The prostaglandin B compounds formed by alkaline treatment were found to inhibit PGDH, noncompetitively.\textsuperscript{11} These inhibitor constants were fairly high, about 210 $\mu$M, which is about 25 times that of $K_m$ of PGF\(_1\). In our method, it was preferable to measure the total prostaglandin (prostaglandin E + prostaglandin F) and each prostaglandin ranging from 10 ng to 50 ng. In this range, the maximum amount of prostaglandin B, formed from prostaglandin E by alkaline treatment, may be about 40 ng. Calculating from the inhibitor constant of prostaglandin B, the prostaglandin B in this concentration may not hinder this enzymic assay, practically.

The proposed method is a modified one for the determination of prostaglandins by the combination of the method of Ånggård, \textit{et al.}\textsuperscript{71} with the enzymic assay of NADH by Guilbault and Kramer.\textsuperscript{12} In their method, the continuous reduction of NAD in the presence of an excess of ethanol and ADH was coupled with resazurin and diaphorase. Their method is more sensitive than the determination of NADH from spectrophotometric measurement of reduced cytochrome c\textsuperscript{13} or of dichlorophenol-indophenol.\textsuperscript{14}

Compared with the method of Ånggård, \textit{et al.},\textsuperscript{71} the present method is more convenient because of simplifying an enzymic cycling system, and more sensitive since the fluorescence of final product, resoruvin, is more intensive than of NADH. Further, it is capable for separatory determination of PGE and PGF.

\textbf{Acknowledgements} The authors wish to express their appreciation to Ono Pharmaceutical Industries, LTD. for presentation of prostaglandins and PGDH.


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\textbf{Reductive Cyclization of o-Nitrobenzylideneacetylacetone Anallogues}

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Reductions of o-nitrobenzylideneacetylacetone analogues (I, II and III) by means of stannous chloride in hydrochloric acid or catalytic hydrogenation of 5\% palladium carbon as catalyst gave the mixture of 3-acylquinaldines and these N-oxides, analysed by gas chromatographies.

Cyclization between aromatic nitro group and ortho side chain to synthesize heterocyclic compounds has widely been investigated by many workers.\textsuperscript{2)} Among them, Loudon and Tennant\textsuperscript{3--5)} reported that the o-nitrobenzylidene derivatives in inert solvent using hydrogen

\textsuperscript{1)} Location: 2-10-65, Kawai, Matsubara, Osaka.
\textsuperscript{2)} F.N. Preston and G. Tennant, \textit{Chem. Rev.}, 72, 627 (1972) and references cited therein.
halides or strongly basic catalyst such as potassium cyanide gave the 1-hydroxyquinolin-4(1H)-ones or quinoline N-oxides. Kametani, et al.,\(^6\) reported the novel synthesis of quinoline derivatives by treatment of ethyl 2-nitrobenzylidenemalonate with triethyl phosphite. In addition Coutts, et al.,\(^7\) have reported the synthesis of 4-hydroxy-3-methoxycarbonyl-2-methylquinoline 1-oxide by reductive cyclization by means of sodium borohydride-palladium charcoal of methyl α-nitrobenzoylacetoacetate. Hereby we wish to report the convenient synthesis of quinoline N-oxide derivatives by simple reductive cyclization of α-nitrobenzylideneacetylacetone analogues.

\[
\begin{align*}
\text{I} : & \quad R_1 = R_2 = H, \quad R_3 = CH_3 \\
\text{II} : & \quad R_1 = R_2 = OCH_3, \quad R_3 = CH_3 \\
\text{III} : & \quad R_1 = R_2 = H, \quad R_3 = C_4H_9
\end{align*}
\]

\[
\begin{align*}
\text{IV} & \quad R_1 = R_2 = H, \quad R_3 = CH_3 \\
\text{V} & \quad R_1 = R_2 = OCH_3, \quad R_3 = CH_3 \\
\text{VI} & \quad R_1 = R_2 = H, \quad R_3 = C_4H_9
\end{align*}
\]

\[\text{Chart I}\]

Reduction of nitrobenzylideneacetylacetone (I) by stannous chloride in hydrochloric acid\(^8\) afforded a mixture (41\% yield) of 3-acetylquinoidaline (IV), mp 78—79°, lit. 78°,\(^9\) and 3-acetylquinoidaline N-oxide (VII), which gave IV by treatment with phosphorus trichloride. The gas chromatography (GLC) showed the ratio of IV and VII to be approximately 5:95. Catalytic hydrogenation of I over 5\% palladium carbon as catalyst gave a mixture of IV and VII (10:90) in 95\% yield. Analogously, reductions of nitropiperonylideneacetylacetone (II) and nitrobenzylidenbenzoylacetonc (III) gave the corresponding quinoline (V and VI, mp 68—69°\(^{10}\)) and quinoline N-oxide (VIII and IX) derivatives shown in Table I.

### Table I. Ratio of Products of Reductive Cyclisation

<table>
<thead>
<tr>
<th>Reduction condition</th>
<th>Yield (%)</th>
<th>Ratio of products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quinolines</td>
<td>Quinoline N-oxides</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (A) SnCl₂-HCl</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>B (B) 5% Pd-C/H₂</td>
<td>95</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>94</td>
<td>14</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>34</td>
<td>41</td>
</tr>
<tr>
<td>B</td>
<td>88</td>
<td>45</td>
</tr>
</tbody>
</table>

Expectedly the fact that in the case of I and II having two acetyl groups on the side chain the overwhelmingly predominant product was the N-oxides, whereas in III having one acetyl and one benzoyl groups the presence of VI and IX was in the ratio of approximately 1:1 clearly shows that the initially formed intermediate hydroxylamine attacks preferentially acetyl carbonyl in relation to cis configuration in former cases and the reduction of nitro group beyond the intermediate hydroxylaminostage happened preferentially in the latter case.

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**Experimental**

**General Method of Reductive Cyclization**

a) Stannous Chloride in Hydrochloric Acid: To a solution of stannous chloride dihydrate (20 g) in conc. HCl (70 ml) was added diketone (0.019 mole). After stirring for 15 hr at room temperature, the mixture was cooled to 0° and made alkaline by NaOH, under vigorous stirring and maintaining the temperature at 0°-5°. The basic solution was stirred for additional 1.5 hr and filtered. The collected pasty residue was dried in vacuum desiccator, combined with sand and extracted with CHCl₃ in a Soxhlet apparatus for 6 days. The CHCl₃ was evaporated to give a crystalline mixture of quinoline and its N-oxide derivatives, which were separated by fractional recrystallization with a suitable solvent.

b) Catalytic Hydrogenation over 5% Pd-C: A solution of diketone (2 g) in MeOH (200 ml) was hydrogenated over 5% Pd-C (0.5 g) under atmospheric pressure and room temperature. After the end of the uptake of hydrogen (required for 4—5 hr), the catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The crystalline residue was treated in the same manner as above.

3-Acetylquinoline N-Oxide (VII), mp 140—140.5° (from ligroin). IR ν_{max} cm⁻¹: 1697 (CO). UV λ_{max} μm (log e): 240 (4.29), 255 (4.35), 325 (3.89). NMR (CDCl₃) δ: 2.70 (3H, s, C₆H₃CH₃), 2.83 (3H, s, COCH₃), 8.78 (1H, d of d, J=8 and 3 Hz, C₆H₃-H). Mass Spectrum m/e: 201 (M⁺); 184 (M²-OH). Anal. Calcd. for C₁₃H₁₂O₂N: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.62; H, 5.60; N, 7.23.

3-Acetyl-6,7-methylenedioxyquinoline N-Oxide (VIII), mp 201—203° (from MeOH). IR ν_{max} cm⁻¹: 1700 (CO). UV λ_{max} μm (log e): 253 (4.55), 255 (4.55), 324 (3.91). NMR (CDCl₃) δ: 2.67 (3H, s, C₆H₃CH₃), 2.80 (3H, s, COCH₃), 6.18 (2H, s, OCH₂O), 7.10 (1H, s, C₆H₃-H), 7.80 (1H, s, C₆H₃-H), 8.10 (1H, s, C₆H₃-H). Anal. Calcd. for C₁₃H₁₃O₂N: C, 63.67; H, 4.52; N, 5.71. Found: C, 63.59; H, 4.49; N, 5.79.

3-Benzylquinoline N-Oxide (IX), mp 258—261° (from benzene-ligroin). IR ν_{max} cm⁻¹: 1690 (CO). UV λ_{max} μm (log e): 234 (4.43), 270 (4.31), 331 (3.74). NMR (CDCl₃) δ: 1.97 (3H, s, C₆H₃CH₃), 8.82 (1H, d of d, J=8 and 3 Hz, C₆H₃-H). Mass Spectrum m/e 263 (M⁺). Anal. Calcd. for C₁₃H₁₂O₂N: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.84; H, 4.95; N, 5.33.

**3.4-Methylenedioxy-6-nitrobenzylidenecycloacetone (II)** After a suspended solution of 6-nitropiperonal (5 g) and acetylacetone (2.57 g) in EtOH (30 ml) containing five drops of piperidine was stirred for 2 days, the precipitate was collected by filtration, washed with cold EtOH, and recrystallized from EtOH to give a yellow needles (6.18 g), mp 115—116°. IR ν_{max} cm⁻¹: 1700, 1670 (CO). UV λ_{max} μm (log e): 245 (4.23), 273 (4.19), 347 (3.83). NMR (CDCl₃) δ: 2.17 and 2.48 (each 3H, each s, 2×COCH₃), 6.20 (2H, s, OCH=O), 7.70 (1H, s, CH=CH₂). Anal. Calcd. for C₁₅H₁₄O₂N: C, 56.32; H, 4.00; N, 5.05. Found: C, 56.12; H, 4.12; N, 4.89.

**Acknowledgement** The authors are grateful to Drs. S. Matsunaga and A. Numata for the measurements of Mass and NMR spectra and to Mrs. H. Takeda for the elemental analyses.

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11) All melting points were uncorrected. The IR and UV spectra were taken with JASCO Model IRA-1 and Shimadzu UV-200 spectrophotometers. NMR spectra were taken with Varian A-60D Spectrometer using tetramethylsilane as the internal standard. Mass spectra were taken with Hitachi Mass Spectrometer RMU-7L. Gas chromatographies were measured on Shimadzu 4-BMPF gas chromatograph, employing SE-30 column.