JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1273–1279

Received January 11, 2001
Accepted May 21, 2001
ISSN 0022-3573

Monoamine oxidase inhibitory activity of some *Hypericum* species native to South Brazil

Carmela Gnerre, Gilsane L. von Poser, Alexandre Ferraz, Alice Viana, Bernard Testa and Stela M. K. Rates

Abstract

The total methanol crude extracts and petroleum ether, chloroform, and methanol fractions obtained from *Hypericum* species, *H. caprifoliatum*, *H. carinatum*, *H. connatum*, *H. cordatum*, *H. myrianthum*, *H. piriai*, *H. polyanthemum* and *H. brasiliense*, all native to South Brazil, were assayed for monoamine oxidase A (MAO A) and MAO B inhibitory activity in rat brain mitochondrial preparations at concentrations ranging from 1 to 20 μg mL^{-1}. Three benzopyrans, HP1 (6-isobutyryl-5,7-dimethoxy-2,2-dimethylbenzopyran), HP2 (7-hydroxy-6-isobutyryl-5-methoxy-2,2-dimethylbenzopyran) and HP3 (5-hydroxy-6-isobutyryl-7-methoxy-2,2-dimethylbenzopyran) isolated from *H. polyanthemum* were also tested at maximal concentrations of 150, 150 and 75 μM, respectively. The lipophilic extracts of *H. polyanthemum*, *H. caprifoliatum* and *H. piriai* displayed MAO A inhibitory activity greater than 50%. Among the benzopyrans, only HP3 showed significant activity, with an IC50 value of 22 μM. The total methanol crude extracts of aerial parts from *H. carinatum*, *H. connatum*, *H. cordatum*, *H. polyanthemum* and *H. piriai* were evaluated for antidepressant activity in the Porsolt's forced swimming test in Wistar rats (270 mg kg^{-1} day^{-1}; i.p); however, none of them showed activity.

Institut de Chimie Thérapeutique Section de Pharmacie, Université de Lausanne, CH-1015, Lausanne-Dorigny, Switzerland

Carmela Gnerre, Bernard Testa

Programa de Pós-Graduaćão em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Gilsane L. von Poser, Alexandre Ferraz, Alice Viana, Stela M. K. Rates

Correspondence: Stela M. K. Rates, Av. Ipiranga, 2752, Porto Alegre, RS, Brazil, CEP 90.610.000. E-mail: ratessmk@farmacia.ufrgs.br

Acknowledgements: The authors thank Dr Sérgio Bordignon for the plant collection and identification and Dr Leandro Rocha for facilitating the first contact between our research groups.

Introduction

The chemical investigation of the genus *Hypericum* (Guttiferae), which encompasses approximately 400 species (Hoelzl 1993), has led to the isolation of more than 100 compounds from about 20 species, with various different biological activity, especially antiviral, antimicrobial and antidepressant activity. *Hypericum perforatum* extracts are widely used in Europe, the USA, and also in Brazil, for the treatment of mild to moderate depression. A large amount of experimental and clinical data clearly demonstrate that this plant is effective as an antidepressant (Wagner & Bladt 1994; Linde et al 1996; Bhattacharya et al 1998; Müller et al 1998; Gaster & Holroyd 2000). However, the mode of action and the identity of the active constituents are not fully known.

Traditionally, the naphthodianthrone hypericin has been considered as the most active constituent of *H. perforatum* (Wagner & Bladt 1994). This substance also has an antiviral action on lipid enveloped and non-enveloped DNA and RNA viruses (Meruelo et al 1988). In fact, some reports still provide evidence for the role of hypericin in the antidepressant activity of *H. perforatum* (Butterweck et al 1998). Nevertheless, most researchers consider that the antidepressant effects are due to a variety of constituents, rather than any single compound (Bhattacharya et al 1998; Chatterjee et al 1998; Butterweck et al 2000). Xanthones (Rocha et al 1994),

flavonoids (Butterweck et al 2000), including amentoflavone and especially hyperforin (Chatterjee et al 1998), a phloroglucinol derivative, are also considered to be pharmacologically relevant compounds.

Regarding the mechanism of antidepressant action, several reports demonstrate that a therapeutically used *H. perforatum* extract is a potent, but non-specific, inhibitor of the neuronal uptake of several biogenic amines and amino acid neurotransmitters and that, albeit at very high concentrations, it also possesses monoamine oxidase (MAO) inhibitory activity (Chatterjee et al 1998).

Study of the genus *Hypericum* could not only lead to more rational uses of *H. perforatum* for the treatment of depression, but could also be helpful in the search for an alternative source of antiviral and antidepressant molecules. To the best of our knowledge, only *H. brasiliense* has been previously investigated among the 20 or so native *Hypericum* species from South Brazil. Xanthone (Rocha et al 1994) and phloroglucinol (Rocha et al 1995) derivatives were isolated from this plant, and its extracts have been found to inhibit MAO (Rocha et al 1994).

Recently, the effect of *H. caprifoliatum* in the forced swimming test, a classic animal model for antidepressant drug screening (Daudt et al 2000), and the antiviral activity against feline immunodeficiency virus of *H. connatum* (Schmitt 2000) were reported. In this work, the aerial parts of *H. caprifoliatum*, *H. brasiliense*, *H. myrianthum*, *H. connatum*, *H. polyanthemum*, *H. carinatum*, *H. cordatum* and *H. piriai*, all native to south Brazil, were evaluated for their MAO inhibitory activity. The species *H. connatum*, *H. carinatum*, *H. cordatum* and *H. piriai* were also evaluated for antidepressant activity in the forced swimming test.

Material and Methods

Chemicals

Kynuramine and clorgyline were obtained from Sigma Chemical Co. (St Louis, MO), and deprenyl from RBI chemicals (Natick, MA). Dimethylsulfoxide (DMSO; highest possible degree of purity $> 99.5\%$) and bovine serum albumin were purchased from Fluka Chemie AG (Buchs, CH, Switzerland). Folin Ciocalteu's phenol reagent was obtained from Merck (Darmstadt, Germany). All solvents were obtained from Merck Kga (Darmstadt, Germany).

Plant material

The aerial parts of *H. caprifoliatum* Cham. & Schltdl were collected in the "Morro Santana", Porto Alegre in May 1998. *H. myrianthum* Cham. & Schltdl and *H. polyanthemum* Klotzsch ex Reichardt were collected in Paraíso do Sul and Caçapava do Sul, in July and August 1998, respectively. *H. connatum* Lam. and *H. piriai* Arechav were collected in Capão do Leão in January 1999. *H. carinatum* Griseb. was collected in Glorinha, RS, in January 1999, and *H. cordatum* (Vell.) N. Robson subsp. *kleinii* N. Robson was collected in São Francisco de Paula, RS, in October 1999. Voucher specimens were deposited in the herbarium of the Federal University of Rio Grande do Sul (ICN): *H. caprifoliatum*, Bordignon, 1400; *H. brasiliense*, Bordignon, 1401; *H. carinatum*, Bordignon et al, 1520; *H. connatum*, Bordignon & Salazar, 1527; *H. cordatum*, Bordignon et al, 1715; *H. myrianthum*, Bordignon, 1402; *H. polyanthemum.*, Bordignon et al 1429; *H. piriai*, Bordignon & Salazar, 1528.

Preparation of plant extracts

The dried and powdered plant material (100 g of aerial parts) was submitted to two distinct extraction processes. A batch of material was extracted with methanol (drug/solvent ratio = 1:10, w/v) by maceration ($3 \times$ 24 h), yielding a total methanol crude extract (TMCE): *H. caprifoliatum* (15%), *H. myrianthum* (11%), *H. piriai* (15%), *H. cordatum* (9%), *H. carinatum* (110%), *H. connatum* (15%), *H. polyanthemum* (10%), and *H. brasiliense* (12%). Another batch (approx. 80 g) was successively extracted in a Soxhlet apparatus with petroleum ether, chloroform, and subsequently with methanol over 12 h, yielding petroleum ether (PET), chloroform (CLF) and methanol (MET) extracts. The extracts were evaporated to dryness under reduced pressure at 45°C. All species afforded concentrations of 3.0–4.0% PET, 2.5–3.5% CLF, and 6.0–7.0% MET.

Chemical characterization of the extracts

To examine whether these native plants produce hypericins, the TMCE from each species was analysed by TLC and HPLC. TLC was performed on silica gel GF254 using toluene/ethylformiate/formic acid (50:40:10) as eluent. The chromatogram was sprayed with pyridine and visualized under UV light at a wavelength of 365 nm. Hypericins appear as light red spots. Analyses were carried out using HPLC L-4000 (Hitachi Ltd,

Tokyo, Japan) by injecting 20-μL samples directly on a μBondapak C_{18} reversed-phase column (3.9 × 150 mm, 10 μm particles; Waters-Millipore Corporation, Milford, MA), and separation was achieved using a mobile phase consisting of 65% (v/v) acetonitrile buffered with 0.02 M potassium phosphate, pH 7.2. A μBondapak C_{18} Guard-Pak insert (Waters-Millipore Corporation) was used to protect the analytical column. A flow rate of 1.0 mL min^{-1} was maintained by an L-6000 solvent delivery system (Hitachi Ltd, Tokyo, Japan). Detection was with an F-1050 fluorescence spectrophotometer (Hitachi Ltd) set at excitation and emission wavelengths of 470 and 590 nm, respectively, and chromatograms were recorded with a D-2500 chromato-integrator (Hitachi Ltd). For reference, purified hypericin (Calbiochem), and the methanolic fraction obtained from a phytomedicine prepared with *H. perforatum* were used.

The PET extracts from each species were analysed by TLC, using chloroform and hexane in different proportions, and Godin's reagent as chromogenic agent characteristic for phloroglucinol derivatives (Rocha et al 1995). Some extracts were also analysed by ^{1}H NMR ($CDCl_3$, 400 MHz).

Isolation of the benzopyrans from *H. polyanthemum*

Air-dried and powdered plant material (80 g) was extracted in a Soxhlet apparatus with $CHCl_3$ for 12 h and the extract was evaporated to dryness under reduced pressure, yielding 2.2 g of a crude extract. From this extract, three benzopyrans, HP1 (78 mg), HP2 (39 mg) and HP3 (46 mg), were isolated by preparative TLC on silica gel using chloroform/hexane (3:1) as eluent.

Forced swimming test

For the forced swimming test, adult male Wistar rats (200–300 g), purchased from the Fundação Estadual de Produção e Pesquisa em Saúde, RS, colony, were used. The animals were housed in plastic cages, five to a cage, with a 12-h light–dark cycle (lights on at 0700 h) and a constant temperature of 23 ± 1°C, and had free access to standard certified rodent diet and tap water. Porsolt's test (Porsolt et al 1978) was used with minor modifications. In this test an acrylic box with four sections of 60 × 60 × 40 cm^3 was used. The external walls and the cover were transparent, but the inside sections were dark, allowing the isolation of each one of the four quadrants. The animals were submitted to swimming for 15 min in water with temperature between 22 and 25°C and height of 30 cm. The surrounding temperature was approximately 22°C. At the end of the swimming exposition, the animals were removed from the water and gently dried. All treatments were administered intraperitoneally (1 mL kg^{-1}) 5 min, 19 and 23 h after the first swimming exposition: the first injection was given between 1400 and 1700 h; the second, between 0900 and 1200 h; and the third, between 1300 and 1600 h. At 1 h after the last injection (24 h after the first swimming session), the animals were submitted to a second swimming exposition (5 min), in which the immobility time was measured.

In this work the following species were tested: *H. connatum*, *H. carinatum*, *H. piriai* and *H. cordatum*. The dried TMCE were reconstituted to a concentration of 90 mg mL^{-1} (dose of 270 mg kg^{-1} day^{-1}) with 0.3% carboxymethylcellulose in saline. The pH of all extracts was adjusted to 7.0. Carboxymethylcellulose (0.3%) in saline was used as the negative control and 10 mg kg^{-1} (30 mg kg^{-1} day^{-1}) imipramine hydrochloride (Galena) was used as the positive control.

The data were evaluated using one-way analysis of variance followed by Student-Newman-Keuls test. P values less than 0.05 were considered statistically significant.

Measurement of MAO inhibitory activity

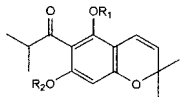
Incubations were carried out in a pH 7.4 buffer (Na_2HPO_4/KH_2PO_4) made isotonic with KCl, in the presence of rat brain mitochondria, the isolation of which is briefly described. Sprague-Dawley rats, 200–250 g, (RCC Ltd, Füllinsdorf, CH, Switzerland) were used. The brains were extracted and transferred in an ice-cold medium (Na_2HPO_4/KH_2PO_4 buffer, 0.15 M, pH 7.4, made isotonic with 0.13 M sucrose). The blood vessels and pial membranes were then carefully removed, and the brains homogenized in buffer with a Dounce tissue grinder (Wheaton, Millville, NJ). Homogenates were centrifuged at 1900 g to remove nuclear pellets, which were washed once and then the supernatants were mixed and centrifuged at 11 800 g. The crude mitochondrial pellet obtained was washed once again with buffer. It was finally resuspended in a definite volume of buffer (9–19 mL), depending on the number of rats killed and the volume of the final pellet, and stored in 1-mL portions at −80°C. For further details see Thull & Testa (1994).

The protein concentration was determined according to the procedure of Lowry et al (1951) and was set to a final value of 1.0 mg mL^{-1}. The protein suspension was pre-incubated at 37°C for 5 min with either 250 nM clorgyline or 250 nM (−)-deprenyl to test for MAO B or

MAO A activity, respectively. The plant extract under study was solubilized in DMSO and added to the suspension to give a final concentration of cosolvent of 5% (v/v). The non-selective substrate kynuramine was then added at a concentration equal to its K_m (90 μM for MAO A; 60 μM for MAO B). Kynuramine is deaminated by MAO to an aldehyde that spontaneously cyclizes to 4-hydroxyquinoline. Formation of this product was monitored spectrophotometrically at 314 nm using a Kontron UVIKON 941 spectrophotometer. The percentage inhibition on MAO A and MAO B was determined for each extract and the standard deviation calculated (n = 3). When a plant extract displayed MAO inhibitory activity greater than 65%, supplementary measurements were made at lower concentrations to evaluate the potency of the inhibition. The IC50 for compound HP3 was determined according to a hyperbolic model. The inhibition degrees for five different inhibitor concentrations were run in duplicate and GraphPad Prism software was used for the IC50 calculation.

Ethical approval

The method used for rat brain mitochondria isolation was in strict accordance with the Swiss legislation and the Ethical Principles and Guidelines for Experiments on Animals, published by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences (1993). The Porsolt's test was approved by the National Commission of Research Ethics and performed following the concepts presented by The National Research Ethical Committee (National Health Council, MS, 1998).



HP1 R1 = CH_3, R2 = CH_3
HP2 R1 = CH_3, R2 = H
HP3 R1 = H, R_2 = CH_3

Figure 1 Chemical structures of benzopyrans isolated from aerial parts of *Hypericum polyanthemum*.

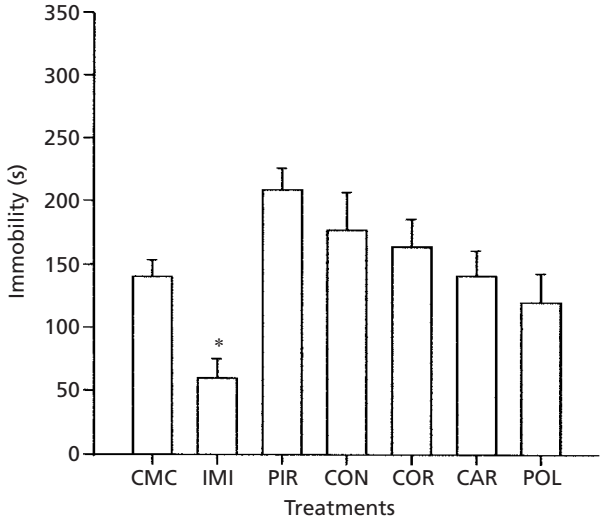


Figure 2 Effects of total methanolic crude extracts (270 mg kg^{-1} day^{-1}, i.p) of aerial parts of *Hypericum piriai* (PIR), *Hypericum connatum* (CON), *Hypericum cordatum* (COR), *Hypericum carinatum* (CAR), *Hypericum polyanthemum* (POL) and imipramine (IMI) (30 mg kg^{-1} day^{-1}, i.p.) in the forced swimming test in rats (second exposition). The results are presented as mean ± s.e.m. $^*P < 0.05$, significant difference compared with the CMC group (saline + 0.3% carboxymethylcellulose; 1 mL kg^{-1}, i.p.) (analysis of variance $F_{(6;75)} = 7.38$).

Results and Discussion

Compounds

The presence of a product with the same chromatographic behaviour as hypericin (TLC and HPLC) could not be detected in any of the crude MET extracts. The taxonomic significance of this finding is discussed by Ferraz et al (2001a).

The PET extracts, analysed by TLC, showed several red spots with the Godin's reagent. In fact, previously studied plants from this genus revealed several phloroglucinol derivatives (Rocha et al 1995) and xanthones (Rocha et al 1994).

In the genus *Hypericum* many phloroglucinol derivatives have been isolated, some of which are derivatives of the well-known hyperforin, isolated from *H. perforatum*, whereas others are pholoroglucinol derivatives with filicinic acid moieties. The NMR spectra of the PET extracts of *H. caprifoliatum*, *H. myrianthum* and *H. carinatum*, gave hydroxyl resonances at approximately δ 18, 16, 11, and 10. The unusually low field shifted signals (approx. δ 18) suggest the presence of hydroxyl protons that participate in rather strong hydrogen bonds. Such a signal is frequently seen in NMR spectra of phloroglucinol derivatives consisting of acyl filicinic acid and phloroglucinol moieties linked by a methylene bridge (Rocha et al 1995). Furthermore, the spectra revealed several signals at approximately δ 18, which could indicate mixtures of tautomeric forms. The purification of these products is in progress.

The benzopyrans HP1, HP2 and HP3 (Figure 1) isolated from *H. polyanthemum* were identified as 6-isobutyryl-5,7-dimethoxy-2,2-dimethylbenzopyran, 7-hydroxy-6-isobutyryl-5-methoxy-2,2-dimethylbenzopyran, and 5-hydroxy-6-isobutyryl-7-methoxy-2,2-di-

methylbenzopyran, respectively. The spectral data are described elsewhere (Ferraz et al 2001b).

Biological activity

Among the extracts tested, none showed activity in Porsolt's forced swimming test (Figure 2). The results of MAO A and MAO B inhibition are presented in Table 1 for the plant extracts, and in Table 2 for the benzopyrans, HP1, HP2 and HP3.

The majority of plant extracts showed MAO A inhibitory activity, which was significantly greater than that toward MAO B. Indeed, the extracts displayed rather low or no MAO B inhibitory activity at the concentrations tested, the highest inhibition value (34%) being observed for the MET extract ($1.5\times$

Table 1 MAO inhibitory activity on rat brain mitochondria of extracts from *Hypericum* species.

Plant species	Extract[a]	Concentration (mg mL^{-1})	MAO inhibition (% ± s.d., n = 3)	
			MAO A	MAO B
Hypericum polyanthemum	TMCE	1.0×10^{-3}	14 ± 1.3	ND
		1.5×10^{-2}	68 ± 3.2	12 ± 1.0
	PET	1.5×10^{-2}	57 ± 2.5	0
	CLF	4.0×10^{-3}	49 ± 2.1	ND
		1.5×10^{-2}	82 ± 2.3	< 5%
	MET	1.5×10^{-2}	21 ± 1.0	0
Hypericum myrianthum	TMCE	1.5×10^{-2}	16 ± 1.2	0
	PET	7.5×10^{-3}	43 ± 2.2	15 ± 1.3
	CLF	1.5×10^{-2}	37 ± 1.8	< 5%
	MET	1.5×10^{-2}	0	23 ± 1.8
Hypericum caprifoliatum	TMCE	1.5×10^{-2}	15 ± 1.5	0
	PET	1.5×10^{-2}	24 ± 1.5	23 ± 2.1
	CLF	4.0×10^{-3}	47 ± 3.2	ND
		1.5×10^{-2}	83 ± 4.2	0
	MET	1.5×10^{-2}	0	0
Hypericum piriai	TMCE	1.5×10^{-2}	67 ± 2.8	0
	PET	1.0×10^{-3}	24 ± 1.4	ND
		5.0×10^{-3}	65 ± 3.5	ND
		1.5×10^{-2}	90 ± 2.8	27 ± 1.8
	CLF	1.0×10^{-3}	11 ± 1.0	ND
		1.5×10^{-2}	70 ± 3.7	28 ± 2.1
	MET	1.5×10^{-2}	0	13 ± 1.3
Hypericum carinatum	TMCE	1.5×10^{-2}	< 5%	0
	PET	1.5×10^{-2}	18 ± 1.5	< 5%
	CLF	1.5×10^{-2}	17 ± 1.7	0
	MET	1.5×10^{-2}	< 5%	0
Hypericum brasiliense	TMCE	1.5×10^{-2}	28 ± 2.3	0
	PET	2.5×10^{-2}	65 ± 3.6	0
	CLF	1.5×10^{-2}	32 ± 3.0	< 5%
	MET	1.5×10^{-2}	15 ± 1.3	18 ± 1.5
Hypericum connatum	TMCE	1.5×10^{-2}	0	< 5%
	PET	1.5×10^{-2}	0	0
	CLF	1.5×10^{-2}	23 ± 1.8	0
	MET	1.5×10^{-2}	0	0
Hypericum cordatum	TMCE	1.5×10^{-2}	21 ± 2.0	15 ± 1.3
	PET	1.5×10^{-2}	0	0
	CLF	1.5×10^{-2}	0	0
	MET	1.5×10^{-2}	31 ± 1.5	34 ± 2.5

[a]TMCE, total methanol crude extract; PET, petroleum ether extract; CLF, chloroform extract; MET, methanol extract. ND, not determined.

Table 2 MAO inhibitory activity on rat brain mitochondria of the benzopyrans from *Hypericum polyanthemum*.

Benzopyran	Concentration (μM)	MAO inhibition (% ± s.d., n = 3)	
		MAO A	MAO B
HP1	150	35 ± 2.8	40 ± 3.3
HP2	150	46 ± 2.1	30 ± 2.5
HP3	75	22.2 ± 0.54 μM[a]	33 ± 3.0

[a]IC50 value obtained from five duplicate inhibitor concentrations.

10^{-2} mg mL^{-1}) of *H. cordatum*. Overall, the MET extract showed the poorest MAO inhibition, whereas the more lipophilic PET and CLF extracts usually gave good results. The highest MAO A inhibition value was observed for the PET extract of *H. piriai* with 90% inhibition at a concentration of 1.5×10^{-2} mg mL^{-1}; this extract was still active below 1×10^{-3} mg mL^{-1}. The *H. piriai* CLF extract also showed good MAO A inhibitory activity (70% at 1.5×10^{-2} mg mL^{-1}), and was still active at 1×10^{-3} mg mL^{-1}. Two other species, *H. polyanthemum* and *H. caprifoliatum*, gave good MAO A inhibition (TMCE extract for *H. polyanthemum* and CLF extract for *H. caprifoliatum*) with MAO inhibitory activity still present at low concentrations (10^{-3} mg mL^{-1}). Practical knowledge indicates that an extract has to inhibit MAO at a concentration of between 5×10^{-4} and 5×10^{-3} mg mL^{-1} in-vitro, in order to lead to possible effects in-vivo (assuming high bioavailability and good blood–brain barrier permeation). Thus, *H. piriai*, *H. polyanthemum* and *H. caprifoliatum* fulfil the first criterion for potential MAO inhibition in-vivo. The extracts of the other species studied, *H. myrianthum*, *H. carinatum*, *H. brasiliense*, *H. connatum* and *H. cordatum*, had modest or no MAO A inhibitory activity (up to 37% at an extract concentration of 1.5×10^{-2} mg mL^{-1}). Again, the extracts displaying the greatest activity were the PET and CLF extracts. As already mentioned, the antidepressant effect of *H. caprifoliatum* was evaluated in the forced swimming test by Daudt et al (2000). The results revealed that among the extracts studied (TCME, PET, CLF and MET), the PET extract was the only one to display an antidepressant-like effect. The same extract submitted to MAO inhibition assays in this study revealed an inhibition of 25% on both MAO A and MAO B, suggesting that the potential antidepressant activity observed by Daudt et al (2000) was only explained in part by MAO inhibition. In addition, the other species that showed MAO inhibitory activity did not display activity in Porsolt's test. Further investigations are warranted to clarify the mechanisms underlying this antidepressant effect. It would also be of interest to isolate and identify compounds from the extracts with high MAO A inhibitory activity.

The MAO inhibition results obtained for the benzopyrans revealed that HP1 and HP2 had moderate MAO A and MAO B inhibitory activity and displayed no selectivity. For HP3, an IC50 of 22 μM was determined for MAO A, with a greater selectivity toward MAO A than that of HP1 and HP2. Interestingly, the most active benzopyrans possess a H-bonded hydroxyl group. A similar case was observed for xanthone derivatives (Rocha et al 1994) revealing that this kind of internal H-bond might play a role in the mechanism of inhibition.

References

Bhattacharya, S., Chakrabarti, A., Chatterjee S. (1998) Activity profiles of two hyperforin-containing *Hypericum* extracts in behavioral models. *Pharmacopsychiatry* **31** (Suppl. 1): 22–29

Butterweck, V., Petereit F, Winterhoff H, Nahrstedt A. (1998) Solubilized hypericin and pseudo-hypericin from *Hypericum perforatum* exert antidepressant activity in the forced swimming test. *Planta Med.* **64**: 291–294

Butterweck, V., Jürgenliemk, G., Nahrstedt, A., Winterhoff, H. (2000) Flavonoids from *Hypericum perforatum* show antidepressant activity in forced swimming test. *Planta Med.* **66**: 3–6

Chatterjee, S. S., Bhattacharya, S. K., Wonnemann, M., Singer, A., Müller, W. E. (1998) Hyperforin as a possible antidepressant component of *Hypericum* extracts. *Life Sci.* **63**: 499–510

Daudt, R., von Poser, G. L., Neves, G., Rates, S. M. K. (2000) Screening for the antidepressant activity of some species of *Hypericum* from South Brazil. *Phytother. Res.* **15**: 344–346

Ferraz, A., Bordignon, S., Mans, D., Schmitt, A., Ravazzolo, A. P., von Poser, G. L. (2001a) Screening for the presence of hypericins in southern Brazilian species of *Hypericum* (Guttiferae). *Pharm. Biol.* In press

Ferraz, A., Bordignon, S., Staats, C., Schripsema, J., von Poser, G. L. (2001b) Benzopyrans from *Hypericum polyanthemum*. *Phytochemistry* **58**: 1227–1231

Gaster, B., Holroyd, J. (2000) St. John's wort for depression – a systematic review. *Arch. Intern. Med.* **160**: 152–156

Hoelzl, J. (1993) Inhaltsstoffe und Wirkmechanismen des Johanniskrauts. *Zeitung Phytother.* **14**: 255–264

Linde, K., Ramirez, G., Mulrow, C. D., Pauls, A., Weidenhammer, W., Melchardt, D. (1996) St. John's Wort for depression – an overview and meta-analysis of randomized clinical trials. *BMJ* **313**: 253–258

Lowry, O. H., Rosebrough, N. J., Farr, L. A., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Meruelo, D., Lavie, G., Lavie, D. (1988) Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. *Proc. Natl Acad. Sci. USA* **85**: 5230–5234

Müller, W. E., Singer, A., Wonemann, M., Hafner, U., Rolli, M., Schafer, C. (1998) Hyperforin represents the neurotransmitter reuptake inhibiting constituent of *Hypericum* extract. *Pharmacopsychiatry* **31** (Suppl. 1): 16–21

Porsolt, R. D., Anton, G., Blavet, N., Jafre, M. (1978) Behavioral despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. Pharmacol.* **47**: 379–391

Rocha, L., Marston, A., Kaplan, M. A. C., Stoeckli-Evans, H., Thull, U., Testa, B., Hostettmann, K. (1994) An antifungal gamma-pyrone and xanthones with monoamino oxidase inhibitory activity from *Hypericum brasiliense*. *Phytochemistry* **6**: 1381–1385

Rocha, L., Marston, A., Potterat, O., Kaplan, M. A., Stoeckli-Evans, H., Hostettmann, K. (1995) Antibacterial phloroglucinols and flavonoids from *Hypericum brasiliense*. *Phytochemistry* **40**: 1447–1452

Schmitt, A. C. (2000) Masters thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil

Thull, U., Testa, B. (1994) Screening of unsubstituted cyclic compounds as inhibitors of monoamine oxidases. *Biochem. Pharmacol.* **47**: 2307–2310

Wagner, H., Bladt, S. (1994) Pharmaceutical quality of *Hypericum* extracts. *J. Geriatr. Psychiatry Neurol.* **7** (Suppl. 1): 65–68