Journal of Medicinal and Pharmaceutical Chemistry
VOL. 3, No. 1 (1961)

Potential Anticancer Agents—XLI.* The Relationship of Chemical Structure to Antitumour Activity in Analogues of *meso*-1,4-Diacetoxymercuri-2,3-dimethoxybutane (NSC–2201)‡

LEON GOODMAN, LEONARD O. ROSS, MARY O. GREENE, JOSEPH GREENBERG and B. R. BAKER, *Department of Biological Sciences, Stanford Research Institute, Menlo Park, California*

Introduction

Among the active compounds discovered by the Cancer Chemotherapy National Service Center in its search for anticancer agents was *meso*-1,4-diacetoxymercuri-2,3-dimethoxybutane (NSC–2201).†‡ This compound showed a variable and borderline activity against Adenocarcinoma 755. We have undertaken a study of the relationship of chemical structure to antitumour activity using analogues of NSC–2201.

The problem was approached by the 'phase-method',[3] so that the maximum amount of information could be obtained with a minimum number of compounds, and changes which led to equal or better activity than NSC–2201 could then be followed up in more detail.

For Phase I evaluation, five classes of compounds were synthesized and evaluated.

* This programme was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contracts No. SA-43-ph-1892 and No. SA-43-ph-1909. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, *cf.* W. W. Lee, A. Benitez, L. Goodman and B. R. Baker, *J. Amer. chem. Soc.*, **82**, 2648 (1960).

† The NSC accession numbers used in this paper were assigned by the CCNSC.

‡ A sample of this compound,[1,2] prepared by B. R. Baker in 1940, was obtained from Professor Roger Adams of the University of Illinois. The Chemical Abstracts name for this compound is *meso*(2,3-dimethoxytetramethylene)-bis[mercury acetate].

Class 1. Replacement of the acetate by other ions

$$\mathrm{RHgCH_2\overset{\overset{\displaystyle OMe}{|}}{C}H\text{—}\overset{\overset{\displaystyle OMe}{|}}{C}HCH_2HgR} \qquad (\textit{meso})$$

NSC no.	R	Chemotherapeutic[a] index
2201	Ac	1
20829	Cl	1
20831	Br	1–2
20830	I	1
22681	$C_6H_5COO^-$	2
23110	n-$C_7H_{15}COO^-$	4
22682	n-$C_{17}H_{35}COO^-$	2·5
30911	·SCN	inactive

[a] The chemotherapeutic index was defined as the ratio of the maximum tolerated dose ($\geqslant$ 7 survivors and $<$ 2 g per mouse weight loss) to the minimum dose that gave $T/C < 0{\cdot}5$; these indices are necessarily approximations at this point.

Class 2. Variations in the alkoxy group

$$\mathrm{R'HgCH_2CH(OR)\text{—}CHOR\text{—}CH_2HgR'}$$

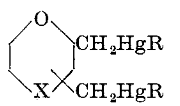
NSC no.	R	R′	Chemotherapeutic index
21289	H	AcO^-	1–2 (variable)
21296	Me (±)	AcO^-	inactive
19952	Et (*meso*)	AcO^-	inactive
23605	Et (±)	AcO^-	inactive
22680	*i*-Pr	Cl	2 (variable)

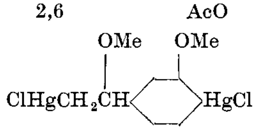
Class 3. Increase or branching of chain between mercury atoms

$R'HgCH_2C(OR)(CH_3)—CH(OR)—CH_2HgR'$

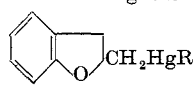
NSC no.	R	R′	Chemotherapeutic index
23103	H	Cl	1·5
20832	Me	Cl (one isomer)	2·5
21292	Et	Cl (*erythro*?)	inactive
21298	Et	AcO^- (*erythro* ?)	inactive
21297	$AcOHgCH_2CH(OMe)—(CH_2)_2—CH(OMe)CH_2HgOAc$		inactive
22690	$IHgCH_2CH(OMe)CH_2OCH_2CH(OMe)CH_2HgI$		inactive

Class 4. Ring compounds to give fixed conformational relationship between mercury atoms



NSC no.	Isomer	R	X	Chemotherapeutic index
21935	2,3	NO_3	O	2
21934	2,3	I	O	2
23106	2,5	NO_3 } OH }	O	1
23105	2,5	I	O	8
21286	2,6	Cl	O	5
21287	2,6	I	O	2
22678	2,6	I	N	1
23108	2,6	AcO	S	2
23109	 (mixed isomers)			inactive

Class 5. Monomercurials—all of these were inactive, but more toxic than dimercurials.

NSC no.	Compound
23601	OMe $CH_3CH—CHCH_3$ HgBr
23104	OMe $CH_3CH—CHCH_3$ HgCl
23604	$CH_3CH_2CHCH_2HgBr$ OMe
23102	$CH_3OCH_2CHCOO^-$ Hg^+
23603	$CH_3OCH_2CHCOOEt$ HgOAc
	
23602	R = AcO$^-$
23107	R = I

Thus, the first phase, consisting of 35 compounds, led to the following conclusions for phase II synthesis.

Class 1. Replacement of the acetate ion in NSC–2201 by other inorganic ions gave no improvement, but replacement by higher organic acids led to an increased chemotherapeutic index, the best compound being the dioctanoate, NSC–23110. The next best compounds were the stearate and benzoate.

Class 2. Alkoxy variations, keeping the acetate group constant, gave poor to no activity. However, (2,3-di-isopropoxytetramethylene)bis[mercury chloride] (NSC–22680) was an exception.

Class 3. Lengthening of the chain gave inactive compounds. Chain branching appeared to give decreased activity, although again a dichloromercuri compound (NSC–20832) was an exception.

Class 4. As a class, compounds built from a dioxan nucleus gave better chemotherapeutic indices than the corresponding salts of NSC–2201. However, a thioxane or morpholine ring gave no better activity than NSC–2201.

Class 5. All of the monomercurials were much more toxic than the corresponding salts of NSC–2201 and were also inactive.

Based on these attempted correlations, a second phase of synthesis of 13 compounds was initiated.

Class 2. Alkoxy variations. Further analogues were synthesized based on the increased chemotherapeutic index of NSC–22680 and the apparent increased chemotherapeutic index of '2201-dibenzoate' (NSC–22681).

$$\begin{array}{c} \quad\;\; OR \quad OR \\ \quad\;\; | \qquad\; | \\ R'HgCH_2CH\text{—}CHCH_2HgR' \end{array}$$

(*meso*)

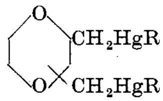
NSC no.	R	R′	Chemotherapeutic index
29442	H	$C_6H_5COO^-$	1
30910	n-C_3H_7-	Cl	1·5
30912	n-C_3H_7-	AcO^-	1·5
30918	n-C_3H_7-	$C_6H_5COO^-$	inactive
30913	n-C_4H_9-	Cl	1
30914	n-C_4H_9-	AcO^-	inactive
30919	n-C_4H_9-	$C_6H_5COO^-$	1·5

Class 3. Branched chain. Two dibenzoates of this series were prepared to see if the chemotherapeutic index could be increased.

$$\begin{array}{c} OR \\ | \\ C_6H_5COOHgCH_2\overset{}{C}H\text{—}CH(OR)\text{—}CH_2HgOOCC_6H_5 \\ | \\ CH_3 \end{array}$$

NSC no.	R	Chemotherapeutic index
30917	Me	inactive
31961	H	1

Class 4. Ring compounds. Three benzoate salts were prepared, as well as one dinitrate. The latter became available as an intermediate to the corresponding benzoate.



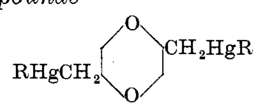
NSC no.	Isomer	R	Chemotherapeutic index
29443	2,3	BzO	8
30915	2,6	BzO	2
30909	2,5	NO_3	2
30916	2,5	BzO	7·5

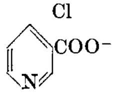
In Phase II, the activity of NSC–22680 in Class 2 was investigated. The di-*n*-propoxy derivative (NSC–30910) had a chemotherapeutic index similar to that of the di-isopropoxy derivative (NSC–22680); the former was easier to synthesize. Higher or lower alkoxy groups gave decreased effectiveness. Variation of the anion of NSC–30910 from chloro to benzoate or acetate did not improve the index.

Two major conclusions can be drawn from the first two phases of work: first, the 2,5-dioxan nucleus, as represented by NSC–30916 (Class 4), probably represents the best carrier group; secondly, the change in anion can make a change in chemo-therapeutic index.

Phase III was initiated to vary the anion of the 2,5-dioxan carrier further; however, these five compounds were less effective than previously evaluated members of this class.

Class 4. Ring compounds



NSC no.	R	Chemotherapeutic index
38186	n-$C_7H_{15}COO^-$	1
38187	Cl	4
40581		inactive
41442	p-$CH_3C_6H_4COO^-$	2
41443	β-naphthoate	inactive

Chemistry

The parent compound, *meso*-1,4-diacetoxymercuri-2,3-dimethoxybutane (I), was prepared according to the procedure described by Johnson, Jobling, and Bodamer,[1] which utilizes the reaction of butadiene with a methanolic suspension of mercuric acetate. A 61 per cent yield of the less soluble (I), designated

Table I. (Class 1). Variation of anion in *meso*-$RHgCH_2CH(OMe)—CH(OMe)CH_2HgR$

Compound no.	NSC no.	R[a]	% Yield[b]	Procedure	m.p., °C	Analysis Calcd. C	Calcd. H	Found C	Found H
I	2201	AcO	61[c]	A	153–154[d]	18·9	2·83[e]	19·1	2·97
XI	20829	Cl	81[c]	B[j]	165–167	12·3	2·04	12·4	2·05
XII	20831	Br	62[f]	B[j]	167–169	10·6	1·77	10·7	1·82
XIII	20830	I	93[g]	B[j]	188–190	52·0[h]		52·0[h]	
XIV	22681	BzO	77[i]	B[j]	185–187	31·6	2·90	31·9	2·90
XV	22682	SrO	53[c]	B	104–105	46·5	7·62	46·2	7·57
XVI	23110	OcO	76[c]	B	91–93	32·8	5·26	32·6	5·16
XVII	30911	SCN	81	B[j]	145–147	15·1	1·98	15·2	1·92

[a] AcO = acetoxy, BzO = benzoyloxy, SrO = stearoxy, OcO = *n*-octanoyloxy. [b] Yield of purified material. [c] Recrystallized from 95% ethanol. [d] Lit.[1] m.p. 148–149°. [e] *Anal.* Calcd.: Hg, 63·1. Found: Hg, 63·4. [f] Recrystallized from acetone. [g] Recrystallized from ethyl acetate. [h] Mercury analysis performed by the method of Walton and Smith[14] which gave satisfactory results for acetoxy and iodo derivatives, but low results for chloro and bromo derivatives. [i] Recrystallized from benzene–methanol. [j] Sodium salt of anion added as aqueous solution.

as the *meso*-isomer by analogy with the corresponding ethoxy compound,[1] was isolated and was accompanied by a 13 per cent yield of the more soluble (±)-isomer (II) which had not previously been isolated in pure form. The *meso*-(III) and (±)-(IV) forms of (2,3-diethoxytetramethylene)bis[mercury acetate] were prepared as described by Johnson *et al.*[1] in 48 per cent and 6 per cent yields, respectively (Table II). The compounds had previously been assigned steric configurations on the basis of dipole moment measurements of the 2,3-diethoxy-1,4-diiodobutane isomers derived from the two acetoxymercuri isomers. The diiodide from (III) had the lower dipole moment, as would be expected

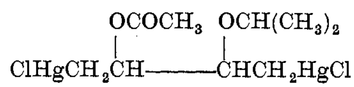
Table II (Class 2). Variations in the alkoxy group

$R'HgCH_2CH(OR)—CH(OR)—CH_2HgR'$

Compound no.	NSC no.	R′[a]	R[a]	% Yield[b]	Procedure	m.p., °C	Analysis Calcd. C	Analysis Calcd. H	Analysis Found C	Analysis Found H
II	21296	AcO	Me[c]	13[d]	A	102–104	18·9	2·83	18·6	2·74
III	19952	AcO	Et[e]	48[f]	A	162–163[g]	60·5[h]		60·4[h]	
IV	23605	AcO	Et[c]	6[i]	A	110–111[j]	60·5[h]		60·3[h]	
V	21289	AcO	H[e]	27[i]	A	220–240(d.)	15·8	2·32	15·8	2·25[k]
VI		AcO	H[c]	4[i]	A	190–260(d.)	15·8	2·32	15·8	2·29[l]
VII	30912	AcO	*n*-Pr[e]	23[m]	A	125–126	24·3	3·79	24·3	3·89
VIII	30914	AcO	*n*-Bu[e]	7[i]	A	115–117	26·6	4·19	26·7	4·30
IX	22680	Cl	*i*-Pr	8[f]	J	144–146	18·6	2·80	18·7	3·30
X		Cl	X[n]	8[f]	J	123–126	16·8	2·50	17·1	2·73
XVIII	30910	Cl	*n*-Pr[e]	70[m]	B[o]	135–136	18·6	3·11	18·7	3·15
XIX	30913	Cl	*n*-Bu[e]	65[m]	B[o]	109–110	21·4	3·59	21·3	3·40
XX	30918	BzO	*n*-Pr[e]	17[m]	B[o]	138–140	35·3	3·70	35·4	3·90
XXI	30919	BzO	*n*-Bu[e]	60[m]	B[o]	135–136	37·0	4·12	36·9	4·12
XXII	29442	BzO	H[e]	83[f]	B[o]	270–280(d.)	29·5	2·48	29·7	2·75

[a] AcO = acetoxy, BzO = benzoyloxy, Pr = propyl, Bu = butyl. [b] Yield of purified material. [c] (±)-isomer. [d] Recrystallized from ethyl acetate. [e] *meso*-Isomer. [f] Recrystallized from 95% ethanol. [g] Lit.[1] m.p. 162–163°. [h] Mercury analysis; see footnote [h] in Table I. [i] Recrystallized from water. [j] Lit.[1] m.p. 111–112°. [k] *Anal.* Calcd.: Hg, 66·1. Found: Hg, 64·8. [l] *Anal.* Calcd.: Hg, 66·1. Found: Hg, 65·7. [m] Recrystallized from methanol. [n] One R = *i*-PrO and the other R = AcO; contained ester C = O absorption in the infrared. [o] Sodium salt of anion added as aqueous solution.

for the *meso*-isomer. The reaction of butadiene with aqueous mercuric acetate gave two crystalline isomers of (2,3-dihydroxytetramethylene)bis[mercury acetate], the less soluble of which was tentatively assigned the *meso*-(V) structure and the more soluble the (±)-(VI) form by analogy with (III) and (IV). The reaction of butadiene with solutions of mercuric acetate in *n*-propyl alcohol and *n*-butyl alcohol gave low yields of sharply melting *n*-propoxy (VII) and *n*-butoxy (VIII), analogues of (I) which are presumed to be *meso* forms. Only very low yields of a diisopropoxy compound (IX) could be isolated from the reaction of butadiene with a solution of mercuric acetate in isopropyl alcohol which was then treated with potassium chloride. Infrared examination of the crude product showed considerable amounts of covalent *O*-acetate and, on one occasion, a product was obtained whose infrared spectrum and analysis indicated it to be compound (X). Obviously, acetic acid formed in the reaction competed well with isopropyl alcohol in the addition to the double bonds; bulky alkoxy groups give poor results in the reaction. Some-



(X)

what better yields of (IX) resulted when mercuric nitrate dissolved in isopropyl alcohol was added to butadiene in the first stage of the reaction. Changes in the anion attached to mercury were readily available by treating the acetoxymercuri compound with aqueous solutions of a suitable anion. Thus, from the *meso* compound (I) were prepared the chloro- (XI), bromo- (XII), iodo- (XIII), benzoyloxy- (XIV), stearoyloxy- (XV), octanoyloxy- (XVI), and thiocyano- (XVII) mercuri analogues of (I) (Table I). Similarly, from (VII) and (VIII) were prepared the chloromercuri and benzoyloxymercuri derivatives (XVIII, XIX, XX, and XXI) and from (V), the benzoyloxymercuri derivative (XXII) (Table II).

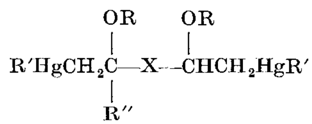
The use of isoprene rather than butadiene in the reactions of mercuric acetate in alcohols and water led to a series of compounds of Class 3 (Table III). The reaction in ethanol gave a low yield of compound (XXIII) as a sharply melting solid, presumed to be the *erythro* diastereoisomer by analogy with the butadiene reaction. The acetoxymercuri compound (XXIII) was converted to the chloromercuri derivative (XXIV). From the reaction of isoprene, mercuric acetate and methanol, an acetoxymercuri compound could not be obtained as a crystalline solid but the crude product was converted to the crystalline chloromercuri (XXV) and benzoyloxymercuri (XXVI) compounds. Similarly, from the reaction of isoprene, mercuric acetate and water, the final isolated solids were the chloromercuri (XXVII) and benzoyloxymercuri (XXVIII) derivatives.

In order to vary the distance between the mercury atoms of (I), 1,5-hexadiene was allowed to react with a methanolic solution of mercuric acetate to give a good yield of a sharply melting, and presumably single, isomer of (2,5-dimethoxyhexamethylene)bis-[mercury acetate] (XXIX) (Table III).

The reaction of aqueous mercuric acetate with diallyl ether,[4, 5] diallylamine,[4] and diallyl sulphide led to a variety of dimercurated cyclic compounds. The reaction with diallyl ether gave a syrup which was converted to the solid 2,6-bis[(chloromercuri)methyl]-*p*-dioxan (XXX) and the analogous iodomercurial (XXXI) (Table IV). Both of these products appeared to be mixtures of *cis*- and *trans*-isomers and recrystallization gave what appeared to be homogeneous products. By further transformations of crude (XXX), Summerbell and Stephens[5] have shown the product to be a mixture of *cis*- and *trans*-isomers. When aqueous mercuric nitrate was allowed to react with diallyl ether and the resulting solution treated with sodium benzoate, a high yield of a sharp-melting, crystalline compound, probably a single isomer of the dibenzoyloxy compound (XXII), was obtained. The reaction between diallylamine, mercuric acetate and water gave a syrup which was converted to the difficultly purifiable 2,6-bis[(iodomercuri)methyl]-morpholine (XXXIII), probably a mixture of *cis*- and *trans*-isomers. The analogous chloromercuri compound has been described by Nesmeyanov and Lutsenko.[4] The reaction of diallyl sulphide, mercuric acetate and water gave a low yield of 2,6-bis[(acetoxymercuri)methyl]-*p*-thioxane (XXXIV) whose sharp melting point suggested that it was a single isomer.

The cyclizations leading to compounds (XXX)–(XXXIV) are representative of a large number of such reactions which accom-

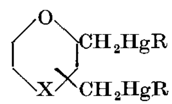
Table III (Class 3). Increase or branching of chain between mercury atoms



Compound no.	NSC no.	R′[a]	R″	R	X	% Yield[b]	Procedure	m.p., °C	Analysis Calcd. C	Calcd. H	Found C	Found H
XXIII	21298	AcO	Me	Et	—	11[c]	A[d]	158–160	23·0	3·57	23·1	3·67
XXIV	21292	Cl	Me	Et	—	72[e]	J[d]	160–161	17·1	2·87	17·0	2·76
XXV	20832	Cl	Me	Me	—	45[c]	J[d]	177–179	13·9	2·33	14·1	2·34
XXVI	30917	BzO	Me	Me	—	8[e]	J[d]	171–172	32·5	3·11	32·6	3·26
XXVII	23103	Cl	Me	H	—	8	J[d]	150–151[f]	11·1	1·87	10·9	1·85
XXVIII	31961	BzO	Me	H	—	10[g]	J[d]	168–170	30·4	2·70	30·5	2·94
XXIX	21297	AcO	H	Me	—CH_2CH_2—	42[c]	A[h]	128–130	21·7	3·34	21·9	3·46
XLIV	22690	I	H	Me	—CH_2OCH_2—	42	[i]	Syrup	11·7	1·97	12·3 12·3	2·17 1·98

[a] These compounds are presumably one pure isomer of unknown configuration; AcO = acetoxy, BzO = benzoyloxy. [b] Yield of purified material. [c] Recrystallized from acetone. [d] Prepared from isoprene. [e] Recrystallized from methanol. [f] Lit.[4], m.p. 147–148°. [g] Recrystallized from dimethylformamide–ethanol–water. [h] Prepared from 1,5-hexadiene. [i] See Experimental.

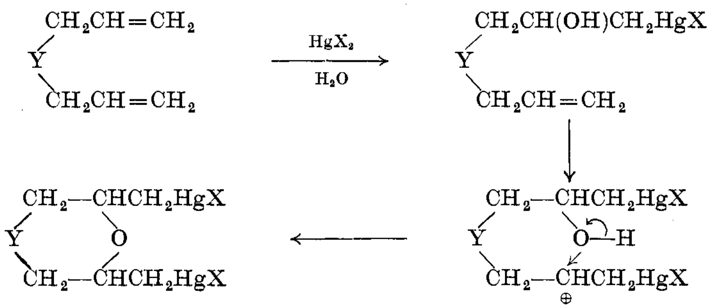
Table IV (Class 4). Ring compounds



Compound no.	NSC no.	Isomer	R[a]	X	% Yield[b]	Procedure	m.p., °C	Analysis Calcd. C	Analysis Calcd. H	Analysis Found C	Analysis Found H
XXX	21286	2.6[c]	Cl	O	31[d]	C	255–260(d.)[e]	12·3	1·72	12·3	1·75
XXXI	21287	2,6[f]	I	O	23[g]	C	241(d.)	9·36	1·31	9·62	1·41
XXXII	30915	2,6[f]	BzO	O	70[h]	C	174–175	31·7	2·78	31·8	2·68
XXXIII	22678	2,6[e]	I	N	27[i]	F[j]	145–160(d.)	9·38	1·44	9·16	1·55
XXXIV	23108	2,6[f]	AcO	S	6[k]	F	191–193(d.)	18·4	2·47	18·6	2·45
XXXV	30909	2,5[l]	NO_3	O	72[m]	D	185–188(d.)	11·2	1·57	11·4	1·70
XXXVI	23106	2,5[l]	[n]	O	64[o]	D	155–158	12·1	1·85	12·1	1·90
XXXVII	23105	2,5[l]	I	O	75[p]	B[q]	273–274(d.)[r]	9·36	1·31	9·64	1·24
XXXVIII	30916	2,5[l]	BzO	O	68[s]	B[q]	205–207	31·7	2·78	31·9	2·68
XXXIX	38187	2,5[l]	Cl	O	77[p]	B[q]	275–276[t]	12·3	1·71	12·4	1·70
XL	38186	2,5[l]	OcO	O	92[d]	B[q]	115–116	32·9	5·02	33·3	5·17
XLI	21935	2,3[c]	NO_3	O	56[d]	E	167–170	11·2	1·57	11·2	1·58
XLII	21934	2,3[c]	I	O	75[u]	B[q]	190–194(d.)	9·36	1·31	9·50	1·42
XLIII	29443	2,3[f]	BzO	O	75[d]	B[q]	181–182	31·7	2·78	31·8	2·74
LIII	41443	2,5[l]	NpO	O	17[p]	B[q]	207–208	39·1	2·82	39·5	3·16
LIV	41442	2,5[l]	TlO	O	55[p]	B[q]	258–260(d.)	33·6	3·07	33·6	3·20
LV	40581	2,5[l]	NcO	O	77[w]	B[q]	253–255(d.)[w]	28·4	2·38	28·8	2·59

[a] AcO = acetoxy, BzO = benzoyloxy, OcO = *n*-octanoyloxy, NpO = β-naphthoyloxy, TlO = *p*-toluyloxy, NcO = nicotinoyloxy. [b] Yield of purified product. [c] Mixture of stereoisomers. [d] Recrystallized from 95% ethanol. [e] Lit.[4], m.p. 116°. Probably pure stereoisomer of *trans* configuration. [g] Recrystallized from ethyl acetate. [h] Recrystallized from methanol. [i] Recrystallized from 2-methoxyethanol–water. [j] Prepared from diallylamine by Procedure F followed by treatment of the resulting aqueous solution with aqueous potassium iodide. [k] Recrystallized from acetone. *trans*-Isomer. [m] Not recrystallized because of resultant dissociation of nitrate group; see compound (XXXVI). [n] One R = OH and the other R = NO_3. [o] Recrystallized from water. [p] Recrystallized from dimethylformamide. [q] By treatment of an aqueous solution of the proper nitratomercury derivative with an aqueous solution of NaR. [r] Lit.[8], m.p. 196–198°. [s] Recrystallized from 2-methoxyethanol. [t] Resolidifies and does not remelt below 300°. [u] Recrystallized from 2-ethoxyethanol. [w] Not recrystallized.

pany mercuration; a number of examples of these are also described in a recent article by Henbest and Nicholls.[6] The strong electrophilicity of mercuric compounds undoubtedly initiates such reactions, whose mechanism is generalized below.



A number of dimercurials based on 2,5-*p*-dioxan (Table IV) were prepared by the reaction of allyl alcohol with aqueous mercuric nitrate solution, a reaction first reported by Hofmann and Sand.[7] Summerbell and Stephens[8] later showed that the direct product of this reaction, 2,5-bis[(nitratomercuri)methyl]-*p*-dioxan (XXXV), was exclusively the *trans*-isomer. Recrystallization of (XXXV) from water gave a compound whose analytical data and infrared spectrum indicated it to be the nitrato-hydroxy compound (XXXVI). Conventional treatment of (XXXV) led to the bis-(iodomercuri)methyl- (XXXVII), bis-(benzoyloxymercuri)-methyl- (XXXVIII), bis-(chloromercuri)methyl- (XXXIX), bis-(octanoyloxymercuri)methyl- (XL), bis-(naphthoyloxymercuri)-methyl- (LII), bis-(*p*-toluyloxymercuri)methyl- (LIV), and bis-(nicotinoyloxymercuri)methyl- (LV) *p*-dioxans. It is interesting that 2,5-bis-(acetoxymercurimethyl)- and 2,5-bis[(hydroxymercuri)methyl]-*p*-dioxan have both been used to link together two molecules of serum mercaptoalbumin.[15, 16]

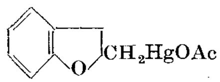
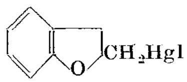
The third series of dimercurated *p*-dioxans was prepared by the reaction of butadiene, ethylene glycol, and aqueous mercuric nitrate solution, as described recently by Summerbell and Lestina,[9] who showed that the product was a mixture of *cis*- and *trans*-isomers of 2,3-bis[(nitratomercuri)methyl]-*p*-dioxan (XLI). The iodo (XLII) and benzoyloxy (XLIII) analogues of (XLI) were also prepared (Table IV).

Two additional dimercurials were prepared by the addition of methanolic mercuric acetate to diallyl ether, giving a compound isolated as 1,7-diiodomercuri-2,6-dimethoxy-4-oxaheptane (XLIV), and by the addition of methanolic mercuric acetate to 4-vinylcyclohexene-1 to give, finally, the dichloromercuri compound (XLV), probably as a mixture of isomers.

A group of monomercurated compounds (Table V) was also prepared as part of the testing programme. The reaction of aqueous mercuric acetate and *o*-allylphenol, as described by Adams, Roman and Sperry,[10] gave mercurated dihydrobenzofurans (XLVI and XLVII). The addition of methanolic mercuric acetate to acrylic acid gave a solid whose infrared spectrum showed strong carboxylate ion absorption at 6·40 μ and 7·25 μ and which is written as the zwitterion, β-methoxy-α-mercuripropionate (XLVIII). The addition of methanolic mercuric acetate to ethyl acrylate gave a syrup which analyzed correctly for ethyl α-acetoxymercuri-β-methoxypropionate (XLIX).[11] The reaction of aqueous mercuric acetate with butene-1 and butene-2, followed by treatment with potassium chloride or potassium bromide, gave compounds (L)–(LII). (2-Methoxybutyl)mercury bromide (L) was a syrup, probably an isomeric mixture, while the related compound (LI) was isolated as a sharply-melting, pure isomer. Compound (LII) had previously been described by Thomas and Wetmore.[12]

Infrared Spectra. Infrared spectra showed that the acetoxymercuri compounds were ionic in the solid state and exhibited acetate-ion bands at 6·25–6·35 μ and 7·10–7·25 μ. These bands disappeared when the acetate was converted to a halomercuri compound. These same carboxylate-ion bands were present in the monomercuri compounds (XLVIII and XLVIX), with the ester (XLIX) also showing its characteristic ester carbonyl at 5·85 μ. The benzoxymercuri compounds (e.g., XIV) were also clearly ionic, showing carboxylate ion absorptions at 6·25–6·45 μ and 7·35–7·55 μ. The stearoyloxymercuri compound (XV) had a strong carboxylate-ion band at 6·05 μ and the octanoyloxymercuri derivative (XIV) a similar band at 6·10 μ, as well as the band near 7·30 μ. The thiocyanomercuri derivative (XVII) had its thiocyanate absorption at 4·72 μ, which is more suggestive of covalent thiocyanate than ionic thiocyanate; the latter generally absorbs in the 4·79–4·95 μ region. The nitratomercuri

Table V (Class 5). Monomercury compounds

Compound no.	NSC no.	Formula	% Yield[a]	Procedure	m.p., °C	Analysis Calcd. C	Analysis Calcd. H	Analysis Found C	Analysis Found H
XLVI	23602		3[g]		79–80[b]				
XLVII	23107		11[h]		104–106[c]				
XLVIII	23102	$MeOCH_2\overset{Hg^{+}}{\overset{\vert}{C}}HCO_2^-$	77[f]	H	149–154[d]	15·9	2·02	15·8	1·99
XLIX	23603	$MeOCH_2CH(HgOAc)COOEt$	45	H	syrup	24·3	3·61	24·3	3·61
L	23604	$CH_3CH_2CH(OMe)CH_2HgBr$	48	J	syrup	16·2	2·92	16·3	3·01
LI	23601	$CH_3CH(OMe)CH(HgBr)CH_3$	26	J[i]	37–38	16·1	2·95	16·3	3·01
LII	23104	$CH_3CH(OMe)CH(HgCl)CH_3$	54	J[h]	65–66[e]				

[a] Yield of recrystallized material. [b] Lit.,[8] m.p. 80–81°. [c] Lit.,[8] m.p. 114–115°. [d] With resolidification followed by decomposition near 200°. [e] Lit.,[9] m.p. 65·5° Not recrystallized. [g] Recrystallized from water. [h] Recrystallized from 95% ethanol. [i] Recrystallized from methanol.

compounds (XXXV and XLI) showed strong bands at 6·63–6·70 μ and 7·80–7·85 μ which are characteristic of ionic nitrate. The C—O—C bands of the cyclic and acyclic ethers appeared in the range of 9·00–9·80 μ.

The *meso*- and (±)-pairs such as (I)–(II) and (III)–(IV) showed characteristic infrared spectral differences that served to distinguish the isomers.

The characterization and analytical data for all the compounds are found in Tables I–V.

Experimental*

A few illustrative procedures are reported in detail below and these serve as examples of the methods used to prepare the compounds listed in Tables I–V.

(2,5-Dimethoxyhexamethylene)bis[mercury acetate] (XXIX). Procedure A. To a stirred suspension of mercuric acetate (20·0 g, 62·6 mmoles) in reagent methanol (60 ml), 1,5-hexadiene (2·70 g, 32·8 mmoles) was added dropwise; a clear solution resulted. The solution was chilled to 15° and 11·0 g (52·8 per cent) of crystalline solid separated. This was recrystallized from acetone (1g/15 ml) to yield 8·8 g (42·3 per cent) of product, m.p. 128–130°; $\lambda_{max(\mu)}^{Nujol}$ 6·08, 6·19 and 6·28 (CO_2^-); 9·22 (C—O—C). Analytical data are listed in Table I.

The ether groups were varied in procedure A by use of the appropriate alcohol in place of methanol.

meso-*(2,3-Dimethoxytetramethylene)bis[mercury stearate] (XV). Procedure B.* A solution of sodium stearate was prepared by adding a solution of sodium hydroxide (1·04 g, 26·0 mmoles) in water (50 ml) to a hot (60–70°), stirred solution of stearic acid (7·44 g, 26·0 mmoles) in 95 per cent ethanol (300 ml). The hot (60–70°) solution of sodium stearate was added to a vigorously stirred solution of *meso*-1,5-diacetoxymercuri-2,3-dimethoxybutane (I) (4·44 g, 7·00 mmoles) in water (300 ml). The solid product was separated and washed well with water. It was recrystallized from 300 ml of 95 per cent ethanol to yield 4·2 g

* Melting points were taken on the Fisher–Johns apparatus and are uncorrected.

(53 per cent) of a crystalline product, m.p. 104·5–105°; $\lambda_{max(\mu)}^{Nujol}$ 6·05 and 7·22 (CO_2^-), 7·68, 7·91, 8·05, 8·18, 8·30, and 8·41 (typical fatty acid bands), 9·22 and 9·76 (C—O—C). Analytical data are listed in Table I.

2,6-Bis[*(benzoyloxymercuri)methyl*]-p-*dioxan* (*XXXII*). *Procedure C*. Diallyl ether (2·0 g, 20·4 mmoles) was added dropwise to a stirred solution of mercuric nitrate (5·0 g, 15 mmoles) in water (150 ml). After a few minutes the solution became yellow and to it was added a solution of sodium benzoate (3·24 g, 22·5 mmoles) in water (30 ml). The product, 5·0 g (88 per cent), m.p. 169–171°, separated and was recrystallized from methanol (40 ml) with the aid of Norite; yield, 4·0 g (70 per cent), m.p. 174–175°; $\lambda_{max(\mu)}^{Nujol}$ 6·25–6·35 (CO_2^- and aryl C—H), 7·45 (CO_2^-), 9·13 (C—O—C), 13·95 (monosubstituted phenyl). Analytical data are listed in Table II.

The use of sodium chloride or iodide in place of sodium benzoate gave (XXX) and (XXXI) respectively.

2,5-Bis[*(nitratomercuri)methyl*]-p-*dioxan* (*XXXV*). *Procedure D*. A solution of mercuric oxide (11·75 g, 54·0 mmoles), concentrated nitric acid (7·5 ml) and water (20 ml) was cooled to 0° and allyl alcohol (30 ml, 0·44 mole) was added dropwise, with stirring. After a few minutes, a product precipitated and was filtered and washed thoroughly with acetone; yield, 12·5 g (72 per cent), m.p. 185–188°(d.); $\lambda_{max(\mu)}^{Nujol}$ 6·63 and 7·81 (NO_3^-), 9·31 (C—O—C). Analytical data are listed in Table II.

2,3-Bis[*(nitratomercuri)methyl*]-p-*dioxan* (*XLI*). *Procedure E*. A solution of mercuric oxide (24·3 g, 0·112 mole), concentrated nitric acid (20 ml), and water (15 ml) was prepared. When solution was complete, 10 ml of water and 50 ml of ethylene glycol were added, then the solution was cooled to 20°. With stirring, gaseous butadiene was added until a small aliquot of the mixture failed to give a yellow precipitate when it was added to aqueous sodium hydroxide solution. A total of 12·0 g (0·22 mole) of butadiene was used. The solution was chilled at 15° and 22 g (61 per cent) of crystalline solid precipitated. After being collected on a filter, the solid was washed with three 50-ml portions of 95 per cent ethanol; yield, 20·0 g (56 per cent), m.p. 167–170°; $\lambda_{max(\mu)}^{Nujol}$ 6·70 and 7·85 (NO_3^-), 9·05 (C—O—C). Analytical data are listed in Table II.

2,6-Bis[(acetoxymercuri)methyl]-p-*thioxane* (*XXXIV*). *Procedure F.* To a cold (10°) and well stirred mixture of diallyl sulphide (5·0 g, 44 mmoles) and water (100 ml) a solution of mercuric acetate (28·0 g, 88 mmoles) in water (100 ml) was added dropwise. The mixture was stirred for a total of about one hour during which time 9·3 g (25 per cent) of solid precipitated. This was recrystallized from 200 ml of acetone; yield, 2·0 g (6 per cent) m.p. 191–193°(d.); $\lambda_{\max(\mu)}^{\text{Nujol}}$ 6·31 and 7·20 (CO_2^-), 9·40 and 9·55 (C—O—C). Analytical data are listed in Table II.

When 40 g of diallyl sulphide was employed in the procedure as described above, 84 g (37 per cent) of (XXXIV) was obtained as the solid precipitate. The infrared spectrum of this material was identical with that of the analytically pure product.

β-Methoxy-α-mercuripropionate (*XLVI*). *Procedure H.* To a stirred solution of mercuric acetate (30·0 g, 62·0 mmoles) in methanol (200 ml), a solution of glacial acrylic acid (4·46 g, 62·0 mmoles) in methanol (50 ml) was added dropwise, stirring well. The solution was chilled at 15° and 15·0 g (77 per cent) of product precipitated. The product was washed with three 50-ml portions of methanol and melted at 149–154° with resolidification followed by decomposition near 220°. In the infrared it had $\lambda_{\max(\mu)}^{\text{Nujol}}$ 6·40 and 7·25 (CO_2^-), 9·25 (C—O—C). Analytical data are listed in Table III.

1,7-Diiodomercuri-2,6-dimethoxy-4-oxaheptane (*XLIV*). Diallyl ether (2·64 g, 27 mmoles) was added dropwise to a stirred suspension of mercuric acetate (17·2 g, 54 mmoles) in absolute methanol (150 ml). The resulting solution gave no precipitate when tested with aqueous sodium hydroxide solution. The methanol was evaporated *in vacuo* at room temperature, and 350 ml of water was added to the residue. To the resulting solution was added with stirring a solution of potassium iodide (8·30 g, 50 mmoles) in water (100 ml). A semi-solid material precipitated and was isolated by decanting the aqueous solution and washing the residue with water. The precipitate was taken up in 50 ml of acetone, the acetone was evaporated *in vacuo* and the residue dried over phosphorus pentoxide leaving 8·0 g (42 per cent) of product; $\lambda_{\max(\mu)}^{\text{Nujol}}$ 9·20–9·30 (C—O—C). See Table III for analytical data.

1 - (Chloromercuri) - 4 - [2 - (chloromercuri) - 1 - methoxyethyl] - 2 - methoxycyclohexane (*XLV*). *Procedure J.* A solution of vinylcyclohexene-1 (3·21 g, 30 mmoles) in methanol (50 ml) was added dropwise to a stirred suspension of mercuric acetate (20 g, 62 mmoles) in methanol (200 ml). The solution was stirred until an aliquot no longer gave a yellow precipitate with aqueous sodium hydroxide solution. To the solution was added dropwise a solution of potassium chloride (9·24 g, 0·12 mole) in water (150 ml). The white precipitate, 18·5 g (86 per cent), was washed thoroughly with cold water. It could not be crystallized from any common solvent. The product had m.p. 140–155° and $\lambda_{max(\mu)}^{Nujol}$ 9·25 (C—O—C).

Anal. Calcd. for $C_{10}H_{18}Cl_2Hg_2O_2$: C, 18·6; H, 2·80. Found: C, 18·8; H, 2·64.

The ether groups were varied in Procedure J by use of the appropriate alcohol in place of methanol.

Biological Activity

Methods

The mercurials were tested against Sarcoma 180 in Swiss mice, Leukemia L–1210 and Adenocarcinoma 755 in (C57Bl × DBA)F1 mice. All tests were done according to the procedures established by the Cancer Chemotherapy National Service Center, National Institutes of Health.[17] Since none of the compounds had any significant activity against the first two tumours, detailed methods and results will be given only for the Adenocarcinoma.

A series, generally of 293 mice, was implanted in the axillary region with 0·1 ml of a tumour brei suspended in Locke's solution. The mice in any series were all of one sex, and all weighed between 18 and 22 g. The mice were distributed randomly in groups of ten, except for one group of 43 animals which served as a control. Treatment was begun 24 h after tumour implantation and continued once daily for eleven days. Drugs were dissolved or suspended in 0·9 per cent sodium chloride containing 0·5 per cent methylcellulose. Animals were weighed at the end of one week. Twenty-four hours after the last treatment, the mice were weighed and sacrificed, then the tumours excised, weighed and averaged.

Compounds were considered sufficiently active to warrant confirmatory tests if, on initial testing, the average tumour

Table VI—*continued*

Compound no.	NSC no.	R[a]	Daily dose mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	*T/C*
XVI	23110	OcO	100	0/10			toxic
			75	3/10			toxic
			50	43/50	−0·7/1·7	438/1462	0·30
			25	16/20	−1·2/0·65	429/1613	0·27
			12·5	19/20	0·7/0·7	747/1613	0·46
			6·3	10/10	1·8/1·2	997/1062	0·94
			3·1	9/10	1·5/1·2	793/1062	0·75
XVII	30911	−SCN	100	0/10			toxic
			75	0/10			toxic
			50	49/90	−0·6/2·3	660/1861	toxic
			37·5	43/60	−0·9/1·8	805/1546	0·52
			25	9/10	−0·9/0·6	1058/1101	0·96

[a] AcO = acetoxy, BzO = benzoyloxy, SrO = stearoyloxy, OcO = *n*-octanoyloxy.

the latter exhibited some tumour-reducing activity though not enough to qualify as significantly active. The octanoate and stearate were the most effective of the salts of (I). It is interesting that the octanoate of the more effective dioxan dimercurials (XL) had a small therapeutic index. This indicates that the effect of the anion varies with the carrier. This is borne out by the observation that of the nine acetates in Tables VI to VIII, only four were active; six of the seven chlorides, eight of the nine halides, and four of the six benzoates were active.

In Table VII the results for a series of open-chain dimercurials, in which the alkoxy groups attached to the two and three carbons were varied, are presented. The variants were hydroxy, methoxy, ethoxy, *n*-propoxy, isopropoxy, and *n*-butoxy. No improvement in the activity resulted from varying this substituent. Eight of the twelve compounds were active.

The result of increasing the chain length as well as branching of the chain is shown in Table VIII. The one compound with more than four carbons in the skeletal chain (XXIX) and the compound with a 3-oxaheptane carrier (XLIV) were inactive. Increased branching of the chain appeared to reduce activity. Three of the six compounds with an additional methyl substituent cyclohexene-1 (3·21 g, 30 mmoles) in methanol (50 ml) was added dropwise to a stirred suspension of mercuric acetate (20 g, 62 mmoles) in methanol (200 ml). The solution was stirred until an aliquot no longer gave a yellow precipitate with aqueous sodium hydroxide solution. To the solution was added dropwise a solution of potassium chloride (9·24 g, 0·12 mole) in water (150 ml). The white precipitate, 18·5 g (86 per cent), was washed thoroughly with cold water. It could not be crystallized from any common solvent. The product had m.p. 140–155° and $\lambda_{max(\mu)}^{Nujol}$ 9·25 (C—O—C).

Anal. Calcd. for $C_{10}H_{18}Cl_2Hg_2O_2$: C, 18·6; H, 2·80. Found: C, 18·8; H, 2·64.

The ether groups were varied in Procedure J by use of the appropriate alcohol in place of methanol.

Biological Activity

Methods

The mercurials were tested against Sarcoma 180 in Swiss mice, Leukemia L–1210 and Adenocarcinoma 755 in (C57Bl × DBA)F1 mice. All tests were done according to the procedures established by the Cancer Chemotherapy National Service Center, National Institutes of Health.[17] Since none of the compounds had any significant activity against the first two tumours, detailed methods and results will be given only for the Adenocarcinoma.

A series, generally of 293 mice, was implanted in the axillary region with 0·1 ml of a tumour brei suspended in Locke's solution. The mice in any series were all of one sex, and all weighed between 18 and 22 g. The mice were distributed randomly in groups of ten, except for one group of 43 animals which served as a control. Treatment was begun 24 h after tumour implantation and continued once daily for eleven days. Drugs were dissolved or suspended in 0·9 per cent sodium chloride containing 0·5 per cent methylcellulose. Animals were weighed at the end of one week. Twenty-four hours after the last treatment, the mice were weighed and sacrificed, then the tumours excised, weighed and averaged.

Compounds were considered sufficiently active to warrant confirmatory tests if, on initial testing, the average tumour weight, treated/controls, was $\leqslant 0 \cdot 50$. Without dwelling on the complexity of the statistics of the confirmatory tests, in general the compound was tested at an active dose in six independent tests.[17] In some tests, after a compound was found to have significant and reproducible activity in three successive tests at a single dose, it was retested at several doses greater and less than the active dose. As will be obvious from the data presented in the following section, the majority of the compounds in the present series were active. In the course of collecting initial and confirmatory evidence of activity of these compounds, a rather unwieldy amount of new data was accumulated. In an attempt to simplify the presentation, data on each compound at each dosage level were averaged; even though this is probably not the most elegant treatment from the statistical viewpoint, it allows a reasonable interpretation of the data without burdening the reader with hundreds of tests.

For purpose of comparative evaluation of the compounds, a compound will arbitrarily be considered active if tumour weight T/C is $\leqslant 0 \cdot 50$, and non-toxic if 70 per cent or more of the mice survived treatment. It is appreciated that loss of body weight is a sign of toxicity and that the tumour growth may be inhibited by attrition (see discussion).

Results and Discussion

None of the monomercurials (Table X) were active against carcinoma 755.

There were 28 open-chain dimercurials tested against carcinoma 755 (Tables VI, VII, VIII). Eighteen of these inhibited the tumours $\geqslant 50$ per cent at dosages tolerated by the mice, reducing tumour size from 9–46 per cent of controls. The maximum reductions in tumour size were observed in small, unconfirmed samples (V, XV, XVIII), or at doses barely tolerated (XV). The therapeutic indices ranged from 1 to 4; all but three compounds (XV, XVI and XXV) had a therapeutic index of less than 2. With the possible exception of (XVI) it is not likely that any of the open-chain compounds are a significant improvement on the parent compound (I).

Table VI shows the results with compounds differing from (I) only in the anion. All but the thiocyanate were active, and even

Table VI. Effect of class 1 mercurials on Adenocarcinoma 755

$$\begin{array}{c} \quad\quad\quad\quad\quad\quad \text{OMe}\ \ \text{OMe} \\ \quad\quad\quad\quad\quad\quad | \quad\quad | \\ \textit{meso}\text{-RHgCH}_2\text{CH—CHCH}_2\text{HgR} \end{array}$$

Compound no.	NSC no.	R[a]	Daily dose, mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	T/C
I	2201	AcO	50	0/30			toxic
			37·5	3/20			toxic
			32	23/30	−0·6/1·9	631/1222	0·52
			25	251/350	−0·2/2·3	544/1075	0·32
			20	47/60	−0·5/1·9	753/1533	0·49
			16	88/100	0·6/1·7	718/1068	0·67
			10	16/20	0·7/1·4	606/1165	0·52
			8	49/50	1·0/1·3	885/1112	0·80
			4	50/50	0·4/1·3	807/1135	0·71
			2	37/40	1·4/1·3	1231/1153	1·07
			1	20/20	0·5/0·5	940/948	0·99
XI	20829	Cl	75	0/10			toxic
			50	23/50	1·1/2·9	337/1853	toxic
			25	8/10	0·4/1·5	401/1267	0·32
			22	10/10	−1·1/2·9	516/2155	0·24
			15	10/10	3·5/3·5	1187/1336	0·89
XII	20831	Br	100	1/10			toxic
			75	29/40	−1·2/1·5	407/1737	0·23
			50	18/20	1·7/3·2	941/1761	0·53
			33	8/10	1·2/2·9	988/2155	0·46
			20	10/10	0·6/0·2	801/938	0·85
XIII	20830	I	150	5/10	0·6/2·0	502/1638	toxic
			100	33/60	−1·5/1·6	472/1714	toxic
			67	9/10	0·5/2·0	730/1638	0·45
			50	10/10	2·7/3·5	1111/1336	0·83
XIV	22681	BzO	50	1/20			toxic
			25	45/50	0·3/2·4	587/1486	0·40
			12·5	18/20	1·7/2·4	573/1418	0·40
			6·25	20/20	2·4/2·4	1005/1418	0·71
XV	22682	SrO	113	6/10	−1·4/1·6	302/1682	toxic
			75	35/41	−1·7/2·2	171/1457	0·12
			50	47/51	−1·8/1·8	438/1528	0·29
			33	21/21	−0·7/1·3	466/1522	0·31
			16	30/30	0·7/2·3	928/1700	0·54
			8	30/30	1·0/2·3	1022/1700	0·60

Table VI—*continued*

Compound no.	NSC no.	R[a]	Daily dose mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	*T/C*
XVI	23110	OcO	100	0/10			toxic
			75	3/10			toxic
			50	43/50	−0·7/1·7	438/1462	0·30
			25	16/20	−1·2/0·65	429/1613	0·27
			12·5	19/20	0·7/0·7	747/1613	0·46
			6·3	10/10	1·8/1·2	997/1062	0·94
			3·1	9/10	1·5/1·2	793/1062	0·75
XVII	30911	−SCN	100	0/10			toxic
			75	0/10			toxic
			50	49/90	−0·6/2·3	660/1861	toxic
			37·5	43/60	−0·9/1·8	805/1546	0·52
			25	9/10	−0·9/0·6	1058/1101	0·96

[a] AcO = acetoxy, BzO = benzoyloxy, SrO = stearoyloxy, OcO = *n*-octanoyloxy.

the latter exhibited some tumour-reducing activity though not enough to qualify as significantly active. The octanoate and stearate were the most effective of the salts of (I). It is interesting that the octanoate of the more effective dioxan dimercurials (XL) had a small therapeutic index. This indicates that the effect of the anion varies with the carrier. This is borne out by the observation that of the nine acetates in Tables VI to VIII, only four were active; six of the seven chlorides, eight of the nine halides, and four of the six benzoates were active.

In Table VII the results for a series of open-chain dimercurials, in which the alkoxy groups attached to the two and three carbons were varied, are presented. The variants were hydroxy, methoxy, ethoxy, *n*-propoxy, isopropoxy, and *n*-butoxy. No improvement in the activity resulted from varying this substituent. Eight of the twelve compounds were active.

The result of increasing the chain length as well as branching of the chain is shown in Table VIII. The one compound with more than four carbons in the skeletal chain (XXIX) and the compound with a 3-oxaheptane carrier (XLIV) were inactive. Increased branching of the chain appeared to reduce activity. Three of the six compounds with an additional methyl substituent

Table VII. Effect of class 2 mercurials on Adenocarcinoma 755

R′HgCH$_2$CH(OR)—CH(OR)—CH$_2$HgR′

Compound no.	NCS no.	R′[a]	R	Daily dose, mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	T/C
II	21296	AcO	Me[b]	20	6/10	−1·1/3·5	707/1336	toxic
				9	10/10	−1·0/−0·5	680/948	0·72
III	19952	AcO	Et[c]	100	0/10			toxic
				50	20/30	−1·7/2·0	269/1681	toxic
				25	29/30	0·3/2·0	616/1700	0·36
				20	9/10	−0·3/0·2	799/938	0·85
IV	23605	AcO	Et[b]	25	3/20	−2·3/2·1	563/1359	toxic
				12·5	0/10			toxic
				6·25	8/10	0·5/3·9	1743/2395	0·73
V	21289	AcO	H[c]	30	2/10	−2·0/1·3	318/1355	toxic
				22·5	4/10	0·0/0·2	378/938	toxic
				20	19/20	0·0/2·4	737/1346	0·55
				13	8/10	−2·4/1·3	159/1355	0·12
				10	20/20	0·3/1·9	690/1439	0·48
				9	9/10	−1·7/1·3	119/1355	0·09
				6	10/10	0·1/1·6	446/1316	0·34
				4	10/10	1·3/1·6	836/1316	0·64
				2·6	9/10	1·5/1·6	936/1316	0·71
VII	30912	AcO	*n*-Pr[c]	50	0/10			toxic
				25	1/10			toxic
				18·7	1/10			toxic
				12·5	57/60	−0·4/2·2	851/2394	0·36
				8	10/10	2·3/3·2	655/2719	0·24
				5·5	10/10	3·6/3·2	1730/2719	0·64
VIII	30914	AcO	*n*-Bu[c]	35	0/10			toxic
				17·5	6/40	−1·7/3·0	1463/3014	toxic
				13	38/40	0·7/2·5	1354/2219	0·61
				9	33/40	0·0/1·3	892/1544	0·58
IX	22680	Cl	*i*-Pr	50	0/10			toxic
				25	30/40	−1·1/2·6	450/1592	0·28
				12·5	19/20	1·4/3·5	1028/1020	1·01
				6·25	10/10	3·3/3·6	847/1774	0·48
				3	10/10	1·4/1·3	1090/1496	0·73
XVIII	30910	Cl	*n*-Pr[c]	50	4/10	−0·6/2·1	265/1313	toxic
				25	6/10	−1·3/2·1	70/1313	toxic
				18·7	9/10	−0·4/3·2	538/2719	0·20
				12·5	53/60	1·9/2·2	995/2393	0·42
				8·0	10/10	2·9/3·2	1429/2719	0·53
				5·5	10/10	2·0/3·2	1670/2719	0·61

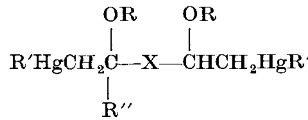
Table VII—*continued*

Compound no.	NCS no.	R′[a]	R	Daily dose, mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	*T/C*
XIX	30913	Cl	*n*-Bu[c]	50	0/10			toxic
				25	4/10	−2·6/0·1	484/2163	toxic
				18·7	0/10			toxic
				12·5	17/20	−1·2/1·8	574/1462	0·39
				8	19/20	−0·5/1·4	1079/1620	0·67
				5·5	10/10	1·9/2·0	1503/1638	0·92
XX	30918	BzO	*n*-Pr[c]	50	0/10			toxic
				25	0/10			toxic
				12·5	10/10	−0·2/1·6	1401/1283	1·09
XXI	30919	BzO	*n*-Bu[c]	100	0/10			toxic
				50	2/10	−4·1/0·3	65/1618	toxic
				37·5	9/10	−1·5/2·0	622/1638	0·38
				25	61/70	−0·6/2·1	538/2204	0·24
				12·5	30/30	0·6/2·0	926/1700	0·54
				6·25	10/10	0·2/2·8	1260/1681	0·75
XXII	29442	BzO	H[c]	50	0/10			toxic
				25	4/10	−3·1/0·3	163/1618	toxic
				18·7	3/10	0·2/2·0	655/1638	toxic
				12·5	49/60	0·9/2·1	1042/2285	0·46
				8	6/10	1·8/2·0	563/1638	toxic
				5·5	10/10	1·6/2·0	899/1638	0·55

[a] AcO = acetoxy, BzO = benzoyloxy. [b] (±)-Isomer. [c] *meso*-Isomer.

on the C-2 carbon were inactive. There were 20 ring compounds (Table IX) and, in contrast to open-chain compounds, all but two of these were active. Therapeutic indices were as high as 7·5 to 8 (XXXVII, XXXVIII, and XLIII). At best, the compounds in Table VI reduced the size of the tumour to 16–43 per cent of the controls; in this respect the ring compounds were no more effective antitumour agents than the open-chain compounds. The variations on the basic structure in this group of compounds were: the position of the substituents in the ring, one of the hetero atoms in the ring, and the anion. Of the three most effective compounds in the series, two were 2,5-substituted dioxans (XXXVII, XXXVIII), and one was a 2,3-substituted dioxan (XLIII). The anion causes considerable difference in activity of the dioxans, but the anion effect again varies with the structure;

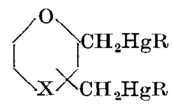
Table VIII. Effect of class 3 mercurials on Adenocarcinoma 755



Compound no.	NSC no.	R′[a]	R″	R	X	Daily dose, mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	T/C
XXIII	21298	AcO	Me	Et	—	25	0/10			toxic
						15	9/10	1·3/3·5	1343/1336	1·01
						8	9/10	−0·8/−0·5	648/948	0·68
						6·25	9/10	0·7/2·8	1049/1681	0·62
XXIV	21292	Cl	Me	Et	—	50	3/10	−3·6/0·3	217/1618	toxic
						25	10/10	−0·1/2·7	974/1166	0·84
						15	10/10	4·1/3·5	1349/1336	1·01
XXV	20832	Cl	Me	Me	—	40	2/10	−2·1/0·2	855/938	toxic
						30	7/10	−1·4/1·6	186/1188	0·16
						20	28/30	−0·3/1·9	620/1370	0·45
						13	30/30	−0·1/1·6	705/1536	0·46
						9	10/10	2·4/1·6	1636/1188	1·38
XXVI	30917	BzO	Me	Me	—	100	5/10	−3·0/2·1	83/1313	toxic
						50	9/10	1·1/2·1	751/1313	0·57
XXVII	23103	Cl	Me	H	—	25	12/30	−1·3/2·1	328/1394	toxic
						12·5	43/60	0·3/2·9	656/2426	0·27
						9·4	38/50	0·8/2·2	708/1541	0·46
XXVIII	31961	BzO	Me	H	—	25	1/10			toxic
						12·5	10/20	0·9/2·2	402/1482	toxic
						8	47/70	−1·3/1·0	448/1159	toxic
						6	35/40	−0·9/0·9	333/1009	0·33
						4	29/30	1·3/2·1	1236/1699	0·73
						2·4	10/10	0·8/0·8	940/1152	0·82
XXIX	21297	AcO	H	Me	$—CH_2CH_2—$	30	0/10			toxic
						20	10/10	1·2/3·5	874/1336	0·65
XLIV	22690	I	H	Me	$—CH_2OCH_2—$	25	7/10	−0·7/2·1	899/1518	0·59

[a] AcO = acetoxy, BzO = benzoyloxy.

Table IX. Effect of class 4 mercurials on Adenocarcinoma 755



Compound no.	NSC no.	Isomer	R[a]	X	Daily dose, mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	*T/C*
XXX	21286	2,6	Cl	O	50	2/10	1·7/2·8	1160/1681	toxic
					37·5	22/30	−0·2/2·0	317/1700	0·30
					26	10/10	−2·1/1·3	392/1355	0·29
					17·5	15/20	−1·8/0·8	465/1147	0·41
					12	7/10	−0·5/1·3	489/1355	0·36
					8	19/20	0·3/1·5	589/1336	0·44
					5·3	28/30	1·1/1·6	992/1578	0·63
					3·5	10/10	1·5/1·6	958/1316	0·73
					2·4	10/10	1·5/1·6	1007/1316	0·77
XXXI	21287	2,6	I	O	150	1/20			toxic
					100	36/70	−1·2/2·0	392/1809	toxic
					75	8/40	−1·9/1·3	291/1322	toxic
					50	23/40	−1·6/1·8	342/1323	toxic
					44	9/10	−0·5/1·6	356/1316	0·27
					25	10/10	1·7/2·7	613/1166	0·53
					17·5	9/10	−1·1/0·2	893/938	0·95
XXXII	30915	2,6	BzO	O	100	0/10			toxic
					50	1/10	−3·5/2·1	25/1313	toxic
					37·5	9/20	−1·9/2·0	231/1667	toxic
					25	76/90	0·1/1·7	724/2224	0·33
					17	16/20	−1·3/2·0	514/2052	0·25
					12·5	9/10	−0·7/1·6	528/1316	0·40
					11	17/20	0·2/2·0	885/2052	0·43
					6·0	20/20	1·5/1·1	791/1351	0·59
					5·0	10/10	1·9/0·7	1120/1385	0·81

XXXIII	22678	2,6	I	N	37·5	0/10			toxic
					25	19/30	0·8/1·8	377/1216	toxic
					17	10/20	−1·8/1·3	275/1345	toxic
					11	14/20	−0·4/1·3	565/1345	0·42
					8	7/10	0·5/1·0	838/1501	0·56
					4	9/10	0·9/1·0	1363/1501	0·91
XXXIV	23108	2,6	AcO	S	25	30/40	−0·6/2·6	938/2405	0·39
					12	62/80	−0·4/1·7	274/1622	0·17
					8	10/10	1·2/1·7	1444/1910	0·76
					5·3	10/10	0·3/2·7	1313/1910	0·69
					3·5	10/10	1·2/1·7	1493/1910	0·78
XXXV	30909	2,5	NO_3	O	75	0/10			toxic
					50	39/90	0·6/2·1	616/2238	toxic
					37·5	52/60	−0·7/1·8	440/1445	0·30
					33	14/20	−1·5/1·5	347/1600	0·22
					25	17/20	−0·9/1·9	525/1612	0·33
					22	18/20	−1·4/1·5	379/1600	0·24
					15	9/10	−0·2/1·9	774/1765	0·44
					7	9/10	−1·2/1·9	1163/1765	0·66
XXXVI	23106	2,5	NO_3 } HO } [b]	O	25	4/10	−1·8/1·8	455/1129	toxic
					12	32/40	1·8/3·6	634/1472	0·43
					6·0	10/10	−2·6/3·1	1408/2010	0·70
XXXVII	23105	2,5	I	O	150	0/10			toxic
					100	59/70	−1·4/3·0	318/1336	0·24
					75	10/10	−1·8/1·2	166/1062	0·16
					50	8/10	−1·2/1·2	194/1062	0·18
					25	29/40	−0·3/1·8	576/1543	0·37
					12·5	40/40	0·5/2·1	577/1516	0·39
					6·25	10/10	2·8/2·8	1270/1681	0·76
					3·1	10/10	1·8/2·8	1667/1681	0·99
XXXVIII	30916	2,5	BzO	O	100	4/20	−3·1/1·6	87/1316	toxic
					75	30/40	−1·4/0·9	319/1060	0·30
					50	96/120	−0·9/1·4	340/1318	0·26
					33	38/40	−0·6/0·8	363/1072	0·34
					25	9/10	0·0/1·6	438/1316	0·33
					22	30/40	0·4/0·8	505/1072	0·47
					15	20/20	−0·3/1·2	707/1648	0·43
					10	28/30	0·8/1·3	716/1510	0·47
					5·0	40/40	0·8/1·7	916/1514	0·61

Table IX—*continued*

Compound no.	NSC no.	Isomer	R[a]	X	Daily dose, mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	*T/C*
XXXIX	38187	2,5	Cl	O	250	2/10	−2·8/−0·6	45/688	toxic
					100	45/50	−0·6/1·3	696/1596	0·44
					50	30/30	−0·1/2·0	858/1700	0·51
					25	30/30	−0·2/2·0	690/1700	0·40
					12·5	10/10	4·3/2·8	1207/1681	0·72
XL	38186	2,5	OcO	O	100	0/10			toxic
					50	4/20	1·4/3·5	1141/1794	toxic
					25	74/90	1·0/2·5	490/1559	0·31
					12·5	20/20	0·9/2·4	1125/1624	0·69
					6·15	19/20	1·6/2·4	1024/1624	0·63
					3·1	10/10	0·7/2·4	1540/1624	0·92
XLI	21935	2,3	NO_3	O	25	20/30	−1·0/1·1	536/1213	toxic
					18	9/10	−1·0/1·6	159/1188	0·13
					12	29/30	1·9/2·2	623/1336	0·47
					8	10/10	0·0/1·6	336/1188	0·28
					5	10/10	1·5/1·6	709/1188	0·60
XLII	21934	2,3	I	O	200	8/20	−1·9/1·2	375/1661	toxic
					150	6/10	−1·5/1·6	231/1188	toxic
					100	57/60	−0·9/1·6	379/1346	0·28
					66	10/10	0·7/1·6	360/1188	0·30
					44	9/10	0·1/1·6	289/1188	0·24
					40	9/10	3·2/3·5	1221/1336	0·91
					25	10/10	−0·1/−0·5	531/946	0·56

XLIII	29443	2,3	BzO	O	50	4/10	−0·4/2·7	144/1166	toxic
					37·5	13/20	−1·3/2·3	198/1786	toxic
					25	72/80	0·3/2·0	519/1795	0·29
					17	10/10	1·9/2·9	683/2155	0·32
					12·5	19/20	−1·2/2·0	337/1499	0·22
					11	10/10	2·5/2·9	898/2155	0·42
					6·25	40/40	0·2/1·9	708/1654	0·48
					3·12	30/30	1·0/2·0	680/1704	0·40
					1·0	10/10	2·2/2·8	1500/1681	0·95
XLV	23109[c]				50	0/10			toxic
					25	0/10			toxic
					12·5	13/30	−0·8/1·8	463/1593	toxic
					6·0	20/30	0·0/1·9	1050/1705	toxic
					3·0	20/20	2·3/1·6	1579/1710	0·92
LIII	40581	2,5	Nc	O	250	0/10			toxic
					100	2/10	−0·2/1·9	383/1162	toxic
					50	39/40	0·7/3·9	1005/1737	0·58
LIV	41442	2,5	TlO	O	200	0/10			toxic
					100	0/10			toxic
					50	57/70	−0·1/2·2	670/1661	0·40
					25	30/30	0·3/2·0	584/1700	0·34
					12·5	30/30	0·8/2·0	1044/1700	0·61
LV	41443	2,5	Np	O	100	0/10			toxic
					50	1/10	−1·9/2·6	160/1631	toxic
					25	8/10	0·8/0·9	421/702	0·60

[a] BzO = benzoyloxy; AcO = acetoxy; OcO = *n*-octanoyloxy; TlO = *p*-toluyloxy; Nc = nicotinate; Np = β-naphthoate. [b] One R = NO_3 and the other R = OH. [c] See introduction of Chemistry for structure.

for example, the dinicotinate (NSC–40581) was inactive while the dibenzoate (NSC–30916) of the same mercuri carrier had a chemotherapeutic index of 7·5. The best results with the 2,6-dioxan carrier was given by the chloride; among 2,5-dioxans, the iodide and benzoate were best, while among 2,3-dioxans the benzoate was the best.

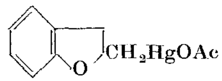
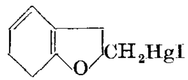
Substitution of an oxygen by N or S, or the elimination of both oxygens in the ring (XLV), did not improve activity.

It would be unfair to the massive amounts of data accumulated and to the concept of rational synthesis not to mention what averages conceal. It was characteristic of the mercurials, but probably not peculiar to them, that there was much variation in results obtained with any one compound in separate tests. Both toxicity and antitumour activity varied markedly from test to test in an entirely unpredictable fashion. These fluctuations did not seem to be associated with the tumour line used or with the rate of growth of the tumours in the test. The mercurials are insoluble in water and the density of the particles, after suspension, is quite high. It is conceivable that the seemingly random fluctuation in activity might be associated with small changes in the size of the particles in the suspension, as prepared in different experiments. Such differences in particle size could result from variation in the tightness of fit of the homogenizer. The explanation is hypothetical, but the variability is real. One statistical implication of the variability is that none of the bismercurials can be dismissed as inactive on the basis of a single experiment. Furthermore, the estimation of a therapeutic index is reliable only if based on a large number of independent tests. Nevertheless, the increased therapeutic effectiveness of the dioxan (XXXVIII) over the parent compound (I) can be demonstrated in concurrent tests run in the same experiment.

Another point that needs to be discussed is the relationship between toxicity and activity. Frequently, toxicity may be reflected in loss of appetite and, if sufficiently severe, the lowered food intake may inhibit tumour growth.

That activity of the mercurials is not due to weight loss can be readily seen by the following break-down of those data in Tables VI to X in which 70 per cent or more of the mice survived in a given test.

Table X. The effect of monomercurials class 5 compounds on Adenocarcinoma 755

Compound no.	NSC no.	Formula	Daily dose, mg/kg	Survivors	Weight change, treated/control	Tumour weight, treated/control	*T/C*
XLVI	23602		25	0/10			toxic
			12·5	6/10	−0·3/3·9	1167/2395	toxic
			6·25	5/10	0·5/3·1	1547/2010	toxic
			3·0	9/10	0·6/1·5	1067/1360	0·78
XLVII	23107		100	0/10			toxic
			50	0/10			toxic
			25	8/10	0·0/2·1	1042/1359	0·77
XLVIII	23102	$MeOCH_2CHCO_2^-$ | Hg^+	25	0/10			toxic
			12·5	0/10			toxic
			6·25	1/10	0·4/0·7	1950/2048	toxic
			3·0	4/10	1·3/3·2	950/1776	toxic
			1·5	10/10	−0·7/1·2	1777/1938	0·92
XLIV	23603	$MeOCH_2CHCOOEt$ | HgOAc	25	0/10			toxic
			12·5	0/10			toxic
			6·25	2/10	0·3/3·1	1528/2010	toxic
			3·0	10/10	1·4/1·5	1811/1360	1·33
L	23604	$CH_3CH_2CHCH_2HgBr$ | OCH_3	25	0/10			toxic
			12·5	0/10			toxic
			6·25	4/10	−2·9/3·1	481/2010	toxic
			3·0	10/10	0·3/1·5	1393/1360	1·02
LI	23601	HgBr | $CH_3CHCHCH_3$ | OMe	50	0/20			toxic
			25	0/10			toxic
			12·5	7/10	0·1/3·9	1422/2395	0·59
LII	23104	HgCl | $CH_3CHCHCH_3$ | OMe	25	0/10			toxic
			12·5	0/10			toxic
			6·25	7/10	1·5/3·9	1642/2395	0·69