藥物食品檢驗局調查研究年報．15: 282-283. 1997.
Ann. Rept. NLFD Taiwan R.O.C. 15: 282-283. 1997.

Acute Toxicity of Higenamine in Mice

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Received: March 1, 1996; Revision accepted: August 3, 1996

Higenamine is a potent cardiotonic benzylisoquinoline alkaloid, isolated from the root of *Aconitum japonicum* Thunb. (Ranunclaceae) (1, 2), and also from several other natural products (3 ~ 7). *Aconitum* has been used as a cardiotonic, diuretic, and analgesic in traditional Chinese medicine. It has been reported that higenamine possesses cardiac stimulation activity mediated by the activation of adenylcyclase and the subsequent elevation of cyclic AMP (8). Higenamine directly interacts with the adrenergic β_1 and β_2 receptors *in vitro* and exhibits both potent inotropic and chronotropic effects in animals and humans (9). It also strongly inhibits human dihydropteridine reductase (10). Intravenous infusion of higenamine to a small group of bradyarrhythmic patients has been reported (11). The root of *Aconitum* is believed to be a narrow therapeutic window herb in traditional Chinese medicine and higenamine may cause toxic effect in animals and humans. There are no toxicology reports on higenamine in the literature. This paper describes the acute toxicity of higenamine in mice.

Higenamine was synthesized as previously reported (12) and confirmed by NMR, FT-IR, and MS. Male and female ICR mice, weighing 15 to 18 g, were used for the acute toxicity LD_{50} test and were supplied by the Animal Center of the Medical College, National Taiwan University (Taipei, Taiwan) and housed in our laboratory. A total of 240 mice was used in this experiment. Higenamine HCl was dissolved in water for injection, and an aliquot of 0.15 to 0.28 ml of higenamine HCl solution (5 mg/ml) was injected into the tail vein of mice. According to the method of Litchfield and Wilcoxon (13), the LD_{50} results (95 % confidence limits) were evaluated after 72 hours, when the drug was administered intravenously. The acute toxicity LD_{50} was estimated using the computer program of PHARM/PCS (14).

The LD_{50} of higenamine HCl was about 50 mg/kg ($p > 0.05$) for both male and female mice and so there were no effects due to differences in gender (Table 1). Most of the mice died of tachycardia and heavy breathing within 5 min after an *i.v.* dose of 60 mg/kg. After autopsy, the appearances of organs and tissue between the test and control mice were not significantly different. However, no animals died when higenamine was administered orally at a dose of 2.0 g/kg. These results indicate that dose-dependent increases in lethality were observed only after intravenous administration of higenamine to mice. The reduction in toxicity after application by the oral route may be due to poor absorption or instability in the GI track or first-pass effect, but the exact mechanism remains to be clarified. The results of this experiment may act as a useful guide for the dose adjustment of higenamine in clinical and animal studies.

Acknowledgements

The authors are grateful to the National Laboratories of Foods and Drugs, Department of Health and Cheng's Foundation of Pharmaceutical Sciences for their support of this work.

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Table 1 LD_{50} of higenamine HCl at 95 % confidence limits in mice (ICR) by *i.v.* administration.

Sex	Dose (mg/kg)	Number of animals tested	Number of animals killed	LD_{50}[b] (mg/kg)	(95 % confidence limits) (mg/kg)
	Control[a]	20	0		
	40	20	2		
	45	20	4		
Male	50	20	13	48.90	46.24–51.71
	55	20	18		
	60	20	16		
	Control[a]	20	0		
	40	20	3		
	45	20	5		
Female	50	20	8	49.51	46.58–52.61
	55	20	15		
	60	20	18		

[a] The control animals were injected with 0.25 ml of water.
[b] Estimation by Lichfield and Wilconson II (14).

原載於 Planta Medica. 1997. 63: 95-96.

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Flavonolacyl Glycosides of the Aerial Parts of *Eryngium campestre*

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Received: February 27, 1996; Revision accepted: August 5, 1996

The roots and herbs of *Eryngium campestre* L. (Umbelliferae) have been used in European traditional medicine as an expectorant, diuretic, and spasmolytic remedy (1). Recently, we reported the isolation and identification of flavonol derivatives from the dried above-ground parts of the plant (2). As a result of our continued studies, we now describe the isolation and characterization of a new acylated flavonol (**1**), together with ten known compounds.

The aerial parts of *E. campestre* were collected in August 1990 in Ásotthalom (Hungary). A voucher specimen (No. 583420) was deposited at the Herbarium of the Museum of Natural Sciences in Budapest. The dried aerial parts of *E. campestre* were extracted with MeOH at room temperature. The crude extract was concentrated *in vacuo* and partitioned between petroleum ether, EtOAc, and H_2O. After repeated chromatography, including CC and preparative TLC, the petroleum ether phase yielded falcarinol, and the EtOAc phase yielded tiliroside, kaempferol 3-*O*-β-D-glucoside-7-*O*-α-L-rhamnoside, rutin, kaempferol, quercetin, isorhamnetin, caffeic acid, chlorogenic acid, mannitol and a mixture of **1** + **2**. The mixture **1** + **2** showed UV maxima at 269, 302(sh), 317, 360(sh) nm indicating flavonol derivatives esterified with a hydroxycinnamic acid. The FAB-mass spectrum exhibited fragment peaks at $m/z = 633$ $[M + K]^+$, 617 $[M + Na]^+$, and 595 $[M + H]^+$, corresponding to a molecular mass of 594. Examination of the ^{1}H-NMR spectrum of **1** + **2** revealed a mixture of *cis*- and *trans*-*p*-coumaroylkaempferol monoglycosides in a ratio of 1:2. ^{1}H-NMR (400 MHz, MeOH-d_4): (**1** = *cis*-isomer) $\delta = 8.00$ (2H, d, $J = 8.6$ Hz, H-2′, 6′), 7.63 (2H, d, $J = 8.5$ Hz, H-2‴, 6‴), 6.88 (1H, d, $J = 12.7$ Hz, H-7‴), 6.87 (2H, d, $J = 8.6$ Hz, H-3′, 5′), 6.69 (2H, d, $J = 8.5$ Hz, H-3‴, 5‴), 6.35 (1H, br. s, H-8), 6.17 (1H, br. s, H-6), 5.86 (1H, d, $J = 12.7$ Hz, H-8‴), 5.64 (1H, d, $J = 8.4$ Hz, H-1″ of Glc), 5.02 (1H, t, $J = 8.4$ Hz, H-2″ of Glc), 3.9–3.3 (5H, m, sugar protons); (**2** = *trans*-isomer) data identical with those reported (3). Alkaline hydrolysis afforded *p*-coumaric acid isomers and kaempferol 3-*O*-β-D-glucoside. The position of attachment of the esterifying acid was determined in a proton decoupling experiment. On irradiation of the proton signals at δ [illegible].02 (**1**) and 5.03 (**2**), which were attributed to the protons on the carbon atoms carrying ester groups, the formation of singlet signals of the anomeric protons [$\delta = 5.64$ (**1**) and 5.65 (**2**)] was observed. Thus, compound **1** was defined as kaempferol 3-*O*-β-D-(2′-*Z*-*p*-coumaroylglucoside) and **2** as its *trans*-isomer. Compound **2** was isolated earlier from *Quercus suber* (3), but the *cis*-isomer (**1**) has previously not been reported.

The light-induced isomerization of cinnamic acid derivatives is known in the literature, so transformations of **1** and **2** were studied (4). Pure *trans*-isomer (**2**), which was separated in a small quantity by analytical HPLC (LiChrospher 100 RP-18, MeCN/H_2O, 2:3 as eluent), after 1 h exposures of UV_{366nm} light yielded the equlibrium mixture (1:2) of **1** and **2**. On this basis it cannot be excluded that **1** was formed during the isolation procedure. Information in detail on the work-up procedure and copies of the original spectra are obtainable from the author of correspondence.

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