Synthesis and Anti-peptic Activity of Compounds Related to the Metabolites of Sodium 3-Ethyl-7-isopropyl-1-azulenesulfonate (KT1-32)

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The metabolites of sodium 3-ethyl-7-isopropyl-1-azulenesulfonate (KT1-32, 1), a candidate as an anti-ulcer drug, and related compounds were synthesized. The effects of the compounds on anti-peptic activity were determined as compared to that of 1.

Keywords metabolite; sodium 3-ethyl-7-isopropyl-1-azulenesulfonate (KT1-32); anti-peptic activity

In previous papers, we described the synthesis and the anti-ulcer activity of new sodium alkylazulenesulfonate derivatives.[1,2] Among them, sodium 3-ethyl-7-isopropyl-1-azulenesulfonate (KT1-32, **1**) was found to possess potent inhibitory action against Shay-ulcer and anti-peptic activity. Furthermore, **1** was extremely stable upon heating. Thus, compound **1** was selected for evaluation in humans as a promising novel agent for the therapy of peptic ulcer. It is now under clinical trial. Metabolic studies are very important in the development of new drugs and are essential to assess the safety and efficacy of medicines. Recently, a study on the metabolism of **1** in biological fluids of rat was presented and three mono-hydroxylated derivatives (**3**, **4** and **5**) and two di-hidroxylated derivatives (**6** and **7**) and a hydrogen sulfated derivative (**8**) were proposed as the metabolites.[3] In this paper, we wish to report the synthesis of compounds (**2**—**7**) related to the metabolites of **1** and their anti-peptic activities.

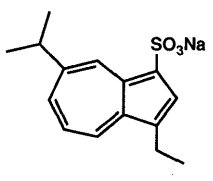
Results and Discussion

Synthesis of Hydroxyazulene Derivatives Oxidation[5] of **1** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in aqueous acetone afforded 3-acetylazulene **9**. Reduction of **9** with sodium borohydride ($NaBH_4$) afforded **2**. Attempts to afford **5** by several oxidizing agents failed. But when a solution of **1** in water was irradiated was 30 W low-pressure Hg lamp,[6] compound **5** was obtained in a 10% yield. Compound **7** was prepared from **3** in the same manner. Deprotection of **10**[1] with aluminium chloride ($AlCl_3$) in anisole afforded **3**. Bromination[4] of **11**[1] with *N*-bromosuccinimide (NBS) in dichloromethane afforded allylic bromide **13** in a 24% yield. Treatment of **13** with sodium acetate in *N*,*N*-dimethylformamide (DMF) afforded allylic acetate **15**. Catalytic hydrogenation of **15** with 5% palladium–carbon (Pd–C) afforded acetate **17**. Decarboxylation of **17** with anhydrous phosphoric acid afforded **19**. Protection

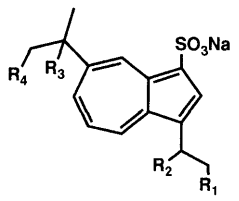
TABLE I. Anti-peptic Activity of the Metabolites **3**—**7** and the Reference Compounds **2** and **1**

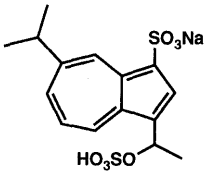
Compd. No.	Concentration (mM)	Inhibition(%)	IC_{50}[a] (mM)
3	1	16.0	3.8
	3	37.2	
	10	100.0	
4	1	1.1	2.9
	3	51.1	
	10	81.9	
5	0.3	13.8	1.5
	1	30.9	
	3	73.4	
6	10	41.5	>10.0
7	10	33.8	>10.0
2	1	7.5	2.6
	3	66.7	
	10	93.6	
1	0.1	12.3	0.5
	0.3	21.7	
	1	80.7	

a) IC_{50} values were calculated from the concentration–inhibition relations by the method of least squares.



1
(KT1-32)





8

	R_1	R_2	R_3	R_4
2	H	OH	H	H
3	OH	H	H	H
4	H	H	H	OH
5	H	H	OH	H
6	OH	H	H	OH
7	OH	H	OH	H

Chart 1

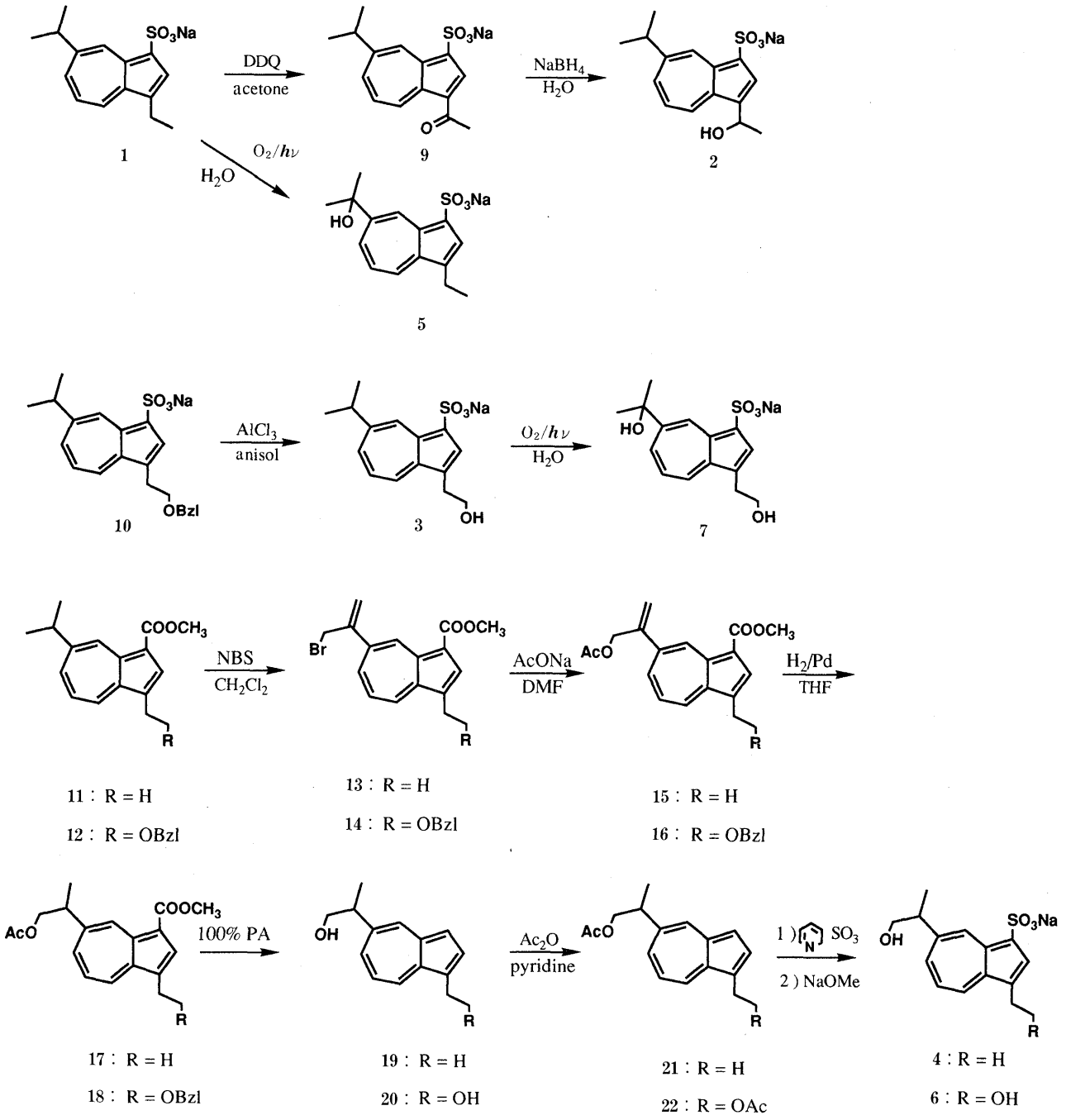


Chart 2

of **19** with acetic anhydride in pyridine afforded acetate **21** in a 93.5% yield. Sulfonation of **21** with a pyridine–sulfur trioxide complex in benzene followed by treatment with a sodium methoxide afforded sodium salt **4**. Compound **6** was prepared from **12** in the same manner as in the case of **4**.

Biological Activities Table I summarizes the anti-peptic activity of the metabolites **3**—**7** and the reference compounds **2** and **1**. All the synthesized derivatives showed lower anti-peptic activity than **1**. Dihydroxylated derivatives (**6** and **7**) exhibited greatly decreased activity. The decrease of anti-peptic activity by the introduction of a hydroxy group at the alkyl side chain on azulene suggests that the alkyl groups without hydroxy groups may play important roles in the manifestation of anti-peptic activity.

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 with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given as values (ppm): s, singlet; d, doublet; dd, double doublet; ddd, doublet of doublets of doublets; t, triplet; q, quartet; br, broad; sept, septet; m, multiplet. Mass spectra (MS) were taken on a Hitachi M-80B spectrometer. For column chromatography, silica gel (Merck, Kieselgel 60, 70—230 mesh) was used.

Sodium 3-Acetyl-7-isopropyl-1-azulenesulfonate (9) 2,3-Dichloro-5,6-dicyano-*p*-bezoquinone (DDQ) (16.6 g, 7.3×10^{-2} mol) was added to a stirred solution of **1**[1] (10.0 g, 3.3×10^{-2} mol) in 10% aqueous acetone (100 ml) and the mixture was stirred at room temperature for 1 h. The solvent was evaporated and dioxane (100 ml) was added to the residual material. DDQ-H was filtered off, and the filtrate was evaporated. The

residue was purified by column chromatography ($CHCl_3$: MeOH = 3 : 1) to give **9** (7.5 g, 72%) as red violet crystals. mp 158—166 °C (dec.). IR (KBr) cm^{-1}: 2950, 1650, 1200. ^{1}H-NMR (90 MHz, CD_3OD) δ: 1.43 (6H, d, iso-Pr–CH_3), 2.66 (3H, s, $COCH_3$), 3.28 (1H, sept, iso-Pr–CH), 7.77 (1H, dd, C_5-H), 7.98 (1H, dd, C_6-H), 8.58 (1H, s, C_2-H), 9.42 (1H, d, C_8-H), 9.75 (1H, d, C_4-H).

Sodium 3-(1-Hydroxyethyl)-7-isopropyl-1-azulenesulfonate (2) $NaBH_4$ (1.0 g, 2.6×10^{-2} mol) was added to a stirred solution of **9** (6.0 g, 1.9×10^{-2} mol) in H_2O (50 ml) at room temperature. The reaction mixture was stirred at room temperature for 4 h, and extracted with *n*-BuOH. After removal of the solvent, the residue was passed through a column of TSK gel (Toyopearl HW-40) (H_2O) to give **2** (5.1 g, 85%)as violet crystals. mp > 280 °C. IR (KBr) cm^{-1}: 3400, 2950, 1420, 1180. ^{1}H-NMR (90 MHz, CD_3OD) δ: 1.39 (6H, d, iso-Pr–CH_3), 1.60 (3H, d, CH–C$\underline{H}_3$), 3.20 (1H, sept, iso-Pr–CH), 5.54 (1H, q, C$\underline{H}$–OH), 7.36 (1H, dd, C_5-H), 7.78 (1H, dd, C_6-H), 8.20 (1H, s, C_2-H), 8.50 (1H, d, C_4-H), 9.27 (1H, d, C_8-H).

Sodium 3-Ethyl-7-(1-hydroxy-1-methylethyl)-1-azulenesulfonate (5) A solution of **1**[1] (10 g, 3.3×10^{-2} mol) in H_2O (500 ml) was irradiated with a 30 W low-pressure Hg lamp for 68 h under O_2-gas bubbling at room temperature and then concentrated *in vacuo*. The residue was purified by Sephadex G-10 column chromatography (H_2O) to give **5** (1.1 g, 10%) as violet crystals. mp > 280 °C. IR (KBr) cm^{-1}: 3400, 1210. ^{1}H-NMR (90 MHz, CD_3OD) δ: 1.36 (3H, t, Et–CH_3), 1.72 (6H, s, C(OH)C$\underline{H}_3$), 3.04 (2H, q, Et–CH_2), 7.32 (1H, dd, C_5-H), 8.05 (1H, s, C_2-H), 8.18 (1H, ddd, C_6-H), 8.36 (1H, dd, C_4-H), 9.46 (1H, d, C_8-H).

Sodium 3-(2-Hydroxyethyl)-7-(1-hydroxy-1-methylethyl)-1-azulenesulfonate (7) Violet crystals. mp > 280 °C. IR (KBr) cm^{-1}: 3450, 1200. ^{1}H-NMR (90 MHz, CD_3OD) δ: 1.70 (6H, s, C(OH)C$\underline{H}_3$), 3.24 (2H, t, C$\underline{H}_2$–CH_2–OH), 3.83 (2H, t, CH_2–C$\underline{H}_2$–OH), 7.32 (1H, dd, C_5-H), 8.05 (1H, s, C_2-H), 8.18 (1H, ddd, C_6-H), 8.38 (1H, dd, C_4-H), 9.46 (1H, d, C_8-H).

Sodium 3-(2-Hydroxyethyl)-7-isopropyl-1-azulenesulfonate (3) A solution of **10** (10.6 g, 2.6×10^{-2} mol) in anisole (50 ml) was added to a suspension of $AlCl_3$ (10.4 g, 7.8×10^{-2} mol) in anisole (50 ml) at room temperature and the mixture was stirred for 2 h. The reaction mixture was poured into ice water (50 ml) and the mixture was extracted with *n*-BuOH. The extract was washed with brine and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography ($CHCl_3$: MeOH = 3 : 1) to give **3** (7.8 g, 95%) as violet crystals. mp > 280 °C. IR (KBr) cm^{-1}: 3400, 2950, 1160. ^{1}H-NMR (90 MHz, CD_3OD) δ: 1.40 (6H, d, iso-Pr–CH_3), 3.20 (1H, sept, iso-Pr–CH), 3.24 (2H, t, C$\underline{H}_2$–CH_2–OH), 3.84 (2H, t, CH_2–C$\underline{H}_2$–OH), 7.30 (1H, dd, C_5-H), 7.73 (1H, ddd, C_6-H), 8.06 (1H, s, C_2-H), 8.36 (1H, dd, C_4-H), 9.23 (1H, d, C_8-H).

Methyl 7-(1-Bromomethylvinyl)-3-ethyl-1-azulenecarboxylate (13) *N*-Bromosuccinimide (NBS) (83 g, 4.6×10^{-1} mol) was added at 0 °C to a stirred solution of **11**[1] (60 g, 2.3×10^{-1} mol) in CH_2Cl_2 (300 ml) and the mixture was stirred at room temperature for 2 h. The solution was poured into water, and the organic layer was washed with brine, dried and evaporated. The residue was purified by column chromatography (hexane : EtOAc = 10 : 1) to give **13** (18.7 g, 24%) as a violet oil. MS *m/z*: 333 (M^+). IR (neat) cm^{-1}: 2950, 1700, 1460. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.35 (3H, t, Et–CH_3), 3.05 (2H, q, Et–CH_2), 3.90 (3H, s, $COOCH_3$), 4.50 (2H, s, Br–CH_2), 5.60 (2H, d, C=CH_2), 7.26 (1H, dd, C_5-H), 7.80 (1H, dd, C_6-H), 8.23 (1H, s, C_2-H), 8.30 (1H, d, C_4-H), 9.78 (1H, d, C_8-H).

Methyl 7-(1-Bromoethylvinyl)-3-(2-benzyloxyethyl)-1-azulenecarboxylate (14) A violet oil, 22.5% yield. MS *m/z*: 440(M^+). IR (neat) cm^{-1}: 2950, 1730, 1450. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.30 (2H, t, C$\underline{H}_2$–CH_2–OBzl), 3.80 (2H, t, CH_2–C$\underline{H}_2$–OBzl), 3.90 (3H, s, $COOCH_3$), 4.52 (2H, s, C$\underline{H}_2$–C_6H_5), 4.53 (2H, s, Br–CH_2), 5.60 (2H, d, C=CH_2), 7.28 (5H, s, C_6H_5), 7.40 (1H, dd, C_5-H), 7.85 (1H, dd, C_6-H), 8.26 (1H, s, C_2-H), 8.38 (1H, d, C_4-H), 9.78 (1H, d, C_8-H).

Methyl 7-(1-Acetoxymethylvinyl)-3-ethyl-1-azulenecarboxylate (15) A solution of sodium acetate (7.3 g, 8.9×10^{-2} mol) in DMF (20 ml) was gradually added to a stirred solution of **13** (15 g, 4.5×10^{-2} mol) in DMF (30 ml) at room temperature. After stirring for 10 h at 50 °C, the solution was poured into water and extracted with EtOAc. The extract was washed with brine, dried and evaporated. The residue was purified by column chromatography (hexane : EtOAc = 10 : 1) to give **15** (12.3 g, 87.0%) as a blue violet oil. MS *m/z*: 313 (M^+). IR (neat) cm^{-1}: 2950, 1740. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.38 (3H, t, Et–CH_3), 2.05, (3H, s, AcO), 3.02 (2H, q, Et–CH_2), 3.90 (3H, s, $COOCH_3$), 5.08 (2H, s, AcO–C$\underline{H}_2$), 5.50 (2H, d, C=CH_2), 7.35 (1H, dd, C_5-H), 7.78 (1H, dd, C_6-H), 8.23 (1H, s, C_2-H), 8.30 (1H, d, C_4-H), 9.78 (1H, d, C_8-H).

Methyl 7-(1-Acetoxymethylvinyl)-3-(2-benzyloxyethyl)-1-azulenecarboxylate (16) A violet oil, 80% yield. MS *m/z*: 418 (M^+), IR (neat) cm^{-1}: 2980, 1750, 1700. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 2.06 (3H, s, OAc), 3.30 (2H, t, C$\underline{H}_2$–CH_2–OBzl), 3.80 (2H, t, CH_2–C$\underline{H}_2$–OBzl), 3.90 (3H, s, $COOCH_3$), 4.52 (2H, s, C$\underline{H}_2$–C_6H_5), 5.10 (2H, s, C$\underline{H}_2$–OAc), 5.58 (2H, d, C=CH_2), 7.28 (5H, s, C_6H_5), 7.40 (1H, dd, C_5-H), 7.80 (1H, dd, C_6-H), 8.26 (1H, s, C_2-H), 8.36 (1H, d, C_4-H), 9.78 (1H, d, C_8-H).

Methyl 7-(2-Acetoxy-1-methylethyl)-3-ethyl-1-azulenecarboxylate (17) A solution of **15** (10 g, 3.2×10^{-2} mol) in EtOH (50 ml) was stirred with 5% Pd–C (200 mg) under H_2 gas at room temperature for 10 h. The catalyst was filtered off, and the solution was evaporated. The residue was purified by column chromatography (hexane : EtOAc = 10 : 1) to give **17** (7.8 g, 78%) as a violet oil. MS *m/z*: 314 (M^+). IR (neat) cm^{-1}: 2950, 1740. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.35 (3H, t, Et–CH_3), 1.42 (3H, d, CH–C$\underline{H}_3$), 1.95 (3H, s, OAc), 3.00 (2H, q, Et–CH_2), 3.35 (1H, m, C$\underline{H}$–CH_3), 3.90 (3H, s, $COOCH_3$), 4.30 (2H, d, CH–C$\underline{H}_2$–OAc), 7.30 (1H, dd, C_5-H), 7.62 (1H, dd, C_6-H), 8.20 (1H, s, C_2-H), 8.26 (1H, d, C_4-H), 9.63 (1H, d, C_8-H).

Methyl 7-(2-Acetoxy-1-methylethyl)-3-(2-benzyloxyethyl)-1-azulenecarboxylate (18) A violet oil, 75% yield. MS *m/z*: 420 (M^+) . IR (neat) cm^{-1}: 2950, 1740, 1700. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.47 (3H, d, CH–C$\underline{H}_3$), 1.98 (3H, s, OAc), 3.30 (2H, t, C$\underline{H}_2$–CH_2–OBzl), 3.32 (1H, m, C$\underline{H}$–CH_3), 3.80 (3H, t, CH_2–C$\underline{H}_2$–OBzl), 3.94 (3H, s, $COOCH_3$), 4.30 (2H, d, C$\underline{H}_2$–OAc), 4.54 (2H, s, C$\underline{H}_2$–C_6H_5), 7.30 (5H, s, C_6H_5), 7.40 (1H, dd, C_5-H) 7.69 (1H, dd, C_6-H), 8.25 (1H, s, C_2-H), 8.38 (1H, d, C_4-H), 9.68 (1H, d, C_8-H).

1-Ethyl-5-(2-hydroxy-1-methylethyl)azulene (19) The treatment of **17** with anhydrous phosphoric acid in the same manner as described previously[1] afforded **19** as violet prisms. mp 62—63 °C. MS *m/z*: 214 (M^+). IR (KBr) cm^{-1}: 3250, 2950. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.34 (3H, d, CH–C$\underline{H}_3$), 1.35 (3H, t, Et–CH_3), 3.05 (2H, q, Et–CH_2), 3.20 (1H, m, C$\underline{H}$–CH_3), 3.78 (2H, d, C$\underline{H}_2$–OH), 7.05 (1H, dd, C_7-H), 7.25 (1H, d, C_3-H), 7.40 (1H, dd, C_6-H), 7.78 (1H, d, C_2-H), 8.16 (1H, d, C_4-H), 8.18 (1H, d, C_8-H).

1-(2-Hydroxyethyl)-5-(2-hydroxy-1-methylethyl)azulene (20) A violet oil, 78% yield. MS *m/z*: 230 (M^+). IR (neat) cm^{-1}: 3350, 2900, 1720. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.35 (3H, d, CH–C$\underline{H}_3$), 3.12 (1H, m, C$\underline{H}$–CH_3), 3.30 (2H, t, C$\underline{H}_2$–CH_2–OH), 3.78 (2H, d, CH–C$\underline{H}_2$–OH), 3.80 (2H, t, CH_2–C$\underline{H}_2$–OH), 7.00 (1H, dd, C_7-H), 7.26 (1H, d, C_3-H), 7.48 (1H, dd, C_6-H), 7.78 (1H, d, C_2-H), 8.20 (1H, d, C_4-H), 8.25 (1H, d, C_8-H).

5-(2-Acetoxy-1-methyl)-1-ethylazulene (21) Acetic anhydride (2.2 g, 2.2×10^{-2} mol) was added to a stirred ice-cooled solution of **19** (2.6 g, 1.1×10^{-2} mol) in dry pyridine (20 ml) and the mixture was stirred at 0 °C for 2 h. The mixture was poured into water, and extracted with EtOAc. The extract was washed with water, dried over anhydrous Na_2SO_4 and evaported. The residue was column chromatography (hexane) to give **21** (2.9 g, 93.5%) as a blue violet oil. MS *m/z*: 256 (M^+). IR (neat) cm^{-1}: 2950, 1730, 1450. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.37 (3H, t, Et–CH_3), 1.38 (6H, d, CH–C$\underline{H}_3$), 1.98 (3H, s, OAc), 3.04 (2H, q, Et–CH_2), 3.17 (1H, m, C$\underline{H}$–CH_3), 4.24 (2H, d, C$\underline{H}_2$–OAc), 7.06 (1H, dd, C_7-H), 7.26 (1H, d, C_3-H), 7.42 (1H, dd, C_6-H), 7.78 (1H, d, C_2-H), 8.16 (1H, d, C_4-H), 8.20 (1H, d, C_8-H).

1-(2-Acetoxyethyl)-5-(2-acetoxy-1-methylethyl)azulene (22) A blue oil, 83% yield. MS *m/z*: 314 (M^+). IR (neat) cm^{-1}: 2960, 1740, 1400. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.40 (3H, d, CH–C$\underline{H}_3$), 1.98 (3H, s, CH–CH_2–OAc), 2.03 (3H, s, CH_2–CH_2–OAc), 3.30 (1H, m, C$\underline{H}$–CH_3), 3.39 (2H, t, C$\underline{H}_2$–CH_2–OAc), 4.30 (2H, t, CH_2–C$\underline{H}_2$–OAc), 7.04 (1H, dd, C_7-H), 7.28 (2H, d, C_3-H), 7.48 (1H, dd, C_6-H), 7.80 (1H, d, C_3-H), 8.21 (1H, d, C_4-H), 8.26 (1H, d, C_8-H).

Sodium 3-Ethyl-7-(2-hydroxy-1-methylethyl)-1-azulenesulfonate (4) The treatment of **21** with pyridine–sulfur trioxide complex in the same manner as described previously[1] afforded **4** as violet crystals. mp > 280 °C. IR (KBr) cm^{-1}: 3400, 1200. ^{1}H-NMR (90 MHz, CD_3OD) δ: 1.37 (3H, t, Et–CH_3), 1.40 (3H, d, CH–CH_3), 3.04 (2H, q, Et–CH_2), 3.17 (1H, m, CH–CH_3), 3.74 (1H, dd, CH_2–OH), 3.86 (1H, dd, CH_2–OH), 7.28 (1H, dd, C_5-H), 7.70 (1H, dd, C_6-H), 8.06 (1H, s, C_2-H), 8.33 (1H, d, C_4-H), 9.15 (1H, d, C_8-H).

Sodium 3-(2-Hydroxyethyl)-7-(2-hydroxy-1-methylethyl)-1-azulenesulfonate (6) Violet crystals, mp > 280 °C. IR (KBr) cm^{-1}: 3400, 1650, 1300. ^{1}H-NMR (90 MHz, CD_3OD) δ: 1.40 (3H, d, CH–C$\underline{H}_3$), 3.16 (1H, m, C$\underline{H}$–CH_3), 3.23 (2H, t, C$\underline{H}_2$–CH_2–OH), 3.75 (2H, d, CH–C$\underline{H}_2$–OH), 3.82 (2H, t, CH_2–C$\underline{H}_2$–OH), 7.29 (1H, dd, C_5-H), 7.70 (1H, dd, C_6-H), 8.05 (1H, s, C_2-H), 8.37 (1H, d, C_4-H), 9.15 (1H, d, C_8-H).

Anti-peptic Activity Anti-peptic activity was measured according to the modified method of Thiemer *et al.*[7] A mixture of bovine serum albumin (BSA) 1 ml (5 mg/ml), 0.3 ml of 0.1 N HCl and 2 ml of water, with or without a test drug, was pre-incubated at 37 °C for 5 min. The reaction

was started by the addition of 0.5 ml of enzyme solution (10 μg pepsin/ml of 0.5 N HCl) and was stopped by the addition of 2 ml of 10% trichloroacetic acid after 10 min incubation at 37 °C. The amount of hydrolyzed BSA was measured according to the method of Udenfriend *et al.*[8] After centrifugation of the reaction mixture at 3000 rpm for 10 min, 0.1 ml aliquot of supernatant fraction was transferred to a test tube and 2 ml of 0.2 M boric acid (pH 9.2) was added followed by 1 ml of fluorescamine solution (0.3 mg fluorescamine/ml of acetone). Then the contents of the tube was mixed immediately and the fluorescence at 470 nm resulting from activation at 390 nm was measured in a spectrofluorometer. The percent inhibition was calculated as follows:

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

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

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