TABLE I. Increase in Specific Activity of Calcium-Binding Substance with Fractionation\(^a\)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Specific activity(^b)</th>
<th>Relative specific activity</th>
<th>Calcium-binding activity (% recovery/step)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heated supernatant</td>
<td>0.89</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>Sephadex G-75 (superfine)</td>
<td>10.62</td>
<td>11.9</td>
<td>50.2</td>
</tr>
<tr>
<td>Sephadex G-50 (superfine)</td>
<td>13.00</td>
<td>14.6</td>
<td>41.5</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>247.69</td>
<td>278.3</td>
<td>17.2</td>
</tr>
</tbody>
</table>

\(^a\) Fractions were assayed by the Caelex method described in text; for each chromatogram, the activity in the peak tube was used for the calculation of specific activity. The percentage recovery per step is based on each preceding step.

\(^b\) As % \(^{45}\)Ca bound/mg of protein.

binding substance in rat liver has a specific calcium binding activity comparable to that of the calcium binding protein isolated from rat intestinal mucosa.\(^b\) What possible physiologic role the calcium binding substance may play in the liver remains to be investigated. It seems likely that the calcium binding substance is involved in calcium transport in the liver cells.

Studies on the Syntheses of Heterocyclic Compounds. DCCLV.\(^1\) A Novel Method for Acetalisation of Formyl Group at the C\(_2\)-Position of 2,3-Dihydro-1H-pyrrrolo[1,2-\(a\)]indole Skeleton

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Reaction of 2,3-dihydro-7-methoxy-6-methyl-1H-pyrrrolo[1,2-\(a\)]indole-9-carboxaldehyde (2), (3), (4), (5), and (6) with thiolacetic acid in the presence of 6\(n\) sulphuric acid at room temperature gave 9-diacetylthiocinnamyl-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrrolo[1,2-a]indoles (12), (13), (14), (15), and (16), respectively. The same reaction of the compound (2) at 0° afforded 1-acetoxy-9-diacetylthiophenyl-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrrolo[1,2-\(a\)]indole (11). Successive treatment of the compound (11) with absolute methanol in the presence of sodium methoxide gave 2,3-dihydro-1-hydroxy-7-methoxy-9-dimethoxyethyl-6-methyl-8-nitro-1H-pyrrrolo[1,2-\(a\)]indole (17).

Keywords—acetalisation; 1H-pyrrrolo[1,2-\(a\)]indole-9-carboxaldehydes; thioacetic acid; diacetylthiolation; mitomycins

Regarding the synthesis of the mitomycins\(^b\) it is necessary to develop a general method for the protection of a formyl group at the C\(_3\)-position of an indole skeleton, because the formyl

2) Location: Aobayama, Sendai 980, Japan.
group of 1-acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde (2) seems to be a proper substituent for the synthesis of mitomycins (1).

The difficulties were encountered in the preliminary experiment for the acetalisation of the compound (2) by using a usual method, namely by heating a solution of 2, ethylene glycol, and p-toluenesulphonic acid in benzene. The compound isolated mainly was not the acetalised one (8), but the compound (7).

Thus, our attention was turned to explore an effective pathway for the acetalisation. Thioacetic acid has been known as a strong nucleophile which causes a 1,4-addition to \( \alpha, \beta \)-unsaturated carbonyl compound\(^4\) and displacement of halogen.\(^5\) On the other hand, N-acetyl-2-acetyltio-3-chloroalanine methyl ester (9) has been transformed into 2-methoxyalanine (10) by the reaction with sodium methoxide in absolute methanol.\(^6\) In our case,

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once the acetylthiomethyl compound (11) is obtained, successive transformation of 11 to the dimethylocetal (17) would be anticipated to proceed readily under the same conditions for the compound (9) because of its vinylogous character of 9.

At first, the carboxaldehydes (2), (3), (4), (5), and (6) were treated with thioacetic acid in the presence of 6 N sulphuric acid under the conditions shown in Table I and the experimental section for each compound (11), (12), (13), (14), (15), and (16) in high yield, respectively.

Acetoxyl group at the C₄-position of the compound (2) and (5) was substituted by acetylthio group to give the compounds (12) and (15) when the reaction was run at room temperature.

Thus we could demonstrate that the diacetylthiolation of 1H-pyrrole[1,2-a]indole-9-carboxaldehyde proceeds smoothly and in a very high yield, and therefore successive conversion of the diacetylthiolated compounds into the acetal was studied.

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Reaction time (hr)</th>
<th>Reaction temperature (°C)</th>
<th>Productb</th>
<th>mp (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>28</td>
<td>0</td>
<td>11</td>
<td>218.5—220</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Room temp.</td>
<td>12</td>
<td>200—201</td>
<td>84</td>
</tr>
<tr>
<td>3(ab)</td>
<td>1</td>
<td>Room temp.</td>
<td>13</td>
<td>175—176</td>
<td>89</td>
</tr>
<tr>
<td>4(ab)</td>
<td>6</td>
<td>Room temp.</td>
<td>14</td>
<td>219—220</td>
<td>92</td>
</tr>
<tr>
<td>5(ab)</td>
<td>2</td>
<td>Room temp.</td>
<td>15</td>
<td>134—135</td>
<td>87</td>
</tr>
<tr>
<td>6(ab)</td>
<td>0.5</td>
<td>Room temp.</td>
<td>16</td>
<td>141—142</td>
<td>89</td>
</tr>
</tbody>
</table>

a) All the reactions were performed in a current of nitrogen.
b) All the products were recrystallized from absolute methanol.

The diacetylthiomethyl compounds (11) was treated with absolute methanol in the presence of sodium methoxide at room temperature and it was found that the reaction occurred very readily to give the dimethyl acetal (17) in a quantitative yield, which lacked a carbonyl absorption in its infrared (IR) spectrum and showed the signals due to two methoxyl groups of dimethylocetal as singlet at 3.20 and 3.42 and signal due to methine proton of dimethylocetal as singlet at 5.65 ppm.

Thus, a novel method for the diacetylthiolation of formyl group at the C₄-position of indole skeleton was developed and successive transformation of the diacetylthiomethyl compound (11) into 17 was found to proceed very readily. The transformation of the diacetylthiomethyl compounds (13) and (16) into the acetalised compounds (19) and (20) was also carried out under the same conditions for the compound (11) and it was found that the only compounds isolated were the aldehydes (3) and (6), respectively, which seemed to be formed from the hydrolysis of 19 and 20. In fact, the acetalised compound (17) was found to be hydrolysed very easily on standing its solution in chloroform containing a trace amount of water or contacting with silica gel to give the aldehyde (18).

![Chart 4](image)

Thus, we could develop a general method for the transformation of formyl group at the C₄-position of indole skeleton into diacetylthiomethyl group and apply this method to the preparation of the acetal (17) from the diacetylthiomethyl compound (11).
Experimental

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole (7)—A mixture of the aldehyde (2) (166 mg), ethylene glycol (32 mg), and a catalytic amount of p-toluenesulphonic acid in benzene (50 ml) was heated for 15 hr under reflux. After cooling to room temperature, this reaction mixture was washed with sat. NaHCO₃ solution, water, and brine, and dried over Na₂SO₄. Evaporation of the solvent gave a tar, which was separated by column chromatography on silica gel (6 g) eluting with benzene to give a yellow waxy crystalline mass. Recrystallisation from ethanol gave 7 as pale yellow needles (37 mg, 24%), mp 98–99°. Anal. Calcd. for C₂₃H₂₃N₂O₅: C, 50.20; H, 5.30; N, 9.21. Found: C, 50.41; H, 5.40; N, 9.11. IR ν max cm⁻¹: 1735 (C=O). NMR (CDCl₃) δ: 6.97 (1H, s, ArH), 6.30 (1H, s, 2-H), 5.87 (1H, dd, J= 6.8 and 2.8 Hz, 1-H), 4.30 (2H, distorted t, 3-H), 3.73 (3H, s, OCH₃), 3.3–2.3 (2H, m, 2-H₂), 2.25 (3H, s, ArCH₃), 1.91 (1H, s, CH₂COO). MS m/z: 304 (M⁺).

Preparation of Acetylated Compounds—The general procedure is as follows: To a solution or a suspension of the aldehyde (0.1–2 mmol) in thioacetic acid (2–10 ml) was added 6 N sulphuric acid (1–5 ml) and stirred under the conditions shown in Table for each compound. The resulting mixture was diluted with cold water and extracted with CHCl₃. The CHCl₃ extract was washed with water, sat. NaHCO₃ solution, water, and brine, and dried over Na₂SO₄. Evaporation of the solvent gave the acetylated compound.

1-Acetoxy-9-diacyethylmethimethyl-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole (11)—From the aldehyde (2) (332 mg), thioacetic acid (5 ml), and 6 N sulphuric acid (2.5 ml), 11 (430 mg, 92%) was obtained as pale yellow needles. Anal. Calcd. for C₂₃H₂₁N₂O₅: C, 51.49; H, 4.75; N, 6.01. Found: C, 51.24; H, 4.55; N, 6.31. IR ν max cm⁻¹: 1735 and 1685 (C=O), 1520 and 1368 (NO₂). NMR (CDCl₃) δ: 7.18 (1H, s, ArH), 6.67 (1H, s, CH(SOCH₃)), 6.52 (1H, dd, J= 6.4 and 3.2 Hz, 1-H), 4.15 (2H, distorted t, 3-H), 3.88 (3H, s, OCH₃), 3.4–2.5 (2H, m, 2-H₂), 2.42 (6H, s, ArCH₃), 2.27 (6H, s, 2×CH₂COS), 2.13 (3H, s, CH₂COO). MS m/z: 466 (M⁺).

1-Acetylthio-9-diacyethylmethimethyl-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole (12)—From the aldehyde (2) (332 mg), thioacetic acid (5 ml), and 6 N sulphuric acid (2.5 ml), 12 (405 mg, 84%) was obtained as pale yellow needles. Anal. Calcd. for C₂₃H₂₁N₂O₅: C, 49.78; H, 4.59; N, 5.80. Found: C, 49.79; H, 4.69; N, 6.19. IR ν max cm⁻¹: 1695 (C=O), 1530 and 1358 (NO₂). NMR (CDCl₃) δ: 7.14 (1H, s, ArH), 6.56 (1H, s, CH(SOCH₃)), 5.44 (1H, dd, J= 7.2 and 4.0 Hz, 1-H), 4.13 (2H, t, J= 6 Hz, 3-H), 3.87 (3H, s, OCH₃), 3.4–2.5 (2H, m, 2-H₂), 2.37 (6H, s, 2×CH₂COS), 2.30 and 2.27 (each 3H, s, CH₂COO). 9-Diacetylthiomethyl-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole (13)—From the aldehyde (3) (55 mg), thioacetic acid (2 ml), and 6 N sulphuric acid (1 ml), 13 (73 mg, 80%) was formed as pale yellow needles. Anal. Calcd. for C₂₃H₂₁N₂O₅: C, 52.93; H, 4.93; N, 6.80. Found: C, 52.91; H, 4.81; N, 6.71. IR ν max cm⁻¹: 1690 (C=O). NMR (CDCl₃) δ: 7.06 (1H, s, ArH), 6.61 (1H, s, CH(SOCH₃)), 4.00 (2H, t, J= 6.6 Hz, 3-H), 3.85 (3H, s, OCH₃), 3.22 (2H, t, J= 6.6 Hz, 1-H₂), 2.69 (2H, distorted q, 2-H₂), 2.30 (3H, s, ArCH₃), 2.27 (6H, s, 2×CH₂). MS m/z: 408 (M⁺).

9-Diacetylthiomethyl-2,3-dihydro-7-methoxy-6-methyl-1-oxo-1H-pyrrolo[1,2-a]indole (14)—From the aldehyde (4) (486 mg), thioacetic acid (10 ml), and 6 N sulphuric acid (5 ml), 14 (605 mg, 92%) was yielded as pale yellow needles. Anal. Calcd. for C₂₃H₂₁N₂O₅: C, 57.29; H, 5.08; N, 3.71. Found: C, 57.65; H, 5.19; N, 3.70. IR ν max cm⁻¹: 1700 (C=O). NMR (CDCl₃) δ: 7.28 (1H, s, ArH), 7.05 (1H, s, ArH), 6.84 (1H, s, CH(SOCH₃)), 4.28 (2H, t, J= 6.0 Hz, 2-H₂), 3.95 (3H, s, OCH₃), 3.13 (2H, t, J= 6.0 Hz, 2-H₂), 2.31 (3H, s, ArCH₃), 2.25 (6H, s, CH₂). MS m/z: 377 (M⁺).

1-Acetylthio-9-diacyethylmethimethyl-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole (15)—From the aldehyde (5) (575 mg), thioacetic acid (10 ml), and 6 N sulphuric acid (5 ml), 15 (762 mg, 87%) was obtained as colourless needles. Anal. Calcd. for C₂₃H₂₂N₂O₅S: C, 54.90; H, 5.30; N, 3.20. Found: C, 54.61; H, 5.11; N, 3.11. IR ν max cm⁻¹: 1690 (C=O). NMR (CDCl₃) δ: 7.03 (1H, s, ArH), 6.98 (1H, s, ArH), 6.64 (1H, s, CH(SOCH₃)), 5.38 (1H, dd, J= 7.8 and 3.1 Hz, CH2SOCH₃), 4.03 (2H, distorted t, 3-H), 3.92 (3H, s, OCH₃), 3.5–2.5 (2H, m, 2-H₂), 2.41, 2.38, 2.30, and 2.26 (each 3H, s, ArCH₃ and 3×CH₂).

9-Diacetylthiomethyl-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole (16)—From the aldehyde (6) (46 mg), thioacetic acid (2 ml), and 6 N sulphuric acid (1 ml), 16 (65 mg, 89%) was yielded as colourless plates. Anal. Calcd. for C₂₃H₂₂N₂O₅S: C, 59.49; H, 5.83; N, 3.86. Found: C, 59.87; H, 5.84; N, 3.93. IR ν max cm⁻¹: 1695 (C=O). NMR (CDCl₃) δ: 6.98 (1H, s, ArH), 6.93 (1H, s, ArH), 6.52 (1H, s, CH(SOCH₃)), 3.95 (2H, t, J= 7.2 Hz, 3-H), 3.91 (3H, s, OCH₃), 3.3–2.4 (4H, m, 1- and 2-H₂), 2.29 (9H, s, 3×CH₃).

2,3-Dihydro-1-hydroxy-7-methoxy-9-dimethoxymethyl-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole (17)—To a yellow stirred suspension of the diacetylthiomethylate (11) (23 mg) in absolute methanol (3 ml) was

7) All melting points are uncorrected and were measured with a Yanagimoto micro melting point apparatus (MP-22). IR spectra were measured with a Hitachi 215 grating spectrophotometer, NMR spectra with a JEOL PMX spectrorometer with (CD₃)₂SO as an internal standard, mass spectra with a Hitachi RMU-7 spectrometer.
added one part (0.15 ml) of sodium methoxide solution, prepared from sodium metal (230 mg) and absolute methanol (10 ml) at 0°, and the resulting mixture was stirred at room temperature for 40 hr. Evaporation of the solvent at room temperature under the reduced pressure gave a syrup, which was extracted with CHCl₃. The CHCl₃ extract was washed with water, dried over Na₂SO₄, and evaporated to give the acetal (17) (16 mg, 96.5%). IR ν(C=O) cm⁻¹: 1375 (NO₂). NMR (CDCl₃) δ: 7.15 (1H, s, ArH), 5.65 (1H, s, C(=O)CH₃), 5.55 (1H, distorted q, 1-H), 4.15 (2H, distorted q, 3-H₂), 3.86 (3H, s, ArOCH₃), 3.42 and 3.20 (each 3H, s, 2×OCH₃), 3.2—2.5 (2H, m, 2-H₂), 2.42 (3H, s, ArCH₃). MS m/z: 288 (M⁺ —OMe—OH).

2,3-Dihydro-1-hydroxy-7-methoxy-6-methyl-8-nitro-1H-pyrrole[1,2-a]indole-9-carboxaldehyde (18)—To a suspension of the aldehyde (2) (1.1 g) in methanol (20 ml) was added dropwise 10% methanolic KOH solution (2.2 ml) and stirred for 30 min at room temperature. Then, the solvent was concentrated and extracted with CHCl₃. The CHCl₃ extract was washed with water and brine, and dried over Na₂SO₄. Evaporation of the solvent gave pale brownish needles, which were recrystallised from methanol to give the aldehyde (18) as colourless needles (904 mg, 94%), mp 188.5—186.5°. Anal. Calcd. for C₁₄H₁₄N₂O₄: C, 57.93; H, 4.86; N, 9.65. Found: C, 57.88; H, 4.80; N, 9.83. IR ν(C=O) cm⁻¹: 1630 (C=O), 1520 and 1355 (NO₂). NMR (CDCl₃) δ: 9.74 (1H, s, CHO), 7.26 (1H, s, 5-H), 5.52 (1H, t, J = 7.0 Hz, 1-H), 4.74 (1H, s, OH, D₂O exchangeable), 4.5—4.1 (2H, m, 3-H₂), 3.87 (3H, s, OCH₃), 3.4—2.5 (2H, m, 2-H₂), 2.42 (3H, s, ArCH₃).

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Studies on the Syntheses of Heterocyclic Compounds. DCCLV.⁵ Iminoketene Cycloaddition. (4)⁵ Alternative Syntheses of 5,6,7,8-Tetrahydro-2,3-dimethoxy-8-oxoisooquinolo[1,2-b]quinazoline and Rutecarpine

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Treatment of the sulfanamide anhydride (2), prepared from anthranilic acid (1), with 3,4-dihydro-6,7-dimethoxysuccinimide (9) gave 5,6,7,8-tetrahydro-2,3-dimethoxy-8-oxoisooquinolo[1,2-b]quinazoline (10), which was also obtained by heating isatoic anhydride (3) with 9. Similarly, rutecarpine (6) was synthesised by the reactions of 1,2,3,4-tetrahydro-1-keto-β-carbolene (13) with the sulfanamide anhydride (2) or isatoic anhydride (3). Heating 3,4-dihydro-β-carbolene (5) with 3 also afforded rutecarpine (6). Furthermore, the phosphites (8a) and (12) were isolated on treatment of the urethanes (7a) and (11) with phosphoryl chloride.

Keywords—5,6,7,8-tetrahydro-2,3-dimethoxy-8-oxoisooquinolo[1,2-b]quinazolone; rutecarpine; iminoketene cycloaddition; sulfanamide anhydride; isatoic anhydride

We have recently reported total syntheses of rutecarpine, evodiamine and other quinazolinone alkaloids by a cycloaddition reaction of the iminoketene (4), generated in situ from anthranilic acid or N-methylanthranilic acid with thionyl chloride via sulfanamide anhydrides

3) Location: Aobayama, Sendai 980, Japan.