# Cycloalkanones. 9. Comparison of Analogues Which Inhibit Cholesterol and Fatty Acid Synthesis

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A number of 2,8-dibenzylcyclooctanone analogues inhibited the HMG-CoA reductase activity of Holtzman male rat liver, whereas only 2-octanone, 2-hexadecanone, 2,8-dibenzylcyclooctanone derivatives, and 2-bis(4-chlorophenyl)-3,5-dimethyltetrahydro-4-pyrone inhibited fatty acid synthetase activity. 2-Octanone significantly lowered serum cholesterol, triglycerides, and glycerol levels in Holtzman male rats and serum cholesterol in male $CF_1$ mice. Serum lipase activity was significantly elevated in rats administered 20 mg/kg/day of 2-octanone for 16 days. The activity of liver HMG-CoA reductase was inhibited in mice administered 10 mg/kg/day of 2-octanone for 10 days and in mouse and rat liver in vitro by 10 mg of 2-octanone. In mice, fecal excretion of [$^{14}$C]cholesterol and tripalmitin was accelerated whereas palmitic acid and cholesteryl oleate were not affected by 10 mg/kg/day of 2-octanone. The $LD_{50}$ in male mice for 2-octanone was 1.6 g/kg.

An extensive structure relationship to hypocholesterolemic and antifertility activities of analogues of 2,8-dibenzylcyclooctanone has previously been reported.[1-6] A simple aliphatic analogue related to 2,8-dibenzylcyclooctanone (2-octanone) is an effective hypocholesterolemic agent. This compound lowered serum cholesterol in male Sprague–Dawley rats by 66% after 16 days of oral dosing at 10 mg/kg/day.[1] 2-Octanone is not estrogenic (rats) nor does it have antifertility activity (mice)[1] which are side effects of substituted analogues of 2,8-dibenzylcyclooctanone.[5,6] Further, SAR study with 2-hexadecanone, the only other significantly active member of the aliphatic series, has shown that the ketone group is required in the 2 position of the carbon chain for maximum activity.[2] At this time we wish to report the ability of selected hypolipidemic agents from the cycloalkanone series to inhibit regulatory enzymes of cholesterol and fatty acid synthesis and an in-depth study of 2-octanone since this compound appears to be the most promising of the series.

## Experimental Section

**Chemical.** Melting points were taken on a Mel-Temp apparatus and are corrected. The synthetic procedures for the substituted cycloalkanones listed in Table I as well as satisfactory combustion analysis data have been reported previously.[1-6] 2-Octanone and 8-pentadecanone were purchased from Calbiochem and used as supplied after TLC and ir data were taken.

**2-Bis(4-chlorophenyl)-3,5-dimethyltetrahydro-4-pyrone (9).** 3-Pentanone (43 g, 0.5 M) and *p*-chlorobenzaldehyde (140 g, 1 M) were dissolved in 600 ml of absolute ethanol and sufficient water was added to produce a slight cloudiness. Potassium hydroxide pellets (10 g, 85% KOH) were dissolved in 10 ml of water and added at one time, after which the reaction was stirred at 60° for 2 h. During the next 2 h, 100 g of potassium hydroxide was added. The reaction was stirred overnight at room temperature, and the crystalline product collected by filtration. After recrystallization from ethanol, 75 g (21%) of colorless needles melting at 162–164° was obtained. Anal. C, H.

**2-Bis(4-methylphenyl)-3,5-dimethyltetrahydro-4-pyrone (8).** This compound was prepared analogously to **9** from 3-pentanone and *p*-tolualdehyde. Stirring for 4 days was required after the final addition of base. The crystalline material collected was recrystallized from ethanol to give 40 g (13%) of colorless needles, mp 184–185°. Anal. C, H.

**Animals and Diet.** Male Holtzman rats (~140 g) and $CF_1$ mice (~30 g) were fed Purina rodent lab chow with water ad libitum for the duration of the experiment. Drugs were suspended in 1% CMC (carboxymethylcellulose)–$H_2O$ and homogenized. Doses were calculated on weekly weights of the animals.

**Serum Hypolipidemic Activity.** All drugs (10 mg/kg/day) were administered to male rats by oral intubation needle (0.2 cc) daily at 11:00 a.m. A dose–response curve for hypocholesterolemic activity of 2-octanone was determined in male rats at 1, 5, 20, 50, and 100 mg/kg/day for 16 days. During this period the average food intake in g/rat/week was determined. After dosing (22–24 h) blood was collected by tail vein bleeding. The blood samples were collected between 9:30 and 10:30 a.m. in alkali free nonheparinized microcapillary tubes which were centrifuged for 3 min to obtain the serum.[5] Duplicate 30-μl samples of nonhemolyzed serum were used to determine the mg % serum cholesterol levels by a modification of the Liebermann–Burchard reaction.[7] 50-μl samples of serum were used to determine triglyceride levels using Calbiochem Fast Packs[8] and 200-μl samples were used to de-

Table I. Percent Control of Serum Cholesterol after Administration of Test Compounds at 10 mg/kg/day to Male Rats[g]

| No. | Compound | Days dosed 4 | 10 | 16 | Synth ref |
|---|---|---|---|---|---|
| 1 | 2-Octanone | 74 ± 11[a] | 59 ± 7[a] | 34 ± 8[a] | 1 |
| 2 | 8-Pentadecanone | 99 ± 7 | 77 ± 10[a] | 59 ± 16[a] | 1 |
| 3 | 2-Hexadecanone | 79 ± 8[a] | 72 ± 19[b] | 58 ± 7[a] | 2 |
| 4 | 1,5-Diphenyl-2,4-methyl-3-pentanone | 56 ± 23[b] | 51 ± 12[a] | 54 ± 14[a] | 1 |
| 5 | 1,3-Bis(*p*-methylphenoxy)-2-propanone | 77 ± 22[c] | 51 ± 13[a] | 59 ± 15[a] | 3 |
| 6 | 2,8-Bis(4-fluorobenzyl)-cyclooctanone | 78 ± 17[b] | 58 ± 18[a] | 41 ± 24[a] | 4 |
| 7 | 2,8-Dibenzylcyclooctanone | 48 ± 13[a] | 50 ± 12[a] | 42 ± 18[a] | 5 |
| 8 | 2-Bis(4-methylphenyl)-3,5-dimethyltetrahydro-4-pyrone | 81 ± 3[b] | 71 ± 7[a] | 54 ± 19[a] | Text |
| 9 | 2-Bis(4-chlorophenyl)-3,5-dimethyltetrahydro-4-pyrone | 102 ± 12 | 99 ± 13 | 97 ± 8 | Text |
| 10 | Clofibrate | 111 ± 16 | 98 ± 21 | 106 ± 9 | 5 |
| 11 | 1% CMC control | 100 ± 12[d] | 100 ± 8[e] | 100 ± 9[f] | |

[a] $p = 0.001$. [b] $p = 0.010$. [c] $p = 0.020$. [d] 72 ± 9 mg %. [e] 76 ± 6 mg %. [f] 75 ± 7 mg %. [g] $N = 8$.

Table II. In Vitro Effects of Hypolipidemic Agents on HMG-CoA Reductase and Acetyl-CoA Synthetase Activities of Rat Liver[d]

| Compound | HMG-CoA reductase | Acetyl-CoA synthetase |
|---|---|---|
| 1 | 33 ± 3[a] | 53 ± 11[a] |
| 2 | 65 ± 4[a] | 98 ± 7 |
| 3 | 59 ± 5[a] | 62 ± 11[a] |
| 4 | 53 ± 6[a] | 82 ± 6[a] |
| 5 | 40 ± 5[a] | 83 ± 5[a] |
| 6 | 62 ± 2[a] | 63 ± 4[a] |
| 7 | 76 ± 5[a] | 74 ± 5[a] |
| 8 | 61 ± 15[a] | 83 ± 5[a] |
| 9 | 77 ± 7[a] | 63 ± 4[a] |
| 10 | 53 ± 6[a] | 88 ± 6 |
| 1% CMC control | 100 ± 8[b] | 100 ± 5[c] |

[a] $p = 0.001$. [b] 1142065 dpm/g of wet tissue. [c] 10.8 mg/g of wet tissue. [d] $N = 6$.

Table III. Percent Control of Serum Cholesterol after Administration of 2-Octanone Orally to Holtzman Rats[c]

| mg/kg/day | Days dosed 4 | 10 | 16 |
|---|---|---|---|
| 1 | 81 ± 7[a] | 71 ± 10[a] | 66 ± 9[a] |
| 5 | 81 ± 7[a] | 66 ± 5[a] | 63 ± 5[a] |
| 20 | 78 ± 7[a] | 60 ± 6[a] | 54 ± 3[a] |
| 50 | 76 ± 4[a] | 60 ± 5[a] | 44 ± 6[a] |
| 100 | 75 ± 3[a] | 51 ± 5[a] | 41 ± 5[a] |
| Control 1% CMC[b] | 100 ± 5 | 100 ± 10 | 100 ± 10 |

[a] $p = 0.001$. [b] 75 mg %. [c] $N = 8$.

termine glycerol level using Calbiochem Fast Pack[5] on day 7. 500-μl of serum was analyzed for serum lipase activity using a Sigma no. 800 kit on day 16.[8]

Periodic animal weights were obtained and expressed as a percentage of the animal's weight on day 0 of the experiment. After dosing for 16 days the animals were sacrificed by cervical dislocation and a number of organs excised and weighed. The organ weights were expressed as a percentage of the body weight of the animal on the day of sacrifice. Tissues were examined for gross malformations, atrophy, pathological changes, and infections.[5]

The livers were analyzed for RNA, DNA glycogen, protein, and lipid content for those animals at doses of 20 mg/kg/day.[9] Liver homogenates were also prepared in 0.25 M sucrose + 0.001 M EDTA and the free and total acid phosphatase activity was determined by the method previously reported.[2]

Serum cholesterol was also determined from $CF_1$ male mice treated with 2-octanone (10 mg/kg/day ip) on day 10. On the 11th day the liver was removed and a sucrose homogenate was prepared (1 g/5 cc of 0.25 M sucrose + 0.001 M EDTA). The incorporation of [$^{14}$C]-1-acetic acid or [$^{14}$C]-2-mevalonic acid into cholesterol was determined by the method of Haven.[10]

**Lipid Regulatory Enzymes.**[11] The in vitro effects of the hypolipidemic drugs on regulatory enzymes for lipid synthesis were examined. For indirect HMG-CoA reductase activity ([$^{14}$C]acetate incorporation into cholesterol),[10] 10 mg of test compounds in 1% CMC was incubated with 2 ml of liver microsomal cytosol suspension from rats or mice, and 1 ml of 1 M phosphate buffer, pH 7.0, containing 2.5 μCi of sodium [$^{14}$C]-acetate 5 μmol, ATP 5 μmol, $MgCl_2 \cdot 6H_2O$ 30 μmol, glucose 1-phosphate 22.5 μmol, reduced glutathione 30 μmol, CoA 0.2 μmol, NAD 1.2 μmol, NADP 1.4 μmol for 1 h at 37° in a total volume of 3 ml.[10] The reaction was stopped with 5 ml of 15% KOH in 95% EtOH heated at 75° for 75 min. 1% digitonin in 50% EtOH was added. The cholesterol was precipitated as the digitonide and was extracted by the procedure of Wada et al.[12]

Acetyl-CoA synthetase (fatty acid synthesis) activity in vitro was determined by the method outlined by Goodridge.[13] A mitochondrial-cytosol preparation (1 ml) was incubated with triethanolamine hydrochloride 200 mM, CoA 0.5 mM, GSH 10 mM, $MgCl_2 \cdot 6H_2O$ 10 mM, ATP 5 mM, alkali free hydroxylamine 400 mM, sodium acetate 10 mM, pyruvate kinase 10 μg and phosphoenol pyruvate 10 mM, and test compounds in a total volume of 2 cc. After 30 min at 37° the reaction was stopped with 3 ml of 6.7% TCA and 20% $FeCl_3 \cdot 6H_2O$ in 0.8 N HCl. After centrifugation of the protein, the supernatant was read at 540 nm using succinic anhydride as a standard.[14,15]

**Oxidative Phosphorylation.** Mouse liver was removed and a homogenate was prepared in 0.25 M sucrose + 0.001 M EDTA, pH 7.2. Oxygen consumption was measured with a Clark oxygen electrode connected to a Gilson oxygraph. The reaction vessel contained sucrose 55 μmol, KCl 22 μmol, $K_2HPO_4$ 22 μmol, test compounds in 1% CMC, and sodium succinate 90 μmol in a total volume of 1.8 cc.[15] After the basal metabolic (state 4) level was obtained, 0.257 μmol of ADP was added to obtain the ADP stimulated rate (state 3).[15,16]

**Fecal Excretion of Lipids.** In other mice treated similarly, on the eighth day of dosing 5 μCi of [$^{14}$C]palmitic acid, cholesterol, tripalmitin, or cholesteryl oleate (New England Nuclear) was

Table IV. Effect of 2-Octanone on Food Intake and Body Weight

| mg/kg/day | Av weekly food intake, g/rat[d] 1st week | 2nd week | Rat[d] body wt (g), days dosed 0 | 7 | 16 |
|---|---|---|---|---|---|
| 1 | 22.2 ± 0.6[a] | 20.7 ± 2.3 | 136 ± 11 | 175 ± 24 | 236 ± 36 |
| 5 | 22.6 ± 0.5[a] | 25.2 ± 2.0[b] | 132 ± 8 | 180 ± 14 | 245 ± 29 |
| 20 | 24.8 ± 0.7[a] | 27.4 ± 1.4[a] | 140 ± 6 | 187 ± 21[c] | 253 ± 30 |
| 50 | 23.1 ± 1.8[a] | 25.6 ± 1.5[a] | 133 ± 14 | 180 ± 21 | 246 ± 26 |
| 100 | 20.7 ± 0.3[a] | 22.1 ± 2.3 | 131 ± 14 | 168 ± 32 | 227 ± 35 |
| Control 1% CMC | 17.8 ± 1.1 | 21.9 ± 2.0 | 130 ± 15 | 158 ± 25 | 235 ± 16 |

[a] $p = 0.001$. [b] $p = 0.005$. [c] $p = 0.050$. [d] $N = 8$.

Table V. Effects of Hypolipidemic Agents on Rat[h] Serum Triglyceride and Glycerol Levels and Serum Lipase and Liver Acid Phosphatase Activities and Lipid Levels

| Compound | mg/kg/day | % of control: Triglycerides on day 7 | Glycerol on day 7 | Lipase on day 16 | Free acid phosphatase on day 16 | Liver lipid |
|---|---|---|---|---|---|---|
| 1 | 20 | 61 ± 19[a] | 76 ± 8[a] | 186 ± 57[b] | 94 ± 21 | 106 ± 38 |
| 3 | 20 | 101 ± 27 | | | 89 ± 3 | 101 ± 24 |
| 4 | 10 | 93 ± 11 | | | | 108 ± 32 |
| 5 | 10 | 70 ± 3[a] | 24 ± 15[a] | 222 ± 36[a] | | 88 ± 10 |
| 6 | 10 | | | | | 130 ± 14 |
| 7 | 10 | 58 ± 18[a] | 69 ± 26[a] | 94 ± 33 | 92 ± 11 | 85 ± 20 |
| Control 1% CMC | | 100 ± 13[c] | 100 ± 8[d] | 100 ± 28[e] | 100 ± 28[f] | 100 ± 24[g] |

[a] $p = 0.001$. [b] $p = 0.010$. [c] 110 mg %. [d] 3.38 mg %. [e] 0.90 σ Tietz unit/ml of serum. [f] 27 mg %. [g] 23.3 mg/g of wet tissue. [h] $N = 8$.

Table VI. Percent Total Rat[d] Body Weight (g) 24 h after 16th Dose of 2-Octanone

| mg/kg/day | Liver | Kidney | Heart | Lung | Brain | Spleen | Testes | Epididymis vas deferens | Vesicular glands |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.19 ± 0.19 | 0.90 ± 0.09 | 0.40 ± 0.12 | 0.68 ± 0.07 | 0.74 ± 0.14 | 0.34 ± 0.04 | 1.35 ± 0.12 | 0.30 ± 0.14 | 0.17 ± 0.11 |
| 5 | 4.10 ± 0.45 | 0.88 ± 0.18 | 0.44 ± 0.05 | 0.79 ± 0.25 | 0.70 ± 0.11 | 0.40 ± 0.04[c] | 1.24 ± 0.25 | 0.31 ± 0.14 | 0.20 ± 0.06 |
| 20 | 5.32 ± 0.32[a] | 0.90 ± 0.21 | 0.38 ± 0.12 | 0.70 ± 0.16 | 0.69 ± 0.12 | 0.39 ± 0.09 | 1.23 ± 0.18 | 0.33 ± 0.07[b] | 0.19 ± 0.05 |
| 50 | 4.23 ± 0.27 | 0.88 ± 0.08 | 0.46 ± 0.04 | 0.80 ± 0.15 | 0.76 ± 0.13 | 0.43 ± 0.07[b] | 1.32 ± 0.24 | 0.33 ± 0.06[b] | 0.26 ± 0.09[b] |
| 100 | 3.65 ± 0.92 | 0.79 ± 0.13 | 0.39 ± 0.05 | 1.10 ± 0.45 | 0.68 ± 0.08 | 0.41 ± 0.06[c] | 1.19 ± 0.37 | 0.27 ± 0.08 | 0.15 ± 0.08 |
| 1% CMC control | 4.28 ± 0.44 | 0.83 ± 0.28 | 0.43 ± 0.10 | 0.86 ± 0.49 | 0.64 ± 0.28 | 0.29 ± 0.11 | 1.21 ± 0.43 | 0.20 ± 0.10 | 0.12 ± 0.05 |

[a] $p = 0.001$. [b] $p = 0.010$. [c] $p = 0.025$. [d] $N = 8$.

Table VII. Percent of Control of RNA, DNA, Protein, Glycogen, and Lipid Levels of Rats[g] Treated 16 Days with 2-Octanone at 20 mg/kg/day

| | DNA | RNA | Protein | Glycogen | Lipid |
|---|---|---|---|---|---|
| 2-Octanone | 96 ± 34 | 99 ± 18 | 117 ± 6[a] | 104 ± 25 | 106 ± 38 |
| Control 1% CMC | 100 ± 29[b] | 100 ± 33[c] | 100 ± 3[d] | 100 ± 19[e] | 100 ± 24[f] |

[a] $p = 0.001$. [b] 1.15 mg/g of wet tissue. [c] 7.44 mg/g of wet tissue. [d] 181 mg/g of wet tissue. [e] 40.8 mg/g of wet tissue. [f] 23.3 mg/g of wet tissue. [g] $N = 8$.

Table VIII. Effects of 2-Octanone (10 mg/kg/day) on Mouse[d] Cholesterol Synthesis on the 10th Day in Vivo

| | [$^{14}$C]Acetic acid | [$^{14}$C]Mevalonic acid |
|---|---|---|
| 2-Octanone | 77 ± 9[a] | 81 ± 22 |
| Control 1% CMC | 100 ± 10[b] | 100 ± 33[c] |

[a] $p = 0.001$. [b] 619 080 dpm/g of wet tissue. [c] 254 573 dpm/g of wet tissue. [d] $N = 8$.

administered ip. Animals were maintained in metabolic cages so that feces and urine could be collected over the next 72 h. Homogenates (1 g/10 cc of water) were prepared and treated with NEN Protosol for 24 h and $^{14}$C content of a sample (0.1 cc) was determined in 10 cc of scintillation fluid containing 2 parts of toluene and 1 part of Triton X-100 with 0.4% PPO and 0.01% POPOP.[3,5]

**$LD_{50}$ Toxicity.** A toxicity screen ($LD_{50}$) was also run in $CF_1$ male mice for 2-octanone according to the Litchfield and Wilcoxon method.[17]

**Statistical Analysis.** In Tables I–X the number of animals in a group, expressed as $N$, and the mean of the percent of control and standard deviation, expressed as $\bar{x} \pm SD$, 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.[18] Data which were not significantly different from the control values are not shown.

## Results and Discussion

Compounds 1–8 significantly lowered serum cholesterol at 10 mg/kg/day in male rats (Table I). All these compounds significantly inhibited HMG-CoA reductase activity of liver in vitro, i.e., the incorporation of [$^{14}$C]acetate into cholesterol (Table II). 2-Octanone (1) resulted in the highest inhibition which correlated well with the serum cholesterol level. Compounds containing the bulky cyclooctanone ring, **6** and **7**, were less effective in inhibiting the enzymatic system than would be expected by comparing serum cholesterol reduction. Compound **7** accelerated lipid excretion in the feces[5] and this could account for the difference in the two observations. Further improvement of HMG-CoA reductase inhibition was seen with elimination of the cyclooctanone ring **4** and shortening of the chain to a propanone **5**. Long-chain ketones **2** and **3** did not improve the inhibition nor did either of the pyrone structures **8** and **9** which would have similar molecular dimensions and shape as the cyclooctanones. Fatty acid synthetase (acetyl-CoA synthetase) activity was significantly reduced by compounds **1**, **3**, **6**, **7**, and **9** (Table II). Compounds 1, **5**, and **7** reduced serum triglycerides (Table V). Thus the inhibition of fatty acid synthesis did not correlate directly to reduction of serum triglycerides. Compounds 1, **5**, and **7** cause a more rapid excretion of triglycerides lipid in the feces.[3,5] Compounds 1 and **3** could possibly be antimetabolites for fatty acid synthesis or elongation. These were more active than **2** which has an uneven numbered carbon chain and the ketone was on carbon 8. The pentanone **4**, propanone **5**, and methyl-substituted pyrone **8** derivatives were inactive. However, the chloro-substituted pyrone **9** possessed significant activity (37%) in inhibition fatty acid synthesis.

Since the 2-octanone was the most potent hypolipidemic in these screens, it was decided that a more in-depth study of the compound was desirable. 2-Octanone significantly lowered the serum cholesterol in male rats over a range of 1–100 mg/kg/day (Table IV). The effect was dose related. The average food consumption of these animals per week was not reduced at any dose; in fact, for the first week food consumption was significantly elevated for all groups (Table IV). The increased food intake was not reflected as an increase in body weight, and after 16 days

Table IX. Fecal Excretion of Lipids in Male Mice[b] after Treatment with 2-Octanone (10 mg/kg) for 10 Days[c]

| | Total DPM | | | |
|---|---|---|---|---|
| | Cholesterol (4-$^{14}$C) | Cholesteryl oleate (1-$^{14}$C) | Palmitic acid (1-$^{14}$C) | Tripalmitin (carboxyl-$^{14}$C) |
| | Control | | | |
| 0-12 h | 9 528 ± 908 | 360 674 ± 33 567 | 187 023 ± 21 230 | 211 533 ± 84 415 |
| 12-24 h | 18 247 ± 4 153 | 74 900 ± 13 271 | 67 493 ± 37 956 | 143 631 ± 55 476 |
| 24-48 h | 11 679 ± 2 065 | 144 795 ± 35 163 | 42 249 ± 3 262 | 28 144 ± 1 151 |
| 48-72 h | 6 588 ± 1 068 | 217 194 ± 35 112 | 40 079 ± 2 203 | 172 523 |
| Total | 46 042 | 797 563 | 336 844 | 555 831 |
| | Treated | | | |
| 0-12 h | 22 271 ± 1 815[a] | 282 475 ± 19 413[a] | 83 523 ± 24 663[a] | 342 055 ± 98 165 |
| 12-24 h | 14 254 ± 892 | 58 202 ± 12 054 | 8 054 ± 3 170[a] | 122 777 ± 65 932 |
| 24-48 h | 13 545 ± 1 548 | 123 223 ± 16 358 | 59 479 ± 8 509[a] | 34 330 ± 3 213[a] |
| 48-72 h | 28 101 ± 4 577[a] | 243 741 ± 33 776 | 39 449 ± 3 039 | 1 274 278 ± 192 433[a] |
| Total | 78 621 | 707 641 | 190 505 | 1 773 441 |

[a] $p = 0.001$. [b] $N = 8$. [c] 5 μCi of labeled lipid injected ip on day 8.

of dosing with 2-octanone, there was no significant change in body weight (Table IV). Thus the lipid lowering effect of 2-octanone cannot be attributed to a loss of appetite.

Treatment with 2-octanone at 20 mg/kg/day for 7 days resulted in a significant reduction of serum triglycerides, i.e., 39%, and serum glycerol, i.e., 24% (Table V). The serum lipase activity on day 16 was significantly elevated which could explain the drastic reduction of triglycerides (Table V). Since 2-octanone is a ketone which could possibly have a detergent effect, we also determined the percent free acid phosphatase activity. This is a hydrolytic lysosomal enzyme bound to the inner membrane of the lysosome. Cellular damage results in a release and an elevated percent of free enzymatic activity. Treatment with 2-octanone caused no changes in activity of this enzyme (Table V). Thus 2-octanone did not lyse cellular membranes after 16 days of dosing.

A dose–response curve demonstrated that there was no infiltration of lipids into the major tissues at dose levels of 1–100 mg/kg. There was no atrophy of the testes, epididymis, vas deferens, and vesicular glands at any dose indicating that this hypolipidemic drug was not estrogenic at high doses (Table VI). At 20 mg/kg/day there was an increase in the weight of the liver. However, examination of the RNA, DNA, glycogen, protein, and lipid content of these livers revealed that the lipid levels were not significantly elevated; however, the protein content was elevated significantly (Table VII).

The $LD_{50}$ for 2-octanone in $CF_1$ male mice was 1.6 g/kg. 2-Octanone at 10 mg/kg/day ip significantly lowered serum cholesterol of male mice to 58 ± 10% of control ($p = 0.001$) after 10 days, whereas in rats this was lowered to 34% after 16 days. In vivo incorporation of [$^{14}$C]acetic acid into cholesterol was significantly reduced (23%), whereas the [$^{14}$C]mevalonic acid incorporation was reduced only 19% after 10 days (Table VIII). These data would indicate that HMG-CoA reductase is not the sole site of inhibition in cholesterol synthesis by these drugs and that other parameters have to be considered. In vitro testing with 10 mg of 2-octanone demonstrated that liver HMG-CoA reductase activity was reduced 31% in mice and 67% in rats (Table II). The in vivo and in vitro reductase activity data correlated well in mouse liver. The enzyme activity was reduced less in the mouse than the rat liver. Furthermore, the acetyl-CoA synthetase (fatty acid synthetase) was reduced 47% by 2-octanone in rat liver at 10 mg in vitro. Elimination of labeled lipids in the feces of male mice treated with 10 mg/kg/day of 2-octanone demonstrated that the drug accelerated the elimination of cholesterol and tripalmitin (Table IX), retarded the elimination of palmitic acid, and had very little effect on cholesteryl oleate elimination in the feces. The drug had no effect on urine lipid excretion.

Table X. In Vitro Effects of Hypolipidemic Agents on Mouse[f] Oxidative Phosphorylation Rates

| Compound | Basal state 4 | ADP stimulated state 3 |
|---|---|---|
| 1 (0.2 mg) | 130 ± 10[c] | 123 ± 22 |
| 1 (10.0 mg) | 144 ± 19[b] | 110 ± 21 |
| 1 (150.0 mg) | 161 ± 35[b] | 70 ± 18[b] |
| **2** (0.2 mg) | 101 ± 9 | 107 ± 18 |
| 3 (0.2 mg) | 124 ± 21 | 115 ± 12 |
| 5 (0.2 mg) | 126 ± 12 | 114 ± 8 |
| 7 (0.2 mg) | 79 ± 7 | 70 ± 19[b] |
| **10** (0.2 mg) | 120 ± 3 | 27 ± 15[a] |
| Control 1% CMC | 100 ± 30[d] | 100 ± 13[e] |

[a] $p = 0.001$. [b] $p = 0.005$. [c] $p = 0.025$. [d] 5.86 μl of $O_2$/mg/h. [e] 11.71 μl of $O_2$/mg/h. [f] $N = 8$.

Clofibrate has been demonstrated to accelerate state 4 oxygen consumption and basal respiration and to inhibit state 3 oxidative phosphorylation respiration coupled to ATP synthesis processes.[16] Our data confirm this finding (Table X). Since HMG-CoA reductase and acetyl-CoA synthetase require ATP for activity, the effects of these drugs on oxidative phosphorylation were examined to determine if their hypolipidemic drugs interfered with available energy of the cell for synthesis of lipids. At 0.2 mg in vitro it was observed that 2-octanone increased basal oxygen consumption and ADP stimulated consumption significantly, but at 150 mg 2-octanone increased basal consumption and reduced ADP stimulated consumption significantly, i.e., followed the pattern of clofibrate. However at 10 mg, a more appropriate dose for hypocholesterolemic activity, there was an increase in basal consumption with little effect on ADP stimulated consumption (Table X). Compounds **3** and **5** cause a slight increase in state 4 which is the normal respiratory rate of the electron-transport system. This may be increased due to slight loosening of the mitochondrial membranes. Compounds 1 (150 mg), **7**, and **10** inhibited state 3 which indicated uncoupling of oxidative phosphorylation (ATP synthesis).

It seems reasonable to conclude that 2-octanone lowers serum cholesterol by inhibiting cholesterol synthesis and accelerating cholesterol excretion in the feces. Triglyceride catabolism by lipase is accelerated by 2-octanone as well as its excretion in the feces, thereby lowering serum triglycerides. It would appear that other medium length ketones and analogues should be examined as potential inhibitors of HMG-CoA reductase and fatty acid

synthetase and/or stimulators of lipase. The low toxicity and lack of side effects of the lead compound also make this series an attractive one from a therapeutic standpoint. Some analogues of 2-octanone are currently under consideration in our laboratories and will be reported in a subsequent communication.

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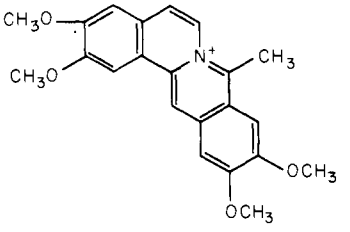
# *Communications to the Editor*

## Coralyne. Intercalation with DNA as a Possible Mechanism of Antileukemic Action

*Sir:*

Coralyne (5,6,7,8,13,13a-hexadehydro-8-methyl-2,3,-10,11-tetramethoxyberbinium chloride, 1) has been shown to exhibit significant antitumor activity against both P388 and L1210 leukemias in mice.[1] The activity coupled with

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relatively low toxicity has created an interest in this compound resulting in synthesis of a number of derivatives,[2] as well as a practical large-scale synthesis of coralyne itself.[3] The fused planar cationic aromatic ring system of coralyne is potentially capable of intercalation with DNA in a manner analogous to berberine,[4] daunorubicin,[5] ethidium bromide,[6] and related compounds.[7,8] Zee-Cheng and Cheng[1] have shown that the electronic absorption spectrum of coralyne is perturbed by DNA and that the visible light induced photohydration[9] of coralyne is inhibited in the presence of DNA. They suggested that such an interaction may account for the antileukemic activity of coralyne as has been demonstrated for other antineoplastic agents.[5] Because of the potential importance of coralyne in chemotherapy of neoplasms and the benefits that can arise from an elucidation of its mode of action as an aid in designing more effective derivatives, we have initiated a detailed investigation of the coralyne–DNA complex.

An indication of the strong interaction between coralyne and DNA is shown in Figure 1 by the dramatic shifts induced into the coralyne spectrum upon addition of DNA. The spectra do not display an isosbestic point which suggests that there is more than one bound species of coralyne. Upon addition of DNA to a dilute coralyne solution, the drug spectrum first changes from the characteristic curve of unbound coralyne (Figure 1, curve 1) to a spectrum displaying hypochromism and a red shift at high ratios of coralyne to DNA (Figure 1, curve 2). On continued addition of DNA, the spectrum again shifts slightly to longer wavelengths and an increase in extinction coefficient relative to curve 2 occurs. The spectrum approaches a constant shape on continued addition of DNA and essentially does not change at ratios of coralyne to DNA of less than 0.03 (Figure 1, curve 3). Similar behavior can be obtained by reversing the titration and adding coralyne to DNA solutions provided care is taken to prevent precipitation.

Acridine orange, proflavin, and similar compounds[10,11] also do not display isosbestic points in their DNA spectrophotometric titration curves at low ionic strength and high ratios of drug to DNA. This has been attributed to stacking of the planar aromatic cations on the periphery of the negatively charged deoxyribose phosphate backbone resulting in multiple bound species. As the ratio of these