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Antitumor Activity of *Rabdosia* and *Teucrium* Diterpenoids against P 388 Lymphocytic Leukemia in Mice[1)]

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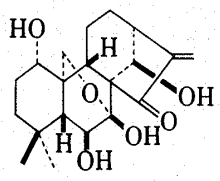
A considerable antitumor activity of *Rabdosia* diterpenoids, oridonin (**1**), enmein (**2**), enmein-3-acetate (**3**), nodosin (**4**), and shikoccin (**5**), was recognized against P 388 lymphocytic leukemia inoculated into mice. *Teucrium* diterpenoids, teucvin (**6**) and teucvidin (**7**), did not show any activity. A brief discussion on the activity is also described.

Keywords——Labiatae plants; *Rabdosia* diterpenoids; *Teucrium* diterpenoids; oridonin; enmein; shikoccin; P 388 lymphocytic leukemia

Labiatae species have been used as medicinal plants for the treatment of cancer in various countries from ancient times.[2)] Recently, we reported that *Rabdosia* (Labiatae) diterpenoids



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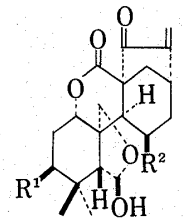
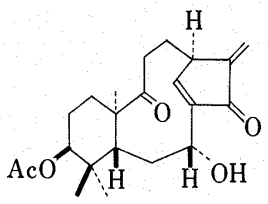


2 : R^1=OH, R^2=H
3 : R^1=OAc, R^2=H
4 : R^1=H, R^2=OH



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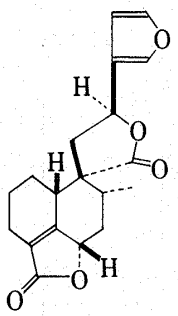

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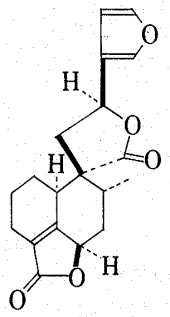

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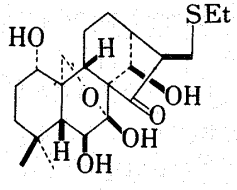

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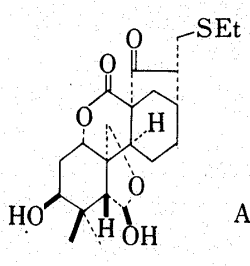

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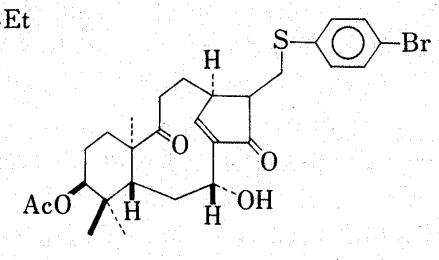

10

and their acyl derivatives showed antitumor activity against Ehrlich ascites carcinoma inoculated into mice, as well as antibacterial activity.[1b,3]

We now report on tumor-inhibitory test of *Rabdosia* dietrpenoids,[4] oridonin (**1**), enmein (**2**), enmein-3-acetate (**3**), nodosin (**4**), and shikoccin (**5**), and *Teucrium* diterpenoids,[4] teucvin (**6**) and teucvidin (**7**), against P 388 lymphocytic leukemia in mice. The testing results are summarized in Table.

Table I. Antitumor Activity of *Rabdosia* and *Teucrium* Diterpenes and Their Thiol Adducts against P 388 Lymphocytic Leukemia in Mice

Entry	Compound	Dose (mg/kg)	Evaluation[a] *T/C* (%)	Entry	Compound	Dose (mg/kg)	Evaluation[a] *T/C* (%)
1	**1**	36	Toxic	7	**5**	60	124
		18	131			30	113
		9	117			15	123
		4.5	76	8	**5**	60	111
2	**1**	27	121			30	114
		18	128			15	120
		12	127			7.5	107
		8	106	9	**6**	100	Toxic
3	**2**	200	120			50	93
		100	112			25	91
		50	121	10	**7**	50	85
		25	131			25	89
4	**2**	50	119			12.5	91
		25	137	11	**8**	90	94
		12.5	125			45	112
		6.25	118			22.5	108
5	**3**	40	118	12	**9**	100	107
		20	118			50	109
		10	124			25	83
		5	109			12.5	103
6	4	80	89			6.25	107
		40	124			3.12	103
		20	118	13	**10**	90	100
		10	106			45	94
		5	120			22.5	100
						11.25	95

a) (M.S.T.[b] treated÷M.S.T.[b] control)×100.
b) Median survival time.

Rabdosia diterpenoids, **1**—**5**, showed considerable activity. Thiol adducts **8**,[3a] **9**, and **10** derived from the diterpenes **1**, **2**, and **5**, respectively, did not show any activity. From these results, the α-methylene cyclopentanone system[5] must be regarded as the active center of the activity against P 388 lymphocytic leukemia, as in the case of activity against Ehrlich ascites carcinoma.[3] In the latter case, however, a subtle dissimilarity of chemical structure has a large effect on the activity. Thus, hydrogen-bonding in oridonin (**1**) played a role as an enhancement factor[5] of the antitumor activity. On the other hand, oridonin (**1**) and emnein (**2**) showed similar activities against P 388 lymphocytic leukemia, and no effect of the hydrogen-bonding was apparent.

The *Teucrium* diterpenes, **6** and **7**, showed no activity against P 388 lymphocytic leukemia inoculated into mice. This result is consistent with a negative biomimetic experiment,[5] in which treatment of teucvin (**6**) with ethanethiol in dimethylformamide (DMF) resulted in recovery of the starting material (see experimental section).[6]

Experimental

1. Preparation of Samples for Assay[7]——Oridonin (**1**), enmein (**2**), enmein-3-acetate (**3**), nodosin (**4**), shikoccin (**5**), teucvin (**6**), teucvidin (**7**), oridonin ethanethiol adduct (**8**), enmein ethanethiol adduct (**9**), and shikoccin *p*-bromobenzenethiol adduct (**10**) were each dissolved in saline–Tween 80 and each solution was used as a test sample for autitumor activity in mice.

2. Assay Method for Antitumor Activity[7]——P 388 lymphocytic leukemia cells, 10^6 cells/mouse, were inoculated intraperitoneally into experimental animals [BDF_1 or CDF_1 mice (male or female)]. From 24 h after the tumor inoculation, each sample was injected into mice intraperitoneally once a day for 9 consecutive days. Observation was continued for 30 d, and evaluation was done by comparison of median survival time (M.S.T.) with that of the control.

3. Organic Reactions——Melting points were determined with a Yanagimoto microapparatus. Infrared (IR) spectra were measured in KBr discs on a Jasco A-202 spectrophotometer. ^{1}H-Nuclear magnetic resonance (NMR) spectra were taken with JEOL JM-FX100 instruments in $CDCl_3$ and d_5-pyridine; signals are given as ppm from tetramethylsilane (TMS), used as an internal standard. Mass spectra (MS) were determined on a JEOL JMS-01SG double-focusing mass spectrometer. Extracts were dried overanhydrous Na_2SO_4. A mixture of Kiesel Gel 60 (70—230 mesh) (Merck) and silicic acid (Mallinckrodt) was used for column chromatography.

(i) Treatment of Enmein (**2**) with Ethanethiol: Into a solution of enmein (100 mg) in EtOH (50 ml) was added dropwise excess ethanethiol (1 ml). After being stirred at room temperature for 1 h, the mixture was concentrated *in vacuo* to give an oily residue, which was crystallized in MeOH to afford the ethanethiol adduct **9** (86 mg, 73.4%) as colorless needles, mp 224—225°C (from MeOH). *Anal.* Calcd for $C_{22}H_{32}O_6S$: C, 62.23; H, 7.61; M, 424. Found: C, 61.90; H, 7.73; M^+ *m/e*: 424. IR ν_{max}: 3440, 1758, 1705 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 1.04, 1.32 (each 3H, s, $CH_3\times2$), 1.08 (3H, t, J=8.0 Hz, $-SCH_2\underline{C}H_3$), 2.40 (2H, q, J=8.0 Hz, $-S\underline{C}H_2CH_3$), 3.80 (1H, m, $1/2W$=4 Hz, C-3-H), 4.36, 4.56 (each 1H, AB type, J=8.5 Hz, C-20-H_2), 5.40 (1H, dd, J=9.0 Hz, 7.0 Hz, C-1-H), 5.90 (1H, br s, C-6-H), 6.82, 8.30 (each 1H, m, $2\times$OH, disappeared with D_2O).

(ii) Treatment of Shikoccin (**5**) with *p*-Bromobenzenethiol: Into a solution of shikoccin (100 mg) in *N*,*N*-dimethylformamide (3.5 ml) was added *p*-bromobenzenethiol (61 mg). After being stirred at room temperature for 42 h, the mixture was extracted with ether after addition of water. The ethereal extract was washed with brine, dried, and concentrated *in vacuo* to give an oily residue, which was chromatographed on a silica gel column with $CHCl_3$ to yield a crude solid. It was purified on a preparative thin layer chromato plate (silica gel 60 F 254, Merck) with $CHCl_3$-acetone (4: 1) to afford the thiol adduct **10** (127 mg, 84.3%) as colorless plates, mp 176—178°C (from ethyl acetate–isopropyl ether). *Anal.* Calcd for $C_{28}H_{35}BrO_5S$: C, 59.67; H, 6.26; M, 562.139. Found: C, 59.30; H, 6.32; M^+ *m/e*: 562.137. IR ν_{max}: 3525, 1735, 1705, 1690, 1623, 1490 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.96, 0.98, 1.02 (each 3H, s, $CH_3\times3$), 2.16 (3H, s, $-COCH_3$) 2.56 (2H, m, C-17-H_2), 3.30 (1H, m, C-13-H), 4.49 (1H, dd, J=11.0 Hz, 6.0 Hz, C-7-H), 4.74 (1H, m, $1/2W$=5.5 Hz, C-3-H), 7.20, 7.38 (each 2H, AB type, J=9 Hz, aromatic protons).

(iii) Treatment of Teucvin (**6**) with Ethanethiol: Into a solution of teucvin (50 mg) in *N*,*N*-dimethylformamide (5 ml) was added excess EtSH (1 ml). After being stirred at room temperature for 24 h, the mixture was treated as usual to recover teucvin (40 mg).

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References and Notes

1) This paper forms Part XXXXVII of the series "Terpenoids" and Part III of "Biological and Physiological Activity" (Kyoto University). For Terpenoids Part XXXXVI and Biological and Physiological Activity Part II, see: E. Fujita, Y. Nagao, T. Kohno, M. Matsuda, and M. Ozaki, *Chem. Pharm. Bull.*, **29**, 3208 (1981).
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7) See the protocol of DR and D, National Cancer Institute, U.S.A.