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MONOTERPENE AND COUMARIN GLUCOSIDES OF *CNIDIUM SILAIFOLIUM*

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Key Word Index—*Cnidium silaifolium*; Apiaceae; monoterpene glycosides; coumarin glycosides; paeoniflorin; Mosher's method.

Abstract—A glycosidic fraction obtained from roots of *Cnidium silaifolium* was shown to contain, as main component, the known monoterpene glucoside, paeoniflorin. The new monoterpene glucoside, (1*S*, 5*R*, 6*R*)-1-(β-D-glucopyranosyloxy)-8-hydroxy-pin-2-en-4-one, and the known coumarin glucosides, *O*-β-D-glucopyranosyl-columbianetin and apterin were also obtained.

INTRODUCTION

Cnidium silaifolium is an umbellifer distributed in Europe in a belt from Southern France in the west to Romania and Greece in the east. The occurrence in this plant of a number of irregular monoterpene aldehydes and of a coumarin, (2′*S*, 3′*R*)-3′-benzoyloxy-columbianetin acetate, has been described [1]. This report deals with the glycosidic constituents of the root. The isolation of two monoterpene glucosides of which one is new, and of two known coumarin glucosides is described.

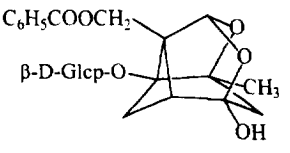
RESULTS AND DISCUSSION

A fraction containing the polar constituents of the root material was chromatographed by a combination of Polyamide 6, Amberlite XAD-2 and silica gel chromatography. Furthermore, some compounds were separated and purified by reversed-phase HPLC on ODS-silica to obtain glycosides **1**, **2**, **9** and **10**.

The main constituent, which was obtained in a high yield (1.3% of the dry root material), was shown to be the unique, highly oxygenated cage-like pinane derivative, paeoniflorin (**1**) [2, 3]. It is the principal physiologically active constituent of roots of *Paeonia* species, used extensively in Chinese and Japanese traditional medicine, and it has been demonstrated to possess mainly anti-allergic, analgesic, antispasmodic and sedative properties [4]. Neither paeoniflorin, nor similar cage-like monoterpenoids, have been reported to occur outside the *Paeonia* genus. The synthesis of paeoniflorin has recently been described [5, 6].

The new glycoside (**2**) upon hydrolysis released 1 mol of D-glucose and a 10-carbon aglycone (**3**). The UV spectra of **2** and **3** showed maxima at 253 and 256 nm, respectively, near the value characteristic for compounds derived from verbenone (2-pinen-4-one) [7]. This relationship was confirmed by comparison of the ^{1}H and ^{13}C NMR data of **2** and **3** with those of verbenone [8, 9], as they were clearly seen to be derived from verbenone by oxygenation of one of the geminal methyl groups and one of the bridgehead carbons. The presence in **2** of a free hydroxyl group at C-8 (and not at C-9) was evident from its coupling pattern in DMSO-d_6 solution, from an observed positive NO effect on the H-7$_{exo}$ signal, and a negative one on the H-7$_{endo}$ signal, upon irradiation of the corresponding carbinol protons; also the low δ-value (δ1.03) of the neighbouring methyl group showed that this must be C-9 [8]. That the glucosyloxy group was positioned at C-1 (and not at C-5) in **2** was evident from the gated ^{13}C NMR spectrum, in which the vinylic methyl group showed up as a quartet of doublets (J = 5 Hz) corresponding to a coupling with H-3 and no coupling with a H-1 (this was later confirmed also by the proton coupling pattern of the reduced derivative, **6**).

Clearly, compound **2** was an 1-*O*-β-D-glucopyranoside of one of the 1,8-dihydroxyverbenone enantiomers. The stereochemistry of the aglycone was determined by means of Mosher's ^{1}H method for secondary alcohols [10]. To this end the pentaacetate (**4**) of **2** was hydrogenated to the saturated ketone (**5**), which was then reduced with sodium borohydride to the secondary alcohol (**6**). Esterification of **6** with the enantiomers of 2-methoxy-2-phenyl-2-(trifluoromethyl) acetic acid (MTPA) yielded the (*S*)-MTPA ester (**7**) and the (*R*)-MTPA ester (**8**). Based upon the $\Delta\nu = \nu_S - \nu_R$ values observed for the aglycone part in the ^{1}H NMR spectra of these esters, an *R*-configuration was determined for C-4 in **6** and accordingly the absolute configuration



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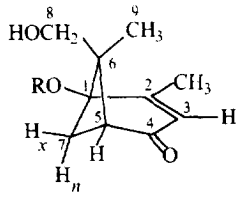
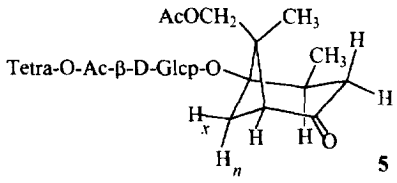


2 R = β-D-Glcp
3 R = H



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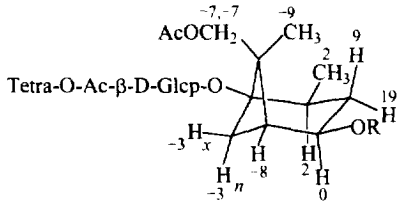

6 R = H
7 R = (*S*)- MTPAc
8 R = (*R*)- MTPAc

With ^{1}H Δν-values (Hz)
(~ $\nu_S - \nu_R$ at 200 MHz)

(1*S*, 2*S*, 4*R*, 5*R*, 6*R*) for the aglycone part of **6**. Thus, compound **2** is (1*S*, 5*R*, 6*R*)-1-(β-D-glucopyranosyloxy)-8-hydroxy-pin-2-en-4-one. Two known coumarin glucosides, apterin (**9**) and columbianetin β-D-glucopyranoside (**10**), were also obtained.

EXPERIMENTAL

Mps: corr.; TLC of glucose: NaH_2PO_4– silica gel [11], with CH_2Cl_2– 96% EtOH–H_2O (10:8:1) as eluent (two runs); ^{1}H and ^{13}C NMR: 200 MHz and 50.3 MHz, respectively, spectra in D_2O with CH_3CN as int. standard (δ 2.00 and δ1.70, respectively). Multiplicities in ^{13}CNMR spectra have been deduced from DEPT spectra.

Plant material. Plants of *C. silaifolium* (Jacq.) Simonk. were cultivated by the Botanic Garden of Copenhagen, Denmark. The identity was verified by Dr K. Rahn of the Botanic Garden. A voucher specimen is deposited at the Department of Pharmacognosy, Royal Danish School of Pharmacy.

Extraction and isolation. The dried and powdered roots (550 g), after extraction with petrol and drying, were extracted in the cold with MeOH (3 × 24 hr). Concn of the combined MeOH solns yielded 65 g of extract. Partitioning between H_2O and EtOAc and evapn of the H_2O-phase then gave 60 g of extract, which was passed through a column of polyamide 6 (80 g) with H_2O (3 l.). Upon evapn, the residue was subjected to CC on macroreticular polystyrene, XAD-2, (160 g) with a CH_2Cl_2–MeOH–H_2O–AcOH (0.25:5:93.7:1) → (4:80:15:1) gradient, rechromatography on XAD-2, and CC on silica gel with CH_2Cl_2–MeOH (95:5) or (92.5:7.5) gradually changed to (85:15). Some compounds were finally separated by RP prep. HPLC on Lichrosorb RP 18, with MeOH–H_2O– AcOH (30:68:2) ~ syst. A, or (34:64:2) ~ syst. B, as eluents.

Yields, listed in order of elution from XAD-2: **2** (0.43 g), **1** (*ca* 7.3 g), **9** (72 mg), **10** (49 mg).

Paeoniflorin (**1**). Amorphous; $[\alpha]_D^{21}$ – 13.2°, $[\alpha]_{436}^{21}$ – 40.8° (MeOH; *c* 3.3), ref. [12]: $[\alpha]_D^{16}$ – 12.8° (MeOH; *c* 4.6); ^{1}H and ^{13}C NMR data (D_2O) as reported [13, 14]. Pentaacetate purified by CC (silica gel, CH_2Cl_2–EtOAc–HCOOH, 95:5:0.05 → 80:20:0.05): mp 162.5 – 163.5°, ref. [5]: 160.5 – 162.5°, $[\alpha]_D^{23}$ + 1.7°, $[\alpha]_{436}^{23}$ + 7.6° (MeOH; *c* 1.5), ref. [6]: $[\alpha]_D^{29}$ + 1.33° (MeOH; *c* 0.9). ^{1}H NMR ($CDCl_3$): δ8.04 (2H, *m*, *o*-H), 7.62 (1H, *m*, *p*-H), 7.49 (2H, *m*, *m*-H), 5.53 (1H, *s*, H-9), 5.18 – 4.95 (3H, *m*, H_G-2, H_G-3, H_G-4), 4.75 (1H, *d*, *J* = 7.6 Hz, H_G-1), 4.61 (1H, *d*, *J* = 12.1 Hz, H-8a), 4.48 (1H, *d*, *J* = 12.1 Hz, H-8b), 4.16 (2H, *d*, apparent *J* = 4.1 Hz, H_G-6), 3.63 (1H, *dt*, *J* = 4.1, 9.6 Hz, H_G-5), 2.82 (1H, *br d*, *J* = 6.8 Hz, H-5), 2.52 (1H, *d*, *J* = 12.3 Hz, H-3a), 2.47 (1H, *dd*, *J* = 6.8, 10.8 Hz, H-7x), 2.18 (1H, *d*, *J* = 12.3 Hz, H-3b), 2.10 (6H, *s*, 2 × AcO–), 2.04, 2.03, 1.99 (3 × 3H, *sss*, 3 × AcO–), 1.96 partially obscured (1H, *br d*, H-7n), 1.36 (3H, *s*, H-10). ^{13}C NMR ($CDCl_3$): δ170.5, 170.2, 169.4, 169.3, 168.5 (5 × *s*, 5 × CO in AcO–), 166.3 (*s*, Ph–CO–O–), 133.5 (*d*, *p*-C), 129.7 (*d*, *o*-C), 129.4 (*s*, *i*-C), 128.7 (*d*, *m*-C), 108.3 (*s*, C-4), 101.4 (*d*, C-9), 96.4 (*d*, C_G-1), 88.2 (*s*, C-1 or C-2), 86.1 (*s*, C-2 or C-1), 73.0 (*d*, C_G-2), 71.9 (*d*, C_G-3 or C_G-5), 71.3 (*d*, C_G-5 or C_G-3), 69.6 (*s*, C-6), 68.5 (*d*, C_G-4), 62.0 (*t*, C_G-6 or C-8), 59.8 (*t*, C-8 or C_G-6), 43.6 (*d*, C-5), 41.3 (*t*, C-3), 23.3 (*t*, C-7) 21.4, 20.7, 20.6 (~ 5 × CH_3 in AcO–), 18.9 (*q*, C-10).

(1*S*, 5*R*, 6*R*)-1-(*β-D-Glucopyranosyloxy*)-8-*hydroxy-pin-2-en-4-one* (**2**). Crystalline (EtOH); mp 166–169°; $[\alpha]_D^{25}$ + 29.9°, $[\alpha]_{436}^{25}$ + 145.1° (MeOH; *c* 0.7). UV λ_{max}^{MeOH} nm (log ε): 253 (3.83). IR ν_{max}^{KBr} cm^{-1}: 1679 (>C=O); 1084, 1056, 1025 (>C–OH). ^{1}H NMR (DMSO-d_6 + 10% CF_3COOD): δ5.65 (1H, *m*, H-3), 4.47 (1H, *d*, *J* = 7.6 Hz, H_G-1), 3.83 (1H, *d*, *J* = 12.2 Hz, H-8a), 3.77 (1H, *d*, *J* = 12.2 Hz, H-8b), 3.66 (1H, *br d*, *J* = 11.5 Hz, H_G-6a), 3.44 (1H, *dd*, *J* = 4.7, 11.5 Hz, H_G-6b), 3.3–3.0 (5H, *m*, H-7x and residual H_G), 2.61 (1H, *dd*, $J_{3,5}$ = 2.4 Hz, $J_{5,7x}$ = 6.7 Hz, H-5), 2.35 (1H, *d*, $J_{7n,7x}$ = 9.2 Hz, H-7n), 2.02 (3H, *d*, $J_{3,10}$ = 1.0 Hz, H-10), 1.03 (3H, *s*, H-9); before addition of CF_3COOD: signals at δ 5.33 (1H, *d*, *J* = 5.3 Hz, sec.-OH), 5.04 (1H, *d*, *J* = 4.9 Hz, sec.-OH), 4.98 (1H, *d*, *J* = 4.7 Hz, sec.-OH), 4.57 (1H, *t*, *J* = 5.9 Hz, C8-OH), 4.41 (1H, *t*, *J* = 5.2 Hz, C_G6–OH). ^{13}C NMR (DMSO-d_6): δ 200.9 (*s*, C-4), 173.2 (*s*, C-2), 120.1 (*d*, C-3), 98.2 (*d*, C_G-1), 82.8 (*s*, C-1), 76.7

(*d*, C_G-3 or C_G-5), 76.6 (*d*, C_G-5 or C_G-3), 73.2 (*d*, C_G-2), 69.9 (*d*, C_G-4), 64.0 (*t*, C-8), 62.8 (*s*, C-6), 60.8 (*t*, C_G-6), 46.5 (*d*, C-5), 42.5 (*t*, C-7), 19.4 (*q*, C-10), 15.5 (*q*, C-9).

(1*S*, 5*R*, 6*R*)-1-(*β*-D-*Glucopyranosyloxy*)-8-*hydroxypin*-2-*en*-4-*one pentaacetate* (**4**). Prepared by the action of pyridine-Ac_2O (1:1) on **2**. Crystalline (Et_2O); mp 156.0–157.5°; $[\alpha]_D^{25}$ + 8.3°, $[\alpha]_{436}^{25}$ + 60.1° (MeOH; *c* 0.4); ^{1}H NMR ($CDCl_3$) : *δ*5.74, (1H, *m*, H-3), 5.24 (1H, *dd*, *J* = 9.3, 9.3 Hz, H_G-3), 5.07 (1H, *dd*, *J* = 7.8, 9.3 Hz, H_G-2), 5.06 (1H, *dd*, *J* = 9.3, 9.8 Hz, H_G-4), 4.72 (1H, *d*, *J* = 7.8 Hz, H_G-1), 4.54 (1H, *d*, *J* = 11.6 Hz, H-8a), 4.22 (1H, *dd*, *J* = 5.6, 12.2 Hz, H_G-6a), 4.08 (1H, *dd*, *J* = 2.5, 12.2 Hz, H_G-6b), 3.90 (1H, *d*, *J* = 11.6 Hz, H-8b), 3.67 (1H, *ddd*, *J* = 2.5, 5.6, 9.8 Hz, H_G-5), 3.06 (1H, *dd*, *J* = 7.1, 9.2 Hz, H-7x), 2.85 (1H, *dd*, $J_{3,5}$ = 2.4 Hz, $J_{5,7x}$ = 7.1 Hz, H-5), 2.43 (1H, *d*, $J_{7n,7x}$ = 9.2 Hz, H-7n), 2.15, 2.09, 2.02 (3 × 3H, *sss*, 3 × CH_3COO-), 2.04 (9H, *br s*, H-10 and 2 × CH_3COO-), 1.10 (3H, *s*, H-9).

Acid hydrolysis of **2**. A soln of **2** (0.4 mg) in 0.25 M H_2SO_4 (0.2 ml) was heated to 100° in a closed vessel for 1.5 hr. After neutralization by heating with $BaCO_3$ (20 mg) and centrifugation, D-glucose was detected in the supernatant by TLC and by a positive D-glucose oxidase test.

Enzymic hydrolysis of **2**. To **2** (30 mg) in H_2O (1.75 ml) was added 0.25 ml of sodium acetate buffer (1.3 M) pH 5.0, and 1.0 ml of *Helix pomatia* *β*-glucuronidase-sulphatase (crude solution, Sigma). After 2 weeks, evapn on silica gel (1.5 g) and CC (silica gel; CH_2Cl_2–MeOH–HCOOH, 95:5:0.1 → 90:10:0.1) yielded **3** (10 mg).

(1*S*, 5*R*, 6*R*)-1,8-*Dihydroxypin*-2-*en*-4-*one* (**3**). Crystalline (Et_2O–$CHCl_3$); mp 121.5–122.5°; $[\alpha]_D^{25}$ + 174°, $[\alpha]_{436}^{25}$ + 536° (MeOH; *c* 0.18); UV λ_{max}^{MeOH} nm (log *ε*) : 256 (3.76). ^{1}H NMR (Me_2CO-d_6): *δ*5.61 (1H, *dq*, *J* = 2.5, 1.6 Hz, H-3), 4.99 (1H, *br s*, 1-OH), 4.01 (3H, *m*, H-8, 8-OH), 2.89 (1H, *dd*, *J* = 6.7, 8.9 Hz, H-7x), 2.60 (1H, *dd*, *J* = 2.5, 6.7 Hz, H-5), 2.36 (1H, *d*, *J* = 8.9 Hz, H-7n), 2.00 (3H, *d*, *J* = 1.6 Hz, H-10), 1.10 (3H, *s*, H-9). The signal at *δ*4.01 upon addition of CF_3COOD (2%) immediately turned into an AB-system, *δ* 4.04, 3.99 (2 × 1H, *doublets*, *J* = 11.3 Hz, H-8a, H-8b) (at the same time acetalisation with Me_2CO-d_6 began). ^{13}C NMR (Me_2CO-d_6): *δ*201.6 (*s*, C-4), 173.1 (*s*, C-2), 121.1 (*d*, C-3), 79.3 (*s*, C-1), 66.7 (*t*, C-8), 62.6 (*s*, C-6), 49.0 (*t*, C-7), 48.4 (*d*, C-5), 18.4 (*q*, C-10), 16.1 (*q*, C-9).

(1*S*, 2*S*, 5*R*, 6*R*)-1-(*β*-D-*Glucopyranosyloxy*)-8-*hydroxy-pinan*-4-*one pentaacetate* (**5**) was prepared from **4** by hydrogenation (EtOH, Pd 10% on diatomaceous earth). Crystalline (EtOH); mp 188–189.5°; $[\alpha]_D^{25}$ − 43.9°, $[\alpha]_{436}^{25}$ − 91.2° (MeOH; *c* 0.75); ^{1}H NMR ($CDCl_3$–Me_2CO-d_6 1:1): *δ*5.25 (1H, *dd*, *J* = 9.4, 9.5 Hz, H_G-3), 5.02 (1H, *dd*, *J* = 9.4, 10.0 Hz, H_G-4), 4.99 (1H, *dd*, *J* = 8.0, 9.5 Hz, H_G-2), 4.82 (1H, *d*, *J* = 8.0 Hz, H_G-1), 4.51 (1H, *d*, *J* = 11.6 Hz, H-8a), 4.19 (1H, *dd*, *J* = 5.3, 12.2 Hz, H_G-6a), 4.14 (1H, *dd*, *J* = 2.9, 12.2 Hz, H_G-6b), 3.90 (1H, *ddd*, *J* = 2.9, 5.3, 10.0 Hz, H_G-5), 3.79 (1H, *d*, *J* = 11.6 Hz, H-8b), 2.97 (1H, *dd*, *J* = 11.5, 19.8 Hz, H-3ax), 2.73, 2.69 (2 × 1H, *mm*, H-2ax′ and H-7x resp., overlapping), 2.61 (1H, *d*, *J* = 7.1 Hz, H-7n), 2.24 (1H, *dd*, *J* = 3.8, 19.8 Hz, H-3eq), 2.14 obscured (H-5), 2.13, 2.05, 2.04, 2.02, 1.98 (5 × 3H, *sssss*, 5 × CH_3COO-), 1.22 (3H, *d*, *J* = 7.1 Hz, H-10), 1.15 (3H, *s*, H-9).

Reduction of **5**. To **5** (38 mg) in 3.5 ml of CH_2Cl_2–EtOH (1:1), $NaBH_4$ (10 mg) was added. After 1 hr, HOAc (20 *μ*l) added, work up as usual and CC (silica gel, CH_2Cl_2– EtOAc– HCOOH, 100:0:0.1 → 50:50:0.1) yielded **6** (23 mg).

(1*S*, 2*S*, 4*R*, 5*R*, 6*R*)-1-(*O*-*Tetraacetyl*-*β*-D-*glucopyranosyloxy*)-8-*acetoxy*-*pinan*-4-*ol* (**6**). ^{1}H NMR ($CDCl_3$–D_2O): *δ*5.19 (1H, *dd*, *J* = 9.4, 9.4 Hz, H_G-3), 5.03 (1H, *dd*, *J* = 9.4, 9.7 Hz, H_G-4), 5.01 (1H, *dd*, *J* = 8.0, 9.4 Hz, H_G-2), 4.45 (1H, *d*, *J* = 8.0 Hz, H_G-1), 4.39 (1H, *d*, *J* = 11.4 Hz, H-8a), 4.18 (1H, *ddd*, *J* = 3, 6, 9.5 Hz, H-4ax′), 4.07 (2H, *m*, H_G-6), 3.72 (1H, *d*, *J* = 11.4 Hz, H-8b), 3.62 (1H, *ddd*, *J* = 3.4, 4.8, 9.7 Hz, H_G-5), 2.62 (1H, *ddd*, *J* = 9.5, 9.5, 15 Hz, H-3ax), 2.35 (1H, *dd*, *J* = 8.5, 9.5 Hz, H-7x), 2.21 partially obscured (1H, *m*, H-2ax′), 2.15 partially obscured (1H, *dd*, *J* = 3, 8.5 Hz, H-5), 2.16, 2.07, 2.07, 2.03, 2.01 (5 × 3H, *sssss*, 5 × CH_3COO-), 1.67 (1H, *ddd*, *J* = 6, 8, 15 Hz, H-3eq), 1.36 (3H, *s*, H-9) 1.26 (1H, *d*, *J* = 9.5 Hz, H-7n), 1.14 (1H, *d*, *J* = 7 Hz, H-10).

Esterification of **6** *with* (−)-(*S*)-*α*-*methoxy*-*α*-*trifluoromethyl*-*phenylacetic acid*. To (−)-(*S*)-*α*-methoxy-*α*-trifluoromethyl-phenylacetic acid (16.4 mg) in toluene (100 *μ*l), thionyl chloride (5 *μ*l) and DMF (0.2 *μ*l) was added. After reflux for 2 hr, **6** (11.5 mg) and 4-dimethylaminopyridine (16 mg) in dry pyridine (100 *μ*l) was added and the mixture was left to stand at room temp. for 1 day. Addition of 1 *μ*l of H_2O, standing for 5 min, work up as usual and evapn, yielded 14.3 mg of crystalline (*S*)-MTPA ester (**7**). ^{1}H NMR ($CDCl_3$): 7.56–7.37 (5H, *m*, arom. H), 5.36 (1H, *ddd*, *J* = 2.5, 6, 10 Hz, H-4), 5.19 (1H, *dd*, *J* = 9.5, 9.5 Hz, H_G-3), 5.03 (1H, *dd*, *J* = 9.5, 9.5 Hz, H_G-4), 5.00 (1H, *dd*, *J* = 8, 9.5 Hz, H_G-2), 4.45 (1H, *d*, *J* = 8 Hz, H_G-1), 4.36 (1H, *d*, *J* = 11.5 Hz, H-8a), 4.14 (2H, *m*, H_G-6), 3.66 (1H, *d*, *J* = 11.5 Hz, H-8b), 3.63 (1H, *ddd*, *J* = 3.5, 5, 9.5 Hz, H_G-5), 3.52 (3H, *br q*, *J* = 1 Hz, $-OCH_3$), 2.72 (1H, *ddd*, *J* = 9.5, 10, 15.5 Hz, H-3ax), 2.45 (1H, *dd*, *J* = 8, 10 Hz, H-7x), 2.33 (1H, *ddd*, *J* = 2, 5, 8 Hz, H-5), 2.28 partially obscured (1H, *m*, H-2ax′), 2.15, 2.06, 2.03, 2.00, 2.00 (5 × 3H, *sssss*, 5 × CH_3COO-), 1.75 (1H, *ddd*, *J* = 6, 7.5, 15.5 Hz, H-3eq), 1.38 (1H, *d*, *J* = 10 Hz, H-7n), 1.13 (3H, *d*, *J* = 7 Hz, H-10), 1.13 (3H, *s*, H-9).

Esterification of **6** *with* (+)-(*R*)-*α*-*methoxy*-*α*-*trifluoromethyl*-*phenylacetic acid*. Preparation of the (*R*)-MTPA ester (**8**) was similar to that of (*S*) -MTPA ester (**7**). They show almost identical ^{1}H NMR spectra. For values of $\Delta\nu = \nu_S - \nu_R$ (at 200 MHz), see formulae.

Apterin (**9**). Crystalline; $[\alpha]_D^{20}$ + 214°, $[\alpha]_{436}^{20}$ + 584° (MeOH; *c* 0.2); isolated by prep. HPLC in syst. A. Identified by comparison with an authentic sample [15] (IR, ^{1}H NMR, $[\alpha]_D$).

Columbianetin *β*-D-*glucopyranoside* (**10**). Amorphous; $[\alpha]_D^{20}$ + 121°, $[\alpha]_{436}^{20}$ + 363° (MeOH; *c* 0.16); $[\alpha]_D^{25}$ + 224°, $[\alpha]_{436}^{25}$ + 664° (H_2O; *c* 0.22); isolated by prep. HPLC in syst. B. Identified by comparison ($[\alpha]_D$, ^{1}H and ^{13}C NMR) with lit. data [16, 17].

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REFERENCES

1. Bohlmann, F. and Grenz, M. (1973) *Chem. Ber.* **106**, 1047.
2. Aimi, N., Inaba, M., Watanabe, M. and Shibata, S. (1969) *Tetrahedron* **25**, 1825.
3. Kaneda, M., Iitaka, Y. and Shibata, S. (1972) *Tetrahedron* **28**, 4309.
4. Wagner, H., Hikino, H. and Farnsworth, N. R. (eds) (1985) *Economic and Medicinal Plant Research*, Vol. I, p. 65, Academic Press, London.
5. Hatakeyama, S., Kawamura, M. and Takano, S. (1994) *J. Am. Chem. Soc.* **116**, 4081.
6. Corey, E. J. and Wu, Y.-J. (1993) *J. Am. Chem. Soc.* **115**, 8871.
7. Meyer, A. Y. and Pasternak, R. (1976) *Tetrahedron* **32**, 2805.
8. Catalán, C. A. N., Iglesias, D. I. A. de, Iñigo, R. P. A. and Retamar, J. A. (1981) *Riv. Ital. E.P.P.O.S.* **63**, 289.
9. Coxon, J. M., Graeme, J. H. and Steel, P. J. (1984) *J. Chem. Soc., Perkin Trans.* **II**, 1351.
10. Ohtani, I., Kusumi, T., Kashman, Y. and Kakisawa, H. (1991) *J. Am. Chem. Soc.* **113**, 4092.
11. Hansen, S. Aa. (1975) *J. Chromatogr.* **107**, 224.
12. Shibata, S. and Nakahara, M. (1963) *Chem. Pharm. Bull.* **11**, 372.
13. Zapesochnaya, G. G., Kurkin, V. A., Avdeeva, E. V., Popov, D. M. and Kolpakova, M. V. (1992) *Khim. Prir. Soedin.* **28**, 55.
14. Yamasaki, K., Kaneda, M. and Tanaka, O. (1976) *Tetrahedron Letters.* **17**, 3965.
15. Lemmich, J. and Havelund, S. (1978) *Phytochemistry* **17**, 139.
16. Shipchandler, M. and Soine, T. O. (1968) *J. Pharm. Sci.* **57**, 747.
17. Bradford, C., van Wagenen, B. C., Huddleston, J. and Cardellina, J. H. (1988) *J. Nat. Prod.* **51**, 136.