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ENT-KAURENE DITERPENES FROM *GOCHNATIA POLYMORPHA* VAR. *POLYMORPHA**

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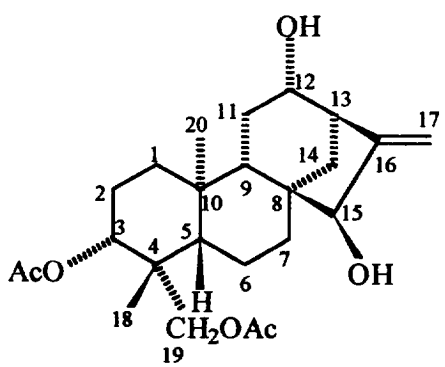
Key Word Index—*Gochnatia polymorpha* var. *polymorpha*; Asteraceae; Mutisieae; diterpenes; *ent*-kaurenes; eudesmanolide.

Abstract—Two diterpenes were isolated from the aerial parts of *Gochnatia polymorpha* var. *polymorpha*. Their structures were established as *ent*-3β,19-diacetoxy-12β,15α-dihydroxy-kaur-16-ene and *ent*-17,19-diacetoxy-3β,16β-dihydroxykaurane, respectively. In addition, the aerial parts afforded cycloart-25-ene-3β,22α-diol and the flavoids genkwanin and desmethoxycentaureidin while the roots furnished taraxerol and the eudesmanolide santamarin. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The large genus *Gochnatia*, traditionally placed in a subtribe (Gochnatiinae [1]) which has recently been absorbed into subtribe Nassauviinae [2], is concentrated in the tropical Andean region and Brazil but also extends into Central America, Mexico, the West Indies and the Southern United States. So far 14 taxa have been studied chemically [3–16]; in addition to common flavonoids [6, 9, 13, 14, 16] and triterpenes [3–5], bisabolenes [6, 8, 11], *ent*-pimaranes [10], *ent*-clerodanes [6] and sesquiterpene lactones of various types [4–9, 11–16] have been reported. We now describe our work on *G. polymorpha* (Less.) Cabr, var. *polymorpha*, The aerial parts furnished two new diterpenes (**1** and **2**) as well as cycloart-25-ene-3β,22α-diol and the flavonoids genkwanin and desmethoxycentaureidin, while the roots gave the eudesmanolide santamarin and taraxerol.



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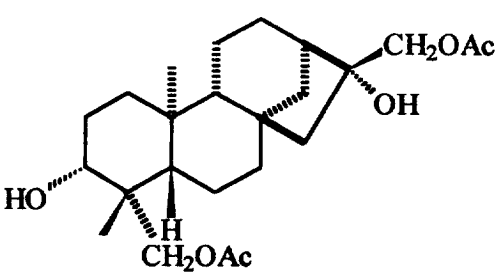

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RESULTS AND DISCUSSION

That compound **1**, $C_{24}H_{36}O_6$, was a diacetoxykaur-12α,15-β-dihydroxy-kaur-16-ene or its enantiomer was apparent from ^{1}H NMR spectrum (Table 1) which exhibited singlets of two acetates and two methyls and showed the presence of an unconjugated olefinic methylene (H-17a, b) as broadened singlets at δ 5.11 and 5.00, both of which were coupled allylically to a slightly broadened triplet at δ 3.83 (H-15), as well as

* Dedicated to the memory of our friend and botanist colleague Prof. Hermógenes de Freitas Leitão Filho who died February 23, 1996.

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Table 1. ^{1}N HMR spectrum of compound **1** (500 MHz, $CDCl_3$)

H		H	
1α	1.86 *dd* (13, 3.5, 3.5)	12β	3.79 *brt* (4.5)
1β	1.05 *ddd* (13, 13, 4.5)	13	2.59 *brt* (4.5)
2α	1.65–1.75 *m*	14α	2.33 *d* (13)
		14β	0.91 *dd* (13, 5)
3β	4.55 *dd* (11.5)	15α	3.83 *brt* (2.5)
5	1.73 *brd* (13)	17α	5.11 *brs*
6α	obsc.	17b	5.00 *brd* (2)
6β	obsc.	18*	1.01 *s*
7α	1.44 *ddd* (13, 3, 3)	19a	4.37 *d* (11.5)
7β	1.55 *ddd* (13, 13, 3)	19b	4.17 *dd* (11.5)
9	1.46 *m*	20*	1.26 *s*
11α	1.65–1.75 *m*		2.05 *s*
11β	1.65–1.75 *m*	Ac*	2.02 *s*

* Intensity three protons.

to a *brt* at δ 2.59 (*J*s = 4.5 Hz, H-13) and homallylically to a broadened triplet (*J*s = 4.5 Hz) at δ 3.79 (H-12) in the manner characteristic of such compounds. H-12 was further coupled to two signals (H-11a, b) submerged in a five proton multiplet at δ 1.65–1.75 while H-13 was coupled further by 13 Hz to only one, as usual, of the protons of the AB system H-14a, b at δ 2.33 and 0.91.

Of the two acetoxy groups one was secondary, equatorial and attached to C-1 and C-3 as shown by a *dd* (*J*s = 11 and 5 Hz at δ 4.55) while the second was primary (*d*s, *J* = 1.15 Hz and δ 4.37 and 4.17). The chemical shifts in the ^{13}C NMR spectrum (Table 2) indicated functionalization at C-3 [17] and C-19 to

Table 2. ^{13}C NMR spectra of compounds **1** and **2** ($CDCl_3$)

C	**1** (67.89 MHz)	**2** (75 MHz)
1	38.9 *t*	38.6 *t*
2	23.5 *t*	26.0 *t*
3	78.9 *d*	79.1 *d*
4	41.1 *s*	42.2 *s*
5	54.9 *d*	55.6 *d*
6	20.6 *t*	20.7 *t*
7	38.4 *t*	41.9 *t*
8	45.2 *s*	44.5 *s*
9	47.7 *d*	56.3 *d*
10	37.2 *s*	38.8 *s*
11	26.0 *t*	18.3 *t*
12	72.0 *d*	27.1 *t*
13	46.9 *d*	45.9 *d*
14	29.5 *t*	36.8 *t*
15	82.4 *t*	52.7 *t*
16	154.6 *s*	79.8 *s*
17	106.6 *t*	64.8 *t*
18	22.8 *q*	22.4 *q*
19	65.2 *t*	65.3 *t*
20	16.0 *q*	17.3 *q*
OCOMe	170.9 *s*, 170.5 *s*	171.1 *s*, 171.2 *s*
OCOMe	21.1 *q*, 21.2 *q*	20.8 *q*, 21.1 *q*

Table 3. NOE difference spectrum of **1**

Irradiated	Observed (% enhancement)
H-1α	H-1β (20.7), H-20 (3.2)
H-1β + H-18	H-1α (4.4), H-3β (5.0)
H-3β	H-1β + H-18 (13.6), H-5 (4.7)
H-12β	H-11α,β (5.3), H-13 (8.7), H-17b (4.0)
H-13	H-12β (9.4), H-14α (4.1), H-14β (5.3), H-17b (5.8)
H-14α	H-13 (5.9), H-14β (25.8), H-20 (9.7)
H-14β	H-13 (8.2), H-14α (23.8), H-15 (6.9)
H-15	H-7α (5.2), H-13 (2.5), H-14β (4.3), H-17a (3.9)
H-17a	H-15 (4.7), H-17b (47.0)
H-17b	H-13 (6.4), H-17a (34.4)
H-18	H-3β (5.6), H-5 (3.8), H-19a (1.6), H19b (1.1), Ac (2.0)
H-19a	H-19b (25.5), H-20 (4.1)
H-19b	H-19a (28.2), H-20 (5.9)
H-20	H-1α (3.0), H-14α (3.4), H-19a (1.7), H-19b (1.1)

produce formula **1**, a conclusion which was confirmed by NOE spectrometry (Table 3).

The ^{1}H NMR spectrum of **2**, $C_{24}H_{56}O_6$ (see Experimental), exhibited two methyl singlets and two singlets of acetates, the latter being attached to two methylenes each of which was in turn attached to a quaternary carbon. An equatorial OH was located at either C-1 or C-3 while the sixth oxygen had to be located as a hydroxyl on C-16 to explain the observation that none of the methylene protons under the acetates was coupled further. Chemical shifts in the ^{13}C NMR spectrum (Table 2) were consistent only with location of the equatorial secondary hydroxyl group on C-3 [17]; as for the stereochemistry at C-16, the chemical shifts of C-13 through C-17 were appropriate for the configuration shown in the formula rather than its opposite [18].

Although the absolute configurations of the two kauranes were not established, we assume that they belong to the *ent*-series like other kauranes of Asteraceae [18]. The flavonoids, triterpenes and santamarin found in our collection were identified by comparison of their spectral properties (IR, MS ^{1}H and ^{13}C NMR, UV) with published data.

The chemistry of our collection of *G. polymorpha* var. *polymorpha* from São Paulo State, Brazil, differs drastically from that of a collection, variety unspecified, from Paraguay which furnished various bisabolenes, costunolide, dehydrocostus lactone and various dimers of guaianolides [11].

EXPERIMENTAL

General. Mps: uncorr: UV: MeOH; IR: KBr disc; NMR $CDCl_3$ and DMSO-d_6 (flavonoids) with TMS as int. standard.

Isolation. The plant was collected in July 1992 along

the main highway to São Paulo 258 km south of Ribeirão Preto, São Paulo State, Brazil, and identified by Hermógenes de Freitas Leitão Filho, Departamento de Botânica, Universidade de Campinas (UNICAMP); voucher specimens are deposited in the Herbarium of UNICAMP. The air-dried and pulverized aerial parts and roots (3.3 and 1.8 kg respectively) were extracted with CH_2Cl_2. The crude extract of the aerial parts amounted to 28.0 g. The material was dissolved in EtOH, and water was added to precipitate fats and pigments. After extraction with $CHCl_3$, the residue (21.0 g) was chromatographed on silica gel D under vacuum, 500 ml fractions being eluted as follows: 1–3 (*n*-C_6H14), 4–7 (*n*-C_6H_{14}-EtOAc, 49:1), 8–18 (*n*-C_6H_{14}-EtOAc, 19:1), 19–33 (*n*-C_6H_{14}-EtOAc, 9:1), 34–53 (*n*-C_6H_{14}-EtOAc, 4:1), 54–83 (*n*-C_6H_{14}-EtOAc, 7:3), 84–110 (*n*-C_6H_{14}-EtOAc, 3:2), 111–133 (*n*-C_6H_{14}-EtOAc, 1:1), 134–149 (*n*-C_6H_{14}-EtOAc, 3:7), 150–167 (EtOAc) and 168–180 (MeOH). The solid of fraction 35 was recrystallized from *n*-C_6H_{14}-EtOAc (4:1) to give crystals (12.2 mg), mp 179–181°, identified as cycloart-25-ene-3β,22α-diol. The material (176 mg) from combined fractions 43–62 was chromatographed on Sephadex LH-20 (MeOH) to give yellow crystals (32.7 mg), mp 287–290°, which were identified as genkwanin. Fractions 63–69 (123.1 mg) were combined, washed with Et_2O and recrystallized from *n*-C_6H_{14}-EtOAc (4:1), giving crystals of **1** (11.4 mg), mp 209–212°. Compound **2** (221.3 mg), mp 160–163°, was obtained from frs. 77–77 in the same fashion. The yellow solid from fractions 78–83 on chromatography on Sephadex LG-20 (MeOH) and then on PVP (MeOH-H_2O 1:1/MeOH/Me_2CO), gave 73.1 mg of desmethoxycentaureidin, yellow crystals, mp 271–273°. The root extract (37.0 g) was chromatographed on silica gel D under vacuum, 500 ml fractions being eluted as follows: 1–5 (*n*-C_6H_{14}), 6–31 (*n*-C_6H_{14}-EtOAc, 9:1), 32–53 (*n*-C_6H_{14}-EtOAc, 4:1), 54–77 (*n*-C_6H_{14}-EtOAc, 7:3), 78–103 (*n*-C_6H_{14}-EtOAc, 3:2), 104–119 (*n*-C_6H_{14}-EtOAc, 1:1), 120–142 (*n*-C_6H_{14}-EtOAc, 3:7), 143–158 (EtOAc) and 159–170 (MeOH). The material (4.63 g) from the combined fractions 10–31 was submitted to vacuum liquid chromatography on a silica gel D column, 40 subfractions being collected. Prep. TLC (C_6H_{14}–$\beta\times$) of subfractions 8–16 (90.6 mg) gave 35.5 mg of taraxerol as crystals, mp 279–281°. Fractions 48–53 (2.12 g) were combined and chromatographed on silica gel D under vacuum, 47 subfractions being collected. HPLC (ODS 4.6 × 250 mm/20 × 250 mm, MeOH-H_2O 3:2, 210 nm) of subfractions 5–19 gave 15.4 mg of santamarin (as crystals, mp 132–134°).

ent-3-β,19-*Diacetoxy*-12β,15α-*dihydroxykaur*-16-*ene* (**1**). Mp 209–212°, $[\alpha]_D = -36.3°$ ($CHCl_3$, *c* 0.1). $IR_{\nu max}$ cm^{-1}: 3480, 2940, 1705, 1720, 1650, 1270; MS PCI (NH_3) *m/z* (rel. int.): 438 $[M+NH_4]^+$(100); ^1H NMR in Table 1; ^{13}C NMR in Table 2.

ent-17,19-*Diacetoxy*-3β,16β-*dihydroxykaurane* (**2**). Mp 160–163°, $[\alpha]_D = -23.8°$ ($CHCl_3$, *c* 0.1); $IR_{\nu max}$ cm^{-1}: 3 380, 2 930, 1 730, 1 700, 1 280, 1 240, 1 050: MS PCI (NH_3) *m/z* (rel. int.): 440 $[M+NH_4]^+$ (18.7), 422 $[M]^+$ (26.6), 404 $[M-H_2O]^+$ (100); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.33 (*d*, *J* = 12 Hz, H-19a), 4.11 (*d*, *J* = 12 Hz, H-19b), 4.21 (2H, *brs*, H-17a, b), 3.25 (*dd*, *J* = 11, 5 Hz, H-3), 2.11 and 2.06 (each *s* and 3H, Ac), 1.12 (*s*, 3H, H-20), 1.02 (*s*, 3H, H-18); ^{13}C NMR: Table 2.

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