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ARGENTILACTONE, A NOVEL 5-HYDROXYACID LACTONE FROM *ARISTOLOCHIA ARGENTINA*

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Key Word Index—*Aristolochia argentina*; Aristolochiaceae; 5-hydroxyacid lactone; (-)-(5*R*)-5-hydroxydodeca-*Z*,*Z*-2,6-dienoic acid lactone; structural determination.

Abstract—Argentilactone, a new constituent of the rhizomes of *Aristolochia argentina*, was isolated and characterized as the (−)-(5*R*)-δ-lactone of 5-hydroxydodeca-*Z*,*Z*-2,6-dienoic acid.

INTRODUCTION

Continuing our chemical study of the constituents of *Aristolochia argentina* Gris. [1], we now report the isolation of the major constituent of the petrol extract of the rhizomes, a skin irritant compound named argentilactone, and its characterization as (−)-(5*R*)-5-hydroxydodeca-*Z*,*Z*-2,6-dienoic acid lactone.†

RESULTS AND DISCUSSION

Argentilactone (1) ($C_{12}H_{18}O_2$, M^+ 194), showed UV and IR absorptions characteristic of an α,β-unsaturated-δ-lactone. The PMR spectrum verified the presence of this moiety and further indicated other features of its structure. The signals of the olefinic protons on C-2 and C-3 appeared as doublets of triplets at δ 6.04 ($J_{2,3}$ = 10 Hz and $J_{2,4}$ = 1.8 Hz) and δ 6.91 ($J_{2,3}$ = 10 Hz and $J_{3,4}$ = 4.2 Hz) respectively [2–4]. Two multiplets centered at δ 2.38 (2H) and δ 2.10 (2H) were assigned to the methylenes at C-4 and C-8 respectively. A broad signal centered at δ 1.30 (6H) and a triplet at δ 0.88 (3H) were assigned to the methylene chain and the terminal Me group of the 1-heptenyl substituent attached at C-5. A broad envelope at δ 5.0–5.8 (3H) was assigned to the oxymethine proton and the two olefinic protons on the unsaturated side chain. The use of lanthanide shift reagents allowed the complete assignment of these protons and the determination of the conformation in the dihydropyrone ring system. The effect on the resonance positions of the different protons upon addition of increasing amounts of $Eu(fod)_3$ and $Pr(fod)_3$ is shown in Table 1. $Pr(fod)_3$ proved a better shift reagent to resolve the signals of the protons on C-5, C-6 and C-7, since a 'bunching' of these signals occurs at lower concentrations of $Eu(fod)_3$. The coupling constant ($J_{6,7}$ = 10.5 Hz) between the olefinic protons on C-6 and C-7 showed that they have a *cis* configuration and the large coupling between the proton on C-5 and one of the protons on C-4 ($J_{4ax,5}$ = 10 Hz) indicated that the 1-heptenyl side chain is pseudo-equatorial, as shown in 2*.

The ^{13}C NMR spectrum supported structure 1. The signals at 163.7, 121.1, 145.1, 73.7, 135.1 and 126.6 ppm were assigned to C-1, C-2, C-3, C-5, C-6 and C-7 respectively, from known chemical shift data [6, 7], their multiplicities in the off-resonance decoupled spectrum and a shift reagent. On using $Eu(fod)_3$ the peaks corresponding to C-2 and C-3 moved downfield and the magnitude of the induced shift was *ca* 3 times larger than the one at C-6 and C-7 [8] (Table 2). The signals at 27.7, 29.1, 31.4, 22.5 and 14 ppm were assigned to C-8, C-9, C-10, C-11 and C-12 using *cis*-2-octene as a model compound. The chemical shift of C-8 (27.7 ppm) is consistent with the *cis*-configuration of the adjacent double bond, since a larger value (*ca* 32.3 ppm) may be expected for the *trans* isomer [9].

Further evidence of structure 1 for argentilactone was obtained by MS analysis and by chemical degradations. The most important fragments are shown in Scheme 1. The oxidation of argentilactone with permanganate–periodate afforded hexanoic acid, and by catalytic hydrogenation apart from dodecanoic acid produced by extensive hydrogenolysis, the saturated lactone 3 was obtained, which was proved identical with an authentic sample of racemic δ-dodecanolactone prepared by the method of Boldingh *et al.* [10]. Saponification of 3 produced the hydroxyacid 4, which without isolation was transformed into a crystalline anilide 5 ($C_{18}H_{29}O_2N$, M^+ 291). Starting from 4, by dehydration and permanganate–periodate oxidation or by direct oxidation with nitric acid, a mixture of C_6, C_7, C_8 and C_9 acids were obtained.

From the positive Cotton effect (Δε = +2 at 256 nm) and by application of Snatzke's rule [11, 5] it follows that the absolute configuration at C-5 of argentilactone

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*Conformation 2 can be assumed for this compound in which C-5 and, in minor extent, the ring oxygen are displaced from the plane determined by C-1, C-2, C-3 and C-4, as shown by X-ray analysis of α,β-unsaturated lactones.

Table 1. PMR spectral data of argentilactone in the presence of Eu(fod)$_3$ and Pr(fod)$_3$

Shift reagent	Mol. equiv.	H_2	H_3	H_{4ax}	H_{4eq}	H_5	H_6	H_7	C_8–H_2
Eu(fod)$_3$	0.000	6.04	6.91	2.38 (2H, *m*)		5.00–5.80 (3H, *m*)			2.10
	0.077	6.55	7.07	2.58 (2H, *m*)		5.25–6.00 (3H, *m*)			2.19
	0.166	7.18 (2H, *s*)		2.82 (2H, *m*)		5.87 (3H, *s*)			2.28
	0.316	8.33	7.46	3.27 (2H, *m*)		6.43	6.23	5.94	2.47
	0.450	9.28	7.80	3.87	3.40	7.05	6.55	6.11	2.64
	0.712	11.1	8.25	4.62	4.03	8.04	7.07	6.33	2.80
	1.018	13.1	8.92	5.45	4.72	9.13	7.60	6.55	3.20
Pr(fod)$_3$	0.220	2.18	5.76	—	—	3.39	4.40	5.17	—

Coupling constants: $J_{2,3} = 10$ Hz, $J_{2,4ax} + J_{2,4eq} = 3.6$ Hz, $J_{3,4ax} = 3.3$ Hz, $J_{3,4eq} = 5.5$ Hz, $J_{4ax,4eq} = -19$ Hz, $J_{4ax,5} = 10$ Hz, $J_{4eq,5} = 5.5$ Hz, $J_{5,6} = 10$ Hz, $J_{6,7} = 10.5$ Hz, $J_{6,8} = 1.5$ Hz, $J_{7,8} = 7$ Hz, $J_{8,9} = 7$ Hz. Chemical shifts given in δ values relate to TMS: CDCl$_3$ was used as solvent.

Table 2. ^{13}C chemical shifts (*) and Eu(fod)$_3$ induced shifts (†) in argentilactone

Carbon no.	1	2	3	4	5	6	7	8	9	10	11	12
*	163.7	121.1	145.1	29.9	73.7	135.1	126.6	27.7	29.1	31.4	22.5	14.0
	(*s*)	(*d*)	(*d*)		(*d*)	(*d*)	(*d*)				(*t*)	(*q*)
†	1.4	2.0	1.9	0.9	1.5	0.6	0.7	0.4	0.3	0.2	0.2	0.1
‡	13.8	122.9	130.3	26.4	29.0	31.2	22.2	13.3	—	—	—	—

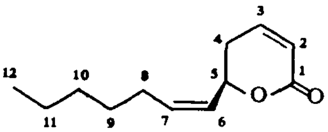
*Chemical shifts are in ppm for TMS; CDCl$_3$ was used as solvent. Signal multiplicities obtained from single frequency off-resonance experiments are given in parentheses; †Differential shift value ($\delta_{Eu} - \delta$) induced by addition of Eu(fod)$_3$, varying from 0.1–0.3 mmol, to a solution of 2.6 mmol of argentilactone in CDCl$_3$; ‡Chemical shift values of *cis*-2-octene taken from ref. [9]; original data converted δ(TMS) = 192.8 − δ(CS$_2$).

is (*R*). Work is in progress to prove this configuration by chemical means.

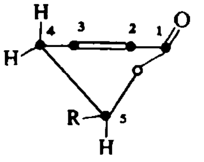
GLC showed that the rhizomes and leaves of *A. argentina* contain 2.4 and 0.8 % (dry wt) of argentilactone, respectively. Several lactones structurally related to argentilactone have been reported in the literature viz. lactone (**6**) and massoilactone (**7**) from the bark of *Cryptocarya massoia* [12] hyptolide (**8**) and boronolide (**9**) from *Hyptis pectinata* and *Tetradenia fruticosa*, respectively [3, 13].

EXPERIMENTAL

Isolation of **1**. Dried powdered rhizomes (2.34 kg) of *A. argentina* (collected in Villa Allende, Córdoba, Argentina) were

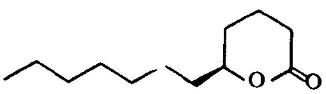


1



R = —CH=CH—(CH$_2$)$_4$—Me

2



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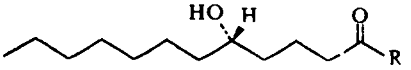
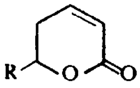
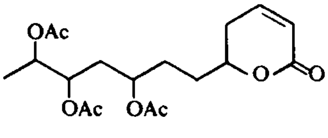


4 R = OH
5 R = NHC$_6$H$_5$



6 R = n-C$_7$H$_{15}$
7 R = *n*-C$_5$H$_{11}$



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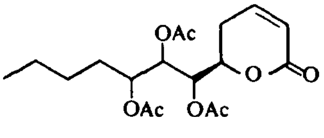

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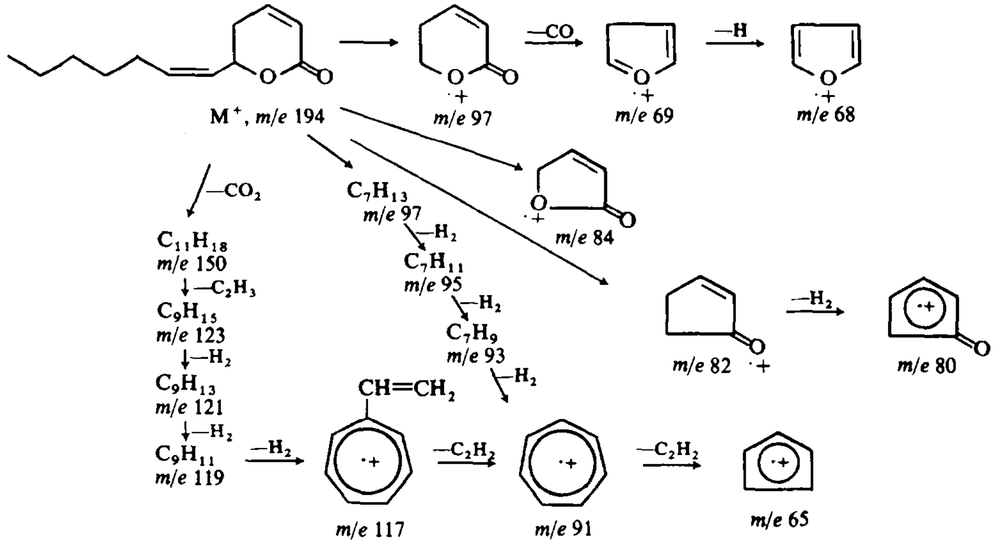

Scheme 1.

exhaustively extracted with petrol. Evaporation of the combined extracts yielded a dark brown oily residue (109.3 g) containing *ca* 50% of **1** as shown by GLC (3% SE-30 column). The crude residue was distilled *in vacuo* and the following fractions were collected: A, bp 60–132°/0.3 mm, pale yellow oil, 6.78 g; B, bp 132–150°/0.3 mm, pale yellow oil, 56.9 g; C, bp 150–165°/0.3 mm, pale yellow oil, 7.73 g. A dark thick oil (33.2 g) remained as residue. White plates, identified as hexadecanoic acid (mmp, IR, GLC), separated from C as well as B on standing at 5°. TLC analysis (Si gel, solvent 1: $CHCl_3$; solvent 2: petrol–Et_2O–HOAc, 80:20:1) revealed that all fractions contained argentilactone but it was heavily contaminated in A and C, and these fractions were discarded. A portion (10 g) of B was chromatographed over Si gel (500 g), 500 ml fractions being collected and monitored by TLC in the following order: 1–2, petrol; 3–6, C_6H_6; 7–10, $CHCl_3$; 11–14, $CHCl_3$–MeOH (99:1). Fractions 5–11, which were shown to consist largely of argentilactone, were rechromatographed over Mg silicate (500 g) and eluted with petrol, C_6H_6 and $CHCl_3$. Argentilactone (7.6 g) recovered from the C_6H_6 fractions was distilled *in vacuo* (139–143°/0.6 mm) yielding an homogeneous product (7 g) by TLC and GLC, $[\alpha]_D$ −21.1 (EtOH; *c* 2.25); $\eta_D^{24^\circ}$ 1.4849; λ_{max}^{EtOH} nm (log ε): 208 (4.00); IR (thin film), ν_{max}cm^{-1}: 3010 (=CH—), 2910 (CH_2), 2850 (CH_2), 1720 (C=O), 1465 (CH_2), 1420 (=CH—), 1380 (Me), 1243 (C—O), 1150, 1055, 1020, 960, 863, 812 and 730; PMR ($CDCl_3$), δ 0.88 ppm (3H, *t*, Me), 1.3 (6H, *m*, 3 × CH_2 groups), 2.10 (2H, *m*, CH_2 group at C-8), 2.38 (2H, *m*, CH_2 group at C-4), 5.0–5.8 (3H, *m*, 5-H, 6-H and 7-H), 6.04 (1H, *ddd*, $J_{2,3}$ 9.8 Hz, $J_{2,4}$ 1.8 Hz, 2-H), 6.91 (1H, *ddd*, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 4.2 Hz, 3-H); MS (rel. int.), *m/e* 194 (M^+, 5%), 152 (9), 150 (15), 123 (16), 121 (68), 119 (99.7), 117 (100), 97 (45), 95 (23), 93 (43), 91 (58), 84 (42), 82 (53), 81 (27), 80 (52), 79 (71), 78 (30), 77 (53), 69 (36), 68 (83), 67 (49), 66 (31), 65 (33), 55 (43), 47 (42), 44 (40), 41 (55), 39 (53), 28 (37), 18 (39), CD (EtOH), λ 300 nm ($[\theta] = 0$), max 256 (+6610), 230 (0), max 226 (−348), 224 (0), 220 (+870).

Permanganate–periodate oxidation of **1**. Argentilactone (1 g) in tert-BuOH (1 l.) was treated with a soln of $NaIO_4$ (16.44 g), $KMnO_4$ (0.39 g) and anhydrous K_2CO_3 (7.56 g) in H_2O (1 l.). After being shaken 18 hr at room temp. [14], the soln was acidified (pH 3) with 2N H_2SO_4 and decolourised with powdered Na_2SO_3. The pH was adjusted to 9 with 5% KOH and the tert-BuOH was evaporated *in vacuo*. The remaining aq. soln (1 l.) was acidified (pH 2) with 2N H_2SO_4 and extracted with $CHCl_3$–MeOH (3:2) (200 ml × 3), the combined organic extracts were washed (H_2O) and evaporated, the oily residue dissolved in EtOH–NH_3 ($\delta = 0.910$) (200:1) was chromatographed on a column of cellulose (100 g) and eluted with a mixture of EtOH–NH_3 (50:1) containing increasing amounts of H_2O until a ratio EtOH–NH_3–H_2O (50:1:10) was obtained. The first fractions afforded an oil which was identified as hexanoic acid by GLC of its Me ester, and the most polar fractions yielded crystalline ammonium oxalate (oxanilide, mp 246°, IR; Me ester, GLC). Other minor cleavage products could not be identified.

Hydrogenation of **1**. A soln of argentilactone (50 g) in EtOH (60 ml) was hydrogenated in the presence of 10% Pd-C (0.5 g) at 3.5 kg/cm^2 (room temp.) for 16 hr. The catalyst was filtered off and the filtrate evaporated to dryness *in vacuo* to yield an oily residue from which needles of dodecanoic acid separated. GLC analysis showed that the product was a *ca* 2:1 mixture of dodecanoic acid and **3** together with some other unidentified minor components. A portion of the crude reaction mixture (10 g) was chromatographed over Si gel (1 kg), 1 l. fractions being collected in the following order: 1–5 ($CHCl_3$); 6–9 ($CHCl_3$–MeOH, 99:1); 10–13 ($CHCl_3$–MeOH, 97:3). Fraction 4 (2.1 g), which was shown by TLC to contain mainly **3**, was distilled at 127–129°/0.2 mm yielding a colourless oil (1.2 g), homogeneous by GLC, $[\alpha]_D$ −32.04 ($CHCl_3$; *c* 3.92), $[\alpha]_D$ +2.2 (N ethanolic KOH; *c* 4.25); IR (thin film), ν_{max} cm^{-1}: 2935 (CH_2), 2870 (CH_2), 1740 (C=O), 1475 (CH_2), 1250 (C—O), 1186, 1055, 940; PMR ($CDCl_3$), δ 0.91 ppm (3H, *t*, Me), 1.31 (16H, *m*, 8 × CH_2 groups), 2.48 (2H, *m*, CH_2CO), 4.26 (1H, *m*, 5-H). Identical IR with an authentic sample of δ-dodecanolactone prepared according to ref. [10].

(−)-*5-Hydroxydodecanilide* (**5**). A crude mixture (3 g) obtained by hydrogenation of argentilactone (**1**), in 10% ethanolic KOH (160 ml) was refluxed for 1 hr. Most of the EtOH was removed by evaporation at low temp. under red. pres. The residue was diluted with H_2O (160 ml), cooled, acidified with aq. HCl and extracted with Et_2O (100 ml × 3). The combined Et_2O extracts were washed, dried, evaporated and the residue in aniline (6 ml), was heated at 160° for 6 hr. After cooling, the reaction mixture was dissolved in Et_2O (100 ml) and washed with 10% HCl, 10% NaOH and H_2O, dried and evaporated to dryness. The residue in hot petrol (50 ml) was decolorized with charcoal and allowed to cool; the crystalline solid that separated (1.9 g) was shown by TLC to be a mixture of two components (R_f 0.39 and 0.88; C_6H_6–Me_2CO–HOAc, 80:20:1) in *ca* equal amounts. After preparative-TLC, from the R_f 0.39 band a solid was obtained which crystallized from C_6H_6 (25 ml) to give (−)-5-hydroxydodecanilide (**5**) (0.885 g) as plates, mp 103°, $[\alpha]_D$ −5.18 ($CHCl_3$; *c* 2.76); UV, λ_{max}^{EtOH} nm (log ε): 243 (4.19); IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 3300 (NH), 2925 (CH_2), 2850 (CH_2) 1678 (CO—N), 1615 (C=C), 1555 (CO—N), 1512 (C=C), 1452, 772, 705; PMR

($CDCl_3$), δ 0.87 ppm (3H, *t*, Me), 1.27 (16H, *m*, 8 × CH_2 groups), 2.39 (2H, *t*, *J* 7.0 Hz, $C\underline{H}_2CO$), 2.63 (1H, *s*, OH, disappears with D_2O), 3.61 (1H, *m*, 5-H), 7.00–7.63 (5H, *m*, aromatic protons), 7.87 (1H, *bs*, NH); MS (rel. int.), *m/e* 291 (M^+, 3.8 %), 273 (4.9), 192 (5.3), 135 (18), 120 (2.5), 114 (5.0), 99 (36), 94 (12), 93 (100), 92 (11), 83 (4.3), 77 (4.9), 71 (12), 70 (9.2), 69 (8.6), 66 (14), 65 (7.4), 57 (9.0), 56 (8.3), 55 (12), 43 (12), 42 (7.5), 41 (14), 29 (5.4). The solid obtained from the R_f 0.88 band was crystallized from petrol (10 ml) yielding needles of dodecanilide (0.554 g), mp 78° identical (mmp, IR, PMR) to an authentic sample.

Dehydration and permanganate–periodate oxidation of 5-hydroxydodecanoic acid (**4**). A portion of **3** (150 mg) was saponified as described above and the freshly prepared hydroxyacid **4** (100 mg) refluxed at 120° for 15 min with 60% H_2SO_4. The reaction mixture was made slightly alkaline with NaOH and oxidized with $KMnO_4$–$NaIO_4$ by the method described previously. Esterification of the acidic product with CH_2N_2 and subsequent GLC analysis showed the presence of the Me esters of hexanoic, heptanoic, octanoic and nonanoic acids in the ratio 1:8:35:10 respectively, along with other unidentified components.

Nitric acid oxidation of 5-hydroxydodecanoic acid (**4**). A portion of freshly prepared hydroxyacid **4** (50 mg) and a mixture of HNO_3 ($\delta = 1.42$)–H_2O (2:1) was heated at 100° for 10 min. H_2O was added and the mixture made alkaline with NaOH, slightly acidified with HCl and extracted with Et_2O. The combined Et_2O extracts were washed, dried, evaporated and the oily residue esterified with CH_2N_2. GLC analysis of the resultant product led to the identification of the Me esters of heptanoic, octanoic and nonanoic acids in a ratio 1:4:1.4 respectively and traces of hexanoic acid.

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