This basic hypothesis remains plausible although the thiazides have not been found to alter the concentration of sodium in the plasma of hypertensive patients (10), or the sodium content of the arterial walls of experimental animals (11).

The studies with intestinal smooth muscle conducted by Riedesel and Combs (4) on the basis of which they postulated that hesperidin methyl chalcone inhibits potassium efflux, and our observations that chalcone R-2803 increased regional perfusion rate and decreased the reactivity of isolated aortic smooth muscle to norepinephrine and angiotensin, prompted an investigation of the effect of R-2803 on the electrolyte composition of vascular tissue. Single intravenous hypotensive doses of R-2803 increased the sodium and potassium content of rabbit aorta and decreased the serum sodium and potassium levels. These data may reflect a shift in the equilibrium of electrolytes between blood and vascular muscle, *i.e.*, alterations in the intracellular/extracellular gradients of sodium and potassium ions. An approximate equidepressor dose of hesperidin methyl chalcone, which was about one-fourth as potent as R-2803 on a milligram basis, produced similar changes in aortic sodium and potassium levels.

The electrolyte alterations in vascular muscle following administration of R-2803 and hesperidin methyl chalcone are apparently not a consequence of the blood pressure reduction as evidenced by the failure of other potent hypotensive agents (*i.e.*, mecamylamine, guanethidine, and chlorothiazide) to similarly alter the aortic electrolyte concentrations.

Sodium and potassium imbalances in vascular smooth muscle may play a role in the initial phases of the hypotensive action of the chalcones, with the inhibition of potassium efflux preventing effective depolarization of the muscle membrane. However, due possibly to compensatory mechanisms, the electrolyte concentrations in vascular tissue revert toward normal levels within a time period during which refractoriness to the blood pressure reducing effect does not develop.

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Keyphrases

Chalcone derivatives—pharmacological activity
Depressor activity—chalcone R-2803
Hypotensive activity—chalcone R-2803
Norepinephrine activity—antagonized
Angiotensin activity—antagonized
Electrolyte changes—smooth muscle, blood

Cyclic α,β-Unsaturated Ketones Related to Ethacrynic Acid

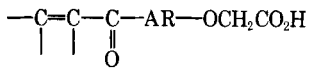
By JOHN G. TOPLISS and LEROY M. KONZELMAN

2-Carboxymethoxy-8,9-dihydro-6-methyl-5*H*-benzocyclohepten-5-one and 5-carboxymethoxy-2-methylindone, which are structurally related to ethacrynic acid, have been synthesized and the acute renal excretory response to intravenous injections of the compounds in the anesthetized dog has been evaluated. Only marginal activity, compared to ethacrynic acid, was observed.

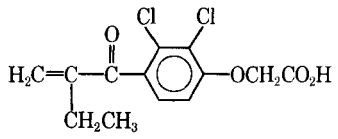
THE DISCOVERY of a new class of diuretic agents, α,β-unsaturated ketone derivatives of aryloxyacetic acids of the general structure I, was reported by Schultz *et al.* in 1962 (1). Subsequently, pharmacological and clinical reports (2) have appeared on a compound in this series, ethacrynic acid (II).

The authors were interested in determining the effect on diuretic activity of the incorporation of the α,β-unsaturated ketonic function in a ring system in compounds of this general structural type. Consequently, it was decided to synthe-

Received October 19, 1967, from the Medicinal Chemical Research Department, Schering Corporation, Bloomfield, NJ 07003
Accepted for publication December 15, 1967.
The authors thank Dr. N. Sperber for his interest and encouragement.

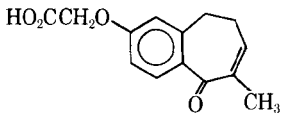


I

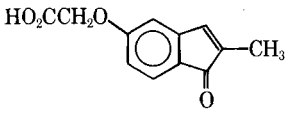


II

size III, and also IV where additional conjugation of the unsaturated ketonic function is present.



III

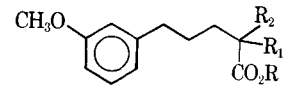


IV

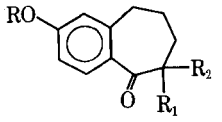
DISCUSSION

The route for the synthesis of the benzocycloheptenone (III) started from *m*-methoxycinnamylidenemalonic acid (Va) which was esterified with methanol in the presence of hydrogen chloride and the resulting dimethyl ester (Vb) was reduced in methanol solution with a palladium catalyst to give dimethyl 3-(*m*-methoxyphenyl)-propylmalonate (VIa). Methylation with methyl iodide and sodium hydride in dimethylformamide gave the malonic ester (VIb) which was then hydrolyzed with alkali and the resulting malonic acid (VIc) was decarboxylated by heating to give 5-(*m*-methoxyphenyl)-2-methylvaleric acid (VId). Cyclization of VId was effected using polyphosphoric acid in high dilution at 70° furnishing 2-methoxy-6-methylbenzocycloheptan-5-one (VIIa). The four-step sequence VIa through VIIa could be performed without purification of the individual intermediates in an overall yield of 80%. Demethylation of VIIa with hydrogen iodide in acetic acid afforded 2-hydroxy-6-methylbenzocycloheptan-5-one (VIIb) which was then transformed into its acetic acid derivative, VIIc, with chloroacetic acid in aqueous alkali. Bromination of VIIc using bromine in methylene chloride yielded the α-bromoketone VIId and this compound on dehydrobromination with refluxing 2,4-lutidine afforded the desired 2-carboxymethoxy-8,9-dihydro-6-methyl-5*H*-benzocyclohepten-5-one (III).

Va, R = H
Vb, R = CH_3

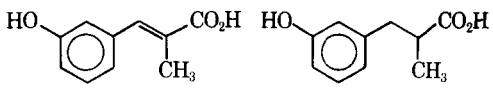


VIa, R = CH_3; R_1 = CO_2CH_3; R_2 = H
VIb, R = CH_3; R_1 = CO_2CH_3; R_2 = CH_3
VIc, R = H; R_1 = CO_2H; R_2 = CH_3
VId, R = H; R_1 = H; R_2 = CH_3

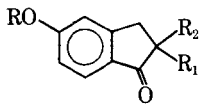


VIIa, R = CH_3; R_1 = H; R_2 = CH_3
VIIb, R = H; R_1 = H; R_2 = CH_3
VIIc, R = CH_2CO_2H; R_1 = H; R_2 = CH_3
VIId, R = CH_2CO_2H; R_1 = Br; R_2 = CH_3

For the synthesis of the indone (IV), *m*-hydroxy-α-methyl cinnnamic acid (VIII) was hydrogenated in dilute sodium hydroxide solution in the presence of a palladium catalyst to give *m*-hydroxy-α-methylhydrocinnamic acid (IX). Cyclization of



VIII IX



a, R = H; R_1 = H; R_2 = CH_3
b, R = CH_2CO_2H; R_1 = H; R_2 = CH_3
c, R = CH_2CO_2H; R_1 = Br; R_2 = CH_3

IX was effected with a boron trifluoride catalyst in chloroform solution to give 5-hydroxy-2-methyl-1-indanone (Xa)[1] which was converted into its acetic acid derivative Xb. The α-bromoketone, Xc, was obtained by bromination of Xb with bromine in methylene chloride solution and Xc was converted into the desired 5-(carboxymethoxy)-2-methylindone (IV) by treatment with refluxing 2,4-lutidine.

Renal Pharmacology[2]—The acute renal excretory response to intravenous injections of the compounds was examined in dogs anesthetized with 50 mg./Kg. of vinbarbital. Physiological saline was infused intravenously at a rate of 1.0 to 3.0 ml./min. continuously except for the experiment on IV. In this instance the infusion fluid (3.0 ml./min.) contained 5% mannitol and 0.25% sodium chloride. Ureters were cannulated with polyethylene tubing to facilitate urine collections. Compounds were dissolved in alkali and, after adjustment of pH, injected into the infusion tube. A standard diuretic, ethacrynic acid, dissolved in 5% sodium bicarbonate was given at a dose of 5 mg./Kg. after at

[1] Cyclization of *m*-hydroxy-α-methylcinnamic acid (VIII) under various conditions to give 5-hydroxy-2-methylindone was unsuccessful.

[2] Determined by the late Dr. R. M. Taylor, Biological Research Division, Schering Corporation.

least 1 hr. had elapsed from the time of injecting the test compound. Sodium and chloride ions were determined in 10-min. urine collections by flame photometery and electrometric titration with silver ion, respectively.

The activity of each compound, included in Table I, represents the percent increase in sodium

TABLE I—NATRIURETIC ACTIVITY OF SOME CYCLIC KETONES RELATED TO ETHACRYNIC ACID

Compd.	Dose, mg./Kg.	Natriuretic Activity[a]
IV	20	30
Xb	10	26
III	10	16
VIIc	10	11
Ethacrynic acid	5	411

[a] Percent increase in sodium excretion based on mean values of three 10-min. control and treated periods. Changes in chloride and urine flow were roughly similar to sodium excretion in magnitude and duration.

excretion over control values using averages for three 10-min. periods before (control) and after (treated) injection. The activity of ethacrynic acid given in Table I is an average value for these experiments.

Biological Results—The compounds showed only marginal diuretic activity compared to ethacrynic acid. The latter appears to exert its diuretic effect through interaction of the α,β-unsaturated acyl group with functionally important sulfhydryl groups (3). Structure-activity studies in the series indicated that for maximum biological activity one position in the aromatic nucleus *ortho* to the unsaturated ketone function must be substituted (1) and that monosubstitution of the methylene of the unsaturated acyl group causes some loss of activity (4). Saturation of the double bond results in almost complete loss of activity (1, 4). With regard to the compounds synthesized in the present study the very low order of activity exhibited by the cyclic saturated ketones VIIc and Xb, was in accord with expectations. In the case of the cyclic unsaturated ketones III and IV, although the known structural requirements for activity appear to be satisfied, the observed diuretic activity was of a marginal character. It seems unlikely that the double bond in these compounds would be chemically unreactive to sulfhydryl groups. However, the relative orientations in space of the double bond, carbonyl group, and phenyl ring are fixed which may interfere with the ability of the compounds to achieve an adequate fit at the receptor site.

EXPERIMENTAL[3]

Dimethyl m - Methoxycinnamylidenemalonate (Vb)—A solution of Va (5) (150 Gm.) in methanol (1,000 ml.) and concentrated HCl (25 ml.) was refluxed for 16 hr. The solution was evaporated *in vacuo* leaving a brown viscous oil which was used in the next step without further purification. Crystallization of the oil from $CHCl_3$-hexane gave the analytical sample, m.p. 61–63°.

[3] Melting points (uncorrected) were determined on a Thomas-Hoover capillary melting-point apparatus and ultraviolet absorption spectra were determined in methanol solution.

Anal.—Calcd. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 65.06; H, 5.89.

Dimethyl 3 - (m - Methoxyphenyl)propylmalonate (VIa)—A solution of crude Vb (161 Gm.) in methanol (600 ml.) was hydrogenated for 16 hr. at 3.4 Kg./cm.^{2} pressure and room temperature in the presence of 5% Pd/C catalyst (6 Gm.). Removal of the catalyst by filtration followed by evaporation of the methanol *in vacuo* gave the product (148 Gm.) as a brown oil which was used without further purification in the next step.

Dimethyl 3 - (m - Methoxyphenyl)propylmethylmalonate (VIb)—Sodium hydride (3.54 Gm., 0.079 mole, of a 53.5% suspension in mineral oil) was added in small portions to a stirred solution of VIa (20.0 Gm., 0.071 mole) in dry DMF (100 ml.) at room temperature. The solution was heated at 90° for 0.5 hr., cooled to room temperature, and methyl iodide (30.4 Gm., 0.214 mole) was then added dropwise. After heating the stirred reaction mixture at 90° for 5 hr., the solvent was removed *in vacuo* to give the crude product which was used without further purification in the next step.

3-(m-Methoxyphenyl)propylmethylmalonic Acid (VIc)—NaOH (20 Gm.) in water (110 ml.) was added to the crude VIb obtained in the previous step and the reaction mixture stirred under reflux for 5 hr. The resulting solution was acidified with concentrated HCl, extracted with $CHCl_3$, and the $CHCl_3$ extract washed with water, dried (Na_2SO_4), and the solvent evaporated. A small portion of the residue solidified on trituration with hexane. This was recrystallized twice from ether-hexane to give the analytical sample, m.p. 93–95°.

Anal.—Calcd. for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 63.20; H, 6.78.

5-(m - Methoxyphenyl) - 2 - methylvaleric Acid (VId)—The crude malonic acid (VIc) was decarboxylated by heating at 160–170° for 1 hr. to give crude VId (17.2 Gm.) characterized as the *S*-benzylisothiuronium salt, m.p. 125–126°.

Anal.—Calcd. for $C_{21}H_{18}N_2O_3S$: C, 64.92; H, 7.26; N, 7.21; S, 8.25. Found: C, 64.89; H, 7.51; N, 7.26; S, 7.83.

2- Methoxy - 6 - methylbenzocycloheptan - 5 - one (VIIa)—Crude VId (16.7 Gm.) was added to polyphosphoric acid at 70° with rapid stirring. The reaction mixture was stirred at 70° for 1.5 hr. and then poured onto ice and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed with 10% NaOH followed by water, dried (Na_2SO_4), and the solvent was evaporated. The residual crude ketone was chromatographed on an alumina column and elution with 1:1 benzene-hexane gave VIIa (10.5 Gm.), $\lambda_{max.}$ 222 mμ (ϵ 11,700); 270 mμ (ϵ 12,400). The semicarbazone crystallized from methanol–water, m.p. 191–192°.

Anal.—Calcd. for $C_{14}H_{19}N_3O_2$: C, 64.34; H, 7.33; N, 16.08. Found: C, 64.01; H, 7.46; N, 16.14.

2- Hydroxy - 6 - methylbenzocycloheptan - 5 - one (VIIb)—A solution of VIIa (6.8 Gm.) in glacial acetic acid (35 ml.) and 47% hydriodic acid (35 ml.) was refluxed for 1 hr. under nitrogen. The reaction mixture was diluted with water (100 ml.) and extracted with ether. The ether extracts were washed with saturated $NaHCO_3$ and then extracted with 10% NaOH. Evaporation of the ether gave recovered VIIa (2.7 Gm.). Acidification of the NaOH extracts gave crude VIIb (3.2 Gm.), m.p. 120–125°.

Recrystallization from ether–hexane gave 2.8 Gm., m.p. 135–137°. The analytical sample had m.p. 136–137°.

Anal.—Calcd. for $C_{12}H_{14}O_2$: C, 75.76; H, 7.42. Found: C, 75.90; H, 7.36.

2-Carboxymethoxy-6-methylbenzocycloheptan-5-one (VIIc)—Chloroacetic acid (2.2 Gm., 0.023 mole) was added to a solution of VIIb (2.9 Gm., 0.015 mole) in NaOH (from 1.7 Gm. NaOH and 25 ml. water) and the resulting solution was refluxed for 5 hr., cooled and acidified with concentrated HCl. The precipitated solid was collected and stirred with saturated $NaHCO_3$ solution (80 ml.) for 1 hr. Filtration gave starting material (0.65 Gm.) and acidification of the filtrate afforded the crude product (2.6 Gm.), m.p. 148–150°. The analytical sample was obtained on recrystallization from ether, m.p. 152–153°.

Anal.—Calcd. for $C_{14}H_{16}O_4$: C, 67.72; H, 6.50. Found: 67.59; H, 6.74.

6-Bromo-2-carboxymethoxy-6-methylbenzocycloheptan-5-one (VIId)—A solution of bromine (1.7 Gm., 0.011 mole) in CH_2Cl_2 (20 ml.) was added dropwise to a stirred refluxing suspension of VIIc (3.0 Gm.) in CH_2Cl_2 (100 ml.). Stirring was continued for 20 min. at room temperature on completion of the bromine addition, and the solvent was then evaporated in an air current giving crude α-bromoketone m.p. 206–209°. Recrystallization from $CHCl_3$ gave 3.1 Gm., m.p. 212–214°. The analytical sample, obtained by recrystallizing once more from the same solvent, had a m.p. 213–214°.

Anal.—Calcd. for $C_{14}H_{15}BrO_4$: C, 51.39; H, 4.62. Found: C, 51.32; H, 4.76.

2-Carboxymethoxy-8,9-dihydro-6-methyl-5H-benzocyclohepten-5-one (III)—A solution of VIId (3.1 Gm.) in 2,4-lutidine (12 ml.) was heated at 160° (oil bath) for 1.5 hr., cooled, diluted with water (40 ml.), acidified with concentrated HCl, and filtered. The crude product, 1.8 Gm., m.p. 158–160°, was recrystallized from $CHCl_3$-hexane to give 1.7 Gm., m.p. 161–162°, unchanged on further recrystallization, $\lambda_{max.}$ 237 mμ (ϵ 10,700); 291 mμ (ϵ 7,060).

Anal.—Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.28; H, 5.63.

m-Hydroxy-α-methylhydrocinnamic acid (IX)—A solution of VIII (6) (90.0 Gm.) in 10% NaOH (300 ml.) was hydrogenated in a Parr shaker over 5% Pd/C (2.0 Gm.) at 3.4 Kg./cm.^{2} pressure and room temperature. After filtration to remove the catalyst, the solution was acidified with concentrated HCl, extracted with ether, and the extracts washed with water and dried (Na_2SO_4). Evaporation of the ether gave the product as a viscous brown oil (89.5 Gm.) which was characterized as the piperidine salt, m.p. 142–144° after crystallization from acetone.

Anal.—Calcd. for $C_{15}H_{22}NO_3$: C, 67.89; H, 8.75. Found: C, 67.70; H, 9.00.

5-Hydroxy-2-methyl-1-indanone (Xa)—Boron trifluoride was bubbled into a cold stirred solution of IX (20.0 Gm.) in $CHCl_3$ (500 ml.) for 4 hr. The reaction mixture was stirred at room temperature for 16 hr. and then 10% Na_2CO_3 (400 ml.) was slowly added, after which stirring was continued at room temperature for 1 hr. more. The chloroform layer was separated, washed with water, dried (Na_2SO_4), and the solvent was evaporated to give the crude indanone (16.0 Gm.) m.p. 136–142°. Recrystallization from ether-hexane gave 11.6 Gm., m.p. 153–155° [reported (7) m.p. 155.5–156.5°, prepared by different method].

5-Carboxymethoxy-2-methyl-1-indanone (Xb)—The procedure for the preparation of VIIc was employed using Xa (7.0 Gm., 0.043 mole), chloroacetic acid (6.2 Gm., 0.065 mole), and NaOH (4.6 Gm.) in water (200 ml.) yielding crude Xb (6.1 Gm.), m.p. 125–135° and recovered Xa (2.7 Gm.). The product was recrystallized from chloroform-hexane giving 4.9 Gm., m.p. 147–149°. The analytical sample had m.p. 148–150°.

Anal.—Calcd. for $C_{12}H_{12}O_4$: C, 65.64; H, 5.49. Found: C, 65.19; H, 5.39.

2-Bromo-5-carboxymethoxy-2-methyl-1-indanone (Xc)—The technique employed for the preparation of VIId was used. Bromination of Xb (31.9 Gm.) gave crude bromoketone (42.0 Gm.) m.p. 110–115°. Recrystallization from ether–hexane gave 37.0 Gm., m.p. 138–140°. The analytical sample had m.p. 138–139°.

Anal.—Calcd. for $C_{12}H_{13}BrO_4$: C, 48.14; H, 3.71; Br, 26.72. Found: C, 48.17; H, 3.90; Br, 27.04.

5-Carboxymethoxy-2-methylindone (IV)—A solution of Xc (18.29 Gm.) in 2,4-lutidine (73 ml.) was heated at 160° for 1.5 hr., poured into dilute HCl and filtered giving crude IV (10.1 Gm.), m.p. 165–170°. Recrystallization from chloroform–hexane furnished 6.1 Gm. of yellow crystals, m.p. 183–185°. The analytical sample had m.p. 187–188°. $\lambda_{max.}$ 218 mμ (ϵ 20,300); 257 mμ (ϵ 52,500); 330 mμ (ϵ 6,900).

Anal.—Calcd. for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 66.39; H, 4.33.

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Keyphrases

α,β–Aryloxyacetic acid ketones, unsaturated—synthesis
Ethacrynic acid related compounds
Structure–activity relationship
UV spectrophotometry—structure
Diuretic activity—α,β-aryloxyacetic acid ketones
Flame photometry—sodium ion analysis
Electrometric titration—chloride ion analysis