JOURNAL OF NATURAL PRODUCTS

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J. Nat. Prod., **1993**, 56 (5), 682-689• DOI: 10.1021/np50095a004 • Publication Date (Web): 01 July 2004

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Journal of Natural Products
Vol. 56, No. 5, pp. 682-689, May 1993

SEARCH FOR BITTER PRINCIPLES IN *CHIRONIA* SPECIES BY LC-MS AND ISOLATION OF A NEW SECOIRIDOID DIGLYCOSIDE FROM *CHIRONIA KREBSII*

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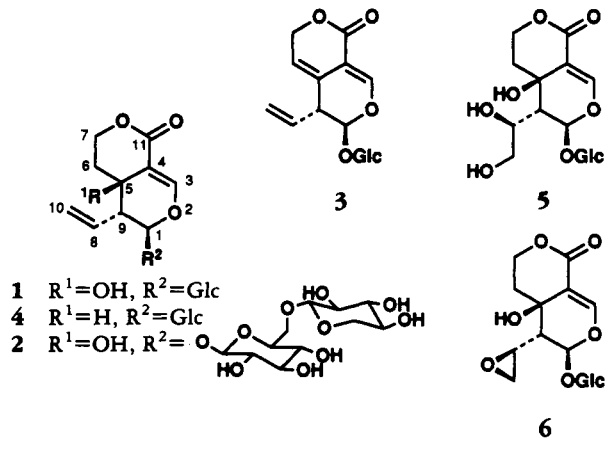
ABSTRACT.—The MeOH root extracts of four *Chironia* species, *Chironia krebsii, Chironia palustris, Chironia purpurascens,* and *Chironia baccifera*, have been screened for secoiridoids by thermospray lc-ms and lc-uv. Swertiamarine [**1**], gentiopicroside [**3**], sweroside [**4**], eustomoside [**5**], and eustomorusside [**6**] were tentatively identified, and the presence of the secoiridoid diglycoside **2** was detected. Compounds **1**–**4** were subsequently isolated from *C. krebsii* and *C. palustris*. The structure of the new secoiridoid **2** (chironioside) was established as swertiamarine-6′-*O*-(β-D-xylopyranoside) by chemical and spectroscopic methods, including double-quantum-filtered phase-sensitive COSY.

Species of the Gentianaceae are known to possess xanthones and secoiridoids (1–3), two classes of compounds with interesting biological activities. Certain xanthones, such as bellidifolin (1,5,8-trihydroxy-3-methoxyxanthone) and gentiacaulein (1,3-dihydroxy-7,8-dimethoxyxanthone), are potent inhibitors of monoamine oxidase (MAO), with a selectivity towards type A MAO (4,5). Secoiridoids are the bitter principles in various gentians which are used as bitter tonics (1). In the course of an investigation of tropical and subtropical Gentianaceae (6,7), we have collected several *Chironia* species. The genus *Chironia* L. is distributed mainly in South Africa, but several species range northward into tropical Africa and Madagascar (8). An initial phytochemical study of the roots and leaves of *Chironia krebsii* Griseb. has afforded 17 xanthones (7), which are currently being tested for their inhibitory activity on monoamine oxidases A and B. Extending the investigation to other classes of secondary metabolites typical of the Gentianaceae family, we have studied the secoiridoid pattern of four *Chironia* species, namely, *C. krebsii, Chironia palustris* Burch., *Chironia purpurascens* Verdoorn, and *Chironia baccifera* L. The MeOH root extracts were first screened by lc-uv and thermospray (tsp) lc-ms (9) for the purpose of rapid identification of known secoiridoids for chemotaxonomic comparison and the search for minor iridoids that would otherwise go unnoticed by conventional uv detection only. Peaks of interest could thus be identified with the aid of on-line uv and ms data, and a targeted isolation procedure afforded three known secoiridoids and a new secoiridoid diglycoside.

RESULTS AND DISCUSSION

Roots of *C. krebsii, C. palustris, C. purpurascens*, and *C. baccifera* were extracted successively with CH_2Cl_2 and MeOH. In order to obtain rapid information on the secoiridoid pattern of the four species, the MeOH extracts were submitted to lc-uv and tsp lc-ms analysis. Satisfactory separation was achieved by reversed-phase hplc (RP18) using a linear MeCN/H_2O gradient. The tsp interface requires aqueous mobile phases for ionization and is thus best suited for reversed-phase hplc.



Lc-uv analysis allowed a preliminary identification of secoiridoid peaks in the chromatogram. In the extract of all four *Chironia* species, the peaks eluted during the first 10 min exhibited uv spectra attributable to secoiridoids. The absorption maxima of compounds **1**, **2**, **4**, **5**, and **6** (Figure 1) were at 238, 238, 244, 235, and 235 nm, respectively. Compounds **5** and **6** (Rt 7.40 and 4.10 min, respectively) were only detectable in the extracts of *C. palustris* and *C. purpurascens*. The uv spectrum of compound **3** showed two maxima at 241 and 270 nm, which were indicative of the presence of an additional conjugated double bond.

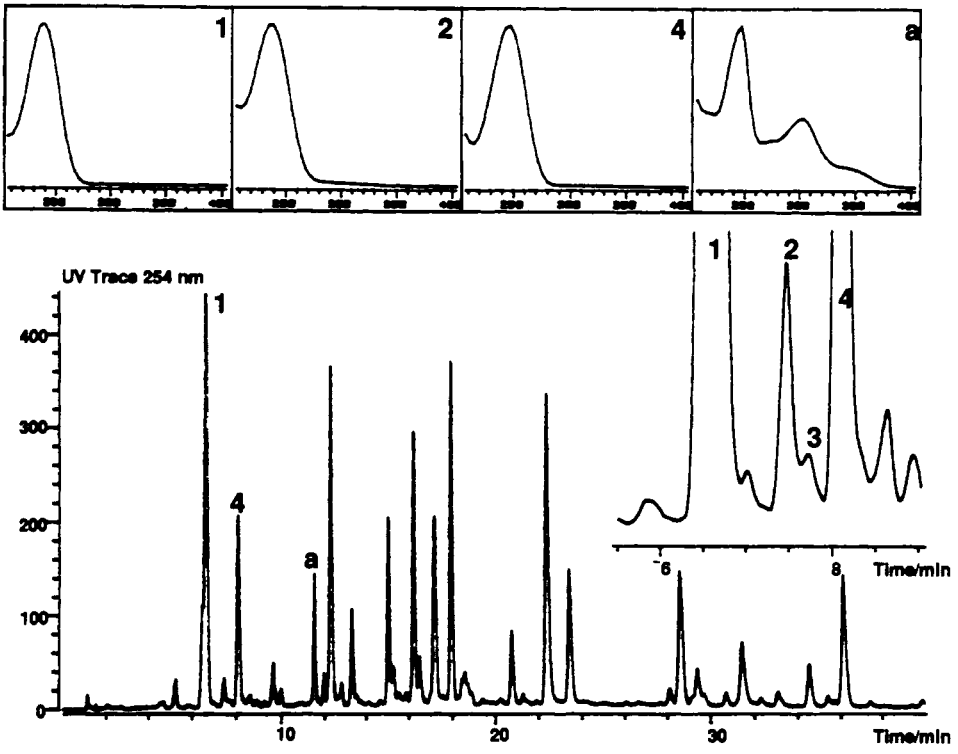


FIGURE 1. Hplc chromatogram of an MeOH root extract of *Chironia krebsii*. For conditions, see Experimental. The inset is an expansion of the chromatogram (Rt 5–9 min). The spectrum of peak a is typical of a xanthone, and all compounds eluted after a are also xanthones (7).

On-line tsp mass spectra of compounds **1–6** were recorded in the positive ion mode. The ionization of the solutes was enhanced by post-column addition of aqueous NH_4OAc (10). Intense pseudomolecular ions $[M+H]^+$ at *m/z* 375, 359, and 391 were observed for **1**, **4**, and **6**, respectively, together with weak $[M+NH_4]^+$ adduct ions at *m/z* 392, 376, and 408. On the other hand, an intense $[M+NH_4]^+$ ion at *m/z* 374 and a weaker $[M+H]^+$ ion at *m/z* 357 were recorded for **3**, whereas the facile dehydration in compound **5** led to a prominent $[M+NH_4-H_2O]^+$ signal at *m/z* 408. Elimination of a hexosyl moiety in compounds **1**, **3**, and **4** was indicated by weak $[M+H-162]^+$ ions at *m/z* 213, 195, and 197, respectively. In the case of glycosides **5** and **6**, the fragment ions at *m/z* 246 and 228 $[M-NH_4-18-162]^+$ result from elimination of a hexosyl moiety and consecutive dehydration. The tsp ms of compound **2** exhibited weak ions at *m/z* 524 and 506 ($[M+NH_4]^+$ and $[M+NH_4-H_2O]^+$). An intense

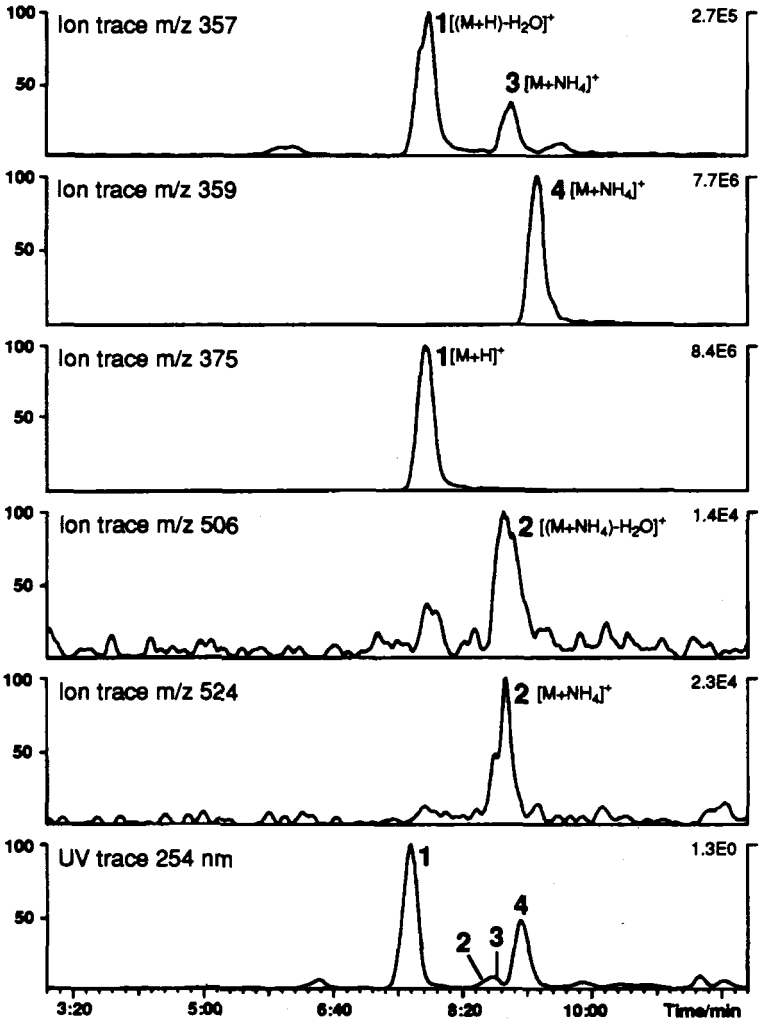


FIGURE 2. Tsp lc-ms analysis of an MeOH root extract of *Chironia krebsii*. Hplc conditions as for Figure 1. For tsp conditions, see Experimental. The display is an expansion of the chromatogram (Rt 3–12 min). The ion traces corresponding to the pseudomolecular ions of **1–4** and the uv trace are displayed.

fragment peak at *m/z* 374 $[M + NH_4 - 132]^+$ and a signal at *m/z* 212 $[M + NH_4 - 132 - 162]^+$ were indicative of the successive elimination of a pentosyl and a hexosyl moiety (Figures 2 and 3).

These on-line spectroscopic data together with chemotaxonomical considerations strongly suggested that compounds **1**, **3**, **4**, **5**, and **6** could be the known secoiridoids swertiamarine, gentiopicroside, sweroside, eustomorusside, and eustomoside, respec-

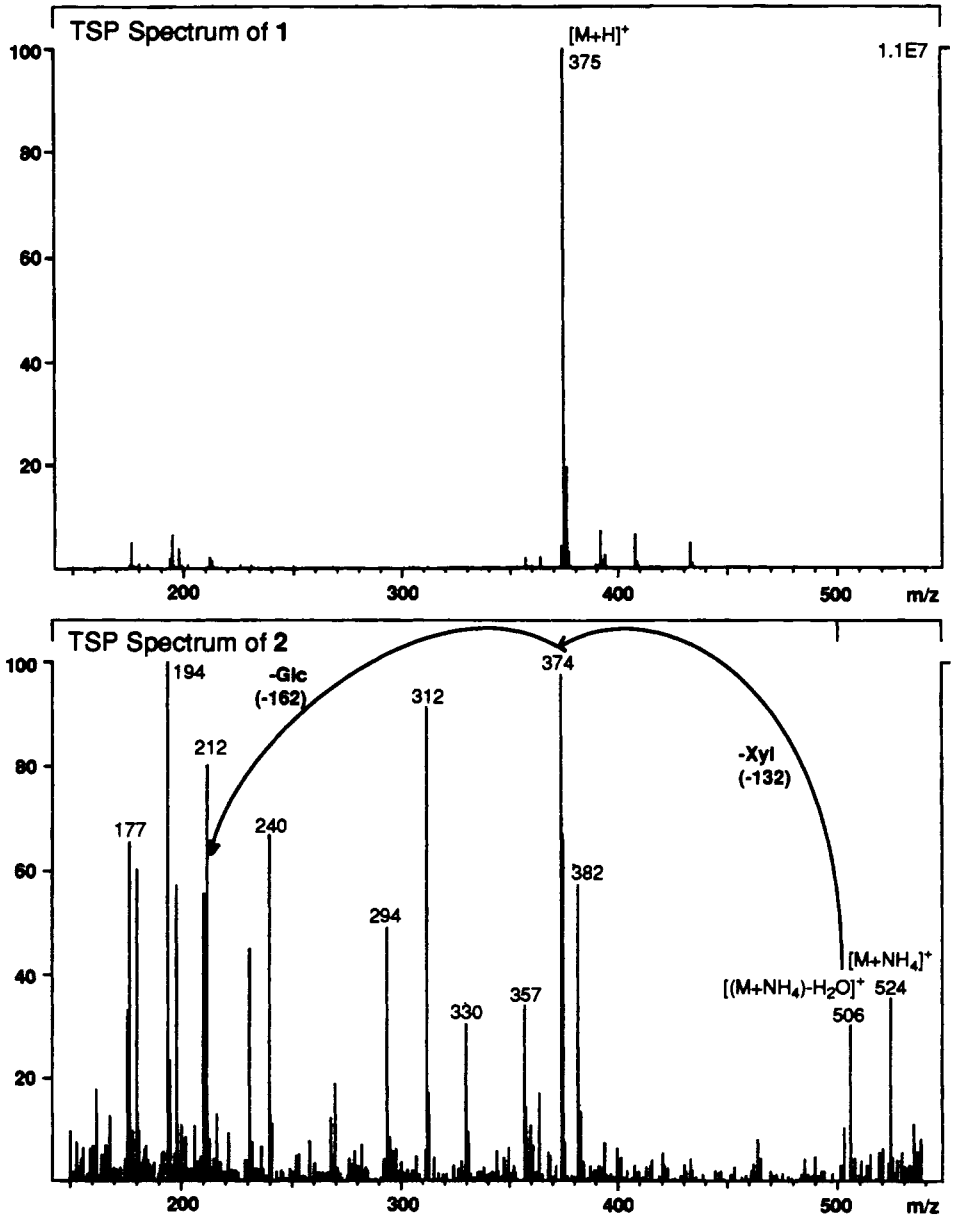


FIGURE 3. Tsp lc-ms spectra of **1** and **2** recorded on-line. Hplc and tsp conditions as in Figure 2. Swertiamarine [**1**] exhibits an intense pseudomolecular ion corresponding to the $[M + H]^+$ adduct at *m/z* 375. Chironioside [**2**] is characterized by two weak pseudomolecular ions $[M + NH_4]^+$ and $[(M + NH_4) - H_2O]^+$ at *m/z* 524 and 506, respectively, and intense fragment ions resulting from the consecutive losses of a pentosyl and of a hexosyl moiety (−132 and −162 amu, respectively).

tively (11,12). Compound **2** exhibited a uv spectrum similar to swertiamarine [**1**], but ms data showed the presence of an additional pentosyl moiety. Thus, it was reasonable to assume secoiridoid **2** to be a diglycoside derived from swertiamarine. In order to verify the identity of the known secoiridoids and to elucidate the structure of the presumably new secoiridoid diglycoside, targeted isolation of these compounds from the MeOH extracts of *C. krebsii* and *C. palustris* was undertaken. The extract of *C. krebsii* was first fractionated by gel chromatography on Sephadex LH-20 with MeOH to afford a secoiridoid fraction. The separation of compounds **1**, **2**, and **4** was subsequently achieved by medium pressure liquid chromatography (mplc) on RP-8 and semi-preparative hplc on RP-18, using MeOH/H_2O mixtures. Following the same strategy, the MeOH extract of *C. palustris* afforded compounds **3** and **4** (see Experimental). The limited sample amount of *C. purpurascens* has so far precluded the isolation of **5** and **6**, which occur in the extract in minute amounts only.

Swertiamarine [**1**], gentiopicroside [**3**], and sweroside [**4**] have all been previously isolated from other Gentianaceae species (13–15). Uv, ^{1}H-nmr, ^{13}C-nmr, dcims, and physical data were in accord with those reported in the literature (11,12).

Acid hydrolysis of compound **2** afforded glucose and xylose. The dcims (NH_3, positive ion mode) and the tsp lc-ms spectra showed two quasimolecular ions at *m/z* 506 $[M + NH_4 - H_2O]^+$ and 524 $[M + NH_4]^+$, together with fragment signals at *m/z* 374 $[M - 132]^+$ and 212 $[M - 132 - 162]^+$, resulting from a consecutive loss of a pentosyl and a hexosyl moiety. The absence of fragment ions at *m/z* 362 $[M + NH_4 - 162]^+$ and 344 $[M - 162]^+$ suggested a disaccharide moiety with xylose as the terminal sugar. In the ^{1}H-nmr spectrum of **2**, the signals of the monoterpene residue were virtually superimposable with those of the aglycone part of swertiamarine. Two anomeric protons at δ 4.45 (1H, d, $J = 7.8$, H-1′) and δ 4.18 (1H, d, $J = 7.4$ Hz, H-1″) were attributable to β-glycosyl and β-xylosyl moieties, respectively. Compared to swertiamarine [**1**], the DEPT spectra of **2** exhibited signals of four additional CH and one CH_2. All the carbon signals observed for **1** are present in the ^{13}C-nmr spectrum of **2**, the downfield shift of the C-6′ resonance in the latter compound (−7.1 ppm) indicating glycosidation at this position (16). Comparison of the ^{13}C-nmr spectral data of **2** with those reported for 3,5,6,7,8-pentamethoxy-1-*O*-primeverosylxanthone isolated from the same plant (7) confirmed that the sugar moiety was 6-*O*-(β-D-xylopyranosyl)-β-D-glucopyranoside (primeveroside). Compound **2**, which has been named chironioside, is therefore swertiamarine-6′-*O*-(β-D-xylopyranoside). To our knowledge, chironioside is the first primeverosyl secoiridoid to be reported, and the second secoiridoid diglycoside found in the Gentianaceae. 6′-*O*-β-D-Glucosylgentiopicroside has been recently isolated from *Gentiana asclepiadea* (17).

Besides xanthones, secoiridoids are the second major class of secondary metabolites characteristic of the family Gentianaceae. Swertiamarine [**1**], gentiopicroside [**3**], and sweroside [**4**] are encountered in all the genera of the family which have been investigated phytochemically. Gentiopicroside [**3**] occurs exclusively in the family Gentianaceae (3), whereas **1** and **4** are also found in indole-alkaloid-producing families such as Apocynaceae (18) and Loganiaceae (19). Comparison of the four *Chironia* species (Table 1) shows that **1**, **3**, and **4** are present in all root extracts. Major secoiridoids are gentiopicroside [**3**] for *C. purpurascens* and *C. baccifera*, sweroside [**4**] in *C. palustris*, and swertiamarine [**1**] in *C. krebsii*. Chironioside [**2**] is present in *C. krebsii*, and tsp lc-ms also allows detection of minute amounts of this secoiridoid in *C. purpurascens* and *C. baccifera*. In the chromatograms of the latter two plants, the retention time of **2** is slightly decreased owing to co-elution with the major secoiridoid gentiopicroside [**3**]. Compounds **5** and **6**, tentatively identified as eustomorusside and eustomoside, respectively, are observed in *C. palustris* and *C. purpurascens* only.

TABLE 1. Secoiridoid Pattern of *Chironia* Species.

Species	Compound					
	1	**2**	**3**	**4**	**5**	**6**
Chironia krebsii	+++	+	+	++	+[a]	−
Chironia palustris	+	−	++	+++	+	+
Chironia purpurascens	+	+	+++	++	+[a]	++
Chironia baccifera	+	+	+++	+	+[a]	−

[a]Partially hidden by swertiamarine [**1**].

Taking advantage of the sensitivity and selectivity of the mass spectrometer as a chromatographic detector, systematic tsp lc-ms analysis of extracts from a broad range of Gentianaceae species will certainly enable discovery of additional secoiridoid diglycosides in the Gentianaceae. The combination of lc-uv and lc-ms provides a powerful tool for the analysis of secondary metabolites in plant extracts. As shown for the case of secoiridoids **1–6**, rapid and reliable peak identification can be obtained with minute amounts of plant material, and hitherto unknown compounds can be localized for further isolation. A systematic screening of other Gentianaceae species for bitter principles and xanthones is presently underway.

EXPERIMENTAL

PLANT MATERIAL.—*C. krebsii* was collected in November 1989 on the Zomba Plateau, Zomba, Malawi, and identified by E.A.K. Banda (National Herbarium, Zomba, Malawi). *C. palustris* and *C. purpurascens* were collected in Zimbabwe and identified in December 1990 by S. Mavi (National Herbarium, Harare, Zimbabwe). *C. baccifera* was collected in South Africa in October 1991 and identified by C. Rogers (University of Durban-Westville, South Africa). Voucher specimens have been deposited at the National Herbarium of Malawi and Zimbabwe, and at the University of Durban-Westville.

EXTRACTION.—Dry roots of *C. krebsii* (384 g), *C. palustris* (162 g), *C. purpurascens* (10 g), and *C. baccifera* (57 g) were ground and extracted at room temperature successively with CH_2Cl_2 and MeOH. The following quantities of CH_2Cl_2 and MeOH extracts were obtained: *C. krebsii*, 13.6 and 89.6 g, *C. palustris*, 2.4 and 46.9 g, *C. purpurascens*, 0.2 and 4.3 g, *C. baccifera*, 2.6 and 9.1 g, respectively.

HPLC UV ANALYSIS.—Reversed-phase hplc of the extract was carried out with a Hewlett-Packard 1090 series II instrument equipped with a photodiode array detector using a NovaPak RP-18 column (5 μm, 150 × 3.9 mm i.d.). Separation was achieved with an MeCN-H_2O gradient (5:95→70:30) (containing 0.1% TFA) over 50 min. The flow rate was 1 ml/min. For crude extracts, the sample amount injected was 200 μg. The uv trace was observed at 254 nm, and uv spectra (190–600 nm) were recorded every 640 msec.

TSP LC-MS ANALYSIS.—Hplc configuration: Waters 600MS solvent delivery system, on-line uv Water 490MS Multiwavelength detector, and Waters 590MS pump for post-column addition of buffer. Chromatographic conditions were identical with those for lc-uv. An aqueous solution of NH_4OAc was added post-column (0.5 M, 0.2 ml/min) to help ionization. A Thermospray 2 (Finnigan MAT) interface was used with the following conditions: source temperature 230°, vaporizer 90°, aerosol 240°, filament off mode. Ms detection was achieved on a Finnigan MAT TSQ 700 triple stage quadrupole instrument. Spectra (150–800 amu) were recorded every 1.2 sec.

ISOLATION.—Gel filtration on Sephadex LH-20 (MeOH) of an MeOH root extract of *C. krebsii* (3 × 10 g) afforded 10 fractions. Fraction 2 (2 g) was separated by mplc on a home packed LiChroprep RP-8 column (15–25 μm, 46 × 2.5 cm i.d.) with MeOH-H_2O (18:82→50:50) at a flow rate of 10 ml/min (30 bar) into 8 fractions (2a–2h). Fractions 2c and 2e consisted of pure **1** (258 mg) and **4** (42 mg). Fraction 2d (9 mg) was purified by semi-preparative hplc on a LiChrosorb RP-18 column (7 μm, 25 × 1.6 cm i.d.) with MeOH-H_2O (19:81) at a flow rate of 10 ml/min to afford **2** (3 mg).

Gel filtration on Sephadex LH-20 (MeOH) of the MeOH root extract of *C. palustris* (2 × 10 g) afforded 8 fractions. Upon concentration of fraction 2 (13 g), a precipitate (5.8 g) consisting of glucose and other sugars formed and was filtered off. The filtrate (7.6 g) was separated into two portions (1.5 g and 3 g each)

by mplc on a home-packed LiChroprep RP-18 column (25–40 μm, 46 × 2.5 cm i.d.) with MeOH-H_2O (1:3) at a flow rate of 10 ml/min (30 bar). Six fractions (2a–2f) were obtained. Fractions 2c and 2e consisted of pure **3** (70 mg) and **4** (560 mg). The purity of the compounds was checked by analytical hplc.

Tlc was carried out on pre-coated Si gel 60 F 254 aluminium sheets or RP-18 $WF_{254}S$ hptlc plates (Merck). The following solvent systems were used: Si gel $CHCl_3$-MeOH (70:30) (system A); RP-18 H_2O-MeOH (80:20) (system B). Detection was at uv 254 nm.

Compound **2** (1 mg) was hydrolyzed with 2 N HCl (reflux, 4 h). Sugars were analyzed by tlc on Si gel with EtOAc-H_2O-MeOH-HOAc (13:3:3:4) and visualized by spraying with *p*-anisidine phthalate.

Secoiridoids **1**, **3**, and **4** have all been isolated previously from other sources (13–15). Therefore, no spectral data are reported here with the exception of those of swertiamarine [**1**].

CHARACTERIZATION.—^1H- and ^{13}C-nmr spectra were measured in DMSO-d_6 or CD_3OD at 200.06 MHz for ^1H and 50.30 MHz for ^{13}C. TMS or the solvent signal was used as internal standard. D/cims (NH_3, positive ion mode) and tsp lc-ms were measured on a Finnigan MAT TSQ 700 triple stage quadrupole instrument.

Swertiamarine [**1**].—Tlc (system A) R_f 0.42; (system B) R_f 0.42; ^1H nmr (200 MHz, DMSO-d_6 + D_2O) δ 5.60 (1H, d, J = 1.4 Hz, H-1), 7.51 (1H, s, H-3), 1.78 (1H, dd, J = 4.5, 13.9 Hz, H_a-6), 1.70 (1H, unresolved, H_b-6), 4.60 (1H, ddd, J = 10.8, 10.8, 4.4 Hz, H_a-7), 4.29 (1H, ddd, J = 10.8, 9.9, 3.2 Hz, H_b-7), 5.35 (1H, unresolved, H-8), 5.35 (1H, unresolved, H_a-10), 5.25 (1H, unresolved, H_b-10), 4.48 (1H, d, J = 7.6, H-1′), 3.00 (1H, dd, J = 8.9, 7.6 Hz, H-2′), 3.10 (1H, unresolved, H-3′), 3.10 (1H, unresolved, H-4′), 3.35 (1H, unresolved, H-5′), 3.69 (1H, dd, J = 11.9, 1.5 Hz, H_a-6′), 3.45 (1H, dd, J = 11.9, 6.3 Hz, H_b-6′); ^{13}C nmr (50 MHz, DMSO-d_6) δ 96.48 (C-1), 151.83 (C-3), 108.16 (C-4), 62.53 (C-5), 32.04 (C-6), 64.06 (C-7), 132.79 (C-8), 49.87 (C-9), 120.28 (C-10), 164.33 (C=O), 98.24 (C-1′), 72.87 (C-2′), 76.10 (C-3′), 69.99 (C-4′), 77.37 (C-5′), 60.92 (C-6′).

Chironioside [**2**].—White amorphous powder: mp 128°; $[\alpha]^{24}D$ −107° (MeOH, c = 0.03); tlc (system A) R_f 0.19, (system B) R_f 0.41; uv λ max (MeOH) (log ε) 235.8 nm (4.01); ^1H nmr (200 MHz, DMSO-d_6 + D_2O) δ 5.51 (1H, d, J = 2.2 Hz, H-1), 7.48 (1H, s, H-3), 1.70 (2H, unresolved, H_{ab}-6), 4.57 (1H, ddd, J = 10.2, 10.2, 3.6 Hz, H_a-7), 4.25 (1H, unresolved, H_b-7), 5.35 (1H, unresolved, H-8), 5.35 (1H, unresolved, H_a-10), 5.23 (1H, dd, J = 7.3, 5.2 Hz, H_b-10), 4.45 (1H, d, J = 7.8, H-1′), 2.98 (1H, unresolved, H-2′), 3.0–3.2 (1H, unresolved, H-3′), 3.15 (1H, unresolved, H-4′), 3.35 (1H, unresolved, H-5′), 3.93 (1H, dd, J = 11.5, 0.7 Hz, H_a-6′), 3.55 (1H, dd, J = 11.5, 6.4 Hz, H_b-6′), 4.18 (1H, d, J = 7.4 Hz, H-1″), 2.93 (1H, unresolved, H-2″), 3.0–3.2 (1H, unresolved, H-3″), 3.00 (1H, unresolved, H-4″), 3.67 (1H, dd, J = 11.0, 5.0, H_a-5″), 3.22 (1H, unresolved, H_b-5″); ^{13}C nmr (50 MHz, DMSO-d_6) δ 96.88 (C-1), 151.94 (C-3), 108.02 (C-4), 62.40 (C-5), 31.99 (C-6), 64.09 (C-7), 132.66 (C-8), 49.94 (C-9), 120.41 (C-10), 98.56 (C-1′), 73.13 (C-2′)^a, 76.19 (C-3′)^b, 69.62 (C-4′)^c, 76.25 (C-5′)^b, 68.02 (C-6′), 103.92 (C-1″), 72.59 (C-2″)^a, 75.71 (C-3″)^b, 69.40 (C-4″)^c, 65.57 (C-5″), (C=O) not detectable ($^{a-c}$assignments with the same superscript are interchangeable); ^{13}C nmr (50 MHz, CD_3OD) δ 99.24 (C-1), 154.84 (C-3), 108.82 (C-4), 65.96 (C-5), 83.21 (C-6), 69.77 (C-7), 133.79 (C-8), 52.05 (C-9), 121.38 (C-10), 168.02 (C=O), 100.41 (C-1′), 74.90 (C-2′)^a, 77.78 (C-3′)^b, 71.22 (c-4′)^c, 77.47 (C-5′)^b, 66.99 (C-6′), 105.63 (C-1″), 74.39 (C-2″)^a, 77.64 (C-3″)^b, 71.15 (C-4″)^c, 65.96 (C-5″) (assignments with the same superscript are interchangeable); dcims (NH_3, positive ion mode) *m/z* $[M + NH_4]^+$ 524, $[M + NH_4 - H_2O]^+$ 506, 382, 312, 270, $[M - 132 - 162]^+$ 212, 210, 194, 177, 150; tsp lc-ms (source 230°, vaporizer 90°, aerosol 240°, filament off, ammonium acetate 0.1 M) $[M + NH_4]^+$ 524, $[M + NH_4 - H_2O]^+$ 506, 382, $[M - 132]^+$ 374, 357, 330, 312, $[M - 132 - 162]^+$ 212.

ACKNOWLEDGMENTS

This work was supported by the Swiss National Science Foundation. The Herbette Foundation of the University of Lausanne and the Directorate for Development Cooperation and Humanitarian Aid (Swiss Federal Department of Foreign Affairs) are acknowledged for travel grants. The authors thank Dr. C. Rogers, University of Durban-Westville, South Africa, for collection of *C. baccifera*.

LITERATURE CITED

1. W.G. Van der Sluis and R.P. Labadie, *Pharm. Weekbl.*, **113**, 21 (1978).
2. K. Hostettmann and H. Wagner, *Phytochemistry*, **16**, 481 (1977).
3. D. Schaufelberger, Ph.D. Thesis, University of Lausanne, Lausanne, Switzerland, 1986.
4. O. Suzuki, Y. Katsumata, M. Oya, V.M. Chari, B. Vermes, H. Wagner, and K. Hostettmann, *Planta Med.*, **42**, 17 (1981).
5. D. Schaufelberger and K. Hostettmann, *Planta Med.*, **54**, 219 (1988).
6. M. Hamburger, M. Hostettmann, H. Stoeckli-Evans, P.N. Solis, M.P. Gupta, and K. Hostettmann, *Helv. Chim. Acta*, **73**, 1845 (1990).

7. J.L. Wolfender, M. Hamburger, J.D. Msonthi, and K. Hostettmann, *Phytochemistry*, **30**, 3625 (1991).
8. R.E.J.R. Weaver and L. Rudenberg, *J. Arnold Arbor. Harv. Univ.*, **56**, 211 (1975).
9. C.R. Blackeley and M.L. Vestal, *Anal. Chem.*, **7**, 339 (1983).
10. A.L. Yergey, C.G. Edmonds, I.A.S. Lewis and M.L. Vestal, "Liquid Chromatography/Mass Spectrometry—Techniques and Applications," Plenum Press, New York, 1990.
11. L.J. El Naggar and J.L. Beal, *J. Nat. Prod.*, **43**, 649 (1980).
12. C.A. Boros and F.R. Stermitz, *J. Nat. Prod.*, **54**, 1173 (1991).
13. T. Kariyone, *J. Pharm. Soc. Jpn.*, **47**, 133 (1927).
14. L. Cannonica, F. Pellizoni, P. Manito, and G. Jommi, *Tetrahedron Lett.*, **16**, 192 (1961).
15. H. Inouye, S. Ueda, and Y. Nakamura, *Tetrahedron Lett.*, 5229 (1966).
16. T. Konishi, A. Tada, J. Shoji, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.*, **26**, 668 (1978).
17. E. Mpondo Mpondo and A.J. Chulia, *Planta Med.*, **54**, 185 (1988).
18. T.A. Van Beek, P.P. Lankhorst, R. Verpoorte, and A. Baerheim Svendsen, *Planta Med.*, **44**, 30 (1982).
19. M. Koch, M. Plat, J. Le Men, and M.M. Janot, *Bull. Soc. Chim. Fr.*, 403 (1964).

Received 20 July 1992