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S-(4-Chlorophenyl) 3-Aryl-3-hydroxypropanethioates as Antibacterial Agents

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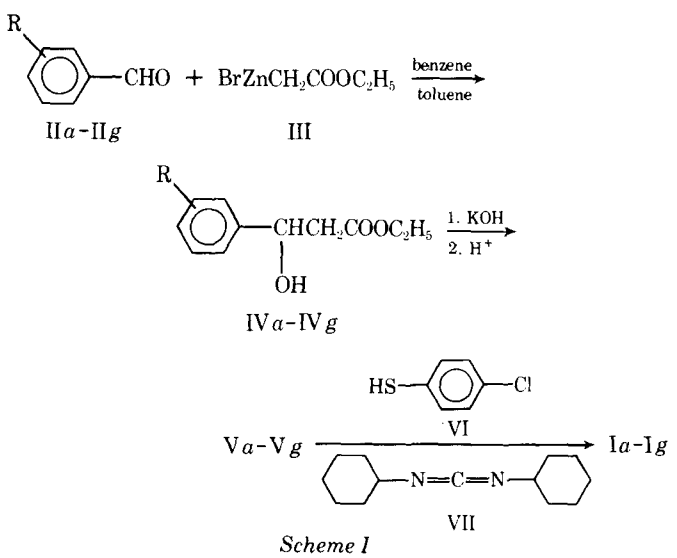
Received March 14, 1977, from the *Research and Development Department, Norwich-Eaton Pharmaceuticals Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication June 1, 1977.

Abstract □ A series of *S*-(4-chlorophenyl) 3-aryl-3-hydroxypropanethioates was prepared and shown to have *in vitro* activity against several selected bacterial species.

Keyphrases □ 3-Hydroxypropanethioates, 3-aryl—series synthesized, antibacterial activity evaluated □ Antibacterial activity—3-aryl-3-hydroxypropanethioates evaluated □ Structure-activity relationships—3-aryl-3-hydroxypropanethioates evaluated for antibacterial activity

The usual pathway for fatty acid (both saturated and unsaturated) synthesis in bacteria is *via* thiol esters of coenzyme A intermediates (1–3). The enzyme responsible for the introduction of the double bond into the fatty acids of *Escherichia coli* is β-hydroxydecanoylthiol ester dehydrase (2, 3). This enzyme is vital for the growth of *E. coli* (2); its inhibition completely stops the growth of the bacterium (4, 5).

The required substrate for dehydrase preferably contains β-hydroxydecanoate as a thiol ester (2–4), which is dehydrated to a β,γ-unsaturated ester. It was postulated that a reasonable structure for a dehydrase inhibitor would possess a thiol ester of a β-hydroxy acid in which the γ-carbon is tertiary and, therefore, incapable of forming a β,γ-double bond. Therefore, a series of *S*-(4-chlorophenyl) 3-aryl-3-hydroxypropanethioates (I*a*–I*g*, Table I) was prepared and tested for activity *in vitro* against several selected bacterial species.



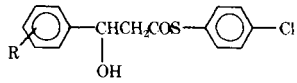
Scheme I

DISCUSSION

Chemistry—The title compounds were prepared by the following reaction sequence (Scheme I). The appropriately substituted benzaldehyde (II*a*–II*g*) was reacted with the zinc complex (III) formed by ethyl bromoacetate and zinc under the conditions of the Reformatsky reaction (6). The resulting crude ethyl 3-aryl-3-hydroxypropanoates (IV*a*–IV*g*) were hydrolyzed directly with alcoholic potassium hydroxide solution to yield the 3-aryl-3-hydroxypropanoic acids (V*a*–V*g*, Table II). The hydroxy acids were coupled with 4-chlorobenzenethiol (VI) using dicyclohexylcarbodiimide (VII) in dichloromethane to yield the desired products. All thiol esters showed a strong OH peak at 2.8–3.0 μm in their IR spectra (mineral oil mull).

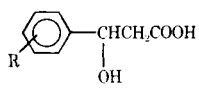
Biological Activity—The propanethioates were screened *in vitro*

Table I—*S*-(4-Chlorophenyl) 3-Aryl-3-hydroxypropanethioates



Compound	R	Melting Point	Yield, %	Recrystallization Solvent	Formula	Analysis, % Calc.	Found
I*a*	H	103–105°	60	Benzene–hexane	$C_{15}H_{13}ClO_2S$	C 61.53	61.86
						H 4.48	4.54
						Cl 12.11	12.08
I*b*	4-CH_3	113–114°	74	Benzene–hexane	$C_{16}H_{15}ClO_2S$	C 62.64	62.92
						H 4.93	4.97
						Cl 11.56	11.66
I*c*	4-C_2H_5	121–123°	60	Methanol	$C_{17}H_{17}ClO_2S$	C 63.64	63.36
						H 5.34	5.22
						Cl 11.05	11.12
I*d*	4-Cl	93–94°	66	Benzene–hexane	$C_{15}H_{12}Cl_2O_2S$	C 55.06	55.45
						H 3.70	3.84
						Cl 21.51	21.50
I*e*	2,4-Cl_2	78–79°	42	Methanol	$C_{15}H_{11}Cl_3O_2S$	C 49.81	49.74
						H 3.07	3.03
						Cl 29.41	29.54
I*f*	3,4-Cl_2	74–75°	77	Benzene–hexane	$C_{15}H_{11}Cl_3O_2S$	C 49.81	50.21
						H 3.07	3.18
						Cl 29.41	29.11
I*g*	3-Cl, 4-CH_3	85–86°	68	Benzene–hexane	$C_{16}H_{14}Cl_2O_2S$	C 56.31	56.35
						H 4.12	4.22
						Cl 20.78	20.81

Table II—3-Aryl-3-hydroxypropanoic Acids



Compound	R	Melting Point	Yield[a], %	Recrystallization Solvent	Formula	Analysis, % Calc.	Found
V*a*	H	89–91°	23	Hexane–benzene	$C_9H_{10}O_3$	C 65.06	65.09
						H 6.07	6.13
						Cl —	—
V*b*	4-CH_3	88–90°	70	Benzene–hexane	$C_{10}H_{12}O_3$	C 66.65	66.53
						H 6.71	6.66
						Cl —	—
V*c*	4-C_2H_5	74–75°	45	Benzene–hexane	$C_{11}H_{14}O_3$	C 68.02	68.11
						H 7.27	7.25
						Cl —	—
V*d*	4-Cl[b]	75–77°	65	Benzene–hexane	$C_9H_9ClO_3$	C —	—
						H —	—
						Cl —	—
V*e*	2,4-Cl_2	124–125°	68	Benzene	$C_9H_8Cl_2O_3$	C 45.98	46.07
						H 3.43	3.55
						Cl 30.17	30.15
V*f*	3,4-Cl_2	74–75°	77	Benzene–hexane	$C_9H_8Cl_2O_3$	C 45.98	45.98
						H 3.43	3.43
						Cl 30.17	30.37
V*g*	3-Cl, 4-CH_3	85–86°	67	Benzene–hexane	$C_{10}H_{11}ClO_3$	C 55.95	56.12
						H 5.17	5.22
						Cl 16.52	16.32

[a] Yield calculated from aldehyde. [b] See Ref. 10.

Table III—*In Vitro* Antibacterial Activity of *S*-(4-Chlorophenyl) 3-Aryl-3-hydroxypropanethioates

Compound	Minimal Inhibitory Concentration Values, μg/ml *Staphylococcus aureus*	*Corynebacterium liquefaciens*	*Haemophilis vaginalis*	*Streptococcus agalactiae*	*Pasteurella multocida*	*Escherichia coli*
I*a*	6.25	6.25	12.50	3.10	6.25	>50
I*b*	12.50	12.50	3.10	3.10	3.10	>50
I*c*	6.25	6.25	6.25	3.10	6.25	>50
I*d*	12.50	6.25	3.10	6.25	6.25	>50
I*e*	6.25	3.10	12.50	12.50	25.00	>50
I*f*	25.00	12.50	25.00	12.50	25.00	>50
I*g*	12.50	12.50	25.00	12.50	25.00	>50

against *Staphylococcus aureus* (Mi-12)[1], *Corynebacterium liquefaciens* (Co-11)[1], *Haemophilis vaginalis* (He-127)[1], *Streptococcus agalactiae* (VStB-1)[1], *Pasteurella multocida* (VPa-6)[1], and *Escherichia coli* (Es-2)[1] according to procedures described previously (7, 8). Compounds I*a*–I*g* possess *in vitro* antibacterial properties against several selected bacterial species (Table III). However, the compounds were ineffective against *E. coli* at the level used.

[1] Eaton Laboratories strain numbers.

EXPERIMENTAL[2]

Crystallization solvents, yields, melting points, and analyses of the compounds synthesized are reported in Tables I and II. All spectra were

[2] The melting points were taken in open capillary tubes on a Mel-Temp melting-point apparatus and are uncorrected. IR spectra (Nujol mull) were obtained on a Perkin-Elmer Infracord model 137, and NMR spectra (deuterated dimethyl sulfoxide using tetramethylsilane as an internal standard) were obtained on a Varian model A-60A spectrometer.

consistent with the assigned structures. Compounds Ia–Ig gave a strong qualitative test for sulfur using the oxygen flask ignition procedure.

The general procedure for the synthesis of the 3-aryl-3-hydroxypropanoic acids (Va–Vg) is illustrated by the preparation of 3-(3-chloro-4-methylphenyl)-3-hydroxypropanoic acid (Vg). The Reformatsky reaction was carried out as described (6) using 3-chloro-4-methylbenzaldehyde (9) (154 g, 1.0 mole), ethyl bromoacetate (150 ml, 1.3 moles), and zinc dust (90 g, 1.35 g-atoms). The crude ester was treated with a solution of 85% potassium hydroxide (80 g, 1.2 moles) in 95% ethanol (1500 ml). The mixture was refluxed for 4 hr and was then evaporated *in vacuo*.

The residue was dissolved in water (1500 ml) and extracted with ether. The aqueous layer was acidified with concentrated hydrochloric acid and extracted with chloroform (2 × 700 ml). The chloroform extracts were combined and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the solvent was removed *in vacuo* to leave the solid product (Vg).

The general procedure for the synthesis of the *S*-(4-chlorophenyl) 3-aryl-3-hydroxypropanethioates (Ia–Ig) is illustrated by the preparation of *S*-(4-chlorophenyl) 3-(3-chloro-4-methylphenyl)-3-hydroxypropanethioate (Ig). A solution of Vg (43 g, 0.2 mole) and VI (29 g, 0.2 mole) in dichloromethane (~600 ml) was stirred at room temperature. A solution of VII (41 g, 0.2 mole) in a small amount of dichloromethane was added in one portion. There was an immediate, very exothermic, reaction with the formation of a white precipitate. After the initial reaction had subsided, the reaction mixture was stirred for 4 hr at room temperature and then filtered. The filtrate was evaporated to dryness under reduced pressure to leave a white, solid product (Ig).

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Assessment of Enterohepatic Circulation of ^{3}H-Digoxin with a Minimal Interruption Technique

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Received March 14, 1977, from the *Departments of Medicine and Surgery, College of Medicine, Ohio State University, Columbus, OH 43210.* Accepted for publication June 8, 1977.

Abstract □ The biliary excretion of ^{3}H-digoxin in rats prepared for bile sampling with minimal interruption of the enterohepatic circulation was compared with that in rats with complete interruption after intraduodenal or intravenous administration. Following dosage by either route, significantly more radioactivity was recovered from animals with nearly intact enterohepatic circulation. The method described allows direct measurement of this cycle in unanesthetized animals without the consequences of bile depletion.

Keyphrases □ ^{3}H-Digoxin—biliary excretion measured by enterohepatic circulation minimal interruption technique, rats □ Excretion, biliary—^{3}H-digoxin, measured by enterohepatic circulation minimal interruption technique, rats □ Enterohepatic circulation—minimal interruption technique used to measure biliary excretion of ^{3}H-digoxin, rats □ Cardiotonic agents—digoxin, biliary excretion measured by enterohepatic circulation minimal interruption technique, rats

The enterohepatic cycle plays a major role in the biotransformation and disposition of some drugs. This aspect of cardiac glycoside metabolism has received considerable attention since it was suggested (1) that enterohepatic recycling is a major determinant of the half-life of these compounds. Direct measurement of the enterohepatic cycle is not usually done; instead, conclusions are drawn from data on fecal excretion or bile fistula preparations.

While these methods are useful for estimating the hepatobiliary contribution to total drug disposition, they allow only indirect conclusions about the extent of recycling. A model previously used for studies of biliary responses to partial hepatectomy and hypoxia (2–4) was adopted to compare the biliary excretion of digoxin in rats with minimal and total interruption of the enterohepatic circulation.

EXPERIMENTAL

^{3}H-Digoxin[1] (specific activity of 1.0 mCi/1.33 μg) was 96% radiochemically pure. Solutions of a specific activity of 0.25 μCi/μg/0.01 ml were prepared by diluting the stock solution with unlabeled digoxin. Aliquots of standards and samples were assayed for radioactivity in a liquid scintillation spectrometer after addition to 10 ml of scintillation medium[2], using automatic external standardization.

Twenty-four female Wistar rats, 250–300 g, were anesthetized with ether, and a midline incision was made. The proximal bile duct was cannulated with polyethylene No. 50 tubing[3]. A duodenostomy was constructed with polyethylene No. 160 tubing, and both duodenal and biliary cannulas were brought through a skin incision in the right flank. The two tubes were connected with a short length of flexible tubing (1.5 mm i.d., 2.4 mm o.d.), the skin was closed with a single suture, and the midline wound was closed.

The animals were allowed to recover in individual metabolic cages; they were observed and allowed free access to food and water for at least 48

[1] New England Nuclear Corp., Boston, Mass.
[2] Scintisol-Complete, Isolab, Akron, Ohio.
[3] Intramedic, Becton, Dickinson and Co., Parsippany, N.J.