Antitumor Agents, 129. Tannins and Related Compounds as Selective Cytotoxic Agents

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ANTITUMOR AGENTS, 129.[1] TANNINS AND RELATED COMPOUNDS AS SELECTIVE CYTOTOXIC AGENTS

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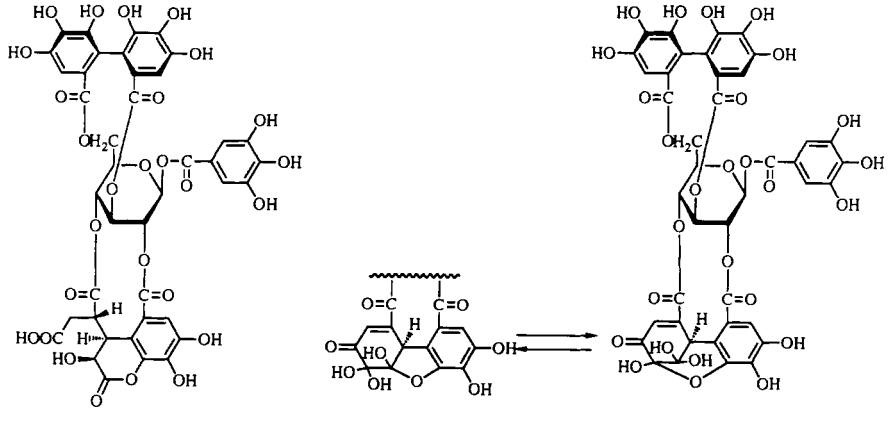
ABSTRACT.—Fifty-seven tannins and related compounds, including gallotannins, ellagitannins, and condensed and complex tannins, were evaluated for their cytotoxicities against human tumor cell lines, including malignant melanoma, lung carcinoma, ileocecal adenocarcinoma, epidermoid carcinoma, malignant melanoma, and medulloblastoma cell lines. Among them, chebulagic acid [**1**], geraniin [**2**], sanguiin H-11 [**3**], 4,5-di-*O*-galloylquinic acid [**12**], 1,3,4,5-tetra-*O*-galloylquinic acid [**15**], 1(β)-*O*-galloylpedunculagin [**24**], furosin [**29**], castalagin [**38**], sanguiin H-2 [**34**], vescalagin [**39**], grandinin [**40**], phyllyraeoidin A [**42**], (−)-epicatechin 3-*O*-gallate [**50**], cinnamtannin B_2 [**55**], and acutissimin A [**56**] exhibited moderate selective cytotoxicity against PRMI-7951 melanoma cells with ED_{50} values in the range of 0.1–0.8 μg/ml. Selective cytotoxicities against the melanoma cells were also observed for strictinin [**22**], pedunculagin [**23**], eugeniin [**25**], elaeocarpusin [**28**], punicacortein C [**37**], casuarinin [**41**], sanguiin H-6 [**43**], procyanidin B-2 3,3′-di-*O*-gallate [**51**], procyanidin C-1 3,3′,3″-tri-*O*-gallate [**52**], and cinnamtannin B_1 [**54**] with ED_{50} values of 1–4 μg/ml. All of the tannins were found to be inactive (>10 μg/ml) against lung carcinoma (A-549), ileocecal adenocarcinoma (HCT-8), epidermoid carcinoma of nasopharnyx (KB), and medulloblastoma (TE-671) tumor cells.

Tannins are widely distributed in the plant kingdom and are classified on the basis of their structures into three groups: hydrolyzable, condensed, and complex tannins.



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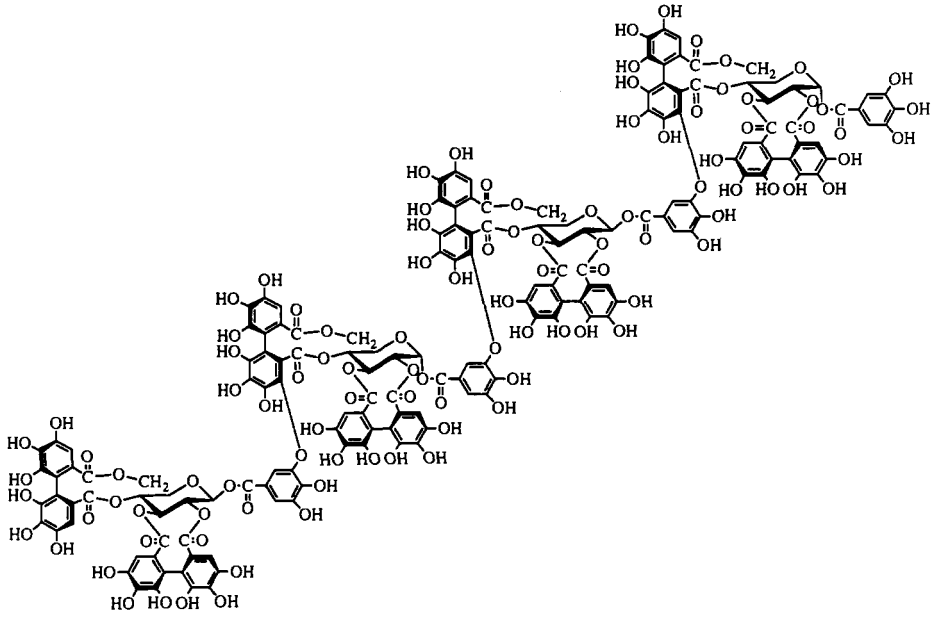
[1]For part 128, see Lee *et al.* (1).



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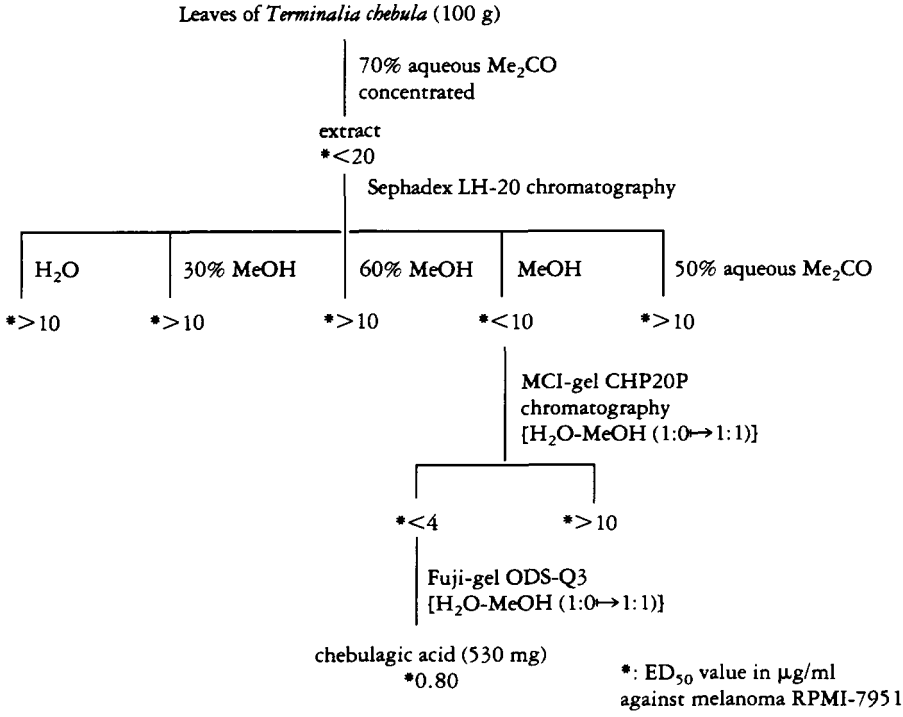
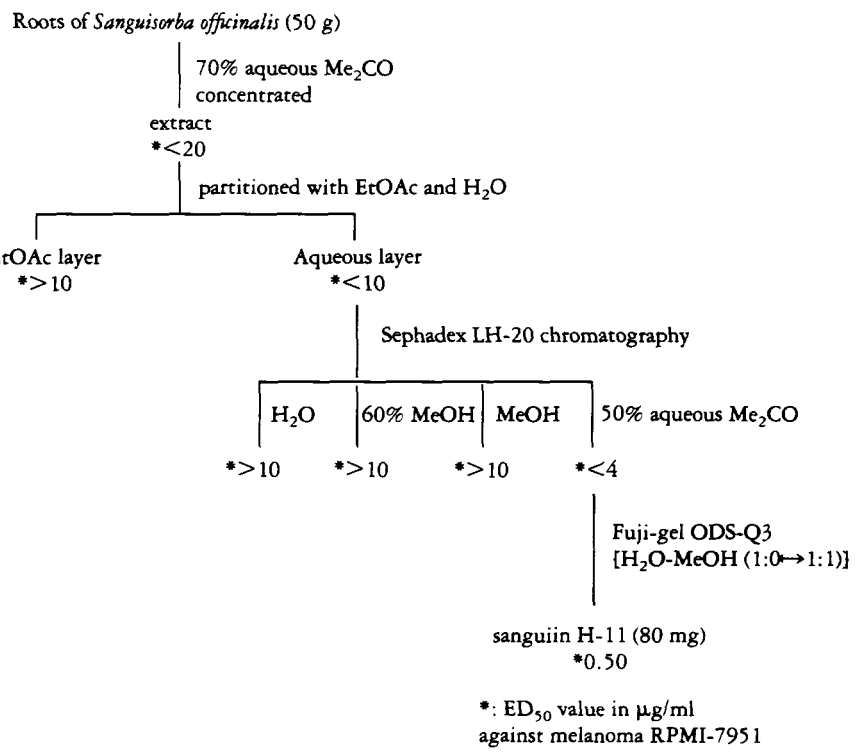
They are known to possess a variety of biological activities, such as lowering the level of blood urea nitrogen (2,3), inhibiting the activity of angiotensin-converting enzyme (4), antiviral and anti-HIV activities (5,6), inhibition of lipid peroxidation (7,8), antimutagenic effects (9), DNA-breaking activity (10,11), and psychotropic activity (12).



SCHEME 1

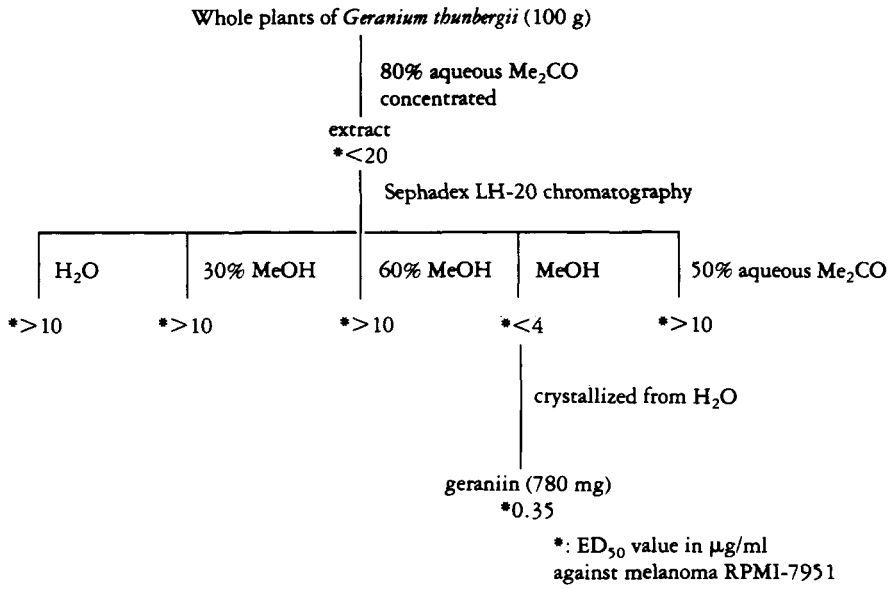
As a result of our continuing search among medicinal plants for novel potent selective cytotoxic agents against solid tumors, the alcoholic extracts of the leaves of *Terminalia chebula* (Combretaceae), the whole plants of *Geranium thunbergii* (Geraniaceae), and the roots of *Sanguisorba officinalis* (Rosaceae) were found to show significant (ED_{50} < 20 μg/ml) cytotoxicity in melanoma tumor cells (RPMI-7951). Subsequent bioassay-directed fractionation led to the isolation of known tannins, chebulagic acid [**1**], geraniin [**2**], and sanguiin H-11 [**3**] from *T. chebula*, *G. thunbergii*, and *S. officinalis*, respectively, as the cytotoxic principles (see Schemes 1–3). Since **1–3** are the first identified tannins to demonstrate potent cytotoxicity against this human cancer cell line, it is important to screen the other tannins as potent selective cytotoxic agents against solid tumor cell lines, although the antitumor activity of tannins against sarcoma-180 has previously been reported (13). We report herein cytotoxicity determinations of fifty-seven tannins against lung carcinoma (A-549), ileocecal adenocarcinoma (HCT-8), epidermoid carcinoma of nasopharnyx (KB), melanoma (RPMI-7951), and medulloblastoma (TE-671) tumor cells.



SCHEME 2

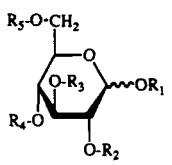
RESULTS AND DISCUSSION

The tannins and related compounds examined for cytotoxicity were classified into four groups, including gallotannins, ellagitannins, and condensed and complex tannins. The gallotannins were further classified into four groups based on their alcoholic core: gallotannins with a glucose core (**4–10**), with a quinic acid core (**11–15**), with a shikimic acid core (**16–18**), and with a hamamelose core (**19,20**). The ellagitannins were classified as ellagitannins with a glucose core (**21–27**), with a modified hexahydroxydiphenoyl moiety and/or related acyl group (**1**, **2**, **28–36**), *C*-glycosidic ellagitannins (**37–41**), ellagitannins dimer (**42,43**), an ellagitannin tetramer (**3**), and el-

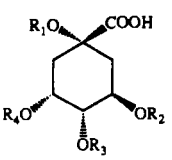


SCHEME 3

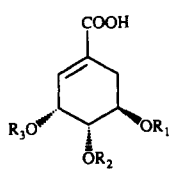
Gallotannins



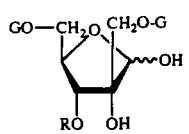
	R_1	R_2	R_3	R_4	R_5
4	G(β)	H	H	H	H
5	G(β)	H	H	H	G
6	G(β)	G	H	H	G
7	G(β)	H	H	G	G
8	G(β)	G	G	H	G
9	H	G	G	G	G
10	G(β)	G	G	G	G



	R_1	R_2	R_3	R_4
11	H	H	G	H
12	H	H	G	G
13	G	H	G	G
14	H	G	G	G
15	G	G	G	G

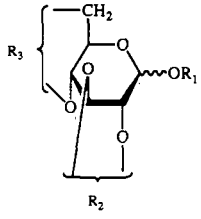


	R_1	R_2	R_3
16	G	H	H
17	G-G	H	H
18	G	H	G

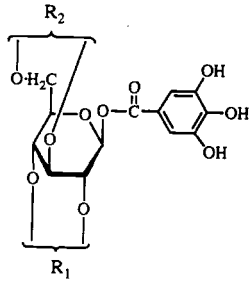


	R
19	H
20	G

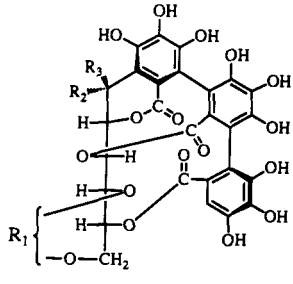
Ellagitannins



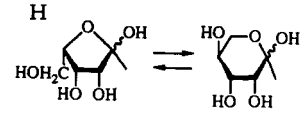
	R_1	R_2	R_3
21	H	*S*-HHDP	H,H
2	G(β)	H,H	*S*-HHDP
23	H	*S*-HHDP	*S*-HHDP
24	G(β)	*S*-HHDP	*S*-HHDP
25	G(β)	G,G	*S*-HHDP
34	G(β)	G,G	San
35	H	H,H	Gal
36	H	*S*-HHDP	Gal

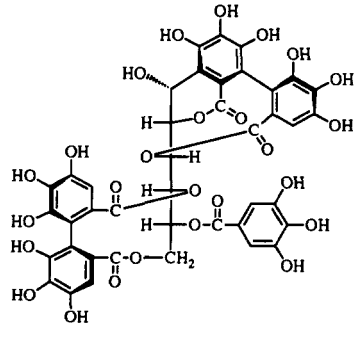


	R_1	R_2
26	H,H	*R*-HHDP
27	G,G	*R*-HHDP
28	Ela	*R*-HHDP
29	DHHDP	H,H
30	DHHDP	G,G
31	Che	G,G
32	H,H	Val
33	DHHDP	Val



	R_1	R_2	R_3
37	Gal	OH	H
38	*S*-HHDP	H	OH
39	*S*-HHDP	OH	H
40	*S*-HHDP	H	





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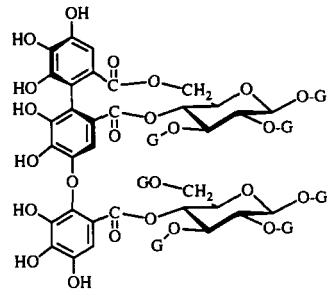

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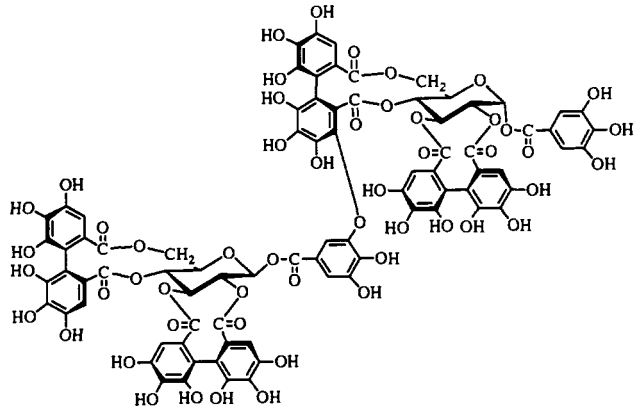

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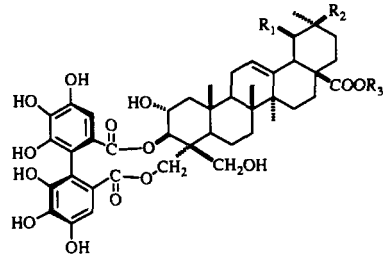


44 R_1=H, R_2=Me, R_3=β-D-Glc
45 R_1=R_3=H, R_2=Me

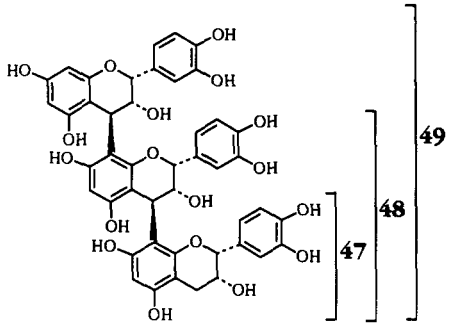
lagitannins with triterpenoid moiety (**44**,**45**). The condensed tannins included monomers (**46**, **47**, and **50**), dimers (**48**, **51**, and **53**), trimers (**49**, **52**, and **54**), and a tetramer (**55**). Compounds **56** and **57** represent the flavanoellagitannins, consisting of the component unit [(+)-catechin] of condensed tannins and *C*-glycosidic ellagitannins moiety-linked through the carbon-carbon bond.

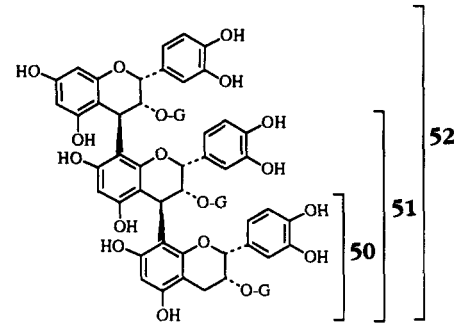
The activity was found only in the melanoma tumor cell line (RPMI-7951), and all compounds tested were inactive against lung carcinoma, ileocecal adenocarcinoma, epidermoid carcinoma, and medulloblostoma tumor cells.

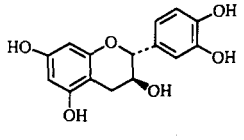
Among the gallotannins, compounds **12** and **15** showed strong cytotoxicity, while **10** showed a little activity. Since both **12** and **15** possess a quinic acid core in the molecule, this core might be important for cytotoxicity.

Among the ellagitannins, **1–3**, **24**, **29**, **34**, **38–40**, and **42** all showed moderate

Condensed Tannins







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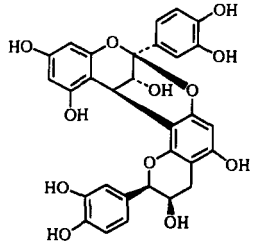

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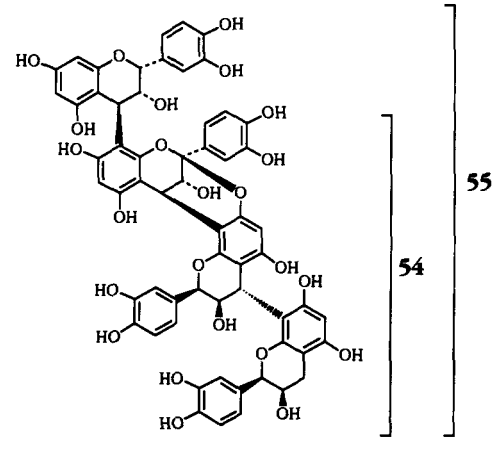
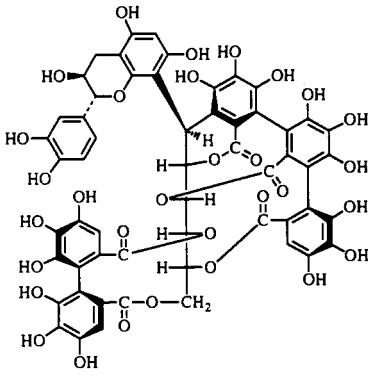


cytotoxic activity with ED_{50} values of 0.1–0.8 μg/ml, while **22**, **23**, **25**, **28**, **37**, and **43** were found to be cytotoxic with ED_{50} values of 1–5 μg/ml. A comparison of the cytotoxicity of compounds **21**–**27** indicated that the location of the hexahydroxydiphenoyl (HHDP) group seems to be important for cytotoxicity and that the 4,6-(*S*)-HHDP group might be essential. Compounds **25**, and **33** are structurally related to each other, but differed only with the HHDP and the sanguisorboyl groups at C-4 and C-6 found in the glucose core. Increased cytotoxicity was observed in **33**, suggesting that the sanguisorboyl group confers some cytotoxicity. Compounds **2**, **29**, **30**, and **33** possess a dehydrohexahydroxydiphenoyl (DHHDP) group at C-2 and C-3 in the glucose core; among these only **2** and **29** showed cytotoxicity. The combination of DHHDP and the other phenol carboxylic acid groups might be important. The *C*-glycosidic ellagitannins (**37**–**41**), especially **38**–**40**, also showed potent cytotoxicity (ED_{50} 0.1–0.8 μg/ml). Since they are structurally quite similar to each other except for the difference of the C-1 substituents of the glucose core, it implies that this structure is essential for cytotoxicity. Compound **42** is composed of **10** and **25**, while **3** is formed from two molecules of **43**. Enhanced cytotoxicity was observed in each case, suggesting that oligomeric ellagitannins might show stronger cytotoxicity.

Complex Tannins



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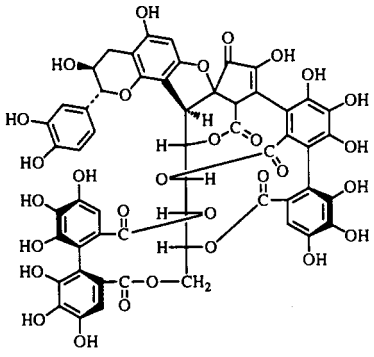

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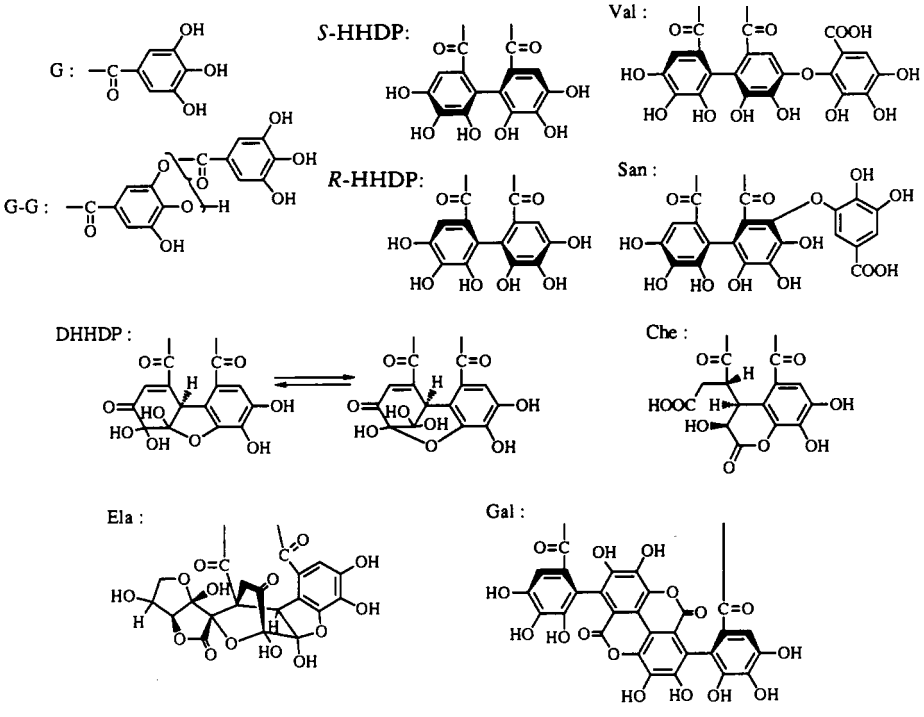

In the case of condensed tannins, a comparison of the cytotoxicity of **47**–**52** indicated that the galloyl group at flavan C-3 increased their cytotoxicity. A comparison of the cytotoxicity of **48** and **53** as well as **49** and **54** revealed that only **53** and **54** are cytotxic, indicating the importance of the presence of an ether linkage at flavan C-2 and C-7 as seen in **53** and **54** for the contribution of the enhanced cytotoxicity. The 5- to 10-fold more potent cytotoxicity demonstrated by **55** compared to that of **53** and **54** is obviously due to an increase of the flavan unit.

Compound **56** showed strong activity similar to that of **37** and **38**. Since these compounds are structurally related to each other, this activity might be based within the *C*-glycosidic ellagitannin moiety.

Studies on the mechanism of action for these tannins are underway.

TABLE 1. Cytotoxicity for Tannins and Related Compounds against Melanoma (RPMI-7951).[a]

Compound	Name	ED_{50} (μg/ml)
Gallotannins		
4	1-*O*-galloyl-β-D-glucose (14)	>10
5	1,6-di-*O*-galloyl-β-D-glucose (15)	>10
6	1,2,6-tri-*O*-galloyl-β-D-glucose (16)	>10
7	1,4,6-tri-*O*-galloyl-β-D-glucose (17)	>10
8	1,2,3,6-tetra-*O*-galloyl-β-D-glucose (18)	>10
9	2,3,4,6-tetra-*O*-galloyl-β-D-glucose (19)	>10
10	1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (20)	5.01
11	4-*O*-galloylquinic acid (21)	>10
12	4,5-di-*O*-galloylquinic acid (21)	0.53
13	1,4,5-tri-*O*-galloylquinic acid (G. Nonaka, unpublished data)	>10
14	3,4,5-tri-*O*-galloylquinic acid (21)	>10
15	1,3,4,5-tetra-*O*-galloylquinic acid (23)	0.43
16	3-*O*-galloylshikimic acid (24)	>10
17	3-*O*-digalloylshikimic acid (24)	>10
18	3,5-di-*O*-galloylshikimic acid (24)	>10
19	hamamelitannin (25)	>10
20	2′,3,5-tri-*O*-galloylhamamelose (25)	>10
Ellagitannins		
1	chebulagic acid (35)	0.80
2	geraniin (31)	0.35
3	sanguiin H-11 (26)	0.50
21	2,3-hexahydroxydiphenoyl-D-glucose (26)	>10
22	strictinin (17)	4.86
23	pedunculagin (27)	2.74
24	1(β)-*O*-galloylpedunculagin (28)	0.21
25	eugeniin (29)	2.74
26	corilagin (30)	>10
27	punicafolin (30)	>10
28	elaeocarpusin (32)	2.62
29	furosin (33)	0.67
30	terchebin (33)	>10
31	chebulinic acid (34)	>10
32	mallotinic acid (33)	>10
33	mallotusinic acid (33)	>10
34	sanguiin H-2 (26)	0.44
35	punicalin (36)	>10
36	punicalagin (36)	>10
37	punicacortein C (37)	3.86
38	castalagin (38)	0.79
39	vescalagin (38)	0.58
40	grandinin (38, 39)	0.10
41	casuarinin (38)	2.24
42	phillyraeoidin A (40)	0.50
43	sanguiin H-6 (26)	5.00
44	castanopsinin A (40)	>10
45	castanopsiniin A (40)	>10
Condensed Tannins		
46	(+)-catechin (42)	>10
47	(−)-epicatechin (43)	>10
48	procyanidin B-2 (43)	>10
49	procyanidin C-1 (43)	>10
50	(−)-epicatechin 3-*O*-gallate (42)	0.55
51	procyanidin B-2 3,3′-di-*O*-gallate (42)	3.45
52	procyanidin C-1 3,3′,3″-tri-*O*-gallate (42)	3.05
53	proanthocyanidin A-2 (44)	5.50

TABLE 1. (Continued)

Compound	Name	ED_{50} (μg/ml)
54	cinnamtannin B_1 (43)	3.57
55	cinnamtannin B_2 (43)	0.66
Complex Tannins		
56	acutissimin A (38, 45)	0.61
57	mongolicain A (38, 46)	>10
	Actinomycin (positive control)	<0.01

[a]All compounds are inactive against epidermoid carcinoma of nasopharnyx (KB), lung adenocarcinoma (A-549), ileocecal adenocarcinoma (HCT-8), and medulloblastoma (TE-671) tumor cell lines.

MATERIALS AND METHODS

MATERIALS.—The 57 tannins and related compounds were isolated from the plant materials as reported previously (14–46) and shown in Schemes 1–3.

BIOLOGICAL ASSAY.—The in vitro cytotoxicity assay was carried out according to a National Cancer Institute protocol (47) as previously described (48). The assay was conducted using a panel of human tumor cell lines. These cell lines are lung carcinoma (A-549), ileocecal adenocarcinoma (HCT-8), epidermoid carcinoma of nasopharnyx (KB), melanoma (RPMI-7951), and medulloblastoma (TE-671). All cell lines were obtained from the American Type Culture Collection, Rockville, MD. In general, the assay methods were the same as those described by Monks *et al.* (49). However, a tetrazolium salt method was used to measure cell survival and proliferation (50).

ACKNOWLEDGMENTS

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