Withanolides from *Aureliana fasciculata* var. *fasciculata*

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In the first phytochemical study of the *Aureliana* genus (Solanaceae), two new withanolides, **1** and **2**, together with two known sterols, were isolated from the MeOH extract of the leaves of *Aureliana fasciculata* var. *fasciculata*. The structures were established as (4*S*,22*R*)-16α-acetoxy-5β,6β-epoxy-4β,17α-dihydroxy-1-oxowitha-2,24-dienolide (aurelianolide A) and (4*S*,22*R*)-16α-acetoxy-4β,17α-dihydroxy-1-oxowitha-2,5,24-trienolide (aurelianolide B). The new compounds possessed the unusual 16α,17α-dioxygenated group and were fully characterized by spectroscopic techniques, including ^{1}H- and ^{13}C-NMR (DEPT), as well as 2D-NMR (HMBC, HMQC, ^{1}H,^{1}H-COSY, NOESY) experiments, and HR-MS.

Introduction. – The withasteroids comprise a group of naturally occurring C_{28}-steroids characterized by an unaltered or a modified ergostane skeleton with a lactone or potential lactone ring in the C_9 side chain [1]. The most abundant type is designated usually as 'withanolide', and these compounds possess an α,β-unsaturated δ-lactone ring in the side chain of the molecule. These steroid derivatives are frequently polyoxygenated, and biogenetic transformations can produce highly modified structures, both in the steroid nucleus and in the side chain, such as physalins, withaphysalins, acnistins, withanolides, ixocarpalactones, and perulactones [2]. To date, over 400 withasteroids have been reported which are restricted in distribution to the plant family Solanaceae occurring primarily in *ca.* 20 genera [1][2]. However, the occurrence of steroidal lactones is not completely restricted to Solanaceae, whereas these compounds have also been found in soft coral from *Minabea* sp. [3], and in plants such as *Tacca plantaginea* [4] and *T. chantrieri* [5] (Taccaceae), *Cassia siamea* (Leguminosae) [6], *Ajuga parviflora* [7], *A. reptans* [8], and *A. bracteosa* (Lamiaceae) [9], and *Eucalyptus globulus* (Myrtaceae) [10].

The isolation and synthesis of withanolides have attracted great interest due to their diverse biological activities such as anti-inflammatory [11], antimicrobial [12], antitumoral [13][14], antistress [15], antiulceral and hepatoprotective [16], antiparasitical [17], immunomodulatory [18], and insect-antifeedant properties [19]. Recently, it has been reported that these compounds exhibit inhibitory activities against cyclooxygenase-1 (COX-1) and -2 (COX-2) [20], acetylcholinesterase, and butyrylcholinesterase enzymes [21], and induction activity on quinone reductase [14][22].

In the course of our search for metabolites from Brazilian Piperaceae and Solanaceae plants [23], we have performed the first phytochemical investigation of the *Aureliana* genus. This study led to the isolation of two new withanolides from the leaves of *Aureliana fasciculata* var. *fasciculata*, together with two known sterols. Here, we describe the isolation and structure elucidation of these new compounds and their chemotaxonomic significance.

Results and Discussion. – The CH_2Cl_2-soluble part of the MeOH extract obtained from the leaves of *A. fasciculata* var. *fasciculata* yielded a mixture of two known sterols and two new withanolides, compounds **1** and **2** (*Fig. 1*), which were isolated and identified. The known sterols were readily identified as stigmasterol and sitosterol by analysis of their NMR data and by comparison with the data reported in the literature [24].

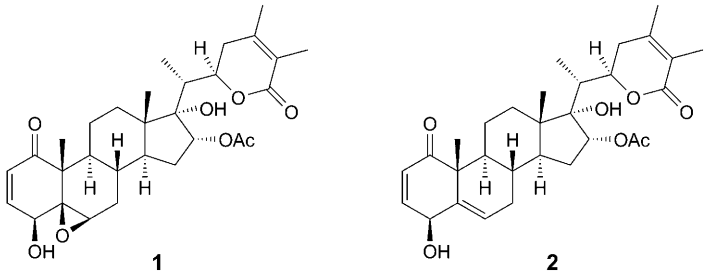

Fig. 1. *Withanolides* **1** *and* **2** *isolated from* Aureliana fasciculata *var.* fasciculata

Compound **1** was obtained as white crystals, and the molecular formula was deduced as $C_{30}H_{40}O_8$ from HR-EI-MS showing an M^+ ion at m/z 528.6323 and an intense fragment at m/z 125, which was indicative of a α,β-unsaturated dimethyl δ-lactone side chain, typical of many withanolides [25]. The IR spectrum exhibited three bands in the CO region at 1689, 1708, and 1740 cm^{-1} due to the presence of an α,β-unsaturated six-membered lactone, an α,β-unsaturated ketone, and an AcO group, respectively. The absorption at 3458 cm^{-1} indicated the presence of a OH group. The UV spectrum exhibited an absorption at 220 nm, attributable to the α,β-unsaturated CO chromophores. The ^{1}H-NMR spectrum (*Table 1*) displayed signals for two mutually coupled α,β-unsaturated olefinic H-atoms at δ(H) 6.20 (*d*, $J = 9.98$) and 6.95 (*dd*, $J = 9.98$ and 5.86) assignable to the H–C(2) and H–C(3) vicinal H-atoms, respectively. The double *doublet* at δ(H) 6.95, and the two *doublet*s at δ(H) 6.20 and 3.76 ($J = 5.86$, H–C(4)) in the ^{1}H-NMR spectrum suggested a 4β-hydroxy-2-en-1-one system in ring *A*. The coupling of the latter to H–C(3) and the absence of an allylic coupling between H–C(4) and H–C(2) indicated the β-configuration of the OH group, identical to that described for withaferin A and other withanolides [13][17][25][26] in which C(5) was quaternary. The β-orientation of OH at C(4) was confirmed by a cross-peak observed between H_α–C(4) and H–C(6) in the NOESY experiment, as previously described for the 18-acetoxywithanolide D [17]. A 5β,6β-epoxy moiety was evident in the ^{1}H-NMR spectrum of **1** from the broad signal at δ(H) 3.20 corresponding to H–C(6), in agreement with the signals at δ(C) 63.7 and 62.2 in

Table 1. *^{1}H-NMR Data of Withanolides* **1** *and* **2**. At 500 MHz, δ in ppm, J in Hz.

Position	**1**[a])	**1**[b])	**2**[c])	**2**[b])
2	6.20 (*d*, *J* = 9.98)	6.20 (*d*, *J* = 9.97)	5.84 (*d*, *J* = 9.98)	5.90 (*d*, *J* = 9.98)
3	6.95 (*dd*, *J* = 9.98, 5.86)	6.90 (*dd*, *J* = 9.97, 5.85)	6.84 (*dd*, *J* = 9.98, 4.58)	6.80 (*dd*, *J* = 9.98, 4.56)
4	3.76 (*d*, *J* = 5.86)	3.80 (*d*, *J* = 5.85)	4.48 (*d*, *J* = 4.58)	4.55 (*d*, *J* = 4.56)
6	3.20 (br. *s*)	3.20 (br. *s*)	5.52 (br. *d*, *J* = 5.55)	5.85 (br. *d*, *J* = 5.53)
7	2.08–2.12 (*m*), 1.34–1.36 (*m*)	2.05–2.09 (*m*), 1.28–1.34 (*m*)	2.02–2.06 (*m*), 1.40–1.42 (*m*)	2.05–2.09 (*m*), 1.45–1.47 (*m*)
8	1.49–1.57 (*m*)	1.49–1.54 (*m*)	1.50–1.60 (*m*)	1.61–1.70 (*m*)
9	1.06–1.08 (*m*)	1.06–1.12 (*m*)	1.04–1.08 (*m*)	1.10–1.16 (*m*)
11	2.15–2.19 (*m*), 1.47–1.49 (*m*)	1.90–1.96 (*m*), 1.44–1.46 (*m*)	2.12–2.18 (*m*), 1.50–1.60 (*m*)	2.15–2.20 (*m*), 1.55–1.57 (*m*)
12	1.93–1.99 (*m*), 1.67–1.73 (*m*)	1.74–1.76 (*m*), 1.63–1.67 (*m*)	1.90–1.94 (*m*), 1.72–1.82 (*m*)	1.90–2.00 (*m*), 1.61–1.70 (*m*)
14	1.75–1.82 (*m*)	1.78–1.82 (*m*)	1.72–1.82 (*m*)	1.80–1.84 (*m*)
15	1.75–1.82 (*m*), 1.49–1.57 (*m*)	1.90–1.96 (*m*), 1.49–1.54 (*m*)	1.72–1.82 (*m*), 1.45–1.47 (*m*)	1.90–2.00 (*m*), 1.50–1.52 (*m*)
16	5.10 (*dd*, *J* = 7.8, 2.4)	5.20 (*dd*, *J* = 7.7, 2.4)	5.00 (*dd*, *J* = 7.9, 2.4)	5.10 (*dd*, *J* = 7.8, 2.4)
18	0.85 (*s*)	0.85 (*s*)	0.83 (*s*)	0.93 (*s*)
19	1.40 (*s*)	1.41 (*s*)	1.34 (*s*)	1.44 (*s*)
20	2.22 (*dq*, *J* = 6.95, 3.80)	2.30 (*dq*, *J* = 6.89, 3.73)	2.14 (*dq*, *J* = 6.87, 3.76)	2.28 (*dq*, *J* = 6.90, 3.75)
21	1.07 (*d*, *J* = 6.95)	1.08 (*d*, *J* = 6.89)	0.97 (*d*, *J* = 6.87)	1.10 (*d*, *J* = 6.90)
22	4.25 (*dt*, *J* = 12.9, 3.80, 3.40)	4.30 (*dt*, *J* = 13.0, 3.73, 3.20)	4.20 (*dt*, *J* = 13.0, 3.76, 3.30)	4.35 (*dt*, *J* = 13.0, 3.75, 3.20)
23	2.56 (*dd*, *J* = 18.0, 12.9), 2.20 (*dd*, *J* = 18.0, 3.40)	2.50 (*dd*, *J* = 18.0, 13.0), 2.20 (*dd*, *J* = 18.0, 3.20)	2.47 (*dd*, *J* = 18.0, 13.0), 2.12 (*dd*, *J* = 18.0, 3.30)	2.42 (*dd*, *J* = 18.0, 13.0), 2.15 (*dd*, *J* = 18.0, 3.20)
27	1.86 (*s*)	1.86 (*s*)	1.84 (*s*)	1.83 (*s*)
28	1.91 (*s*)	1.91 (*s*)	1.90 (*s*)	1.98 (*s*)
MeCO	2.04 (*s*)	2.03 (*s*)	2.00 (*s*)	2.00 (*s*)

[a]) In $CDCl_3$. [b]) In CD_3OD. [c]) In (D_6)DMSO.

Table 2. *^{13}C-NMR Data of Withanolides* **1** *and* **2**. At 125 MHz, δ in ppm.

Position	**1**[a])	**1**[b])	**2**[c])	**2**[b])
1	202.0 (*s*)	201.9 (*s*)	203.5 (*s*)	202.3 (*s*)
2	132.1 (*d*)	132.3 (*d*)	128.8 (*d*)	131.4 (*d*)
3	141.9 (*d*)	143.9 (*d*)	146.3 (*d*)	146.4 (*d*)
4	69.7 (*d*)	71.2 (*d*)	67.6 (*d*)	70.1 (*d*)
5	63.7 (*s*)	65.0 (*s*)	138.6 (*s*)	140.0 (*s*)
6	62.2 (*d*)	63.2 (*d*)	127.3 (*d*)	129.5 (*d*)
7	30.9 (*t*)	31.5 (*t*)	30.5 (*t*)	32.3 (*t*)
8	29.5 (*d*)	30.0 (*d*)	32.1 (*d*)	33.8 (*d*)
9	43.4 (*d*)	44.0 (*d*)	42.4 (*d*)	44.3 (*d*)
10	47.4 (*s*)	48.7 (*s*)	47.9 (*s*)	50.6 (*s*)
11	21.3 (*t*)	22.0 (*t*)	22.1 (*t*)	23.4 (*t*)
12	31.8 (*t*)	32.5 (*t*)	31.9 (*t*)	34.0 (*t*)
13	48.3 (*s*)	49.0 (*s*)	48.7 (*s*)	49.5 (*s*)
14	48.3 (*d*)	49.5 (*d*)	47.8 (*d*)	50.0 (*d*)
15	33.4 (*t*)	34.5 (*t*)	33.2 (*t*)	34.8 (*t*)
16	78.7 (*d*)	79.8 (*d*)	77.9 (*d*)	79.8 (*d*)
17	83.1 (*s*)	83.9 (*s*)	82.6 (*s*)	84.8 (*s*)
18	14.7 (*q*)	14.9 (*q*)	15.0 (*q*)	15.9 (*q*)
19	17.3 (*q*)	17.8 (*q*)	22.0 (*q*)	23.2 (*q*)
20	42.2 (*d*)	43.5 (*d*)	41.6 (*d*)	44.8 (*d*)
21	9.3 (*q*)	9.5 (*q*)	9.0 (*q*)	9.8 (*q*)
22	77.9 (*d*)	79.5 (*d*)	78.0 (*d*)	79.7 (*d*)
23	33.0 (*t*)	34.4 (*t*)	32.2 (*t*)	34.0 (*t*)
24	149.1 (*s*)	152.5 (*s*)	151.0 (*s*)	153.4 (*s*)
25	121.7 (*s*)	123.3 (*s*)	120.2 (*s*)	122.3 (*s*)
26	166.3 (*s*)	167.9 (*s*)	166.0 (*s*)	169.3 (*s*)
27	12.3 (*q*)	12.0 (*q*)	12.2 (*q*)	12.5 (*q*)
28	20.3 (*q*)	20.6 (*q*)	20.2 (*q*)	20.6 (*q*)
AcO	168.9 (*s*), 21.0 (*q*)	170.3 (*s*), 21.3 (*q*)	169.6 (*s*),20.9 (*q*)	171.9 (*s*), 21.2 (*q*)

[a]) In $CDCl_3$. [b]) In CD_3OD. [c]) In $(D_6)DMSO$.

the ^{13}C-NMR spectrum (*Table 2*). Thus, the signals at δ(C) 69.7, 63.7, and 62.2 were assigned to C(4), C(5), and C(6), respectively, of a 4β-hydroxy-5β,6β-epoxywithanolide, further corroborated and supported by HMBCs from H–C(3) to C(5), and from H–C(4) to C(5) and C(6) (*Fig. 2*). In 5,6-epoxywithanolides, the β-orientation of the

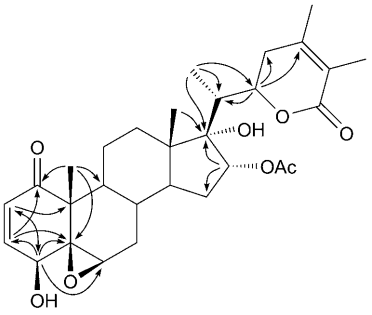

Fig. 2. *Key HMBC interactions in* **1**

epoxy group is favored almost exclusively, except in the presence of a 7α-OH directing group, which **1** does not contain [27].

Additionally, the coupling constants between H–C(6) and H–C(7) can be used to differentiate the *cis*- or *trans-A*/*B* ring junction. From literature reports, a *cis*-junction shows two similar coupling constant values for H–C(6) (0–2 Hz), while a *trans*-junction shows two different coupling constant values (0–2 and 4–6 Hz) [28]. The H–C(6) H-atom signal appeared as a broad *singlet*; therefore, the *A*/*B* ring conformation should be *cis*. On the other hand, the NOESY correlations observed between H–C(9) and H–C(2), Me(19) and H–C(8), H_β–C(11) and H–C(8), H_β–C(11) and Me(19), H_β–C(11) and Me(18), and Me(18) and H–C(20) of **1** confirmed the *trans*-conformation for *B*/*C* and *C*/*D* rings.

The ^{1}H-NMR spectrum of **1** showed characteristic signals for six Me groups at δ(H) 0.85, 1.07, 1.40, 1.86, 1.91, and 2.04 (*Table 1*), which were assigned to Me(18), Me(21), Me(19), Me(27), Me(28), and Me(Ac) groups, respectively, through the HMBC experimental data. The appearance of the Me(21) signal as a *doublet* at δ(H) 1.07 (J = 6.95), which showed a correlation with the resonance at δ(C) 42.19 (C(20)) in the HMBC spectrum (*Fig. 2*), suggested that the neighboring C(20) had one H-atom. This evidence was confirmed through the signals at δ(H) 2.22 (*dq*, J = 6.95, 3.70) and 4.25 (*dt,* J = 3.20, 3.70, 13.0) assigned to H–C(20) and H–C(22), respectively. The two vinylic Me signals at δ(H) 1.91 and 1.86 in the ^{1}H-NMR spectrum (*Table 1*), and the chemical shifts at δ(C) 149.1, 121.7, and 166.3, assigned to C(24), C(25), and C(26), respectively, in the ^{13}C-NMR spectrum (*Table 2*), indicated the presence of the dimethyl α,β-unsaturated lactone side chain of the withasteroids [1].

The HMBC data of **1** (*Fig. 2*) allowed placement of the tertiary OH group at C(17), once both H-atom signals at δ(H) 0.85 (H–C(18)) and 1.07 (H–C(21)) correlated with the O-bearing quaternary C-atom at δ(C) 83.1 in ^{13}C-NMR spectrum. As the O-bearing CH group H-atom (δ(H) 5.10) showed correlations with a CH_2 C-atom at δ(C) 33.4 (C(15)) and with O-bearing quaternary C-atom C(17) at δ(C) 83.1, the secondary AcO group must be located at C(16), adjacent to the OH group. The orientation of the AcO group at C(16) was established from the NOESY spectrum, which showed a strong interaction between Me(18) and H–C(16), characteristic of the 16α-substituted derivatives [29][30], and confirming that these groups (Me(18) and H–C(16)) were encountered on the β-face of the molecule, once the shielded position of Me(18) was typical for the normal β-side-chain configuration of 17-hydroxylated withanolides [27]. The ^{1}H- and ^{13}C-NMR data of ring *D* and the side chain of **1** (*Tables 1* and *2*) agree well with those reported for other 16α,17α-dioxygenated withanolides [29].

The configuration at C(22) was assigned as (*R*), since the α-oriented H-atom at C(22) is known to give rise to J(22,23) values of 0.5–4.0 and of 9.0–13.8 Hz, whereas the β-oriented H-atom at C(22) leads to values of 2.5–7.0 and 2.0–5.0 Hz [30]. The observed coupling constants for H–C(22) in compound **1** were J = 3.2 and 13.0 Hz, indicating an α-orientation, *i.e.*, (22*R*)-configuration. CD-Spectroscopic analysis was also used to establish the absolute configuration of the compound, because, according to literature [31], withanolides with (*R*)-configuration at C(22) show a positive *Cotton* effect at *ca.* 250 nm in the CD spectrum of the α,β-unsaturated δ-lactone. The observation of a characteristic positive *Cotton* effect at 255 nm in the CD spectra confirmed the (22*R*)-configuration in the compound.

The absolute configuration of C(4) in compound **1** was determined by preparing *Mosher* esters [32]. The OH groups at C(4) were converted to (+)-(*S*)- and (−)-(*R*)-α-methoxy-α-(trifluoromethyl)(phenyl)acetate (MTPA) derivatives. Distribution of the positive and negative δ values of the MTPA esters (*Table 3*) established the configuration at C(4) as (*S*), since negative values ($\Delta\delta_{S-R}$) were obtained for H−C(2) and H−C(3) [32]. Therefore, the structure of **1** was elucidated as (4*S*,22*R*)-16α-acetoxy-5β,6β-epoxy-4β,17α-dihydroxy-1-oxowitha-2,24-dienolide, and was given the trivial name aurelianolide A.

Table 3. *The* ^{1}*H-NMR Data of* Mosher*'s Esters of Compounds* **1** *and* **2** *in (*D_5*)Pyridine*

Position	Compound **1**			Compound **2**		
	(*S*)-MTPA	(*R*)-MTPA	$\Delta(\delta_S-\delta_R)$	(*S*)-MTPA	(*R*)-MTPA	$\Delta(\delta_S-\delta_R)$
2	6.431	6.518	−0.087	6.083	6.175	−0.092
3	7.179	7.234	−0.055	7.085	7.138	−0.053
4	5.417	5.453	−0.036	5.567	5.598	−0.031
6	3.665	3.572	+0.093	6.213	6.117	+0.096
19	1.498	1.409	+0.089	1.496	1.403	+0.093

Compound **2**, also identified as a withanolide, was obtained as an amorphous white solid. The molecular-ion peak at *m*/*z* 512.6335 is compatible with the molecular formula $C_{30}H_{40}O_7$, and an intense fragment-ion peak at *m*/*z* 125, resulting from the cleavage of the C(20)−C(22) bond, suggested the presence of a six-membered lactone at C(20). The IR spectrum revealed the presence of OH group at 3467cm^{-1} and included bands at 1692, 1706, and 1743 cm^{-1}, typical for a six-membered cyclic ketone, an α,β-unsaturated δ-lactone, and an AcO group, respectively. The UV spectrum showed an absorption at 219 nm, attributable to the α,β-unsaturated CO chromophores. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Tables 1* and *2*) were very similar to those of **1**, and the major difference in the ^{1}H-NMR spectrum of **2** as compared to that of **1** was the presence of typical signals of a 2,5-dien-1-one system with a substituent at C(4) [33]. The signals for two α,β-unsaturated olefinic H-atoms at δ(H) 5.84 (*d*, *J* = 9.98), 6.84 (*dd*, *J* = 9.98, 4.58) were assignable to the H−C(2) and H−C(3) vicinal H-atoms, respectively. A 4β-OH group was inferred from the presence of a *doublet* at δ(H) 4.48 (*J* = 4.58) corresponding to H−C(4). The coupling of the latter to H−C(3) confirmed the β-orientation of this substituent [25]. The ^{1}H-NMR spectrum of **2** closely resembled that of 5,6-deoxywithaferin A, and other analogue withanolides revealing the same substitution pattern in rings *A* and *B* [7][17][25]. Thus, an unsaturated deoxy structure in **2** was confirmed by the ^{1}H-NMR spectrum with the broad *doublet* at δ(H) 5.52 (*J* = 5.55) corresponding to H−C(6), in agreement with the signals at δ(C) 138.6 and 127.3 in the ^{13}C-NMR spectrum. The signals at δ(C) 203.5, 128.8, 146.3, 67.6, 138.6, and 127.3 were assigned to C(1)−C(6), respectively, of a 4β-hydroxy-2,5-dien-1-one withanolide (*Table 2*), supported by HMBCs from H−C(2) to C(4) and C(10); from H−C(3) to C(1) and C(5); and from H−C(4) to C(2), C(3), C(5), C(6), and C(10) (*Fig. 3*). The other ^{1}H- and ^{13}C-NMR assignments of **2** are in agreement with the proposed structure and in resemblance with compound **1** described above. The configuration at C(22) was assigned as (*R*) through the ^{1}H-NMR data, since the observed coupling constants for

H–C(22) in compound **2** were $J = 3.3$ and 13.0 Hz, indicating an α-orientation. The (22*R*)-configuration was confirmed by the CD spectroscopic analysis, with the compound **2** showing a positive *Cotton* effect at 254 nm. The absolute configuration at C(4) of compound **2** was determined as (*S*) through the same procedure described for the compound **1** (*Table 2*). Therefore, the structure of **2** was elucidated as (4*S*,22*R*)-16α-acetoxy-4β,17α-dihydroxy-1-oxowitha-2,5,24-trienolide and was given the trivial name aurelianolide B.

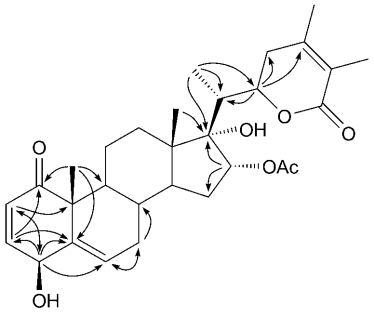

Fig. 3. *Key HMBC interactions in* **2**

Conclusions. – *Aureliana* is a genus pertaining to Solanaceae tribe grouped in the subfamily Solanoideae, characterized by the production of the withasteroids in the Solanaceae family. This genus is represented by seven species that grow in South America [34], and it has been related to *Athenaea*, *Capsicum*, *Witheringia*, and *Vassobia* species, and, considering the different opinions concerning the systematic position of this plant at the trivial level, identification of the withasteroids may be important from a chemotaxonomic point of view. Both withanolides isolated from *A. fasciculata* var. *fasciculata* share the pattern of substitution of the *A* and *B* rings as found in compounds previously isolated from the various genera [1][2]. Moreover, a number of withanolides substituted at C(16) have been isolated recently from Solanaceae plants, such as 16α-substituted derivatives isolated from *Acnistus arborescens* [30], *Discopodium penninervum* [29], *Dunalia brachyacantha* [26], *Exodeconus maritimus* [29], *Hyoscyamus niger* [35], *Iochroma coccineum* [26], *Iochroma gesnerioides* [26], *Salpichroa origanifolia* [35], and *Tubocapsicum anomalum* [32], and 16β-substituted derivatives isolated from *Deprea subtriflora*, *Physalis angulata*, *Physalis philadelphica*, and *Withania somnifera* [36]. However, the aurelianolides A and B (**1** and **2**, resp.) are rare examples of the 16α,17α-dioxygenated derivatives which are encountered, up to now, only in the *Discopodium penninervum* [29], *Exodeconus maritimus* [29], and *Tubocapsicum anomalum* [32].

In this first phytochemical study of *Aureliana* genus, the discovery of withanolides is, at the moment, only suggestive of its chemical relationship to allied genera and in agreement with the close taxonomic affinity exhibited by the genera of the Solanaceae tribe. Further chemical studies of additional species of this group of the Solanaceae, which have been difficult to classify on purely morphological data, can help to determine the correct taxonomic position of the genus, once the proposed pathway for the biogenesis of withanolides [1], such as **1** and **2**, suggests compounds with the 2,5-

dien-1-one system as the key intermediates, which lead to different functionalizations of the *A*- and *B*-rings.

The authors are grateful to the financial support from *CNPq*, *FAPERJ*, and *FAPESP*. We also thank *CNPq* scientific research award to *M. J. P. F.*, *M. A. C. K.*, and *V. P. E.*

Experimental Part

General. Prep. TLC: silica gel *60 F_{254}* (*Merck*); the spots and bands were detected by spraying with 2% ceric sulfate in conc. H_2SO_4 and sulfuric anisaldehyde. HPLC: *Shimadzu class-LC10*; with two pumps *LC10AT*, UV detector *SPD-M10A*, *Supelcosil LC18* column (250 mm × 4.6 mm × 5 μm) and MeCN/H_2O + TFA 0.05% as mobile phase, initiating with MeCN 5% and eluting in increasing polarities up to 90% MeCN. M.p.: *MQAPF-301* equipment. UV Spectra: *Shimadzu UV-1601*; λ_{max} in nm (log ε). CD Spectra: in MeOH, *JASCO ORD/UV-6* spectropolarimeter. IR Spectra: *Perkin-Elmer 599B*; in cm^{-1}. ^{1}H- and ^{13}C-NMR: *Bruker-Avance DRX 500* spectrometer operating at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR in CD_3OD for both compounds, and in $CDCl_3$ and DMSO for compounds **1** and **2**, resp., with TMS as internal standard. HR-MS: *Micromass* spectrometer, *VG-Autospec* model.

Plant Material. Leaves of *Aureliana fasciculata* (VELL.) SENDTNER var. *fasciculata* were collected in Juiz de Fora, Minas Gerais State, Brazil, in November 2001. The plant was identified by *R. C. A.-L., Federal University of Rio de Janeiro. A voucher sample (RFA: 28261) was deposited with the Herbarium of Rio de Janeiro Federal University, Rio de Janeiro, Brazil.*

Extraction and Isolation. The leaves of *A. fasciculata* var. *fasciculata* (420 g) were initially extracted with hexane for remove the apolar material and, subsequently, with MeOH. The cold MeOH extract (43.3 g) was partitioned with hexane, CH_2Cl_2, AcOEt, and BuOH. The CH_2Cl_2-soluble part (10 g) was subjected to flash liquid chromatography (SiO_2; binary mixtures of hexane, AcOEt, and MeOH of increasing polarities). The fractions eluted with AcOEt and AcOEt/MeOH 9 : 1 were purified through a *Sephadex LH-20* column to yield compounds **1** (25 mg) and **2** (35 mg), resp. The sterols stigmasterol and sitosterol were isolated from the CH_2Cl_2 fraction as a mixture of 5 mg.

Aurelianolide A (= *(4*S*,22*R*)-16α-Acetoxy-5β,6β-epoxy-4β,17α-dihydroxy-1-oxowitha-2,24-dienolide* = *(4β,5β,6β,16α,22*R*)-4,17-Dihydroxy-1,26-dioxo-5,6 : 22,26-diepoxyergosta-2,24-dien-16-yl Acetate*; **1**). White crystals. M.p.: 185°. HPLC: t_R 34.496 min. UV (MeOH): 220 (2.36). CD: +10230 (255). IR (KBr): 3458, 2955, 1740, 1708, 1689, 1452, 1383, 1340, 1238. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 528 (10, M^+), 510 (5, $[M-H_2O]^+$), 492 (5, $[M-2\,H_2O]^+$), 403 (25, $[M-125]^+$), 343 (36, $[M-125-AcOH]^+$), 125 (100). HR-EI-MS: 528.6323 (M^+, $C_{30}H_{40}O_8^+$; calc. 528.6338).

Aurelianolide B (= *(4*S*,22*R*)-16α-Acetoxy-4β,17α-dihydroxy-1-oxowitha-2,5,24-trienolide* = *(4β,16α,22*R*)-4,17-Dihydroxy-1,26-dioxo-22,26-epoxyergosta-2,5,24-trien-16-yl acetate*; **2**). Amorphous white solid. M.p. 230°. HPLC: t_R 39.072 min. UV (MeOH): 219 (2.25). CD: +10270 (253). IR (KBr): 3467, 2954, 1743, 1706, 1692, 1453, 1388, 1237, 1127. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 512 (12, M^+), 494 (8, $[M-H_2O]^+$), 476 (8, $[M-2\,H_2O]^+$), 387 (30, $[M-125]^+$), 327 (45, $[M-125-AcOH]^+$), 125 (100). HR-EI-MS: 512.6335 (M^+, $C_{30}H_{40}O_7^+$; calc. 512.6344).

*Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of Compounds* **1** *and* **2**. Two portions (each 2.0 mg) of compound **1** were treated with (+)-(*S*)- and (−)-(*R*)-α-methoxy-α-(trifluoromethyl)(phenyl)-acetyl chloride (10 μl) in anh. pyridine (1.0 ml) at r.t. for 8 h, to afford the (*R*)- and (*S*)-MTPA ester derivatives. The *Mosher* esters of **2** were prepared by the same procedure.

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Received February 28, 2010