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Anti-ulcer Effect of Isoprenyl Flavonoids. III.[1] Synthesis and Anti-ulcer Activity of Metabolites of 2′-Carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone[2]

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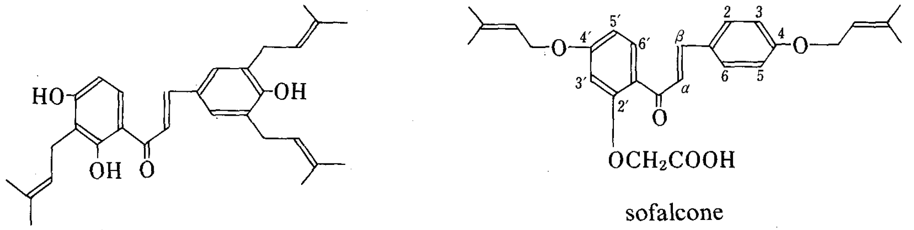
Five dihydrochalcone derivatives, 2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)dihydrochalcone(M-6-b), 2′,4-bis(carboxymethoxy)-4′-(3-methyl-2-butenyloxy)dihydrochalcone(M-2), 2′,4-bis(carboxymethoxy)-4′-(3-carboxy-2-butenyloxy)dihydrochalcone(M-1-a), 2′,4-bis(carboxymethoxy)-4′-(3-hydroxymethyl-2-butenyloxy)dihydrochalcone(M-1-b), and 2′-carboxymethoxy-4-hydroxy-4′-(3-methyl-2-butenyloxy)dihydrochalcone(M-4-b), which are the main metabolites in rats of a new anti-ulcer drug "sofalcone" (2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone) were synthesized and the anti-ulcer activities of the major metabolites in humans, M-6-b, M-2, and M-1-a, were examined by Shay's and Takagi's methods and also by the use of rats with histamine-induced ulcer. The most active compound was M-6-b, which showed activity equal to or slightly weaker than that of sofalcone.

Keywords——anti-ulcer effect; isoprenyl flavonoid; chalcone; dihydrochalcone; sofalcone; metabolite

Sophoradin has been isolated[3] from a Chinese crude drug, Guang-dou-gen (or Shan-dou-gen), which consists of the roots of *Sophora subprostrata* (Legminosae). Sophoradin has been found[4] to be effective against experimental ulcers. However, it is obtainable synthetically[5] only in poor yield. In the course of our screening of sophoradin analogs for anti-ulcer activity, we[1] succeeded in finding a new anti-ulcer compound, 2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)chalcone (general name, sofalcone; code No., SU-88), which could be prepared in good yield.



sophoradin

sofalcone

Fig. 1

Sofalcone has been used clinically in Japan since March 1984. The main metabolites of sofalcone in the rat were reported[6] to be 2′,4-bis(carboxymethoxy)-4′-(3-methyl-2-butenyloxy)dihydrochalcone (M-2), 2′,4-bis(carboxymethoxy)-4′-(3-hydroxymethyl-2-butenyloxy)dihydrochalcone (M-1-b), 2′,4-bis(carboxymethoxy)-4′-(3-carboxy-2-butenyloxy)-dihydrochalcone (M-1-a), 2′-carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)dihydrochalcone (M-6-b), and 2′-carboxymethoxy-4-hydroxy-4′-(3-methyl-2-butenyloxy)dihydrochalcone (M-4-b). The main metabolites of sofalcone in human plasma were reported[7] to be M-6-b and M-2, and those in human urine were reported[7] to be M-2 and M-1-a. However, the syntheses and anti-ulcer effects of these metabolites have not yet been reported.

Here, we describe the syntheses of M-2, M-1-b, M-1-a, M-6-b and M-4-b, and the anti-ulcer effects of M-2, M-1-a and M-6-b.

Synthesis

The synthetic routes used for the preparation of compounds M-2, M-1-b and M-1-a are shown in Chart 1 and described in detail in the experimental section. Treatment of 4′-hydroxy-2′,4-bis(methoxycarbonylmethoxy)dihydrochalcone (VIII) with 3-methyl-2-butenyl bromide (IX) followed by hydrolysis of the methyl ester gave M-2. Similarly, M-1-b was

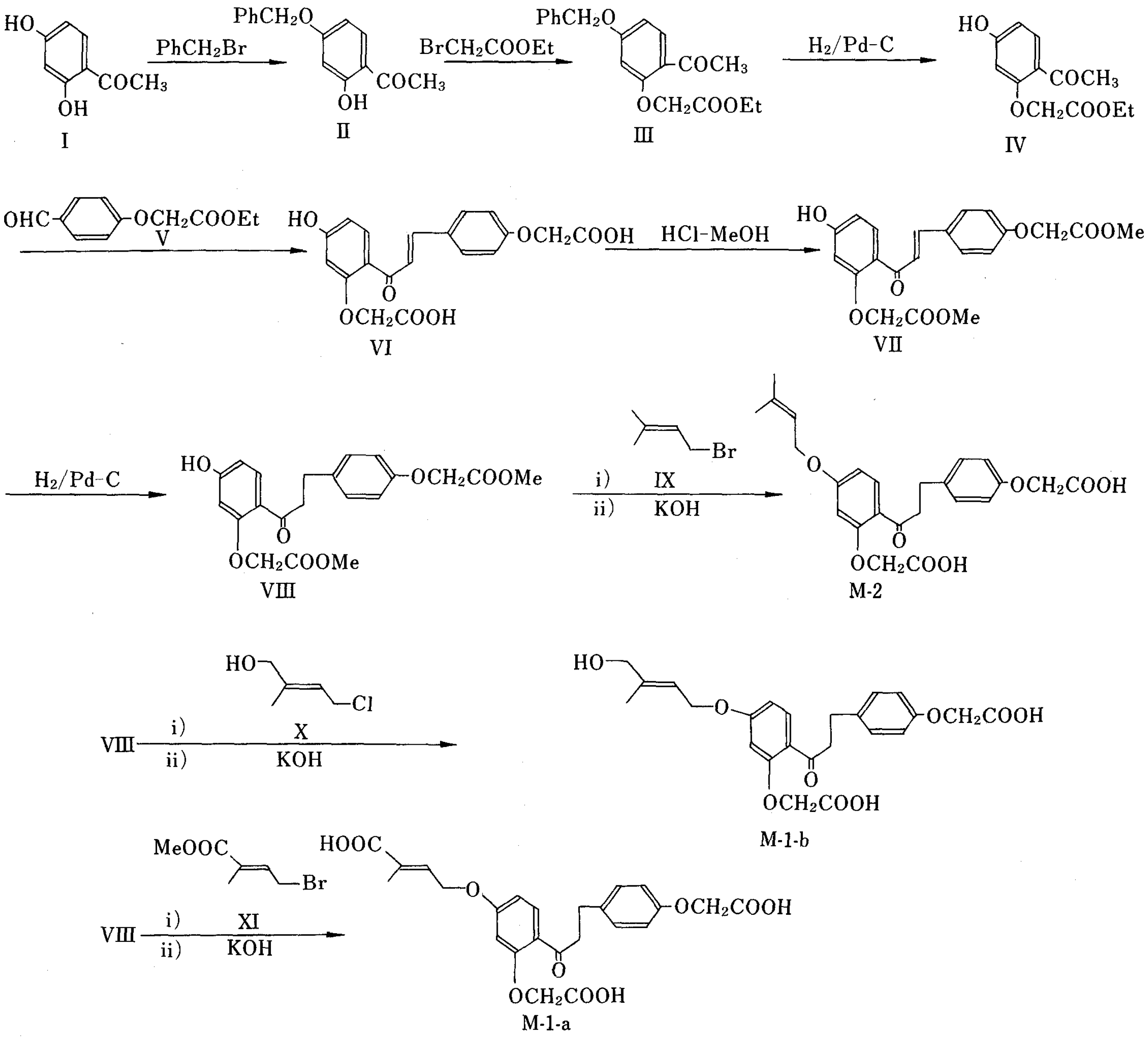


Chart 1

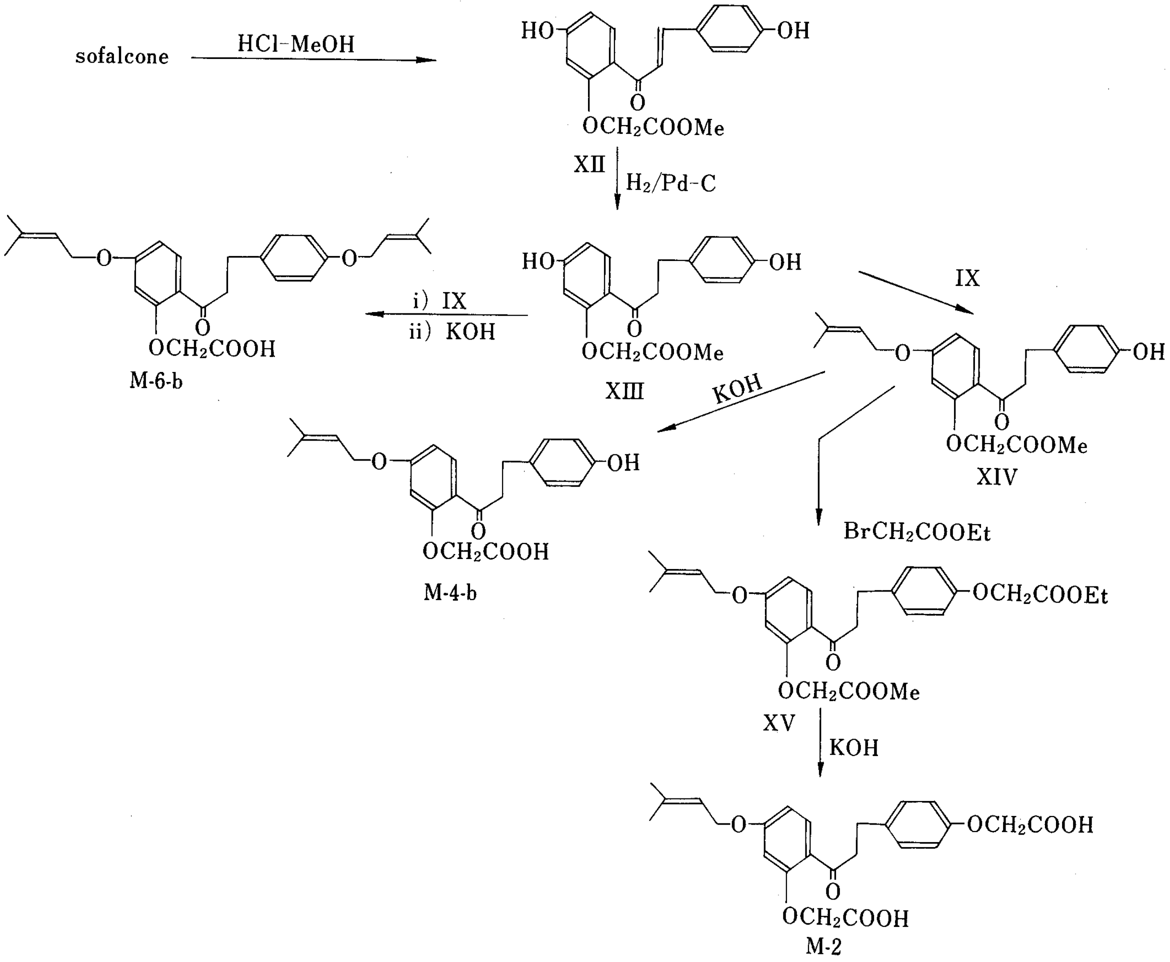


Chart 2

Table I. Anti-ulcer Effect of the Metabolites of Sofalcone on Shay's Pylorus-Ligated Rats, Takagi's Water-Immersed Restraint-Stressed Rats and Rats with Histamine-Induced Ulcer

	Shay's rats		Stress rats		Histamine rats	
	Dose	Inhibitory ratio	Dose	Inhibitory ratio	Dose	Inhibitory ratio
Sofalcone	100	+++	100	+++	100	++
M-6-b	100	++	100	++	100	++
M-2	100	++	100	+	100	++
M-1-a	100	+	100	+	100	−
Atropine sulfate	3	++	0.1	+++	10	+++

Each *i.p.*, $n=8$, dose in mg/kg.
Inhibitory ratio: +++ (71—100%), ++ (41—70%), + (11—40%), − (<10%).

prepared by treatment of VIII with 3-hydroxymethyl-2-butenyl chloride[8] (X) followed by hydrolysis. Compound M-1-a was prepared by treatment of VIII with 3-methoxycarbonyl-2-butenyl bromide[9] (XI) followed by hydrolysis. On the other hand, M-6-b, M-4-b, and M-2 were prepared from sofalcone as shown in Chart 2. The key intermediate compound, 2′-methoxycarbonylmethoxy-4,4′-dihydroxydihydrochalcone (XIII), was prepared by hydrolysis of sofalcone with methanolic hydrochloride followed by hydrogenation over palladium carbon. Compounds M-6-b, M-4-b, and M-2 were each derived from XIII.

Anti-ulcer Effect

Anti-ulcer activity was examined in Shay's pylorus-ligated rats, water-immersed and restraint-stressed rats, and rats with histamine-induced ulcer. Samples and a positive control (atropine sulfate) were given intraperitoneally (*i.p.*). As shown in Table I, the extent of inhibition is expressed as $-$ ($<10\%$), $+$ (11—40%), $++$ (41—70%), and $+++$ (71—100%).

The results are shown in Table I. Compounds M-6-b and M-2, two of the main metabolites in human plasma, showed a considerable anti-ulcer effect, and M-1-a, which is excreted in human urine, showed a somewhat weaker effect.

Experimental

All melting points were measured using a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a JASCO DS-701 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were taken at 60 MHz with trimethylsilane as an internal standard, using a JEOL PMX-60 spectrometer. Mass spectra (MS) were obtained on a Shimadzu LKB GC-MS 9000, and field desorption-mass spectrum (FD-MS) of M-1-b was obtained on a Hitachi M-80A instrument. The developing solvent used in thin layer chromatography (TLC) on precoated Silica gel 60F 254 plates (Merck) was benzene–tetrahydrofuran (THF)–HCOOH (40 : 15 : 2 v/v%). Abbreviation: arom. means aromatic.

4-Benzyloxy-2-hydroxyacetophenone (II)——Benzyl bromide (56.3 g) was added to a mixture of 2,4-dihydroxyacetophenone (I) (50 g) and K_2CO_3 (68.1 g) in acetone (500 ml), then the mixture was stirred at 50 °C for 4 h. After removal of the insoluble matter, the solvent was evaporated off. The residue was recrystallized from EtOH to give 63.9 g (80%) of colorless plates (II). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1620 (C=O). MS *m/z*: 242 (M^+). NMR ($CDCl_3$) δ: 2.52 (3H, s, $COCH_3$), 5.03 (2H, s, $PhCH_2O$), 6.3—7.7 (8H, m, arom. H), 12.61 (1H, s, OH). *Anal.* Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83. Found: C, 74.20; H, 5.99.

4-Benzyloxy-2-ethoxycarbonylmethoxyacetophenone (III)——Ethyl bromoacetate (45.0 g) was added to a mixture of II (63.0 g) and KOH (18.0 g) in acetone (900 ml), then the mixture was stirred at room temperature for 8 h. After removal of the insoluble matter, the solvent was evaporated off. The residue was recrystallized from EtOH to give 77.6 g (91%) of colorless plates (III), mp 121—123 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1751 (COO), 1652 (C=O). NMR ($CDCl_3$) δ: 1.26 (3H, t, $J=7$ Hz, $OCH_2C\underline{H}_3$), 2.62 (3H, s, $COCH_3$), 4.20 (2H, q, $J=7$ Hz, $OC\underline{H}_2CH_3$), 4.58 (2H, s, OCH_2COO), 4.99 (2H, s, OCH_2Ph), 6.2—7.8 (8H, m, arom. H). MS *m/z*: 328 (M^+). *Anal.* Calcd for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 69.51; H, 6.22.

2-Ethoxycarbonylmethoxy-4-hydroxyacetophenone (IV)——Compound III (50.0 g) was dissolved in a mixture of MeOH (500 ml) and dioxane (100 ml) and hydrogenated over 10% Pd–C (5.0 g). After removal of the catalyst, the solvents were evaporated off. The residue was recrystallized from toluene to give 33.8 g (93%) of colorless needles (IV), mp 120—122 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3160 (OH), 1767 (COO), 1629 (C=O). NMR ($CDCl_3$) δ: 1.26 (3H, t, $J=7$ Hz, $OCH_2COOCH_2C\underline{H}_3$), 2.66 (3H, s, $COCH_3$), 4.23 (2H, q, $J=7$ Hz, $COOC\underline{H}_2CH_3$), 4.61 (2H, s, OCH_2COO). MS *m/z*: 238 (M^+). *Anal.* Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92. Found: C, 60.48; H, 5.98.

4′-Hydroxy-2′,4-bis(methoxycarbonylmethoxy)chalcone (VII)——A solution of NaOH (85.0 g) in H_2O (300 ml) was added to a mixture of IV (25.0 g) and 4-ethoxycarbonylmethoxybenzaldehyde (V)[1] (21.6 g) in EtOH (450 ml), then the mixture was stirred under an N_2 atmosphere at room temperature for 8 h. The reaction mixture was acidified with dil. HCl to precipitate crystals. After removal of the solvent, the resulting crystals were collected, washed with H_2O, and dried to give 32.9 g (85%) of VI. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1758, 1730 (COO × 2), 1663 (C=O). MS *m/z*: 372 (M^+). NMR (DMSO-d_6) δ: 4.67 (2H, s, CH_2COO), 4.74 (2H, s, CH_2COO).

A solution of VI (32.5 g) in MeOH (1000 ml) and conc. HCl (500 ml) was refluxed for 7 h, then cooled. The resulting crystals were collected and washed with chilled MeOH, then recrystallized from MeOH to give 26.7 g (76%) of orange-yellow needles (VII), mp 176—178 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3140 (OH), 1770, 1752 (COO × 2), 1632 (C=O). NMR (DMSO-d_6) δ: 3.67 (6H, s, $COOCH_3 \times 2$), 4.78 (2H, s, OCH_2COO), 4.84 (2H, s, OCH_2COO), 6.3—7.8 (9H, m, arom. H, C_α-H, C_β-H), 10.14 (1H, s, OH). MS *m/z*: 400 (M^+). *Anal.* Calcd for $C_{21}H_{20}O_8$: C, 62.99; H, 5.04. Found: C, 62.91; H, 5.01.

4′-Hydroxy-2′,4-bis(methoxycarbonylmethoxy)dihydrochalcone (VIII)——Compound VII (10 g) was dissolved in a mixture of MeOH (500 ml) and dioxane (150 ml) and hydrogenated over 10% Pd–C (1 g). After removal of the catalyst, the solvents were evaporated off and the residue was recrystallized from EtOH to give 6.34 g (63%) of colorless needles (VIII), mp 139—141 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3220 (OH), 1770, 1756 (COO × 2), 1627 (C=O). NMR (DMSO-d_6) δ: 2.76 (2H, m, $PhC\underline{H}_2CH_2$), 3.20 (2H, m, $PhCH_2C\underline{H}_2$) 3.62 (6H, s, $COOCH_3 \times 2$), 4.63 (2H, s, OCH_2COO), 4.76 (2H, s, OCH_2COO), 6.2—7.6 (7H, m, arom. H), 10.1 (1H, s, OH). MS *m/z*: 402 (M^+). *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found: C, 62.43; H, 5.55.

2′,4-Bis(carboxymethoxy)-4′-(3-methyl-2-butenyloxy)dihydrochalcone (M-2)——3-Methyl-2-butenyl bromide (IX) (0.56 g) was added dropwise to a mixture of VIII (1.0 g) and K_2CO_3 (0.7 g) in acetone (20 ml), then the mixture was stirred at room temperature for 3 h. After filtration of the insoluble matter, the solvent was evaporated off. The residue was recrystallized from isopropyl ether to give 0.84 g (72%) of colorless needles (dimethyl ester of M-2), mp 83—86 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1754, 1735 (COO × 2), 1658 (C = O). NMR ($CDCl_3$) δ: 1.73 (3H, s, CH_3), 1.79 (3H, s, CH_3), 2.90 (2H, m, $PhC\underline{H}_2CH_2$), 3.37 (2H, m, $PhCH_2C\underline{H}_2$), 3.70 (3H, s, $COOCH_3$), 3.76 (3H, s, $COOCH_3$), 4.50 (2H, d, J = 7 Hz, $OC\underline{H}_2CH=C$), 4.56 (2H, s, OCH_2COO), 4.63 (2H, s, OCH_2COO), 5.43 (1H, t, J = 7 Hz, $OCH_2C\underline{H}=C$), 6.3—7.8 (7H, m, arom. H). MS m/z: 470 (M^+). *Anal.* Calcd for $C_{26}H_{30}O_8$: C, 66.37; H, 6.43. Found: C, 66.16; H, 6.42.

A solution of KOH (0.3 g) in H_2O (2 ml) was added to a solution of dimethyl ester of M-2 (0.36 g) in MeOH (4 ml), then the mixture was refluxed for 1 h and acidified with dil. HCl to precipitate crystals. The resulting crystals were recrystallized from acetone–hexane to give 0.23 g (68%) of colorless needles (M-2), mp 150—151 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1742 (COO), 1637 (C = O). MS m/z: 442 (M^+). NMR (DMSO-d_6) δ: 1.72 (6H, s, CH_3 × 2), 2.85 (2H, t, J = 7 Hz, $PhC\underline{H}_2CH_2$), 3.32 (2H, t, J = 7 Hz, $PhCH_2C\underline{H}_2$), 4.58 (2H, d, J = 7 Hz, $C\underline{H}_2CH=C$), 4.59 (2H, s, OCH_2COO), 4.83 (2H, s, OCH_2COO), 5.43 (1H, t, J = 7 Hz, $OCH_2C\underline{H}=C$), 6.5—7.7 (7H, m, arom. H). MS m/z: 442 (M^+). *Anal.* Calcd for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92. Found: C, 65.10; H, 5.93.

2′,4-Bis(carboxymethoxy)-4′-(3-hydroxymethyl-2-butenyloxy)dihydrochalcone (M-1-b)——*trans*-3-Hydroxymethyl-2-butenyl chloride[8] (X) (0.5 g) was added to a mixture of VIII (0.8 g), K_2CO_3 (1.2 g) and KI (10 mg) in acetone (10 ml), and the mixture was stirred at room temperature for 10 h. After removal of the insoluble matter, the solvent was evaporated off. The residue was recrystallized from EtOH to give 0.74 g (76%) of colorless needles (dimethyl ester of M-1-b), mp 103—106 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3440 (OH), 1752, 1740 (COO × 2), 1640 (C = O). NMR ($CDCl_3$) δ: 1.73 (3H, s, CH_3), 1.99 (1H, s, OH), 2.86 (2H, m, $PhC\underline{H}_2CH_2$), 3.34 (2H, m, $PhCH_2C\underline{H}_2$), 3.64 (3H, s, $COOCH_3$), 3.70 (3H, s, $COOCH_3$), 3.98 (2H, s, $C\underline{H}_2OH$), 4.50 (2H, s, OCH_2COO), 4.53 (2H, d, J = 7 Hz, $OC\underline{H}_2CH=C$), 4.56 (2H, s, OCH_2COO), 5.63 (1H, t, J = 7 Hz, $OCH_2C\underline{H}=C$), 6.2—7.3 (7H, m, arom. H). MS m/z: 486 (M^+). *Anal.* Calcd for $C_{26}H_{30}O_9$: C, 64.18; H, 6.22. Found: C, 64.10; H, 6.35.

A mixture of the dimethyl ester of M-1-b (0.24 g) in MeOH (4 ml) and KOH (0.3 g) in H_2O (2 ml) was stirred for 1 h. After addition of dil. HCl, the resulting precipitate was collected and recrystallized from MeOH–H_2O to give 0.17 g (75%) of colorless needles (M-1-b), mp 127—130 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3460 (OH), 1730 (COO), 1657 (C = O). FD-MS m/z: 459 ($M^+ + 1$). NMR (DMSO-d_6) δ: 1.66 (3H, s, CH_3), 2.83 (2H, m, $PhC\underline{H}_2CH_2$), 3.31 (2H, m, $PhCH_2C\underline{H}_2$), 3.85 (2H, s, $C\underline{H}_2OH$), 4.59 (2H, s, OCH_2COO), 4.65 (2H, d, J = 7 Hz, $OC\underline{H}_2CH=C$), 4.83 (2H, s, OCH_2COO), 5.64 (1H, t, J = 7 Hz, $OCH_2C\underline{H}=C$), 6.6—7.7 (7H, m, arom. H). *Anal.* Calcd for $C_{24}H_{26}O_9$: C, 63.87; H, 5.72. Found: C, 63.62; H, 5.63.

2′,4-Bis(carboxymethoxy)-4′-(3-carboxy-2-butenyloxy)dihydrochalcone (M-1-a)——*trans*-3-Methoxycarbonyl-2-butenyl bromide[9] (XI) (2.7 g) was added to a mixture of VIII (4.7 g) and K_2CO_3 (3.5 g) in acetone (180 ml), then the whole was stirred at room temperature for 15 h. After removal of the insoluble matter, the solvent was evaporated off. The residue was recrystallized from MeOH to give 5.49 g (91%) of colorless needles (trimethyl ester of M-1-a), mp 104—105 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1770, 1733, 1715 (COO × 3), 1658 (C = O). MS m/z: 514 (M^+). NMR ($CDCl_3$) δ: 1.90 (3H, s, CH_3), 2.89 (2H, m, $PhC\underline{H}_2CH_2$), 3.35 (2H, m, $PhCH_2C\underline{H}_2$), 3.64 (3H, s, $COOCH_3$), 3.68 (3H, s, $COOCH_3$), 3.71 (3H, s, $COOCH_3$), 4.51 (2H, s, OCH_2COO), 4.58 (2H, s, OCH_2COO), 4.65 (2H, d, J = 7 Hz, $OC\underline{H}_2CH=C$), 6.2—7.8 (8H, m, arom. H × 7 and $OCH_2C\underline{H}=C$). *Anal.* Calcd for $C_{21}H_{30}O_{10}$: C, 63.03; H, 5.88. Found: C, 63.07; H, 6.02.

A solution of the timethyl ester of M-1-a (3.7 g) in THF (100 ml), MeOH (200 ml), and 10% KOH (40 ml) was stirred for 7 h. After evaporation of the solvents, the reaction mixture was acidified with dil. HCl under cooling with ice to precipitate crystals. The resulting crystals were recrystallized from MeOH to give 2.1 g (62%) of colorless needles (M-1-a), mp 193—194 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 1732, 1680 (COO × 2), 1660 (C = O). MS m/z: 472 (M^+). NMR (DMSO-d_6) δ: 1.87 (3H, s, CH_3), 2.85 (2H, t, J = 7 Hz, $PhC\underline{H}_2CH_2$), 3.34 (2H, t, J = 7 Hz, $PhCH_2C\underline{H}_2$), 4.59 (2H, s, OCH_2COO), 4.84 (4H, br, OCH_2COO and $OC\underline{H}_2CH=C$), 6.6—7.7 (8H, m, arom. H and $OCH_2C\underline{H}=C$). *Anal.* Calcd for $C_{24}H_{24}O_{10} \cdot H_2O$: C, 58.77; H, 5.34. Found: C, 58.77; H, 5.23.

4,4′-Dihydroxy-2′-methoxycarbonylmethoxychalcone (XII)——A mixture of sofalcone (20 g) and conc. HCl (10 ml) in MeOH (300 ml) was refluxed for 2 h. The solution was concentrated and the residue was extracted with AcOEt. The AcOEt layer was washed with H_2O, and dried over anhydrous Na_2SO_4. The solvent was evaporated off, and the residue was recrystallized from hexane–acetone to give 10.3 g (71%) of yellow amorphous crystals (XII), mp 191—194 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3440 (OH), 1742 (COO), 1632 (C = O). NMR (DMSO-d_6) δ: 3.68 (3H, s, CH_3), 4.85 (2H, s, OCH_2COO), 6.35 (1H, d, J = 2 Hz, $C_{3'}$-H), 6.43 (1H, dd, J = 8, 2 Hz, $C_{5'}$-H), 6.74 (2H, d, J = 8 Hz, C_3, C_5-H), 7.22—7.79 (5H, m, $C_{2,6,6'}$-H and $C_{\alpha,\beta}$-H). MS m/z: 328 (M^+). *Anal.* Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.60; H, 4.70.

4,4′-Dihydroxy-2′-(methoxycarbonylmethoxy)dihydrochalcone (XIII)——Compound XII (10 g) was dissolved in MeOH (150 ml) and hydroganated over 10% Pd–C (1 g). After removal of the catalyst, the solvent was evaporated off. The residue was recrystallized from ether to give 8.2 g (82%) of colorless needles (XIII), mp 141—142 °C. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3300 (OH), 1750, 1731 (COO × 2), 1639 (C = O). MS m/z: 330 (M^+). NMR (DMSO-d_6) δ: 2.72 (2H, m,

$\mathrm{PhC\underline{H}_2CH_2}$), 3.18 (2H, m, $\mathrm{PhCH_2C\underline{H}_2}$), 3.61 (3H, s, $\mathrm{CH_3}$), 4.78 (2H, s, $\mathrm{OCH_2COO}$), 6.28 (1H, d, $J=1.5$ Hz, $\mathrm{C_{3'}}$-H), 6.41—6.68 (3H, m, $\mathrm{C_{3,5,5'}}$-H), 6.90 (2H, d, $J=8$ Hz, $\mathrm{C_{2,6}}$-H), 7.45 (1H, d, $J=8$ Hz, $\mathrm{C_{6'}}$-H), 7.84 (1H, s, $\mathrm{C_4}$-OH), 8.93 (1H, s, $\mathrm{C_{4'}}$-OH). *Anal.* Calcd for $\mathrm{C_{18}H_{18}O_6}$: C, 65.44; H, 5.49. Found: C, 65.60; H, 5.40.

2′-Carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy)dihydrochalcone (M-6-b)——3-Methyl-2-butenyl bromide (IX) (6.0 g) was added dropwise to a solution of XIII (4.0 g) in acetone (100 ml), then the mixture was stirred at room temperature for 3 h. After removal of the insoluble matter, the solvent was evaporated off. The residue was dissolved in hexane and cooled with dry ice in acetone. The resulting crystals were recrystallized from hexane to give 4.0 g (71%) of colorless needles (methyl ester of M-6-b), mp 72—74 °C. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm^{-1}}$: 1765 (COO), 1665 (C=O). NMR (acetone-d_6) δ: 1.72 (12H, s, $\mathrm{C(CH_3)_2\times 2}$), 2.92 (2H, m, $\mathrm{PhC\underline{H}_2CH_2}$), 3.26 (2H, m, $\mathrm{PhCH_2C\underline{H}_2}$), 3.67 (3H, s, $\mathrm{COOCH_3}$), 4.45 (2H, d, $J=7$ Hz, $\mathrm{C_4\text{-}OC\underline{H}_2CH=C}$), 4.56 (2H, d, $J=7$ Hz, $\mathrm{C_{4'}\text{-}OC\underline{H}_2CH=C}$), 4.81 (2H, s, $\mathrm{OCH_2COO}$), 5.4 (2H, m, $\mathrm{OCH_2C\underline{H}=C\times 2}$), 6.47 (1H, s); 6.6 (1H, m); 6.7 (2H, d, $J=8$ Hz); 7.10 (2H, d, $J=8$ Hz); 7.60 (1H, d, $J=8$ Hz); arom. H × 7. MS *m/z*: 466 ($\mathrm{M^+}$). *Anal.* Calcd for $\mathrm{C_{28}H_{34}O_6}$: C, 72.08; H, 7.35. Found: C, 72.28; H, 7.11.

A mixture of the methyl ester of M-6-b (3.9 g) in acetone (50 ml) and KOH (3 g) in $\mathrm{H_2O}$ (30 ml) was refluxed for 30 min. The solution was concentrated and the residue was acidified with dil. HCl. The resulting crystals were recrystallized from hexane–acetone to give 3.2 g (85%) of amorphous crystals (M-6-b), mp 132—133 °C. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm^{-1}}$: 1745 (COO), 1655 (C=O). MS *m/z*: 452 ($\mathrm{M^+}$). NMR (DMSO-d_6) δ: 1.70 (12H, s, $\mathrm{C(CH_3)_2\times 2}$), 2.85 (2H, d, $J=7$ Hz, $\mathrm{PhC\underline{H}_2CH_2}$), 3.20 (2H, d, $J=7$ Hz, $\mathrm{PhCH_2C\underline{H}_2}$), 4.41 (2H, d, $J=7$ Hz, $\mathrm{C_{4'}\text{-}OC\underline{H}_2CH=C}$), 4.51 (2H, d, $J=7$ Hz, $\mathrm{C_4\text{-}OC\underline{H}_2CH=C}$), 4.75 (2H, s, $\mathrm{OCH_2COO}$), 5.35 (2H, m, $\mathrm{OCH_2C\underline{H}=C\times 2}$), 6.50 (2H, m); 6.67 (2H, d, $J=8$ Hz); 7.02 (2H, d, $J=8$ Hz); 7.48 (1H, d, $J=8$ Hz); arom. H × 7. *Anal.* Calcd for $\mathrm{C_{27}H_{32}O_6}$: C, 71.66; H, 7.13. Found: C, 71.57; H, 7.16.

4-Hydroxy-2′-methoxycarbonylmethoxy-4′-(3-methyl-2-butenyloxy)dihydrochalcone (XIV)——3-Methyl-2-butenyl bromide (IX) (11 g) was added dropwise to a mixture of XIII (20 g) and $\mathrm{K_2CO_3}$ (20 g) in acetone (300 ml), then the whole was stirred at room temperarure for 4 h. After removal of the insoluble matter, the solvent was evaporated off. The residue was extracted with AcOEt. The AcOEt layer was washed with $\mathrm{H_2O}$, and dried over anhydrous $\mathrm{Na_2SO_4}$. After the evaporation of the solvent, the resulting residue was recrystallized from hexane–acetone to give 17.0 g (70%) of colorless needles (XIV), mp 108—109 °C. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm^{-1}}$: 3300 (OH), 1739 (COO), 1647 (C=O). MS *m/z*: 398 ($\mathrm{M^+}$). NMR (acetone-d_6) δ: 1.70 (6H, s, $\mathrm{C(CH_3)_2}$), 2.86 (2H, d, $J=7$ Hz, $\mathrm{PhC\underline{H}_2CH_2}$), 3.24 (2H, d, $J=7$ Hz, $\mathrm{PhCH_2C\underline{H}_2}$), 3.63 (3H, s, $\mathrm{COOCH_3}$), 4.54 (2H, d, $J=7$ Hz, $\mathrm{OC\underline{H}_2CH=C}$), 4.78 (2H, s, $\mathrm{OCH_2COO}$), 5.4 (1H, m, $\mathrm{OCH_2C\underline{H}=C}$), 7.83 (1H, s, OH), 6.43 (1H, s); 6.6 (3H, m); 6.97 (2H, d, $J=8$ Hz); 7.58 (1H, d, $J=8$ Hz); arom. H × 7. *Anal.* Calcd for $\mathrm{C_{23}H_{26}O_6}$: C, 69.33; H, 6.58. Found: C, 69.05; H, 6.60.

4-Ethoxycarbonylmethoxy-2′-(methoxycarbonylmethoxy)-4′-(3-methyl-2-butenyloxy)dihydrochalcone (XV)——Ethyl bromoacetate (10 g) was added dropwise to a mixture of XIV (17 g) and $\mathrm{K_2CO_3}$ (17 g) in acetone (500 ml), then the mixture was refluxed for 2 h. After removal of the insoluble matter, the solvent was evaporated off. The residue was dissolved in hexane and cooled with dry ice in acetone. The resulting crystals were recrystallized from hexane to give 18.5 g (90%) of colorless needles (XV), mp 84—87 °C. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm^{-1}}$: 1761, 1740 (COO × 2), 1669 (C=O). MS *m/z*: 484 ($\mathrm{M^+}$). NMR (acetone-d_6) δ: 1.21 (3H, t, $J=7$ Hz, $\mathrm{COOCH_2C\underline{H}_3}$), 1.73 (6H, s, $\mathrm{C(CH_3)_2}$), 2.92 (2H, d, $J=7$ Hz, $\mathrm{PhC\underline{H}_2CH_2}$), 3.26 (2H, d, $\mathrm{PhCH_2C\underline{H}_2}$), 3.65 (3H, s, $\mathrm{COOCH_3}$), 4.14 (2H, q, $J=7$ Hz, $\mathrm{COOC\underline{H}_2CH_3}$), 4.55 (2H, d, $J=7$ Hz, $\mathrm{OC\underline{H}_2CH=C}$), 4.60 (2H, s, $\mathrm{C_4\text{-}OCH_2COO}$), 4.80 (2H, s, $\mathrm{C_{2'}\text{-}OCH_2COO}$), 5.4 (1H, m, $\mathrm{C_{4'}\text{-}OCH_2C\underline{H}=C}$), 6.46 (1H, s); 6.6 (1H, m); 6.73 (2H, d, $J=8$ Hz); 7.11 (2H, d, $J=8$ Hz), 7.61 (1H, d, $J=8$ Hz); arom. H × 7. *Anal.* Calcd for $\mathrm{C_{27}H_{32}O_8}$: C, 66.92; H, 6.66. Found: C, 67.10; H, 6.41.

2′-Carboxymethoxy-4-hydroxy-4′-(3-methyl-2-butenyloxy)dihydrochalcone (M-4-b)——A mixture of XIV (1 g) in acetone (30 ml) and KOH (2 g) in $\mathrm{H_2O}$ (30 ml) was refluxed for 0.5 h. After evaporation of the acetone, the residue was recrystallized from hexane or subjected to column chromatography on silica gel with acetone–hexane (1 : 1). However, pure crystals could not be obtained and only 0.5 g of crude crystals (M-4-b) resulted. IR $\nu_{\max}^{\mathrm{KBr}}\,\mathrm{cm^{-1}}$: 3300 (OH), 1730 (COO), 1651 (C=O). MS *m/z*: 384 ($\mathrm{M^+}$) for $\mathrm{C_{22}H_{24}O_6}$. NMR (acetone-d_6) δ: 1.73 (6H, s, $\mathrm{C(CH_3)_2}$), 2.90 (2H, m, $\mathrm{PhC\underline{H}_2CH_2}$), 3.25 (2H, m, $\mathrm{PhCH_2C\underline{H}_2}$), 4.55 (2H, d, $J=7$ Hz, $\mathrm{OC\underline{H}_2CH=C}$), 4.80 (2H, s, $\mathrm{OCH_2COO}$), 5.4 (1H, m, $\mathrm{OCH_2C\underline{H}=C}$), 6.35—6.75 (4H, m); 6.97 (2H, d, $J=8$ Hz); 7.58 (1H, d, $J=8$ Hz); arom. H × 7.

Hydrolysis of XV to Produce M-2——A mixture of XV (18 g) in acetone (300 ml) and KOH (10 g) in $\mathrm{H_2O}$ (80 ml) was refluxed for 1 h. After evaporation of the acetone, the residue was acidified with dil. HCl. The resulting crystals were recrystallized from hexane–acetone to give 14.5 g (88%) of colorless needles (M-2). Compound M-2 was identical with an authentic sample of M-2 obtained from VIII (Chart 1) on the basis of IR, NMR and mass spectral comparisons, and elemental analysis. This was confirmed by mixed melting point measurement.

Bioassay——Male Wister strain rats weighing 180—200 g were used. Samples (sofalcone and atropine sulfate) were suspended in 0.4% (w/v) sodium carboxymethylcellulose solution, and the suspension was given *i.p.*

$$\text{Inhibitory ratio (\%)} = \frac{\text{ulcer index (control)} - \text{ulcer index (sample)}}{\text{ulcer index (control)}}$$

Shay's Pylorus-Ligated Rats[6]: Animals were deprived of food for 48 h before the experiments. Under ether anesthesia, the animals were laparotomied and the pylorus was ligated. Samples were given immediately, then the abdomen was closed. The animals were sacrificed 18 h after the pylorus ligation by means of an overdose of ether. The stomach was excised, and lesions developing in the forestomach were grossly examined. The sum of the areas (mm^2) of lesions for each rat was determined as the ulcer index.

Takagi's Water-Immersed and Restraint-Stressed Rats[6]: Animals were derived of food for 18 h before the experiments. After administration of test samples, the animals were placed in a stress cage and immersed in a water bath (23 °C) for 7 h to the xiphoid level. At the end of the stress, the animals were sacrificed by means of a blow on the head, then cut open and examined grossly for lesions in the glandular portion. The ulcer index was calculated as the sum of the areas (mm^2) of lesions for each rat.

Histamine-Induced Ulcer[6]: Animals were deprived of food for 18 h before the experiments. Histamine dihydrochloride 300 mg/kg was given *i.p.* 0.5 h after the administration of the samples. The animals were sacrificed 4 h after the administration of histamine by means of an overdose of ether, then the stomach was excised. The stomach was cut open and examined grossly for lesions in the glandular portion. The ulcer index was calculated as the sum of the areas (mm^2) of lesions for each rat.

References and Notes

1) Part II: K. Kyogoku, K. Hatayama, S. Yokomori, R. Saziki, S. Nakane, M. Sasajima, J. Sawada, M. Ohzeki and I. Tanaka, *Chem. Pharm. Bull.*, **27**, 2943 (1979).
2) A part of this work was reported at the 101st Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, April 1981 and at the 28th Congress of the International Union of Pure and Applied Chemistry, Vancouver (Canada), August 1981.
3) M. Komatsu, T. Tomimori, K. Hatayama and N. Mikuriya, *Chem. Pharm. Bull.*, **18**, 602 (1970).
4) M. Sasajima, S. Nakane, R. Saziki, H. Saotome, H. Hatayama, K. Kyogoku and I. Tanaka, *Yakugaku Zasshi*, **74**, 897 (1978).
5) K. Kyogoku, K. Hatayama, S. Yokomori, T. Seki and I. Tanaka, *Agric. Biol. Chem.*, **38**, 229 (1974); *idem, ibid.*, **39**, 133 (1975); *idem, ibid.*, **39**, 667 (1975).
6) T. Nozu, T. Suwa, K. Fukushima and M. Ohzeki, *Oyo Yakuri*, **18**, 835 (1979).
7) Y. Hayashi and T. Umehara, *Yakuri To Chiryo*, **10**, 2697 (1982).
8) P. L. Stotter and K. A. Hill, *Tetrahedron Lett.*, **1975**, 1679.
9) D. S. Letham and H. Young, *Phytochemistry*, **10**, 2077 (1971).