anhyd Et_2O was added dropwise to a mixt of 0.3 g (0.008 mole) of LAH and 150 ml of anhyd Et_2O. The mixt was refluxed for 3 hr. After cooling, 2 ml of H_2O, 4 ml of 5 *M* NaOH, and 6 ml of H_2O were added, and the mixt was stirred vigorously for 20 min. The Et_2O was decanted from the solid $Al(OH)_3$, which was washed with 2 × 100 ml of Et_2O. The Et_2O solns were dried ($MgSO_4$) and evapd to give 1.90 g of a colorless oil. Purification on a column of 80 g of neutral Al_2O_3 (Woelm), activity grade III, and elution with 500 ml of C_6H_6, 500 ml of C_6H_6-Et_2O (1:1), and 250 ml of Et_2O afforded 1.75 g (91%) of a colorless oil: bp 144° (0.7 mm).

Bis(3-cyanopropyl)amine was prepd by a modification of the procedure of Iorio, *et al.*[15] 4-Chlorobutyronitrile and liq NH_3 were kept in an autoclave for 3.5 days. After work-up including fractional distn and sepn on a neutral Al_2O_3 column, 2.74 g of a colorless oil was obtd (bp 120–128° (0.3 mm) (vapor temp)]. Tlc showed 1 major and 2 minor spots. Prepn of the hydrochloride in Et_2O and recrystn from abs EtOH gave 2.78 g of white needles: mp 172–173°. *Anal.* ($C_8H_{14}ClN_3$).

The base was liberated by addn of 10 *M* NaOH, the mixt was satd with anhyd K_2CO_3, extd with 3 × 50 ml of $CHCl_3$, dried ($MgSO_4$), and evapd to give 2.19 g (15%) of a colorless oil: homogeneous on tlc; bp 121–124° (0.2 mm) (vapor temp) [lit.[15] 119–122° (0.12 mm)].

N,N-Bis(3-cyanopropyl)hexadecanamide was prepd from bis(3-cyanopropyl)amine and palmitoyl chloride (99% yield) following the general procedure. The colorless crystals were recrystd from EtOH-H_2O: mp 66–68°; bp 250° (0.1 mm). *Anal.* ($C_{24}H_{43}N_3O$).

N,N-Bis(4-aminobutyl)hexadecanamide (36). A soln of 0.60 g (0.0015 mole) of *N,N*-bis(3-cyanopropyl)hexadecanamide in 300 ml of AcOH was hydrogenated with 0.225 g of 30% Pd/C as catalyst. The H_2 pressure was kept at 4.2 kg/cm² for 2.5 days. Evapn of the solvent gave an oil, which was dissolved in 10 ml of H_2O and extd with 15 ml of $CHCl_3$ to remove unreacted starting material. The aq soln was made alk by dropwise addn of 10 *M* NaOH, satd with anhyd K_2CO_3, and extd with 3 × 40 ml of $CHCl_3$. After drying ($MgSO_4$) and evapn, 0.72 g of amorphous solid was obtd. The solid was dissolved in Et_2O and treated with an Et_2O soln of oxalic acid, whereupon the dioxalate salt sepd. Recrystn from abs EtOH-Et_2O yielded (16%) colorless crystals, mp 188–190° dec. *Anal.* ($C_{28}H_{55}N_3O_9$).

Bismethiodide Salt of *N,N*-Bis(4-dimethylaminobutyl)dodecanamide (37). To 0.50 g (0.0013 mole) of *N,N*-bis(4-dimethylaminobutyl)dodecanamide in 50 ml of abs EtOH was added 3.55 g (0.025 mole) of MeI. The mixt was stirred for 24 hr at room temp. Anhyd Et_2O was added and a yellow-white ppt was formed. The ppt was washed thoroughly with anhyd Et_2O. After drying in a desiccator 0.65 g (76.5%) of yellow crystals was obtd: mp 126–128°. Tlc on a silica plate in MeOH-Me_2CO-2 *N* HCl-AcOH (14:3:6:3) showed 1 spot. *Anal.* ($C_{26}H_{57}I_2N_3O$).

Bismethiodide Salt of Solapalmitine (38). Solapalmitine was quaternized as above and gave light yellow crystals in 67.4% yield: mp 234–235°. Tlc as above showed one spot. *Anal.* ($C_{30}H_{65}I_2N_3O$).

N-(4-Dimethylaminobutyl)butyramide was prepd from 1.16 g (0.01 mole) of 4-dimethylaminobutylamine (K&K 3044) and 1.17 g (0.011 mole) of PrCOCl in 100 ml of Et_2O in the presence of 10.1 g (0.10 mole) of Et_3N according to the general procedure described above. After purification on an Al_2O_3 column, 1.30 g (70%) of a colorless oil was obtd: bp 115° (0.5 mm). The monooxalate salt was prepd in Et_2O: mp 125–126°. *Anal.* ($C_{12}H_{24}N_2O_5$).

N-Butyl-*N*-4-dimethylaminobutylamine was obtd by LAH reduction of *N*-(4-dimethylaminobutyl)butyramide according to the general procedure. After purification on neutral Al_2O_3, a colorless oil (49%) was obtd: bp 75° (1.0 mm). The dioxalate salt was prepd in Et_2O: mp 152–154° dec. *Anal.* ($C_{14}H_{28}N_2O_8$).

N-Butyl-*N*-(4-dimethylaminobutyl)hexadecanamide (39) was synthesized from *N*-butyl-*N*-4-dimethylaminobutylamine and palmitoyl chloride in 72% yield according to the general procedure. The colorless oil had bp 171° (0.4 mm). *Anal.* ($C_{26}H_{54}N_2O$).

N,N-Di-*n*-butylpalmitamide (40). Acylation of *n*-Bu_2NH (1.42 g) with palmitoyl chloride (1.38 g) in anhyd Et_2O (200 ml) by the usual procedure, and chromatography on Al_2O_3 (100 g, Woelm, grade III) gave a homogeneous colorless oil (1.86 g). The oil had bp 235° (1.5 mm). *Anal.* ($C_{24}H_{49}NO$).

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Mixed Bifunctionality. 4. Antitumor Activity of Alkylating Derivatives of Polycyclic Aromatic Hydrocarbons as a Function of Structure and of Vehicle†

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The activity of this class of hydrophobic antitumor agent is dependent on its *in situ* availability as well as on the geometry of the aromatic moiety and to a lesser extent on the reactivity of the alkylating function. 9,10-Bis(chloromethyl)anthracene is curative for the Ehrlich ascites mouse tumor at a total dosage of less than two-thirds of a microgram when given in the colloidal state.

Simple chloromethyl derivatives of polynuclear aromatic hydrocarbons are extremely potent antitumor agents. We have previously shown marked increases in potency following administration of these solutions in sesame oil over those given as fine saline dispersions.[1] Other vehicles were considered, partly with a view to finding a procedure adaptable to intravenous injection.

An emulsion in saline was found to be stabilized for hours (or longer, as a function of concentration) by a minimal amount of sesame oil. As a measure of agent availability *in situ* in such dispersions, testing of a representative group of previously tested chloromethyl hydrocarbons and of some

†Supported in part by Research Grants CA-06927 and RR-05539 from the National Institutes of Health, U. S. Public Health Service, and by an appropriation from the Commonwealth of Pennsylvania.

Table I. Emulsion-Dispersed Alkylating Agents *vs.* the Ehrlich Ascites Tumor

No.	Compd	Antitumor activity[a] Range, μmoles/kg	Degree	Yield, %	Mp, °C	Formula[b]
	A. Monofunctional					
1	9-Chloromethylanthracene	1-25 (0.5-15)	2.2			$C_{15}H_{11}Cl$
2	9-Bromomethylanthracene[c]	1-25	2.2			$C_{15}H_{11}Br$
3	10-Chloromethyl-9-methylanthracene	4-30[d] (1.5-50)	2.4			$C_{16}H_{13}Cl$
4	10-Bromomethyl-9-methylanthracene	5-30[d]	1.9	53	183-185.5	$C_{16}H_{13}Br$
5	7-Chloromethylbenz(*a*)anthracene	0.2-8 (0.2-4)	2.1			$C_{19}H_{13}Cl$
6	7-Bromomethylbenz(*a*)anthracene	0.4-15[d]	2.2	60	203-205 dec	$C_{19}H_{13}Br$
7	7-Chloromethyl-12-methylbenz(*a*)anthracene	0.4-20 (0.2-10)	2.3			$C_{20}H_{15}Cl$
8	7-Bromomethyl-12-methylbenz(*a*)anthracene	1-20[d] (0.4-8)	2.3	9	141-142	$C_{20}H_{15}Br$
9	1-Chloromethylpyrene	0.25-4 (0.1-6)	2.1			$C_{17}H_{11}Cl$
10	1-Bromomethylpyrene	0.4-6	1.8	31	134.5-137	$C_{17}H_{11}Br$
11	10-Chloromethyl-9-bromoanthracene	0.4-12	2.1	9	190-193	$C_{15}H_{10}BrCl$
12	10-Bromomethyl-9-chloroanthracene	0.4-15	2.1	83	182.5-184	$C_{15}H_{10}BrCl$
13	1-(10-Chloro-9-anthrylmethyl)aziridine[e]	(40-80)	2.0	6	~150	$C_{17}H_{14}ClN$[f]
14	1-(10-Methyl-9-anthrylmethyl)aziridine[e]			24	95-97	$C_{18}H_{17}N$
	B. Bifunctional					
1	9,10-Bis(chloromethyl)anthracene	0.03-4 (0.1-4)	2.4			$C_{16}H_{12}Cl_2$
2	9,10-Bis(bromomethyl)anthracene[g]	0.04-1.8[d]	2.2	56	>300	$C_{16}H_{12}Br_2$
3	9-Bromomethyl-10-chloromethylanthracene	0.03-3[d]	2.2	82	245-250 dec	$C_{16}H_{12}BrCl$
4	9-Bromomethyl-10-chloromethylphenanthrene[h]	Inactive				$C_{16}H_{12}BrCl$
5	9,10-Bis(chloromethyl)phenanthrene[i]	Inactive				$C_{16}H_{12}Cl_2$

[a]Values in parentheses, given for comparison, are activity ranges previously found for the same compound given as a sesame oil solution. These data and compound sources are given in the preceding paper.[1] [b]Compounds reported herein, for which yield and melting point are tabulated, were analyzed for C, H, and halogen, with the exception of 13 and 14 which were analyzed for C, H, and N. The results obtained were within ±0.4% of the theoretical values, except where noted. Melting points are uncorrected. [c]This compound was previously synthesized (*via* PBr_3) by Meek, *et al.*;[7] mp 137.5-142.5° dec. Our preparation (*via* HBr), obtained in 60% yield, melted at 137.5-139°. [d]Toxic levels were not reached because of emulsion instability at higher doses. [e]Compounds 13 and 14 were tested only in sesame oil due to the high levels necessary; 14 showed only borderline activity at 150-200 μmoles/kg. [f]C; calcd, 76.00; found, 75.28. [g]Previously synthesized by other methods; see reference 8. [h]Synthesized according to Stille and Foster.[9] [i]Synthesized according to Hauptmann.[10]

newly synthesized alkylating derivatives (Table I) has defined further structure-activity relationships and raised new questions. The new compounds in Table I were synthesized by conventional methods as given in the Experimental Section.

Experimental Section

10-Bromomethyl-9-methylanthracene. In a typical procedure for prepn of bromomethyl compounds in Table IA, except for 11 as described below, 100 ml of abs EtOH was added to a suspension of 3.0 g of 10-hydroxymethyl-9-methylanthracene in 150 ml of C_6H_6 and a rapid stream of dry HBr was passed in through a gas dispersion tube for 20 min. The soln was concd *in vacuo* to a small vol, cooled, and filtered, and the ppt was washed with EtOH and petr ether (30-60°) and dried to give 3.1 g of crude product. Crystn from C_6H_6-petr ether gave **4** in Table IA.

7-Bromomethyl-12-methylbenz(*a*)anthracene.‡ A soln of 2.3 g of 7-methoxymethyl-12-methylbenz(*a*)anthracene[3] in 100 ml of warm sodium-distd dioxane was stirred and 20 ml of 48% HBr was added. Stirring was contd, and the flask was warmed in an oil bath to 98-100° for 1.5 hr and cooled. The mixt was dild with H_2O and extd with Et_2O, and the extracts were washed, dried, concd, and cooled. The crude product was filtered (1.25 g) and crystd from C_6H_6-petr ether. An unknown side product of higher melting point formed in yellow rosettes hand separable from the massive crystals of product. (The rosettes, not identified, melted at 172-192° dec.). Two more crystallizations from benzene gave **8** in Table IA.

10-Chloromethyl-9-bromoanthracene. A mixture of 4 g of paraformaldehyde and 45 ml of AcOH was satd with dry HCl and added to a stirred suspension of 10 g of 9-bromoanthracene in 85 ml of AcOH. After stirring at 40-50° for 3.5 hr, the mixt was poured on ice and filtered. The product was washed, dried, and crystd from C_6H_6-petr ether to give only 1.9 g, mp 178-183°; a large proportion of 9-bromoanthracene was recoverable from the filtrate. Two recrystns were required to obtain **11** in Table IA.

1-(10-Methyl-9-anthrylmethyl)aziridine. To a stirred soln of 7.3 ml of aziridine and 9.5 ml of triethylamine in 75 ml of C_6H_6 cooled in an ice bath was added 10 g of 10-iodomethyl-9-methylanthracene,[4] which quickly dissolved. The mixt was stirred 1 hr without cooling, and the soln was decanted from an insol residue. After an addl 3 hr standing, it was concd *in vacuo*, taken up in 10 ml each of C_6H_6 and petr ether, filtd, and cooled with addn of more petr ether to give 3.5 g of product, mp 88-93°. Crystn from hexane gave **14** in Table IA. The corresponding 1-(10-chloro-9-anthrylmethyl)aziridine (**13**) was prepared correspondingly, in low yield, from 10-bromomethyl-9-chloroanthracene.

9-Bromomethyl-10-chloromethylanthracene. A mixt of 5.0 g of 10-chloromethyl-9-methylanthracene, 3.7 g of NBS, and 150 ml of warm CCl_4 was stirred and 50 mg of benzoyl peroxide was added. The mixture was refluxed 2.5 hr, stirred overnight without heat, partially concd, and cooled with addn of 100 ml of petr ether to give 8.4 g of product plus succinimide. Leaching with hot EtOH left a residue of 6.4 g (98%), mp 245-250°. A sample was recrystd from C_6H_6 to give 3 in Table IB. The same method was employed for prepn of **2** in Table IB.

Preparation of Saline-Dispersed Emulsion. A 2.8-mg sample of 9,10-bis(chloromethyl)anthracene was dissolved in approximately 7 ml of purified dioxane (sodium distilled), 0.1 ml of sesame oil was added, and dioxane was added up to 10 ml. One milliliter of this stock was slowly added through a 23-gauge needle to 99 ml of rapidly stirred saline. This was made up fresh each day; the stock was kept frozen. Emulsions so prepared then contained 1% dioxane, 0.01% sesame oil, and 0.28 μg/0.1 ml of test compound (0.04 μmole/kg based on a 25-g mouse). For more concd stock solutions, it might be necessary to warm the thawed stock to effect complete soln before prepn of the emulsion.

Results and Discussion

The protocol of antitumor testing and expression of data are given fully in the last paper,[1] and in previous publications. The *average* degree of effectiveness within the whole of the active range is based on a scale between 1.8 and 3.0.

It should be noted that control mice in the emulsion experiments had a mean survival time indistinguishable from those where the vehicle was saline. However, controls in the sesame oil solution experiments showed a shortened survival, apparently caused by alteration of the peritoneal environment in a manner conducive to a more rapid multiplication of tumor cells.[1] In this sense the emulsion vehicle gave re-

‡Also prepd (from the corresponding hydroxymethyl compound) by Pataki, *et al.*[2]

sults more comparable with previous experiments where water-soluble compounds were tested.[5]

Two salient features of the data in Table IA are: (1) comparisons between $BrCH_2$ and $ClCH_2$ pairs of compounds, and (2) comparisons between results obtained from sesame oil solution *vs.* those from an emulsion.

Comparing activities of $BrCH_2$ compounds with analogous $ClCH_2$ compounds gives a picture counter to that expected from the chemically more active Br, since the Br compounds (**2**, **4**, **6**, 8, **10**) are usually both *less potent* and *less toxic* than their Cl analogs (**1**, **3**, **5**, **7**, **9**). However, this is consistent with the apparent anomaly pointed out previously in comparing the active ranges of 7-iodomethyl-12-methylbenz(*a*)anthracene and 7-chloromethyl-12-methylbenz(*a*)anthracene.[1] Compound **8**, the $BrCH_2$ analog, has now been shown to be intermediate in potency. Under identical experimental conditions (administration in sesame oil solution), the minimum effective doses of the three analogs are: $ClCH_2$–0.2 μmole/kg; $BrCH_2$–0.4 μmole/kg; and ICH_2–0.8 μmole/kg.

This anomaly would support our physical picture of the biochemical mechanism of killing of tumor cell; that is, alkylation with this class of compounds does not occur appreciably *in vivo* until the hydrocarbon moiety has, as a first step, become incorporated into the DNA helix by intercalation.§ At this point, an intimate approach between nucleophilic groups in DNA and the XCH_2 alkylating group results in irreversible formation of a covalent bond (cross-linkage in a hybrid sense). Although the alkylating agents in clinical use are generally characterized (unfavorably) as *nonspecific* cytotoxic agents, *i.e.*, not dependent on cell cycle, agents such as these halomethyl hydrocarbons may well be more active during transcription and/or replication. Their mode of action, rather than cross-linkage as a result of two identical alkylating centers, is dependent on *mixed* bifunctionality. In this case intercalation, which relies on "available" DNA is required as the first step. Larger halogen atoms would only sterically impede the initial step of intercalation and react no more completely than the smaller Cl in the intimate environment resulting from intercalation.

Comparisons of sesame oil *vs.* emulsion administration present an interesting empirical relation. Examination of the range values *vs.* the parenthetical values in sesame oil in Table I shows that toxicities were comparable (where the toxic level was reached). Interestingly, the minimum effective dose was somewhat lower in sesame oil with *monofunctional* XCH_2 compounds whereas it was lower in emulsion with the most highly active *bifunctional* XCH_2 compounds. This means that the most potent compounds we have ever tested are further potentiated; mice can be cured with 1 in Table IB with a total dose of less than two-thirds of a *microgram.*

The very active 9,10-bis(halomethyl)anthracenes (1, **2**, and **3** in Table IB) assume a unique position in this study of vehicle influence. Whereas all compounds tested have been potentiated by administration in sesame oil compared with saline suspension, only these compounds show a further potentiation when given in an aqueous emulsion.

The two bifunctional halomethyl compounds (**4** and **5** in Table IB) showed no more than borderline activity up to 75 μmoles and 20 μmoles, respectively, in sesame oil compared with a comparable activity range of 10–250 μmoles for 10-chloromethyl-9-methylphenanthrene.[1] This contrasts with the manyfold potentiation achieved by addition of a second chloromethyl group in the para position of anthracene. Further models must be tested to see whether an optimum spatial separation of alkylating groups can be attained, possibly corresponding to a complementary spatial separation of nucleophiles in the DNA structure.

The two aziridine derivatives (**13** and **14** in Table IA) gave only borderline activity at relatively high dosages in sesame oil; suitable concentrations could not be attained in an emulsion.

In an experiment not shown in the Table, **1** in Table IB was administered intravenously to tumor-bearing mice in dosages from 0.04 μmole/kg to 5 μmoles/kg without curative effect; toxicity occurred at 2 μmoles/kg. It appears that little if any of such hydrophobic molecules penetrate the peritoneal wall; even a few per cent would suffice in view of the very large therapeutic index exhibited by this compound.

§ This is also our physical picture of the mechanism for those monofunctional alkylating agents showing unique mutagenic properties; see reference 6.

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