Notes

Inhibition of Leukotriene Biosynthesis by Stilbenoids from *Stemona* Species

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Fifteen stilbenoids and two alkaloids from *Stemona collinsae, S. tuberosa*, and *S. peirrei* were tested alongside the commercially available stilbenoids resveratrol and pinosylvin for inhibition of leukotriene formation in an ex vivo test system based on activated human neutrophilic granulocytes. The stilbenoids resveratrol (**1**), pinosylvin (**2**), dihydropinosylvin (**3**), stilbostemin A (**4**), stilbostemin B (**5**), stilbostemin D (**6**), stilbostemin F (**7**), stilbostemin G (**8**), stemofuran B (**9**), stemofuran C (**10**), stemofuran D (**11**), stemofuran G (**12**), stemofuran J (**13**), stemanthrene A (**14**), stemanthrene B (**15**), stemanthrene C (**16**), and stemanthrene D (**17**) showed structure-dependent activities with IC_{50} values ranging from 3.7 to >50 μM. The alkaloids tuberostemonine (**18**) and neotuberostemonine (**19**) were inactive at a concentration of 50 μM.

Due to the ever-increasing incidence of leukotriene-related inflammatory disorders such as asthma, there is a constant demand for new nonsteroidal anti-inflammatory lead compounds. Commercial anti-inflammatory drugs such as corticosteroids often provoke serious side effects on mineral levels and hormonal functions.[1] Experience has shown secondary plant metabolites, especially those from medicinal herbs long used against inflammatory disorders, to be a highly rewarding field for the discovery of new anti-inflammatory compounds.[1,2] Various species from the genus *Stemona* (Stemonaceae) have long been used in traditional Asian medical practices for the treatment of inflammatory-related diseases. "Baibu", the dried root tuber of *Stemona sessilifolia* (Miq.) Miq., *S. japonica* (Bl.) Miq., or *S. tuberosa* Lour., is listed in the Chinese Pharmacopoeia[3] and used to relieve cough and kill insects and worms. In Vietnamese folk medicine, *S. tuberosa* Lour., *S. collinsae* Craib, *S. saxorum* Gagnep., *S. pierrei* Gagnep., *and S. cochinchinensis* Gagnep. have been used for cough relief and as antiasthmatics.[4,5] The root tubers of members of the genus have been examined phytochemically and were shown to contain a number of antifungal stilbenoids as well as various pyrido[1,2-*a*]azepine alkaloids with high insect toxicity or insect-repelling activity.[6−11]

Here we report the testing of 15 stilbenoids and two alkaloids isolated from *Stemona* species,[6−8] as well as two further commercially available stilbenes in an ex vivo leukotriene biosynthesis inhibition assay using human neutrophile granulocytes.

The stilbenes resveratrol (**1**), pinosylvin (**2**), dihydropinosylvin (**3**), stilbostemin A (**4**), stilbostemin B (**5**), stilbostemin D (**6**), stilbostemin F (**7**), stilbostemin G (**8**), stemofuran B (**9**), stemofuran C (**10**), stemofuran D (**11**), stemofuran G (**12**), stemofuran J (**13**), stemanthrene A (**14**), stemanthrene B (**15**), stemanthrene C (**16**), and stemanthrene D (**17**) as well as the alkaloids tuberostemonine (**18**) and neotuberostemonine (**19**) were tested for inhibitory effects on leukotriene metabolism at test concentrations of 50, 25, 10, 5, and 1 μM. The alkaloids showed no activity at the highest test concentration of 50 μM, whereas the stilbenoids showed clear structure-related activity in a dose-dependent manner. Their IC_{50} values ranged from under 10 μM (**12**, **14**, **17**) to compounds with less than 10% inhibition at 50 μM (**1**, **4**, **6**, **7**). Substances **3**, **5**, and **10** showed inhibition between 10 and 50% at a test concentration of 50 μM. Therefore, their IC_{50} values could not be determined correctly (Table 1). The three most active stilbenoids showed inhibitory effects notably higher than the commercial specific 5-lipoxygenase inhibitor zileuton, which was used as a positive control and was shown to have an IC_{50} value of 10.4 μM (Table 1). The substances **15** and **16**, which initially showed very high inhibition with 100% inhibition at 25 μM, lost activity during storage due to degradation. The promising first test results could not be

Table 1. IC_{50} Values (μM) of Stilbenoids **1**−**17** and Alkaloids **18** and **19** [shown with 95% fiducial limits (FL)]

compound	IC_{50}	FL
1	>50	
2	22.7	20.8−24.9
3	>50	
4	>50	
5	>50	
6	>50	
7	>50	
8	25.8	23.1−28.7
9	23.3	21.3−25.5
10	>50	
11	30.3	27.3−33.1
12	3.7	3.2−4.2
13	26.3	24.2−28.5
14	8.5	7.3−8.8
15		
16		
17	4.8	4.3−5.3
18	>50	
19	>50	
zileuton[a]	10.4	9.0−11.7

[a] Positive control substance.

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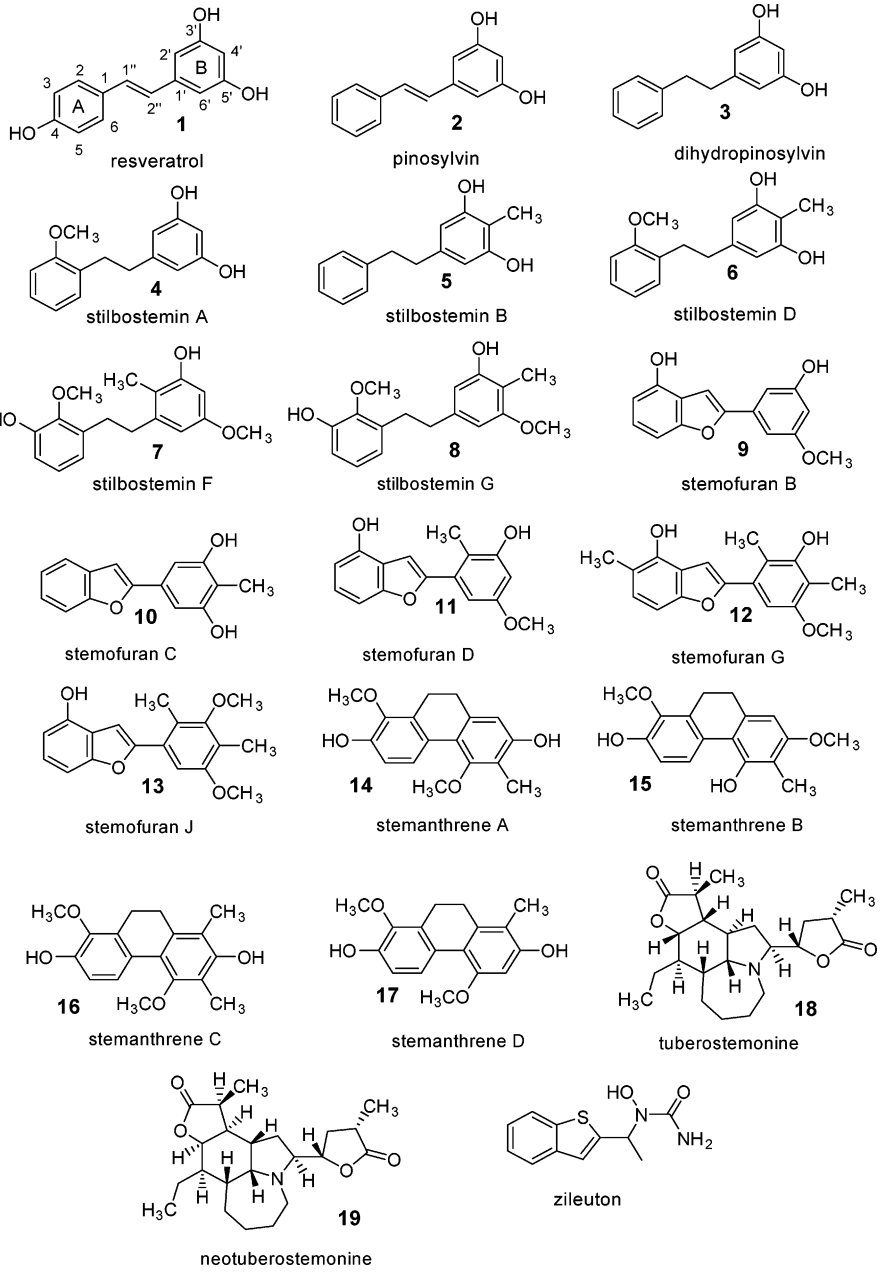
Chart 1



reproduced with the same samples 2 months later. The test samples were examined by LC-MS and were shown to have been degraded. Therefore, IC_{50} values could not be determined. The instability of these compounds has already been described by Kostecki et al.[8] The similar compounds **14** and **18**, however, remained active. It is notable that **2** (IC_{50} 22.7 μM) was significantly more active than **3** (IC_{50} > 50 μM). The only difference between the substances is the C-1″–C-2″ double bond (the stilbenoid atom numbering follows Kostecki et al.[8]). All other substances saturated at C-1″–C-2″ (**4**–**7**) except for **8** showed only minimal inhibition at 50 μM. The stemofurans **9** and **11**–**13** all showed significant activity, whereas **10**, a stemofuran with no substitution on ring A, was less active. All the stemanthrenes (**14**–**17**) were shown to be active. It seems reasonable that stabilization of the steric position of rings A and B due to the covalent C-6–C-6′ bond found in the stemanthrenes is beneficial for the observed activity.

The potency of various stilbenoids in our biological test system suggests that these substances might represent the anti-inflammatory and antiasthmatic principles of *Stemona* species and could be leads for further drug development.

Experimental Section

Test Compounds. Compounds **1** and **2** and the positive control zileuton were purchased from Sequoia Research Products, Ltd., Oxford, UK. Substances **4**–**7**, **9**–**13**, and **17** were isolated as previously described[6] from *Stemona collinsae* Craib, collected in Southeast Thailand near Chonburi, Khao Khieo, close to the Chanthathen waterfall and Sri Racha. Voucher specimens (HG 840, HG 841, HG 842, and HG 860) are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU). Compound **2** also occurs naturally in *S. collinsae*.[6] Compound **3** was isolated from slowly dried tubers of *S. tuberosa* obtained from a local market in Bangkok, Thailand, following the same method as previously reported,[6] and its structure was confirmed by comparison of NMR results with literature data.[12] Compounds **8** and **14**–**16** were isolated as described by Kostecki et al.[8] from *Stemona* cf. *pierrei* Gagnep. collected in East Thailand, Sri Sa Ket Province, between Sri Sa Ket and Surin. A voucher specimen (HG 910)

is deposited at the Herbarium of the Institute of Botany, WU. Compound **18** was isolated from *S. tuberosa* as described by Brem et al.[7] A voucher specimen (HG 910) is deposited at the Herbarium of the Institute of Botany, WU. Compound **19** was isolated from a commercial *Stemona* sp. preparation from Thailand. Its identity was confirmed by comparison of 1D and 2D NMR data with literature data.[13] The purity of the test compounds was evaluated by two TLC methods (Merck Kieselgel 60, detection: UV, Dragendorff-reagent for the alkaloids and anise-aldehyde sulfuric-acid reagent for the stilbenoids) as well as HPLC-DAD (see ref 6), where no impurities were visible. The LC-MS system for examining the degradation of substances **15** and **16** was a Thermo Finnigan Surveyor liquid chromatograph equipped with a Merck Lichrospher 100, RP 18 (5 μm), 125 $\times$ 4 mm column interfaced with a Finnigan LCQ Deca XP Plus mass detector operating in the ESI positive mode. The mass spectra were recorded in a scan mode. Mobile phase: acetonitrile−water from 10:90 to 95:5 in 30 min. PDA detection was at 230 nm.

Ex Vivo Leukotriene Bioassay. The bioassay for inhibition of leukotriene biosynthesis was performed as described by Adams et al.[2] Neutrophile granulocytes with 5-LOX activity were isolated by separation techniques based on sedimentation rates and lysis tolerance. They were activated with a calcium ionophore and incubated with a defined concentration of test sample and arachidonic acid. After stopping the enzymatic reaction by addition of formic acid and separation from cellular particles by centrifugation, the supernatant was diluted 50-fold and LTB_4 was quantified by a leukotriene B_4 EIA kit (Cayman Chemical, Ann Arbor, MI). The competitive enzyme immunoassay kit was used according to the manufacturer's instructions. The plate was incubated for 18 h at 4 °C in the dark. The EIA kit was emptied, rinsed, and developed with Ellmans Reagent on an orbital shaker (MS 1 Minishaker, IKA Works, Wilmington, NC) developed for 150 min at room temperature in the dark, before measuring the absorption at 412 nm using a photometric ELISA plate reader (Tecan RAIN BOW, Tecan Austria) and processing with EASYIN-Fitting 4.0 a software (Tecan). The inhibition was expressed as a percent in relation to a control.

Each substance was tested at the concentrations 50, 25, 10, 5, and 1 μM and quantified in duplicate. Mean values and the standard deviations were determined (Microsoft, Excel). IC_{50} values with fiducial limits were calculated by probit-log analysis as described for quantitative bioassays by Finney[14] using SPSS 6.0 for MS Windows. To ensure that the observed activity was not caused by unspecific cytotoxic effects, cells were dyed with trypan blue solution after incubation and examined by light microscopy. The test substances did not cause a significant loss of cell viability at the used test concentrations after 10 min.

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References and Notes

(1) Bauer, R. In *Bioassay Methods in Natural Product and Drug Development*; Bohlin, L., Bruhn, J. C., Eds.; Kluwer Academic Publishers: Dordrecht, 1999; pp 119−141.
(2) Adams, M.; Kunert, O.; Bauer, R. *Planta Med.* **2004**, *70*, 904−908.
(3) *Pharmacopoeia of the Peoples Republic of China*, English Edition; Chemical Industry Press: Beijing, 2000; Vol. 1, pp 199−200.
(4) Do, T. L. *Vietnamese Traditional Medicinal Plants and Drugs*, 3rd ed.; Publishing House of Medicine: Hanoi, 2001; p 180.
(5) Vo, V. C. *A Dictionary of Traditional Vietnamese Medicinal Plants*; Bio-Pharmaceutical Publisher: Hanoi, 2001; p 162.
(6) Pacher, T.; Seger, C.; Engelmeier, D.; Vajrodaya, S.; Hofer, O.; Greger, H. *J. Nat. Prod*. **2002**, *65*, 820−827.
(7) Brem, B.; Seger, C.; Pacher, T.; Hofer, O.; Vajrodaya, S.; Greger, H. *J. Agric. Food Chem.* **2002**, *50*, 6383−6388.
(8) Kostecki, K.; Engelmeier, D.; Pacher, T.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2004**, *65*, 99−106.
(9) Pilli, R. A.; Ferreira de Oliveira, M. C. *Nat. Prod. Rep*. **2000**, *17*, 117−127.
(10) Kaltenegger, E.; Brem, B.; Mereiter, K.; Kalchhauser, H.; Kaehlig, H.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2003**, *63*, 803−816.
(11) Zhao, W.; Qin, G.; Ye, Y.; Xu, R.; Le, X. *Phytochemistry* **1995**, *38*, 711−713.
(12) Takasugi, M.; Kawashima, S.; Monde, K.; Katsui, N.; Masamune, T.; Shirata, A. *Phytochemistry* **1987**, *26*, 371−375.
(13) Ye, Y.; Qin, G. W.; Xu, R. S. *Phytochemistry* **1994**, *37*, 1201−1203.
(14) Finney, J. D. *Statistical Methods in Biological Assay*; Charles Griffin: London, 1978; pp 58−65.

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