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# Synthesis and Anti-ulcer Activities of Sodium Alkylazulene Sulfonates

TAKASHI YANAGISAWA,*,[a] SHUICHI WAKABAYASHI,[a] TSUYOSHI TOMIYAMA,[a] MASAFUMI YASUNAMI[b] and KAHEI TAKASE[b]

*Kotobuki Pharmaceutical Co., Ltd.,[a] Sakaki 6351, Nagano 389–07, Japan and Department of Chemistry, Faculty of Science, Tohoku University,[b] Aramaki-aza-Aoba, Sendai 980, Japan*

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Many derivatives of sodium alkylazulene sulfonates were newly synthesized and their anti-ulcer activities were examined in Shay pylorus-ligated rats. The values of lipophilicity (log *P*), a physicochemical parameter, of these new azulene derivatives were also examined in order to study the structure–activity relationship. The optimum value of log *P* which gave maximum anti-ulcer activity was about −1.0. Among the derivatives of azulene examined, 3-ethyl-7-isopropylazulene-1-sulfonic acid sodium salt (KT1-32) exhibited an extremely potent inhibitory action against Shay ulcer, and its antipeptic activity was more potent than that of guaiazulene sodium sulfonate (GAS). Furthermore, KT1-32 was extremely stable on heating as compared to GAS.

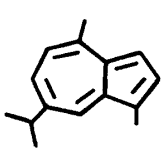
**Keywords**——anti-ulcer activity; stability; lipophilicity; Shay pylorus-ligated rat; azulene derivative; structure–activity relationship; antipeptic activity

Guaiazulene (GA) (Fig. 1) is an active component of the essential oil of the plant *Guaiacum officinalis*, and its chemical structure was determined in 1949.[1] There are a number of reports describing anti-allergic, anti-inflammatory and anti-ulcer activities of GA.[2] In recent years, guaiazulene sodium sulfonate (GAS), a hydrophilic derivative of GA, was synthesized and it has been widely used clinically as an anti-inflammatory and anti-ulcer agent.[3] Both GA and GAS, however, are known to be unstable to heat and sunlight.

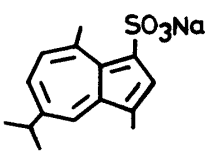
We recently described a new method to synthesize alkylazulenes.[4] Using this method, we synthesized new sodium alkylazulene sulfonates. The inhibitory effects of the synthesized compounds against Shay ulcer were examined, and the structure–activity relationship between anti-ulcer activity and lipophilicity was also examined.

## Synthesis of Alkyl-, Aralkyl- and Alkenylazulene Sodium Sulfonates (Chart 1)

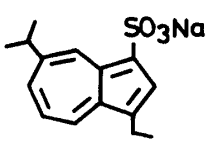
Reaction of 2-tosyloxytropone (Ia) with two molar equivalents of dimethyl malonate in the presence of sodium methoxide on ice afforded 3-methoxycarbonyl-2*H*-cyclohepta[*b*] furan-2-one (IIa) good yield. Similarly, the 5-isopropyl derivative (IIb) was also obtained by reacting 5-isopropyl-2-tosyloxytropone (Ib) with dimethyl malonate. Reaction of IIa or IIb with *in situ*-generated morpholino enamines of aldehydes in ethanol under reflux resulted in



guaiazulene (GA)




guaiazulene sodium sulfonate (GAS)




KT1-32

Fig. 1

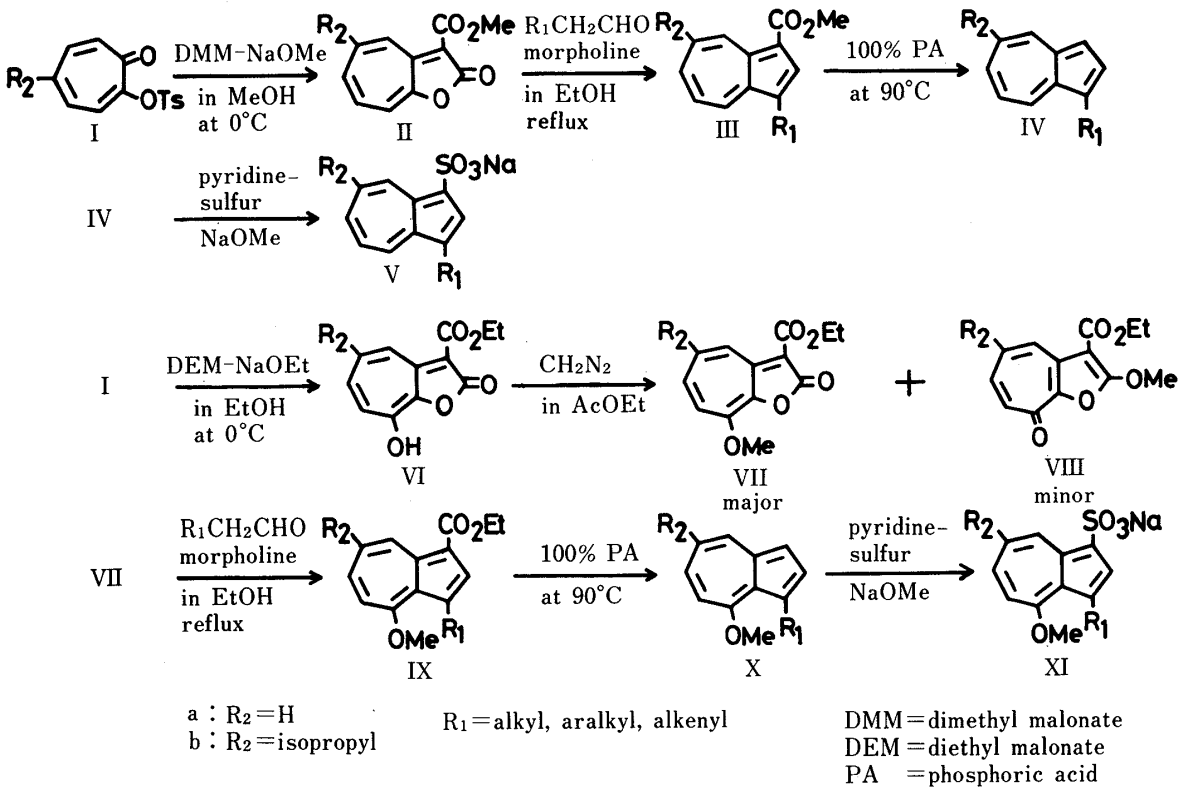



Chart 1. Synthetic Route to Azulene Derivatives

the formation of the 3-alkyl derivatives of methyl azulene-1-carboxylates (IIIa—h) or the 3,7-dialkyl derivatives (IIIi—s), respectively, in the yield of over 90%. Heating of III with anhydrous phosphoric acid (PA) at 90—95 °C for 15—30 min afforded 1-alkyl-(IVa—h) and 1,5-dialkylazulenes (IVi—r) in good yields. Sulfonation of IV with dioxane–sulfur trioxide or pyridine–sulfur trioxide complex in benzene followed by treatment with sodium methoxide yielded the corresponding azulene sodium sulfonates (Va—r) as bluish violet crystals.

Reaction of Ia with diethyl malonate in the presence of sodium ethoxide in ethanol yielded 8-hydroxy-3-ethoxycarbonyl-2*H*-cyclohepta[*b*]furan-2-one (VIa).[5] Methylation of VIa with diazomethane in ethyl acetate afforded 8-methoxy-3-ethoxycarbonyl-2*H*-cyclohepta-[*b*]furan-2-one (VIIa) as a major product together with 2-methoxy-3-ethoxycarbonyl-8*H*-cyclohepta[*b*]furan-8-one (VIIIa).[5] Similarly, reaction of Ib with diethyl malonate followed by methylation with diazomethane afforded 5-isopropyl-8-methoxy-3-ethoxycarbonyl-2*H*-cyclohepta[*b*]furan-2-one (VIIb). Reaction of VII with *in situ*-generated morpholino enamines of aldehydes yielded the 4-methoxyazulene derivatives (IXa—k). Deethoxycarbonylation of IX by treatment with PA yielded X. Sulfonation with sulfur trioxide complex followed by treatment with sodium methoxide afforded the sodium 3-alkyl-4-methoxyazulene-1-sulfonates (XIa—k).

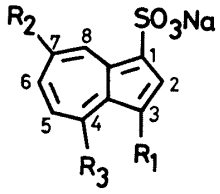
**Stability**

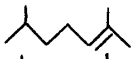
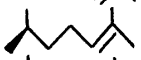
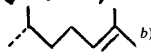
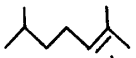
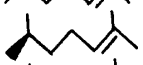
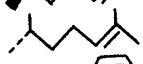
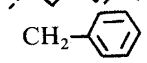
The thermal stabilities of KT1-32 and GAS were compared. Samples of KT1-32 and GAS were placed in test tubes, and heated at 60 °C followed by thin layer chromatographic (TLC) analysis on silica gel plates. GAS was decomposed after heating for 20 h, whereas KT1-32 was not affected. These results indicate that KT1-32 is significantly more stable than GAS.

**Structure–Activity Relationships**

The anti-ulcer activity of azulene derivatives synthesized for this study was evaluated in terms of the ability to inhibit Shay ulcer in rats. The activity was expressed as inhibition

TABLE I. Anti-ulcer Activities of Azulene Derivatives




| Compound | $R_1$ | $R_2$ | $R_3$ | Yield (%) | mp (°C) | Formula | Shay ulcer inhibition (%) | log $P$ |
|---|---|---|---|---|---|---|---|---|
| Va | $CH_3$ | H | H | 88 | 156—158 (dec.) | $C_{11}H_9O_3SNa$ | 52.4 | −1.49 |
| Vb | $C_2H_5$ | H | H | 85 | 160—162 (dec.) | $C_{12}H_{11}O_3SNa$ | 54.0 | −1.07 |
| Vc | $C_3H_7$ | H | H | 85 | 210—212 (dec.) | $C_{13}H_{13}O_3SNa$ | 78.5 | −1.11 |
| Vd | $C_4H_9$ | H | H | 70 | 215—217 (dec.) | $C_{14}H_{15}O_3SNa$ | 54.2 | −0.05 |
| Ve | $C_5H_{11}$ | H | H | 65 | 217—219 (dec.) | $C_{15}H_{17}O_3SNa$ | 64.3 | −0.25 |
| Vf |  | H | H | 62 | 78—80 (dec.) | $C_{18}H_{21}O_3SNa$ | 49.0 | — |
| Vg |  *a)* | H | H | 63 | 78—80 (dec.) | $C_{18}H_{21}O_3SNa$ | 59.8 | −0.77 |
| Vh |  *b)* | H | H | 63 | 78—80 (dec.) | $C_{18}H_{21}O_3SNa$ | 69.9 | −0.29 |
| Vi | H | Isopro | H | 32 | 241—243 (dec.) | $C_{13}H_{13}O_3SNa$ | 62.0 | −2.13 |
| Vj | $CH_3$ | Isopro | H | 90 | 91—93 (dec.) | $C_{14}H_{15}O_3SNa$ | 69.7 | −0.88 |
| Vk*e)* | $C_2H_5$ | Isopro | H | 90 | 152—154 (dec.) | $C_{15}H_{17}O_3SNa$ | 92.7 | −0.74 |
| Vl | $C_3H_7$ | Isopro | H | 87 | 138—140 (dec.) | $C_{16}H_{19}O_3SNa$ | 31.2 | −0.45 |
| Vm | $C_4H_9$ | Isopro | H | 80 | 150—152 (dec.) | $C_{17}H_{21}O_3SNa$ | 17.2 | 0.10 |
| Vn | $C_5H_{11}$ | Isopro | H | 75 | 168—170 (dec.) | $C_{18}H_{23}O_3SNa$ | −10.3 | 0.75 |
| Vo |  | Isopro | H | 63 | 113—115 (dec.) | $C_{21}H_{27}O_3SNa$ | 7.1 | 0.59 |
| Vp |  *c)* | Isopro | H | 61 | 114—116 (dec.) | $C_{21}H_{27}O_3SNa$ | 14.5 | 0.70 |
| Vq |  *d)* | Isopro | H | 60 | 114—116 (dec.) | $C_{21}H_{27}O_3SNa$ | −19.5 | 0.71 |
| Vr |  | Isopro | H | 83 | >250 | $C_{20}H_{19}O_3SNa$ | 9.8 | 0.21 |

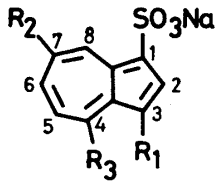
*a)* $[\alpha]_D^{25} = +252°$ ($c=0.01$ MeOH). *b)* $[\alpha]_D^{25} = -316$ ($c=0.01$ MeOH). *c)* $[\alpha]_D^{25} = +289$ ($c=0.01$ MeOH). *d)* $[\alpha]_D^{25} = -274$ ($c=0.01$ MeOH). *e)* KT1-32.

percent (%) and these data are shown in Tables I and II, where the partition coefficients ($P$) are expressed as $\log P = C_O/C_W$ ($C_O$ is the concentration of azulene derivatives in the $n$-octanol phase, and $C_W$ is the concentration of azulene derivatives in the aqueous phase). Substitution of more than two or three carbon atoms at the $C_3$ position led to a gradual decrease in the activity as follows: $n$-propyl(Vc) > $n$-butyl(Vd), ethyl(Vk) > $n$-propyl(Vl) > $n$-butyl(Vm) > $n$-pentyl(Vn), ethyl(XIh) > $n$-propyl(XIi) = $n$-butyl(XIj) > $n$-pentyl(XIk), ethyl(XIb) = $n$-propyl-(XIc) > $n$-butyl(XId) > $n$-pentyl(XIe) > $n$-hexyl(XIf). Therefore, two carbon atoms as alkyl substituents at the $C_3$ position seem to be optimal for anti-ulcer activity. The inhibitory effect of compounds with OMe at the $C_4$ position ($R_3$) on Shay ulcer was almost parallel to that of the parent compounds.

Among the compounds synthesized in this study KT1-32 showed the most potent anti-ulcer activity and its inhibitory effect was greater than that of GAS.

The values of lipophilicity (log $P$) of compounds, such as Vc, Vj, Vk, XIb and XIc, which had high inhibitory potency against Shay ulcer, were in the range of −1.2——0.6 as shown in Tables I and II. These findings indicate that lipophilicity (log $P$) of compounds may play an important role in enhancement of anti-ulcer activity. log $P$ values and inhibition percentages (Shay ulcer) of azulene derivatives are summarized in Tables I and II. Equation 1 was derived from a regression analysis.

TABLE II. Antiulcer Activities of Azulene Derivatives




| Compound | $R_1$ | $R_2$ | $R_3$ | Yield (%) | mp (°C) | Formula | Shay ulcer inhibition (%) | log *P* |
|---|---|---|---|---|---|---|---|---|
| XIa | $CH_3$ | H | OMe | 65 | 112—113 (dec.) | $C_{12}H_{11}O_4SNa$ | 63.4 | −1.29 |
| XIb | $C_2H_5$ | H | OMe | 60 | 71—73 (dec.) | $C_{13}H_{13}O_4SNa$ | 75.6 | −0.98 |
| XIc | $C_3H_7$ | H | OMe | 54 | 52—54 (dec.) | $C_{14}H_{15}O_4SNa$ | 75.6 | −0.63 |
| XId | $C_4H_9$ | H | OMe | 55 | 45—47 (dec.) | $C_{15}H_{17}O_4SNa$ | 66.3 | −0.31 |
| XIe | $C_5H_{11}$ | H | OMe | 42 | 48—49 (dec.) | $C_{16}H_{19}O_4SNa$ | 42.0 | −0.05 |
| XIf | $C_6H_{13}$ | H | OMe | 48 | 52—53 (dec.) | $C_{17}H_{21}O_4SNa$ | 38.0 | −1.31 |
| XIg | $CH_3$ | Isopro | OMe | 73 | 95—97 (dec.) | $C_{15}H_{17}O_4SNa$ | 58.7 | −0.78 |
| XIh | $C_2H_5$ | Isopro | OMe | 75 | 108—110 (dec.) | $C_{16}H_{19}O_4SNa$ | 67.6 | −0.37 |
| XIi | $C_3H_7$ | Isopro | OMe | 75 | 188—190 (dec.) | $C_{17}H_{21}O_4SNa$ | 44.1 | −0.01 |
| XIj | $C_4H_9$ | Isopro | OMe | 63 | 166—169 (dec.) | $C_{18}H_{23}O_4SNa$ | 64.8 | — |
| XIk | $C_5H_{11}$ | Isopro | OMe | 60 | 123—125 (dec.) | $C_{19}H_{25}O_4SNa$ | −14.6 | 0.20 |

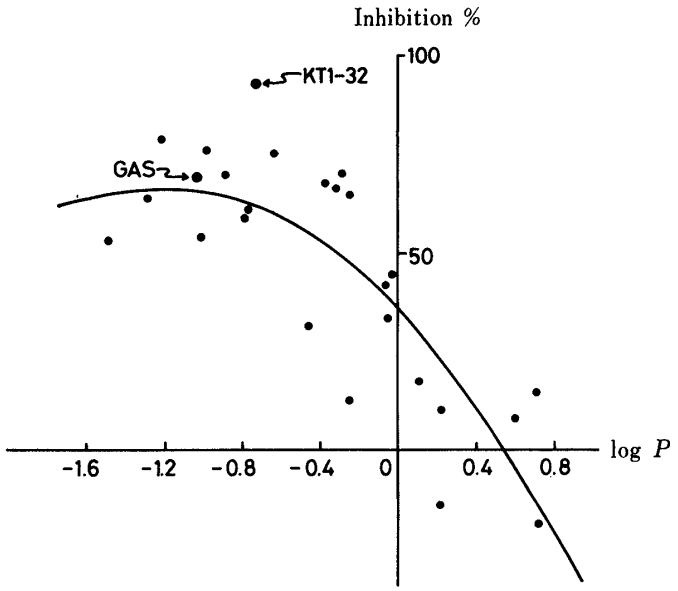



Fig. 2. Anti-ulcer Activity (Shay Ulcer) of the Compounds Plotted against log *P*

$$\text{Inhibition } (\%) = -23.0(\log P)^2 - 53.7(\log P) + 36.4 \quad (1)$$
$$(n=26,\ r=0.821,\ s=18.7,\ F_{23}^2=23.35)$$

*n*, *r*, *s* and $F_{23}^2$ represent the number of compounds used, correlation coefficient, standard deviation and value in the *F* test, respectively.

Equation 1 gave a high correlation coefficient ($r=0.821$), which was statistically significant at the 99.99% level with an *F* value of 23.35 ($F_{23}^2$ ($=0.001$)$=9.95$). Expected values of inhibition percentage calculated from Eq. 1 were in good agreement with those observed for the compounds, as shown in Table III. Consequently, the potency (inhibition percentage) of compounds can be estimated easily by the use of Eq. 1 with log *P* as the parameter, as shown

TABLE III. Comparison of Expected Values of Inhibition Percentage from Eq. 1 with Those Observed for the Compounds

| Compound No. | log $P^{a)}$ | Inhibition % | | |
|---|---|---|---|---|
| | | Obsd[b)] | Calcd[c)] | \|obsd − calcd\| |
| Va | −1.49 | 52.4 | 65.4 | 13.0 |
| Vb | −1.07 | 54.0 | 67.6 | 13.6 |
| Vc | −1.11 | 78.5 | 68.0 | 10.8 |
| Vd | −0.06 | 54.2 | 39.6 | 14.6 |
| Ve | −0.25 | 64.3 | 48.2 | 16.1 |
| Vg | 0.77 | 59.8 | 64.1 | 4.2 |
| Vh | −0.29 | 69.9 | 50.0 | 20.0 |
| Vi | −2.13 | 62.0 | 46.4 | 15.6 |
| Vj | −0.88 | 69.7 | 65.9 | 3.8 |
| Vk | −0.74 | 92.3 | 63.6 | 28.7 |
| Vl | −0.45 | 31.2 | 55.9 | 24.7 |
| Vm | 0.10 | 17.2 | 30.9 | 13.7 |
| Vn | 0.75 | −10.3 | −16.8 | 6.5 |
| Vo | 0.59 | 7.1 | −2.9 | 10.0 |
| Vp | 0.70 | 14.5 | −12.7 | 27.2 |
| Vq | 0.71 | −19.5 | −13.5 | 6.0 |
| Vr | 0.21 | 9.8 | 24.1 | 14.4 |
| XIa | −1.29 | 63.4 | 67.4 | 4.0 |
| XIb | −0.98 | 75.6 | 67.0 | 8.6 |
| XIc | −0.63 | 75.6 | 61.0 | 14.6 |
| XId | −0.31 | 66.3 | 50.9 | 15.4 |
| XIe | −0.05 | 42.0 | 38.9 | 3.1 |
| XIf | −1.31 | 38.0 | 67.2 | 29.2 |
| XIg | −0.78 | 58.7 | 64.4 | 5.7 |
| XIh | −0.37 | 67.6 | 53.3 | 14.3 |
| XIi | −0.01 | 44.1 | 37.1 | 7.0 |
| GAS | −1.00 | 70.0 | 67.1 | 2.9 |

*a*) From Tables I and II. *b*) From Tables I and II. *c*) The calculated values were obtained from Eq. 1.

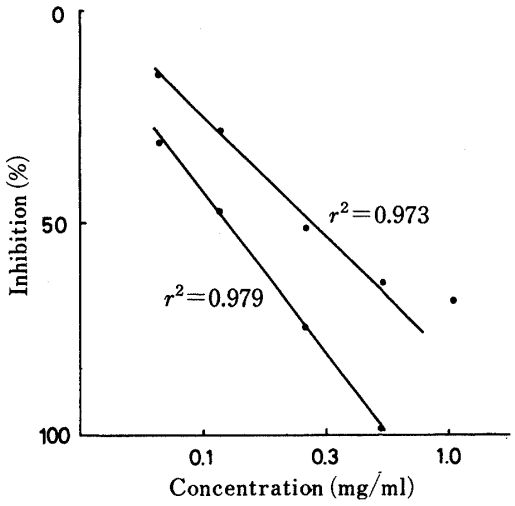



Fig. 3. Antipeptic Activity of KT1-32 and GAS

●—●, KT1-32; ○—○, GAS. The $IC_{50}$ values of KT1-32 and GAS were 0.126 and 0.284 mg/ml respectively.

in Fig. 2. The optimum log *P* value is about −1.2 based on Eq. 1, and adequate prediction of the potency of anti-ulcer activity of compounds is possible.

**Antipeptic Activity**

Dose–response curves for antipeptic activity of azulene derivatives and GAS are shown

in Fig. 3. KT1-32 (0.07—0.53 mg/ml) showed inhibitory activity against pepsin in a dose-dependent manner. KT1-32 (0.53 mg/ml) completely inhibited the peptic activity. The inhibitory effect of KT1-32 was more potent than that of GAS. The $IC_{50}$ (concentrations which inhibit peptic activity by 50%) values of KT1-32 and GAS were 0.126 and 0.284 mg/ml, respectively.

## Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 270-30. Proton nuclear magnetic resonance ($^1$H-NMR) spectra were measured at 90 MHz on a Hitachi R-90H Fourier-transform NMR spectrometer. Chemical shifts are quoted in parts per million (ppm) with tetramethylsilane as an internal standard, and coupling constants ($J$) are given in Hz. The following abbreviations are used: s=singlet, d=doublet, q=quartet, m=multiplet and br s=broad singlet. Ultraviolet (UV) spectra were recorded on a Hitachi 150-20 spectrometer. Optical rotations were measured on a JASCO DIP-4 polarimeter. For column chromatography, silica gel (Merck, Kieselgel 60, 70—230 mesh) was used. TLC was performed on Silica gel 60 $F_{254}$ plates (Merck).

**Methyl 3-Methylazulene-1-carboxylate**——A mixture of propionaldehyde (5.2 g, 90 mmol), morpholine (7.8 g, 90 mmol), and 3-methoxycarbonyl-2*H*-cyclohepta[*b*]furan-2-one (6 g, 30 mmol) in EtOH (120 ml) was heated under reflux for 4 h. The solvent was removed, and the residue was extracted with benzene. The organic layer was washed with $H_2O$, and dried over $Na_2SO_4$. The solvent was removed, and the residue was purified by column chromatography with benzene to give methyl 3-methylazulene-1-carboxylate (5.4 g, 27 mmol 91%) as violet crystals, mp 61—62 °C. IR (KBr): 2950, 1690, 1450, 1440, 1202, 1100 cm$^{-1}$. $^1$H-NMR ($CDCl_3$, 90 MHz): 2.58 (3H, s, Me), 7.28 (1H, dd, $J$=9.6, 9.6, $C_5$-H), 7.37 (1H, dd, $J$=9.6, 9.6, $C_7$-H), 7.67 (1H, dd, $J$=9.6, 9.6, $C_6$-H), 8.14 (1H, s, $C_2$-H), 8.24 (1H, d, $J$=9.6, $C_4$-H), 9.49 (1H, dd, $J$=9.6, $C_8$-H).

**1-Methylazulene (IVa)**——A mixture of methyl 3-methylazulene-1-carboxylate (6.0 g, 30 mmol), and PA (60 ml) was heated with stirring at 90—95 °C for 15 min. The reaction mixture was then poured into $H_2O$ (300 ml), extracted with benzene, washed with $H_2O$, and dried over $Na_2SO_4$. The solvent was removed, and then the residue was purified by chromatography with benzene to give 1-methylazulene (4.0 g, 28 mmol 93.3%) as a blue oil. IR (neat): 3012, 2930, 1577, 1510, 1455 cm$^{-1}$. $^1$H-NMR ($CDCl_3$, 90 MHz): 2.64 (3H, s, Me), 7.00 (2H, dd, $J$=9.8, 9.8, $C_{5,7}$-H), 7.31 (1H, d, $J$=3.6, $C_3$-H), 7.51 (1H, dd, $J$=9.8, 9.8, $C_6$-H), 7.75 (1H, d, $J$=3.6, $C_2$-H), 8.21 (2H, d, $J$=9.8, $C_{4,8}$-H).

**3-Ethyl-7-isopropylazulene Sodium Sulfonate (KT1-32)**——A mixture 1-ethyl-5-isopropylazulene (20 g, 10 mmol), and pyridine–sulfur trioxide complex (32 g, 20 mmol) in benzene (120 ml) was heated under reflux for 6 h. Precipitates were collected by filtration, and dissolved in $H_2O$ (100 ml). Next 15 ml of sodium hydroxide solution (6.4 g, 16 mmol) was added at 10—15 °C, and the reaction mixture was stirred at 30—35 °C for 1 h, and extracted with *n*-butanol. The extract was washed with saturated NaCl solution. After the removal of the solvent, the crude product was recrystallized from ethanol to give KT1-32 (27 g, 90 mmol 90%) as bluish violet crystals, mp 152—154 °C (dec). IR(KBr): 3600, 1460, 1420, 1390, 1200, 1060 cm$^{-1}$. $^1$H-NMR (DMSO, 90 MHz): 1.35 (3H, t, $J$=7.5, Et-Me), 1.40 (6H, d, $J$=7.5, isoprMe), 3.04 (2H, q, $J$=7.5, Et-$CH_2$), 3.18 (1H, m, $J$=7.5, isoprCH), 7.26 (1H, dd, $J$=10.0, 10.0, $C_5$-H), 7.71 (1H, ddd, $J$=10, 2.0, 1.0, $C_6$-H), 8.06 (1H, s, $C_2$-H), 8.34 (1H, dd, $J$=1.0, $C_4$-H), 9.22 (1H, d, $J$=2.0, $C_8$-H).

**Pharmacological Method**——1) Shay Ulcer: Donryu rats weighing 180 to 220 g were deprived of food but allowed free access to water for 48 h prior to the experiments. Under ether anesthesia, the pylorus was ligated according to the method described by Shay *et al.*[6] After 16 h, each animal was sacrificed and the stomach was removed. Each stomach was fixed in formalin solution (1%) for 10 min. Gastric mucosa was then exposed by opening the stomach along the greater curvature and gastric ulcers that had developed in the forestomach were observed. The degree of ulceration was estimated using the ulcer index according to Okabe *et al.*[3] Inhibition ratio was calculated as follows;

$$\text{inhibition ratio (\%)} = \frac{\text{ulcer index (control)} - \text{ulcer index (sample)}}{\text{ulcer index (control)}} \times 100$$

Each drug was administered orally immediately after ligation.

2) Antipeptic Activity: Bovine serum albumin (BSA) and pepsin were purchased from Wako Co. Antipeptic activity was measured as described previously by Thiemer *et al.*[7] A mixture of BSA (25 mg/ml), 0.2 ml of 0.1 N HCl and 2 ml of water, with or without a test drug, was preincubated at 37 °C for 5 min. Then 0.5 ml of enzyme solution (100 $\mu$g pepsin/1 ml of 0.5 N HCl) was added, and the whole was incubated at 37 °C for 30 min. Two milliliters of 10% trichloroacetic acid was then added to this mixture. The pellet obtained after centrifugation at 3000 rpm for 5 min was used to determine the amount of residual BSA. The amount of BSA was measured according to the method of Lowry *et al.*[8]

The percentage inhibition was calculated as follows;

$$\% \text{ inhibition} = 100 \times (A - B)/A$$

where $A$ is the amount of digested BSA in the absence of test drug and $B$ is that in the presence of the drug.

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