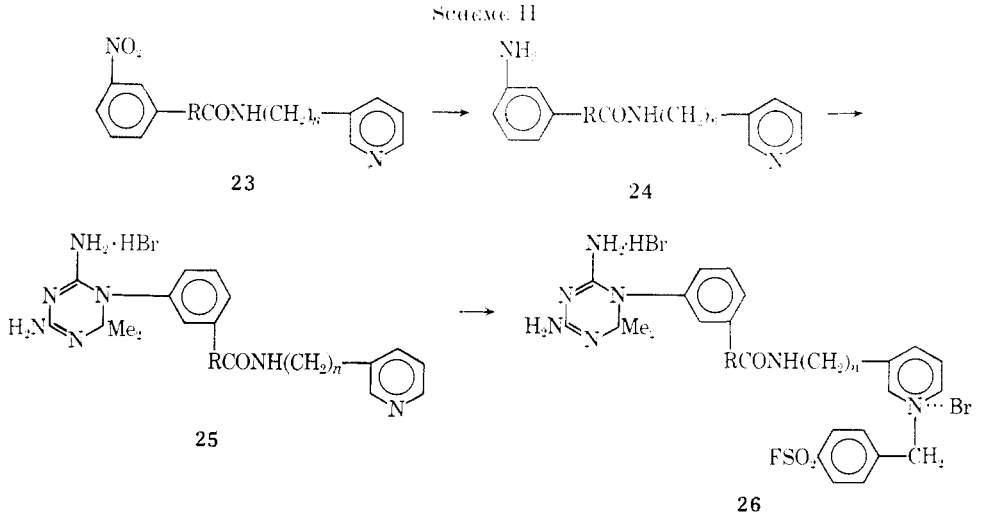
Scheme II



of the gum in hot *i*-PrOH, then cooling gave an amorphous solid that was collected by centrifugation and immediately dried over P_2O_5 *in vacuo;* yield, 1.00 g (64%), of white solid that gradually decomposes over 130° without melting. Two more precipitations from hot *i*-PrOH by cooling gave 0.7 g (42%) with no definite melting point; tlc on polyamide with EtOH showed one spot. See Table V for additional data and other compounds prepared in this way.

Mixed Bifunctionality. II. Relation of Antitumor Activity to Structure in Alkylating Agents Derived from Polynuclear Aromatic Hydrocarbons[1]

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Received August 18, 1969

Investigations of the antitumor properties of a variety of polynuclear aromatic hydrocarbon derivatives containing a single alkylating function have been continued. The existence of a parallelism between the carcinogenic and carcinostatic potentialities of several anthracene and benzanthracene moieties is indicated from studies with their S and N mustard derivatives. Various halomethyl aromatic hydrocarbons have also been found to be highly effective against ascites tumors in mice.

Antitumor activity due to mixed bifunctionality has been shown to occur when a "carrier" group embodying an acridine (or related) nucleus or an anthracene (or related) nucleus is convalently joined, preferably through a basic side chain, with a 2-chloroethylamine or a 2-chloroethyl sulfide alkylating function.[2-4]

Independent variation of each of these two structural features has already led to distinct new classes of antitumor agents. Further variations of the "carrier" portion have been made and, as shown in Table I, this feature when combined with a simple alkylating function as its complement has given agents with properties superior in many respects to any we have ever tested.

Two synthetic routes to the hydroxy precursors of the mustards in Table II were found to be superior to those previously employed.[4] In the first (method A in the Experimenal Section), the anil formed from the aromatic aldehyde and 2-(2-aminoethylthio)ethanol was reduced with $LiBH_4$. In the second (method B), the iodomethyl derivatives, available from the corresponding anthraquinones by the method of Badger and Pierce,[5] on reaction with the side chain gave the precursor in one step. In spite of generally poor yields and sometimes difficult isolations, this was, in some cases, the most convenient synthesis; often the choice of route was dictated by the accessibility of intermediates. Where two isomers are theoretically possible in the synthesis of the iodomethylanthracenes, the position of the side chain as determined by that of the iodomethyl group is tabulated as being at the *meso* position *not* adjacent to the substituent in the outer ring. This conclusion is not rigorously proved but is deduced from two lines of evidence both of which harmonize with the obvious rationale of steric hindrance: (1) Sandin and Fieser proved[6] that the iodomethyl group so introduced occupies the 7-position of benz[*a*]anthracene and (2) in contrast to the eight anthraquinones successfully subjected to the conditions of synthesis in this laboratory (not all of these are reported herein), only 1,4-dimethylanthraquinone, where there is a

(1) Supported by Research Grants CA-02975, CA-06927, and FR-05539 from the National Institutes of Health, U. S. Public Health Service, and by an appropriation from the Commonwealth of Pennsylvania.

(2) (a) R. K. Preston, R. M. Peck, E. R. Breuninger, A. J. Miller, and H. J. Creech, *J. Med. Chem.*, **7**, 471 (1964); (b) R. M. Peck, E. R. Breuninger, A. J. Miller, and H. J. Creech, *ibid.*, **7**, 480 (1964).

(3) R. M. Peck, A. P. O'Connell, and H. J. Creech, *ibid.*, **9**, 217 (1966).

(4) R. M. Peck, A. P. O'Connell, and H. J. Creech, *ibid.*, **10**, 37 (1967).

(5) G. M. Badger and R. S. Pierce, *J. Chem. Soc.*, 2314 (1950).

(6) R. B. Sandin and L. F. Fieser, *J. Amer. Chem. Soc.*, **62**, 3098 (1940).

TABLE I
HALOMETHYL AROMATIC HYDROCARBONS

	Antitumor activity[a]			
	Range, μmol/kg	Degree	Mp, °C	Formula[b]
A. Monofunctional				
4-Chloromethyl-1-methylnaphthalene[c]	60–300	2.0		$C_{12}H_{11}Cl$
10-Chloromethyl-9-methylphenanthrene[d]	35–300	2.3	155.5–156.5	$C_{16}H_{13}Cl$
9-Chloromethylanthracene[e]	1–125	2.1	142–144	$C_{15}H_{11}Cl$
10-Chloromethyl-9-methylanthracene[f]	4–100	2.4	193–197 dec	$C_{16}H_{13}Cl$
10-Iodomethyl-9-methylanthracene[g]	20–300	2.6	Dec	$C_{16}H_{13}I$
7-Chloromethylbenz[a]anthracene[h]	0.8–20	2.0	190–192, s 185	$C_{19}H_{13}Cl$
7-Chloromethyl-12-methylbenz[a]anthracene[i]	2–175	2.2	144–147	$C_{20}H_{15}Cl$
7-Iodomethyl-12-methylbenz[a]anthracene[j]	20–350	2.3	Dec	$C_{20}H_{15}I$
1-Chloromethylpyrene[k]	0.8–35	2.3	138–142 dec	$C_{17}H_{11}Cl$
B. Bifunctional				
1,4-Bis(chloromethyl)benzene[c]	Inactive; toxic at 100			$C_8H_8Cl_2$
3,6-Bis(chloromethyl)durene[c]	50–180	2.3		$C_{12}H_{16}Cl_2$
α,α'-2,3,5,6-Hexachloro-*p*-xylene[c]	Inactive; toxic at 30			$C_8H_4Cl_6$
9,10-Bis(chloromethyl)anthracene[c]	0.3–30	2.4		$C_{16}H_{12}Cl_2$

[a] See section on biological results. [b] Although all of these compounds have been reported in the literature, crystallization to constant composition was carried out on all the compounds in part A apart from the first compound (commercially available) and the unstable iodomethyl derivatives. In part B, the first three were commercial products and used directly. The last commercial product was found to be impure and was crystallized three times from benzene. [c] Commercially available. [d] S. Hauptmann, *Chem. Ber.*, **93**, 2604 (1960). [e] W. T. Hunter, *et al.*, *J. Org. Chem.*, **21**, 1512 (1956). [f] J. L. Adelfang and G. H. Daub, *J. Amer. Chem. Soc.*, **80**, 1405 (1958). [g] G. M. Badger and R. S. Pierce, *J. Chem. Soc.*, 2314 (1950). [h] Although this compound was described by Badger and Cook, *ibid.*, 802 (1939), we found that the action of dry ethanolic hydrogen chloride on the methylol gave a cleaner product. [i] J. Pataki, R. Wlos, and Y. Cho, *J. Med. Chem.*, **11**, 1083 (1968). [j] W. E. Bachmann and M. Carmack, *J. Amer. Chem. Soc.*, **63**, 2494 (1941).

TABLE II
DERIVATIVES OF $RCH_2NHCH_2CH_2SCH_2CH_2Cl \cdot HCl$

	Antitumor activity[a]		Yield,		
R	Range, μmol/kg	Degree	%	Mp, °C	Formula[b]
9-Anthryl	40–150	2.5	41	185–187	$C_{19}H_{20}ClNS \cdot HCl$
9-Phenanthryl	60–275	2.4	85	140–140.5	$C_{19}H_{20}ClNS \cdot HCl$
10-Methyl-9-anthryl[c]	1.6–90	2.6	58	194–196.5	$C_{20}H_{22}ClNS \cdot HCl$
10-Ethyl-9-anthryl[d]	80–500	2.1	70	183–185 dec	$C_{21}H_{24}ClNS \cdot HCl$
4,10-Dimethyl-9-anthryl[e]	30–180	2.1	76	190–192 dec	$C_{21}H_{24}ClNS \cdot HCl$[f]
4-Fluoro-10-methyl-9-anthryl	1–15	2.3	86	207–209	$C_{20}H_{21}ClFNS \cdot HCl$
4-Chloro-10-methyl-9-anthryl	2.5–100	2.3	62	194–195.5	$C_{20}H_{21}Cl_2NS \cdot HCl$[g]
7-Benz[a]anthryl	12–160	2.5	79	195–197	$C_{23}H_{22}ClNS \cdot HCl$
12-Methyl-7-benz[a]anthryl	5–250	2.6	61	183–186 dec	$C_{24}H_{24}ClNS \cdot HCl$

[a] See section on biological results. [b] All compounds were analyzed for C, H, S, Cl. [c] By methods I and II. In the course of the animal tests it was found that the two samples of this compound, apparently chemically identical, gave differing antitumor results. We could find no rationale for this discrepancy. [d] By methods I and II. [e] By methods II and III. [f] C: calcd, 63.94; found, 64.39, 64.37. [g] Cl: calcd, 25.65; found, 24.84, 24.95.

blocking group adjacent to both *meso* positions, failed to yield a methyliodomethylanthracene.

Replacement of OH by Cl to produce the compounds in Table II was carried out by the method routinely used in the past, *i.e.*, excess $SOCl_2$ as a solvent at 5–30° (method I in the Experimental Section). Where the terminal ring(s) carried an alkyl substituent however, only intractable tars were obtained, and two other methods were found to yield clean products. Method II consisted of a slight molar excess of $SOCl_2$ in dioxane solution on the steam cone[7] and method III, successful only with certain hydroxyethyl sulfides, utilized a large excess of 12 *N* HCl as a solvent and dilution after standing overnight.

Two of the unstable iodomethyl hydrocarbons not of analytical purity[5,6] and a variety of corresponding chloromethyl compounds (Table I) were included in the antitumor screen. For comparison with these unexpectedly potent compounds, four commercially available bis(chloromethyl) hydrocarbons were tested.

(7) W. T. Hunter, J. S. Buck, F. W. Gubitz, and C. H. Bolen, *J. Org. Chem.*, **21**, 1512 (1956).

The alcohols corresponding to the nitrogen mustards in Table III were synthesized from these halomethyl compounds by condensation with the necessary 2-[(ω-aminoalkyl)ethylamino]ethanol.

Experimental Section

Melting points were taken in open capillary tubes in a Hershberg apparatus using total immersion thermometers and are reported as uncorrected values. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

2-[2-[(9-Anthrylmethyl)amino]ethylthio]ethanol (Method A).—A mixture of 4.12 g (0.02 mol) of 9-anthraldehyde and 2.7 g (0.022 mol) of 2-(2-aminoethylthio)ethanol in 20 ml of EtOH was refluxed gently for 2 hr, diluted with hexane, cooled overnight, and filtered. The product was recrystallized from EtOH–H_2O to give 4.9 g of the product in Table IV (79%). This was dissolved in 50 ml of warm C_6H_6 and added in portions over a period of 0.5 hr to a stirred solution of 1 g of $LiBH_4$ in 1 l. of dry Et_2O. The resulting suspension was stirred for an additional 3.5 hr and decomposed with ice and AcOH. The aqueous layer was filtered and made alkaline to yield 1.9 g of product. Further extraction of the Et_2O with 100 ml of 20% AcOH gave an additional 0.25 g. The combined product was reprecipitated from solution in dilute AcOH in the presence of a little petroleum ether (bp 30–60°) to give 2.1 g of the product in Table V.

Table III
Derivatives of $RCH_2NH(CH_2)_nN(C_2H_5)CH_2CH_2Cl\cdot 2HCl$

R	Antitumor activity[a] Range, μmol/kg	Degree	Yield, %	Mp, °C	Formula[b]
9-Anthryl, $n = 3$	8–15	2.0	78	147–152	$C_{22}H_{27}ClN_2\cdot 2HCl\cdot 0.75H_2O$
10-Methyl-9-anthryl, $n = 2$	18–80	1.9	50	166.5–169 dec	$C_{23}H_{27}ClN_2\cdot 2HCl\cdot 0.5H_2O$
10-Methyl-9-anthryl, $n = 3$	3–10	2.2	86	135–138 dec	$C_{23}H_{29}ClN_2\cdot 2HCl\cdot H_2O$
7-Benz[*a*]anthryl, $n = 3$	6–10	1.9	76	162–168	$C_{26}H_{29}ClN_2\cdot 2HCl\cdot 0.5H_2O$
12-Methyl-7-benz[*a*]anthryl, $n = 3$	5–20	2.3	100	55–65	$C_{27}H_{31}ClN_2\cdot 2HCl$

[a] See section on biological results. [b] Analyzed for C, H, N, Cl. [c] This product resisted crystallization; the ethereal precipitate from EtOH was rubbed with fresh dry Et_2O to give a tan powder with analytical results. Calcd: C, 65.95; H, 6.77; N, 5.70; Cl, 21.60; found: C, 64.06; H, 6.83; N, 5.24; Cl, 20.30.

Table IV
Anil Intermediates
$RCH{=}NCH_2CH_2SCH_2CH_2OH$

R	Yield, %	Mp, °C	Formula[a]
9-Anthryl	79	97–98.5	$C_{19}H_{19}NOS$
9-Phenanthryl	80	91–92	$C_{19}H_{19}NOS$
10-Methyl-9-anthryl	75	109–110	$C_{20}H_{21}NOS$
10-Ethyl-9-anthryl	75	91.5–92.5	$C_{21}H_{23}NOS$
7-Benz[*a*]anthryl	82	103.5–105.5	$C_{23}H_{21}NOS$

[a] Compounds were analyzed for C, H, S.

2-[2-[(4-Chloro-10-methyl-9-anthrylmethyl)amino]ethylthio]-ethanol (Method B).—Following the method of Badger and Pierce,[9] 10 g of 1-chloroanthraquinone was added in portions to a stirred solution of 70 ml of 3 *M* ethereal MeMgI and 120 ml of C_6H_6. The solution was heated 1 hr at gentle reflux, stored overnight at 5°, and added slowly to a stirred mixture of 120 ml of 55% HI and 400 ml of MeOH maintained at −5° to −10° in a cooling bath. The mixture was diluted with 500 ml of AcOH and allowed to stand 4 hr at −20°, whereupon the precipitate was removed and washed with ice–water and a little petroleum ether. The crude product after overnight air drying at 5° weighed 9.6 g. A 2.4-g portion was added to a stirred, cooled mixture of 3 g of 2-(2-aminoethylthio)ethanol in 10 ml of C_6H_6 and 5 ml of EtOH. After the solid had dissolved, the mixture was stirred in an ice bath for 45 min, at room temperature for 1 hr, then partitioned (H_2O–Et_2O), and separated, and the organic layer was washed (H_2O) and then extracted with two 15-ml portions of 5 *N* AcOH. The combined extracts were made alkaline in the presence of a small amount of C_6H_6–petroleum ether and then cooled overnight to give 1.0 g of crude product. Crystallization from C_6H_6–petroleum ether gave 0.80 g of product (Table V).

If the chloromethyl hydrocarbon was utilized, no solvent was used and the reaction was carried out at 70–90°.

Except where footnotes specify an exception, the following method, I, was the means of synthesis of all the compounds in Table II. The only variation in conditions for those compounds in Table III was that the overnight reaction period was carried out at room temperature.

***N*-[2-(2-Chloroethylthio)ethyl]-10-methyl-9-anthracenemethylamine Hydrochloride.**—To 60 ml of stirred $SOCl_2$ cooled in an ice bath was added, in portions, 3.1 g of 2-[2-[(10-methyl-9-anthrylmethyl)amino]ethylthio]ethanol (Table V). After solution was complete, the flask was stored overnight at about 5° and at room temperature for 4 hr; then volatile material was removed at water pump vacuum. The residue was decomposed with 5–10 ml of EtOH, from which the product crystallized. Solvent was again removed *in vacuo*, the product was stirred with more EtOH, ether was added, and the product was filtered and washed (Et_2O) to yield 2.8 g, mp 193–194 dec. Recrystallization (EtOH–Et_2O) gave 2.1 g of the product in Table II.

Method II was devised for compounds unstable to the above conditions. It was also applied to a resynthesis of the above compound, as follows. To a solution of 5.55 g of 2-[2-[(10-methyl-9-anthrylmethyl)amino]ethylthio]ethanol in pure dry dioxane was added a solution of 2.5 g (25% molar excess) of $SOCl_2$ in 25 ml of dioxane, with stirring. The solution was immediately heated on the steam cone, and crystals soon began to form. Although the mass became quite thick, heating was continued 20 min; then volatile material was removed *in vacuo*. The residue was filtered from EtOH–Et_2O to give 6.5 g of product. Crystallization (EtOH–Et_2O) gave 5.5 g (85%), mp 200–201°.

7-Chloromethyl-12-methylbenz[*a*]anthracene.[8]—To 20 ml of $SOCl_2$, stirred in an ice bath, was added 700 mg of 7-methoxymethyl-12-methylbenz[*a*]anthracene.[6] The solution was allowed to stand 2 hr at 0° and 1 hr at room temperature and then concentrated *in vacuo*. The residue was decomposed with EtOH, again taken to dryness, and filtered from fresh EtOH to give 0.60 g, mp 138–140.5°. Recrystallization from C_6H_6–EtOH gave 0.45 g (63%) of the product in Table I.

Biological Results and Discussion

The Ehrlich ascites tumor in ICR Swiss mice has been used in this laboratory for many years for the quantitative assay of the antitumor activities of a variety of bifunctional and monofunctional alkylating agents. The procedures, which have been described in detail in previous publications,[2,3,4] involve comparisons of the survival times of the treated and the control tumor-bearing mice. The experiments were terminated at the end of a period equal to three times the mean survival time of the controls in order to conserve space in our animal colony, and because it has been our experience that mice surviving to this point (usually 45–50 days) seldom showed recurrence of tumor when held for much longer periods.

Compounds are characterized by two values: the effective dosage range and the degree of antitumor activity. Our criteria for activity are stringent and require a minimum 80% increase (a relative degree of 1.8) in mean survival time over that of the control animals. The "degree" figure given in Tables I through III consists of the average of two minimum values of 1.8 or more at the lowest effective dosage and one at the maximum effective dosage, where concomitant toxicity is the limiting factor) and of the higher values found at 3–8 intermediate dosages. Since 3.0 is the maximum value (signifying that all the mice were alive at three times the mean survival time of the controls), a "degree" figure of 2.2–2.5 denotes a highly effective compound, and 1.8–2.1 indicates a moderately active one.

In 1961 we discovered that compounds with a single alkylating function displayed potent antitumor activity provided that the molecule contained certain polycyclic aromatic components. This activating property was at first apparently limited to the acridines,[9] but was later extended to several other heterocyclic aromatics[2,3] and recently to polynuclear hydro-

(8) Also prepared by J. Paraki, R. Wise, and Y. Cho, *J. Med. Chem.*, **11**, 1083 (1968).

(9) R. M. Peck, R. K. Preston, and H. J. Creech, *J. Org. Chem.*, **26**, 3409 (1961).

TABLE V
HYDROXY PRECURSORS OF MUSTARDS

R	Method[a]	Yield, %	Mp, °C	Formula	Analyses
A. From Table II: $RCH_2NHCH_2CH_2SCH_2CH_2OH$					
9-Anthryl	A	42	111.5–113	$C_{19}H_{21}NOS$	C, H, S
9-Phenanthryl	A	36	68.5–70.5	$C_{19}H_{21}NOS$	C, H, S
10-Methyl-9-anthryl	B[b]	42	185–186.5	$C_{20}H_{23}NOS \cdot HCl$	C, H, S, Cl
10-Ethyl-9-anthryl	A	36	105–106	$C_{21}H_{25}NOS$	C, H, N, S
4,10-Dimethyl-9-anthryl	B	52	84.5–86	$C_{21}H_{25}NOS$[c]	C, H, N, S
4-Fluoro-10-methyl-9-anthryl	B	41	113–114.5	$C_{20}H_{22}FNOS$	C, H, S
4-Chloro-10-methyl-9-anthryl	B	29	79–80	$C_{20}H_{22}ClNOS$	C,[d] H, S, Cl
7-Benz[*a*]anthryl	A	25	112–113	$C_{23}H_{23}NOS$	C, H, N, S
12-Methyl-7-benz[*a*]anthryl	B[e]	47	74–76	$C_{24}H_{25}NOS$	C, H, S
B. From Table III: $RCH_2NH(CH_2)_nN(C_2H_5)CH_2CH_2OH$					
9-Anthryl, $n = 3$	B	69	188.5–190	$C_{22}H_{28}N_2O \cdot 2HCl$	C, H, N, Cl
10-Methyl-9-anthryl, $n = 2$	B	20	123–127 dec	$C_{22}H_{28}N_2O \cdot 2HCl \cdot 0.5H_2O$	C, H,[f] N, Cl[f]
10-Methyl-9-anthryl, $n = 3$	B[g]	25	165–166.5	$C_{23}H_{30}N_2O \cdot 2HI$	C,[h] H, N, I[h]
7-Benz[*a*]anthryl, $n = 3$	B	43	230–233 dec	$C_{26}H_{30}N_2O \cdot 2HCl$	C, H, N, Cl
12-Methyl-7-benz[*a*]anthryl, $n = 3$	B	23	158–162	$C_{27}H_{32}N_2O \cdot 2HI$	C,[i] H, N, I[i]

[a] See Experimental Section; method B1 utilized the chloromethyl hydrocarbon at 70–90° without solvent. [b] Also by method A. [c] The hydrochloride was also prepared, mp 174–177°, and analyzed for C, H, S, Cl. [d] C: calcd, 66.80: found, 67.48. [e] First precipitated as the hydriodide from AcOH extracts and recrystallized from EtOH–H_2O, mp 137.5–139°. [f] Calcd: H, 7.48; Cl, 16.94; found: H, 7.95; Cl, 16.42. [g] Also prepared by reaction of 3-(10-methyl-9-anthrylmethyl)amino-1-chloropropane[4] with excess ethylaminoethanol. [h] Calcd: C, 45.57; I, 41.82; found: C, 44.73; I, 42.33. [i] Calcd: C, 49.40; I, 38.68; found: C, 48.82, 49.03; I, 38.31, 38.21.

carbons;[4] in these studies, the alkylating function consisted of a 2-chloroethylamine or a 2-chloroethyl sulfide.

In addition to the sulfur mustards, which are injected in animals as homogenized suspensions, where a substantial partial solubility nevertheless exists, data on 5 soluble nitrogen mustard derivatives and on 13 halomethyl hydrocarbons, whose suspensions contain essentially no dissolved material, are given (Tables I and III).

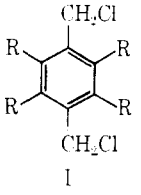
The length of the side chain and the nature of the amine joining the arylalkyl portion of the molecule to the mustard moiety were found to be important. As was the general case with the structures studied to date, compounds containing a "propyl" side chain displayed antitumor activity at lower dosage levels than those with the "ethyl" side chain. The latter were considerably less toxic than the propyl analogs, however, with the result that a more favorable therapeutic index was shown by the ethyl series of N- and S-mustards.

This suggested the use of the side chain in Table II for evaluating the structure–activity relationships of various condensed polynuclear hydrocarbons. An impressive illustration of a large biological effect due to a small alteration in chemical structure is shown by the first, third, and fourth compounds in which the *meso* substituent changes from H to Me to Et.

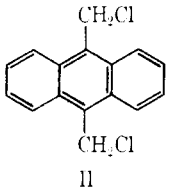
Although an additional Cl or F substituent at the 4-position of the anthracene nucleus had little influence on the antitumor activity of the S mustard derivative, a Me in this position caused a significant decrease in activity. Enlargement of the condensed ring system by one benzenoid nucleus resulted in the lowering of the dosage needed to demonstrate activity (lines 8 and 1). A Me at the other *meso* position had a less pronounced effect in the S mustard of benz[*a*]-anthracene than of anthracene in causing increased antitumor effectiveness (lines 9 and 3). These data confirm and extend the previous observations[4] with several polycyclic hydrocarbon derivatives containing the "propyl" side chain and again indicate the existence of a parallelism between the carcinogenic and carcinostatic potentialities of the aromatic hydrocarbon moieties.

The antitumor activities of the N mustard derivatives of several polynuclear aromatic hydrocarbons are listed in Table III. Since these compounds, in contrast to their corresponding sulfur mustards, have appreciable solubilities in aqueous media, they were administered to the mice as solutions in saline. Comparisons of the dosage ranges of the two types of mustards show that the N mustards are toxic at much lower dosage levels than the S mustards which exhibit superior therapeutic indices. The importance of a Me on the other *meso* position of anthracene and of benz[*a*]anthracene is again evident since this substitution results in an appreciably greater degree of antitumor activity at a somewhat lower dosage level and over a broader dosage range. The length of the alkyl side chain is of significance, with the "ethyl" form showing lower activity than the "propyl" form, but a higher therapeutic index.

Table I lists alkylating derivatives which have essentially no solubility in H_2O; the high potency of a number of these compounds appears remarkable in the light of this property. All of these compounds have been described in the literature; it was found, however, that exhaustive recrystallization of the products was usually necessary to obtain a sample that was pure by the criteria of both elementary composition and biological activity. The procedure used to obtain the fourth compound in particular at first gave samples displaying greater potency than that listed in the table. The presence of a second CH_2Cl as an impurity was suspected; this led to the testing of the extremely potent final compound in Table I, a bifunctional alkylating agent. An indication pertinent to the great importance of geometric configuration of the aromatic moiety is given by the comparative biologic properties of three



I



II

other *p*-bis(chloromethyl) models. All may be represented by I; the simple benzene derivative (I, R = H) is inactive but the durene derivative (I, R = CH_3) has enough steric resemblance to the highly active II to impart activity to the simple benzene ring. In the hexachloro compound (I, R = Cl) the bulky halogen atoms have an equivocal effect; the trend to higher toxicity may mask any latent antitumor activity.

Two additional points of interest concerning the properties of the compounds in Table I arise: (1) the ICH_2 derivatives are less active biologically than the corresponding $ClCH_2$ hydrocarbons under the testing conditions, although much more reactive chemically as alkylating agents; (2) the $ClCH_2$ compounds of Table I (A) as well as the last compound in Table I (B) show strong activity *vs.* the S-37 ascites tumor, against which N mustards including HN2, have never shown more than borderline activity (a "degree" of 1.5 to 1.8). The S mustards in Table II are also much more active as a group in this test than any N mustards ever tested in this laboratory, an indication that a wider spectrum of activity may be realized in this area.

When our initial studies demonstrated the exceptional antitumor activity of some of our monofunctional alkylating agents, we postulated that this behavior was actually following a bifunctional pattern in which cross-linkage was being accomplished through the reactivity of the monofunctional mustard moiety combined with the action of the polycyclic components of these agents. This belief has been supported by the work of Lerman[10] and of Boyland and Green,[11] among others, who present evidence for intercalation of comparable moieties into the DNA helix. Further support has come from the discovery, by a large number of workers, of strong mutagenic activity of our monofunctional nitrogen mustard derivatives of polynuclear heterocyclics in various organisms; Ames and Whitfield's work[12] reviews some of these. It has also been found that monofunctional S mustards derived from polynuclear hydrocarbons possess mutagenic frame shift activity in *Neurospora*[13] comparable with that of the N mustard-heterocyclic aromatic combination.

Acknowledgment.—The authors thank Mrs. G. Bates for assistance in the animal studies.

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(13) Personal communication from Dr. H. V. Malling.

Notes

Nitrofuryl Heterocycles. X.[1] Analogs of 6-(5-Nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione

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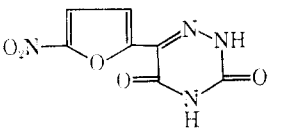
Received August 8, 1969

In a previous note from this laboratory, Hayes[2] reported the synthesis and antibacterial activity of 6-(5-nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione (I). Since I was also excreted to a significant extent in the urine of the mouse, rat, and monkey, the compound was of interest as a potential urinary tract antibacterial agent. On this basis, the structure of I was modified at positions 2 through 5 in an attempt to enhance biologic activity.

Most of the compounds reported herein were prepared by modifications of the general *as*-triazine-3,5-dione synthesis used by Hayes to prepare I. The



I

remaining compounds were prepared by modifications of the procedures reviewed by Erickson.[3] Table I summarizes the compounds prepared.

Compounds **15–36** were screened for urinary tract activity by measuring the per cent of the oral dose excreted in rat urine following a 24-hr collection period using both antibacterial (*Escherichia coli*) and uv spectrometric assay techniques. These data are summarized in Table II. Using I as a standard, it can be seen from the data in Table II that none of the analogs of I was excreted as well as the parent compound in the urine of rats. The most active analog prepared was the 3-thio compound, **29**, which showed about one-half the urinary excretion of I. Compounds **17**, **21**, **22**, **24**, and **28** were excreted significantly in the urine as measured by uv assay but not by antibacterial assay, an observa-

(1) For the previous paper in this series see H. R. Synder, Jr., and L. E. Banjamin, *J. Med. Chem.*, **13**, 164 (1970).
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