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3-Acetylaltholactone and related styryl-lactones, mitochondrial respiratory chain inhibitors

Eva Peris[a], Ernesto Estornell[b], Nuria Cabedo[a], Diego Cortes[a], Almudena Bermejo[a,*]

[a]*Departamento de Farmacología, Laboratorio de Farmacognosia, Universidad de Valencia, 46100 Burjassot, Valencia, Spain*
[b]*Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Valencia, 46100 Burjassot, Valencia, Spain*

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Abstract

A novel furano-pyrone, 3-acetylaltholactone, and two other known styryl-lactones, altholactone and 5-acetoxyisogoniothalamin oxide, have been isolated from *Goniothalamus arvensis* (Annonaceae) stem bark. We report here the isolation and structural elucidation of these compounds with furane-pyrone and styryl-pyrone skeletons, postulating also for the first time their mechanism of cytotoxicity based on inhibition on mammalian mitochondrial respiratory chain. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Goniothalamus arvensis*; Annonaceae; Furano-pyrones; 3-Acetylaltholactone; Mitochondrial respiratory chain inhibitors

1. Introduction

Styryl-lactones are an interesting group of bioactive agents with significant cytotoxicities against several human tumor cell lines, many of which have been isolated from *Goniothalamus* species (Annonaceae) (Blázquez et al., 1999). In previous publications, we have described for the first time several styryl-lactones from *Goniothalamus arvensis* stem bark. Four furano-pyrones, (+)-goniotharvensin (Bermejo et al., 1995), (−)-etharvensin (Bermejo et al., 1997), arvensin and (+)-2-epialtholactone (Bermejo et al., 1999a); two styryl-pyrones, (+)-garvensintriol and (+)-etharvendiol (Bermejo et al., 1998a); and two heptolides, (+)-almuheptolide-A and (+)-almuheptolide-B (Bermejo et al., 1998b). Several furano-pyrone semisynthetic derivatives have been found both cytotoxic and inhibitors of the cell-cycle on L-1210 leukemia line (Bermejo et al., 1999b). In a recent biochemical work, we have reported the ability of the bioactive heptolide-type-styryl-lactones to inhibit complex **I** of the mitochondrial respiration chain (Bermejo et al., 1998b).

We describe herein both the isolation from *G. arvensis* stem bark methanolic extract, and the structure elucidation of the novel natural 3-acetylaltholactone (**1**), with a furano-pyrone skeleton, and two other known compounds previously isolated from several *Goniothalamus* species, altholactone (**2**) (Loder and Nearn, 1977) and 5-acetoxyisogoniothalamin oxide (**3**) (Hasan et al., 1994), with furano-pyrone and styryl-pyrone skeletons, respectively. In addition, to gain new insights on their cytotoxicity mechanism of action, these natural compounds (**1**–**3**) were tested for inhibition of the mitochondrial respiratory chain. Our results indicate that they are highly effective to block this important bioenergetic process, and thus, they might be exploited for biomedical research, antitumor therapy and agrochemical pest control like other respiratory chain inhibitors.

* Corresponding author. Tel.: +34-96-386-4975; fax: +34-96-386-4943.

E-mail address: bermejoa@uv.es (A. Bermejo).

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2. Results and discussion

2.1. Isolation and chemistry

The biological active compound **1**, which we have assigned the trivial name of 3-acetylaltholactone, was isolated as transparent needles, m.p. 140–142°C and $[\alpha]_D$ + 166.6° (*c* 0.3, EtOH). Analysis of the UV, ^{1}H- and ^{13}C-NMR spectra of **1**, revealed that it has a characteristic furano-pyrone skeleton, closely related to that of altholactone (**2**), a 3-hydroxy-2-phenyl-tetrahydrofurano-pyr-5-one derivative with an α,β-unsaturated δ-lactone (Loder et al., 1977; El-Zayat et al., 1985).

Molecular weight of **1** was indicated in the HRCIMS by a prominent peak at *m/z* 275.09178 $[M+H]^+$ (calc. 275.09194), corresponding to the molecular formula $C_{15}H_{14}O_5$, which is in agreement with 15 carbon signals observed in the ^{13}C-NMR spectrum. The mass fragmentation pattern of **1** was very close to that of **2** (Bermejo et al., 1995). The presence of two carbonyl groups in **1** was established by carbon resonances at δ_C 160.4 (α,β-unsaturated δ-lactone) and δ_C 169.4 (acetyl group), and by two strong IR absorptions at 1742 and 1722 cm^{-1}. The acetyl group in **1** was evidenced in HRCIMS by successive losses of $COCH_3$ and $OCOCH_3$ from $[M+H]^+$, and by a singlet proton resonance at δ_H 2.15 (3H, $COCH_3$) and a deshielded *dd* proton at δ_H 5.38. The existence of a phenyl group in **1** was suggested by the proton resonances at δ_H 7.34 (*m*, 5H, H-9 to H-13), IR absorptions at 763 and 700 cm^{-1}, and by the carbon signals at δ_C 137.4 (C-8), 126.2 (C-9 and C-13), 128.4 (C-11) and 128.6 (C-10 and C-12). Four resonances due to oxygen-bearing carbons (δ_C 86.1: C-3a; δ_C 83.6: C-2; δ_C 83.5: C-3; δ_C 69.1: C-7a) and the corresponding four carbinol protons between δ_H 4.62 and 5.38, were observed in the NMR spectra (Fig. 1).

The results consigned above are in agreement with a furano-pyrone skeleton in **1**. The relative stereochemistry of the four stereogenic centers in the tetrahydrofuran ring was evidenced by the ^{1}H–^{1}H coupling constant data and 2D homonuclear experiments. COSY 45 spectrum of **1** showed correlations between olefinic protons (H-6 at δ_H 6.28 and H-7 at δ_H 7.04) and the consecutive methine oxygenated ones, consistent with the placement of the acetyl group at C-3 position (see Fig. 2). Therefore, a 2,3-*trans* ($J_{2,3}$ = 3.5 Hz), 3,3a-*trans* ($J_{3,3a}$ = 1.0 Hz) and 3a,7a-*cis* ($J_{3a,7a}$ = 4.1 Hz) relative configuration was established for **1**, as it occurs in altholactone (**2**) and other furano-pyrone compounds (Blázquez et al., 1999). The acetylation of **2** gave **1** identical to natural one in all respects, suggesting the structure of **1** as 3-acetylaltholactone.

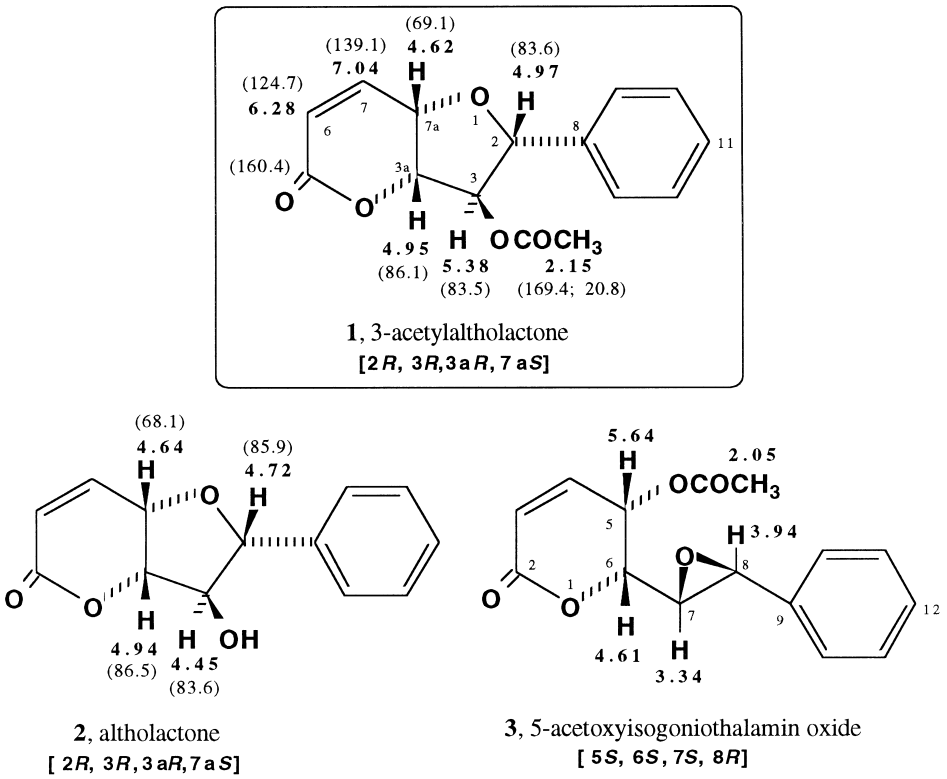

Fig. 1. ^{1}H- and ^{13}C-NMR (in parentheses) of compounds **1**–**3**.

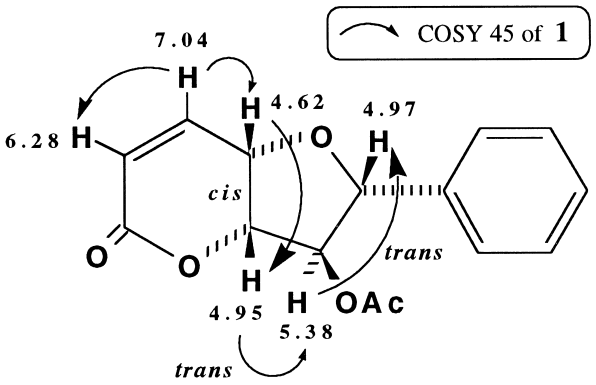

Fig. 2. ^{1}H–^{1}H COSY 45 of **1**.

Thus, the absolute configuration of **1** is [2*R*, 3*R*, 3a*R*, 7a*S*], as occurs in **2**, whose configuration was determined by X-ray crystallographic analysis (El-Zayat et al., 1985).

The known compounds **2** and **3** (Fig. 1) were isolated as yellowish oils. Comparison of their spectroscopic properties with those related compounds previously isolated supported their structural assignments (Loder et al., 1977; El-Zayat et al., 1985; Hasan et al., 1994; Bermejo et al., 1995).

2.2. Bioactivity

Natural and synthetic styryl-lactone derivatives have been found to possess significant cytotoxic activities against several human tumor cell lines (Cao et al., 1998; Tsubuki et al., 1999). We have recently reported the enantiospecific semisynthesis of almuheptolide-A, a novel natural heptolide, a selective inhibitor of the complex **I** of the mammalian mitochondrial respiratory chain. Almuheptolide-A, a 8-phenyl-2-oxocanone derivative also isolated from *G. arvensis* was synthesized from altholactone (**2**) via the 6,7-dihydro-7-ethoxy-altholactone (etharvensin) (Bermejo et al., 1997, 1998b). Taking into account such precedents, styryl-lactone derivatives obtained here were expected to inhibit the mitochondrial respiratory chain as cytotoxicity mechanism, and thus, we assayed compounds **1**–**3** against the NADH oxidase activity of beef-heart submitochondrial particles, a model of mammalian respiratory chain.

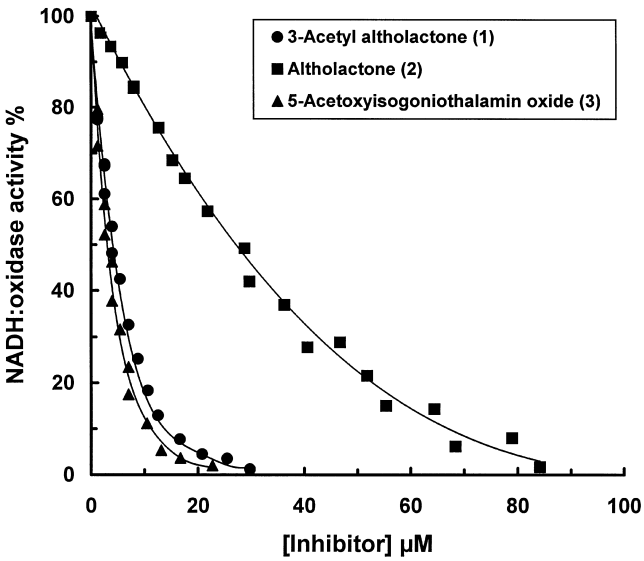

Fig. 3. Inhibition of NADH:oxidase activity in beef-heart submitochondrial particles by: 3-acetylaltholactone (**1**), altholactone (**2**), and 5-acetoxyisogoniothalamin oxide (**3**).

Table 1 shows both the half-inhibition (IC_{50}) and full-inhibition concentrations (IC_{100}) of each compound **1** and **3** gave IC_{50} in the μM range, and thus, they were effective inhibitors of the respiratory chain with similar potency to other inhibitors with potential biomedical interest. Instead, **2** was less potent. Titration curves showed in Fig. 3 evidenced that **1** and **3** yielded a rapid decay of the NADH oxidase activity with increasing concentration, but final inhibition stages, below 15% activity, were slower. Contrarily, compound **2** gave a more hyperbolic curve and thus, IC_{100} of the three compounds were closer than IC_{50}.

Comparison of compounds **1** and **2** shows that acetylation of C-3 hydroxy group clearly increases the inhibitory potency. Compound **3** is as potent as **1** and it also bears an acetyl group spatially near to epoxy ring. It is thought that these functional groups placed in this part of the molecule play an important role to modulate the inhibitory action of this type of compounds against the mitochondrial respiratory chain, accounting for the cytotoxic and antitumor activities previously described for several styryl-lactone derivatives.

Table 1
Inhibitory potency of compounds **1**–**3** against the NADH oxidase activity of mammalian respiratory chain

Compound	Half inhibition IC_{50} (μM)	Full inhibition IC_{100} (μM)
3-Acetylaltholactone (**1**)	4.7 ± 1.6	32 ± 4
Altholactone (**2**)	25 ± 7	84 ± 6
5-Acetoxyisogoniothalamin oxide (**3**)	3.0 ± 0.3	22 ± 2

3. Experimental

3.1. General experimental procedures

Optical rotations were determined with a Perkin-Elmer 241 polarimeter. IR spectra were run in film on a Perkin-Elmer 843 spectrometer. UV spectra were obtained on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer. Mass spectra were performed with direct injection at 70 eV on a Nermay-Sidar instrument or a VG Auto Spec Fisons spectrometer. ^{1}H-NMR and ^{13}C-NMR 400 MHz Variant (Unity 400). Multiplicities of ^{13}C-NMR resonances were obtained by DEPT experiments. COSY 45 correlations were run using a Bruker AC-250 (250 MHz).

3.2. Plant material

Goniothalamus arvensis Scheff. was collected in the National Park of Varirata, located in the Central Province of Papua New Guinea. A voucher specimen (No. 517 Varirata plot) was deposited in the herbarium of the University of Papua New Guinea.

3.3. Extraction and isolation

Dried and powdered stem barks of *G. arvensis* (368 g) were macerated with methanol at room temperature. The crude methanolic extract was partitioned between hexane and 50% aqueous methanol. After partial evaporation, the aqueous solution was fractioned with CH_2Cl_2 to obtain 8 g of organic extract which was applied to a flash silica gel (Merck 9385) column and eluted with CH_2Cl_2/EtOAc (8:2). This afforded us the altholactone (**2**, 4.5 g) and a fraction (200 mg) that was submited to a subsequent 60H silica gel (Merck 7736) column with CH_2Cl_2/EtOAc (99:1), which led us to the isolation of the new compound 3-acetylaltholactone (**1**, 7 mg) and the known 5-acetoxyisogoniothalamin oxide (**3**, 5 mg).

3.4. (+)-3-Acetylaltholactone *1*

$C_{15}H_{14}O_5$; recrystallized from CH_2Cl_2/EtOAc m.p. 140–142°C; $[\alpha]_D$ +166.6° (*c* 0.3, EtOH); UV λ_{max} EtOH nm (log ε): 216 (2.89) and 256 (2.33); IR ν_{max} (film) cm^{-1}: 2969, 2345, 2334, 1742, 1722, 1630, 1602, 1491, 1454, 1375, 1360, 1316, 1300, 1288, 1250, 1221, 1046, 1099, 1069, 1054, 1021, 917, 882, 846, 817, 763, 745, 700, 667, 638; HRCIMS *m/z* (%): $[M + H]^+$ 275.09178 (calc. for $C_{15}H_{15}O_5$ 275.09194) (100), $[M + H-COCH_2]^+$ 233.08191 (calc. for $C_{13}H_{13}O_4$ 233.08138) (23), $[M + H-OHCOCH_3]^+$ 215.06440 (calc. for $C_{13}H_{11}O_3$ 215.07081) (83), 199.06669 (calc. for $C_9H_{11}O_5$ 199.06064) (29), 163 (48), 107 (51); ^{1}H- and ^{13}C-NMR spectral data were unambiguously assigned by 1D and 2D NMR experiments: see Figs. 1 and 2.

3.5. Acetylation of *2*

Fifty mg of altholactone (**2**) were treated with Ac_2O (2 ml) and pyridine (1 ml) at room temperature for 3 h. Usual work up gave a compound in quantitative yield identical to **1**.

3.6. Bioactivity assays

Inhibition of mitochondrial respiratory chain was assayed using titration of the compounds against the aerobic oxidation of 75 μM NADH using beef-heart submitochondrial particles obtained by ultrasonic disruption (6–7 μg/ml). Reaction rates were calculated from the linear decrease of NADH concentration ($\lambda = 340$ nm, $\varepsilon = 6.22$ mM^{-1}cm^{-1}) in presence of increasing amount of the compound in an end-window photomultiplier spectrophotometer ATI-Unicam UV4-500 (Tormo et al., 1999a). The inhibitory concentration (IC_{50}) was the final compound concentration in the assay medium that yielded 50% inhibition of NADH oxidase activity. Full-inhibition concentration (IC_{100}) was taken when reaction rate was less than 3% of control activity. Given values are means ± SD of four assays for each compound (Tormo et al., 1999b).

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