Novel Protecting Groups. III. A Study of the Reaction of 2-Picolyl 1-Oxide Derivatives with Acetic Anhydride (1).

Kazuyoshi Ikeda, Kiyomi Tsuchida, Taeko Monma, and Yoshihisa Mizuno

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

Received August 17, 1973

2-Picolyl 1-oxides (I-VIII) were prepared and the reaction of I-VII with acetic anhydride was followed by the gas chromatography. In this reaction, the increasing order of the rate of disappearance of the 2-picolyl 1-oxide derivatives was found to be IV>III>VII>I>VI>II>V. Under comparable conditions, a half-life of VIII is midway between that of I and VI. The 2-picolyl 1-oxide group was found to be potentially useful for the blocking of hydroxyl functions of phosphates (*viz.*, VIII), although *ca.* 4% of the organic phosphate remained unblocked after the deblocking procedure (acetic anhydride treatment and subsequent hydrolysis).

In the preceding papers it has been shown that the 2-picolyl 1-oxide group (po-group) (2) attached to imino functions of purines and pyrimidines was stable in both acidic and basic media and could be easily deblocked with acetic anhydride treatment and hydrolysis (1,3). The mechanistic scheme of each process of deblocking can be written as shown in equations 1 and 2 (4,5).

Most of the first process (equation 1) follows the second-order rate equation (5):

$$v=K[N\text{-Oxide}][Ac_2O].$$

The second process (equation 2) proceeds rapidly under very mild basic conditions. Therefore, the ease with which the deblocking of the po-group is achieved depends on the proton abstraction by the acetoxy group (equation 1). In order to come up with the best "protective type group", the reaction of some 2-picolyl 1-oxide derivatives with acetic anhydride was kinetically examined primarily by following the disappearance of the substrate. In the case of 2-picolyl phosphate 1-oxide, the progress of the reaction was followed by the determination of inorganic phosphate released after acetic anhydride treatment and hydrolysis.

Substrates examined were 1-oxides of 2-picolyl chloride (I), 2-picolyl acetate (II), 2-picolylthiol acetate (III), 2-picolyl cyanide (IV), 2-picolylmethyl ether (V), 2-picolyldimethylamine (VI), *N*-(2-picolyl)acetamide (VII), and 2-picolyl dihydrogen phosphate (VIII).

The 1-oxides: I(6), II(7), III(8), IV(8) and V(9) are known compounds and were prepared according to reported procedures by some modifications. The compound VI was prepared by the reaction of I with dimethylamine and the compound VII was prepared by the reaction of 2-picolylamine 1-oxide (IX, 3b) with ethyl acetate in the presence of an catalytic amount of sodium methoxide. 2-Picolyl dihydrogen phosphate 1-oxide (VIII) was prepared by the reaction of 2-picolylalcohol 1-oxide with polyphosphoric acid in satisfactory yield. Ultraviolet and nmr spectral data of these compounds (I-IX) are shown in Table I. Progress of the reactions of the substrates (I-VII) with acetic anhydride was followed by gas chromatography (Fig. I A, B, and C).

As shown in Fig. IA and IC, with III and VII, a couple of peaks other than that of the substrate appeared among which peak b (in Fig. IA) or peak a (in Fig. IC) was found to correspond to the respective rearranged product (see equation 1). It is highly probable that a remaining peak (peak a in Fig IA or peak b in Fig. IC) corresponds to an intermediate of unknown structure, because inspection of respective time-course chromatograms revealed that a decrease in peak area (a) or (b) was accompanied by concomitant increase in peak area (b) or (a).

The reaction of I with acetic anhydride showed quite a similar chromatographic profile as in the case of IV, whereas the reaction of II or V showed a chromatographic profile similar to that of III or VII. In the case of VI, a peak corresponding to the rearranged product was overlapped with a peak of the solvent (chloroform) under the conditions employed. However, silica gel tlc revealed the presence of two main products whose structure assignment has not been done.

Residual-time profiles of I through VII were obtained (those of I, IV, VI and VII are shown in Fig. II). A half-life of each compound was estimated from the curves and is summarized in Table I.

As shown, the increasing order of reactivity in the first process (equation 1) was found to be IV>III>VII>I>VI>II>V.

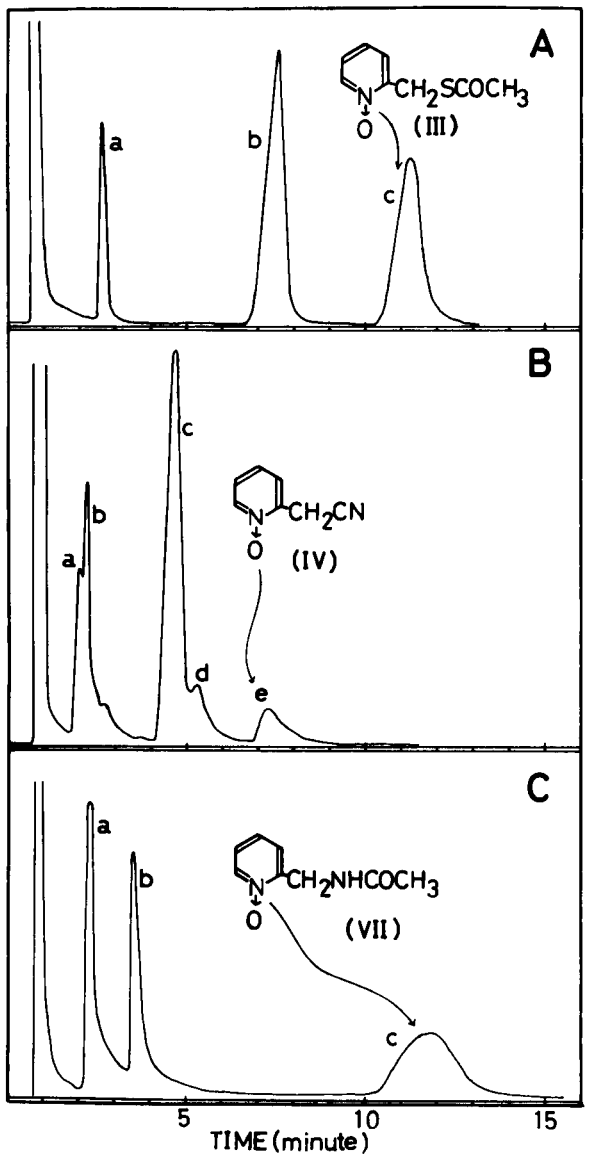


Figure 1. Gas chromatographic analysis of the products from reaction of III, IV or VII with acetic anhydride at 50°. A; reaction of III for 51 minutes, B; reaction of IV for 50 minutes, and C; reaction of VII for 81 minutes.

As expected the reaction of IV with acetic anhydride was unimolecular with respect to acetic anhydride (see Fig. III and equation 3), whereas that of VII with acetic anhydride proceeds by bimolecular kinetics with respect with the anhydride. These data strongly suggested that with VII the corresponding diacetylamino derivative was initially formed and was in turn converted into bis(acetamido)-acetoxy-2-pyridylmethane.

Since by analogy with the findings of Oae and coworkers (5), the rate determining step of the above-mentioned

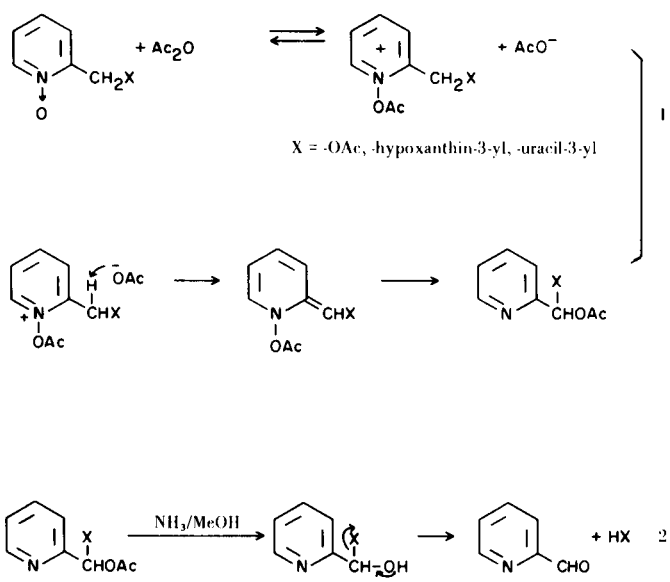
TABLE I

Spectral Data and Half-Life Periods of 2-Picolyl 1-Oxide Derivatives

(RX, R = 2-Picolyl 1-Oxide)

Compound number	= X	Uv λ max nm (ε)			Nmr (δ)		Ir cm^{-1}	Half-life Periods (b)	
		0.1*N* HCl	*p*H 7.0	0.1*N* NaOH	$-CH_2-$	$-CH_3$	$N \rightarrow O$	1.6M of Acetic Anhydride	5.0M of Acetic Anhydride
I	-Cl	259.5 (9400)	259.5 (9900)	259.5 (9800)	4.84		1257	280 (min.)	1 (hr.)
II	$-OCOCH_3$	255.5 (9500)	255.5 (11000)	255.5 (10000)	5.20	2.15	1230		23
III	$-SCOCH_3$	258.0 (9200)	257.5 (10000)	251.5 (12000)	4.19	2.30	1242	30	
IV	-CN	260.0 (11000)	256.0 (11000)	256.0 (11000)	4.09		1255	20	
V	$-OCH_3$	254.5 (8300)	254.5 (10000)	254.4 (10000)	4.55	3.42	1250		56
VI	$-N(CH_3)_2$	260.0 (10000)	260.0 (9500)	256.0 (10000)	3.62	2.31	1245		5.8
VII	$-NHCOCH_3$	255.0 (9600)	255.0 (11000)	255.0 (11000)	4.40	1.95	1223	90	
VIII	$-OPO_3H_2$	254.0 (7900)	252.5 (9000)	252.5 (9000)	4.96 (a)		1210		2.5
IX	$-NH_2$	254.0	254.0	250.0	3.90		1240		

(a) In deuterium oxide. (b) Chloroform at 50° (in the case of VIII. DMF).



reactions of I-VII is presumably the proton removal by acetate anion, the above sequence must be closely associated with the acidity of the methylenic protons, which in turn must be related to the electron-withdrawing properties of the group X (CN, $SCOCH_3$, $NHCOCH_3$, Cl, $N(CH_3)_2$, $OCOCH_3$ and OCH_3). The cyano group is a most powerful electron withdrawing group. Hydrogen in the methylthio grouping is more acidic than that in the methoxy group (10). A methylene group adjacent to the diacetylamino group (rather than monoacetylamino) is more acidic than that linked to an acetoxy group. The fact that VI was rearranged more rapidly than II can be explained by the added electron-attracting property of the dimethylamino group protonated by acetic acid. Thus, the above sequence can be resonably interpreted within the framework of the proposed mechanism.

In the case of VIII, after acetic anhydride treatment (2.5 hours at 50°) and hydrolysis inorganic phosphate reached

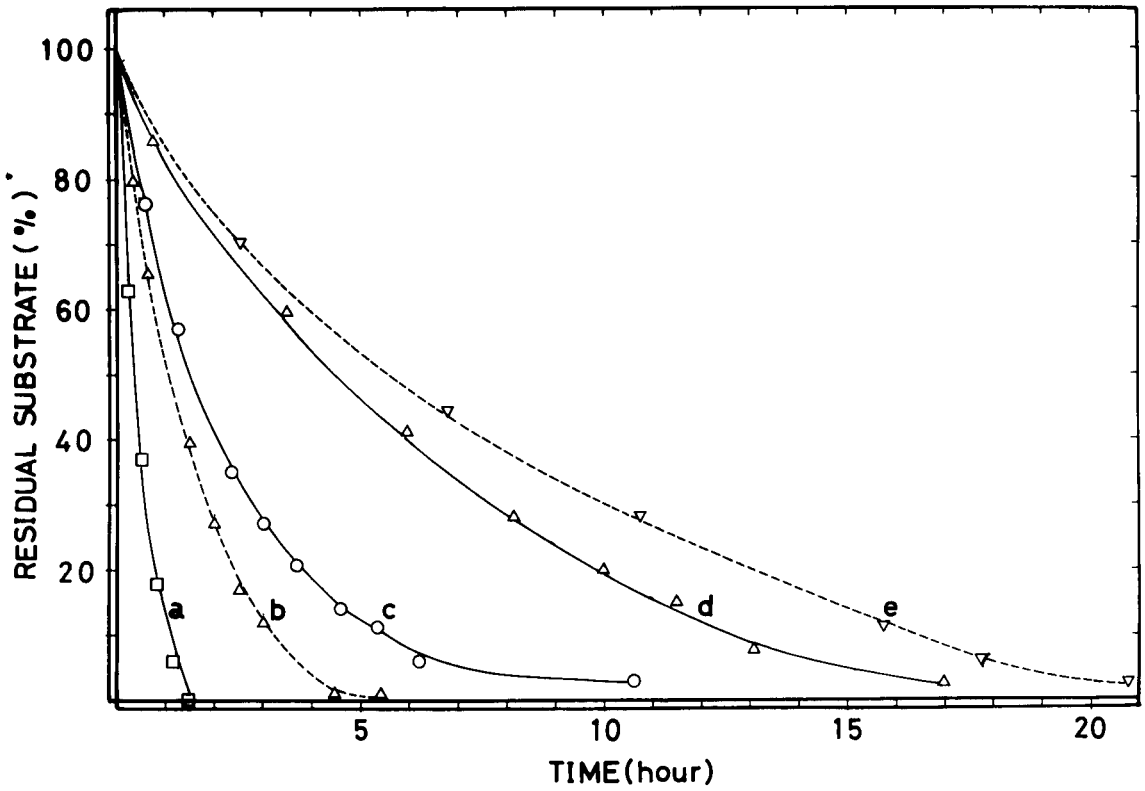


Figure 2. Residual substrate-time profile on reaction of *N*-oxides with 1.6*M* or 5.0*M* of acetic anhydride in chloroform. a; the reaction of IV with 1.6*M* of acetic anhydride, b; the reaction of I with 5.0*M* of acetic anhydride, c; the reaction of VII with 1.6*M* of acetic anhydride, d; the reaction of I with 1.6*M* of acetic anhydride, e; the reaction of VI with 5.0*M* of acetic anhydride.

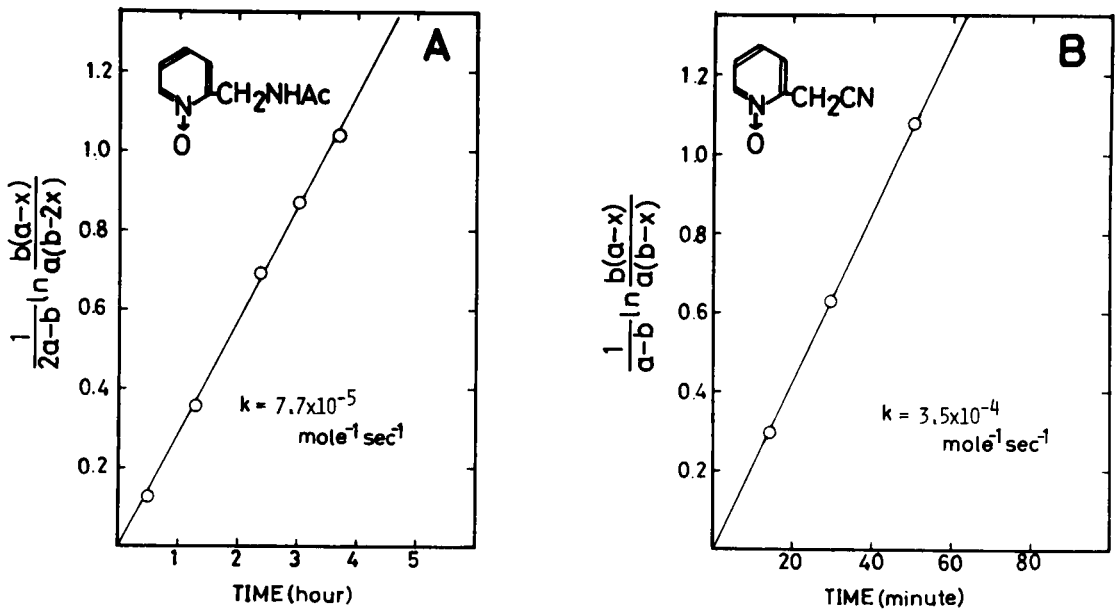


Figure 3. Plot of the kinetic equation for reactions of acetic anhydride and *N*-oxides. A; compound VII, *a* is the initial concentration of VII and *b* is the initial concentration of acetic anhydride. B: compound IV. The velocity constants K were calculated from slopes of these linear rate plots.

50% of the theoretical value, indicating that under the comparable conditions the rearrangement of VIII proceeded more rapidly (half-life, 2.5 hr) than VI or II whose half-life periods were *ca* 6 and 23 hours, respectively. After 40 hours, VIII was almost completely converted to inorganic phosphate and 2-formylpyridine after hydrolysis. Recovery of inorganic phosphate was 96%. The rest of the phosphate(s) (*ca*. 4% of the total phosphate) was examined by a combination of DEAE-cellulose column chromatography and enzymatic degradation. The phosphate esters which were not unblocked were found to be mainly 2-picolyl mono-(X) (1.5%), pyro-(XI) (2.47%) and triphosphate (XII) (0.04%). These had been formed by reduction of the corresponding 1-oxide. Similar observations were made also by Cohen and coworkers (11).

These data suggest that in order to achieve clean chromatographic separation prior hydrolysis of higher phosphates to the monophosphate was required so as to minimize the number of phosphates.

EXPERIMENTAL

General

Uv spectra were determined on a Hitachi Spectorphotometer Model 3T and ir spectra were run on a JASCO Infrared Spectrophotometer DS-701G in potassium bromide tablets. Nmr spectra were determined on a Hitachi NMR Spectrometer Model R-24 in deuterochloroform-deuterodimethyl sulfoxide (1:3 v/v) or in deuterium oxide. The chemical shifts were reported in parts per million downfield from tetramethylsilane as the internal standard. Paper electrophoresis was carried out on Toyo Roshi No 51 A paper impregnated with 0.05 *M* triethylammonium bicarbonate (*p*H 8.0) using 700 volts or with 0.05 *M* acetate buffer (*p*H 3.7) using 1000 volts conducted on a flat bed apparatus. Paper chromatography was carried out by the descending technique on Toyo Roshi No 51A paper using the following systems: solvent A, 2-propanol-28% ammonium hydroxide-water (7:2:1); solvent B, ethanol-1*M* ammonium acetate buffer, *p*H 3.7 (5:2). Unless otherwise stated, the solvent was removed under reduced pressure either with a rotating evaporator or an oil pump. Gas chromatography was carried out by a Shimadzu Gas Chromatograph Model GC4-APF and a glass column (3 x 4 cm) packed with 3% silicone OV-1 on chromosorb WAW was used. The conditions used were as follows: flow rate of nitrogen was 40 ml. per minute and flow rates of air and hydrogen were optimized for the maximum response. Column temperature were 125°, 135°, and 150°. 1,2,3-Trinitrobenzene, anthracene, or estrance were used as the internal standard.

2-Picolylmethylether 1-Oxide (V).

A mixture of 2-picolychloride 1-oxide hydrochloride (5.1 g.) and 1*N* methanolic sodium methoxide (66 ml.) was stirred for 8 hours at room temperature and filtered. The filtrate was neutralized with acetic acid and evaporated to dryness (3.9 g.). The dried residue was purified with silica gel chromatography (solvent system, chloroform-methanol 7:1). The eluate was concentrated to leave an oily residue which was further purified by fractionation; yield, 2.3 g. b.p. 121° (1.5 mm Hg) and m.p. 37°.

Anal. Calcd. for $C_7H_9NO_2$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.26; H, 6.43; N, 10.00.

The picrate (m.p. 97.5°) was crystallized from methanol-ether.

Anal. Calcd. for $C_{13}H_{12}N_4O_9$: C, 42.40; H, 3.28; N, 15.22. Found: C, 42.48; H, 3.31; N, 15.18.

2-Picolylchloride 1-Oxide (1).

A mixture of 2-picolyl chloride 1-oxide hydrochloride (5.0 g.), powdered potassium carbonate (5.0 g.), ethyl acetate (40 ml.) and 3 drops of water was stirred for an hour at room temperature. The mixture was filtered and the solid was washed with ethyl acetate (120 ml.). The combined filtrate and washings were dried over sodium sulfate, and filtered. The filtrate was evaporated to dryness and the residue was crystallized from ethyl acetate, yield, 3.8 g., m.p. 82-83°.

Anal. Calcd. for C_6H_6ClNO: C, 50.19; H, 4.21; N, 9.76. Found: C, 50.05; H, 4.21; N, 9.76. Found: C, 50.05; H, 4.36; N, 9.59.

2-Picolyl Dimethylamine 1-Oxide (VI).

2-Picolyl Chloride 1-Oxide hydrochloride (5.1 g.) was treated with 30% aqueous dimethylamine (100 ml.) at room temperature overnight. The mixture was concentrated to dryness. The residue was dissolved in 20 ml. of water and the solution was then applied to a Dowex column (1x8; 50-100 mesh, 2x50 cm; OH^- form) and washed with water. The eluate was evaporated to leave an oily residue (4.0 g.). The residue was then applied to a silica gel column (solvent system, chloroform-ethanol 9:1). The oil obtained after evaporation of the eluate was fractionated to yield 3.1 g. of VI, b.p. 135-140° (1.5 mm Hg). M.p. of the picrate, 156°.

Anal. Calcd. for $C_{14}H_{15}N_5O_8$: C, 44.10; H, 3.97; N, 18.37. Found: C, 43.85; H, 3.86; N, 18.24.

N-(2-Picolyl)acetamide 1-Oxide (VII).

2-Picolylamine 1-oxide hydrochloride (0.9 g.) was dissolved in 7 ml. of 1*N* methanolic sodium methoxide. The solution was concentrated to dryness. The residue was dissolved in ethyl acetate (30 ml.) and the insoluble material was filtered off. The filtrate was refluxed for 2 hours. The cooled solution was neutralized with acetic acid and then was concentrated to dryness. The residue was applied to a silica gel chromatography (solvent, chloroform-methanol 20:1). The eluate containing VII was concentrated to leave an oily residue which was crystallized from ethyl acetate ether, yield 420 mg. (60%), m.p. 97-101°.

Anal. Calcd. for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.86. Found: C, 58.04; H, 6.15; N, 16.93.

2-Picolyl Dihydrogen Phosphate 1-Oxide (VIII).

A mixture of 2-picolyl alcohol 1-oxide (2.5 g.) and polyphosphoric acid prepared from phosphorus pentoxide (5.0 g.) and 85% phosphoric acid (5.0 g.) was heated at 70° for 12 hours (12). After cooling, water (10 ml.) was added to the mixture which was then heated on a boiling water bath for 30 minutes. The cooled solution was applied to a Dowex column (50W x 8, H^+ form, 50-100 mesh, column size: 3 x 60 cm). Fractions 11-30 (fraction size: 15 ml.) containing VIII were concentrated to dryness. The residue was recrystallized from water-ethanol (1:2). The crystal was dried at 70° for 10 hours, yield, 3.1 g., m.p. 173° (72%).

Anal. Calcd. for $C_6H_8NO_5P$: C, 35.13; H, 3.91; N, 6.83. Found: C, 34.90; H, 4.14; N, 6.75.

2-Picolyl Pyrophosphate (XI).

A mixture of 2-picolyl hydrogen phosphate (190 mg.) (13), tri-*n*-butylamine (0.4 ml.), and diphenyl phosphorochloridate (0.2 ml.) in DMF (2.0 ml.) was allowed to stand at room temperature for 3 hours. The mixture was concentrated to dryness. There was then added ether (10 ml.) and the resulting mixture was shaken vigorously. Precipitates deposited were collected, washed with ether and then dissolved in DMF (1.0 ml.). Phosphoric acid (85% 170 mg.) was separately dissolved in pyridine (2.0 ml.) containing tri-*n*-butylamine (0.4 ml.) and was rendered anhydrous by three co-distillations with pyridine (2 ml.). The DMF solution of the mixed anhydride and the pyridine solution of tributylammonium phosphate were mixed and allowed to stand at room temperature for 3 hours and then in an ice box for 2 days. The solution was concentrated to dryness. The residue was dissolved in cold water (10 ml.) and then *p*H of the solution was adjusted to *p*H 8 with 10% ammonium hydroxide. The muddy mixture was extracted with ether (10 ml. x 3). The aqueous solution was applied to a DEAE-cellulose column (column size: 3 x 50) and eluted by a linear gradient of 0.3 *M* triethylammonium bicarbonate (*p*H 7.8, 1.5 ℓ.) and water (1.5 ℓ). The fractions containing XI were concentrated. Triethylammonium bicarbonate was removed by co-distillations with methanol. The final residue was dissolved in water (2.0 ml.) and stored. This solution contained 0.38 mmole of XI as determined by optical density at 261 nm. This sample gave a single spot on paper chromatograms and paper electrophoresis and had 3200 of ϵ_p at *p*H 6.5.

2-Picolyl Triphosphate (XII).

A mixture of X (190 mg.), tri-*n*-butylamine (2.0 ml.), 85% phosphoric acid (1.0 g.) and DCC (7.0 g.) in pyridine (20 ml.) was allowed to stand at room temperature for 3 days. Dicyclohexylurea was filtered off. The filtrate was concentrated to dryness The residue was dissolved in water (120 ml.) and then extracted with ether. The aqueous layer was separated and mixed with charcoal (15 g.). The charcoal was collected by filtration and washed with water (1.5 ℓ.) until almost no inorganic phosphate was detected in the washings. 2-Picolyl phosphates were eluted from the charcoal with 50% aqueous ethanol containing 0.7% of ammonia (1ℓ.). The eluate was concentrated to 20 ml. and adjusted to *p*H 8 with 10% aquoeus ammonia. The solution was applied to a DEAE-cellulose column and the column was washed with a linear gradient of 0.3 *M* triehtylammonium bicarbonate (*p*H 7.8, 1.5 ℓ.) and water (1.5 ℓ.). Fractions containing XII were concentrated to dryness. Triethylammonium bicarbonate was removed by co-distillations with methanol. The final residue was dissolved in water (2.0 ml.) and stored. This solution contained 0.25 mmole of XII as determined by optical density at 261 nm. This sample gave a single spot on a paper chromatogram and paper electrophoresis and had 2300 of ϵp at *p*H 6.5.

Quatitative Studies of the Reaction of Oxides (I-VII) with Acetic Anhydride by the Use of Gas Chromatographic Technique.

A solution (1.0 ml.) containing 0.1 mmole of substrate (I-VII), 1.6 or 5.0 mmoles of acetic anhydride and 0.1 mmole of internal standard in chloroform, was each kept in a sealed flask (5 ml.) at 50 ± 2°. At appropriate intervals, an aliquot was removed and passed through the column of the gas chromatograph. The decrease in amount of substrate was estimated from the peak-area ratios of substrate to internal standard. For comparison of reaction of VIII with II and VI, a solution (2.0 ml.) containing 58 μ moles of II or VI, 5.24 mmoles of acetic anhydride and 58 μ moles of the internal standard in DMF was used.

Quantitative Study of the Reaction of 2-Picolyl Dihydrogen Phosphate 1-Oxide (VIII) with Acetic Anhydride.

A solution (2.0 ml.) containing 58 μ moles of VIII and 5.24 mmoles of acetic anhydride in DMF was kept in a sealed flask (5 ml.) at 50 ± 2°. At appropriate intervals, an aliquot (100 μl) was removed and treated with 0.18 *N* perchloric acid (1.0 ml.) for 10

minutes on a boiling water bath. Inorganic phosphate released was determined by Allen's methos (14). After 40 hours, recovery of inorganic phosphate was 96% of the theoretical value. The initial concentration of VIII was also estimated from the total phosphate was 96% of the theoretical value. The initial concentration of VIII was also estimated from the total phosphate.

Investigation of Products in the Reaction Mixture of VIII after Acetic Anhydride Treatment and Hydrolysis.

Separation and Purification of Products: A mixture of VIII (205 mg.) and acetic anhydride (20 ml.) in DMF (20 ml.) was heated to 50° for 40 hours. The solution was concentrated to dryness below 30°. The residue was dissolved in water (10 ml.) and extracted with chloroform (10 ml. x 4). The aqueous layer was adjusted to *p*H 8 with ammonium hydroxide and applied to a column of DEAE-cellulose. The column was washed with water until no more uv-absorbing material was eluted. Elution was then continued with a linear gradient of 0.3*M* triehtylammonium bicarbonate (2ℓ.) and water (2ℓ.). The fraction size was 18 ml. The elution was monitored by optical density at 261 nm. The chromatogram showed the presence of seven peaks (peak I-VII). Each peak was separately pooled, and concentrated to dryness and freed of triethylammonium bicarbonate by co-evaporations with methanol. Each residue was dissolved in water (0.5 ml.) and stored. Each phosphate on peaks IV, V, VI, and VII was then purified by preparative paper chromatography (solvent A), and analyzed by uv, paper electrophoresis and enzymatic degradation.

Enzymatic degradation: E. *coli* alkaline phosphatase (product of Worshington Biochemical Co., N. J.), was made up to a concen - tration of 1.0 mg./ml. About 10 optical density units of each phosphate were incubated at room temperature with 10 μ of 1*M* triethylammonium bicarbonate (*p*H 8.5), and 10 μl of E. *coli* alkaline phosphatase overnight and then examined by paper chromatography in oslvent B and by tlc on silica gel in chloroform-ethanol (7:1).

Structural Assignment of Each Compound: The phosphates in peaks IV, V, VI, and VII were completely degraded by the alkaline phaosphatase to afford 2-picolyl alcohol and inorganic phosphate. The compounds in peaks IV, V, and VI were assigned the 2-picolyl-mono- (X), -pyro-(XI), and -triphosphate (XII) structure on the basis of paper chromatographic and electrophoretic comparison with authentic samples. A compound in peak II whose uv absorption maxima appeared at λ max (*p*H 1) (nm) 262, 268 (sh) and λ max (*p*H 12) (nm) 261, 268 (sh) and 280 (sh), was completely degraded with the alkaline phosphatase, but was not examined further, because of a minute amount of this sample. No phosphates were detected in peaks I and III.

Acknowledgements.

We wish to thank Dr. Nobuo Ikekawa of the Tokyo Institute of Technology for valuable suggestions concerning the gas chromatographic technique. We are also grateful to Dr. Sadahiko Toma of this Faculty of Pharmaceutical Sciences for the gift of estrane. Thanks are also due to the staff in the analytical Laboratory of this Faculty for elemental analyses.

REFERENCES

(1) Part II; T. Endo, Y. Kawamura, K. Ikeda, and Y. Mizuno, *Chem. Commun.*, 673 (1973).

(2) Hereafter, the 2-picolyl 1-oxide group will be referred to as the po-group.

(3a) Y. Mizuno, W. Linn, K. Tsuchida, and K. Ikeda, *J. Org. Chem.*, 37, 39 (1972); (b) Y. Mizuno, T. Endo, T. Miyaoka, and K. Ikeda, *ibid.*, 39, in press.

(4a) V. J. Traynelis in "Mechanism of Molecular Migrations," Vol 2, B. S. Thyagarajan, Ed., Interscience Publishers, New York, 1969, p. 1; (b) E. Ochiai, "Aromatic amine Oxides", Elsevier Company, New York, 1967, p. 290.

(5) S. Oae, S. Tamagaki, T. Negoro, K. Ogino, and S. Kozuka, *Tetrahedron Letters*, 917, (1969).

(6) P. T. Sullivan, M. Kester, and S. J. Norton, *J. Med. Chem.*, 11, 1172 (1968).

(7a) G. Kobayashi, *Chem. Pharm. Bull.* (Japan), 1, 347(1953); (b) V. Boekelheide and W. J. Linn, *J. Am. Chem. Soc.*, 76, 1286 (1954).

(8) P. Majewski, R. Rodalski, and J. Michalski, *Synthesis*, 140 (1971).

(9) W. S. Chilton and A. K. Buther, *J. Org. Chem.*, 32, 1270 (1967).

(10) W. J. Brehm and T. Levenson, *J. Am. Chem. Soc.*, 76, 5389 (1954).

(11) T. Cohen and G. L. Deets, *ibid.*, 89, 3939 (1967).

(12) R. H. Hall and H. G. Khorana, *ibid.*, 77, 1871 (1955).

(13) Y. Murakami, M. Takagi, and H. Nishi, *Bull. Chem. Soc. Japan*, 39, 1197 (1966).

(14) R. J. L. Allen, *Biochem. J.*, 34, 858 (1940).