Registry No.—(±)-**2**, 61664-47-9; (+)-**2**, 57525-74-3; (±)-**3**, 61664-45-7; (±)-**4**, 61664-46-8; (−)-**4**, 61664-48-0; (+)-**4**, 57525-75-4; (±)-**6**, 61664-49-1; (+)-**6**, 61664-50-4; (−)-**6**, 61664-51-5; (±)-**7**, 61604-83-9; (±)-**8**, 61664-52-6; (+)-**8**, 61664-53-7; (+)-**9**, 61604-84-0; (−)-**9**, 61664-54-8; 3-phenylcyclohexene, 15232-96-9; *m*-chloroperbenzoic acid, 937-14-4; 2-phenyladipinaldehyde, 61604-85-1; (−)-(*R*)-2-phenyladipic acid, 61604-86-2; (−)-(*S*)-3-phenylcyclohexene, 61604-88-4; (+)-(*R*)-3-phenylcyclohexene, 17540-19-1; 8-methoxy-8-methyl-2-phenyl-7,9-dioxa[4.3.0]nonane, 61604-87-3.

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Stereochemistry of Nucleophilic Addition Reactions. 2.[1] Kinetically Controlled Reaction of Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside with Hydrogen Cyanide. Important Role of Electrostatic Interaction

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The reaction of title compound **1** with hydrogen cyanide gave the 2-cyano-3-nitroglucopyranoside **5** and the 2-cyanohex-2-enopyranoside **6** in good yield. The data suggest that cyanide ion adds irreversibly from the equatorial side of **1** and that the intermediate of **6** is the 2-cyano-3-nitroallopyranoside **7**. The additions of hydrazoic acid and *p*-toluenesulfinic acid to **1** also gave the adducts with the gluco configuration. The stereochemistry of nucleophilic addition reactions to **1** and its α anomer **2** was discussed in terms of electrostatic interaction, stereoelectronic control, and steric hindrance.

Under the conditions of kinetic control axial attack of a nucleophile generally predominates in the nucleophilic addition reactions of conformationally rigid cyclohexene derivatives.[2] This is attributed to stereoelectronic control;[3] almost continuous overlap between the developing σ bond and the conjugated system in the formation of the transition state leads to a product with the newly attached substituent in an axial orientation on a chair form rather than the alternative quasi-axial on a boat conformation. Exceptions to this are reported by Abramovitch and co-workers in the reactions of diethyl malonate to 4-*tert*-butyl-1-cyanocyclohexene[4] and 4-*tert*-butylcyclohexene-1-carboxylate,[5] in which preferred equatorial attack of the bulky diethyl malonate was attributed to large diaxial nonbonded interactions in the transition state for axial addition. On the other hand, regardless of the bulkiness various kinds of nucleophiles added from the equatorial side of methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (**1**);[6-8] these results do not appear to be explained by steric hindrance only. From the facts that some reactions of methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-α-D-*erythro*-hex-2-enopyranoside (**2**) (the α anomer of **1**) gave the thermodynamically less stable α-D-mannopyranoside[7,9] we cannot immediately conclude that the corresponding reactions of **1**, which gave the more stable β-D-glucopyranoside, are also controlled kinetically, because the β-D-mannopyranoside should be much less stable than the α-D-mannopyranoside due to Δ^2 effect[10] and easily epimerize to the stable β-D-glucopyranoside. In fact, predominance of axial attack was found in the reactions of **1** with *o*-aminobenzoic acid[11] and hydrogen cyanide,[12] where the less stable β-mannopyranoside were isolated in 56 and 15% yield, respectively. On the contrary, we have shown that the reaction of phenyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside with hydrazoic acid in THF–deuterium oxide exclusively gave the adduct with the gluco configuration, of which about 50% were deuterated at C-3, indicating that it is controlled kinetically, at least partially.[8]

In addition, methyl 4,6-*O*-benzylidene-2-deoxy-2-nitro-β-D-glucopyranoside (**3**) and the corresponding 3-nitro sugar were obtained in 14 and 70% yield, respectively, in the reaction of **1** with nitrous acid,[13] where a dinitro intermediate should be involved, at least, in the formation of the 2-nitro sugar **3**. If nitrite ion added to the C-2 position from the equatorial side, the dinitro intermediate has the allo and/or gluco structure. The former should give predominantly 2-nitrohex-2-enopyranoside since the nitro group at C-3 is in a trans-diaxial relationship with H-2; however, the latter seems to lack such a high degree of selectivity. The yield of the 2-nitro sugar **3**, therefore, should be affected by stereochemistry

of protonation to intermediary nitronate ion. But the ambident character of nitrite ion should make the reaction complicated.

These facts prompted us to reinvestigate the reaction of **1** with hydrogen cyanide,[14] which had been also investigated by Paulsen and co-worker,[12] in order to obtain some stereochemical information about protonation of a nitronate intermediate and to elucidate whether the reaction of **1** with hydrogen cyanide proceeds under conditions of kinetic control or not.

Results and Discussion

When the nitro olefin **1** was treated with potassium cyanide in the presence of acetic acid in acetonitrile–water, the 2-*O*-acetyl glucopyranoside **4** was obtained in 94% yield. The similar treatment of **1** in the presence of *p*-toluenesulfonic acid instead of acetic acid afforded methyl 4,6-*O*-benzylidene-2-cyano-2,3-dideoxy-3-nitro-β-D-glucopyranoside (**5**) in 54% yield along with the order of benzaldehyde. Treatment of **1** with hydrogen cyanide in the presence of a catalytic amount of potassium cyanide in acetonitrile at 40–50 °C for 3 h gave a mixture of **5** and methyl 4,6-*O*-benzylidene-2-cyano-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranoside (**6**), in a ratio of 4:1 by NMR spectroscopy, in 79% yield (Table I, expt 4). These compounds were in good agreement with an authentic sample obtained previously and with the spectral data reported by Paulsen et al.[12] Although the long-range couplings of **6** were assigned to be $J_{1,3}$ = 2.0 and $J_{1,4}$ = 1.0 Hz,[12] they ought to be corrected to 1.0 and 2.5 Hz, respectively, since secondary irradiation at δ 6.83 (quartet, H-3) led the signal at δ 5.33 (H-1) to be a doublet with J = 2.5 Hz; this was confirmed by the spectrum of the 3-deuterated derivative of **6**.

The reaction of **1** with hydrogen cyanide in the presence of a catalytic amount of potassium cyanide in acetonitrile was carried out at 0 °C for 3 h yielding **5** only (expt 3). Without potassium cyanide, the reaction did not proceed even after 48 h (expt 1). But when acetonitrile was replaced by a more basic solvent such as Me_2SO, it finished within 3 h and exclusively gave **5**, indicating that the rate-determining step is the nucleophilic attack of cyanide ion on the nitro olefin moiety (expt 7 and 8).

To obtain further information, we carried out this reaction using deuterium oxide. If the reaction involves the following path, deuteration at C-3 should go to completion: (1) the reverse Michael-type reaction occurs with ease, (2) the reaction proceeds through 1,4 addition to afford the *aci*-nitro form, which isomerizes to the corresponding nitro form by an intermolecular proton exchange, and/or (3) the rate of deuterium exchange between deuterium oxide and hydrogen cyanide is much more rapid than that of addition.

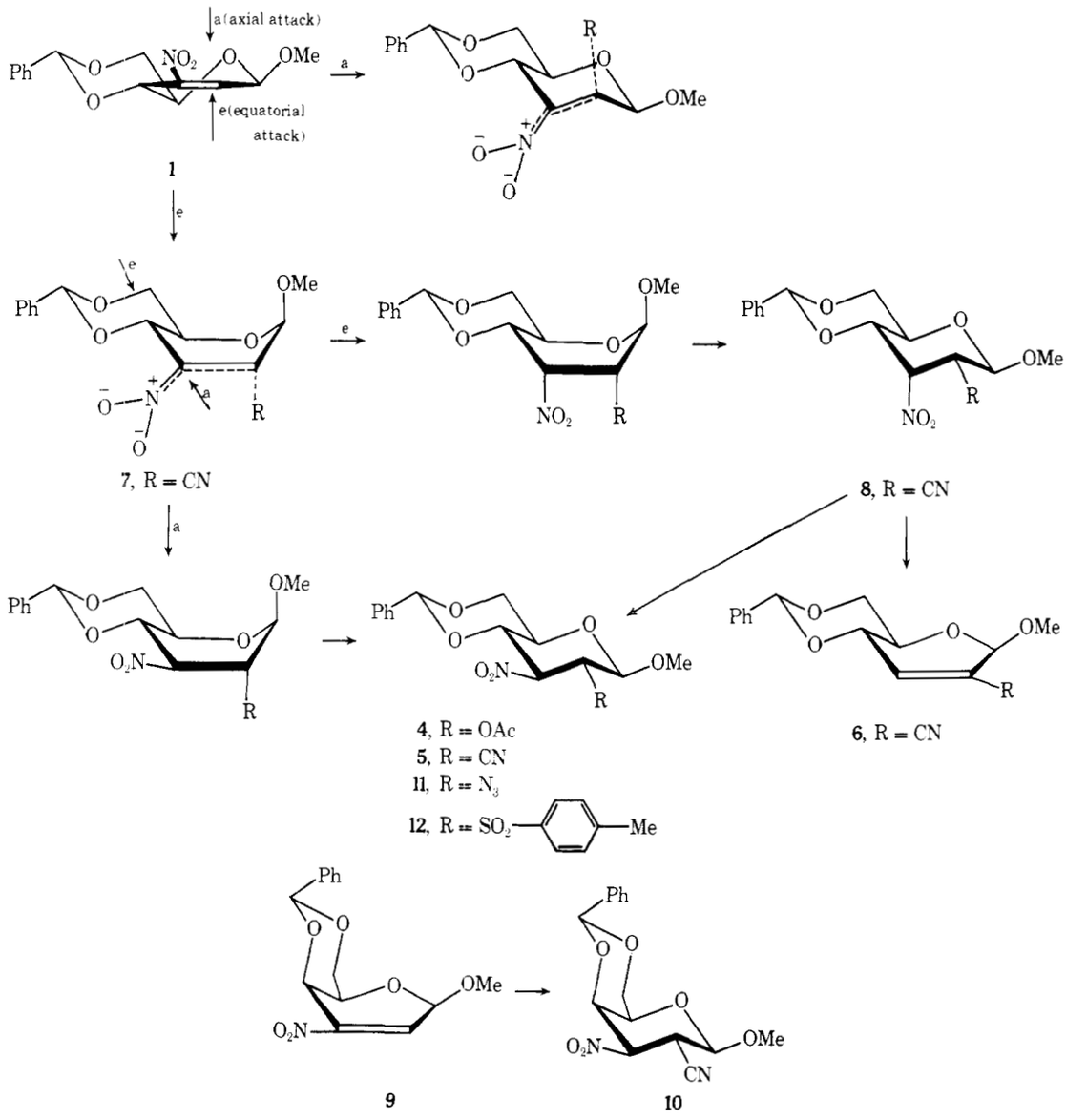

Table I. Reactions of 1 or 5 with Hydrogen Cyanide

Expt	Starting material (mmol)[a]	HCN in CH_3CN, mmol	KCN, mg	Solvent, mL	Reaction temp, °C	Reaction time, h	Ratios of the products[b] 5:6
1	1 (0.1)	0.6	0	CH_3CN (0.3)	RT[c]	48	Recovered of 1
2	1 (1)	6	2.1	CH_3CN (8)–H_2O (0.5)	RT	3	q—
3	1 (1)	6	2.1	CH_3CN (8)	0	3	q—
4	1 (1)	6	2.1	CH_3CN (8)	40–50	3	4:1
5	5 (1)	6	2.1	CH_3CN (8)	40–50	3	q—[d]
6	5 (0.5)	0	39	CH_3CN (4)–H_2O (0.3)	50	1	Degradation[e]
7	1 (0.1)	0.6	0	Me_2SO-d_6 (0.2)	RT	3	q—
8	1 (0.1)	0.6	0	DMF (0.2)	RT	3	q—
9	1 (0.1)	0.6	0.2	Me_2SO-d_6 (0.2)–H_2O (0.05)	RT	1	q—

[a] In the case of 0.1-mmol scale, the reactions were carried out in a NMR sample tube and directly measured by NMR spectroscopy. [b] Ratios of the products were determined by NMR spectroscopy: q, quantitative or almost so; —, not detected. [c] RT means room temperature. [d] NMR spectrum of the crude product showed the absence of **6**, but that of the residue obtained after removal of **5** (252 mg) revealed the presence of small amounts of **6** (≤2.5%). [e] The reaction mixture turned brown and its NMR spectrum was very complicated.

$$HCN + D_2O \rightleftarrows DCN + HOD$$

Treatment of **1** with hydrogen cyanide in THF–deuterium oxide in the presence of a catalytic amount of potassium cyanide at room temperature for 2 h exclusively gave **5**, of which only 30% was deuterated as seen from NMR spectroscopy. Under the same conditions compound **5** did not undergo deuteration. The ratio of the deuterated derivative of **5** was up to 75% when **1** was added 2 h later in the above reaction. These results indicate that the incorporation of deuterium is not mainly attributed to 1 and 2, but to 3 and that the possibility of formation of the manno and altro isomer as an unstable intermediate may be neglected.[15]

The present data, namely, that under the kinetically controlled conditions cyanide ion added at C-2 from the equatorial side, are in good agreement with those obtained by the reactions of **1** with *S*-ylides[6b] and hydrogen peroxide.[7a] The latter two reactions seem to be kinetically controlled because the reverse Michael-type reactions of products once formed are impossible and the nucleophilic attack is considered to be the rate-determining step.

When a nucleophile approaches from the equatorial side, the original half-chair conformation changed to a boatlike transition state in which the incoming nucleophile approached from the stereoelectronically acceptable axial direction as shown. The alternative chairlike transition seems to be disfavorable for not only stereoelectronic control but also $A^{(1,3)}$ strain between nitronate oxygen and the equatorial cyano group. Furthermore, the required boatlike transition can be easily achieved because of the favorable anomeric effect[10] such an arrangement affords.

If the hindrance due to the axial cyano group is a decisive factor, the allo isomer **8**, via the change of its conformation,[16] is formed. The allo isomer **8** with the axial nitro group should be unstable and subject to epimerization to the gluco isomer **5** and/or elimination of nitrous acid to give the cyano olefin **6**. If the hindrance from the axial methoxy group is predominant or even similar to that from the cyano group, the gluco isomer should be predominantly formed because the nitro group approaching axial orientation encounters severe steric hindrance from the cyano group.[17] In this reactions the gluco isomer seems to be the preferred product because the steric hindrance due to these two groups appears to be compatible, but formation of the allo isomer is not excluded. In fact the allo isomer under the conditions employed by us appears to be an intermediate of the cyano olefin **6** from the following evidence: (1) As already described, the axial attack of cyanide ion appears to be unlikely. In fact no evidence for formation of the manno isomer, of which conversion to the cyano olefin **6** had proved to be much slower than that of the gluco isomer,[12] was obtained by NMR spectroscopy under the conditions employed by us.[18] (2) The gluco isomer **5** was almost quantitatively recovered (the yield of **6** ≤ 2.5%) under the conditions used for the preparation of **5** and **6** (expt 5). (3) Treatment of the gluco isomer **5** with triethylamine in acetone–deuterium oxide quantitatively afforded the 3-deuterated derivative of **6** as seen from NMR spectroscopy. This showed that before the elimination of nitrous acid abstraction of H-3 occurred to give nitronate ion (from which the formation of the allo isomer is possible).[19] (4) The allo isomer **8** has a suitable configuration for E2 reaction of nitrous acid.[20]

Furthermore, if we take into consideration the epimerization of the allo isomer to the gluco isomer,[21] the equatorial attack of proton on **7** may become considerable. The stereochemistry of protonation of the nitronato ion **7**, therefore, would not be determined from the present data.

The similar reaction of methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*threo*-hex-2-enopyranoside (**9**) with hydrogen cyanide gave the galactopyranoside **10** in good yield. Similarly, addition of hydrazoic acid and *p*-toluenesulfinic acid on the nitro olefin **1** afforded the glucopyranoside **11** and **12** in good yield, respectively.

Hitherto the stereochemistry of nucleophilic addition reactions to C-2 of pyranosides was explained in terms of steric hindrance[6a,22] and stereoelectronic control.[22] The preference of axial attack in the α anomer **2** appears to be explained by these factors, but they afford no reasonable explanation about the facts that *o*-aminobenzoic acid[11] predominantly added from the axial side of **1** to give the β-mannopyranoside, whereas hydrogen cyanide or hydrazoic acid added from the equatorial side to give the β-glucopyranoside. Furthermore, in the reactions of nitro olefins with hydrazoic acid[9a] and *S*-ylides[6b,23] the β anomer **1** showed a higher degree of stereoselectivity than the α anomer **2**, which is contrary to results predicted by stereoelectronic control and steric hindrance. Regardless of an anomeric configuration, axial attack is preferred owing to stereoelectronic control. Furthermore, equatorial attack in the α-glycoside should be retarded by steric hindrance to a much larger extent than axial attack in the β-glycoside since the glycosidic methoxy group occupies pseudoaxial and pseudoequatorial positions in α- and β-glycoside, respectively.[24] In order to explain these stereochemical results, we wish to propose the important role of electrostatic interaction between a nucleophile and both the C_1–O_1 and C_1–O_5 bonds especially in the case of β-glycosides. If a nucleophile is an anion or has nonbonded electron pairs, electrostatic repulsion becomes the decisive factor in determining

the approach of the nucleophile and would completely retard axial attack, giving the β-gluco isomer; this is the case for most reactions of **1** with nucleophiles such as activated hydrazoic acid and *S*-ylides. But if a nucleophile is electronically neutral, it becomes less important than combined effects of stereoelectronic control and steric hindrance; this may be the case for the reaction of *o*-aminobenzoic acid[11] because under appropriate conditions *o*-aminobenzoic acid should be neutralized intramolecularly and affected strongly by stereoelectronic control giving the β-mannopyranoside and almost neutral conditions suppressed the reverse reaction.[9a] Furthermore, if a nucleophile is acidic, an attractive force such as a hydrogen bond should be operative; the reaction of the α anomer **2** with hydrazoic acid in chloroform, where the α-glucopyranoside was exceptionally formed as a major product, may be the case.

Experimental Section

Melting points were determined in capillaries and are uncorrected. IR spectra were recorded for potassium bromide disks and NMR spectra were determined either at 100 MHz with a JNM-4H-100 (JEOL) or 60 MHz with a Varian T-60 spectrometer in chloroform-*d* or Me_2SO-d_6 using tetramethylsilane as internal standard.

Treatment of Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (1) with Potassium Cyanide in the Presence of the Following Acids. A. Acetic Acid. To a solution of nitro olefin **1**[25] (293 mg, 1 mmol) in acetonitrile (12 mL) and acetic acid (3 mL) was added a solution of potassium cyanide (78 mg, ca. 1.2 mmol, purity 95%) in water (2 mL). The mixture was stirred for 17 h at room temperature and then evaporated in vacuo. The solid residue was washed with water and crystallized from ethanol–acetone to afford methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucopyranoside (**4**, 94%). A mixture melting point with an authentic sample[25] was undepressed, and the IR and NMR spectra were identical with those of the sample.

B. *p*-Toluenesulfonic Acid. To a solution of nitro olefin **1** (147 mg, 0.5 mmol) and *p*-toluenesulfonic acid (95%, ca. 0.5 mmol) in acetonitrile (12 mL)–water (1 mL) was added potassium cyanide (33 mg, 0.5 mmol). The mixture was stirred for 23 h at room temperature and then evaporated in vacuo. The remaining material was washed with water and crystallized from ethanol to give **5** (87 mg, 54%), which was identified by IR and NMR data of an authentic sample.[12,14]

Reaction of 1 with Hydrogen Cyanide. To a solution of **1** (293 mg, 1 mmol) in acetonitrile (5 mL) in the presence of a catalytic amount of potassium cyanide (2.1 mg) was added an acetonitrile solution of hydrogen cyanide (ca. 6 mmol) at 40 °C. The mixture was stirred for 3 h at 40–50 °C and evaporated in vacuo to afford a solid residue (290 mg), which comprised **5** and **6** in a ratio of 4:1 by NMR spectroscopy. Recrystallization from ethanol gave two kinds of crystals; the first crop was **5** (205 mg, 64%), the second crop was **6** (41 mg, 15%), which was identical with an authentic sample[12,14] by IR and NMR spectra. When the same reaction was carried out at 0 °C, compound **5** was isolated in 87% (278.4 mg) yield and the cyano olefin **6** was not detected by NMR spectroscopy.

Reaction of 1 with Hydrogen Cyanide in the Presence of Deuterium Oxide. To a solution of **1** (147 mg, 0.5 mmol) in absolute THF (16 mL)–deuterium oxide (1 mL) in the presence of potassium cyanide (1.1 mg) was added an acetonitrile solution of hydrogen cyanide (0.25 mL, ca. 3 mmol). The mixture was stirred for 2 h at room temperature and then evaporated in vacuo to afford a solid residue (158 mg), whose NMR spectrum showed that the product was **5**, of which 30% was deuterated at the C-3 position.

Conversion of 5 into 6. To a solution of **5** (32 mg) in acetone-d_6 (0.15 mL)–deuterium oxide (0.1 mL) in a NMR sample tube was added triethylamine (40 mg). The mixture was allowed to stand for 30 min at room temperature and then measured by NMR spectroscopy. It showed that the starting material **5** had disappeared and that methyl 4,6-*O*-benzylidene-2-cyano-3-deuterio-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranoside was quantitatively formed.

Methyl 4,6-*O*-Benzylidene-2-cyano-2,3-dideoxy-3-nitro-β-D-galactopyranoside (10). To a solution of the threopyranoside **9**[26] (147 mg, 0.5 mmol) in acetonitrile (2 mL) in the presence of a catalytic amount of potassium cyanide (1.1 mg) was added an acetonitrile solution of hydrogen cyanide (ca. 3 mmol) at 0 °C. The mixture was allowed to stand for 3 h at 0 °C, and then evaporated in vacuo to give a NMR spectroscopically pure residue (155 mg). Recrystallization from ethanol gave 130 mg (81%) of **10**: mp 194 °C dec; $[\alpha]^{20}_D$ +25.5° (*c* 0.5, Me_2SO); IR 2240 (CN) and 1555 cm^{-1} (NO_2); NMR data (Me_2SO-d_6) δ 4.83 (1 H, d, $J_{1,2}$ = 8.8 Hz, H-1), 3.52 (1 H, q, $J_{2,3}$ = 11.3 Hz, H-2), 5.57 (1 H, q, $J_{3,4}$ = 3.8 Hz, H-3), 3.58 (3 H, s, OMe), and 5.71 (1 H, s, PhCH).

Anal. Calcd for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.04; N, 8.75. Found: C, 56.33; H, 5.02; N, 8.95.

Methyl 2-Azido-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-glucopyranoside (11). To a solution of **1** (147 mg, 0.5 mmol) in THF (16 mL) and water (1 mL) was added a chloroform solution of hydrazoic acid (2 mL, ca. 1.6 N). The mixture was stirred for 7 h at room temperature and then evaporated in vacuo to give a crystalline residue. Recrystallization from ethanol afforded **11** in almost quantitative yield, which was found to be identical with an authentic sample.[27]

Methyl 4,6-*O*-Benzylidene-2,3-dideoxy-3-nitro-2-(*p*-toluenesulfinyl)-β-D-glucopyranoside (12). Sodium *p*-toluenesulfinate (356 mg, 2 mmol) was dissolved in ethanol and deionized by passing through a column of cation-exchange resin (Mitsubishi Diaion SK1, H^+ form, Japan, 1 g) with ethanol as eluent, and the eluate was directly added to a solution of **1** (293 mg, 1 mmol) in acetonitrile (6 mL) with stirring. The mixture, after having been gently stirred for 3 h at room temperature, was evaporated in vacuo to afford a residue, which crystallized from ethanol. Recrystallization from ethanol–acetone gave 382 mg (85%) of **12**: mp 158.0–158.5 °C; $[\alpha]^{20}_D$ −113° (*c* 1, $CHCl_3$); IR 1550 (NO_2), 1325 and 1170 cm^{-1} (SO_2); NMR data δ 4.34 (1 H, d, $J_{1,2}$ = 8.1 Hz, H-1), 4.15 (1 H, q, $J_{2,3}$ = 11.3 Hz, H-2), 4.84 (1 H, q, $J_{3,4}$ = 9.4 Hz, H-3), 3.95 (1 H, q, $J_{4,5}$ = 10 Hz, H-4), 3.50 (1 H, sextet, $J_{5,6a}$ = 10, $J_{5,6e}$ = 4.4 Hz, H-5), 3.74 (1 H, t, $J_{6e,6a}$ = 10 Hz, H-6a), 4.36 (1 H, q, H-6e), 3.42 (3 H, s, OMe), 2.43 (3 H, s, Me), and 5.48 (1 H, s, PhCH).

Anal. Calcd for $C_{21}H_{23}NO_8S$: C, 56.12; H, 5.12; N, 3.12; S, 7.13. Found: C, 56.03; H, 5.27; N, 3.07; S, 6.95.

Registry No.—**1**, 61664-96-8; **5**, 61664-97-9; **9**, 61664-98-0; **10**, 61664-99-1; **12**, 61617-97-8; HCN, 74-90-8.

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(14) We had already obtained the nitro cyanide **5** and cyano olefin **6** by treatment of nitro olefin **1** with potassium cyanide in the presence of cation-exchange resin, and these results involving the structural determination were presented at the 26th Spring Meeting of the Japanese Chemical Society, Kanagawa Prefecture, April 1972.
(15) But the present experiment has not completely excluded the following possibility: in the reverse Michael-type reaction of the kinetically formed manno (or altro) product, abstraction of H (or D) from C-3 and cyanide departure would be followed by readdition mainly from the pool of nonexchanged, excess hydrogen cyanide.
(16) Before the change of conformation, epimerization at C-3 via deprotonation of H-3 followed by readdition of proton from the axial side appears not to be ruled out.
(17) In extensive studies on protonation of sodium nitronates derived from methyl 3-deoxy-3-nitropentopyranoside, Baer and J. Kovář, *Can. J. Chem.*, **49**, 1940 (1971), present that the transition state does not resemble the nitronate ion but the product. But it is difficult to predict the degree of resemblance of transition state to the product. If the transition state resembles completely the product, 2-phenyl- and 2-methylcyclohexane nitronate ion should afford the trans isomer, but, in fact, cis isomer is formed exclusively: F. G. Bordwell and K. C. Yee, *J. Am. Chem. Soc.*, **92**, 5939 (1970). Fur-

thermore, we recently isolated a less stable product with the axial nitro group in the reaction of **2** with acetylacetone (unpublished data).

(18) Although the reason why our conditions, different from the conditions employed by Paulsen et al., gave no manno isomer is not clear, it was noteworthy that the reaction of **2** (the α anomer of **1**) as well as the analogous α-acetate with the excess of hydrazoic acid in chloroform solutions afforded exceptionally large amounts of cis adduct (as a major product) with respect to the aglycone; these conditions used excess of reagent in nonpolar solvent to be close to those employed by Paulsen et al. rather than those described here.

(19) Nitronate ion derived from the gluco isomer might exist in the chair conformation. Equatorial attack of proton on this ion may occur, at least to a considerable extent, because the $A^{(1,3)}$ strain would force both the cyano group and oxygen at C-4 up and block approach of proton from the axial side.[5,21]

(20) Nitrite ion is not a poor leaving group and basicity of this reaction is not high; therefore, the possibility of Elcb reaction may be neglected. The anti elimination of the allo isomer should predominate over syn elimination of the gluco isomer owing to the torsional strain and the principle of least motion; J. E. Bunnett. *Surv. Prog. Chem.*, **5**, 53 (1969).

(21) Bordwell and co-worker, *J. Am. Chem. Soc.*, **92**, 5933 (1970), found that *cis*-2-phenyl-1-nitrocyclohexane was deprotonated 350-fold more rapidly than its trans isomer. Angyal and co-worker, *Aust. J. Chem.*, **23**, 1485 (1970), showed that *cis*-2-nitrocyclohexane-*cis*-1,3-diol rapidly epimerized in solution to the more stable trans,trans isomer. These facts suggest that abstraction of H-3 of the allo isomer should be much more rapid than that of the gluco isomer. As already described, abstraction of H-3 of the gluco isomer was much easier than that of H-2. Therefore it is likely that before a base works on H-2 of the allo isomer deprotonation of H-3 should occur to give a nitronate ion, from which the more stable gluco isomer is formed.

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(24) Combined steric hindrance due to H-4 (1,3-nonbonded interaction) and H-5 (1,4-nonbonded interaction), the former retarding axial attack and the latter equatorial attack, appears to be less important than that due to the glycosidic methoxy group.

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(26) H. H. Baer, F. Kienzle, and T. Neilson, *Can. J. Chem.*, **43**, 1829 (1965).

(27) T. Sakakibara, R. Sudoh, and T. Nakagawa, *Bull. Chem. Soc. Jpn.*, submitted for publication.

The Chemistry of a Method for the Determination of Carboxyl-Terminal Residues in Peptides

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We here describe in detail our method whereby the carboxyl-terminal residue of a peptide may be determined. The procedure involves, first, formation of an O-substituted hydroxamic acid by reaction of the peptide C-terminal carboxyl group with a water-soluble carbodiimide and a nucleophilic O-substituted hydroxylamine. Considerations leading to the choice of *O*-pivaloylhydroxylamine (**10**, OPHA) as the nucleophile are described, and optimization of the conditions for this reaction are discussed. The O-substituted hydroxamic acid then undergoes, at higher pH, a Lossen rearrangement, leading to degradation of the carboxyl-terminal residue and observed loss of that residue upon subsequent amino acid analysis. The fate of the rearranged residue has been thoroughly explored, and the rates of the Lossen rearrangement have been characterized for representative amino acids. Potential interferences from aromatic amino acids, the amino-terminal residue, carboxyl-terminal dicarboxylic amino acids, and residues with nucleophilic side chains have been characterized. In some cases, these do not occur; in cases in which such interferences do occur, these have been characterized, and most do not affect the utility of the method. Only carboxyl-terminal Asp and Glu do not degrade satisfactorily, and the reasons for this observation have been investigated.

Rapid experimental development has provided the protein chemist with a variety of useful analytical tools. A number of reagents are now available for the specific modification and cleavage of peptide chains,[2,3] and highly efficient amino terminal[3,4] and sequential[5] methods have been described. However, "no entirely satisfactory chemical method of carboxyl-terminal analysis exists".[3] Of the several carboxyl-terminal methods that have been proposed,[4,6] all suffer limitations, and few have been useful in actual practice. Vigorous conditions, solvent restrictions, and failure at certain amino acid residues have all contributed to their lack of general utility. It becomes important to search for new methods of carboxyl-terminal analysis which complement other methods now in use. We have developed a method of carboxyl-terminal residue analysis of peptides which not only circumvents some of the usual limitations, but is also mild, efficient, and easily used in aqueous solution, mixed solvents, or 8 M urea. We here wish to describe this method and the detailed chemistry of its development.

The concept of this method is shown in Scheme I, and involves the activation of the C-terminal carboxyl group followed by reaction with a nucleophile NH_2X. When X is a good leaving group, the resulting amide **5** is susceptible to rearrangement leading to eventual loss of the C-terminal residue as an aldehyde. Although the Lossen and related rearrangements have had substantial appeal for such a degradation,[7,8] until now such a degradation has been impractical because of the large number of reactions hitherto required to generate the intermediate **5** which is capable of rearrangement. Once the rearrangement occurs, the carboxyl terminal residue will be absent in the amino acid analysis, and subsequent reactions determine its ultimate fate, but not the fact of its loss. Critical to the fulfillment of Scheme I, then, is the quantitative conversion of peptide carboxyl-terminal carboxyl groups to a derivative such as **5**. This objective required careful selection of the nucleophile, NH_2X.

Choice of the Nucleophile. In order to be useful, the nucleophile NH_2X is required to be stable, reasonably water soluble, nucleophilic enough to compete with hydrolysis of a carbodiimide-derived activated intermediate, and, most importantly, it must incorporate a critical substituent, X, a potential leaving group which would be compatible with a reasonably facile rearrangement. Rearrangements of the type illustrated in Scheme I include the Hofmann,[9] Curtius,[10] Wawzonek,[11] and Lossen[12,13] reactions. Consideration of these types of reactions led to the synthesis and investigation of a variety of potential nucleophiles shown in Table I.[14] Of all these, only *O*-pivaloylhydroxylamine (OPHA, **10**) satisfactorily fulfilled the requisite criteria.

O-Pivaloylhydroxylamine was first prepared by Marmer and Maerker so that they could study its potential as an aminating agent.[15] These workers showed that its hydrochloride