

锌二氢卟吩 e_4 的合成及初步抗胃溃疡活性和对急性肝损伤的保护作用

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摘要: 目的 研究锌二氢卟吩 e_4 (1) 的合成及实验性抗溃疡活性和对急性肝损伤的保护作用。方法 蚕沙叶绿素粗品经酸碱降解反应制得二氢卟吩 e_6 (3), 3 经吡啶回流降解制得二氢卟吩 e_4 (2), 2 与醋酸锌络合制得 1; 并测定 1 对消炎痛诱发的大鼠胃溃疡的保护作用及对硫代乙酰胺、四氯化碳所致小鼠急性肝损伤的防治作用。结果 1 为新化合物。生物活性实验结果表明, 1 能显著降低消炎痛诱发的大鼠胃溃疡指数和溃疡个数; 能显著降低小鼠硫代乙酰胺或四氯化碳急性肝损伤后升高的 SGPT 活性。结论 1 对消炎痛诱发的大鼠胃溃疡和硫代乙酰胺、四氯化碳所致小鼠急性肝损伤具有显著的保护作用。

关键词: 蚕沙; 叶绿素; 降解; 锌二氢卟吩 e_4 ; 胃溃疡; 肝损伤

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叶绿素衍生物如铜叶绿素钠(sodium copper chlorophyllin, Cu-Chl-Na) 等有促进组织愈合、抗溃疡和保肝等多方面生物活性^[1], 但以往报道大都为多组份叶绿素衍生物的混合物且包括未知结构的化合物, 从而影响研究结果的可重现性, 因此, 本文以我国特有的廉价蚕沙叶绿素为原料, 制备叶绿素 a 稳定降解产物的单体金属络合物, 在确证化学结构的基础上, 研究其保肝和抗溃疡活性, 这将直接为发展不同用途的新一代天然结构新药提供科学依据。

Zn^{2+} 是人体所必需的微量元素, 参与多种酶的合成, 能加速生长发育, 增强创伤组织的再生能力^[2], 二氢卟吩 e_4 (2) 衍生物的化学和生物活性迄今尚未见报道。作者以蚕沙叶绿素为原料, 按文献^[3]方法制得二氢卟吩 e_6 (3), 3 在吡啶中回流脱羧制得 2^[4], 2 与 $Zn(OAc)_2$ 络合反应制得锌二氢卟吩 e_4 (1) (图 1), 其中, 1 为新化合物, 其结构经 UV, IR, ¹HNMR, FAB-MS 和元素分析确证。药理试验结果表明, 1 能显著降低消炎痛诱发的大鼠胃溃疡指数和溃疡个数, 且作用优于西咪替丁(表 1); 1 可显著抑制小鼠因硫代乙酰胺、四氯化碳急性肝损伤所致 SGPT 活性升高, 且作用稍优于铜叶绿素钠, 值得深入研究。

实 验 部 分

蚕沙糊状叶绿素、叶绿素铜钠: 浙江省海宁市天然色素厂生产; 柱色谱硅胶 H(60 型): 青岛海洋化工集团公司出品; 所用试剂均为分析纯。消炎痛: 上海第十七制药厂生产, 用 0.5% CMC 配成 0.2% 的混悬液; 西咪替丁: 上海第六制药厂生产, 用 0.5% CMC 配成 1% 的混悬液; 四氯化碳: AR, 上海长江化工厂生产, 配成 0.3% 的橄榄油溶液; 硫代乙酰胺: CP, 上海陶湾化工厂生产, 配成 0.3% 的生理盐水溶液。昆明种小鼠(18-22 g)、Wistar 大鼠(180-220 g): 第二军医大学动物实验中心提供。HPLC 用 Water600 型高效液相色谱仪(美国)测定, 分离柱: ZORBAX ODS 柱(0.46 cm × 25 cm), 流动相: 60% THF 水溶液 + 10% 0.2 mol·L⁻¹ HAc-NaAc 缓冲液, 流速: 1 mL·min⁻¹, 检测波长: 401 nm, 灵敏度: 0.1 AUFS。¹HNMR 用 MSL-300 型核磁共振谱仪(德国, Bruker)测定。FAB-MS 用 4510-GC-MS-DS 质谱仪(美国, Finnigan MAT)测定。UV 用 UV-160A 紫外分光光度计(日本, 岛津)测定。IR 用 270-50 型红外分光光度计(日本, 日立)测定, KBr 压片。

脱镁叶绿酸 a(4) 和二氢卟吩 e_6 (3) 按文献^[3]方法制得。

1 二氢卟吩 e_4 (2) 的合成

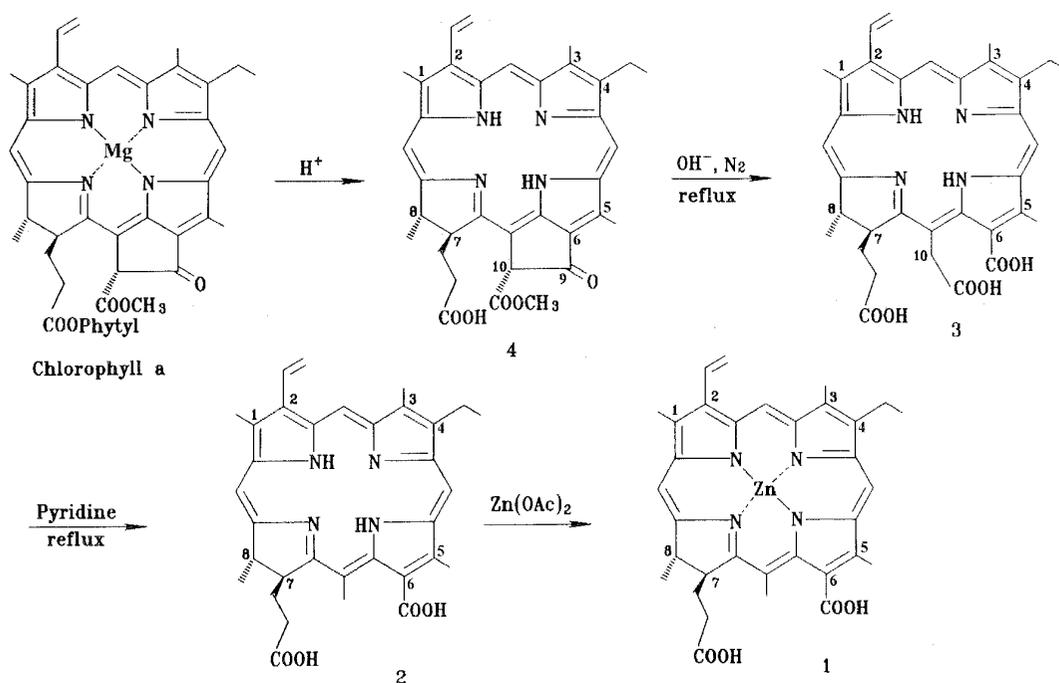
3 (10 g, 16.78 mmol) 溶于吡啶(250 mL), 回流 45 min, 稍冷, 加水(1000 mL), 用 10%(w/v) 硫酸中

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Fig 1 Degradation reaction of chlorophyll a and synthesis of zinc chlorin e₄

和至 pH 5 - 6, 过滤, 干燥后经硅胶 H 柱色谱分离得黑色粉末 2 (7.6 g, 82.1%), 纯度 99% (HPLC)。C₃₃H₃₆N₄O₄, 计算值: C 71.74, H 6.52, N 10.15。实测值: C 71.96, H 6.49, N 10.13。UVλ_{max} nm: 665, 608, 530, 501, 401 (soret) (THF)。IR (KBr) cm⁻¹: 3450 (OH), 2950 - 2870, 1700 (C=O), 1600 (C=C), 1510 (chlorin skeleton), 1440, 1210, 1050, 985。¹H NMR (CDCl₃) δ: 9.81 (1H, s, β-H), 9.67 (1H, s, α-H), 8.94 (1H, s, δ-H), 8.04 (1H, dd, J = 17.9, 11.5 Hz, 2α-H_x), 6.33 (1H, d, J = 17.9 Hz, 2β-H_B), 6.19 (1H, d, J = 11.5 Hz, 2β-H_A), 4.72 (1H, m, 7-H), 4.56 (1H, q, J = 7.2 Hz, 8-H), 4.10 (3H, s, γ-Me), 3.77 (2H, q, J = 7.5 Hz, 4α-CH₂), 3.67 (3H, s, 5-Me), 3.50 (3H, s, 1-Me), 3.32 (3H, s, 3-Me), 2.50 - 2.20 (4H, m, 7a, 7b-CH₂), 1.79 (3H, d, J = 7.2 Hz, 8-Me), 1.67 (3H, t, J = 7.5 Hz, 4β-Me), -1.97 (2H, s, 环内 NH × 2)。MS (FAB) m/z: 554 (M⁺ + 2) (37%), 553 (M⁺ + 1) (100%), 552 (M⁺) (53%), 509 (M⁺ + 1 - CO₂) (13%), 307 (chlorin skeleton) (26%)。

2 锌二氢卟吩 e₄ (1) 的合成

2 (5 g, 9.06 mmol) 溶于四氢呋喃 300 mL, 加 1.7% (w/v) 醋酸锌甲醇液 (100 mL), 水浴回流 30 min, 加水 (1200 mL), 过滤, 干燥后经硅胶 H 柱色谱分离得草绿色粉末 1 (3.7 g, 66.5%), 纯度 98%

(HPLC)。C₃₃H₃₄N₄O₄Zn, 计算值: C 64.50, H 5.54, N 9.12, Zn 10.42。实测值: C 64.71, H 5.51, N 9.08, Zn 10.27。UVλ_{max} nm: 628 (17%), 602 (2%), 588 (3%), 548 (2%), 414 (soret, 100%) (THF)。IR (KBr) cm⁻¹: 3400 (OH), 2950 - 2850 (C-H), 1700 (C=O), 1610 (C=C), 1550 (chlorin skeleton), 1445, 1210, 1180, 1065, 975, 915。¹H NMR (DMSO-d₆) δ: 12.10 (2H, s, 2 × COOH), 9.51 (1H, s, β-H), 9.48 (1H, s, α-H), 8.64 (1H, s, δ-H), 8.24 (1H, dd, J = 17.8, 11.5 Hz, 2α-H_x), 6.21 (1H, dd, J = 17.8, 1.8 Hz, 2β-H_B), 5.99 (1H, dd, J = 11.5, 1.8 Hz, 2β-H_A), 4.46 (2H, m, 7, 8-H), 3.85 (1H, s, γ-Me), 3.81 (2H, q, J = 7.5 Hz, 4α-CH₂), 3.47 (3H, s, 5-Me), 3.38 (3H, s, 1-Me), 3.33 (3H, s, 3-Me), 2.56 - 2.27 (4H, m, 7a, 7b-CH₂), 1.81 (3H, d, J = 7.2 Hz, 8-Me), 1.68 (3H, t, J = 7.5 Hz, 4β-Me)。MS (FAB) m/z: 618 (M⁺ + 4) (24%), 616 (M⁺ + 2) (38%), 614 (M⁺) (40%), 307 (chlorin skeleton) (100%)。

3 1 对消炎痛诱发的大鼠胃溃疡的保护作用

参考文献^[5]方法, 健康 Wistar 大鼠 (180 - 220 g), ♀ ♂ 各半, 禁食不禁水 24 h 后, 按体重随机分组, 以 ip 生理盐水 10 mL · kg⁻¹ 或 0.5% CMC 10 mL · kg⁻¹ 为阴性对照组; ip 西咪替丁 100 mg · kg⁻¹ 为阳性对照组; 试药组 ip 1 (100 mg · kg⁻¹)。给药 30 min 后 ip 消炎痛 (20 mg · kg⁻¹), 5 h 后断颈处死

大鼠,取出胃,用10%甲醛溶液灌注,置10%甲醛溶液固定1h,沿胃大弯剪开冲净内容物,测定每只胃所有溃疡长径总和(溃疡指数)和溃疡个数,计算每组大鼠的溃疡指数和溃疡个数的平均值,与阴性对照组比较算出溃疡指数和溃疡个数抑制率,并作统计学t检验,结果见表1。

表1结果显示,ip消炎痛(20 mg·kg⁻¹)5h后,生理盐水和0.5%CMC对照组大鼠胃粘膜损伤较严重,溃疡指数依次为(13.47 ± 10.74) mm 和

(12.29 ± 10.68) mm,溃疡个数依次为(12.38 ± 7.68)个和(12.50 ± 8.59)个,经预先ip1的大鼠,溃疡指数和溃疡个数与生理盐水对照组相比,分别降低了91.0%和82.4%。预先ip西咪替丁的大鼠,溃疡指数和溃疡个数与0.5%CMC对照组相比,分别降低了78.5%和78.4%;说明1对消炎痛诱发的大鼠胃溃疡具有显著的保护作用,且作用优于西咪替丁。

Tab 1 Protective effects of compound 1 on indomethacin induced gastric ulcer in rats

Group	Dose/ mg·kg ⁻¹	Number of rats	Ulcer index/ mm	Number of ulcer	Inhibiting rate / %	
					Ulcer index	Number of ulcer
1	100	10	1.21 ± 1.36 ^{***}	2.18 ± 1.74 ^{***}	91.0	82.4
Cimetidine	100	10	2.64 ± 3.31 ⁺	2.70 ± 3.71 ⁺⁺⁺	78.5	78.4
0.9% NaCl	-	16	13.47 ± 10.74	12.38 ± 7.68	-	-
0.5% CMC	-	12	12.29 ± 10.68	12.50 ± 8.59	-	-

*** P < 0.001 vs 0.9% NaCl group, +++ P < 0.005, + P < 0.02 vs 0.5% CMC group

4 1对硫代乙酰胺、四氯化碳所致小鼠急性肝损伤的防治作用

参考文献^[6,7]方法,昆明种小鼠(18 - 22 g),♀♂各半,按体重随机分组,正常组每次ip生理盐水10 mL·kg⁻¹,未染毒,不给药;阴性对照组每次ip生理盐水10 mL·kg⁻¹;阳性对照组每次ip1%铜叶绿素钠100 mg·kg⁻¹;试药组每次ip1100 mg·kg⁻¹。首先,ip药物,16h后,ip0.3%硫代乙酰胺(TAA)30 mg·kg⁻¹或0.3%四氯化碳(CCl₄)30 mg·kg⁻¹染毒,2及8h后各ip药物1次,染毒24h后,断头采血,取血清,按赖氏法测定SGPT活性,并作统计学t检验。

结果显示,ip硫代乙酰胺(30 mg·kg⁻¹)染毒24h后,对照组小鼠的SGPT活性(赖氏单位)(193.5 ± 120.6)较正常组(14.5 ± 5.5)高,经ip1(100 mg·kg⁻¹ × 3)的小鼠SGPT活性降低(62.6 ± 43.9),而ip铜叶绿素钠(Cu·Chl·Na,100 mg·kg⁻¹ × 3)的小鼠SGPT活性降低(78.5 ± 49.7);ip四氯化碳(30 mg·kg⁻¹)染毒24h后,对照组小鼠的SGPT活性(110.7 ± 68.9)较正常组(14.5 ± 5.5)高,经ip1(100 mg·kg⁻¹ × 3)的小鼠SGPT活性降低(29.5 ± 30.4),而ip铜叶绿素钠(Cu·Chl·Na,100 mg·kg⁻¹ × 3)的小鼠SGPT活性降低(44.9 ± 38.6);表明1可显著阻抑小鼠因硫代乙酰胺、四氯化碳急性肝损伤所致SGPT活性升高,且作用稍优

于铜叶绿素钠。

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REFERENCES:

[1] Hu LQ. Use of chlorophyll derivatives in medicine [J]. *World Pharm* (in Chinese), 1987, 8(3):146 - 153.

[2] Miao J, Gao Q, Xu SL. *Trace Elements and Relative Diseases* [M]. Henan: Henan University Press, 1997. 111 - 114.

[3] Yao JZ, Shen WD, Chen WH, et al. Synthesis and tumor photobiological activity of Chlorin f [J]. *Chin J Pharm* (in Chinese), 2000, 30(5):215 - 217.

[4] Fischer H, Heckmaier J, Plots E. Uber Chlorin e₄, Chlorin e₅ und Iso-phaeoporphyrin a₅ [J]. *Ann Chem* (in German), 1933, 500:215 - 252.

[5] Zhang SR, Shao JY, Yu YW. The protective effects of furazolidone and some commonly used antiulcer drugs on several gastric ulcer models in rats [J]. *Acta Pharm Sin* (in Chinese), 1984, 19(1):5 - 11.

[6] Zhu LQ, Shen NC, Xu DY. Experimental study on protective effect of sodium copper chlorophyllin on acute and chronic liver injuries in rats [J]. *Chin J Pharm* (in Chinese), 1990, 21(10):442 - 445.

[7] Tan YX, Li WH, Yao ZZ, et al. Protection of ebselen on liver damage induced by CCl₄ and lipopolysaccharide + D-galactosamine *in vivo* and *in vitro* [J]. *Acta Pharm Sin* (in Chinese), 1999, 34(2):99 - 102.

SYNTHESIS, PRELIMINARY ANTIGASTRELCOSES ACTIVITY AND THE PROTECTIVE EFFECT ON ACUTE LIVER INJURY OF ZINC CHLORIN E₄

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ABSTRACT: AIM To study the synthesis of zinc chlorin e₄ (**1**), its experimental antigastrelcosis activity as well as the protection against acute liver injuries. **METHODS** Chlorin e₆ (**3**) was prepared through acidic and alkaline oxidative degradation using silkworm excrement crude chlorophyll extracts as starting material. Compound **1** was synthesized via Zn(OAc)₂ complex action with Chlorin e₄ (**2**) which was prepared by refluxing **3** in pyridine. Gastric ulcers were induced by abdominal injection of 0.2% indomethacin at 20 mg•kg⁻¹ in rats. The ulcer indexes and ulcer numbers in gastric mucosa were determined. Acute liver injuries were induced by abdominal injection of 0.3% thioacetamide (TAA) or 0.3% CCl₄ at 20 mg•kg⁻¹ in mice, and activities of SGPT in mice were determined. **RESULTS** Compound **1** is previously unknown. Compared with control group, abdominal administration of **1** at 100 mg•kg⁻¹ reduced significantly the gastric ulcer index ($P < 0.001$) and the number of ulcer ($P < 0.001$) induced by indomethacin in rats. Abdominal administration of **1** at 100 mg•kg⁻¹ × 3 exhibited marked inhibitory effects on elevated activities of SGPT induced by TAA ($P < 0.02$) or CCl₄ ($P < 0.01$) in mice. **CONCLUSION** These results show that **1** has significant protective effect against indomethacin-induced gastric lesion in rats and TAA or CCl₄ induced acute liver injuries in mice. It is suggested that **1** may be a promising new drug candidate for antigastrelcosis and liver injury protection.

KEY WORDS: silkworm excrement; degradation; chlorophyll; zinc chlorin e₄; gastrelcosis; liver injury