New constituents of *Baccharis genistelloides* (Lam.) Pers.

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Dedicated to Prof. Heinz Heimgartner on the occasion of his 70th birthday

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Abstract

A phytochemical investigation of the constituents of the aerial parts of *Baccharis genistelloides* has resulted in the isolation of the new piquerol derivative (*E*)-4-acetoxy-6-methylene-5-(prop-1-en-2-yl)cyclohex-2-enyl 2-methylbut-2-enoate **4**, along with two other compounds [foliasalacin A_4 **5**, and (*E*)–icosyl 3-(4-hydroxy-3-methoxyphenyl)-acrylate **6**], which have not been reported as constituents of this plant until now.

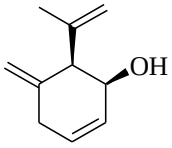
Keywords: *Baccharis genistelloides*, piquerol, foliasalacin A_4, icosyl ferulate

Introduction

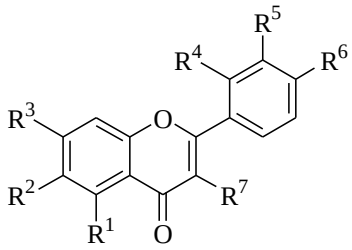
Baccharis is a New World genus belonging to the Asteraceae (tribe Astereae). The genus includes more than 400 species, about 90% of which are located in South America. Many of these species have been used since ancient times as folk remedies for some treatment purposes. Over 100 *Baccharis* species have been investigated to reveal that they contain many classes of secondary metabolites, e.g. terpenoids, diterpenoids, sesquiterpenes, triterpenoids, essential oils, flavonoids, coumarins, and other phenolic compounds. During the intensive investigation of the chemical components of *Baccharis*, much attention has been paid to its bioactive constituents.[1]
Baccharis genistelloides (Lam.) Pers. ("Carqueja", "Charara") is used for liver disorders and as an antithermic. Other popular uses include digestive disorders, malaria, diabetes, ulcers, sore throat and tonsillitis, anaemia, diarrhea, indigestion, intestinal worms and leprosy. *B. genistelloides* is also widely used in folk medicine in the form of infusion for its anti-inflammatory properties.[2-4]

β-Pinene (15%), carquejyl acetate (55%) and carquejol **1** (6-7%) were identified by Naves as the main components of the essential oil of this plant and numerous derivatives have been synthesized to confirm the structure of carquejol.[5] The relative and absolute configuration of **1** was determined by Thomas *et al*.[6]

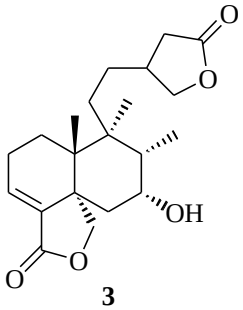
In an investigation of the chemical constituents of. *B. genistelloides*, flavones named eupatrin **2a**, cirsimaritin **2b**, cirsiliol **2c**, hispidulin **2d** and genkwanin **2e**, together with the known apigenin **2f** were isolated.[7-10] It is thought that these flavones are the major active substances in this plant. Quercetin **2g**, luteolin **2h**, and nepetin **2i** also have been found.[11] Other flavonoids, e.g. rutin **2j**, have been isolated as anti-inflammatory constituents.[12] Dos Santos *et al.*[13] reported the isolation of eupatorine **2k** and the diterpene lactone **3** which showed antimicrobial activity. In another study[14a] the biological activity of the dilactonic clerodane-diterpene **3**[14b,c] from *B. genistelloides* has also been described.



1



2a-k



3

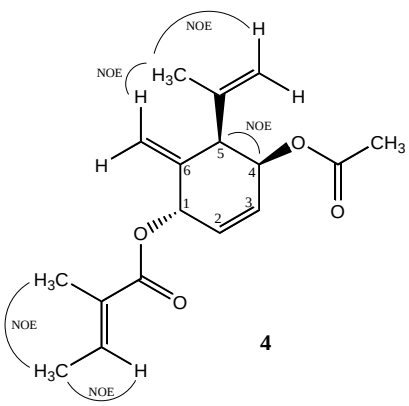
		R^1	R^2	R^3	R^4	R^5	R^6	R^7
2a	Eupatrin	OH	OMe	OMe	OH	OMe	OH	H
2b	Cirsimaritin	OH	OMe	OMe	H	H	OH	H
2c	Cirsiliol	OH	OMe	OMe	H	OH	OH	H
2d	Hispidulin	H	OMe	OH	H	H	OH	H
2e	Genkwanin	OH	H	OMe	H	H	OH	H
2f	Apigenin	OH	H	OH	H	H	OH	H
2g	Quercetin	OH	H	OH	H	OH	OH	OH
2h	Luteolin	OH	H	OH	H	OH	OH	H
2i	Nepetin	OH	OMe	OH	H	OH	OH	H
2j	Rutin	OH	H	OH	H	OH	OH	H
2k	Eupatorin	OH	OMe	OMe	H	OH	OMe	H

In the present study, we describe a new piquerol derivative and two other unknown compounds of *Baccharis genistelloides*.

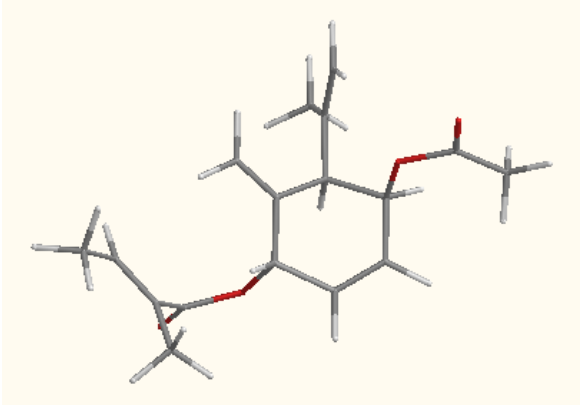
Results and Discussion

From the n-hexane extract of the aerial part of *Baccharis genistelloides* (collected in Huamachuco, Northern Peru) we have isolated three new constituents of this plant after careful chromatography.

First of all, the n-hexane extract was purified by column chromatography (silicagel; n-hexane-acetone 4:1) to give different fractions. A part of it (fractions 22-38) was then purified with n-hexane-ethyl acetate (5:2) followed by n-hexane-acetone (10:1) to give (*E*)-4-Acetoxy-6-methylene-5-(prop-1-en-2-yl)cyclohex-2-enyl 2-methylbut-2-enoate **4** as a colourless oil.

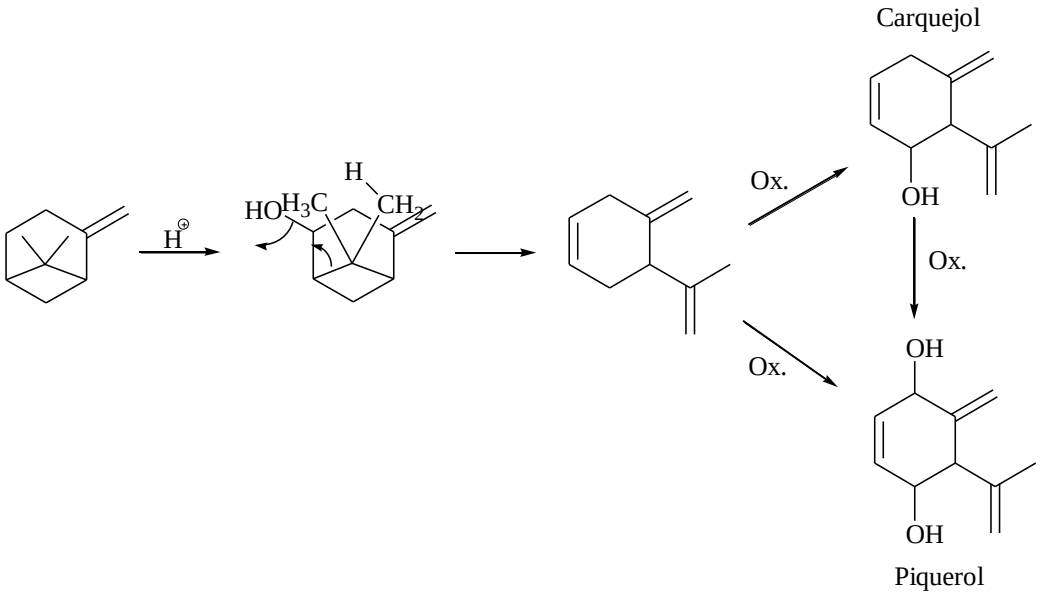


4



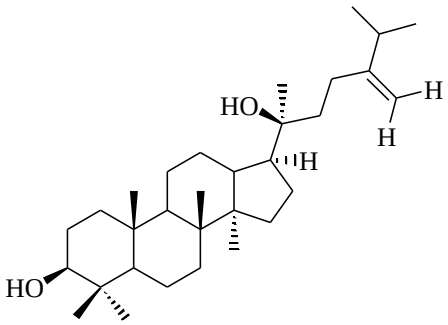
The structure of **4** has been identified by a combination of spectroscopic methods. The full NMR assignment was made by ^{1}H, ^{13}C, APT, gCOSY, gHSQC, gHMBC and NOESY experiments and is given in the experimental section and the supplementary material. In the HMBC experiment long-range correlations between the proton in 5-position (3.38 ppm) and the ring carbon atoms C-4, C-6, C-1, C-3 and all carbons of the side chains in 5- and 6-position were observed. NOE correlations have been found between the ring protons H-4 and H-5 at 5.44 and 3.38 ppm, respectively, but not between H-1 and H-5. Other important NOE correlations are shown in the formula. The *E*-configuartion of the substituent in 1-position can be concluded from these NOE´s. Therefore, the stereostructure can be described best with the model shown above.

Two similar terpene derivatives already have been found by F. Bohlmann *et al*. in the roots of *Baccharis trimera* (Less.) DC.[15] Both compounds with unusual terpene carbon skeleton are derivatives of piquerol A, and one of them was also identified as a constituent in the roots of *Piqueria trinervia* Cav.[16] Piquerol A represents a hydroxy substituted carquejol with high biological potential.[17] Its structure was determined with different spectroscopic methods[18] and X-ray analysis.[19] Piquerol A and carquejol as well are probably formed starting from β-pinene (Scheme 1).



Scheme 1

Furthermore, we isolated from the aerial part of *Baccharis genistelloides* Foliasalacyin A_4 **5** as a new constituent of this plant. The triterpene Foliasalacyin A_4 **5** was obtained as a white powder after column chromatography with n-hexane-ethyl acetate (5:3) followed by n-hexane-ethyl acetate (5:4). Other triterpenoid compounds (phytol, β-sitosterol and stigmasterol) already have been identified during the investigation of *B. serrifolia* DC. and *B. genistelloides*.[20,21] The combination of different NMR spectra of **5** showed eight methyl groups, an olefinic methylene group, a methine carbon bearing a hydroxy group, and a quaternary carbon which is also connected to a hydroxy group (Supplementary Material). The final structure could be proved by comparison with literature results.

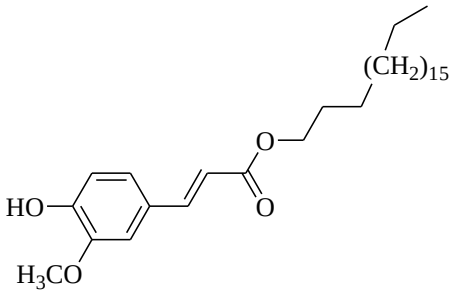


5

Foliasalacin A_4 is a dammarane-type triterpene which recently was isolated for the first time from the leaves of *Solacia chinensis* L. collected in Thailand.[22] All our recorded spectra of **5** are

in total agreement with the spectra and characterization of the stereostructure described in literature.[22]

Finally, purification of fractions 95-108 of the n-hexane-acetone 4:1 eluate furnished the icosyl ferulate **6** (column chromatography with n-hexane-acetone 2:1) as a slightly yellow solid. The structure of **6** was proved by NMR, IR, UV-Vis and HRMS. The elemental constitution could be calculated from the molecular ion and is in accordance with the NMR spectra. A coupling constant of 16.0 Hz of the olefinic protons in the proton spectrum showed (*E)*-configuration. The position of hydroxy and methoxy group in the aromatic ring system was identified by long-range correlations in the HMBC experiment.



6

The antioxidant ferulic acid derivatives are ubiquitous in plants and abundant in fruits and vegetables. For example, different ferulates, including the icosyl ferulate **6,** were isolated from cork layers of *Solanum tuberosum* L. and *Pseudotsuga menziesii* (Mirb.) Franco.[23] Ferulic acid esters have been tested for tumor cell proliferation, cyclooxygenase enzymes (COX-1 and COX-2) and lipid peroxidation inhibitory activities in vitro. In the tumor cell proliferation assay, some of these esters showed excellent growth inhibition of colon cancer cells. In COX enzyme assays, ferulic acid esters significantly inhibited both COX-1 and COX-2 enzymes.[24]

Experimental Section

General. NMR spectra were recorded with a Varian Mercury Plus 400 spectrometer. Residual solvent signals were used as internal chemical shift references for proton (DMSO-d_6: δ 2.50 ppm; $CDCl_3$: δ 7.26 ppm) and carbon (DMSO-d_6: δ 39.52 ppm; $CDCl_3$: δ 77.16 ppm). The full assignment is attached in the supplementary information. Mass spectra were measured on a VG ZAB-HSQ (VG Analytics) and HRMS spectra in positive mode on a FT-ICR-MS APEX II (Bruker Daltonics) spectrometer. IR spectra were recorded on a spectrophotometer Genesis FTIR Unicam Analytical System (ATI Mattson) with KBr pellets. Elemental analyses were performed on a Heraeus CHNO Rapid Analyzer. UV-Vis spectra were recorded on a Beckman DU 650; λ_{max} in nm (log ε). All separations were carried out in carefully purified and dried solvents and were monitored by thin-layer chromatography (TLC) on plates of Silufol UV/VIS 254 nm.

Preparative column chromatography was carried out on neutral silica gel (MERCK 70–230 mesh) in gradient regime.

Plant material

The aerial part of *B. genistelloides* (Lam.) Pers. was collected in March 2009 at Huamachuco (José Faustino Sánchez Carrión Province – Perú) and identified by Botanist Eric Rodriguez-Rodriguez [National University of Trujillo; Herbarium Truxillense (HUT)]. A voucher specimen No. 50002 (HUT) has been deposited at the Herbarium Truxillense (HUT) of the National University of Trujillo (Perú).

Isolation

The air-dried aerial parts of the plant (103.52 g) were extracted with n-hexane (5 L) by cold maceration for 5 days. The resulting extract was concentrated *in vacuo* to afford a residue of 2.61 g. This residue was stored at -10 ºC until use for column chromatography (CC).

The n-hexane extract (2.61 g) was purified by CC on silcagel with n-hexane-acetone (4:1) according to the TLC profile resulting in 115 fractions. Fractions 22-38 were eluted by repeated CC with n-hexane-ethyl acetate (5:2) to give six new fractions. Out of these the second fraction gave 33.8 mg of **4** (Rf. 0.48) using CC with n-hexane-acetone (10:1). The sixth fraction was purified with n-hexane-ethyl acetate (5:3) to give four other fractions. Out of these fractions the first one was eluted with n-hexane-ethyl acetate (5:4) to give 21.5 mg of **5** (Rf. 0.44). Fractions 95-108 from the original n-hexane-acetone 4:1 eluate were purified by CC with n-hexane-acetone (2:1) to give 40.0 mg of **6** (Rf. 0.5).

(*E*)-4-Acetoxy-6-methylene-5-(prop-1-en-2-yl)cyclohex-2-enyl 2-methylbut-2-enoate (4). Colourless oil; $C_{17}H_{22}O_4$ (290.35). ^{1}H NMR ($CDCl_3$) δ 1.73 (s (br), 3 H, CH_3), 1.79 (d, *J* = 6.9 Hz, 3 H, CH_3), 1.82 (s (br), 3 H, CH_3), 2.05 (s, 3 H, CH_3COO), 3.38 (d, *J* = 5.2 Hz, 1 H, H-5), 4.92 (s (br), 1 H, $=CH_2$), 4.97 (s (br), 1 H, $=CH_2$), 5.44 (m, 1 H, H-4), 5.21 (s, 1 H, $=CH_2$), 5.32 (s, 1 H, $=CH_2$), 5.82 (m, 1 H, H-2), 5.83 (1 H, H-1), 6.00 (m, 1 H, H-3), 6.86 (q, *J* = 6.9 Hz, 1 H, =CH-). ^{13}C NMR ($CDCl_3$) δ 12.11(CH_3), 14.57 (CH_3), 21.29 (CH_3COO), 23.24 (CH_3), 50.10 (C-5), 69.38 (C-1), 70.95 (C-4), 114.61 ($=CH_2$), 116.84 ($=CH_2$), 128.52 (C-2), 128.71 (=Cq), 130.64 (C-3), 137.97 (=CH-), 141.07 (=Cq), 141.34 (C-6), 167.45 (COO), 170.58 (COO); IR ν_{max} 1738, 1709, 1650 cm^{-1}; HRMS *m/z* 313.14108 (M^+ + Na).

(3*S*,8*R*,10*R*,14*R*,17*S*)-17-((*S*)-2-hydroxy-6-methyl-5-methyleneheptan-2-yl)-4,4,8,10,14-pentamethyl-hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-ol (Foliasalacin A$_4$) (5). White powder; $C_{31}H_{54}O_2$ (458.76). ^{1}H NMR ($CDCl_3$) δ 0.73 (1 H; H-5), 0.77 (s, 3 H, CH_3-28); 0.85 (s, 3 H, CH_3-19), 0.88 (s, 3 H, CH_3-18), 0.96 (s, 3 H, CH_3-30), 0.96/1.70 (m, 2 H, CH_2-1), 0.97 (s, 3 H, CH_3-29), 1.04 (d, *J* = 6.8 Hz, 6 H, CH_3-26,27), 1.08/1.48 (m, 2 H, CH_2-15), 1.16 (s, 3 H, CH_3-21), 1.25/1.51 (m, 2 H, CH_2-11), 1.28/1.53 (m, 2H, CH_2-7), 1.32 (m, 1 H, CH-9), 1.35/1.81 (m, 2 H, CH_2-16), 1.44/1.52 (m, 2 H, CH_2-6), 1.51/1.76 (m, 2 H, CH_2-12), 1.58/1.63 (m, 2 H, CH_2-2), 1.59/1.61 (m, 2 H, CH_2-22), 1.65 (m, 1 H, CH-13), 1.76 (m, 1 H, CH-17),

2.08/2.10 (m, 2 H, CH_2-23), 2.26 (sept, *J* = 6.8 Hz,1 H, CH-25), 3.20 (dd, *J* = 11.2 Hz, *J* = 5.2 Hz, 1 H, CH-3), 4.69 (s (br), 1 H, =CH_2), 4.75 (s (br), 1H, =CH_2). ^{13}C NMR ($CDCl_3$) δ 15.51 (CH_3-28), 15.62 (CH_3-30), 16.37 (CH_3-19), 16.64 (CH_3-18), 18.44 (CH_2-6), 21.71 (CH_2-11), 22.08 (CH_3-27), 22.11 (CH_3-26), 24.94 (CH_2-12), 25.53 (CH_3-21), 27.57 (CH_2-2), 27.69 (CH_2-16), 28.15 (CH_3-29), 28.54 (CH_2-23), 31.37 (CH_2-15), 34.18 (CH-25), 35.38 (CH_2-7), 37.29 (C-10), 39.13 (C-4), 39.20 (CH_2-1), 39.56 (CH_2-22), 40.53 (C-8), 42.51 (CH-13), 49.93 (CH-17), 50.50 (C-14), 50.79 (CH-9), 56.02 (CH-5), 75.49 (C-20), 79.12 (CH-3), 106.31 (CH_2-31), 156.69 (C-24); IR ν_{max} 3434, 2955, 2869, 1642 cm^{-1}.

(*E*)–Icosyl 3-(4-hydroxy-3-methoxyphenyl)-acrylate (6). Slightly yellow solid; $C_{30}H_{50}O_4$ (474.72). ^{1}H NMR ($CDCl_3$) δ 0.86 (t, *J* = 6.8 Hz, 3 H, CH_3), 1.22 (m, 30 H, 15xCH_2), 1.29 (m, 2 H, CH_2), 1.39 (m, 2 H, CH_2), 1.70 (m, 2 H, CH_2), 3.94 (s, 3 H, OCH_3), 4.19 (t, *J* = 6.8 Hz, 2 H, OCH_2), 5.84 (s (br), 1 H, OH), 6.29 (d, *J* = 16.0 Hz, 1 H, =CH-), 6.91 (d, *J* = 8.4 Hz, 1 H, Ph), 7.03 (d, *J* = 2.0 Hz, 1 H, Ph), 7.07 (dd, *J* = 8.4 Hz, J = 2.0 Hz, 1 H, Ph), 7.61 (d, *J* = 16.0 Hz, 1 H, =CH-). ^{13}C NMR ($CDCl_3$) δ 14.27 (CH_3), 22.84 (CH_2), 26.15 (CH_2), 28.94 (CH_2), 29.46 (CH_2), 29.52 (CH_2), 29.70 (CH_2), 29.75 (CH_2), 29.81 (2xCH_2), 29.85 (9xCH_2), 56.09 (OCH_3), 64.77 (OCH_2), 109.44 (C-2-Ph), 114.84 (C-5-Ph), 115.87 (=CH-), 123.19 (C-6-Ph), 127.23 (C-1-Ph), 144.77 (=CH-), 146.90 (C-3-Ph), 148.04 (C-4-Ph), 167.54 (CO); HRMS *m/z* 497.36180 (M^+ + Na), 971.73813 ($2M^+$ + Na); IR ν_{max} 3427, 2917, 2849, 1713 cm^{-1}; UV-Vis ($CHCl_3$) λ = 320 (4.37), 295 (4.10), 243 (4.00).

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