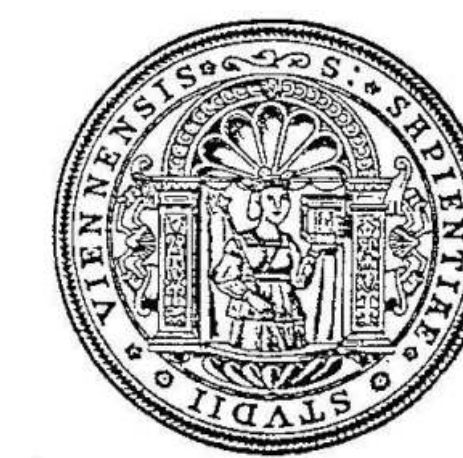


STEMOFURANS, A NEW CLASS OF ANTIFUNGAL STILBENOIDS FROM *STEMONA COLLINSAE*

Christoph Seger,^{1,4} Doris Engelmeier,² Thomas Pacher,² Srunja Vajrodaya,³ Franz Hadacek,² Otmar Hofer,¹ and Harald Greger²



¹Institute of Organic Chemistry, University of Vienna, Währinger Straße 38, A-1090 Vienna

²Department of Comparative and Ecological Phytochemistry, Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna

³Department of Botany, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

⁴Institute of Pharmaceutical Chemistry and Pharmaceutical Technology, Universität of Graz, Universitätsplatz 1, A-8010 Graz; current adress: Institute of Pharmacy, Department of Pharmacognosy, University of Innsbruck, Innrain 52, A-6020 Innsbruck, email: Christoph.Seger@uibk.ac.at

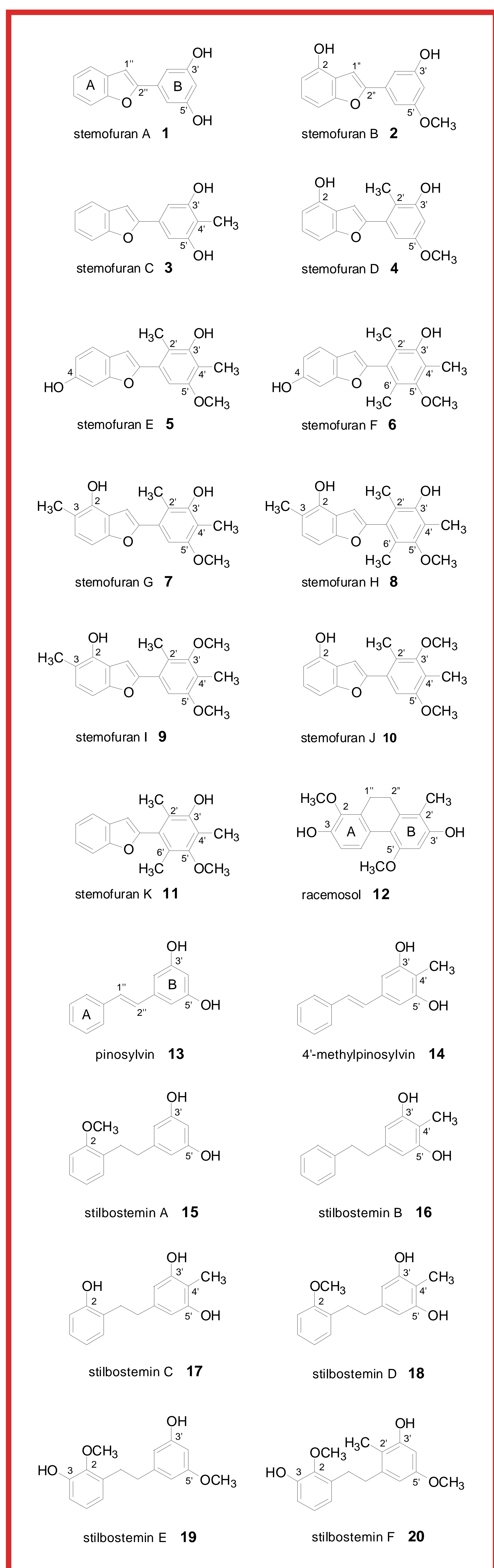


Fig. 1: Blooming *Stemona collinsae* in the natural habitate.



Fig. 2: TLC *Cladosporium herbarum* bioassay performed on purified fractions (10µg/spot, n.a. not analysed).

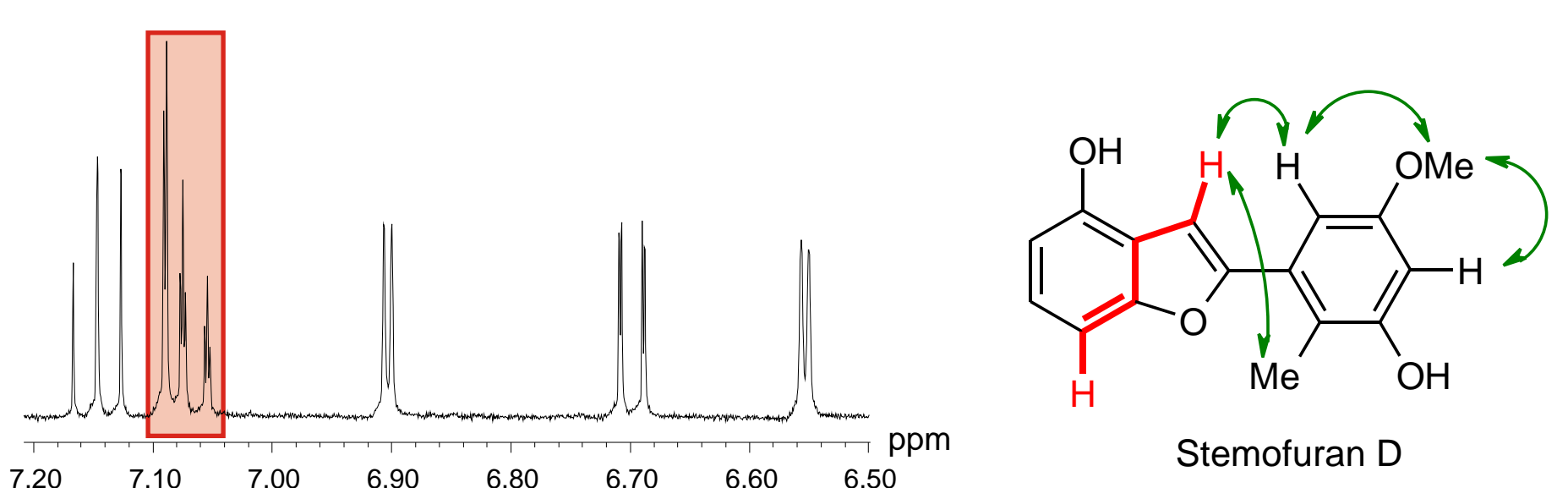


Fig. 3: Aromatic region of the ¹H NMR spectrum and selected COSY (red) and NOESY crosspeaks (green) in stemofuran D (4).

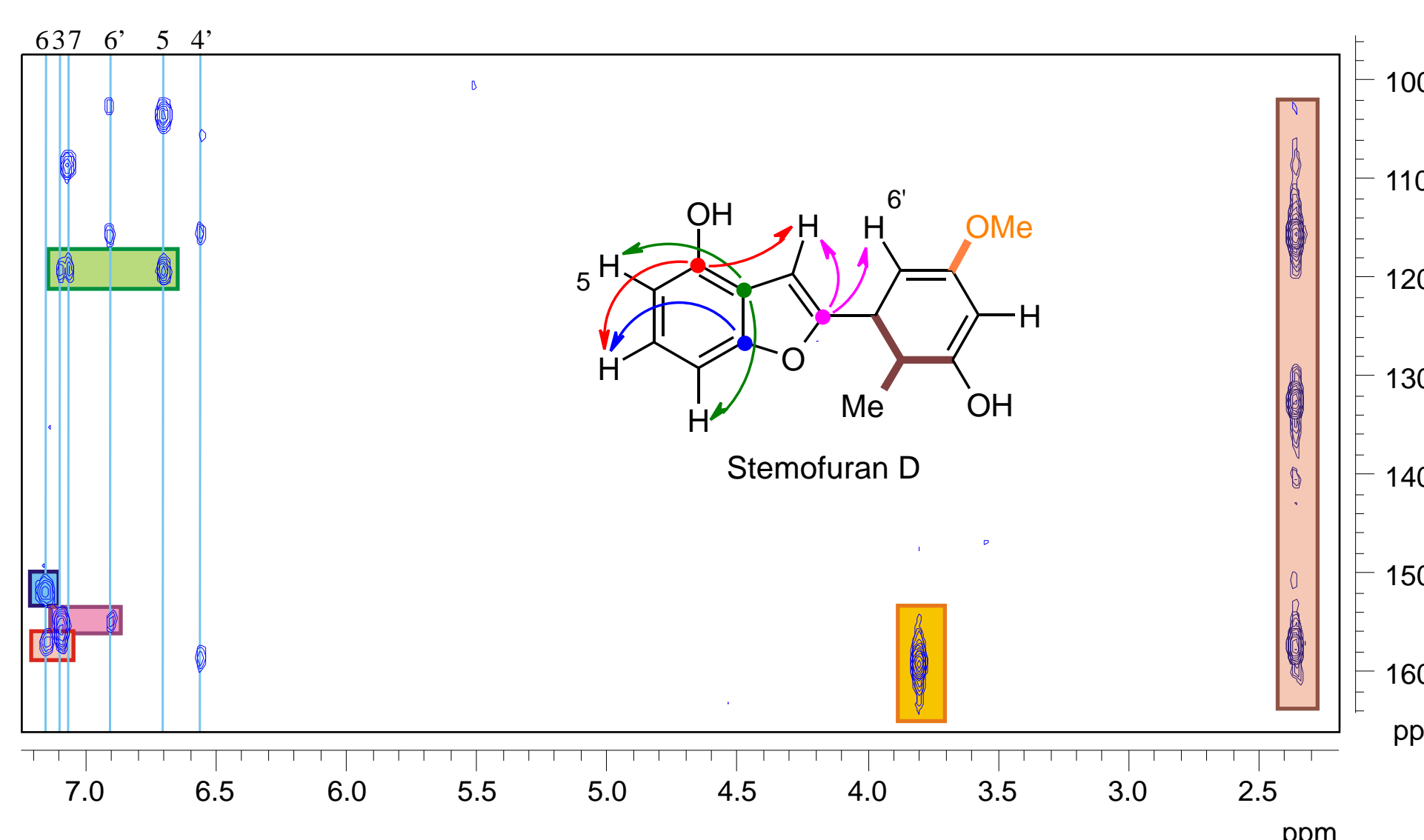


Fig. 4: HMBC spectrum and selected HMBC crosspeaks (insert) proofing the substitution pattern of stemofuran D (4).

REFERENCES

- T. Pacher, C. Seger, D. Engelmeier, S. Vajrodaya, O. Hofer, H. Greger, *J. Nat. Prod.*, **65**, 820 (2002).
 F. Hadacek, H. Greger, *Phytochem. Anal.*, **11**, 137 (2000).
 D. Engelmeier, F. Hadacek, T. Pacher, S. Vajrodaya, H. Greger, *J. Agri. Food Chem.*, **48**, 1400 (2000).
 C. Cobas, J. Cruces, F. J. Sardinia
 MestRe-C 2.3 and MestRe-C nD 1.1.1, <http://qobruue.usc.es/>



Summary

Fifteen new stilbenoids including 11 phenylbenzofurans, the stemofurans A-K (1-11), and four dihydrostilbenes, the stilbostemins A (15), C (17), E (19), and F (20), were isolated and identified from a methanolic extract of *Stemona collinsae* roots together with five known derivatives, the stilbenes pinosylvin (13) and 4'-methylpinosylvin (14), the dihydrostilbenes, stilbostemins B (16) and D (18), and the dihydrophenanthrene racemosol (12) as well as (+)-sesamin, conferyl alcohol, and stigmasterol.

Bioautographic tests (TLC) with *Cladosporium herbarum* displayed antifungal activity for stilbenoids of all four structural types.

Ten derivatives were tested against five microfungi using the microdilution technique linked with digital image analysis of germ tubes.

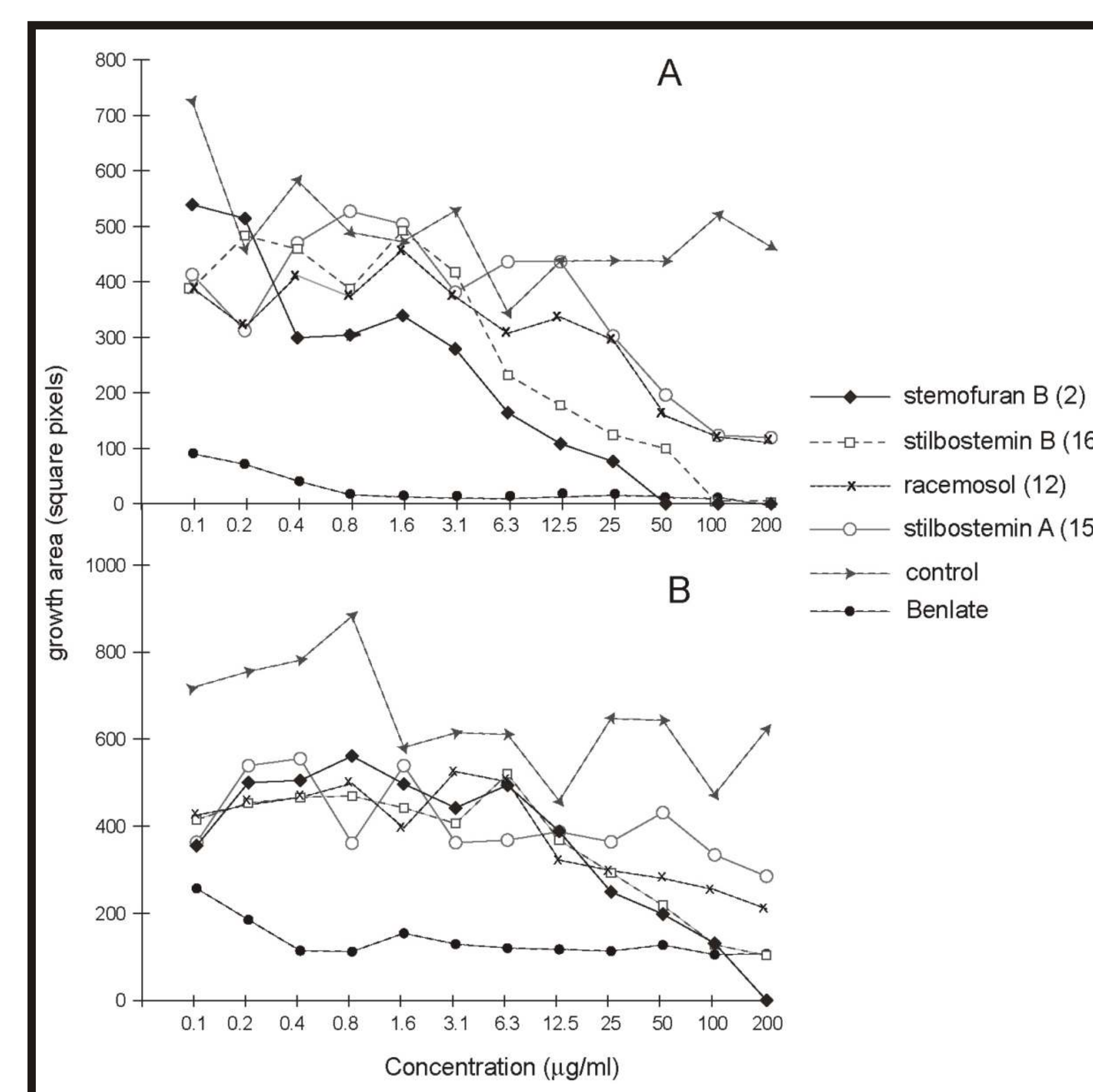


Fig. 5: Germ tube inhibition assay. A: *Pyricularia grisea* B: *Botrytis cinerea*

Isolation

Roots from *Stemona collinsae* (collected near Chonburi/Thailand), were extracted with methanol followed by partitioning of the aqueous concentrate with chloroform. The HPLC detected phenolics were separated by MPLC and TLC. Chromatographic separations of some derivatives were difficult and did not always lead to pure compounds. Altogether 23 compounds could be isolated, 15 of which were shown to be hitherto unknown derivatives.

Structure Elucidation

NMR spectra were run on a Bruker DRX400WB using standard pulse sequences. All compounds were characterized by NOE and HMBC data. Besides the chemical shifts and the coupling patterns of the aromatic systems, the ¹H and ¹³C chemical shifts of the methyl and methoxy groups were of diagnostic value for this series of compounds. The spectra in Figure 3 and 4 were obtained with a 1.5 mg sample. The graphical representation was done with the freeware programs MestRe-C nD 1.1.1 and MestRe-C 2.3 [Cobas 2000].

Bioactivity

Bioautography on TLC plates tests with conidiospore suspensions of *Cladosporium herbarum* paralleling the isolation showed that most of the isolated compounds possessed remarkable antifungal activity (Figure 2). Eight stilbenoids were tested in microwells against *Alternaria citri*, *Fusarium avenaceum*, *Pyricularia grisea*, *Botrytis cinerea*, and *Cladosporium herbarum* (Figure 5). The germ tube inhibition assay was used and compared with Benlate (50% benomyl) as positive control [Engelmeier 2000, Hadacek 2000]. Stemofuran B (2) showed the highest antifungal activity. High activity was also observed for stemofuran E (5) and stilbostemin B (16), showing strong antifungal properties even against *C. herbarum*. All three derivatives represented major components of the root extract of *Stemona collinsae*.