Degradation and Isomerization of Nileprost, 5-Cyano-16-methyl-prostacyclin, in Aqueous Solution

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Nileprost is a new prostacyclin analogue stabilized by introduction of the cyano group at its 5-position. The acidic and alkaline degradation and the structure determination of the degradation products were investigated. The degradation of nileprost is very slow in comparison with that of prostacyclin. Although prostacyclin is easily decomposed to 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) through the hydrolysis of a vinyl ether moiety, nileprost gives little of such hydrolysis product but many isomers and dehydrates. The structures of the products were determined by high-performance liquid chromatography (HPLC), negative ion fast atom bombardment mass spectra (N-FABMS) and nuclear magnetic resonance (NMR) studies. It was found that the vinyl ether moiety of nileprost is converted from 5*Z* form to 5*E* form in both media, and that the ω-side chain also undergoes transfer of hydroxyl group or dehydration in acid medium. These results indicate that introduction of the cyano group into the 5-position of prostacyclin is extremely effective for the stabilization of the vinyl ether moiety. Furthermore, on the basis of the structural elucidation, the reaction mechanism of nileprost in both media was clarified.

Keywords nileprost; prostacyclin analogue; acidic degradation; alkaline degradation; degradation mechanism

It is well known that prostacyclin (PGI_2) has remarkable biological activity but is very easily hydrolyzed to give biologically inactive 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) because of the extreme instability of the vinyl ether moiety.[1] Various more stable analogues have therefore been investigated in order to utilize PGI_2 for clinical treatment without the unstable limitation.[2–4] Nileprost (NP) is one of these, and for the stabilization an electron withdrawing cyano group is introduced into its 5-position.[2] The degradation and/or the kinetics of vinyl ether moiety in PGI_2 or in its analogues have been widely studied, but the degradation of other moieties has been generally neglected due to the very fast vinyl ether hydrolysis.[1,4–6] In the stabilized analogues or other type prostaglandins,[7] however, the chemical reactivity of other moieties becomes relatively important. We investigated the degradation of NP in aqueous solution, and found that the vinyl ether hydrolysis of NP is very minor but the isomerization and dehydration are major. These results are reported in this paper.

Experimental

Instruments Nuclear magnetic resonance (NMR) spectra were measured with a JEOL JNM-GSX 270 spectrometer with CD_3OD as solvent and using tetramethylsilane (TMS) as an internal standard. The chemical shifts and coupling constants (*J*) are given in ppm from TMS and Hz, respectively. Negative ion fast atom bombardment mass spectra (N-FABMS) were measured with a JEOL JMS-D300 mass spectrometer equipped with FAB gun (Xe, 6 kV) at the accelerating voltage of 3 kV. *m*-Nitrobenzyl alcohol was used as a matrix.

Materials and Reagents Nileprost and its 15β-isomer were supplied by Schering AG (Berlin, Germany). 0.02 M Phosphate buffer solution (pH 3.5) was prepared by mixing 0.02 M KH_2PO_4 and 0.02 M H_3PO_4. 0.1 M Phosphate buffer solutions (pH 5.0 and 7.0) were prepared by mixing 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4, respectively. 0.1 M HCl–KCl buffer solution (pH 1.2) was prepared by mixing 0.1 N HCl and 0.1 M KCl. Deionization water was used in all experiments. Deuterated solvents and reagents used were of NMR reagent grade. Other reagents used were of analytical reagent grade.

High Performance Liquid Chromatography (HPLC) HPLC was performed with a JASCO TRI ROTAR-III equipped with a JASCO UVIDEC-100III ultraviolet (UV) absorption detector, a JASCO 830RI refractive index (RI) detector and a KYOWASEIMITSU AUTO SAMPLER KSP-600. A column oven was maintained at 30 °C.

Analytical HPLC Conditions: Column, μBondapak-C_{18} (3.9 i.d. × 300 mm, Waters Assoc.); mobile phase, water–MeCN–AcOH (100 : 50 : 1); flow rate, 1.5 ml/min.; detector, UV 240 nm; and injection volume, 100 μl.

Preparative HPLC Conditions: Column, μBondapak-C_{18} (8 i.d. × 300 mm, Waters Assoc.); mobile phase, 0.02 M phosphate buffer solution (pH 3.5)–MeCN (5 : 2) for shorter retention products or (1 : 1) for longer retention products; flow rate, 4.0 ml/min; and detector, UV 240 nm and RI.

Degradation of Nileprost in Aqueous Solution i) Acidic Solution: A solution of nileprost in MeCN (10 mg/ml) was diluted with 0.1 M HCl–KCl buffer solution (pH 1.2) to a concentration of 20 μg/ml. After standing at 37 °C for 2—48 h, the solution was adjusted to pH 4—6 by addition of an equal volume of 0.1 M phosphate buffer solution (pH 7.0) and then injected into analytical HPLC.

ii) Alkaline Solution: Nileprost was dissolved in 0.1 N NaOH to a concentration of 20 μg/ml. After standing at 37 °C for 1—10 d, the solution was adjusted to pH 4—6 by addition of an equal volume of a mixture of 0.2 N HCl and 0.1 M phosphate buffer solution (pH 5.0) (1 : 1) and injected into analytical HPLC.

The quantification of each degradation product was carried out by comparison with a standard solution which was prepared using authentic sample and the isolated degradation products.

Isolation of Degradation Products i) Acidic Degradation: Nileprost (0.5 g) was dissolved in MeCN (35 ml) and then 0.2 N HCl (65 ml) was added. After standing at 40 °C for 48 h, the solution was concentrated to a half volume *in vacuo* and then extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* to give an oily residue.

ii) Alkaline Degradation: Nileprost (0.2 g) was dissolved in 0.2 N NaOH (50 ml). After standing at 60°C for 5 d, the solution was acidified with HCl and then extracted with $CHCl_3$. The extract was treated in a manner similar to that of acidic degradation.

The residue was dissolved in MeCN–water (1 : 1) and injected into preparative HPLC. A fraction of each peak was collected, concentrated to a half volume *in vacuo*, and then extracted with $CHCl_3$. The extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* to give oily residue. This preparation procedure was repeated as necessary.

A mixture of the products **11** and **12** prepared by HPLC was separated to **11** (upper band) and **12** (lower band) by preparative silica gel thin-layer chromatography (TLC) with AcOEt–EtOH–AcOH (40 : 2 : 1) as the developing solvent.

Results and Discussion

HPLC of Degradation Products in Aqueous Solution Figure 1 shows liquid chromatograms of the degradation products of NP under acidic and alkaline conditions. Many peaks appeared under acidic condition but only one peak was observed under alkaline condition. In acidic degradation, in addition to the peaks detected by UV, other peaks were observed in the chromatogram monitored by RI but those intensities were weak. In this study, the

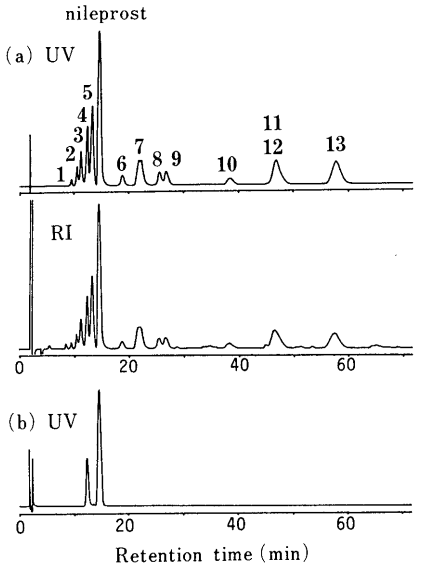


Fig. 1. Liquid Chromatograms of Nileprost and Its Degradation Products (a) in 0.1 M HCl–KCl Buffer Solution (pH 1.2) after 48 h and (b) in 0.1 N NaOH after 6 d at 37 °C

structures of the numbered peaks in Fig. 1 were determined.

Structure Determination of the Degradation Products In N-FABMS studies, all the products gave very intense peaks considered to be $[M-H]^-$ ions. The ions of the products **1**—**9** were the same mass number of *m/z* 390, which is equal to the $[M-H]^-$ ion of NP, and those of the products **10**—**13** were also the same mass number of *m/z* 372, which is 18 mass units less than the $[M-H]^-$ ion of NP. These MS data indicated that **1**—**9** are isomers of NP, and **10**—**13** isomers of a dehydrate of NP. An ion at *m/z* 408 was also observed in the spectra of **1** and **2** when those were not yet pure enough, but the intensity was relatively weak (less than about 10%) in comparison with that at *m/z* 390. Although the hydrate could not be isolated sufficiently during the purification of **1** or **2**, the appearance of the *m/z* 408 ion suggested the formation of a hydrate such as 6-keto-$PGF_{1\alpha}$ type compound.

Proton nuclear magnetic resonance (^{1}H-NMR) data of the products **1**—**13** are shown in Table I. ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{13}C–^{1}H COSY were used for the assignment.

The degradation product **5** gave a retention time corresponding to that of the authentic 15*β*-isomer of NP on HPLC, and the ^{1}H-NMR spectrum of **5** was also identical to that of the authentic sample. Thus the product **5** was determined to be 15*β*-isomer of NP.

In comparing the ^{1}H-NMR spectrum of **4** with that of NP, it was observed that 4-, 7*α*- and 7*β*-protons of **4** were 0.10, 0.09 and 0.14 ppm upper field of those of NP, respectively, while the chemical shifts of other protons of **4** were coincident to those of NP. The upfield shifts of 7*α*- and 7*β*-protons, which are most influenced by the cyano group, suggested that the product **4** is 5*Z* isomer of NP (the geometric configuration of NP at the 5-position is *E*) because a proton in the direction of C≡N bonding axis is shifted to upfield, but that around the C≡N bond to downfield. The nuclear Overhauser effect (NOE) difference spectra were measured to confirm this observation, and showed that the NOE between H-4 and H-7 in the product **4** is positive, but that in NP is negative. Although the 5-position of **4** was determined to be *Z*, the configuration at C-15 was not determined in the NMR studies. However, **4** was revealed to be identical to the alkaline degradation product by HPLC, N-FABMS and NMR studies. A chromatogram (Fig. 1b) indicates that the degradation product is only **4** and the rearrangement occurs only at C-5 of NP without any change at C-15 in alkaline solution because of the absence of a peak corresponding to the 15*β*-isomer of NP. From this consideration, the configuration of the product **4** at C-15 must be *α*. In fact, the treatment of the isolated **4** under alkaline condition gave only NP. Therefore, the product **4** is 5*Z* isomer of NP.

^{1}H-NMR spectrum of the product **3** also closely resembled that of **4**. The configuration at C-15 of **3** was considered to be *β* from the chromatographic behavior, similar to the relationship between NP and **5**. The treatment of **3** under alkaline condition gave only **5**. Therefore, the product **3** is 5*Z*-15*β*-isomer of NP.

In comparing the spectrum of **1** with that of NP, the following changes were observed: for 16-methyl group the change from overlapping signal with 20-methyl group at 0.9 ppm to separated singlet at 1.13 ppm, the conversion at the 15-position from methine at 3.9 ppm to methylene at 2.2 ppm, loss of the signal of methine proton at the 16-position, and change of the signal pattern of methine at the 14-position from a double doublet at 5.58 ppm to a double triplet at 5.63 ppm. These changes indicated the transfer of the hydroxyl group from C-15 to C-16. The coupling constant between H-13 and H-14 is 15.4 Hz, similar to that of NP (15.8 Hz), indicating *trans*-form.^{1}H-NMR spectrum of **2** was similar to that of **1**, and the structure of the *ω*-side chain of **2** was also determined to be similar to that of **1**. The geometric configuration at the 5-position was determined by analysis of the chemical shifts of H-4, H-7*α* and H-7*β* between **1** and **2**, *i.e.* **1** is 5*Z* form, and **2**, 5*E* form, because those protons of **1** are higher field than those of **2**, similar to the relationship between NP and **4**. Therefore, **1** is 5*Z*-16-hydroxy-isomer and **2** its 5*E*-isomer.

^{1}H-NMR spectrum of the product **6** was similar to that of NP. The signals of two protons at 4.1 ppm were, however, assigned to be 11-H and 13-H, and those of two protons at 5.48 and 5.54 ppm, 14-H and 15-H, where in NP the former signals correspond to 11-H and 15-H, and the latter signals to 13-H and 14-H. These assignments indicate the transfers of the double bond from C-13, 14 in NP to C-14, 15 and of the hydroxyl group from C-15 in NP to C-13. The coupling constant between H-14 and H-15 is 15.9 Hz, indicating *trans*-form.^{1}H-NMR spectra of **7**—**9** were also similar to that of **6**, and the structures of the *ω*-side chain of **7**—**9** were also determined to be similar to that of **6**. The geometric configurations of the products **6**—**9** at the 5-position were determined by analysis of the chemical shifts of H-4, H-7*α* and H-7*β*. Products **7**—**9** all show similar chemical shifts of those protons, whereas the signals of those protons of **6** are higher field than those of **7**—**9**, indicating that **6** is *Z* and **7**—**9** are *E*, similar to the relationship between NP and **4**. Therefore, **6** is 5*Z*-13-hydroxy-isomer, and **7**—**9** its 5*E*-isomers. The peak of **7** in Fig. 1 is found to be an overlapping peak. Now, NP consists of 16*R*- and 16*S*-isomers, which can be resolved by a change of HPLC conditions, for example, a decrease of acetonitrile in the

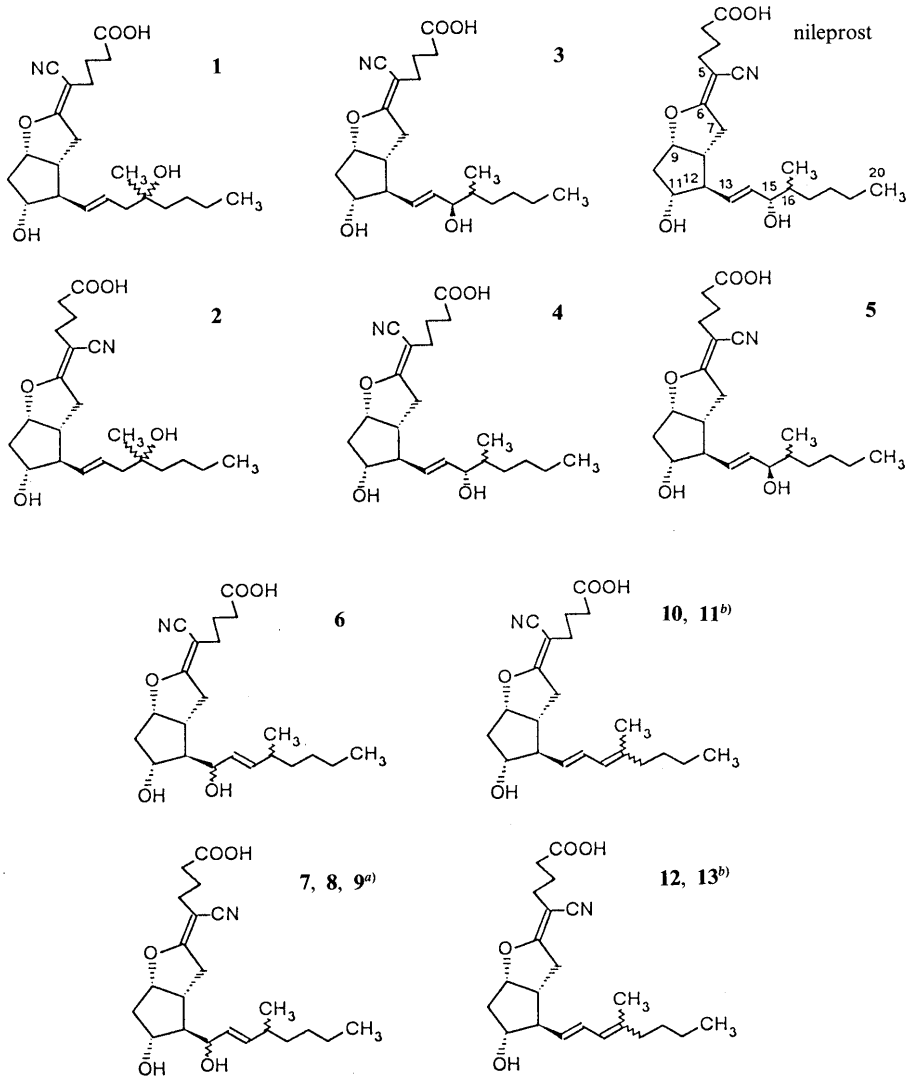
TABLE I. ^{1}H-NMR Data of Nileprost and Its Degradation Products

Product	Chemical shifts δ(ppm)
NP	0.90 (m, 16-CH_3, 20-H_3), 1.0—1.6 (m, 16-H, 17-H_2, 18-H_2, 19-H_2), 1.77 (q, J=7.3, 3-H_2), *ca.* 1.80 (m, 10β-H), 2.08 (dt, J=7.2, 10.0, 12-H), 2.24 (t, J=7.3, 4-H_2), 2.31 (t, J=7.3, 2-H_2), 2.55 (m, 8-H, 10α-H), 2.79 (d, J=17.2, 7α-H), 2.99 (dd, J=8.5, 17.2, 7β-H), *ca.* 3.90 (m, 11-H, 15-H), 4.94 (dt, J=3, 6.5, 6.5, 9-H), 5.55 (dd, J=7.2, 15.8, 13-H), 5.58 (dd, J=6, 15.8, 14−H)
1	0.93 (t, J=7.0, 20-H_3), 1.13 (s, 16-CH_3), 1.3—1.5 (m, 17-H_2, 18-H_2, 19-H_2), 1.79 (q, J=7.2, 3-H_2), *ca.* 1.80 (overlap, 10β-H), 2.04 (q, J=8, 12-H), 2.15 (t, J=7.3, 4-H_2), 2.20 (d, J=7.2, 15-H_2) 2.33 (t, J=7.3, 2-H_2), *ca.* 2.55 (m, 8-H, 10α-H), 2.72 (d, J=17.6, 7α-H), 2.82 (dd, J=8.1, 17.6, 7β-H), 3.89 (q, J=8 11-H), *ca.* 4.9 (overlap, 9-H), 5.38 (dd, J=7.8, 15.4, 13-H), 5.63 (dt, J=7.2, 15.4, 14-H)
2	0.93 (t, J=7.0, 20-H_3), 1.14 (s, 16-CH_3), 1.3—1.5 (m, 17-H_2, 18-H_2, 19-H_2), *ca.* 1.8 (m, 3-H_2, 10β-H), 2.05 (q, J=8, 12-H), *ca.* 2.2 (m, 4-H_2, 15-H_2), 2.31 (t, J=7.2, 2-H_2), *ca.* 2.55 (m, 8-H, 10α-H), 2.80 (d, J=17.3, 7α-H), 2.98 (dd, J=8.1, 17.3, 7β-H), 3.88 (q, J=8, 11-H), *ca.* 4.9 (overlap, 9-H), 5.39 (dd, J=8.1, 15.4, 13-H), 5.64 (dt, J=7.3, 15.4, 14-H)
3	0.90 (m, 16-CH_3, 20-H_3), 1.0—1.6 (m, 16-H, 17-H_2, 18-H_2, 19-H_2), 1.78 (q, J=7.3, 3-H_2), *ca.* 1.80 (overlap, 10β-H), 2.09 (dt, J=7.2, 10.0, 12-H), 2.14 (t, J=7.3, 4-H_2), 2.33 (t, J=7.3, 2-H_2), 2.55 (m, 8-H, 10α-H), 2.73 (d, J=17.5, 7α-H), 2.85 (dd, J=8.3, 17.5, 7β-H), *ca.* 3.90 (m, 11-H, 15-H), 4.91 (dt, J=2.7, 6.5, 6.5, 9H), 5.55 (dd, J=7, 15, 13-H), 5.58 (dd, J=6, 15, 14-H)
4	0.90 (m, 16-CH_3, 20-H_3), 1.0—1.6 (m, 16-H, 17-H_2, 18-H_2, 19-H_2), 1.79 (q, J=7.3, 3-H_2), *ca.* 1.80 (m, 10β-H), 2.09 (dt, J=7.2, 10.0, 12-H), 2.14 (t, J=7.3, 4-H_2), 2.33 (t, J=7.3, 2-H_2), 2.55 (m, 8-H, 10α-H), 2.70 (d, J=17.4, 7α-H), 2.85 (dd, J=8.3, 17.4, 7β-H), *ca.* 3.90 (m, 11-H, 15-H), 4.91 (dt, J=2.6, 6.5, 6.5, 9-H), 5.55 (dd, J=7, 15.5, 13-H), 5.58 (dd, J=6, 15.5, 14-H)
5	0.90 (m, 16-CH_3, 20-H_3), 1.0—1.6 (m, 16-H, 17-H_2, 18-H_2, 19-H_2), 1.79 (q, J=7.3, 3-H_2), *ca.* 1.80 (m, 10β-H), 2.09 (dt, J=7.2, 10.0, 12-H), 2.24 (t, J=7.3, 4-H_2), 2.31 (t, J=7.3, 2-H_2), 2.55 (m, 8-H, 10α-H), 2.82 (d, J=17.3, 7α-H), 3.00 (dd, J=8.3, 17.3, 7β-H), *ca.* 3.90 (m, 11-H, 15-H), 4.94 (dt, J=3, 6.5, 6.5, 9-H), 5.55 (dd, J=7, 15.4, 13-H), 5.58 (dd, J=6, 15.4, 14-H)
6	0.90 (t, J=6.5, 20-H_3), 1.00 (d, J=6.8, 16-CH_3), 1.30 (br s, 17-H_2, 18-H_2, 19-H_2), *ca.* 1.75 (overlap, 12-H), 1.77 (q, J=7.3, 3-H_2), 1.92 (ddd, J=2.7, 5, 14.8, 10β-H), 2.11 (t, J=7.3, 4-H_2), *ca.* 2.15 (overlap, 16-H), *ca.* 2.25 (m, 10α-H), 2.32 (t, J=7.3, 2-H_2), 2.81 (d, J=17.5, 7α-H), *ca.* 2.85 (overlap, 8-H), 2.95 (dd, J=8.3, 17.5, 7β-H), 4.10 (m, 11-H, 13-H), 4.95 (dt, J=2.7, 6.5, 6.5, 9-H), 5.48 (dd, J=6, 15.9, 14-H), 5.54 (dd, J=5, 15.9, 15-H)
7	0.90 (t, J=6.5, 20-H_3), 1.01 (d, J=6.8, 16-CH_3), 1.30 (br s, 17-H_2, 18-H_2, 19-H_2), *ca.* 1.75 (overlap, 12-H), 1.75 (q, J=7.2, 3-H_2), 1.90 (ddd, J=2.7, 5.1, 14.6, 10β-H), 2.15 (m, 16-H), 2.22 (t, J=7.2, 4-H_2), *ca.* 2.30 (overlap, 10α-H), 2.31 (t, J=7.2, 2-H_2), *ca.* 2.85 (overlap, 8-H), 2.92 (d, J=17.3, 7α-H), 3.07 (dd, J=9, 17.3, 7β-H), 4.10 (m, 11-H, 13-H), 4.97 (dt, J=2.7, 6.5, 6.5, 9-H), 5.48 (dd, J=6, 15.7, 14-H), 5.55 (dd, J=5, 15.7, 15-H)
8	0.91 (t, J=6.6, 20-H_3), 1.01 (d, J=6.8, 16-CH_3), 1.30 (br s, 17-H_2, 18-H_2, 19-H_2), *ca.* 1.75 (overlap, 12-H), 1.76 (q, J=7.2, 3-H_2), 1.91 (ddd, J=2.2, 5.4, 14.9, 10β-H), 2.15 (m, 16-H), 2.22 (t, J=7.2, 4-H_2), *ca.* 2.30 (overlap, 10α-H), 2.31 (t, J=7.2, 2-H_2), 2.65 (m, 8-H), 2.97 (d, J=17.3, 7α-H), 3.04 (dd, J=8.5, 17.3, 7β-H), 4.03 (t, J= 6.8, 13-H), 4.13 (q, J=6.3, 11-H), 4.96 (dt, J=2.7, 6.5, 6.5, 9-H), 5.46 (dd, J=7.0, 15.4, 14-H), 5.57 (dd, J=7.6, 15.4, 15-H)
9	0.90 (t, J=6.6, 20-H_3), 1.01 (d, J=6.8, 16-CH_3), 1.30 (br s, 17-H_2, 18-H_2, 19-H_2), *ca.* 1.75 (overlap, 12-H), 1.76 (q, J=7.2, 3-H_2), 1.91 (ddd, J=2.4, 5.5, 14.9, 10β-H), 2.15 (m, 16-H), 2.22 (t, J=7.2, 4-H_2), *ca.* 2.30 (overlap, 10α-H), 2.31 (t, J=7.2, 2-H_2), 2.65 (m, 8-H), 2.97 (d, J=17.4, 7α-H), 3.04 (dd, J=8.4, 17.4, 7β-H), 4.04 (t, J=6.8, 13-H), 4.12 (q, J=6.3, 11-H), 4.96 (dt, J=2.7, 6.5, 6.5, 9-H), 5.46 (dd, J=7.0, 15.4, 14-H), 5.58 (dd, J=7.6, 15.4, 15-H)
10	0.91 (t, J=6.5, 20-H_3), 1.25—1.45 (m, 18-H_2, 19-H_2), 1.74 (s, 16-CH_3), *ca.* 1.8 (m, 3-H_2, 10β-H), 2.0—2.2 (m, 12-H, 17-H_2), 2.15 (overlap, 4-H_2), 2.34 (t, J=7.2, 2-H_2), *ca.* 2.55 (m, 8-H, 10α-H), 2.71 (d, J=16.2, 7α-H), 2.84 (dd, J=8.1, 16.2, 7β-H), 3.90 (q, J=6.9, 11-H), 4.90 (dt, J=2, 6, 6, 9-H), 5.42 (dd, J=7.9, 16.0, 13-H), 5.82 (d, J=13.5, 15-H), 6.39 (dd, J=13.5, 16.0, 14-H)
11	0.92 (t, J=6.5, 20-H_3), 1.25—1.45 (m, 18-H_2, 19-H_2), 1.74 (s, 16-CH_3), *ca.* 1.8 (m, 3-H_2, 10β-H), 2.0—2.2 (m, 12-H, 17-H_2), 2.15 (overlap, 4-H_2), 2.34 (t, J=7.2, 2-H_2), *ca.* 2.55 (m, 8-H, 10α-H), 2.69 (d, J=16.2, 7α-H), 2.84 (dd, J=8.1, 16.2, 7β-H), 3.90 (q, J=6.9, 11-H), 4.90 (dt, J=2, 6, 6, 9-H), 5.40 (dd, J=7.8, 16.0, 13-H), 5.82 (d, J=13.5, 15-H), 6.39 (dd, J=13.5, 16.0, 14-H)
12	0.93 (t, J=6.5, 20-H_3), 1.25—1.45 (m, 18-H_2, 19-H_2), 1.75 (s, 16-CH_3), *ca.* 1.8 (m, 3-H_2, 10β-H), 2.07 (q, J=8, 12-H), 2.1—2.3 (overlap, 17-H_2), 2.25 (overlap, 4-H_2), 2.32 (t, J=7.6, 2-H_2), *ca.* 2.55 (m, 8-H, 10α-H), 2.77 (d, J=17.6, 7α-H), 2.99 (dd, J=7.6, 17.6, 7β-H), 3.88 (q, J=7.8, 11-H), 4.92 (dt, J=2, 6, 6, 9-H), 5.41 (dd, J=7.8, 14.5, 13-H), 5.84 (d, J=10.8, 15-H), 6.38 (dd, J=10.8, 14.5, 14-H)
13	0.91 (t, J=6.5, 20-H_3), 1.25—1.45 (m, 18-H_2, 19-H_2), 1.75 (s, 16-CH_3), *ca.* 1.8 (m, 3-H_2, 10β-H), *ca.* 2.1 (m, 12-H, 17-H_2), 2.24 (t, J=7.8, 4-H_2), 2.32 (t, J=7.6, 2-H_2), *ca.* 2.55 (m, 8-H, 10α-H), 2.77 (d, J=17.8, 7α-H), 2.99 (dd, J=7.2, 17.6, 7β-H), 3.88 (q, J=7.8, 11-H), 4.92 (dt, J=2, 6, 6, 9-H), 5.42 (dd, J=7.8, 14.9, 13-H), 5.83 (d, J=10.8, 15-H), 6.39 (dd, J=10.8, 14.9, 14-H)

mobile phase described in Experimental. In this change, each of the peaks **2**—**5** can be partially resolved to two peaks, indicating that each of **2**—**5** is a mixture of 16*R*- and 16*S*-isomers. Peak **7** was also considered to be such an overlapping peak. Although the products **8** and **9** were separated on HPLC, there was no significant difference between ^{1}H-NMR spectra of the two. These products are also considered to be isomers at the 16-position. Concerning the relationship between the 13-hydroxyl compounds **7** and **8** (or **9**), since the 15α- and 15β-hydroxyl isomers could be resolved chromatographically, as described above, it was considered that the separation between 13-hydroxyl compounds was also similarly effected on HPLC, *i.e.* the separation between 13α- and 13β-forms.

All the degradation products **10**—**13** are isomers of a dehydrate of NP from mass spectrum (MS) data. Comparing the ^{1}H-NMR spectra of the product **10** with that of NP, the following downfield shifts were observed: for 16-methyl group from 0.9 ppm to 1.74, for H-15 from 3.9 ppm to 5.82, and for H-14 from 5.58 ppm to 6.39. These shifts indicated the dehydration between the 15- and 16-positions of NP and the formation of conjugated double bond at the same positions. The coupling constant between H-13 and H-14 is 16 Hz and that between H-14 and H-15, 13.5 Hz, indicating *trans*-form. ^{1}H-NMR spectra of **11**—**13** are similar to that of **10**, and the structures of the ω-side chain of **11**—**13** were also determined to be similar to that of **10**. The geometric configurations of the products **10**—**13** at the 5-position were determined to be *Z*, *Z*, *E* and *E*, respectively, by analysis of the chemical shifts of H-4, H-7α and H-7β. Now, both compounds **10** and **11** are *5Z* form. These products are therefore considered to be geometric

TABLE II. Nileprost and Its Degradation Products



a) Configuration isomers at the 13-position: **7** and **8** (or **9**). Configuration isomers at the 16-position: **8** and **9**. b) Geometric isomers at the 16-position.

isomers of each other at the 16-position. The relationship between the products **12** and **13** is similar to that between **10** and **11**.

Elucidated structures of the degradation products are listed in Table II.

Degradation Mechanism of Nileprost On the basis of the structure determination of the degradation products it was learned that there are two main degradation pathways, (a) a geometric rearrangement of the vinyl ether moiety and (b) one pathway *via* the formation of carbonium ion in the ω-side chain under acidic condition (Chart 1) and one under alkaline condition (Chart 2), similar to that (a) for acidic degradation.

To confirm the mechanism, the deuterium exchange reaction of NP was examined as follows: NP was treated with a mixture of 0.1 N deuterated hydrochloric acid and deuterated acetone (1 : 1) for 2 days or with 0.1 N deuterated sodium hydroxide for 6 d at 37 °C, and then extracted according to the method in the Experimental section. The N-FABMS of the extract from the acidic solution gave very intense peaks at m/z 390 and 372 corresponding to the $[M-H]^-$ ions of NP and the dehydrate of NP, respectively, indicating no proton exchange reaction. On the other hand, the spectrum of the extract from alkaline solution gave a very intense peak at m/z 392, which is 2 mass units higher than the $[M-H]^-$ ion of NP. The ^{1}H-NMR of the extract was similar to that of NP but no signal at 2.6—3.1 ppm was observed, indicating the exchange of protons of H-7α and H-7β under alkaline condition. These results revealed that the mechanism of the geometric rearrangement of the vinyl ether moiety in acid medium is different from that in alkaline medium in spite of the appearance of the same product **4** corresponding to d in the charts. In acid medium, the protonation to nitrogen of cyano group induces the mesomeric structure b whose bond between C-5 and C-6 is a single bond. Then, the rotation from b to c occurs and the geometric isomer d appears (Chart 1a). In alkaline medium, deprotonation from the 7-position by an attack of hydroxyl anion induces the mesomeric structure l. The isomer d is also given *via* the rotation of ths single bond between C-5 and C-6 (Chart 2). These reactions are reversible. Proton exchange is, therefore, possible in alkaline

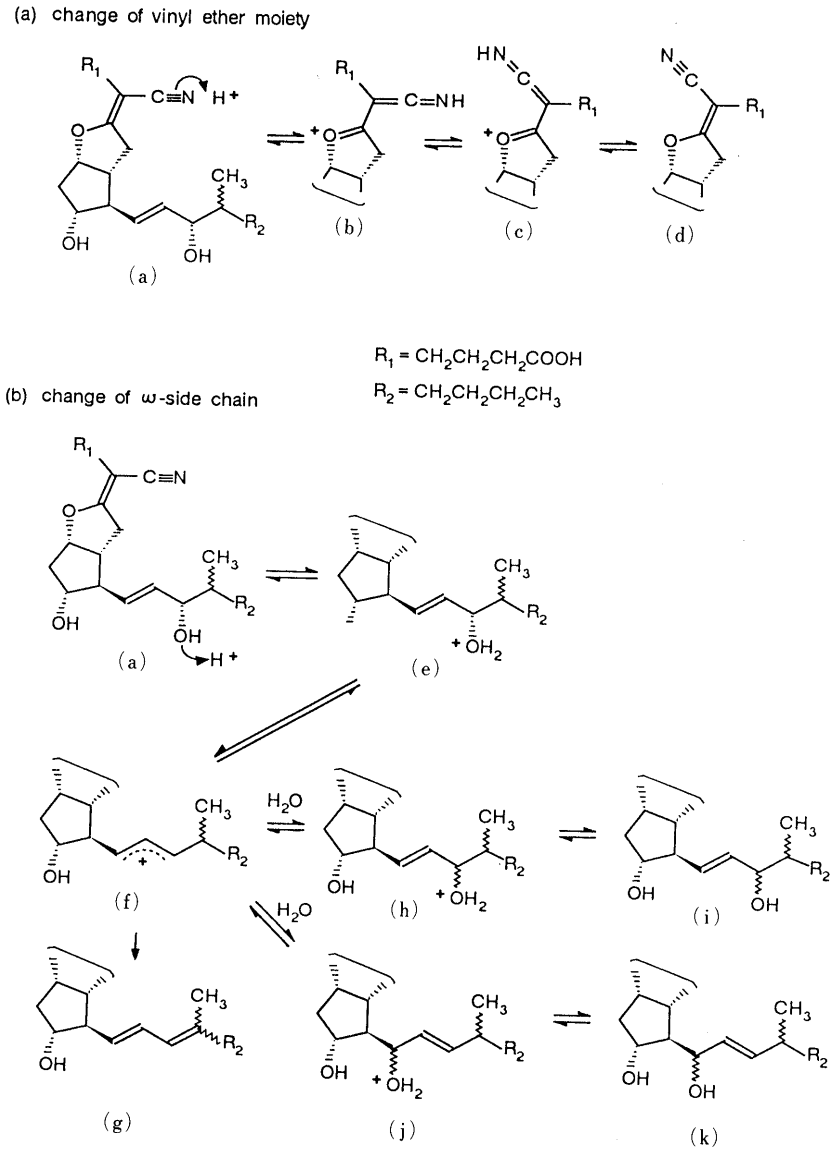


Chart 1. Acidic Degradation

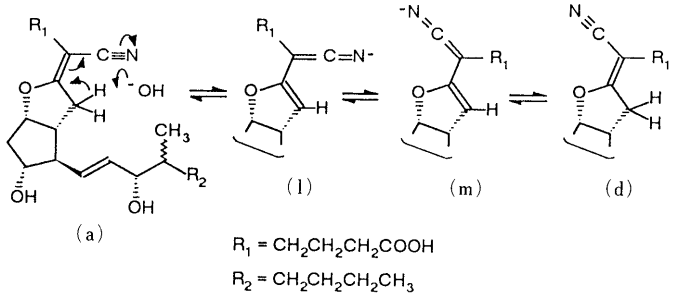


Chart 2. Alkaline Degradation

medium. Furthermore, the changes of the ω-side chain occur in acid medium (Chart 1b), *i.e.* the dehydration, the transfers of the double bond and of the hydroxyl group and the change of the configuration of hydroxyl group. These changes can be explained by the formation of carbonium ion f which is produced by the elimination of water from the species e protonated to oxygen of 15-hydroxyl group. The dehydrogenation from the 16-position gives the conjugated dienes g corresponding to the products **10**—**13**.

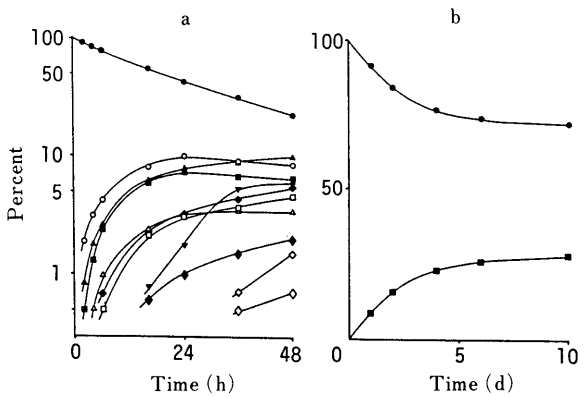


Fig. 2. Degradation Profiles of Nileprost (a) in 0.1 M HCl–KCl Buffer Solution (pH 1.2) and (b) in 0.1 N NaOH at 37 °C

NP, ●; **1**, ◇; **2**, ◆; **3**, □; **4**, ■; **5**, ○; **6**, ▼; **7**, ▲; **8** and **9**, △; **10**, ◇; **13**, ◆.

The addition of water at the 15-position of f, followed by the deprotonation from it gives the species i corresponding to the original NP and its isomers **3**—**5**, and the addition of water at the 13-position similarly gives the species k

corresponding to the 13-hydroxyl isomers **6**—**9**. The products **1** and **2** are also considered to be produced *via* the carbonium ion f, although the pathway is not shown in Chart 1b.

Degradation Profiles of Nileprost The degradation profiles for NP in acidic and alkaline solutions are shown in Fig. 2, where the products **11** and **12** were excepted from the profile Fig. 2a because they overlapped on HPLC. The acidic degradation of NP was very slow in comparison with that of PGI_2, whose lifetime is only a few minutes at low pH values. The alkaline degradation of NP was also very slow and NP reached equilibrium with the product **4** in the ratio about 2:1.

Conclusion

Degradation of nileprost, 5-cyano-16-methyl-prostacyclin, in acid and alkaline media was investigated. Although prostacyclin is very unstable in solution and easily decomposed to 6-keto-$PGF_{1\alpha}$ through hydrolysis of its vinyl ether moiety, nileprost is extremely stable in both media and gives little of such hydrate but many isomers and dehydrates. These results indicate that the stability of nileprost is due to the introduction of the electron-withdrawing cyano group at the 5-position in its structure. Major degradation is geometric rearrangement of the vinyl ether moiety in both media and, in acid medium, rearrangement of a hydroxyl group and dehydration in the ω-side chain. The combination of the changes at the two different moieties gave many acidic degradation products. On the basis of the structure determination of the degradation products of nileprost, the mechanism was clarified. Our results are the first to show that the vinyl ether moiety of prostacyclin derivative undergoes geometric isomerization, and that the ω-side chain, which is common to many prostaglandins, also undergoes isomerization and dehydration. There is a possibility of similar degradation for other prostaglandins and/or related compounds.

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