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# Mutagenic Principles in Sinomeni Caulis *et* Rhizoma. II.[1] The Mutagenicity of Liriodenine in the Basic Fraction of the Methanol Extract

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A mutagenic principle in the basic fraction of the methanol extract from Sinomeni Caulis *et* Rhizoma (Menispermaceae) was isolated and identified as an oxoaporphine alkaloid, liriodenine. Liriodenine exhibited potent mutagenic activity towards *Salmonella typhimurium* strains TA98 and TA100 in the presence of liver homogenate (S9 mix) (10.9 and 90.7 revertants/nmol, respectively) and accounted for about 40% of the mutagenicity of the total methanol extract.

**Keywords**——mutagenicity; Sinomeni Caulis *et* Rhizoma; liriodenine; oxoaporphine alkaloid; Menispermaceae

In the previous paper[1] we reported the mutagenic activity of *N*-demethyl-*N*-formyldehydronuciferine in the neutral fraction of the methanol extract from Sinomeni Caulis *et* Rhizoma, which consists of the root, root-stalk, and stem of *Sinomenium acutum* REHDER *et* WILSON (Menispermaceae; Japanese name, oh-tsuzurafuji). In that report the presence of other kinds of mutagens in the extract was suggested.

This paper reports the mutagenic activity of liriodenine in the basic fraction of the extract, as well as its contribution to the mutagenicity of the extract as a whole.

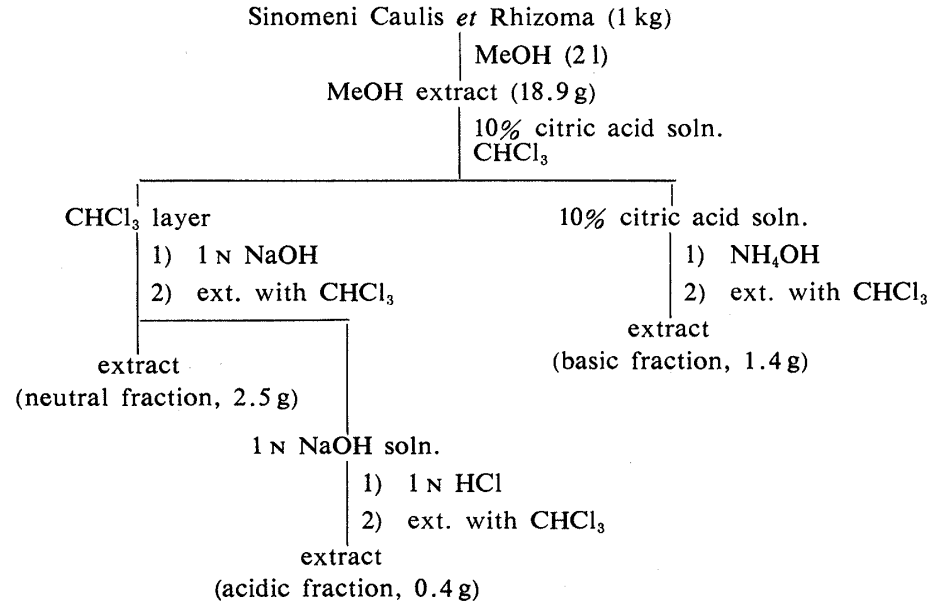


Chart 1

The methanol extract of Sinomeni Caulis *et* Rhizoma was fractionated into the acidic, basic, and neutral fractions as shown in the chart. The mutagenicity of the fractions was examined by the use of Ames' test.[2] In the test, the number of revertants produced by the fractions did not increase to twice the control value in the absence of S9 mix (−S9), whereas in the presence of S9 mix (+S9), all the fractions caused a significant increase in revertants.

Of the fractions mentioned above, the basic fraction showed the most potent mutagenic activity and the mutagenic principle in the fraction was further fractionated as described in Experimental to give yellow needles in about 0.0003% yield. The ultraviolet (UV) absorption spectrum ($\lambda_{max}^{EtOH}$: 248, 269, 311 nm) suggested that this product might be an oxoaporphine alkaloid[3] and it was identified as liriodenine (Fig. 1.) by spectroscopic comparison with an authentic specimen.

Liriodenine has been reported in a variety of plants, including *Neolitsea sericea* (BLUME) KOIDZ. (Lauraceae), *Nelumbo nucifera* GAERTN. (Nymphaeaceae) and *Magnolia ovobata* THUNB. (Magnoliaceae),[3] but this is the first report of the isolation of liriodenine from *Sinomenium acutum* REHDER *et* WILSON (Menispermaceae).

Liriodenine showed potent mutagenic activities towards TA98 and TA100 in the presence of S9 mix, but did not show mutagenicity in the absence of S9 mix. The relationship between the amount of S9 added and the number of revertants colonies induced has been reported in the case of benzo[*a*]pyrene.[4] The S9 mixture used in our experiments had the same composition as that used by Yahagi,[2] and we used 150 μl of S9 per plate (required for optimal mutagenicity with liriodenine). The dose–response curves of the mutagenicity of liriodenine are shown in Fig. 2. From these results, the specific mutagenic activities were calculated to be 330 revertants per microgram towards TA100 and 40 revertants per microgram towards TA98 at the dose of 4 μg per plate. As shown in Fig. 2, the number of revertants increased almost linearly with increasing dose from 2 to 10 μg when tested on TA98 with S9 mix, whereas in the

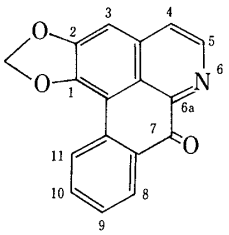


Fig. 1. Structure of Liriodenine

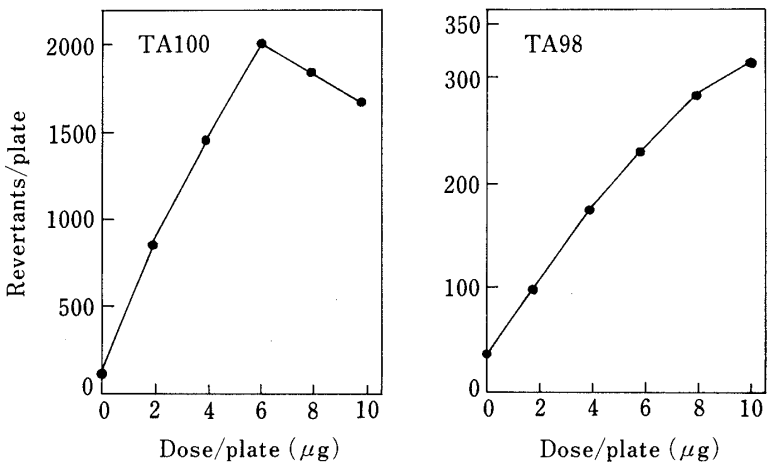


Fig. 2. Dose–Response Curves of Liriodenine with TA98 and TA100

Mutagenicity was assayed by the preincubation method. For activation of liriodenine by liver homogenate, S9 mix was used. Each point is the average of 3 plates. Spontaneous revertants have not been subtracted.

TABLE I. Yield and Mutagenicity of Samples (TA100+S9[a])

| Sample | Yield[b] (g) | Specific mutagenic activity ($His^+$/mg) |
|---|---|---|
| Methanol extract | 18.9 | 117 |
| Acidic fraction | 0.4 | 255 |
| Neutral fraction | 2.5 | 103 |
| Basic fraction | 1.4 | 1215 |
| Liriodenine | 0.003 | $3.3 \times 10^5$ |
| *N*-Demethyl-*N*-formyl dehydronuciferine | 0.006 | $2 \times 10^4$ |
| Sinomenine | 0.01 | (—)[c] |

*a*) For activation of samples by liver homogenate, S9 mix was used. *b*) From 1000 g of Sinomeni Caulis *et* Rhizoma. *c*) (—), not mutagenic.

case of TA100 with S9 mix an apparently linear relationship was observed between dose and response in the range of doses from 2 to 6 $\mu$g, but the number of revertants decreased at doses of over 6 $\mu$g (8 $\mu$g per plate and 10 $\mu$g per plate). The reduction in mutagenicity was not affected by the amount of S9 used (50—300 $\mu$l of S9 per plate) and moreover toxicity was not observed. The mutagen may have an inhibitory effect on bacterial cell growth at doses of over 6 $\mu$g per plate.

As reported previously,[1] *N*-demethyl-*N*-formyldehydronuciferine obtained from the neutral fraction in a yield of about 0.0006% exhibited specific mutagenic activities of 2 and 19 revertants per microgram towards TA98 and TA100, respectively, at the dose of 10 $\mu$g per plate with S9 mix.

Although the acidic fraction of the methanol extract also gave 400 revertants per milligram per plate in the presence of S9 mix, the mutagenic principle of this fraction has not yet been isolated.

Sinomenine is a major alkaloid contained in Sinomeni Caulis *et* Rhizoma[5] and has been reported to show pharmacological activities.[5] We have isolated sinomenine from the methanol extract of Sinomeni Caulis *et* Rhizoma, and examined its mutagenicity. In our experiment, no mutagenic activity towards TA98 and TA100 was observed at doses of 10 $\mu$g to 1 mg per plate in the presence or absence of S9 mix.

Yield and mutagenicity of samples are summarized in Table I. Liriodenine and *N*-demethyl-*N*-formyldehydronuciferine accounted for about 40% and 5% of the mutagenicity of the total methanol extract of Sinomeni Caulis *et* Rhizoma, respectively, and their combined mutagenicity represented about half of the whole mutagenic activity in the methanol extract. The mutagenicity of liriodenine was about 20 times that of *N*-demethyl-*N*-formyldehydronuciferine. The mutagenic potency of liriodenine is comparable to that of benzo[*a*]pyrene.

## Experimental

Melting points were determined on a Yazawa BY-2 apparatus and are uncorrected. Shimadzu IR 435 and Hitachi model 330 spectrometers were used for the measurements of infrared (IR) and ultraviolet (UV) spectra, respectively. The mass spectra (MS) were recorded on a Shimadzu LKB-9000 GC-MS high-resolution spectrometer. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were measured in $CDCl_3$ or $CD_3OD$ with a JEOL TNM-GX 400 instrument and chemical shifts are given in $\delta$ (ppm) with tetramethylsilane (TMS) as an internal reference and coupling constants ($J$) in Hz (s, singlet; d, doublet; dd, double doublet; m, multiplet).

**Plant Materials**——Commercial Sinomeni Caulis *et* Rhizoma collected at Shikoku was purchased from Kinokuniya Kanyakkyoku Ltd. (Japan).

**Mutagenicity Assay**——Mutagenicity was tested by the use of Ames' method with the modification of preincubation[2] using *Salmonella typhimurium* TA98 and TA100 in the presence or absence of S9 mix. S9 was pre-

pared from the liver of male Sprague–Dawley rats pretreated with phenobarbital and β-naphthoflavone. Benzo[*a*]-pyrene was used as a positive control for TA98 and TA100 with S9 mix and furylfuramide (trade name AF-2) for TA98 and TA100 without S9 mix. Spontaneous revertants amounted to 28 (TA98, −S9), 31 (TA98, +S9), 140 (TA100, −S9) and 145 (TA100, +S9). Furylfuramide (0.2 μg) yielded 485 $His^+$ revertants/plate from TA98 without S9 mix and it (0.02 μg) yielded 631 $His^+$ revertants/plate from TA100 without S9 mix. Benzo[*a*]pyrene (5μg) yielded 273 $His^+$ revertants/plate from TA98 and 801 $His^+$ revertants/plate from TA100 with S9 mix. Numbers of revertants are the averages of 3 plates.

**Preparation of the Methanol Extract**——Dried and ground material (1.0 kg) was extracted with MeOH (2 l) at room temperature for 2 d. The MeOH extract (18.9 g) was fractionated into the acidic, basic, and neutral fractions as shown in Chart 1. All the fractions were screened for mutagenicity.

**Liriodenine**——Basic fraction (1.4 g) was applied to a Sephadex LH-20 column (3 × 60 cm) with MeOH. The sample was separated into two bands (brown and yellow) on the column. The latter band, showing mutagenicity in Ames' test, was collected and further purified by preparative thin-layer chromatography (TLC) ($CHCl_3$: ether = 9 : 1) to give liriodenine (3 mg), yellow needles from $CHCl_3$, mp 281°C dec. UV $\lambda_{max}^{EtOH}$ nm: 248, 269, 311. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1660, 1420, 1302, 1255, 1052, 962. MS *m/z*: 275 ($M^+$). $^1$H-NMR ($CDCl_3$) δ: 6.48 (2H, s, $CH_2O_2$–), 7.42 (1H, s, $C_3$-H), 7.64, 7.85 (each 1H, m, $C_9$, $C_{10}$-H), 8.02 (1H, d, $J$ = 6 Hz, $C_4$-H), 8.50 (1H, dd, $J$ = 3 and 8 Hz, $C_8$-H), 8.76 (1H, d, $J$ = 6 Hz, $C_5$-H), 8.78 (1H, dd, $J$ = 3 and 8 Hz, $C_{11}$-H).

**Sinomenine**——Basic fraction (1.4 g) was separated by preparative TLC (MeOH:$H_2O$ = 97 : 3). The UV-absorbing band (*Rf*, 0.2) was extracted with MeOH from the TLC plate and further purified on a column of Sephadex LH-20 with MeOH to give sinomenine (10 mg), colorless needles from MeOH, mp 161°C. UV $\lambda_{max}^{MeOH}$ nm: 260. MS *m/z* 329 ($M^+$). $^1$H-NMR ($CD_3OD$) δ: 2.43 (N-$CH_3$), 3.49 (O-$CH_3$), 3.81 (O-$CH_3$).

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