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CHEMICAL PROPERTIES AND BIOLOGICAL EFFECTS OF 2-HALOETHYL SULFONATES*

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Abstract—Data for the alkylating activities, DNA cross-linking activities, and proliferation-inhibitory activities toward cultured L1210 cells for twenty-four 2-haloethyl sulfonates are reported. Previously reported activities against P388 leukemia *in vivo* are also presented to permit correlation of *in vitro* and *in vivo* properties. Since these compounds are believed to be 2-haloethylating agents, their properties and effects were compared with those of chlorozotocin, which is a recognized 2-chloroethylating agent. 2-Chloroethyl chloromethanesulfonate, which was the most effective compound against P388 leukemia, had a moderate level of alkylating activity and a low level of cross-linking activity, but it was quite active in inhibiting proliferation of cultured L1210 cells. Although its alkylating activity was about the same as that of chlorozotocin, it caused much less cross-linking of DNA. The *in vitro* tests were useful for gaining information relating structure to the individual properties, but results obtained for one of the properties might not be predictive of the relative values obtained for other properties nor for *in vivo* activity against P388 leukemia. These results indicate that additional experiments to define the mechanism of action of these agents are needed.

In the course of preparing and testing 2-chloroethyl amines as anticancer agents, Haddow and Ross [1] considered the use of 2-chloroethyl methanesulfonate as a chemical agent for attaching 2-chloroethyl groups to the nitrogen atoms of amines under mild conditions, and they also reasoned that this compound might add 2-chloroethyl groups to the amino groups of biological materials under physiological conditions. They found that the agent was active against Walker rat carcinoma and that the ethyl and the 2-fluoroethyl esters of methanesulfonic acid were also active. Fahmy and Fahmy [2] tested 2-chloroethyl methanesulfonate for mutagenic activity in *Drosophila melanogaster* and concluded that the "extraordinary efficiency of chloroethyl methanesulfonate in mutating morphogenesis loci is unprecedented among all known mutagens."

Consoli and coworkers [3–5] reported the activities of 2-chloroethyl methanesulfonate and 2-fluoroethyl methanesulfonate against several experimental neoplasms. They observed lesions in the cytoplasm and the nucleus of the neoplastic cells and suggested [6] that the compounds interfered with the synthesis of nucleic acids and that electrophilic radicals derived from the compounds might react with nucleophilic centers, such as amino groups and carboxyl groups, in biological systems. Ross and Davis [7] synthesized and tested a number of other esters of methanesulfonic acid and concluded that none of them was more active than the 2-chloroethyl ester, although the methyl, ethyl, and 2-fluoroethyl esters were of the same order of activity, if higher doses were administered. Schmidt [8] compared the effects of 2-chloroethyl methanesulfonate and a variety of alkylating agents on a spectrum of experimental neoplasms and observed that this agent differed from the others in being specifically active against Lymphoma 8. This specificity led him to state that "this compound has antitumor activity and pharmacological properties different from those of the usual alkylating agent". In a broader study [9], in which a number of alkyl esters of methanesulfonic acid were evaluated in a variety of experimental cancer systems, the specificity of the 2-haloethyl esters for Lymphoma 8 was confirmed, and the 2-chloroethyl ester was more active than the 2-fluoroethyl or 2-bromoethyl esters. Sandberg *et al.* [10] presented the experimental anticancer data for methanesulfonates that the Division of Cancer Treatment, NCI, had accumulated. Of twenty-nine alkyl or cycloalkyl esters that were tested against several neoplasms, only five were active, and not all of these were active against the same neoplasms. These five were the methyl, the 2-chloroethyl, the 2-fluoroethyl, the 4-hydroxybutyl, and the 2,3-dibromo-2-propen-1-yl esters. Because of the limited anticancer activity of the 2-haloethyl esters of methanesulfonic acid, these compounds have evoked little interest for cancer chemotherapy.

The clinical anticancer activities of 5-[3,3-bis(2-chloroethyl)-1-triazenyl]-1*H*-imidazole-4-carboxamide and the *N*-(2-chloroethyl)-*N*-nitrosoureas, which are believed to act by 2-chloroethylation of macromolecules, led Shealy and co-workers to synthesize a series of 2-haloethyl sulfonates that

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included some that would be expected to be chemically more active than the methanesulfonates and to test their antileukemic activities against murine leukemia P388 [11]. Several of the compounds had improved antileukemic activity. The purpose of the experiments reported here was to examine the interrelationships of the structure, the alkylating activity, the cross-linking activity, the antiproliferative activity against cultured L1210 cells, and the activity against P388 leukemia *in vivo*. The antileukemic activities were reported previously [11], but they are repeated here to facilitate correlation of these properties.

METHODS

Determination of antileukemic activity. Activity against P388 leukemia was determined by the protocol of the National Cancer Institute [12], and compounds were considered to be active if they caused a 20% or greater increase in lifespan of the mice [13].

Determination of the effects of the test compounds on the rate of proliferation of cultured L1210 cells. A suspension of cultured L1210 cells in Fischer's medium containing 10% horse serum was dispensed into sets of triplicate screw-cap test tubes to give a cell density of 50,000–100,000 cells/ml. The test compound was dissolved in ethanol or dimethyl sulfoxide (DMSO) to give a stock solution, portions of which were added to water to give the desired concentrations, and portions of the dilute solutions were added to the suspension of cells. All operations with the dilute solutions were performed as rapidly as possible to minimize decomposition of the compounds prior to their coming in contact with the cells. After incubation of the sealed tubes for 48 hr, 1-ml samples of the cell suspensions were removed and diluted with SRI salts solution containing formalin [14], and the cells were counted with a model Z_h Coulter Particle Counter. The IC_{50} was approximated from a plot of cell count versus the concentration of agent. The IC_{50} is the concentration for which

$$\frac{N_{treated} - N_O}{N_{control} - N_O} = 0.5$$

$N_{treated}$ and $N_{control}$ are the cell counts of the treated and control cultures after incubation for 48 hr, and N_O is the cell count at the beginning of the incubation.

Determination of alkylating activity. The alkylating activity was determined by a method that has been used extensively in this laboratory for a number of years [15]. The test compound is dissolved in ethanol immediately before the test is performed, and an equal volume of the solvent is added to the control test mixture. The test compound (12 μmoles) is incubated with 4-(*p*-nitrobenzyl)pyridine (74 μmoles) in Tris buffer, pH 7.4, at 37° for 2 hr, the mixture is made alkaline, the colored reaction product is extracted into ethyl acetate, and the absorbance at 540 nm of the extract is determined.

Measurement of cross-linking of DNA. Two methods were used to detect the cross-linking activity. Both methods are based upon the enhancement of the fluorescence of ethidium upon its becoming complexed with double-stranded DNA. In the first step of each method, calf thymus DNA (1 mg/ml) and the test compound (5 μmoles) were incubated in 0.1 M phosphate buffer (pH 7.4) for 24 hr.

By the first method [16], a portion of the incubated solution was added to an aqueous solution of ethidium bromide at pH 11.4, the fluorescence was determined, and the mixture was heated at 100° for 4 min and then quenched in ice water for 15 min. The fluorescence was again measured. The enhancement of fluorescence in the treated sample in comparison to the control sample is indicative of the renaturation of the DNA resulting from cross-linking.

By the second method [17], a portion of the incubated solution was added to an aqueous solution of ethidium bromide at pH 8.5, the fluorescence was determined, and the mixture was heated at 100° for 4 min and then quenched in ice water for 15 min. The fluorescence of the cooled solution was measured, and then the fluorescence was measured at stepwise higher temperatures. The fluorescence decreased as the DNA "melted", and supposedly the extent of melting as the temperature was raised would be influenced by the extent of cross-linking of the DNA. The difference in the temperature required to cause an 80% decrease in the fluorescence of the control and treated samples was taken to be indicative of the extent of cross-linking, although other causative phenomena may be involved.

RESULTS AND DISCUSSION

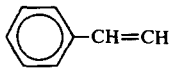
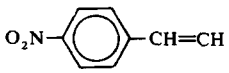
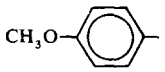
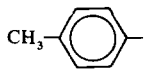
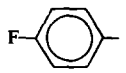
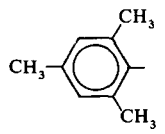
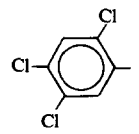
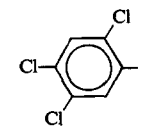
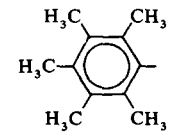
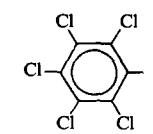
Table 1 contains data for the structures, alkylating activities, DNA cross-linking activities, cytotoxicities for cultured L1210 cells, and antileukemic activities against P388 leukemia for twenty-five sulfonates and for chlorozotocin. The syntheses of the new sulfonic esters have been reported previously [11].

Comparison of the data for compounds 1–3 with those for compounds 4–8 shows that the 2-haloethyl esters of halo- or cyanomethanesulfonic acids have higher alkylating activities and are more cytotoxic to cultured cells than the corresponding esters of methanesulfonic acid. Compound 4 was the most cytotoxic and had the greatest therapeutic effect against leukemia P388. Compound 5 was also quite active. The greater cytotoxicity and antileukemic activity of the 2-chloroethyl chloromethanesulfonate (compound 4) in comparison with that of the 2-fluoroethyl ester (compound 8) are consistent with the results of Kohn and co-workers [18, 19], who observed that *N*-(2-fluoroethyl)-*N*-nitrosoureas caused less cross-linking than the corresponding *N*-(2-chloroethyl)-*N*-nitrosoureas. The results obtained with the ethanesulfonates (compounds 10 and 11) were similar to those obtained with the corresponding methanesulfonates (compounds 1 and 3).

The 2-chloroethyl ester of ethenesulfonic acid (compound 12) was quite active in the *in vitro* tests, but it had only marginal therapeutic activity against P388 leukemia. The reason for this low activity *in vivo* is not known. The 2-chloroethyl ester of 1-bromoethenesulfonic acid (compound 13) had high alkylating activity and high cytotoxicity but lacked *in*

Table 1. Properties of 2-haloethyl sulfonates and related compounds*

$XCH_2CH_2OSO_2R$

Compound No.	X	R	Alkylating activity, A_{540}	Cross-linking activity†		Cytoxicity, IC_{50} (μM)	Best T/C for P388
				Percent renaturation	$\Delta T_{0.2}$		
1	Cl	CH_3	0.03	(1.8)	(1.3)	> 20	134
2	Br	CH_3	0.02	1.7	(4.5)	> 20	116
3	F	CH_3	0.0	1.5		> 20	111
4	Cl	$ClCH_2$	1.10	1.8	3.6	< 0.9	214 (177 + 1 survivor)
5	Cl	$BrCH_2$	1.50	4.4	(0.5)	4.5	200
6	Cl	ICH_2	0.77	4.0	(3.0)	6.7	110
7	Cl	$NCCH_2$	>3.0	2.6	(0.2)	4.8	142
8	F	$ClCH_2$	1.25	(3.1)	(1.1)	>20	117
9	H	$ClCH_2$	>3.0	(1.1)		>20	112
10	Cl	C_2H_5	0.04	(1.7)	(1.8)	>20	116
11	F	C_2H_5	0.08	1.4	(4.0)	>20	111
12	Cl	$CH_2{=}CH$	1.56	0.2	5.7	2.8	125
13	Cl	$CH_2{=}C(BR)$	>3.0	3.4	(6.0)	<0.9	105
14	Cl		0.13	(0.5)	(1.0)	10	107
15	Cl		0.06	0.4	(6.0)	9.3	115
16	Cl		0.07	1.5	(3.0)	>20	100
17	Cl		0.20	0.1	(3.7)	20	
18	Cl		0.20	(0.9)	(1.3)	>20	109
19	Cl		>3.0	2.0	(5.0)	<0.4	110
20	Cl		0.02	0.2	(3.5)	>20	106
21	Cl		1.17	0.6	(3.5)	17	114
22	F		2.75	1.2	(5.3)	>20	113
23	Cl		0.03	(1.0)		>20	98
24	Cl		0.24	(0.7)	1.0	>20	96

(*continued*)

Table 1 (*continued*)

Compound No.	X	R	$XCH_2CH_2OSO_2R$ Alkylating activity, A_{540}	Cross-linking activity† Percent renaturation	Cross-linking activity† $\Delta T_{0.2}$	Cytoxicity, IC_{50} (μM)	Best T/C for P388
25	Cl	O_2N-C$_6$H$_4$-CH_2	0.25	(2.2)	1.0	>20	98
Chlorozotocin			1.11	19.7 ± 2.1 (N = 4)	26.1 ± 12.5 (N = 4)	10.5 ± 4.2 (N = 6)	Cures

* Abbreviations and definitions: Chlz., chorozotocin, 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose; A_{540}, absorbance at 540 nm; IC_{50}, the concentration of the compound that inhibits the proliferation of cultured L1210 cells 50% during an exposure period of 48 hr; $\Delta T_{0.2}$, difference between the temperatures at which the fluorescence of the DNA–ethidium complexes is decreased 80% for the treated and the control samples; and T/C, median life span of the treated mice/median life span of the control mice × 100.

† Values in parentheses are negative differences in comparison with the control samples.

vivo activity. The esters of the 2-arylethenesulfonic acids (compounds 14 and 15) were moderately cytotoxic in the absence of high alkylating activity and were ineffective against P388 leukemia. The 2-chloroethyl ester of *p*-nitrophenylmethanesulfonic acid (compound 25) had low activities in all of the tests.

None of the 2-haloethyl esters of the arenesulfonic acids (compounds 16–24) had significant antileukemic activity, although some of them did have significant alkylating and cytotoxic activities. Some of these cytotoxic activities might have been due to the free sulfonic acids that were generated.

As a first approximation, a reasonable explanation of the enhanced activity of 2-chloroethyl chloromethanesulfonate (compound 4), relative to the methanesulfonate (compound 1), is that compound 4 is more reactive because the chloromethanesulfonate group should be a better leaving group than the methanesulfonate group. The inductive effect (−I) of the chloro, bromo, iodo, and cyano substitutents of compounds 4–7 is expected to confer greater leaving-group capability on these sulfonate groups. However, it should be noted that the increased leaving-group potential of the chloromethanesulfonate group did not confer significant activity, *in vitro* or *in vivo*, on the fluoroethyl and ethyl esters (8 and 9 respectively). Also, the arenesulfonates represent a range of reactivities, some of the arenesulfonate groups being better leaving groups than is the methanesulfonate group of compound 1. (Highly reactive compounds may decompose before they can reach target sites.) As stated, none of the arenesulfonates were active except for the activity *in vitro* of the pentafluorobenzene sulfonate. In addition to chemical reactivity, other factors, such as transport properties or steric effects, may influence cytotoxicity or activity *in vivo*.

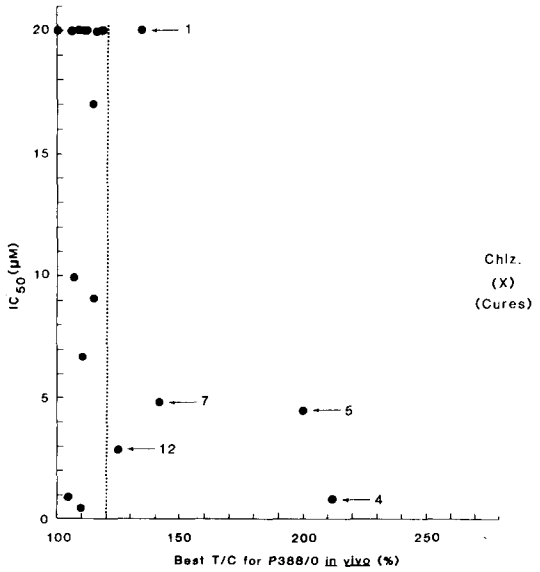


Fig. 1. Plot of IC_{50} values versus antileukemic activity against P388 leukemia. The numbers refer to the compounds in Table 1. Chlz. = chlorozotocin.

To examine the degrees of correlation among the various properties for which data are given in Table 1, the data are plotted in a series of graphs.

Figure 1 shows that only five of the compounds had significant antileukemic activity. The cytotoxicity for cultured L1210 cells was not an accurate predictor of activity against P388 leukemia *in vivo*, but the four therapeutically active 2-chlorethyl esters (compounds 4, 5, 7 and 12) were rather cytotoxic and were more cytotoxic than chlorozotocin, which caused cures of mice bearing P388 leukemia.

Panels A and B of Fig. 2 show that the *in vitro* alkylating activity is not a predictor of either antileukemic activity or cytotoxicity to cultured L1210 cells. Compound 1 had a low, but significant antileukemic activity, but it had a low alkylating activity and a low level of *in vitro* cytotoxicity. Although several of the compounds had high alkylating activities, they had low levels of cytotoxicity and therapeutic efficacy. The compounds with the greatest cytotoxic and therapeutic activities (compounds 4 and 5, and chlorozotocin) had medium levels of alkylating activity. It is interesting that the value in Fig. 2B for chlorozotocin, which effects cures of P388 leukemia, lies near the center of the scatter of values.

It is presently believed that 2-chloroethylation of the bases of DNA by *N*-(2-haloethyl)-*N*-nitrosoureas

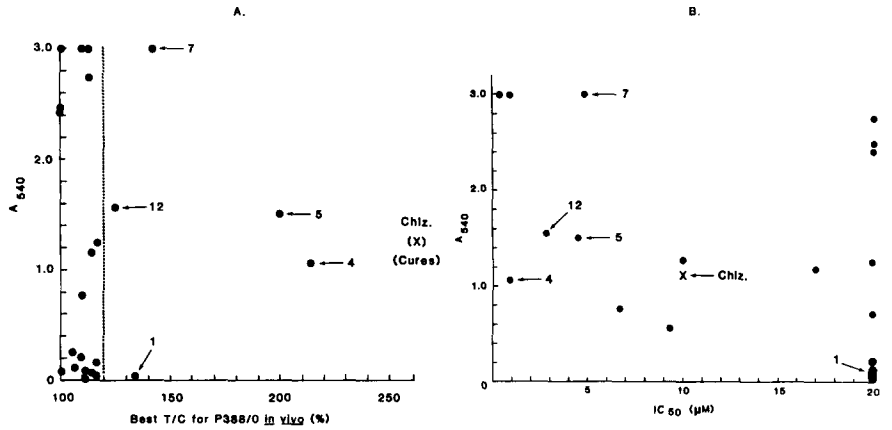


Fig. 2. (A) Plot of the alkylating activity (A_{540}) versus antileukemic activity against P388 leukemia. (B) Plot of the alkylating activity (A_{540}) versus IC_{50} values. The numbers refer to the compounds in Table 1.

[20–22] followed by cross-linking of the strands of DNA [23, 24] is the major mechanism causing cytotoxicity. Since there is reason to expect that the 2-haloethyl sulfonates might act as 2-chloroethylating agents, it is reasonable to seek correlation between the cross-linking activity and the other properties given in Table 1. Figure 3 shows that the alkylating activities of the compounds cover the whole range of values of A_{540} that can be obtained in the test (0 to 3.0), and that there is poor correlation between alkylating activity and renaturation activity. Although chlorozotocin had only a moderate alkylating activity in these tests, it caused much more renaturation of the DNA than any of the 2-haloethyl esters of the sulfonic acids. In Fig. 4 the values obtained for the therapeutically active compounds in the two tests for cross-linking are graphed with the corresponding values for chlorozotocin. The values for chlorozotocin were obtained in four experiments, and the mean values and the standard deviations are plotted. Although the standard deviations of the values for chlorozotocin are rather large, particularly for $\Delta T_{0.2}$, it is evident that chlorozotocin apparently caused much more cross-linking than the sulfonates. This difference was notable in all of the experiments. In view of the large standard deviations obtained in the tests with chlorozotocin, it is questionable whether or not significant extents of cross-linking were caused by the sulfonates.

It is recognized that caution must be exercised in trying to interrelate the results obtained in experiments with chemicals, with isolated DNA, with cultured cells, and with ascites cells *in vivo*. Nevertheless comparison of the data for the more therapeutically active 2-chloroethyl sulfonates with those for chlorozotocin arouses certain questions. Why does chlorozotocin cause so much more cross-linking than the sulfonates even though the two

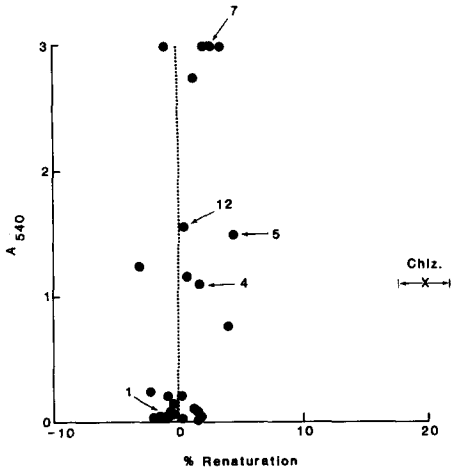


Fig. 3. Plot of the alkylating activity (A_{540}) versus percent renaturation. The numbers refer to the compounds in Table 1.

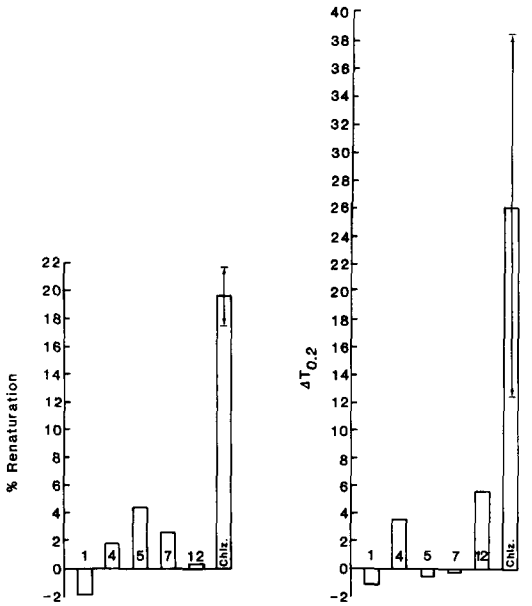


Fig. 4. Comparison of the DNA-cross-linking activities of the therapeutically active 2-haloethyl sulfonates and chlorozotocin. The methods of measuring the percent renaturation and the $\Delta T_{0.2}$ are described in the text. The arrows show the standard deviation for the data for chlorozotocin. The numbers refer to the compounds in Table 1.

types of compounds might have similar alkylating activities? If cross-linking of DNA is a major determinant of cytotoxicity, why is the IC_{50} for chlorozotocin higher than those of several of the sulfonates that cause less cross-linking? Although the reactions of the test compounds with 4(*p*-nitrobenzyl)-pyridine occurred at 37° and pH 7.4, the relative extents of alkylation by the agents might differ for alkylation of free native DNA and of DNA in the chromatin of intact cells. It is possible that the two types of agents alkylate DNA by different mechanisms to cause alkylation at different sites of the DNA with resulting differences in the ease and extent of subsequent strand scission and cross-linking. Other investigators have reported (reviewed in Refs. 25 and 26) that ethyl methanesulfonate reacts mainly by an S_N2 mechanism, but *N*-ethyl-*N*-nitrosourea reacts mainly by an S_N1 mechanism, and that the ratio of alkylation of O–6 to N–7 of guanine in DNA is about twenty-three times greater for the nitrosourea than for the methanesulfonate. The experiments reported here do not provide information about the chemical kinetics of the reactions or of the sites of alkylation. It is also possible that different extents of strand scission and cross-linking might alter the secondary and tertiary structures of the DNA in such ways that the binding of ethidium is affected.

Although the experiments presented here leave a number of questions unanswered, they do provide useful data for relating structure and several types of activity. The above questions and facts will be addressed in future experiments, and in-depth studies of the mechanisms of action will be performed with the compounds that have demonstrated good chemotherapeutic activity.

REFERENCES

1. A. Haddow and W. C. J. Ross, *Nature, Lond.* **177**, 995 (1956).
2. O. G. Fahmy and M. J. Fahmy, *Nature, Lond.* **177**, 996 (1956).
3. G. Consoli, S. Ricciardi, L. Talarico and P. Altucci, *Boll. Soc. ital. Biol. sper.* **33**, 682 (1957) [*Chem. Abstr.* **52**, 4027f (1958)].
4. G. Consoli, S. Ricciardi, L. Talarico and P. Altucci, *Boll. Soc. ital. Biol. sper.* **33**, 1091 (1957) [*Chem. Abstr.* **53**, 14324e (1959)].
5. S. Ricciardi, P. Altucci, L. Talarico, C. Riccardi and G. Consoli, *Boll. Soc. ital. Biol. sper.* **35**, 276 (1959) [*Chem. Abstr.* **56**, 13500h (1962)].
6. S. Riccardi, L. Talarico, P. Altucci, C. Riccardi and G. Consoli, *Boll. Soc. ital. Biol. sper.* **35**, 278 (1962) [*Chem. Abstr.* **56**, 13500i (1962)].
7. W. C. J. Ross and W. Davis, *J. chem. Soc.* 2420 (1957).
8. L. H. Schmidt, *Cancer Chemother. Rep.* **9**, 56 (1960).
9. L. H. Schmidt, R. Fradkin, R. Sullivan and A. Flowers, *Cancer Chemother. Rep.* (Suppl. 2), 78 (1965).
10. J. S. Sandberg, H. B. Wood, Jr., R. R. Engle, J. M. Venditti and A. Goldin, *Cancer Chemother. Rep.* (Part 2), **3**, 137 (1972).
11. Y. F. Shealy, C. A. Krauth, R. F. Struck and J. A. Montgomery, *J. med. Chem.* **26**, 1168 (1983).
12. R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacker and B. J. Abbott, *Cancer Chemother. Rep.* (Part 3, No. 2), **3**, 1 (1972).
13. Drug Evaluation Branch, National Cancer Institute, *Instruction 14—Screening Data Summary Interpretation and Outline of Current Screen*, p. 18, Revised June, 1978.
14. G. G. Kelley, M. H. Vail, D. J. Adamson and E. A. Palmer, *Am. J. Hyg.* **73**, 231 (1961).
15. G. P. Wheeler, B. J. Bowdon, J. A. Grimsley and H. H. Lloyd, *Cancer Res.* **34**, 194 (1974).
16. J. W. Lown, A. Begleiter, D. Johnson and A. R. Morgan, *Can. J. Biochem.* **54**, 110 (1976).
17. A. R. Morgan and V. Paetkau, *Can. J. Biochem.* **50**, 210 (1972).
18. K. W. Kohn, *Cancer Res.* **37**, 1450 (1977).
19. R. A. G. Ewig and K. W. Kohn, *Cancer Res.* **38**, 3197 (1978).
20. B. S. Kramer, C. C. Fenselau and D. B. Ludlum, *Biochem. biophys. Res. Commun.* **56**, 783 (1974).
21. D. B. Ludlum and W. P. Tong, in *Nitrosoureas: Current Status and New Developments* (Eds. A. W. Prestakyo, S. T. Crooke, L. H. Baker, S. K. Carter and P. S. Schein), p. 85. Academic Press, New York (1981).
22. D. B. Ludlum and W. P. Tong, in *Nitrosoureas in Cancer Treatment* (Eds. B. Serrou, P. S. Schein and J-L. Imbach), p. 21. INSERM Symposium No. 19. Elsevier/North Holland Biomedical Press, Amsterdam (1981).
23. K. W. Kohn, L. C. Erickson, G. Laurent, J. Ducore, N. Sharkey and R. A. Ewig, in *Nitrosoureas: Current Status and New Developments* (Eds. A. W. Prestayko, S. T. Crooke, L. H. Baker, S. K. Carter and P. S. Schein), p. 69. Academic Press, New York (1981).
24. K. W. Kohn, L. C. Erickson and G. Laurent, in *Nitrosoureas in Cancer Treatment* (Eds. B. Serrou, P. S. Schein, and J-L. Imbach), p. 33. INSERM Symposium No. 19. Elsevier/North-Holland Biomedical Press, Amsterdam (1981).
25. P. D. Lawley, in *Biology of Radiation Carcinogenesis* (Eds. J. M. Yuhas, R. W. Tennant and J. D. Regen), p. 165. Raven Press, New York (1976).
26. P. D. Lawley, in *Chemical Carcinogens* (Ed. C. E. Searle), ACS Monograph 173, p. 83. American Chemical Society, Washington, DC (1976).