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Two novel triterpenoids from the stemwood of *Herrania cuatrecasana*

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Abstract

From the wood of *Herrania cuatrecasana* two novel triterpenoids have been isolated and structurally elucidated mainly by 1D and 2D NMR spectroscopy and X-ray crystallography. The two new compounds have been identified as 3β,25-epoxy-25-hydroxy-14-taraxerene-1-one (**1**) and 6α,25-dihydroxy-3β,25-epoxy-14-taraxerene-1,16,21-trione (**2**) and were named as herranone (**1**) and herrantrione (**2**). © 1999 Elsevier Science Ltd. All rights reserved.

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Herrania cuatrecasana García-Barriga is indigenous to tropical South America and called *sacha cacao* by the Amazonian Indians (Iglesias, 1985). The internal part of the bark is used in traditional medicine as an aqueous suspension against snake bite, whereas the ash of the bark's external part, suspended in alcohol, is applied orally against throat irritation and dry cough.

Besides lipids from the seeds (Carpenter, Hammerstone, Romanczyk, Aitken, & Martin, 1994) and traces of purine alkaloids isolated from *Herrania sp*. (Gurney, Evans, & Robinson, 1991) no other compounds are known that could explain the ethnomedical use of the plant. In this paper we report on the isolation and structure elucidation of two novel taraxerene type triterpenoids from the lipophilic extracts of the bark and wood of *H. cuatrecasana*.

1. Results and discussion

After Soxhlet extraction of the chopped wood with hexane, followed by EtOAc and MeOH, the dried extracts were subjected to repeated silica column chromatography. Compound **1** could be isolated from the hexane extract, whereas the fractionation of the EtOAc extract yielded compound **2**.

The ^{13}C spectrum and the MS ($C_{30}H_{46}O_3$, HRMS m/z 454.3417 $[M]^+$) of compound **1** indicated the presence of 30 carbons and suggested a triterpenoid structure. Among the triterpenoids reported on the *Sterculiaceae* family those of the friedelan, ursene and taraxerene type are dominating (Hegnauer, 1990). The chemical shifts δ 158.25 and 117.71 ppm of **1** for the C-14 and C-15 olefinic resonances led to taraxerene as the most likely basic structure (taraxerene: $C_{30}H_{50}$ m/z 410) (Karrer, 1958). The location of the double bond at C-14 and C-15 was confirmed by the retro-Diels-Alder fragments (EIMS) at m/z 330 and 124 in the mass spectrum. The 30 carbons were characterized by the DEPT experiments and showed seven methyl-, nine methylene groups, six methine and eight quarternary carbons. In contrast to the taraxerene skeleton, which originally has eight methyl groups, one methyl was missing and the number of the various carbon types

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Table 1
^{13}C NMR spectral data of compound **1** and **2**

C	Taraxerol $CDCl_3$[a]	Herranone pyridine	Herrantrione $CDCl_3$
1	38.1 t	211.25 s	207.94 s
2	27.3 t	42.74 t	41.11 t
3	79.2 d	78.52 d	79.10 d
4	39.1 s	37.04 s	37.62 s
5	55.7 d	51.03 d	54.47 d
6	19.0 t	22.81 t	66.77 d
7	35.3 t	40.08 t	48.96 t
8	38.9 s	38.46 s	39.81 s
9	48.9 d	37.92 d	35.77 d
10	37.9 s	57.84 s	57.72 s
11	17.7 t	18.43 t	17.04 t
12	35.9 t	34.66 t	31.67 t
13	37.9 s	36.03 s	37.42 s
14	158.1 s	158.25 s	174.34 s
15	117.0 d	117.71 d	119.60 d
16	36.9 t	38.00 t	203.75 s
17	38.1 s	38.00 s	42.41 s
18	49.4 d	49.56 d	46.44 d
19	41.4 t	33.59 t	36.76 t
20	29.0 s	29.12 s	46.05 s
21	33.9 t	37.04 t	217.98 s
22	33.2 t	35.48 t	44.29 t
23	28.1 q	28.71[b] q	23.68[b] q
24	15.6 q	23.90[b] q	29.54[b] q
25	15.6 q	93.51 d	93.16 d
26	30.1 q	25.19 q	27.41 q
27	26.0 q	22.41 q	27.41 q
28	30.1 q	30.25 q	33.27 q
29	33.5 q	33.53[b] q	28.46[b] q
30	21.5 q	30.07[b] q	24.25[b] q

[a] Mahato & Kundu, 1994.
[b] Interchangeable values in each vertical column.

differed. Additionally the ^{13}C shift pattern and the mass of 454 suggested a substituted taraxerene derivative with three oxygenes.

The HMBC spectrum showed two different types of methyl groups: three were located at three different quarternary carbons (C-26–C-8, C-27–C-13, C-28–C-17), whereas each of two further quarternary carbons must bear two methyl groups (C-23,24–C-4, C-29,30–C-20). The HMBC coupling of C-23 and C-24 and its high shift assigned the signal at δ 78.52 as C-3 with a linkage to an oxygen. This was also consistent with the coupling of H-25 (δ 6.21) and C-3 in the HMBC spectrum. The same spectrum indicated an additional cyclisation between one methine- (δ 93.51, C-25) and another methine group (δ 78.52, C-3) with inclusion of an oxygen. In agreement with the characteristic fragmentation of a C-3 hydroxy substituted taraxerene (e.g. taraxerol), the EIMS (m/z 204 and m/z 189) did not show any functional group in the rings D and E (Baas, 1983). The HMBC couplings of the isolated methyl groups in position C-25 suggested the participation of C-25 (HMBC: H-3, H-5, H-9) in the new ring. Because of the high shift of C-25 (δ 93.51) the

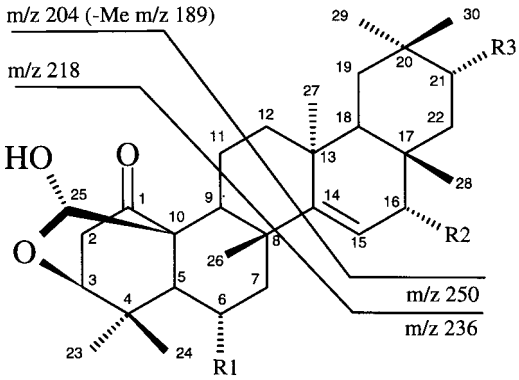

Fig. 1. Mass spectral fragments (EI) of herranone and numbering scheme of **1** and **2**.

new ring should be an oxygen heterocycle, implying a semiacetalic structure.

In the ^1H–^1H-COSY spectrum a three proton system was found for H-3 (δ 2.71, t) and H-2 (δ 3.26, 2.65, dd) in ring A. Because of missing further couplings with H-2 C-1 must be assigned as a quart. carbon bearing a carbonyl function. The same spectrum showed also clearly the five protons system of H-9 (δ 2.68, dd), H-11 (δ 2.52, 2.90, dm) and H-12 (δ 1.59, 1.58) and the similar coupling system of H-5, H-6 and H-7 (Table 1 and Figs 1 and 2).

A similar shift pattern, found in the NMR spectras of **2**, suggested clearly a related structure to **1**. The ^{13}C spectrum showed the same 30 carbons as in herranone, but differed in the ratios of the various multiplicities: seven methyl- and six methylene groups, seven methines and ten quart. carbons. In addition the mass spectrum with a higher mass at m/z 498 showed new functional groups, which were confirmed by ^{13}C NMR and DEPT and indicated three carbonyl groups at δ 217.98, 207.94, 203.75 and a new hydroxy group at δ 66.77. The C=C double bond (δ 174.34, 119.60) showed a stronger shift, whereas the semiacetalic carbon (δ 93.16) was similar. In the COSY the corre-

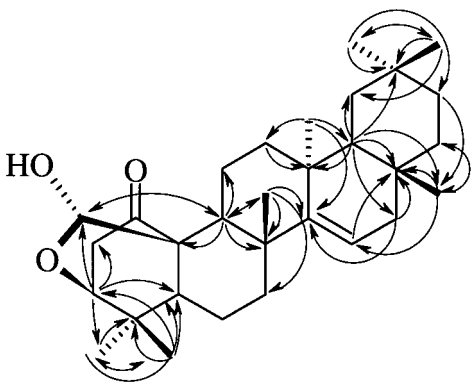

Fig. 2. HMBC couplings of herranone.

sponding shifts and coupling patterns for H-2, H-3 and the coupling system of H-9, H-11 and H-12 were displayed. This suggested the rings A and C without any substituents. The HMQC showed one proton at δ 4.01, split in a dt, for the hydroxy bearing carbon (δ 66.77) indicating a methine- and a methylene group as neighbours. Since this could be confirmed by a COSY spectrum, the location of the hydroxy group can be assigned to C-6 position. The higher shift of the C=C unit must have been generated by a carbonyl substitution of C-16 (δ 203.75), whereas the H-15 (δ 5.96) in the ^{1}H and HMQC spectra was displayed as a singlet. In the ^{1}H–^{1}H-COSY spectrum also a three protons coupling system for H-18 and H-19 and a exclusive geminal coupling of a methylene group could be assigned. Therefore the location of the third carbonyl carbon was supposed to be at C-21 or C-22. The final assignment of this C=O group in ring E could be achieved by a X-ray experiment, which at the same time confirmed the suggested conformation of the hexacyclic structure and the substituent pattern of **1** and **2**. Therefore, the structures of the two novel taraxerenes of *Herrania cuatrecasana* can be assigned to the following molecules (Fig. 3).

2. Experimental

2.1. General

Solvents and chemicals were of analytical grade and purchased from Merck, Darmstadt, Germany. TLC was performed on silica gel 60 F 254 coated glass plates (Merck, Darmstadt, Germany); detection with Godin reagent. Sephadex LH-20 was purchased from Pharmacia Biotech AB, Uppsala, Sweden. IR spectrum was obtained from KBr disks on a Beckman Acculab®1. Mass spectrometry was performed on a Kratos MS 80 RFA spectrometer with NBA (7 kV); NMR: **1**: all spectra were measured on a Bruker DXR 500 MHz with TMS as internal standard using 10 mg of **1** in 0.6 ml pyridine-d_5 at room temperature; **2**: 1D measurements were done on a Bruker AM-360 (360.13 MHz, 90.6 MHz), 2D on a Jeol GSX 400 N (399.79 MHz; 100.5 Mhz), all with TMS at room temperature using 3 mg of **2** in 0.4 ml in $CDCl_3$. X-ray structural analysis: Siemens R3m diffractometer.

2.2. Plant material

The drug was collected by *F. Ghia* in the Ecuadorian province *del Napo* in 1990 and identified by the collector. A voucher specimen is deposited at the Herbario Economico, Escuela Politecnica Nacional, Quito, Ecuador. We purchased the material from L. Bohlin, Biomedicinska Centrum, Institutionen för Farmakognosi, Uppsala, Sweden.

2.3. Extraction and isolation of compounds

The dried and chopped wood (400 g) was extracted in a 1 l Soxhlet apparatus with hexane, followed by EtOAc and MeOH (1600 ml for 32 h each).

2.4. Compound *1* (herranone)

The hexane extract was dried under red. pres. yielding 0.96 g of dry extract. It was prefractionized over

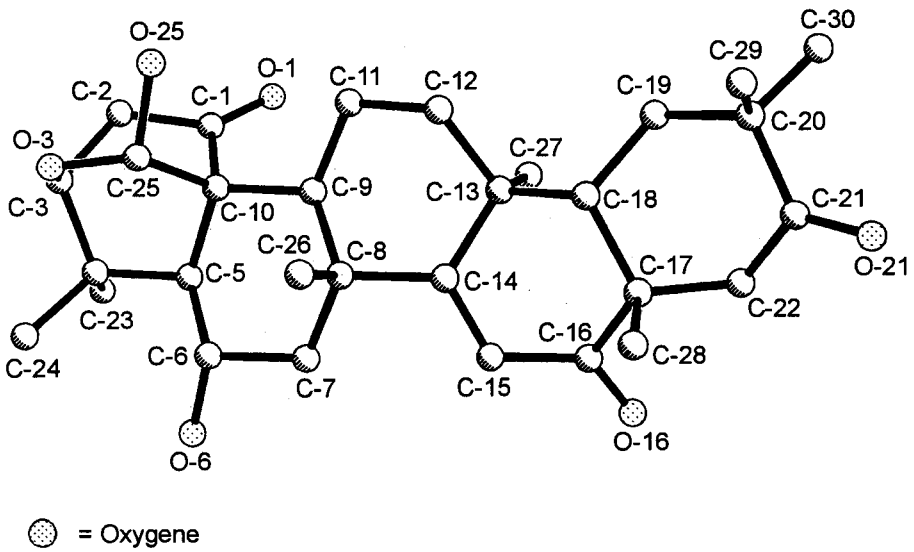

Fig. 3. ORTEP diagram of herrantrione showing the crystalline conformation and numbering system.

silica gel 60 by MPLC with the gradient system hexane, EtOAc and MeOH (130 ml each in order of increasing polarity). 180 fractions each containing 2.2 ml were obtained. On the basis of TLC ($CHCl_3$) the fractions 59–75 were pooled and the solvent removed under red. pres. After liquid extraction of the residue between hexane and acetonitrile 12 mg of pure herranone were obtained in the hexane phase. Compound **2** (herrantrione): The EtOAc extract was dried under red. pres., the residue (1.03 g) prefractionized by MPLC over silica gel 60 using a gradient system of increasing polarity (hexane, toluene–EtOAc (1:1), $CHCl_3$, MeOH equal parts). 60 fractions containing 15 ml were obtained. On the basis of TLC ($CHCl_3$:MeOH (80:20)) the fractions 1–7 were pooled and the solvent evaporated. The residue was subjected to CC over Sephadex LH-20 with a $CHCl_3$– MeOH gradient (increasing polarity, 400 ml all). The fractions 21–27 were combined based on TLC, reduced to dryness and rechromatographed over silica gel 60 with the same gradient system. The fractions 9 and 10 yielded 5 mg of pure herrantrione.

2.5. Herranone (*1*)

Needles (pyridine), 12 mg, m.p. 280°C. IR ν^{KBr} cm^{-1}: 3450 (O–H), 2950 (C–H), 1710 (C=O), 1460(C–H); HRMS *m/z* 454.3417 $[M]^+$ ($C_{30}H_{46}0_3$); EIMS *m/z* (rel. int.): 454 $[M]^+$ ($C_{30}H_{46}0_3$) (47), 436 $[M–H_2O]^+$ ($C_{30}H_{44}0_2$) (77), 421 $[M–H_2O–Me]^+$ ($C_{29}H_{41}0_2$) (26), 408 $[M–HCOOH]^+$ ($C_{29}H_{44}0$) (39), 393 $[M–HCOOH–Me]^+$ ($C_{28}H_{41}0$) (18), 330 [M-ring E]$^+$ ($C_{21}H_{30}0_3$) (43), 315 $[330\text{-}Me]^+$ ($C_{20}H_{27}0_3$) (24), 312 $[330\text{-}H_2O]^+$ ($C_{21}H_{28}0_2$) (70), 297 $[330\text{-}H_2O–Me]^+$ ($C_{20}H_{25}0_2$) (23), 284 $[330\text{-}HCOOH]^+$ ($C_{20}H_{28}0$) (100), 269 $[330\text{-}HCOOH–Me]^+$ ($C_{19}H_{25}0$) (30), 250 [ring A, B, part C]$^+$ ($C_{15}H_{22}0_3$) (21), 236 [ring A, B]$^+$ ($C_{14}H_{22}0_3$) (10), 218 [ring C, D, E]$^+$ ($C_{16}H_{26}$) (24), 204 [ring E, D, part C]$^+$ ($C_{15}H_{24}$) (62), 189 $[204\text{-}Me]^+$ ($C_{14}H_{21}$) (33), 124 $[M\text{-}330]^+$ (C_9H_{16}); ^{1}HNMR: δ 6.21 ppm (1H, s, H-25), 5.64 (1H, dd, $J_1=8.1$ Hz, $J_2=3.1$ Hz, H-15), 3.86 (1H, t, J = 2.7Hz, H-3), 3.26 (1H, dd, $J_1=18$ Hz, $J_2=3.1$ Hz, H-2a), 2.90 (1H, m, H-11a), 2.68 (1H, dd, $J_1=8.4$ Hz, $J_2=10.9$ Hz, H-9), 2.65 (1H, dd, $J_1=18$ Hz, $J_2=3.1$ Hz, H-2b), 2.52 (1H, m, H-11b), 2.05 (1H, dt, $J_1=13$ Hz, $J_2=3$ Hz, H-7a), 2.00 (1H, dd, $J_1=14.7$ Hz, $J_2=2.4$ Hz, H-16a), 1.68 (1H, m, H-12a), 1.67 (1H, dd, $J_1=14.7$ Hz, $J_2=2.4$ Hz, H-16b), 1.65 (1H, m, H-6a), 1.59 (1H, m, H-12b), 1.35 (1H, H-5), 1.35 (H-22a), 1.34 (H-19a), 1.34 (1H, dd, $J_1=13$ Hz, $J_2=3$ Hz, H-7b), 1.30 (H-21a), 1.28 (3H, s, H-26), 1.22 (H-19b), 1.16 (3H, s, H-24), 1.14 (3H, s, H-27), 1.02 (H-18), 1.01 (H-22b), 0.98 (H-21b), 0.95 (3H, s, H-29), 0.92 (3H, s, H-30), 0.88 (3H, s, H-28), 0.84 (3H, s, H-23).

2.6. Herrantrione (*2*)

Platelets (pyridine), 5 mg, m.p. 261°C. CIMS *m/z* (rel. int.): 499 $[M+H]^+$ ($C_{30}H_{42}O_6$) (65), 481 $[M–H_2O+H]^+$ ($C_{30}H_{40}O_5$) (25), 453 $[M–HCOOH+H]^+$ ($C_{29}H_{40}O_4$) (100), 435 $[M–HCOOH–H_2O+H]^+$ ($C_{29}H_{38}O_3$) (57). EIMS *m/z* (rel. int.): 360 [M-ring E]$^+$ ($C_{21}H_{28}O_5$) (5), 342 [M-ring E–H_2O]$^+$ ($C_{21}H_{26}O_4$) (5), 296 $[342\text{-}HCOOH]^+$ ($C_{20}H_{24}O_2$) (54), 281 $[342\text{-}HCOOH–Me]^+$ ($C_{19}H_{21}O_2$) (14), 168 [ring A]$^+$ ($C_9H_{12}O_3$) (100), 123 [ring A–HCOOH]$^+$ ($C_8H_{10}O$) (13). ^{1}HNMR: δ 5.96 ppm (1H, s, H-15), 5.71 (1H, s, H-25), 4.01 (1H, dt, $J_1=11$ Hz, $J_2=4$ Hz, H-6), 3.76 (1H, t, J = 3.8 Hz, H-3), 2.84 (1H, dd, $J_1=20$ Hz, $J_2=3.5$ Hz, H-2a), 2.69 (1H, d, J = 14 Hz, H-22a), 2.62 (1H, dd, $J_1=20$ Hz, $J_2=3.5$ Hz, H-2b), 2.52 (1H, d, J = 14 Hz, H-22b), 2.35 (1H, d, J = 3.4 Hz, H-7a), 2.34 (1H, t, J = 4.0 Hz, H-9), 2.19 (1H, m, H-11a), 2.06 (1H, m, H-11b), 1.93 (1H, H-19a), 1.80 (1H, H-12a), 1.79 (1H, H-18), 1.67 (1H, H-19b), 1.65 (1H, H-12b), 1.45 (3H, s, H-23/24), 1.38 (1H, d, J = 3.4 Hz, H-7b), 1.29 (3H, s, H-29/30), 1.23 (1H, H-5), 1.18 (3H, s, H-26), 1.14 (3H, s, H-27), 1.12 (3H, s, H-29/30), 1.09 (3H, s, H-28), 1.05 (3H, s, H-23/24). X-Ray crystallographic analysis: a colourless transparent platelet was crystallised from $CHCl_3$ having a size of $0.4 \times 0.15 \times 0.05$ mm. Preliminary investigations on the crystal were performed on a Polaroid XR7 land diffraction cassette #57-4 using a Polaroid 4×5 instant sheet high speed film. A total of 3668 ($R_{int}=2.60\%$) independent reflections were measured on a Siemens R3m diffractometer at 298 K (Ω-scan, scan speed 2–29.3°/min in ω, 2Θ range up to 114.0°, balanced filtered CuK_α radiation. 3871 Reflections were treated as observed with $F>4\sigma(F)$. An empirical absorption correction was applied to the measurements. Crystal data: $C_{30}H_{42}O_6$, monoclinic, space group: C2, unit cell dimensions: a = 31.66 (2) Å, b = 7.380 (3) Å, c = 12.071 (6) Å, $\beta=104.87$ (2)°, volume: 2726.210 (0) $Å^3$, Z 4, formula weight: 498, density (calc.): 1.254 mg/m^3, absorption coefficient: 0.712 mm^{-1}, F(000): 1112. The structure was dissolved by direct methods using Siemens SHELXTL PLUS (PC Version) (Baas, 1983); refinement method: full-matrix least-squares, quantity minimized: $\sum w(F_0-F_c)^2$, absolute structure: N/A, extinction correction: N/A, hydrogen atoms: riding model, fixed isotropic U, weighting scheme: $w^{-1}=\sigma^2(F)+99.0000F^2$, number of parameters refined: 333, final R indices (obs. data): R = 6.99%, wR = 6.78%, R indices (all data): R = 7.89%, wR = 7.23%, goodness-of-fit: 2.07, largest and mean Δ/σ: 0.018, 0.004, data-to-parameter ratio: 9.6:1, largest difference peak: 0.42 e $Å^{-3}$, largest difference hole: −0.49 e $Å^{-3}$. Tables of the atomic coordinates as well as distances and angles will be deposited at the Cambridge Crystallographic Data Centre. These tables

may be obtained, on request, from the Director, Cambridge Crystallographic Data centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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