Acta Tropica, 48(1991)251–261
Elsevier

ACTROP 00120

Constituents of *Bidens pilosa* L.: Do the components found so far explain the use of this plant in traditional medicine?

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(Received 27 February 1990; accepted 22 June 1990)

The dried aerial parts of *Bidens pilosa* L. were extracted with petrol ether, chloroform, methanol, and methanol/water. The petrol ether and the methanol/water extracts showed some antimicrobial activity. Fractionation of the extracts yielded well known substances, most of which have, however, not yet been described as constituents of *Bidens pilosa*. Several of these substances have previously been shown to be biologically active. Thus, phenylheptatriyne, linolic acid and linolenic acid have antimicrobial activities. On the other hand, friedelin and friedelan-3β-ol, as well as several of the flavonoids found are anti-inflammatory agents. The detection of these compounds in extracts from *B. pilosa* may rationalize the use of this plant in traditional medicine in the treatment of wounds, against inflammations and against bacterial infections of the gastrointestinal tract.

Key words: *Bidens pilosa* L.; Traditional medicine; Antimicrobial compounds; Anti-inflammatory compounds; Plant constituents

Introduction

Biden pilosa L. (Asteraceae, Heliantheae) is an annual, erect, branched herb originating from South America, which today is spread all over the world. It grows to a height of up to 1.5 m and has yellow flowers of 5–15 mm diameter. The plant is mainly found in tropical and subtropical regions and is widely used in traditional medicine.

In Africa, aqueous preparations of the plant are used for the treatment of wounds, against hyperemesis gravidarum (E. Lugakingira, Nansio, personal communication), as well as against stomach ache, constipation, intestinal worms (Kokwaro, 1976), dysentery, diarrhoea and colics (Watt and Breyer-Brandwijk, 1962). The juice of the plant is applied to burns (Haerdi, 1964) or used to treat conjunctivitis (Haerdi, 1964, Kokwaro, 1976, Dalziel, 1937) and otitis (Dalziel, 1937, Githens, 1949). Furthermore, it serves as a styptic to stop bleeding from a wound (Githens, 1949). The juice obtained by chewing or cooking of the roots is said to be effective against malaria (Kokwaro, 1976), whereas the young shoots are chewed by the Zulu as a remedy for rheumatism (Watt and Breyer-Brandwijk, 1962).

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In Chinese traditional medicine *B. pilosa* is also known and used against enteritis, bacillary dysentery, and pharyngitis (Wong-Leung, 1988). The crushed fresh plant or a decoction of it are used for the treatment of wounds and chronic ulcers (Wat et al., 1980).

In the Middle American islands, the plant juice is used against eye irritations; ulcers are treated with *Bidens pilosa* in Venezuela and Brazil (Wat et al., 1980). The Kallawaya in the Bolivian Andes take it in mate in combination with the juice of *Valeriana officinalis* and of peas to lower blood pressure (Bastien, 1987); they also use *Bidens pilosa* as a diuretic and choleretic and to lower the fever in case of rubella and scarlatina (Girault, 1984).

Although *Bidens pilosa* L. has been used as a medicinal plant for a long time and the antimicrobial activity of its juice and aqueous extracts has been proven (Bushnell et al., 1950; Wong-Leung, 1988), no systematic investigation with respect to biologically active constituents of the plant has been undertaken hitherto. Yet, a number of natural products from *B. pilosa* have been isolated and identified, mainly polyacetylenes and flavonoids. Bohlmann et al. (1964), investigated several *Bidens* species* for their polyacetylenes. The fresh aerial parts of the plant contained the polyynes **1** (main constituent), **2**, **3** and **4** (in traces), as well as the thiophene derivative **5**. Fresh roots of *B. pilosa* contained **6**, **7**, **8**, **9**, and **10**. Several flavonoids (aglycones and glycosides) have also been isolated (Ballard, 1975; Hoffmann and Hölzl, 1988), among them the chalcones okanin (**11**) and butein (**12**), as well as quercetin 3-*O*-glucoside (**13**), the flavones luteolin (**14**) and apigenin (**15**) and a series of corresponding glucosides. We tried to isolate additional biologically active principles of the medicinal plant.

Results

The dried aerial parts of *Bidens pilosa* L. were ground and extracted successively with petrol ether, chloroform, methanol, and methanol/water. The antimicrobial activity of the crude extracts was determined with nine non pathogenic microorganisms (see Table 1). Only the petrol ether and the methanol/water extracts showed an activity, mainly against Gram-positive bacteria, which was, however, rather modest compared with chloramphenicol.

Several constituents (see Table 2) could be isolated from the crude extracts by repeated column chromatography. They were identified by chromatographic and spectroscopic comparison with authentic samples and with data from the literature. With the exception of 1-phenyl-1,3,5-heptatriyne (**1**) (Bohlmann et al., 1964), stigmasterol, β-sitosterol and the long chain alkanes (Chen et al., 1975), the compounds found have not yet been described as constituents of *Bidens pilosa*; they are, however, rather ubiquitously found in plants.

Discussion

The antimicrobial activity of the petrol ether extract is certainly due to its content of phenylheptatriyne (**1**), linolic acid (**20**) and linolenic acid (**21**). The latter two

*For chemotaxonomic studies on *B. pilosa* see e.g. Ballard (1975), Bohlmann and Zdero (1975), Crawford and Stuessy (1981), Harborne and Turner (1984), Marchant et al. (1984).

TABLE 1

Antimicrobial activities of the crude extracts from *Bidens pilosa* L.[a]

Test organism	Petrol ether extract	$CHCl_3$ extract	MeOH extract	MeOH/H_2O extract	Chloramphenicol
M. luteus	(7)	–	–	(7)	–
B. megaterium	7	–	–	–	19
C. glutamicum	–	–	–	–	23
S. griseus	(6)	–	–	(7)	29[b]
E. coli	–	–	–	–	23
A. faecalis	(7)	–	–	–	9
B. catarrhalis	–	–	–	–	45
X. vesicae	–	–	–	(5.5)	–
S. cerevisiae	(8)	–	–	–	–

[a]Diameters of the inhibition zones in mm; values in parentheses: partial inhibition only; –: inactive; diameter of the holes and the discs: 5 mm; amount of substance applied: 1 mg of the crude extracts, 30 µg of chloramphenicol.
[b]*S. coelicolor.*

compounds are known bacteriostatics even at concentrations as low as 5–50 ppm (Nieman, 1954; Hattori et al., 1987). This is in the range of the solubility of these compounds in aqueous media. It seems therefore possible that the fresh juice of the plant or a concentrated aqueous extract may contain sufficient amounts of unsaturated fatty acids to be active against bacteria. It is not known so far, however, whether these compounds are present as free fatty acids in the fresh plant, or whether they are formed from oils or fats by enzymatic degradation when the plant material is dried (Wagner, 1980, p. 264).

Phenylheptatriyne (**1**), the main polyacetylene from the petrol ether extract of *B. pilosa*, is rather active against Gram-positive bacteria and shows only a weak activity against Gram-negative organisms, dermatophytes, yeasts and molds (Bondarenko et al., 1985). Compound **1** is phototoxic, which means that it is only active against microorganisms when it is irradiated with ultraviolet light with a wavelength of 360–370 nm (Wat et al., 1979). The polyacetylenes **4** and **6** are also phototoxic; they show, however, even in the dark a certain bacteriostatic and fungistatic activity, which may be the reason why parts of *B. pilosa* are able to inhibit the growth of *Candida albicans* in the dark (Towers et al., 1977). 1-Phenylhepta-1,3,5-triyne (**1**) was further shown to have anthelmintic and protozoocidal properties in vitro and in infected mice (N'Dounga et al., 1983). Cercaries of schistosomal and echinostomal trematodes are paralyzed irreversibly in the dark or under UV light within 1–15 min at concentrations of 0.3 ppm (Towers et al., 1984).

The phytosterols and *n*-alkanes that were isolated from the petrol ether extract are also said to have a certain antibacterial activity (Goyal and Gupta, 1988). It is, however, questionable, whether these almost water insoluble compounds can reach sufficiently high concentrations in the juice from fresh plants or in aqueous extracts to give such preparations a significant activity.

The pentacyclic triterpenes friedelin (**22**) and friedelan-3β-ol (**23**) have anti-inflammatory and anticonvulsant properties (Chaturvedi et al., 1974). Similarly, the flavonoids luteolin 7-*O*-β-D-glucoside (**17**) and quercetin 3-*O*-β-D-galactoside (**18**), as

TABLE 2

Constituents isolated from *Bidens pilosa* L. extracts

Compound	Content (%)[a]	Biological activity[b]
Petrol ether extract		
Phenylheptatriyne (**1**)	3×10^{-3}	antimicrobial, fungicidal (Bondarenko et al., 1985), anthelmintic, protozoocidal (N'Dounga et al., 1983), cercaricidal (Towers et al., 1984)
Linolic acid (**20**)	5×10^{-3}	bacteriostatic (Niemann, 1954; Hattori et al., 1987), fungicidal (Baker et al., 1970)
α-Linolenic acid (**21**)	6×10^{-3}	
Squalene	8×10^{-3}	
Friedelin (**22**)	7×10^{-3}	anti-inflammatory, anticonvulsant (Chaturvedi et al., 1974), slightly fungistatic (Nes and Patterson, 1981)
Friedelan-3β-ol (**23**)	4×10^{-3}	anti-inflammatory, anticonvulsant (Chaturvedi et al., 1974)
Stigmasterol, β-Sitosterol, Campestrol[c]	3×10^{-3}	antibacterial (Goyal and Gupta, 1988)
Mixture of waxes[d]	2×10^{-3}	
Mixture of triglycerides[e]	0.4	
Mixture of *n*-alkanes[f]	0.03	antibacterial (Goyal and Gupta, 1988)
Methanol extract		
Luteolin 7-*O*-β-D-glucopyranoside (**17**)	5×10^{-3}	anti-inflammatory (Alcaraz and Jimenez, 1988)
Quercetin 3-*O*-β-D-glucopyranoside (**13**)	0.01	anti-inflammatory (Maki, 1966)
Quercetin 3-*O*-β-D-galactopyranoside (**18**)	6×10^{-3}	anti-inflammatory (Alcaraz and Jimenez, 1988)
Methanol/water extract		
Quercetin 3-*O*-β-D-glucuronopyranoside (**16**)	0.02	

[a]Minimal content with respect to dried plant material.
[b]Only those activities are listed which are in relation to the use of *B. pilosa* in traditional medicine.
[c]Isolated as a mixture.
[d]Consisting according to MS mainly of the C_{16}-, C_{18}-, C_{20}- and C_{22}-fatty acids and the C_{22}-, C_{24}-, C_{26}-, and C_{28}-fatty alcohols.
[e]Main fatty acid components (from methanolysis followed by GC/MS): palmitic (36%), linolic (44%), oleic (13%), stearic (5%), myristic (< 1%), arachidic (< 1%).
[f]$C_{21}H_{24}$ to $C_{33}H_{68}$; compounds with an odd number of carbon atoms were predominant; main constituents: $C_{23}H_{48}$ and $C_{29}H_{60}$.

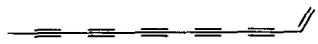
well as luteolin (**14**), apigenin (**15**) and apigenin 7-*O*-glucoside (**19**) (Ballard, 1975) are known anti-inflammatory agents (Alcaraz and Jiménez, 1988). However, *B. pilosa* contains only rather small amounts of these compounds. Again, it is not yet known, whether the plant juice or strong aqueous extracts contain enough of these compounds to have anti-inflammatory properties.

The compounds recognized so far as constituents of *Bidens pilosa* L. include quite a number of substances which could rationalize the use of this plant in the treatment of wounds, against inflammations and against bacterial infections of the gastrointestinal tract, provided these compounds were also present in the traditional preparations, which has not been tested so far.

1: R = CH_3

2: R = CH_2OH

3: R = CH_2OAc



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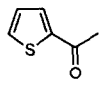

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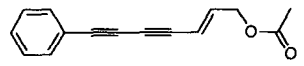
6: R = CH_3

7: R = CHO

8: R = CH_2OH

9: R = CH_2OAc



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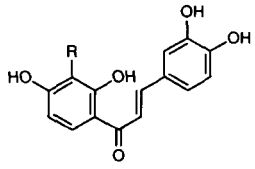
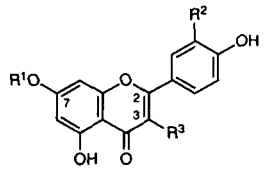
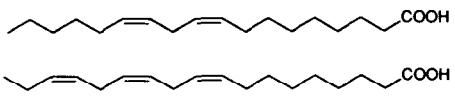


11: R = OH

12: R = H



	R^1	R^2	R^3
13:	H	OH	Oglucose
14:	H	OH	H
15:	H	H	H
16:	H	OH	Oglucuronic acid
17:	glucose	OH	H
18:	H	OH	Ogalactose
19:	glucose	H	H



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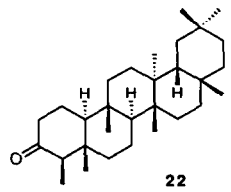

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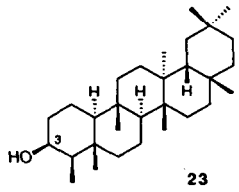

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Materials and Methods

General remarks

NMR: Bruker WH 90 (22.63 MHz ^{13}C); Varian VXR 400 (400 MHz ^{1}H and 101 MHz ^{13}C). *MS*: VG 70-250; GC/MS; Hewlett-Packard GC HP 5790A with MSD HP 5970A. Hewlett-Packard dimethylsilicone capillary column (12 m). *UV/VIS*: Beckman model 25; the UV spectra of the flavonoids were measured in MeOH and after the addition of the usual shift reagents (Mabry, 1970, p. 35 ff). *Optical rotations*: polarimeter Perkin-Elmer 141 at 589 nm. *GC*: Hewlett-Packard GC HP 5790A; Hewlett-Packard dimethylsilicone capillary column (12 m); injector temperature 250 °C, detector and interface temperature 300 °C, temperature program: 150° (3 min)→ + 10°/min→300°C (10–20 min). *HPLC*: Spectra Physics pump and gradient mixer SP 8700, UV/VIS detector SP 8400; prepacked column (Dr. H. Knauer, Berlin, 25 × 1.6 cm, LiChrosorb RP-18, 7 µm, E. Merck). *Low pressure liquid chromatography (LPLC)*: Pump Duramat-80 (Chemie und Filter, Regensdorf); Lobar LiChroprep RP-8 prepacked columns (40–63 µm, E. Merck). *Adsorbents for column chromatography*: cellulose, microcrystalline (E. Merck), silica gel 60, 40–60 µm, or 30–75 µm (Chemische Fabrik Uetikon), silica gel H according to Stahl for TLC, 5–25 µm (E. Merck), MN-Polyamide SC 6 (polycaprolactam), 50–160 µm (Macherey-Nagel), Sephadex LH-20 (Pharmacia Fine Chemicals).

Plant material

The plant material was harvested and dried by E. Lugakingira in May 1984 in Nansio (Ukerewe Island, Tanzania). It was identified botanically as *Bidens pilosa* L. by Dr. F. Haerdi (F. Hoffmann-La Roche & Co. AG, Basel, and the Pharmazeutisches Institut der Universität Basel). Seeds of this material were cultivated in Birsfelden near Basel in summer 1987; in fall 1987 two plants of 1.5 m height with flowers and fruit were harvested, pressed and deposited as voucher specimens with the Botanisches Institut der Universität Basel.

Extraction

The dried plant material consisting of stems, leaves, flowers and seeds was ground to a fine powder, 115 g of which were extracted in a 2 l conical flask with 5 × 740 ml of petrol ether, 6 × 670 ml of $CHCl_3$, 6 × 820 ml of MeOH and 6 × 810 ml of MeOH/H_2O 1:1, leading after evaporation of the solvents to 1.94 g of petrol ether extract, 1.64 g of $CHCl_3$ extract, 17.5 g of MeOH extract and 17.6 g of MeOH/H_2O extract.

Constituents of the petrol ether extract

A sample of the petrol ether extract (3.5 g) was dissolved in 5 ml of petrol ether and subjected to flash chromatography on 150 g of silica gel 60 (column diameter 50 mm; elution successively with 0.5% AcOEt in petrol ether (250 ml), 1% AcOEt in petrol ether (100 ml), 2→5→10→20→40→100% AcOEt in petrol ether (500 ml each), 10→20→50% MeOH in AcOEt (200 ml each)). A total of 95 fractions of 40–45 ml each was collected. They were pooled as follows: Fractions 1–5: 2.1 mg; fr. 6–9:

66 mg; fr. 10–11: 9 mg; fr. 12–16: 9.5 mg; fr. 17–22: 12.5 mg; fr. 23–28: 15 mg; fr. 29–31: 516 mg; fr. 32–39: 857 mg; fr. 40–42: 83 mg; fr. 43–45: 54 mg; fr. 46–50: 388 mg; fr. 51–54: 312 mg; fr. 55–59: 275 mg; fr. 60–65: 180 mg; fr. 66–76: 155 mg; fr. 77–95: 47 mg.

1-Phenylhepta-1,3,5-triyne (**1**): A portion (9 mg) of the material from fractions 17–22 of the petrol ether extract was rechromatographed on 1 g of silica gel in an open column (diameter 8 mm; pentane) to give 3.2 mg of **1** as a pale yellow solid. Identification by UV (Sörensen and Sörensen, 1958), IR, ^{1}H-NMR (Wat et al., 1979), and MS.

Linolic acid (**20**) *and α-linolenic acid* (**21**): A portion (0.26 g) of the material from fractions 51–54 of the petrol ether extract was prepurified on 25 g of silica gel in an open column (diameter 25 mm; elution with 400 ml of $CHCl_3$, then 130 ml of MeOH in $CHCl_3$ (0.5→2%); fractions of 15–20 ml). Rechromatography of 155 mg of the material from the fractions 8–11 with LPLC on a RP-8 column (31 × 2.5 cm; 900 ml of MeOH with 40→0% H_2O, then 100 ml of MeOH/CH_2Cl_2 1:1; fractions of 25–30 ml) gave 66 mg of a mixture of **20** and **21** in fractions 16–21. Separation was achieved by HPLC on the RP-18 column (acetonitrile/H_2O 9:1 (5 ml/min); detection at 200 nm). α-Linolenic acid (2.4 mg, **21**) and linolic acid (1.8 mg, **20**, slightly contaminated with **21**) were obtained. Identification by TLC with authentic samples and by ^{1}H-NMR and MS.

Squalene: The fractions 23–28 of the petrol ether extract consisted to 90% of squalene according to GC. Identification by GC and TLC with an authentic sample and by MS.

Friedelin (**22**): The fractions 29–31 of the petrol ether extract were further separated by column chromatography on 50 g of silica gel (diameter 25 mm; 1.56 l of petrol ether with 95→0% of CH_2Cl_2). The fractions 33–36 of this column were subjected to flash chromatography (5 g of silica gel H; 320 ml of petrol ether/toluene 75:25→50:50). Fractions 12–22 of this separation gave after three additional purifications with small amounts of silica gel 9 mg of friedelin (**22**). Identification by ^{1}H-NMR (Kikuchi et al., 1980), ^{13}C-NMR (Patra et al., 1981), and MS (Hirota et al., 1975).

Friedelan-3β-ol (**23**): The fractions 40–42 of the petrol ether extract were further separated by column chromatography on 8 g of silica gel (diameter 15 mm; 310 ml of petrol ether with 50→100% of CH_2Cl_2). The fractions 9–10 of this column were rechromatographed with CH_2Cl_2 on 5 g silica gel to give 6.3 mg of friedelan-3β-ol (**23**). Identification by ^{1}H-NMR (Kikuchi et al., 1980), ^{13}C-NMR (Patra et al., 1981), and MS (Hirota et al., 1975), and by acetylation with Ac_2O in pyridine to friedelan-3β-yl acetate ($[\alpha]_D = +41° \pm 4°$ ($c = 0.13$, $CHCl_3$), Pradhan et al. (1985): $[\alpha]_D = +38°$). Identification by ^{1}H-NMR (Kikuchi et al., 1980).

Mixture of stigmasterol, β-sitosterol and campesterol: A portion (0.17 g) of the material from fractions 60–65 of the petrol ether extract was chromatographed on 20 g of silica gel (column diameter 25 mm; 300 ml of CH_2Cl_2, then 560 ml of CH_2Cl_2 with 0.2→5% of MeOH). Fractions 10–12 of this column (5.6 mg) were dissolved in warm petrol ether. Upon cooling, 3.1 mg of a mixture of stigmasterol, β-sitosterol and campesterol precipitated. Identification by GC/MS comparison with authentic samples.

Mixture of saturated esters: The fractions 29–31 of the petrol ether extract were chromatographed on 50 g of silica gel (column diameter 25 mm; 1.56 l of CH_2Cl_2

with 95→0% of petrol ether). Part (67 mg) of the material (80 mg) obtained in fractions 10–12 of this column was subjected first to flash chromatography on 13.8 g of silica gel H (column diameter 18 mm; 130 ml of petrol ether/CH_2Cl_2 83:17) and then to LPLC on RP-8 (column 24 × 1 cm; 20 ml of MeOH, 20 ml of MeOH/AcOEt 1:1, 20 ml of AcOEt, 60 ml of CH_2Cl_2). The fractions 16–18 (4.5 mg) from this column were finally purified on 5 g of silica gel (petrol ether/CH_2Cl_2 8:2) to give 3.5 mg of a mixture of saturated esters. Identification by ^{1}H-NMR, ^{13}C-NMR, and MS.

Triglyceride mixture: A sample (0.1 g) of the material from fractions 32–39 of the petrol ether extract was chromatographed on 10 g of silica gel (column diameter 17 mm; 100 ml of CH_2Cl_2/petrol ether 3:1) to give in fractions 4–6 90 mg of a mixture of triglycerides as a slightly yellowish oil. Identification by ^{1}H-NMR and MS. *Methanolysis*: The triglyceride mixture (2.4 mg) was dissolved in 0.2 ml of heptane (p.a., Merck) and 0.1 ml of a solution of 0.1 g of Na in 10 ml of MeOH (absolute, Baker) was added. The reaction mixture was refluxed for 25 min. After cooling, 1 ml of heptane and 1 ml of MeOH were added. The heptane layer was separated and the methyl esters contained in it were identified by GC and GC/MS comparison with a commercial reference mixture.

n-Alkanes: GC/MS comparison of the fractions 6–9 of the petrol ether extracts with a sample obtained by extraction of 'parafilm' (Gaskin et al., 1971) and addition of a trace of tetracosane allowed the following constituents to be identified; $C_{21}H_{44}$ (0.7% of the total alkane mixture), $C_{22}H_{46}$ (0.6%), $C_{23}H_{48}$ (6.3%), $C_{24}H_{50}$ (1.0%), $C_{25}H_{52}$ (1.8%), $C_{26}H_{54}$ (0.2%), $C_{27}H_{56}$ (1.9%), $C_{28}H_{58}$ (0.5%), $C_{29}H_{60}$ (6.9%), $C_{30}H_{62}$ (0.7%), $C_{31}H_{64}$ (6.6%), $C_{32}H_{66}$ (0.3%) and $C_{33}H_{68}$ (0.8%).

Constituents of the MeOH extract

The MeOH extract (12 g in 5 portions) were fractionated by DCCC (Hostettmann, 1983; Hostettmann et al., 1986) (Büchi 670 DCC Chromatograph, 294 columns 2.7 mm diameter; $CHCl_3$/MeOH/PrOH/H_2O 50:50:19:40; descending mode; 30 ml/h) into 85 fractions of 20 ml each.

*Luteolin 3-O-β-*D*-glucopyranoside* (**17**), *quercetin 3-O-β-*D*-glucopyranoside* (**13**) *and quercetin 3-O-β-*D*-galactopyranoside* (**18**). A sample (0.25 g) of the material obtained in fractions 50–64 of the DCCC was fractionated on 26 g of polyamide (column diameter 26 mm; 670 ml of MeOH with 50→0% of H_2O). The material eluted with ca. 90% MeOH (77 mg) was dissolved in 3 ml of MeOH/H_2O 2:1 and rechromatographed on 15 g of cellulose (column diameter 20 mm; 545 ml of H_2O with 0→100% of MeOH). The fractions 14–20 (9.2 mg) were pooled, evaporated and dissolved in a small amount of MeOH. *Luteolin 3-O-β-*D*-glucopyranoside* (**17**, 3 mg) was obtained as a yellow, amorphous precipitate. Fractions 2–7 contained 29 mg of a slightly contaminated mixture of **13** and **18**. Three successive chromatographies on 20 g of Sephadex LH-20 each (column 17 × 2.5 cm; MeOH) finally gave *quercetin 3-O-β-*D*-glucopyranoside* (**13**, 7 mg, contaminated with some **18**), *quercetin 3-O-β-*D*-galactopyranoside* (**18**, 3.8 mg, contaminated with some **13**) and 6 mg of a mixture of **13** and **18**. Identification by UV (Mabry et al., 1970, p. 96 and 128; Barberán et al., 1985), ^{1}H-NMR (Barberá et al., 1986), ^{13}C-NMR (Markham et al., 1978), and FAB-MS. *Acid hydrolyses of* **13** *and* **18**: 1–2 mg of the glycosides or of their mixture were refluxed for 1.5 h with 1 ml of 2N HCl. Then 3 ml of H_2O were added and the

aglycone was extracted into 2 × 10 ml of AcOEt. The aqueous phase containing the sugar was evaporated and the sugar identified on TLC with authentic samples of glucose and galactose. The aglycone in the organic layer was identified by TLC comparison with an authentic sample of quercetin and by UV/VIS spectroscopy (Mabry et al., 1970, p. 126).

Constituents of the MeOH/H_2O extract

The MeOH/H_2O extract (17.5 g) was partitioned between H_2O and 1-BuOH. The organic layer was filtered and evaporated to give 0.785 g of an orange-brown amorphous solid, 0.74 g of which were dissolved in 7 ml of MeOH containing some H_2O and chromatographed on Sephadex LH-20 (column 16.5 × 2.6 cm; MeOH; 32 fractions of 10 ml each).

*Quercetin 3-O-β-*D*-glucuronopyranoside* (**16**): The fractions 17–20 contained 66 mg, 46 mg of which were chromatographed on 4.5 g of polyamide (280 ml of acetone/H_2O 8:2 with 0→1% of 25% aqueous NH_3). Fractions 16–18 of this column were purified on Sephades LH-20 (column 14 × 2.6 cm; MeOH/H_2O 85:15) to give 13 mg of **16** as a yellow solid. Identification by UV, ^{1}H-NMR, ^{13}C-NMR (Merfort and Wendisch, 1988), and FAB-MS. *Hydrolysis*: Compound **16** (4.5 mg) was refluxed with 1 ml of 2 M HCl for 2 h. Then 3 ml of H_2O were added and the solution was extracted twice with 5 ml of AcOEt. Upon evaporation, the aqueous layer yielded ca. 2 mg of D-glucuronic acid. The organic layer contained cs. 2.8 mg of quercetin. Both compounds were identical with authentic samples on TLC. Specific rotation of the sugar: $[\alpha]_D = +12° \pm 6°$ ($c = 0.15 \pm 0.04$, H_2O), Rehorst (1929): $[\alpha]_D = +12.4 \rightarrow +35.9°$ ($c = 2.8$, H_2O).

Tests for antimicrobial activity

The microbiological tests were carried out in the laboratories of the Department of Microbiology, Biocenter, University of Basel. Test organisms: Gram-positive: *Micrococcus luteus*, *Bacillus megaterium*, *Corynebacterium glutamicum*, *Streptomyces coelicolor*, *or griseus*; Gram-negative: *Escherichia coli* K 12, *Alcaligenes faecalis*, *Branhamella catarrhalis*, *Xanthomonas vesicae;* fungus: *Saccharomyces cerevisiae*. The substances to be tested were applied to paper discs according to the method of Kirby and Bauer (Bauer et al., 1966; von Graevenitz et al., 1984), or were deposited as solutions in petrol ether or H_2O in holes of 5 mm diameter punched into the agar plates (Geissberger, 1988).

Acknowledgements

The authors wish to thank E. Lugakingira, Nansio (Ukerewe, Tanzania), for collecting and drying *Bidens pilosa*, Dr. F. Haerdi, Basel, for botanical identification of the plant material, and PD Dr. J. Meyer and Mrs. M. Carlemalm, Basel, for their help with the microbiological tests. Financial support of this work was provided by the Swiss National Science Foundation (grant No. 20-5098.86) and the Ciba-Stiftung, Basel.

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