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Synthesis of argentatin A derivatives as growth inhibitors of human cancer cell lines in vitro

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Abstract—The syntheses of nine argentatin A analogs are described. These compounds were assessed for their ability to inhibit growth in vitro in four human cancer cell lines. Our results showed that the presence of either a double bond at C-1/C-2, or a bromine atom or formyl moiety at C-2 as well as the presence of an isoxazol ring in argentatin A enhanced its potency in all cell lines tested. In addition, an X-ray study of (16*S*,17*R*,20*S*,24*R*)-3-oxime-20,24-epoxy-16,25-dihydroxy-cycloartan-3-one led to the determination of the correct stereochemistry of argentatin A.
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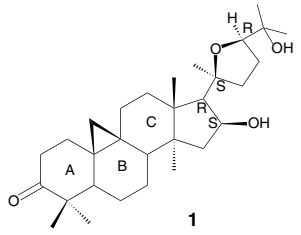
Triterpenoids form a large class of compounds derived biosynthetically from the cyclization of squalene with the retention of all 30 carbon atoms.[1] Many of these naturally occurring compounds have interesting biological and pharmacological properties.[2] For example, ursolic acid, an ursane-type triterpene, inhibits tumorigenesis,[3] and induces tumor cell differentiation.[4] It has also proven effective in the inhibition of angiogenesis[5] and invasion,[6] which are important events involved in tumor metastasis.

On the other hand, some cycloartane-type triterpenes, such as methyl quadrangularates B and D, and actein, have also shown cytotoxic activity on several cancer cell lines.[7]

Argentatin A (**1**), a cycloartane-type triterpene, is an important component of the resin of *Parthenium argentatum* Gray (Guayule).[8] Guayule is a common desert shrub in Northern Mexico and Southwestern USA. It has been intensively studied as a renewable native source of natural rubber, and it is known that for each kilogram of natural rubber obtained, 1 kg of a by-product named resin is also produced.[8] Taking into account that the argentatins, including argentatin A, comprise 20% of the resin, it can be inferred that these compounds will be available in large amounts.



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Our studies on the argentatins have demonstrated that argentatin A (**1**) possesses anti-microbial properties.[9] Additionally, in a previous paper, we reported that **1** inhibited 100% of cell growth in six cancer human cell lines (HeLa, K562, U251, PC-3, MCF-7, and HCT15) at 100 μM.[10]

As a strategy to elucidate structural requirements involved in the cytotoxic activity of **1**, we now wish to report the synthesis and biological evaluation of nine derivatives of argentatin A.

Argentatin A was obtained from the resin of *P. argentatum* (Gray) as previously reported[11] and identified by comparison of its physical and spectroscopic constants (mp, ^{1}H and ^{13}C NMR) with those reported in

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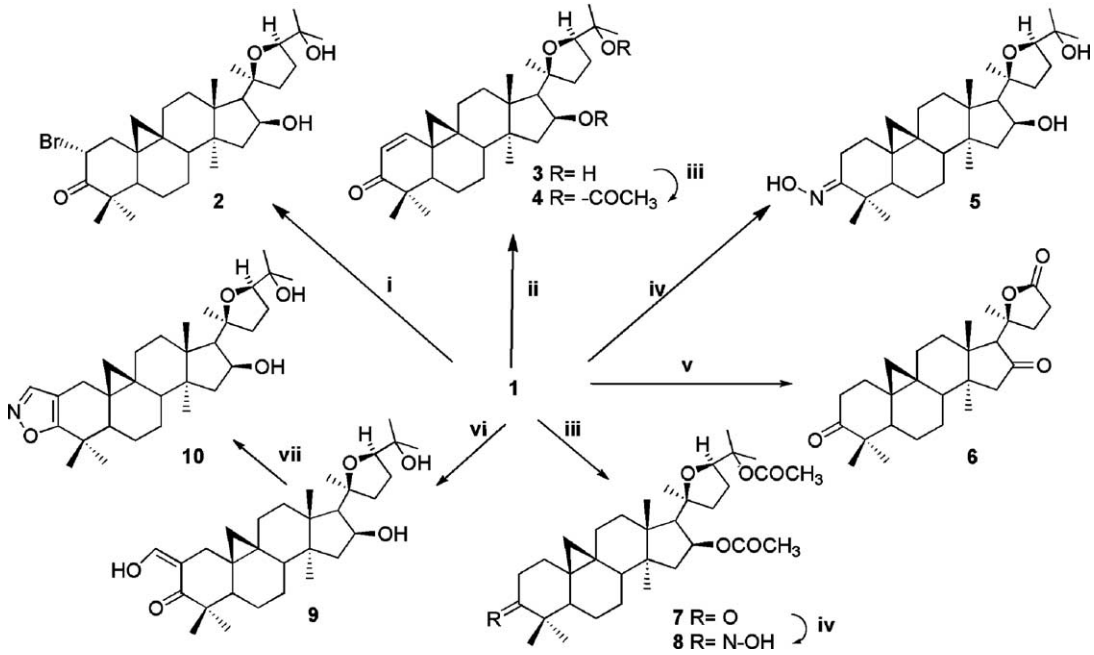

Scheme 1. Reagents and conditions: (i) Br/CH_3COOH, 98%; (ii) (a) PhSeCl, EtOAc, (b) THF, H_2O_2 70%; (iii) AcONa/$(CH_3CO)_2O$, 99%; (iv) $NH_2OH{\cdot}HCl$, C_5H_5N, 90%; (v) CH_3COOH, CrO_3, H_2O, 40%; (vi) HCO_2Et, C_5H_5N, Na/MeOH, 89%; (vii) $NH_2OH{\cdot}HCl$, CH_3COOH, 80%.

literature, as well as by comparison with an authentic sample.[8]

Syntheses of argentatin A derivatives (**2**–**10**) are described in Scheme 1.

The 2-bromo derivative **2** was obtained from Argentatin A (**1**) when treated with bromine liquid in acetic acid, while treatment of **1** with phenyl selenium chloride in ethyl acetate followed by addition of tetrahydrofuran and hydrogen peroxide yielded **3**.

Compound **3** was transformed into the unsaturated acetate **4** by treatment with sodium acetate in acetic anhydride. Oxime **5** was synthesized by treatment of **1** with hydroxylamine hydrochloride in pyridine.

Lactone **6** was obtained from **1**, when it was dissolved in acetic acid and treated with chromium trioxide in water.

The treatment of **1** with sodium acetate in acetic anhydride gave **7**. The resulting acetate was transformed into its corresponding oxime **8** by treatment with hydroxylamine hydrochloride in pyridine.

Formyl derivative **9** was obtained from **1** by treating with sodium methoxide prepared in situ and ethyl formate. In addition, isoxazol derivative **10** was obtained from **9**, when treated with hydroxylamine hydrochloride in acetic acid.

All the compounds were characterized by spectroscopic and analytical tools. Additionally, adequate crystals of **5**, suitable for X-ray analysis were obtained. This analysis permitted the assignment of the structure as (16*S*, 17*R*,20*S*,24*R*)-3-oxime-20,24-epoxy-16,25-dihydroxy-cycloartan-3-one (Fig. 1).[12]

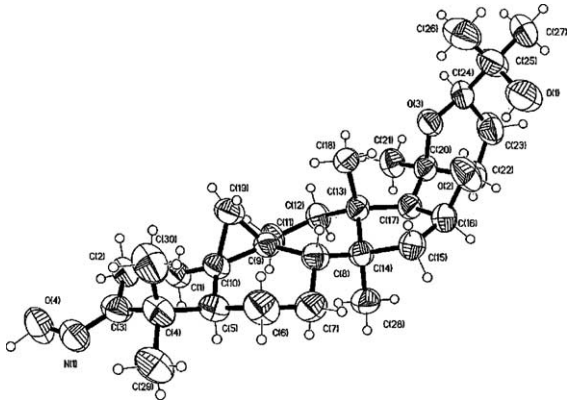

Figure 1. Crystal structure of **5**.

The view of molecule **5** in Figure 1 shows the *trans*, *cis*, *trans*, *cis* junctions for rings A–D similar configuration to those previously described for some argentatins.[13] In **5**, the ring A adopts a chair conformation, while the ring B displays a half-chair conformation due to the presence of the cyclopropane ring. The central ring, C, is present as intermediate between half chair and twist, while the five-member ring D adopts a twist conformation.

Even though argentatin A has been isolated since 1970 from *P. argentatum* and from other *Parthenium* species,[14] its stereochemistry has not been accurately determined. For instance, some authors assigned an *R* configuration to the stereogenic center at C-20,[15] while

Table 1. Effect of argentatin A derivatives on the growth of human cancer cell lines (concentration causing 50% cell growth inhibition)

Compound	IC_{50} (SEM), μM			
	HCT-15 (colon)	K562 (leukemia)	PC-3 (prostate)	U251 (CNS[a])
1	31.70 ± 1.10	38.61 ± 4.47	20.22 ± 3.44	27.34 ± 1.00
2	3.23 ± 1.10	4.34 ± 0.75	11.06 ± 0.66	13.89 ± 0.44
3	16.66 ± 1.50	16.84 ± 3.46	15.26 ± 1.70	18.88 ± 0.36
4	>100	>100	13.93 ± 0.324	58.44 ± 4.00
5	35.80 ± 1.98	44.43 ± 5.57	46.91 ± 2.75	26.79 ± 2.39
6	>100	>100	>100	>100
7	40.68 ± 2.49	68.39 ± 8.30	22.69 ± 2.50	30.51 ± 3.45
8	28.87 ± 3.17	15.18 ± 2.35	12.56 ± 2.42	21.37 ± 1.67
9	9.82 ± 0.21	14.38 ± 0.78	5.69 ± 0.05	5.88 ± 1.03
10	10.24 ± 0.77	11.02 ± 1.05	13.00 ± 3.56	11.30 ± 1.55

Each data is given as the mean and its standard error (SEM) of at least three independent experiments. Doxorubicin: HCT-15: 0.23 ± 0.01 μM; K562: 0.28 ± 0.01 μM; PC-3: 0.32 ± 0.02 μM; U251: 0.09 ± 0.02 μM.
[a] Central Nervous System.

others did not specify the stereochemistry of the same center. Since no stereogenic center was modified in the reaction to obtain **5** from **1**, then the stereochemistry of **1** was established as (16*S*,17*R*,20*S*,24*R*)-20,24-epoxy-16,25-dihydroxy-cycloartan-3-one.

The cytotoxic activity of argentatin A derivatives on K562 (leukemia), PC-3 (prostate), U251 (central nervous system), and HCT-15 (colon) cancer human cell lines was determined using the sulforhodamine B test.[16] The concentrations required to inhibit cell growth by 50% (IC_{50}) were calculated according to the protocol previously established.[16]

The cytotoxic activity of argentatin A (**2**–**10**) derivatives is shown in Table 1.

An analysis of the results of cytotoxic activity of the synthetic argentatin A derivatives led to the establishment of an important fact with respect to their structure–activity relationship. The presence of either a double bond at C-1/C-2, or a bromine atom or formyl moiety at C-2, as well as the presence of an isoxazol moiety linked to ring A enhanced the potency of argentatin A in all cell lines tested (**2**, **3**, **9**, and **10** are more active than **1**).

Lactone derivative **6** was inactive in all cell lines tested; this fact suggests that the presence of an isopropyl alcohol group at C-24 is essential for the inhibitory activity.

In conclusion the modification of argentatin A afforded four compounds that resulted in more active than **1** on all cell lines tested.

Acknowledgements

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11. The resin, a by-product of the industrial process to obtain natural rubber from *P. argentatum* was donated by CONAZA Company (Coahuila, Mexico). The resin (157.39 g) was dissolved in a small volume of hexane and percolated over tonsil. The first fractions collected

were reunited and chromatographed on Silica gel. This procedure resulted in the isolation of argentatin A (4.2 g), which was purified by conventional procedures.

12. X-ray data were collected on a Siemens P4 diffractometer. Space group $P2_12_12_1$, $a = 27.458$ (2), $b = 7.9900$ (10), $c = 13.0030$ (10). Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Center, CCDC-254670. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 (1223)336 033; e-mail for inquiry: fileserv@ccdc.cam.ac.uk).
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