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Antitumor Activity of Acylated Oridonin[1)]

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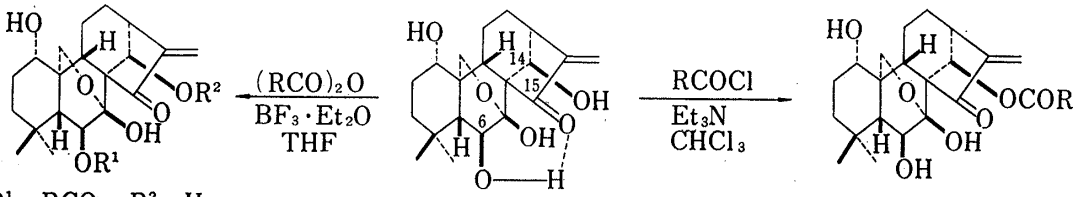
14-*O*-Acyl derivatives of oridonin were prepared and tested for antitumor activity against Ehrlich ascites carcinoma cells in the mouse. In a series of derivatives **7**—**12**, the activity increased with increase of the acyl carbon chain length. The 14-*O*-benzoyl derivative **5** was shown to have the same order of activity as oridonin. 6-*O*-Acyl derivatives were also prepared and tested. In a series of derivatives **13**—**15**, no activity was observed at the doses of 5 mg/kg and 10 mg/kg in mice, at which doses oridonin did exhibit activity. Thus, the importance of the ester side chain and the hydrogen-bonding for antitumor activity was demonstrated in oridonin and its derivatives.

Keywords——Oridonin; antitumor activity; acylation; enhancement factor; ester moiety; hydrogen-bonding; structure–activity relationship

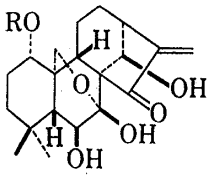
In the previous paper, we reported on the antitumor activity of the *Rabdosia* diterpenoids oridonin (**1**), lasiokaurin (**2**), enmein (**3**), enmein-3-acetate (**4**), and related compounds against Ehrlich ascites carcinoma inoculated into mice,[1b,2)] and showed the active center to be the



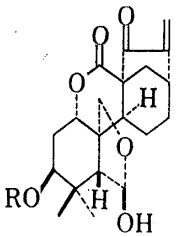
R^1=RCO, R^2=H
R^1=R^2=RCO
13—**15**

1

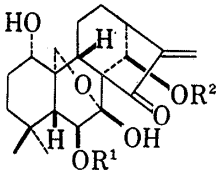
5—**12**



1: R=H
2: R=Ac



3: R=H
4: R=Ac



5: R^1=H, R^2=COPh
6: R^1=H, R^2=COCH=CHPh
7: R^1=H, R^2=COC_3H_7
8: R^1=H, R^2=COC_5H_{11}
9: R^1=H, R^2=COC_9H_{19}
10: R^1=H, R^2=$COC_{11}H_{23}$
11: R^1=H, R^2=$COC_{13}H_{27}$
12: R^1=H, R^2=$COC_{15}H_{31}$
13: R^1=Ac, R^2=H
14: R^1=R^2=Ac
15: R^1=$COC_{11}H_{23}$, R^2=H

Chart 1

α-methylenecylopentanone system in these molecules.[1b,2,3] The stronger activity of oridonin (**1**) than of enmein (**3**) was suggested to be attributable to the hydrogen-bonding between the C-6 hydroxyl group and the C-15 carbonyl group in the oridonin molecule.[1b,2]

We report here some interesting new findings on the following two subjects: (1) the synthesis of C-14-*O*-acylated derivatives of oridonin (**1**) and testing of their antitumor activity; (2) confirmation of the proposed relationship between the antitumor activity and the foregoing hydrogen-bonding in the oridonin molecule.

The synthesis of several acylated oridonin derivatives, **5**—**15**, was carried out by two methods[4] as shown in Chart 1. All of the synthetic samples were subjected to testing for antitumor activity.

Experimental

Melting points were determined with a Yanagimoto microapparatus. Infrared (IR) spectra were measured on a Jasco A-202 spectrophotometer. ^{1}H-Nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were taken with Varian T-60 and JEOL JM-FX100 instruments in $CDCl_3$ and d_5-pyridine; signals are given as ppm from TMS as an internal standard. Mass spectra were determined on a JEOL JMS-O1SG double-focusing mass spectrometer. Extracts were dried over anhydrous Na_2SO_4. A mixture of kieselgel 60 (70—230 mesh) (Merck) and silicic acid (Mallinckrodt) was used for column chromatography.

1. Synthesis of Test Samples——A typical procedure for the synthesis of 14-*O*-acyl derivatives of oridonin (**1**) was as follows. Triethylamine (2.272 g, 4.5 mol. equiv.) was added dropwise at 0°C to a suspension of oridonin (**1**) (1.820 g) in benzoyl chloride (3.16 g, 4.5 mol. equiv.) and $CHCl_3$ (50 ml) with stirring. The mixture was stirred at room temperature for 45 min, with monitoring of the reaction progress by thin layer chromatography. During the reaction the suspension gradually changed to a solution. The solution was repeatedly washed with cold 5% Na_2CO_3 aqueous solution and then with water. The $CHCl_3$ layer was dried and concentrated *in vacuo* to give crude crystals which were recrystallized from $CHCl_3$–ether to afford the 14-benzoate **5** (1.801 g) in 76.9% yield.

Physical Data for 14-*O*-Acyl Derivatives of Oridonin: 14-*O*-Benzoyl Derivative **5**: Colorless needles (from $CHCl_3$–ether), mp 244—245°C. *Anal.* Calcd for $C_{27}H_{32}O_7 \cdot 1/2H_2O$: C, 67.92; H, 6.91; M, 468. Found: C, 68.29; H, 6.95; M$^+$ *m/e*: 468. IR ν_{max}^{KBr}: 3425, 3350, 1705, 1638, and 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.11 (6H, s, $CH_3 \times 2$), 3.27 (1H, d, J=10.0 Hz, C-13-H), 3.48 (1H, t, J=7.0 Hz, C-1-H), 3.75 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.06, 4.35 (each 1H, AB, J=10.0 Hz, C-20-H_2), 4.09 (1H, s, OH, disappeared with D_2O), 5.48, 6.05 (each 1H, s, C-17-H_2), 6.10 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O), 6.16 (1H, s, C-14-H), 7.30—7.95 (5H, m, aromatic protons).

14-*O*-Cinnamoyl Derivative **6**: Yield 98.1%, colorless plates (from $CHCl_3$–ether), mp 270—271°C. *Anal.* Calcd for $C_{29}H_{34}O_7$: C, 70.42; H, 6.93; M, 494. Found: C, 70.03; H, 6.97; M$^+$ *m/e*: 494. IR ν_{max}^{KBr}: 3425, 3350, 1705, 1635, and 1500 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.13 (6H, s, $CH_3 \times 2$), 3.27 (1H, d, J=9.5 Hz, C-13-H), 3.51 (1H, t, J=7.0 Hz, C-1-H), 3.79 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.09, 4.33 (each 1H, AB, J=11.0 Hz, C-20-H_2), 5.50, 5.96 (each 1H, s, C-17-H_2), 6.13 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O), 6.18 (1H, s, C-14-H), 6.32 (1H, d, J=16.0 Hz, $-\overset{O}{\overset{\|}{C}}-C\underline{H}=CH-Ph$), 7.30—7.52 (5H, m, aromatic protons), 7.64 (1H, d, J=16.0 Hz, $-\overset{O}{\overset{\|}{C}}-CH=C\underline{H}-Ph$).

14-*O*-Butanoyl Derivative **7**: Yield 72.9%, colorless oil. *Anal.* Calcd for $C_{24}H_{34}O_7$: M, 330.183. Found: M$^+$ *m/e*: 330.180. IR $\nu_{max}^{CHCl_3}$: 3540, 3360, 1740, 1715, and 1638 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7.5 Hz, $-CH_2C\underline{H}_3$), 1.12 (6H, s, $CH_3 \times 2$), 3.20 (1H, d, J=9.0 Hz, C-13-H), 3.55 (1H, t, J=8.0 Hz, C-1-H), 3.78 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.09, 4.30 (each 1H, AB, J=9.0 Hz, C-20-H_2), 5.48, 5.82 (each 1H, s, C-17-H_2), 6.12 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O), 6.13 (1H, s, C-14-H).

14-*O*-Hexanoyl Derivative **8**: Yield 40.7%, colorless oil. *Anal.* Calcd for $C_{26}H_{38}O_7$: M, 462.262. Found: M$^+$ *m/e*: 462.263. IR $\nu_{max}^{CHCl_3}$: 3525, 3350, 1740, 1707, and 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7.0 Hz, $-CH_2C\underline{H}_3$), 1.12 (6H, s, $CH_3 \times 2$), 3.19 (1H, d, J=9.0 Hz, C-13-H), 3.50 (1H, t, J=8.3 Hz, C-1-H), 3.77 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.06, 4.29 (each 1H, AB, J=10.3 Hz, C-20-H_2), 5.47, 5.80 (each 1H, s, C-17-H_2), 6.12 (1H, s, C-14-H), 6.16 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O).

14-*O*-Decanoyl Derivative **9**: Yield 67.5%, colorless oil. *Anal.* Calcd for $C_{30}H_{46}O_7$: M, 518.324. Found: M$^+$ *m/e*: 518.322. IR $\nu_{max}^{CHCl_3}$: 3540, 3355, 1740, 1710, and 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.5 Hz, $-CH_2C\underline{H}_3$), 1.11 (6H, s, $CH_3 \times 2$), 3.19 (1H, d, J=9.0 Hz, C-13-H), 3.50 (1H, t, J=8.0 Hz, C-1-H), 3.78 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.07, 4.28 (each 1H, AB, J=10.0 Hz, C-20-H_2), 5.46, 5.80 (each 1H, s, C-17-H_2), 6.12 (1H, s, C-14-H), 6.19 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O).

14-*O*-Dodecanoyl Derivative **10**: Yield 75.9%, colorless oil. *Anal.* Calcd for $C_{32}H_{50}O_7$: M, 546.355. Found: M$^+$ *m/e*: 546.353. IR $\nu_{max}^{CHCl_3}$: 3540, 3350, 1740, 1715, and 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, t,

J=7.0 Hz, $-CH_2C\underline{H}_3$), 1.12 (6H, s, $CH_3\times2$), 3.20 (1H, d, J=9.0 Hz, C-13-H), 3.50 (1H, t, J=8.0 Hz, C-1-H), 3.79 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.06, 4.29 (each 1H, AB, J=10.0 Hz, C-20-H_2), 5.47, 5.80 (each 1H, s, C-17-H_2), 6.12 (1H, s, C-14-H), 6.14 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O).

14-*O*-Tetradecanoyl Derivative **11**: Yield 71.2%, colorless oil. *Anal.* Calcd for $C_{34}H_{54}O_7$: M, 574. Found: M$^+$ *m/e*: 574. IR $\nu_{max}^{CHCl_3}$: 3530, 3355, 1740, 1710, and 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, t, J=6.0 Hz, $-CH_2C\underline{H}_3$), 1.12 (6H, s, $CH_3\times2$), 3.19 (1H, d, J=9.0 Hz, C-13-H), 3.51 (1H, t, J=8.0 Hz, C-1-H), 3.78 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.08, 4.29 (each 1H, AB, J=10.0 Hz, C-20-H_2), 5.47, 5.80 (each 1H, s, C-17-H_2), 6.13 (1H, s, C-14-H), 6.14 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O).

14-*O*-Hexadecanoyl Derivative **12**: Yield 59.5%, colorless plates (from MeOH), mp 78—80°C. *Anal.* Calcd for $C_{36}H_{58}O_7$: C, 71.72; H, 9.70; M, 602. Found: C, 71.54; H, 10.09; M+ *m/e*: 602. IR ν_{max}^{KBr}: 3440, 3360, 1730, 1718, and 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, t, J=7.0 Hz, $-CH_2C\underline{H}_3$), 1.13 (6H, s, $CH_3\times2$), 3.19 (1H, d, J=8.5 Hz, C-13-H), 3.51 (1H, t, J=8.0 Hz, C-1-H), 3.78 (1H, dd, J=11.0 Hz, 7.0 Hz, C-6-H), 4.08, 4.27 (each 1H, AB, J=13.0 Hz, C-20-H_2), 5.47, 5.80 (each 1H, s, C-17-H_2), 6.13 (1H, s, C-14-H), 6.15 (1H, d, J=11.0 Hz, C-6-OH, disappeared with D_2O).

Test samples **13**—**15** were synthesized by an improved acylation method which was reported in the preceding paper.[4]

2. Preparation of Samples for Assay——Oridonin (**1**) and its *O*-acyl derivatives **5**—**15** were each dissolved in 0.5% sodium carboxymethyl cellulose–ethanol (4: 1) and each solution was employed as a sample for testing antitumor activity in mice (see footnote to Table I).

3. Assay Method for Antitumor Activity——Ehrlich ascites carcinoma cells, 2×10^6 cells/mouse, were inoculated intraperitoneally into experimental animals (Slc: ddY male mice weighing 20±0.5 g) in groups of 9. From 24 h after the inoculation, each sample was injected into mice intraperitoneally once a day for 7 consecutive days. The mice were observed for 40 days and the mean survival time (days) was compared with that of the control.

Results

The results of the antitumor test are shown in Tables I and II. In a series of oridonin 14-*O*-acyl derivatives **7**—**12**, the activity increased with increase of the acyl carbon chain length. 14-*O*-Dodecanoyl (**10**), tetradecanoyl (**11**), and hexandecanoyl (**12**) derivatives were found to have stronger activity than oridonin (**1**), although **11** and **12** showed some toxicity. The benzoyl derivative **5** was shown to have the same order of activity as oridonin (**1**), while the cinnamoyl derivative **6** had no activity. The derivatives **8** and **9** were shown to have slightly stronger activity at a dose of 5 mg/kg in mice than oridonin (**1**) (see Table I). The 6-*O*-acyl derivatives **13**—**15** were shown to have no activity at doses of 5 mg/kg and 10 mg/kg in mice (see Table II).

Table I. Antitumor Activity of Oridonin (**1**) and Its 14-*O*-Acyl Derivatives against Ehrlich Ascites Carcinoma in Mice

Compound	Dose (mg/kg)	M.S.D.[a] (d)	Evaluation[b] *T/C* (%)	Activity-increasing effect *vs.* oridonin[c] (%)
5	2.5	16.7±0.7	118.4	6.4
	5	17.6±0.7	124.8	−0.6
	10	22.3±1.9	158.2	10.9
6	2.5	15.1±1.0	107.1	−3.8
	5	14.7±0.7	104.3	−16.9
	10	14.6±0.5	103.5	−27.4
7	2.5	16.3±0.5	115.6	3.8
	5	18.0±1.4	127.7	8.6
	10	16.6±0.6	117.7	−17.4
8	2.5	16.4±0.5	116.3	4.5
	5	18.7±0.6	132.6	5.6
	10	18.7±1.1	132.6	−7.0
9	2.5	16.0±0.6	113.5	1.9
	5	19.3±2.1	136.9	9.0
	10	19.1±1.9	135.5	−5.0

Compound	Dose (mg/kg)	M.S.D.[a] (d)	Evaluation[b] T/C (%)	Activity-increasing effect *vs.* oridonin[c] (%)
10	2.5	17.4±1.2	123.4	10.8
	5	20.1±0.9	142.6	13.6
	10	20.0±1.5	141.8	−0.5
11	2.5	17.6±1.3	124.8	12.1
	5	22.1±1.7	156.7	24.9
	10	22.8±2.0	161.7	13.4
12	2.5	17.3±2.0	122.7	10.2
	5	24.9±2.5	176.6	40.7
	10	26.8±1.3	190.1	33.3
1	2.5	15.7±0.5	111.3	—
	5	17.7±0.6	125.5	—
	10	20.1±1.9	142.6	—
Control	—	14.1±2.6	—	—

a) M.S.D.: mean survival days.
b) T/C (%)=(M.S.D. (treated)÷M.S.D. (control))×100.
c) [(T/C(14-*O*-acyl deriv)÷T/C(oridonin))×100]−100.

TABLE II. Antitumor Activity of Oridonin (**1**) and Its 6-Acyl Derivatives against Ehrlich Ascites Carcinoma in Mice

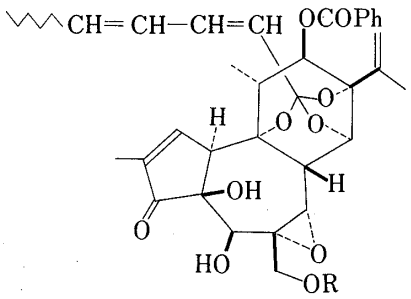
Compound	Dose (mg/kg)	M.S.D.[a] (d)	Evaluation[a] T/C (%)	Activity-increasing effect *vs.*oridonin[a] (%)
13	5	13.4±1.0	102.3	−24.7
	10	14.9±0.9	113.3	−35.5
14	5	14.0±0.7	106.9	−21.3
	10	14.3±0.8	109.2	−38.1
15	5	11.6±0.6	88.5	−34.8
	10	13.3±0.6	101.5	−42.4
1	5	17.8±1.2	135.9	—
	10	23.1±2.8	176.3	—
Control	—	13.1±0.9	—	—

a) See footnotes to Table I.

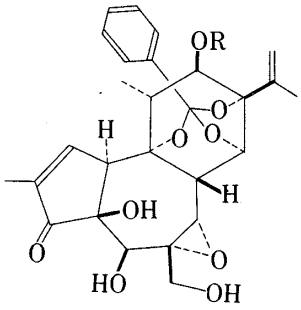
Discussion

In a review article,[5] we described the importance of "the ester side chain" and "hydrogen-bonding" for the antitumor activity of oridonin. The present findings that the esters with higher fatty acids, **10**—**12**, showed stronger activity that those with lower fatty acids, **7**—**9**, and also than oridonin (**1**) itself, and that there was an increase of the activity with increase of carbon chain length of the acyl groups are interesting; they suggest that the activity increases in proportion to the lipophilicity of the oridonin derivatives. Thus, it was strongly suggested that the ester side chain in oridonin 14-*O*-acyl derivatives may play a carrier role in the process(es) related to penetration into cells. Several examples in which an ester side chain enhances antitumor activity in natural products have been reported;[5] gnidilatidin-20-palmitate (**16**), gnididin (**17**), bruceantin (**18**), and maytanvaline (**19**) have stronger activity against P338 lymphocytic leukemia in mice than gnidilatidin (**20**) (no activity), 12-hydroxydaphnetoxin **21**) (no activity), bruceolide (**22**) (marginal activity), and maytansinol (**23**) (no activity), respectively.[6]

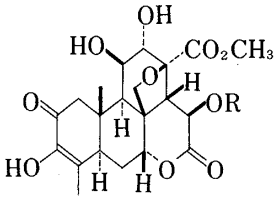
The results shown in Table II confirmed the correctness of our hypothesis of the importance of hydrogen-bonding in the oridonin molecule as a factor enhancing the antitumor



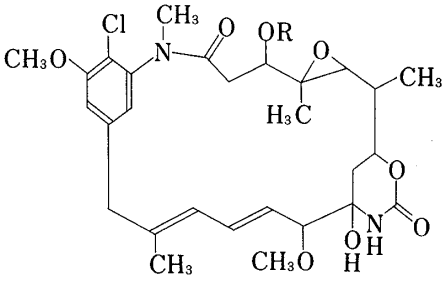
16: R=$COC_{15}H_{31}$
20: R=H



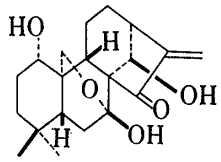
17: R=$COCH{=}^{t}CHCH{=}^{t}CH(CH_2)_4CH_3$
21: R=H



18: R=$COCH{=}^{t}CHCH(CH_3)_2$
22: R=H



19: R=$COCH(CH_3)NCOCH_2CH(CH_3)_2$
23: R=H



24

activity. Although comparison of the activity of compound **24** with that of oridonin (**1**) was not possible, because of the difficulty in deriving this derivative from oridonin (**1**), all of the C-6-*O*-acyl derivatives synthesized from oridonin (**1**) were shown to have no activity at the doses of 5 mg/kg and 10 mg/kg, at which doses oridonin did exhibit activity.

This hydrogen-bonding must result in δ^+ nature at the C-15 and C-17 atoms in oridonin (**1**); the ^{13}C-NMR spectra support this (see Table III).[7] The increased electrophilicity of the C-17 atom would then make the addition of *e.g.* the mercapto group of enzymes in the tumor cells easier. Thus, the ability of oridonin (**1**) to act as an alkylating agent is enhanced (see Figure 1).

TABLE III. The ^{13}C-NMR Chemical Shifts[a] of the C-15, C-16, and C-17 Atoms in Oridonin (**1**) and the 6-Acyl Derivatives **13** and **15**

	Oridonin (**1**) ppm	Oridonin-6-acyl derivative **13** ppm	**15** ppm	Values of $\Delta\delta$ (1−13) and $\Delta\delta$ (1−15) ppm	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.

The enhancement of antitumor activity by hydrogen-bonding has been reported in cucurbitacin B (**25**) and dehydroailanthinone (**26**).[6]

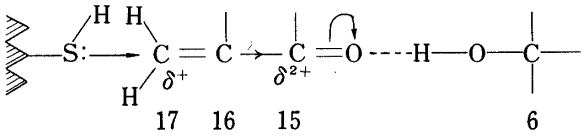
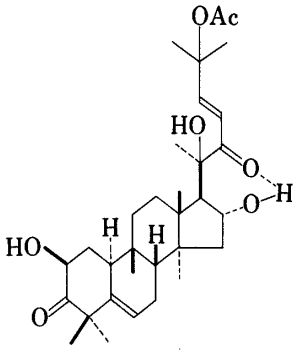
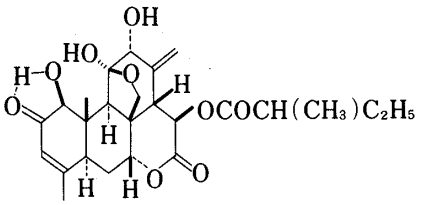


Fig. 1

Recently the interesting results were obtained that DNA synthesis was 74% inhibited by 10 μg/ml of oridonin (**1**) and 91% inhibited by 10 μg/ml of enmein (**3**) in a test system using cells of the ascitic form of Ehrlich carcinoma and that RNA and protein syntheses were somewhat less affected.[8] The details of these findings will be published elsewhere.



25



26

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References and Notes

1) Dedicated to Prof. C.H. Eugster, University of Zürich, on the occasion of his sixtieth birthday. This paper forms Part 46 of the series "Terpenoids" and Part II of "Biological and Physiological Activity" (Kyoto University). *a*) For Terpenoids Part 45, see: M. Node, M. Sai, and E. Fujita, *Phytochemistry*, **20**, 757 (1981); *b*) For Biological and Physiological Activity Part I, see: E. Fujita, Y. Nagao, K. Kaneko, S. Nakazawa, and H. Kuroda, *Chem. Pharm. Bull.*, **24**, 2118 (1976).
2) E. Fujita, Y. Nagao, M. Node, K. Kaneko, S. Nakazawa, and H. Kuroda, *Experientia*, **32**, 203 (1976).
3) Y. Nagao and E. Fujita, *Proc. Symp. Wakan-yaku*, **13**, 79 (1980).
4) Y. Nagao, E. Fujita, T. Kohno, and M. Yagi, the preceding paper.
5) E. Fujita and Y. Nagao, *Bioorg. Chem.*, **6**, 287 (1977).
6) See the references cited in ref. 5.
7) Cf. A.K. Bose and P.R. Srinivasan, *Tetrahedron Lett.*, **1975**, 1571.
8) Private communication from Dr. T. Anke, University of Tübingen.