under physiological conditions, also proved ineffective as inhibitors of the Walker carcinoma in rats.[10] Since the iodoacetyl grouping reacts more readily than does the nitrogen mustard grouping with thiol groups, the ineffectiveness against the Walker tumor of the iodoacetyl derivative (VI) and of *N,N'*-bis(2-iodoacetyl)-*o*-phenylenediamine[11] may be related to an extensive reaction with extracellular or extranuclear thiol groupings, thus preventing their reaction at the required site of action, assuming this to be DNA and its associated macromolecules.

Experimental Section†

N-(2-Chloroethyl)-*N*-(2-hydroxyethyl)aniline (I). A solution of *N,N*-bis(2-chloroethyl)aniline[5] (5 g) in CH_3CN-H_2O (3:2 v/v) (50 ml) was refluxed for 3 hr, then concd under reduced pressure. The concentrate was partitioned between HCl (1 *N*, 50 ml) and Et_2O (50 ml). The organic phase contained starting material (3 g). The aqueous phase was treated with Et_2O (50 ml) and then with satd aqueous $NaHCO_3$ (*ca.* 50 ml) to neutrality. The dried ($MgSO_4$) organic phase was concd and applied to a column of silicic acid (25 cm × 3 cm^2) which was eluted with the same solvent (10-ml fractions). Concn of fractions 10–19, which contained the reqd product (R_f 0.6 on tlc in Et_2O; starting material, R_f 0.8), gave a colorless oil (1.13 g, 25%: 62% based on unrecovered starting material) which turned blue on exposure to light. *Anal.* ($C_{10}H_{14}ClNO$) C, H, Cl, N.

N-(2-Chloroethyl)-*N*-(2-hydroxyethyl)-4-hydroxyaniline (II). A soln of *N,N*-bis(2-chloroethyl)-4-hydroxyaniline (from the hydrochloride,[6] 10 g) in CH_3CN-H_2O (1:1 v/v) (100 ml) was refluxed for 2 hr, and the product isolated as for I (column 25 cm × 8 cm^2, product in fractions 21–56, R_f 0.45 in Et_2O; starting material, R_f 0.7). On crystn from PhH, the product formed pale pink needles, (3.35 g, 42%), mp 83–85°. *Anal.* ($C_{10}H_{14}ClNO_2$) C, H, Cl, N.

4-[4-*N*-(2-Chloroethyl)-*N*-(2-hydroxyethyl)aminophenyl]butyric Acid (III). A soln of 4-[4-bis(2-chloroethyl)aminophenyl]butyric acid[1] (5 g) in CH_3CN-H_2O (1:1 v/v) (100 ml) was refluxed for 2 hr. After concn and extn with Et_2O from a soln in 1 *N* HCl, the pH was adjusted to *ca.* 1.0 with 1 *N* NaOH. Further extn with Et_2O (50 ml) removed some product (III) together with all remaining starting material (which otherwise chromatographed in partial admixture with the product). The aqueous phase was further basified to pH 3.0 at which value the CO_2H group is still largely un-ionized and then extd with Et_2O (50 ml). The concd organic phase was chromatographed as for I (column 35 cm × 4 cm^2, product in fractions 8–35, R_f 0.35 in Et_2O; starting material, R_f 0.6). On crystn from *i*-Pr_2O (40 ml) at −15°, the product formed hemispherical clusters of colorless needles (0.82 g, 17%), mp 56–58°. *Anal.* ($C_{14}H_{20}ClNO_3$) C, H, Cl, N.

N-(2-Chloroacetoxyethyl)-*N*-(2-chloroethyl)aniline (IV). To a stirred soln of *N*-(2-chloroethyl)-*N*-(2-hydroxyethyl)aniline (2 g) in 2,6-lutidine (5 ml) at −10° was added dropwise chloroacetyl chloride (1.13 g). After 0.5 hr, the soln was stirred at room temperature for a further 2 hr, then poured into ice-cold dil H_2SO_4, and then extd with $CHCl_3$. The organic phase was washed (10% aqueous Na_2CO_3), dried ($MgSO_4$), and then concd. A soln of the concentrate in PhH was applied to a column of silicic acid (18 cm × 9 cm^2) which was eluted with the same solvent (5-ml fractions). On concn, the fractions containing the component of R_f 0.5 (tlc in PhH) gave a colorless oil (1.65 g, 60%), $n^{23.5}$D 1.5578°. *Anal.* ($C_{12}H_{15}Cl_2NO_2$) C, H, Cl, N.

N-(2-Bromoacetoxyethyl)-*N*-(2-chloroethyl)aniline (V). The title compound, prepared as for IV, using bromoacetyl chloride, was obtained as a colorless oil (2.08 g, 65%) of the same R_f in PhH, $n^{22.9}$D 1.5734°. *Anal.* $C_{12}H_{15}BrClNO_2$) C, H, Br, Cl, N.

N-(2-Chloroethyl)-*N*-(2-iodoacetoxyethyl)aniline (VI). To a stirred soln of the bromo derivative (V, 3.2 g) in Me_2CO (10 ml) was added a soln of NaI (2.3 g) in this solvent. NaBr separated during 0.5 hr. The filtered soln was concd, and a soln of the concentrate in PhH was chromatographed as for IV. On concn, the fractions containing the component of R_f 0.7 in PhH gave a slightly colored oil (2.13 g, 58%), $n^{21.8}$D 1.5875°. *Anal.* ($C_{12}H_{15}ClINO_2$) C, H, Cl, N; I: calcd, 34.5; found, 34.0.

†Melting points, which were corrected, were determined with a Kofler hot-stage apparatus. Merck Kieselgel G was used for column chromatography and for thin-layer chromatography (tlc) on coated microscope slides.

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ω-(*N,N*-Diethylamino)-*n*-alkyl 3,4,5-Trimethoxybenzoates as Local Anesthetics†

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A series of ω-(*N,N*-diethylamino)-*n*-alkyl 3,4,5-trimethoxybenzoates (TMB) were prepared by the esterification of the appropriate ω-(*N,N*-diethylamino)-*n*-alkanol with 3,4,5-trimethoxybenzoyl chloride (I) according to the general synthetic method described by Sastry and Lasslo.[2] These compounds have the structural characteristics of a typical local anesthetic:[3] a lipophilic part and a hydrophilic part connected by an intermediate chain. Therefore, they were tested for their local anesthetic activities.

Experimental Section‡

Synthetic Methods. Each TMB was prepd as a white cryst HCl salt and purified by recrystn from C_6H_6 unless otherwise stated.

2-(*N,N*-Diethylamino)ethyl 3,4,5-trimethoxybenzoate·HCl (II) was prepd from 10.6 g (0.05 mole) of I and 6.6 g (0.05 mole) of 2-(*N,N*-diethylamino)ethanol, yield 12.4 g (72%), mp 153–154.5° (lit.[4] 152–155°).

3-(*N,N*-Diethylamino)propyl 3,4,5-trimethoxybenzoate·HCl (III) was prepd from 10.6 g (0.05 mole) of I and 7.0 g (0.05 mole) of 3-(*N,N*-diethylamino)-1-propanol, yield 14.1 g (80%), mp 169.5–171° (lit.[5] 172°).

†A part of this investigation[1] was presented orally at the Meetings of the American Society for Pharmacology and Experimental Therapeutics in Pittsburgh, Pa., Aug 1969. This investigation was supported by a Graduate Traineeship to one of the authors (C.P.R.), Grant No. GZ-604 from the National Science Foundation; U. S. Public Health Service Training Grant No. GM 00058 from the National Institute of General Medical Sciences and U. S. Public Health Service Research Grant No. NS-04699 from the National Institute of Neurological Diseases and Stroke.

‡Microanalysis was reported by International Chemical and Nuclear Corp., City of Industry, Calif. Analytical results obtained were within ±0.4% of the theoretical values. All melting points were uncorrected.

4-(*N,N*-Diethylamino)butyl 3,4,5-trimethoxybenzoate-HCl (IV) was prepd from 10.6 g (0.05 mole) of I and 7.3 g (0.05 mole) of 4-(*N,N*-diethylamino)-1-butanol, yield 14.1 g (70%), mp 138–139° (lit.[2] 139–140°).

5-(*N,N*-Diethylamino)amyl 3,4,5-trimethoxybenzoate-HCl (V) was prepd from 10.6 g (0.05 mole) of I and 7.6 g (0.05 mole) of 5-(*N,N*-diethylamino)-1-pentanol, yield 13.8 g (76%), mp 129–130° *Anal.* ($C_{19}H_{32}ClNO_5$) C, H, N.

6-(*N,N*-Diethylamino)hexyl 3,4,5-trimethoxybenzoate-HCl (VI) was prepd from 10.6 g (0.05 mole) of I and 7.8 g (0.05 mole) of 6-(*N,N*-diethylamino)-1-hexanol, and recrystd from Et_2O–C_6H_6, yield 12.7 g (69%), mp 96–97.5°. *Anal.* ($C_{20}H_{34}ClNO_5$) C, H, N.

Pharmacological Methods. The duration of surface anesthesia in the rabbit cornea,[6] the duration of infiltration anesthesia in the guinea pig skin,[7] and the blockade of conduction in the desheathed frog (*Rana pipiens*) sciatic nerve bundle[8,9] were determined according to the methods in literature with only minor modifications.

Results and Discussion

Surface Anesthesia in the Rabbit Cornea. All TMB's, procaine, and cocaine caused corneal anesthesia (Figure 1 and Table I). All TMB's except II and III were more potent than procaine. Cocaine was more potent than all TMB's except VI which was not different ($p > 0.05$) at the concentration tested (10 m*M*). Potency increased with increasing length of the connecting chain. Corneal anesthesia with procaine and IV has been reported previously.[10,11]

Lacrimation and pitting were observed with 10 m*M* III. Higher concentrations of TMB's and procaine caused signs of irritation (licking, lacrimation, and withdrawal movements on application).

Infiltration Anesthesia in the Guinea Pig Skin. All TMB's and procaine caused local anesthesia by this method (Figure 2 and Table I). Surface anesthesia by procaine was reported previously.[12,13] With TMB's, potency generally increased with the increasing length of the connecting chain. High concentration of TMB's and procaine given intracutaneously often caused excitation followed by hypoactivity, defecation, ptosis, and decreased reactivity to pin pricks on the posterior extremities.

Blockade of Impulse Conduction in the Frog Sciatic Nerve Bundle. TMB's and procaine decreased the height of the action potentials and slowed nerve conduction. Potency increased with increasing length of the connecting chain (Table I). Usually the height of the action potentials could be restored to control levels by washing. The time required for the restoration of the action potential increased with increasing length of the intermediate chain. With VI, washing even for 2 hr did not always result in complete recovery of the action potential. The blockade of nerve conduction by procaine has been previously demonstrated.[9,14,15]

It is evident from the above results that potencies for local anesthetic activities of TMB's increased with increasing length of the intermediate chain between the lipophilic part (trimethoxybenzoyl moiety) and the hydrophilic part (*N,N*-diethylamino radical). The chloroform-aqueous sodium phosphate buffer partition coefficients of TMB's were higher than that of procaine (Table I). They increased with increasing length of the intermediate chain. These observations suggest that high lipoid solubilities of TMB's would contribute significantly to their high local anesthetic potencies.

Table I. Local Anesthetic Activities and Lipoid Solubilities of ω-(*N,N*-Diethylamino)-*n*-alkyl 3,4,5-Trimethoxybenzoates, Procaine, and Cocaine[a]

Compd	Duration of anesthesia, min, resulting from the application of 10 m*M* (cornea) or 30 m*M* (skin) soln		Time, min, required for 50% reduction in the action potential in the frog sciatic nerve bundle after 1 m*M* soln	Partition coefficients between chloroform and aqueous sodium phosphate buffer[b]
	Rabbit cornea	Guinea pig skin		
II	5 ± 2.3	18 ± 2.4	126 ± 18.2	21.6 ± 3.2
III	9 ± 1.6	21 ± 2.1	118 ± 5.0	55.2 ± 4.7
IV	27 ± 3.8	27 ± 1.6	91 ± 3.5	97.3 ± 4.6
V	33 ± 4.6	34 ± 3.7	70 ± 3.5	>200.0[c]
VI	37 ± 4.1	55 ± 4.6	34 ± 3.7	>200.0[c]
Procaine	11 ± 5.5	19 ± 3.2	334 ± 4.7	14.1 ± 0.2
Cocaine	43 ± 5.4			

[a] Values were mean ±SE from 5 to 8 experiments. [b] Each compound (2.0 μmoles) was partitioned between equal volumes (20 ml) of chloroform and 0.1 *M* sodium phosphate buffer. Procaine was analyzed by the uv spectrophotometric method described by Kalow.[16] A similar method was used for the determination of TMB's. [c] TMB's V and VI were highly soluble in chloroform and gave insignificant standard errors.

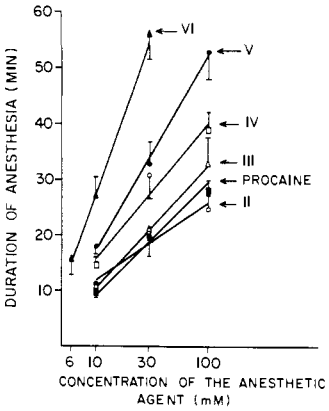

Figure 2. Duration of anesthesia of the guinea pig skin following intracutaneous injection of ω-(*N,N*-diethylamino)-*n*-alkyl 3,4,5-trimethoxybenzoates and procaine. Each mean is from 6 expt. Each vertical bar represents one SE.

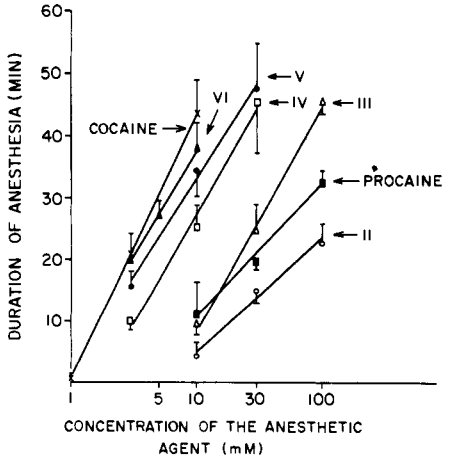

Figure 1. Duration of anesthesia of the rabbit cornea following topical administration of ω-(*N,N*-diethylamino)-*n*-alkyl 3,4,5-trimethoxybenzoates, procaine, and cocaine. Each mean is from 6 expt. Each vertical bar represents one SE.

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Antitumor Activity of Some Derivatives of Daunorubicin at the Amino and Methyl Ketone Functions†

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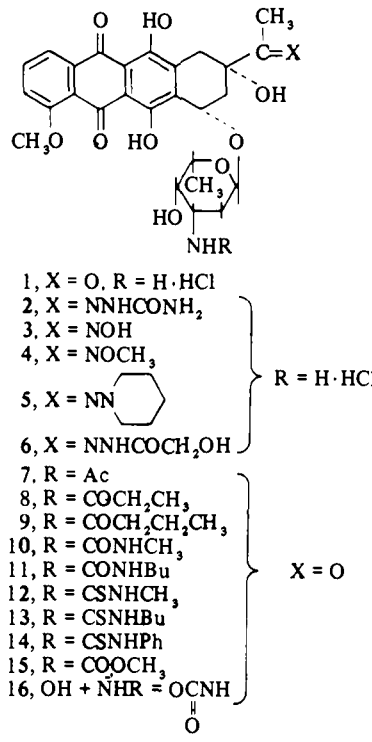
The antitumor-antibiotic daunorubicin‡ (1) is a clinically effective agent for remission induction in acute leukemia and for treatment of other kinds of cancer.[1-3] Antitumor activity has also been reported for three derivatives of daunorubicin. The analogous hydroxymethyl ketone adriamycin (1, with $COCH_2OH$ in place of $COCH_3$) was reported to be superior to 1 in clinical trial[4] as well as in tests on experimental tumors in mice and rats.[5,6] In addition, the semicarbazone **2** and thiosemicarbazone have been reported in the patent literature[7] as antitumor agents. Since daunorubicin and adriamycin both show toxic side effects, additional analogs are of considerable interest for either greater activity or reduced toxicity.

Synthesis. Compds **2-16** were all prepared directly from daunorubicin by use of the standard reagents for derivatizing amines and ketones, in procedures modified to allow for the low solubility and limited stability of daunorubicin. It was often convenient to start from daunorubicin as the free base, which could be isolated from the HCl salt and stored in the cold for several weeks without deterioration. The ketone derivatives **2-6** were isolated as the mono-HCl salts, generally after extraction of the corresponding free bases and treatment with equivalent amounts of HCl. Physical properties of *N*-acetyldaunorubicin (**7**) have been described,[8] but without the details of its preparation.§ Some biological properties of the oxime **3** have been reported,[9] but with no mention of its synthesis or physical properties.

Biological Data. The compounds were evaluated for antitumor and/or cytotoxic properties by the Drug Research and Development Branch of the National Cancer Institute



(formerly the Cancer Chemotherapy National Service Center) according to its protocols.[10] Initial tests have employed leukemia L1210 in mice and, in some cases, KB cells in culture. All the ketone derivatives (**2**, **3**, **5**, **6**), except the *O*-methyl oxime **4** and three (**7**, **13**, **16**) of the ten amine derivatives, gave positive results in one of these systems. Table I shows results against L1210 with a 3-injection schedule. The *N*-piperidinoimine **5** was active at doses comparable with those of daunorubicin **1**. The semicarbazone **2** and oxime **3** were active at somewhat higher doses.

The most extensive tests with any single compound have been done with the acetamide **7**. Results in Table II show the activity of **7** in varied regimens, and the importance of dose timing and dose level in these evaluations. Although **7** is less efficacious than **1**, it displayed no acute toxicity under any of the regimens given in Table II. In contrast, the LD_{50} for daunorubicin in the mouse is 6-12 mg/kg (single ip injection).[2] These results are also of considerable interest because acetamide **7** is devoid of the deleterious effects on coronary vasculature (isolated dog heart) that are characteristic of daunorubicin and adriamycin.[11] This suggests that **7** or other derivatives of daunorubicin may be free of the cardiac toxicity that has been associated with clinical use of the parent antibiotic.[2,3] The homologous amides **8** and **9** were inactive against L1210, but the tests to date have been mostly at lower dosages than for **7** (see Table I for **8**; **9** was inactive at doses up to 4 mg/kg in 9 daily injections). Because a limited quantity of *N*-carboxy δ-lactam **16** was available, it was tested with only a single-dose regimen. However, substantial activity was observed against L1210; at 200 mg/kg the *T/C* ratio was 148%, and at 400 mg/kg the *T/C* was 132%.

In addition to the L1210 results, activity against KB cells in culture was confirmed for the glycoloylhydrazone **6** ($ED_{50} < 0.63$ μg/ml) and for the *N*-butylthiourea **13** (ED_{50} 0.49 μg/ml).

These preliminary results demonstrate that significant levels of antitumor activity are retained among derivatives of daunorubicin. Further evaluation of these substances is

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‡Previously called daunomycin or rubidomycin.

§The authors are indebted to Dr. James E. Christensen for the initial preparation of *N*-acetyldaunorubicin in our laboratories.