NOTES

Hypolipidemic Activity of Substituted 2-Pyrrolidinones in Rodents

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Abstract ☐ A series of substituted 2-pyrrolidinones was evaluated for hypolipidemic activity at 20 and 30 mg/kg/day in CF_1 male mice. 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone was the most potent compound at 30 mg/kg/day, reducing serum triglyceride levels 52% after 14 days of dosing and serum cholesterol levels 48% after 16 days of dosing. 4-Phenyl-5-carbethoxy-2-pyrrolidinone and 4-phenyl-3,5,5-tricarbethoxy-2-pyrrolidinone also demonstrated significant activity. Those compounds which contained a phenyl substituent were more potent than either the unsubstituted, the alkyl, or the dicarbethoxy 2-pyrrolidinone analogues.

Keyphrases ☐ 2-Pyrrolidinones—synthesis of substituted analogues, hypolipidemic activity in mice ☐ Antihyperlipidemic agents—substituted 2-pyrrolidinone analogues, synthesis, hypolipidemic activity in mice ☐ Succinimide—2-pyrrolidinone congeners, synthesis of substituted analogues, hypolipidemic activity in mice

Studies on the antihyperlipidemic activity of *N*-substituted phthalimides (1) and related imide analogues (2) revealed a wide variety of imides which caused a reduction in serum cholesterol and triglyceride levels in rodents. Succinimide was observed to be active at a dose of 20 mg/kg/day ip, affording a 27% reduction in serum cholesterol levels after 16 days and a 32% reduction of serum triglyceride levels after 14 days in mice (2). As an extension of these studies, the hypolipidemic activity of a series of 2-pyrrolidinone derivatives as congeners of succinimide was examined; those results are reported herein.

EXPERIMENTAL

Chemistry—Of the 18 compounds studied (Table I), 2-pyrrolidinone[1] (I) and L-pyroglutamic acid[2] (IV) were purchased commercially. 5-Carboxy-2-pyrrolidinone (III), 4-phenyl-5-carboxy-2-pyrrolidinone (used to prepare XIV), 5,5-dicarbethoxy-2-pyrrolidinone (V), and 5,5-dicarboxy-2-pyrrolidinone (VI) were prepared by the method of Cocolas and Hartung (3). 5-Carbethoxy-2-pyrrolidinone (II) was prepared by esterification of D,L-5-carboxy-2-pyrrolidinone (III) with ethanol–hydrochloric acid. The four alkyl-5,5-dicarbethoxy-2-pyrrolidinones (VII–XIII) were prepared by a Michael addition of an α,β-unsaturated ester to diethyl acetamidomalonate and subsequent cyclization as reported by Kim and Cocolas (4).

4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone (XIV)—The method of Kim and Cocolas (4) was used. To 400 mg (0.019 g-atom) of sodium dissolved in 200 ml of absolute ethanol of alcohol was added 21.7 g (0.1 mole) of diethyl acetamidomalonate and 22 g (0.125 mole) of ethyl cinnamate. The mixture was refluxed overnight (10–12 hr), cooled to room temperature, and then neutralized with glacial acetic acid. The solvent was removed under reduced pressure to give solid material which was recrystallized from toluene to give 16 g of product, mp[3] 96–97°.

Anal.—Calc.[3] for $C_{16}H_{12}NO_5$: C, 62.95; H, 6.23; N, 4.59. Found[3]: C, 62.82; H, 6.19; N, 4.74.

4-Phenyl-5-carbethoxy-2-pyrrolidinone (XV)—4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone (XIV), 26.9 g (0.088 mole), was dissolved in 125 ml of absolute ethanol, and 250 ml of 20% ethanolic potassium hydroxide solution was added slowly. The resulting solution was refluxed for 9 hr and allowed to cool, and the white solid precipitate was collected and dissolved in 150 ml of hot water. The solution was made strongly acidic with 6 *N* HCl and the resulting precipitate filtered to give 10.8 g of 4-phenyl-5,5-dicarboxy-2-pyrrolidinone. Decarboxylation at 160–165° under vacuum and recrystallization from ethanol gave 3.04 g of 4-phenyl-5-carboxy-2-pyrrolidinone, mp 180°. Esterification with ethanolic hydrochloric acid yielded 4-phenyl-5-carbethoxy-2-pyrrolidinone, mp 140–141°.

Anal.—Calc. for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.79; H, 6.67; N, 6.03.

4-Phenyl-3,5,5-tricarbethoxy-2-pyrrolidinone (XVI)—The method of Kim and Cocolas (4) was used. A solution containing 1.15 g (0.05 g-atom) of sodium metal, 10.85 g (0.05 mole) diethyl acetamidomalonate, and 12.5 g (0.05 mole) diethyl benzalmalonate in 100 ml absolute ethanol was maintained at room temperature for 3 days. The reaction mixture was then neutralized with glacial acetic acid and cooled to precipitate 20 g of XVI. The product was recrystallized from 95% ethanol, mp[3] 107–109°.

Anal.—Calc.[3] for $C_{19}H_{23}NO_7$: C, 60.47; H, 6.14; N, 3.71. Found[3]: C, 60.81; H, 6.22; N, 3.54.

4-Isopropyl-3,5-dicarbethoxy-2-pyrrolidinone (XVII)—Compound XVII was prepared according to the procedure of Kim and Cocolas (5).

3-Methyl-5,5-dicarbethoxy-2-pyrrolidinone (XVIII)—Compound XVIII was prepared according to the method of Cocolas *et al.* (6).

Biological Testing—Compounds were tested at 20 and 30 mg/kg/day ip in CF_1 male mice (~25 g). On days 9 and 16, blood was collected *via* a tail vein, and the serum was separated by centrifugation. Serum cholesterol was determined by a modification of the Leibermann–Burchard reaction (8). A separate group of mice were bled on day 14 and their serum triglyceride levels were determined using a commercial kit[4].

RESULTS AND DISCUSSION

The 2-pyrrolidinone derivatives were potent hypolipidemic agents in mice after dosing for 14–16 days at 20 and 30 mg/kg/day (Table II). The unsubstituted compounds demonstrated very poor activity. 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone (XIV) gave the best anticholesteremic activity resulting in a 42% reduction of serum cholesterol levels at 20 mg/kg/day and a 48% reduction at 30 mg/kg/day. At the latter dose, serum triglycerides were reduced 52%. The other two derivatives containing a phenyl substituent, XV and XVI, also reduced cholesterol serum levels significantly but to a lesser degree. 4-Phenyl-5-carbethoxy-2-pyrrolidinone XV was more effective than 4-phenyl-3,5,5-tricarbethoxy-2-pyrrolidinone XVI, causing cholesterol reductions of 37 and 24%, respectively, at 20 mg/kg/day and 35 and 42%, respectively, at 30 mg/kg/day. Compound XV reduced serum triglyceride content 44%.

4-Alkyl substitution on the 5,5-dicarbethoxy-2-pyrrolidinone nucleus improved the reduction of serum triglycerides and cholesterol in mice.

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[1] Aldrich Chemical Co.
[2] Mann Chemical Co.
[3] Determined at M-H-W Laboratories, Phoenix, Ariz.

[4] Fisher, Hycel Triglyceride Test (1975), Hycel, Inc.

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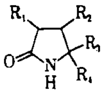
Table I—Physical Characteristics of 2-Pyrrolidinone Analogues



Compound	Name	R_1	R_2	R_3	R_4	Melting Point Found	Melting Point Lit. (ref.)
I	2-Pyrrolidinone	H	H	H	H	—	—
II	5-Carbethoxy-2-pyrrolidinone	H	H	$COOC_2H_5$	H	57–59°	61° (7)
III	D,L-5-Carboxy-2-pyrrolidinone	H	H	COOH	H	187–189°	183–185° (4)
IV	L-5-Carboxy-2-pyrrolidinone	H	H	COOH	H	158–159°	—
V	5,5-Dicarbethoxy-2-pyrrolidinone	H	H	$COOC_2H_5$	$COOC_2H_5$	82–83°	82–83° (3)
VI	5,5-Dicarboxy-2-pyrrolidinone	H	H	COOH	COOH	152–153°	152.5–153° (3)
VII	4-Methyl-5,5-dicarbethoxy-2-pyrrolidinone	H	CH_3	$COOC_2H_5$	$COOC_2H_5$	77–78°	77.5–78° (3)
VIII	4-Ethyl-5,5-dicarbethoxy-2-pyrrolidinone	H	C_2H_5	$COOC_2H_5$	$COOC_2H_5$	113–115°	113–115° (4)
IX	4-*n*-Propyl-5,5-dicarbethoxy-2-pyrrolidinone	H	n-C_3H_7	$COOC_2H_5$	$COOC_2H_5$	88–89°	88–89° (4)
X	4-Isopropyl-5,5-dicarbethoxy-2-pyrrolidinone	H	i-C_3H_7	$COOC_2H_5$	$COOC_2H_5$	98–99°	98–99° (4)
XI	4-*n*-Butyl-5,5-dicarbethoxy-2-pyrrolidinone	H	n-C_4H_9	$COOC_2H_5$	$COOC_2H_5$	104–105°	104–105° (4)
XII	4-Isobutyl-5,5-dicarbethoxy-2-pyrrolidinone	H	i-C_4H_9	$COOC_2H_5$	$COOC_2H_5$	108–109°	109° (4)
XIII	4-*n*-Pentyl-5,5-dicarbethoxy-2-pyrrolidinone	H	n-C_5H_9	$COOC_2H_5$	$COOC_2H_5$	104–105°	104–105° (4)
XIV	4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone	H	C_6H_5	$COOC_2H_5$	$COOC_2H_5$	96–97°	—
XV	4-Phenyl-5-carbethoxy-2-pyrrolidinone	H	C_6H_5	$COOC_2H_5$	H	140–141°	—
XVI	4-Phenyl-3,5,5-tricarbethoxy-2-pyrrolidinone	$COOC_2H_5$	C_6H_5	$COOC_2H_5$	$COOC_2H_5$	107–109°	—
XVII	4-Isopropyl-3,5-dicarbethoxy-2-pyrrolidinone	$COOC_2H_5$	i-C_3H_7	$COOC_2H_5$	H	107–109°	106–108° (5)
XVIII	3-Methyl-5,5-dicarbethoxy-2-pyrrolidinone	CH_3	H	$COOC_2H_5$	$COOC_2H_5$	113–115°	113–115° (6)

Table II—Effects of 2-Pyrrolidinone Analogues on Serum Cholesterol and Triglyceride Levels of CF_1 Male Mice [a]

Compound	Dose	Serum Triglyceride, % of Control Day 14	Serum Cholesterol, % of Control Day 9	Serum Cholesterol, % of Control Day 16
Succinimide		68 ± 7^b	78 ± 9^b	73 ± 10^b
I	20 mg/kg/day	80 ± 6^b	86 ± 7^c	83 ± 6^c
II	20 mg/kg/day	62 ± 6	89 ± 6	72 ± 5
III	20 mg/kg/day	85 ± 7^c	80 ± 6^b	83 ± 7^c
IV	20 mg/kg/day	88 ± 9	84 ± 7^c	84 ± 6^c
V	20 mg/kg/day	87 ± 10	94 ± 8	82 ± 5^b
VI	20 mg/kg/day	82 ± 4^b	92 ± 5	71 ± 3^b
VII	20 mg/kg/day	76 ± 4^b	84 ± 4^b	79 ± 4^b
VIII	20 mg/kg/day	75 ± 3^b	81 ± 7^b	73 ± 9^b
	30 mg/kg/day	68 ± 6^b	72 ± 6^b	58 ± 5^b
IX	20 mg/kg/day	63 ± 7^b	77 ± 8^b	73 ± 5^b
X	20 mg/kg/day	70 ± 3^b	78 ± 6^b	75 ± 3^b
	30 mg/kg/day	54 ± 4^b	91 ± 8	55 ± 6^b
XI	20 mg/kg/day	57 ± 7	80 ± 8	68 ± 3
XII	20 mg/kg/day	70 ± 7^b	96 ± 7	62 ± 5^b
	30 mg/kg/day	67 ± 6^b	80 ± 7^b	58 ± 6^b
XIII	20 mg/kg/day	72 ± 3^b	79 ± 7^b	79 ± 7^b
	30 mg/kg/day	61 ± 5^b	86 ± 8^c	68 ± 4^b
XIV	20 mg/kg/day	83 ± 9	94 ± 4	58 ± 8^b
	30 mg/kg/day	48 ± 5^b	55 ± 6^b	52 ± 5^b
XV	20 mg/kg/day	82 ± 7^c	91 ± 6	63 ± 9^b
	30 mg/kg/day	56 ± 6^b	97 ± 7	65 ± 6^b
XVI	20 mg/kg/day	88 ± 4^c	101 ± 5	76 ± 3^b
	30 mg/kg/day	89 ± 6	89 ± 9	58 ± 5^b
XVII	20 mg/kg/day	98 ± 9	85 ± 6^c	68 ± 3^b
	30 mg/kg/day	62 ± 7^b	83 ± 8^c	70 ± 7^b
XVIII	20 mg/kg/day	84 ± 8^c	94 ± 5	88 ± 10
1% Carboxymethyl cellulose		100 ± 6	100 ± 5	100 ± 6

[a] $n = 6$. [b] $p \leq 0.001$. [c] $p \leq 0.010$.

Reduction of serum cholesterol ranged from 21 to 38% at 20 mg/kg/day and 32 to 48% at 30 mg/kg/day compared with the control. 4-Isobutyl-5,5-dicarbethoxy-2-pyrrolidinone (XII) caused the greatest reduction of these 4-alkyl derivatives, 38% at 20 mg/kg/day and 42% at 30 mg/kg/day. The serum triglyceride levels were reduced 33% by XII at 30 mg/kg/day, 46% by X at 30 mg/kg/day, and 43% by XI at 20 mg/kg/day.

4-Isopropyl-3,5-dicarbethoxy-2-pyrrolidinone (XVII) reduced serum cholesterol levels by 32% on day 16 at 20 mg/kg/day, being slightly more active than the 4-isopropyl-5,5-dicarbethoxy analogue (X) which suppressed serum cholesterol levels by 25% at this dose level. However, X was more active than XVII at 30 mg/kg/day in lowering serum cholesterol.

3-Methyl-3,5-dicarbethoxy-2-pyrrolidinone (XVIII) was less effective in reducing both triglyceride and serum cholesterol levels than the 4-methyl analogue (VII).

The 4-*n*-propyl-5,5-dicarbethoxy-2-pyrrolidinone (IX) gave the best antitriglyceridemic activity of all the compounds when tested at 20 mg/kg/day, reducing serum levels by 37%. L-Pyroglutamic acid (IV) was one of the less effective compounds tested and had the same level of hypolipidemic activity as the racemic compound, III.

These studies demonstrate that the 2-pyrrolidinones possess antihyperlipidemic effects in mice. The compounds appear to be more potent than clofibrate which is inactive in the 20–30 mg/kg/day dose range. The hypolipidemic activity of these pyrrolidinones is, in general, equal to succinimide and similar imide-containing structures.

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Potential Antitumor Agents IX: Synthesis and Antitumor Activity of Two Analogues of Ketocaine

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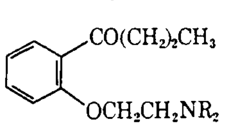
Abstract ☐ The reaction between *o*-hydroxybutyrophenone and tris(2-chloroethyl)amine gave two analogues (II, V) of the well-known local anesthetic ketocaine (I). Compounds II and V showed interesting antitumor activity in mice implanted with Ehrlich ascites tumor cells (% T/C = 149 at 5 mg/kg and 171 at 50 mg/kg, respectively). Further studies on the pharmacological behavior of these new compounds are in progress.

Keyphrases ☐ Antitumor agents—synthesis and antitumor activity of two analogues of ketocaine, potential ☐ Ketocaine—potential antitumor agents, synthesis and antitumor activity of two analogues

The antimitotic properties associated with the bis(2-chloroethyl)amino group is well known, and a number of compounds bearing this group are of therapeutic interest. The choice of a suitable supporting moiety for this pharmacophoric group is very important (1). The local anesthetic ketocaine (I) (2) at high concentrations reduces the oxygen consumption by cerebral tissue, while at lower concentrations is able to stimulate intensely this consumption, not only by cerebral tissue, but also by tissues with prevailing anaerobic metabolism (HeLa cells, KB cells) (3–6). In particular, ketocaine inhibits the mitosis of lymphocytes in culture stimulated by phytohemoagglutinine, a process which has been correlated with the stimulating effect of this local anesthetic on oxygen consumption by tissues with prevailing anaerobic metabolism (7).

These observations prompted us to attach the bis(2-chloroethyl)amino group to the phenolic group in *o*-hydroxybutyrophenone, in order to potentiate and/or specialize the antimitotic action of the parent drug, ketocaine. Such a compound also is in agreement with the N—O—O triangulation hypothesis recently proposed for some antineoplastic agents (8).



I R = —CH(CH$_3$)$_2$
II R = —CH$_2$CH$_2$Cl

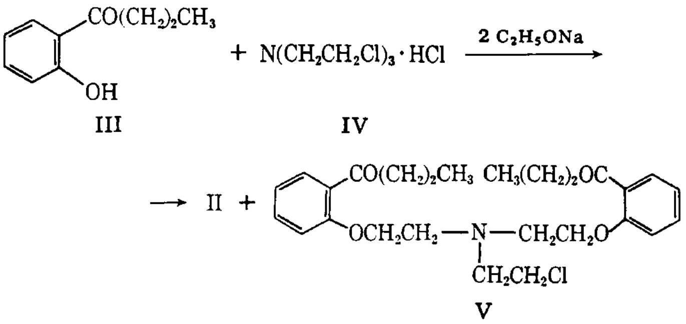
RESULTS AND DISCUSSION

Replacement of the two isopropyl groups of ketocaine (I) with two 2-chloroethyl groups was effected by treating *o*-hydroxybutyrophenone(III) with one equivalent of tris(2-chloroethyl)amine (IV) in ethanolic sodium ethoxide. This not only furnished the expected *N*-[2-(2-butanoyl)phenoxyethyl]-*N*,*N*-di(2-chloroethyl)amine (II) but also *N*,*N*-di[2-(2-butanoyl)phenoxyethyl]-*N*-(2-chloroethyl)amine (V) (Scheme I). The IR and ^{1}H-NMR spectra of compounds II and V are in agreement with the assigned structures (see *Experimental*).

For antitumor testing, female Swiss mice (average weight 21 ±1 g) were implanted on day 0 with 10^6 Ehrlich ascites tumor cells from donor mice. After 24 hr the animals were treated with compound II dissolved in water (1, 5, or 20 mg/kg, i.p.) or compound V dissolved in dimethyl sulfoxide (10, 50, or 200 mg/kg, i.p.). The amount of dimethyl sulfoxide used was shown previously, in analogous experiments, not to affect tumor growth. Deaths were recorded for the 60-day period. The activities were measured as the ratio of the mean survival time of the test animals to that of the control animals expressed as a percentage (% T/C). Significant activity is achieved with an increased life span of 25% ($T/C \geq 125$).

Both compounds prolonged the life span of mice bearing Ehrlich ascites carcinoma beyond that of untreated animals: increase for compound II was 48.7% at a 5 mg/kg, i.p. dose and for compound V was 71.2% at 50 mg/kg, 24 hr after tumor implantation (Table I).

The activity of compounds II and V is related to toxicity. While compound II may act as a cross-linking agent (similar to melphalan, chlorambucil, and cyclophosphamide), the antitumor activity of compound V may be due only to its alkylating properties; its lower toxicity is in agreement with the data reported for other monofunctional agents (1).



Scheme I

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