Pergamon

PII: S0031-9422(97)00228-8

Phytochemistry, Vol. 46, No. 1, pp. 161–164, 1997

0031-9422/97 $17.00+0.00

DITERPENES AND SESQUITERPENES FROM *MIKANIA BANISTERIAE**

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(*Received in revised form* 3 *February* 1997)

Key Word Index—*Mikania banisteriae*; Asteraceae; diterpenes; *ent*-kauranes; sesquiterpenes; eudesmane; eudesmanolides; biological studies.

Abstract—Two new *ent*-kaurane diterpenes, 18,19-diacetoxy-*ent*-kaur-16-ene and 17-oxo-*ent*-kaur-15(16)-en-18-oic acid, were isolated from the aerial parts of *Mikania banisteriae*, together with the known *ent*-kauranes *ent*-kaur-16-en-18-oic acid and *ent*-kaur-16-en-18-ol. The structures were established by mass spectrometry, ^{1}H NMR and partly ^{13}C NMR spectroscopy. In addition, the sesquiterpene lactones eudesma-4(15),7(11)-dien-8β,12-olide and eudesma-4(15),7(11),8(9)-trien-12-olide as well as the sesquiterpenes 1β,6α-dihydroxyeudesm-4(15)-ene and caryophyllenoxide were identified by direct comparison (TLC, GC, GC-MS) with authentic samples. The chemotaxonomic significance is briefly discussed. *ent*-Kaur-16-en-18-oic acid and *ent*-kaur-16-en-19-oic acid showed no antifeedant, antimicrobial and antiinflammatory properties.

INTRODUCTION

The large genus *Mikania* (tribe Eupatorieae) with nearly 430 species is distributed over the tropical parts of America, Africa and Asia [1]. Some *Mikania* species are used in the traditional medicine of Costa Rica because of their antiinflammatory and antimicrobial effects [2]. Looking for further *Mikania* species with biological activities, we have studied *M. banisteriae* DC., from which only kaurane derivatives have been reported up to now [3].

RESULTS AND DISCUSSION

From the lipophilic extract of the aerial parts of *M. banisteriae* the kaurane derivatives *ent*-kaur-16-en-18-oic acid (**1**), *ent*-kaur-16-en-18-ol (**2**), 18,19-diacetoxy-*ent*-kaur-16-ene (**3**) and 17-oxo-*ent*-kaur-15(16)-en-18-oic acid (**4**) were isolated. To the best of our knowledge, compounds **3** and **4** are described for the first time in nature. Additionally, the eudesmane, 1β, 6α-dihydroxyeudesm-4(15)-ene (**5**), the eudesmanolides eudesma-4(15),7(11)-dien-8β,12-olide (**6**) and eudesma-4(15),7(11),8(9)-trien-12-olide (**7**) as well as caryophyllenoxide (**8**) were found to be present. With the exception of diterpene **1** all compounds are new for the genus *Mikania*.

The structure of compound **3** was deduced from the mass and NMR (^{1}H, ^{1}H-^{1}H COSY) spectra. The EI mass spectrum displayed a $[M]^+$ peak at *m/z* 388. Peaks at *m/z* 328 and 268 were derived from the successive loss of two acetic acid molecules. In accordance with this, the ^{1}H NMR spectrum exhibited singlets at δ 2.07 and 2.04 due to acetoxy methyl groups, which in turn were attached to C-18 and C-19, since only one singlet for a tertiary methyl group occurred at δ 1.07 (H-20). The remaining methyl equivalents were found to be the AB system from two substituted hydroxymethyl groups (H-18/18′: δ 4.03 and 3.94; H-19/19′: δ 4.30 and 4.04). Additionally, the spectra contained the typical low field signals of a conjugated exocyclic H-17/17′ at δ 4.80 and 4.74 and a deshielded proton at δ 2.65, characteristic for kaurenoid diterpenes. All signals agreed well with those reported for 18-acetoxy-*ent*-kaur-16-ene [3], already known from *M. banisteriae*, with the exception of protons at C-18 and C-19.

The structure of compound **4** followed from its mass and NMR (^{1}H, ^{1}H-^{1}H COSY) spectra. The EI mass spectrum showed, besides a $[M]^+$ at *m/z* 316, the typical fragment ions for a kaurene diterpene with a carboxyl group at C-4 [4]. The ^{1}H NMR spectrum contained singlets for two methyl groups and its chemical

* Part I in the series 'Phytochemical and Biological Studies of Costa Rican Asteraceae'.

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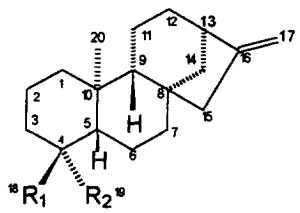
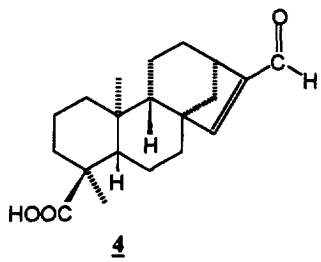


	R_1	R_2
1	COOH	CH_3
2	CH_2OH	CH_3
3	CH_2OAc	CH_2OAc



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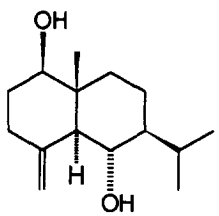

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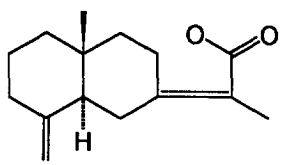

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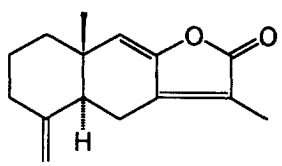

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Table 1. ^{1}H NMR spectral data of diterpenes **3** and **4** (500 MHz, $CDCl_3$, TMS as int. standard)

H	3	4
13α	2.65 *s*(*br*)	3.05 *m*
15	2.00 *m*	6.58 *s*
15′		—
17	4.80 *s* (*br*)	9.74 *s*
17′	4.74 *s* (*br*)	—
18	4.03 *d* [a]	—
18′	3.94 *d* [a]	
19	4.30 *d* [a]	1.18 *s*
19′	4.04 *d* [a]	
20	1.07 *s*	1.11 *s*
C-18-Ac	2.04 *s*[b]	
C-19-Ac	2.07 *s*[b]	

[a,b] Assignments may be interchanged.
J (Hz): compound **3**: 18,18′ = 11.2; 19,19′ = 11.6.

shifts agreed nicely with those from compound **1**, indicating the same configuration at C-4. The presence of a 15(16)-double bond conjugated with a carbonyl group followed from the downfield shift of the signal for H-13 by δ 0.5, the absence of signals for the exo-methylene protons and the occurrence of a singlet for a vinylic proton at δ 6.58, which must be assigned to H-15. In the low field a singlet appeared at δ 9.74 due to an aldehyde group, which had to be located at C-16. NMR data have been reported for the methyl ester of 17-oxo-*ent*-kaur-15(16)-en-19-oic acid [5]. Our data differed only from those due to the methyl group at C-4.

The kaurene derivatives **1** and **2** were identified by comparison of their spectral data (^{1}H and ^{13}C NMR, mass) with those reported in the literature [6–9]. Compounds **5–8** occurred in such low quantities in the extract that identification was done from the mixture by direct comparison (TLC, GC, GC-MS) with the respective authentic samples. The obtained data agreed with those reported in the literature [10–13].

The *ent*-kaurane derivatives **1–4** and the sesquiterpene lactones **6** and **7** are interesting in chemotaxonomic terms. The large genus Mikania is considered to form two groups of species: one contains highly oxygenated sesquiterpene lactones and the other only diterpenes. *M. banisteriae* has been thought to belong to the latter group [3]. The identification of compounds **6** and **7** shows that the absence or occurrence of compounds often depends on the analytical methods used. Therefore, the strict division within this genus should be handled carefully. Additionally, *M. banisteriae* is the only species within this genus from which only *ent*-kaurene derivatives with an oxidized C-18 methyl group have been found up to now. This type occurs more rarely in nature compared to that with an oxidized C-19 methyl group.

Diterpenes are supposed to play a role in the defence system of plants [14]. Therefore, diterpene **1**, being the main compound of the lipophilic extract, was examined for its antiherbivorous effect towards the notorious polyphagous pest insect *Spodoptera littoralis* [15]. At concentrations of 0.3 and 0.8 mg g^{-1} dry weight no effect was observed. Compound **1** and *ent*-kaur-16-en-19-oic acid showed no inhibition in the agar diffusion test against *Staphylococcus aureus* at a concentration of 100 μg per filter paper. On the other hand, antimicrobial activity against several other gram positive bacteria and antifungal activity is shown by the C-19 isomer [16–17]. Both *ent*-kaurenic acids were tested for their antiinflammatory activity in the

cyclooxygenase test [18]. No significant effects could be observed with concentrations of 100 and 150 μM.

EXPERIMENTAL

Plant material. Leaves and stems of *M. banisteriae* were collected in Tapanti, National Park, Costa Rica in February 1994 and identified by L. Poveda, Professor of Botany, Universidad Nacional, Costa Rica. Voucher specimens (no. 3613) are deposited at the Nacional Herbarium of Costa Rica.

Extraction and isolation. Dried powdered leaves and stems from *M. banisteriae* (867 g) were extracted with *n*-hexane–Et_2O–MeOH (1:1:1) at room temp. The crude extract (36.4 g) was treated with MeOH at −20°. After removal of ppts and solvent, the soluble part (22 g) was sepd by flash chromatography (silica gel) yielding five frs. CC of fr. 2 (hexane–Et_2O (1:1); 4.57 g) on silica gel with mixts of CH_2Cl_2–EtOAc (9:1 and 7:3) afforded 10 subfrs. Fr. 2.3 (2.5 g) was purified by CC on Sephadex LH-20 with C_6H_{12}–CH_2Cl_2–MeOH (7:4:1) followed by CC on silica gel with CH_2Cl_2–EtOAc–MeOH (8:1:1) and MPLC (RP 18, 15–25 μm, using MeOH–H_2O mixts) yielding compound **4** (2 mg) and in a further subfr. compound **5**, together with unidentified substances. From subfr. 2.4, compound **3** (1 mg) was isolated by repeated CC, first on silica gel with toluene and increasing amounts of CH_2Cl_2 and then afterwards with C_6H_{12}–EtOAc (9:1). Final purification was done by prep. TLC (C_6H_{12}–Et_2O, 2:1). Further subfrs contained the terpenes **5** and **6–8** as well as unidentified compounds. Compound **2** (7 mg) was obtained by CC on silica gel with toluene and increasing amounts of CH_2Cl_2 from subfr. 2.5. Subfr. 2.7 afforded 1529 mg of compound **1**.

GC-MS: OV-1-DF capillary (25 m × 0.25 mm i.d.; film: 0.25 μm), carrier N_2 at 65 ml min^{-1} (total flow), split 1:50, gradient: 150–270°, FID; TLC: silica 0.2 mm on alumina sheets.

18,19-*Diacetoxy-ent-kaur*-16-*ene* (**3**). Mp 274–276° (decomp.); EIMS 70 eV, *m/z* (rel. int.): 388 $[M]^+$ (16), 373 $[M-Me]^+$ (5), 345 $[373-CO]^+$ (4), 328 $[M-HOAc]^+$ (18), 313 $[328-Me]^+$ (9), 300 $[328-CO]^+$ (3), 285 $[300-Me]^+$ (11), 268 $[328-HOAc]^+$ (40), 253 $[268-Me]^+$ (36), 240 $[268-CO]^+$ (18), 225 $[240-Me]^+$ (44), 211 (16), 197 (23), 185 (21), 173 (20), 159 (26), 145 (29), 131 (36), 119 (39), 105 (56), 91 (71), 79 (68), 67 (55), 55 (56), 43 (100).

17-*Oxo-ent-kaur*-15(16)-*en*-18-*oic acid* (**4**). Mp 213–215°; EIMS 70 eV, *m/z* (rel. int.): 316 $[M]^+$ (7), 301 $[M-Me]^+$ (3), 298 (3), 283 (3), 272 $[M-CHO-Me]^+$ (5), 271 (5), 270 $[M-COOH_2]^+$ (5), 255 $[270-Me]^+$ (8), 241 $[270-CHO]^+$ (4), 237 (11), 227 (5), 213 (4), 199 (9), 195 (9), 185 (6), 173 (8), 159 (11), 147 (15), 131 (19), 121 (40), 105 (46), 91 (85), 79 (77), 67 (68), 55 (81), 43 (100).

Eudesma-4(15),7(11)-*dien*-8β,12-*olide* (**6**): EIMS 70 eV; *m/z* (rel. int.): 232 $[M]^+$ (66), 217 $[M-Me]^+$ (18), 204 $[M-CO]^+$ (12), 199 $[217-H_2O]^+$ (6), 189 $[204-Me]^+$ (10), 187 (5), 176 (10), 171 (5), 161 (16), 147 (17), 133 (16), 121 (33), 119 (21), 110 (19), 108 (25), 105 (40), 93 (51), 77 (48), 67 (39), 65 (30), 55 (34), 53 (56), 41 (100) [12].

Eudesma-4(15),7(11),8(9)-*trien*-12-*olide* (**7**). EIMS 70 eV, *m/z* (rel. int.): 230 $[M]^+$ (75), 215 $[M-Me]^+$ (100), 201 $[M-HCO]^+$ (40), 187 $[215-CO]^+$ (34), 174 (29), 162 (17), 159 (29), 145 (22), 131 (25), 117 (21), 115 (25), 105 (29), 91 (67), 77 (56), 65 (31), 53 (63), 41 (71) [12].

Acknowledgements—The authors are grateful to the DFG for financial support (Me 1389/1-1), to Dr D. Wendisch, Abt. ZF-DZA-Strukturforschung der Bayer-AG, Leverkusen and H. Mathew, Institut für Pharmazeutische Chemie, Universität Düsseldorf, for measuring the NMR spectra, Prof. L. Poveda, Universidad Nacional, Costa Rica for his support in collection of the plant material, Prof. R. Bauer and Mrs A. Seibt, Institut für Pharmazeutische Biologie, Universität Düsseldorf, for the cyclooxygenase test, Prof. P. Proksch, Julius-von-Sachs-Institut für Biowissenschaften, Universität Würzburg, for the antifeedant test, Prof. Zhong-liang, Shanghai Institute of Materia Medica, China for the eudesmanolides, and INBio, Costa Rica, for the support in collection and extraction of the plant material.

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