Products from Nileprost, 5-Cyano-16-methylprostacyclin, and Its β -Cyclodextrin Complex under Heating

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Products from nileprost (NP) and its β -cyclodextrin (CD) complex under heating was investigated. Stability of NP under thermal conditions was improved by complexation. The product from the complex is an ester between the carboxyl group of NP and the hydroxyl group of CD, and the NP moiety is fixed at the interior of the cavity of the CD moiety. Main products from NP are also esters between two molecules.

Keywords nileprost; prostacyclin analogue; inclusion complex; β -cyclodextrin; heating

It is well known that prostacyclin (PGI_2) has remarkable biological activity, but its vinyl ether moiety is easily hydrolyzed in aqueous solution.¹⁾ This chemical instability has limited the utilization of PGI_2 for clinical treatment. Nileprost (NP, Fig. 1) with an electron withdrawing cyano group at the 5-position is one of the analogues synthesized in order to improve the stability of PGI_2 .²⁾ In the previous paper, we noted that the introduction of the cyano group is very effective for the stabilization of the vinyl ether moiety and that the degradation mode of NP is different from that of PGI_2 .³⁾ Furthermore, we investigated the stability of NP under thermal conditions and found that NP gradually changes to different compounds, even under normal storage conditions (room temperature). We also investigated the stability of a complex between NP and β -cyclodextrin (CD). Recently, many studies of complexes of various drugs with CDs have been done for improvement of the stability and solubility and for many other aims.⁴⁾

This paper describes the structure determination of products from NP and its inclusion complex with CD under heating.

Experimental

Instruments Nuclear magnetic resonance (NMR) spectra were measured with a JEOL JNM-GSX 270 spectrometer. Tetramethylsilane (TMS) was used as an internal standard. The chemical shifts are given in ppm from TMS. ^1H - ^1H correlation spectroscopy (COSY) and ^{13}C - ^1H -COSY were used for the assignment. Fast atom bombardment mass spectra (FABMS) were measured with a JEOL JMS-D300 mass spectrometer equipped with a FAB gun (Xe, 6 kV) at an accelerating voltage of 1.5 kV. *m*-Nitrobenzyl alcohol (NBA) and glycerol were used as matrices, and a mixture of NBA, polyethylene glycol (PEG) 1000, PEG 400 and 5% NaCl (16:4:2:1) as both the matrix and the standard for positive high-resolution measurements. Infrared (IR) absorption spectra were measured by the KBr disc method with a JASCO FT/IR-3 spectrophotometer. High performance liquid chromatography (HPLC) was performed with a Hitachi 655A-12 equipped with a Hitachi 655A-21 ultraviolet (UV) absorption detector.

Materials and Reagents NP and its CD complex⁵⁾ were supplied by

Fig. 1

Schering AG (Berlin, Germany). A 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 . Deionized water was used in all experiments. Other reagents used were of an analytical reagent grade.

Isolation of Products Product 1 was isolated by preparative HPLC. The conditions are as follows: column, $\mu\text{Bondapak-C}_{18}$ (8 i.d. $\times$ 300 mm, Waters Assoc.); oven, 30 $^\circ\text{C}$; mobile phase, water–MeCN (3:1); flow rate, 3.0 ml/min; and detector, UV 240 nm. Products 2–4 were separated by preparative thin-layer chromatography (TLC) with CHCl_3 –MeOH–conc. NH_4OH (12:6:1) as the developing solvent. *R_f*-Values of NP, 2 and a mixture of 3 and 4 are about 0.2, 0.3 and 0.5, respectively. The mixture of 3 and 4 was separated to 3 (upper band) and 4 (lower band) by preparative silica gel TLC with AcOEt–AcOH (20:1) as the developing solvent.

Results and Discussion

HPLC of Products Figure 2 shows liquid chromatograms of NP and its CD complex before and after heating. Three peaks from NP and one peak from the complex appeared after heating. The stability of NP under thermal conditions was fairly improved by the complexation, although NP gradually changed to different compounds even at room temperature. The structures of the numbered peaks in Fig. 2 were determined.

Structure Determination of the Products Negative FAB-MS of product 1 gave the $[\text{M}-\text{H}]^-$ ion at m/z 1506 corresponding to $[\text{NP} + \text{CD} - \text{H}_2\text{O} - \text{H}]^-$. The integral value of the ^1H -nuclear magnetic resonance (^1H -NMR) spectrum of 1 also indicated that 1 consists of NP moiety and CD moiety in 1:1 ratio. IR spectra of 1 gave absorption

Fig. 2. Liquid Chromatograms of NP and Its CD Complex

A, the complex in a refrigerator; B, the complex at 60 $^\circ\text{C}$ for a week; C, NP in a refrigerator; D, NP at 60 $^\circ\text{C}$ for a week. HPLC conditions: column, TSK gel ODS-80TM 4.6 mm $\times$ 150 mm (TOSOH); detector, UV 240 nm; oven, 40 $^\circ\text{C}$; mobile phase, (a) 0.02 M phosphate buffer (pH 3.5)–MeCN (3:2), (b) 0.02 M phosphate buffer (pH 3.5)–MeCN (3:7); flow rate, 1 ml/min.

bands at 2203 and 1732 cm^{-1} , indicating the existence of a cyano group and an ester carbonyl group, respectively, whereas the carboxyl band of NP is at 1711 cm^{-1} . Analytical data of 1 was as follows: C, 49.84; H, 6.86; N, 0.94 (Calcd for $\text{C}_{64}\text{H}_{101}\text{NO}_{39} \cdot 2\text{H}_2\text{O}$: C, 49.77; H, 6.85; N, 0.91). These results indicate the formation of ester-linking between NP and CD. For the structure of the ester, there are two possibilities: (a) NP moiety is at the interior of the cavity of CD or (b) at outside of the cavity. If

the structure is the former, NMR signals of protons at the interior of the cavity should shift from the chemical shifts of corresponding protons of NP alone and CD alone and exhibit no concentration-dependent change. By contrast, if the latter is true, the concentration-dependent change is expected.^{4a)} Figure 3 shows the chemical shifts of ^1H -NMR spectra of product 1 measured in the range of 1.4×10^{-2} — 3.6×10^{-4} mol, and those of a 1:1 mixture of NP and CD measured in a similar concentration range.

Fig. 3. Proton Chemical Shifts of (A) Product 1, (B) a 1:1 Mixture of NP and CD, (C) NP and (D) CD
 ■, broad overlapping peak. ^1H -NMR spectra were measured with D_2O using tetramethylsilane as an external standard.

Fig. 4. ^{13}C -NMR Spectra of Products 2—4 and NP in CD_3OD
 A, product 2; B, product 3; C, product 4; D, NP.

Fig. 5. Structure of the Products

In the spectra of the mixture of NP and CD, while the concentration increased, protons of NP at 7 α -, 7 β -, 8-, 9- and 12-positions shifted to a lower field, and those of the glucose units of CD at 3- and 5-positions shifted to a higher field (Fig. 3B), indicating a concentration-dependent interaction between NP and the cavity of CD. On the other hand, the spectra of **1** showed no concentration-dependence and, even under the lowest concentration of **1**, proton chemical shifts of the NP moiety of **1** at 4-, 8-, 9-, 13- and 14-positions were lower field and that of the CD moiety at 5-position higher field than those of corresponding protons in the highest concentration of the mixture of NP and CD (Fig. 3A), indicating that the interaction between NP and CD moieties of **1** is large and constant. To explain the above results, the NP moiety of **1** must be fixed at the interior of the cavity of CD moiety. The integral value of the signal of **1** at *ca.* 4.2 ppm corresponds to four protons. Two of those were considered to be assigned to the methylene of a glucose unit ester-linked to the NP moiety. Hydrolysis of **1** gave NP. From these results, product **1** was determined to be the structure shown in Fig. 5.

In FABMS of product **2**, the molecular ion region in the negative mode exhibited an $[M-H]^-$ ion at m/z 388 and that in the positive mode an $[M+Na]^+$ ion at m/z 412, which was confirmed as $[C_{22}H_{31}NO_5Na]^+$ ($[NP-H_2+Na]^+$) by m/z 412.2099. IR spectrum of **2** gave absorption bands at 2203, 1711 and *ca.* 1680 cm^{-1} ,⁶⁾ indicating the existence of a keto-carbonyl group. The ^{13}C -nuclear magnetic resonance (^{13}C -NMR) spectrum is shown in Fig. 4A. The signal of C-15 shifted from 77.8 ppm in NP to 206.1 ppm, indicating a conversion from the hydroxyl group of NP at C-15 to a carbonyl group. Thus, product **2** was determined to be a 15-keto compound.

In FABMS studies of product **3**, the molecular ion region in the negative mode exhibited an $[M-H]^-$ ion at m/z 763 and that in the positive mode an $[M+Na]^+$ ion at m/z 787, which was confirmed as $[C_{44}H_{64}N_2O_9Na]^+$ ($[2NP-$

$H_2O+Na]^+$) by m/z 787.4523. Similarly, product **4** gave an $[M-H]^-$ ion and an $[M+Na]^+$ ion at the same mass numbers to those of **3**, respectively, and the formula of the $[M+Na]^+$ ion was also confirmed to be the same as that of **3** by m/z 787.4535. IR spectrum of **3** gave absorption bands at 2203, 1732 and 1711 cm^{-1} , indicating the existence of an ester carbonyl group. Product **4** also gave bands similar to those of **3**. These results indicate the ester formation between the carboxyl group and hydroxyl group (11- or 15-positions) of another molecule. ^{13}C -NMR spectra of **3** and **4** are shown in Figs. 4B and 4C, respectively. The chemical shifts of signals of carbons numbered without a prime (Fig. 5) correspond closely to those of NP excepting a carbonyl group. It is known that esterification of secondary aliphatic alcohol causes a 4 ppm downfield shift for α -carbon, a 3 ppm upfield shift for β -carbon and a 2 ppm downfield shift for γ -carbon.⁷⁾ Similar shifts, which are noted by the arrows in the figures, were observed for **3** and **4**. The spectrum of **3** shows a change around the 11-position and of **4** a change around the 15-position. Thus, **3** and **4** were determined to be dimers esterified at the 11- and 15-positions, respectively.

Determined structures of the products are shown in Fig. 5. The product from the complex is an ester between NP and CD and has a structure where the NP moiety is fixed at the interior of the cavity of CD. This result is the first to show that such a compound is produced from the CD complex. The main products from NP are esters between the carboxyl group of NP and the hydroxyl group of another molecule.

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References and Notes

- 1) Y. Chiang, M. J. Cho, B. A. Euser and A. J. Kresge, *J. Am. Chem. Soc.*, **108**, 4192 (1986).

- 2) W. Skuballa, B. Radüchel and H. Vorbrüggen, *Tetrahedron Lett.*, **29**, 4285 (1988).
- 3) M. Yoneda and Y. Ohkawa, *Chem. Pharm. Bull.*, **38**, 2507 (1990).
- 4) For example: a) K. Uekama, *Yakugaku Zasshi*, **101**, 857 (1981); b) A. Yoshida, M. Yamamoto, T. Irie, F. Hirayama and K. Uekama, *Chem. Pharm. Bull.*, **37**, 1059 (1989); c) K. Nakanishi, M. Masada, T. Nadai and K. Miyajima, *ibid.*, **37**, 211 (1989); d) R. D. Voyksner, F. P. Williams, C. S. Smith, D. L. Koble and H. H. Seltzman, *Biomed. Environ. Mass Spectrom.*, **18**, 1071 (1989).
- 5) The ratio of NP and CD is 2:5 and the ratio was confirmed by titration with 0.1 N NaOH.
- 6) The wave numbers of carbonyl groups of products 2—4 are slightly inaccurate because of broad overlapping bands.
- 7) F. W. Wehrli and T. Wirthlin, "Interpretation of Carbon-13 NMR Spectra," Heydon and Son Ltd., London, 1978.