Table I. Increase in Specific Activity of Calcium-Binding Substance with Fractionation[a)]

Fraction	Specific activity[b)]	Relative specific activity	Calcium-binding activity (% recovery/step)
Heated supernatant	0.89	1.0	100
Sephadex G-75 (superfine)	10.62	11.9	50.2
Sephadex G-50 (superfine)	13.00	14.6	41.5
DEAE-cellulose	247.69	278.3	17.2

a) Fractions were assayed by the Chelex 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.[13)] 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.

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Studies on the Syntheses of Heterocyclic Compounds. DCCLIV.[1)] A Novel Method for Acetalisation of Formyl Group at the C_3-Position of 2,3-Dihydro-1H-pyrrolo[1,2-*a*]indole Skeleton

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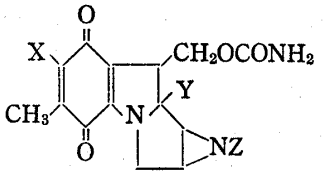
Reaction of 2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-*a*]indole-9-carboxaldehydes (**2**), (**3**), (**4**), (**5**), and (**6**) with thiolacetic acid in the presence of 6 N sulphuric acid at room temperature gave 9-diacetylthiomethyl-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-*a*]indoles (**12**), (**13**), (**14**), (**15**), and (**16**), respectively. The same reaction of the compound (**2**) at 0° afforded 1-acetoxy-9-diacetylthiomethyl-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[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-dimethoxymethyl-6-methyl-8-nitro-1H-pyrrolo[1,2-*a*]indole (**17**).

Keywords——acetalisation; 1H-pyrrolo[1,2-*a*]indole-9-carboxaldehydes; thioacetic acid; diacetylthiolation; mitomycins

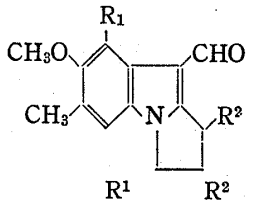
Regarding the synthesis of the mitomycins[3)] it is necessary to develop a general method for the protection of a formyl group at the C_3-position of an indole skeleton, because the formyl

1) Part DCCLIII: T. Kametani, C.V. Loc, M. Ihara, and K. Fukumoto, *Heterocycles*, 9, 863 (1978).
2) Location: *Aobayama, Sendai 980, Japan.*
3) *a*) T. Kametani, K. Takahashi, M. Ihara, and K. Fukumoto, *Heterocycles*, 3, 691 (1975); *b*) T. Kametani, K. Takahashi, M. Ihara, and K. Fukumoto, *J. Chem. Soc. Perkin I*, **1976**, 389; *c*) T. Kametani, T. Ohsawa, K. Takahashi, M. Ihara, and K. Fukumoto, *Heterocycles*, 4, 1637 (1976); *d*) T. Kametani, K. Takahashi, Y. Kigawa, M. Ihara, and K. Fukumoto, *J. Chem. Soc. Perkin I*, **1977**, 28.



1

	X	Y	Z
mitomycin A	CH_3O	CH_3O	H
mitomycin B	CH_3O	HO	CH_3
mitomycin C	NH_2	CH_3O	H
porfiromycin	NH_2	CH_3O	CH_3

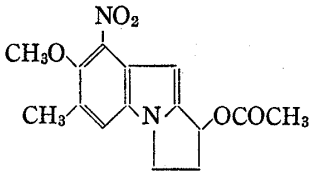


	R^1	R^2
2:	NO_2	$OCOCH_3$
3:	NO_2	H
4:	H	O=
5:	H	$OCOCH_3$
6:	H	H

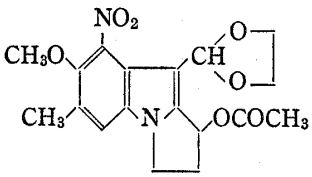
Chart 1

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**).



7



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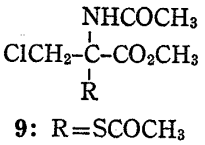
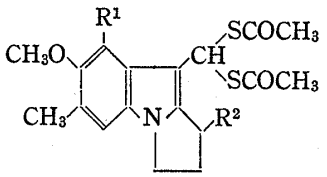


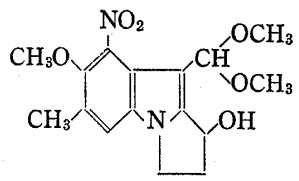
9: R=$SCOCH_3$
10: R=OCH_3

Chart 2

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 α,β-unsaturated carbonyl compound[4] and displacement of halogen.[5] On the other hand, N-acetyl-2-acetylthio-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,



	R^1	R^2
11:	NO_2	$OCOCH_3$
12:	NO_2	$SCOCH_3$
13:	NO_2	H
14:	H	O=
15:	H	$SCOCH_3$
16:	H	H



17

Chart 3

4) *a*) M.W. Bullock, J.A. Brockman, Jr., E.L. Patterson, J.V. Pierce, M.H. von Saltza, F. Sanders, and E.L.R. Stokstad, *J. Am. Chem. Soc.*, **76**, 1828 (1954); *b*) E. Walton, A.F. Wagner, F.W. Bachelor, L.H. Peterson, F.W. Holly, and K. Folkers, *ibid.*, **77**, 5144 (1955); *c*) R.M. Dodson and R.C. Tweit, *ibid.*, **81**, 1224 (1959); *d*) J.A. Cella and R.C. Tweit, *J. Org. Chem.*, **24**, 1109 (1959).
5) *a*) D. Horton and M. L. Wolfrom, *J. Org. Chem.*, **27**, 1794 (1962); *b*) S.M. Patel, J.O. Currie, Jr., and R.K. Olsen, *ibid.*, **38**, 126 (1973).
6) R.K. Olsen and A.J. Kolar, *Tetrahedron Lett.*, **1975**, 3579.

once the acetylthiomethyl compound (**11**) is obtained, successive transformation of **11** to the dimethylacetal (**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 C_1-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-pyrrolo[1,2-*a*]indole-9-carboxaldehydes proceeds smoothly and in a very high yield, and therefore successive conversion of the diacetylthiolated compounds into the acetal was studied.

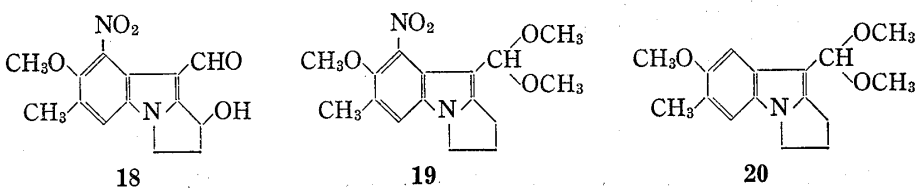
Table I. Diacetylthiolation of 1H-Pyrrolo[1,2-*a*]indole-9-carboxaldehyde Derivatives[a)]

Aldehyde	Reaction time (hr)	Reaction temperature (°C)	Product[b)]	mp (°C)	Yield (%)
2[c)]	28	0	**11**	218.5—220	92
2	9	Room temp.	**12**	200—201	84
3[3b)]	1	Room temp.	**13**	175—176	89
4[4d)]	6	Room temp.	**14**	219—220	92
5[4d)]	2	Room temp.	**15**	134—135	87
6[4d)]	0.5	Room temp.	**16**	141—142	89

a) All the reactions were performed in a current of nitrogen.
b) All the products were recrystallized from absolute methanol.
c) G. Leadbet"er, D.L. Fost, N.N. Ekwuribe, and W.A. Remers, *J. Org. Chem.*, **39**, 3580 (1974).

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 dimethyl acetal as singlet at 3.20 and 3.42 and signal due to methine proton of dimethyl acetal as singlet at 5.65 ppm.

Thus, a novel method for the diacetylthiolation of formyl group at the C_3-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**).



18 **19** **20**

Chart 4

Thus, we could develop a general method for the transformation of formyl group at the C_3-position of indole skeleton into diacetylthiomethyl group and apply this method to the preparation of the acetal (**17**) from the diacetylthiomethyl compound (**11**).

Experimental[7)]

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. $NaHCO_3$ solution, water, and brine, and dried over Na_2SO_4. Evaporation of the solvent gave a tar, which was separated by column chromatography on silica gel (6 g) eluting with benzene to give a yellowish crystalline mass. Recrystallisation from ethanol gave **7** as pale yellowish needles (37 mg, 24%), mp 98—99°. *Anal.* Calcd. for $C_{15}H_{16}N_2O_5$: C, 59.20; H, 5.30; N, 9.21. Found: C, 59.41; H, 5.40; N, 9.11. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (C=O). NMR (CCl_4) δ: 6.97 (1H, s, ArH), 6.30 (1H, s, 9-H), 5.87 (1H, dd, J=6.8 and 2.8 Hz, 1-H), 4.30 (2H, distorted t, 3-H_2), 3.73 (3H, s, OCH_3), 3.3—2.3 (2H, m, 2-H_2), 2.25 (3H, s, $ArCH_3$), 1.91 (1H, s, CH_3COO). MS *m/e*: 304 (M^+).

Preparation of Acetylthiolated 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 $CHCl_3$. The $CHCl_3$ extract was washed with water, sat. $NaHCO_3$ solution, water and brine, and dried over Na_2SO_4. Evaporation of the solvent gave the acetylthiolated compound.

1-Acetoxy-9-diacetylthiomethyl-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 6N sulphuric acid (2.5 ml), **11** (430 mg, 92%) was obtained as pale yellowish needles. *Anal.* Calcd. for $C_{20}H_{22}N_2O_7S_2$: C, 51.49; H, 4.75; N, 6.01. Found: C, 51.24; H, 4.55; N, 6.31. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 and 1685 (C=O), 1520 and 1368 (NO_2). NMR ($CDCl_3$) δ: 7.18 (1H, s, ArH), 6.67 (1H, s, C<u>H</u>$(SCOCH_3)_2$), 6.52 (1H, dd, J=6.4 and 3.2 Hz, 1-H), 4.15 (2H, distorted t, 3-H_2), 3.88 (3H, s, OCH_3), 3.4—2.5 (2H, m, 2-H_2), 2.42 (3H, s, $ArCH_3$), 2.27 (6H, s, $2\times CH_3COS$), 2.13 (3H, s, CH_3COO). MS *m/e*: 466 (M^+).

1-Acetylthio-9-diacetylthiomethyl-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 yellowish needles. *Anal.* Calcd. for $C_{20}H_{22}N_2O_6S_3$: C, 49.78; H, 4.59; N, 5.80. Found: C, 49.79; H, 4.69; N, 6.19. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1695 (C=O), 1530 and 1358 (NO_2). NMR ($CDCl_3$) δ: 7.14 (1H, s, ArH), 6.56 (1H, s, C<u>H</u>$(SCOCH_3)_2$), 5.44 (1H, dd, J=7.2 and 4.0 Hz, 1-H), 4.13 (2H, t, J=6 Hz, 3-H_2), 3.87 (3H, s, OCH_3), 3.4—2.5 (2H, m, 2-H_2), 2.37 (6H, s, $2\times CH_3$), 2.30 and 2.27 (each 3H, s, $2\times CH_3$).

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, 89%) was formed as pale yellowish needles. *Anal.* Calcd. for $C_{18}H_{20}N_2O_5S_2$: C, 52.93; H, 4.93; N, 6.80. Found: C, 52.91; H, 4.81; N, 6.71. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1690 (C=O). NMR ($CDCl_3$) δ: 7.06 (1H, s, ArH), 6.61 (1H, s, C<u>H</u>$(SCOCH_3)_2$), 4.00 (2H, t, J=6.6 Hz, 3-H_2), 3.85 (3H, s, OCH_3), 3.32 (2H, t, J=6.6 Hz, 1-H_2), 2.69 (2H, distorted q, 2-H_2), 2.30 (3H, s, $ArCH_3$), 2.27 (6H, s, $2\times CH_3$). MS *m/e*: 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** (695 mg, 92%) was yielded as pale yellowish needles. *Anal.* Calcd. for $C_{18}N_{19}NO_4S_2$: C, 57.29; H, 5.08; N, 3.71. Found: C, 57.65; H, 5.15; N, 3.70. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1700 (C=O). NMR ($CDCl_3$) δ: 7.28 (1H, s, ArH), 7.05 (1H, s, ArH), 6.84 (1H, s, C<u>H</u>$(SCOCH_3)_2$), 4.28 (2H, t, J=6.0 Hz, 3-H_2), 3.95 (3H, s, OCH_3), 3.13 (2H, t, J=6.0 Hz, 2-H_2), 2.31 (3H, s, $ArCH_3$), 2.25 (6H, s, CH_3). MS *m/e*: 377 (M^+).

1-Acetylthio-9-diacetylthiomethyl-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 $C_{20}H_{23}NO_4S_3$: C, 54.90; H, 5.30; N, 3.20. Found: C, 54.61; H, 5.11; N, 3.11. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1690 (C=O). NMR ($CDCl_3$) δ: 7.03 (1H, s, ArH), 6.96 (1H, s, ArH), 6.64 (1H, s, C<u>H</u>$(SCOCH_3)_2$), 5.38 (1H, dd, J=7.8 and 3.1 Hz, C<u>H</u>$SCOCH_3$), 4.03 (2H, distorted t, 3-H_2), 3.92 (3H, s, OCH_3), 3.5—2.5 (2H, m, 2-H_2), 2.41, 2.33, 2.30, and 2.26 (each 3H, s, $ArCH_3$ and $3\times CH_3$).

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 $C_{18}H_{21}NO_3S_2$: C, 59.49; H, 5.83; N, 3.86. Found: C, 59.87; H, 5.64; N, 3.93. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1695 (C=O). NMR ($CDCl_3$) δ: 6.98 (1H, s, ArH), 6.93 (1H, s, ArH), 6.52 (1H, s, C<u>H</u>$(SCOCH_3)_2$), 3.95 (2H, t, J=7.2 Hz, 3-H_2), 3.91 (3H, s, OCH_3), 3.3—2.4 (4H, m, 1- and 2-H_2), 2.29 (9H, s, $3\times CH_3$).

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 spectrometer with $(CH_3)_4Si$ 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 $CHCl_3$. The $CHCl_3$ extract was washed with water, dried over Na_2SO_4, and evaporated to give the acetal (**17**) (16 mg, 96.5%) as a brownish syrup. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1375 (NO_2). NMR ($CDCl_3$) δ: 7.15 (1H, s, ArH), 5.65 (1H, s, C<u>H</u>$(OCH_3)_2$), 5.35 (1H, distorted q, 1-H), 4.15 (2H, distorted q, 3-H_2), 3.86 (3H, s, $ArOCH_3$), 3.42 and 3.20 (each 3H, s, $2\times OCH_3$), 3.2—2.5 (2H, m, 2-H_2), 2.42 (3H, s, $ArCH_3$). MS *m/e*: 288 (M^+ −OMe−OH).

2,3-Dihydro-1-hydroxy-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[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 $CHCl_3$. The $CHCl_3$ extract was washed with water and brine, and dried over Na_2SO_4. 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 $C_{14}H_{14}N_2O_5$: C, 57.93; H, 4.86; N, 9.65. Found: C, 57.88; H, 4.80; N, 9.83. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1630 (C=O), 1520 and 1355 (NO_2). NMR ($CDCl_3$) δ: 9.74 (1H, s, C<u>H</u>O), 7.26 (1H, s, 5-H), 5.52 (1H, t, $J=7.0$ Hz, 1-H), 4.74 (1H, s, O<u>H</u>, D_2O exchangeable), 4.5—4.1 (2H, m, 3-H_2), 3.87 (3H, s, OCH_3), 3.4—2.5 (2H, m, 2-H_2), 2.42 (3H, s, $ArCH_3$).

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

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Treatment of the sulfinamide anhydride (**2**), prepared from anthranilic acid (**1**), with 3,4-dihydro-6,7-dimethoxyisocarbostyril (**9**) gave 5,6,7,8-tetrahydro-2,3-dimethoxy-8-oxoisoquinolo[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-carboline (**13**) with the sulfinamide anhydride (**2**) or isatoic anhydride (**3**). Heating 3,4-dihydro-β-carboline (**5**) with **3** also afforded rutecarpine (**6**). Furthermore the phosphates (**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-oxoisoquinolo[1,2-*b*]quinazolone; rutecarpine; iminoketene cycloaddition; sulfinamide 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* sulfinamide anhydrides

1) Part DCCLIV: T. Kametani, Y. Kigawa, K. Takahashi, H. Nemoto, and K. Fukumoto, *Chem. Pharm. Bull.* (Tokyo), **26**, 1918 (1978).
2) Part 3: T. Kametani, C.V. Loc, T. Higa, M. Ihara, and K. Fukumoto, *J. C. S. Perkin I*, **1977**, 2347.
3) Location: *Aobayama, Sendai 980, Japan.*