262° dec (uncor); R_f 0.56-0.57; λ_{max} (H_2O) 268 (ϵ 38,424), 359 (9852), 446 (12,315); λ_{min} 307 (2266), 391 (5468). *Anal.* ($C_{17}H_{19}ClN_4O_6$) C, H, Cl, N.

Biology. The procedures used for the biological evaluation of the new analogs in rats and *L. casei* were the same as those used by us on earlier occasions[2,14] for similar analogs.

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Cycloalkanones. 2. Synthesis and Biological Activity of α,α'-Dibenzylcycloalkanones

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A number of mono- and disubstituted cycloalkanones have been synthesized by condensation of the cyclic ketones with the appropriate aldehyde in the presence of sodium ethoxide. The hypocholesterolemic activity has been studied and the most potent compound was found to be *trans*-2,8-dibenzylcyclooctanone which showed an ED_{50} of 10 mg/kg per day which lowered rat blood cholesterol levels to 50% of the normal levels.

Research involving studies on the chemotherapy of atherosclerosis has dealt with compounds that lower specific serum lipids, *i.e.*, cholesterol and triglycerides. The present investigation describes the synthesis and initial biological studies on a series of substituted cycloalkanone derivatives. These were tested for hypocholesterolemic effects in rats and mice. The series studied included pentanone, hexanone, heptanone, octanone, nonanone, and dodecanone derivatives. Compound series including cyclohexane and indan derivatives have been studied by others.[1]

Of the cyclic ketones tested, the 2,8-dibenzylcyclooctanone was found to be most effective as a potential hypocholesteremic agent. At the screening dose of 10 mg/kg orally, 2,8-dibenzylcyclooctanone effectively lowered the serum cholesterol levels to 50% as compared to the controls.

Isomeric dimethoxy- and trimethoxybenzylidenecycloalkanones have been reported by Mattu and Manca,[2] and some diarylidenecycloalkanones have been described by Farrell and Read.[3] The photochemical properties of 2,5-dibenzylidene-3-cyclopentanone have been studied by Chapman and Pasto.[4] However, the aryl methylene compounds have not been reported. Recently, Irvine, *et al.*,[5] have investigated the stereochemistry of α,α'-dibenzylcycloalkanones.

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Experimental Section

The disubstituted cycloalkanones were prepared by condensation of cyclic ketones with the appropriate aldehyde in the presence of NaOEt. The resulting dibenzylidene cycloalkanones were then treated with palladium-charcoal catalyst in the presence of EtOAc to give the disubstituted dibenzylcycloalkanone. All melting points are corrected and were obtained on a Mel-Temp apparatus. Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich., or Atlantic Microlab, Atlanta, Ga., and where indicated by symbols were within ±0.4% of the theoretical values.

Clofibrate. The oil from commercial capsules of clofibrate (Atromid-S, Ayerst) was distilled, and the fraction boiling at 145-146° (0.005 mm) was collected.

Cyclopentanone, cyclohexanone, cycloheptanone, cyclododecanone, and cyclononanone were used as received from the supplier after thin-layer chromatography which showed no contaminants.

Cyclooctanone was sublimed at 0.6 mm prior to testing.

2,6-Dibenzylidenecyclohexanone. This compound was prepared according to the published method for dibenzalacetone on a 0.5 *M* scale:[6] yield 91 g (67%); mp 118-121° (lit.[3] 118-119°). *Anal.* ($C_{20}H_{18}O$) C, H.

2,7-Dibenzylidenecycloheptanone. Condensation of cycloheptanone and benzaldehyde was carried out according to the method given below for cyclooctanone: yield 12.4 g (43%); yellow needles from MeOH; mp 106-110° (lit.[3] 107°). *Anal.* ($C_{21}H_{20}O$) C, H.

2,8-Dibenzylidenecyclooctanone. Na (5 g) was dissolved in 125 ml of absolute EtOH. When the solution had cooled to room temperature, 12.6 g (0.1 mol) of cyclooctanone (Aldrich) dissolved in 21.2 g (0.2 mol) of benzaldehyde was added in one batch. There was an immediate rise in temperaure of 10-15°, and formation of a light yellow color in the solution, and a solid soon began to form. After about 3 hr of stirring, the odor of benzaldehyde was still present in the reaction flask but was absent after 4 hr. At this time the reaction mixture was filtered. Filtration was slow (about 45 min) due to the gummy nature of the solid material. The solid was then stirred with approximately 300 ml of water for 30-45 min followed with

collection by filtration and recrystallization from 75 ml of 95% EtOH: yield 10.1 g (33%) of pale yellow platelets; mp 108–110° (lit.[3] 111°). *Anal.* ($C_{22}H_{22}O$) C, H.

If the reaction was allowed to proceed for a longer period of time, the product was found to be contaminated with the product of addition of EtOH across one of the double bonds. Reaction times of 24 hr or longer resulted in isolation of only the EtOH addition product, mp 116–118°. Use of 100 ml of EtOH resulted in lower yields. If 200 ml of EtOH was used the condensation was greatly slowed. After 8 hr with 200 ml of EtOH, the smell of benzaldehyde was still present and the isolated product was a mixture of 2,8-dibenzylidenecyclooctanone and the EtOH addition product. These two compounds were not separable by recrystallization.

2,12-Dibenzylidenecyclododecanone. Cyclododecanone (9 g) and 10.6 g of freshly distilled benzaldehyde were dissolved in 100 ml of toluene containing 0.2 g of boric acid and refluxed with a Dean–Stark trap for 40 hr. The reaction was filtered and washed with aqueous $NaHSO_3$, and toluene was removed to give an oil from which the product was isolated by chromatography on silica gel (benzene–chloroform, 95:5). The ylidene solidified when stirred with MeOH: mp 88–98°; yield 5 g (55%). *Anal.* ($C_{26}H_{34}O$) C, H.

General Hydrogenation Procedure. Bis(benzylidene)cycloalkanone (10 g) was dissolved in 50–200 ml of EtOAc (in some cases, only partial solution occurred). Palladium on charcoal (1 g, 5%) was added and the suspension shaken on a Parr hydrogenator until hydrogen uptake stopped. Catalyst was filtered, solvent removed, and the residual oil purified by chromatography on 60–200 mesh silica gel or recrystallization.

***cis*-2,6-Dibenzylcyclohexanone** was recrystallized from MeOH to give white plates, mp 119–122° (lit.[7] 122°). *Anal.* ($C_{20}H_{22}O$) C, H.

***trans*-2,6-Dibenzylcyclohexanone** was isolated by the method of Corey[8] from the mother liquors of the cis crystallization, mp 49–51° (lit. 49–51°). *Anal.* ($C_{20}H_{22}O$) C, H.

2,7-Dibenzylcycloheptanone was obtained as an oil after chromatography on silica gel (benzene) and was shown to be a mixture of isomers by vpc. *Anal.* ($C_{21}H_{24}O$) C, H.

***trans*-2,8-Dibenzylcyclooctanone.** Recrystallization of the crude product from MeOH gave a white solid, mp 82–83°, later shown to be trans. *Anal.* ($C_{22}H_{26}O$) C, H.

***cis*-2,8-Dibenzylcyclooctanone.** This was obtained by isomerization of the trans isomer. *trans*-2,8-Dibenzylcyclooctanone (5 g), mp 82–83°, was dissolved in 150 ml of 0.1 *M* NaOEt in EtOH and left standing at room temperature for 5 days. Acetyl chloride was added dropwise until the solution was neutral to pH paper. After the EtOH was reduced to half volume, the solution was filtered through a fine sintered glass funnel to remove precipitated NaCl. The solution was then cooled at 0° for several days. A solid which was mostly trans ketone was collected and recrystallized several times to give 1.7 g of pure trans compound. The mother liquors of these recrystallizations were combined and concentrated giving 1.0 g of cis isomer, mp 72–75°. Several recrystallizations from MeOH raised the mp to 84–85°. Tlc showed only the cis isomer present. The melting point was depressed over 15° when mixed with the trans isomer, mp 82–83°. *Anal.* ($C_{22}H_{26}O$) C, H.

2,12-Dibenzylcyclododecanone was purified by chromatography (60–200 mesh silica gel) (benzene eluent) to give a colorless oil. *Anal.* ($C_{26}H_{38}O$) C, H.

Table I. % of Control of Serum Cholesterol after Administration of 10 mg/kg of Test Compound[a]

	Days dosed		
	4th	10th	16th
Orally			
Control 1% CMC	100 ± 16	100 ± 10	100 ± 13
Clofibrate	111 ± 16	98 ± 21	106 ± 9
Cyclopentanone	92 ± 18	92 ± 5	83 ± 11**
Cyclohexanone	81 ± 15*	100 ± 9	81 ± 23*
Cycloheptanone	95 ± 13	78 ± 8***	81 ± 10***
Cyclooctanone	83 ± 24	114 ± 26	116 ± 18
trans-2,8-Dibenzylcyclooctanone	48 ± 13***	50 ± 12***	42 ± 18***
2,7-Dibenzylcycloheptanone	77 ± 9***	61 ± 13***	75 ± 9***
Intraperitoneal			
Control 1% CMC	100 ± 24	100 ± 17	100 ± 18
2,8-Dibenzylcyclooctanone			
Trans	59 ± 9***	62 ± 14***	64 ± 9***
Cis	80 ± 14	92 ± 7	64 ± 11***
2,6-Dibenzylcyclohexane			
Trans	88 ± 15	94 ± 7	81 ± 14*
Cis	65 ± 17***	106 ± 17	92 ± 16
Cholesterol-Induced Hypercholesterolemia[§]			
Orally		7th day	
1% cholesterol		100 ± 4	
2,8-Dibenzylcyclooctanone			
5 mg		69 ± 3***	
50 mg		52 ± 4***	

[a] ***, $P = 0.001$; **, $P = 0.010$; *, $P = 0.025$.

Biological Studies. Procedures. Animals and Diet. Male Sprague-Dawley rats (Zivic-Miller, Allison Park, Pa.) and CF_1 mice (Carworth Farms) were fed Purina rodent lab chow with water *ad libitium* for the duration of the experiment.

Administration of Drugs. Each test compound was suspended in 1% CMC (carboxymethylcellulose)-H_2O and homogenized. Doses (mg/kg) were calculated on weekly weights of the rats and daily weights of the mice. All drugs were administered to the animals by oral intubation needle (0.2 cc) daily at 11:00 a.m. In the structure-activity studies a 10 mg/kg dose was used for all compounds administered.

Serum Collection. After dosing (22–24 hr), blood was collected by tail vein bleeding. The blood was collected in nonheparinized microcapillary tubes. These tubes were centrifuged 3 min in an International microcapillary tube centrifuge in order to obtain the serum.

Serum Cholesterol. The method used for serum cholesterol levels was a modification of the Liebermann Burchard reaction.[9] Duplicate 20- or 25-μl samples of serum were used to determine the mg % serum cholesterol levels.

Cholesterol-Induced Hypercholesterolemia.[§] Rats were fed a diet containing 1% cholesterol together with 5 or 50 mg/kg of 2,8-dibenzylcyclooctanone daily for 7 days.

Serum Triglycerides and Glycerol. Calbiochem Stat Packs were used to measure serum triglycerides and glycerol after 5 days and 3 weeks in male rats administered 10 mg/kg/day of 2,8-dibenzylcyclooctanone.

Fructose-Induced Hypertriglyceridemia.[§] Rats were administered by oral intubation 3 mg/kg/day of 2,8-dibenzylcyclooctanone for 7 days. During the last 24 hr of treatment, the drinking water of one group was replaced with 10% fructose solution.

Animal Weight and Autopsy. Periodic animal weights were obtained and expressed as a percentage of that animal's weight on day 0 of the experiment. After dosing the animals for 16 days, half of the animals were sacrificed (ether) and a number of organs excised and weighed. These organs were expressed as a percentage of the body weight of that animal on the day of sacrifice. Tissues were examined for gross malformation, atrophy, pathological changes, infection, etc. The remaining animals were allowed to recover for 16 days, the serum cholesterol was determined, and an autopsy was performed as before.

Food Intake.[§] The average food intake in g/rat/day was determined over a 6-week period when 2,8-dibenzylcyclooctanone was administered to male rats at 9.3 or 31 mg/kg/day.

Estrogenic Activity. Weaned rats were ovariectomized by the procedure outlined by Emmens, *et al.* (1970).[10] Three days were allowed to pass before treatment was commenced with drugs. The animals were treated for 3 days with 17-ethinylestradiol or 2,8-dibenzylcyclooctanone and sacrificed. The uterus was removed, trimmed, and weighed.

Toxicity Study. In an attempt to determine the LD_{50} of 2,8-dibenzylcyclooctanone for CF_1 mice, 1,2, and 4 g/kg orally and ip and 6 g/kg orally were administered to eight females and eight males for each dose. The number of deaths was recorded. The Litchfield and Wilcoxon technique was used.[11]

Liver Lipid, Glycogen, Protein, and Nucleic Acid Levels. Male mice which were treated with 1% CMC, or 10 mg/kg of 2,8-dibenzylcyclooctanone or cyclooctanone in 1% CMC, orally, for a total of 9 weeks were sacrificed and liver, kidney, brain, adrenal, and testes were excised. A 10% homogenate in 0.25 *M* sucrose + 0.001 *M* EDTA was prepared from each tissue. Quantitative separation and determination of protein, RNA, DNA, lipid, and glycogen was carried out by the technique of Shibko, *et al.*[12]

§ We also wish to thank Dr. Claude E. Teague, Jr., and R. J. Reynolds, Inc., for support of some of the biological studies and for the studies done in hyperlipidemic rats performed in their laboratories.

Table II. % of Initial Body Weight after 16 Days of Treatment

	% increase	P
10 mg/kg orally		
Control 1% CMC	156 ± 2	
Clofibrate	166 ± 9	n.s.
Cyclopentanone	151 ± 2	0.025
Cyciohexanone	151 ± 3	0.025
Cycloheptanone	143 ± 8	0.010
Cyclooctanone	143 ± 9	0.010
2,8-Dibenzylcyclooctanone	116 ± 8	0.001
2,7-Dibenzylcycloheptanone	151 ± 2	0.025
2,5-Dibenzylidenecyclopentanone	143 ± 8	0.002
10 mg/kg ip		
Control 1% CMC	156 ± 4	
2,8-Dibenzylcyclooctanone		
Trans	112 ± 7	0.001
Cis	134 ± 5	0.001

Average Food Intake during Treatment, g/rat/day §	1 week	3 weeks	6 weeks
Control	17.3	17.3	17.3
2,8-Dibenzylcyclooctanone			
9.3 mg/kg/day	12.8	15.3	12.0
3.1 mg/kg/day	13.9	14.0	11.8

Glucose milligram percentage was determined in paired animals: a control receiving 1% CMC and a treated receiving 10 mg/kg of 2,8-dibenzylcyclooctanone in 1% CMC for 5 days. The glucose levels were measured on serum obtained by tail vein bleeding using the Calbiochem Stat Pack for glucose.

Adenylcyclase activity was measured by the technique of Krishna, *et al.*,[13] in brain and liver after treatment of rats as described above.

***In Vitro* Lipid Mobilization from Adipose Tissue.** § Rat epididymal fat pad minces were incubated in the presence of 2,8-dibenzylcyclooctanone ($5 \times 10^{-4} M$) with and without norepinephrine ($1 \times 10^{-5} M$) and the effect of the compound on basal- and norepinephrine-induced increase in free fatty acid (FFA) release was tested by the method of Cayen. §

Statistical Analysis. In Tables I–X the number of animals in a group, expressed as N, and the mean of the per cent of control and standard deviation, expressed as $\bar{x} \pm$ S.D., are noted. The probable significant level (P) between each test group and the control group was determined by the Student's t test according to Snedecor.[14] Data which were not significantly different from the control values are not shown.

[^{14}C]Cholesterol Distribution. Twelve male rats were divided into two groups. One group was used as a control and was administered 1% CMC orally; the other group was treated with 10 mg of 2,8-dibenzylcyclooctanone in 1% CMC daily for 5 days. On the fifth day 10 mg/kg of [^{14}C]cholesterol (C-4) was administered orally (4,228,000 dpm in 1 cc) to each rat who received no further food or water. Each rat was placed in a separate plastic cage. Excreted urine and feces were collected at 6, 12, and 24 hr and pooled for each animal over the 24-hr period. After 24 hr, the animals were sacrificed, the blood was collected, and the heart, liver, kidney, chyme (small intestine), testes, and spleen were excised.

A 10% homogenate in water was made from these tissues and the feces. A portion of the blood was centrifuged to obtain the plasma. An aliquot of 0.1 cc of each of the homogenates of urine, plasma, and blood was plated on 2.4-cm Whatman No. 1 filter paper. These were placed in scintillation fluid (2 parts of toluene, 1 part of triton X-100, 0.4% PPO, 0.01% POPOP). Each sample was plated in duplicate, counted on a scintillation counter using the channels ratio technique to correct for quenching. The DPM were corrected for total organ weight.

In other paired animals, bile cannulation was performed on each animal. After bile excretion was established and clear (free of blood), each animal of the pair was administered 0.5 cc of [^{14}C]-cholesterol (C-4) (2,114,000 dpm). This was noted as time zero. Bile was collected over 30-min intervals on each animal. The bile was plated on 2.4-cm Whatman No. 1 filter paper. These were placed in scintillation fluid and the results expressed as dpm for total bile output for that interval of time.

Incorporation of [1-^{14}C]Palmitic Acid of [1-^{14}C]Acetyl Coenzyme A in Lipid Fractions. Male Sprague–Dawley rats were paired: a control receiving 1% CMC and a treated received 10 mg/kg of 2,8-dibenzylcyclooctanone in 1% CMC daily by intubation needle for 5 days. Prior to sacrifice (20 hr), each pair was administered either 20 μCi of [1-^{14}C]palmitic acid or 2 μCi of [1-^{14}C]acetyl coenzyme A. The animals were sacrificed and the liver was excised and weighed. Tissue lipids were extracted by modification of the Bligh and Dyer[15] and Folch techniques.[16] A 20% homogenate in $CHCl_3$-MeOH (2:1) was prepared. The homogenate was centrifuged for 20 min at 2000 rpm. The supernatant was decanted, and the pellet was washed twice by resuspension in the same volume of $CHCl_3$-MeOH and centrifuged. All three supernatants were pooled and filtered through a Büchner funnel. The supernatant was then washed with 20% of its volume in 0.03% $MgCl_2$. The organic phase was separated and the aqueous phase was back-washed twice with equal volumes of $CHCl_3$-MeOH (2:1). The organic phases were pooled, allowed to stand at 0° overnight, and evaporated to dryness and weighed. The residues were diluted to a known volume with hexane, and duplicate samples were taken to determine radioactivity. Aliquots equivalent to 100 mg of extracted lipid were placed on columns containing 6 g of silicic acid (−325 mesh, lipid chromatography grade, Sigma) in 2% $CHCl_3$–98% hexane. The cholesterol and cholesterol esters fraction was eluted with 130 ml of 2% $CHCl_3$–98% hexane. Neutral lipids and fatty acids and alcohols were eluted with 150–160 ml of $CHCl_3$ and the phospholipids were eluted with 160–180 ml of MeOH (Freeman, *et al.*, 1957).[17]

The fractions from the column were evaporated to dryness, their weight was obtained, and they were diluted to a known volume in $CHCl_3$. Duplicate samples were taken to determine radioactivity and were plated on 5 × 20 cm activated silica gel G plates. These plates were eluted with hexane, anhydrous ether, and glacial acetic acid (80:20:1). The plates were sprayed with 50% sulfuric acid and heated until charred areas appeared. Each plate was divided into seven specific areas established by reference standards' R_f values, *i.e.*, into areas of the polar-lipid, phospholipid, cholesterol, fatty alcohol, fatty acids, triglycerides, and cholesterol esters (Snyder, 1969, 1971).[18,19] These areas were scraped into scintillation vials and 10 cc of scintillation fluid was added. These vials were counted in a Packard scintillation counter using the channel ratio technique to correct for quenching. The DPM's were corrected for total volume.

Results

Reduction in Serum Cholesterol and Body Weight. Cyclooctanone, cyclononanone, cyclododecanone, 2,8-dibenzylidenecyclooctanone, 2,7-dibenzylidenecycloheptanone, 2,6-dibenzylcyclohexanone (cis and trans), 2,6-dibenzylidenecyclohexanone, 2,5-dibenzylcyclopentanone,[5] 2,5-dibenzylidenecyclopentanone,[5] 2,12-dibenzylcyclododecanone, and 2,12-dibenzylidenecyclododecanone were inactive in the cholesterol study after 16 days at 10 mg/kg. *trans*-2,8-Dibenzylcyclooctanone caused a drastic reduction (50%) of the mg % serum cholesterol both after oral and ip administration and significantly reduced serum cholesterol levels which had been elevated by dietary cholesterol. 2,7-Dibenzylcycloheptanone caused a moderate reduction (25%) in mg % serum cholesterol (Table I) in male rats.

There was a reduction in the per cent body weight in-

Table III. Mg % Serum Cholesterol after Treatment with 2,8-Dibenzylcyclooctanone

Daily	N	4 doses, $\bar{x} \pm$ S.D.	P	6 doses, $\bar{x} \pm$ S.D.	P	10 doses, $\bar{x} \pm$ S.D.	P	12 doses, $\bar{x} \pm$ S.D.	P	16 doses, $\bar{x} \pm$ S.D.	P
Control (1% CMC)	9	104 ± 16		107 ± 26		106 ± 11		93 ± 19		97 ± 13	
1 mg/kg	10	65 ± 14	0.001	77 ± 14	0.006	91 ± 17	0.050	51 ± 19	0.001	61 ± 17	0.001
5 mg/kg	10	47 ± 7	0.001	44 ± 21	0.001	65 ± 18	0.001	29 ± 19	0.001	42 ± 11	0.001
10 mg/kg	10	48 ± 13	0.001	25 ± 15	0.001	50 ± 21	0.001	28 ± 16	0.001	42 ± 18	0.001
50 mg/kg	10	26 ± 17	0.001	13 ± 8	0.001	46 ± 12	0.001	16 ± 13	0.001	41 ± 15	0.001

Table IV. % Body Weight after Treatment with 2,8-Dibenzylcyclooctanone

Daily	N	6 doses, $\bar{x}$ ± S.D.	P	12 doses, $\bar{x}$ ± S.D.	P	16 doses, $\bar{x}$ ± S.D.	P
Control (1% CMC)	9	103 ± 3		111 ± 3		113 ± 4	
1 mg/kg	10	100 ± 2	0.040	102 ± 4	0.001	101 ± 2	0.001
5 mg/kg	10	96 ± 5	0.002	95 ± 4	0.001	95 ± 5	0.001
10 mg/kg	10	93 ± 6	0.001	90 ± 8	0.001	88 ± 7	0.001
50 mg/kg	10	91 ± 5	0.001	88 ± 4	0.001	88 ± 4	0.001

Table V. % Total Body Weight (g) 24 hr after 16 Doses of Dibenzylcyclooctanone

	Control, $\bar{x}$ ± S.D.	10 mg/kg, $\bar{x}$ ± S.D.	P	50 mg/kg, $\bar{x}$ ± S.D.	P
Liver	4.95 ± 1.35	4.55 ± 1.28	n.s.	4.31 ± 0.61	n.s
Spleen	0.25 ± 0.05	0.26 ± 0.05	n.s.	0.20 ± 0.04	n.s.
Kidney	0.82 ± 0.10	0.93 ± 0.16	n.s.	0.73 ± 0.09	n.s.
Heart	0.32 ± 0.03	0.40 ± 0.10	0.060	0.31 ± 0.00	n.s.
Vesicular glands	0.26 ± 0.06	0.06 ± 0.02	0.001	0.06 ± 0.00	0.002
Vas deferens and epididymis	0.34 ± 0.04	0.25 ± 0.09	0.030	0.023 ± 0.03	0.003
Testes	0.86 ± 0.13	0.91 ± 0.14	n.s.	0.060 ± 0.12	0.050

Table VI. Estrogenic Activity of 2,8-Dibenzylcyclooctanone

	No. of animals	% control, uterine wt	P
Control OV[a] (1% CMC)	13	100 ± 19	
10 mg/kg OV 2,8-dibenzylcyclooctanone	13	260 ± 22	0.001
10 μg/kg OV ethinylestradiol	13	259 ± 9	0.001

[a] OV = ovariectomized.

crease over 16 days with 2,8-dibenzylcyclooctanone and 2,5-dibenzylidenecyclopentanone and a slight reduction in the per cent body weight increase with cyclooctanone and 2,7-dibenzylcycloheptanone (Table II). 2,8-Dibenzylcyclooctanone lowered food intake by 35%.[§]

Tables III and IV demonstrate a dose-response effect of 2,8-dibenzylcyclooctanone on rat mg % serum cholesterol and increased body weight over the dose range of 1-50 mg/kg daily. The vesicular glands, vas deferens and epididymis, and testes expressed as per cent of total body weight at autopsy after 16 days of 2,8-dibenzylcyclooctanone administration were significantly reduced (Table V), while there was a 78% increase in adrenal weight.

In the animals who were allowed to recover from the 2,8-dibenzylcyclooctanone treatment 16 days after the last dose, the mg % serum cholesterol and organ weights had returned to normal. The body weight still lagged behind the control value by some 10-15% but was on a parallel rate of increase with the control animals.

The cis isomer of 2,8-dibenzylcyclooctanone was not as active as the trans isomer in reducing the mg % serum cholesterol. However, both significantly reduced the per cent increase in body weight.

Mouse Studies. Similar studies were performed on mice which resulted in a 22% decrease in mg % serum cholesterol after treatment for 9 weeks with 2,8-dibenzylcyclooctanone. There was a 12% reduction in body weight over the 9 weeks with a significant reduction in liver, vesicular glands, and vas deferens expressed as per cent of total body weight.

Toxicity. In mice, there were no observable toxic effects from 2,8-dibenzylcyclooctanone at 1, 2, 4, and 6 g/kg. There were no abnormalities in behavior patterns, with the exception that at doses of 4-6 g/kg there was drowsiness for the first 24 hr, but no ill effects were observed after 2 days. None of the animals died after administration of any of the drugs.

Estrogenic Activity. The 2,8-dibenzylcyclooctanone possesses estrogenic (Table VI) activity; however, it is on the order of $^1/_{1000}$ of that of ethinylestradiol when given orally for 3 days in ovariectomized Sprague-Dawley rats. 2,8-Dibenzylcyclooctanone (20 mg/kg/day) was required to produce 90% inhibition of gonadotrophin release in parabiotic rats.[§]

Liver Protein, Glycogen, Lipids, and Nucleic Acid Levels. Results are shown in Table VII. In male mice who were administered 10 mg/kg of 2,8-dibenzylcyclooctanone daily for 9 weeks, there was a significant reduction in lipid and glycogen level (% mg/g wet liver) and there was a rise in the protein level. The RNA and DNA content remained at the same level as the control liver values.

Serum Glucose Levels. In rats treated with 2,8-dibenzylcyclooctanone, there was a slight elevation (9%) in serum glucose levels over the control.

Adenylcyclase activity was not altered in the brain but elevated 245% in the liver (Table VIII).

Serum Triglyceride Levels. Serum triglyceride and glycerol were lowered by 2,8-dibenzylcyclooctanone along with fructose-induced hypertriglyceridemia (Table IX). 2,8-Dibenzylcyclooctanone has no effect on basal- or norepinephrine-induced lipolysis (Table X).

Cholesterol Distribution. The cholesterol which was removed from the serum after 2 8-dibenzylcyclooctanone treatment was not being deposited in the major organs for the rat, *i.e.*, liver, kidney, heart, spleen, or testes. The [^{14}C]-cholesterol level was reduced in the blood approximately 50% after treatment with 2,8-dibenzylcyclooctanone which supported the biochemical assay. The [^{14}C]cholesterol was significantly higher in the excreted feces of the treated animals after 24 hr (Table XI). There is a significant increase in the

Table VII. Protein, Glycogen, and Lipid Levels after Treatment with 2,8-Dibenzylcyclooctanone

Male mouse liver, % mg/g	N	Protein, $\bar{x}$ ± S.D.	P	Glycogen, $\bar{x}$ ± S.D.	P	Lipid, $\bar{x}$ ± S.D.	P
Control (1% CMC)	6	100 ± 2		100 ± 2		100 ± 1	
Cyclooctanone	6	102 ± 4	n.s.	104 ± 4	0.060	104 ± 2	0.004
2,8-Dibenzylcyclooctanone	6	110 ± 4	0.001	76 ± 1	0.001	77 ± 1	0.001

Table VIII. Adenylcyclase Activity, cpm/10 mg of Tissue

	Brain	Liver
Control	51 ± 21	11 ± 9
2,8-Dibenzylcyclooctanone	60 ± 19	27 ± 15**

Table IX

	N	Triglyceride	Glycerol
Serum Triglycerides and Glycerol			
Control	8	100%	100%
2,8-Dibenzylcyclooctanone	8	58%***	69%*
Frustose-Induced Hypertriglyceridema[§]			
Fructose	8	136%	
Fructose + 2,8-dibenzylcyclo-octanone	8	84%**	

Table X. FFA Release (μmol)/g of Tissue/hr[§]

	Without epinephrine	With epinephrine
Control	1.63 ± 0.14	9.42 ± 0.43
2,8-Dibenzylcyclooctanone	1.59 ± 0.11	7.61 ± 0.65

Table XI. Total dpm per Organ 24 hr after [^{14}C]Cholesterol Administration

	Total dpm		% dose administration	
	Control	Treated	Control	Treated
Spleen	18,899	14,301	0.45	0.34
Heart	8,656	7,774	0.21	0.18
Testes	980	426	0.02	0.01
Kidney	36,708	32,819	0.87	0.78
Liver	158,286	123,370	3.74	2.92
Blood[a]	88,910	53,720	2.12	1.26
Plasma[a]	25,900	12,779	0.61	0.30
Chyme	184,361	144,462	4.36	3.42
Feces[b]	580,688	725,571	13.62	17.18
Urine	41,648	36,257	0.98	0.86

[a]Corrected for total blood volume in rat, *i.e.*, 17.00 cc. [b]Feces and urine represent sample collection for entire 24 hr.

cholesterol excretion by the biliary system in the first 6 hr after treating with 2 8-dibenzylcyclooctanone (Table XII).

Incorporation of [1-^{14}C]Palmitic Acid or [1-^{14}C]Acetyl Coenzyme A. The rats treated with 10 mg/kg of 2,8-dibenzylcyclooctanone showed a 22% reduction in their incorporation of palmitic acid and a 16% reduction in acetyl CoA from the control value into tissue lipids. The milligrams of lipid extracted from the control and treated animals were identical. Table XIII denotes the per cent reduction of fatty acid incorporation in the neutral lipid and per cent increase into the phospholipid (-serine, -choline, -ethanolamine, -inositol) of animals treated with 2,8-dibenzylcyclooctanone. Tlc demonstrated that the decrease in incorporation of palmitic acid into neutral lipids was due to reduced amounts of radioactivity in the glycoethers, monoglycerides, diglycerides, fatty alcohol, and triglyceride fractions. The results with [1-^{14}C]acetyl CoA showed a slight reduction in incorporation of acetyl CoA into both neutral and phospholipids and an increase into cholesterol and cholesterol esters. During the lipid extraction process less than 0.1% of the total radioactivity of [^{14}C]palmitic acid was found in the aqueous $MgCl_2$ phase of control and treated animals. On the other hand, for the control animals treated with [1-^{14}C]-acetyl CoA, 7.5% of the total radioactivity appeared in the aqueous $MgCl_2$ phase and 2.2% of the total radioactivity was found in the aqueous $MgCl_2$ phase of animals treated with 2,8-dibenzylcyclooctanone.

Table XII. dpm per Total Bile Output after [^{14}C]Cholesterol Administration

		2,8-Dibenzylcyclooctanone	
Hr	1% CMC control	Dosed/day at 10 mg/kg for 1 day	Dosed/day at 10 mg/kg for 3 days
0.5	39	206	188
1	99	230	341
1.5	129	293	381
2	104	406	402
2.5	129	464	486
3	141	628	450
3.5	125	936	438
4	140	953	414
4.5	140	1162	462
5	152	1131	431
5.5	143	1317	364
6	153	1177	345

Table XIII. Lipid Extraction after Treatment with 2,8-Dibenzylcyclooctanone

		% wt (mg) of 100 mg of lipid extracted collected in the column fraction containing		
	N	Cholesterol	Neutral lipids	Phospho-lipids
1% CMC	10	3.1	31.4	65.5
2,8-Dibenzylcyclo-octanone	10	2.8	29.1	67.7
		% [1-^{14}C]palmitic acid (dpm) incorporated into each of the column fractions of 100 mg of lipid extracted from liver		
		Cholesterol	Neutral lipids	Phospho-lipids
1% CMC	6	6.1	34.0	59.8
2,8-Dibenzylcyclo-octanone	6	5.3	18.5	76.2

	% [1-^{14}C]palmitic acid (dpm) scraped from specific areas of tlc plates	
	1% CMC	2,8-Dibenzylcyclooctanone
Phospholipids (origin)	57.4	80.2
Polar lipid (glycoethers, monoglycerides, and diglycerides)	9.3	2.0
Cholesterol	3.0	2.1
Fatty alcohols	5.3	3.2
Fatty acids	7.9	8.4
Triglycerides	14.3	1.7
Cholesterol esters	1.0	0.5
Total recovery of tlc plate	98.2	98.2

		% of [1-^{14}C]acetyl coenzyme A incorporated into each of the column fractions of 100 mg of extracted lipid from liver		
		Cholesterol	Neutral lipids	Phospho-lipids
1% CMC	4	0.9	31.8	67.2
2,8-Dibenzylcyclo-octanone	4	3.8	30.6	65.6

Discussion

Thorp and Waring (1962)[20] reported that ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate (clofibrate) was active in reducing total lipid and cholesterol by 15–20% of the control value in blood and liver at a dose 100–200 mg/kg daily by oral or subcutaneous administration for 11 days. Best and Duncan (1963)[21] treated hypercholesterolic rats with 0.25% of their diet with clofibrate and observed a 28% reduction in serum cholesterol. In man there was only a 10% reduction in serum cholesterol after administration of 1.5 g

of clofibrate daily with no reduction of serum cholesterol in some patients.

Gordon and Cekleniak (1968)[22] tested a very potent hypocholesteremic agent, boxidine, which probably inhibits reabsorption of 7-dehydrocholesterol in the biliary system and lowered serum sterol to less than 30% of the control value. Gilfillan, *et al.* (1971),[23] reported that 2-acetamidoethyl (*p*-chlorophenyl)(*m*-trifluoromethylphenoxy)acetate administered to rats at 0.00625–0.05% of their daily diet for 9 days caused a significant reduction in plasma cholesterols.

In rats 2,8-dibenzylcyclooctanone is 10–20 times more potent than clofibrate in producing a reduction in serum cholesterol. Only small quantities of 2,8-dibenzylcyclooctanone were needed to bring about an effective reduction in serum cholesterol (5 mg/kg) which was almost immediate (24 hr) and lowered increased dietary cholesterol levels also. All effects brought about by the drug appear to be reversible. The drug was not toxic nor did it have any adverse side effects, but the compounds did possess slight estrogenic activity. Weak estrogens at very high doses are known to lower serum cholesterol in rodents which normally have a lower serum cholesterol content (75 mg %) than man. However, estrogens are not known to lower serum triglycerides; in fact, estrogens usually elevate triglyceride levels.[24] Thus, it is conceivable that the hypolipidemic activity of 2,8-dibenzylcyclooctanone is unrelated to its estrogenic properties. Currently further SAR studies are under way to separate the two characteristics. Reduced food intake could also account for the lowered serum cholesterol and triglycerides levels over a long period of time, but it would not account for the immediate reduction in serum cholesterol levels. In addition, if 2,8-dibenzylcyclooctanone inhibited appetite and lowered food intake, then fatty acids mobilization and elevated serum glycerol levels would be expected, events which did not occur. Thus, it would appear that 2,8-dibenzylcyclooctanone has its site of action at some stage in lipid metabolism. 2,8-Dibenzylcyclooctanone and 2,8-dibenzylidenecyclopentanone caused decided reduction in body weight. Possibly these agents could be used in the treatment of obesity, particularly the latter one, since it is not associated with hypocholesterolemic activity.

Not only did 2,8-dibenzylcyclooctanone lower serum cholesterol and triglycerides, but there was a reduction in the total lipids and glycogen of the liver with a concomitant rise in serum glucose. This raised the question whether or not one site of action of 2,8-dibenzylcyclooctanone is a key regulatory enzyme, *e.g.*, adenylcyclase, which also demonstrated a slight increase in activity in the liver. Elevation of the activity of this enzyme would account for elevated serum glucose and lower serum triglyceride levels.

Treatment with 2,8-dibenzylcyclooctanone did not result in deposits of cholesterol in any of the body tissue analyzed at 10 mg/kg but did at high doses of the drug (31 mg/kg). From the results, *i.e.*, the increased [^{14}C]cholesterol in the bile and the feces, one can postulate that the drug caused an increase in the transport of cholesterol and its metabolites into the biliary system and through the small intestinal tract for the purpose of excretion. Administration of [1-^{14}C]acetyl CoA to treated and control animals demonstrated that acetyl CoA incorporated into the cholesterol–cholesterol ester fraction of the lipids was not altered by the drug. This indicated that synthesis of cholesterol after treatment with 2,8-dibenzylcyclooctanone was not impaired at the initial stages, but preliminary data indicate that the site of action is at the premevalonate stage of cholesterol synthesis.[§]

Incorporation of [1-^{14}C]palmitic acid and [1-^{14}C]acetyl CoA into the liver tissue was reduced in the treated animals, as was the uptake of the fatty acid into triglycerides, diglycerides, and monoglycerides, while incorporation into phospholipids was enhanced. Preliminary data indicate that the activity of the enzyme acylglycerol-3-phosphate acyltransferase may be reduced by treatment with 2,8-dibenzylcyclooctanone.[#]

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