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The Antitumor and Antibacterial Activity of the *Isodon* Diterpenoids[1]

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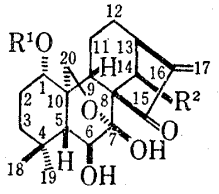
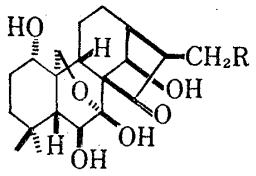
Oridonin (**1**), lasiokaurin (**2**), enmein (**8**), and enmein-3-acetate (**9**) and related compounds (**3** and **10**,) all of which have α-methylene cyclopentanone function in their molecule, have been shown to have antitumor activity against Ehrlich ascites carcinoma inoculated into mice. These compounds have also indicated specific activity against gram-positive bacteria. On the other hand, oridonin dihydro-derivative (**4**), compound (**5**), trichokaurin (**6**), and dihydroenmein (**11**) show any activity against neither tumor nor bacteria. Thus, it is concluded that the α-methylene-cyclopentanone system must be an important active center. Biomimetic reactions of oridonin and enmein with several thiols *etc.* support this conclusion.

Isodon japonicus Hara (Japanese name: Hikiokoshi) and *I. trichocarpus* Kudo (Kurobana-hikiokoshi) have been used as the home remedy in Japan, but their essential physiological activity has not yet been clarified.

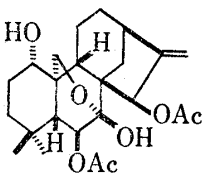
Recently, Fujita, *et al.* isolated many kinds of diterpenoids of the kaurene- and B-secokaurene-types from the foregoing plants and *I. lasiocarpus* (Hayata) Kudo (Taiwan-hikiokoshi) and determined their structures.[3] Now, we explored the antitumor and antibacterial activity



1 : R^1=H, R^2=OH
2 : R^1=Ac, R^2=OH
3 : R^1=R^2=H



4 : R=H
5 : R=S$(CH_2)_3CH_3$



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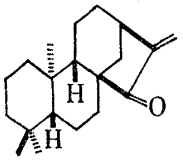

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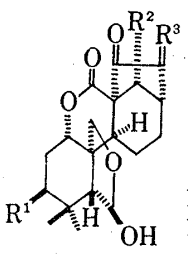

8 : R^1=OH, R^2=H, R^3=CH_2
9 : R^1=OAc, R^2=H, R^3=CH_2
10 : R^1=H, R^2=OH, R^3=CH_2
11 : R^1=OH, R^2=H, R^3=α-CH_3, β-H

1) Preliminary communication on antitumor activity: E. Fujita, Y. Nagao, M. Node, K. Kaneko, S. Nakazawa, and H. Kuroda, *Experientia*, **32**, 203 (1976). This paper forms Part XXXVI of the series "Terpenoids" and Part I of "Biological and Physiological Activity" (Kyoto University). Terpenoids. Part XXXV: E. Fujita, M. Ochiai, I. Uchida, A. Chatterjee, and S.K. Desmukh, *Phytochemistry*, **14**, 2249 (1975).
2) Location: *a*) *Uji, Kyoto, 611, Japan*; *b*) *Higashiyama-ku, Kyoto, 607, Japan.*
3) E. Fujita, M. Node, Y. Nagao, and T. Fujita, *Yakugaku Zasshi*, **94**, 788 (1974) and references cited therein.

on some available diterpenoids and their derivatives. The test samples are oridonin (**1**),[4] lasiokaurin (**2**),[5] trichokaurin (**6**),[6] enmein (**8**),[7] enmein-3-acetate (**9**),[7] and dihydroenmein (**11**) as the natural products, 14-deoxy-derivative (**3**),[8] dihydro-derivative (**4**),[4] butane thiol adduct (**5**), and compound (**10**) as the derivatives of oridonin, and *ent*-15-oxo-16-kaurene (**7**).[9]

Experimental

1. Preparation of Samples for Assay——Oridonin (**1**), lasiokaurin (**2**), oridonin 14-deoxy-derivative (**3**), oridonin dihydro-derivative (**4**), butane thiol adduct (**5**), trichokaurin (**6**), compound (**7**), enmein (**8**), enmein-3-acetate (**9**), compound **10**, and dihydroenmein (**11**) were respectively dissolved in water–ethanol (4:1) or water–ethanol–Tween 80 (40:9:1) and the each solution was employed as the testing sample for antitumor activity in mice (see footnote in Table I). Likewise, solutions of these diterpenes (**1**—**11**) (except compound (**7**)) in water-dimethylformamide, water–acetone, and water–ethanol were subjected to antibacterial screening test (see footnote in Table II and III).

2. Assay Method for Antitumor Activity——Ehrlich ascites carcinoma cells, 2×10^6 cells/mouse, were inoculated intraperitoneally to experimental animals (ddYS male mice weighing 20 ± 0.5 g) divided into groups of 10 each. From 24 hours after the inoculation, each sample was injected to mice intraperitoneally once a day for consecutive 7 days. Observation was continued for 40 days, and change of the body weight, life-prolongation effect and survival rate were compared with those of the controls.

3. Assay Method for Antibacterial Activity——The antibacterial activity *in vitro* of each sample was determined against gram-positive and gram-negative bacteria which had been preserved in Department of Microbiology, Kyoto College of Pharmacy. The determination was made according to agar dilution method authorized by the Japan Society of Chemotherapy.

4. Biomimetic Reactions——Adenosine and cytidine were purchased from Kohjin Co., Ltd., Tokyo, Japan. L-Cysteine presented by Dr. K. Sohda (Institute for Chemical Research, Kyoto University) was employed. L-Lysine, L-serine, ethane thiol, propane thiol, butane thiol, and 2-butane thiol were purchased from Nakarai Chemicals Co., Ltd., Kyoto Japan.

Melting points were determined with a Yanagimoto micro-apparatus. Infrared (IR) spectra were measured in KBr discs on a Hitach model EPS-3 Spectrophotometer. Nuclear magnetic resonance (NMR) spectra were taken with a Varian A-60 and T-60 spectrometers in d_5-pyridine; signals are reported in ppm from tetramethylsilane (TMS) as internal standard. The mass spectra were determined on a JMS-OISG double-focusing mass spectrometer. Extracts were dried over anhydrous sodium sulfate. Merck Kieselgel 60 (70—230 mesh) was used for column chromatography. Merck Kieselgel 60 F_{254} pre-coated plates (layer thickness 0.25 mm) and Merck Cellulose F pre-coated plates (layer thickness 0.10 mm) were employed for thin-layer chromatography. (TLC). pH 7.2—7.3 potassium phosphate buffer solution (0.1 mol concentration) was prepared by dilu tion of the mixture of 1 mol K_2HPO_4 (2 liter) and 1 mol $KHPO_4$ (0.9 liter) with 10 times volume of H_2O.

(i) Treatment of Oridonin (**1**) with Adenosine (**15**): Oridonin (100 mg) and adenosine (71 mg, 1.2 mol equiv.) were dissolved in a mixture of pH 7.2—7.3 potassium phosphate buffer solution (3 ml) and ethanol (3 ml). The solution was stirred at room temp. for 7 days and then evaporated *in vacuo* to remove ethanol. The remaining aqueous solution was extracted with excess EtOAc. The extract was treated as usual to recover the starting material (60 mg).

(ii) Treatment of Oridonin (**1**) with Cytidine (**16**): Oridonin (30 mg) and cytidine (40 mg, 2 mol equiv.) were treated as mentioned above to recover oridonin (26 mg).

(iii) Michael-type Reaction of Oridonin (**1**) with Alkane Thiols (Ethane-, Propane-, Butane-, and 2-Butanethiols): General procedure in dimethylformamide (DMF): Into a solution of oridonin (200 mg) in DMF (5 ml) was dropped excess ethane thiol.

The mixture was stirred at room temperature for 12 hr and then evaporated *in vacuo* to give an oily residue, which was crystallized in MeOH to afford a colorless needles **17a** (204 mg). Yields are listed in Table IV.

Physical data of each products; Compound (**17a**): mp 188—190° (decomp.). *Anal.* Calcd. for $C_{22}H_{34}O_6S$: mol. wt., 426.208. Found: Mass Spectrum *m/e*: 426.209 (M^+). IR ν_{max} cm^{-1}: 3200 and 1705. NMR δ: 1.12 (3H, s), 1.16 (3H, t, $J=7$ Hz, -S-CH_2-C$\underline{H}_3$), 1.24 (3H, s), 2.50 (2H, q, $J=7$ Hz, -S-C$\underline{H}_2$-CH_3), 3.66 (1H, t, $J=8$ Hz, C-1-H), 4.14 (1H, d, $J=5.5$ Hz, C-6-H), 4.38, 4.70 (each 1H, AB type, $J=10.5$ Hz, C-20-H_2), and 5.40 br (1H, s, 1/2 $W=2.5$ Hz, C-14-H). Compound (**17b**): colorless needles, mp 179—180° (decomp.). *Anal.* Calcd. for $C_{23}H_{36}O_6S$: C, 62.69; H, 8.23; mol. wt., 440.223. Found: C, 62.40; H, 8.49, Mass Spectrum *m/e*: 440.227 (M^+).

4) E. Fujita, T. Fujita, H. Katayama, M. Shibuya, and T. Shingu, *J. Chem. Soc.* (*C*), 1970, 1674.
5) E. Fujita and M. Taoka, *Chem. Pharm. Bull.* (Tokyo), **20**, 1752 (1972).
6) E. Fujita, T. Fujita, M. Shibuya, and T. Shingu, *Tetrahedron*, **25**, 2517 (1969).
7) E. Fujita, T. Fujita, and M. Shibuya, *Yakugaku Zasshi*, **87**, 1076 (1967).
8) E. Fujita, T. Fujita, and Y. Nagao, *Chem. Pharm. Bull.* (Tokyo), **18**, 2343 (1970).
9) M.F. Barnes and J. MacMillan, *J. Chem. Soc.* (*C*), **1967**, 361.

IR ν_{max} cm^{-1}: 3250 and 1710. NMR δ: 0.90 (3H, t, J=7 Hz, -SCH$_2$CH$_2$C<u>H$_3$</u>), 1.15, 1.25 (each 3H, s), 2.50 (2H, t, J=8 Hz, -SC<u>H$_2$</u>-CH$_2$-CH$_3$), 3.68 (1H, t, J=8 Hz, C-1-H), 4.42, 4.73 (each 1H, AB type, J=10 Hz, C-20-H$_2$), and 5.42 br (1H, s, 1/2 W=3.5 Hz, C-14-H). Compound (**5**): colorless needles, mp 189—190° (decomp.). *Anal.* Calcd. for $C_{24}H_{38}O_6S$: C, 63.40; H, 8.42; mol. wt., 454.239. Found: C, 63.12; H, 8.70, Mass Spectrum *m/e*: 454.238 (M$^+$). IR ν_{max} cm^{-1}: 3250 and 1715. NMR δ: 0.82 (3H, triplet like, J=7 Hz, -S-CH$_2$-CH$_2$-CH$_2$-C<u>H$_3$</u>), 1.13, 1.25 (each 3H, s), 3.68 (1H, t, J=7 Hz, C-1-H), 4.15 (1H, m, C-6-H, in d_5-pyr.–D$_2$O d, J=6 Hz), 4.39, 4.73 (each 1H, AB type, J=10.5 Hz, C-20-H$_2$), and 5.42 br (1H, s, 1/2 W=2 Hz, C-14-H). Compound (**17c**): colorless needles, mp 194.5—196° (decomp.). *Anal.* Calcd. for $C_{24}H_{38}O_6S$: C, 63.40; H, 8.42; mol. wt., 454.239. Found: C, 63.40; H, 8.72, Mass Spectrum *m/e*: 454.240 (M$^+$). IR ν_{max} cm^{-1}: 3350 and 1710. NMR δ: 0.92 (3H, t, J=7 Hz, S-CH(CH$_3$)CH$_2$C<u>H$_3$</u>), 1.13 (3H, s), 1.22, 1.23 (3H, d, J=6.5 Hz, -S-CH(—C<u>H$_3$</u>)CH$_2$-CH$_3$), 1.25 (3H, s), 4.16 (1H, dd, J=10.5 Hz, 6 Hz, C-6-H), 4.38, 4.72 (each 1H, AB type, J=10 Hz, C-20-H$_2$), 5.39 br (1H, s, 1/2 W=2 Hz, C-14-H), and 6.42 (1H, d, J=10.5 Hz, C-6-OH, disappeared with D$_2$O).

(iv) Reduction of Compound (**5**) with Raney Ni: To a solution of **5** (30 mg) in acetone (3 ml) was added 1 ml of ethanol solution of Raney-Ni W-2 (*ca.* 600 mg), and the mixture was refluxed for 12 hr. Filtration from the catalyst and evaporation of the solvent *in vacuo* afforded a dark oily residue, which was eluted through a silica gel short column with CH_2Cl_2 to yield a fine needles (14 mg) (from MeOH–ether). This compound was identified with the authentic sample (**4**)[4] (mmp, IR, and TLC).

(v) Conversion of Compound (**5**) into Oridonin (**1**): Compound (**5**) (50 mg) was dissolved in tetrahydrofuran (4 ml) and then a solution of *m*-chloroperbenzoic acid (49.4 mg, 2.6 mol equiv.) in CH_2Cl_2 (1 ml) was dropped. The mixture was stirred in ice-cooling bath for 2 hr and allowed to be evaporated *in vacuo* to give a solid residue, which was poured into cold Na_2CO_3 solution and extracted with EtOAc. Usual work-up afforded colorless needles (35 mg). These crystals were chromatographed on silica gel with $CHCl_3$ to yield oridonin (17 mg) identified with the authentic sample (**1**)[4] (mmp, IR, and TLC).

(vi) Treatment of Oridonin (**1**) with L-Cysteine: Oridonin (100 mg) was dissolved in EtOH (5 ml) and a solution of L-cysteine (37 mg, 1.1 mol equiv.) in pH 7.2—7.3 potassium phosphate buffer (5 ml) was dropped with stirring. After 3 min at room temperature, a white precipitation came out. The reaction medium was further stirred at room temperature for 5 min and subjected to filtration to give a white solid, which was repeatedly washed with excess water, excess ethanol, and excess ether to yield a fine needles **17d** (127 mg). mp 203—205° (decomp.). *Anal.* Calcd. for $C_{23}H_{35}O_8NS\cdot 2H_2O$: C, 52.96; H, 7.54; N, 2.69. Found: C, 53.08; H, 7.74; N, 2.84. IR ν_{max} cm^{-1}: 3355, 3175, 1710, and 1620. TLC [Cellulose F, H_2O–acetone (1: 4)] showed one spot (ninhydrin reagent, pinky spot); *Rf* 0.48 (*cf.* 0.59 for L-cysteine). TLC [Kiesel gel 60 F_{254}, CH_2Cl_2–acetone (2: 3)] showed one spot (Ceric sulfate reagent and combustion treatment, black-brown spot); *Rf* 0.00 (*cf.* 0.42 for oridonin).

(vii) Reduction of **17d** with Raney Ni: To a suspension of **17d** (38 mg) in acetone (10 ml) was added Raney-Ni W-2 (*ca.* 600 mg) in EtOH (1 ml). The mixture was refluxed for 8 hr and then filtrated. The filtrate was evaporated off *in vacuo* to give a dark gum, which was purified by column chromatography on silica gel using $CHCl_3$ to yield fine needles (15 mg) (from MeOH–Ether). The mp, IR, and TLC data were identical with those of the authentic sample (**4**)[4].

(viii) Treatment of Oridonin (**1**) with L-Lysine: To a suspension of oridonin (50 mg) in EtOH (3 ml) was dropwise added a solution of L-lysine (22 mg, 1.1 mol equiv.) in pH 7.2—7.3 potassium phosphate buffer (3 ml). The mixture was stirred at room temperature for 5 hr and then extracted with excess EtOAc. The extract was treated as usual to recover oridonin (**1**) (26 mg).

(ix) Treatment of Oridonin (**1**) with L-Serine: The similar treatment of oridonin (50 mg) with L-serine (16 mg, 1.1 mol equiv.) as described in (viii) resulted in recovery of oridonin (35 mg).

(x) Treatment of Enmein (**8**) with Butane Thiol: To a solution of enmein (200 mg) in DMF (5 ml) was dropped excess butane thiol. The mixture was allowed to stir at room temperature for 12 hr. The usual work-up of the reaction mixture gave adduct (**18**) as a prism, mp 169—170° (decomp.) (168 mg) (from MeOH). *Anal.* Calcd. for $C_{24}H_{36}O_6S$: C, 63.68; H, 8.01; mol. wt., 452.223. Found: C, 63.39; H, 8.31, Mass Spectrum *m/e*: 452.223 (M$^+$). IR ν_{max} cm^{-1}: 3450, 3270, 1757, and 1723. NMR δ: 0.80 (3H, t like, J=6.5 Hz, -S-CH$_2$-CH$_2$CH$_2$C<u>H$_3$</u>), 1.03, 1.15 (each 3H, s), 3.80 (1H, m, 1/2 W=4 Hz, C-3-H), 4.34, 4.49 (2H, AB type, J=8.5 Hz, C-20-H$_2$), 4.80 (1H, m, OH, disappeared with D$_2$O), 5.37 (1H, dd, J=11 Hz, 6.5 Hz, C-1-H), 5.85 (1H, C-6-H), and 6.58 (1H, m, OH, disappeared with D$_2$O).

(xi) Reduction of Compound (**18**) with Raney-Ni: To a solution of **18** (30 mg) in acetone (3 ml) was added a suspension of Raney-Ni W-2 (*ca.* 600 mg) in EtOH (1 ml). The mixture was refluxed for 8 hr, and the usual work-up gave a white needles **11** (14 mg), whose physical data (mp, IR, and TLC) were identical with those of the authentic dihydroenmein.

Results

1. Antitumor Activity

The experimental results are shown in Table I and Fig. 1.

Thus, oridonin (**1**) and lasiokaurin (**2**) showed a fairly high activity. Enmein (**8**), enmein-3-acetate (**9**), compound (**10**) and oridonin 14-deoxy-derivative (**3**) showed activity at the higher dose than that of oridonin. Oridonin dihydro-derivative (**4**), butane thiol adduct (**5**), dihydro-enmein (**11**) and trichokaurin (**6**), however, did not show any activity.

The fact suggests that the important active center is the α-methylene-cyclopentanone function.

Table I. Antitumor Activity of the *Isodon* Diterpenoids and the Related Compounds against Ehrlich Ascites Carcinoma in Mice

Series	Compound	Dose (mg/kg)	Change of body weight(g)	Number of survival	M.S.D.[a] (day)	I.L.S.[b] (%)
I	**1**[c,d]	10	+1.8	4/7	33.8	115
	2[c,d]	10	+2.1	3/7	34.1	117
	4[d]	10	+5.6	0/7	17.1	9
	10[d]	10	+4.9	0/7	16.4	4
	6[d]	10	+5.8	0/7	18.3	17
	control	—	+6.9	0/8	15.7	—
II	**5**[e]	10	+7.0	0/8	19.5	7
	3[e]	10	+1.6	0/8	20.6	13
	1[e]	5	+5.1	0/8	18.9	3
		10	+1.2	4/8	31.3	71
		15	+0.2	5/8	33.8	85
		20	−2.1	toxic	—	—
	control	—	+7.3	0/8	18.3	—
III	**1**[d]	10	+1.3	4/7	34.0	95
	7[d,f]	10	+6.6	0/7	18.0	2
	control	—	+6.9	0/7	17.7	—
IV	**8**[c,e]	10	+1.2	1/8	24.1	39
	control	—	+7.8	0/8	17.3	—
V	**8**[e]	25	+0.8	2/8	28.0	66
	control	—	+6.9	0/8	16.8	—
VI	**8**[e]	20	+2.5	2/8	27.9	55
		40	−0.6	4/8	33.3	86
	1[d]	10	+2.0	3/7	31.2	74
		20	−1.8	toxic	—	—
	3[d]	10	+2.8	0/8	23.1	29
		20	+3.2	1/8	28.9	61
	4[d]	20	+5.7	0/8	19.3	8
		40	+4.5	0/8	16.8	−6
	10[d]	20	+2.7	1/8	19.0	6
		40	+2.7	3/8	29.6	65
	control	—	+7.3	0/8	17.9	—
VII	**1**[d]	5	+5.1	2/10	20.5	19
		10	+4.1	4/10	29.4	71
		15	+0.3	4/10	30.6	78
	8[e]	20	+3.0	3/10	25.7	49
		40	+1.7	3/10	24.4	48
		80	+0.8	toxic	—	—
	9[e]	40	+2.1	4/10	26.8	56
		80	+1.8	3/10	23.8	38
		160	−2.5	toxic	—	—
	11[e]	40	+5.0	0/10	16.2	−5
		80	+6.5	1/10	18.4	7
	control	—	+9.0	0/10	17.2	—

a) M.S.D.: means of survival days
b) I.L.S.: increase of life span
c) LD_{50} values oridonin: 37.5 mg/kg; lasiokaurin and enmein: >80 mg/kg
d) dissolved in water-ethanol (4: 1)
e) dissolved in water-ethanol-Tween 80 (40: 9: 1)
f) Testing for higher dose was not carried out, because of its limited availability and less solubility.

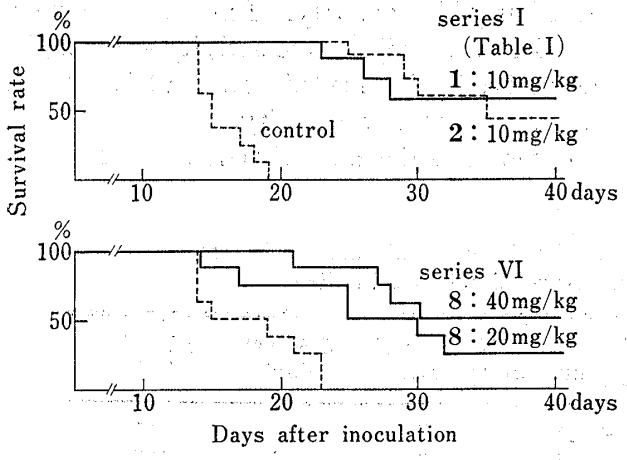

Fig. 1. Effect of Oridonin (1), Lasiokaurin (2), and Enmein (8) on Ehrlich Ascites Carcinoma in Mice

2. Antibacterial Activity

The results of the screening test of oridonin (1) for 19 kinds of bacteria are shown in Table II. It indicated a moderate activity specifically for gram-positive bacteria.

Subsequently, the antibacterial test of lasiokaurin (2), compound (3), enmein (8), enmein-3-acetate (9), oridonin derivatives (4) and (5), trichokaurin (6), and dihydroenmein (11) was carried out. The results are shown in Table III. Thus, compounds (2, 3, 8, and 9) showed activity against gram-positive bacteria, while compounds (4, 5, 6, and 11) did not indicate any activity. The antibacterial activity of oridonin (1), lasiokaurin (2), and compound (3) was higher than that of enmein (8) and enmein-3-acetate (9).

Table II. Antibacterial Activity of Oridonin[a]

Microorganism		M.I.C.[b] value (μg/ml)
Gram-positive bacteria	*Staphylococcus aureus* 209-P JC	31.25
	Staphylococcus aureus Smith	31.25
	Staphylococcus aureus Terajima	31.25—15.6
	Staphylococcus aureus Newmann	31.25
	Staphylococcus aureus E-46	31.25
	Staphylococcus aureus No. 80	31.25
	Staphylococcus epidermidis	31.25
	Sarcina lutea ATCC-9341	15.6 — 7.8
	Bacillus subtilis ATCC-6633	31.25
	Bacillus anthracis	31.25
Gram-negative bacteria	*Escherichia coli* NIH JC-2	500
	Enterobacter aerogenes	500
	Salmonella typhi T-287	250
	Salmonella paratyphi A	250
	Klebsiell pneumoniae	62.5
	Shigella flexneri 2a EW-10	500
	Shigella sonnei EW-33	500
	Proteus vulgaris OX-19	125
	Pseudomonas aeruginosa	500

a) Solution of oridonin (5000 μg/ml) in EtOH–H_2O (1: 9) was prepared and used under dilution as shown.
b) minimum inhibitory concentration

3. Biomimetic Reactions

As described in the experimental part, the reactions *in vitro* of oridonin with nucleic acids, with thiols, and with amino acids were carried out. The results are shown in Table IV. Oridonin (1) easily reacted only with thiols and L-cysteine to yield adducts (5 and **17a—d**).

Discussion

1. Antitumor Activity

Previously, it was reported by Arai, *et al.*[10] that the crude crystalline substance obtained from *Isodon japonicus* and *I. trichocarpus* indicated an antitumor activity. Subsequently,

10) T. Arai, Y. Koyama, T. Morita, and H. Kaji, *Chemotherapy*, 9, 403, 404 (1961).

Table III. Antibacterial Effect of *Isodon* Diterpenoids

Compound	Microorganism			
	Staphylococcus aureus 209-P JC	*Sarcina lutea* ATCC-9341	*Escherichia coli* NIH JC-2	*Proteus vulgaris* OX-19
1[b]	31.25[a]	15.6	>250	125
2[b]	15.6	7.8	>250	>250
3[c]	50	6.25	>50	>50
8[d]	125	31.25	>250	31.25
9[d]	125	62.5	>250	>250
4[b]	250	250	>250	>250
5[c]	250	62.5	>250	250
6[c]	>250	>250	>250	250
11[b]	>250	>250	>250	>250

a) M.I.C. (minimum inhibitory concentration) value (μg/ml)
b) dissolved in acetone and adjusted to 2500 μg/ml in acetone–H_2O (1: 9)
c) dissolved in ethanol and adjusted to 2500 μg/ml in EtOH–H_2O (1: 9)
d) dissolved in DMF and adjusted to 2500 μg/ml in DMF–H_2O (1: 9)

Table IV. Biomimetic Reactions of Oridonin with Nucleic Acid Model Compounds and with Enzyme Model Compounds

Model compound	Solvent system	Product and yield[a] (%)	
Adenosine (**15**)	pH 7.2—7.3 potassium phosphate buffer solution–EtOH (1: 1)	recovery of oridonin	
Cytidine (**16**)	pH 7.2—7.3 potassium phosphate buffer solution–EtOH (1: 1)	recovery of oridonin	
Ethane thiol	DMF	**17a**	87
Propane thiol	DMF	**17b**	100
Butane thiol	DMF	**5**	77
	EtOH	**5**	43
sec-Butane thiol	DMF	**17c**	68
L-Cysteine	pH 7.2—7.3 potassium phosphate buffer solution–EtOH (1: 1)	**17d**	100
L-Lysine	pH 7.2—7.3 potassium phosphate buffer solution–EtOH (1: 1)	recovery of oridonin	
L-Serine	pH 7.2—7.3 potassium phosphate buffer solution–EtOH (1: 1)	recovery of oridonin	

a) Isolating yield calculated on the basis of oridonin

it was shown by the same group[11] that purified enmein and its diacetate indicated an antitumor activity, while dihydroenmein indicated no activity, and the exocyclic methylene group attached to five-membered cyclic ketone in enmein was suggested to be essential to this biological activity, although only the partial structure had been proposed for enmein in that time. Our present results based on the detailed investigations on compounds of much wider range not only support the foregoing suggestion, but lead to a conclusion that α-methylene cyclopentanone system is really the active center, that is, an essential structural factor required for activity.

Many antitumor sesquiterpenoids possessing the α-methylene-γ-lactone system as an active center in the molecule have been reported,[12] but the antitumor natural products of

11) T. Arai, Y. Koyama, T. Suenaga, and T. Morita, *J. Antibiotics Ser. A*, **16**, 132 (1963), and references cited therein.
12) *a*) S.M. Kupchan, *Pure and Applied Chemistry*, **21**, 227 (1970); *b*) R.W. Doskotch, C.D. Hufford, and F.S. El-Feraly, *J. Org. Chem.*, **37**, 2740 (1972); *c*) S.M. Kupchan, V.H. Davies, T. Fujita, R. Cox, R.J. Restivo, and R.F. Bryan, *J. Org. Chem.*, **38**, 1853 (1973) and references cited therein.

α-methylene-cyclopentanone system had not been known except sarcomycin (**12**).[13,14] Now, a new group of kaurene-related compounds containing an active center which is the same as that of sarcomycin (**12**) has been found.

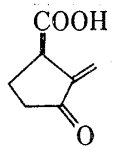

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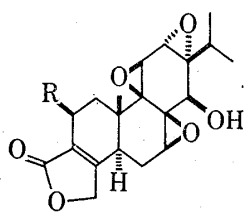
Table V. The C-17 Protons Chemical Shifts of Oridonin (**1**), Enmein (**8**), Enmein-3-acetate (**9**) and Compound (**10**) in Their NMR Spectra[a]

Compound	Chemical shifts of methylene protons at C-17 (δ_{ppm})	
Oridonin (**1**)	5.53	6.31
Enmein (**8**)	5.43	5.98
Enmein-3-acetate (**9**)	5.33	5.99
Compound (**10**)	5.40	6.12

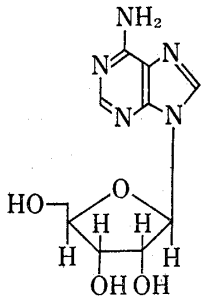
a) taken in d_5-pyridine

Intensity of the activity and its relation with structure are subsequently discussed. Since oridonin (**1**) and lasiokaurin (**2**) showed almost the same intensity of the activity, the hydroxy group at C-1 has no direct effect for the activity. The toxicity of lasiokaurin, however, is lower than that of oridonin, which is perhaps due to the difference of their solubility. An important role of the hydroxy group at C-6 is clarified by the following discussion. The activities of enmein (**8**), enmein-3-acetate (**9**), and compound (**10**) are one fourth or lesser when compared with that of oridonin (**1**). The presence of a hydrogen-bonding between the carbonyl group at C-15 and the hydroxy group at C-6 in the oridonin molecule has been confirmed by its IR, UV, and NMR spectra.[4] Hence, the C-17 atom is polarized to δ^+ and its reactivity with nucleophile must be increased. In fact, it is supported by the lower chemical shifts of C-17 methylene protons of oridonin (**1**) than those of enmein (**8**), enmein-3-acetate (**9**), and compound (**10**) that the electron density at C-17 of oridonin (**1**) is lower than those of **8**, **9**, and **10** (see Table V).

Kupchan, *et al.*[15] described that the hydrogen-bonding between 14β-hydroxy group and 9,11-oxirane in each molecule of the antitumor diterpenoids, triptolide (**13**) and tripdiolide (**14**) facilitated the nucleophilic attack of propane thiol to the C-9 atom see (Fig. 2). The compounds which had no such a hydrogen-bonding, for instance, the 14α-hydroxy isomer and the 14-oxo derivative, did not show the antitumor activity.



13 : R=H
14 : R=OH



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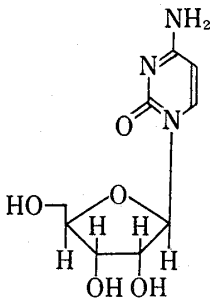

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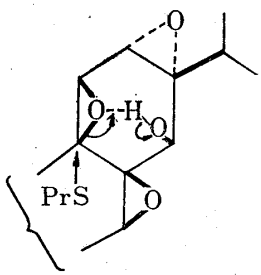

Fig. 2

13) H. Umezawa, T. Yamamoto, T. Takeuchi, T. Osato, Y. Okami, S. Yamaoka, T. Okuda, K. Nitta, K. Yagishita, R. Utahara, and S. Umezawa, *Antibiot. Chemotherapy*, **4**, 514 (1954).
14) R.K. Hill, P.J. Foley, Jr., and L.A. Gardella, *J. Org. Chem.*, **32**, 2330 (1967).
15) S.M. Kupchan and R.M. Schubert, *Science*, **185**, 791 (1975).

The activity of oridonin 14-deoxy derivative (**3**) was lower than that of oridonin (**1**) and almost the same as that of enmein (**8**), enmein-3-acetate (**9**), and compound (**10**). Thus, the 14β-hydroxy group of oridonin (**1**) must play such an important role for increasing the activity, for instance, as the binding site to a special biologically important substance in a tumor cell. The hydroxy group at C-7 is located in close to the active center and in parallel to the C-14 hydroxy group. Hence it may act some role cooperating with the C-14 hydroxy group.

In conclusion, the appearance of the antitumor activity in oridonin (**1**) and lasiokaurin (**2**) is attributed to the satisfaction of the following necessary conditions.

1) An α-methylene-cyclopentanone system is present in the molecule.

2) Some hydroxy group(s) is located in the suitable position for contact with and binding to a special enzyme carrying a nucleophile in a tumor cell.

3) A hydrogen-bonding is present for increasing the electrophilicity of the C-17 atom.

2. Antibacterial Activity

There have been reported on the antibacterial activity of *Isodon* diterpenoids by Arai, *et al.*[11] and Kubota, *et al.*[16] We have also independently investigated their antibacterial activity in relation to antitumor activity. The results are as described above. It was also shown that the active center was the α-methylene-cyclopentanone function and the hydrogen-bonding between the C-6 hydroxy group and the C-15 carbonyl group acted an important role for increasing the activity.

3. Activity of Sarcomycin

Sarcomycin (**12**) is an antibiotic[13] produced by *Streptomyces erythrochromogens* Strain W-115-C and shows antitumor and antibacterial activities. It is an α-methylene-cyclopentanone derivative carrying carboxy group as an only substituent. The results of the screening tests[17,19] on many related synthetic derivatives are consistent with the structure-activity correlation described in this paper. Hooper, *et al.*[18] had reported that "hydrogenation yields an optically active 2-methyl-3-oxocyclopentane carboxylic acid which is an active antitumor agent but devoid of antibacterial activity," but later Capto, *et al.*[19] published the antitumor inactivity of "dihydrosarcomycin."

4. Biomimetic Reactions

Adenosine (**15**) and cytidine (**16**), as the nucleic acid model compounds, and four kinds of alkane thiols, L-cysteine, L-lysine, and L-serine, as the enzyme model compounds, were allowed to react with oridonin (**1**) and enmein (**8**). The reaction conditions and the results are shown in the experimental part and Table V, respectively.

Oridonin did not react with adenosine (**15**) and cytidine (**16**) under the conditions shown, but was recovered. The reactions of oridonin with the SH enzyme model compounds easily proceeded under mild conditions to give alkane thiol adducts (**5**, **17a**, **17b**, and **17c**). The reaction with L-cysteine also took place smoothly to yield adduct (**17d**) quantitatively.

The chemical structures of these products were determined by the spectroscopic evidence and also the reverse reaction of compound (**5**) into oridonin, and the stereochemistry of the C-16 atom was chemically confirmed (see Experimental). These processes are shown in Chart 1.

Enmein (**8**) also easily gave adduct (**18**) by the reaction with butane thiol. The stereochemistry was chemically determined (see Experimental). The processes are shown in Chart 2.

16) *a*) I. Kubo, T. Kamikawa, and T. Kubota, *Tetrahedron*, **30**, 615 (1974); *b*) I. Kubo, M. Taniguchi, Y. Satomura, and T. Kubota, *Agr. Biol. Chem.*, **38**, 1261 (1974).

17) M. Kinoshita, S. Nakada, and S. Umezawa, *Bull. Chem. Soc. Japan*, **36**, 860 (1963) and references cited therein.

18) I.R. Hooper, L.C. Cheney, M.J. Cron, O.B. Fardig, D.A. Johnson, D.L. Johnson, F.M. Palermiti, H. Schmitz, and W.B. Wheatley, *Antibiot. Chemotherapy*, **5**, 585 (1955).

19) A. Caputo, M. Brunori, and R. Giuliano, *Cancer Research*, **21**, 1499 (1961).

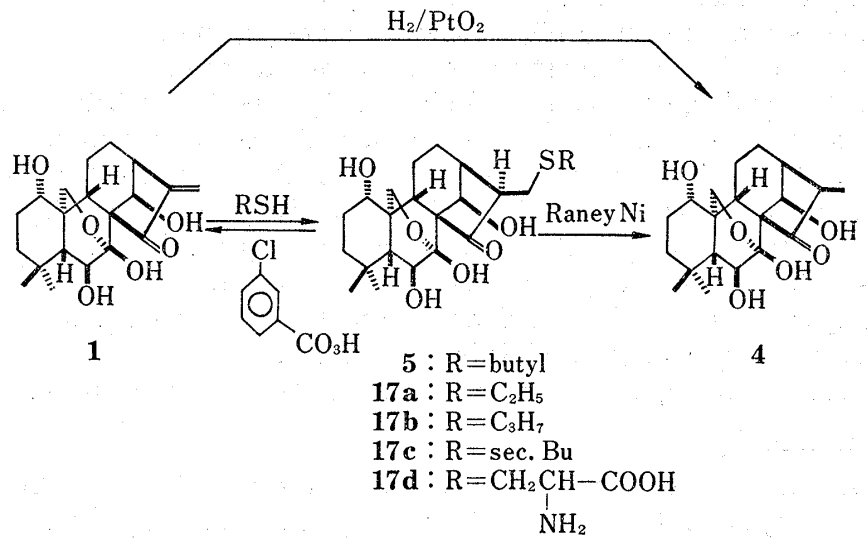

Chart 1

Kubota, *et al.*[16b] reported in their recent communication the easy formation of addition products in the reactions of L-cysteine with oridonin (**1**), enmein (**8**), and umbrosin A. The biomimetic reactions of α-methylene-γ-butyrolactones with L-cysteine and alkyl thiols have been reported by van Tamelen, *et al.*[20] and Kupchan, *et al.*[12a,21] The reactions of L-cysteine with some unsaturated lactones, methyl vinyl ketone, and phenyl vinyl ketone have been reported by Black.[22]

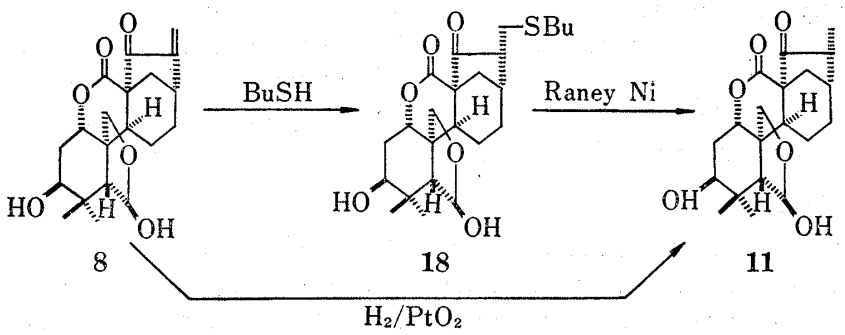

Chart 2

Finally the reactions of oridonin with L-lysine and L-serine did not take place, but the material was recovered.

Kupchan, *et al.*[23] confirmed that the tumor inhibitors, *e.g.* taxodone, taxodione(diterpenes), vernolepin and euparotin acetate (sesquiterpenes), inhibit phosphofructokinase and the inhibition results from their reaction with the SH groups of this enzyme. They[24] also reported a similar inactivation of glycogen synthase by vernolepin.

In conclusion, our biomimetic reactions support the physiologically active center of *Isodon* diterpenoids to be the α-methylene-cyclopentanone function and the hypothesis that the appearance of the physiological activity may be due to the *in vivo* deactivation of the SH enzymes by these diterpenoids. A hypothetical illustration is presented in Fig. 3.

20) E.E. van Tamelen and S. Rosenberg, *J. Am. Chem. Soc.*, **77**, 4683 (1955).
21) S.M. Kupchan, M.A. Eakin, and A.M. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).
22) D.K. Black, *J. Chem. Soc.*, (*C*), **1966**, 1123.
23) R.L. Hanson, H.A. Lardy, and S.M. Kupchan, *Science*, **168**, 378 (1970).
24) C.H. Smith, J. Larner, A.M. Thomas, and S.M. Kupchan, *Biochim. Biophys. Acta*, **276**, 94 (1972).

Such easy Michael type reactions with thiols without any catalysts as described above are reasonably explainable as the reactions of "soft" acids with "soft" bases.[25] Thus, the selective reactions of α-methylene-cyclopentanone system with only SH-group among many nucleophilic groups (*e.q.* –OH, $-NH_2$, $-COO^-$ *etc.*) in the enzyme can be rationalized by the "Hard Soft Acids Bases" principle,[25] and experimentally supported by our biomimetic reactions.

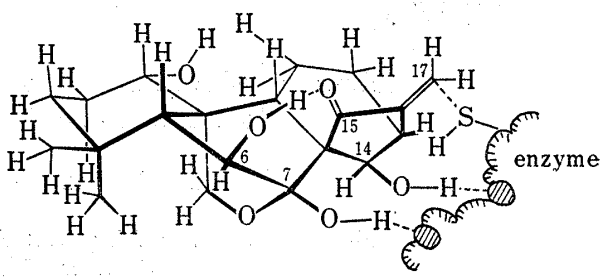

Fig. 3. Hypothetical Transition State between Oridonin and a Specific Enzyme in a Cancer Cell

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25) *a*) P.G. Pearson, *J. Am. Chem. Soc.*, **85**, 3533 (1963); *b*) Review article: T. -L. Ho, *Chem. Rev.*, **75**, 1 (1975).