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ACKNOWLEDGMENTS AND ADDRESSES

Received September 10, 1973, from the *Departments of Biochemical Pharmacology, Pharmaceutics, and Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication August 9, 1974.

Supported by U.S. Public Health Service Research Grant RO-CA06695-11 from the National Cancer Institute and in part by Grant GM-20852-01 from the Institute of General Medical Sciences, National Institutes of Health.

[x] To whom inquiries should be directed. Present address: Research Laboratories, Astra Pharmaceutical Products, Worcester, MA 01606

Cycloalkanones V: Synthesis, Distribution, and Effects on Triglyceride Metabolism

IRIS H. HALL [x], ROBERT G. LAMB [*], MEI-HENG H. MAR, GERALD L. CARLSON, and CLAUDE PIANTADOSI

Abstract □ The ^{14}C-labeled 2,8-dibenzylcyclooctanone was synthesized to study its absorption, distribution, and excretion in rats. Maximum drug absorption from the GI tract occurred between 12 and 14 hr after administration. The major organs possessed maximum amounts of the drug in 1 hr, with the liver concentrating the most with 6.56% ^{14}C and the muscle mass reaching a maximum of 41% ^{14}C after 14 hr. The drug remained in the GI tract over the first 6 hr and was associated with the lipid and glycogen fractions. Eighty-seven percent was eliminated in the feces after 72 hr. 2,8-Dibenzylcyclooctanone caused a significant reduction *in vitro* of dihydroxyacetone phosphatase acyltransferase and *sn*-glycerol-3-phosphate acyltransferase, which is the proposed mechanism for the observed *in vivo* reduction of hepatic, intestinal, and serum triglycerides and total glycerolipids. *In vivo* administration of the drug resulted in a depression of liver acid phosphatidyl phosphatase, acid phosphatase and lipase, and adipose lipase. The drug increased the rates of excretion of exogenous cholesterol, palmitic acid, and progesterone.

Keyphrases □ Cycloalkanones—synthesis of radiolabeled 2,8-dibenzylcyclooctanone, tissue distribution, effects on triglyceride metabolism, rats □ 2,8-Dibenzylcyclooctanone, radiolabeled—synthesis, distribution to tissues, effects on triglyceride metabolism, rats □ Triglyceride metabolism—effects of 2,8-dibenzylcyclooctanone □ Hypolipidemic activity—2,8-dibenzylcyclooctanone

Previously, the synthesis and hypolipidemic activity of cyclooctanone derivatives were reported (1). In these studies, 2,8-dibenzylcyclooctanone (I) lowered serum cholesterol by 50%, triglyceride by 42%, and glycerol by 31% in Sprague–Dawley rats. The structure–activity relationship of some derivatives was reported (2), delineating the minimum requirement for hypocholesterolemic activity in this series of compounds.

In a continuing effort to study the mechanism of action, I was labeled with ^{14}C and studies were conducted on absorption, distribution, and excretion. Studies also were performed on the effects of I on triglyceride metabolism.

EXPERIMENTAL

Organic Synthesis—The chemical method used for isotopic labeling of I was derived from that of Piantadosi *et al.* (1). Sodium (0.5 g) was dissolved in 12.5 ml of absolute ethanol, and the solution was cooled to room temperature. Four milliliters of this solution was added to a mixture of benzaldehyde (650 mg, 6.13 mmoles) and cyclooctanone (386.8 mg, 3.065 mmoles) in a 10-ml round-bottom flask equipped with a condenser protected by a drying tube. The mixture was stirred at room temperature for 4 hr. At this time, 5 ml of water was added and the mixture was extracted three times with 10 ml of benzene.

The combined extracts were evaporated to yield a viscous residue, and the residue was purified by silica gel column chromatography (35 g, 2.4 × 30 cm). Benzene was used as an eluent, and the first 130 ml of eluent contained no product. The desired 2,8-dibenzylidenecyclooctanone was obtained in the next 60 ml, and this eluate was evaporated to yield 320 mg (35%) of pure 2,8-dibenzylidenecyclooctanone as a pale-yellow solid, mp 108–110° [lit. mp 108–110° (1) and mp 111° (3)].

Ten percent palladium-on-carbon (50 mg) and sodium methylate (30 mg) were added to a solution of 2,8-dibenzylidenecyclooctanone (320 mg, 1.06 mmoles) in 25 ml of ethyl acetate. The mixture was hydrogenated until exactly 2.12 mmoles of hydrogen was absorbed. The catalyst was filtered and washed with 5 ml of hot ethyl acetate. The filtrate was evaporated to yield a viscous residue, which was purified by silica gel column chromatography (12 g, 1.2 × 40 cm), using benzene as the elution solvent. Fractions of 2 ml were collected. After a forerun of 56 ml, 32 ml was collected and evaporated to dryness. All fractions were shown to contain only I by TLC [R_f 0.72 in benzene–chloroform (95:5)]. The yield was 190 mg (62%), mp 82–83° [lit. (1) mp 82–83°].

^{14}C-2,8-Dibenzylidenecyclooctanone and ^{14}C-2,8-Dibenzylcyclooctanone—The identical procedure as described for the preparation of unlabeled I was utilized. For the first run, 581 mg of benzaldehyde and 69.15 mg (1.5 mCi) of benzaldehyde-(carbonyl-^{14}C)[1] (2.25 mCi/mmole) were used. The labeled compound obtained was identical to the unlabeled compound in R_f value. The ^{14}C-labeled I (192 mg) (22% yield based on the starting material) was obtained with a specific activity of 460 μCi/mmole (1.5 μCi/mg).

For the second run, 31.3 mg (2 mCi) of benzaldehyde-(carbonyl-

[1] Amersham Searle.

Table I—Percent Recovery (Mean ± Standard Deviation) of Radioactivity in the GI Tract of Male Sprague–Dawley Rats following Oral Administration of ^{14}C-2,8-Dibenzylcyclooctanone (20 mg/kg)

Sample (n = 6)	0.083 hr	0.25 hr	0.50 hr	1 hr	2 hr	6 hr
Esophagus content	0.501 ± 0.159	0.194	0.086 ± 0.032	0.152 ± 0.102	0.107 ± 0.064	0.123 ± 0.066
Stomach content	85.17 ± 2.65	53.41 ± 10.32	52.97 ± 6.25	31.54 ± 9.91	21.64 ± 5.43	7.21 ± 0.81
Stomach	2.66 ± 0.53	13.7 ± 2.61	5.70 ± 4.03	7.80 ± 5.13	4.72 ± 1.53	6.99 ± 3.56
Small intestine content	0.615 ± 0.171	4.91 ± 1.87	9.72 ± 7.18	20.2 ± 5.22	32.7 ± 9.14	29.5 ± 2.42
Small intestine	0.358 ± 0.130	8.70 ± 2.10	8.92 ± 7.35	14.45 ± 2.25	19.6 ± 2.65	7.81 ± 2.79
Cecum content	0.020 ± 0.024	0.160 ± 0.060	0.042 ± 0.048	0.103 ± 0.063	0.216 ± 0.116	22.7 ± 3.67
Cecum	0.025 ± 0.023	0.160 ± 0.081	0.048 ± 0.024	0.035 ± 0.027	0.069 ± 0.051	1.03 ± 0.241
Large intestine content	0.017 ± 0.016	0.205 ± 0.049	0.032 ± 0.0236	0.042 ± 0.010	0.0947 ± 0.0118	7.03 ± 1.50
Large intestine	0.078 ± 0.041	0.057 ± 0.013	0.044 ± 0.036	0.241 ± 0.194	0.179 ± 0.103	0.512 ± 0.356
Head (site of administration)	8.31 ± 0.65	3.66 ± 1.52	7.04 ± 2.10	5.06 ± 3.66	4.89 ± 0.33	2.50 ± 0.42
Feces	—	—	0.182 ± 0.147	0.730 ± 0.589	0.762 ± 0.681	1.63 ± 0.652
	94.754	85.156	84.734	80.363	84.977	87.035

Sample (n = 6)	14 hr	18 hr	24 hr	48 hr	72 hr
Esophagus content	0.005 ± 0.001	0.089 ± 0.021	—	0.001 ± 0	0.003 ± 0.001
Stomach content	2.86 ± 1.35	4.42 ± 2.76	0.530 ± 0.014	0.014 ± 0.012	0.061 ± 0.031
Stomach	0.615 ± 0.133	0.596 ± 0.451		0.021 ± 0.025	0.144 ± 0.097
Small intestine content	10.0 ± 3.01	26.6 ± 9.78	5.74 ± 0.63	2.40 ± 1.40	1.73 ± 2.02
Small intestine	1.31 ± 0.81	7.81 ± 3.72	0.400 ± 0.156	0.556 ± 0.406	0.625 ± 0.32
Cecum content	11.8 ± 2.03	15.4 ± 3.78	1.96 ± 0.36	0.356 ± 0.047	0.625 ± 0.605
Cecum	0.326 ± 0.263	3.68 ± 1.72	0.020 ± 0.023	0.055 ± 0.008	0.053 ± 0.056
Large intestine content	4.03 ± 2.04	0.032 ± 0.026	0.115 ± 0.010	0.271 ± 0.098	0.487 ± 0.337
Large intestine	0.201 ± 0.205	0.131 ± 0.117	0.130 ± 0.127	0.032 ± 0.028	0.127 ± 0.119
Head (site of administration)	1.79 ± 0.88	2.91 ± 0.24	3.89 ± 0.82	1.69 ± 0.49	2.07 ± 0.95
Feces	18.8 ± 11.7	21.79 ± 2.97	57.6 ± 9.62	76.1 ± 10.6	86.7 ± 20.2
	51.737	83.458	70.385	81.496	92.653

^{14}C)[2] (6.8 mCi/mmole) and 619 mg of unlabeled benzaldehyde were used. The yield of product, Compound I, was 270 mg (31% based on the starting material) with a specific activity of 450 μCi/mmole (1.47 μCi/mg).

Radioactivity—The radioactivity was measured by placing a known volume of material in a scintillation vial; then 10 ml of the following scintillation fluid was added: 2,5-diphenyloxazole[3] and 1,4-bis[2-(5-phenyloxazolyl)]benzene[4] in toluene. These vials were counted in a scintillation counter[5], using the channel ratio technique to correct for quenching.

Animals—Male Sprague–Dawley rats[6], 120 g, were used. Prior to the experiment, the animals were maintained on lab chow[7] and water *ad libitum*.

Compound I was suspended in 1% carboxymethylcellulose solution. Each animal was administered 20 mg/kg of ^{14}C-2,8-dibenzylcyclooctanone (specific activity 450–460 μCi/mmole) orally by an esophageal intubation needle in 0.2 ml of 1% carboxymethylcellulose.

Collection of Samples—At appointed time periods (0.083, 0.25, 0.5, 1, 2, 6, 12 or 14, 18, 24, and 48 or 72 hr), the rats were killed by decapitation; the blood was drained from the carotid artery and immediately heparinized (0.2 ml heparin sodium[8]). The liver, kidneys, brain, heart, testes, adrenal glands, spleen, and each section of the GI tract with its contents were excised. The tissues were weighed and homogenized with 10 volumes of distilled water (w/v). The urine and feces were collected at 0.25, 0.5, 1, 2, 6, 12, 18, 24, 48, and 72 hr.

Radioactivity of Freeze-Dried Tissue Samples—These samples were lyophilized using a freeze dryer[9]. Dried samples (~50 mg wet weight) were combusted[10], or dried samples were weighed into scintillation vials (~10 mg) and 1 ml of tissue solubilizer[11] was added to each vial. These vials were closed tightly and heated to 55° for 20 hr. Ten milliliters of the following scintillation fluid was added: 10 g of 2,5-bis[2-(5-*tert*-butylbenzoxazolyl)]thiophene (scintillation grade), 200 g of naphthalene, 1500 ml of toluene, 1000 ml of methylcellulose, and 89 ml of water[12].

Radioactivity of Wet Homogenate—Aliquots (1 ml) of the wet homogenate were placed in scintillation vials, and 2 ml of tissue solubilizer was added to each vial; the vials were closed tightly and heated to 55° for 20 hr. After cooling to room temperature, 10 ml of scintillation fluid was added containing 1,4-bis[2-(5-phenyloxazolyl)]benzene[4] and 10.8 g of 2,5-diphenyloxazole[3] in 2 liters of toluene and 1.2 liters of octoxynol[13]. Aliquots (0.1 ml) of the tissue and feces homogenates were placed on a filter disk[14] and dried. The head and body were solubilized separately by heating in 2 volumes of 30% potassium hydroxide at 80° overnight (4). Aliquots were taken to determine the radioactivity in scintillation fluid.

***In Vitro* Absorption**—The stomach and the first half of the small intestine were removed from male rats. The contents of each portion of the gut were washed out with pH 7.2 Hanke's buffer. The radiolabeled drug was dissolved in 1% carboxymethylcellulose and placed in the lumen of the gut with an intubation needle. The gut was tied at both ends with No. 40 cotton thread so that no leakage of materials occurred. Then the gut was placed in 20 ml of pH 7.2 Hanke's buffer reinforced with 1 g/liter of dextrose and placed in a shaker[15] at 37°.

At intervals of 1 hr, aliquots were taken from the medium bathing the tissue to determine radioactivity. At the end of 6 hr, the radioactivity of the contents and a 10% homogenate in water of each segment of gut was determined.

***In Vivo* Absorption**—Male rats were administered 20 mg/kg of ^{14}C-2,8-dibenzylcyclooctanone orally. The animals were sacrificed after 2 hr, and the stomach and duodenum were removed with

[2] ICN, Pharmaceutical, Inc.
[3] PPO, New England Nuclear.
[4] POPOP, Packard.
[5] Packard Tri-Carb model 3320.
[6] Zivic Miller, Allison Park, Pa.
[7] Purina rodent lab chow.
[8] Riker Lab Inc., 5000 USP units/ml.
[9] Virtis automatic model 10-010.
[10] Packard Tri-Carb model 305 sample oxidizer.
[11] Protosol, 0.5 *M*, New England Nuclear.
[12] BBT.
[13] Triton X-100, Rohm and Haas Co.
[14] Whatman No. 1.
[15] Dubnoff.

Table II—Percent Recovery (Mean ± Standard Deviation) of Radioactivity in the Tissue of Male Sprague–Dawley Rats following Oral Administration of ^{14}C-2,8-Dibenzylcyclooctanone (20 mg/kg)

Sample (n = 6)	0.083 hr	0.25 hr	0.50 hr	1 hr	2 hr	6 hr
Liver	0.120 ± 0.009	0.722 ± 0.172	1.580 ± 0.248	6.563 ± 3.63	3.565 ± 2.02	2.221 ± 0.958
Kidneys	0.015 ± 0.002	0.202	0.225 ± 0.192	0.359 ± 0.081	0.147 ± 0.131	0.112 ± 0.075
Brain	0.026 ± 0.024	0.097 ± 0.014	0.047 ± 0.041	0.371 ± 0.331	0.071 ± 0.065	0.013 ± 0.010
Lungs	0.073 ± 0.056	0.268 ± 1.05	0.048 ± 0.030	0.214 ± 0.195	0.027 ± 0.022	0.151 ± 0.064
Heart	0.015 ± 0.014	0.253 ± 0.248	0.028 ± 0.029	0.080 ± 0.100	0.035 ± 0.034	0.009 ± 0.014
Spleen	0.004 ± 0	0.327 ± 0.227	0.021 ± 0.020	0.068 ± 0.050	0.009 ± 0.010	0.006 ± 0.010
Testes	0.003 ± 0	0.493 ± 0.329	0.049 ± 0.009	0.362 ± 0.051	0.047 ± 0.023	0.027 ± 0.030
Blood	0.001 ± 0	0.167 ± 0.158	0.015 ± 0.003	0.160 ± 0.032	0.045 ± 0.041	0.018 ± 0.004
Adrenals	0.011 ± 0.014	0.395 ± 0.231	0.027 ± 0.022	0.130 ± 0.150	0.033 ± 0.030	0.008 ± 0.001
Adipose tissue	0.377 ± 0.230	0.473 ± 0.300	0.356 ± 0.129	0.881 ± 0.797	0.767 ± 0.365	0.708 ± 0.631
Muscle mass	0.897 ± 0.085	5.05 ± 0.037	12.298 ± 1.632	12.504 ± 6.761	7.63 ± 5.43	5.943 ± 1.832
	1.54	8.447	14.694	21.692	12.376	9.215
Total recovery	96.296	93.603	99.428	102.05	97.353	96.251

Sample (n = 6)	14 hr	18 hr	24 hr	48 hr	72 hr
Liver	0.247 ± 0.246	2.58 ± 1.12	0.165 ± 0.233	0.242 ± 0.046	0.007 ± 0.033
Kidneys	0.009 ± 0.001	0.111 ± 0.103	0	0.022 ± 0.003	0.058 ± 0.009
Brain	0	0.071 ± 0.069	0.055 ± 0.028	0.014 ± 0.017	0.041 ± 0.044
Lungs	0.070 ± 0.072	0.040 ± 0.042	0	0	0.066 ±
Heart	0	0.060 ± 0.010	0	0.006 ± 0.001	0.012 ± 0.003
Spleen	0	0.042 ± 0.006	0	0.002 ± 0	0.051 ± 0.007
Testes	0.016 ± 0.003	0.069 ± 0.066	0	0.021 ± 0.031	0.128 ± 0.114
Blood	0	0.026 ± 0.005	0	0.021 ± 0.024	0
Adrenals	0	0.031 ± 0.011	0	0.006 ± 0	0.050 ± 0.008
Adipose tissue	0.434 ± 0.213	0.432 ± 0.329	0	0.238 ± 0.117	0.018 ± 0.016
Muscle mass	40.91 ± 1.29	7.98 ± 1.64	21.107 ± 9.072	7.566 ± 3.814	10.788 ± 9.532
	41.686	11.242	21.327	8.131	11.319
Total recovery	93.423	94.692	91.712	89.627	102.972

their contents. The chyme was removed from the GI tract, and a 10% homogenate of the tissue or food in 0.25 *M* sucrose plus 0.001 *M* ethylenedinitrilotetraacetic acid was prepared. Aliquots of the whole homogenates were extracted three times with chloroform and once with 10% methanol–90% chloroform.

Aliquots were taken from the organic and aqueous extract to determine radioactivity. Forty milliliters of the aqueous extract was treated with 10 ml of 20% trichloroacetic acid and centrifuged to obtain a supernate and pellet. Ten milliliters of the whole homogenates was extracted by the method of Shibko *et al.* (5) to obtain the ribonucleic acid, deoxyribonucleic acid, lipid, glycogen, and protein fractions.

***In Vivo* Hepatic and Intestinal Glycerolipid Formation from 1,3-^{14}C-Glycerol**—Male Sprague–Dawley rats, 300–350 g, were divided into two groups which received either 0.5 ml of 1% carboxymethylcellulose or 10 mg/kg of I suspended in 1% carboxymethylcellulose daily for 5 days by an oral intubation needle. After 5 days, both control and experimental animals were injected intraperitoneally with 1 ml of isotonic saline containing 5 μCi of 1,3-^{14}C-glycerol (6.5 μCi/μmole). Seventeen minutes later, the animals were anesthetized with ether, the abdomen was opened, and blood was removed from the abdominal aorta. The liver and small intestine were removed, washed with 0.05 *M* tromethamine–hydrochloric acid (pH 7.5), quickly frozen in dry ice–acetone, and stored at −40°.

Lipids from duplicate 1-g samples of tissue were extracted by the Bligh and Dyer procedure (6). Serum, hepatic and intestinal triglyceride, and serum glycerol levels and the rate of glycerolipid formation from 1,3-^{14}C-glycerol were determined by techniques described previously (7). The time course of 1,3-^{14}C-glycerol incorporation into intestinal triglycerides and total hepatic and intestinal glycerolipids was similar to that described for hepatic triglycerides (7). Therefore, the 17-min time point was used to estimate *in vivo* hepatic and intestinal triglyceride and total glycerolipid formation.

***sn*-Glycerol-3-phosphate Acyltransferase**—Rat liver microsomes were prepared as previously described (8) and incubated with 30 m*M* tromethamine–hydrochloric acid (pH 8.0), 3.3 m*M* magnesium chloride, 0.7 m*M* dithiothreitol, 50 μ*M* coenzyme A, 0.5 m*M* *sn*-1,3-^{14}C-glycerol-3-phosphate, 3.3 m*M* adenosine triphosphate, 0.60 m*M* ammonium palmitate, and 1.25 mg of albumin in a total volume of 0.40 ml. These conditions were optimum for total glycerolipid formation (8). After a 30-min incubation, reactions were stopped by the addition of 3 ml of chloroform–methanol (1:2).

The lipids were extracted by the Bligh and Dyer procedure (6) and separated into phosphatidic acid and neutral lipid by TLC in a solvent system containing chloroform–methanol–3.5 *N* ammonium hydroxide (65:35:8) (8). Areas of the TLC plates which corresponded to known lipid standards were scraped into scintillation vials, and the radioactivity was determined with a scintillation counter.

Dihydroxyacetone Phosphate Acyltransferase—The incubation mixture optimum for dihydroxyacetone phosphate esterification contained 1.5 m*M* dihydroxyacetone phosphate, 1.3 m*M* reduced nicotinamide adenosine dinucleotide, 25 m*M* tromethamine–hydrochloric acid (pH 7.5), 1.5 m*M* magnesium chloride, 40 μ*M* coenzyme A, 2.8 m*M* adenosine triphosphate, 0.3 m*M* dithiothreitol, 0.7 m*M* 1-^{14}C-palmitate (0.1 μCi), 1.25 mg of fatty acid-poor albumin, and 0.3 mg of rat liver microsomal protein in a total volume of 0.5 ml. Reactions were started by the addition of microsomal protein and stopped after a 30-min incubation by the addition of 3 ml of chloroform–methanol (1:2). Lipids were extracted and identified as described for *sn*-glycerol-3-phosphate acyltransferase.

Lipase, Acid Phosphatase, and Phosphatidyl Phosphatase Activity—Rats, 180 g, were treated in the identical manner as outlined in the *in vivo* studies. After 5 days, the liver and adipose tissue were excised and blood was collected so that the following enzymatic assays could be performed: liver phosphatidyl phosphatase at pH 7.0 (control value = 0.152 mg phosphate released in 30 min/g wet liver) and at pH 5.0 (0.448 mg phosphate released in 30 min/g wet liver) (9), free acid phosphatase (0.598 mg phosphate released in 10 min/g wet tissue), total acid phosphatase (11) (1.798 mg phosphate released in 10 min/g wet liver), hormone sensitive lipase (10) (6.4 ml of 0.025 *N* sodium hydroxide/ml of serum) and lipolysis (12) (1.7 ml of 0.05 *N* sodium hydroxide/ml of serum), and adipose hormone sensitive lipase (10) (22.5 ml of 0.025 *N* sodium hydroxide/g wet tissue). Inorganic phosphate was determined by the procedure of Chen *et al.* (11).

Table III—Effect of 2,8-Dibenzylcyclooctanone on Serum, Hepatic, and Intestinal Triglycerides, Serum Glycerol, and Liver Weight

Experiment (n)	Triglycerides				
	Serum, mg % ± *SEM*	Hepatic, mg/g ± *SEM*	Intestinal, mg/g ± *SEM*	Serum Glycerol, nmoles/ml ± *SEM*	Liver Weight, g ± *SEM*
Control (5)	128.0 ± 22.9	6.48 ± 0.63	14.8 ± 1.9	154.6 ± 24.7	13.1 ± 0.6
2,8-Dibenzylcyclooctanone	65.5 ± 11.1[a]	5.02 ± 0.73	10.9 ± 2.3	38.2 ± 4.2[b]	12.7 ± 1.2

[a] $p \leq 0.05$ (level of significance from control group). [b] $p \leq 0.001$ (level of significance from control group).

Table IV—Effect of 2,8-Dibenzylcyclooctanone on *In Vivo* Hepatic, Intestinal, and Serum Triglycerides and Total Glycerolipid Formation from 1,3-^{14}C-Glycerol

Experiment (n)	Intestine, nmoles/17 min/g ± *SEM*		Liver, nmoles/17 min/g ± *SEM*		Serum, nmoles/17 min/ml ± *SEM*
	Triglycerides	Total	Triglycerides	Total	Triglycerides
Control (5)	21.4 ± 3.3	33.1 ± 2.5	95.9 ± 10.8	131.5 ± 14.7	21.3 ± 4.9
2,8-Dibenzylcyclooctanone	7.5 ± 1.2[a]	13.3 ± 3.8[b]	30.9 ± 3.5[a]	45.3 ± 4.7[b]	5.8 ± 0.8[a]

[a] $p \leq 0.01$ (level of significance from control group). [b] $p \leq 0.001$ (level of significance from control group).

Lipid Excretion—Other rats, treated identically, were given ^{14}C-cholesterol (1 μCi), ^{14}C-palmitic acid (2 μCi), or ^{3}H-7-progesterone (60 μCi) orally on the 5th day; the feces were collected after 24 hr. Homogenates of the feces in water were prepared, and aliquots were taken for radioactive determination. The homogenates were extracted three times with chloroform and once with chloroform–methanol (90:10) to obtain the lipid fraction; this fraction was dried and weighed.

RESULTS

Distribution of 2,8-Dibenzylcyclooctanone (I)— The freeze-dried sample and wet homogenate sample had no significant accumulation of ^{14}C-2,8-dibenzylcyclooctanone or its metabolites[16] in the kidneys, heart, brain, testes, or adrenal glands. Since there was such a low recovery of ^{14}C in the freeze-dried samples, studies were performed on the total animals to determine the percent recovery of the administered radioactive dose.

Table I notes the percent recovery of radioactivity in the GI tract after oral administration of ^{14}C-2,8-dibenzylcyclooctanone. Very high concentrations of ^{14}C were found in the GI tract and its contents, representing 75–89% of the ^{14}C administered over the first 6 hr, with the largest percentage present in the stomach and small intestine. After 6 hr, the ^{14}C-2,8-dibenzylcyclooctanone began to decrease in the upper GI tract and gradually built up in the excreted feces. Table I shows that about 58, 76, and 87% of the ^{14}C was excreted in the feces 24, 48, and 87 hr, respectively, after an oral dosing.

The total carcass (muscle mass) of the rat reached 41% ^{14}C-2,8-dibenzylcyclooctanone after 14 hr (Table II). The major organs of the body reached a maximum absorption of I at 1 hr. A second peak was reached at 18 hr, especially in the liver. Fourteen hours probably represents the maximum absorption of the drug from the GI tract, since only 31% of the ^{14}C-2,8-dibenzylcyclooctanone remained in the GI tract at this time.

Organ distribution of ^{14}C-2,8-dibenzylcyclooctanone after oral administration is shown in Table II. These data on whole homogenates reflect the same conclusions as with the freeze-dried samples, whether combusted or not. Only a small percentage (9.2% at 1 hr) of ^{14}C-2,8-dibenzylcyclooctanone was found in the tissues, and the concentration found was in the following order: liver ≫ adipose tissue > testes = kidneys = brain > lungs > blood > adrenal > heart > spleen. The blood was corrected for a total blood volume of 17.5 ml for rats.

Absorption—Since a large amount of ^{14}C-2,8-dibenzylcyclooctanone was observed in the upper GI tract during the first 6 hr of the percent recovery studies, absorption studies were undertaken to determine why the drug remained there. It had been expected that the drug would be lipophilic and be absorbed rapidly. However, *in vitro* incubation studies indicated that after 6 hr 83% of ^{14}C-2,8-dibenzylcyclooctanone remained in the stomach in an isolated preparation and 84% remained in the small intestine in an isolated preparation, indicating that the drug was poorly absorbed from either site, which was congruent with the *in vivo* distribution data.

When homogenates were extracted, it was observed that of the total ^{14}C-2,8-dibenzylcyclooctanone present in the GI contents, 5.6% was in the deoxyribonucleic acid fraction, 10.9% in the ribonucleic acid, 4.1% in the protein, 38.2% in the lipid, 40.6% in the glycogen, and 0.5% in the water. Of the total ^{14}C-2,8-dibenzylcyclooctanone present in the GI tissue homogenate, 5.0% was in the deoxyribonucleic acid, 18.0% in the ribonucleic acid, 1.5% in the protein, 33.8% in the lipid, 38.8% in the glycogen, and 2.9% in the water. The ^{14}C-labeled drug or its metabolite appears to be isolated predominantly with the glycogen and lipid fractions in both the GI contents (79%) and the GI tissue (73%).

When whole homogenates were extracted (chloroform–methanol), it was observed that 65% of the ^{14}C-2,8-dibenzylcyclooctanone was in the aqueous fraction of the GI contents and 86% was in the aqueous fraction of the GI tissue. When the aqueous fraction was treated with 20% trichloroacetic acid, the ^{14}C-2,8-dibenzylcyclooctanone remained in the supernate rather than the pellet. These results indicate that the ^{14}C-drug is binding to a component in the glycogen and lipid fractions.

Tissue Triglyceride, Serum Glycerol, and Liver Weight—The administration of 10 mg/kg of I to rats for 5 days reduced mean serum, liver, and intestinal triglyceride concentrations to 49, 22.5, and 26.5%, respectively (Table III). Serum glycerol concentrations were reduced 75.3%, and there was no change in mean liver weight in rats receiving I for 5 days. During the 5-day period, rats receiving I gained 22.0 ± 3.6 g/kg and those receiving 1% car-

Table V—*In Vitro* Effect of 2,8-Dibenzylcyclooctanone on Hepatic *sn*-Glycerol-3-phosphate Acyltransferase and Dihydroxyacetone Phosphate Acyltransferase Activity

2,8-Dibenzylcyclooctanone, m*M*	*sn*-Glycerol-3-phosphatase Acyltransferase Relative Rate	Dihydroxyacetone Phosphate Acyltransferase Relative Rate
0	1.00	1.00
0.75	0.44	0.43
1.5	0.37	0.34
2.3	0.33	0.28
3.0	0.32	0.22

[16] Although no metabolites have been identified to date, the ^{14}C-isotope would also be distributed in any metabolites.

Table VI—Effects of 2,8-Dibenzylcyclooctanone on Enzyme Activities of Sprague–Dawley Rats *In Vivo*[a]

	1% Carboxymethylcellulose (n = 8)	2,8-Dibenzylcyclooctanone, 10 mg/kg/day for 5 days	p
Liver:			
Phosphatidyl phosphatase			
pH 7.0	100 ± 12	104 ± 11	—
pH 5.0	100 ± 6	87 ± 7	0.050
Lipase	100 ± 11	64 ± 14	0.001
Acid phosphatase			
Free	100 ± 15	65 ± 16	0.001
Total	100 ± 13	80 ± 14	0.050
Serum:			
Lipase	100 ± 29	94 ± 33	—
Lipolysis test[b]	100 ± 25	41 ± 21	0.001
Adipose tissue:			
Lipase	100 ± 17	64 ± 13	0.001

[a] The number of animals in the group is expressed as n. All values are expressed as percent of control noted as mean and standard deviation, $\bar{x} \pm SD$. The probable significant level (p) between each test group and the control group was determined by the Student t test. [b] Reference 12.

boxymethylcellulose gained 68.3 ± 8.2 g/kg. The difference in weight gain may be partially explained by a decrease in food intake for animals receiving I (1).

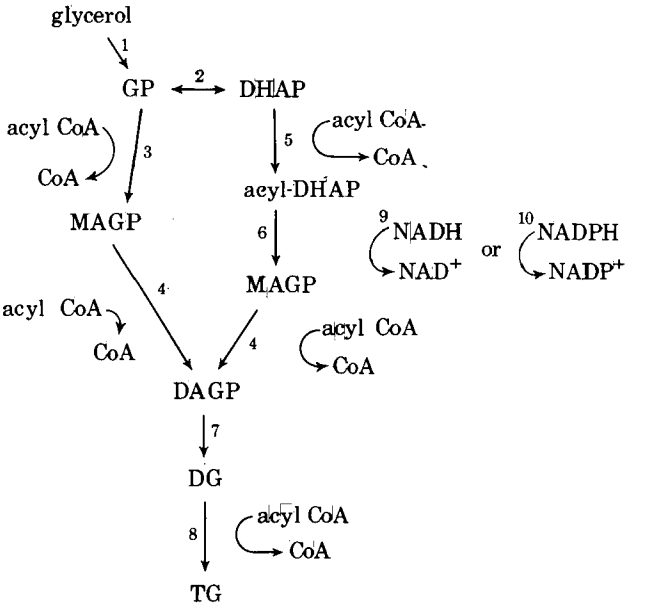
***In Vivo* Glycerolipid Synthesis**—Table IV shows the effect of I on the *in vivo* incorporation of 1,3-^{14}C-glycerol into hepatic, intestinal, and serum triglycerides and total glycerolipids. These calculations are based on the specific activity of serum glycerol 17 min after intraperitoneal injection of 1,3-^{14}C-glycerol. The methodology used for these calculations was presented previously (7).

The administration of I produced marked decreases in hepatic and intestinal triglyceride and total glycerolipid synthesis from 1,3-^{14}C-glycerol. The amount of labeled triglyceride found in the serum was also reduced 73% in animals treated with I. These results suggest that I may lower serum triglyceride levels by inhibition of hepatic and intestinal triglyceride synthesis. Since both total glycerolipid formation and triglyceride formation from 1,3-^{14}C-glycerol are reduced to the same degree by administration of I, this agent may reduce triglyceride formation by inhibiting an early step of glycerolipid metabolism (Scheme I).

Both the liver and intestine produce serum triglycerides. However, in the postabsorptive state, the liver is the major source of serum triglycerides. Therefore, the effect of I on individual reactions of hepatic glycerolipid metabolism was studied in more detail.

***In Vitro* Hepatic Glycerolipid Metabolism**—The effect of increasing concentrations of I on dihydroxyacetone phosphate acyltransferase and *sn*-glycerol-3-phosphate acyltransferase is shown in Table V. Compound I produced greater than 50% inhibition of both reactions at 0.75 m*M*. Since both reactions govern initial steps of glycerolipid metabolism (Scheme I) (13), I may exert its lipid-lowering action by inhibition of these steps *in vivo*.

***In Vivo* Lipase and Phosphatase Activity**—Compound I had



Scheme I—Pathways for Synthesis of Triglycerides: Step 1, glycerol kinase; Step 2, sn-*glycerol-3-phosphate (GP) dehydrogenase; Step 3,* sn-*glycerol-3-phosphate (GP) acyltransferase; Step 4, monoacylglycerophosphate (MAGP) acyltransferase; Step 5, dihydroxyacetone phosphate (DHAP) acyltransferase; Step 6, acyldihydroxyacetone phosphate reductase; Step 7, phosphatidate (DAGP) phosphatase; Step 8, diglyceride (DG) acyltransferase; Step 9, nicotinamide adenine dinucleotide; and Step 10, nicotinamide adenine dinucleotide phosphate.*

no effect on neutral phosphatidyl phosphatase in the liver but caused a reduction in the activity of liver acid phosphatidyl phosphatase, free and total acid phosphatase, and lipase (Table VI). Serum lipase was unaltered, but adipose lipase was significantly reduced. The lipolysis test was drastically reduced by I, indicating low levels of triglycerides and fatty acids in the plasma. The drug increased the rates of excretion of orally administered, labeled, exogenous cholesterol, progesterone, and palmitic acid (Table VII).

DISCUSSION

The results presented here and in a previous report (1) demonstrate that I lowers serum triglyceride and glycerol levels in rats. This agent may reduce serum triglycerides by inhibiting hepatic and intestinal triglyceride formation *in vivo*, since the incorporation of 1,3-^{14}C-glycerol into hepatic, intestinal, and serum triglycerides was markedly reduced by I. The fall in serum triglyceride levels is accompanied by a decrease in hepatic and intestinal triglyceride concentrations, which also may indicate a reduction in triglyceride formation by these tissues.

Scheme I outlines the basic biosynthetic pathways of hepatic glycerolipid metabolism. In this study, 1,3-^{14}C-glycerol was used to estimate *in vivo* glycerolipid metabolism. Labeled glycerol is taken up by tissues and converted to *sn*-glycerol-3-phosphate (GP) by the action of glycerokinase (Step 1, Scheme I). *sn*-Glycerol-3-phosphate may be converted to dihydroxyacetone phosphate

Table VII—Percent Recovery (Mean ± Standard Deviation) of Radioactivity in Fecal Lipid Excretion after Treatment with 2,8-Dibenzylcyclooctanone

	Control		Treated	
n = 6	6 hr	24 hr	6 hr	24 hr
Lipid extracted, mg	—	100		129
Exogenous				
^{14}C-Palmitic acid	100 ± 2	100 ± 6	190 ± 1[a]	297 ± 9[a]
^{14}C-Cholesterol	100 ± 15	100 ± 9	225 ± 14[a]	124 ± 12
^{3}H-Progesterone	100 ± 11	100 ± 9	178 ± 11[a]	237 ± 21[a]

[a] $p \geq 0.001$.

(DHAP) by *sn*-glycerol-3-phosphate dehydrogenase (Step 2, Scheme I). Both *sn*-glycerol-3-phosphate and dihydroxyacetone phosphate are converted to triglyceride (TG) by the sequential esterification steps.

Since hepatic total glycerolipid and triglyceride formation from 1,3-^{14}C-glycerol were reduced to the same degree by I (Table IV), this agent may inhibit an early step of glycerolipid metabolism *in vivo*. *In vitro* studies demonstrated that I inhibits *sn*-glycerol-3-phosphate acyltransferase (Step 3, Scheme I) and dihydroxyacetone phosphate acyltransferase (Step 5, Scheme I). An *in vivo* study also demonstrated that acid phosphatidate (DAGP) phosphatase (Step 7, Scheme I) was slightly reduced by I.

Other effects of I were decreased liver and adipose tissue lipase activity, reduced free and total acid phosphatase activity, and increased excretion of cholesterol, palmitic acid (triglyceride), and steroid hormone. Reduced lipase activity may explain the marked reduction in serum glycerol levels (Table III).

A number of agents effectively reduce serum triglycerides in humans and experimental animals (7, 14–26). However, the mode of action of these agents has not been elucidated. The hypolipidemic effect of I may partially be explained by inhibition of triglyceride formation, reduced lipolysis, and increased excretion of lipids. The effect of this agent on other factors that would lower serum lipids such as fatty acid biosynthesis, triglyceride clearance and release, and lipoprotein synthesis still remains to be studied.

The absorption and distribution data demonstrated that only minute amounts of I were being absorbed from the GI tract. The drug or its metabolites remained associated with the glycogen and lipid component(s) of the digestive tract. Only minimal concentrations of the drug were found in the blood. The muscle mass possessed more drug than the major organs of the body. The liver concentrated 6.5% of the drug in 1 hr, and a second peak (2.6%) in the liver was reached at 18 hr. This second peak may represent recirculation of the drug since the muscle mass decreased from this point in time and the fecal content increased. The drug and its metabolites were essentially eliminated from the body *via* the feces after 72 hr. Thus, it can be concluded that only very small quantities of the drug actually reached the cellular level.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 3, 1974, from the *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514*

Accepted for publication August 19, 1974.

Abstracted in part from a thesis submitted by M.-H. H. Mar to the University of North Carolina in partial fulfillment of the Master of Science degree requirements.

Supported in part by R. J. Reynolds trust fund and National Institutes of Health Grant 1-R01-HL16464-01.

* Present address: Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

[x] To whom inquiries should be directed.