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CARDENOLIDES FROM *NIEREMBERGIA ARISTATA*[1]

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ABSTRACT.—The EtOAc extract of the whole plant of the Argentinian species *Nierembergia aristata* showed significant cytotoxicity against eleven different cancer cell lines. In addition to several known compounds, bioassay-guided fractionation led to the isolation of three new cardenolides, 17-*epi*-11α-hydroxy-6,7-dehydrostrophanthidin-3-*O*-β-boivinopyranoside [**1**], 6,7-dehydrostrophanthidin-3-*O*-β-boivinopyranoside [**2**], and 6,7-dehydrostrophanthidin-3-*O*-β-oleandropyranoside [**3**], of which the latter demonstrated activity against all the cell lines tested. To our knowledge, this is the first report of the isolation of cardiac glycosides from a species in the Solanaceae.

Nierembergia, a genus in the family Solanaceae, comprises 21 species (A.T. Hunziker, personal communication, September, 1994), and has been subjected to only limited phytochemical investigation. The most highly studied species is *N. hippomanica*, which is reported as being toxic to livestock (2). Investigations reported on this species have included the isolation of flavonoids (3–7), alkaloids (8), the toxic compound pyrrole-3-carbamidine (2), an evaluation of seed oils (9), and the effect of exogenous flavonoids on peroxidases (10). The calcinogenic potential of an aqueous extract of *N. veitchii* was studied, but no activity was observed (11). Evaluation of the seed oil was also conducted with an Argentinian collection of *N. aristata* (9). However, some confusion has existed regarding the nomenclature of *Nierembergia* species. As described by Cocucci and Hunziker (1), the name *Nierembergia aristata* was misapplied to the species *N. rigida* Miers. Thus, studies previously reported as being conducted with *N. aristata* may have actually been conducted on *N. rigida*.[2]

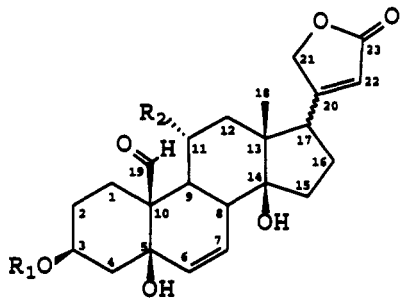
As part of a continuing program aimed at the discovery of novel anticancer agents, it was found that the EtOAc extract of the whole plant of *Nierembergia aristata* D. Don displayed potent cytotoxic activity (Table 1). In this work, we report the bioassay-guided fractionation of this extract using a panel of cancer cell lines. From the active fractions we have isolated scopoletin, ursolic acid, lariciresinol and 5′-methoxylariciresinol (12), and three new cardenolides, **1–3**. These three cardiac glycosides demonstrated activity against all cell lines tested (Table 1). Structure elucidation of the new cardiac glycosides was performed by a series of ir, fabms, ^{1}H-nmr, ^{13}C-nmr, DQF-COSY, HOHAHA, ROESY, HETCOR, and selective INEPT nmr experiments (13–15).

RESULTS AND DISCUSSION

Compound **1** gave a positive Kedde (16) test for cardenolides. Its positive-ion hrfabms revealed a molecular formula of $C_{29}H_{40}O_{10}$, with the molecular ion $M^+ + 1$ at *m/z*

[1]Some of the results presented herein were given at the 34th Annual Meeting of the American Society of Pharmacognosy, San Diego, CA, July 18–22, 1993. At that time, the subject plant was referred to as *Nierembergia stricta* Miers. However, subsequent botanical work by Cocucci and Hunziker (1) corrected the name of this and another species of the genus *Nierembergia*. Accordingly, *N. rigida* Miers is the correct name for the plant previously referred to as *N. aristata*. Prior to clarification, *N. aristata* D. Don received four additional names, with *N. stricta* being one of them.

[2]The voucher specimen of the plant material studied by Maestri and Guzmán (9) was revised by Dr. A.A. Cocucci, who confirmed the identity of the plant as *Nierembergia rigida* Miers.



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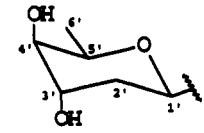

B=

1 R_1=A; R_2=OH; H-17β
2 R_1=A; R_2=H; H-17α
3 R_1=B; R_2=H; H-17α

TABLE 1. Evaluation of the Cytotoxic Activity of the EtOAc-Soluble Extract of *Nierembergia aristata* and Compounds **1–3**.[a]

Human Cancer Cell Line	*N. aristata* EtOAc-soluble extract	Compounds		
		1	**2**	**3**
BC1	6.3	0.3	0.04	0.3
HT	3.0	2.3	0.05	0.4
LU1	11.0	0.09	0.03	0.1
Mel2	>20	4.1	0.05	0.8
Col2	4.4	0.5	0.07	0.9
KB	3.5	0.3	0.04	0.3
KB-V(+VLB)	2.8	0.6	0.05	0.3
KB-V(−VLB)	2.0	1.6	0.06	0.5
A-431	10.2	0.5	0.09	0.7
LNCaP	7.1	0.3	0.10	0.5
ZR-75-1	4.7	0.4	0.08	0.2
U373	3.3	0.4	0.09	0.8

[a]ED_{50} values given in μg/ml; BC1=human breast cancer; HT=human fibrosarcoma; LU1=human lung cancer; Mel2=human melanoma; Col2=human colon cancer; KB=human oral epidermoid; KB-V(+VLB)=drug resistant KB+vinblastine (1 μg/ml); KB-V(−VLB)=drug-resistant KB (no vinblastine); A-431=human epidermoid carcinoma; LNCaP=human prostate cancer; ZR-75-1=hormone-dependent human breast cancer; U373=human glioma.

549, and a fragment at *m/z* 419 as a product of the loss of 130 mass units. The fragment lost represents a molecular formula of $C_6H_{10}O_3$, indicative of the presence of a dideoxyhexose, with a steroid nucleus $C_{23}H_{30}O_7$, possessing eight degrees of unsaturation also being evident.

In the ir spectrum, bands for hydroxyl groups at 3440 cm^{-1}, and two carbonyl peaks at 1734 cm^{-1} (butenolide) and 1717 cm^{-1} were apparent, and the uv spectrum showed an absorption maximum at 215 nm, characteristic of a carbonyl group conjugated with a double bond.

The ^{1}H-nmr spectrum (Table 2) presented the typical profile of a steroidal glycoside. A broad, one-proton singlet at 6.12 ppm coupled with the two-proton doublet (J=2 Hz) at 5.12 ppm, is indicative of the butenolide ring and these signals correspond to H-22 and H_2-21, respectively. This was confirmed by signals in the ^{13}C-nmr spectrum (Table 3) at 174.31 ppm (C-20), 74.02 ppm (C-21), 117.01 ppm (C-22), and 173.53 ppm (C-

TABLE 2. ^{1}H-Nmr Data of Compounds **1–3**.[a]

Proton	Compound		
	1	**2**	**3**
1α (e)	2.84 (br dt, 14, 3)	2.09 obsc.	2.05 obsc.
1β (a)	2.61 (td, 14, 3)	2.47 (td, 15, 2.5)	2.47 (td, 15, 3)
2α (a)	2.51 (tt, 14, 13)	1.64 (br tt, 14, ~2.5)	1.65 (br tt, 14, ~2.5)
2β (e)	2.37 (br d quint, 14, 3)	2.23 (br d, 14.5)	2.17 (br d, 14)
3	4.42 (br quint, ~3)	4.34 (br quint, ~3)	4.33 (br quint, ~3)
4α (a)	2.07 (dd, 15, 3)	1.96 obsc.	2.00 obsc.
4β (e)	2.30 (br dt, 15, 3)	2.23 (br d, 14.5)	2.35 (br d, 14)
6	6.14 (dd, 10.5, 3)	6.05 (dd, 10.5, 2.5)	6.14 (dd, 10, 3)
7	6.37 (dd, 10.5, 2)	6.37 (br d, 10.5)	6.40 (d, 10)
8	2.76 (br dt, 12.5, ~2)	2.61 (br d, 12)	2.63 (br d, 12)
9	2.50 (dd, 12.5, 8)	2.15 (br t, 12)	2.16 (br t, 12)
11α (e)	—	1.62 (ddd, 13, 3, 2)	1.62 obsc.
11β (a)	4.55 obsc.	1.44 (br q, 13)	1.44 (br q, 13)
12α (a)	1.96 obsc.	1.35 (td, 13, 3)	1.35 (td, 13, 3)
12β (e)	1.82 (dd, 14.5, 4.5)	1.49 (br d, 13)	1.49 (br d, 13)
15a	2.19 obsc.	1.98 obsc.	1.99 obsc.
15b	1.94 obsc.	1.82 (br t, 9.5)	1.85 (br t, 10)
16a	2.02 obsc.	2.08 obsc.	2.10 obsc.
16b	1.80 obsc.		
17	3.46 (dd, 9.5, 6.5)	2.77 (dd, 8, 6)	2.77 (dd, 8, 6)
18	1.02 (s)	0.96 (s)	0.96 (s)
19	10.46 (s)	10.37 (s)	10.39 (s)
21a	5.12 (d, 2)	5.25 (dd, 18, 1.5)	5.25 (dd, 18, 1.5)
21b		5.02 (dd, 18, 1.5)	5.02 (dd, 18, 1.5)
22	6.12 (br s)	6.15 (br s)	6.12 (br s)
1′	5.45 (dd, 10, 3)	5.43 (dd, 10, 3)	4.80 (dd, 9.5, 2)
2′α (a)	2.41 (ddd, 13.5, 10, 3)	2.39 (ddd, 13.5, 10, 3)	1.59 obsc.
2′β (e)	2.12 (dt, 13.5, 3)	2.11 obsc.	2.38 (br d, obsc., ~13)
3′	4.57 (br q, 3)	4.57 (br q, 3)	3.36 (td, 9, 5)
4′	3.78 (br dd, 3, 1)	3.79 (br dd, 3, 1)	3.44 (t, 9)
5′	4.46 (qd, 6.5, 1)	4.46 (qd, 6.5, 1)	3.51 (dq, 9, 6)
6′	1.46 (d, 6.5)	1.51 (d, 6.5)	1.52 (d, 6)
3′-OMe	—	—	3.43 (s)

[a]In pyridine-d_5 at 500 MHz; chemical shifts are given in ppm using TMS as internal reference; signal multiplicity and coupling constants (Hz) are in parentheses; (a)=axial, (b)=equatorial; obsc. indicates overlapping resonances.

23). The presence of only one three-proton singlet at 1.02 ppm and a proton resonance at 10.46 ppm suggested that one of the angular methyl groups of the steroid nucleus was oxidized to an aldehyde.

Apart from the signals corresponding to the butenolide ring, the ^{13}C-nmr spectrum presented 25 more signals, constituted by a carbonyl doublet at 209.11 ppm corresponding to the aldehyde group; two doublets at 136.08 and 127.58 ppm indicating the presence of a non-terminal, disubstituted double bond; an anomeric carbon doublet at 98.68 ppm; and seven carbinol signals (two singlets and five doublets) between 65 and 85 ppm. The two singlets at 73.91 and 83.19 ppm suggested hydroxylation at C-5 and C-14, respectively. The higher field aliphatic region was composed of two quartets, seven triplets, three doublets, and two singlets. The complete assignments of the ^{1}H- and ^{13}C-nmr spectra of **1** were achieved using a combination of DQF-COSY, HETCOR, ROESY, HOHAHA, and selective INEPT experiments.

The following strategy was used to position the disubstituted, non-terminal double

TABLE 3. ^{13}C-Nmr Data of Compounds **1–3**.[a]

Carbon	Compounds		
	1	**2**	**3**
1	19.72	18.03	17.95
2	27.02	25.88	25.79
3	74.87	74.36	74.12
4	39.60	39.83	39.76
5	73.91	73.42	73.36
6	136.08	135.95	135.97
7	127.58	127.09	127.08
8	44.99	44.41	44.40
9	43.36	39.46	39.43
10	54.69	53.96	53.90
11	66.59	22.36	22.33
12	45.28	39.77	39.76
13	48.96	50.23	50.24
14	83.19	83.58	83.57
15	34.56	32.51	32.52
16	26.96	27.66	27.66
17	49.01	50.96	50.96
18	19.45	16.63	16.63
19	209.11	208.13	208.37
20	174.31	175.29	175.29
21	74.02	73.68	73.68
22	117.01	117.82	117.84
23	173.53	174.38	174.34
1′	98.68	98.78	99.04
2′	35.13	35.10	36.97
3′	70.06	70.06	81.37
4′	71.32	71.35	75.94
5′	70.16	70.16	73.00
6′	17.47	17.52	18.67
3′-OMe	—	—	56.80

[a]In pyridine-d_5 at 90.8 MHz; chemical shifts given in ppm using TMS as internal reference.

bond in the aglycone. On irradiation of the three-proton singlet at 1.02 ppm in a differential nOe experiment, the signals corresponding to H-21 and H-22 were enhanced, indicating that this singlet corresponds to H_3-18 and that C-19 was the carbon oxidized to an aldehyde. In the selective INEPT experiment (Table 3), irradiation of H_3-18 produced enhancement in the carbon signals at 45.28 (t, C-12), 48.96 (s, C-13), 49.01 (d, C-17), and 83.19 ppm (s, C-14). The fact that enhancement was observed in the carbon signal at 45.28 ppm (CH_2, C-12), and not in a signal corresponding to a sp^2 carbon, ruled out the possibility of a double bond at C-11, C-12. The signal corresponding to C-17 showed a cross-correlation peak with the proton signal at 3.46 ppm (dd, 9.5 and 6.5 Hz, H-17) in the HETCOR spectrum. Following the connections in the DQF-COSY spectrum, the signal of H-17 led to assignment of the signals corresponding to H-16a, H-16b, H-15a, and H-15b at 2.02, 1.80, 2.19, and 1.94 ppm, respectively. We have also observed a relay cross-correlation peak between H-17 and H-15a,b in the HOHAHA experiment. This evidence eliminated the possibility of a double bond at C-15, C-16.

The following series of selective INEPT experiments (Table 3) led to the confirmation of the unusual position of the double bond at C-6, C-7 in the molecule of **1**. Irradiation of H-6 produced enhancement of the signal of C-10 (54.69 ppm); this signal

was also enhanced when H-19 (10.46 ppm) was irradiated. Due to the proximal chemical shifts of H-6 and H-22, irradiation of H-6 partially affected the signal of H-22, resulting in the enhancement of C-20 (174.31 ppm) and C-23 (173.53 ppm). Irradiation of H-8 enhanced the signals of C-14 (83.19 ppm) and C-7 (127.58 ppm), and the irradiation of H-7 enhanced the signals of C-14 (83.19 ppm), C-5 (73.91 ppm), and C-9 (43.36 ppm), indicating without ambiguity that the disubstituted double bond is located at C-6, C-7. Although neither enhancement of C-1 on irradiation of H-19, nor enhancement of C-4 on irradiation of H-6 was observed, irradiation of the signal corresponding to H-3 (4.42 ppm) enhanced the carbon signal of C-5 (73.91 ppm), showing a common enhancement with the irradiation of H-7.

The ^{13}C-nmr signal at 98.68 ppm corresponding to the anomeric carbon C-1′ showed a cross-correlation peak with the proton signal at 5.45 ppm (dd, J=10 and 3 Hz, H-1′) in the HETCOR spectrum of **1**. This multiplicity not only indicated that the anomeric proton is in a β-configuration, but also that H-2′ is a methylene group, suggesting the lack of an OH group at C-2. The DQF-COSY spectrum led to the assignment of H-2′, H-3′, H-4′, and H-5′, and also showed a cross-correlation peak between H-5′ and the doublet (J=6.5 Hz) corresponding to a CH_3 group at 1.46 ppm (H-6′). This indicated the lack of a hydroxy group at C-6′. From the vicinal coupling constants (Table 2) involving the sugar protons and the assistance of the ROESY spectrum, the sugar portion was identified as 2,6-dideoxy-β-xylo-hexose, commonly known as β-boivinose. Irradiation of the anomeric proton in the selective INEPT experiment produced enhancement of the signal corresponding to C-3 (74.87 ppm) confirming the position of attachment of the sugar moiety. Enhancement was also observed in the signal of C-2′ at 35.13 ppm.

The two remaining carbinol signals indicated a high degree of hydroxylation in the cardenolide skeleton of **1**. The observed doublet at 74.87 ppm was assigned to C-3, because this signal showed a cross-correlation peak in the HETCOR spectrum with the proton signal at 4.42 ppm (br quint, J=3 Hz), and the C-3 signal also showed a nOe cross-correlation peak in the ROESY spectrum with the signal corresponding to the anomeric proton. The signal at 66.59 ppm was assigned to C-11, with the OH group attached in an α-orientation, and correlated with the signal of H-11β at 4.55 ppm in the HETCOR spectrum. This assignment was made on the basis of the following evidence from the DQF-COSY experiment: (a) starting from the signal corresponding to H-3, there was no correlation with the signal at 4.55 ppm; (b) with the double bond located at C-6, C-7, and following the cross-correlation peaks from the signals of H-6 and H-7, correlations were observed of H-6 with H-8, H-7 with H-8, H-8 with H-9, and finally H-9 with the signal at 4.55 ppm. A value of 8 Hz for the vicinal coupling constant between H-9 and H-11 supported their trans-diaxial relationship, and therefore the α configuration of the OH group at C-11; and (c) in the selective INEPT experiment, irradiation of the signal of H-11 (although overlapped with H-3′) enhanced, through a three-bond connection, the signals of C-10 (54.69 ppm) and C-13 (48.96 ppm).

Almost all the known cardenolides are characterized by a cis-junction of rings C and D and the presence of a 14β-hydroxy group. The stereochemistry at C-5 must be determined, however, but is frequently β (17,18). If hydroxylation occurs at this position, it is also β in almost all cases. As mentioned earlier, in the case of compound **1**, hydroxylation at C-5 and C-14 was confirmed by the selective INEPT experiments on irradiation of the proton signals of H_3-18 and H-3 (Table 4). The coupling pattern involving H-1, H-2, H-3, and H-4 (Table 2) indicated that H-3 adopts an equatorial orientation giving rise to two possible configurations of the OH groups at C-3 and C-5, as follows: 3β,5β or 3α,5α. In our case, the chemical shifts of the carbons belonging

to ring A agreed very well with those reported for the strophanthidin skeleton (3β,5β-diOH) (19). With the help of the DQF-COSY and HETCOR experiments the signal of C-1 was located at 19.72 ppm. Thus far, in publications regarding the ^{13}C-nmr assignment of strophanthidin derivatives (18,20,21), this carbon was assigned at ca. 25 ppm and the signal at ca. 19 ppm was assigned to either C-7 or C-11. Our results confirm the speculations made by Pauli *et al.* (19), who reported that the assignments of the signals at 25.91 and 18.94 ppm to C-7 and C-1, respectively, are more consistent with glycosidation effects calculated for several strophanthidin glycosides. In our case the chemical shifts of the carbon atoms belonging to ring A are not as close as expected due to the presence of the double bond at C-6,C-7.

TABLE 4. Selective INEPT Experiments on Compound **1**.[a]

Proton(s) irradiated	δ (ppm)	Carbon(s) enhanced
3	4.42	73.91 (C-5)
6 (and 22)	6.14	54.69 (C-10), 173.53 (C-23), 174.31 (C-20)
7	6.37	43.36 (C-9), 73.91 (C-5), 83.19 (C-14)
8	2.76	83.19 (C-14), 127.58 (C-7)
11 (and 3′)	4.57	54.69 (C-10), 48.96 (C-13), 99.01 (C-1′), 71.32 (C-4′)
17	3.46	48.96 (C-13)
18	1.02	45.28 (C-12), 48.96 (C-13), 49.01 (C-17), 83.19 (C-14)
19	10.46	54.69 (C-10)
21	5.12	117.01 (C-22), 173.53 (C-23)
1′	5.45	35.13 (C-2′), 74.87 (C-3)
4′	3.78	35.13 (C-2′), 70.06 (C-3′)
6′	1.46	70.16 (C-5′), 71.32 (C-4′)

[a]Recorded in $CDCl_3$ at 90.8 MHz.

The literature is replete with nmr data for 17β-cardenolides because this is the predominant configuration. It has been observed that the inversion of the configuration of C-17 from a 17β- to 17α-cardenolide produces a change of ca. −8 ppm in the ^{13}C-nmr chemical shift of C-12 (22–24), which normally falls at ca. 40 ppm in the 17β-epimer when there is no substitution at C-11. The 17α-epimers are not abundant, hence nmr data for these isomers are scarce. However, it appears that the ^{1}H-nmr chemical shift of H-17 is also indicative of the stereochemistry at C-17, when no substitution is present at C-12, C-15, and C-16. In the case of 17β-cardenolides, the chemical shift of H-17α falls at ca. 2.8 ppm, and in the case of the 17α-isomer the chemical shift of H-17β falls at ca. 3.40 ppm (24,25). The downfield shift observed in the signal of H-17 when the lactone moiety changed its orientation from β to α, is due to the 1,3-pseudodiaxial interaction of H-17β with the 14β-OH group. In compound **1**, H-17 appeared at 3.46 ppm, indicating its β-configuration. Hence, **1** is a 17α-cardenolide. No nOe effect was observed between H-17 and H_3-18 in either ROESY or differential nOe nmr experiments. However, the lactone ring protons H_2-21 and H-22 showed a nOe effect with H_3-18 and H-17. In addition, no nOe effect was observed between H-17 and H_2-12. As mentioned earlier, the normal chemical shift for C-12 in the 17β-cardenolides is ca. 40 ppm. Inversion of the configuration at C-17 lowers this chemical shift to ca. 32 ppm. The effect of a 11α-OH at C-11 is +10.9 ppm (18) giving rise to a chemical shift of ca. 43 ppm for C-12. In the ^{13}C-nmr spectrum of **1** the signal of C-12 was found at 45.28 ppm, in good agreement considering the conformational changes that the double bond at C-6,C-7 may produce to the ring system. This value is much closer to the value reported

for the 17α-sarmentogenin derivatives (42.6 ppm) than to those reported for their 17β-isomers (50.4 ppm) (24).

Compound **2** revealed a molecular formula of $C_{29}H_{40}O_9$ by positive-ion hrfabms, representing one oxygen atom less than structure **1**. Similar to compound **1**, the positive fabms also showed the loss of 130 mass units from the $M^+ + 1$ ion at *m/z* 533 to give an ion at *m/z* 403, indicating the presence of a dideoxyhexose sugar. Its uv and ir spectra were practically identical to those shown by **1**.

The ^{1}H-nmr (Table 2) and ^{13}C-nmr (Table 3) spectra of **2** revealed the presence of the same sugar (boivinose) as compound **1** attached to the aglycone. The most important spectroscopic differences between **1** and **2** were: (a) the absence of the H-11 and C-11 signals of **1** in the nmr spectra of **2**, and the appearance of one more CH_2 signal at 22.36 ppm in the ^{13}C-nmr (DEPT) spectrum; and (b) in the ^{1}H-nmr spectrum of **2**, the signal corresponding to H-17 was shifted upfield compared to compound **1**, appearing at 2.77 ppm (dd, J=8 and 6 Hz). The first difference, together with the absence of one oxygen atom observed in the molecular formula, suggested the absence of the OH group at C-11. The second piece of evidence indicates that H-17 is in an α-configuration. Moreover, the signal of C-12 was observed at 39.77 ppm indicating the β-configuration of the lactone chain, and the signal of H-17α showed nOe cross-correlation peaks with the signals of H-12a,b in the ROESY experiment. The same experiments used for compound **1**, except for the selective INEPT technique, were used for the complete nmr assignments of compound **2**. The 6,7-dihydro derivative of **2** is known as corchoroside-A (=strophanthidin-3-*O*-β-D-boivinoside) (26). Its reported ^{13}C-nmr data (21) agree very well with those obtained for **2**, except for the resonances in the environment of the double bond, such as C-5, C-6, C-7, C-8 and C-9. As in compound **1**, we observed that the CH_2 signal at 18.02 ppm in **2** belongs to C-1, and therefore the resonance at 22.36 ppm was assigned to C-11.

The third new cardenolide, **3**, showed a molecular formula of $C_{30}H_{42}O_9$ by positive-ion hrfabms. Its uv and ir spectra were identical with those of compound **2**. The carbon signals of the aglycone moiety of compound **3** were nearly superimposable with those of compound **2**. Differences were observed only in the signals belonging to the sugar portion, which also showed the characteristic signals of a 2,6-dideoxyhexose in the ^{1}H-nmr spectrum, but with the presence of a OMe group at C-3′. From the vicinal coupling constants (Table 2) involving the sugar protons, the nOe effects observed in the ROESY spectrum and the carbon chemical shifts, the sugar moiety was identified as 2,6-dideoxy-3-*O*-methyl-β-arabino-hexose, commonly known at β-oleandrose. The ^{13}C-nmr resonances of this sugar in compound **3** match very well with those reported for the sugar portion of glaucogenin-B-3-*O*-β-D-oleandropyranoside (27).

We did not determine the series of the sugar moieties, but we assume that the sugar attached to compounds **1** and **2** is D-boivinose, considering that the L-isomer does not occur as a natural product. In the case of oleandrose, we have observed that the D-isomer normally occurs linked in the β-form and the L-isomer in the α-form. But we do not have conclusive evidence to prove that in the case of compound **3** the sugar belongs to the D-series.

Compounds **1–3** displayed potent, general cytotoxic effects against eleven human cancer cell lines. The activities observed for the isolates were substantially greater than those observed for the crude extract. The data indicate that an 11-hydroxy group detracts from optimum cytotoxic activity (**1** vs. **2**), and that minor modification to the sugar moiety may have a profound effect on the cytotoxicity (**2** vs. **3**).

At the outset of this project we did not expect the isolation of cardiac glycosides, considering that the plant belongs to the Solanaceae, a family which is noted for the

occurrence of tropane and steroidal alkaloids, as well as withanolides. Hence, it is important to highlight that, without the assistance of the bioassay-guided fractionation, the isolation of these active cardenolide principles would have been highly unlikely, considering their presence in such low abundance. As a result of this experimental approach, this is the first report of this type of compound in the Solanaceae. The unusual position of the double bond at C-6,C-7, thus far not observed in other cardenolides, may be characteristic of the cardenolides produced by this family.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H-nmr, DQF-COSY, ROESY, HETCOR, and HOHAHA spectra were recorded at 500.12 MHz employing a GE Omega 500 instrument, using standard GE programs. The ^{13}C-nmr (BB and DEPT) spectra and selective INEPT experiments were recorded at 90.8 MHz with a Nicolet NMC-360 instrument. For the selective INEPT experiments, data sets of 16 K covering a spectral width of 10 KHz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% C_6D_6 (^1J=6.7 Hz) in a 5-mm nmr tube (28). The radiofrequency fieldstrength for the soft pulse was on the order of 25 Hz for these experiments. Seven Hz was used as $^3J_{C\text{-}H}$ for the aromatic protons and 5 Hz for aliphatic protons. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. Fabms were obtained using a Finnigan MAT 90 instrument in the positive- and negative-ion mode using a glycerol matrix. Uv spectra were taken in a Beckman DU-7 spectrometer. Ir spectra were recorded in a KBr pellet on a Midac Ft-ir interferometer. Mps were determined on a Kofler hot-stage apparatus and are uncorrected.

PLANT MATERIAL.—*Nierembergia aristata* D. Don was collected near the shore of San Roque Lake, near Bialet Masé, Departamento Punilla, Province of Córdoba, Argentina. The plant material was identified by Dr. Andrea A. Coccuci and Ing. Armando T. Hunziker, and the voucher specimen No. ARIZA 3117 (CORD) is deposited in the Museo Botánico, Córdoba, Argentina.

EXTRACTION AND ISOLATION.—The whole plant of *N. aristata* (520 g) was air-dried at room temperature, ground, and macerated with MeOH (3×1250 ml) at room temperature. The MeOH extract was concentrated at reduced pressure (40°) to one-third of the original volume and H_2O was added to obtain a MeOH-H_2O (9:1) extract. This extract was defatted by partition with an equal volume of petroleum ether and then concentrated under reduced pressure (40°). Additional H_2O (750 ml) was added to the remaining H_2O extract which was partitioned with EtOAc (2×500 ml). The H_2O extract was lyophilized and the EtOAc extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness at reduced pressure. The amber residue (3.5 g) obtained after evaporation of the solvent showed significant cytotoxicity against a panel of human cancer cell lines with ED_{50} values ranging from 0.95 to 6.3 μg/ml, as summarized in Table 1.

The EtOAc fraction was adsorbed onto Si gel and fractionated on a column packed with the same adsorbent (150 g). The column was eluted with $CHCl_3$ (350 ml), increasing eluent polarity every 350 ml, using the following percentages of MeOH: 2, 5, 10, 20, 40, 60, and 100. Fractions of 100 ml were collected and the activity was monitored against a human breast cancer cell line (BC1). The active fractions 14–20 were combined, and, following a combination of chromatographic techniques including cc, prep. tlc, and flash cc (Si gel 40–60 μm), the known compounds myristic acid (12 mg, 0.0023%), scopoletin (5 mg, 0.00096%), ursolic acid (12 mg, 0.0023%), 5′-methoxylariciresinol (5 mg, 0.00096%), lariciresinol (3 mg, 0.00058%), and the new cardenolide monoglycosides **1** (15 mg, 0.0029%), **2** (9 mg, 0.0017%), and **3** (5 mg, 0.00096%) were isolated. Myristic acid, ursolic acid, and scopoletin were identified by comparison of their physical and spectroscopic data with those of authentic samples; and 5′-methoxylariciresinol and lariciresinol were identified by comparison of their physical and spectroscopic data with those reported in the literature (13).

CELL-BASED BIOLOGICAL EVALUATION PROCEDURES.—The EtOAc-soluble extract and the pure isolates **1–3** were tested against a panel of human cancer cell lines (Table 1) using established protocols (29).

Compound **1**.—White solid, mp 153–155°, $[\alpha]_D$ −55° (c=0.03, MeOH); uv λ max (MeOH) (log ε) 215 nm (4.2); ir ν max (KBr) 3440 (OH), 2932, 1734 (butenolide), 1717 (C=O), 1624 (C=C), 1036 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; positive fabms *m/z* 641 (24) M^++glycerol+1, 549 (92) M^++1, 419 (97) M^++1−$C_6H_{10}O_3$, 113 (100) $C_6H_8O_2$+1; negative fabms *m/z* 639 (21) M^++glycerol−1, 547 (100) M^+−1; positive hrfabms *m/z* 549.2705 (calcd for $C_{29}H_{41}O_{10}$, 549.2700).

Compound **2**.—White solid; mp 128.5–130.5°; $[\alpha]_D$ −48° (c=0.04, MeOH); uv λ max (MeOH) (log ε) 211 nm (4.2); ir ν max (KBr) 3442 (OH), 2931, 1735 (butenolide), 1717 (C=O), 1624 (C=C), 1042 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; positive fabms *m/z* 625 (2) M^++glycerol+1,

533 (22) $M^+ + 1$, 403 (16) $M^+ + 1 - C_6H_{10}O_3$, 131 (71) $C_6H_{10}O_3 + 1$, 113 (100) $C_6H_8O_2 + 1$; negative fabms *m/z* 623 (28) M^+ + glycerol − 1, 531 (100) M − 1, 401 (7) $M - 1 - C_6H_{10}O_3$, 383 (23) $M - 1 - C_6H_{12}O_4$; positive hrfabms *m/z* 533.2753 (calcd for $C_{29}H_{41}O_9$, 533.2751).

Compound **3**.—Gum; $[\alpha]_D$ −19° (*c*=0.05, MeOH); uv λ max (MeOH) (log ε) 211 nm (4.2); ir ν max (KBr) 3441 (OH), 2931, 1735 (butenolide), 1717 (C=O), 1625 (C=C), 1070 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; positive fabms *m/z* 639 (6) M^+ + glycerol + 1, 547 (37) $M^+ + 1$, 403 (18) $M^+ + 1 - C_7H_{12}O_3$, 145 (100) $C_7H_{12}O_3 + 1$, 113 (94) $C_6H_8O_2 + 1$; negative fabms *m/z* 637 (21) M^+ + glycerol − 1, 545 (100) $M^+ - 1$, 383 (5) $M^+ - 1 - C_7H_{14}O_4$; positive hrfabms *m/z* 547.2909 (calcd for $C_{30}H_{43}O_9$, 547.2907).

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