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Cycloalkanones. 4. Antifertility Activity

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A number of mono- and disubstituted cycloalkanones were tested in female rodents for their antifertility activity. 2,8-Dibenzylcyclooctanone, 2,4-dibenzyl-3-pentanone, 2-(2-fluorobenzyl)-8-(α-ethoxy-2-fluorobenzyl)cyclooctanone, and 2,8-bis(2-methylbenzyl)cyclooctanone were found to inhibit pregnancy completely at 50 mg/kg. The MED_{100} for 2,8-dibenzylcyclooctanone in CF_1 mice was 28 mg/kg while the MED_{100} for Sprague–Dawley rats was <1 mg/kg. These compounds had no effect on male fertility.

For continual use of an oral contraceptive it is important that the agent be nontoxic, essentially nonestrogenic, nonteratogenic, and 100% effective in preventing pregnancy. Currently estrogens and progestrogens are being used which, because of their adverse effects, are not desirable for extended periods of time. Presented below is a series of substituted cycloalkanones which possess substantial antifertility activity in rodents but possess a different mode of action than estrogens.

Procedures and Methods

Chemical. Melting points were taken on a Mel-Temp apparatus and are corrected. Satisfactory elemental analyses were obtained (Atlantic Microlab) for all compounds and are indicated by element. The synthetic procedures for many of the substituted cycloalkanones listed in Table I have been reported previously.[1-3] However, further related compounds have now been synthesized which are described below and their biological activities are reported in Table I.

2,3:6,7-Dibenzosuberone (8) was used as received from Aldrich Chemical Co. after tlc showed no contamination.

7-Benzyl-2,3-benzosuberone (9) was prepared by sodium ethoxide catalyzed condensation of 8 g (0.05 mol) of 2,3-benzosuberone with 5.3 g (0.05 mol) of benzaldehyde using the method described previously.[3] The yield after hydrogenation and chromatography (silica–benzene) was 8 g (64%). The ylidene has a melting point of 81–82° and the reduced material is an oil. *Anal.* ($C_{18}H_{18}O$) C, H.

1-Methyl-2,8-dibenzylcyclooctanol (24). A 2.31 *M* solution (7 ml) of methyllithium in ether was added to 2.06 g (0.0061 mol) of 2,8-dibenzylcyclooctanone in 50 ml of ether under a nitrogen atmosphere. The mixture was stirred at room temperature for 24 hr. Acetone was added slowly, followed by water, until no reaction was visible upon further addition. The mixture was extracted with three 20-ml portions of water and dried, and the ether was removed. The remaining oil was chromatographed on 60 g of 70–325 mesh silica gel [benzene–petroleum ether (bp 30–60°), 50:50] to give 1.9 g (97%) of solid, mp 53–55°. *Anal.* ($C_{23}H_{30}O$) C, H.

1-Methyl-2,8-dibenzylcyclooctene (25). 24 (6 g, 0.019 mol) was dissolved in 50 ml of glacial acetic acid and 1 ml of 45% BF_3·Et_2O was added. The solution was allowed to stand 30 hr at room temperature during which time an oil separated. The mixture was extracted four times with ligroine and the combined extracts were washed with aqueous sodium bicarbonate and then with water until neutral to pH paper. The organic layer was dried, solvent removed, and the oil chromatographed (silica gel, ligroine) to give 5.6 g (98%) of colorless oil. *Anal.* ($C_{23}H_{18}$) C, H.

2,8-Bis(*N*-morpholinomethyl)cyclooctanone Dihydrochloride (31). A mixture of 12.6 g (0.1 mol) of cyclooctanone, 6 g (0.2 mol) of paraformaldehyde, and 24.7 g (0.2 mol) of morpholine hydrochloride in 40 ml of glacial acetic acid was maintained at 95° while stirring for 2.5 hr. The solvent was removed under reduced pressure and 70 ml of acetone added to the residue which slowly dissolved. After 4 hr at room temperature the precipitate was filtered and recrystallized from ethanol to give 12.3 g (31%) of colorless crystals, mp 171–173° dec. *Anal.* ($C_{18}H_{34}Cl_2N_2O_3$) C, H.

2-Benzyl-8-(α-methoxybenzyl)cyclooctanone (32) was synthesized by hydrogenation of the product of the condensation of cyclooctanone with benzaldehyde in MeOH instead of the usual EtOH:[3] mp 107–109° (30% overall yield). *Anal.* ($C_{23}H_{29}O_2$) C, H.

2-(4-Methylbenzyl)-8-(α-methoxy-4-methylbenzyl)cyclooctanone (33) was prepared *via* our previously reported method[3] (ylidene: mp 149–151°; 27%): mp 95–97° (87%). *Anal.* ($C_{25}H_{32}O_2$) C, H.

2,8-Bis(4-phenylbenzyl)cyclooctanone (38) was prepared by our general method.[3] The ylidene had a melting point of 166–168° (20% yield) and the reduced compound had mp 121–123° (62%). *Anal.* ($C_{34}H_{30}O$) C, H.

2-(2-Fluorobenzyl)-8-(α-ethoxy-2-fluorobenzyl)cyclooctanone (43) was made by the general method to give an ylidene (mp 126–128°, 8%) and reduced compound, mp 63–65° (15% yield). *Anal.* ($C_{24}H_{26}F_2O_2$) C, H.

1-Benzoyl-7-(α-hydroxybenzyl)-8-oxabicyclo[5.1.1]nonan-9-one (53). Diepoxydibenzylcyclooctanone[3] (10 g, 0.03 mol) was dissolved in 100 ml of dry DMSO. BF_3·Et_2O (1 ml) was added and the solution stirred at 100° for 12 hr. The reaction was poured into 2 l. of H_2O and extracted with ether in a continuous extractor until the aqueous layer was clear. The ether layer was washed with water, dried, and flash-evaporated. The remaining oil was recrystallized from methanol. The yield was 3.4 g (32%), mp 181–183°. *Anal.* ($C_{22}H_{22}O_4$) C, H.

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Table I. Antifertility Activity in Female CF_1 Mice[a]

Compd no.	Compd	N	% pregnant	% viable fetus per litter	% reabsorption per litter	Source or synthetic ref
	Control (1% CMC)	62	100	100	100	
1	Diethylstilbestrol	8	0	0	0	
2	Cyclopentanone	8	88	76	345	2
3	Cyclohexanone	7	100	83	46	2
4	Cycloheptanone	7	100	88	0	2
5	Cyclooctanone	8	88	109	170	2
6	Cyclononanone	8	88	100	23	2
7	Cyclodecanone	6	100	93	76	2
8	2,3:6,7-Dibenzosuberone	6	50	65	0	
9	7-Benzyl-2,3-benzosuberone	8	88	99	228	
10	2,5-Dibenzylcyclopentanone	8	100	78	195	1
11	2,6-Dibenzylcyclohexanone	8	63	78	165	1
12	2,7-Dibenzylcycloheptanone	8	88	76	115	1
13	2,8-Dibenzylcyclooctanone (trans)	8	0	0	0	2
14	2,12-Dibenzylcyclododecanone	8	88	82	112	2
15	2,5-Dibenzylidenecyclopentanone	8	100	98	200	1
16	2,6-Dibenzylidenecyclohexanone	8	100	88	165	2
17	2,7-Dibenzylidenecycloheptanone	8	88	101	58	2
18	2,8-Dibenzylidenecyclooctanone	8	75	72	172	2
19	2,12-Dibenzylidenecyclododecanone	8	88	91	300	2
20	2,5-Dibenzylidene-*trans*-3,4-dimethylcyclopentanone	8	100	92	75	3
21	2,5-Dibenzylcyclopentanol	7	57	92	0	3
22	2,6-Dibenzylcyclohexanonol	8	63	87	120	1
23	2,8-Dibenzylcyclooctanol	8	100	75	105	3
24	1-Methyl-2,8-dibenzylcyclooctanol	8	100	68	144	
25	1-Methyl-2,8-dibenzylcyclooctene	8	88	99	91	
26	Diepoxydibenzylcyclooctanone	16	93	85	263	3
27	1,3-Dibenzylcyclooctane	8	100	60	72	3
28	2,8-Dicyclohexylmethylenecyclooctanone	7	100	94		3
29	2-Benzylcyclooctanone	8	175	76	57	3
30	2-Benzoylcyclooctanone	8	50	101	278	3
31	2,8-Bis(*N*-morpholinomethyl)cyclooctanone dihydrochloride	9	33	43	0	
32	2-Benzyl-8-(α-methoxybenzyl)cyclooctanone	8	75	84	0	
33	2-(4-Methylbenzyl)-8-(α-methoxy-4-methylbenzyl)cyclooctanone	8	87	54	340	
34	2-(α,4-Dimethoxybenzyl)-8-(4-methoxybenzyl)cyclooctanone	8	100	93	62	3
35	2-(α-Ethoxybenzyl)-8-benzylcyclooctanone	8	100	81	0	3
36	2,8-Bis(α-naphthylidene)cyclooctanone	8	75	79	35	3
37	2,8-Bis(α-naphthylmethyl)cyclooctanone	8	88	82	90	3
38	2,8-Bis(4-phenylbenzyl)cyclooctanone	8	88	81	133	
39	2,8-Bis(diphenylmethyl)cyclooctanone	8	88	64	145	3
40	2,8-Bis(2-chlorobenzylidene)cyclooctanone	8	88	102	100	3
41	2,8-Bis(4-chlorobenzylidene)cyclooctanone	8	75	84	250	3
42	2,8-Bis(2-chlorobenzyl)cyclooctanone	7	43	31	300	3
43	2-(2-Fluorobenzyl)-8-(α-ethoxy-2-fluorobenzyl)cyclooctanone	8	0	0	0	
44	2,8-Bis(2-methylbenzyl)cyclooctanone	8	0	0	0	3
45	2,8-Bis(2-methoxybenzyl)cyclooctanone	8	25	48	300	3
46	2,8-Bis(4-methylbenzyl)cyclooctanone	8	100	80	148	3

Table I *(Continued)*

Compd no.	Compd	N	% pregnant	% viable fetus per litter	% reabsorption per litter	Source or synthetic ref
47	2,8-Bis(4-methoxylbenzyl)cyclooctanone	8	87	90	90	3
48	2,8-Bis(2,4,6-trimethylbenzyl)-cyclooctanone	8	88	28	68	3
49	2,8-Bis[(α-phenylthio)benzyl]-cyclooctanone	8	88	64	312	3
50	2,8-Bis[(α-benzylthio)benzyl]cyclooctanone	8	100	88	125	3
51	3,7-Bis(phenylthio)cyclooctanone	8	88	73	200	3
52	2-Carbethoxy-2-(β-phenylethyl)-cyclooctanone	8	75	54	450	3
53	1-Benzoyl-7-(α-hydroxybenzyl)-8-oxabicyclo[5.-1.1]nonan-9-one	8	63	71	66	
54	*cis*-1,5-Bis(*p*-chlorobenzyloxy)-cyclooctanone	8	100	87	26	
55	2,4-Dibenzyl-3-pentanone	8	0	0	0	3

[a] A change of 25% was considered to be significant.

cis-**1,5-Bis(4-chlorobenzyloxy)cyclooctane** (54). Potassium shot (5.4 g, 0.14 mol) was prepared in 50 ml of refluxing dry dioxane. *cis*-1,5-Cyclooctanediol (12.0 g, 0.083 mol) in 100 ml of dioxane was added dropwise and the reaction stirred under reflux until all the potassium had reacted. *p*-Chlorobenzyl chloride (24 g, 0.149 mol) was added dropwise and the reaction refluxed for 24 hr. After cooling, the mixture was filtered and the filter cake washed with 150 ml of $CHCl_3$. Solvent was removed and the residue chromatographed on 300 g of 70–230 mesh silica. The column was eluted with 70 ml of benzene, followed by 700 ml of benzene–ether (9:1) and a further 700 ml of benzene–ether (8:2). Impure product (5 g) from the second fraction was recrystallized from MeOH: yield 2.2 g (8.7%); mp 65–67°. *Anal.* ($C_{22}H_{26}Cl_2O_2$) C, H, Cl.

Biological. Preparation of Drugs. Each of the 55 test drugs was suspended in 1% carboxymethylcellulose (CMC) and homogenized. All drugs were administered in 0.2 cc ip or orally. Dosages were corrected for weight gains during the experiment.

Female Mouse Fertility Screen. CF_1 mice were divided into groups of eight animals each. They were weighed and administered 50 mg/kg of test drug daily ip for a total of 28 days. On the tenth day of dosing, the females were exposed to males (two females/male) for the remainder of the experiment. The males were rotated every fifth day to avoid male infertility. After 28 days of dosing, the females were sacrificed. The number of pregnancies, viable fetuses, dead in uterine, and reabsorptions were recorded[4] (Table II).

Female mice were also dosed daily ip at 10, 20, 30, and 50 mg/kg with **13** and **55** to obtain a MED_{100} for the antifertility activity.

Female Rat Fertility Screen. Sprague–Dawley rats were divided into groups of eight animals and 0.1–50 mg/kg/day of **13** was administered daily by intubation needle (orally) for 30 days. From this stage, the procedure was identical with the mouse studies.

Table II. Antifertility Activity in Sprague–Dawley Female Rats

Drug	No. examined	% pregnant	% of fetuses per litter of control	% of reabsorptions per litter of control
Control	16	100	100	100
Compound **13**				
50 mg/kg	7	0	0	0
30 mg/kg	7	0	0	0
20 mg/kg	6	0	0	0
10 mg/kg	8	0	0	0
5 mg/kg	7	0	0	0
1 mg/kg	6	0	0	0
0.75 mg/kg	8	12	0	450
0.50 mg/kg	8	50	83	45
0.25 mg/kg	8	50	83	43
0.10 mg/kg	8	50	114	9
Compound **55**				
10 mg/kg	8	0	0	0
1 mg/kg	8	50	28	90
0.5 mg/kg	8	75	90	49
Norethynodrel (control)				
0.600 mg/kg	24	0	0	0
0.300 mg/kg	17	35	39	185
0.150 mg/kg	17	88	89	120
0.075 mg/kg	5	100	105	0

Table III. cAMP Levels ($\times 10^{-8}$ *M*) after 5 Days of Treatment at 10 mg/kg/day in Female Sprague–Dawley Rats

	Uterus	Liver
Control (1% CMC)	184 ± 37	62 ± 28
2,8-Dibenzylcyclooctanone	827 ± 34[a]	408 ± 202[a]

[a] $p = 0.001$.

Table IV. Effects of 2,8-Dibenzylcyclooctanone on the Release of Gonadotrophins

	FSH, %	LH, %
Control (1% CMC)	100	100
2,8-Dibenzylcyclooctanone, 10 mg/kg/day	101	102
Ethinylestradiol, 10 mg/kg/day	25[a]	101

[a] $p = 0.001$.

Male Mouse Fertility Screen. Compounds **5**, **11**, and **13** were tested for antifertility activity in male CF_1 mice at 10 mg/kg/day according to the method of Coppola.[5]

Serum Androst-4-ene-3,17-dione–Testosterone Levels. Male steroid hormone levels were determined by the Sachs method[6] using 20–25 μl of serum obtained by tail vein bleeding from mice and rats which had been treated with **13** ip (10 mg/kg/day) between 5 days and 7 weeks.

Cyclic AMP Levels. Female Sprague–Dawley rats (180 g) were divided into a control group receiving 1% CMC and a treated group receiving 10 mg/kg/day for 5 days of **13** orally. The uterus and liver were excised and analyzed for cAMP levels using the Schwarz-Mann cyclic AMP radioimmunoassay kit (Table III).

FSH and LH Release. Animals were treated as above. In addition, a third group was treated with 10 μg/kg/day of ethinyl estradiol for 5 days. Blood was collected through the carotid artery and centrifuged to obtain the serum. The follicle-stimulating hormone (FSH) and lutenizing hormone (LH) were determined using

Table V. Effects of 2,8-Dibenzylcyclooctanone on Liver and Uterus DNA, RNA, Lipid, Protein, and Glycogen Levels Expressed as Percentages

	N	Lipid	Protein	Glycogen	RNA	DNA
Liver						
Control	8	100 ± 39	100 ± 6	100 ± 8	100 ± 15	100 ± 21
2,8-Dibenzyl-cyclooctanone	8	85 ± 20	76 ± 5[a]	67 ± 5[a]	263 ± 33[a]	296 ± 49[a]
Uterus						
Control	8	100 ± 13	100 ± 5	100 ± 9	100 ± 14	100 ± 9
2,8-Dibenzyl-cyclooctanone	8	105 ± 4	110 ± 14	64 ± 10[a]	165 ± 13[a]	242 ± 23[a]

[a] $p = 0.001$.

Table VI. Effects of 2,8-Dibenzylcyclooctanone on [^{14}C]-Tyrosine Incorporation into Protein of Liver

	N	dpm [^{14}C] tyrosine incorporated in protein
Control (1% CMC)	8	511
2,8-Dibenzylcyclooctanone	8	518

the Calbiochem radioimmunoassay with New England Nuclear ^{131}I. The γ-radiation was counted on a Baird atomic spectrometer Model 530 (Table IV).

Uterus and Liver DNA, RNA, Protein, Lipid, and Glycogen Levels. For 3 weeks female rats were treated with 10 mg/kg/day of 13 or 1% CMC. The liver and uterus were excised and analyzed for DNA, RNA, protein, glycogen, and lipid levels by the method of Shibko, *et al.*[7] (Table V).

Protein Synthesis. *In vitro* protein synthesis was determined[8] on the liver of females after 5 days of treatment with 13 or 1% CMC (Table VI).

RNA Polymerase and DNase Activities. After 5 days of treatment with 13 or 1% CMC, the liver was excised. The DNase activity of the liver was measured by the technique of deDuve, *et al.*[9] The hormone-sensitive RNA polymerase, the nucleolar RNA polymerase, and nuclear RNA polymerase of the liver and uterus were measured[10] after 5 days of treatment. Statistical analysis was determined by Student's t test (Table VII).

Table VII. Effects of 2,8-Dibenzylcyclooctanone on DNase and RNA Polymerase Activity of the Liver and Uterus

	N	1% CMC	2,8-Dibenzylcyclooctanone, 10 mg/kg/day, for 5 days	p
Liver DNase	8	100 ± 15	136 ± 16	0.005
Liver RNA polymerase				
Hormone-sensitive III	8	100 ± 16	196 ± 32	0.001
Nucleolar (rRNA) I	8	100 ± 10	52 ± 3	0.001
Nuclear (mRNA) II	8	100 ± 24	65 ± 3	0.001
Uterus RNA polymerase				
Hormone-sensitive III	6	100 ± 31	230 ± 93	0.001
Nucleolar (rRNA) I	6	100 ± 51	29 ± 11	0.001
Nuclear (mRNA) II	6	100 ± 80	107 ± 95	

Results

In our animal quarters, the average gestation time for rodents was 19.4 days with some seasonal variation. The average number of fetuses and reabsorption sites including uterine deaths for the CF_1 mice is 12 ± 3 and 0.48 ± 0.12 per litter, respectively, and for the Sprague-Dawley rats were 10.7 and 0.42 per litter, respectively. The effects of the drugs were expressed as a percentage of the control animals which were administered 1% CMC daily. Thus, the above values are equivalent to 100% for the controls of each species.

The antifertility activity of the substituted cycloalkanones was noted in Table I. At a dose of 50 mg/kg/day in female CF_1 mice compounds **13, 43, 44,** and **55** completely inhibited pregnancy. Compounds **8, 30, 31, 42,** and **45** decrease the per cent pregnant to 50% or less. Compounds **8, 31,** and **43** lowered the number of pregnancies; however, there was respectively a 25, 33, and 50% death rate in the treated females. The remaining drugs demonstrated no toxicity at this dose. Compounds **13, 31, 33, 42, 43, 44, 45, 48, 52,** and **55** reduced the number of fetuses per litter to 54% or less. Of these compounds, **33, 42, 45,** and **52** resulted in a large number of reabsorptions (three- to fourfold increase) and uterine deaths which are indicative of the drug interfering with the ability of the mother to carry the litter to term. The MED_{100} in rats for **13** was <1 mg/kg/day to inhibit pregnancy. In CF_1 mice the MED_{100} for **13** was 28 mg/kg/day. The MED_{100} for compound **55** was 10 mg/kg/day.

The cycloalkanones tested for antifertility activity in male mice demonstrated no activity, *i.e.*, treated males copulated successfully with fertile females and had viable offspring despite the fact that **13** caused atrophy of the vas deferens, epididymis, and vesicular glands.[2] The serum androst-4-ene-3,17-dione–testosterone levels remained within normal limits between 5 days and 7 weeks of testing with **13** in both male rats and mice.

There was a 66-fold increase over the control of cAMP levels in the liver and a 4.5-fold increase of cAMP in the uterus with compound **13**. This compound caused an elevation of the RNA and DNA levels and a depression of the glycogen levels of the liver and the uterus. In addition, the hormone-sensitive RNA polymerase activity was elevated while rRNA (I) and mRNA (II) polymerases activities were depressed. Protein synthesis was unaffected by compound **13** while DNase activity was elevated. Compound **13** had no effect on the supression of FSH and LH release from the pituitary.

Discussion

Other nonsteroidal compounds have previously been reported.[11-22] Compounds **13** and **55** inhibit pregnancy 100%; however, these compounds were reported to be estrogenic, *i.e.*, positive uterotropic effect, atrophy of vesicular, vas-deferens, and epidiymis, and inhibition of gonadotropin release in parabiotic rats at high doses.[2] Since then the effects of compound **13** on FSH and LH release have been determined at 10 mg/kg/day for 5 days and this drug (**13**) caused no repression of either gonadotropin.

The estrogen component of oral contraceptives (17β-estradiol, stilbestrol, ethinylestradiol, mestranol) causes an elevation of serum triglycerides,[23-29] cholesterol,[24,30] and glucose,[31] synthesis of triglycerides in the liver, phospholipid, glycogen, protein, RNA, DNA, and cAMP levels in the uterus,[27,28,32-36] DNA-dependent RNA polymerase II,[37] nucleolar RNA polymerase I,[38] and hormone-sensitive RNA polymerase III[39] activities of the uterus and protein synthesis.[37-39] Whereas, these agents caused no change in serum fatty acids, glycerol or D-β-hydroxybutyrate,[23,24,26,27] or hormone-sensitive lipase.[27,40] The latter would result in a lack of removal of triglycerides from the serum.[24,41,42]

In comparing the effect of compound 13 on metabolism with estrogen, 13 lowers serum triglycerides, fatty acids, glycerol, and cholesterol.[2] Furthermore, compound 13 interferes with triglyceride synthesis of the liver and depresses liver and adipose lipase activity.[43] Compound 13 causes no significant change in protein levels of the uterus or liver or protein synthesis of the liver; however, it did cause elevations of RNA, DNA of the liver, and uterus.[38]

An increase in the activities of RNA polymerases I and II is responsible for the late hypertrophy of the uterus and protein synthesis.[37-39] Hormone-sensitive RNA polymerase III is elevated by cAMP and estrogens[36,39,44] which results in an early increase in RNA levels[44] prior to weight increase or protein synthesis in the uterus.[45] Increased levels of cAMP directly stimulate hormone-sensitive RNA polymerase[46,47] and cause an increase in uterine weight.[48] Treatment with compound 13 resulted in increased levels of cAMP which would cause the observed stimulation of hormone-sensitive RNA polymerase III. However, RNA polymerase I and II activities were depressed by 13 which explains the fact that protein levels and protein synthesis were not altered. Both RNA and DNA content was elevated in the liver and uterus. DNase activity is increased during DNA replication. The activity of this enzyme is elevated by 13. Thus perhaps the elevated nucleic acid levels represent increased numbers of cells. From these studies it can be concluded that compound 13 is not acting in the same manner as estrogen on cellular metabolism. The previous positive uterotropic test with 13 could be due to elevated levels of cAMP and thus increase uterine weight with electrolyte and water shifts[48] as opposed to a true stimulation of the estrogen receptor, *i.e.*, the cytoplasmic 4S receptor. cAMP itself has antifertility activity[49] and could account for the antifertility activity of compound 13.

Compounds 42 and 44 possess antifertility activity and a high retention of fluid in the peritoneal cavity but do not possess as high a hypocholesterolemic activity as 13 and 55. This bis(β-phenethyl) ketone system was necessary for antifertility as well as hypocholesteremic activity.[3] These two activities subsequently have been separated and will be the subject of a future communication.

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