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

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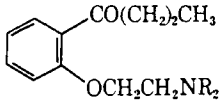
Abstract □ The reaction between *o*-hydroxybutyrophenone and tris(2-chloroethyl)amine gave two analogues (II, V) of the well-known local anesthetic ketocaine (I). Compounds II and V showed interesting antitumor activity in mice implanted with Ehrlich ascites tumor cells (% T/C = 149 at 5 mg/kg and 171 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_2CH_2Cl

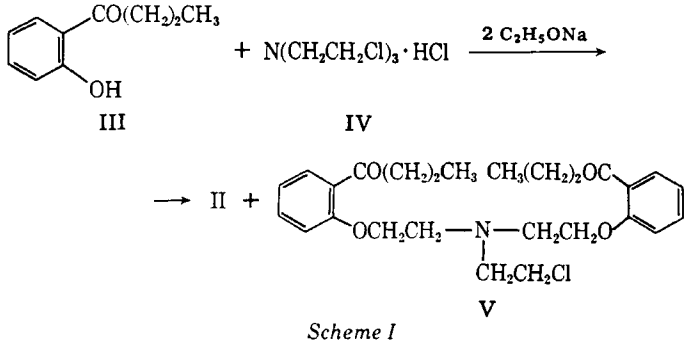
RESULTS AND DISCUSSION

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 ≥ 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).



III IV

II + V

Scheme I

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Table I—Antitumor Activity of Ketocaine Analogues Against Ehrlich Ascites Carcinoma in Mice

Compound	Dose, mg/kg i.p.	Number per Group	MST[a]	%,T/C[b]
Control[c]	—	10	16.0	—
II	1	5	15.0	93.7
	5	5	23.8	148.7
	20	5	—[d]	—
V	10	5	19.6	122
	50	5	27.4	171.2
	200	5	—[d]	—

[a] Mean Survival Time (days). [b] % T/C ≥ 125 denotes significant activity. [c] Saline. [d] Death due to drug-related toxicity.

EXPERIMENTAL[1]

Synthesis of Compounds II and V—*o*-Hydroxybutyrophenone (III: 16.4 g, 0.1 mole) and tris(2-chloroethyl)amine hydrochloride (IV: 24.1 g, 0.1 mole) were added to a solution of sodium ethoxide (0.2 mole) in absolute ethanol (150 ml). The mixture was refluxed for 3 hr, and the solvent was evaporated under reduced pressure. The residue was treated with water and a few drops of 34% ammonium hydroxide until alkaline, then extracted with chloroform, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. Column chromatography of the residue gave 6 g of starting material (III), then 12 g of II, and finally 3 g of V. Compounds II and V, oily at room temperature, were then converted into the corresponding hydrochlorides: II HCl: mp 113–115° (absolute ethanol).

Anal.—Calc. for $C_{16}H_{23}Cl_2NO_2$·HCl: C, 52.11; H, 6.56; N, 3.80. Found: C, 52.19; H, 6.44; N, 3.72. IR (cm^{-1}): 2400–2300, 1690, 1600, 1580, 975, 760. ^{1}H-NMR (δ): 0.96 (t, 3, CH_3); 1.68 (m, 2, CH_2—CH_3); 2.84 (t, 2, $COCH_2$); 3.86 (m, 6, 3 $CH_2N^{(+)}\lesssim$), 4.10 (m, 4, 2 CH_2Cl); 4.61 (t, 2, O—CH_2); 6.9–7.7 (m, 4 aromatic).

V·HCl: mp 83–85° (toluene). *Anal.*—Calc. for $C_{26}H_{34}ClNO_4$·HCl: C, 62.90; H, 7.11; N, 2.82. Found: C, 63.18; H, 7.32; N, 2.81. IR (cm^{-1}): 2400–2300, 1670, 1600, 1580, 1125, 750. ^{1}H-NMR (δ): 0.95 (t, 6, 2 CH_3); 1.66 (m, 4, 2 CH_2—CH_3); 2.82 (t, 4, 2 $COCH_2$); 3.88 (m, 6, 3 $CH_2N^{(+)}\lesssim$); 4.12 (t, 2, CH_2Cl); 4.67 (t, 4, 2 O—CH_2); 6.9–7.7 (m, 8, aromatic).

[1] Melting points are uncorrected. Bakerflex plates (Silica-gel IB2-F) were used for TLC. For column chromatography, Kieselgel 60 (Merck) was used, activated at 120° for 2 hr: the eluent was a mixture of petroleum ether (bp 60–80°)–acetone 80:20. The IR spectra were recorded in Nujol with a Perkin-Elmer 298 spectrometer. ^{1}H-NMR spectra were recorded in $CDCl_3$ (10% w/v) with a 90-MHz spectrometer (Varian EM 390) using TMS as an internal standard.

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GLC Determination of (−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine in Human Plasma and Urine

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Abstract □ (−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine (MK-160) was extracted from human plasma and urine with petroleum ether and quantitated by GLC using a nitrogen-sensitive detector. A homologue of the drug served as the internal standard. The method is specific for the drug in the presence of potential metabolites and is capable of measuring concentrations in plasma as low as 6 ng/ml.

Keyphrases □ GLC determination—(−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohept-5-ylidene)piperidine (MK-160) in plasma and urine

(−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine (I)[1] shows stereospecific antipsychotic, antidopaminergic, and anticholinergic activities in the mouse, rat, and squirrel monkey (1, 2). Clinical trials in human subjects necessitated an analytical method capable of measuring low nanogram concentrations of I in plasma without interference from potential metabolites of the drug. This report describes a GLC method using a nitrogen–phosphorus detector which meets these requirements with acceptable precision and accuracy.

EXPERIMENTAL

Chemicals and Reagents—Heptane, petroleum ether (35–60°), and methanol were reagent grade; isoamyl alcohol was spectroscopic quality. Stock solutions of I HCl and II were prepared in methanol and serially diluted with methanol to the desired concentrations. All concentrations are expressed as the free base of I.

Apparatus—GLC analysis was performed with a gas chromatograph[2] equipped with a nitrogen–phosphorus detector and a 91-cm × 2-mm column packed with 3% OV-17 on Gas Chrom Q (80–100 mesh)[3]. The

[1] This compound is designated as MK-160 by Merck & Co., Inc.

[2] Hewlett-Packard Model 5840A.

[3] Supelco.

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