Supplementary information

Proton-independent activation of acid-sensing ion channels by alkaloid lindoldhamine from *Laurus nobilis*

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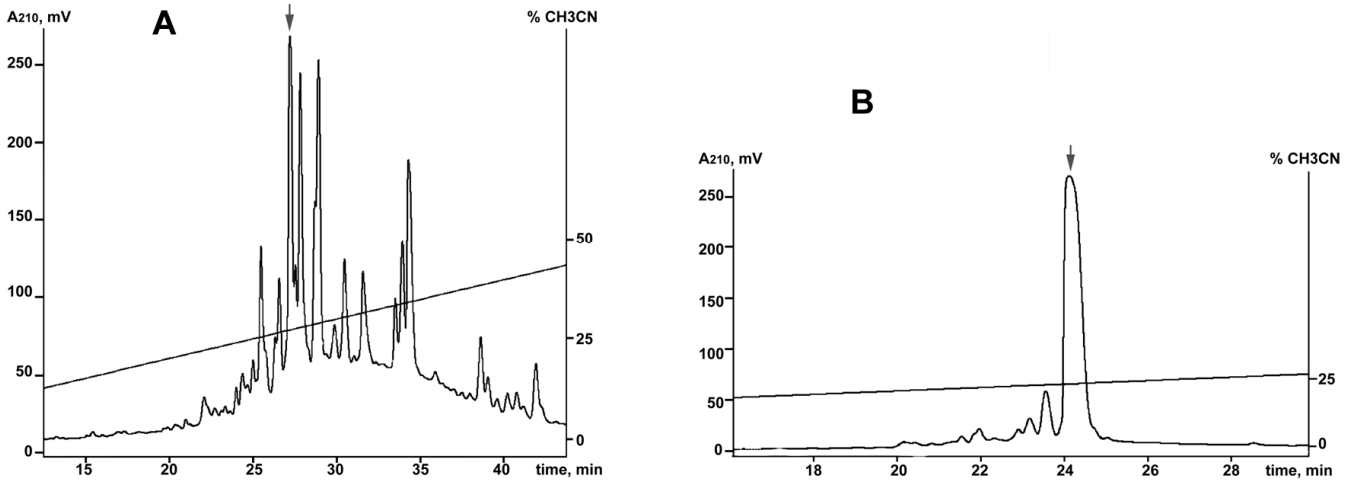
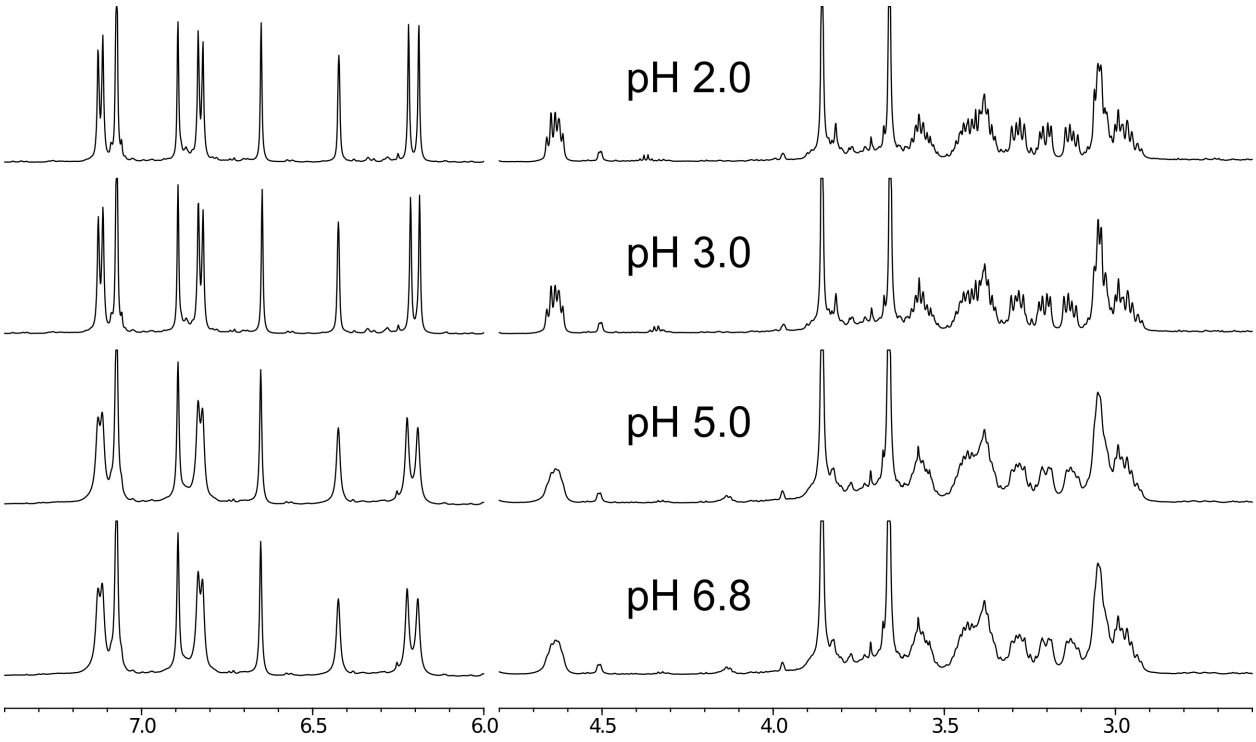


Supplementary Figure 1. Purification of LIN. *A,* The first separation stage of the acetic extract of *L. nobilis* leaves on a reverse-phase column LunaC18 (10x250mm) in 0.1% TFA with a flow rate 5 ml/min using a linear gradient of acetonitrile concentration. *B,* Final purification on a column VydacC18 (4.6x250mm) in 0.1% TFA with a flow rate of 1 ml/min using a linear gradient of acetonitrile concentration.

Fractions containing the active component are marked by an arrow.

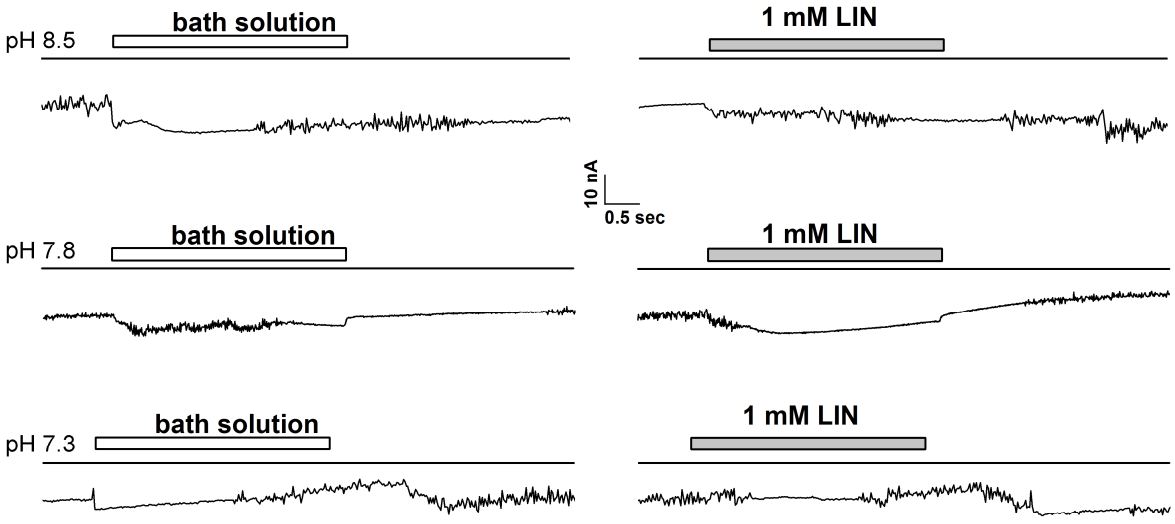
Atom	^{1}H ppm (J Hz)	^{13}C(^{15}N) ppm	NMR contacts[a)]	Atom	^{1}H ppm (J Hz)	^{13}C(^{15}N) ppm	NMR contacts[a)]
α	3.233 dd(6.0;13.9) 3.064 dd(6.8;13.9)	38.145	***1***,*8a,9,**10,14***	α'	3.314 dd(7.6,13.8) 3.167 dd(7.4,13.8)	38.496	***1'***,*8a',9',**10'14'*
1	4.643 dd(6.0;6.8)	55.20	***α***,*3,4a,**8**,8a,9,* **10,14**	1'	4.662 t(7.5)	55.64	***α'***,***3'***,*4a',**8'**,8a',* *9',***10'14'**
2	. [b)]	^{15}N 43.8	*4*	2'	.	^{15}N 43.8	*4'*
3	3.452 m 3.388 m	38.16	***4***,***1***,*4a*	3'	3.584 m 3.412 m	38.392	***4'***,***1'***,*4a'*
4	3.002 m 2.969 m	23.71	*2,**3**,**5**,4a,8a*	4'	3.051 m	23.99	*2',**3'**,**5'**,4a',8a'*
4a	.	123.42	*1,3,4,8*	4a'	.	123.12	*1',3',4',8'*
5	6.648 s	111.60	***4***,*6,7,8a,**6OMe***	5'	6.909 s	111.69	***4'***,*6',7',8a',* **6'OMe**
6	.	147.03	*5,8*	6'	.	147.21	*5',8'*
6OMe	3.657 s	55.21	*6,***5**	6'OMe	3.867 s	55.47	*6',***5'**
7	.	142.71	*5,8*	7'	.	143.04	*5',8'*
8	6.193 s	113.40	***α***,***1***,*4a,5,6,7*	8'	6.201 s	113.21	***α'***,***1'***,*4a',6',7'*
8a	.	122.37	*α,1,4,5*	8a'	.	122.90	*α',1',4',5'*
9	.	127.66	*α,1,2*	9'	.	129.73	*α',1',11'13'*
10	6.449 s	121.51	***α***,**1**,*11,12,14,* **11'13'**	10'14'	7.133 d(8.3)	130.78	**1'**,***α'***,**11'13'**,*12'*
11	.	143.20	*13,10*	11'13'	6.841 d(8.3)	117.49	**10'14'**,*9'*,**10**
12	.	146.09	*14,10*	12'	.	155.91	*10'14'*
13	7.086 s	117.17	*9,11*				
14	7.083 s	125.83	***α***,**1**,*10,12*				

Supplementary Table 1.
NMR chemical shifts (ppm), multiplicities (Hz) and NMR HMBC/ROESY/COSY contacts in LIN. Conditions: D_2O, pH 3.0, 14ºC, Bruker Avance III 600MHz. Atom numbering follows Fig. 1B.
[a)] Heavy atom number of corresponding NMR cross-peak outline HMBC contacts (*italic*), ROESY contacts (**bold**) and COSY ones (underlined)
[b)] Not applicable



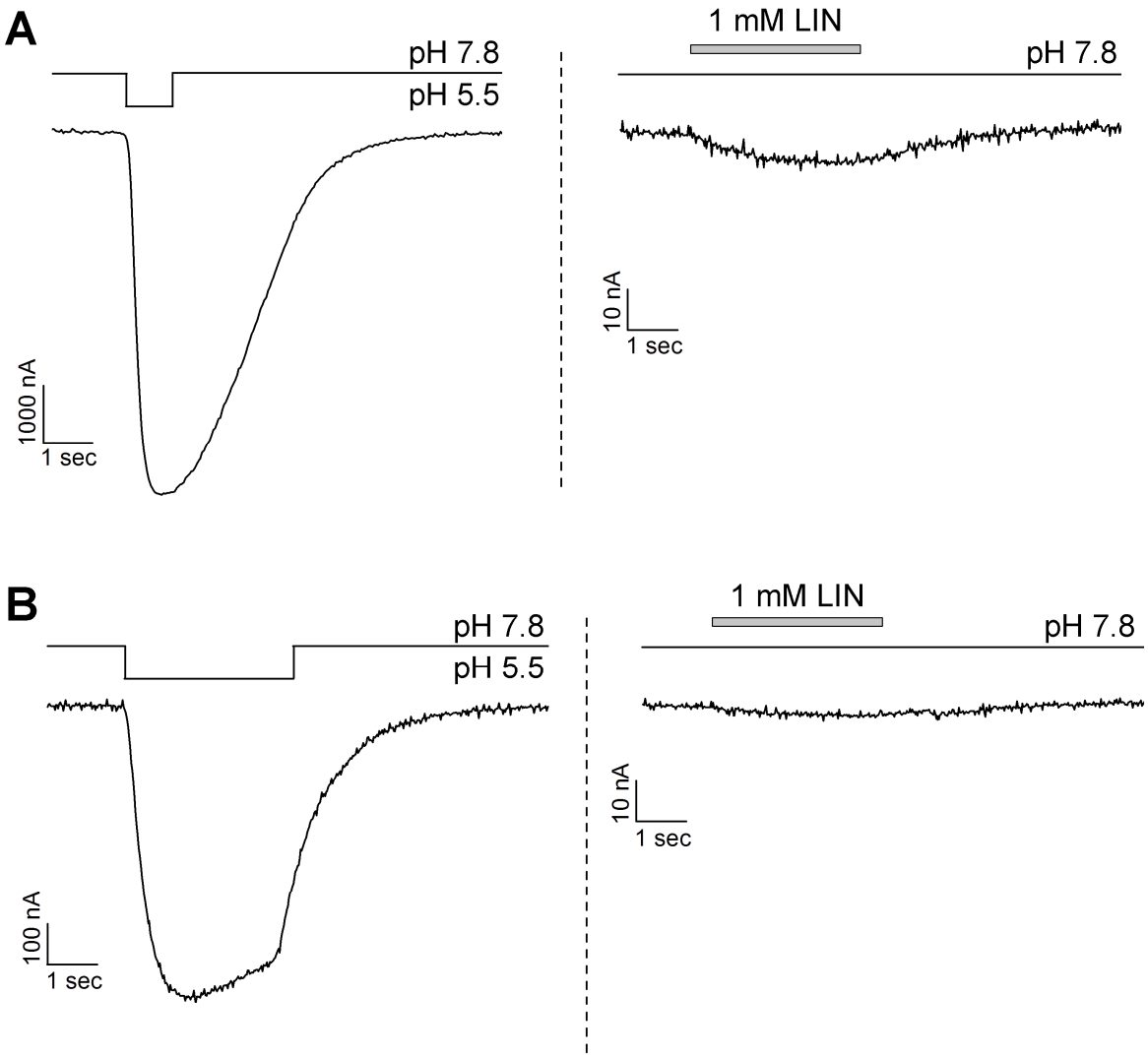
Supplementary Figure 2.

The ^{1}H NMR spectra of LIN at different pH values (D_2O, 14°C, 600 MHz). Absence of any observable chemical shift changes in ^{1}H NMR and 2D ^{13}C-HSQC/^{13}C-HMBC NMR spectra (data not shown) proves absence of ionogenic groups with pKa in range 1..8 in LIN.



Supplementary Figure 3. LIN application on uninjected oocytes.

Whole-cell current traces recorded from oocytes held at pH 8.5, 7.8 and 7.3 in response to corresponding bath solution (left panel) and to 1 mM LIN (right panel).



Supplementary Figure 4. LIN application on rat ASIC1a and rat ASIC2a channels.

(A) Whole-cell current traces recorded from rat ASIC1a channels held at pH 7.8 in response to pH 5.5 stimulus (left panel) and to 1 mM LIN (right panel). The mean of LIN-induced current was 0.55 ±0.13% of pH 5.5-induced current, n = 6. Data are mean ± SEM.

(B) Whole-cell traces recorded from rat ASIC2a channels held at pH 7.8 in response to pH 5.5 stimulus (left panel) and to 1 mM LIN (right panel). The mean of LIN-induced current was 0 ± 0 % of pH 5.5-induced current, n = 5.