Communications to the Editor

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INDUCTION OF DIFFERENTIATION IN MURINE ERYTHROLEUKEMIA CELLS BY FLAVONOIDS[1)]

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Some flavonoids which occur widely in nature have been found to induce hemoglobin synthesis in murine erythroleukemia cells (B8). Most of them are more potent in inducing differentiation of B8 cells than dimethyl sulfoxide.

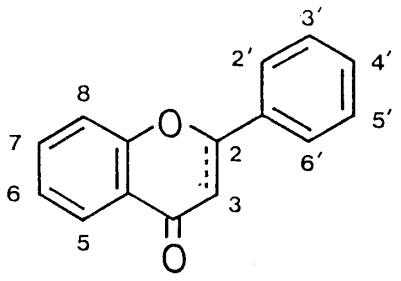
KEYWORDS —— cell differentiation; erythroleukemia cell; flavonoid; isoflavonoid

As a part of our current work on the differentiation-inducing substances of animal tumor cells, we screened naturally occurring substances for their effect on murine leukemia cells and found that a phenolic compound structurally related to flavonoids induces differentiation-associated properties in these cell.[2)] To define more fully the structural features involved in the differentiation-inducing activity of flavonoids, we have investigated the potency of the activity in a number of flavonoids using murine erythroleukemia cells.

A murine erythroleukemia cell line (B8), which was obtained from Dr. K. Nose, was cultured and maintained in Ham's F12 medium supplemented with 15% fetal bovine serum and kanamycin (60 μg/ml) at 37 °C in a humidified incubator with 8% CO_2 atmosphere. The cells were cultured for 3-7 days with samples at concentrations of 1-125 μg/ml, and then stained with benzidine. The percentage of stained cells was determined under a light microscope.[3)]

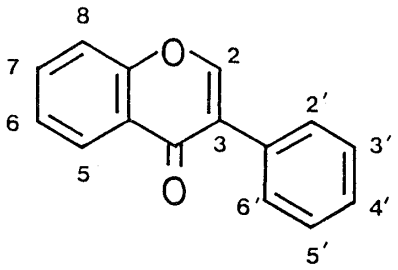
The structural features of the compounds tested and their differentiation-inducing activity are presented in Table 1 and Fig. 1. The activity was expressed in two values as the minimum differentiation inducible dose (μg/ml) and the maximum potency of differentiation induction. The latter refers to the maximun percentage of differentiated cells among viable cells after incubation with the samples.

Nearly all types of flavonoids including isoflavonoids were found to cause greater induction of hemoglobin synthesis in B8 cells than dimethyl sulfoxide.[4] However, derivatives with large functional groups, such as glycosyl and isoprenyl, were entirely devoid of inducing activity, or showed a large loss of activity as indicated by the active/inactive pairs such as apigenin (1)/licoflavone B (7), apigenin (1)/saponaretin (11) and naringenin (16)/licoflavanone (14). The weak but significant activity of daidzin (19) can be viewed as a result of hydrolysis of glycoside to its active aglycone daidzein (18). Both the reduction of the pyrone ring and the fixation of the side phenyl group which inhibits its free rotation did not interfere with activity significantly as shown by the potent activity of an isoflavan, flavanone and pterocarpan. Highly

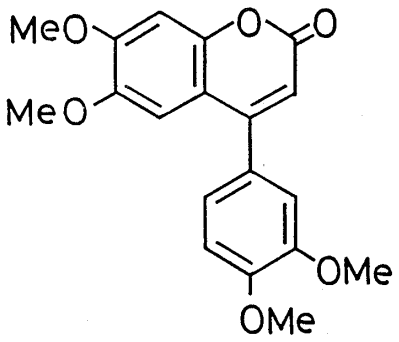


flavone (Δ 2,3)

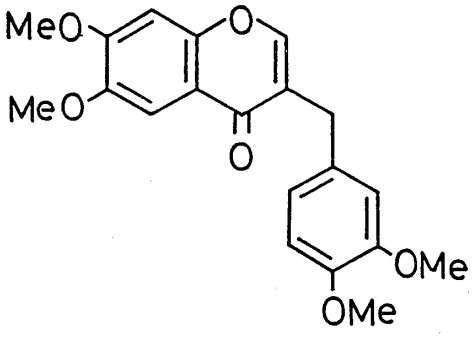
and flavanone



isoflavone

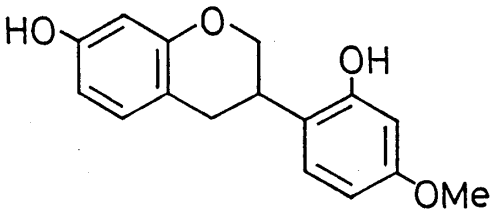


dimethyl melannein

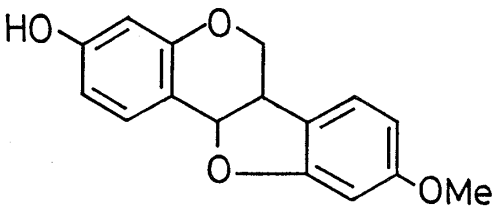


tetramethoxy homo-

isoflavone



vestitol (isoflavan)



medicarpin (pterocarpan)

Fig. 1. Structural Features of Flavonoids Tested for Differentiation Induction of Erythroleukemia Cells

Table 1. Differentiation Inducing Activity of Flavonoids in Murine Erythroleukemia Cells

Compounds	Substituents								Minimum inducing dose	Maximum inducing potency
	3	5	6	7	8	3'	4'	5'	g/ml	%
(Flavone)										
Apigenin (1)	-	OH	-	OH	-	-	OH	-	8	80
Baicalein (2)	-	OH	OH	OH	-	-	-	-	32	40
Chrysin (3)	-	OH	-	OH	-	-	-	-	4	70
Fisetin (4)	OH	-	-	OH	-	OH	OH	-	16	50
Galangin (5)	OH	OH	-	OH	-	-	-	-	4	50
Kaempferol (6)	OH	OH	-	OH	-	-	OH	-	16	70
Licoflavone B (7)	-	OH	iPr	OH	-	-	OH	-	ne	ne
Myricetin (8)	OH	OH	-	OH	-	OH	OH	OH	32	30
Nobiletin (9)	-	OMe	OMe	OMe	OMe	OMe	OMe	-	63	55
Quercetin (10)	OH	OH	-	OH	-	OH	OH	-	8	40
Saponaretin (11)	-	OH	Glu	OH	-	-	OH	-	ne	ne
Tetramethoxyflavone (12)	-	-	OMe	OMe	-	OMe	OMe	-	ne	ne
Wogonin (13)	-	OH	-	OH	OMe	-	-	-	2	ne
(Flavanone)										
Licoflavanone (14)	-	OH	iPr	OH	iPr	-	OH	-	8	35
Liquiritigenin (15)	-	OH	-	OH	-	-	OH	-	4	75
Naringenin (16)	-	OH	-	OH	-	-	OH	-	32	80
(Isoflavone)										
Calycosin (17)	-	-	-	OH	-	OH	OMe	-	16	65
Daidzein (18)	-	-	-	OH	-	-	OH	-	<1	55
Daidzin (19)	-	-	-	OGlu	-	-	OH	-	63	60
Isoformononetin (20)	-	-	-	OMe	-	-	OH	-	4	55
Puerarin (21)	-	-	-	OH	Glu	-	OMe	-	ne	ne
Tetramethoxyisoflavone (22)	-	-	OMe	OMe	-	OMe	OMe	-	ne	ne
(Other flavonoids)										
Vestitol (23)									8	ne
Dimethylmelannein (24)									ne	ne
Tetramethoxy homoisoflavone (25)				See Fig. 1.					32	65
Medicarpin (26)									8	70

(1) Maximum inducing potency is defined as the percentage of benzidine-positive cells among viable cells.
(2) iPr: isoprenyl; Glu: glucosyl.
(3) <1: the value was less than 1 μg/ml.
(4) ne indicates no inducing effects on cells at doses up to 125 μg/ml.

hydroxylated flavones such as myricetin (8) and quercetin (10) were very weakly active, whereas apigenin (1), chrysin (3) and kaempferol (6) were among the most effective differentiation inducers tested. Such differences in the activity of compounds with the same skeleton may depend in part on physicochemical properties which determine such factors as solubility and penetration into test cells as well as the compounds' intrinsic potential for interaction with target sites responsible for differentiation. On the other hand, polymethoxy flavonoids, which are considered to penetrate cells more easily due to their hydrophobicity, were largely inactive.

With regard to the mechanism of terminal differentiation in animal cells, which has not been fully understood, Nomura and Oishi revealed that erythroid differentiation in mouse Friend cells is a result of two fundamentally different

cellular reactions which are mediated by the plasma membrane and cellular DNA respectively.[5] In view of the interaction between DNA and flavonoids, such flavonoids as kaempferol (6), quercetin (10) and so on are known to be mutagenic in bacteria cells, which is ascribed to the mutagens' DNA damaging property.[6] However, the damage to DNA may not be essential for the induction of differentiation in animal cells since nonmutagenic apigenin (1) was one of the most effective differentiation inducers tested. It should be noted that hydroxy flavones have strong affinity for the nucleotide involving stacking interaction between base pairs and are thus expected to affect DNA function in cells.[7] In addition, flavonoids are known to interact with membranes in vivo as shown by their inhibition of such membrane-bound enzymes as ATPase, phospholipase A_2 and prostaglandin cyclooxygenase.[8] These dual effects on DNA and membrane, in line with Oishi's experimental results, may well be the basis for the differentiation induction of B8 cells by flavonoids.

In view of the flavonoids' wide occurrence in free or conjugated form in many plant products used for food,[9] it is of particular interest to determine whether these flavonoids can influence the embryogenesis as well as terminal differentiation of animal cells. Further studies of this matter and the effect of these flavonoids on other differentiable tumor cells, including human leukemia cells, are currently under way.

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