(9) R. E. Pyke, J. B. Cohn, J. P. Feighner, and W. T. Smith, *Psychopharmacol. Bull.* **19**, 96 (1983).
(10) M. Konishi, K. Hirai, and Y. Mori, *J. Pharm. Sci.*, **71**, 1328 (1982).
(11) N. Inotsume and M. Nakano, *Chem. Pharm. Bull.* **28**, 2536 (1980).

Potential Antitumor Agents X: Synthesis and Antitumor Activity of Two Nitrogen Mustard Derivatives Related to Ketocaine

ALDO ANDREANI *x, DANIELA BONAZZI *, MIRELLA RAMBALDI *, IRAKLIS GALATULAS ‡, and ROSARIA BOSSA ‡

Received July 19, 1982, from the **Istituto di Chimica Farmaceutica e Tossicologica, University of Bologna* and the ‡*Istituto di Farmacologia, University of Milan, Italy.* Accepted for publication May 23, 1983.

Abstract □ The synthesis of two nitrogen mustard derivatives (VIII and IX) related to the well-known local anesthetic ketocaine (III) is reported. These compounds were tested in mice implanted with Ehrlich ascites tumor cells, and the antitumor activity was compared with that of two previously synthesized analogues (I and II) lacking the nitro group and with that of doxorubicin. The monofunctional compound IX was inactive, but the bifunctional compound VIII showed potent antitumor activity (%T/C > 254 at 20 mg/kg).

Keyphrases □ Antitumor agents—potential, nitrogen mustard derivatives, Ehrlich ascites carcinoma screen □ Nitrogen mustard derivatives—antitumor agents, potential, Ehrlich ascites carcinoma screen

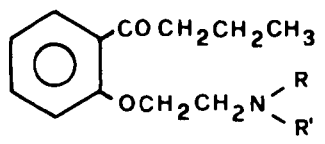
In a previous paper of this series (1), we reported the synthesis of I and II, two analogues of the well-known local anesthetic ketocaine (III). This work was based on the hypothesis that the *in vitro* antimitotic activity of ketocaine is related to its effect on oxygen consumption by tissues with prevailing anaerobic metabolism; this, in turn, demonstrates the ability of ketocaine to pass through the cell membrane (2). While ketocaine does not show antitumor activity, I and II were active (1). On the other hand, a series of butyrophenone derivatives showed that ketocaine and one of its nitro derivatives had analogous behavior to oxygen consumption (3). This study describes the nitration of *o*-hydroxybutyrophenone and the subsequent alkylation of the nitro derivative with tris(2-chloroethyl)amine hydrochloride, as previously described (1). The antitumor activity and the effect on oxygen consumption of these substances were compared with those of the previously described I and II.

RESULTS AND DISCUSSION

The nitration of *o*-hydroxybutyrophenone always gave a mixture of IV–VI. These materials were separated by column chromatography and identified by means of spectrometric data (see *Experimental Section*). The reaction between the nitro derivative (IV) and tris(2-chloroethyl)amine hydrochloride (VII) gave VIII and IX, as well as X (Scheme I). Compounds VIII and IX were tested, as the hydrochlorides, for antitumor activity.

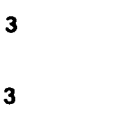
The bifunctional compound VIII inhibited tumor growth and prolonged the life span of mice bearing Ehrlich ascites carcinoma beyond that of the untreated animals. The %T/C at 4 mg/kg ip was 144, and it was >254 at 20 mg/kg. The monofunctional compound IX was inactive. Table I reports the antitumor activity of VIII and IX compared with that of the previously reported I and II (1) and doxorubicin.

Compounds I, II, VIII, and IX also affected the oxygen consumption of tumor cells. Figure 1 shows a difference in the respiratory inhibition that may be related to the different lipophilicity of the four compounds: in fact respiratory inhibition is a linear function of lipophilicity in a series of 4-hydroxyquinoline-3-carboxylic acids (4). The potent antitumor activity of VIII under the experimental conditions employed is evident. From the results so far available it appears that VIII is also active against other experimental tumors.

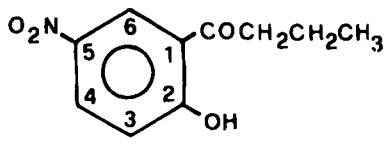


I R = R' = $-CH_2CH_2Cl$

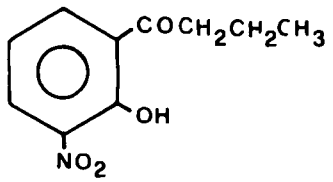
II R = $-CH_2CH_2Cl$; R' = $-CH_2CH_2O-$ (with $COCH_2CH_2CH_3$)



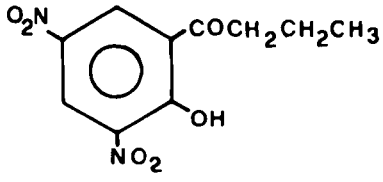
III R = R' = $-CH(CH_3)_2$



IV

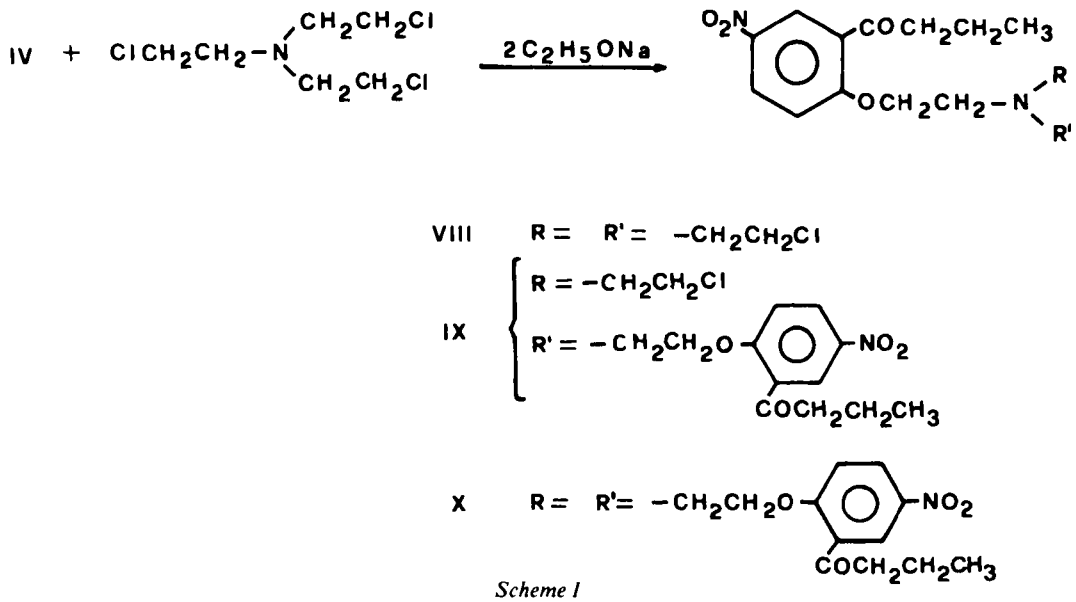


V



VI

0022-3549/84/0800-1175$01.00/0



Scheme I

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

Compound	Dose on Day 1, mg/kg ip	n^a	MST[b]	%T/C[c]	LTS[d]
Control[e]	—	10	16.0	—	
I	1	5	15.0	93.7	
	5	5	23.8	148.7	
	20	5	†[f]	—	
II	10	5	19.6	122	
	50	5	27.4	171.2	
	200	5	†[f]	—	
VIII	4	10	23.1	144.3	
	20	10	40.7	>254	4
	100	10	†[f]	—	
IX	20	10	16.6	103.7	
	100	10	15.6	97.5	
	200	10	17.6	110	
Doxorubicin	1	10	36.6	>228	3

[a] Number of animals per group. [b] Mean survival time (d). [c] %T/C ≥125 denotes significant activity. [d] Long-term survivors (>60 d). [e] Saline. [f] †— Death due to drug-related toxicity.

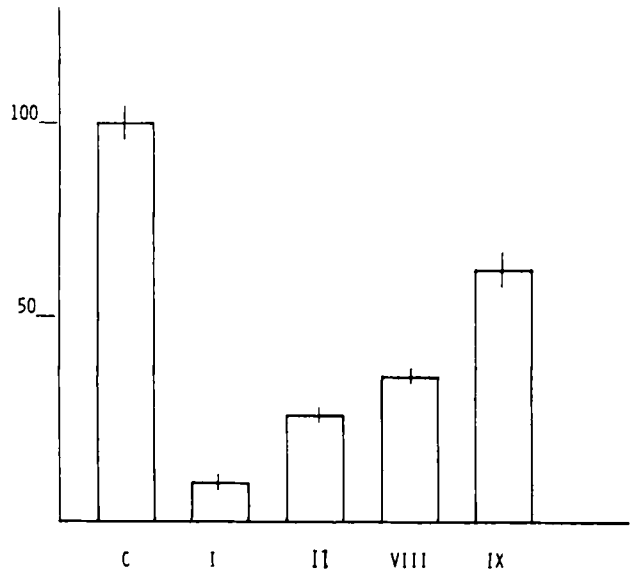


Figure 1—*Effect of oxygen consumption induced in Ehrlich ascites tumor cells by I, II, VIII, and IX at 50 μg/10⁶ cells. Controls (C) = 100 (means of at least six determinations).*

EXPERIMENTAL SECTION[1]

Chemistry—*Nitration of o-Hydroxybutyrophenone*—A cold solution of 90% HNO_3 (5.2 mL, 110 mmol) in acetic anhydride (15 mL) was slowly added with stirring to a cold solution of *o*-hydroxybutyrophenone (14.8 g, 90 mmol) in 20 mL of acetic anhydride. The temperature was maintained below 15°C. The mixture was then stirred for 1 h at room temperature and poured onto ice. A densitometric TLC analysis of the precipitate gave the following percentages: 60% IV, 30% V, and 10% VI similar results were obtained by using acetic acid or sulfuric acid in place of acetic anhydride. The three compounds were isolated by means of column chromatography.

2-Hydroxy-5-nitrobutyrophenone (IV)—Compound IV was obtained as yellow needles, mp 108–110°C (petroleum ether). IR (nujol): 3080, 1638, and 810 cm^{-1}; ^{1}H-NMR: δ 8.85 (d, 1, J = 3.0 Hz, H-6); 8.43 (q, 1, J = 9.6 Hz, 3.0 Hz, H-4); 7.15 (d, 1, J = 9.6 Hz, H-3); and 3.15, 1.83, and 1.06 ppm (7, $—CH_2CH_2CH_3$).

Anal.—Calc. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.70; H, 5.31; N, 6.69.

2-Hydroxy-3-nitrobutyrophenone (V)—Compound V was obtained as yellow needles, mp 43–45°C (petroleum ether). IR (nujol): 3080, 1642, and 1030 cm^{-1}; ^{1}H-NMR: δ 8.26 (q, 1, J = 8.0 Hz, 2.0 Hz, H-6); 8.20 (q, 1, J = 9.0 Hz, 2.0 Hz, H-4); 7.10 (q, 1, J = 9.0 Hz, 8.0 Hz, H-5); and 3.08, 1.83, and 1.03 ppm (7, $—CH_2CH_2CH_3$).

Anal.—Calc. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.18; H, 5.35; N, 6.60.

2-Hydroxy-3,5-dinitrobutyrophenone (VI)—Compound VI was obtained as yellow needles, mp 118–120°C (ethanol). IR (nujol): 3070, 1645, and 1610 cm^{-1}; ^{1}H-NMR: δ 8.96 (d, 2, J = 2.4 Hz, H-4 + H-6) and 3.15, 1.83, and 1.06 ppm (7, $—CH_2CH_2CH_3$).

Anal.—Calc. for $C_{10}H_{10}N_2O_6$: C, 47.25; H, 3.96; N, 11.02. Found: C, 47.08; H, 3.76; N, 10.82.

Synthesis of VIII–X—Compound IV (6.3 g, 30 mmol), dissolved in 60 mL of absolute ethanol, was added to a solution of C_2H_5ONa (60 mmol) in absolute ethanol (50 mL). Tris(2-chloroethyl)amine hydrochloride (VII; 7.3 g, 30 mmol) was then added with stirring. The mixture was refluxed for 5 h, and then the solvent was removed under reduced pressure. The residue was extracted with chloroform. The organic phase was washed with water, dried (Na_2SO_4), and the solvent was removed under reduced pressure. A densitometric TLC analysis of the residue gave the following percentages: 35% (IV), 40% (VIII), 20% (IX), and 5% (X). The four compounds were separated by column chromatography and VIII and IX were converted into the corresponding hydrochlorides.

N-[2-(2-Butanoyl-4-nitro)phenoxyethyl]-N,N-bis(2-chloroethyl)amine Hydrochloride (VIII-HCl)—The compound was a white powder, mp 113–115°C (absolute ethanol); IR (KBr): 1695, 1610, and 1580 cm^{-1}.

[1] Melting points are uncorrected. Bakerflex plates (silica-gel IB2-F) were used for the TLC. For column chromatography Kieselgel 60 (Merck) was used, activated at 120°C for 2 h, in the proportion of 40 g/g of mixture; the eluant was petroleum ether (bp 60–80°C)/acetone (80:20). The IR spectra were recorded on a Perkin-Elmer 298 spectrometer. The ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Varian XL-100 instrument using Me_4Si as internal standard.

Anal.—Calc. for $C_{16}H_{22}Cl_2N_2O_4 \cdot HCl$: C, 46.45; H, 5.60; N, 6.77. Found: C, 46.46; H, 5.70; N, 6.59.

N,N-*Bis*[*2-(2-butanoyl-4-nitro)phenoxyethyl*]-N-(*2-chloroethyl*)*amine Hydrochloride* (*IX·HCl*)—The compound was a white powder, mp 160–165°C (absolute ethanol); IR (KBr): 1685, 1605, and 1580 cm^{-1}.

Anal.—Calc. for $C_{26}H_{32}ClN_3O_8 \cdot HCl$: C, 53.25; H, 5.67; N, 7.16. Found: C, 53.49; H, 5.83; N, 7.05.

N,N,N-*Tris*[*2-(2-butanoyl-4-nitro)phenoxyethyl*]*amine* (*X*)—Compound X was a white powder, mp 87–90°C (ethanol); IR (KBr): 1670, 1605, and 1580 cm^{-1}.

Anal.—Calc. for $C_{36}H_{42}N_4O_{12}$: C, 59.82; H, 5.86; N, 7.75. Found: C, 59.56; H, 5.80; N, 7.63.

Pharmacology—*Antitumor Test*—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 h the animals were treated with VIII (4, 20, or 100 mg/kg ip) or IX (20, 100, or 200 mg/kg ip) dissolved in dimethyl sulfoxide; the amount of dimethyl sulfoxide, previously used in analogous experiments, did not affect tumor growth. Doxorubicin (1 mg/kg ip) was used as a positive control. Deaths were recorded for a period of 60 d. The activity was measured as the ratio of the mean survival time of the test animals to that of the control expressed as a percentage (%T/C). Significant activity is achieved with an increased life span of 25% (T/C ≥ 125).

Respirometric Test—Oxygen consumption by Ehrlich ascites tumor cells was performed by placing in each flask of a Warburg's apparatus 4×10^7 Ehrlich carcinoma cells suspended in 1.8 mL of Ringer's solution and 0.2 mL of distilled water or the same volume of solution of the test compound (the final concentration in each flask was 1 mg/mL). Ehrlich ascites carcinoma cells were obtained from the ascitic fluid removed from mice 7–9 d after transplantation (female Swiss mice with weekly transplantations of 10^6 cells). Viability of cells was assayed by trypan blue dye test. Flasks containing the cell suspension were equilibrated for 10 min and respiration was measured for 60 min (temperature: 37°C; gas phase: air).

REFERENCES

(1) A. Andreani, G. Scapini, I. Galatulas, and R. Bossa, *J. Pharm. Sci.*, **72**, 814 (1983).

(2) R. Bossa, F. Dubini, I. Galatulas, and M. Taniguchi, *Arch. Ital. Pat. Clin. Tumori*, **13**, 135 (1970).

(3) F. Ardizzone, I. Galatulas, A. Longhi, and P. Pomarelli, *Arzneim.-Forsch.*, **20**, 1794 (1970).

(4) E. A. Coats, K. J. Shah, S. R. Milstein, C. S. Genther, D. M. Nene, J. Roesener, J. Schmidt, M. Pleiss, E. Wagner, and J. K. Baker, *J. Med. Chem.*, **25**, 57 (1982).

ACKNOWLEDGMENTS

Presented in part at the XXI Congress of the Italian Pharmacological Society in Naples, June 1982.

Gas Chromatographic–Mass Spectrometric Assay for the Ultra-Short-Acting β-Blocker Esmolol

CHECK Y. SUM ˣ and AVRAHAM YACOBI *

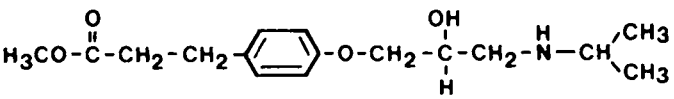
Received November 15, 1982, from *Research and Development, American Critical Care, McGaw Park, IL 60085.* Accepted for publication May 19, 1983. *Present address: Lederle Laboratories, Pearl River, NY 10965.

Abstract □ Esmolol is an ultra-short-acting β-blocker currently in Phase II clinical trials. The ester functionality in esmolol results in rapid metabolism of the β-blocker into an acidic metabolite and methanol. Dichloromethane was used to denature blood esterases and quantitatively extract esmolol from the blood. A deuterated analogue of esmolol was selected as the internal standard, and both compounds were chromatographed as the trimethylsilyl derivatives. Blood levels of esmolol were quantitated by gas chromatography–mass spectrometry with selective-ion monitoring, focusing on specific ions corresponding to esmolol and the internal standard. The lower limit of sensitivity of the assay was 2.5 ng/mL. Using the assay, blood samples from a dose-ranging study in humans were analyzed for concentrations of esmolol. Steady-state blood levels of esmolol after intravenous infusion rates of 40, 100, 200, 300, 450, and 650 μg/kg/min were 0.202, 0.464, 0.977, 1.31, 1.92, and 2.97 μg/mL of blood. The elimination $t_{1/2}$ and total body clearance were estimated to be ~10 min and 220 mL/kg/min, respectively. The high clearance of esmolol suggested that metabolism by blood esterase(s) was the primary determinant of the duration of action of the drug.

Keyphrases □ Esmolol—GC-MS, ultra-short-acting β-blocker □ Beta-blocker—ultra-short-acting, esmolol, GC-MS.

The concept and advantages of ultra-short-acting β-adrenergic receptor blockers have been presented recently (1). Briefly, in critical care settings like emergency rooms and intensive cardiac-care units, an ultra-short-acting β-blocker would be superior to conventional β-blockers with long durations of action, since therapeutic effects of the drug can be altered rapidly in either direction to meet changing cardiovascular responses.

Development of esmolol, methyl-3-[4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl]propionate hydrochloride, as a novel ultra-short-acting β-blocker is based on the enzymatic instability of an ester function and incorporation of this functionality into a chemical structure that has β-blocking properties. Hydrolysis of the ester linkage in esmolol by blood and tissue esterases transforms the β-blocker into an acidic metabolite and methanol.



ESMOLOL

Esmolol has been shown to be cardioselective and to possess a duration of action of ~15 min in dogs (1). Since blood levels of esmolol were anticipated to decrease very rapidly after stopping administration of the drug, a sensitive assay was needed in order to conduct detailed preclinical and clinical pharmacodynamic and pharmacokinetic studies. This report describes a gas chromatographic–mass spectrometric (GC-MS) method for the quantitation of esmolol in whole blood. Application of the method to the analysis of blood samples from a dose-ranging study in humans is also presented.

EXPERIMENTAL SECTION

Chemicals—Esmolol and the deuterated internal standard, methyl-3-[4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl][1,2-$^2H_2$]propionate hydrochloride, were synthesized in-house[1]. Dichloromethane[2] was spectro grade.

[1] Medicinal Chemistry Section, American Critical Care, McGaw Park, Ill.
[2] Matheson, Coleman, and Bell, Norwood, Ohio.

0022-3549/84/0800-1177$01.00/0