BIOORGANIC CHEMISTRY 6, 287–309 (1977)

Tumor Inhibitors Having Potential for Interaction with Mercapto Enzymes and/or Coenzymes[1]

A Review

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Received April 20, 1977

On the basis of extensive information on *in vivo* metabolism as well as biomimetic reactions using simple SH compounds and some enzymes, numerous chemical functions which react with SH groups are divided into two classes; i.e., one which involves electrophilic addition (EA) to an SH group and the other which features displacement reactions (DR) by the SH group (see Tables 1 and 2). The known tumor inhibitors are accordingly classified into EA and DR types. Biomimetic reactions of tumor inhibitors with model compounds of SH enzymes (or coenzymes) and with some SH enzymes themselves are described. Finally, as enhancement factors for the antitumor activity, the roles of hydrogen-bonding, neighboring group participation, and effect of ester side chains are introduced. These discussions may serve in the development of the new SH alkylating antitumor agents.

INTRODUCTION

To date, many natural and synthetic tumor inhibitors have been found and developed (*1*). In particular, magnificient research on tumor inhibitors from natural products has been contributed by Kupchan and co-workers. Generally, the tumor inhibitors are classified into several groups on the basis of their mechanism of activity or their structure. Constituting a group of "alkylating agents", many tumor inhibitors expected to alkylate SH compounds (enzymes or coenzymes) have recently been found. The chemistry and biochemistry of the SH group, on the other hand, has developed significantly, and much interesting information has been obtained (*2*).

Although the attempt may be bold and somewhat speculative, we now attempt classification of the related tumor inhibitors into two groups according to their chemical behavior with SH units. One is a group which easily causes the electrophilic addition to SH groups, and another is a group which is subject to displacement by SH groups.

Subsequently, several biomimetic reactions of tumor inhibitors with SH enzymes, cysteine and its derivatives, alkylthiols, and arylthiols are described. Finally, enhancement factors on the antitumor activity are mentioned.

CLASSIFICATION OF THE FUNCTIONAL GROUPS WHICH ARE REACTIVE TO THE SH GROUP

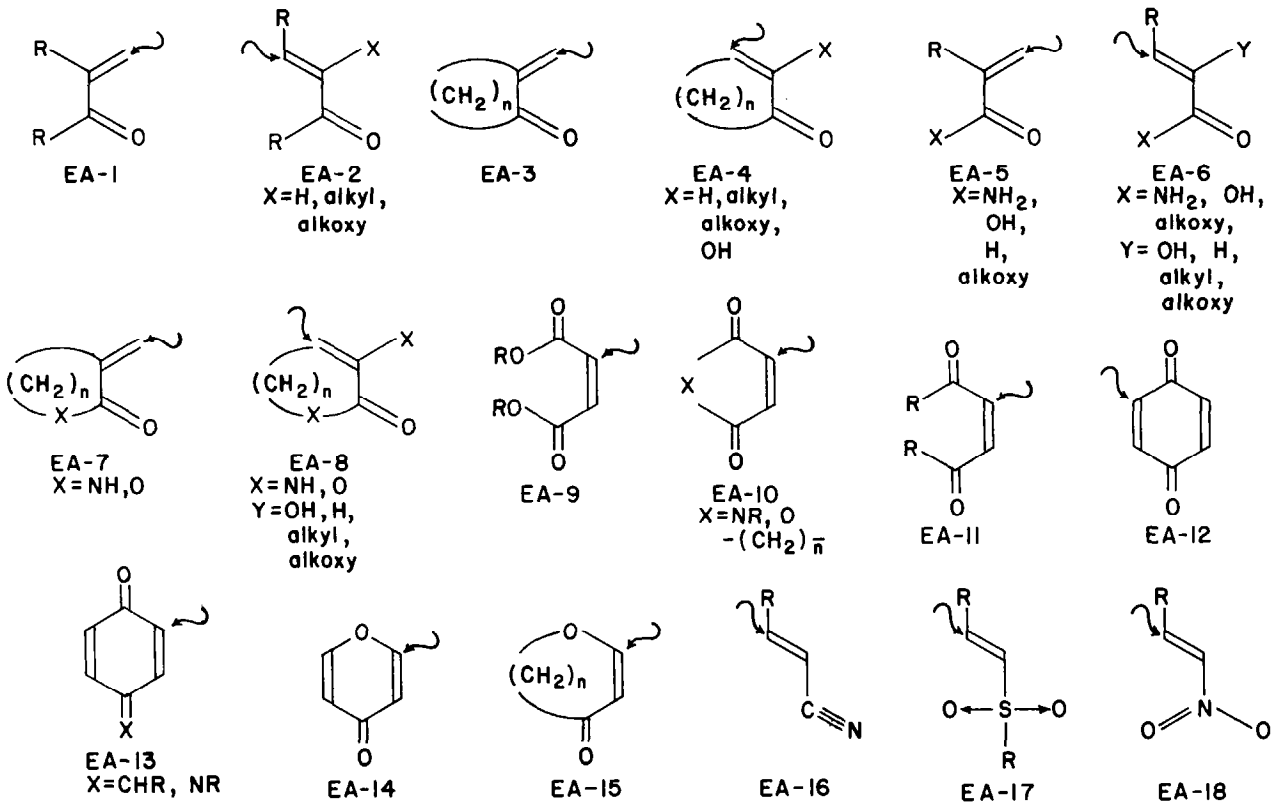
Since the sulfur atom has a large polarizability and "soft base" character (*3*), it exhibits a strong nucleophilicity. Thus its conjugate addition, S_N2 reaction on a sp^3

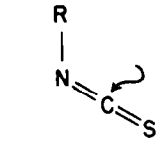
[1] Dedicated to the memory of Professor S. Morris Kupchan.

ISSN 0045–2068

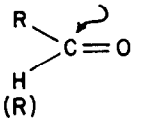
TABLE 1

Functional Groups Causing "Electrophilic Addition" (EA) to SH Group[a,b]





EA-19



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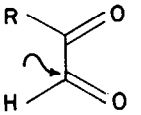

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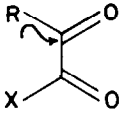
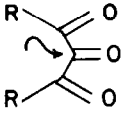


EA-22
X=OH, alkyl



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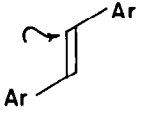
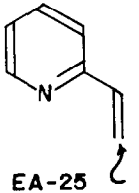


EA-24



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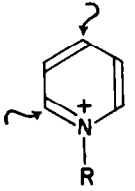

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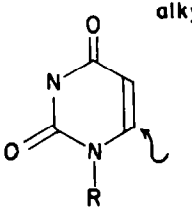

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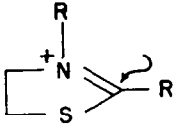

EA-28

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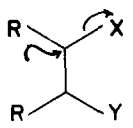
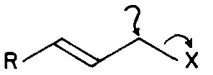
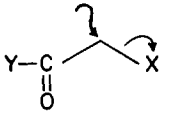
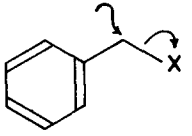
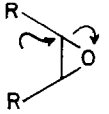
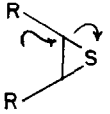
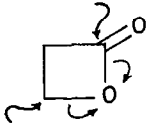
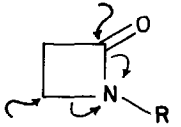
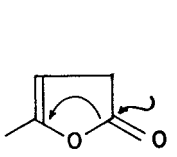
[a] R = H, alkyl, aryl.

[b] ⤳ : Position of addition.

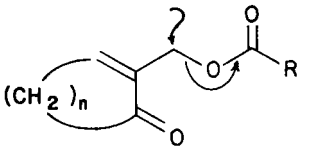
TABLE 2

Functional Groups Causing "Displacement Reaction[a]" (DR) with SH Group[b,c]

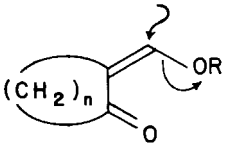
 DR-1 X = halogen Y = halogen, $N<^{R}_{R}$	 DR-2 X = halogen, acyloxy	 DR-3 X = halogen Y = OH, NH_2, alkoxy, aryl	 DR-4 X = halogen
 DR-5	 DR-6	 DR-7 X = CN, $-\overset{\text{I}}{\underset{\text{I}}{\text{P}}}=O$, electron withdrawing groups	 DR-8
 DR-9			



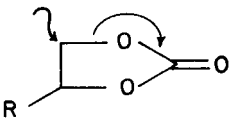
DR-10



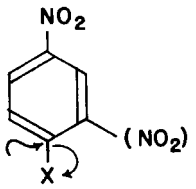
DR-11



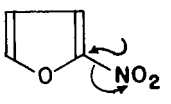
DR-12



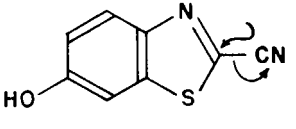
DR-13



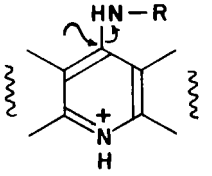
DR-14
X=halogen, $N_2^+Cl^-$



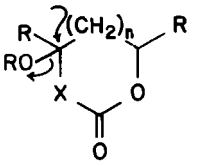
DR-15



DR-16



DR-17



DR-18
X=O,NH

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[a] Including substitution, addition-elimination, and elimination-addition.

[b] R=H, alkyl, aryl.

[c] ↷: Attacking position of SH group.

carbon, and displacement on a carbon atom of the aromatic compounds are well known (*2*, *4*). Now we classify the functional groups into two types; i.e., one which involves electrophilic addition (EA) to SH and another which effects displacement reactions (DR) with SH as shown in Tables 1 and 2, on the basis of information from purely organic chemical as well as biomimetic reactions *in vitro* using simple SH compounds and also from *in vivo* detoxication of several organic compounds (e.g., cysteine and glutathione conjugation) (*2*, *4*).

Thus, either natural or synthetic compounds having such functional group(s) as shown in Tables 1 and 2 may be expected to have some biological (e.g., antitumor, cytotoxic, antibacterial, antifungal, antiviral, plant growth inhibitory, etc.) activity. However, these functional groups involve only the active centers, hence the cooperative assistance of the enhancement factors (*vide infra*) is necessary for effective biological activity *in vivo*. Nevertheless, these tables are useful for planning the syntheses of potential antitumor compounds, the search for antitumor materials from the store of known compounds, and elucidation of the mechanism of the activity.

CLASSIFICATION OF TUMOR INHIBITORS INTO "EA" TYPE AND "DR" TYPE

We selected various tumor inhibitors and classified them into "EA" and "DR" types, as shown in Tables 3 and 4. The compounds which have been subjected to biomimetic reaction with any SH compound are also included.

BIOMIMETIC REACTIONS OF TUMOR INHIBITORS WITH MODEL COMPOUNDS OF SH ENZYMES AND OF SH COENZYMES

Black (*4b*) investigated the reactions of many types of compounds with L-cysteine at pH 7.0. Some results are cited in Tables 1 and 2.

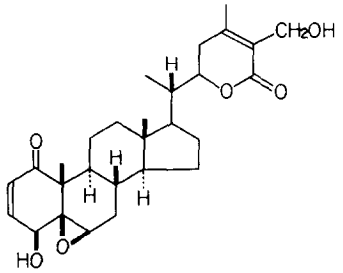
The reactions of endocyclic α,β-unsaturated γ-lactones, (**45**)–(**48**), with thiols were investigated (*53*), and a methyl substituent in the α or β position was shown to reduce remarkably the reactivity of the butenolide toward addition of thiols. Thus, addition of cysteine to lactones (**46**) and (**47**) hardly occurred, giving only a very poor yield of the adduct. Neither 1-propanethiol nor α-toluenethiol gave adducts. In contrast, addition of L-cysteine to the exocyclic unsaturated lactone in elephantopin (**12**) occurred very much faster (10^3 times) than additions to the endocyclic lactones, (**46**), (**47**), or (**48**). Alternatively, the elimination reactions of cysteine adducts into endocyclic unsaturated lactones (**46**), (**47**), and (**48**) proceeded much faster than the similar reaction of the cysteine adduct of elephantopin (**12**), which revealed the particularly unstable nature of the former adducts.

Treatment of α-methylene lactone tumor inhibitors, vernolepin (**11**), elephantopin (**12**), and eupatundin (**13**), with aqueous solution of L-cysteine at pH 7.4 afforded cystine adducts (**49**), (**50**), and (**51**). The rapid rates were spectrophotometrically measured to determine the second-order rate constants for the reactions of the lactones with L-cysteine (*19*).

TABLE 3

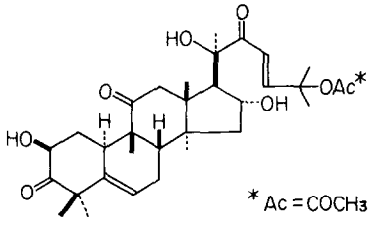
Tumor Inhibitors Classified into "EA" Type[a,b]

[Steroidal Compounds]



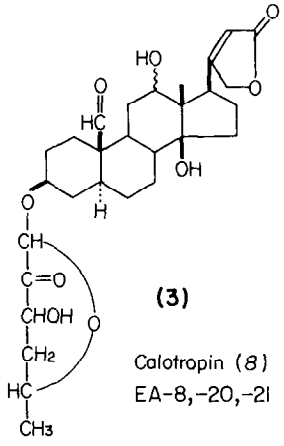
(1)

Withaferin-A (5)
EA-8 BM (6)
(DR-5)



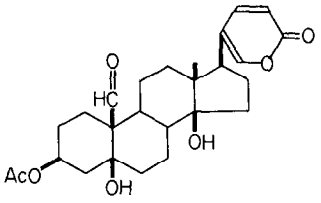
(2)

Cucurbitacin B (7)
EA-2



(3)

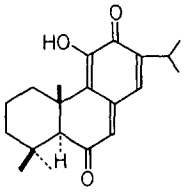
Calotropin (8)
EA-8,-20,-21



(4)

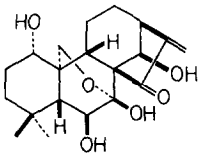
Hellebrigenin acetate (9)
EA-8,-20

[Diterpenes]



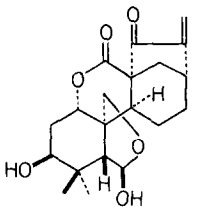
(5)

Taxodione (10)
EA-13 BM (11)



(6)

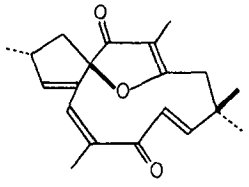
Oridonin (12,13)
EA-3 BM (13)



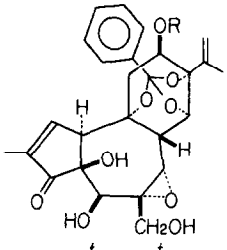
(7)

Enmein (12,13,14)
EA-3 BM (13)

TABLE 3—*continued*

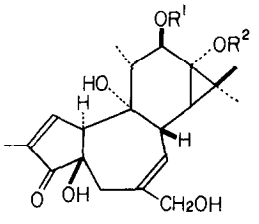


(8)
Jatrophone (*15*)
EA-4 BM (*15*)



R=COCH=^{t}CHCH=^{t}CH(CH$_2$)$_4$CH$_3$

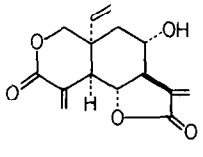
(9)
Gnididin (*16*)
EA-4 (*DR-5*)



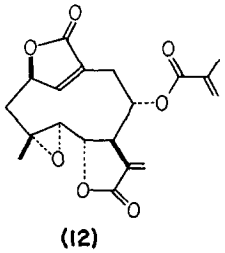
R^1=COC(CH$_3$)C=CHCH$_3$
R^2=COC(CH$_2$)$_8$CH$_3$

(10)
Phorbol 12-tiglate 13-decanoate (*17*)
EA-4

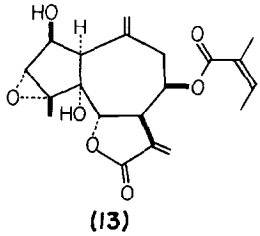
[Sesquiterpenes]



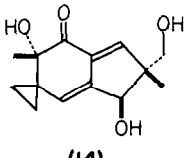
(11)
Vernolepin (*18*)
EA-7 BM (*11,19,20*)



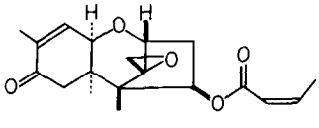
(12)
Elephantopin (*21*)
EA-7 (DR-5) BM (*19*)



(13)
Eupatundin (*22*)
EA-7 (DR-5) BM (*19*)

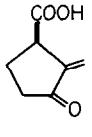


(14)
Illudin S (*23*)
EA-2, -4

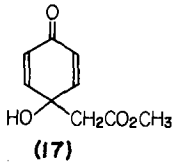


(15)
Trichotecin (*24*)
EA-2 (*DR-5*)

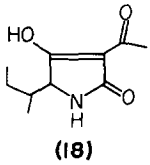
[Others]



(16)
Sarcomycin (*25*)
EA-3

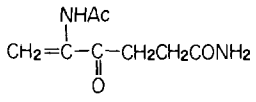


(17)
Jaccaranone (*1e,26*)
EA-4

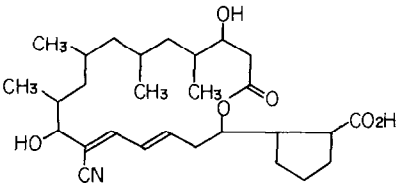


(18)
Tenuazonic acid (*1a*)
EA-8

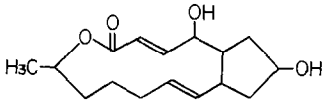
TABLE 3—*continued*



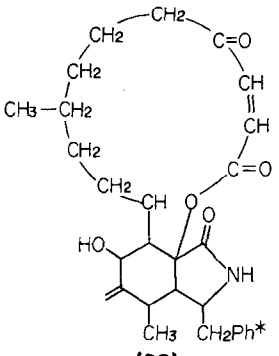
(19)
Primocarcin (*1a*)
EA-1



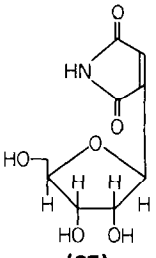
(20)
Borellidin (*27*)
EA-16



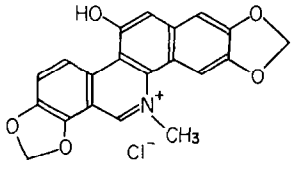
(21)
Brefeldin (*28*)
EA-8



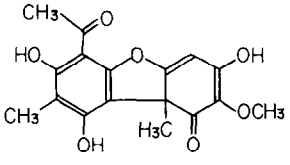
(22)
Cytochalasin A (*29*)
EA-10 *Ph = C_6H_5



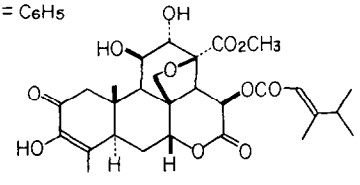
(23)
Showdomycin (*30*)
EA-10



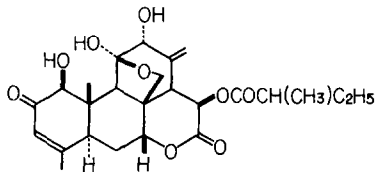
(24)
Sanguinarine chloride (*1e*)
EA-26



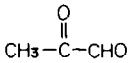
(25)
l-Usnic acid (*31*)
EA-4



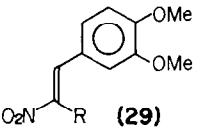
(26)
Bruceantin (*32*)
EA-4



(27)
Dehydroailanthinone (*33*)
EA-4



(28)
Methylglyoxal (*34*)
EA-21 BM (*34*)



(29)
R=H, CH_2CH_3, or $CH_2CH_2CCH_3$ (*35,36*)
O
EA-18 BM (*36*)

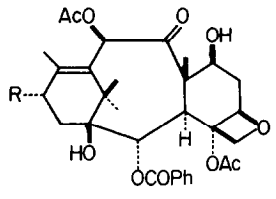
a See Tables 1 and 2 for EA-x and DR-x

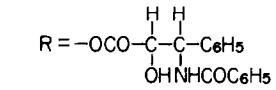
b BM means that the said compound has been tested for the biomimetic reaction.

TABLE 4

Tumor Inhibitors Classified into "DR" Type

[Diterpenes]

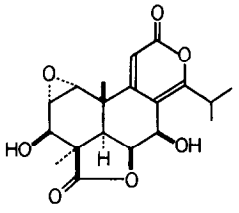




(30)

Taxol (*37*)

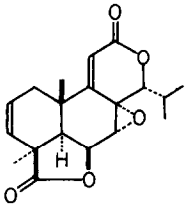
DR-2



(31)

Nagilactone C (*38*)

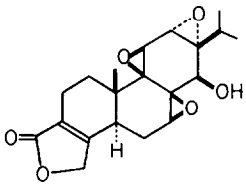
DR-5 (EA-8)



(32)

Podolide (*39*)

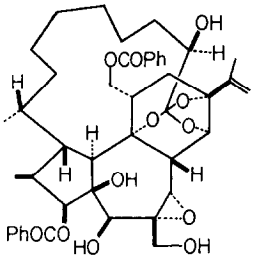
DR-5 (EA-8)



(33)

Triptolide (*40*)

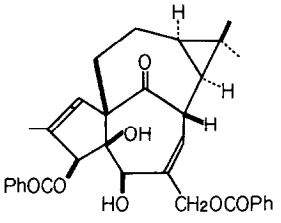
DR-5 BM (*41*)



(34)

Gnidimacrin (*42*)

DR-5

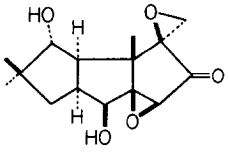


(35)

Ingenol 3,20-dibenzoate (*17*)

DR-2

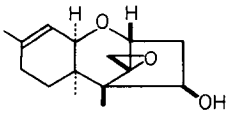
[Sesquiterpenes]



(36)

Corriolin (*43*)

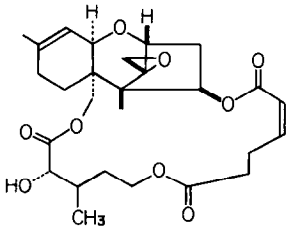
DR-5



(37)

Roridin C (*44*)

DR-5



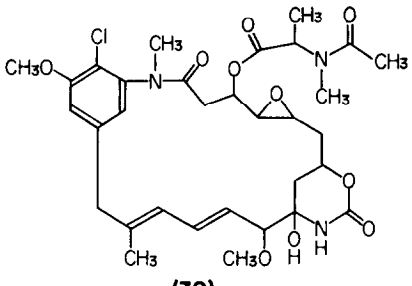
(38)

Verrucarin A (*44,45*)

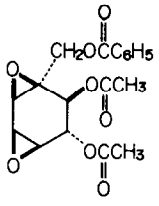
DR-5 (EA-8)

TABLE 4—*continued*

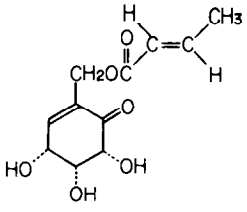
[Others]



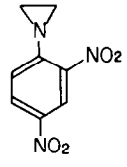
(39)
Maytansine (*46,47*)
DR-5, -18



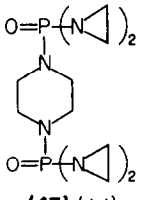
(40)
Crotepoxide (*48*)
DR-5



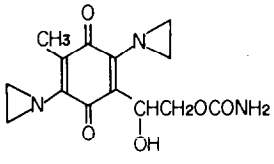
(41) (*49,50*)
DR-11 BM (*50*)



(42)
CB 1837 (*51*)
DR-7

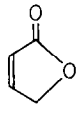


(43) (*1d*)
DR-7

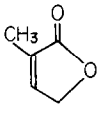


(44) (*52*)
DR-7

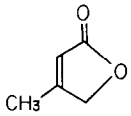
Inhibition of an SH enzyme, phosphofructokinase, by taxodone (**52**), taxodione (**5**), vernolepin (**11**), euparotin acetate (**53**), eupacunin (**54**), and some standard inhibitory reagents was examined in the absence and presence of the substrates (fructose-6-phosphate, ATP) (*11*).



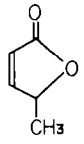
(45)



(46)

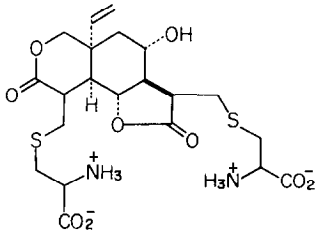


(47)

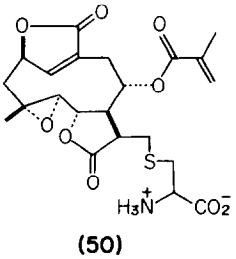


(48)

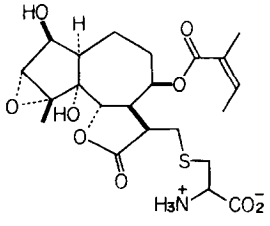
Addition of only 1.6 mol of taxodione (**5**) per protomer of enzyme reduced activity by 50%. The other quinone methide, taxodone (**52**), inhibited 50% at a relative concentration of 32; and the α,β-unsaturated lactones, euparotin acetate (**53**), eupacunin (**54**),



(49)

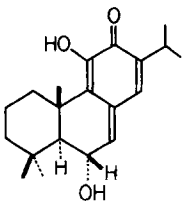


(50)

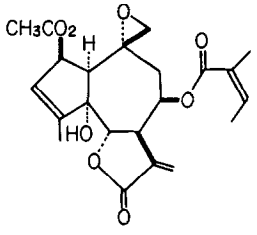


(51)

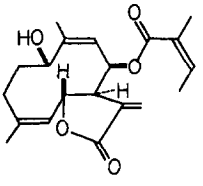
and vernolepin (**11**) inhibited 50% at relative concentrations of 1000 to 2000. Taxodone (**52**) was about as effective an inhibitor as *N*-ethylmaleimide (**55**) and Ellman's reagent (**56**), whereas the α,β-unsaturated lactones [(**11**), (**63**), and (**54**)] were about ten times as effective as iodoacetamide (**57**). The substrates, fructose-6-phosphate and ATP, were shown to protect the enzyme from each of the inhibitors.



(52)



(53)

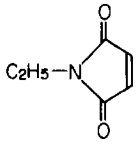


(54)

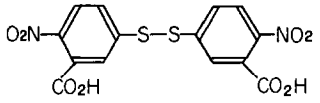
The direct evidence for a reaction of taxodione (**5**) with the SH groups of phosphofruktokinase was provided. When phosphofructokinase was denatured with sodium dodecyl sulfate, 10 molecules of taxodione per enzyme protomer resulted in the loss of about 12 SH groups, indicating about a 1 : 1 reaction (*11*).

Inactivation of glycogen synthase by vernolepin (**11**) was reported (*20*). Reaction with 3 mol of radioactive vernolepin per 90,000-dalton subunit caused virtually complete loss of activity. The concurrent disappearance of three titrable thiol groups (out of six) indicates that thioether formation is the major mode of binding to the protein.

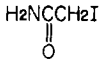
Triptolide (**33**) and tripdiolide (**58**) on treatment with a 100-fold excess of 1-propanethiol in a mixture of methanol and phosphate buffer (pH 7.4) gave regio- and stereo-selectively thiol adducts (**59**) and (**60**) in 78 and 79% yields, respectively. In contrast, the 14α-alcohol (**61**) remained intact. Because of characteristic hydrogen-bonding between the 9,11-epoxide and the 14β-hydroxyl group in (**33**) and (**58**),



(55)



(56)



(57)

addition may proceed via a process (**62**) which involves opening of the epoxide function with neighboring hydroxyl assistance. Biological studies rationalize the foregoing *in vitro* reactions. Triptolide (**33**) and tripdiolide (**58**) at 0.1 mg/kg showed impressive life-prolonging effects (T/C $\geqq$ 230) in mice afflicted with the L-1210 lymphoid leukemia. Triptonide (**63**), 14-epitriptolide (**61**), and the thiol adducts, (**59**) and (**60**), did not show antileukemic activity at doses up to 0.4 mg/kg (*41*).

A glyoxalase I inhibitor (cf. Chart 8), 2-crotonyloxymethyl-4,5,6-trihydroxy-cyclohex-1-en-3-one (**41**), isolated from a culture broth of *Streptomyces griseosporeus*, showed antitumor activity against HeLa cells in cell culture, and also against Ehrlich ascites carcinoma and L-1210 cells inoculated in mice (*49*). As shown in Chart 2, the crotonyloxy group of (**41**) was very easily displaced by 2-hydroxyethanethiol or *p*-

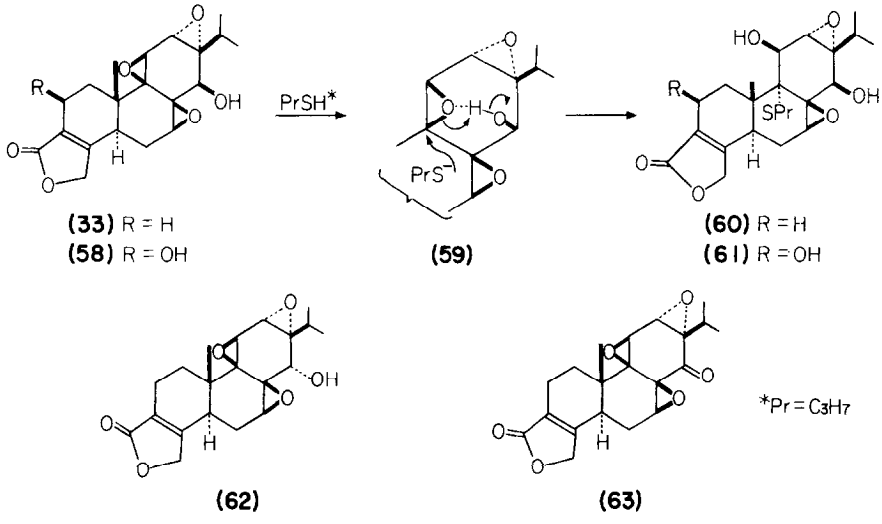


Chart 1

bromobenzenethiol, giving the SH adduct, (**64**) or (**65**), while the hydrolyzed alcohol (**66**) did not react with thiols. These biomimetic displacements with (**41**) may provide a rationalization for its antitumor activity. Compounds (**64**), (**65**), and (**66**) did not show any biological activity (*50*).

Isodon diterpenoids (*55*), oridonin (**6**) (*56*), lashiokaurin (**67**) (*57*), and enmein (**7**) (*58*), showed significant antitumor activity against Ehrlich ascites carcinoma inoculated in mice. Biomimetic reactions of oridonin (**6**) and enmein (**7**) with several biological model compounds were investigated in detail. The results are shown in Table 5 (*13*).

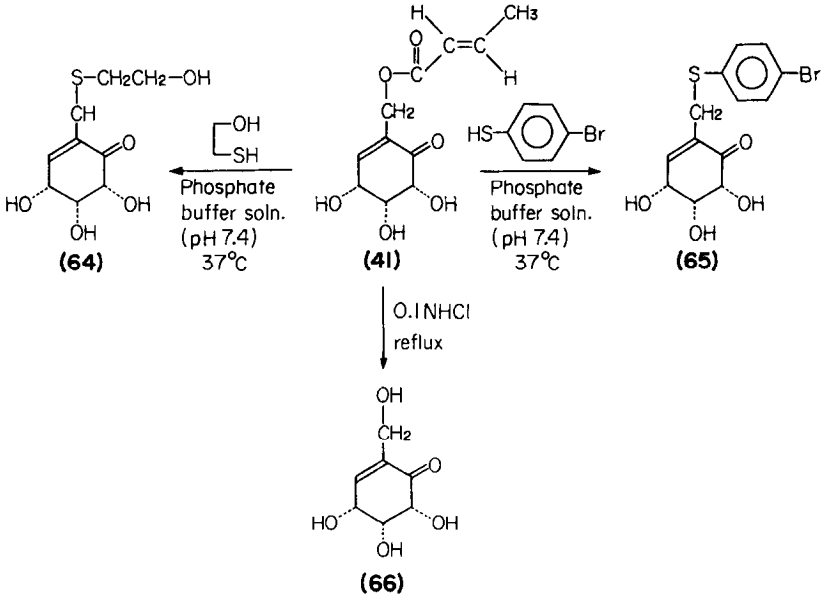


Chart 2

TABLE 5

BIOMIMETIC REACTIONS OF ORIDONIN AND ENMEIN WITH NUCLEIC ACID MODEL COMPOUNDS AND WITH ENZYME MODEL COMPOUNDS

Model compound	Solvent system	Product and yield[a] (%)	
Oridonin			
Adenosine	pH 7.2–7.3; potassium phosphate buffer solution–EtOH (1 : 1)	None[b]	
Cytidine	pH 7.2–7.3; potassium phosphate buffer solution–EtOH (1 : 1)	None[b]	
Ethanethiol	DMF	(**68**)	87
1-Propanethiol	DMF	(**69**)	100
1-Butanethiol	DMF	(**70**)	77
	EtOH	(**70**)	43
2-Butanethiol	DMF	(**71**)	68
L-Cysteine	pH 7.2–7.3; potassium phosphate buffer solution–EtOH (1 :)	(**72**)	100
L-Lysine	pH 7.2–7.3; potassium phosphate buffer solution–EtOH (1 : 1)	None[b]	
L-Serine	pH 7.2–7.2; potassium phosphate buffer solution–EtOH (1 : 1)	None[b]	
Enmein			
1-Butanethiol	DMF	(**73**)	67

[a] Isolated yield calculated on the basis of oridonin and enmein.

[b] Recovery of oridonin.

Oridonine (**6**) did not react with adenosine and cytidine, the nucleic acid model compounds, but was recovered. The reactions of oridonin with the SH enzyme model compounds proceeded easily under mild conditions to give alkane thiol adducts (**68**), (**69**), (**70**), and (**71**). The reaction with L-cysteine also took place very rapidly to yield adduct (**72**) quantitatively. The chemical structures of these products were determined by spectroscopic evidence and also by carrying out the reverse reaction on compound (**68**), giving oridonin (**6**). The stereochemistry of the C-16 atom was chemically confirmed. These processes are shown in Chart 3. Enmein (**7**) also easily gave adduct (**73**) on the reaction with butanethiol. The stereochemistry was chemically determined as shown in Chart 4. The reactions of oridonin with L-lysine and L-serine, however, did not take place. Such easy addition reactions of thiols without any catalysts as described above are reasonably explainable as the reactions of "soft" acids with "soft" bases. Thus, the selective reactions of the α-methylene–cyclopentanone system with only the SH group of many nucleophilic groups (e.g., –OH, $-NH_2$, $-COO^-$, etc.) in the enzyme can be rationalized by the "Hard Soft Acids Bases" principle (*3*) and experimentally supported by our biomimetic reaction.

The reactions of jatrophone (**8**), an antileukemic active macrocyclic diterpenoid isolated from *Jatropha gossypiifolia*, with thiophenols in neutral solution occurred quickly to give single-component adducts. However, isolation of the adducts was unsuccessful, because they were unstable and underwent retro-reactions readily. The reaction of jatrophone with cysteine also took place, but it was recognized only by the

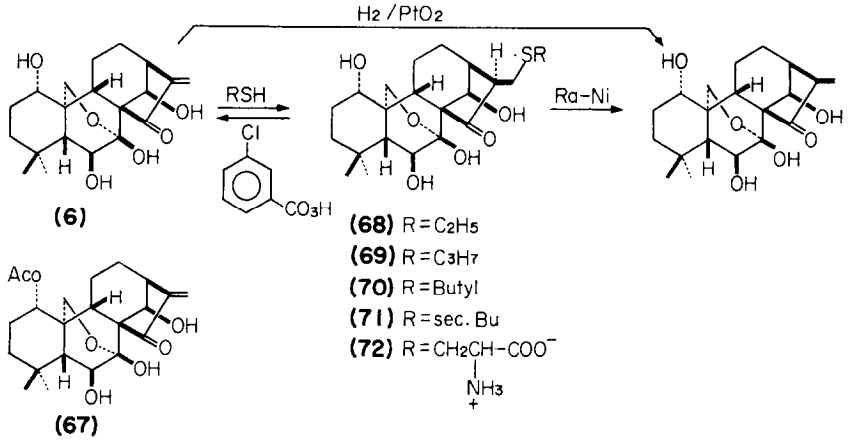


Chart 3

change in the ultraviolet chromophore at 285 nm. In contrast, its reaction with 1-propanethiol in borate buffer afforded a stable adduct (**74**) (see Chart 5) (*15*). Jatrophone was also observed to react with thiol groups on proteins such as bovine serum albumin and DNA-dependent RNA polymerase from *Escherichia coli* (*54*).

It is known that β-nitrostyrenes are remarkably reactive toward thiols to give adducts, which show powerful antifungal and antibacterial activity (*59*). We developed

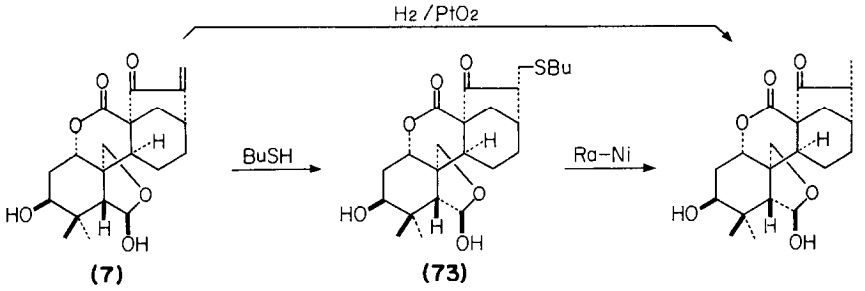


Chart 4

a new stereoselective synthesis of 3,4-dimethoxy E-β-nitrostyrenes and examined their antitumor and antibacterial activity. The synthetic sequence is shown in Chart 6 (*35*, *36*). The reactions of (**75**) with other thiols (MeSH, PrSH, BuSH, s-BuSH, t-BuSH, and PhSH) also took place similarly, and a quantitative yield of the adduct was obtained in every case. Nitrostyrene (**75**), on treatment with benzenethiol without a basic catalyst, also easily afforded the adduct. Compounds (**76**) and (**82**) showed considerable anti-

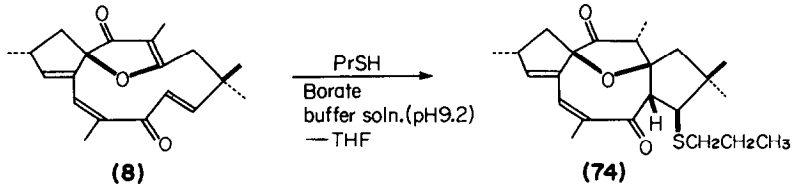


Chart 5

bacterial and tumor inhibitory activity (*35*). The facile addition of thiols shown in Chart 6 may be regarded as a biomimetic reaction.

Withaferin-A (**1**), a biologically active principle isolated from leaves of *Withania somnifera* (*5*), possesses three likely positions [C-3, C-5 (or -6), and C-24] which may be sensitive to attack *in vivo* of biologically important SH compounds. Hence, compound (**1**) was subjected to reaction with ethanethiol, benzenethiol, and L-cysteine ethyl

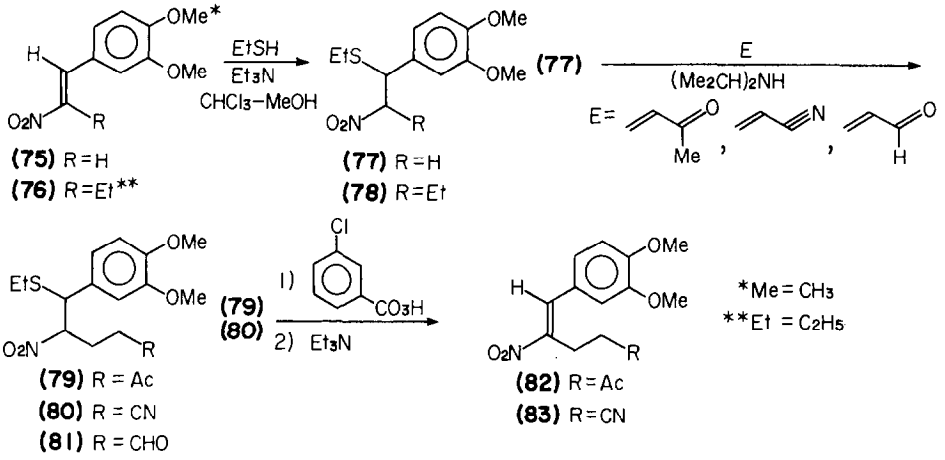


Chart 6

ester, respectively (see Chart 7). As a result, the products (**84**), (**85**), and (**86**), resulting from exclusive reaction at the C-3 position, were obtained in every case. Discussions on the selective addition at C-3 *in vitro* have been presented (*6*). An analogous A-ring compound (**87**) of withaferin-A exhibited inhibitory activity against sarcoma 180 in mice (*60*).

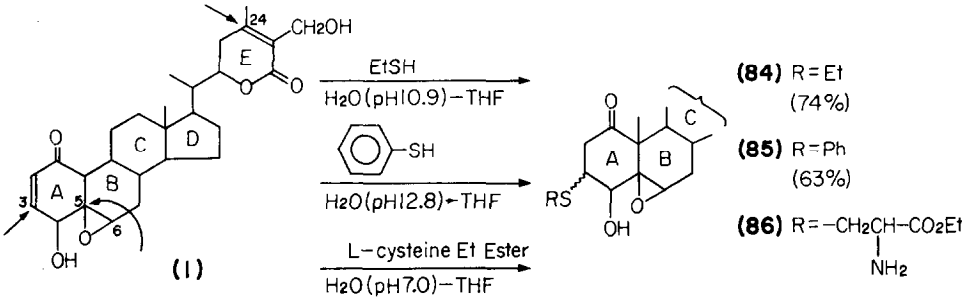
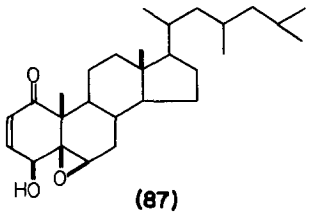


Chart 7



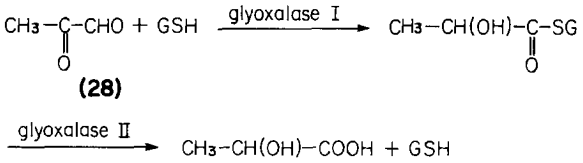


Chart 8

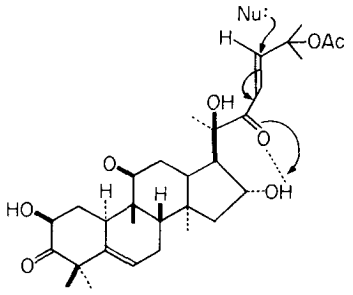
Almost all of living cells have the glyoxalase system which consists of glyoxalase I, the coenzyme glutathione (GSH), and glyoxalase II. This system catalyzes the conversion of methylglyoxal (**28**) into lactic acid (**88**) (see Chart 8).

Szent-Györgyi *et al.* suggested that the glyoxalase system may play a key role in the regulation of cell division. If α-ketoaldehydes interact with SH groups which are involved in cell division, inhibition of cell division can be expected. Thus, inhibitions of the proliferation of *Escherichia coli* by some α-ketoaldehydes (methylglyoxal, β-ethoxy-α-ketobutyraldehyde, and hydroxymethylglyoxal) were observed (*34a*, *2a*, *2b*). Vince *et al.* assumed that *S*-alkyl and *S*-aryl glutathiones may combine with glyoxalase I, and this may result in inhibition of the enzyme activity and a buildup of methylglyoxal in tumor cells, thus causing an inhibition of cellular growth. Actually, they demonstrated inhibition of glyoxalase I by certain *S*-alkyl glutathiones (*61*) and *S*-aryl glutathiones (*34b*). They also showed *S*-aryl glutathiones to inhibit the cellular growth of L-1210 and KB (*34b*).

ENHANCEMENT FACTORS ON THE ANTITUMOR ACTIVITY

Hydrogen-Bonding

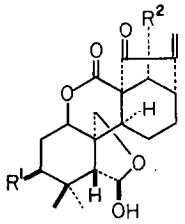
While cucurbitacin B (**2**) exhibited a very high cytotoxicity [$ED_{50} = 10^{-6}$ μg/ml (KB)], its C-16 acetate, fabacein, showed only a weak activity ($ED_{50} = 1$ μg/ml). The remarkable diminution in cytotoxicity suggests that the free hydroxyl group on C-16 may be important for the reactivity of the conjugated ketone. Thus, the hydrogen-bonding interaction between the C-16 hydroxyl group and the C-22 ketone could activate the α,β-unsaturated ketone toward nucleophilic attack by a biological macromolecule as shown in formula (**A**) (*62*).



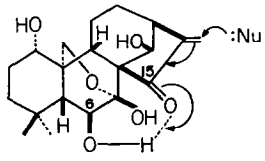
(**A**)

The importance of hydrogen-bonding in triptolide (**33**) and tripdiolide (**58**) was previously discussed (*41*).

An important role of hydrogen-bonding was identified also in oridonin (**6**) as shown by the following facts. Enmein (**7**), enmein-3-acetate (**89**), and compound (**90**), incapable of hydrogen-bonding such as that in (**6**), exhibited less activity even at four-fold doses or more of oridonin. The existence of hydrogen-bonding between the carbonyl group at C-15 and the hydroxyl group at C-6 in the oridonin molecule has been confirmed by its ir, uv, and nmr spectra (*56*). Hence, the C-17 atom is polarized to



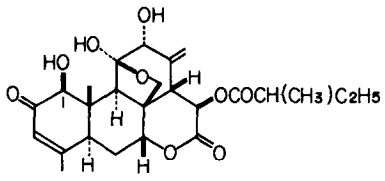
(7) R^1=OH, R^2=H
(89) R^1=OAc, R^2=H
(90) R^1=H, R^2=OH



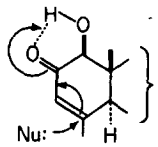
(B)

δ^+, and its reactivity with a nucleophile must be increased [see Formula (**B**)]. In fact, it is supported by the lower chemical shifts of C-17 methylene protons of oridonin (**6**) than those of enmein (**7**), enmein-3-acetate (**89**), and compound (**90**), showing that the electron density at C-17 of oridonin (**6**) is lower than those of the latter compounds. In addition, this view was also supported by the fact that oridonin (**6**) was observed to react with butanethiol faster than enmein (**7**) in the competitive reaction in one flask by tlc analysis (*63*).

Dehydroailanthinone (**91**), isolated from *Pierrodendron kerstingii*, showed significant antileukemic activity against P-388. However, methylation of the C-1 alcohol of (**91**)



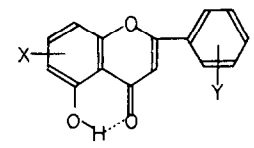
(91)



(C)

resulted in a diminution of cytotoxicity and of *in vivo* antileukemic activity. Possibly the hydroxyl group enhances the reactivity of the conjugated ketone toward biological nucleophiles through intramolecular hydrogen bonding as shown in formula (**C**) (*33*).

There are many natural products possessing intramolecular hydrogen-bonding such as that mentioned above, e.g., flavonoids (**92**), isoflavonoids (**93**), and chromones (**94**). Inhibition of a released anaphylactoid mediator with baicalein (**95**) is antagonized by cysteine (*64*). Isoflavonoid (**96**) and its related compounds affect germination of red clover seeds (*65*). The isoflavonoid (**97**) isolated from *Lupinus luteus* shows antifungal activity (*66*). These interesting observations suggest the potential antitumor and other biological activities of flavonoids, isoflavonoids, and chromones. We plan to test their activity using several samples.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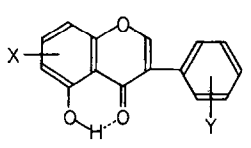
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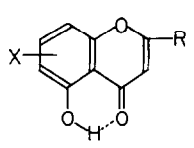
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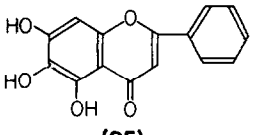
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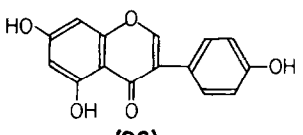
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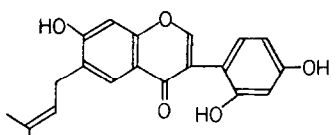
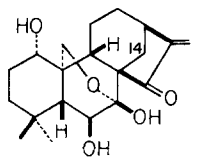
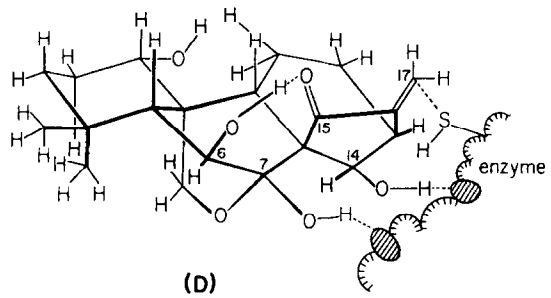

(97)

Neighboring Group Participation

Neighboring OH or *O*-acyl group participation was recognized by the addition of cysteine to cytotoxic sequiterpene lactones. However, attempts to find a direct correlation between the rate of cysteine addition and cytotoxicity were unsuccessful (*67*).

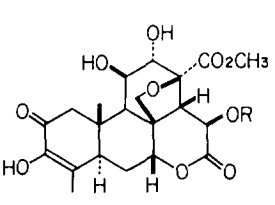
The antitumor activity of oridonin 14-deoxy derivative (**98**) was weaker than that of oridonin (**6**) and almost the same as those of enmein (**7**) and enmein-3-acetate (**89**). Thus, the 14β-hydroxy group of oridonin (**6**) must play an important role in increasing the activity, such as constituting the binding site to a special biologically important nucleophile in a tumor cell [see Formula (**D**)]. The hydroxyl group at C-7 is located close to the active center and in parallel to the C-14 hydroxyl group. Hence it may play some role in cooperating with the C-14 hydroxyl group, as shown in formula (**D**) (*12*, *13*).


(98)


(D)

Significance of Ester Side Chains

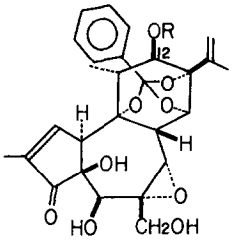
The antileukemic activity of the brucenolide derivatives varies greatly with the nature of the ester substituents. Thus, bruceantin (**26**) and bruceantinol (**99**), which possess α,β-unsaturated esters, exhibited potent antileukemic activity. Bruceantarin (**100**), having a benzoate ester, and dihydrobruceantin (**101**), having a saturated aliphatic ester, showed moderate activity. In contrast, bruceine B (**102**), which bears the smaller acetate ester, and bruceolide (**103**), which bears no ester at all, showed only marginal antileukemic activity. The ester moiety may serve as a carrier group involved in processes such as transport or complex formation (*32*, *68*).



(26) R= CO
(99) R= CO OH
(100) R= COC_6H_5
(101) R= CO
(102) R= $COCH_3$
(103) R= H

Gnididin (**9**), gniditrin (**104**), and gnidicin (**105**), diterpenoid esters from *Gnidia lamprantha*, showed potent antileukemic activity against P-388 leukemia in mice. However, 12-hydroxydaphnetoxin (**106**), possessing no ester moiety at C-12, showed no antileukemic activity. Benzoate ester derivative (**107**) showed potent activity of the same order as the naturally occurring esters. Thus, the hypothesis that the ester affixed at C-12 may act as a carrier moiety (e.g., in processes concerned with cell penetration or selective molecular complex formation) was proposed (*16*).

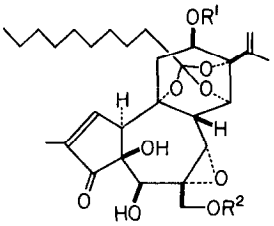
Gnidilatin 20-palmitate (**108**) and gnidilatidin 20-palmitate (**109**) exhibited substantial inhibitory activity at optimal doses against P-388 leukemia in mice. Gnidilatin (**110**)



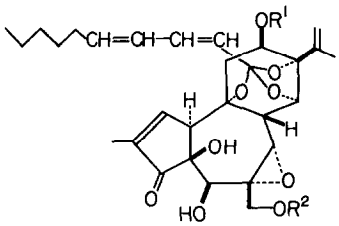
(9) R = $COCH\overset{t}{=}CHCH\overset{t}{=}CH(CH_2)_4CH_3$
(104) R = $COCH\overset{t}{=}CH(CH=CH_2)_2(CH_2)_2CH_3$
(105) R = $COCH\overset{t}{=}CHC_6H_5$
(106) R = H
(107) R = COC_6H_5

showed moderate activity. In contrast, gnidiglaucin (**111**) and gnidilatidin (**112**) did not show inhibitory activity (*69*).

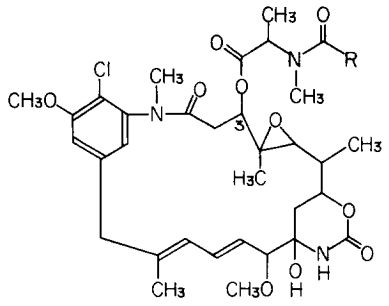
Maytansine (**39**) and its analogs, novel ansa macrolides from *Maytenus* species (*46*, *70*), *Putterlickia verrucosa* (*47*), and *Colubrina texenis* (*71*), are excellent antileukemic agents. Maytansine (**39**), maytanprine (**113**), maytanbutine (**114**), and maytanvaline (**115**), which bear an ester side chain at the C-3 position, exhibited highly antileukemic activity. On the other hand, maysine (**116**), normaysine (**117**), and maysenine (**118**), which bear no ester side chain at C-3, lacked antileukemic activity and showed about



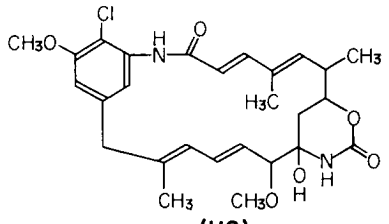
(108) $R^1 = COC_6H_5$; $R^2 = COC_{15}H_{31}$
(110) $R^1 = COC_6H_5$; $R^2 = H$
(111) $R^1 = COCH_3$; $R^2 = H$



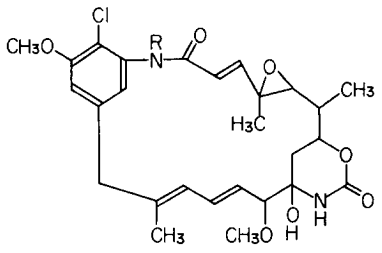
(109) $R^1 = COC_6H_5$; $R^2 = COC_{15}H_{31}$
(112) $R^1 = COC_6H_5$; $R^2 = H$



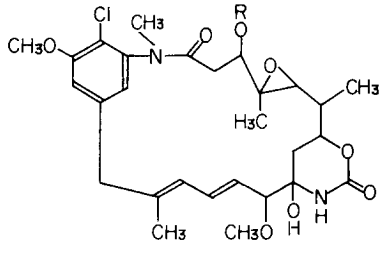
(39) R = CH_3
(113) R = CH_2CH_3
(114) R = $CH(CH_3)_2$
(115) R = $CH_2CH(CH_3)_2$



(118)



(116) R = CH_3
(117) R = H



(119) R = $COCH_3$
(120) R = $COCH_2CH_3$
(121) R = $COCH_2Br$
(122) R = $COCH=CHCH_3$
(123) R = H

1/10 000 the cytotoxicity of maytanside esters such as (**115**) (*70*). Maytanacine (**119**) from *P. verrucosa* and several semisynthetic esters, (**120**), (**121**), and (**122**), derived from maytansinol (**123**), showed antileukemic activity comparable to naturally occurring substituted alanyl esters. In contrast, maytansinol (**123**) showed no antileukemic activity (*47*). Thus, the ester function in the antileukemic maytansinoids may play a key role in the formation of highly selective molecular complexes with growth-regulatory biological macromolecules.

CONCLUSION

On the basis of the foregoing discussion, a rational approach from the standpoint of organic chemistry toward development of the effective new SH-alkylating antitumor agents is suggested: (1) search for compounds including active center(s) (Tables 1 and 2) among known compounds, or synthesis of new compounds bearing active center(s) through consideration of enhancement factors: (2) trying biomimetic reactions with simple SH compounds; (3) biological testing for positive compounds in (2); (4) chemical modification for enhancement of activity.

This approach may be more economic and more reasonable than the direct bioassay in animals alone. We hope the active center and enhancement factors discussed in this review conserve as an effective guide for the development of more potent anticancer agents.

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