

[Chem. Pharm. Bull.]
34(11)4825—4828(1986)

Isolation of an Anti-histaminic Substance from Green-Lipped Mussel (*Perna canaliculus*)

TAKUO KOSUGE, KUNIRO TSUJI,* HITOSHI ISHIDA
and TOMOHIKO YAMAGUCHI

Shizuoka College of Pharmacy, 2-2-1, Oshika, Shizuoka 422, Japan

(Received June 14, 1986)

The anti-histaminic principle in green-lipped mussel (*Perna canaliculus*) was isolated by a combination of countercurrent distribution and column chromatographies on silica gel and Sephadex LH-20, and identified as lysolecithin.

Keywords—anti-histaminic; green-lipped mussel; *Perna canaliculus*; lysolecithin; anti-inflammatory activity

There is some evidence that the green-lipped mussel (*Perna canaliculus*) may be of benefit in the treatment of inflammatory joint disease, including allergy.¹⁾ Subsequently, animal studies using the carrageenan-induced rat footpad edema model have shown that the mussel possesses moderate anti-inflammatory activity.²⁻⁵⁾ Recently, it was reported that the mussel also contains an inhibitor of prostaglandin biosynthesis.⁶⁾ However, no detailed chemical studies to identify the active principle have yet been reported. It has been considered that various factors such as histamine, serotonin, slow-reacting substance, bradykinin and prostaglandins may contribute to the inflammatory response. The present paper describes the isolation of an anti-histaminic principle from green-lipped mussel.

During the isolation process, anti-histaminic activity test in isolated guinea pig ileum was used for screening of active fractions. This test allowed simple and rapid detection of the anti-histaminic substance. Isolation of the active principle was achieved by a combination of countercurrent distribution, silica gel column chromatography and Sephadex LH-20 column chromatography. The procedures are summarized in Chart 1.

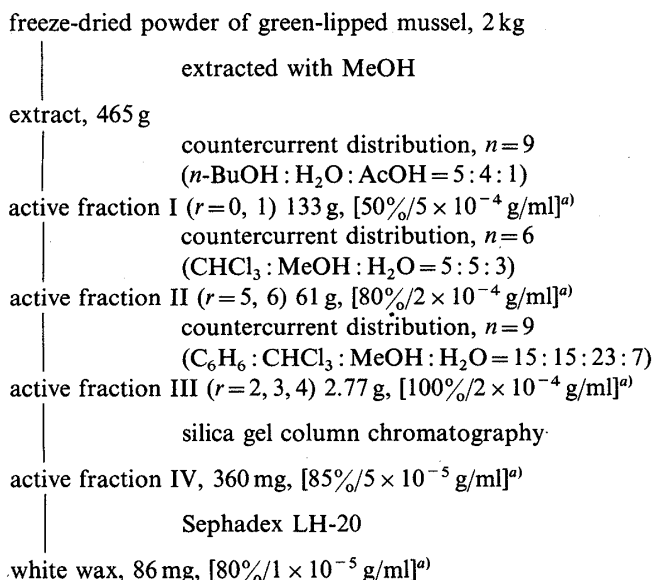


Chart 1. Isolation of the Active Principle

a) [] indicates anti-histaminic activity.

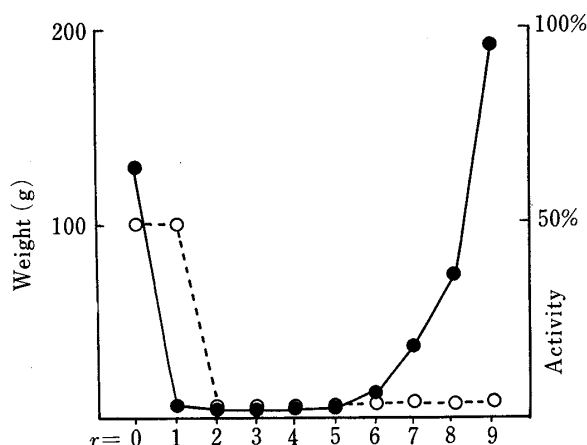


Fig. 1. Countercurrent Distribution Pattern of the MeOH Extract with *n*-BuOH-H₂O-AcOH (5:4:1) (*n*=9)

●—●: distribution pattern (weight). ○---○: anti-histaminic activity in each tube (5×10^{-4} g/ml) in terms of contractile response of isolated guinea pig ileum to histamine (1×10^{-7} g/ml).

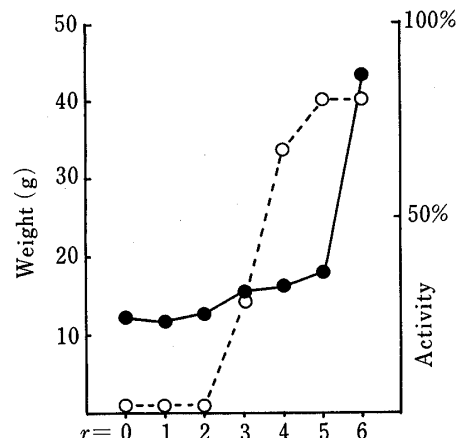


Fig. 2. Countercurrent Distribution Pattern of Active Fraction I with CHCl₃-MeOH-H₂O (5:5:3) (*n*=6)

●—●: distribution pattern (weight). ○---○: anti-histaminic activity in each tube (2×10^{-4} g/ml) in terms of contractile response of isolated guinea pig ileum to histamine (1×10^{-7} g/ml).

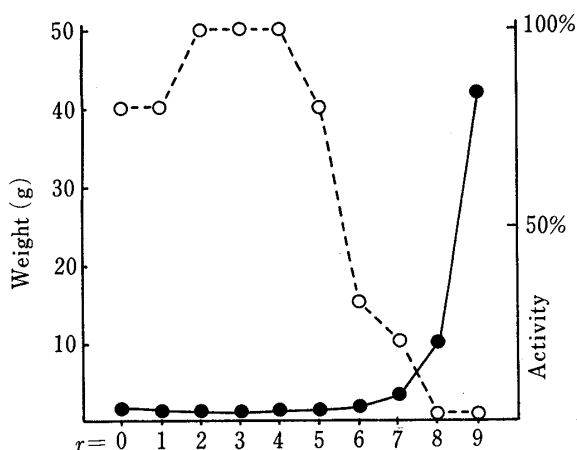


Fig. 3. Countercurrent Distribution Pattern of Active Fraction II with C₆H₆-CHCl₃-MeOH (15:15:23:7) (*n*=9)

●—●: distribution pattern (weight). ○---○: anti-histaminic activity in each tube (2×10^{-4} g/ml) in terms of contractile response of isolated guinea pig ileum to histamine (1×10^{-7} g/ml).

Freeze-dried powder of green-lipped mussel was extracted with methanol at room temperature. The extract was separated by countercurrent distribution, using the solvent system of *n*-butanol-H₂O-AcOH (5:4:1). The distribution pattern and activities are shown in Fig. 1. Tubes 5, 6 and 7 showed activity to contract isolated guinea pig ileum. As the anti-histaminic activity was located in tubes 0 and 1 (active fraction I), this fraction was subjected to countercurrent distribution using a solvent system of CHCl₃-MeOH-H₂O (5:5:3). Figure 2 shows the results. The activity emerged in tubes 3—6. The most active fraction (tubes 5 and 6; active fraction II) was further separated by countercurrent distribution using a solvent system of C₆H₆-CHCl₃-MeOH-H₂O (15:15:23:7). Figure 3 shows the distribution pattern and activities. The most active fraction (tubes 2—4; active fraction III) was subjected to silica gel column chromatography with CHCl₃-MeOH and methanol as eluents. The active compound was eluted with methanol. Final purification of the active compound was achieved by gel filtration through Sephadex LH-20. Elution with methanol afforded the active compound as a white wax.

The purified substance inhibited the contractile response of isolated guinea pig ileum to 1×10^{-7} g/ml of nicotine by about 75% at a concentration of 1×10^{-5} g/ml. The active substance was unstable in an alkaline medium (1 N NH₄OH, 20°C, 24 h) but was relatively

stable in acid (0.01 N HCl, 20 °C, 24 h) and to heat (80 °C, 3 h).

The active substance showed a single spot on a thin layer chromatogram and was visualized by spraying Dittmer–Lester and Dragendorff reagents. The proton nuclear magnetic resonance ($^1\text{H-NMR}$) and the infrared (IR) spectral data showed the presence of aliphatic acid ester, hydroxyl group and *N*-trimethyl moieties in the molecule of the active compound. These observations indicated that the active compound might be a phospholipid, and it was compared with standard phospholipids by thin layer chromatography (TLC). The active compound showed the same mobility as standard lysolecithin in two different solvent systems. In order to determine the fatty acid composition of lysolecithin from green-lipped mussel, it was methanolized with methanolic HCl, and the fatty acid methyl esters thus formed were analyzed by gas chromatography (GC). The major fatty acids of the lipid were $\text{C}_{16:0}$, 80.2%, $\text{C}_{16:1}$, 3.2%, $\text{C}_{18:0}$, 12.0% and $\text{C}_{18:1}$, 1.9%. It was shown by elemental analysis that the ratio of C:N:P is about 24:1:1. From these results, the active compound was concluded to be lysolecithin. The anti-histaminic activity of an authentic sample (obtained from Wako (Pure Chemical Co.)) was equal to that of the natural product.

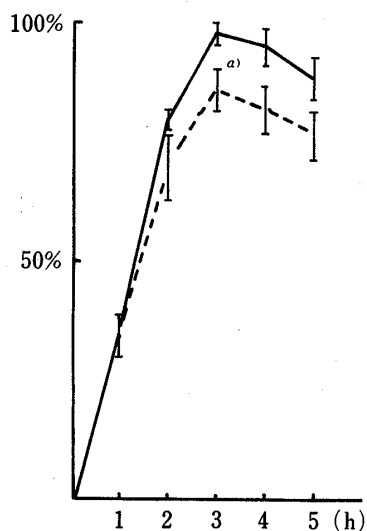


Fig. 4. Anti-inflammatory Effect of Lysolecithin on Carrageenan Induced Footpad Edema
 — control, - - - - lysolecithin (250 mg/kg, *p.o.*).
 a) $p < 0.05$.

Inhibitory activity of lysolecithin on the gut-stimulating actions of histamine and acetylcholine has been reported.⁷⁻¹⁰⁾ Middleton and Phillips also demonstrated a similar effect of lysolecithin on the guinea pig ileum-stimulating action of anaphylactic slow-reacting substance, bradykinin and 5-hydroxytryptamine.¹⁰⁾ According to these reports, the anti-inflammatory activity of lysolecithin was examined on carrageenan-induced hind paw edema in rats. The results (Fig. 4) show that lysolecithin was effective at 3 h after the carrageenan treatment. Attempts to isolate other biologically active principles from green-lipped mussel are in progress.

Experimental

Assay of Anti-histaminic Activity—Male guinea pigs (300–400 g) were sacrificed and a segment of the ileum was dissected out. The preparation was suspended in oxygenated, stirred Tyrode solution (37 °C) in a bath of 5 ml working volume. The isotonic contractile response of the ileum to histamine (1×10^{-7} g/ml) was recorded. Samples were added to the organ bath 3 min before addition of histamine.

Extraction—Freeze-dried powder of green-lipped mussel (2 kg, obtained from McFarlane Laboratories, Ltd., Auckland, NZ) was extracted 3 times with methanol (5 l) for 3 h under reflux. The combined extracts were concentrated under reduced pressure to give a brown gum (465 g).

Countercurrent Distribution with *n*-BuOH-H₂O-AcOH (5:4:1) Solvent System—A portion (100 g) of the crude extract was distributed between the lower phase (500 ml) and upper phase (500 ml) of the solvent system described above with 9 lower phase transfers. In total, 133 g of active fraction I ($r=0, 1$) was obtained by repeated countercurrent distribution.

Countercurrent Distribution with CHCl₃-MeOH-H₂O (5:5:3) Solvent System—Active fraction I (133 g) was successively distributed between the lower phase (500 ml) and upper phase (500 ml) of the solvent system described above by 6 lower phase transfers to afford active fraction II (61 g, $r=5,6$).

Countercurrent Distribution with C₆H₆-CHCl₃-MeOH-H₂O (15:15:23:7) Solvent System—Active fraction II (61 g) was distributed between the lower phase (500 ml) and the upper phase (500 ml) of the solvent system described above by 9 lower phase transfers to afford active fraction III (2.77 g, $r=2,3,4$).

Silica Gel Column Chromatography—Active fraction III (2.77 g) was subjected to column chromatography on silica gel (3 × 100 cm) using CHCl₃-MeOH (1:1) and then methanol as eluents to afford active fraction IV (360 mg) from the methanol fraction.

Column Chromatography on Sephadex LH-20—Active fraction IV (360 mg) was dissolved in 5 ml of methanol and subjected to gel filtration on Sephadex LH-20 (1.5 × 70 cm) using methanol as an eluent to afford a white wax (86 mg).

TLC—Precoated Silica gel 60 thin layer plates (Merck) were used. As the developing solvent mixtures for separation, CHCl₃-MeOH-H₂O (65:25:3) and CHCl₃-MeOH-2.5 N NH₄OH (70:30:5) were used.

Physical Data of Lysolecithin from Mussel—The ¹H-NMR spectrum was measured with a JEOL FX-90 spectrometer. Chemical shifts are reported in δ -values downfield from internal tetramethylsilane. ¹H-NMR (in CDCl₃ + CD₃OD) (ppm): 0.89 (brt, -(CH₂)_n-(CH₃)), 1.04–1.40 (m, -(CH₂)_n-), 1.44–1.78 (m, -CH₂CH₂-C(=O)), 2.31 (brt, -CH₂-CH₂-C(=O)), 3.21 (s, -N-(CH₃)₃), 3.44–3.78 (brt, -CH₂-CH₂-N-), 3.80–4.04 (-O-CH₂-CH₂-N-), 4.06–4.44 (m). IR ν_{\max}^{KBr} cm⁻¹: 3400 (OH), 2930, 2865, 1752, 1470, 1242, 1098, 1058, 973. Elemental analysis: C, 55.56; H, 10.03; N, 2.55; P, 5.98.

Fatty Acid Analysis by GC—Lysolecithin was methanolized with 3% methanolic HCl for 3 h at 100 °C in a sealed tube. Fatty acid methyl esters were extracted with hexane three times. The fatty acid methyl esters thus obtained were analyzed by GC on a 2-m column of 10% Silar 10C coated on Gas-Chrom Q (100–200 mesh), programed from 160 to 220 °C at a rate of 2 °C/min.

Assay for Anti-inflammatory Activity—The carrageenan-induced inflammatory edema model described by Winter *et al.*¹¹ was used in these experiments. Five male Wistar strain rats weighing 130–150 g were used. The sample was dissolved in saline and administered *p.o.* at 1 h before the carrageenan treatment. The subplantar injection of 0.1 ml of 1% carrageenan (lambda carrageenan type IV, Sigma Chemical Co.) in saline solution was performed, and the volume of the foot was measured with an Ugo Basile plethysmometer every 1 h for 5 h. The percent swelling (foot edema) was calculated.

References

- 1) G. R. Gibson, S. L. Gibson, V. Conway and D. Chappell, *Practitioner*, **224**, 955 (1980).
- 2) K. D. Rainsford and M. W. Whitehouse, *Arzneim.-Forsch.*, **30**, 2128 (1980).
- 3) T. E. Miller and D. Ormrod, *N. Z. Med. J.*, **92**, 187 (1980).
- 4) D. G. Palmer, *N. Z. Med. J.*, **92**, 328 (1980).
- 5) R. A. F. Couch, D. Ormrod and T. E. Miller, *N. Z. Med. J.*, **95**, 803 (1982).
- 6) T. Miller and H. Wu, *N. Z. Med. J.*, **97**, 355 (1984).
- 7) W. Feldberg, H. F. Holden and C. H. Kellaway, *J. Physiol. (London)*, **94**, 232 (1938).
- 8) C. H. Kellaway and E. R. Trethewie, *Q. J. Exp. Physiol.*, **30**, 121 (1940).
- 9) M. Rocha e Silva and W. T. Beraldo, *J. Pharmacol. Exp. Ther.*, **93**, 457 (1948).
- 10) E. Middleton and G. B. Phillips, *Nature (London)*, **198**, 758 (1963).
- 11) C. A. Winter, E. A. Risley and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).