UDC 547.823.07

121. Shigehiko Sugasawa and Makoto Kirisawa: Oxidation of 4-(1,1-Ethylenedioxyethyl)- and 4-(Ethylenedioxymethyl)-1-methylpyridinium Salts.**

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The preparation of 1-substituted 4-methyl- and 4-ethyl-2(1*H*)-pyridones through direct oxidation of 4-methyl- and 4-ethylpyridinium salts appeared to be difficult, because the alkyl group on 4-position was also involved in the oxidation reaction.[1]

The alkaline ferricyanide oxidation of 1-substituted 3-ethylpyridinium salts gives 1-substituted 3-ethyl-2(1*H*)-pyridone as the main product, the isomeric 5-ethyl-2(1*H*)-pyridone being produced simultaneouly only in a minor quantity.[2] To obtain the latter in quantity the present authors[3] prepared quaternary salts of 3-(1,1-ethylenedioxyethyl)pyridine (ethylene-ketal of 3-acetylpyridine) and subjected them to alkaline ferricyanide oxidation. The resultant pyridones produced in excellent yields were hydrolyzed with an acid and the ketones thus recovered were then reduced according to the method of Huang-Minlon to yield 1-substituted 5-ethyl-2(1*H*)-pyridones as the sole product.

This technique was now applied to 4-acetyl- and 4-formylpyridine (Ia and Ib). These pyridines gave the corresponding ethylenedioxy derivatives (IIa and IIb) in excellent yields by the standard method, which were then quaternarized by heating with dimethyl sulfate to give (IIIa) and (IIIb), which were then oxidized as usual. The oxidation proceeded smoothly and the pyridones (IVa and IVb) were produced in good yields. By acid hydrolysis they gave 4-acetyl- and 4-formylpyridones (Va and Vb) respectively in quantitative yields and their structures were proved beyond doubt by converting the former by haloform reaction and the latter through oxidizing with silver oxide to 1-methyl-2-oxo-1,2-dihydroisonicotinic acid (VII), which was identified with an authentic sample.

When (Va) and (Vb) were reduced according to the method of Huang-Minlon 4-ethyl- and 4-methyl-2(1*H*)-pyridones[6] (VIa and VIb) were obtained again in good yields.

This oxidation method will be applicable to various other 4- and 3-acylpyridinium salts, provided they are available, and acylpyridones thus prepared will form suitable intermediates for synthetical work and their application to the synthesis of some benzoquinolizine derivatives of possible pharmacological interest will be the subject of forthcoming communication from this laboratory.

The authors are grateful to the members of the Central Analysis Room of this Faculty, and to Mrs. F. Hisamichi and Mr. T. Yoda of Tanabe Seiyaku & Co. for micro-analytical data.

Experimental

4-(1,1-Ethylenedioxyethyl)pyridine (IIa)—4-Acetylpyridine[4] (12.1 g.) in 80 cc. of benzene was mixed with anhyd. ethylene glycol (8 g.) and *p*-toluenesulfonic acid (18 g.) and the mixture was heated in an oil bath kept at 120°; H_2O being azeotropically removed with benzene as it was formed. After 5 hrs.' heating the cooled reaction mixture was poured into 200 cc. of 10% K_2CO_3 soln. The aq. layer was extracted repeatedly with benzene and this solution was mixed with the original benzene layer,

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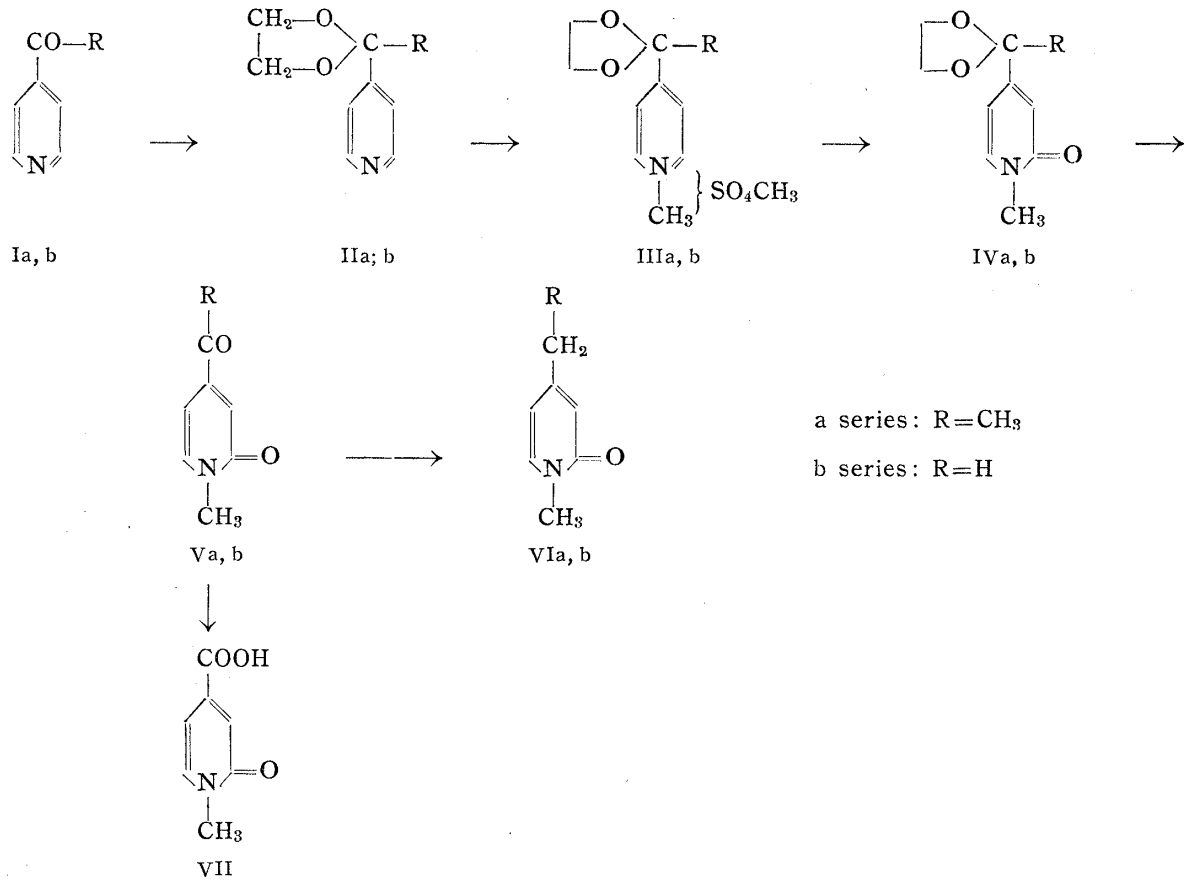
1) From methyl methosulfate of 4-ethylpyridine, 1-methyl-2-oxo-1,2-dihydroisonicotinic acid was obtained in a small yield, when an excess of potassium ferricyanide was used.

2) S. Sugasawa, Y. Ban: Yakugaku Zasshi, **72**, 1336 (1952); cf. also This Bulletin, **4**, 139 (1956) by the present authors.

3) S. Sugasawa, M. Kirisawa: This Bulletin, **3**, 190 (1955).



dried over K_2CO_3, and the solvent was evaporated. The residue distilled at 137~138°/34 mm. and soon solidified on standing. The yield was 15 g. or 95%. This melted at 49~50°. *Anal.* Calcd. for $C_9H_{11}O_2N$: C, 65.4; H, 6.7; N, 8.5. Found: C, 65.9; H, 6.4; N. 8.6.
Picrate: Yellow needles of m.p. 141~143° from EtOH. *Anal.* Calcd. for $C_{15}H_{14}O_9N_4$: C, 45.7; H, 3.6; N, 14.2. Found: C, 45.35; H, 3.2; N, 14.3.

1-Methyl-4-(1, 1-ethylenedioxyethyl)-2(1*H*)-pyridone (IVa)—The foregoing base (8.4 g.) was mixed with freshly purified Me_2SO_4 (7 g.) with cooling and the mixture was then heated on a steam bath for 1 hr. to form the methyl methosulfate (IIIa), which gave a clear soln. when dissolved in 30 cc. of H_2O. The resultant aq. soln. was mixed with a soln. of $K_3Fe(CN)_6$ (50 g.) in 200 cc. of H_2O, to which was now introduced KOH (27 g. in 30 cc. of H_2O) during 20 mins. with stirring and ice-cooling. Benzene (150 cc.) was then added and the whole was stirred in an ice-bath for 1 hr. and at room temp. for 2 hrs., and then allowed to stand overnight. The aq. layer was extracted with benzene, which was combined with the original benzene layer, dried, and the solvent was removed. The residue was distilled *in vacuo* to give a fraction of $b.p_3$ 168~169°, which soon solidified on standing. The yield of the distilled product was 7.3 g. or 74% based on (IIa). When purified from hexane this formed somewhat deliquescent colorless needles of m.p. 62~63°. *Anal.* Calcd. for $C_{10}H_{13}O_3N$: C, 61.5; H, 6.7; N, 7.2. Found: C, 61.2; H, 6.7; N, 7.1. Picrate: Yellow plates of m.p. 105~107° from EtOH. *Anal.* Calcd. for $C_{16}H_{16}O_{10}N_4$: C, 45.3, H, 3.8; N. 13.2. Found: C, 44.7; H, 3.7; N, 13.0.

1-Methyl-4-acetyl-2(1*H*)-pyridone (Va)—The foregoing (IVa) (6.9 g.) was warmed with 40 cc. of 3% HCl at 50~60° for 1 hr. On cooling the ketone formed was salted out by adding powdered K_2CO_3 with cooling and extracted with $CHCl_3$, which was dried and evaporated. The crude ketone thus recovered weighed 5.3 g. (quantitative) and was purified from benzene, forming colorless needles of m.p. 146~148°. *Anal.* Calcd. for $C_8H_9O_2N$: C, 63.6; H, 6.0; N, 9.3. Found: C, 63.8; H, 6.4; N, 9.35. Semicarbazone: Colorless scales of m.p. 236~238°(decomp.) from MeOH. *Anal.* Cacld. for $C_9H_{12}O_2N_4$: C, 51.9; H, 5.8; N, 26.9. Found: C, 51.8; H, 5.7; N, 26.5.

1-Methyl-4-ethyl-2(1*H*)-pyridone (VIa)—A mixture of the foregoing ketone (2 g.), hydrazine hydrate (1.8 g. of 80%), KOH (1.5 g.), and ethylene glycol (10 cc.) was heated with stirring in an oil bath kept at 110~120° for 1.5 hrs. The temp. was now raised to 180° in 30 mins. and kept at 180~190°

4) H. G. Kalloff, J. H. Hunter: J. Am. Chem. Soc., **63**, 490 (1941).

for additional 2 hrs. surmounted by a descending condenser; the reaction mixture was stirred all the time. The residue was diluted with ca. 20 cc. of H_2O and was salted out by adding K_2CO_3, separating an oily layer, which was collected in $CHCl_3$, dried, and evaporated. The oily residue distilled at 127~128°/7 mm. to give a colorless liquid in a yield of 1.7 g. or 94%, which gave yellow needle-shaped picrate of m.p. 130~132° from EtOH. *Anal.* Calcd. for $C_{14}H_{14}O_8N_4$: C, 45.9; H, 3.85; N, 15.3. Found: C, 45.9; H, 3.7; N, 15.15.

Compounds of b-series were prepared in like manner as in a-series.

4-Ethylenedioxymethylpyridine (IIb)—This was prepared from isonicotinaldehyde[5] (2 g.), ethylene glycol (1.8 g.), and *p*-toluenesulfonic acid (4 g.), and was obtained in a yield of 2.5 g. or 89%. This boiled at 116~118°/9 mm. and solidified on standing to form a deliquescent plates of m.p. 35~37°, and was characterized as picrate, which formed yellow needles of m.p. 128~129° from EtOH. *Anal.* Calcd. for $C_{14}H_{12}O_9N_4$: C, 44.2; H, 3.2; N, 14.7. Found: C, 43.95; H, 3.1; N, 14.4.

1-Methyl-4-ethylenedioxymethyl-2(1*H*)-pyridone (IVb)—The foregoing base (2.2 g.) was quaternized with Me_2SO_4 (1.9 g.) and the quaternary salt was oxidized with 11 g. of $K_3Fe(CN)_6$ and 8 g. of KOH in aq. soln. as described above. After being allowed to stand overnight, the aq. layer was extracted with $CHCl_3$, which was combined with the original benzene layer, dried, and the solvent was removed. The crude product was obtained as a colorless oil of $b.p_3$ 170~171°, which solidified to colorless plates of m.p. 63~65°. The yield was 1.6 g. or 61% based on (IIb). The picrate was prepared for characterization purpose, which separated in yellow scales of m.p. 115~117° from EtOH. *Anal.* Calcd. for $C_{15}H_{14}O_{10}N_4$: C, 43.9; H, 3.4; N, 13.7. Found: C, 43.8; H, 3.3; N, 14.0.

1-Methyl-4-formyl-2(1*H*)-pyridone (Vb)—The acid hydrolysis of the foregoing ketal proceeded with a quantitative yield of (Vb), which formed colorless needles of m.p. 130~132° from benzene. *Anal.* Calcd. for $C_7H_7O_2N$: C, 61.3; H, 5.15; N, 10.2. Found: C, 61.1; H, 5.0; N, 10.2.
Semicarbazone: Colorless prisms of m.p. 235~236°(decomp.) from MeOH. *Anal.* Calcd. for $C_8H_{10}O_2N_4$; C, 49.5; H, 5.2; N, 28.85. Found: C, 49.0; H, 5.0; N, 28.3.

1,4-Dimethyl-2(1*H*)-pyridone (VIb)—The Huang-Minlon reduction of the foregoing aldehyde gave (VIb) in a yield of 78% of the distilled product of $b.p_7$ 120~121°, which solidified on standing to form deliquescent crystalline solid of m.p. 56~59° (reported $b.p_1$ 110° and m.p. 59°[6]). This was characterized as picrate. The latter formed yellow plates of m.p. 166~167° from EtOH (reported m.p. 168~169° (corr.)[6]). *Anal.* Calcd. for $C_{13}H_{12}O_8N_4$: C, 44.3; H, 3.4; N, 15.9. Found: C, 44.5; H, 3.3; N, 15.6.

1-Methyl-2-oxo-1,2-dihydroisonicotinic Acid (VII)—i) From (Va): An alkaline hypobromite soln. was prepared from NaOH (2 g.), H_2O (15 cc.), and Br_2 (2.7 g.) as usual. (Va) (0.75 g.) was now added to this soln. with stirring at 5° during 10 mins. The whole was kept at −5° for 1 hr. and then stirred for additional 3 hrs. at room temp. $CHBr_3$ formed was then distilled off, 1 g. of $NaHSO_3$ in H_2O was added to the residual soln., and the solution was acidified with HCl, when colorless minute needles separated, which were collected on a filter, washed, and dried to give 0.47 g. of solid of m.p. 254~258° (yield, 62%). When purified from hot H_2O, this formed colorless needles of m.p. 256~259°, which was not depressed on admixture with the authentic sample prepared as mentioned below.

ii) From (Vb): 0.1 g. of (Vb) was treated with Ag_2O, prepared from 0.26 g. of $AgNO_3$ and 0.12 g. of NaOH dissolved in 2 cc. of H_2O. The mixture was allowed to stand for some time with occasional shaking, when the solid (Vb) passed into soln. After being allowed to stand overnight at room temp., the reaction mixture was worked up as usual and there were obtained minute crystalline needles in a yield of 0.08 g. or 72%, which melted at 254~258°. The m.p. was raised to 256~259° by purifying from hot H_2O, which remained unchanged on admixture with the authentic sample.

iii) From methyl isonicotinate.: This furnished an authentic specimen of (VII). The ester (6.6 g.) was quaternized with Me_2SO_4 (7 g.) and the quaternary salt obtained was oxidized with 50 g. of $K_3Fe(CN)_6$ and 28 g. of KOH in aq. soln. as usual. The reaction mixture was acidified with HCl to precipitate crystals, which were filtered and dissolved in 10% NaOH. To the resultant solution was introduced H_2S-gas, separating FeS which was filtered off. The clear solution thus obtained was again acidified with HCl to furnish colorless needles (VII) of m.p. 256~259° in a yield of 5.5 g (75%). *Anal.* Calcd. for $C_7H_7O_3N$: C, 54.9; H, 4.6; N, 9.15. Found: C, 55.0; H, 4.55; N. 9.4.

Summary

The preparation of 4-ethyl- and 4-methyl-2(1*H*)-pyridones from 4-acetyl- and 4-formylpyridine, respectively, was described. Thus the 4-acylpyridines were converted to their etheylene ketal derivatives by the standard method, which were then treated with dimethyl sulfate to give the corresponding 1-methylpyridinium salts. The quaternary salts thus ob-

5) S. Sugasawa, K. Mizukami: This Bulletin, **2**, 341 (1954).
6) R. Adams, A. W. Schrecker: J. Am. Chem. Soc., **71**, 1186 (1949).

tained underwent smooth oxidation by alkaline potassium ferricyanide to furnish the 2(1*H*)-pyridones, from which 4-acetyl and 4-formyl compounds were recovered through mild acid hydrolysis, and were then reduced according to Huang-Minlon, thus yielding 4-ethyl- and 4-methyl-2(1*H*)-pyridones in good yields. Yields were good throughout the entire series of reactions.

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122. Satoru Kuwada, Toru Masuda, Toyokazu Kishi, and Mitsuko Asai: Application of Chromatography. XXXVI.* Biosynthesis of Riboflavin. (2).** Riboflavin-synthesizing Enzyme extracted from *Eremothecium ashbyii*.

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The assumption that production of riboflavin by *Er. ashbyii* probably goes through 6,7-dimethylribolumazine came from the fact that one of the authors (T.M.) once prepared riboflavin by the *in vitro* reaction of this lumazine derivative with diacetyl or acetoin. Katagiri, *et al.*[1] thereafter confirmed the formation of riboflavin from the 6,7-dimethylribolumazine sent from the authors, using a cell-free extract of *E. coli* and resting cells of the acetone·butanol-producing microörganism (*Clostridium acetobutyricum*), as well as a solution of the crude enzyme extracted from *Er. ashbyii*, confirming the assumption to a certain extent. They reported, however, that while addition of pyruvic acid in their reaction increased the formation of riboflavin, addition of diacetyl did not cause such a phenomenon. Of course, it is natural to think that a reaction *in vitro* does not necessarily take the same course *in vivo*, but as the components of the crude enzyme of *Er. ashbyii* were considered to be varied depending on the method of preparing the enzyme, attempts were made to reëxamine the result of Katagiri, *et al.*, and some new findings were obtained which are presented in this report.

Experimental

1) Preparation of a Solution of the Crude Enzyme of *Er. ashbyii*—*Er. ashbyii*** was cultivated in the peptone medium described in the previous paper* for 64 hrs. with shaking and the filtered mycelium was lyophilized. Five grams of the lyophilized mycelium was thawed in a mortar and ground with 7 g. of alumina (W-800, commercial preparation of Wako Pure Chemicals, Ltd.) and 20 cc. of Sörensen-Palitzsch's phosphate buffer (pH 7.0) for 15~30 mins. under ice-cooling. The mixture thus prepared was centrifuged for 30 mins., at $6.7\times10^3 g$, in the International Refrigerated Centrifuge, the supernatant was dialyzed in a cellophane tube against phosphate buffer (pH 7.0) in the cold, and finally the content of the tube was diluted to 50 cc. with the same buffer solution. The crude enzyme solution was freshly prepared in this manner immediately before each experiment and the riboflavin contained in each lot was determined to obtain the blank test value for subsequent experiments. The content of the whole protein in the solution, as determined by the Folin's method, was ca. 300 γ/cc. when calculated as N (serum albumin was used as the standard).

2) Effect of the Crude Enzyme Solution on 6,7-Dimethylribolumazine—A 0.2-cc. portion of a $2\times10^{-3}M$ aqueous solution of 6,7-dimethylribolumazine was placed in a brown micro-test tube, 0.2 cc. of the above-mentioned crude enzyme solution was added, and incubated at 37° for 1 hr. The tube was immersed in a boiling water for 10 mins. to interrupt the reaction and the content was applied in a straight line on Toyo Roshi No. 5B filter paper, 47×40 cm., and developed with EtOH·BuOH·H_2O

* Part XXXV: This Bulletin, **6**, 523(1958).

** "Biosynthesis of Riboflavin by *Er. ashbyii*" (This Bulletin, **5**, 136(1957)) by T. Masuda is considered as Part (1) of this work.

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1) H. Katagiri, *et al.*: Vitamins (Japan), **12**, 480(1957); **14**, 164, 695, 702(1958).