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REVIEW

Design and Study of New Tumor Inhibitors*

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On the basis of attracting information concerning metabolism (e.g., cysteine or gulutathione conjugation), biomimetic reactions using simple SH compounds and enzymes, and sulfur chemistry, numerous functional segments which exihibit good reactivity with SH groups are arranged into two categories; i.e., one which performs electrophilic addition (EA) to an SH group and the other which possesses functional groups causing displacement reaction (DR) with the SH group. With a view to design new SH-alkylating tumor inhibitors the introduction of various EA and DR type functional segments as listed in Tables 1 and 2 into simple compounds would lead to antitumor activity. The enhancement factors on the tumor inhibitory activity, the importance of hydrogen-bonding, lipophilic ester side chains, and bifunctional and multifunctional effects by combination of various functional segments are discussed. Biomimetic model reactions of some tumor inhibitors and related compounds using simple SH-containing compounds and SH enzymes are explained. Studies for the development of the antitumor active diterpenoids of "Enmei-so" (*Rabdosia japonica* Hara and *R. trichocarpa* Kudo) and related plants are described in detail from several points of view. Finally, recent extensive studies on DNA-interacting tumor inhibitors are summarized and the design for a new type of radiosensitizers for cancer radiotherapy is introduced.

KEY WORDS: SH-Alkylating tumor inhibitor/ Functional segment/ Hydrogen-bonding/ Lipophilic ester side chain/ Bifunctional and multifunctional effect/ SH Enzyme/ Rabdosia diterpene/ DNA-Interacting tumor inhibitor/ Hypoxic cell radiosensitizer

1. INTRODUCTION

The study of the naturally occurring and synthetic tumor inhibitors is of the current interest. Previously, Fujita and co-workers have reported new antitumor active diterpenes which readily react with SH compounds under the mild conditions.[1,2] During the course of this study, a review article concerning classification of the functional segments which are reactive to the SH group and tumor inhibitors having potential for interaction with SH enzymes and/or coenzymes was published by Fujita and Nagao.[3] In the meantime, we have been interested in development of the tumor inhibitors particularly with the appearance of the antitumor activities which is attributed to the cleavage of cellular DNA or to the perturbation of the DNA replication by DNA-drug binding. Thus, we have developed new polyamine-containing

* Dedicated to Professor Emeritus Eiichi Fujita on the occasion of his retirement.

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compunds for cancer radiotherapy that would involve the sensitization of hypoxic radioresistant cells in tumor tissues.[4,5] In this context we wish to mainly the papers concerned with the SH-alkylating and DNA-binding tumor inhibitors.

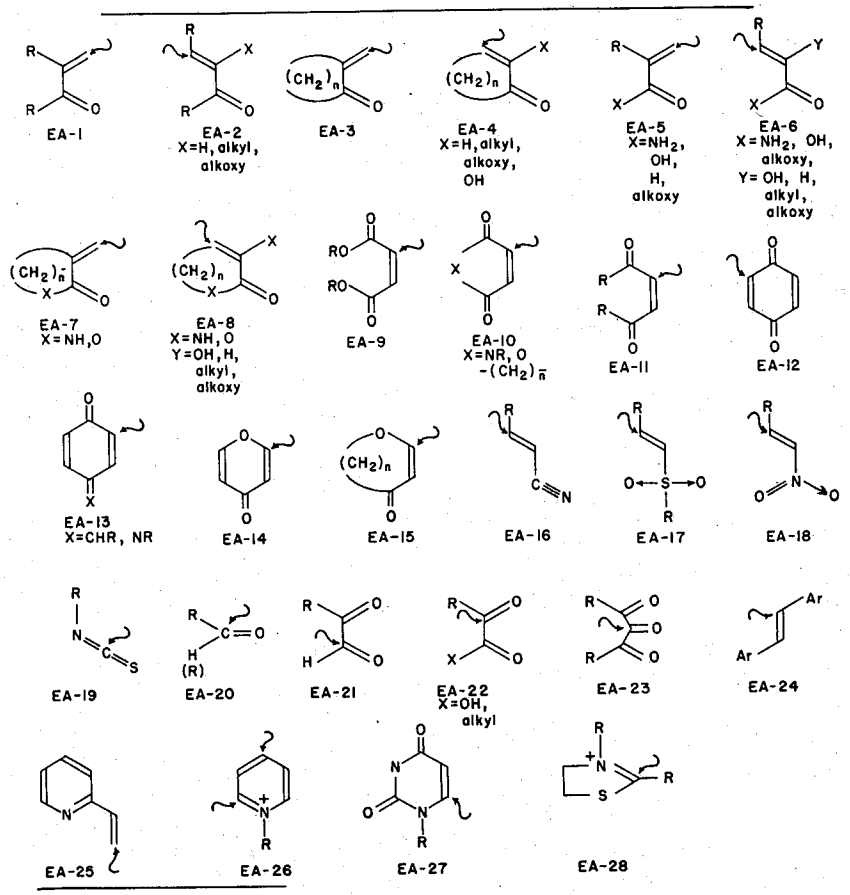
2. SH-ALKYLATING TUMOR INHIBITORS

2-1. Design of Active Centers of SH-Alkylating Tumor Inhibitors

It is well known that DNA and RNA polymerase and other biologically important enzymes have several SH groups in their molecules.[6,7] Compounds containing SH group such as glutathione, cysteine, and SH enzymes are significantly enriched in caner cells. Therefore, one can readily realize that the particular compounds reacting with an SH group may be expected to exhibit an antitumor activity.[3]

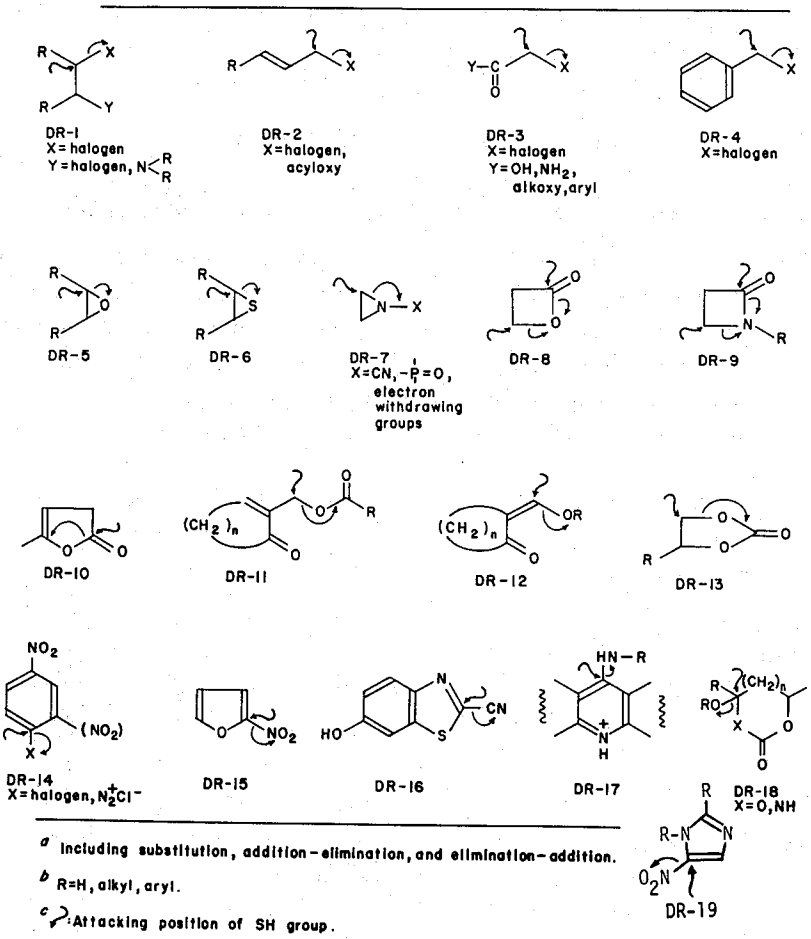
On the basis of various information on *in vivo* metabolism as well as biomimetic

Table 1. Functional Segments Causing Electrophilic Addition (EA) to SH Group[a,b]



[a] R=H, alkyl, aryl.
[b] : Position of addition.

Table 2. Functional Segments Causing Displacement Reaction[a](DR) with SH Group[b,c]



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

[b] R=H, alkyl, aryl.

[c] : Attacking position of SH group.

reactions with simple SH compounds and some enzymes,[8~12] numerous functional segments which react with SH groups are arranged into two categories; i.e., one which involves electrophilic addition (EA) to an SH group and the other which causes displacement reaction (DR) between the suitable leaving groups and the SH group at the δ^+ carbon atom in the functional segments (see Tables 1 and 2).[3] Among some reactive centers in a functional segment, the more "soft" δ^+ carbon atom should be attacked slectively by an SH group, because the sulfur atom has generally a large polarizability and "soft base" character.[13~15]

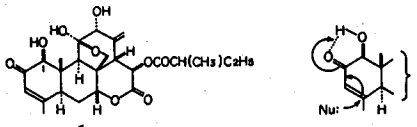
Thus, either natural or synthetic compounds having such functional segments as shown in Tables 1 and 2 may be expected to exhibit some biological activity (e.g., tumor inhibitory, cytotoxic, antibacterial, antifungal, antiviral, or plant growth inhibitory activity). The functional segments listed in Tables 1 and 2 seem to be very suggestive and useful for design of new tumor inhibitors, the search for antitumor active lead compounds from the store of known compounds, and investigation of the mecha-

nisms of biological activities. In fact, many naturally occurring and synthesized tumor inhibitors possess these functional segments in their molecules.[3,16~22] Of course, these functional segments involve only the active centers, therefore the cooperative assistance of the activity-enhancement factors or of the hydrophilic factors is very important for effective biological activity *in vivo*.

2–2. Enhancement Factors on the Tumor Inhibitory Activity

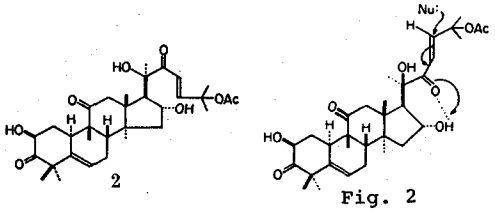
1) Hydrogen-Bonding

Dehydroailanthinone (**1**), isolated from *Pierrodendron kerstingii* by Kupchan and co-workers, showed significant antileukemic activity against P–388 in mice. However, methylation of the C–1 OH group of **1** resulted in a diminution of cytotoxicity and of *in vivo* antileukemic activity. Plausibly the hydroxyl group enhances the reactivity of the conjugated ketone toward biolobical nucleophiles through intramoleculra hydrogen-bonding as shown in Fig. 1.[23]



1 Fig. 1

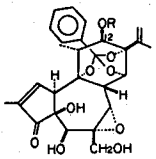
Although cucurbitacin B (**2**) showed a remarkably high cytotoxicity, its C–16 acetate, fabacein, showed only a weak activity. The remarkable diminution in cytotoxicity suggests that the free hydroxyl group on C–16 may be important for the reactivity of the conjugated ketone and the hydrogen-bonding between the C–16 hydroxyl group and the C–22 ketone could activate the $\alpha\beta$-unsaturated ketone toward nucleophilic attack by a biological macromolecule as shown in Fig. 2.[24]



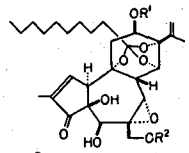
2 Fig. 2

2) Lipophilic Ester Side Chains

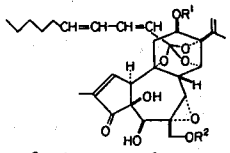
Gnididin (**3**), gniditrin (**4**), and gnidicin (**5**), diterpenoid esters isolated from *Gnidia lamprantha* by Kupchan and co-workers, showed potent antileukemic activity against P–388 leukemia in mice. However, 12-hydroxydaphnetoxin (**6**), possessing no ester moiety at C–12, showed no antileukemic activity. Benzoate ester derivative (**7**) showed potent activity of the same order as the naturally occurring esters.[25] Gnidilatin 20-palmitate (**8**) and gnidilatidin 20-palmitate (**9**) exhibited substantial inhibitory activity at optimal doses against P–388 leukemia in mice. Gnidilatin (**10**) showed moderate activity. Gnidiglaucin (**11**) and gnidilatidin (**12**) did not show inhibitory activity.[26] Thus, the ester moiety may serve as a carrier group in process concerned with cell penetration or selective molecular complex formation.



3: R = COCH=CHCH=CH(CH2)4CH3
4: R = COCH=CH(CH=CH2)2(CH2)2CH3
5: R = COCH=CHC6H5
6: R = H
7: R = COC6H5

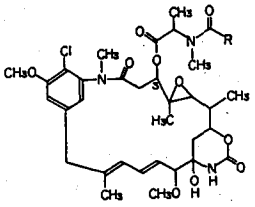


8: R1 = COC6H5 ; R2 = COC15H31
10: R1 = COC6H5 ; R2 = H
11: R1 = COCH3 ; R2 = H

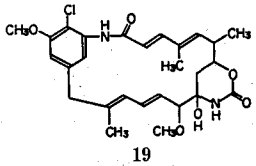


9: R1 = COC6H5 ; R2 = COC15H31
12: R1 = COC6H5 ; R2 = H

Maytansin (**13**) and its analogous compounds, novel ansamacrolides from *Maytenus* species,[27~30] *Putterlickia verrucosa*,[29,31] and *Colubrina texenis*,[32] are powerful antileukemic agents. Maytansin (**13**), maytanprine (**14**), maytanbutine (**15**), and maytanvaline (**16**), which possess an ester side chain at C–3 position, showed highly antitumor activity. However, maysine (**17**), normaysine (**18**), and maysenine (**19**), which have no ester group at C–3, did not show activity *in vivo* and showed about 1/10000 cytotoxicity of maytanside esters such as **16**. Maytanacine (**20**) from *P. verrucosa* and several semi-synthetic esters, **21**, **22**, and **23**, prepared from maytansinol (**24**), showed antitumor activity comparable to naturally occurring substituted alanyl esters. Maytansinol (**24**) showed no antitumor activity *in vivo*.[31] Hence, it may be correct that the ester function in the antileukemic maytansinoids may play a key role *in vivo* system. It is noteworthy that ansamytosin antileukemic antibiotics, novel maytansinoids **25~27**, were obtained from a fermentation broth of *Nocardia* species by Takeda chemical industries group.[33]



13: R = CH3
14: R = CH2CH3
15: R = CH(CH3)2
16: R = CH2CH(CH3)2



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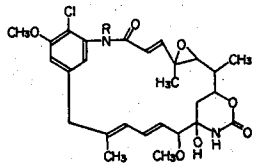
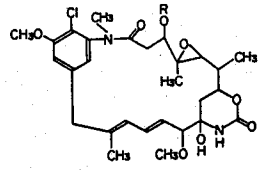


17: R = CH3
18: R = H

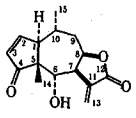


20: R = COCH3
21: R = COCH2CH3
22: R = COCH2Br
23: R = COCH=CHCH3
24: R = H
25: R = COCH(CH3)2
26: R = CO(CH2)2CH3
27: R = COCH2CH(CH3)2
28: R = COCF3

3) Bifunctional or Multifunctional Effect

The bifunctional or multifunctional effect due to two or several functional segments in the molecule of tumor inhibitors is essentially important for the enhancement of the antileukemic activity. Lee and co-workers demonstrated it by the systematic investigation of the structure-activity relationship among the sesquiterpene lactones and related compounds.

The comparison of the ED_{50} values (μg/ml) for the cytotoxicity (against the growth of KB tissue culture cells) of helenalin (**29**) and its chemically modified compounds disclosed that both the 2,3 double bond of **29** (ED_{50}=0.10) and the 2,3-epoxide of compound **30** (ED_{50}=0.11) gave equally effective cytotoxicity. The 1,2-epoxide of compound **31** (ED_{50}=0.53) is five times less active in comparison with **29** and **30**. The corresponding saturated compound **32** (ED_{50}=3.84) gave a 35-fold decrease in activity. Significant cytotoxicity could also be maintained when two potential alkylating centers, such as the $\alpha\beta$-unsaturated carbonyl system in the conjugated cyclopentenone and the α-methylene γ-lactone segments of helenalin (**29**), were converted to the $\alpha\beta$-epoxy carbonyl system, although the diepoxide **33** (ED_{50}=0.50) was five times less active in comparison with **29**. Compound **34** (ED_{50}>40.00) was more than an 80-fold diminution in cytotoxicity in comparison with **33**.[34]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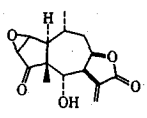
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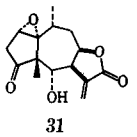
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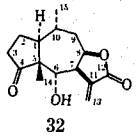
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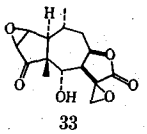
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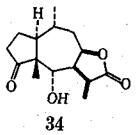
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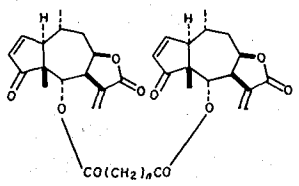
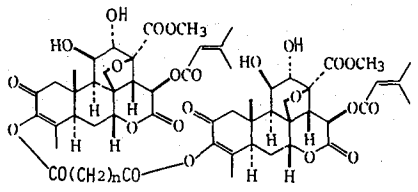
The diesters **35**~**38** were assayed for their *in vivo* antileukemic activity against P–388 lymphocytic leukemia in mice by Lee and his group. Examination of the *in vivo* data demonstrated that at 8 (mg/kg)/day compounds **35** and **36** afforded a higher percent T/C value (**35**: T/C=195%, **36**: T/C=173%) than **29** (T/C=162%), whereas at 15 mg/kg compound **37** extended the life expectancy of the mice two fold (T/C=261%) compared to compound **29** (T/C=123%). At 25 mg/kg, compound **38** afforded a percent T/C=178, compared to **29** with a percent T/C=127. These data would suggest that the bis(helenalinyl) esters (**35**~**38**) are more potent than the



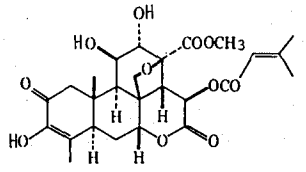
35: n = 3
36: n = 4
37: n = 1
38: n = 2

parent alcohol helenalin (**29**) at specific doses and several alkylating centers in the particular ester delivatives may cooperatively affect on their activities.[35]

Bisbrusatolyl esters **39**~**43** showed equal or more potent activity against a quassinoid-sensitive strain of P-388 lymphocytic leukemia than brusatol (**44**) at 0.6 mg/kg (compare T/C% of 272, 217, 176, 176, and 143 for **39**, **40**, **41**, **42**, and **43**, respectively, to 149 for **44**).[36,37]



39: n = 1
40: n = 2
41: n = 3
42: n = 4
43: n = 8



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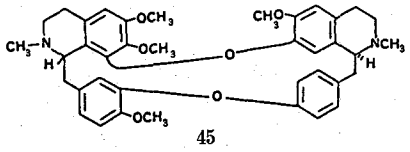
Tetrandrine (**45**), a bisbenzylisoquinoline alkaloid from *Cyclea peltata* Diels,[38] and vincristine (**46**) and vinblastine (**47**), dimeric indole alkaloids,[19] are known to be clinically useful antitumor agents. Activities of these alkaloids may be explainable by the effect of bifunctional segments.



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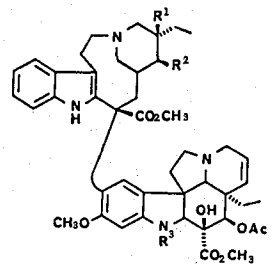


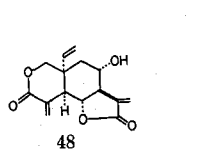
	R^1	R^2	R^3
47:	OH	H	CH_3
46:	OH	H	CHO

2-3. Biomimetic Reactions of Tumor Inhibitors and Related Compounds with SH Enzymes and Coenzyme and Their Model Compounds

Informations obtained from the biomimetic reactions of tumor inhibitors and related compounds with simple SH compounds, SH enzymes, and glutathione (co-

enzyme) can be useful for the better understanding of their active mechanisms and for design of new SH-alkylating tumor inhibitors.

Treatment of α-methylene lactone tumor inhibitors, vernolepin **48**, elephantopin **49**, and eupatundin **50**, with aqueous solution of L-cysteine at pH 7.4 afforded each corresponding cysteine adduct **51**, **52**, and **53**, respectively.[39]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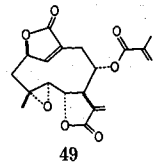
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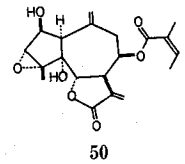
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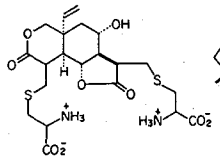
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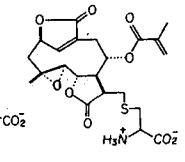
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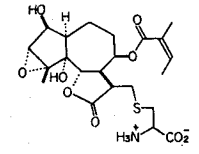
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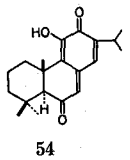
Inhibition of an SH enzyme, phosphofructokinase, by taxodione (**54**), taxodone (**55**), vernolepin (**48**), euparotin acetate (**56**), eupacunin (**57**), and some standard inhibitory reagents **58**~**60** against SH enzymes was investigated by Kupchan and his group in the absence and presence of the substrates (fructose-6-phosphate and ATP). Addition of only 1.6 mol of taxodione (**54**) per protomer of the enzyme diminished its enzyme activity by 50%. Taxodone (**55**) inhibited 50% at a relative concentration of 32. Euparotin acetate (**56**), eupacnin (**57**), and vernolepin (**48**) inhibited 50% at relative concentrations of 1000 to 2000. Taxodone (**55**) exhibited same order inhibition as that of *N*-ethylmaleimide (**58**) and Ellman's reagent (**59**). However, $\alpha\beta$-unsaturated lactones (**48**, **56**, and **57**) were only about ten times more active in comparison with iodoacetamide (**60**). The substrates, fructose-6-phosphate and ATP, were shown to protect the enzyme from each of the inhibitors.[40] Thus, the quinone methide segment seems to be more reactive to the SH groups of enzymes than the α-methylene lactone segment.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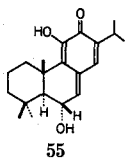
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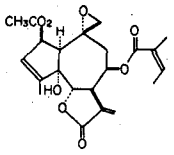
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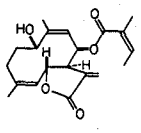
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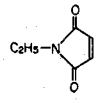
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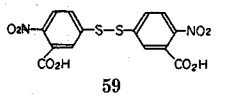

59


60

Inactivation of glycogen synthase by vernolepin (**48**) was examined by Kupchan group.[41] Reaction with 3 mol of radioactive vernolepin per 90000-dalton subunit

caused complete loss of activity. The concurrent disappearance of three titrable SH groups (out of six) indicates that thioether formation is the major model of binding to the protein.[41]

2-Crotonyloxymethyl-4,5,6-trihydroxycyclohex-1-en-3-one (**61**), isolated from a culture broth of *Streptomyces griseosporeus* as a component of glyoxalase I inhibitor, exhibited tumor inhibitory activities against HeLa cells *in vitro*, and also against Ehrlich ascites carcinoma and L-1210 cells inoculated in mice.[42] As shown in Chart 1, the crotonyloxy group of **61** was readily displaced by 2-hydroxyethanethiol or *p*-bromobenzenethiol to afford the SH adduct, **62** or **63**, while the hydrolyzed alcohol **64** did not react with thiols. These biomimetic reactions may provide a rationalization for the antitumor activities of **61**. Compounds **62**, **63**, and **64** did not show any biological activity as expected.[43]

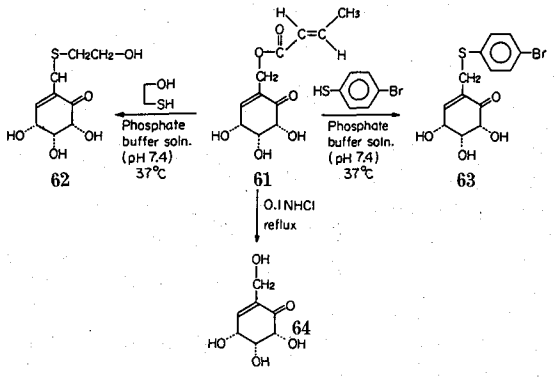


Chart 1

The reactions of jatrophone (**65**), an antileukemic macrocyclic diterpenoid isolated from *Jatropha gossypiifolia* by Kupchan group, with 1-propanethiol in a borate buffer solution gave the SH adduct **66** (Chart 2).[44] Jatrophone (**65**) was shown to react with SH groups on proteins such as bovine serum albumin and DNA-dependent RNA polymerase from *Escherichia coli*.[45]

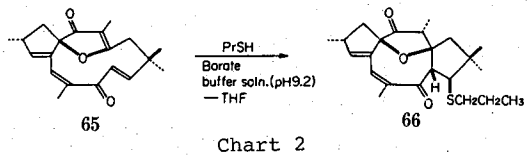
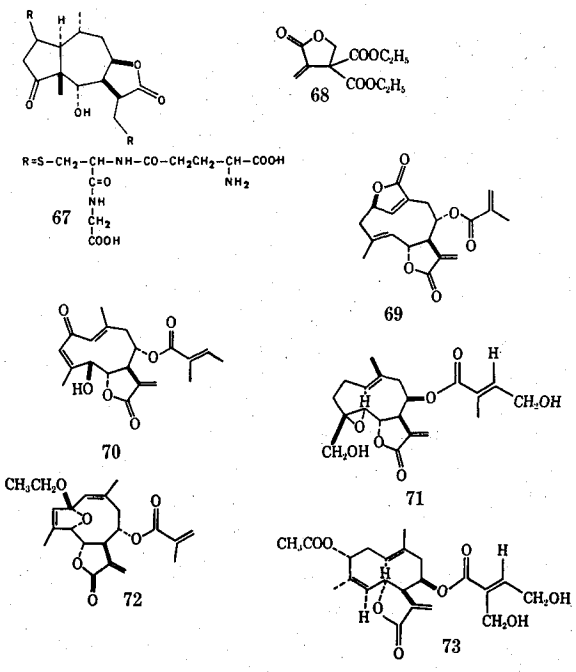


Chart 2

Helinalin (**29**) was homogenized with reduced glutathione in deuterated water and the formation of glutathione adduct **67** was confirmed by its NMR spectrum.[46] Helinalin was shown to be an effective inhibitor of DNA polymerase activity of Ehrlich ascites cells *in vivo*.[47] Presumably, the DNA polymerase alpha and gamma enzymes contain exposed SH groups which can be alkylated.

In vitro DNA polymerase activity of Ehrlich ascites cells was inhibited drastically (>50%) by helinalin (**29**), compounds **30**, **35** and **68**~**72** at 0.75 μmole. *In vitro* thymidylate synthetase activity was inhibited (at least 40%) by compounds **29** and

35 and also inhibited (at least 30%) by **71** and **73**, respectively at 0.75 μmole. Thymidylate synthetase was reported to be a sulfhydryl-bearing enzyme.[48]



Other similar enzyme inhibitions using various tumor inhibitors were reported by Lee group.[49~52]

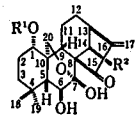
2–4. Studies on the *Rabdosia* Tumor Inhibitory Diterpenoids

Labiatae species have been used as medicinal plants for the treatment of cancer in various countries from ancient times.[53] *Rabdosia japonica* HARA (Labiatae) and *R. trichocarpa* KUDO (Labiatae) have also been used as the home remedy in Japan. However, their biological activities have not yet been clarified. Hence, Fujita and Nagao investigated in detail the antitumor and antibacterial activity on some available diterpenoids and related compounds.[1,2,54,55]

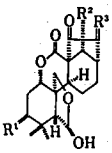
Oridonin (**74**) (10 mg/kg, T/C=215%) and lasiokaurin (**75**) (10 mg/kg, T/C=217%) showed a fairly high activity against Ehrlich ascites carcinoma inoculated in mice. Enmein (**76**) (25 mg/kg, T/C=166%), enmein-3-acetate (**77**) (40 mg/kg, T/C=156%), compound **78** (40 mg/kg, T/C=165%), and oridonin 14-deoxy-derivative **79** (20 mg/kg, T/C=161%) showed activity at the higher dose than that of oridonin (**74**). Oridonin dihydro-derivative **80**, butanethiol adduct **81**, dihydro enmein (**82**), and trichokaurin (**83**), however, did not show any activity. Thus, the α-methylene-cyclopentanone segment was shown to be an important active center of the tumor inhibitory *Rabdosia* diterpens.[1,2]

Many antitumor sesquiterpenes possessing the α-methylene-γ-lactone segment as an active center in the molecule have been reported, but the antitumor natural products

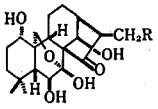
having α-methylene-cyclopentanone segment had not been known except for sarcomycin (**84**).[56,57]



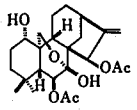
74: R^1=H, R^2=OH
75: R^1=Ac, R^2=OH
79: R^1=R^2=H



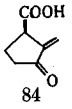
76: R^1=OH, R^2=H, R^3=CH_2
77: R^1=OAc, R^2=H, R^3=CH_2
78: R^1=H, R^2=OH, R^3=CH_2
82: R^1=OH, R^2=H, R^3=α-CH_3, β-H



80: R=H
81: R=S$(CH_2)_3CH_3$



83



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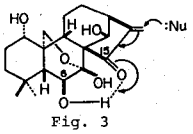


Fig. 3

Intensity of the activity and its relation with structure are also investigated. Since oridonin (**74**) and lasiokaurin (**75**) showed almost the same intensity of the activity, the OH group at C–1 has no direct effect for the activity. An important role of the OH group at C–6 is clarified by the following fact. The activities of enmein (**76**), enmein-3-acetate (**77**), and compound **78** are 1/4 or lesser in comparison with that of oridonin (**74**). The hydrogen-bonding between the carbonyl group at C–15 and the OH group at C–6 in oridonin (**74**) has been confirmed by its IR, UV, and NMR spectra. Hence, the C–17 atom is polarized to δ^+ and its reactivity with nucleophile must be increased (Fig. 3). In fact, it is supported by the lower chemical shifts of C–17 methylene protons of oridonin (**74**) than those of enmein (**76**), enmein-3-acetate (**77**), and compound **78**, that the electron density of C–17 of oridonin is lower than those of **76**, **77**, and **78**.[2]

In order to validate the argument mentioned above, we planned the examination of antitumor activity of C–6 acyloxyl derivatives of oridonin (**74**). We investigated preparation of the desired acyl derivatives of oridonin. After several attempts, we found an efficient method for selective acylation of the OH groups of **74**. On treatment with excess Ac_2O and a catalytic amount of $BF_3 \cdot Et_2O$ under ice-cooling, oridonin (**74**) was converted to the 6-acetate **85** in 25.1% yield and the diacetate **86** in 63.8% yield. Oridonin (**74**) was treated with the more bulky dodecanoic anhydride in the presence of a catalytic amount of $BF_3 \cdot Et_2O$ at room temperature to give only the 6-dodecanoate **87** in 83% yield. On the other hand, oridonin (**74**) was reacted with several acyl chlorides in the presence of Et_3N to afford each corresponding 14-*O*-acyl derivative **88**~**95** exclusively (Chart 3).[58]

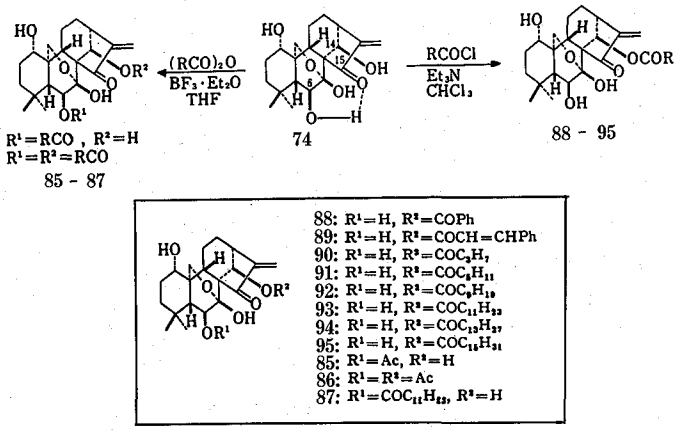


Chart 3

Thus, 6-*O*-acyl derivatives **85**~**87** were submitted to the antitumor test against Ehrlich ascites carcinoma inoculated in mice. All of the tested compounds were found to be inactive at the dose of 5 mg/kg and 10 mg/kg, at which doses oridonin (**74**) did exhibit activity.[59]

It was again supported by the ^{13}C–NMR analysis of oridonin (**74**) and the 6-*O*-acyl derivatives (**85** and **87**) that the hydrogen-bonding between the carbonyl group at C–15 and the OH group at C–6 must result in δ^+ nature at the C–15 and C–17 atoms in oridonin (Table 3). The increased soft electrophilicity of the C–17 atom would then make the addition of e.g. the SH group of enzymes in the tumor cells easier. Therefore, the ability of oridonin (**74**) to act as an alkylating agent is enhanced (Fig. 4).[59]

Table 3. The ^{13}C–NMR Chemical Shifts[a] of the C–15, C–16, and C–17 Atoms in Oridonin (**74**) and the 6-Acyl Derivatives **85** and **87**

	Oridonin **74** ppm	Oridonin-6-acyl derivative **85** ppm	**87** ppm	Values of $\Delta\delta$ (**74**–**85**) ppm	and $\Delta\delta$ (**74**–**87**) ppm
H H \ / C_{17}	119.0	115.2	115.2	+3.8	+3.8
–C_{16}	153.0	153.5	153.6	−0.5	−0.6
O=C_{15}	209.0	200.8	200.9	+8.2	+8.1

a) Taken in d_5-pyridine; ppm from TMS as an internal standard.

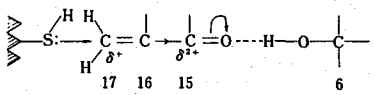


Fig. 4

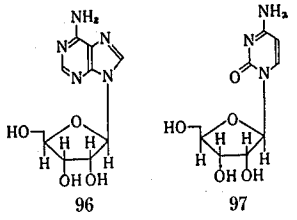
The antileukemic test of a series of oridonin 14-*O*-acyl derivatives **88**~**95** was carried out in order to clarify the significant effect of the ester side chains on the tumor inhibitory activity of oridonin. In compounds **90**~**95**, the activity was found to be increased with increase of the acyl carbon chain length. The activity of 14-*O*-dodecanoyl **93**, tetradecanoyl **94**, and hexadecanoyl **95** derivatives was shown to be stronger than that of oridonin (**74**), although **94** and **95** exhibited some toxicity. The benzoyl derivative **88** was shown to have the same order of acitivy as **74**, while the cinnamoyl derivative **89** had no activity. The derivatives **91** and **92** were shown to have slightly stronger activity at a dose of 5 mg/kg in mice than oridonin (**74**). The esters with higher fatty acids, **93**~**95**, showed stronger activity than those with lower fatty acids, **90**~**92**, and also than oridonin (**74**) it self. The observation that there was an increase in the activity with the increase in carbon chain length of the acyl groups is interesting. They suggest that the activity increase is in proportion to the lipophilicity of the oridonin derivatives.[59] Thus, the ester side chain in oridonin 14-*O*-acyl derivatives may play a carrier role in the process(es) related to the penetration into cells.

Biomimetic reactions of oridonin (**74**) and enmein (**76**) were investigated in detail using adenosine (**96**) and cytidine (**97**), as the nucleic acid model compounds, and four kinds of alkane thiols, L-cysteine, L-lysine, and L-serine, as the enzyme model compounds.[2]

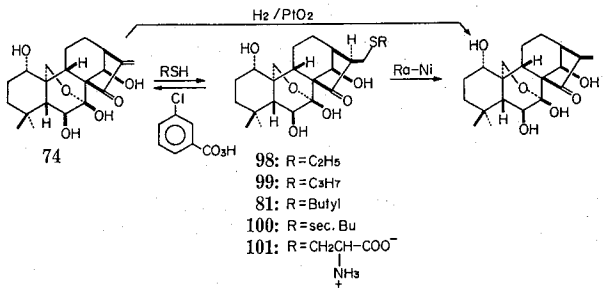
Oridonin (**74**) did not react with adenosine (**96**) and cytidine (**97**), and was recovered. The reactions of oridonin (**74**) with the SH enzyme model compounds readily proceeded under mild conditions to give alkane thiol adducts (**81**, **98**, **99**, and **100**). The reaction with L-cysteine also took place smoothly to yield adduct **101** quantitatively (Chart 4). Enmein (**76**) also easily gave adduct **102** by the reaction with 1-butanethiol. This process is shown in Chart 5. Interestingly, it was observed that oridonin (**74**) reacts with 1-butanethiol with much faster rate than that with enmein (**76**) in the competitive reaction in one flask which was followed by tlc analysis. The reactions of oridonin with L-lysine and L-serine, however, did not take place. Such easy addition reactions of thiols as described above without any catalysts are rationally explainable as the reactions of "soft" acids with "soft" bases.[13~15] Thus, the selective reactions of the α-methylene-cyclopentanone systems with only the SH group of enzyme containing many other nucleophilic groups (e.g., —OH, —NH_2, —COOH, etc.) can be rationalized by the "Hard Soft Acids Bases" principle[13,15] and experimentally supported by our biomimetic reaction.

It was also confirmed by Anke that DNA synthesis was 74% inhibited by 10 μg/ml of oridonin (**74**) and 91% inhibited by 10 μg/ml of enmein (**76**) in a test system using cells of the ascitic form of Ehrlich carcinoma and the RNA and protein syntheses were somewhat less affected.

A considerable antitumor activity of oridonin (**74**) (18 mg/kg, T/C=131%), enmein (**76**) (25 mg/kg, T/C=131%), enmein-3-acetate (**77**) (10 mg/kg, T/C=124%), nodosin (**103**), and shikoccin (**104**) (15 mg/kg, T/C=123%) was recognized against P388 lymphocytic leukemia inoculated into mice. Thiol adducts **98**, **105**, and **106** derived from the diterpenes **74**, **76**, and **104**, respectively, did not exhibit any activity.



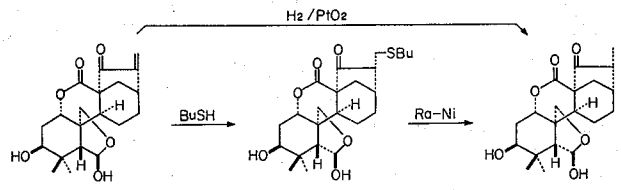
96 97



74

98: R=C_2H_5
99: R=C_3H_7
81: R=Butyl
100: R=sec. Bu
101: R=$CH_2CH(NH_3^+)-COO^-$

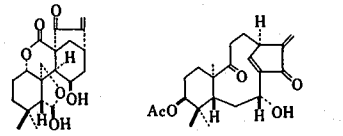
Chart 4



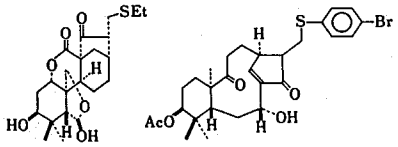
76 102

Chart 5

Therefore, the α-methylene-cyclopentanone segment must be regarded as the important active center of the activity against P388 lymphocytic leukemia, as in the case of activity against Ehrlich ascites carcinoma.[60]



103 104



105 106

In the related study on design for new SH-alkylating tumor inhibitors, we developed a new method for the synthesis of α-methylene-cyclopentanone segment utilizing a new bissulfenylation reagent, methyl 2-nitrophenyl disulfide (MNPDS) (**107**). A proposed transition state **108** for sulfenylation and the synthetic procedure

of the model compounds **109** and **110** having α-methylene-cyclopentanone segment are illustrated in Chart 6.[55,61]

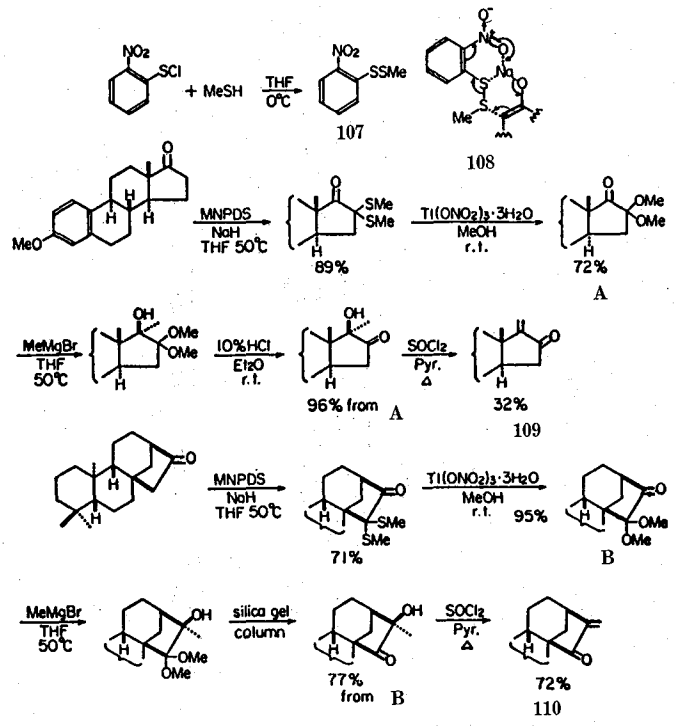


Chart 6

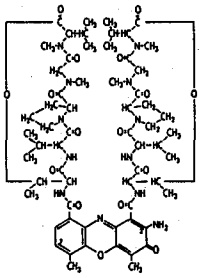
The recent advances in the field of *Rabdosia* antitumor diterpenoids are well reviewed by Fujita and Node,[62] and Fuji[63] independently.

3. DNA-INTERACTING TUMOR INHIBITORS

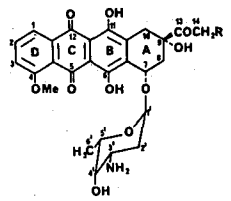
3-1. DNA-Binding Compounds

Some particular antitumor agents such as actinomycin D (**111**), adriamycin (**112**), daunomycin (**113**), ellipticine (**114**), nogalamycin (**115**), bleomycins (**116**), and echinomycin (**117**) interact with DNA by intercalation between two adjacent base pairs.[20,22] The mutual relation between DNA-binding (e.g., intercalation) and antitumor activity is not clear. However, the molecular design and synthesis of DNA-binding compounds with high affinity are useful not only for the development of new powerful anti-cancer drugs but also for the supply of probes for the DNA structure and function studies.[64]

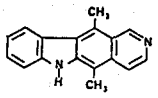
Actinomycin D (**111**) has been known for its interaction with DNA, which causes a specific inhibition of RNA polymerase, seems to be responsible for its antitumor activity.[20,65] Two peptide rings of actinomycin D (**111**) are located in the minor



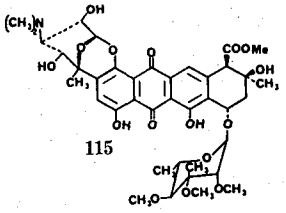
111



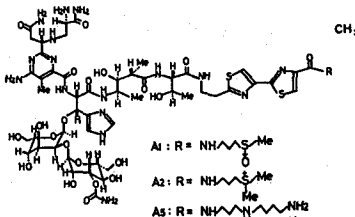
112: R = OH
113: R = H



114

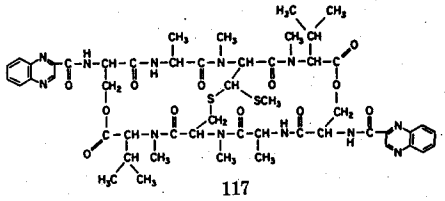


115



A1 : R= NH
A2 : R= NH
A3 : R= NH
A6 : R= NH
B2 : R= NH

116



117

groove of DNA when the flat ring moiety of **111** is intercalated.[66] Actinomycin D (**111**) exhibited specific intercalation between G–C base pairs,[67] and Sobell[68] postulated that this is due to specific hydrogen-bonding between the peptide ring of **111** and guanine on the basis of X-ray crystallographic analysis[66] of actinomycine-nucleotide crystals.

The antibiotic adriamycin (**112**) and daunomycin (**113**) are highly cytotoxic substances isolated from a cultured broth of *Streptomyces peucetius*.[69,70] Adriamycin (**112**) exhibited very wide spectrum of antileukemic activity and displayed considerable effects against various solid tumors.[71] The *in vivo* binding of daunomycin (**113**) and its analogs to DNA was extensively investigated[22,72] and the spectroscopic methods were efficiently employed. For example, the 480 nm visible absorption maximum of **113** was decreased on addition of DNA and was shifted to longer wavelength.[73,74] The fluorescence of the anthraquinone chromophore in the anthracycline antibiotics was quenched when binding to DNA took place.[73,75] A progressive increase in the relative viscosity of DNA was produced upon binding of daunomycin (**113**),[73,74,76]

which was analogous to that produced by other intercalating compounds.

Ellipticine (**114**) and its derivatives showed antileukemic activity against L-1210 lymphocytic leukemia in mice.[77] This flat molecule compound **114** strongly binds, by intercalation, to helical DNA.[78,79] The DNA-binding property of various ellipticine derivatives was investigated in an attempt to correlate DNA affinity with antitumor activity.[80]

Bleomycins (BLMs) (**116**), isolated as the Cu(II) complexes from a culture of *Streptomyces verticillus*,[81,82] are clinically used in the treatment of squamous cell carcinoma and malignant lymphoma.[83] Cleavage of cellular DNA by BLM seems to be responsible for the antitumor activity.[84] Fluorescence and ^{1}H-NMR spectral data indicated that BLM binds preferentially to the guanine base of DNA with its bisthiazole moiety.[84] The terminal amine moiety of BLM may play an electrostatic binding role to DNA. Interestingly, BLM promoted cleavage at G-T and G-C sequences of DNA in the presence of ferrous ion and molecular oxygen.[84]

Echinomycin (**117**), a naturally occurring antitumor active antibiotic, consists

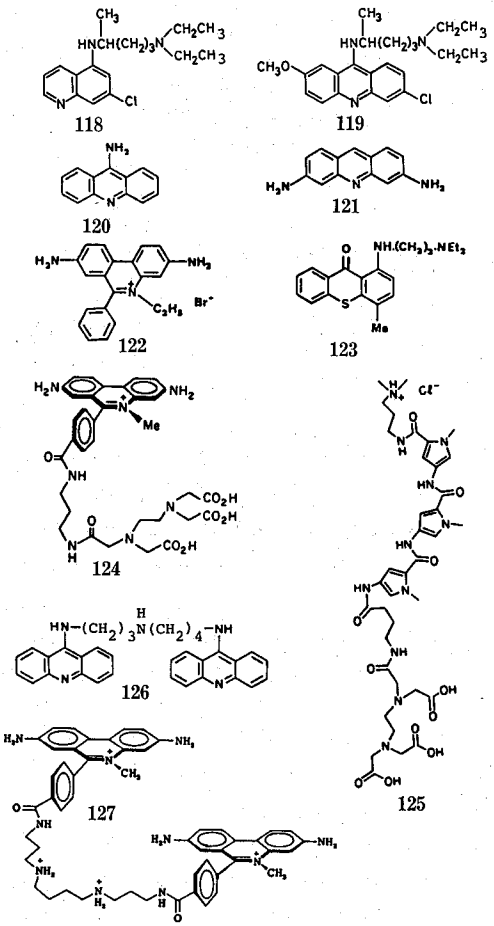


Chart 7

of two planar quinoxaline moieties connected by an octapeptide bridge.[85,86] Waring and co-workers reported echinomycin (**117**) and its analogs (triostin A and des-*N*-tetramethyltriostin A) to bind to various naturally occurring and synthesized DNA by a fassion involving bis-intercalation of both quinoxaline chromophores common to the antibiotics.[87~90] Conformational studies of echinomycine (**117**) and triostin A are reported by Williams group.[91,92]

Other DNA-binding compounds are listed in Chart 7.[64,93,94] These are chloroquine (**118**), quinacrine (**119**), 9-aminoacridine (**120**), proflavine (**121**), ethidium bromide (**122**), miracil D (**123**), methidiumpropyl-EDTA (**124**),[95] distamycine-EDTA (**125**),[96] spermine diacridine (**126**),[97] and bis (methidium) spermine (**127**).[98]

The particular compounds (**124** and **125**) were designed by Dervan for cleavage of double helical DNA in the presence of ferrous ion and oxygen. Spermine-containing compounds (**126** and **127**) proved to be powerful bis-intercalators.

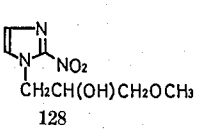
On the basis of the suggestive information obtained from the bioorganic chemistry of the DNA-binding compounds, we newly designed several hypoxic cell sensitizers[4,5] in antitumor radiotherapy, which is reviewed in the following paragraph.

3-2. Design of New Hypoxic Cell Radiosensitizers

Considerable efforts have been made to develop effective compounds for cancer radiotherapy that would involve the sensitization of hypoxic radioresistant cells in tumor tissues. Among them, misonidazole (**128**) has been known to be an efficient radiosensitizer for hypoxic cells. However, due to its neurotoxicity, its clinical applicability at doses sufficient to produce optimum sensitization limited.

Thus, we developed a new type of hypoxic cell radiosensitizers: FNT-1 (**129**), -2 (**130**), -3 (**131**), -4 (**132**) and —SS-1 (**133**).[4,5]

These compounds were designed on the basis of the following reson and information. a) Chemistry of polyamine-containing compounds is familiar to us.[99~102] b) The biogene-polyamines [spermidine (**134**) and spermine (**135**)] exhibit remarkable charge-charge affinity to the phosphate moiety of the nucleic aicd.[97,98,103] c) Some aromatic compounds bind to nucleic acids by intercalation, i.e., the insertion of a flat molecule between the base pairs of a double helix by charge-transfer complex.[104] d) Most excellent hypoxic cell sensitizers possess one or two nitro group(s) as the



128

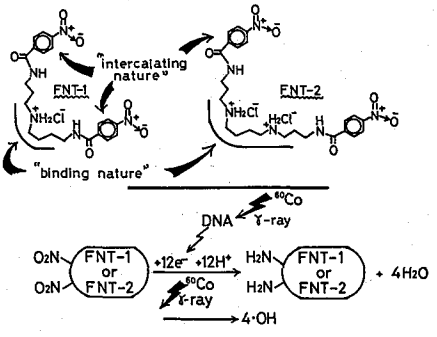


Fig. 5

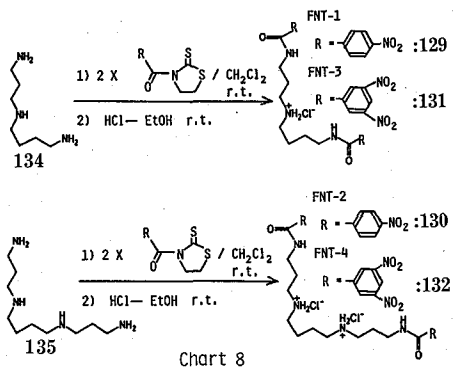


Chart 8

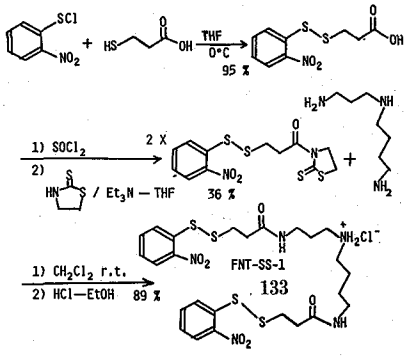


Chart 9

electron-accepting group.[105,106] Thus, a speculative intercalator, the 4-nitro- or 3,5-dinitro-benzoyl group was linked to the polyamines, spermidine (**134**) and spermine (**135**). The whole of the molecular design of these compounds is shown in Fig. 5.

Synthesis of FNT-series compounds **129**~**133** was efficiently done utilizing a highly chemoselective acylating reagent, 3-acyl-1,3-thiazolidine-2-thione (Charts 8 and 9).[101]

A radiosensitizing effect of FNT-series compounds on hypoxic cells (HeLa S3 cells) in vitro was investigated according to the method which was previously reported by Mori group.[107] Enhancement ratios (ER) of the sensitization of hypoxic HeLa S3 cells irradiated *in vitro* at room temperature were determined for each compound over a range of concentrations. The ER was obtained from the Do ratio in the absence and presence of the radiosensitizer. However, the maximum concentration was limited by the solubility of the compounds. Figure 6 shows the concentration dependance of the ER for each compound; the ER for misonidazole (**128**) is also presented for comparison. All curves show an increase in the ER with the increasing concentration of compounds, and it is obvious that FNT series compounds are more effective than misonidazole (**128**) (Table 4). FNT-1 (**129**) and FNT-3 (**131**) especially were found to have good sensitizing effects; 1 mM both compounds have about 1.6 times sensitizing effect of misonidazole (**128**).

Half-wave reduction potentials ($E_{1/2}$) were measured with a Yanaco P-1000 polarographic analyser using a dropping mercury electrode and standard calomel electrode (SCE). Values for the reduction potential were obtained with a Tast polarograph. A higher electron-affinity was observed with FNT-1~3 (**129**: $E_{1/2}$= −350 mV, **130**: −330 mV, and **131**: −270 and −380 mV) with that (−395 mV) of misonidazole.[5]

Since spermine and spermidine themselves do not show any sensitization, it is reasonable that the radiosensitizing abilities of FNT-series compounds are mainly due to the nitrobenzoyl group in their molecules. FNT-series compounds carry ionizable basic groups and, as shown by Adams,[105] basic nitro aromatic compounds are unusually effective radiosensitizers. In addition, these compounds are more electron-affinic than misonidazole (**128**), and therefore more effective in radiosensitizing under the hypoxic conditions than misonidazole (**128**).

Table 4. *Sensitizer Enhancement Ratios*

Compound	Concentration (mM)	ER
FNT-1	0.1	1.18
	1.0	2.10
FNT-2	0.1	1.00
	1.0	1.45
FNT-3	0.1	1.17
	1.0	1.99
FNT-4	0.1	1.11
	1.0	1.22
FNT-SS-1	0.1	1.20
	1.0	1.85
Misonidazole	0.1	1.05
	1.0	1.32

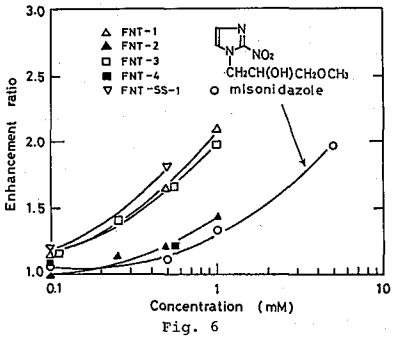


Fig. 6

Recently, we have found an interesting experimental result in the interaction between the FNT-series compounds and NDA, which will be reported very soon.[108]

CONCLUSION

We reviewed on the design and study of new tumor inhibitors having SH-alkylating function and DNA-binding function. From the foregoing discussions, we would offer some variable suggestions about a strategy for the development of new tumor inhibitors. Thus, for instance, the following process may be served: (1) search for compounds including SH-alkylating segment(s) among known compounds; (2) synthesis of the lead compounds possessing SH-alkylating segment(s) and/or DNA-binding planer segment(s); (3) chemical modification of the lead compounds on the basis of activity-enhancement factors (e.g., hydrogen-bonding, lipophilic ester side chains, and bifunctional or multifunctional effect); (4) introduction of hydrophilic moiety into the lead compounds for diminution of the toxicity; (5) trying biomimetic reactions with simple compounds including an SH group or with DNA; (6) biological testing. We eagerly hope this strategy to be utilized as an efficient guide for the development of more potent and less toxic cancer drugs.

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