acetone solution containing the residue of fraction F was concentrated to a small volume and allowed to stand in a refrigerator overnight to give a white powdery material. The material was separated, redissolved in acetone, and treated in the same manner as described above, giving 20 mg of white powdery material. The chromatographical purity was 98.4% by area normalization. This compound showed a mp of 257–263 °C (decomposition); UV (nm in methanol) $\lambda_{max}$ 239, 265 and $\lambda_{min}$ 210, 255; fluorescent spectrum (nm in methanol): $E_{m\ max}$ 428 ($\lambda_{ex}$ 330); IR (KBr) $\nu$ 3430, 3180, 3020, 2980, 2900, 1985, 1710, 1670, 1610, 1548, 1510, 1435, 1380, 1365, 1350, 1338, 1310, 1270, 1245, 1185, 1160, 1130, 1110, 1025, 955, 908, 885, 845, 838, 808, 785, 760, 730, 685, 650, 640, 590, 520, 495, and 470 $cm^{-1}$; EI-MS *m/z* (relative intensity) 282 (100, $M^+$), 267 (4, $M^+ - CH_3$), 251 (70, $M^+ - OCH_3$), 223 (15, $M^+ - COOCH_3$), 180 (8), 153 (8), 127 (8), 102 (3), 77 (5), 42 (3), 28 (7), and 15 (5); LCMS *m/z* (relative intensity) 283 [100, $(M + H)^+$], 251 (8), 223 (3); $^1H$ NMR ($CDCl_3$) $\delta$ 2.66 (s, 3H, $CH_3$), 3.19 (s, 3H, $CH_3$), 4.00 (s, 3H, $OCH_3$), and 7.07–7.86 (m, 5H, aromatic H and amide NH); HRMS spectrum $C_{16}H_{14}O_3N_2(M^+)$ requires 282.1005, found 282.1005.

*Anal.*—Calc. for $C_{16}H_{14}O_3N_2$: C, 68.06; H, 5.00; N, 9.93. Found: C, 68.23; H, 5.08; N, 9.49.

All procedures for preparation of samples, HPLC analysis, separation, and isolation of photodegradation product were performed under the irradiation of a photo-flood red lamp in a dark room.

## Results and Discussion

**HPLC Analyses of the Preparation Samples**—Photodegradation of 1 proceeds more markedly when it is in solution than when it is in a solid form.[12] In a previous study,[7] however, we found that 1 in various solid-state preparations decomposed completely in about 5 days under the exposure to normal room light. Therefore, in the first series of experiments, photostability of 1 in either pulverized tablets or other preparations were examined by the use of HPLC before and after the exposure to light for 30 days. (The reason for selecting 30 days for the light exposure is that a period of 30 days is the longest period for which a prescription can be made for outpatients in Japan.) During the 30 days of exposure to light, the color of the 1 preparations changed from yellow to brown. Typical HPLC chromatograms of the extracts from pulverized Adalat-L tablets before and after the 30-day period are shown in Figure 1. Other preparations containing 1 also showed the same patterns of HPLC chromatograms before and after the exposure to light. These results indicate that the photodegradation of 1 occurs even when 1 is in a solid state. Prior to exposure to light, the 1 preparations had only a peak for 1 (peak 1) on the HPLC chromatograms (Figure 1a). After exposure to light for 30 days, the HPLC chromatogram exhibited about 6 peaks (peak 2 through peak 7) which were attributable to the photodegradation products of 1 (Figure 1b). The retention time of peak 3 (8.1 min) and that of major peak 2 (9.9 min) were shorter than the retention time of peak 1 (12.4 min), and the two peaks 3 and 2 coincided with those of the authentic samples of 3 and 2, respectively. The compounds corresponding to other peaks, peaks 4–7, are unknown. Peak 4 (12.6 min) appeared immediately after the peak 1, and therefore it can be mistaken for peak 1, namely, recovered 1. This is an important information when the analysis of 1 is performed by HPLC.

**Correspondence of the PLC Separation to HPLC Analysis**—The extract from the pulverized Adalat-L tablets after the exposure to light for 30 days was composed of at least 11 compounds by the separation of PLC (Figure 2a). These compounds were visualized under UV light (254 and 365 nm). Among these components, there were six prominent parts (fractions A–F). Each of the extracted fractions was checked

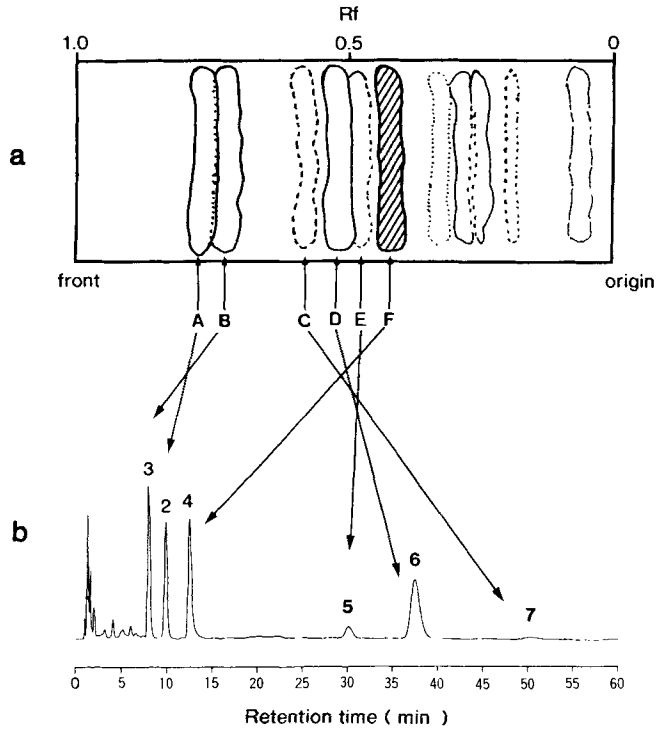



**Figure 2**—(a) Preparative thin-layer chromatogram of the photodegradation products of nifedipine, and the correspondence of each of the fractions (A–E) to (b) the peaks analyzed by HPLC. Both thin-layer and high-performance liquid chromatograph conditions are discussed in the text. The chromatogram of a was visualized under UV light (254 nm) and the striped band (fraction F) was also visualized brightly under that of 365 nm. The key to the chromatogram of b are the same as that given in Figure 1.

for its correspondence to the peak analyzed by HPLC, and the correspondence to the HPLC chromatogram is shown in Figure 2b. Two fractions, A (retardation factor, $R_f$ = 0.76) and B ($R_f$ = 0.71) matched with peak 2 (9.9 min) of **2** and peak 3 (8.1 min) of **3**, respectively. Fraction C ($R_f$ = 0.57), D ($R_f$ = 0.51), E ($R_f$ = 0.47), and F ($R_f$ = 0.41) matched with unknown peak 7 (49.6 min), 6 (37.2 min), 5 (30.0 min), and 4 (12.6 min), respectively. The fraction F, which was brightly visual under UV light of 365 nm, was far from 1 in the $R_f$ value (0.63 for **1**). This result also indicates that the compound of fraction F is not the recovered **1** mentioned above.

**Identification of Compounds 2 and 3**—Besides the retention times for the compounds **2** and **3** by HPLC analysis, the mp and spectral data of these compounds gave excellent agreements with those of the authentic compounds and of the results reported previously.[3,9,13–15] Thus compound **2**, a major photodegradation product of **1**, and compound **3** were identified as the known photodegradation products **2** and **3**, respectively.

**Establishments of Chemical Structures of Compounds 4–7**—Compound **4** showed a UV absorption minimum at 293 nm and a broad, long wavelength absorption maximum at 335 nm ($\lambda_{max}$). The latter absorption band stretched on past 440 nm which is responsible for the brownish color. The EI-MS spectrum (Figure 3a) showed a base peak of molecular ion at *m/z* 640 ($M^+$). The fragment ions detected at *m/z* 625 (2, $M^+ - CH_3$), 609 (5, $M^+ - OCH_3$) and 581 (18, $M^+ - COOCH_3$) resulted from a stepwise cleavage of the carboxymethyl moiety. The peaks at *m/z* 624 (6) and 610 (2) were due to the loss of an oxygen atom and NO. Other major peaks were at *m/z* 342 (7, $M^+ - C_{17}H_{16}O_4N_1$), 314 (72, $M^+ - C_{17}H_{16}O_4N_3$) and 298 (97, $M^+ - C_{17}H_{16}O_5N_3$). These peaks indicated the presence of an azoxy moiety (—N=N→O) in this compound. Furthermore, the $^1H$ NMR spectrum (Figure 4a) indicated eight aromatic protons

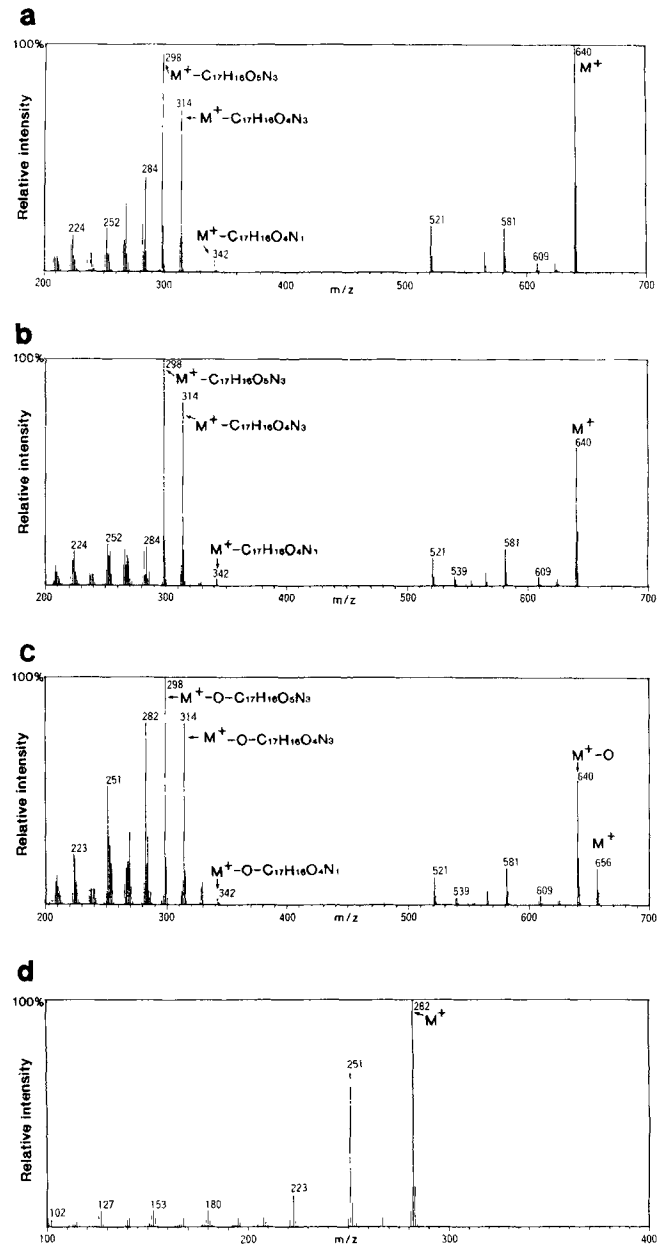



**Figure 3**—Electron-impact (70 eV) mass spectra of photodegradation products of nifedipine: (a) compound **4**, (b) compound **5**, (c) compound **6**, and (d) compound **7**.

(6.72–7.60 ppm), four methyl (2.63 and 2.65 ppm), and four methoxyl (3.39 and 3.43 ppm) groups. The IR spectrum showed a similar pattern to that of **2** above 1600 $cm^{-1}$, and indicated the presence of a carbonyl group (3450 and 1725 $cm^{-1}$), aromatic ring (3000 and 2950 $cm^{-1}$), and a methyl group conjugated to aromatic ring (2850 $cm^{-1}$). A strong absorption band of the azoxy group appeared at 1558 (—N=N—) and 1240 $cm^{-1}$ (N→O) in the IR spectrum. It is, therefore, assumed that the chemical structure of compound **4** is an azoxy derivative, 2,2′-bis[2,6-dimethyl-3,5-bis(methoxycarbonyl)pyridine-4-yl]azoxybenzene ($C_{34}H_{32}O_9N_4$: 640.2171), which may be derived from two molecules of **2** by dehydrocondensation. HRMS spectrum (*m/z* 640.2151) and elementary analysis also supports the above assumption regarding the chemical structure.

The EI-MS spectrum of the compound **5** (Figure 3b) showed a similar pattern to that of the compound **4** in fragmentation and intensity. However, the base peak was not at *m/z* 640 ($M^+$), relative intensity of which was 62%, but it appeared at *m/z* 298

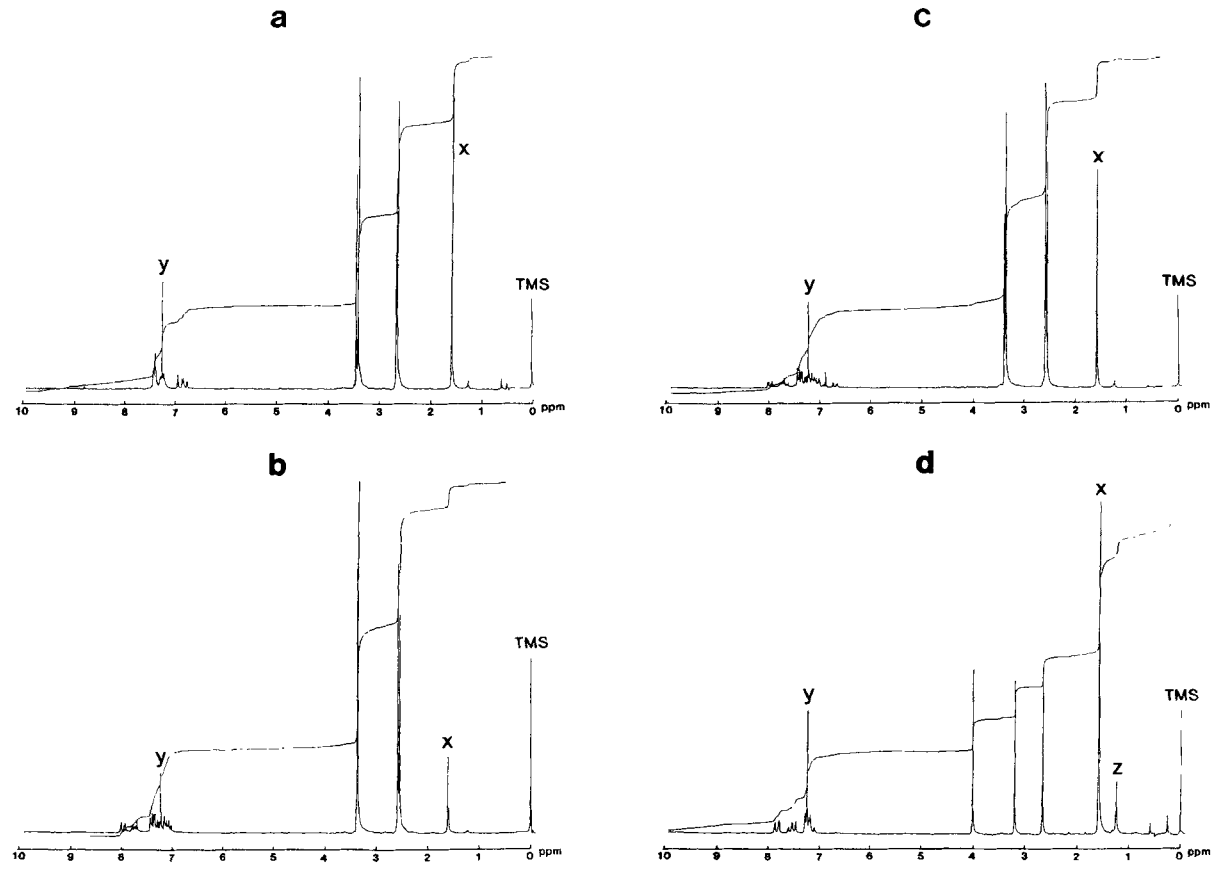



**Figure 4**—The $^1$H NMR spectra of photodegradation products: (a) compound **4**, (b) compound **5**, (c) compound **6**, and (d) compound **7**. Key: (x) water; (y) chloroform ($CHCl_3$); (z) signal due to a free fatty acid mixed into the tube during the preparation for measurement; (TMS) tetramethylsilane as an internal reference ($\delta$ 0.00).

($M^+ - C_{17}H_{16}O_5N_3$). This compound also had a molecular ion peak in the HRMS spectrum at $m/z$ 640.2160, which corresponded to the elemental composition $C_{34}H_{32}O_9N_4$ (calcd 640.2171). This formula was supported by elementary analysis. In the $^1$H NMR spectrum (Figure 4b), each of the chemical shift values of the compound was a little different from that of the compound **4**. Their assignments, however, showed the presence of eight aromatic protons (6.80–8.05 ppm), four methyl (2.55 and 2.59 ppm), and four methoxyl (3.36 and 3.37 ppm) groups, being identically to that of the compound **4**. Furthermore, these spectral data agreed excellently with the results obtained by Matsuda and co-workers[9] who reported a new photodegradation product of **1**, an azoxy derivative, under intense light exposure conditions. These results suggest that the chemical structure of this compound is identical to that of the compound **4**, namely, an azoxy derivative, 2,2′-bis[2,6-dimethyl-3,5-bis(methoxycarbonyl)pyridine-4-yl]azoxybenzene. Compounds **4** and **5** are a pair of stereoisomers based on the double bond of azoxy group. In the UV spectrum of compound **5**, the values of $\lambda_{max}$ (325 nm in methanol) and its molar extinction coefficient ($\log \epsilon = 4.01$) were lower than those (335 nm, $\log \epsilon = 4.13$) of the compound **4**. This result is unanalogous to the trans series.[16] Compound **5**, however, gave a higher yield and a lower mp than the compound **4**. In general, it is noted that the trans-isomer of azoxy compound is more stable and shows a lower mp and a higher yield than cis-isomer.[17] Compounds **4** and **5** possessed similar patterns of IR spectra, except for the absorption bands of 1490, 1455, 1405, 965, 918, 850, and 690 $cm^{-1}$, which were observed only in compound **5** and also in the spectra of *trans*-azoxybenzene (data not shown). The absorption band of 1490 $cm^{-1}$ is characteristics of trans azoxy compounds.[18,19] Therefore, it is considered that these bands are related to trans conformational vibrations in the azoxy group. Consequently, compound **5** is a trans-form of azoxy derivative, and compound **4** is the so-called cis-form of the derivative. Lower solubility[19] in methanol and the absorption band at 1510 $cm^{-1}$ in the IR spectrum of compound **4** also indicated the cis-conformation. Thus, the cis–trans isomerization[20] of the azoxy derivative of **1** was demonstrated for the first time by the isolation of the two compounds in the present study. The thermochemical reconversion of the cis-isomer to trans-isomer[20] had no bearing on isolation and crystallization because purities of these isolated compounds were found to be high by the HPLC analysis.

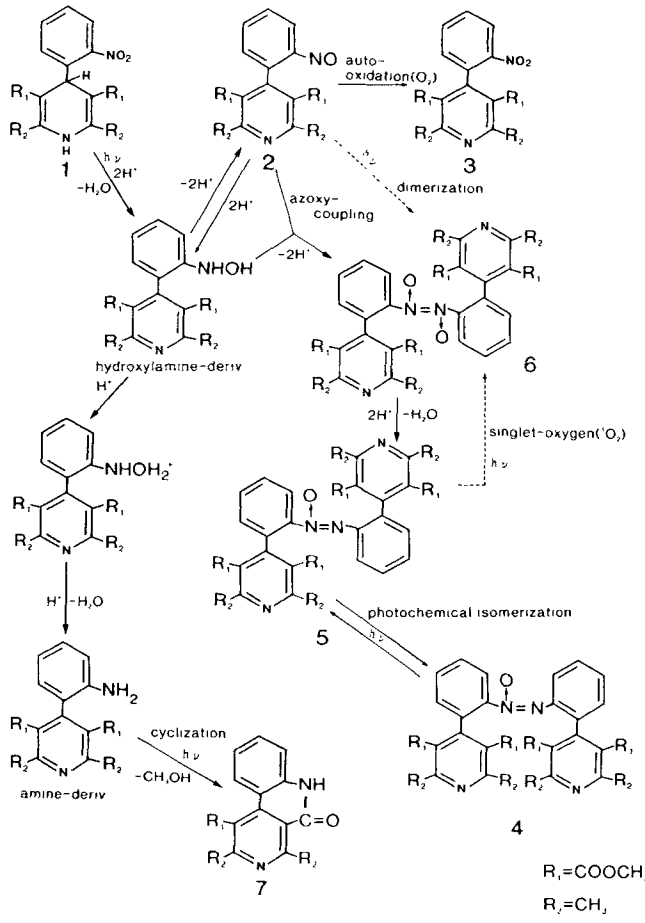
Compound **6** was a brownish crystal, showing a mp of 155–157 °C, and the UV spectrum of the compound in methanol solvent presented a striking similarity to that of the compound **5**. The EI-MS spectrum (Figure 3c) also showed that compound **6** was almost identical to compound **5** in the portion below $m/z$ 640. The molecular ion peak of compound **6** appeared at $m/z$ 656 (17, $M^+$). This molecular ion fragment was also ascertained by the LCMS spectrum at 655 [100, $(M - H)^+$]. In the $^1$H NMR spectrum (Figure 4c), only the assignment of aromatic ring resonance (6.64–8.05 ppm) of compound **6** was a little different from that of compound **5**. The IR spectrum revealed that the chemical structure of this compound was very similar to that of compound **5**. From these results, compound **6** is presumed to have a structure that possesses one more oxygen than does compound **5**, corresponding to a *trans-N,N′*-dioxide derivative, *N,N′*-bis[2,6-dimethyl-3,5-bis(methoxycarbonyl)pyridine-4-phenyl]diimide dioxide ($C_{34}H_{32}O_{10}N_4$ 656.2120). HRMS spectrum ($m/z$ 656.2109), and elementary analysis also support this structure.

Compound **7** was a white powdery substance and showed a high melting point temperature [257–263 °C dec]. The UV

spectrum of compound 7 in methanol solvent showed that the compound was quite different from 1 and other photodegradation products (compound 2–6). The $\lambda_{max}$ and $\lambda_{min}$ were 239, 265 nm and 210, 255 nm, respectively. Furthermore, this compound showed a strong fluorescent spectrum at 428 nm ($\lambda_{ex}$ = 330 nm). The above data indicate that this compound has a striking feature in its chemical structure. In the EI-MS (Figure 3d) or LCMS spectra, the base peak at $m/z$ 282 and 283 showed a molecular ion of $M^+$ and $(M + H)^+$, respectively. The molecular weight of this compound was also estimated from HRMS spectrum at $m/z$ 282.1005, corresponding to the elemental composition $C_{16}H_{14}O_3N_2$ (calcd 282.1005). This formula was supported by the elementary analysis. Other major fragment ions at $m/z$ 267 (5), 251 (70) and 223 (15) in the EI-MS spectrum indicated the presence of methyl and carboxymethyl groups in this molecule. The $^1H$ NMR spectrum (Figure 4d) indicated there were four aromatic protons and one proton corresponding to amide NH (five protons as a total, 7.07–7.86 ppm), two methyl (2.66 and 3.19 ppm), and methoxyl (4.00 ppm) groups. Since the molecular weight of this compound was smaller than 2 and the $^1H$ NMR spectrum indicated only one signal from methoxyl group (4.00 ppm) in comparison with 2, a carboxymethyl group ($COOCH_3$) on the pyridine ring was presumed to be condensed with a nitroso group, forming a δ-lactam ring. The presence of δ-lactam ring was confirmed by the assignment of absorption bands at 1675 and 1595–1610 $cm^{-1}$ characteristic of amide I (>C=O) and II (—NH—) in the IR spectrum and was also suggested from the fluorescent absorption spectrum described above and the positive color tests with the *p*-(dimethylamino)benzaldehyde[21] in toluene and ethanol and fluorescein chloride.[22] The signal of —NH— of the lactam ring could not be detected by the $^1H$ NMR spectrum, because this signal overlapped with that of an aromatic ring. It is therefore deduced from these data that compound 7 is a lactam derivative, 3,2′-(4-phenyl-2,6-dimethyl-5-(methoxycarbonyl)pyridine)lactam.

**A Mechanism and Pathway of Photogegradation of 1**—In summation of the above, a mechanism and pathway as shown in Scheme 1 is proposed to account for the degradation pathway of 1. In the present study, 1, even in the solid state, decomposed to at least six compounds upon exposure to normal room light (Figure 1). This finding is not in accord with the results obtained by earlier investigations,[1–5] which indicated that 1 decomposed only to 2 and 3.

There are facts that nitrosobenzene can be obtained by the oxidation of phenylhydroxylamine and that the reduction of nitrosobenzene to phenylhydroxylamine occurs more easily (at more positive potentials of electrolytic reduction) than that of nitrobenzene.[23] That is, 1 was photochemically reduced in a four-electron process resulting predominantly in the hydroxylamine derivative[24] (see Scheme 1). This chemical change in irradiated 1 involves the oxidation of the dihydropyridine ring to the pyridine ring, which decreases the density over the nitro group and makes the reduction process easier.[25] The hydroxylamine derivative in the presence of air dioxygen was easily oxidized to 2, and 3 could be formed by autoxidation of 2. When phenylhydroxylamine and nitrosobenzene are allowed to interact, the product of the reaction is azoxybenzene[26] (the so-called azoxy coupling reaction). This reaction is analogous to the aldol-type condensation of nitrosobenzene with hydroxylamine.[19,27] Therefore, it is possible that condensation of hydroxylamine derivative and 2 can produce an azoxy derivative (4 and 5) of 1. In this case, some of the 2 is reduced to a hydroxylamine derivative, which is then condensed with new 2 to form the azoxy derivative. The prior formation of a symmetrical *N,N′*-diol intermediate has long been regarded as a mechanism in the coupling reaction.[28,29] It has also been proposed by Russel and coworkers[30,31] on the basis of ESR studies that the bis-anion [N(O⁻)N(O⁻)], formed from two nitrosobenzene anion radicals (Ar-NO·⁻), is an intermediate in the condensation reaction,




**Scheme 1**—Proposed photodegradation pathway of nifedipine. Key: (1) nifedipine; (2) nitroso derivative; (3) nitro derivative; (4) *cis*-azoxy derivative; (5) *trans*-azoxy derivative; (6) *trans-N,N′*-dioxide derivative; (7) lactam derivative

yielding azoxybenzene. We, therefore, presume that compound 6 is the intermediate that is to be reduced to produce the azoxy derivative. And furthermore, 2 might be conjugated directly with itself to form *N,N′*-dioxide derivatives (6), a dimer product.[11,19] Dimerization of aromatic nitroso compounds, however, has not been well documented, as opposed to aliphatic ones.[19] On oxidation with a photosensitizer of singlet oxygen, which is generated from 1 under irradiation conditions in the presence of molecular oxygen,[15] 4 or 5 (probably 5) could be converted into 6. The nitrogen–nitrogen double bond in 4–6 can give rise to cis–trans isomerization by optical pumping.[17] In addition to this isomerization, the Wallach rearrangement to form a hydroxyazobenzene derivative can also occur,[20] but appears to be a negligible reaction in the solid state. It is considered that the ordinary stable form of trans-isomer was converted partially into the cis-isomer by irradiation. On the other hand, if the hydroxylamine derivative were changed to a protonated form ($Ar–NHOH_2^+$), it is relatively easily reduced to the amine.[24] The protons which should be generated from the oxidation of either the dihydropyridine ring of 1 to pyridine ring or the hydroxylamine derivative to 2, mentioned above, may lead the protonation (see Scheme 1). It is well known that the end reduction product of nitrobenzene is aniline. The amine derivative of 1 might be converted into a lactam derivative (7) by intramolecular condensation, that is, the primary amine residue in the benzene ring condensed with 3-methoxycarbonyl residue in the pyridine ring to form δ-lactam ring. This cyclization of carbonyl–amine is catalyzed by light.

In previous studies,[7] the sum of the molar fractions of 1, and its photodegradation products (both 2 and 3) did not remain

constant but decreased gradually during exposure to light. The fractional formation of **2** reached the maximum, but it decreased gradually. These facts indicate that there is further decomposition of **2** and formation of other photoproducts. As mentioned above, it is assumed that **2** is the main intermediate, from which minor new photoproducts are formed.

The possibility of occurrence of intramolecular as well as intermolecular condensation of **2** on exposure to light was thus demonstrated by isolating four new photoproducts of **1**. Furthermore, the isolation of compound **6** is considered important, because compound **6** is the key substance to understand the degradation process of **1** including the azoxy-coupling mechanism.

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## Acknowledgments


We are greatly indebted to Dr. T. Ohta (Science University of Tokyo) for obtaining the EI-MS, HRMS, $^1$H NMR spectra and valuable discussion. Thanks are also due to Dr. E. Sakurai (Tohoku College of Pharmacy) for elemental analysis and to Mr. Y. Hamano and Mr. K. Toyama (Techno-research Laboratory, Hitachi Instrument's Engineering Co. Ltd. Japan) for measuring the LCMS spectra.