Note

Antimutagenic Diterpenoids from
a Crude Drug Isodonis
Herba (Enmei-So)

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Recently trials have been carried out to detect the natural antimutagens of plant and animal sources, and to study the mechanism and implications of their action.¹⁾ Described in this paper are the results of one such screening of natural sources, in which we were able to isolate three diterpenoids as antimutagens.²⁾

The bioassay system used was a paper disk-agar plate method, adopted from the one originally described.³⁾ After brief irradiation with ultraviolet light, a cell suspension of the test organism *Escherichia coli* WP2 B/r (tryptophan auxotroph) was spread on agar plates of semi-enriched (MB) medium.³⁾ Paper disks (Toyo Filter Paper, 8 mm diameter, 0.7 mm thickness) absorbing a chemical or an extract were placed on the inoculated agar plates and incubation was carried out at 37°C for 3 days. Reverse mutations induced by UV irradiation resulted in the forming of colonies of tryptophan prototrophs on the plates. However, if a paper disk contained an antimutagenic factor which inhibited reversion to Trp⁺, no growth of revertant colonies was observed around the disk. This inhibition zone was proportional to the antimutagenic activity.

We examined the antimutagenic activity of aqueous and organic extracts of fifty commercially available crude drugs and found activities in eight. Among them, further attempts to isolate active principles were made with *Isodon japonicus* HARA since preliminary experiments showed that they were thermally stable and extractable with an organic solvent as well. With the guidance of the above-mentioned bioassay, active substances were extracted, fractionated and purified by solvent extraction, solvent partition, repeated silica gel adsorption chromatography

and crystallization. Starting from 1 kg of the pulverized crude drug, 430 mg of Crystal A, 1.85 g of Crystal B, 81 mg of Crystal C and 870 mg of Crystal D were obtained. Although a fraction from which Crystal A was obtained was active in this assay, Crystal A was inactive and the activity was found in the mother liquor of crystallization whose active principle is still unknown. All the bioactive compounds were analyzed by physicochemical and spectroscopic methods and then identified with authentic samples.

The major active constituent, Crystal B, mp 303°C (decomp.); MS: m/z 362 (M^+ , $C_{20}H_{26}O_6$) and m/z 344 ($M^+ - H_2O$); UV (EtOH): λ_{max} 232 nm (log ϵ 4.04); IR (KBr): ν_{max} 3510, 3460, 1755, 1710, 1650 and 910 cm^{-1} , was determined to be enmein on the basis of the physicochemical data,⁴⁾ and was positively identified with an authentic specimen by comparing their 400 MHz ¹H-NMR spectra (pyridine- d_5): δ^{TMS} 8.18 (1H, br. d), 6.66 (1H, d, $J=4$ Hz), 5.95 (1H, s), 5.86 (1H, br. s), 5.38 (1H, dd, $J=7$ and 11 Hz), 5.25 (1H, s), 4.52 (1H, d, $J=9$ Hz), 4.34 (1H, d, $J=9$ Hz), 3.80 (br.), 3.12 (1H, dd, $J=5$ and 12 Hz), 2.88 (1H, m), 2.46 (1H, d, $J=11$ Hz), 1.80~2.40 (m), 1.32 (3H, s) and 1.04 ppm (3H, s).

The component with the highest antimutagenic activity was Crystal C, mp 276.5~279.5°C (decomp.); MS: m/z 362 (M^+ , $C_{20}H_{26}O_6$) and m/z 344 ($M^+ - H_2O$); UV (EtOH): λ_{max} 231 nm (log ϵ 3.84); IR (KBr): ν_{max} 3500~3300, 1740, 1690, 1640, 1040 and 880 cm^{-1} , which was designated as nodosin.⁵⁾ Again, its identity with an authentic sample was confirmed by comparing the 400 MHz ¹H-NMR spectra (pyridine- d_5): δ^{TMS} 8.24 (1H, br. s), 6.76 (1H, d), 5.96 (1H, s), 5.79 (1H, s), 5.82 (1H, t, $J=6$ Hz), 5.30 (1H, s), 5.19 (1H, q, $J=4$ Hz), 4.58 (1H, d, $J=9$ Hz), 4.34 (1H, d, $J=9$ Hz), 3.72 (1H, d, $J=11.5$ Hz), 3.24

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TABLE I. ANTIMUTAGENIC ACTIVITIES OF
DITERPENOIDS ISOLATED FROM
Isodon japonicus

Each chemical was impregnated with a paper disk and placed on the surface of agar medium on which UV-exposed cells of *E. coli* B/r WP2 *trp*⁻ had been previously spread (see the text for details). The diameter (mm) of zones without mutant on the layer of the auxotroph cells colony was measured.

Compound	Dose (mg) per disk				
	3.0	1.5	1.0	0.5	0.25
Isodonal		0	0	0	0
Enmein	25	22	16	14	12
Nodosin	40	34	21	19	10
Oridonin		25	16	14	

(1H, dd, $J=4$ and 10 Hz), 3.09 (1H, d, $J=2$ Hz), 2.50 (1H, dd, $J=10$ and 14 Hz), 2.21 (1H, dd, $J=4$ and 12 Hz), 1.8 (m), 1.53 (m), 1.36 (1H, dt, $J=4$ and 14 Hz), 1.00 (3H, s) and 0.96 ppm (3H, s).

Another minor active principle was Crystal D, mp 236~238°C; MS: m/z 364 (M^+ , $C_{20}H_{28}O_6$), m/z 346 ($M^+ - H_2O$) and m/z 328 ($M^+ - 2H_2O$); UV (EtOH): λ_{max} 237 nm ($\log \epsilon$ 4.01); IR (KBr): ν_{max} 3460, 3250, 2950, 1715, 1655, 1070 and 920 cm^{-1} .⁶⁾ The 400 MHz 1H -NMR spectrum (pyridine- d_5) of Crystal D, δ^{TMS} 6.94 (1H d, $J=10$ Hz), 6.28 (1H, s), 5.98 (1H, br. s), 5.50 (1H, s), 5.05 (1H, br. s), 4.79 (1H, d, $J=10$ Hz), 4.41 (1H, d, $J=10$ Hz), 4.27 (1H, dd, $J=10$ and 7 Hz), 3.84 (1H, m), 3.20 (1H, d, $J=9$ Hz), 1.30 (3H, s) and 1.14 ppm (3H, s), was superimposable with that of authentic oridonin.

Biologically inactive Crystal A was also a diterpenoid, mp 242.5~248.5°C; MS: m/z 404 (M^+ , $C_{22}H_{28}O_7$), m/z 386 ($M^+ - H_2O$) and m/z 344 ($M^+ - AcOH$); UV (EtOH): λ_{max} 231 nm ($\log \epsilon$ 3.85); IR (KBr): ν_{max} 3435, 2750, 1758, 1710, 1645, 1270 and 855 cm^{-1} , which was assigned as isodonal based on these data as well as on the 1H -NMR spectrum (100 MHz, DMSO- d_6), δ^{TMS} 9.85 (1H, d, $J=4$ Hz), 5.90 (1H, s), 5.62 (1H, s), 5.21 (m), 4.65 (1H, br. d, $J=12$ Hz), 4.22 (broad), 3.40 (m), 1.88 (br. s), 1.09 (3H, s) and 1.00 (3H, s).⁷⁾

The antimutagenic activities of these diterpenoids are summarized in Table I. While antibacterial activities of related diterpenoids were previously reported against *Bacillus subtilis*,⁸⁾ oridonin showed bactericidal activity at higher doses in the present assay. Worth noting is that antitumor activities of enmein and oridonin on Ehrlich ascites carcinoma in mice have been previously reported^{9,10)} and this seems interesting in relation to their antimutagenic activities shown in the present study.

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