New Oleanan-Type Triterpene and Cincholic Acid Glycosides from Peruvian "Uña de Gato" (*Uncaria tomentosa*)

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A new oleanan-type triterpene (1) and three new cincholic acid glycosides (2—4) were isolated from Peruvian "Uña de Gato" (Cat's claw, plant of origin: *Uncaria tomentosa*), a traditional herbal medicine in Peru. Their structures were determined by spectroscopic analysis.

Key words triterpene; *Uncaria tomentosa*; Uña de Gato (Cat's claw); Rubiaceae

The Peruvian traditional herbal medicine, "Uña de Gato" (Cat's claw),[1,2] is used for treating various ailments, including arthritis, inflammation and cancer. The plants of origin of "Uña de Gato" are *Uncaria tomentosa* (WILLD.) D.C. and *U. guianensis* (AUBL.) GMEL. (Rubiaceae). To date, chemical[3—5] and biological studies[6,7] have been conducted by many researchers. Our recent pharmacological studies on oxindole alkaloids, which are the constituents of this herbal medicine, suggested that pteropodine and isopteropodine, which are Heteroyohimbine-type oxindole alkaloids, act as positive modulators of muscarinic M_1 and 5-HT_2 receptors,[8] and rhynchophylline and isorhynchophylline, which are Corynanthé-type oxindole alkaloids, act as noncompetitive antagonists of the *N*-methyl-D-aspartate (NMDA) receptor in *Xenopus* oocytes.[9] We have reported the isolation of new triterpenoids[10,11] and alkaloids[12,13] from Peruvian "Uña de Gato" (plant of origin: *Uncaria tomentosa*). As a continuation of our chemical study, we isolated one new oleanan-type triterpene (**1**) and three new cincholic acid glycosides (**2**—**4**) from Peruvian "Uña de Gato", which are described herein.

From the MeOH extract of "Uña de Gato" (plant of origin: *Uncaria tomentosa*), four new triterpenes (**1**—**4**) were isolated together with five known quinovic acid glycosides. The structures of the known compounds were deduced from spectroscopic data and confirmed by comparison with reported data.

The high resolution (HR)-FAB-MS spectrum of **1** measured in the positive ion mode gave a quasi-molecular ion peak at *m*/*z* 503.3343 ($[M+H]^+$) that corresponded to the molecular formula $C_{30}H_{47}O_6$ (*m*/*z* 503.3372). The ^{1}H-NMR spectrum (CD_3OD) showed five singlets due to six methyl groups at δ 1.44, 1.06, 0.96 (6H), 0.93 and 0.78, one broad singlet at δ 3.07, signals assignable to two methine protons bearing a hydroxyl group at δ 3.26 (br d, J=3.9 Hz) and 3.54 (dd, J=11.2, 4.4 Hz), one pair of doublets due to a hydroxymethyl group at δ 3.51 and 3.38 (each d, J=10.7 Hz), and one signal of an olefinic proton at δ 5.36 (br t). In the ^{13}C-NMR spectrum (CD_3OD), thirty carbons, including one carboxyl carbon at δ 182.3, two sp^2 carbons of a trisubstituted olefin at δ 144.2 and 124.5, two methine carbons bearing a hydroxyl group at δ 82.4 and 72.4, one hydroxymethyl carbon at δ 66.1, and six methyl carbons (δ 28.7, 25.7, 25.0, 18.1, 17.3, 12.8), were observed. Therefore, **1** was deduced to be an oleanan-type triterpenoid possessing two secondary hydroxyl groups, one hydroxymethyl group and one carboxyl group. The HMBC cross-peaks between the oxymethine proton at δ 3.54 and the methyl carbon at δ 12.8 and between both the methine proton at δ 2.61 and the methyl protons at δ 1.06 and the oxymethine carbon at δ 72.4 indicated the existence of a hydroxyl group at C-3, as common triterpenes. Furthermore, a signal assignable to a ketone carbon was observed at δ 215.8 in the ^{13}C-NMR spectrum. HMBC correlations were observed between the ketone carbon (δ 215.8) and a singlet proton assignable to H-5 (δ 2.61), and between the same carbon and methylene protons assignable to H_2-7 (δ 2.69 and 1.77, each d), indicating that the ketone was located at C-6. Cross-peaks between the hydroxymethyl protons and C-3 (δ 72.4), C-4 (δ 42.4), C-5 (δ 59.1) and methyl carbon (δ 12.8) indicated that the hydroxymethyl group was located at C-4. In the differential NOE experiment, irradiation of the methyl protons located at C-4 (δ 1.06) led to enhancement of the signal intensities of the H-25 methyl protons at δ 0.96 (5%) and the hydroxymethyl protons at δ 3.51 (3%) and δ 3.38 (2%), indicating that the

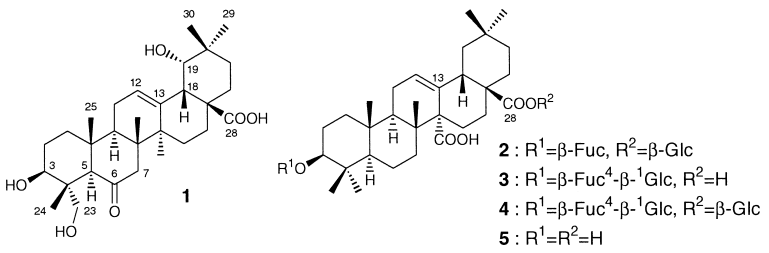

Fig. 1

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hydroxymethyl group at C-4 was α-oriented; C-23 was oxidized to hydroxymethyl. HMBC cross-peaks between H-18 (δ 3.07) and both the carboxyl carbon (δ 182.3) and the olefinic carbons (δ 144.2) demonstrated that C-28 was oxidized to a carboxylic acid. Irradiation of the olefinic proton (δ 5.36) led to 4% enhancement of the signal intensity of H-19 (δ 3.26) in the differential NOE experiment, indicating that the hydroxyl group at C-19 was α-oriented. From the above data, the structure of **1** was deduced to be 3β,19α,23-trihydroxy-6-oxo-olean-12-en-28-oic acid.

The HR-FAB-MS spectrum of **2** measured in the negative ion mode gave a quasi-molecular ion peak at *m/z* 793.4348 ($[M-H]^-$) that corresponded to the molecular formula $C_{42}H_{65}O_{14}$ (*m/z* 793.4375). Acid hydrolysis of **2** gave cincholic acid (**5**),[14] D-glucose and D-fucose. The ^{1}H-NMR spectrum of **2** showed six singlets assignable to the methyl groups of the triterpene aglycon at δ 1.00 (H_3-23), 0.95 (H_3-25), 0.91 (H_3-30), 0.87 (H_3-26), 0.86 (H_3-29) and 0.82 (H_3-24), one olefinic proton signal at δ 5.65 (H-12) and one characteristic proton signal due to H-18 in oleanan-type triterpenes at δ 2.90. In addition, signals assignable to two β-linked anomeric protons at δ 5.40 (d, J=7.9 Hz, H-1‴) and 4.22 (d, J=7.3 Hz, H-1′) and one doublet methyl signal of fucose at δ 1.24 (H_3-6′) were observed. The ^{13}C-NMR spectrum (CD_3OD) showed forty-two carbons, including one carboxyl carbon at δ 179.9 (C-27), one ester carbonyl carbon at δ 178.0 (C-28), two sp^2 carbons of a trisubstituted olefin at δ 137.4 (C-13) and 127.8 (C-14), two anomeric carbons at δ 107.1 (C-1′) and 95.6 (C-1‴), and six methyl carbons at δ 33.5 (C-29), 28.5 (C-23), 24.0 (C-30), 19.0 (C-26) and 16.9 (C-24, C-25). The sugar linkages were deduced from NMR chemical shifts and HMBC experiments. From the chemical shifts of the anomeric protons and carbons, one sugar was found to be attached through an ether linkage and the other sugar unit was attached through an ester linkage. HMBC correlations between H-1′ of fucose at δ 4.22 and C-3 of the aglycon at δ 90.6 and between H-1‴ of glucose at δ 5.40 and the C-28 ester carbonyl carbon at δ 178.0 revealed that fucose and glucose were attached to C-3 and C-28, respectively. Therefore, the structure of **2** was deduced to be cincholic acid 3β-*O*-β-D-fucopyranosyl-28-*O*-β-D-glucopyranosyl ester.

The HR-FAB-MS spectrum of **3** measured in the negative ion mode gave a quasi-molecular ion peak at *m/z* 793.4348 ($[M-H]^-$) that corresponded to the molecular formula $C_{42}H_{65}O_{14}$ (*m/z* 793.4375), which was the same as that of **2**. Acid hydrolysis of **3** gave cincholic acid (**5**), D-glucose and D-fucose. The ^{1}H-NMR spectrum showed two doublets with a large coupling constant due to anomeric protons at δ 4.54 (J=7.6 Hz, H-1″) and 4.23 (J=7.6 Hz, H-1′), one doublet methyl signal of fucose at δ 1.29 (H_3-6′), six methyl singlets (δ 1.01, 0.96, 0.92, 0.88, 0.86, 0.82) and one olefinic proton signal (δ 5.63). The ^{13}C-NMR spectrum (CD_3OD) showed two carboxyl carbons at δ 182.1 and 180.0, two sp^2 carbons of a trisubstituted olefin at δ 138.1 and 127.3, two anomeric carbons at δ 107.0 (C-1′) and 105.9 (C-1″) and six methyl carbons at δ 33.6, 28.5, 24.0, 18.9, 17.1 and 16.8. From the above data, **3** was deduced to be cincholic acid possessing two β-linked sugar units attached through ether linkages. The sugar linkages were deduced by HMBC experiments. The HMBC correlation between H-1′ of fucose at δ 4.23 and C-3 of the aglycon at δ 90.8 revealed that fucose was attached to C-3 of the aglycon. The HMBC correlation between both H-1″ of glucose at δ 4.54 and methyl protons (H_3-6′) of fucose at δ 1.29 and C-4′ of fucose at δ 82.4 revealed that a terminal glucose was attached to C-4′ of an inner fucose. Therefore, the structure of **3** was deduced to be cincholic acid 3β-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside.

The HR-FAB-MS spectrum of **4** measured in the positive ion mode gave a sodiated molecular ion peak at *m/z* 979.4908 ($[M+Na]^+$) that corresponded to the molecular formula $C_{48}H_{76}O_{19}Na$ (*m/z* 979.4878). Acid hydrolysis of **4** gave cincholic acid (**5**), D-glucose, and D-fucose. The existence of three sugar units was revealed by the three β-linked anomeric proton signals at δ 5.40 (d, J=8.2 Hz, H-1‴), 4.54

Table 1. ^{13}C-NMR Data for **1**—**4** in CD_3OD

	1	**2**	**3**	**4**
1	39.7	39.9	39.9	39.9
2	27.0	27.1	27.0	27.0
3	72.4	90.6	90.8	90.8
4	42.4	40.1	40.1	40.1
5	59.1	57.1	57.0	57.0
6	215.8	19.3	19.3	19.3
7	51.7	37.8	37.8	37.8
8	47.7	40.7	40.6	40.7
9	*a)*	48.3	48.3	48.3
10	44.1	37.9	37.9	37.9
11	25.1	24.0	23.9	24.0
12	124.5	127.8	127.3	127.8
13	144.2	137.4	138.1	137.4
14	43.0	57.2	57.3	57.3
15	29.5*b)*	25.6	25.6	25.6
16	28.4	25.1	25.2	25.1
17	46.7	*a)*	*a)*	*a)*
18	45.2	44.6	44.8	44.6
19	82.4	44.6	44.7	44.6
20	36.1	31.5	31.6	31.5
21	29.4*b)*	34.7	34.8	34.7
22	33.9	32.7	33.4	32.7
23	66.1	28.5	28.5	28.5
24	12.8	16.9	17.1	17.1
25	17.3	16.9	16.8	16.9
26	18.1	19.0	18.9	19.0
27	25.7	179.9	180.0	180.0
28	182.3	178.0	182.1	178.0
29	28.7	33.5	33.6	33.5
30	25.0	24.0	24.0	24.0
Fuc-1′		107.1	107.0	107.0
2′		72.9	73.5	73.5
3′		75.3	75.8*b)*	75.8*b)*
4′		73.1	82.4	82.3
5′		71.6	71.2	71.2
6′		17.0	17.4	17.4
Glc-1″			105.9	105.9
2″			75.9*b)*	75.9*b)*
3″			78.1	78.1
4″			71.6	71.6
5″			78.3	78.2
6″			62.9	62.9
Glc-1‴		95.6		95.6
2‴		73.9		73.9
3‴		78.3		78.3
4‴		71.2		71.2
5‴		78.7		78.7
6‴		62.5		62.5

a) Under CD_3OD signal. *b*) Interchangeable.

(d, J=7.6 Hz, H-1″) and 4.23 (d, J=7.3 Hz, H-1′) in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum was very similar to that of **3** except for the signals due to one extra glucose unit including the anomeric carbon at δ 95.6 (C-1‴). HMBC correlations revealed that **4** has the same sugar chain as **3** at C-3 position and an extra glucose unit at C-28 through an ester linkage. Therefore, the structure of **4** was deduced to be cincholic acid 3β-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl-28-*O*-β-D-glucopyranosyl ester.

Experimental

General Experimental Procedures Optical rotation: JASCO DIP-140. IR: JASCO FT/IR-230. ^{1}H- and ^{13}C-NMR spectra: at 500 (^{1}H-NMR) and 125.65 (^{13}C-NMR) MHz, respectively, JEOL JNM A-500. FAB-MS and HR-FAB-MS: JEOL JMS-HX110. TLC: Precoated silica gel 60 F_{254} plates (Merck, 0.25 mm thick). Column chromatography: Silica gel 60 (Merck, 70—230 mesh for open column chromatography and 230—400 mesh for flash column chromatography), DIAION HP20 (Mitsubishi Chemical), Sephadex LH-20 (Pharmacia Biotech). MPLC: C. I. G. prepacked column CPS-HS-221-05 (SiO_2) and CPO-HS-221-20 (ODS) (Kusano Kagakukikai). HPLC: Shodex RSpak DC-613 (Showa Denko).

Plant Material "Uña de Gato" used in this study was imported from Peru through Coperunix Japan, Inc. (Tokyo, Japan) in 1996. The plant of origin was confirmed to be *Uncaria tomentosa* (stem and stem bark) by the company. A voucher specimen (no. 961001) was deposited at the herbarium of the Graduate School of Pharmaceutical Sciences, Chiba University.

Extraction and Isolation "Uña de Gato" (835 g, dry weight) was extracted with hot MeOH six times (11×6) to give the MeOH extract (total: 95 g). Fifty grams of the MeOH extract (1st, 2nd and 3rd extracts were combined) was subjected to column chromatography on DIAION HP20 to give seven fractions, as described previously.[10] Fraction D (12.44 g) eluted with MeOH was separated by SiO_2 gel open column chromatography (MeOH/$CHCl_3$ gradient). The 30—35% MeOH/$CHCl_3$ eluate was purified by flash column chromatography and MPLC (SiO_2, 10% MeOH/$CHCl_3$), followed by SiO_2 flash column chromatography (*n*-hexane/AcOEt gradient) to give new compound **1** (3.5 mg). Fraction E (13.43 g) eluted with MeOH/H_2O=7 : 3 on a DIAION HP20 column gave quinovic acid 3β-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl-(28→1)-*O*-β-D-glucopyranosyl ester[15] (1078.7 mg).

The *n*-BuOH extract (25 g), which was obtained from a portion (40 g) of the MeOH extract (4th, 5th and 6th extracts were combined), was subjected to column chromatography on DIAION HP20 to give six fractions, as described previously.[12] Fraction J (9.50 g) eluted with MeOH was purified by SiO_2 gel flash column chromatography (MeOH/$CHCl_3$ gradient) and MPLC (SiO_2, 20% MeOH/$CHCl_3$), followed by MPLC (ODS, H_2O–MeOH, 2 : 5) to afford new compound **3** (28.8 mg). Quinovic acid 3β-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside[15] (186.9 mg), quinovic acid 3β-*O*-β-D-glucopyranoside[16] (9.1 mg) and quinovic acid 3β-*O*-β-D-glucopyranosyl-(1→4)-β-L-rhamnopyranoside[15] (19.8 mg) were also obtained from fraction J. Fraction I (5.24 g) eluted with MeOH/H_2O=7 : 3 on a DIAION HP20 column gave new cincholic acid glycosides **2** (45.3 mg) and **4** (85.4 mg), together with quinovic acid 3β-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl-(28→1)-*O*-β-D-glucopyranosyl ester[15] (759.9 mg) and quinovic acid 3β-*O*-β-D-quinovopyranosyl-(28→1)-*O*-β-D-glucopyranosyl ester[17] (14.8 mg). Fraction I was separated by SiO_2 gel flash column chromatography (MeOH/$CHCl_3$ gradient). The 40% MeOH/$CHCl_3$ eluate (1.56 g) was purified by Sephadex LH-20 chromatography (MeOH) and by MPLC (ODS, H_2O–MeOH, 1 : 1.5) repeatedly to afford new glycoside **2**. The combined fraction of 50—90% MeOH/$CHCl_3$ and MeOH eluates was separated by Sephadex LH-20 chromatography (MeOH) and by MPLC (ODS, H_2O–MeOH, 1 : 1.5) repeatedly to give new glycoside **4**.

3β,19α,23-Trihydroxy-6-oxo-olean-12-en-28-oic Acid (1) Colorless amorphous powder. $[\alpha]_D^{25}$ +17.2° (c=0.11, MeOH); selected ^{1}H-NMR (CD_3OD, 500 MHz) δ: 5.36 (1H, br t, J=3.5 Hz, H-12), 3.54 (1H, dd, J=11.2, 4.4 Hz, H-3), 3.51 and 3.38 (each 1H, d, J=10.7 Hz, H_2-23), 3.26 (1H, br d, J=3.9 Hz, H-19), 3.07 (1H, br s, H-18), 2.69 (1H, d, J=12.3 Hz, H-7), 2.61 (1H, s, H-5), 2.38 (1H, dd, J=10.4, 7.0 Hz, H-9), 2.31 (1H, m), 2.11 and 2.01 (each 1H, m, H_2-11), 1.77 (1H, d, J=12.3 Hz, H-7), 1.44 (3H, s, H_3-27), 1.06 (3H, s, H_3-24), 0.96 (6H, s, H_3-25, H_3-30), 0.93 (3H, s, H_3-29), 0.78 (3H, s, H_3-26); ^{13}C-NMR: Table 1; FAB-MS (NBA, positive) m/z 503 $[M+H]^+$; HR-FAB-MS (NBA, positive) m/z 503.3343 $[M+H]^+$ (Calcd for $C_{30}H_{47}O_6$, 503.3372).

Cincholic Acid 3β-*O*-β-D-Fucopyranosyl-28-*O*-β-D-glucopyranosyl Ester (2) Colorless amorphous powder. $[\alpha]_D^{25}$ +36.0° (c=1.21, MeOH); IR (KBr) ν_{max} 3367, 2942, 1733, 1699, 1067 cm^{-1}; selected ^{1}H-NMR (CD_3OD, 500 MHz) δ: 5.65 (1H, m, H-12), 5.40 (1H, d, J=7.9 Hz, H-1‴), 4.22 (1H, d, J=7.3 Hz, H-1′), 3.08 (1H, dd, J=11.4, 4.4 Hz, H-3), 2.90 (1H, br dd, J=13.6, 3.5 Hz, H-18), 1.24 (3H, d, J=6.4 Hz, H_3-6′), 1.00 (3H, s, H_3-23), 0.95 (3H, s, H_3-25), 0.91 (3H, s, H_3-30), 0.87 (3H, s, H_3-26), 0.86 (3H, s, H_3-29), 0.82 (3H, s, H_3-24), 0.71 (1H, d, J=11.3 Hz, H-5); ^{13}C-NMR: Table 1; FAB-MS (NBA+MeOH, negative) m/z 793 $[M-H]^-$; HR-FAB-MS (NBA+MeOH, negative) m/z 793.4348 $[M-H]^-$ (Calcd for $C_{42}H_{65}O_{14}$, 793.4375).

Cincholic Acid 3β-*O*-β-D-Glucopyranosyl-(1→4)-β-D-fucopyranoside (3) Colorless amorphous powder. $[\alpha]_D^{25}$ +31.2° (c=0.82, MeOH); IR (KBr) ν_{max} 3320, 2942, 1687, 1069 cm^{-1}; selected ^{1}H-NMR (CD_3OD, 500 MHz) δ: 5.63 (1H, m, H-12), 4.54 (1H, d, J=7.6 Hz, H-1″), 4.23 (1H, d, J=7.6 Hz, H-1′), 3.08 (1H, dd, J=11.4, 4.5 Hz, H-3), 2.89 (1H, br dd, J=13.4, 3.4 Hz, H-18), 1.29 (3H, d, J=6.6 Hz, H_3-6′), 1.01 (3H, s, H_3-23), 0.96 (3H, s, H_3-25), 0.92 (3H, s, H_3-30), 0.88 (3H, s, H_3-26), 0.86 (3H, s, H_3-29), 0.82 (3H, s, H_3-24), 0.72 (1H, d, J=11.5 Hz, H-5); ^{13}C-NMR: Table 1; FAB-MS (NBA, negative) m/z 793 $[M-H]^-$; HR-FAB-MS (NBA, negative) m/z 793.4348 $[M-H]^-$ (Calcd for $C_{42}H_{65}O_{14}$, 793.4375).

Cincholic Acid 3β-*O*-β-D-Glucopyranosyl-(1→4)-β-D-fucopyranosyl-28-*O*-β-D-glucopyranosyl Ester (4) Colorless amorphous powder. $[\alpha]_D^{25}$ +26.0° (c=1.01, MeOH); IR (KBr) ν_{max} 3340, 2941, 1731, 1698, 1069 cm^{-1}; selected ^{1}H-NMR (CD_3OD, 500 MHz) δ: 5.64 (1H, m, H-12), 5.40 (1H, d, J=8.2 Hz, H-1‴), 4.54 (1H, d, J=7.6 Hz, H-1″), 4.23 (1H, d, J=7.3 Hz, H-1′), 3.08 (1H, dd, J=11.4, 4.4 Hz, H-3), 2.90 (1H, br dd, J=13.6, 3.5 Hz, H-18), 1.29 (3H, d, J=6.4 Hz, H_3-6′), 1.00 (3H, s, H_3-23), 0.95 (3H, s, H_3-25), 0.91 (3H, s, H_3-30), 0.87 (3H, s, H_3-26), 0.86 (3H, s, H_3-29), 0.81 (3H, s, H_3-24), 0.71 (1H, d, J=11.3 Hz, H-5); ^{13}C-NMR: Table 1; FAB-MS (glycerol, positive) m/z 979 $[M+Na]^+$; HR-FAB-MS (NBA+NaCl, positive) m/z 979.4908 $[M+Na]^+$ (Calcd for $C_{48}H_{76}O_{19}Na$, 979.4878).

Acid Hydrolysis of 2 A solution of **2** (4.7 mg) in 5% aqueous H_2SO_4 (0.25 ml) and 1,4-dioxane (0.25 ml) was heated at 115 °C for 2 h under Ar. Water was added to the reaction mixture and the entire mixture was extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel open column chromatography (MeOH/$CHCl_3$ gradient) to afford cincholic acid (**5**, 1.6 mg), as identified from ^{1}H- and ^{13}C-NMR spectra.[14] The aqueous layer was neutralized by passage through Amberlite IRA-93 and elution with H_2O. This was followed by evaporation *in vacuo* to give a sugar fraction. The identity and configuration of the sugars were determined by comparison with authentic D-(+)-fucose (t_R, 8.6 min) and D-(+)-glucose (t_R, 11.2 min) on HPLC. HPLC conditions: column, Shodex RSpak DC-613 (6.0×150 mm i.d.); solvent, CH_3CN–H_2O, 7 : 3 (v/v); flow rate, 0.5 ml/min; temperature, 70 °C; RI detection, Shodex RI-72 and chiral detection, JASCO OR-1590. The sugar fraction gave corresponding peaks of D-(+)-fucose (t_R, 8.6 min) and D-(+)-glucose (t_R, 11.2 min).

Acid Hydrolysis of 3 A solution of **3** (5.0 mg) in 5% aqueous H_2SO_4 (1.0 ml) was heated at 110 °C for 4 h under Ar. Water was added to the reaction mixture and the entire mixture was extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel open column chromatography (MeOH/$CHCl_3$ gradient) to afford cincholic acid (**5**, 2.6 mg), as determined from ^{1}H- and ^{13}C-NMR spectra.[14] The aqueous layer was neutralized by passage through Amberlite IRA-93 and elution with H_2O. This was followed by evaporation *in vacuo* to give a sugar fraction. The identity and configuration of the sugars, D-(+)-fucose and D-(+)-glucose, were determined by comparison with authentic sugars on HPLC under the same conditions as those described above.

Acid Hydrolysis of 4 A solution of **4** (5.0 mg) in 5% aqueous H_2SO_4 (1.0 ml) was heated at 110 °C for 4 h under Ar. Water was added to the reaction mixture and the entire mixture was extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel open column chromatography (MeOH/$CHCl_3$ gradient) to afford cincholic acid (**5**, 2.3 mg), as determined from ^{1}H- and ^{13}C-NMR spectra.[14] The aqueous layer was neutralized by passage through Amberlite IRA-93 and elution with H_2O. This was followed by evaporation *in vacuo* to give a sugar fraction. The identity and configuration of the sugars, D-(+)-fucose and D-(+)-glucose, were determined by comparison with authentic sugars on HPLC.

Acknowledgement One of the authors (M.K.) would like to thank Takeda Science Foundation for financial support.

References

1) Obregon L. E., "Cat's Claw, Uncaria Genus. Botanical, Chemical and Pharmacological Studies of *Uncaria tomentosa* (Willd.) D.C. (Rubiaceae) and *Uncaria guianensis* (Aubl.) Gmel.," Instituto de Fitoterapia Americano, Lima, 1995.
2) Cabieses F., "The Saga of the Cat's Claw," Via Lactea Editores, Lima, 1994.
3) Laus G., Keplinger K., *Phyton*, **43**, 1—8 (2003) and references cited therein.
4) Aquino R., De Tommasi N., De Simone F., Pizza C., *Phytochemistry*, **45**, 1035—1040 (1997) and references cited therein.
5) Wirth C., Wagner H., *Phytomedicine*, **4**, 265—266 (1997) and references cited therein.
6) Sandoval M., Okuhara N. N., Zhang X.-J., Condezo L. A., Lao J., Angeles F. M., Musah R. A., Bobrowski P., Miller M. J. S., *Phytomedicine*, **9**, 325—337 (2002) and references cited therein.
7) Sheng Y., Li L., Holmgren K., Pero R. W., *Phytomedicine*, **8**, 275—282 (2001) and references cited therein.
8) Kang T.-H., Matsumoto K., Tohda M., Murakami Y., Takayama H., Kitajima M., Aimi N., Watanabe H., *Eur. J. Pharmacol.*, **444**, 39—45 (2002).
9) Kang T.-H., Murakami Y., Matsumoto K., Takayama H., Kitajima M., Aimi N., Watanabe H., *Eur. J. Pharmacol.*, **455**, 27—34 (2002).
10) Kitajima M., Hashimoto K., Yokoya M., Takayama H., Aimi N., *Tetrahedron*, **56**, 547—552 (2000).
11) Kitajima M., Hashimoto K., Yokoya M., Takayama H., Sandoval M., Aimi N., *J. Nat. Prod.*, **66**, 320—323 (2003).
12) Kitajima M., Hashimoto K., Yokoya M., Takayama H., Aimi N., *Chem. Pharm. Bull.*, **48**, 1410—1412 (2000).
13) Kitajima M., Yokoya M., Takayama H., Aimi N., *Nat. Med.*, **55**, 308—310 (2001).
14) Rumbero-Sanchez, A., Vazquez, P., *Phytochemistry*, **30**, 623—626 (1991).
15) Lamidi M., Olliver E., Faure R., Debrauwer L., Nze-Ekekang L., Balansard G., *Planta Med.*, **63**, 284—285 (1997).
16) Aquino R., De Simone F., Pizza C., Cerri R., De Mello J. F., *Phytochemistry*, **27**, 2927—2930 (1988).
17) Aquino R., De Simone F., Pizza C., Conti C., Steon M. L., *J. Nat. Prod.*, **52**, 679—685 (1989).