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CHAMAEDRYDIOL, AN URSANE TRITERPENE FROM *MARSYPIANTHES CHAMAEDRYS*

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Key Word Index—*Marsypianthes chamaedrys*; Lamiaceae; triterpenoids; chamaedrydiol.

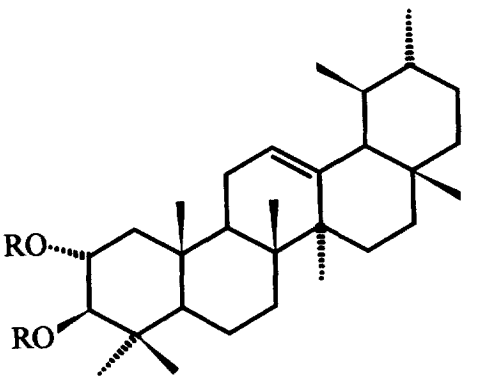
Abstract—A mixture containing a new triterpene, chamaedrydiol (urs-12-ene-2α,3β-diol), and three known triterpenes (lup-20(29)-ene-2α,3β-diol, castanopsol and epigermanidiol) was isolated from *Marsypianthes chamaedrys*. The components in the mixture were identified using spectroscopic methods. In addition, a mixture of four less polar known triterpenes consisting of α-amyrin, β-amyrin, lupeol and germanicol, was also isolated.

INTRODUCTION

Marsypianthes chamaedrys (Vahl.) Kuntze, a species belonging to the subfamily Ocimoideae, occurs in the North and Northeast regions of Brazil where it is known as paracarí, erva de cobra, bóia-caá or betônica brava and is used by the local population to treat several conditions, including snake venom poisoning [1]. The first chemical investigation on *M. chamaedrys* has shown the presence of steroids (sitosterol and stigmasterol), triterpenes (including oleanolic, ursolic and tormentic acids) as well as the flavonoid rutin [2]. In the present paper, we report the identification of a new triterpene (**1**) in mixture with castanopsol, epigermanidiol and lup-20(29)-ene-2α,3β-diol in addition to another mixture containing α-amyrin, β-amyrin, lupeol and germanicol.

RESULTS AND DISCUSSION

The hexane extract of *M. chamaedrys* stems was filtered on a silica gel column to give two main fractions apparently pure on TLC. These fractions were analysed by GC revealing that they were actually mixtures. The more polar mixture, eluted with hexane-EtOAc (1:1), consisted of four substances. GC-MS analysis of this mixture showed for compound **1** (R_t = 49.158 min) a mass spectrum having $[M]^+ = m/z$ 442 in agreement with $C_{30}H_{50}O_2$. The chromatogram of the peracetylated mixture showed a signal relative to the R_t of 68.762 min with a mass spectrum having $[M]^+ = m/z$ 526 in agreement with the mass observed for the peracetylated product **1a**. The 1D ^{1}H NMR of this mixture showed signals for methyl groups (δ 0.73–1.11), for two carbinol methine protons at δ 3.0 (*d*, *J* 9.6 Hz, 3β-H) and 3.7 ppm (*ddd*, *J* 11.2 Hz, 9.6 Hz, 4.6 Hz) and for one olefinic proton at δ 5.19 (*t*, *J* 3.6 Hz). These data suggested **1** to be a triterpene with an ursane skeleton. Confirmation of some aspects of the chamaedrydiol structure was made by comparison of its ^{1}H NMR data with those



1- R = H
1a- R = Ac

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Table 1. ^{1}H NMR data for compounds **1** and **1a**. Comparison with data published for methyl 2α,3β-dihydroxyurs-12-en-28-oate (**2**) and its diacetate (**2a**)

Assignments	**1**	**2**	**1a**	**2a**
H-2β	3.7 *ddd*	3.68 *ddd*	5.02 *ddd*	5.10 *ddd*
	(*J* 11.2, 9.6, 4.6)	(*J* 11.0, 10.0, 4.5)	(*J* 10.3, 9.5, 4.6)	(*J* 11.0, 10.5, 4.5)
H-3α	3.0 *d*	2.99 *d*	4.7 *d*	4.75 *d*
	(*J* 9.6)	(*J* 10.0)	(*J* 9.5)	(*J* 10.5)
H-12	5.19 *t*	5.24 *t*	5.14 *t*	5.23 *t*
	(*J* 3.6)	(*J* 3.5)	(*J* 3.6)	(*J* 3.5)
H-18	2.2 *d*	2.22 *d*	2.2 *d*	2.23 *d*
	(*J* 11.0)	(*J* 11.0)	(*J* 11.0)	(*J* 11.0)
O*Ac*	—	—	1.93 *s*	1.97 *s*
			2.02 *s*	2.05 *s*

proposed for a similar compound (Table 1) [3]. This ^{1}H NMR spectrum also contained signals corresponding to the other three triterpenes present in the mixture. Some of those signals were very characteristic of the three different triterpene skeletons: δ 5.21 (*t*, *J* 3.6 Hz) for castanopsol [4], δ 4.84 (*br s*) for germanidiol [5], and δ 4.66 (*d*, *J* 2.4 Hz) together with δ 4.57 (*dd*, *J* 2.4 Hz, 1.4 Hz) for lup-20(29)-ene-2α,3β-diol [5]. The ^{13}C NMR spectrum confirmed **1** to be urs-12-ene-2α,3β-diol. The presence of signals at δ 139.3 and at δ 124.0 as well as signals at δ 83.2 and at δ 68.7 support this proposal. A comparison between the ^{13}C NMR data obtained for chamaedrydiol and its diacetate with data collected in the literature for α-amyrin and for methyl 2α,3β-dihydroxyurs-12-en-28-oate and its diacetate is shown in Table 2. The characterization of the constituents of this triterpene mixture as well as the characterization of the constituents of the less polar triterpene mixture was simplified by the assignment of the carbon atoms in the ^{13}C NMR. As the chemical shift of a sp^2 carbon atom is very characteristic for each triterpenoid skeleton, ^{13}C NMR spectroscopy has been very frequently employed for the structural analysis of triterpene mixtures [6]. To distinguish carbon types (multiplicity), the attached proton test (APT) was used. In this way, it was possible to identify in the less polar triterpene mixture the presence of α-amyrin, β-amyrin, lupeol and germanicol (Table 3) by comparison with previously published ^{13}C NMR data [7]. This result was also confirmed by CG-MS analysis. The mass spectra corresponding to the signals that appeared in the chromatogram, show for each constituent $[M]^+ = m/z$ 426, in agreement with $C_{30}H_{50}O$. It is interesting to note that the two triterpene mixtures isolated in sequence from the hexane extract of *M. chamaedrys* characterize an oxidative evolutionary biosynthetic sequence. The less polar mixture consisted of monohydroxylated derivatives while the more polar mixture consisted of dihydroxylated derivatives with the same skeletons.

Table 2. ^{13}C NMR data for compounds **1** and **1a**. Comparison with data published for α-amyrin [7], methyl 2α,3β-dihydrours-12-en-28-oate (**2**) and its diacetate (**2a**) [3]

C	**1**	**2**	α-Amyrin	**1a**	**2a**
1	38.4	48.6	38.7	34.2	44.1
2	68.7	68.9	27.2	70.1	70.1
3	83.1	83.9	78.3	80.5	80.7
4	38.4	39.2	38.7	38.2	39.3
5	55.2	55.3	55.2	55.0	54.9
6	18.3	18.3	18.3	18.0	18.2
7	32.4	32.8	32.9	32.5	32.8
8	39.9	39.5	40.0	39.6	39.5
9	47.7	47.5	47.7	47.4	47.5
10	36.9	38.2	36.9	36.9	38.1
11	23.4	23.3	23.3	23.4	23.4
12	124.0	125.3	124.3	123.8	125.1
13	139.3	138.2	139.3	139.5	138.3
14	42.0	42.2	42.0	42.6	42.1
15	28.7	28.0	28.7	27.8	28.0
16	26.8	24.2	26.6	26.7	24.2
17	33.6	48.1	33.7	33.4	48.1
18	58.9	52.8	58.9	57.9	52.8
19	39.6	39.0	39.6	39.4	39.0
20	39.6	38.8	39.6	39.3	38.9
21	31.3	30.6	31.2	31.0	30.6
22	41.6	36.6	41.5	41.5	36.6
23	28.2	28.6	28.1	28.2	28.5
24	16.0	16.8	15.6	16.9	17.7
25	16.0	16.7	15.6	15.8	16.5
26	16.9	16.9	16.8	16.6	16.9
27	23.3	23.6	23.3	23.5	23.6
28	28.4	178.0	28.1	28.1	178.0
29	17.4	17.0	17.4	17.0	17.0
30	21.3	21.2	21.3	21.2	21.2
O*Ac*	—	—	—	170.8	170.8
	—	—	—	170.5	170.5

EXPERIMENTAL

General

EIMS 70eV; ^{1}H NMR (200 MHz) and ^{13}C NMR (50.2 Mhz): $CDCl_3$ using TMS as int. standard.

Table 3. ^{13}C NMR data of the constituents of the less polar triterpene mixture isolated from the hexanic extract of *M. chamaedrys* stems [7]

C	Triterpenes of the Less Polar Mixture			
	Lupeol	β-amyrin	α-amyrin	Germanicol
1	38.6	38.6	38.6	38.6
2	27.4	27.3	27.1	27.4
3	78.8	78.8	78.8	78.8
4	38.7	38.7	38.6	38.8
5	55.2	55.1	55.1	55.4
6	18.2	18.2	18.2	18.2
7	34.2	32.8	32.8	34.5
8	40.6	38.6	39.9	40.6
9	50.3	47.6	47.5	51.1
10	37.2	37.6	37.0	37.2
11	20.9	23.6	23.2	21.2
12	25.1	121.6	124.3	26.4
13	37.9	145.0	139.4	38.8
14	42.6	41.4	41.9	43.2
15	27.4	26.0	28.6	27.8
16	35.5	27.1	26.5	37.6
17	43.1	32.5	33.6	34.5
18	48.2	47.5	58.9	142.6
19	47.8	46.7	39.5	129.5
20	150.7	31.1	39.5	32.3
21	29.7	34.6	31.1	32.2
22	39.8	37.2	41.6	37.5
23	27.9	28.3	27.9	27.9
24	15.4	15.5	15.6	15.4
25	15.9	15.6	15.6	15.9
26	15.8	16.8	16.8	16.7
27	14.4	26.0	23.3	14.4
28	18.1	28.3	27.9	25.2
29	109.2	33.2	17.3	31.2
30	19.2	23.6	21.3	29.5

Separation. CC: Silica gel (Merck); GC: Varian Star 3400 gas chromatograph, fused silica capillary column (DB-1, 30 m × 0.20 mm), H_2 as carrier gas and temp. programming from 40° to 270° (5°min); CG-MS HP5890 SII gas chromatograph coupled to a VG Autospect mass spectrometer at 70 eV, fused silica capillary column (DB-1, 30 m × 0.20 mm), H_2 as carrier gas and temp. programming from 40° to 270° (5° min). The temp. programming used for the separation of the peracetylated compounds was 150° to 270° (3° min).

Plant material. Stems of *M. chamaedrys* were collected near Fortaleza, Ceará State, Brazil in December, 1995. A herbarium sample (Voucher number = 24856) has been deposited at the Department of Biology, Herbário Prisco Bezerra, Universidade Federal do Ceará.

Extraction and isolation. The dried and powdered plant material (1 Kg) was submitted to successive extraction with hexane and MeOH at room temp. The extracts were evapd. to dryness under red. pres. The hexane extract of stems (11.6 g) of *M. chamaedrys* was fractionated by silica gel CC eluted with mixtures of hexane-EtOAc and EtOAc-MeOH. The fraction eluted from this column with hexane-EtOAc (92.5:7.5) was filtered in the presence of active charcoal to give a mixture containing α-amyrin, β-amyrin, lupeol and germanicol. The fraction eluted with hexane-EtOAc (1:1) was re-chromatographed on silica gel CC to give a mixture which contained **1** together with castanopsol, epigermanidiol and lup-20(29)-ene-2α,3β-diol.

Chamaedrydiol (**1**). ^1H NMR (200 MHz, $CDCl_3$, TMS): δ 0.73–1.11 (signals for methyl groups), 3.0 (*d*, *J* 9.6 Hz, H-3), 3.7 (*ddd*, *J* 11.2 Hz, 9.6 HZ., 4.6 Hz, H-2). and 5.19 (*t*, *J* 3.6 Hz, H-12); ^{13}C NMR: Table 2; IE-MS *m/z* (rel. int.): 442 $[M]^{+\cdot}$ (13.1), 427 (8.1), 218 (63.1), 204 (73.1), 189 (66.9), 177 (62.5), 161 (18.8), 147 (20.0), 133 (26.3), 121 (44.4), 109 (53.8), 95 (81.3), 69 (62.5), 55 (100), 43 (87.5).

Acetylation of the mixture containing **1**. This mixture was acetylated (C_5H_5N-Ac_2O) overnight at room temp. and the reaction mixture treated in the usual way. The mixture of acetates was further analysed by physical methods.

Chamaedrydiol diacetate (**1a**). ^1H NMR (200 MHz, $CDCl_3$, TMS): δ (ppm) 0.70–1.10 (signals for methyl groups). 1.93 (*s*, 3H, CH_3COO). 2.2 (*s*, 3H, CH_3COO). 4.70 (*d*, *J* 9.5 Hz, H_3). 5.02 (*ddd*, *J* 10.3; 9.5; 4.6, H_2). 5.14 (*t*, *J* 3.6 Hz, H_{12}); ^{13}C NMR: Table 2; EM-IE *m/z* (rel. int.): 526 $[M]^{+\cdot}$ (2.5), 511 (1.9), 229 (1.9), 218 (7.5), 204 (22.5), 190 (21.2), 177 (18.8), 161 (5.0), 147 (5.6), 133 (9.4), 109 (14.4), 95 (21.9), 69 (19.4), 55 (18.8), 43 (100).

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