A New *ent*-Kaurane Diterpene from Stems of *Alibertia macrophylla* K. SCHUM. (Rubiaceae)

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Phytochemical investigations of the stems of a specimen of *Alibertia macrophylla* led to the isolation and characterization of the new diterpene *ent*-kaurane-2β,3α,16α-triol (**1**), along with triterpenes **2**–**8**, iridoids **9**–**12**, and phenolic acids **13**–**15**. The structure of **1** was established based on spectroscopic studies (^{1}H- and ^{13}C-NMR, IR, and HR-ESI-MS). This is the first report of the isolation of a diterpene from the *Alibertia* genus in Rubiaceae.

Introduction. – Plants of the *Alibertia* genus are known to be a rich source of pentacyclic triterpenes [1–3]. Moreover, *Alibertia* belongs to the Ixoroideae subfamily in Rubiaceae, these being known for the occurrence of iridoids as main secondary metabolites [4]. Previous investigation of the leaves of *A. macrophylla* resulted in the isolation of non-glycosidic iridoids 1β- and 1α-hydroxydihydrocornin aglycones, and the caffeic acid esters 2-phenylethyl caffeoate and 2-methyl-4-hydroxybutyl caffeoate [5].

This paper reports the occurrence of a new *ent*-kaurane diterpene (**1**) from *A. macrophylla* stems in addition to seven triterpenes **2**–**8**, four glucosidic iridoids **9**–**12**, three phenolic acids **13**–**15**, and two steroids **16** and **17**. It is the first report of a diterpene from any *Alibertia* species, but *ent*-kauranes were previously isolated from *Chiococca alba* [6] and *Tricalysia dubia* [7] species of the family Rubiaceae.

Results and Discussion. – Dried and powdered stems of *Alibertia macrophylla* were successively extracted with hexane, AcOEt and EtOH. The hexane extract was submitted to chromatography (column and preparative TLC) to afford lupenone (**2**), germanicone (**3**), α-amirenone (**4**), β-amirenone (**5**), lupeol (**6**), and oleanolic (**7**) and ursolic (**8**) acids. The AcOEt was fractionated by using a combined chromatographic column on silica gel and *RP18*, preparative TLC and preparative RP-HPLC to afford 6α- (**9**) and 6β-hydroxygeniposide (**10**), gardenoside (**11**), shanziside methyl ester (**12**), and protocatechuic (**13**), vanilic (**14**), and caffeic (**15**) acids, sitosterol (**16**) stigmasterol (**17**), and the new *ent*-kaurane-2β,3α,16α-triol (**1**).

Compounds **2**–**17** were identified by comparing their ^{1}H- and ^{13}C-NMR data with those previously published [1][8–13].

Compound **1** was isolated as a yellow amorphous powder. The HR-ESI-MS of **1** exhibited its $[M+Na]^+$ peak at m/z 345.2574 (calc. 345.2502), consistent with the

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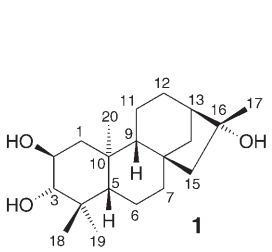

molecular formula $C_{20}H_{34}O_3$. The IR spectrum of **1** revealed OH absorptions (3373 cm^{-1}). The 1D and 2D NMR data (*Table*) revealed the presence of four Me groups (δ(H) 0.69/δ(C) 16.8, δ(H) 0.90/δ(C) 28.9, δ(H) 1.00/δ(C) 18.7, and δ(H) 1.22/δ(C) 24.3), two oxygenated CH groups (δ(H) 3.45/δ(C) 67.2 and δ(H) 2.72/δ(C) 82.3), and one oxygenated quaternary C-atom (δ(C) 76.7). The ^{13}C-NMR spectrum also showed the presence of seven CH_2 and three more CH groups, and three additional quaternary C-atoms in the molecule. Based on these data, **1** was assumed to be a trihydroxy-kaurane diterpene. On the basis of its negative specific rotation value ($[\alpha]_D^{27} = -1.36$), it is suggested that **1** belongs to the *ent*-kaurane series, similarly to

Table. *^{1}H- and ^{13}C-NMR data of* **1**, *together with* g*HMBC* (H → C) *correlations.* At 500 and 126 MHz, respectively, in (D_6)DMSO; δ in ppm, J in Hz.

Position	δ(H)	δ(C)	*g*HMBC
H_{ax}–C(1)	0.67 (br. *dd*, *J* = 12.0, 12.0)	47.0 (*t*)	C(2), C(5)
H_{eq}–C(1)	1.95 (*dd*, *J* = 12.0, 4.5)		C(3)
H_{ax}–C(2)	3.45 (*ddd*, *J* = 12.0, 9.5, 4.5)	67.2 (*d*)	
H_{ax}–C(3)	2.72 (*d*, *J* = 9.5)	82.3 (*d*)	C(2), C(4), C(18), C(19)
C(4)	–	38.8 (*s*)	
H_{ax}–C(5)	0.76 (br. *d*, *J* = 12.0)	54.6 (*d*)	C(6), C(10), C(18)
CH_2(6)	1.50–1.52 (*m*)	17.7 (*t*)	C(8)
CH_2(7)	1.54–1.55 (*m*)	41.6 (*t*)	
C(8)	–	44.4 (*s*)	
H–C(9)	0.89–0.95 (*m*)	56.1 (*d*)	
C(10)	–	39.0 (*s*)	
CH_2(11)	1.46–1.50 (*m*)	19.8 (*t*)	
CH_2(12)	1.44–1.48 (*m*)	26.4 (*t*)	C(16)
H–C(13)	1.71–1.74 (*m*)	47.8 (*d*)	C(12), C(15), C(16)
CH_2(14)	1.54–1.59 (*m*), 1.72–1.75 (*m*)	37.0 (*t*)	C(16)
CH_2(15)	1.38–1.48 (*m*)	57.5 (*t*)	
C(16)	–	76.7 (*s*)	
Me(17)	1.22 (*s*)	24.3 (*q*)	C(13), C(15), C(16)
Me(18)	0.90 (*s*)	28.9 (*q*)	C(3), C(4), C(5), C(19)
Me(19)	0.69 (*s*)	16.8 (*q*)	C(3), C(4), C(5), C(18)
Me(20)	1.00 (*s*)	18.7 (*q*)	C(1), C(9), C(10)

other analogous diterpenes [14][15]. The assignments of C(2) and C(3) were confirmed by the cross-peak correlations in the *g*HMQC spectrum of H–C(2) (δ 3.45) and H–C(3) (δ 2.72) with the ^{13}C signals at δ 67.2 and δ 82.3, respectively, as well as the long-range connectivities shown by the *g*HMBC spectrum between the signals ascribed to H–C(18) (δ 0.90) and δ 82.3, and between H–C(19) (δ 0.69) and δ 82.3. The Me(20) group and H–C(5) are α- and β-oriented, respectively, on biogenetic grounds [16]. The configuration at C(16) was determined as (*R*), the OH group at which has an equatorial orientation (α) and the Me(17) group has an axial orientation (β). In this case, a chemical shift at C(16) ((*R*)-configuration) appears at δ(C) 76–79, and if C(16) has the configuration (*S*), the chemical shift would be δ(C) 81–82. NOESY Interactions observed between H–C(5) (δ(H) 0.76) and H–C(3) (δ(H) 2.72), and also between H–C(5) (δ(H) 0.76) and H–C(9) (δ(H) 0.89–0.95) indicated the axial orientations (β) of H–C(5), H–C(3), and H–C(9) (*Fig.*). The configuration at C(2) was determined from the multiplicity and coupling constant observed for H–C(2) and H–C(3) from the 1D TOCSY spectrum, and from interactions found for

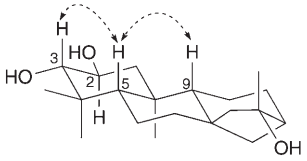

Figure. *Important NOESY interactions in compound* **1**

H–C(3) in NOESY spectrum. From the NOESY spectrum, H–C(3) was defined as being in an axial position (β), and its coupling constant of 9.5 Hz indicated that H–C(2) is also in an axial position but with an α-orientation. Compound **1** was, therefore, identified as *ent*-kaurane-2β,3α,16α-triol.

Thus, this is the first report of this *ent*-kaurane in literature, and it is the first report of the isolation of a diterpene from the *Alibertia* genus in Rubiaceae. The occurrence of triterpenes **2**–**8**, iridoids **9**–**12**, and phenolic acids **13**–**15** in *Alibertia* is in agreement with chemosystematic correlations and botanical positioning of this genus in the subfamily Ixoroideae [17].

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Experimental Part

General. Column chromatography (CC): silica gel (35–70 and 70–200 mesh; *Acros*) and silica *RP18* (15–25 μm; *Merck*). TLC: silica gel *60 G* F_{254} plates; visualization under UV light, I_2 vapor and by spraying 0.5% anisaldehyde (in H_2SO_4), followed by heating. HPLC Separations were performed on a *Varian PrepStar Dynamax SD-1* system with a UV/VIS detector and a *RP18* column (*Phenomenex Luna C18*), eluting with MeOH/H_2O. Optical rotations: *Polamat A Carl Zeiss Jena* polarimeter. IR Spectra: *Nicolet 730 FT-IR* spectrometer, with KBr discs; in cm^{-1}. NMR Spectra: *Varian INOVA 500* instrument; at 500 (^1H) and 126 MHz (^{13}C); δ in ppm rel. to TMS, *J* in Hz. HR-ESI-MS: *Bruker Daltonics UltrOTOF-Q*; in *m*/*z*.

Plant Material. Alibertia macrophylla K. SCHUM. was collected in the Mogi Guaçú Ecological and Experimental Reserve in the São Paulo State (Estação Ecológica e Experimental de Mogi-Guaçú, São Paulo – SP), Brazil in November 2003 by Dr. *M. C. M. Young* and identified by Dr. *I. Cordeiro*. The voucher specimen (SP 370.915) was deposited at the Botanical Institute Herbarium in São Paulo city, São Paulo State (Instituto de Botânica, São Paulo – SP), Brazil.

Extraction and Isolation. The dried and powdered stems (550.0 g) of *A. macrophylla* were successively extracted with hexane, AcOEt, and EtOH at r.t. After evaporation, the hexane extract (2.80 g) was submitted to CC (silica gel (70–200 mesh); gradient of hexane/AcOEt) to give ten *Fractions* after TLC analysis. *Fr. 2* (350.0 mg) was subjected to a CC (silica gel (35–70 mesh); hexane/AcOEt 98:02 (*v*/*v*)) to afford a mixture of **2**–**5** (133.6 mg). *Fr. 4* (140.0 mg) was purified by a prep. TLC (hexane/AcOEt 4:1 (*v*/*v*)) to afford **6** (54.8 mg). *Fr. 7* (103.0 mg) was submitted to prep. TLC (hexane/AcOEt 7:3 (*v*/*v*)) to afford a mixture of **7** and **8** (15.0 mg). The AcOEt extract (8.0 g) was subjected to CC (*RP18*; gradient of MeOH/H_2O and EtOAc/MeOH) to give 13 *Fractions* after TLC analysis. *Fr. 3* (380.0 mg) was purified by prep. HPLC (*C18* column; MeOH/H_2O 3:7 (*v*/*v*); 10 ml/min; $\lambda = 238$ nm) to afford **9** (48.0 mg), **10** (13.2 mg), a mixture of **11** and **13** (15.0 mg), and a mixture of **14** and **15** (9.0 mg). *Fr. 4* (1.5 g) was submitted to CC (*RP18*; gradients of MeOH/H_2O and EtOAc/MeOH) to give eight subfractions after TLC analysis. *Fr. 4.4* (70.0 mg) was purified by prep. HPLC (*C18* column; MeOH/H_2O/$MeCO_2H$ 34:165:1 (*v*/*v*/*v*); 10 ml/min; $\lambda = 254$ nm) to afford **9** (8.3 mg), **10** (12.5 mg), **11** (5.8 mg), and **14** (3.0 mg). *Fr. 4.5* (70.0 mg) was submitted to a purification by CC (*RP18*; MeOH/H_2O gradient) to afford **12** (16.4 mg) and **14** (8.7 mg). *Fr. 6* (800.0 mg) was submitted to a CC (silica gel (35–70 mesh); gradient of AcOEt/MeOH) to afford **1** (130.0 mg). *Fr. 7* (300.0 mg) was purified by CC (silica gel (35–70 mesh); gradient of hexane/AcOEt) to afford a mixture of **16** and **17** (23.6 mg).

ent-*Kaurane-2β,3α,16α-triol* (**1**). Yellow amorphous powder. $[\alpha]_D^{27} = -1.36$ ($c = 0.122$, MeOH). IR (KBr): 3373, 2938, 2856, 1461, 1387, 1042, 1116. ^1H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 345.2574 ($[M + Na]^+$, $C_{20}H_{34}NaO_3^+$; calc. 345.2502).

Lupenone (**2**), *Germanicone* (**3**), *α-Amirenone* (**4**), *and β-Amirenone* (**5**). The ^1H- and ^{13}C-NMR (in $CDCl_3$) data of the mixture were in accordance with those reported in [1].

Lupeol (**6**), *Ursolic Acid* (**7**), *and Oleanolic Acid* (**8**). The ^{1}H- and ^{13}C-NMR (in $CDCl_3$) data of these compounds were in accordance with those reported in [8].

6α-Hydroxygeniposide (**9**) *and 6β-Hydroxygeniposide* (**10**). The ^{1}H- and ^{13}C-NMR (in CD_3OD) data of theses compounds were in accordance with those reported in [9].

Gardenoside (**11**). The ^{1}H- and ^{13}C-NMR (in (D_6)DMSO) data of this compounds was in accordance with those reported in [10].

Shanziside Methyl Ester (**12**). The ^{1}H- and ^{13}C-NMR (in (D_6)DMSO) data of this compounds was in accordance with those reported in [11].

Protocatechuic Acid (**13**), *Vanilic Acid* (**14**), *and Caffeic Acid* (**15**). The ^{1}H- and ^{13}C-NMR (in (D_6)DMSO) data of these compounds were in accordance with those reported in [12].

Sitosterol (**16**) *and Stigmasterol* (**17**). The ^{1}H- and ^{13}C-NMR (in $CDCl_3$) data of these compounds were in accordance with those reported in [13].

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