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Trypanocidal activity of bergenin, the major constituent of *Flueggea virosa*, on *Trypanosoma brucei*

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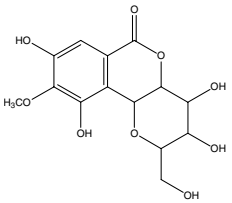
The presence of bergenin in substantial amounts in the methanol leaves extract of *Flueggea virosa* (Euphorbiaceae) was established as a strong chemotaxomic point of differentiation between *Flueggea virosa* and *Securinega virosa*. Bergenin showed an inhibitory effect on the growth of the bloodstream form of *Trypanosoma brucei* with an IC_{50} value of 1 μM.

Despite a reference work (Webster 1984) which should have already resolved problems related to the distinction between *Flueggea* and *Securinega* (Euphorbiaceae) there are still some misleading reports suggesting that *Flueggea virosa* is identical to *Securinega virosa* (Pu 2002; Dehmlow 1999). Moreover, the genus *Securinega* is known to produce a group of tetracyclic compounds classified as the securinega alkaloids (Han 2000; Tatematsu 1991; Golebriewski 1976; Nakano 1963) whereas *Flueggea* mainly produces isocoumarins such as bergenin in substantial amounts (Ahmad 1972).

As a continuation of our programme aimed at the isolation of naturally occurring compounds with potential antiprotozoal activities it was deemed necessary to study the chemical composition of *Flueggea virosa* which is locally used to treat sleeping sickness or related symptoms in order to corrolate its components with the use of plant as a trypanocidal remedy. In contrast to previous results (Pu et al. 2002; Dehmlow et al. 1999), only bergenin (**1**) was isolated in substantial amounts from *Flueggea virosa* and no securinega alkaloid could be detected.

Bergenin (**1**) is an isocoumarin found in a number of plant species including *Flueggea microcarpa*, *Mallotus japonicus*, *Astilbe thunbergii* and *Ardisia japonica*. It has a long history in Asian and Indian natural medicine, where it can be found in various herbal extracts purported



to exhibit anti-arrhythmia, bronchitis treating, astringent, tonic, anti-obesity and laxative properties. In recent years, it has been looked at in Western medicine as purified bergenin, with early studies reporting potential benefits to this agent including anti-hepatotoxic (Kim 2000; Lim 2001), antiarrhythmic (Pu 2002) and antiulcer (Goel 1997) activities. Bergenin has also been shown to be capable of augmenting the lipolytic action of agents acting on the adrenergic system. Studies have confirmed that while the compound itself does not directly stimulate lipolysis or have measurable adrenergic activity, it markedly enhances lipolysis induced by an adrenergic hormone such as norepinephrine, and even opposes the lipogenic (fat building) actions of insulin (Han 1998). Its exact mode of action is unknown at this time, but it is believed to involve increased norepinephrine binding to fat cells.

In our experiments, the inhibitory activity of bergenin isolated from *Flueggea virosa*, on the growth of *Trypanosoma brucei* bloodstream form was established as well as its effects on three glycolytic enzymes of *T. brucei* (GAPDH, PFK and PGK). In fact, bergenin exhibited an inhibitory activity on the growth of the bloodstream form of *T. brucei brucei* with an IC_{50} value of 1 μM when suramin (IC_{50} 0.22 μM) was used as the reference drug. Under the experimental conditions used, bergenin was considered as a moderate trypanocidal compound since its IC_{50} value was never below 0.5 μM.

In an attempt to understand its modes of action and taking into consideration the fact that bergenin is a C-glycopyranosyl derivative of 4-O-methylgallic acid (Hay and Haynes 1958), the effects of bergenin were also evaluated on three glycolytic enzymes, the inactivation of which will inevitably lead to the death of the parasite. In this context, GAPDH (glyceraldehyde-3-phosphate dehydrogenase), PFK (Phosphofructokinase) and PGK (phosphoglycerate kinase) from both *Trypanosoma brucei* and the rabbit muscle (mammalian host equivalent) were considered. The results obtained are displayed in the Table.

Our results did not show any inhibition of PGK at a 2 mM concentration of bergenin which was the maximal concentration tested and above which there was a very strong and troublesome absorbance of the reaction medium and its precipitation.

The results on GAPDH and PFK were in the same range (IC_{50} 1 to 2 mM) and did suggest that there was no better selectivity of bergenin for the enzymes of *T. brucei* with respect to those from the rabbit muscle.

In conclusion, bergenin is a moderate trypanocidal compound which is isolated in substantial amounts from *Flueggea virosa* and could serve as a tag compound for the differentiation between *Securinega* and *Flueggea* genera. However, the presence of bergenin in *Flueggea virosa* could not explain alone the anti-sleeping sickness uses of this plant by traditional healers in Africa. For any ultimate use as an acceptable trypanocide in the fight against sleeping sickness, some chemical modifications of the structure of bergenin will be necessary in order to improve its biological activity in this context.

Table: Inhibitory effects of bergenin of some glycolytic enzymes

Glycolytic enzyme	Inhibitory effect on enzymes from rabbit muscle: IC_{50} (mM)	Inhibitory effect on enzymes from *T. brucei*: IC50 (mM)
GAPDH	2.00 ± 0.04	1.00 ± 0.02
PFK	2.70 ± 0.11	1.20 ± 0.04
PGK	–	–

Experimental

1. General experimental procedures

M.ps were determined on a Gallenkamp apparatus and are not corrected. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60 F_{254}) with the mobile phase CH_2Cl_2/MeOH 4/1. TLC spots were visualized under UV light and preferentially either by iodine vapour spray or by 50% sulfuric acid and subsequent heating (black spot). CC was carried out on Merck Kieselgel 60 (70–230 mesh). NMR spectra were recorded on a 500 MHz Brucker spectrometer at 25 °C. IR by a Mattson Polaris FTIR spectrometer in the solid state (KBr).

2. Plant material

The leaves of *Flueggea virosa* subsp. *virosa* were collected along the road side in Obala (Center Province, Cameroon) in March 1999 and identified by one of us (Sonké) by comparison with the material available at the National Botanic Garden (Belgium) (BR) and the National Herbarium, Yaoundé (YA). A voucher specimen of this plant has been deposited in the National Herbarium, Yaoundé (Sonké 2247). The abbreviations of herbaria follow those suggested by Holmgren et al. (1990).

3. Extraction and isolation

Dried leaves (2.5 kg) of *F. virosa* were powdered and defatted with hexane. The residual powder was then extracted at room temperature with methanol for 48 h. The methanol extract was concentrated and upon standing at room temperature overnight deposited a brown solid which was filtered to yield impure bergenin (20 g). The filtrate and the methanol soluble-extract were pooled and taken to dryness. This resulting fraction was chromatographed on a silica gel pad and yielded pure bergenin (**1**, 3 g) under elution with CH_2Cl_2/MeOH 4 : 1. This material was further recrystallized in a methanol-water mixture (4 : 1) to give colourless crystals.

Bergenin (**1**, colourless crystals, $C_{14}H_{16}O_9.H_2O$): m.p. 132–135 °C; ^{13}C NMR δ (^{13}C): 61.33 (CH_3O-); 63.07 ($-CH_2OH$); 72.28 (C3); 74.64 (C10b); 76.03 (C4); 81.78 (C4a); 83.42 (C2); 111.48 (C7); 117.69 (C10a); 119.81 (C7a); 142.67 (C9); 149.81 (C8); 152.71 (C10); 166.19(C6). These ^{13}C-data have been published before for this compound.

4. Activity against Trypanosoma brucei

Primary screening on *Trypanosoma brucei brucei* was carried through the WHO/TDR/DDR Supported network of screening and evaluation laboratories.

5. Inactivation studies of GAPDH, PFK and PGK

Inhibitory effects of bergenin on glycolytic enzymes were made by spectrophotometry following published procedures by Claustre et al. (2002) for PFK, Willson et al. (1994) for GAPDH and Misset et al. (1984) for PGK. The glycolytic enzymes from *Trypanosoma brucei* were produced and purified according to Hannaert et al. (1995). GAPDH from Rabbit muscle, auxiliary enzymes and substrates were purchased from Boehringer Mannheim and Sigma-Aldrich Chemical Company.

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