6.05–6.45 (m, 5, C-7, C-8, and C-12 HC=CH, C-10 and C-14 C=CH), 7.01 (dd, J = 15 and 11 Hz, 1, C-11 HC=CH), 13.5 (very broad s, 1, NH); ^{13}C NMR ($CDCl_3$) 12.9 (C-19), 15.4 (C-20), 19.3 (C-3), 21.7 (C-18), 29.0 (C-16 and C-17), 33.2 (C-4), 34.3 (C-1), 39.8 (C-2), 109.0 (C-14), 128.7, 129.5, 130.0, and 130.5 (C-5, C-7, C-10, and C-11), 134.7 (C-12), 137.3, 137.8, and 139.5 (C-6, C-8, and C-9), 147.9 and 154.3 ppm (C-13 and C-15); UV λ_{max} (EtOH) 341 nm (ϵ 5.48 × 10^4). MS Calcd for $C_{20}H_{28}N_4$: 324.2314. Found: 324.2312.

The *all-E* configuration was assigned by ^{1}H and ^{13}C NMR comparison with the retinoic acid isomers. The proton chemical shifts of H-12 and H-8 (δ <6.4) are not in agreement with those of the 13-(*Z*) and 9-(*Z*) acids, respectively. The chemical shift (δ 2.51) of the singlet due to the methyl on C-13 is indicative of the 13-(*E*) configuration, and the 11-(*E*) geometry is apparent from the coupling constants (15 and 11 Hz) of H-11.[32h] The ^{13}C NMR spectrum provides further support for a 9-(*E*) assignment, since C-8 falls in the range 137.3–139.5 ppm, and the C-9 methyl (C-19) shift (12.9 ppm) is in exact agreement with that of (*all-E*)-retinoic acid and not that of the 9-(*Z*) acid (20.5 ppm).

ODC Assay. The ODC assay is rapid, reliable, and inexpensive. Female Charles River CD-1 mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass., and were used when 7 to 9 weeks old. The dorsal hair of the mice was shaved 1 to 2 days before testing, and only mice showing no hair regrowth were used. A single dose of TPA (10.5 μg, 17 nmol) in 0.2 mL of acetone was applied topically. The synthetic retinoid at one of three dose levels (1.7, 17, and 170 mmol), dissolved in 0.2 mL of acetone, was applied 1 h before the TPA treatment. The mice were killed by cervical dislocation 5 h after TPA treatment.

The epidermis was obtained essentially as described by Raineri et al.[38] To obtain sufficient material, we pooled the dorsal skins from the five mice in each treatment group. The depilatory agent Nudit (Helena Rubinstein, N.Y.) was applied to the shaved area of the skin; after 5 min, it was washed off thoroughly with cold tap water. Then the skin was excised and plunged immediately into ice-cold water; it then was placed in a 55 °C water bath for 30 s and reimmersed in ice-cold water for at least another 30 s. The skin was placed epidermis side up on a cold plate, and the epidermis was scraped off with a razor blade. The pooled epidermal sheets were homogenized (Polytron PT-10 homogenizer) at 0 to 4 °C for 15–20 s in 50 mM sodium phosphate buffer containing 0.1 mM pyridoxal phosphate and 0.1 mM EDTA. The supernatant fraction remaining after centrifugation of the homogenate at 30 000g for 30 min at 0 °C was used for the enzyme assay.

Enzyme activity was determined according to the procedure of O'Brien et al.,[39] i.e., by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine (58 mCi/mmol) after incubation with the 30 000g supernatant. The incubations were carried out in 25-mL Erlenmeyer flasks equipped with a rubber stopper–polyethylene center well assembly (Kontes Glass Co., Vineland, N.J.) containing 0.2 mL of ethanolamine/2-methoxyethanol (2:1, v/v). The incubation mixtures consisted of a final volume of 2.0 mL, containing 0.4 μmol of pyridoxal phosphate, 1.0 μmol of dithiothreitol, 0.2 μmol of L-ornithine, 100 μmol of sodium phosphate (pH 7.2), 0.5 mL of the 30 000g supernatant, containing 0.5–1.0 mg of protein, and approximately 0.5 μCi of DL-[1-^{14}C]ornithine (58 mCi/mmol). Incubations routinely were carried out at 37 °C for 30 to 60 min. The reaction was stopped by the addition of 1.0 mL of 2 M citric acid, and incubation was then continued for an additional 30 min to ensure complete absorption of $^{14}CO_2$. We measured radioactivity using a toluene-based scintillant (4 g of PPO and 50 mg of POPOP/L of toluene) in a Beckman LS-250 liquid scintillation counter. Enzyme activity was determined in triplicate and expressed as nmol of CO_2 in 30 min per mg of protein. Enzyme activity was linear for the protein concentration used. The protein concentrations of the epidermal extracts were determined by the Lowry procedure, using bovine serum albumin as the standard.

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(38) R. Raineri, R. C. Simsiman, and R. K. Boutwell, *Cancer Res.*, **33**, 134 (1973).

(39) T. G. O'Brien, R. C. Simsiman, and R. K. Boutwell, *Cancer Res.*, **35**, 1662 (1975).

Evaluation of Rotenone and Related Compounds as Antagonists of Slow-Reacting Substance of Anaphylaxis

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Rotenone (1), dihydrorotenone (2), isorotenone (3), mutarotenone (4), and deguelin (12) were found to be potent antagonists of slow-reacting substance of anaphylaxis (SRS-A) in vitro. However, these compounds were also shown to inhibit histamine, serotonin, and acetylcholine at only ten times their IC_{50} concentrations for SRS-A antagonism. Rotenone (1) and several related compounds were also evaluated in an in vivo guinea pig anaphylaxis model. Several of these compounds and FPL 55712 (I) were effective in prolonging collapse times of animals which received an aerosol challenge of an antigen to which they had been sensitized.

Rotenone (1) is an abundant natural product, occurring in the roots of tropical plants belonging to the Leguminosae family. These natural sources have supplied the rotenone which has been and still is widely used as a fish and insect poison. Rotenone is a useful insecticide, effective against mosquitoes and mosquito larvae, and is nonpersistent in the environment.

Rotenone was first isolated in 1895.[1] Its structure, however, was not elucidated until 1933,[2] which is not surprising in view of the structural complexity (pentacyclic; three asymmetric centers). Since then, partial and total syntheses of 1 have been reported.[3]

Pharmacology, which has been performed with rotenone, includes several studies dealing with its effects on various muscle preparations. Rotenone, at a concentration of 0.1 μg/mL, has been shown to inhibit the contractions of isolated guinea pig atria.[4] Another related study showed

(1) E. Geoffrey, *Ann. Inst. Colon. Marseille*, **2**, 1 (1895).

(2) F. B. LaForge, H. L. Haller, and L. E. Smith, *Chem. Rev.*, **12**, 181 (1933).

(3) T. J. Haley, *J. Environ. Pathol. Toxicol.*, **1**, 315 (1978).

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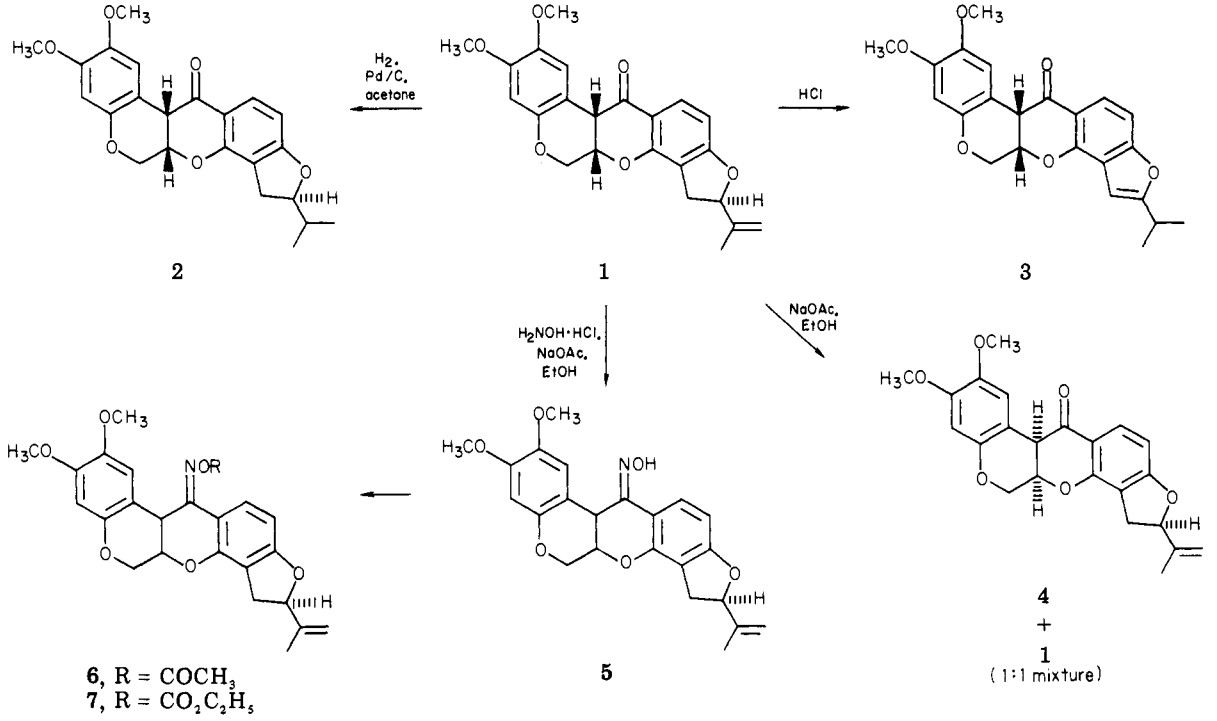
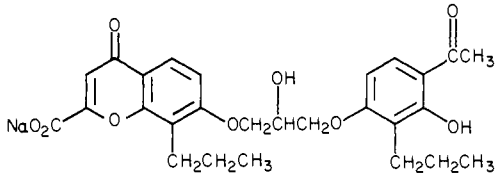
Scheme I



that isolated guinea pig atria, when treated with 1–8 μM concentrations of rotenone, developed negative ionotropic and chronotropic effects.[5] Rotenone, at 10^{-8} to 10^{-9} M concentrations, inhibited the responses of rabbit and guinea pig intestine to histamine and acetylcholine.[6] In addition, rotenone blocked the barium chloride and bradykinin induced contractions of guinea pig and rabbit ileum, as well as the potassium chloride induced contraction of guinea pig taenia and the epinephrine-induced contraction of the guinea pig seminal vesicle.[7] Another study demonstrated that rotenone, at concentrations of 5×10^{-9} to 10^{-8} g/mL, caused a relaxation of spontaneously active guinea pig taenia coli.[8] Rotenone at 0.33 to 1.7 $\times$ 10^{-5} M concentrations has also been shown to dilate the vessels in the perfused rabbit ear.[9] Thus, the literature is replete with documentation regarding the inhibitory effects of rotenone on contractile responses in isolated muscle preparations.

In the present study we examined the effects of rotenone and related compounds on the contractile responses of isolated guinea pig ileum induced by chemical mediators of hypersensitivity. Since rotenone (a dihydrochromone) is structurally related to FPL 55712[10] (I) (a chromone) and



I (FPL 55712)

its congeners[11] which are known to specifically inhibit slow-reacting substance of anaphylaxis (SRS-A),[12,13] we investigated its potential for specifically inhibiting SRS-A. SRS-A has been identified as an important chemical mediator of immediate hypersensitivity and may be more important than histamine in causing bronchospasm in asthma.[14-17] A specific inhibitor of SRS-A could be useful in the treatment of human asthma.

Chemistry. Most of the benzopyrones which were evaluated as antagonists of SRS-A were synthesized from rotenone (1). Thus, catalytic reduction of 1 gave a 90% yield of dihydrorotenone (2),[2] as shown in Scheme I. Treatment of 1 with sulfuric acid gave isorotenone (3) as reported,[2,18,19] but in very low (3%) yield. We were able to obtain 3 in 34% yield by treating 1 with concentrated hydrochloric acid for an extended period. [Interestingly, the latter procedure is reported[20] to produce rotenone hydrochloride (?), mp 194 °C. Our sample of 3 melted at 174–176 °C.] Isomerization of 1 with sodium acetate in

(4) C. E. Toth, M. Ferrari, A. Bruni, and R. Santi, *Arch. Ital. Sci. Farmacol.*, **15**, 109 (1965).
(5) M. Liu, M. Siess, and P. C. Hoffman, *Biochem. Pharmacol.*, **19**, 197 (1970).
(6) R. Santi, M. Ferrari, and E. Toth, *J. Pharm. Pharmacol.*, **15**, 697 (1963).
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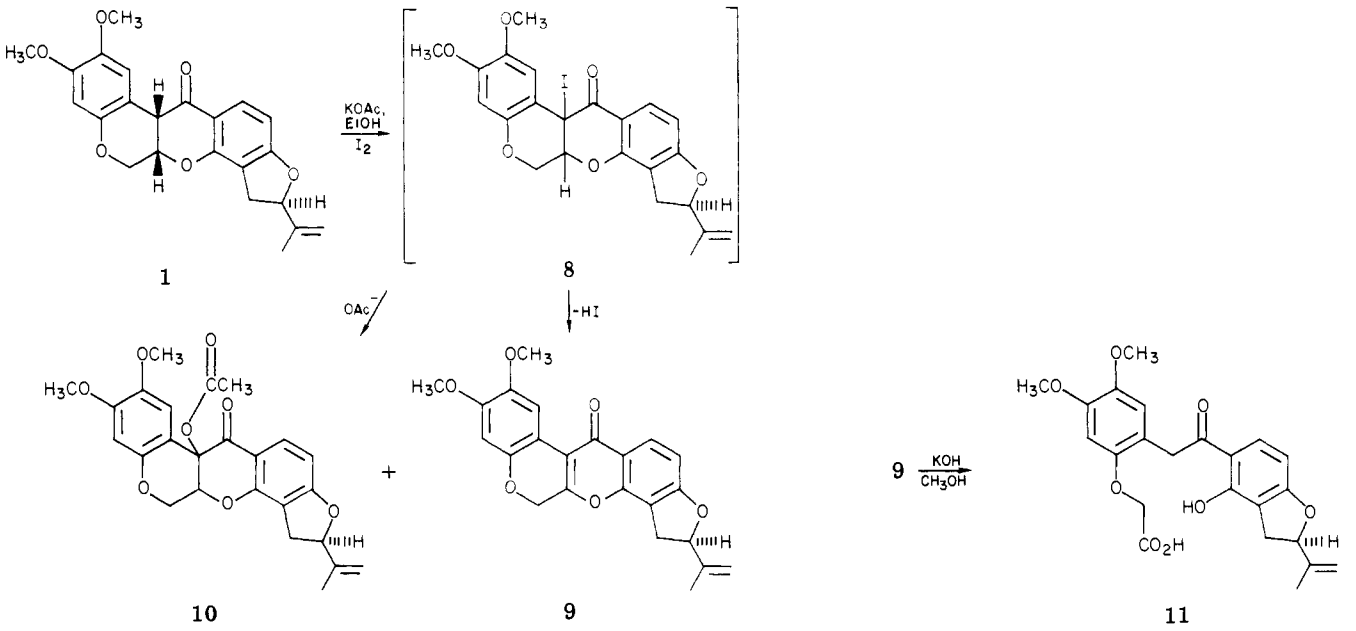
(9) M. Ferrari, *J. Pharm. Pharmacol.*, **22**, 71 (1970).
(10) J. Augstein, J. B. Farmer, T. B. Lee, P. Sheard, and M. L. Tattersall, *Nature (London), New Biol.*, **245**, 215 (1973).
(11) R. A. Appleton, J. R. Bantick, T. R. Chamberlain, D. N. Hardern, T. B. Lee, and A. D. Pratt, *J. Med. Chem.*, **20**, 371 (1971).
(12) Other investigators have also examined the antagonism of SRS-A by compounds structurally related to I. For example, see D. R. Buckle, D. J. Outred, J. W. Ross, H. Smith, R. J. Smith, B. A. Spicer, and B. C. Gasson, *J. Med. Chem.*, **22**, 158 (1979).
(13) A proposed structure for SRS-A has recently been reported. See *Chem. Eng. News*, **57**(24), 19 (1979); *ibid.*, **58**(7), 28 (1980).
(14) C. H. Kellaway and E. R. Trethewie, *Q. J. Exp. Physiol.*, **30**, 121 (1940).
(15) W. E. Brocklehurst, *J. Physiol.*, **128**, 1 (1955).
(16) R. P. Orange and K. F. Austen in "Immunobiology", R. A. Good and D. W. Fisher, Eds., Sinauer Associates, Stamford, Conn., 1971, pp 115–121.
(17) W. E. Brocklehurst, *Biochem. Pharmacol.*, **12**, 431 (1963).
(18) F. B. LaForge and L. E. Smith, *J. Am. Chem. Soc.*, **51**, 2574 (1929).
(19) Dihydrorotenone (2) displays dimorphism. Reference 2 reports that 2 usually melts at 216 °C, while ref 18 records a melting point of 164 °C for dihydrorotenone.
(20) R. Verhe and N. Schamp, *Experientia*, **31**, 759 (1975).

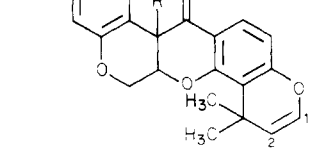
Scheme II



ethanol as reported by Crombie et al.[21] gave an 82% yield of mutarotenone (4), which is a 50:50 mixture of 1 and its 6a-α, 12a-α epimer. Rotenone oxime (5) was prepared in 78% yield from 1 and hydroxylamine in ethanol.[22] Subsequent treatment of 5 with acetic anhydride produced the *O*-acetyl derivative 6 quantitatively, while treatment of 5 with ethyl chloroformate gave the *O*-carbethoxy derivative 7 in 85% yield. Compounds 6 and 7 have been previously described by Brown and Wright.[23]

Treatment of 1 with potassium acetate and iodine in ethanol (Scheme II) led to a mixture of dehydrorotenone (9) and acetylrotenolene (10).[24] The formation of both 9 and 10 from 1 strongly suggests the intermediacy of structure 8, as shown in Scheme II.[25] Treatment of dehydrorotenone (9) with methanolic potassium hydroxide afforded a sample of derrisic acid (11),[26] which provided a nonrigid structure related to the rotenoids with ruptured B and C rings for biological evaluation.

Racemic deguelin (12)[27] and hydroxydeguelin or tephrosin (13)[27] were isolated, using a combination of separation and purification techniques, from a commercial powdered root of *Longocarpus nicou*.



12, R = H; deguelin (racemic)
13, R = OH; tephrosin

Table I. SRS-A Antagonism in the Guinea Pig Ileum

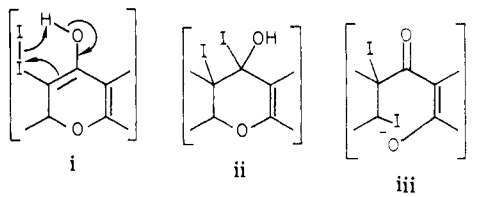
compd	SRS-A IC_{50}, g/mL	% inhibition[a] histamine	serotonin	acetylcholine
I	4.2×10^{-8} [c]	0	0	0
1	$1.55 \pm 0.83 \times 10^{-8}$ [d]	38	92	15
2	$2.38 \pm 0.19 \times 10^{-8}$ [d]	29	60	25
3	6.2×10^{-8} [c]	30		28
4	$2.47 \pm 1.68 \times 10^{-8}$ [d]	28	87	11
5	4.0×10^{-7} [c]			
6	4.0×10^{-7} [c]			
7	4.0×10^{-7} [c]			
9	NA[b]	0		0
10	NA			
11	NA	0		0
12	$4.96 \pm 1.64 \times 10^{-8}$ [d]	20	89	13
13	2.0×10^{-7} [c]			

[a] At 10 times the IC_{50} dose. [b] Not active. [c] IC_{50} values are the average obtained by interpolation from two dose-response curves. [d] IC_{50} values were obtained from a minimum of three dose-response curves.

Biological Results and Discussion. In Table I are listed the IC_{50} values displayed by rotenone (1) and related compounds for SRS-A antagonism in the isolated guinea pig ileum. Many of these compounds were found to be potent inhibitors of SRS-A induced contractions. The IC_{50} value determined for I in this preparation resides in the middle of the IC_{50} value range defined by compounds 1–7, 12, and 13.

The dose–response curve for rotenone (1) in the SRS-A induced contraction of the guinea pig ileum is shown in

(21) L. Crombie, P. J. Godin, D. A. Whiting, and K. S. Siddalingaiah, *J. Chem. Soc.*, 2876 (1961).
(22) S. H. Harper, *J. Chem. Soc.*, 1424 (1939). This author claims that 5 is dimorphic, displaying melting points of 237 and 249 °C. Our sample of 5 melted at 245–246 °C.
(23) G. R. Brown and B. Wright, *Experientia*, **32**, 277 (1976).
(24) F. B. LaForge and L. E. Smith, *J. Am. Chem. Soc.*, **52**, 1091 (1930).
(25) Intermediate 8 could arise in several ways. Iodination α to the carbonyl group via the 6-membered transition state depicted by i could lead to 8, as could direct iodination of the double



bond of 1 in the enol form to give ii, which could then dehydrohalogenate. Alternatively, one could envision iii as a precursor to 8. Structure iii could arise from iodination of the corresponding enone-phenolate anion, which is presumably an intermediate in the acetate-catalyzed preparation of 4 from 1.
(26) A. Butenandt, *Justus Liebigs Ann. Chem.*, **464**, 253 (1928).
(27) E. P. Clark, *J. Am. Chem. Soc.*, **53**, 729 (1931).

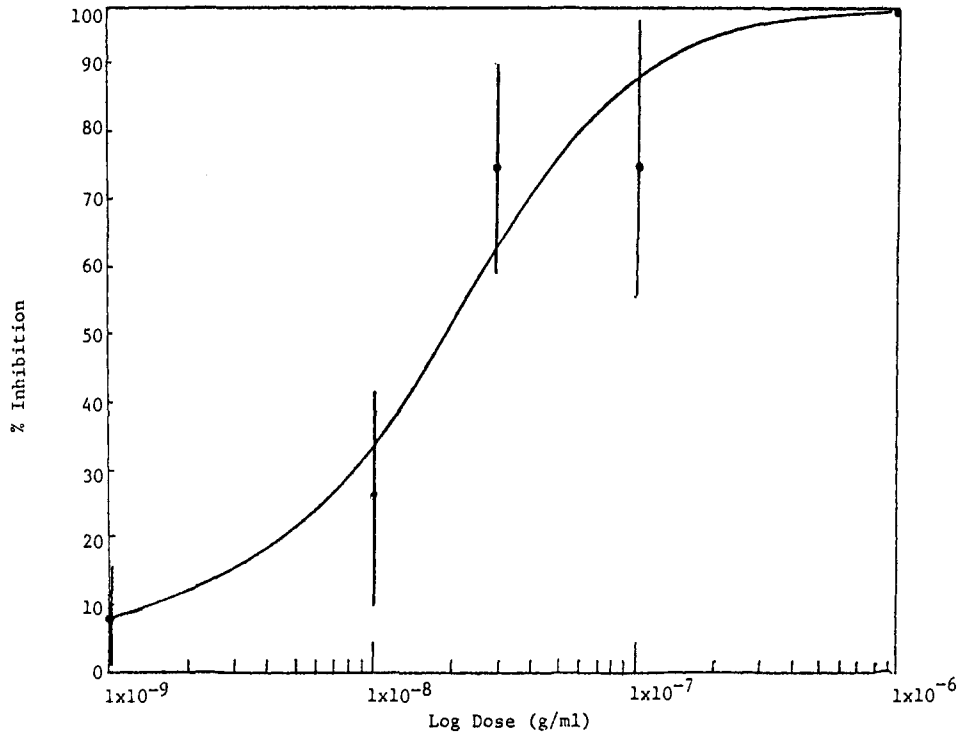


Figure 1. The dose–response curve for rotenone (1) in the SRS-A induced guinea pig ileum contraction. Each point represents the mean value of at least three different tissue preparations ± SD. Ordinate: percent inhibition of the SRS-A induced contractile response; abscissa: the concentration of rotenone (g/mL).

Figure 1. This curve is typical of the dose–response curves displayed by the other active compounds (**2**, **4**, and **12**) in Table I. Compounds **3**, **5**–**7**, and **13** were less effective in antagonizing SRS-A.

It is instructive to note which compounds were ineffective in blocking the SRS-A induced contractions. Derrisic acid (**11**) was not effective, which indicated the necessity of intact B and C rings in the polycyclic system. Dehydrorotenone (**9**), which is an almost planar pentacyclic system in contrast to rotenone (**1**), which is V shaped, was also inactive. Acetylrotenolone (**10**) was inactive, although tephrosin (**13**), a close relative of rotenolone, was active.

The spasmolytic activity of rotenone (**1**) and related compounds, however, was not specific for SRS-A. In Table I is listed percent inhibition of three other mediators (histamine, serotonin, and acetylcholine) by rotenone (**1**), dihydrorotenone (**2**), isorotenone (**3**), mutarotenone (**4**), and deguelin (**12**), when tested at ten times the IC_{50} concentration for SRS-A induced contractions. Using these conditions, I displayed no inhibition of histamine, serotonin, or acetylcholine. The five compounds (**1**–**4** and **12**) which were evaluated showed inhibition of these additional three mediators in the following order: serotonin > histamine > acetylcholine. The nonspecific action of these compounds in blocking guinea pig ileum contractile responses may be due to inhibition of mitochondrial respiration.[3,28]

Many of the compounds in Table I were also evaluated in vivo, using a guinea pig anaphylaxis model. Guinea pigs were sensitized to ovalbumin, challenged with an oral dose of the antagonist, and then exposed to an aerosol of ovalbumin. Collapse times recorded for the animals treated with the various antagonists are listed in Table II. Compound I was active at 10 mg/kg in this model. The most effective compound in prolonging collapse time was deguelin (**12**) at 10 mg/kg. The lack of significant collapse time prolongation for deguelin (**12**) at 5 mg/kg indicated a dose–response relationship in this test, as did the prolongation of collapse times for dehydrorotenone (**9**) at 5 and 10 mg/kg.

Table II. Guinea Pig Anaphylaxis

compd	dose, mg/kg	n^a	collapse time, s ± SD[b]	tD^c
control		15	145 ± 76.8	
I	10	12	253 ± 134.6	2.54[d]
1	5	12	288 ± 107.2	3.35[d]
2	5	9	214 ± 113.3	1.48
3	10	7	211 ± 140.7	1.31
4	10	11	240 ± 132.4	1.64
5	10	12	248 ± 124.9	2.55[d]
9	5	4	216 ± 104.7	1.14
9	10	12	248 ± 112.3	2.40
11	10	12	167 ± 101.5	0.52
12	5	8	172 ± 78.9	0.55
12	10	10	360 ± 0	4.67[d]

[a] Number of test animals. [b] Standard deviation. [c] Dunnett's t value. [d] These values, where tD is greater than 2.50, are significant (95% confidence level).

We conclude that the lack of specificity of rotenone and related compounds for SRS-A does not warrant their further development as SRS-A antagonists. However, we feel that the information generated in this study is useful in furthering our understanding of the pharmacology of rotenone.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. The IR spectra were recorded with a Perkin-Elmer Model 727B spectrophotometer, NMR spectra were determined using Varian T-60 and EM360 spectrometers, and mass spectra were obtained with a Finnigan GC/MS Model 3000D (electron impact and chemical ionization) mass spectrometer at 70 eV. Combustion analyses for C, H, and N were performed by Dow Analytical Laboratories.

Rotenone (**1**) was purchased from the Aldrich Chemcial Co. Samples of **1** used for biological evaluation were twice recrystallized from ethanol. Dihydrorotenone (**2**), mutarotenone (**4**),

(28) S. Tsuda, N. Urakawa, and J. Fukami, *Jpn. J. Pharmacol.*, **27**, 855 (1977).

rotenone oxime (5), acetylrotenone oxime (6), carbethoxyrotenone oxime (7), dehydrorotenone (9), acetylrotenolone (10), and derrisic acid (11) were prepared using the procedures documented under "Chemistry Section". Verification and purity of these compounds were established by combustion analysis and spectral data, using the instrumentation described above.

6a,12a-Dihydro-8,9-dimethoxy-2-(1-methylethyl)[1]benzopyrano[3,4-*b*]furo[2,3-*h*][1]benzopyran-6(12*H*)-one (Isorotenone; 3). A slurry of 1 (25.0 g, 63.4 mmol) in 300 mL of concentrated HCl was heated at reflux for 20 min. Gas evolution (HCl) was copius. The mixture was cooled and the precipitate was collected. TLC (silica gel; 9:1 $CHCl_3$–CH_3OH) indicated mostly 1, with a minor amount of 3 which displayed an R_f slightly higher than 1. The solid was suspended in 400 mL of concentrated HCl and 100 mL of water and heated at reflux. After 60 h, TLC indicated mainly 3, with some 1 present. After an additional week at reflux, TLC indicated the absence of 1. The mixture was cooled and the solid was collected and recrystallized from methanol (600 mL) to yield 8.50 g (34%) of 3 as white prisms: mp 174–176 °C, lit.[29] mp 177–178 °C; IR (Nujol) 1780 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.27 [d, J = 7 Hz, 6 H, $CH(CH_3)_2$]. Anal. ($C_{23}H_{22}O_6$) C, H, N.

Isolation of Dequelin (12) and Tephrosin (13). The powdered root of *Lonchocarpus nicou*[30] (384 g) was exhaustively extracted with 500 mL of ether using a Soxhlet apparatus. On cooling, the solution deposited crystals of rotenone which were collected (20 g). The filtrate was concentrated and the dark residue was reconstituted in 200 mL of CCl_4, whereupon another crop of rotenone (12 g) was deposited. The CCl_4 filtrate was concentrated to leave 58 g of syrup, which was dissolved in 200 mL of ethanol. Sodium acetate (8 g) was added and reflux was maintained for 2 h. The ethanol was removed by evaporation, and the residue was suspended in CH_2Cl_2 and filtered. The filtrate was concentrated and the residue was dissolved in 100 mL of acetone. The resulting yellow crystals which formed were collected (6.7 g) and successively crystallized from ether, ethyl acetate, and acetonitrile. The resulting white crystals were shown by high-performance LC to be a mixture of 12 and 13. A Varian Model 8500 high-performance LC system was used for this and subsequent composition determinations. A two-column system was employed; a 4 × $^1/_8$ in. o.d. precolumn packed with C_{18} Porasil (Waters Associates; 37–50 μm) was followed by a 30 cm × 4 mm i.d. column of μBondapak C_{18} (Waters Associates). Elution was accomplished with 3:2 CH_3CN–H_2O.

A portion of the mixture of 12 and 13 (1.4 g) was chromatographed on a silicic acid column. Elution with 9:1 CH_2Cl_2–$CHCl_3$ yielded 12 (457 mg), mp 169–173 °C, lit.[27] mp 171 °C. Subsequent elution with 4:1 CH_2Cl_2–$CHCl_3$ initially afforded mixtures of 12 and 13 and then pure 13 (446 mg), mp 197–198 °C, lit.[27] mp 197–198 °C.

Pharmacological Methods. Measurement of SRS-A Antagonism. The SRS-A used in the present study was obtained by collection of peritoneal shock fluid from rats subjected to passive peritoneal anaphylaxis as described previously.[31] The material was assayed on a strip of guinea pig ileum suspended in a tissue bath containing Tyrode's solution, maintained at 37 °C, and continuously aerated with 95% O_2–5% CO_2. Responses were checked in the presence of atropine (5×10^{-7} M) and mepyramine maleate (10^{-6} M),[32] as well as the selective SRS-A antagonist FPL 55712, to assure pharmacological activity and selectivity.

A dose–response study allowed us to select a dose of SRS-A which produced a contraction which was ca. 60% of the maximum. This dose was employed in the evaluation of all antagonists. All antagonists were added to the tissue bath 2 min prior to challenge with SRS-A. Activity was recorded by calculating the percent inhibition induced by the antagonist.

In vivo measurement of inhibition of allergic reactions was done using the method of Greig and Griffin.[33] Guinea pigs were immunized to ovalbumin with a 1-mL subcutaneous and a 1-mL intraperitoneal administration of a 10% solution of ovalbumin. After sensitization developed (ca. 4 weeks), the animals were challenged with aerosolized antigen (ovalbumin). Time of collapse was the end point. Failure to collapse within a 6-min exposure to the antigen was considered maximal protection. The collapse times for animals receiving the antagonists were compared with collapse times of control animals. Bartlett's test was employed to delineate the homogeneity of variances among the treatment groups. Since the variances were homogeneous, a one-way analysis of variance and a Dunnett's test were utilized to determine any significant differences between experimental and control groups.

Acknowledgment. The authors are grateful to Fisons Pharmaceutical Ltd., Loughborough, Leics., for supplying a sample of FPL 55712. We also thank Ben J. Cerimele for statistical analyses.

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(31) H. J. Rapp, *J. Physiol. (London)*, 158, 35 (1961).
(32) J. W. Ross, H. Smith, and B. A. Spicer, *Int. Arch. Allergy Appl. Immunol.*, 51, 226 (1976).
(33) M. E. Greig and R. L. Griffin, *J. Med. Chem.*, 18, 112 (1975).

Bronchodilator and Antiulcer Phenoxypyrimidinones[1]

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Series of 5-phenoxy-2(1*H*)-pyrimidinones, 5-phenoxy-4(3*H*)-pyrimidinones, and related compounds were prepared in a follow-up of a lead prepared as a potential cyclic nucleotide regulating agent. Compounds were evaluated for bronchodilator activity in histamine-challenged guinea pigs and for antiulcer activity in a cold-restraint, stressed rat ulcer model. Bronchodilator activity comparable to, or greater than, that of theophylline was found in both the 2(1*H*)- and 4(3*H*)-pyrimidinone series and was most prominent in analogues containing either an electron-withdrawing or -donating substituent in the para position of the phenoxy ring. Significant antiulcer activity was observed only in the 2(1*H*)-pyrimidinone series among three closely related analogues. One of these, 5-(*m*-methylphenoxy)-2(1*H*)-pyrimidinone (3), exhibited more potent antiulcer effects than the clinically useful antiulcer agent carbenoxolone, without demonstrating bronchodilator activity.

Series of chemical prototypes having certain structural features in common with the clinically useful bronchodilator theophylline were prepared as potential cyclic nucleotide regulating agents. One of these, 5-phenoxy-2-

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