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Liposomal encapsulation of lauroyl-indapamide in the presence of divalent or trivalent cations

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Divalent or trivalent cations such as Ca^{2+}, Ba^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+} and Fe^{3+} can cause a significant increase in the entrapment efficiency of lauroyl-indapamide in liposomes, from about 5% to more than 90%, which suggests that the presence of these ions plays an important role in the encapsulation of lauroyl-indapamide.

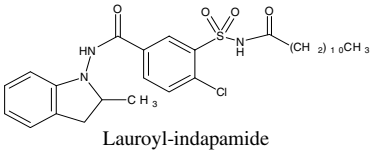
Lauroyl-indapamide is a new compound derived from indapamide, an antihypertensive diuretic (Gerber et al. 1985; Pruss and Wolf 1983; Weiss et al. 1994), which is still a preferred option for the first-line treatment of hypertension (Clarke 1991; De Leeuw 2001). Lauroyl-indapamide was synthesized for the purposes of increasing the elimination half-life of indapamide *in vivo* and obtaining a long-term antihypertensive action.

Liposomes have been tried to make lauroyl-indapamide suitable for the injection route to achieve the above aims. Lauroyl-indapamide is a lipophilic drug, soluble in methanol, ethanol and ethylacetate, partially soluble in ethyl ether and sparingly dissolved in petroleum ether and water. There should be no problem in encapsulating this lipophilic compound with a long saturated hydrocarbon chain substituted at the free terminal amino into liposomes. But as a matter of fact, it is quite difficult to encapsulate it by the ethanol injection method, and only in the presence of divalent or trivalent cations such as Ca^{2+}, Ba^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+}, Fe^{3+} etc. could lauroyl-indapamide be encapsulated into liposomes. Liposomes with different membrane compositions have also been tried. The results are shown in the Table, from which we can see that liposomes with different membrane compositions have no influence on the entrapment efficiency of lauroyl-indapamide, as although a cationically charged lipid enhanced it a little, it is still much lower compared with that achieved by the addition of divalent or trivalent cations, which indicates that cations play an important role in the liposomal encapsulation of lauroyl-indapamide, a lipophilic weak acid. With regard to liposomal encapsulation of weak acids, it has been reported that a calcium acetate gradient has been used for remote loading of weak acids and a much higher entrapment efficiency was achieved (Clerc and Barenholz 1995; Hwang et al. 1999). Bailey even used calcium and ethanol to condense DNA plasmids to encapsulate them into liposomes by incubating the preformed liposomes with DNA in an ethanol and calcium solution (Bailey and Sullivan 2000). But all these methods differ from that presented in this study. The mechanism of liposomal encapsulation of lauroyl-indapamide in the presence of divalent or trivalent cations using the ethanol injection method still deserves further investigation.

Table: Influence of divalent and trivalent ions on entrapment efficiency (n = 3)

Membrane composition	Molar ratio	Ions	Entrapment efficiency (% ± SD)
sPC/CH	4 : 1	−*	5.2 ± 2.1
sPC/CH/PS	4 : 1 : 0.25	−*	6.5 ± 1.4
sPC/CH/PG	4 : 1 : 0.25	−*	3.4 ± 1.3
sPC/CH/OA	4 : 1 : 0.25	−*	11.8 ± 3.5
sPC/CH	4 : 1	Ca^{2+}	92.4 ± 6.8
sPC/CH	4 : 1	Ba^{2+}	94.3 ± 5.6
sPC/CH	4 : 1	Mg^{2+}	97.8 ± 10.2
sPC/CH	4 : 1	Fe^{2+}	95.6 ± 8.3
sPC/CH	4 : 1	Zn^{2+}	96.2 ± 7.1
sPC/CH	4 : 1	Fe^{3+}	90.6 ± 6.9
sPC/CH	4 : 1	Al^{3+}	95.7 ± 5.2
sPC/CH/PS	4 : 1 : 0.25	Ca^{2+}	94.5 ± 4.8
sPC/CH/PG	4 : 1 : 0.25	Ca^{2+}	93.4 ± 7.2
sPC/CH/OA	4 : 1 : 0.25	Ca^{2+}	92.9 ± 6.4

* – denotes no added divalent or trivalent cationics



Lauroyl-indapamide

Experimental

1. Materials

Lauroyl-indapamide was synthesized from indapamide and lauroylchloride and purified by silica column chromatography, and its identity and purity were confirmed by HPLC, UV, IR and ^{1}H NMR; HPLC grade methanol and tetrahydrofuran were purchased from Concord (Tianjin, China). Epikuron 200 soybean phosphatidylcholine (sPC), phosphatidylserine (PS) and phosphatidylglycerol (PG) were purchased from Degussa Bioactives (Germany), and cholesterol (CH) was purchased from Bodi Chemical Company (Tianjin, China) and octadecylamine (OA) from Fluka Chemika (Germany). Unless stated otherwise all the chemicals were analytical grade.

2. Preparation of liposomal lauroyl-indapamide

Liposomal lauroyl-indapamide was prepared by the normal ethanol injection method; the only difference was that the buffer solution contained divalent or trivalent cations. Briefly: lauroyl-indapamide (about 1/25 the weight of lipid) and lipid (sPC, CH, PS, PG and OA) were dissolved in absolute ethanol, which was then injected into acetate buffer solution (50 mM, pH 6) containing 0.5% divalent or trivalent cations (Ca^{2+}, Ba^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+}, Fe^{3+} etc.) thermostated at 55 °C under magnetic stirring, and the ethanol was evaporated off under reduced pressure, the liposomal preparation was then extruded through Millipore membranes of 0.8 μm, 0.45 μm, 0.22 μm in sequence.

3. Determination of the entrapment efficiency of liposomal lauroyl-indapamide

The separation of liposomal lauroyl-indapamide was achieved on a Sephadex G-50 column (16 × 2.5 cm) eluted with acetate buffer solution (50 mM, pH 6) at a flow rate of 0.5 ml/min, the liposome part was pooled. Before loading the samples, the column was presaturated with blank liposomes.

Lauroyl-indapamide was assayed by HPLC against a calibration curve, the separation was achieved on a Kromasil ODS column (250 × 4.6 mm, 5 μm) with methanol-tetrahydrofuran-0.2% trifluoroacetic acid (170 : 15 : 20, v/v/v) as the mobile phase flowing at 0.8 ml/min and the detection wavelength was set at 240 nm.

The entrapment efficiency (EE) was calculated according to the following equation:

$$EE\ \% = W_{in}/W_{total} \times 100\% \quad (1)$$

Where W_{in} is the amount of lauroyl-indapamide in liposomes and W_{total} is the total amount of lauroyl-indapamide in the liposomal preparation.

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An unusual new sulfated triterpene saponin from *Arenaria juncea*

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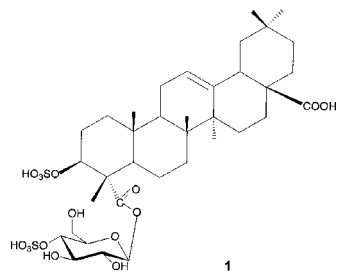
A novel triterpene saponin, 3-*O*-sulfate-gypsogenic acid-23-*O*-β-D-4-*O*-sulfate-glucopyranoside (junceoside D), has been isolated from the roots of *Arenaria juncea*. The structure was characterized mainly by a combination of 2D-NMR techniques, mass spectrometry and chemical methods.

Arenaria juncea Bieb. roots endemic to China (Family: Caryophyllaceae) is well-known in the Traditional Chinese Medicine in the Shanxi, Gansu Hubei province as a substitute of the Chinese drug Yin-Chai-Hu (root of *Stellaria dichotoma* var. *lanceolata* Bge) (Cui et al. 1992) and is used to treat fever due to Yin-deficiency and fever in infant malnutrition. Our previous phytochemical studies on the methanolic extract of *Arenaria juncea* roots led to the isolation of five triterpene-saponins (Gaidi et al. 2001). A detailed further investigation of the same extract has led to the isolation of an additional triterpene-glycoside **1**. The concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *Arenaria juncea* Bieb. (Caryophyllaceae) was purified by precipitation with diethyl ether. The crude saponin mixture was further dialysed and subjected to multiple chromatographic steps over Sephadex LH-20 and medium pressure liquid chromatography (MPLC) over reversed-phase Si RP-18 yielding a saponin, junceoside D (**1**). Its structure was elucidated mainly by FABMS, MALDITOFMS, HRESIMS and by 600 MHz NMR spectroscopic analysis including 1D and 2D NMR experiments ($^1H-^1H$ COSY, TOCSY, NOESY, HSQC, and HMBC).



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Compound **1** was obtained as a yellow powder. The FABMS (negative-ion mode) exhibiting a quasi-molecu-