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Mitomycin Antibiotics. Synthesis and Activity of 1,2-Disubstituted Mitosenes

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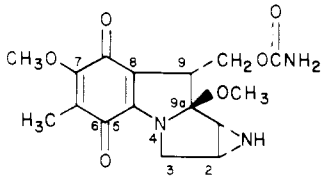
cis-1-Acetamido-2-acetoxy-7-methoxy-*N*-methylmitosene was prepared in 11 steps from 7-methoxy-6-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-one by a route involving bromination of the pyrrolidineenamine or trimethylsilyl enol ether of starting material, displacement of bromide by acetate, oxime formation, and reductive acetylation, followed by elaboration of the quinone and methyl carbamate functions according to previously established methods. An unsubstituted carbamate could not be prepared. The mitosene thus synthesized differs from previously reported 1,2-disubstituted mitosenes, which are derived from the solvolysis of mitomycins, in that it has the opposite arrangement of oxygen and nitrogen substituents at the 1 and 2 positions. It showed antibacterial activities in disk-plate assays superior to those of *cis*-diacetylapomitomycin A and equivalent to those of certain 1-substituted mitosenes; however, it was less active than mitomycin A in these assays. It was inactive in inducing λ-bacteriophage in *Escherichia coli* and inactive against P388 leukemia in mice. In contrast, certain 1-substituted mitosenes were active in prophage induction and **2b** and mitomycin A were active in both assays.

The mitosene family of compounds represents analogues (e.g., **2**) of the naturally occurring mitomycins in which the elements of methanol have been lost from the 9 and 9a positions.[1] Mitosenes are obtained from the mitomycins (for example, mitomycin A, 1) by procedures such as catalytic reduction followed by air oxidation[2] or acid-catalyzed solvolysis.[1,3] In addition, certain 1-substituted mitosenes have been prepared by total synthesis.[4] The biological activities of mitosenes depend upon substituents at the 7 position, which determine the ease of their reductive bioactivation,[5] and substituents at the 1 and 2 positions, which appear to involve binding to DNA.[6] Compounds such as 7-methoxymitosene (**2a**) and its 1-substituted analogues (e.g., **2c**) show antibacterial activities in culture, but they lack antitumor activity.[7] In contrast, 7-methoxyaziridinomitosene (**3**) has both antibacterial and antitumor activities, presumably because it is more reactive in binding to DNA after bioactivation.[5]



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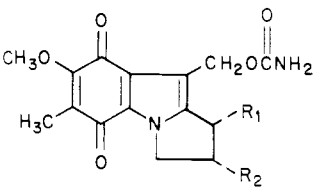
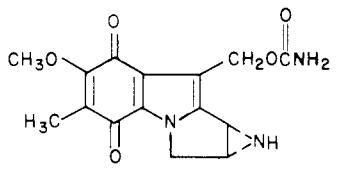


2a, $R_1 = R_2 = H$
b, $R_1 = OAc$; $R_2 = H$
c, $R_1 = OH$; $R_2 = H$
d, $R_1 = OH$; $R_2 = NH_2$
e, $R_1 = OH$; $R_2 = NHAc$
f, $R_1 = OAc$; $R_2 = NHAc$
g, $R_1 = OH$; $R_2 = NCH_3Ac$



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Biological activities of 1,2-disubstituted 7-methoxymitosenes such as the *cis*-2-amino-1-hydroxy compound (apomitomycin A, **2d**) and its *N*-acetyl derivative **2e** appear to be somewhere between those of the aziridinomitosene (**3**) and the 1-substituted analogues; however, they have not been thoroughly studied. Thus, antibacterial activities in culture have been briefly reported for 1,2-disubstituted mitosenes by several groups.[5,7] The activity of **2e** against experimental sarcomas in mice was reported,[8,9] but no data on its activity against P388 and L1210 leukemia or other tumors were given. However, diacetyl derivative **2f** and *N*-methyl analogue **2g** showed very poor activity against L1210 leukemia.[10]

Since our continuing effort in the synthesis of mitomycin analogues had resulted in a reliable route to 1-substituted mitosenes,[4] we sought to extend this kind of route to 1,2-disubstituted mitosenes. The main problem in this extension appeared to lie in functionalizing the 2 position with a substituent that was stable to the chemical reactions required to elaborate the quinone and carbamate groups and that would lead to useful biological activity. The target compound of our synthesis became 1-acetamido-2-acetoxy-7-methoxymitosene (**15**, no methyl group on the carbamate nitrogen). This compound has an arrangement of 1- and 2-substituents opposite to that found in mitomycin solvolysis products such as **2d** and **2f**.[3] Hence, it is particularly interesting for structure–activity relationships. Earlier studies on pyrrolo[1,2-*a*]indoles had shown that in the 7-benzyloxy series of compounds it was possible to prepare the oxime of a 2-acetoxy 1-ketone.[11] If this same type of compound (**12**) could be prepared in the 7-methoxy-6-methyl series and reduced to the corresponding acetylated aminohydrin (**7**), we felt that there was a good chance that a synthesis of **15** could be achieved (Scheme I).

Starting with tricyclic ketone **4**,[4,12] the corresponding 2-bromo derivative **10** was prepared by way of the pyrrolidineenamine **5** in yields of 0–68%. As previously shown in the 7-benzyloxy series, direct bromination of ketones like **4** produces the 9-bromo derivative;[13] therefore, it is essential to activate the 2 position by making the enamine. In agreement with our previous report[4] the 7-methoxy-6-methylpyrrolo[1,2-*a*]indoles were less stable than their 7-benzyloxy analogues and special reaction conditions had to be developed. Thus, acid catalysts could not be used in preparing enamine **5**. Furthermore, hydrolysis of the

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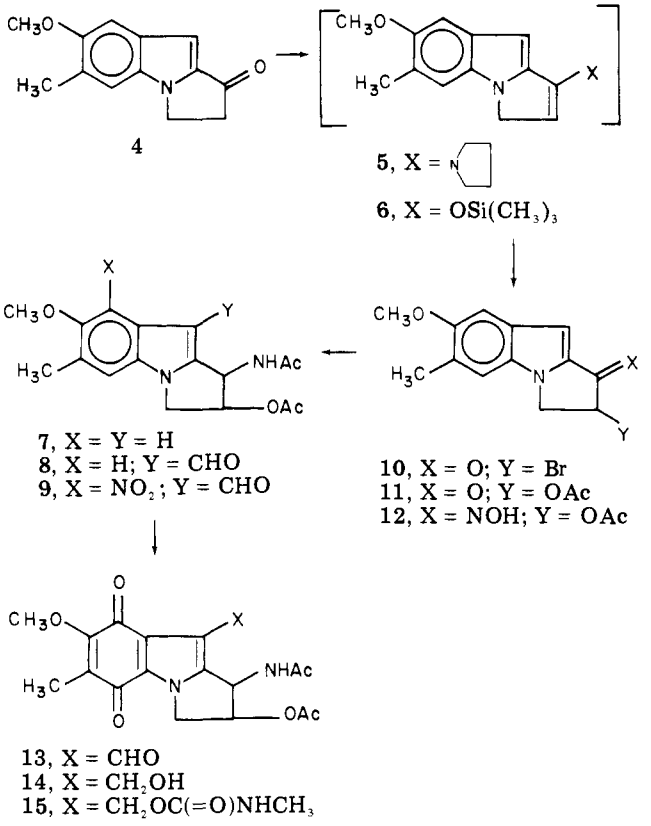
Scheme I



intermediate obtained upon treatment of **5** with *N*-bromosuccinimide required a special acetate buffer. Since the resulting procedures still gave erratic yields, we investigated the preparation of **10** by way of trimethylsilyl ether **6**. This route was successful, but the yield was only 7%.

Acetolysis of bromo ketone **10** went in good yield and oximation of the resulting acetoxy ketone **11** was straightforward. However, reduction of acetoxy oxime **12** presented difficulties. In the 7-benzyloxy series, reduction of the corresponding acetoxy oxime gave a mixture of a 1-acetamido-2-hydroxy compound, presumed to have cis stereochemistry because of the O → N acetyl migration, and an oxazolidine, also presumed to be cis.[11] Initial attempts at catalytic reduction of **12** gave unstable products which could not be characterized. When the reduction was run in the presence of acetic anhydride, the 1-acetamido-2-acetoxy compound **7** could be obtained in moderate yield. Analogy with the 7-benzyloxy series[11] suggests that **7** has cis stereochemistry. However, we obtained direct evidence on its cis stereochemistry by alkaline hydrolysis to a product containing no carbonyl groups, which afforded a cyclic carbamate (oxazolidinone) upon treatment with *N,N*-carbonyldiimidazole (Experimental Section).

Elaboration of the quinone ring and hydroxymethyl groups, affording **14**, was accomplished by procedures developed for 1-substituted mitosenes.[4] This involved in sequence (**7** → **8** → **9** → **13** → **14**) Vilsmeier–Haack formylation, nitration, iron-in-acetic acid reduction followed by potassium nitrosodisulfonate oxidation, and sodium borohydride reduction followed by ferric chloride oxidation. As previously encountered with 1-acetamido analogues, but not with 1-acetoxy analogues, carbamate formation with **14** was difficult.[4] A phenyl carbonate derivative of **14** was prepared, but it decomposed without forming a carbamate when treated with ammonia.

Table I. Microbiological Characterization of Mitosenes Compared with Mitomycin A

Bacterial species	Compound and diameter of zone of inhibn which it produces (mm)[a]					
	1	2b	2c	2d	2f	15
E. coli K12	8	0	0	0	0	0
E. coli B A9885	13	1	0	2	0	0
B. subtilis	>22	23	19	8	0	14
K. pneumonia	16	0	0	0	0	0
Staph Smith P	24	17	17	11	5	15
Staph 209P	23	15	17	9	5	13
S. faecalis P1377	21	16	17	6	0	13
S. lutea	>25	14	14	9	9	13
Myco No. 607	21	7	0	4	0	0

[a] Abbreviations for microorganisms: *E. coli* = *Escherichia coli*, *B. subtilis* = *Bacillus subtilis*, *K. pneumonia* = *Klebsiella pneumonia*, *Staph* Smith = *Staphylococcus aureus* strain Smith, *S. faecalis* = *Streptococcus faecalis*, *S. lutea* = *Sarcina lutea*, *Myco* = *Mycobacterium smegmatis*. For a complete description of the assay procedure, see A. W. Bauer, W. M. Kirby, J. C. Sheris, and M. Turck, *Am. J. Clin. Pathol.*, 45, 493 (1966).

Table II. Induction of λ-Bacteriophage in *E. coli*[a] Mitosenes Compared with Mitomycin A

Compd	Ratio of plaques induced by test compd at μg/ml to those induced in control[a]					
	3.1	0.8	0.2	0.05	0.0125	0.003
1		ta, T[b]	ta, T	ta, T	31.1, T	7.4
2b	24.5	13.2	4.3			
2c	6.9	2.9	1.3			
2d	16.7, T	4.1, T	1.4			
2f	1.6	1.1	0.8			
15		1.6	1.1	1.4	1.2	1.6

[a] For a complete description of the assay procedure, see K. E. Price, R. E. Bush, and J. Lein, *Appl. Microbiol.*, 12, 428 (1964). [b] ta means too active to measure; T means toxic to culture.

Treatment of **14** with chlorosulfonyl isocyanate gave a vigorous reaction with possible formation of a chlorosulfonylcarbamate, but hydrolysis of the crude product was unsuccessful. Finally, an *N*-methyl carbamate (**15**) was prepared by treating **14** with methyl isocyanate and triethylamine.

Biological Activity. The antibacterial activity of *cis*-1-acetamido-2-acetoxy-7-methoxy-*N*-methylmitosene (**15**) is compared with that of related 1-substituted mitosenes and 1,2-disubstituted mitosenes in Table I. This table presents the zones of inhibition in a disk-plate assay and mitomycin A (**1**) is included as a reference compound. Antibacterial activities of compounds **2b** and **2c** by the serial dilution method were given previously.[7] From Table I it is evident that compound **15** has antibacterial activity comparable to that of the 1-substituted mitosenes **2b** and **2c** and somewhat better than that of the 1,2-disubstituted mitosenes **2d** and **2f**. Mitomycin A is superior to these mitosenes against all of the bacteria listed in Table II. Since **15** has a methyl-substituted carbamate we must consider the possibility that this methyl group confers an advantage to its antibacterial activity. This possibility seems improbable in view of the report that the *N*-methyl carbamate is somewhat less active than its unsubstituted analogue in related indoloquinones.[14]

In Table II the abilities of these mitosenes and mitomycin A to induce the expression of λ-bacteriophage in *Escherichia coli* are presented. This assay has value in the preliminary screening of potential antitumor agents. It shows that neither **15** nor **2f** has significant activity in

prophage induction. In contrast, 1-substituted mitosenes **2b** and **2c** and 1,2-disubstituted mitosene **2d** show significant activities in the microgram per milliliter range. Mitomycin A is more potent than any of these compounds by a factor of about 100.

Compound **15** was inactive against P388 leukemia in CDF, male mice at a dose level of 12.8 mg/kg given on days 1 and 5 following the tumor inoculum of 10^6 ascites cells implanted intraperitoneally.[15] Mitomycin A gave an 80% increase in maximum survival time over saline control at a dose of 3.2 mg/kg in this assay, and **2b** gave a 65% increase at 6.4 mg/kg. Groups of six mice were used for each compound and the control group had ten mice.

Experimental Section

Melting points were determined on a calibrated Mel-Temp apparatus. Infrared spectra were determined in KBr disks on a Perkin-Elmer 137B Infracord spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian A-60A spectrometer using tetramethylsilane as a standard. Mass spectra were recorded with a CEC 21-110B mass spectrometer. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Purdue University. Analytical results were within ±0.4% of theoretical values. Each compound had ir and NMR spectra compatible with its structure.

2-Bromo-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indol-1-one (10). (A) By Way of Enamine 5. A mixture of 4.7 g (23.2 mmol) of ketone **4**, 400 ml of anhydrous benzene, and 6.1 ml (74 mmol) of pyrrolidine was heated at reflux for 24 h. During this time water was removed by a Dean-Stark trap. The mixture was concentrated under reduced pressure and the brown residual enamine, which showed no carbonyl absorption in its ir spectrum, was converted directly to the bromo ketone.

A solution of the crude enamine **5** in 200 ml of pure tetrahydrofuran was treated at 0 °C under nitrogen with a solution of 4.45 g (25 mmol) of *N*-bromosuccinimide in 150 ml of tetrahydrofuran during 15 min. After 90 min more, 40 ml of an acetate buffer (containing 4.0 g of NaOAc and 8 ml of HOAc) was added at 0 °C. The brown mixture was stirred 30 min and then allowed to warm to room temperature. Water (300 ml) was added and the mixture was heated on a steam bath for 15 min, cooled, and extracted with dichloromethane. Concentration of the organic phase under reduced pressure gave a dark residue which was purified by chromatography on silica gel with benzene as solvent. The first yellow band eluted contained 2.48 g (36%) of bromo ketone **10**, which did not melt below 300 °C. It had an ir band at 5.90 μ; NMR δ 4.9 ppm (dd, 1, CHBr); *m/e* 293. In other experiments the yield of **10** ranged from 0 to 68%.

(B) By Way of Trimethylsilyl Ether 6. A suspension of 8.2 mg (0.06 mmol) of zinc chloride (ground and dried) in 0.67 ml of anhydrous triethylamine, under nitrogen, was treated with 431 mg (2.0 mmol) of **4** in 25 ml of hot benzene. Freshly distilled chlorotrimethylsilane (0.52 ml, 4.0 mmol) was added and the mixture was stirred for 14.5 h. Since TLC showed starting material present, additional zinc chloride was added and the mixture was heated at 55 °C for 26 h. It was cooled, treated with ether, and filtered to remove triethylamine hydrochloride. The filtrate was evaporated to dryness and the brown solid residue was dissolved in 20 ml of tetrahydrofuran (TLC showed **4** and silyl ether **6**). This solution was cooled to 0 °C and 0.35 g (2.0 mmol) of *N*-bromosuccinimide in 20 ml of tetrahydrofuran was added during 15 min. The mixture was stirred at 0–10 °C for 1.5 h, stirred at room temperature for 3 h, and concentrated under reduced pressure. The black residue was chromatographed on silica gel with benzene as solvent. Concentration of the first yellow fraction gave 42 mg (7.1%) of bromo ketone **10**, identical by TLC and infrared spectrum with the sample prepared above.

2-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indol-1-one (11). A mixture of 2.94 g (10 mmol) of bromo ketone **10**, 9.0 g (92 mmol) of potassium acetate, 200 ml of acetonitrile, and 30 ml of methanol was stirred at room temperature for 24 h. It was concentrated under reduced pressure and the residue was washed well with water, dried, and treated with 10 ml of acetic anhydride in 25 ml of pyridine for 15 min. Methanol was added and the mixture was concentrated under reduced pressure. Crystallization of the residue from benzene (charcoal) gave 2.2 g (80.5%) of acetoxy ketone **11** as pale yellow needles: mp 152–153 °C; ir 5.73 and 5.90 μ; NMR δ 5.81 ppm (dd, 1, CHOAc).

***cis*-1-Acetamido-2-acetoxy-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole (7).** A mixture of 800 mg (3.1 mmol) of acetoxy ketone **11**, 220 mg (3.2 mmol) of hydroxylamine hydrochloride, 10 ml of pyridine, and 10 ml of ethanol was heated on a steam bath for 2 h and concentrated under reduced pressure and the resulting crude oxime **12** was dissolved in ethanol and precipitated with water. The dried precipitate (658 mg) was shown by TLC and NMR to be a mixture containing about 68% of the oxime. It was converted into **4** without further purification.

A mixture of 200 mg of oxime **12**, 200 mg of 5% palladium on charcoal, 50 ml of methanol, and 2 ml (excess) of acetic anhydride was shaken with hydrogen at an initial pressure of 50 psi for 2 days. The catalyst was removed by filtration and washed with methanol, and the combined filtrate and wash was concentrated to a greenish solid. This solid was washed with carbon tetrachloride and water and crystallized from hot carbon tetrachloride to give 39 mg of **7** as white crystals that melted with decomposition at 244 °C: ir 5.75 and 6.12 μ; R_f 0.49 in 8% methanol in chloroform on silica gel.

Determination of Stereochemistry of 7. A small portion of **7** was heated at reflux temperature with a mixture of dioxane and aqueous barium hydroxide for 24 h. The resulting mixture was diluted with water and extracted with ethyl acetate. Concentration of this extract gave a nearly white solid which had only one spot (R_f 0.05) on TLC (8% methanol in chloroform on silica gel) and showed no carbonyl absorption in the ir spectrum. This solid was treated with excess *N,N*-carbonyldiimidazole in tetrahydrofuran for 2 h at 40 °C, poured into water, and extracted with ethyl acetate. Concentration of the dried extract gave a yellowish semisolid which showed three spots on TLC. Preparative TLC on silica gel (8% methanol in chloroform) gave first two major bands and then a minor one. From the first band (R_f 0.81) was obtained a small amount of whitish solid that showed an ir peak at 5.75 μ and no absorption at 6.45 μ, which is characteristic of a cyclic carbamate (oxazolidinone). The second band (R_f 0.67) gave a very small amount of yellowish semisolid which showed ir absorption at 5.69, 6.15, and 6.45 μ (not a cyclic carbamate). The minor band did not afford sufficient material for characterization.

***cis*-1-Acetamido-2-acetoxy-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-9-carboxyaldehyde (8).** To 1.0 ml of redistilled *N,N*-dimethylformamide at 0 °C was added 76.7 mg (0.50 mmol) of phosphorus oxychloride. The resulting complex was treated dropwise during 1 h with a solution of 94.9 mg (0.30 mmol) of **7** in 2 ml of *N,N*-dimethylformamide. After an additional 1 h at 0 °C the mixture was added to a saturated aqueous sodium acetate solution at 0 °C during 30 min and stirred another 30 min, and the product was collected by filtration and washed with water. After drying there was obtained 78 mg (68%) of **8**, which gave white flakes of mp 260–263 °C dec after crystallization from ethanol: ir 5.69 and 6.02–6.17 μ (ester, amide, and aldehyde carbonyls); NMR δ 10.02 ppm (s, 1, CHO).

***cis*-1-Acetamido-2-acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (9).** A stirred solution of 300 mg of **8** in 8 ml of glacial acetic acid at 10 °C was treated with 0.35 ml of 90% nitric acid. After 35 min 100 ml of water was added and the precipitated product was collected by filtration, washed with ethanol, and dried. Analytically pure **9** (265 mg, 78%) was obtained as a tan solid: mp 255–260 °C dec; ir 6.49 (NO_2), 5.68 (ester), 6.02–6.17 μ (amide and aldehyde carbonyls).

***cis*-1-Acetamido-2-acetoxy-2,3-dihydro-7-methoxy-6-methyl-5,8-dioxo-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (13).** To a solution of 265 mg of nitroaldehyde **9** in 20 ml of 50% aqueous acetic acid was added 380 mg (excess) of iron filings. The mixture was stirred 2 h at 80 °C, cooled to room temperature, and diluted with water. The mixture was extracted with dichloromethane and this extract was shaken with 6 N hydrochloric acid. The acid layer was cooled in ice, neutralized (pH 7) with 5% sodium carbonate solution, and extracted with dichloromethane. This extract was dried (Na_2SO_4) and concentrated to give 170 mg of the corresponding 8-aminoaldehyde, which was

converted directly to the quinone.

A solution of 170 mg (0.47 mmol) of the aminoaldehyde in 32.5 ml of acetone was treated with a solution of 612 mg (2.3 mmol) of potassium nitrosodisulfonate in 16 ml of 0.167 M potassium dihydrogen phosphate and 32.5 ml of acetone. After 20 h the resulting solution was diluted with water and extracted with dichloromethane. This extract was dried (Na_2SO_4) and concentrated and the residue was chromatographed on silica gel with chloroform and then ethyl acetate as eluents. Concentration of the main yellow band gave 50 mg (28%) of quinone 13: glistening orange crystals from ethanol; mp 247 °C dec; ir 5.68 (ester), 5.9–6.2 μ (quinone, amide, and aldehyde carbonyls); NMR δ 10.30 (s, 1, CHO), 8.35 (m, 1, NH), 5.80 ppm (broad, 2, CHNAc and CHOAc).

cis-1-**Acetamido-2-acetoxy-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione Methyl Carbamate (1-Acetamido-2-acetoxy-7-methoxy-*N*-methylmitosene, 15).** A stirred solution of 60 mg of 13 in 10.5 ml of ethanol and 10.5 ml of tetrahydrofuran, at 0 °C under nitrogen, was treated with 120 mg (excess) of sodium borohydride. After 30 min, acetone (3 ml) was added. The mixture was stirred 10 min, treated with 0.40 ml of 1 M ferric chloride in 0.1 M hydrochloric acid, stirred another 5 min, and diluted with water. The resulting mixture was extracted with dichloromethane and this extract was dried and concentrated to give 60 mg of crude hydroxymethylquinone 14. This product, which showed only one spot on TLC (R_f 0.08 in 3:1 chloroform–acetone), was used directly to prepare the methyl carbamate.

A mixture of 30 mg of crude 14, 5 ml (excess) of methyl isocyanate, 5 ml of tetrahydrofuran, and 0.1 ml of triethylamine was kept at room temperature for 4 h and then concentrated under reduced pressure. The residual solid was chromatographed on a silica gel column (15 mm × 30 cm) with 1:1 chloroform–acetone as solvent. Following a blue impurity and a small yellowish band, the main yellow band eluted. Concentration of this main eluate gave a yellow solid which was recrystallized from ethanol. Golden crystals (10 mg) were obtained: mp 226 °C dec; ir 3.1 (NH), 5.65–5.8 (carbamate and ester), 6.0–6.2 μ (amide and quinone carbonyls).

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Studies on Antianaphylactic Agents. 5.[1] Synthesis of 3-(1*H*-Tetrazol-5-yl)chromones, a New Series of Antiallergic Substances

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A number of 3-(1*H*-tetrazol-5-yl)chromones were synthesized and found to have antiallergic activity in the rat passive cutaneous anaphylaxis (PCA) test. These compounds are active when administered orally in rats and of possible value for the treatment of asthma.

Disodium cromoglycate (DSCG, 1) has been established as being of use in the treatment of some types of bronchial asthma.[2] It has been shown to inhibit the liberation of mediators of immediate type allergic reactions initiated by reaginic antibody–antigen interactions.[3] It inhibits homologous passive cutaneous anaphylaxis (PCA) reactions in the rat induced by reaginic antibody and this reaction has been used as a routine screen for compounds with similar biological activities.[4] Although many kinds of compounds possessing a similar activity have been reported, much attention is being devoted to the antiallergic agents which can be administered orally.

Following the observation that introduction of a carbonyl group at the 3 position enhanced the antiallergic activity of chromones,[5] we began a program of investigation of 3-substituted chromone derivatives. As part of this program we have reported the activity of 3-(4-oxo-4*H*-1-benzopyran-3)acrylic acids (2).[1] We have also found, in sharp contrast to DSCG-type 4-oxo-4*H*-1-benzopyran-2-carboxylic acids (3), that some 4-oxo-4*H*-1-benzopyran-3-carboxylic acids (4) are inactive in inhibiting PCA in rats.[6] Inactivity of 4 was attributed to its weak acidity (4, R = H; $pK_a' = 8.85$[6]) due to intramolecular hydrogen bond formation.