Antihyperlipidemic Activity of Saccharin Analogues in Rodents

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Abstract □ Saccharin analogues were observed to be potent antihyperlipidemic agents at 20 mg/kg/day in rodents, significantly reducing both serum cholesterol and triglyceride levels in both normal and atherogenic mice. The saccharin analogues suppressed *in vitro* and *in vivo* liver enzymatic activity of acetyl-CoA synthetase, citrate lyase, and mitochondrial citrate exchange leading to a reduction of available cytoplasmic acetyl-CoA, which is required for the synthesis of cholesterol and fatty acids. Liver acetyl-CoA carboxylase, phosphatidate phosphohydralase, and glycerol-3-phosphate acyl transferase activities were markedly reduced by the saccharin analogues. Suppression of these enzymes would lead to a reduction of triglyceride synthesis. The saccharin analogues accelerated bile excretion of cholesterol metabolites and increased the fecal excretion of the cholesterol, triglycerides, neutral lipids, and phospholipids. The liver and plasma lipoprotein lipid content (including cholesterol, triglycerides, and neutral lipids) was markedly reduced by the saccharin analogues, whereas phospholipid content was elevated. The reduction of lipid content of serum chylomicron, very low-density, low-density, and high-density lipoprotein fractions by the saccharin analogues indicates that these agents may be useful in controlling hyperlipidemic diseases where specific lipoprotein fractions are elevated.

Keyphrases □ Saccharin—analogues, antihyperlipidemic activity, cholesterol and triglyceride reduction □ Antihyperlipidemic agents—saccharin analogues, cholesterol and triglyceride reduction □ Cholesterol—antihyperlipidemic effect of saccharin analogues □ Triglycerides—antihyperlipidemic effect of saccharin analogues

The antihyperlipidemic activity of saccharin and 1-*N*-(*o*-benzosulfimido)butan-3-one has been reported previously (1) in mice at 20 mg/kg/day ip. Saccharin afforded a 33% reduction in serum cholesterol after 16 days and a 49% reduction in serum triglyceride levels after 14 days, whereas 1-*N*-(*o*-benzosulfimido)butan-3-one resulted in a 38% reduction of serum cholesterol and a 49% reduction of serum triglyceride levels (1). Saccharin derivatives were examined for their hypolipidemic effects because of their structural similarity to phthalimide (2) and *N*-phenyl-bridged isoindoline ketones (3), which have been reported previously to have hypolipidemic action in rodents. The purpose of this study was to examine the antihyperlipidemic activity of saccharin and its derivatives in detail, as well as their mode of action on cellular lipid metabolism in rodents.

EXPERIMENTAL

Source of Compounds—Saccharin was purchased commercially[1]. The syntheses and chemical characterizations of 1-*N*-(*o*-benzosulfimido)butan-3-one(1) and 3-*N*-(*o*-benzosulfimido)propionic acid have been reported previously (2).

Antihyperlipidemic Screens in Normal Rodents—Compounds were suspended in 1% carboxymethylcellulose–water and administered to male CF_1 mice (~25 g in weight) intraperitoneally or Holtzman male rats (~200 g in weight) orally by an intubation needle for 16 days. On days 9 and 16, blood was obtained by tail vein bleeding, and the serum was separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann–Burchard reaction (4). Serum was also collected on day 14, and the triglyceride content was determined using a commercial kit[2].

Testing in Atherogenic Mice—Male CF_1 mice (~25 g) were placed on a commercial diet[3] that contained butterfat (400 g), cellulose[4] (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt mixture oil[5] (40 g), sodium cholate (20 g), sucrose (223 g), vitamin-free casein (200 g), and total vitamin supplement for a 2-week period. After the cholesterol and triglyceride levels were assayed and observed to be elevated, the mice were administered test drugs at 20 mg/kg/day for an additional 2-week period. Serum cholesterol and triglyceride levels were measured after 14 days of drug administration.

Animal Weights and Food Intake—Periodic animal weights were obtained during the experiments and expressed as a percentage of the animal's weight on day 0. After dosing for 16 days with test drugs, selected organs were excised, trimmed of fat, and weighed. The organ weights were expressed as a percentage of the total body weight of the animal. The average food intake[6] in g/rat/day was determined over the 16-day dosing period.

Toxicity Studies—The acute toxicity (LD_{50} value) (5) was determined in male CF_1 mice by administering test drugs intraperitoneally from 100 mg to 2 g/kg as a single dose. The number of deaths recorded in the group over 7 days was determined for each dosage.

Enzymatic Studies—*In vitro* enzymatic studies were determined using 10% homogenates of male CF_1 mouse liver with 2.5 μmoles of the test drugs and male Holtzman rat livers with 0.100–10 m*M* concentration of the test drugs. *In vivo* enzymatic studies were determined using 10% homogenates of liver from male CF_1 mice obtained after administering the agents for 16 days at a dose of 10–60 mg/kg/day. The liver homogenates for both *in vitro* and *in vivo* studies were prepared in 0.25 *M* sucrose and 0.001 *M* EDTA[(ethylenedinitrilo)tetraacetic acid].

Acetyl-CoA synthetase (6) and adenosine triphosphate-dependent citrate lyase (7) activities were determined spectrophotometrically at 540 nm as the hydroxamate of acetyl-CoA formed after 30 min at 37°. Mitochondrial citrate exchange was determined by the procedure of Robinson *et al.* (8, 9) using sodium [^{14}C]bicarbonate (41 mCi/mmole) incorporated into mitochondrial [^{14}C]citrate after isolating the mitochondria (9000×*g* for 10 min) from the homogenates. The exchange of the [^{14}C]citrate was determined after incubating the mitochondrial fraction, which was loaded with labeled citrate, and the test drugs for 10 min. Then the radioactivity was measured in the mitochondrial and supernatant fractions in scintillation fluid[7] and expressed as a percentage. Cholesterol side-chain oxidation was determined by the method of Kritchevsky and Tepper (10) using [26-^{14}C]cholesterol (50 mCi/mmole) and mitochondria isolated from rat liver homogenates. After an 18-hr incubation at 37° with the test drugs, the generated $^{14}CO_2$ was trapped in the center well in [2-[2-(*p*-1,1,3,3-tetramethylbutylcresoxy)ethoxy]-ethyl]dimethylbenzylammonium hydroxide[8] and counted[7]. 3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) was measured using [1-^{14}C]acetate (56 Ci/mmole) using a postmitochondrial supernatant (9000×*g* for 20 min) incubated for 60 min at 37° (11). The digitonide derivative of cholesterol was isolated and counted (12). Acetyl CoA carboxylase activity was measured by the method of Greenspan and Lowenstein (13). Initially, the enzyme had to be polymerized for 30 min at 37°, and then the assay mixture containing sodium [^{14}C]bicarbonate (41.0 mCi/mmole) was added and incubated for 30 min at 37° with the test drugs. Fatty acid synthetase activity was determined by the method

[1] Ruger Chemical Co., Inc.

[2] Hycel Triglyceride Test Kit; Fisher Scientific Co.
[3] Basal Atherogenic Test Diet; U.S. Biochemical Corp.
[4] Celufil.
[5] Wesson.
[6] Wayne Blox Rodent Chow.
[7] Fisher Scintiverse in a Packard Scintillation Counter.
[8] Hyamine hydroxide; New England Nuclear.

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Table I—Effects of Saccharin Analogues on Serum Cholesterol and Triglyceride Levels of Male Holtzman Rats and Male CF_1 Mice [a]

Compound	Rats: Dose, mg/kg/day	Rats: Serum Cholesterol Day 9	Rats: Serum Cholesterol Day 16	Rats: Serum Triglyceride Day 14	Mice: Dose, mg/kg/day	Mice: Serum Cholesterol Day 9	Mice: Serum Cholesterol Day 16	Mice: Serum Triglyceride Day 14
Control (1% carboxymethylcellulose)		100 ± 9[d]	100 ± 7[e]	100 ± 8[f]		100 ± 5[g]	100 ± 6[h]	100 ± 6[i]
Saccharin					10	81 ± 8[b]	76 ± 4[b]	47 ± 4[b]
	20	65 ± 6[b]	56 ± 7[b]	55 ± 6[b]	20	77 ± 6[b]	68 ± 7[b]	48 ± 6[b]
					40	66 ± 5[b]	65 ± 3[b]	54 ± 3[b]
					60	63 ± 5[b]	62 ± 4[b]	61 ± 4[b]
1-*N*-(*o*-Benzosulfimido)butan-3-one	20	64 ± 5[b]	56 ± 6[b]	65 ± 6[b]	20	60 ± 8[b]	62 ± 6[b]	51 ± 7[b]
3-*N*-(*o*-Benzosulfimido)propionic Acid					10	85 ± 6[c]	76 ± 7[b]	84 ± 5[b]
	20	72 ± 6[b]	63 ± 7[b]	48 ± 7[b]	20	70 ± 7[b]	56 ± 7[b]	58 ± 5[b]
					40	77 ± 6[b]	65 ± 5[b]	60 ± 4[b]
					60	68 ± 8[b]	62 ± 6[b]	58 ± 5[b]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$ [c] $p \leq 0.005$. [d] 73 ± 7 mg%. [e] 78 ± 6 mg%. [f] 110 ± 9 mg%. [g] 118 ± 6 mg%. [h] 122 ± 7 mg%. [i] 137 ± 8 mg%.

of Brady *et al.* (14) using [2-^{14}C]malonyl-CoA (37.5 mCi/mmole) incorporated into newly synthesized fatty acids, which were extracted with ether and counted. Acyl transferase activity was determined with L-[2-^{3}H]glycerol-3-phosphate (7.1 Ci/mmole) and the microsomal fraction of the liver homogenates (15). The reaction was terminated after 10 min, and the lipids were extracted with chloroform–methanol (1:2) containing 1% concentrated HCl and counted. Phosphatidate phosphohydralase activity was measured as the inorganic phosphate released after 30 min from phosphatidic acid by the method of Mavis *et al.* (16). The released inorganic phosphate after development with ascorbic acid and ammonium molybdate was determined at 820 nm.

The *in vitro* oxidative phosphorylation process in male CF_1 mouse liver was also examined with an oxygen electrode[9] connected to an oxygraph[10] at 37°. The reaction vessel typically contained 55 μmoles of sucrose, 22 μmoles of monobasic potassium phosphate, 22 μmoles of potassium chloride, 90 μmoles of succinate or 60 μmoles of α-ketoglutarate as substrate, 2 μmoles of adenosine triphosphate, and 2.5 μmoles of the test compounds in a total volume of 1.8 ml. After the basal metabolic (state 4) rate was obtained, 0.257 μmole of adenosine diphosphate was added to obtain the adenosine diphosphate-stimulated respiration (state 3) rate (17). The rates were calculated as μl of oxygen consumed/mg of liver/hr.

Liver, Small Intestine, and Fecal Lipid Extraction—In male CF_1 mice that had been administered test drugs for 16 days, the liver, small intestine, and fecal materials (24-hr collection) were removed and a 10% homogenate in 0.25 *M* sucrose and 0.001 *M* EDTA was prepared. An aliquot (2 ml) of the homogenate was extracted by the methods of Floch *et al.* (18) and Bligh and Dyer (19) and the number of milligrams of lipid weighed. The lipid was taken up in methylene chloride, and the cholesterol level (4), triglyceride levels[11], neutral lipid content (20), and phospholipid content (21) were determined.

[^{14}C]Cholesterol Distribution in Mice and Rats—Male CF_1 mice (~25 g) were administered test agents intraperitoneally for 14 days and rats were administered test agents orally. On day 13, 10 μCi of [4-^{14}C]-cholesterol (52.5 mCi/mmole) was administered intraperitoneally in mice and orally in rats, and feces were collected for 0–6, 6–12, and 12–24 hr postadministration. Twenty-four hours after cholesterol administration, the major organs were excised and samples of blood, chyme, and urine were obtained. Homogenates (10%) were prepared of the tissues which were combusted[12] and counted. Some tissue samples were plated on filter paper[13], dried, and digested for 24 hr in base[8] at 40° and counted. Results were expressed as dpm/mg of wet tissue and dpm/mg of total organ.

Cholesterol Absorption Study—Male Holtzman rats (~400 g) were administered test drug intraperitoneally for 14 days at 20 mg/kg/day. On day 13, 10 μCi of [1,2-^{3}H]cholesterol (40.7 Ci/mmole) was administered to the rat orally. Twenty-four hours later, the blood was collected and the serum was separated by centrifugation (22). Both the serum and the precipitate were counted.

Bile Cannulation Study—Male Holtzman rats (~400 g) were treated with test drugs at 20 mg/kg/day orally for 14 days. The rats were anesthetized with chlorpromazine[14] (25 mg/kg) followed after 30 min by pentobarbital[15] (22 mg/kg ip). The duodenum section of the small intestine was isolated and ligatures were placed around the pyloric sphincter and at a site distally approximately one-third of the way down the duodenum. Sterile isotonic saline was injected into the sectioned off duodenum segment. The saline expanded the duodenum and the common bile duct. After the bile duct was identified, a loose ligature was placed around the duct, an incision was made, the plastic tubing[16] was introduced into the duct. Once past the ligature, the tubing was tied in place and the ligatures around the duodenum were removed. When bile was freely moving down the cannulated tube, [1,2-^{3}H]cholesterol (40.7 Ci/mmole) was injected intravenously into the rats. The bile was collected over the next 6 hr and measured (in ml). Aliquots were counted as well as analyzed for cholesterol content (4).

Plasma Lipoprotein Fractions—Male Holtzman rats (~400 g) were administered test drugs at 20 mg/kg/day for 14 days. On day 14, blood was collected from the abdominal aorta. Serum was separated from whole blood by centrifugation at 3500 rpm. Aliquots (3 ml) were separated by density gradient ultracentrifugation according to the methods of Hatch and Lees (23) and Havel *et al.* (24) into the chylomicrons, very low-density lipoproteins, high-density lipoproteins, and low-density lipoproteins. Each of the fractions was analyzed for cholesterol (4), triglyceride[11], neutral lipids (20), phospholipids (21), and protein levels (25).

RESULTS

Saccharin, 1-*N*-(*o*-benzosulfimido)butan-3-one, and 3-*N*-(*o*-benzosulfimido)propionic acid markedly reduced the serum lipid levels in normal rodents after intraperitoneal administration. In CF_1 mice, saccharin afforded a 38% reduction of serum cholesterol after 16 days of dosing at the 60-mg/kg dose (Table I). In the triglyceride screen in mice, the 10- and 20-mg/kg doses resulted in a reduction of 53 and 52%, respectively. Saccharin was also active as a hypolipidemic agent orally in rats at 20 mg/kg, suppressing serum cholesterol levels 44% and serum triglyceride levels 45%. 3-*N*-(*o*-Benzosulfimido)propionic acid was active in the mouse screen. The maximum suppression of serum cholesterol levels in mice was at 20 mg/kg/day, with 44% reduction. In the triglyceride screen, the 20-, 40-, and 60-mg/kg/day doses caused >40% reduction. 3-*N*-(*o*-Benzosulfimido)propionic acid was active orally in the rat. The serum cholesterol levels were suppressed 37% at 20 mg/kg/day in the rat, while triglyceride levels were suppressed 52%. 1-*N*-(*o*-Benzosulfimido)-butan-3-one in the mouse screen reduced serum cholesterol 38% and triglyceride 49%, whereas in the rat, serum cholesterol was reduced 44% and triglyceride level was reduced 35%. In mice that had been rendered atherogenic by a high lipid diet, after 2 weeks of administration it can be observed that saccharin and the propionic analogue suppressed the induced cholesterol levels by >170% resulting in serum cholesterol only slightly above normal levels (Table II). In the triglyceride screen, levels

[9] Clark oxygen electrode for measuring pO_2 tension.
[10] Gilson Instruments.
[11] Bio-Dynamics/bmc Triglyceride Kit.
[12] Packard Tissue Oxidizer.
[13] Whatman No. 1.
[14] Thorazine, chlorpromazine hydrochloride; Smith, Kline and French Laboratories.
[15] Nembutal, sodium pentobarbital; Abbott Laboratories.
[16] PE-10 Intramedic polyethylene tubing.

Table II—Effects of Saccharin Analogues on Serum Cholesterol and Triglyceride Levels in Normal and Atherogenic Mice [a]

Compound	Serum Cholesterol		Serum Triglyceride	
	2-Week Diet +	14-Day Dosing	2-Week Diet +	14-Day Dosing
Control (1% Carboxymethylcellulose)	100 ± 6[d]	100 ± 7[e]	100 ± 5[f]	100 ± 4[g]
Control Atherogenic Diet	289 ± 9[b]	290 ± 9[b]	131 ± 3[b]	131 ± 5[b]
Saccharin (20 mg/kg/day)	289 ± 9[b]	116 ± 6[c]	131 ± 6[b]	100 ± 4
3-*N*-(*o*-Benzosulfimido)propionic Acid (20 mg/kg/day)	290 ± 10[b]	119 ± 7[c]	129 ± 8[b]	88 ± 5[c]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$ as related to 1% carboxymethylcellulose values. [c] $p \leq 0.005$. [d] 118 ± 6 mg%. [e] 122 ± 7 mg%. [f] 136 ± 7 mg%. [g] 139 ± 5 mg%.

Table III—Effect of Saccharin Derivatives on the Weight of Major Organs and Body Weight of Rats After 16 Days of Dosing at 20 mg/kg/day

Compound	Mean Weight on Day Zero, g	Body Weight Increase on Day 16, %	Food Consumption, gm/day
Control (1% carboxymethylcellulose)	439 ± 8	138 ± 3	22.5
Saccharin	378 ± 9	141 ± 4	19.0
3-*N*-(*o*-Benzosulfimido)propionic Acid	421 ± 8	139 ± 3	20.5

	Percent of Total Body Weight				
	Liver	Lung	Heart	Kidney	Spleen
Control (1% carboxymethylcellulose)	3.46 ± 0.83	0.48 ± 0.04	0.34 ± 0.03	0.71 ± 0.09	0.25 ± 0.05
Saccharin	3.12 ± 0.95	0.50 ± 0.03	0.34 ± 0.04	0.66 ± 0.08	0.21 ± 0.06
Weight of organ, g	15.21 ± 3.59	2.10 ± 0.17	1.51 ± 0.13	3.12 ± 0.39	1.11 ± 0.22

	Brain	Adrenal	Stomach	Small Intestine	Large Intestine
Control (1% carboxymethylcellulose)	0.36 ± 0.04	0.011 ± 0.005	0.68 ± 0.22	2.35 ± 0.65	1.09 ± 0.22
Saccharin	0.34 ± 0.06	0.014 ± 0.008	0.83 ± 0.28	2.28 ± 0.53	1.19 ± 0.18
Weight of organ, g	1.59 ± 0.17	0.047 ± 0.021	2.99 ± 0.96	10.32 ± 2.86	4.78 ± 0.97

[a] Mean ± *SD*; $n = 6$.

Table IV—*In Vitro* Effects of Saccharin Analogues on Mouse Liver Enzyme Activities at 2.5 μmoles [a]

Compound	Mitochondrial Citrate Exchange	Acetyl-CoA Synthetase	Citrate Lyase	HMG-CoA Reductase	Cholesterol Side-Chain Oxidation
Control (1% carboxymethylcellulose)	100 ± 10[c]	100 ± 11[d]	100 ± 9[e]	100 ± 7[f]	100 ± 8[g]
Saccharin	7 ± 5[b]	61 ± 7[b]	65 ± 6[b]	71 ± 7[b]	94 ± 6
1-*N*-(*o*-Benzosulfimido)butan-3-one	7 ± 6[b]	74 ± 9[b]	47 ± 8[b]	98 ± 5	72 ± 4[b]
3-*N*-(*o*-Benzosulfimido)propionic Acid	20 ± 7[b]	53 ± 7[b]	—	95 ± 5	61 ± 3[b]

	Acetyl-CoA Carboxylase	Fatty Acid Synthetase	Phosphatidate Phosphohydrolase	Acyl Transferase
Control (1% carboxymethylcellulose)	100 ± 6[h]	100 ± 7[i]	100 ± 7[j]	100 ± 8[k]
Saccharin	9 ± 2[b]	93 ± 5	52 ± 5[b]	15 ± 3[b]
1-*N*-(*o*-Benzosulfimido)butan-3-one	12 ± 3[b]	104 ± 7	61 ± 4[b]	25 ± 6[b]
3-*N*-(*o*-Benzosulfimido)propionic Acid	43 ± 5[b]	98 ± 6	49 ± 5[b]	8 ± 4[b]

	α-Ketoglutarate		Succinate	
	State 4	State 3	State 4	State 3
Control (1% carboxymethylcellulose)	100 ± 6[l]	100 ± 7[m]	100 ± 5[n]	100 ± 6[o]
Saccharin	69 ± 6[b]	68 ± 4[b]	78 ± 6[b]	65 ± 3[b]
1-*N*-(*o*-Benzosulfimido)butan-3-one	82 ± 7[c]	80 ± 3[b]	64 ± 5[b]	63 ± 4[b]
3-*N*-(*o*-Benzosulfimido)propionic Acid	77 ± 7	61 ± 4[b]	76 ± 5[b]	66 ± 5[b]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$. [c] 30.8 ± 3.1 mg% exchange of mitochondrial citrate. [d] 28.5 ± 3.14 mg of acetyl-CoA formed/g wet tissue/30 min. [e] 30.5 ± 2.74 mg of citrate hydrolyzed/g wet tissue/30 min. [f] 384,900 ± 26,943 dpm cholesterol formed/g wet tissue/60 min. [g] 6080 ± 5.58 dpm CO_2 formed/g wet tissue/18 hr. [h] 32,010 ± 1921 dpm/g wet tissue/30 min. [i] 37,656 ± 2635 dpm/g wet tissue/30 min. [j] 16.70 ± 1.16 μg Pi/g wet tissue/15 min. [k] 537,800 ± 43,024 dpm triglyceride formed/g wet tissue/10 min. [l] 3.51 ± 0.21 μl of oxygen consumed/hr/mg of tissue. [m] 5.21 ± 0.36 μl of oxygen consumed/hr/mg of tissue. [n] 5.92 ± 0.30 μl of oxygen consumed/hr/mg of tissue. [o] 11.31 ± 0.67 μl of oxygen consumed/hr/mg of tissue.

were reduced to normal levels by saccharin and 12% below normal (control) levels by the propionic acid derivative.

Saccharin and the propionic acid derivative did not affect the daily food intake over the 16-day dosing period, nor was the increase in body weight significantly different from the control rats (Table III). The acute toxicity studies demonstrated that the LD_{50} values in mice as a single intraperitoneal injection exceeded 2 g/kg which was the limit of solubility of the agents in carboxymethylcellulose. *In vitro* enzymatic studies of liver from CF_1 mice at 2.5 μmoles of the test agents, demonstrated that mitochondrial [^{14}C]citrate exchange was inhibited markedly by all three agents: 93% by saccharin and the butanone derivative and 80% by the propionic acid derivative (Table IV). Acetyl-CoA synthetase activity was suppressed 26–47% by these agents. Saccharin suppressed citrate lyase activity 35%, and the butanone derivatives suppressed citrate lyase activity 53%. HMG-CoA reductase activity was inhibited 29% by saccharin, but was essentially unaffected by the other two compounds. Cholesterol side-chain oxidation was inhibited 28% by the butanone derivative and 30% by the propionic analogue, but was unaffected by saccharin. Saccharin was more active and caused a 91% reduction of acetyl-CoA carboxylase activity; butanone caused 38% reduction, whereas 3-*N*-(*o*-benzsulfimido)propionic acid caused only 57% reduction. Fatty acid synthetase activity was not affected by any of the saccharin agents. Phosphatidate phosphohydralase activity was reduced 39–51%, and acyl transferase activity was reduced 75–92% by these test agents.

The saccharin agents suppressed basal respiration (state 4) rates. When succinate was used as substrate, saccharin derivatives reduced basal respiration 22–36%. When α-ketoglutarate was used as substrate, a reduction of 18–31% in basal respiration occurred. For the adenosine diphosphate-stimulated respiration (state 3) rate using succinate as substrate, a reduction of 34–37% was observed; with α-ketoglutarate substrate, a reduction of 20–39% was noted.

ID_{50} values obtained for *in vitro* enzymatic studies on rat liver ho-

Table V—*In Vivo* Effects of Saccharin Analogues on CF_1 Male Mouse Liver Enzyme Activities After 16 Days of Administration [a]

Compound	Dose, mg/kg/day	Acetyl-CoA Synthetase	HMG-CoA Reductase	Acetyl-CoA Carboxylase	Fatty Acid Synthetase	Phosphatidate Phosphohydralase	Liver Lipids
Control (1% carboxymethylcellulose)	—	100 ± 7[d]	100 ± 6[e]	100 ± 5[f]	100 ± 6[g]	100 ± 8[h]	100 ± 9[i]
Saccharin	10	73 ± 6[b]	80 ± 7[b]	41 ± 3[b]	73 ± 7[b]	30 ± 4[b]	57 ± 5[b]
	20	75 ± 6[b]	76 ± 6[b]	44 ± 4[b]	85 ± 7[c]	45 ± 3[b]	48 ± 4[b]
	40	71 ± 7[b]	77 ± 6[b]	56 ± 7[b]	85 ± 5[c]	82 ± 5[c]	54 ± 5[b]
	60	77 ± 5[b]	85 ± 6[c]	68 ± 6[b]	88 ± 5[c]	102 ± 7	61 ± 6[b]
3-*N*-(*o*-Benzosulfimido)propionic Acid	10	80 ± 5[b]	82 ± 8[c]	55 ± 4[b]	79 ± 6[b]	63 ± 5[b]	84 ± 6[c]
	20	71 ± 4[b]	75 ± 3[b]	45 ± 3[b]	83 ± 5[b]	73 ± 4[b]	58 ± 6[b]
	40	66 ± 5[b]	79 ± 5[b]	52 ± 5[b]	83 ± 6[c]	83 ± 6[c]	60 ± 7[b]
	60	64 ± 7[b]	72 ± 4[b]	72 ± 4[b]	78 ± 6[b]	93 ± 5	58 ± 6[b]

[a] Expressed as percentage of control (mean ± *SD*); $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.005$. [d] 28.5 ± 3.14 mg of acetyl-CoA formed/g wet tissue/30 min. [e] 384,900 ± 26,943 dpm cholesterol formed/g wet tissue/60 min. [f] 32,010 ± 1921 dpm/g wet tissue/30 min. [g] 37,656 ± 2635 dpm/g wet tissue/30 min. [h] 16.70 ± 1.16 μg Pi/g wet tissue/15 min. [i] 79.5 ± 5.56 mg/g wet tissue.

Table VI—Effects of Saccharin Analogues on Liver and Small Intestinal Lipid Content After 16 Days of Dosing [a]

Compound	Dose, mg/kg/day	Lipid, mg	Cholesterol	Neutral Lipids	Triglyceride	Phospholipids
				Liver, % of control		
Control (1% carboxymethylcellulose)	—	100 ± 6	100 ± 7[d]	100 ± 4[e]	100 ± 5[f]	100 ± 8[g]
Saccharin	10	47 ± 4[b]	34 ± 5[b]	19 ± 3[b]	54 ± 4[b]	116 ± 7[c]
	20	48 ± 6	55 ± 5[b]	20 ± 2[b]	28 ± 3[b]	105 ± 6[c]
	40	54 ± 3[b]	63 ± 4[b]	13 ± 3[b]	54 ± 3[b]	120 ± 6[c]
	60	61 ± 4[b]	60 ± 7[b]	32 ± 4[b]	64 ± 4[b]	129 ± 9[b]
3-*N*-(*o*-Benzosulfimido)propionic Acid	10	84 ± 5[c]	51 ± 5[b]	63 ± 3[b]	75 ± 4[b]	129 ± 9[b]
	20	58 ± 5[b]	54 ± 5[b]	41 ± 5[b]	74 ± 7[b]	126 ± 5[b]
	40	60 ± 4[b]	58 ± 4[b]	81 ± 3[b]	49 ± 3[b]	99 ± 6
	60	58 ± 5[b]	52 ± 6[b]	105 ± 6	83 ± 8[b]	96 ± 7
				Small Intestine, % of control		
Control (1% carboxymethylcellulose)	—	100 ± 5	100 ± 7[h]	100 ± 5[i]	100 ± 6[j]	100 ± 8[k]
Saccharin	10	85 ± 4[b]	104 ± 8	78 ± 7[b]	74 ± 4[b]	97 ± 7
	20	53 ± 3[b]	157 ± 9[b]	130 ± 7[b]	29 ± 3[b]	111 ± 8
	40	75 ± 5[b]	175 ± 9[b]	70 ± 8[b]	59 ± 5[b]	114 ± 6[c]

[a] Mean ± *SD*; $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.005$. [d] 12.24 ± 0.86 mg cholesterol/g tissue. [e] 28.35 ± 1.13 mg neutral lipid/g tissue. [f] 4.77 ± 0.24 mg triglyceride/g tissue. [g] 4.39 ± 0.35 mg phospholipid (P)/g tissue. [h] 7.81 ± 0.54 mg cholesterol/g tissue. [i] 7.18 ± 0.36 mg neutral lipid/g tissue. [j] 1.06 ± 0.06 mg triglyceride/g tissue. [k] 2.02 ± 0.16 mg phospholipid (P)/g tissue.

Table VII—Effects of Saccharin on the Fecal Excretion of Lipids After Administration for 16 Days [a]

Compound	Lipid, mg/g	Cholesterol	Neutral Lipids	Triglyceride	Phospholipids
			0 to 6-hr Fecal Sample, % of control		
Control (1% carboxymethylcellulose)	100 ± 6	100 ± 7[d]	100 ± 7[e]	100 ± 6[f]	100 ± 5[g]
Saccharin					
10 mg/kg/day	100 ± 7	115 ± 6[c]	107 ± 6	100 ± 5	113 ± 6
20 mg/kg/day	174 ± 9[b]	166 ± 8[b]	159 ± 5[b]	146 ± 8[b]	140 ± 8[b]
40 mg/kg/day	91 ± 5	74 ± 5[b]	114 ± 7	133 ± 7[b]	114 ± 6[c]
			6 to 12-hr Sample, % of control		
Control (1% carboxymethylcellulose)	100 ± 6	100 ± 7[h]	100 ± 8[i]	100 ± 6[j]	100 ± 6[k]
Saccharin					
10 mg/kg/day	118 ± 9[c]	108 ± 8	91 ± 7	90 ± 5	108 ± 5
20 mg/kg/day	131 ± 7[b]	115 ± 7[b]	105 ± 7	150 ± 7[b]	143 ± 8[b]
40 mg/kg/day	110 ± 6	100 ± 6	104 ± 8	108 ± 7	133 ± 7[b]
			12 to 24-hr Sample, % of control		
Control (1% carboxymethylcellulose)	100 ± 7	100 ± 8[l]	100 ± 6[m]	100 ± 7[n]	100 ± 6[o]
Saccharin					
10 mg/kg/day	124 ± 8[b]	102 ± 7	143 ± 7[b]	225 ± 12[b]	120 ± 7[b]
20 mg/kg/day	141 ± 8[b]	105 ± 6	143 ± 8[b]	277 ± 10[b]	120 ± 5[b]
40 mg/kg/day	126 ± 6[b]	100 ± 7	150 ± 8[b]	255 ± 9[b]	111 ± 6[c]

[a] Mean ± *SD*; $n = 6$. [b] ≤ 0.001. [c] $p \leq 0.005$. [d] 19.77 ± 1.38 mg/g. [e] 17.62 ± 1.23 mg/g. [f] 1.74 ± 0.10 mg/g. [g] 1.85 ± 0.09 mg/g. [h] 29.47 ± 2.06 mg/g. [i] 33.94 ± 2.72 mg/g. [j] 1.86 ± 0.11 mg/g. [k] 1.61 ± 0.10 mg/g. [l] 28.47 ± 2.27 mg/g. [m] 33.94 ± 2.04 mg/g. [n] 1.86 ± 0.13 mg/g. [o] 1.39 ± 0.08 mg/g.

mogenate were calculated from a semilogarithmic plot. In the acetyl-CoA synthetase assay, saccharin afforded an $ID_{50} \simeq 6.61$ m*M*. For the acetyl-CoA carboxylase assay, an $ID_{50} \simeq 1.26$ m*M* was obtained for saccharin, and an $ID_{50} \simeq 1.36$ m*M* was obtained for the propionic acid derivative. For the phosphatidate phosphohydralase assay, saccharin afforded an $ID_{50} \simeq 3.56$ m*M* and the propionic acid derivative an $ID_{50} \simeq 1.68$ m*M*. For the acyl transferase assay, saccharin gave an ID_{50} value of 3.26 m*M* and the propionic analogue gave a value of 1.93 m*M*.

In vivo studies on the enzymatic activities of liver from mice treated with test agents for 16 days showed that saccharin at doses from 10 to 60 mg/kg suppressed acetyl-CoA synthetase activity ~25% (Table V). *In vivo* HMG-CoA reductase activity was suppressed 23–24% at 20 and 40 mg/kg/day. Acetyl-CoA carboxylase activity was suppressed maximally at the lower doses; *i.e.*, 10 and 20 mg/kg/day afforded a 50–56% reduction. Fatty acid synthetase activity was not affected by *in vivo* administration of the drugs. Phosphatidate phosphohydralase activity was reduced 70% by saccharin at 10 mg/kg/day and 55% at 20 mg/kg/day. Liver lipids were reduced 53–39% by *in vivo* administration of 10–60 mg/kg/day of saccharin. 3-*N*-(*o*-Benzosulfimido)propionic acid *in vivo* administration resulted in a dose-related reduction of acetyl-CoA synthetase activity, with 60 mg/kg/day causing the maximum inhibition, *i.e.*, 36%. HMG-Co-A reductase activity was reduced 25 and 28% at 20 and 60 mg/kg/day, respectively. The propionic acid derivative caused marked reduction of acetyl-CoA carboxylase activity, with 20 mg/kg/day resulting in the maximum inhibition of 55%. Fatty acid synthetase activity was not affected by the propionic acid analogue. Phosphatidate phosphohydralase

Table VIII—[^{3}H]Cholesterol Content 24 hr After Intraperitoneal Injection of 10 μCi in CF_1 Mice Administered Saccharin for 16 Days at 20 mg/kg/day with Imides

Organ	Control		Saccharin	
	Total dpm[a]	Tritium Recovered, %	Total dpm[a]	Tritium Recovered, %
Brain	8305 ± 306	0.124	8048 ± 417	0.120
Lung	33949 ± 1982	0.506	30113 ± 1147	0.449
Heart	23774 ± 2112	0.354	7645 ± 902	0.114
Liver	436066 ± 4508	6.502	365175 ± 3721	5.445
Spleen	54303 ± 3291	0.810	57878 ± 2886	0.863
Kidney	82954 ± 1789	1.237	61030 ± 948	0.910
Stomach	266464 ± 5621	3.973	161898 ± 3277	2.414
Small intestine	607318 ± 9241	9.056	63445 ± 1112	9.460
Large intestine	791297 ± 7432	11.799	319288 ± 6456	8.614
Subtotal		34.361		28.389
Feces				
0–6 hr	374855 ± 14399	5.589	1738384 ± 18451	25.920
6–12 hr	2126220 ± 27861	31.703	1542185 ± 22231	22.995
12–24 hr	1901108 ± 36986	28.333	1524749 ± 28564	22.735
Total excreted in feces in 24 hr		65.625		71.650
Plasma/ml	256730 ± 5366		197092 ± 5382	

[a] Mean ± *SD*; $n = 6$.

Table IX—Effects of Saccharin on [^{3}H]Cholesterol Distribution in Holtzman Rats After 14 Days of Dosing[a]

Organ	Control		Saccharin	
	Total Organ dpm[b]	Recovery, %	Total Organ dpm[b]	Recovery, %
Brain	42412 ± 1171	1.21	43591 ± 986	1.26
Heart	37638 ± 3215	1.07	20412 ± 1836	0.59
Lung	100584 ± 7431	2.87	70230 ± 3233	2.02
Liver	901785 ± 7886	25.77	451828 ± 3954	13.06
Spleen	67760 ± 1431	1.93	69885 ± 1879	2.02
Kidney	69192 ± 986	1.97	59505 ± 1203	1.72
Stomach	127446 ± 8321	3.64	70230 ± 6121	2.03
Small intestine	851406 ± 70012	24.33	919916 ± 53202	26.59
Large intestine	246924 ± 12104	7.05	468088 ± 29998	13.53
Chyme	163977 ± 8421	4.68	444908 ± 12562	12.86
Feces	889892 ± 43265	25.43	841037 ± 12008	24.31
Total	3499016		3459630	

[a] At 20 mg/kg/day. [b] Mean ± *SD*; $n = 6$.

activity was reduced 37 and 27% at 10 and 20 mg/kg/day, respectively.

Liver lipids were reduced by the propionic acid derivative, with doses >20 mg/kg/day causing at least 50% reduction. The liver cholesterol and neutral lipids, including triglyceride levels, were reduced by saccharin and the propionic acid derivative (Table VI). Saccharin at 10 mg/kg reduced liver cholesterol levels 66% and at 20 mg/kg caused 45% reduction. Liver neutral lipid levels at 20–40 mg/kg were reduced >80% by saccharin. Liver triglyceride levels were reduced maximally at 20 mg/kg of saccharin, resulting in 72% reduction. Liver phospholipid levels were elevated significantly by saccharin at 40 and 60 mg/kg/day. The propionic acid analogue at all doses employed caused >40% reduction of liver cholesterol content. Liver neutral lipids were reduced 50% at 20 mg/kg/day by the propionic acid derivative, whereas liver triglyceride levels were reduced 51% at 40 mg/kg/day. Liver phospholipid levels were elevated at the lower doses (*i.e.*, 10 and 20 mg/kg/day) by the propionic acid analogue. Extracted lipids from the small intestine of mice treated for 16 days with saccharin at 20 mg/kg/day showed a reduction of triglyceride, but an elevation of cholesterol and neutral lipids. The phospholipid content was essentially not altered in the small intestine tissue.

Table X—Effect of Saccharin on Bile Secretion and Cholesterol Absorption in Rats After Administration of Saccharin for 15 Days[a]

	Bile Secretion Over 6 Hr		
	Bile flow, ml/hr	Total cpm for 6 hr	Cholesterol Content, mg%
Control (1% carboxymethylcellulose)	0.560 ± 0.042	1016 ± 81	111 ± 8
Saccharin (20 mg/kg/day)	0.675 ± 0.036	1858 ± 129[c]	194 ± 10[c]

	[^{3}H]Cholesterol Absorption 24 Hr After Administration	
	Plasma dpm[b]	Percent of Control
Control (1% carboxymethylcellulose)	14280 ± 385	100 ± 5
Saccharin (20 mg/kg/day)	9568 ± 463[c]	67 ± 5[c]

[a] Mean ± *SD*; $n = 6$. [b] Plasma volume assumed to be 17 ml for rats. [c] $p \leq 0.001$.

Examination of the lipid content of the fecal material (Table VII) excreted after administration of saccharin at 20 mg/kg/day indicated that the amount of lipid per gram of fecal material as well as cholesterol, triglyceride, and phospholipid contents were elevated in 0 to 6-, 6 to 12-, and 12 to 24-hr fecal collections. The fecal triglyceride content was markedly increased in the 12 to 24-hr sample. The distribution study in mice with [^{3}H]cholesterol (Table VIII) indicated that the cholesterol in bile was elevated 5.5 to 25.92% of the total recovered cholesterol content in the 0 to 6-hr fecal sample. The excretion of cholesterol in the feces tended to equilibrate over the 24-hr period so that the control mice excreted 65.5% of the cholesterol or bile acids in 24 hr, whereas the saccharin-treated mice excreted 71.6% of the labeled cholesterol or bile acids. From Table VIII, it can also be seen that after administration of saccharin, there was less accumulation of radiolabeled cholesterol in the major organs (brain, lung, heart, liver, kidney, stomach, and large intestine) compared with the control. Increases in labeled cholesterol were observed in the spleen and small intestines. The distribution of cholesterol in rats after 14 days of saccharin administration, again showed that there was no accumulation of cholesterol in the major organs (Table IX). Higher concentrations of cholesterol were found in the intestine and chyme after oral administration of the labeled cholesterol compared with control values.

The cannulation studies (Table X) demonstrate that after saccharin administration, there is an increase in bile flow from 0.560 to 0.675 ml/hr with an increase in cholesterol content, *i.e.*, 82% in dpm and 75% increase in mg%. After administering saccharin for 14 days to rats, it can be observed that there was a 33% reduction of cholesterol absorption from the gut over a 24-hr period.

The lipoprotein fractions of rat blood (Table XI) collected after a 2-week administration of saccharin demonstrated that cholesterol, neutral lipid, and triglyceride contents were reduced in the chylomicrons and very low-density, low-density, and high-density lipoprotein fractions. The phospholipid content of the individual fractions was not reduced significantly in all fractions; in fact, the high-density lipoprotein fraction showed a 148% increase in phospholipid content. The protein content of all fractions was reduced; particularly significant were the chylomicron, very low-density, and high-density lipoprotein fractions.

Data are expressed in the Tables I–XI as percent of control ± the standard deviation. The probable significant level (p) between each test group and the control group was determined by the Student's t test.

DISCUSSION

Saccharin, 1-*N*-(*o*-benzosulfimido)butan-3-one, and 3-*N*-(*o*-benzosulfimido)propionic acid were demonstrated to be very potent antihyperlipidemic agents in rodents at 20 mg/kg/day. The dosage required to induce reductions of plasma lipids was low compared with clofibrate

Table XI—Effect of Saccharin on Lipoprotein Fractions of Blood from Holtzman Rats After 14 Days of Dosing [a]

Compound	Cholesterol	Neutral Lipids	Triglyceride	Phospholipids	Protein
			Chylomicrons		
Control (1% carboxymethylcellulose)	100 ± 9[b]	100 ± 8[c]	100 ± 6[d]	100 ± 10[e]	100 ± 7[f]
Saccharin (20 mg/kg/day)	30 ± 4[v]	36 ± 5[v]	51 ± 6[v]	112 ± 9	61 ± 6[v]
			Very Low-Density Lipoproteins		
Control (1% carboxymethylcellulose)	100 ± 8[g]	100 ± 9[h]	100 ± 7[i]	100 ± 8[j]	100 ± 8[k]
Saccharin (20 mg/kg/day)	61 ± 7[v]	21 ± 4[v]	49 ± 5[v]	86 ± 9	64 ± 5[v]
			Low-Density Lipoproteins		
Control (1% carboxymethylcellulose)	100 ± 9[l]	100 ± 7[m]	100 ± 8[n]	100 ± 7[o]	100 ± 8[p]
Saccharin (20 mg/kg/day)	38 ± 4[v]	59 ± 6[v]	65 ± 6[v]	83 ± 8[w]	84 ± 9[w]
			High-Density Lipoproteins		
Control (1% carboxymethylcellulose)	100 ± 8[q]	100 ± 9[r]	100 ± 4[s]	100 ± 6[t]	100 ± 8[u]
Saccharin (20 mg/kg/day)	58 ± 6[v]	66 ± 7[v]	79 ± 8[v]	248 ± 12[v]	29 ± 4[v]

[a] Expressed as percent of control (mean ± *SD*); $m = 6$. [b] 337 ± 30 μg/ml. [c] 67 ± 5.6 μg/ml. [d] 420 ± 25 μg/ml. [e] 145 ± 15 μg/ml. [f] 3.0 ± 0.2 μg/ml. [g] 190 ± 15 μg/ml. [h] 98 ± 9 μg/ml. [i] 221 ± 15 μg/ml. [j] 26 ± 2 μg/ml. [k] 50 ± 4 μg/ml. [l] 210 ± 19 μg/ml. [m] 10 ± 0.7 μg/ml. [n] 45.1 ± 3.6 μg/ml. [o] 41 ± 3 μg/ml. [p] 0.681 ± 0.54 μg/ml. [q] 544 ± 44 μg/ml. [r] 620 ± 56 μg/ml. [s] 27 ± 1 μg/ml. [t] 153 ± 9 μg/ml. [u] 5.68 ± 0.45 μg/ml. [v] $p \leq 0.001$. [w] $p \leq 0.005$.

(100–200 mg/kg), which induces only marginal changes (15–20%) in serum and liver cholesterol and total lipid levels (26). The dose required for antihyperlipidemic activity of the saccharin analogues was in a safe therapeutic range compared with the observed LD_{50} values. The saccharin analogues were observed to be equally effective in rats and mice by either the oral or intraperitoneal administration routes. The agents were markedly effective in atherogenic mice in lowering the blood lipids, which approached normal levels after 2 weeks of drug administration.

The saccharin analogue did not bring about this reduction of lipids due to suppression of appetite in rats. Rather, saccharin analogues suppressed key enzymes in the early synthesis of cholesterol and fatty acids and in the synthesis of triglycerides. The enzymes include acetyl-CoA synthetase, citrate lyase, and mitochondrial citrate exchange. All of the enzymes play a role in synthesizing cytoplasmic acetyl-CoA, an intermediate precursor in cholesterol and fatty acid syntheses. The inhibition of these enzymes appeared to be related more with the suppression of serum cholesterol levels. The inhibition of mitochondrial citrate exchange by the saccharin analogues is a key regulatory site for the conversion of excess carbohydrates in the diet to lipids for storage. The inhibition of the acetyl-CoA carboxylase enzyme by saccharin analogues reduces the available fatty acids for triglyceride and cholesterol ester syntheses. The suppression of the acyl transferase and phosphatidate phosphohydralase enzymes correlates with the reduction of serum triglyceride levels, since the former enzyme is responsible for the addition of fatty acid to glycerol-3-phosphate for *de novo* triglyceride synthesis and the latter enzyme allows the synthesis of triglycerides from phospholipids. The same enzymes were inhibited in both rat and mice livers. Furthermore, the same enzymes were inhibited *in vivo* after 16 days of dosing with the saccharin analogues in mice. The ID_{50} estimates obtained from the rat liver enzyme assay are realistic values considering the dose required in the *in vivo* hydrolipidemic screen.

Clofibrate has been shown to accelerate basal respiration (state 4) and to inhibit adenosine diphosphate-stimulated respiration, thus uncoupling oxidative phosphorylation and reducing available energy for synthetic process in the cell (17). This may be due to the detergent-type effect of clofibrate. The saccharin analogues did not uncouple oxidative phosphorylation. They did reduce both states 3 and 4 respiration, interfering with the availability of adenosine triphosphate for enzymatic reaction, which requires energy.

It is interesting to note that the lipids being removed from the plasma compartment are not deposited in the major organs. Clearly the cholesterol, neutral lipids, and triglyceride levels are being reduced in the liver; however, the phospholipid content is elevated, probably indicative of inhibition of phosphatidate phosphohydralase activity by the saccharin analogues. In the treated animal, there was no indication of major changes in the body weight or major organ weights after administering the agents for 16 days. Rather, the cholesterol or bile acids were excreted in the bile at a fast rate and were cleared in the feces at an early time segment. Saccharin did decrease cholesterol absorption from the gut, which may explain the reduction of cholesterol in the tissues and the increase in the small intestine and feces.

Human chylomicrons and very low-density lipoproteins contain a high concentration of triglycerides. The low- and high-density lipoprotein fractions contain high concentrations of cholesterol ester and phospholipids. The saccharin analogues in the future may be helpful in Type I hyperlipidemic disease which is a state of hyperchylomicronemia. Types IIa, IIb, IV, and V hyperlipidemic states have increased levels of either low-density or very low-density lipoproteins, or both. Saccharin analogues were shown to suppress both of these lipoprotein fractions.

It would appear that saccharin analogues are more potent antihyperlipidemic agents in rodents than many of the current agents on the market today. A relatively low dose of the analogues was required to observe the lipid-lowering effect. It has been reported previously (1) that no deleterious side effects were observed in the rodent after short-term administration of these agents.

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Photostability of Solid-State Ubidecarenone at Ordinary and Elevated Temperatures under Exaggerated UV Irradiation

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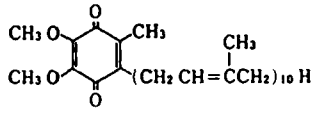
Abstract □ The photostability of ubidecarenone was investigated. Two irradiation apparatus, a grating monochromator and a high-pressure mercury vapor lamp, were employed at ordinary and elevated temperatures. Both physicochemical and chemical stabilities were significantly affected by irradiation wavelength, with UV light causing the greatest changes. The degree of degradation was a function of the light absorption properties of the substrate and markedly increased when the absorption became >30%. The photolytic degradation followed apparent first-order kinetics at all wavelengths and was promoted with temperature elevation. The Arrhenius plot gave an activation energy in the solid state different from that in the liquid state. These activation energies linearly decreased with increasing intensity of UV light.

Keyphrases □ Ubidecarenone—photostability, ordinary and elevated temperatures, exaggerated UV irradiation, activation energies □ Photostability—solid-state ubidecarenone, ordinary and elevated temperatures, exaggerated UV irradiation, activation energies □ Degradation—ubidecarenone photostability, ordinary and elevated temperatures, exaggerated UV irradiation

Preformulation study is of prime importance in the rational development of dosage forms for drug substances labile against various environmental factors. In designing a solid dosage form, it is necessary to know the inherent stability of the drug substance. There have been many reports concerning the behavior of organic compounds when subjected to heat or moisture. Photochemical mechanisms of solid-state reactions also have been reviewed (1), but not from the viewpoint of stabilization. Because of the complexity of photochemical reactions, there has been very little reported on the photostability of solid dosage forms (2–9).

Ubidecarenone [2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone (I)], a lipid-soluble benzoquinone derivative with a melting point of ~48° (10), is widely used in Japan for the treatment of angina. It is a yellow or orange crystalline powder; on exposure to light, I gradually decomposes and the color changes to dark yellow (10). The dosage forms commercially available are tablets, granules, and hard or soft gelatin capsules; these are photo-protected with a package system using light-resistant films.

The objective of the present investigation was to obtain useful informations on the behavior of I in the presence of light and heat under ordinary and accelerated storage conditions as the first step toward photostabilization.



I

Emphasis was placed on the photostability of the drug itself.

EXPERIMENTAL

Samples—Ubidecarenone, 170 mg, was accurately weighed and compressed into a flat-faced tablet 15 mm in diameter, using a compression-tension testing machine[1]. To keep the surface condition constant, a fixed compression force of 200 kg was used. Tablets were used for the quantification of appearance change by light irradiation. For the kinetic study 50 mg of ubidecarenone was dissolved in 50 ml of *n*-hexane–ether (1:1). Sixty microliters was placed on a quartz-glass plate (26 × 38 mm) and evaporated at room temperature. The oily sample was then cooled to 0–4° for 24 hr and allowed to crystallize. A 60-μg sample (<70 μm in diameter) was dispersed over the plate to illuminate all molecules as uniformly as possible. Samples were stored over silica gel in a desiccator in the dark until the irradiation test.

UV Irradiation—Two irradiation apparatus were employed. To investigate the effect of irradiation wavelength on the appearance change or photolytic degradation, a grating monochromator[2] with a 5-kW xenon lamp adjusted for 290–500 nm, a 5- to 21-nm intervals, was used (8). A band width of 5 nm was employed at all wavelengths. The amount of energy irradiated to each sample was calculated from the counts of the integrating photometer attached to the monochromator. Tablets or crystalline samples were attached to the front of the sample holder of the monochromator and exposed to UV rays. Elevation of temperature in the monochromator was prevented by a cooling water jacket surrounding the light source; the surface of samples was maintained at 25°.

In the accelerated irradiation test at ordinary and elevated temperature, the samples were placed in a thermostated jacket (Fig. 1). Water at the prescribed temperature was allowed to circulate through the jacket, and the temperature in the sample chamber was monitored with a thermocouple sensor. The jacket was placed in a fading tester[3] equipped with a 400-W mercury vapor lamp for color fading, as reported previously (11), and exposed to UV rays. The distance between the light source and the sample was 30 cm. The temperature in the fading tester was maintained below 27° by a constant-operating fan. To control the UV intensity irradiated to the sample, several optical filters[4] having various light transmission properties were attached to the front of the sample chamber.

[1] Autograph model IS-5000; Shimadzu Co., Kyoto, Japan.
[2] Model CRM-50; Japan Spectroscopic Co., Tokyo, Japan.
[3] Model MH-1; Mitsubishi Electric Co., Tokyo, Japan.
[4] Toshiba Kasei Kogyo Co., Tokyo, Japan.

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