The Relation of Anticancer Activity to Chemical Structure

A Review*

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This discussion of structure-activity relationships is confined to two or the more important classes of anticancer agents—the alkylating agents and the antimetabolites—and to results obtained with these compounds in experimental animal therapy.

An attempt is made to point to correlations between the anticancer activity and the structure of these compounds. From these structure-activity correlations, limited deductions can be made concerning the mechanism of action of some of the compounds.

This is not a comprehensive review. Emphasis has been placed on studies that have at least partially defined the structural requirements necessary for the anticancer activity of the various types of agents.

DISCUSSION

A. THE ALKYLATING AGENTS

The material presented here on the alkylating agents has been gathered from many sources, the most comprehensive and helpful source being the recently published monograph entitled "Comparative Clinical and Biological Effects of Alkylating Agents" (100).

The alkylating agents, being electrophilic, react most readily with nucleophilic groups, such as amino and mercapto groups, and the anions of organic and inorganic acids. They react with these groups by two generally recognized mechanisms (48):

1. first-order nucleophilic substitution (SN1)

\[ RX \rightarrow R^+ + X^- \]

or

\[ R^+ + HY \rightarrow RY + H^+ \]

rate = \( k_1[RX] \)

2. second-order nucleophilic substitution (SN2)

\[ RX + Y^- \rightarrow RY + X^- \]

or

\[ RX + HY \rightarrow RY + X^- + H^+ \]

rate = \( k_2[RX][Y^-] \) or \( HY \)

From these equations it is obvious that the rate of reaction of the alkylating agents that react by the first mechanism (such as nitrogen mustard) is dependent only on the concentration of the agent, whereas the rate of reaction of those that react by the second mechanism (such as Myleran) is dependent on the concentrations of both the agent and the nucleophilic sites.

There are many possible \textit{in vivo} sites for reaction of these agents so that much, if not most, of any agent employed in therapy may be wasted in the cell in reactions that are not specific insofar as tumor inhibition is concerned. The exact nature of the reaction which is important for the anticancer activity of the alkylating agents has not yet been established. Many investigators agree that this attack is probably on the gene chemicals (cellular polynucleotides), but there are at least two schools of thought concerning the specific site on the polynucleotides at which this reaction takes place. One group believes, on the basis of \textit{in vitro} experiments, that the important reaction is the esterification of the phosphate groups of the polynucleotides (1,
whereas the other group feels that the significant attack may be on the nitrogens of the polynucleotide purines and pyrimidines (see Chart 1) (53, 64, 84).

![Chart 1](image)

**CHART 1.**—Some possible sites of reaction of the alkylating agents and polynucleotides.

A generally assumed requirement for cytotoxic activity of the alkylating agents is bifunctionality (i.e., at least two alkylating groups in the molecule), although there are some notable exceptions to this rule: for example, 1-(2,4-dinitrophenyl)-aziridine (I) and 2-chloroethyl methanesulfonate (II) (1). This requirement of bifunctionality is the basis of the cross-linking hypothesis, which states that a bifunctional alkylating agent can react with two nucleophilic centers of a biological macromolecule such as deoxyribonucleic acid and that the resulting bridge alters the chromosomes, causing cytotoxicity (1, 88). The cycloalkylating hypothesis of Timmis is another attempt to explain this general requirement (96).

Four main classes of alkylating agents are in use today: (a) nitrogen mustards, (b) ethylenimines, (c) epoxides, and (d) esters of sulfonic acids. The oldest and best established of these classes—the nitrogen mustards—is discussed first.

1. **Nitrogen mustards.**—The aliphatic mustards, as exemplified by nitrogen mustard itself (IV), react under physiological conditions via the ethylenimmonium ion (V) (5).

Nitrogen mustard (HN₂), although the first mustard to be used in man, is still considered by many clinicians as the agent of choice for the treatment of a variety of disseminated cancers.

One early modification of nitrogen mustard was its oxidation to the amine oxide (Nitromin, III). The oxide is a weaker base than HN₂ and therefore less reactive; as a result, this compound has no vesicant action. It is also reported to have greater antitumor efficacy and less toxicity than HN₂ (29).

As might be expected, replacement of the methyl group of HN₂ by other groups such as substituted phenyl groups has a marked effect on both the chemical reactivity and the biological activity of nitrogen mustard (70).

From the data in Table 1 it is apparent that a certain degree of chemical reactivity is necessary for biological activity. Presumably the less reactive analogs simply do not alkylate under physiological conditions. In the series under discussion, the reactivity is enhanced by an electron-donating group on the phenyl ring and is decreased by an electron-withdrawing group. This effect on reactivity is due to the influence that the group in question has on the electron density at the nitrogen atom or, in other words, due to the effect on the basic strength of the amine nitrogen. It might also be pointed out that there is no evidence that the cyclic ethylenimmonium intermediate exists in the reactions of any of the aryl nitrogen mustards (70). An explanation of this fact is that all these compounds are much less basic than nitro-
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...gen mustard itself. However, the reactivity of the chloro group of these compounds, as compared with simple alkyl chlorides, is undoubtedly due to the assistance of the &-nitrogen, even if the cyclic structure is not formed.

The data presented in Table 2 emphasize that chemical reactivity is only one factor in the biological activity of the mustards. Here are some closely related compounds of approximately the same chemical reactivity, which is well above the supposed minimal required reactivity (see Table 1), and yet there are large differences in the capacity of these compounds to inhibit Walker rat carcinoma 256. Ross attributes these differences to the so-called transport characteristics of these compounds (70).

The differences just mentioned lead to a discussion of the search that has been and is being made for mustards (and other alkylating agents) which show some specificity of action. The introduction of certain groups in the nitrogen mustard structure may influence the arrival of the alkylating agent at its site of action without affecting its chemical reactivity. An interesting example of this type of modification is 3-(p-[bis(2-chloroethyl)amino]phenyl)alanine (VII) (7). The L-isomer of this phenylalanine mustard showed intense inhibition of Walker rat carcinoma 256; the D-isomer showed only slight inhibition under the conditions of the test; and the DL-form showed an intermediate degree (6, 35). The reason for the lack of activity of the D-isomer is not clear, and further study of this problem would appear to be of considerable importance.

Ross and Warwick synthesized p-[bis(2-chloroethyl)amino]azobenzene (VIII, X=H) in hopes that this compound, in itself a poor alkylating agent, might be selectively reduced in vivo to N,N-bis(2-chloroethyl)-p-phenylenediamine (IX), which is a good alkylating agent (72). In fact, they prepared a series of these azobenzenes (VIII) and found that their biological activity is directly proportional to their ease of reduction by the xanthine-xanthine oxidase system (73, 74).

TABLE 2

VARIATION IN BIOLOGICAL ACTIVITY OF COMPOUNDS OF COMPARABLE CHEMICAL REACTIVITY*  

<table>
<thead>
<tr>
<th>R</th>
<th>Per cent hydrolysis in 1 hr. in 50% per cent acetone at 66°C</th>
<th>Biological activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOOC(CH₃)₂O</td>
<td>42</td>
<td>++</td>
</tr>
<tr>
<td>HOOC(CH₃)₂O</td>
<td>44</td>
<td>++</td>
</tr>
<tr>
<td>HOOC(CH₃)₂O</td>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td>HOOC(CH₃)₂O</td>
<td>59</td>
<td>±</td>
</tr>
</tbody>
</table>

* Data from W. C. J. Ross (71).
† Inhibition of Walker rat carcinoma 256.
‡ Chlorambucil (CB1846).

An extensive investigation of the steric aspects of the necessity of bifunctionality for anticancer activity of the nitrogen mustards (and the other alkylating agents) has been carried out by a number of workers. Kon and Roberts (50) found that for the general structure (X) activity was confined to two compounds in which \( m = n - 2 \) and \( n = 3 \).

This work is related to that of Vargha (98), whose “mannitol mustard” (XI) has been reported to possess a high degree of activity in spite of the fact that closely related compounds (XII and XIII) are inactive.

Among other variations of nitrogen mustard that have shown anticancer activity are the pyrimidine-containing “mustard,” 5-[bis(2-chloroethyl)amino]6-methyl-2,4-pyrimidinediol (XIV) (52); a derivative of pyridoxine, 5-[bis(2-chloro-
ethyl)aminomethyl][4-(methoxymethyl)-2-methyl-3-pyridinol (XV) (90); the antimalarial analogs of Jones (49) and Creech (21) (XVI–XVIII); and

the benzimidazole mustard of Gellhorn (XIX) (41). Because of the variation in methods of testing, it is difficult to compare the efficacy of these various types of "mustards."

2. Ethylenimines.—The first ethylenimine derivative to show marked anticancer activity was 2,4,6-tris(1-aziridinyl)-s-triazine (XX) (11, 13). A related compound—2-(1-aziridinyl)-4,6-dimethoxy-s-triazine (XXXI)—and 1-(2,4-dinitrophenyl)-aziridine (XXII) (70) are outstanding examples of active monofunctional alkylating agents.

A number of phosphoramides have been prepared and have been found to be active (XXIII–XXV), (12, 14).

More recently, Heidelberger has obtained complete regressions of the established Flexner-Jobling carcinoma with other phosphoramides (XXVI and XXVII) (38).

Domagk (24) has investigated still another type of ethylenimine—namely, a 2,5-bis(1-aziridinyl)phenylazoquinone, E 39 (XXVIII).

The ethylenimines, which have not been studied as extensively as the nitrogen mustards, are of interest to the chemist in search of structure-activity relationships, chiefly because some are examples of monofunctional active alkylating agents (XXI and XXII).

### TABLE 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reactivity (Kthio)</th>
<th>Biological activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₂-CH₂CH₂</td>
<td>85</td>
<td>+</td>
</tr>
<tr>
<td>CH₂CH₂CH₂OCH₂CH₂</td>
<td>69</td>
<td>+</td>
</tr>
<tr>
<td>CH₂CH₂CH₂CH₂</td>
<td>40</td>
<td>+</td>
</tr>
<tr>
<td>CH₂-CH₂-CH₂</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>CH₂CH₂</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>CH₂CH₂CH₂-CH₂</td>
<td>2.3</td>
<td>-</td>
</tr>
</tbody>
</table>

* Data from W. C. J. Ross (71).
† Inhibition of Walker rat carcinoma 256.

3. Epoxides.—Although the epoxides have not proved as effective as the other alkylating agents, some investigation of structure-activity relationships in this series has been carried out.

Ross (71) has shown that, as expected, a certain minimal chemical reactivity is necessary for biological activity of the epoxides, as has been noted for the nitrogen mustards (see Table 3).

Everett and Kon, who studied the effect of separation of the epoxide groups, found that antitumor activity decreases as the number of methylene groups between the epoxide groups increases from one to six (28, 70). These results relate to some of the studies on the nitrogen mus-
tards mentioned above and to some of the studies on the sulfonic acid esters (see below).

4. Sulfonic acid esters.—Myleran (XXIX, n = 4) is probably the most studied and most used of the sulfonic acid esters. A study of the effect of the variation of n (3 to 10) on the anticancer activity of this series has shown that peak activity occurs when n = 4 and 5, although there is essentially no change in the chemical reactivity in the series. Timmis has advanced his hypothesis of cycloalkylation to explain these results, but he points out that XXX is active against Walker rat carcinoma 256, although the triple bond of its structure precludes the possibility of cycloalkylation (96).

Timmis also prepared Dimethylmyleran (XXXI, n = 4) and related compounds, since these compounds should alkylate by an SN1 mechanism rather than SN2 (96). This series showed the same peak activity at n = 4 and 5 as the Myleran series, but also showed increased reactivity and water solubility. This greater solubility and quicker onset of action of Dimethylmyleran compared with Myleran has led to clinical trials of this compound (96).

Recently, a series of monosulfonic acid esters (XXXII) has shown anticancer activity (67). It probably should be emphasized, however, that, although this last example and the ones given above (see ethylenimines) have shown that bifunctionality is not essential to the activity of alkylating agents, the bifunctional compounds appear to have more antitumor activity than do the monofunctional ones.

\[
\begin{align*}
\text{CH}_3\text{SO}_2\text{O}(&\text{CH}_2)_n\text{OSO}_2\text{CH}_3 & \quad \text{CH}_3\text{SO}_2\text{O}-\text{CH}_2-\text{C}(-\text{CH}_2)_n\text{C\text{C}}-\text{CH}_3\text{OSO}_2\text{CH}_3 \quad \text{XXIX} \\
\text{XX} & \quad \text{CH}_3\text{SO}_2\text{O}(&\text{CH}_2)_n\text{OSO}_2\text{CH}_3 \\
\text{XXX} & \quad \text{CH}_3\text{SO}_2\text{O}(&\text{CH}_2)_n\text{OSO}_2\text{CH}_3
\end{align*}
\]

In summary, certain points have been established concerning the relation of chemical structure to the biological activity of the alkylating agents:

1. Candidate alkylating agents must either have a certain minimal chemical reactivity or be converted in vivo to structures that do.

2. In general, the most active alkylating agents are bifunctional, although bifunctionality is not an essential requirement for activity. The degree of separation of the two alkylating groups of the molecule is critical. These facts, which are general for all the classes of alkylating agents, point to in vivo reaction at two sites a fixed distance apart, as suggested by the cross-linking hypothesis.

3. In certain instances, activity may be restricted by highly specific structural requirements as exemplified by Melphalan (VII) and the mannitol mustard (XI).

B. ANTIMETABOLITES

The antimetabolite theory has been well established for some time, and analogs of a variety of metabolites have been investigated for anticancer activity. Emphasis has been placed on analogs of the metabolites involved in the de novo synthesis of nucleic acids, and of purine- and pyrimidine-containing co-factors (see Charts 2 and 3). This emphasis is logically justifiable, since cancer is a disease of abnormal metabolism and mitosis, and the nucleic acids, as polynucleotides, control mitosis. It is also justifiable practically, since some of the best anticancer agents in use today are analogs of these metabolites (for example, amethopterin, 6-mercaptopurine, and 5-fluorouracil).

Antimetabolites of miscellaneous metabolites
are discussed first, followed by a more extensive section on the antimetabolites which interfere with nucleotide or nucleic acid metabolism.

1. Antagonists of miscellaneous metabolites.—
   a) Pyridoxine (Vitamin B6): Deoxypyridoxine (II) coupled with a B6-deficient diet inhibits a number of types of neoplasms (91, 92). Synergism with the acid hydrazides (III) and
   
   b) Riboflavin: A riboflavin-deficient diet has produced regression of established lymphosarcomas and this regression is enhanced by isoriboflavin (VII) and galactoflavin (VIII) (93). This observation of Stoerk and Emerson led other investigators to prepare compounds structurally related to riboflavin (45). One such compound—7,8-dichloro-10-D-sorbitylisoalloxazine (IX)—has been effective in producing regressions of lymphosarcomas in mice on a deficient diet (45).

c) Nicotinamide: At least two nicotinamide antagonists—3-ethylamino-1,3,4-thiadiazole (XI) (39) and 6-aminonicotinamide (XII) (37, 81)—are known to inhibit various forms of experimental neoplasms.

d) Amino acids: Nitrogen mustards containing the amino acid structure are mentioned above. These compounds do not function primarily as amino acid antagonists.

Although D,L-ethionine, an antagonist of methionine, has shown the ability to inhibit the
Jensen sarcoma (54), few other amino acid antagonists are effective as anticancer agents.²

2. Antagonists of metabolites involved in the de novo synthesis of nucleic acids.—

a) Folic acid antagonists: Although the structure of folic acid has been modified in many ways, giving rise to a number of compounds which have shown antifolic acid activity in different biological test systems (19, 20, 25, 34, 46, 47, 62, 75, 76, 80, 102), only the more potent of these have shown marked anticancer activity in experimental animals (63, 89).

Thus, the slight changes in the pteroylglutamic acid structure (XIII) shown (XIVa-e) (20, 46, 47, 62) gave rise to weak to moderate antifolics, none of which has shown anticancer activity (57).

In contrast, when the 4-hydroxy group of folic acid was replaced by an amino group (80), a potent antifolic with anticancer activity resulted (aminopterin, XVa) (57, 63, 89, 94). This structure when modified further gave several active compounds (XVb-d) (23, 80), including amethopterin (XVb) (80). Less effective as an anticancer agent was the aspartic acid analog of aminopterin (XVe) (57, 67).

Recently, Goldin and co-workers (33) have found that amethopterin chlorinated in the benzene ring (XVI) (19) is less toxic and more effective than is amethopterin itself. Other changes in aminopterin or in folic acid itself have not resulted in active anticancer agents (19, 20, 34, 47, 75, 76, 102). Indeed, most of the other changes have resulted, at best, in only weak antifolics, for example, XVI–XXI.

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A variety of other structures not so closely related to folic acid have also shown weak antifolic but no anticancer activity (XXII–XXVI) (22, 26, 43, 101). It would appear that the structure:

\[
\begin{align*}
&\text{COOH} \\
&\text{CH}_2\text{NH}_2 \quad \text{N} \quad \text{CONHCH}_2\text{CH}_2\text{COOH}
\end{align*}
\]

\[
\text{N} \quad \text{H} \quad \text{CONHCH}_2\text{CH}_2\text{COOH}
\]

COOH

\[
\text{CH}_2\text{NH}_2 \quad \text{N} \quad \text{CONHCH}_2\text{CH}_2\text{COOH}
\]

\[
\text{N} \quad \text{H} \quad \text{CONHCH}_2\text{CH}_2\text{COOH}
\]

is a feature essential for any significant degree of antifolic and anticancer activity in the folic acid derivatives.

The necessity for this structural feature carries over to the 2,4-diamino-5-phenylpyrimidines that show antifolic and anticancer activity. One of the most potent compounds of this series (XXVI) is 2,4-

\[
\text{NH}_2 \quad \text{Cl} \quad \text{N} \quad \text{NH}_2 \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{Ar}
\]

\[
\text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{R}
\]

\[
\text{COOH}
\]

\[
\text{R} \quad \text{CONHCH}_2\text{CH}_2\text{COOH}
\]

of folic to folinic acid; in addition, these antagonists probably directly interfere with folinic acid metabolism (95).

In its role of co-factor in one-carbon metabolism, folic acid is thought to undergo reduction and then the sequence of reactions shown in Chart 4 (9).

![Chart 4](#)

\[
\text{CHO} \quad \text{CHO} \quad \text{CHO}
\]

\[
\text{CH}_2\text{NR} \quad \text{CHO} \quad \text{CHO}
\]

\[
\text{N} \quad \text{R} \quad \text{CONHCH}_2\text{CH}_2\text{COOH}
\]

The function of the N10-methyl group in N10-methylpteroylglutamic acid (XIVc) is obviously that of blocking the one-carbon transfer shown above. The role of the 4-amino group of aminopterin, which apparently is so much more important that the N10-methyl group of XIVc, is not clear. Still, it is known that aminopterin and amethopterin in some way prevent the conversion of folic to folinic acid; in addition, these antagonists probably directly interfere with folinic acid metabolism (95).

The necessity for this structural feature carries over to the 2,4-diamino-5-(3,4-dichlorophenyl)-6-methyl-pyrimidine (DDMP) (29, 44). Hitchings has attributed the activity of this compound to its resemblance to part of the folic acid molecule (44).

Recently Timmis et al. (97) and Modest et al. (59) have found antifolic activity in a related series (XXVII). However, Timmis found that the most active compound prepared in his investigation was an 8-azapurine (XXVIII), which was 1/50 as active as amethopterin in the same test; the most active ary lazoprimidines (XXVII, R=p-bromophenyl) was only 1/300 as active as amethopterin (97). No evaluation of anticancer activity is given in Timmis's report.

Modest and Foley have prepared a series of dihydrotriazines (XXIX) which also show potent antifolic activity in microbial systems (29). The resemblance of the 2,4-diamino-portion of this structure to that of the previous two is obvious. Maximum activity occurs with meta and para halogens in the phenyl ring and with one or two carbon atoms at the 6-position of the triazine ring. These compounds have shown activity against mouse leukemias and solid tumors, but, unfortunately, they have also shown a high degree of toxicity, i.e., a low chemotherapeutic index.

It is known that antifolics interfere with the
formation of coenzyme F and that this interference in turn blocks one-carbon metabolism (see above). Thus, the de novo synthesis of purines and of thymine is inhibited, and it is the inhibition of one or both of these synthetic processes that is thought to inhibit growth (88). From his work with bacteria, Cohen has presented evidence that it is the inhibition of thymine synthesis and, therefore, DNA synthesis which is important in the action not only of the antifolics but of many other anticancer agents as well (18). However, recent evidence indicates that the action of folic acid antagonists is directed chiefly against tissues in which there is rapid cell division, and not specifically against neoplastic tissues (9).

b) Glutamine antagonists: The metabolism of glutamine (XXX), which is involved in at least three steps on the de novo pathway to nucleic acids (85), is inhibited strongly by the two closely related antibiotics, L-azaserine (XXXI) (61) and 6-diazo-5-oxo-L-norleucine (DON, XXXII) (58).

Through their inhibition of glutamine metabolism, these two compounds inhibit certain neoplastic tissues (40).

\[
\begin{align*}
\text{HOOCCHCH}_2 \text{CH}_2 \text{C-NH}_2 & \quad \text{HOOCCH}_2 \text{CH}_2 \text{O-C-CHN}_2 \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{XXX} & \quad \text{XXXI} \\
\text{HOOCCHCH}_2 \text{CH}_2 \text{C-NH}_2 & \quad \text{HOOCCH}_2 \text{CH}_2 \text{C-O-NHH}_2 \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{XXXII} & \quad \text{XXXIII} \\
\text{HOOCCH}_2 \text{O-C-NH}_2 & \quad \text{NH}_2 \\
\text{XXXIV} & \\
\end{align*}
\]

Glutamic acid, 5-hydrazide (XXXIII) shows activity against bacteria and weak activity against tumors.\(^5\) L-Serine carbamate (XXXIV) was reported by Skinner et al. to show the same order of activity against bacteria as azaserine, but no report on anticancer activity is given (82). The many observed differences in the biological activity of azaserine and DON indicate that these compounds are acting at multiple metabolic sites. It may be that XXXIII and XXXIV are acting at some, but not all, of these sites.

c) Pyrimidine antagonists:

(1) Unnatural pyrimidines: The anticancer activity of a pyrimidine containing an alkylating group (Dopan) has already been mentioned, as well as pyrimidines which show antifolic activity. With the exception of these, and a few pyrimidine antagonists such as 6-(methylsulfonyl)uracil (XXXV) (35) and 5-hydroxymuridine (XXXVI) (99), the search for anticancer activity in the pyrimidine series produced few promising compounds until the 5-fluoropyrimidines (XXXVII) and their nucleosides were investigated (25, 39). These compounds have been found to be potent inhibitors of a number of animal tumors (39).

There is evidence that 5-fluorouracil, the most potent compound of this group, specifically blocks the methylation of deoxyuridyl acid and also that 5-fluorouracil is incorporated into nucleic acids intact. There is, however, no evidence that this incorporation is the basis of its anticancer activity (85).

A number of other pyrimidines interfere with pyrimidine metabolism, but none has shown significant anticancer activity.

\[
\begin{align*}
\text{HOOCCHCH}_2 \text{CH}_2 \text{C-NH}_2 & \quad \text{HOOCCH}_2 \text{CH}_2 \text{O-C-CHN}_2 \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{XXXV} & \quad \text{XXXVI} \\
\text{CH}_3 & \quad \text{OH} \\
\text{OH} & \quad \text{OH} \\
\text{XXXVII} & \quad \text{XXXVIII} \\
\end{align*}
\]

Several “fraudulent” pyrimidine nucleosides have been synthesized (30, 31), but their lack of inhibitory action has been disappointing.

(2) Ring analogs of pyrimidines: 6-Azauracil (XXXVIII) inhibits Sarcoma 180 in vivo but not in tissue culture; its ribonucleoside, however, is active in tissue culture (78, 79). (This difference contrasts the activity in vivo and in vitro of 6-mercaptopurine and its ribonucleoside.)

\[
\begin{align*}
\text{COOH} & \\
\text{CH}_2 & \\
\text{CH}_2 \text{NHC-NH}_2 & \quad \text{C}_2 \text{H}_5 \text{O-C-NH}_2 \\
\text{H-C-NH}_2 & \quad \text{H-C-NHCH}_3 \\
\text{COOH} & \\
\text{XXXIX} & \quad \text{XL} \quad \text{XLI} \quad \text{XLII} \\
\end{align*}
\]

6-Azathymine, 6-azathymidine, and 6-azathymidyllic acid have shown activity in bacterial systems but not against neoplastic tissue (36, 65, 66).

(3) Urethan and formamides: Recent evidence (27, 69) has led to the speculation that urethan (XL), formamide (XLI), and N-methylformamide (XLII) all interfere with de novo pyrimidine synthesis, possibly at the ureidosuccinic acid (XXXIX) stage, and that this interference is the basis of their anticancer activity (85).
d) Purine antagonists:  
(1) Unnatural purines (including nucleosides and nucleotides): Many purines and their ribonucleosides have been synthesized and tested for anti-cancer activity by Hitchings et al. (56), by Brown (10), Bendich et al. (32), by Robins et al. (51), by Montgomery (60), Schaeffer et al. (77), and by others. These extensive investigations have led to the discovery of a few useful anti-cancer agents and are providing some information concerning their mechanism of action. It is reasonable to hope that the continuation of these studies may lead to the discovery of new anticancer agents and to modifications of the more effective known agents that will provide drugs of greater utility.

The results obtained in screening a large number of purines against Adenocarcinoma 755 in C57BL mice are discussed below (86). This tumor was chosen as a test system because it is more sensitive to the action of purine antagonists than any of the other test systems commonly employed (86).

The many variations in functional groups and the positions thereof on the purine ring have produced, so far, only two basic structures showing strong activity against Ad-755. These two structures are 6-chloropurine and 6-mercaptopurine. Neither 2,6-diaminopurine and 6-mercaptopurine, which has shown activity against leukemia in AKR mice, nor any of its derivatives has shown more than suggestive activity. Purine itself, 6-methylpurine, and 6-hydrazinopurine, a close relative of adenine, have shown slight but consistent activity. Other adenine derivatives, 6-amino-2-purinethiol, 2-(methylthio)-adenine, and isoguanine have shown some activity, but the activity is not consistent, and minimum effective doses are close to the maximum tolerated doses. The results obtained with a number of the purines which have been studied are given in Table 4.

Turning our attention to the two highly active purines, 6-chloropurine and 6-mercaptopurine, we find that some changes in their structure cause a complete loss of activity, whereas other changes affect the toxicity and the “therapeutic index” of the compounds. The therapeutic index of each compound was determined by a “titration” procedure (86) and is defined as the ratio of the maximum tolerated dose to minimum effective dose, so that the net effect is to reduce the chemotherapeutic index. Two compounds, 6-(2-naphthylthio)purine and 6-(methoxy-2-naphthylthio)purine, fail to show any significant activity at the highest level tested, although they do not show toxicity either, at that level.

2. The nature of the group in the 2-position is all important. An amino group produces a compound—thioguanine—with much greater toxicity but with high activity. A mercapto group or the hydroxyl group causes a decrease in toxicity but also in chemotherapeutic index. An ethyl group produces a compound that is completely inactive.

3. Blocking the 9-nitrogen of 6-mercaptopurine with an ethyl group decreases the maximum tolerated dose and minimum effective dose, but this balancing effect leaves the chemotherapeutic index unchanged. This result is of particular interest, since the 9-nitrogen of this compound is not available for in vivo ribonucleotidation unless deethylation occurs. Other groups in the 9-position reduce the chemotherapeutic index while raising the maximum tolerated dose and the minimum effective dose.

4. & 5. Data on 6-mercaptopurines substituted in the 7- and 8-position are more limited but indicate that such substitution reduces the effectiveness of the drug.

Studies on 6-chloropurine show the same pattern presented for 6-mercaptopurine, but 6-chloropurine appears much more sensitive to structural changes. Thus, groups in the 9-position reduce the chemotherapeutic index to a lower value than in the case of 6-mercaptopurine, and in no case does a change increase the index (see Table 4).

It is interesting that changes in the less effective purines almost always result in complete loss in the chemotherapeutic index. The results obtained in screening a large number of purines against Adenocarcinoma 755 in C57BL mice are discussed below (86).
### TABLE 4
**INHIBITION OF ADENOCARCINOMA 755 BY PURINES***

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Degree of inhibition</th>
<th>Therapeutic index</th>
<th>Toxic dose (mg/kg/day)</th>
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<tbody>
<tr>
<td>SH</td>
<td>H</td>
<td>H</td>
<td>++</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>SCH₂</td>
<td>H</td>
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<td>&gt; 80</td>
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<tr>
<td>S-n-C₃H₇</td>
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<td>++</td>
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<td>&gt; 250</td>
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<tr>
<td>S-n-C₄H₂₁</td>
<td>H</td>
<td>H</td>
<td>++</td>
<td>4</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>S-n-C₅H₁₀</td>
<td>H</td>
<td>H</td>
<td>+</td>
<td>1</td>
<td>&gt; 250</td>
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<td>250</td>
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<tr>
<td>SCH₂C=CH</td>
<td>H</td>
<td>H</td>
<td>++</td>
<td>8</td>
<td>125</td>
</tr>
<tr>
<td>SCH₂COCH₂</td>
<td>H</td>
<td>H</td>
<td>++</td>
<td>8</td>
<td>125</td>
</tr>
<tr>
<td>SCH₂CH₂H₂</td>
<td>H</td>
<td>H</td>
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<td>6</td>
<td>125</td>
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#### 6-Mercaptopurine and S-substituted derivatives

<table>
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<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Degree of inhibition</th>
<th>Therapeutic index</th>
<th>Toxic dose (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH</td>
<td>H</td>
<td>H</td>
<td>++</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>S-n-C₃H₇</td>
<td>H</td>
<td>H</td>
<td>++</td>
<td>&gt; 80</td>
<td>150</td>
</tr>
<tr>
<td>S-n-C₄H₂₁</td>
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<td>H</td>
<td>++</td>
<td>25</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>S-n-C₅H₁₀</td>
<td>H</td>
<td>H</td>
<td>+</td>
<td>4</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>S-n-C₆H₁₄</td>
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<td>H</td>
<td>+</td>
<td>1</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>SCH₂CN</td>
<td>H</td>
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<td>++</td>
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<td>250</td>
</tr>
<tr>
<td>SCH₂C=CH</td>
<td>H</td>
<td>H</td>
<td>++</td>
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<td>125</td>
</tr>
<tr>
<td>SCH₂COCH₂</td>
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<td>H</td>
<td>++</td>
<td>8</td>
<td>125</td>
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<tr>
<td>SCH₂CH₂H₂</td>
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<td>H</td>
<td>++</td>
<td>6</td>
<td>&gt; 500</td>
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#### 8-Substituted derivatives of 6-mercaptopurine

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Degree of inhibition</th>
<th>Therapeutic index</th>
<th>Toxic dose (mg/kg/day)</th>
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</thead>
<tbody>
<tr>
<td>SH</td>
<td>NH₃</td>
<td>H</td>
<td>++</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>SCH₂</td>
<td>NH₃</td>
<td>H</td>
<td>++</td>
<td>8</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>SH</td>
<td>SH</td>
<td>H</td>
<td>+</td>
<td>2.5</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>SH</td>
<td>OH</td>
<td>H</td>
<td>++</td>
<td>1</td>
<td>500</td>
</tr>
<tr>
<td>SH</td>
<td>C₆H₅</td>
<td>H</td>
<td>-</td>
<td></td>
<td>500</td>
</tr>
</tbody>
</table>

#### 9-Substituted derivatives of 6-mercaptopurine

<table>
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<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Degree of inhibition</th>
<th>Therapeutic index</th>
<th>Toxic dose (mg/kg/day)</th>
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<tr>
<td>SH</td>
<td>H</td>
<td>C₆H₅</td>
<td>++</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>SH</td>
<td>H</td>
<td>n-C₆H₁₄</td>
<td>++</td>
<td>20</td>
<td>125</td>
</tr>
<tr>
<td>SH</td>
<td>H</td>
<td>cyclo-C₆H₁₀</td>
<td>++</td>
<td>4</td>
<td>250</td>
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</tbody>
</table>

#### 6-Chloropurine and derivatives

<table>
<thead>
<tr>
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<th>R₂</th>
<th>R₃</th>
<th>Degree of inhibition</th>
<th>Therapeutic index</th>
<th>Toxic dose (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>++</td>
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<td>200</td>
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<td>H</td>
<td>C₆H₅</td>
<td>++</td>
<td>5</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>Cl</td>
<td>H</td>
<td>n-C₆H₁₄</td>
<td>++</td>
<td>1</td>
<td>&gt; 250</td>
</tr>
<tr>
<td>Cl</td>
<td>H</td>
<td>cyclo-C₆H₁₀</td>
<td>++</td>
<td>1</td>
<td>125</td>
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<td>H</td>
<td>-</td>
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<tr>
<td>Cl</td>
<td>Cl</td>
<td>H</td>
<td>--</td>
<td></td>
<td>&gt; 175</td>
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#### Purine, 6-methylpurine, and derivatives of adenine

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>Degree of inhibition</th>
<th>Therapeutic index</th>
<th>Toxic dose (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>H</td>
<td>+</td>
<td>&lt; 2</td>
<td>500</td>
</tr>
<tr>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>+</td>
<td>&lt; 2</td>
<td>3</td>
</tr>
<tr>
<td>NHH₂</td>
<td>H</td>
<td>H</td>
<td>+</td>
<td>&lt; 2</td>
<td>750</td>
</tr>
<tr>
<td>NH₂</td>
<td>OH</td>
<td>H</td>
<td>+</td>
<td>&lt; 2</td>
<td>100</td>
</tr>
<tr>
<td>NH₂</td>
<td>SH</td>
<td>H</td>
<td>+</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>NH₂</td>
<td>SCH₂</td>
<td>H</td>
<td>+</td>
<td>1</td>
<td>100</td>
</tr>
</tbody>
</table>

* Data from Skipper, Montgomery, Thomson, and Schabel (86).

† Based on ratio of average tumor weights, treated/control: ++ < 6 per cent, +6-25 per cent, ± 26-42 per cent, > 42 per cent.

‡ Maximum tolerated dose/minimum effective dose.
activity. For example, purine and 6-hydrazino-purine substituted in the 9-position are both inactive.

Another phase of investigation is the comparison of the activity of purines and their ribonucleosides. There is now ample evidence that ribonucleosidation can markedly affect activity, even though one cannot predict what the effect will be. Thus, while 6-mercaptopurine ribonucleoside is less toxic and has a greater chemotherapeutic index than 6-mercaptopurine against Adenocarcinoma 755, the converse is true for 6-(methylthio)purine ribonucleoside. 6-(Benzylthio)purine stands between 6-(methylthio)purine and 6-mercapto-purine with respect to the effect of ribonucleosidation (see Table 5). Even though we know of no cases in which ribonucleosidation has converted an inactive compound to an active one, the activity of 6-mercaptopurine ribonucleoside would suggest the desirability of the preparation and testing of the ribonucleoside of any active purine or purine analog.

At the present time only limited data are available on the so-called fraudulent nucleosides of purines. The "aminonucleoside" of puromycin (XLIII) is active against Ad-755 and other forms of neoplasia (8). This activity is associated with the aminosugar of the molecule, since the ribonucleoside of 6-dimethylaminopurine is less active than the aminoribonucleoside. However, when other purines were substituted for 6-dimethylaminopurine, the activity is reduced and to a greater degree than in the previous case (4). Baker and co-workers have more recently prepared other fraudulent nucleosides such as 9-β-D-xylofuranosyladenine (XLIV) which have shown suggestive, but not great, anticancer activity (2).

Clark, Elion, Hitchings, and Stock have recently published a study of the anticancer activity of purines related to 6-mercaptopurine (17). The results discussed here are in substantial agreement with theirs, except that Sarcoma 180 (employed by them) is less sensitive to "purine therapy" than is Adenocarcinoma 755, and therefore fewer derivatives of 6-mercaptopurine showed activity against that tumor.

(9) Ring analogs of purines:

(a) 8-Azapurines: Among the ring analogs of the purines, the best known anticancer agent is probably 8-azaguanine (5-amin-o-triazolo[d]pyrimidin-7-ol) (68). It is active against several transplantable tumors including Ad-755 and is of particular interest because the Ad-755/MP tumor is not cross-resistant to it. 8-Azahypoxanthine and 8-aza-2,6-diaminopurine are both active against Ad-755, although less active than 8-azaguanine. 6-Azahypoxanthine seems to be slightly more effective against the 755/MP tumor than against 755/O (see Table 6).

(b) Pyrazolopyrimidines: A large number of the isomeric pyrazolopyrimidines (XLV and LXVI) have been prepared by Robins et al. (16) and tested against Ad-755 and certain leukemias (87). Although the pyrazolo[4,3-d]pyrimidines (XLVI) were inactive, some of the isomeric pyrazolo[3,4-d]pyrimidines (XLV)—which have one nitrogen in the position corresponding to the 9-nitrogen of purines—were quite active. The most effective compound was found to be 4-amino pyrazolo[3,4-d]pyrimidine (XLVII), a compound which is also active against Ad-755/MP. A number of derivatives of this compound were active, although their chemotherapeutic index is only 1-2, and it is of interest to compare this series (XLIX) with the derivatives of 6-mercaptopurine (XLVIII).

TABLE 5
A COMPARISON OF THE ACTIVITIES OF SOME PURINES AND THEIR RIBONUCLEOSIDES AGAINST ADENOCARCINOMA 755*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approx. therapeutic index</th>
<th>Toxic dose (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Mercaptopurine</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>6-Mercaptopurine ribonucleoside</td>
<td>&gt;200</td>
<td>125</td>
</tr>
<tr>
<td>6-Methylthiopurine</td>
<td>&gt;80</td>
<td>150</td>
</tr>
<tr>
<td>6-Methylthiopurine ribonucleoside</td>
<td>1</td>
<td>&gt;25</td>
</tr>
<tr>
<td>6-Benzylthiopurine</td>
<td>120</td>
<td>200</td>
</tr>
<tr>
<td>6-Benzylthiopurine ribonucleoside</td>
<td>&gt;4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Thioguanine</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Thioguanosine</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>6-Chloropurine</td>
<td>13</td>
<td>&gt;200</td>
</tr>
<tr>
<td>6-Chloropurine ribonucleoside</td>
<td>&gt;1</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

* Data from Skipper, Montgomery, Thomson, and Schabel (86).
† Maximum tolerated dose/minimum effective dose.

Alkylation of the mercapto group of 6-mercapto-purine (XLIII) is active against Ad-755 and other forms of neoplasia (8). This activity is associated with the aminosugar of the molecule, since the ribonucleoside of 6-dimethylaminopurine is less active than the aminoribonucleoside. However, when other purines were substituted for 6-dimethylaminopurine, the activity is reduced and to a greater degree than in the previous case (4).
captopurine or of the amino group of 4-amino-pyrazolo[3,4-d]pyrimidine gives rise to active derivatives; the same is true of alkylation of the corresponding nitrogens of the five-membered ring (the 9 and 1 nitrogens, respectively). In the case of 6-mercaptopurine, these alkylations raised the maximum tolerated dose, the minimum effective dose, and in two cases the chemotherapeutic index; the 4-amino-pyrazolo[3,4-d]pyrimidine derivatives, in contrast, appear very similar in activity to their parent. Alkylation of the corresponding carbons of the pyrimidine rings (the 2 and 6 carbons, respectively) in both cases results in compounds devoid of activity.

Table 6 shows the inhibition of Adenocarcinoma 755 and 755/MP by 8-azapurines.

A comparison of purine antagonists of the three series—purines, N-triazolo[d]pyrimidines, and pyrazolo[3,4-d]pyrimidines—is worth while. The most active member of each heterocyclic series—6-mercaptopurine, 4-aminopyrazolo[3,4-d]pyrimidine, and 8-azaguanine—is inactive in the other two, providing no carry-over of knowledge. In an investigation of a new heterocyclic system for anticancer activity it would seem necessary to prepare a large variety of compounds in order to evaluate thoroughly the potential of that system. Further, although the two purine ring analogs are active against the Ad-755/MP tumor, they probably still differ in their mechanism of action, since 4-aminopyrazolo[3,4-d]pyrimidines substituted on the 1-nitrogen are still active, as are the 9-substituted derivatives of 6-mercaptopurine, whereas 9-ethyl-8-azaguanine is completely inactive. It is possible that incorporation into the nucleic acids is essential to the action of 8-azaguanine and not to the action of 6-mercaptopurine and 4-aminopyrazolo[3,4-d]pyrimidine.

The effects of some of the purine antagonists on Ad-755, 755/MP, L1210 leukemia, and L1210/MP are shown in Table 7. There are some notable differences in the activity of these compounds in the four test systems. First, although ribonucleosidation increases the effectiveness of 6-mercaptopurine against Ad-755 (see also Table 5) and decreases its effectiveness against L1210, the reverse is true in the case of 6-((methylthio)purine. S-Substitution of 6-mercaptopurine and thioguanine increases or does not change their effectiveness against Ad-755 but decreases their activity against L1210. None of the purines including the "amino-nucleoside" is effective against either Ad-755/MP or L1210/MP.

In the case of the ring analogs of purines, we find that 8-azaguanine is effective against Ad-755/MP but not against L1210/MP, that 8-aza-2,6-diaminopurine is effective against neither resistant neoplasm, and that 4-aminopyrazolo[3,4-d]pyrimidine is effective against both.

Although a number of other heterocyclic ring systems which resemble the purines have been prepared, none of these shows marked in vivo anti-cancer activity. Among these are the benzimidazoles, the 1- and 3-deazapurines, the 2-azapurines, the thiazolopyrimidines, and the oxazolopyrimidines (42). Recently, the synthesis and activity of 4-aminimidazo[4,5-d]pyridazine have been reported (15).

In summary, the data on the activity of the
purine antimetabolites show that changes in the structure of the active purines and purine analogs produce a great variation in biological activity; and, more important, certain derivatives of 6-mercaptopurine exhibit a greater chemotherapeutic index than 6-mercaptopurine itself in the particular test system employed.

SUMMARY

The material reviewed in this paper supports the view that one practical approach to the search for more effective anti-cancer agents is the modification of drugs of known biological activity. Certainly the development of amethopterin, and more recently the even more effective 3',5'-dichloro- and 3'-fluoropurines is the result of this approach to the cancer problem. One other approach to the search for effective anti-cancer agents is the modification of drugs of known biological activity. Certain derivatives of 6-mercaptopurine exhibit a greater chemotherapeutic index than 6-mercaptopurine itself in the particular test system employed. The derivatives of 6-mercaptopurine may also be cited as an example of the success of this approach. The anti-folic activity of N10-methylfolic acid, may be cited as an example of the success of this approach. The derivatives of 6-mercaptopurine may also be cited as at least a partially successful venture of the same type.

It may be hoped that the basic biochemical approach to the cancer problem will provide clues to more fruitful ways to modify known drugs and perhaps to entirely new metabolic areas in which the synthetic chemist may work with some measure of success in his endeavor to find truly effective anticancer agents.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Dr. Howard E. Skipper and Dr. Leonard L. Bennett for their helpful suggestions and criticisms concerning this review.

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The Relation of Anticancer Activity to Chemical Structure A Review

J. A. Montgomery