The Unusual Ability of α-Angelicalactone to Form Adducts: A Kinetic Approach

ESTRELLA FERNÁNDEZ-RODRÍGUEZ, JOSÉ A. MANSO, M. TERESA PÉREZ-PRIOR, M. DEL PILAR GARCÍA-SANTOS, EMILIO CALLE, JULIO CASADO

Departamento de Química Física, Universidad de Salamanca, E-37008 Salamanca, Spain

Received 7 March 2007; revised 26 May 2007; accepted 27 May 2007

DOI 10.1002/kin.20273
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Since α-angelicalactone (AAL) substantially inhibits the formation of tumors, here its chemical reactivity was compared with that of carcinogenic lactones. Investigation of the electrophilic potential of AAL was carried out by studying the capacity of this lactone to form adducts with NBP, 4-(*p*-nitrobenzyl)pyridine, a substrate with nucleophilic characteristics similar to DNA bases. The formation of the AAL–NBP adduct occurs about 900,000-fold faster than with β-propiolactone, the most effective carcinogenic lactone ($\Delta G^{\#}_{35}$ =52 and 87 kJ mol^{-1}, respectively). A stopped-flow technique was required for this reaction to be monitored. It was concluded that the formation of AAL–NBP adducts takes place through an entropy-strain-catalyzed mechanism caused by early lactone ring cleavage. The kinetic results are consistent with the AAL potential as a chemoprotective agent. © 2007 Wiley Periodicals, Inc. Int J Chem Kinet 39: 591–594, 2007

INTRODUCTION

The nitrosation of amino acids with an $-NH_2$ group gives lactones as products [1–3]. Some lactones give alkylating reactions with any of a number of nucleophilic sites in tissues. Because alkylating agents are considered to be archetypal carcinogens [4], considerable effort has been devoted to addressing the chemical carcinogenesis caused by these species [5].

Because the behavior of lactones in their hydrolysis reactions is a good indicator of their reactivity as electrophilic molecules, previously the alkylating potential of some four- to six-membered lactones was investigated [6,7].

Five-membered lactones such as α-angelicalactone and γ-butyrolactone fail to produce tumors [4]. Indeed, α-angelicalactone (AAL), a naturally occurring compound, substantially inhibits the formation of tumors and was investigated as a chemoprotective agent [8]. The effects of AAL on the amount of α-benzopyrene (BP) metabolite-DNA adduct, formed in the forestomach, lung, and liver of ICR/Ha mice, are discussed elsewhere [9]. Results suggest that one of the mechanisms of inhibition of BP-induced neoplasia by AAL occurs through inhibition of the formation of some BP–metabolites–DNA adducts.

Correspondence to: J. Casado; e-mail: jucali@usal.es.
Contract grant sponsor: Spanish Ministerio de Educación y Ciencia.
Contract grant number: CTQ2004-05048/BQU.
Contract grant sponsor: Spanish Junta de Castilla y León.
Contract grant number: SA011A05.
Contract grant sponsor: Junta de Castilla y León for Ph.D. grants (to José A. Manso and M. Teresa Pérez-Prior).

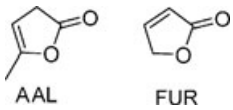


Figure 1 Chemical structures of α-angelicalactone and furanone.

Since (i) a correlation has been observed between the alkylating capacity of lactones and their carcinogenicity [7]; (ii) AAL inhibits the formation of tumors [8]; and (iii) to our knowledge, the chemical reactivity of AAL as an electrophilic reagent has not been investigated, the present study addresses the latter issue. In addition, since furanone (FUR) is also an unsaturated molecule (Fig. 1), its alkylating capacity was also investigated.

EXPERIMENTAL

Because the molecule 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents [10], has nucleophilic characteristics similar to DNA bases [11], it was used as the alkylation substrate.

Formation of the AAL–NBP adduct (Scheme 1) was visualized kinetically by adding Et_3N reagent, which stops the alkylation reaction and induces the formation of a blue color. Absorbance was measured at the wavelength of maximum absorption. Adduct formation was investigated under mimicked cellular conditions [11,12] at neutral pH.

The NBP molecule is quite sensitive not only to effective electrophilic molecules such as β-propiolactone (BPL) or β-butyrolactone (BBL) [7] but also to much weaker alkylating molecules, such as sorbates [13].

To render NBP soluble, the AAL + NBP alkylation mixtures were prepared in 7:3 (vol) water/dioxane medium. No NBP alkylation by FUR was observed after 2 weeks.

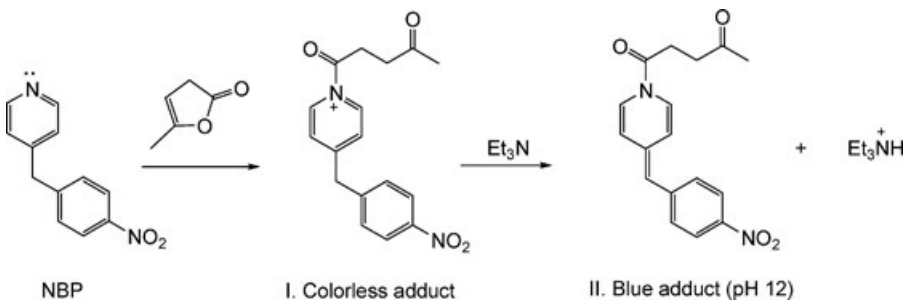
Since AAL–NBP adduct formation occurs very rapidly, a stopped-flow technique [14] was required to monitor this reaction. A modular three-syringe Bio-Logic sequential mixing stopped-flow spectrometer SFM300 was used.

Both reagents, AAL and NBP, were maintained separately in two of the apparatus' syringes, Et_3N being present in the third syringe. The method was as follows: Syringe A contained $[AAL]_0 = 0.2$ M dissolved in 7:3 water/dioxane, whereas a solution of $[NBP]_0 = 6 \times 10^{-4}$ M, also in 7:3 water/dioxane, was maintained in syringe B. Since lactone was present in large excess, it may be assumed that all the NBP was consumed in the alkylation reaction. 12.5 μL of Et_3N was rapidly injected from syringe C to mixtures of 50 μL of lactone and 50 μL of NBP, prepared by injections from syringes A and B. The very fast proton transfer [15] from the sp^3-C atom that interlinks both adduct phenyl groups to the Et_3N molecule stops the NBP alkylation reaction due to the drastic loss of NBP nucleophilicity. As a consequence of its deprotonation, that C atom passes into the sp^2 hybridization state; an internal rearrangement occurs with π-delocalization between phenyl groups, and a blue color develops, which permits the reaction to be monitored (Scheme 1).

When the NBP alkylation reaction by AAL was stopped, the blue color induced by the addition of Et_3N showed maximum absorption at $\lambda = 583$ nm. After an alkylation time of 17 ms (Fig. 2), curves t_i and t_{i+1} were superimposed, showing that the alkylation reaction ends in that time.

Acquisition and analysis of kinetic signals were carried out using the Bio-Kine software from Bio-Logic. Temperatures were kept constant to ±0.05°C using a Lauda Ecoline RE112 thermostat. Kinetic runs were performed in quintuplicate.

98% AAL, 98% FUR, and 99% triethylamine (Et_3N) were obtained from Aldrich (Steinheim, Germany), all of them being used without further purification. NBP was obtained from Sigma (St. Louis, MO). Dioxane was purchased from Panreac (Barcelona, Spain).



Scheme 1

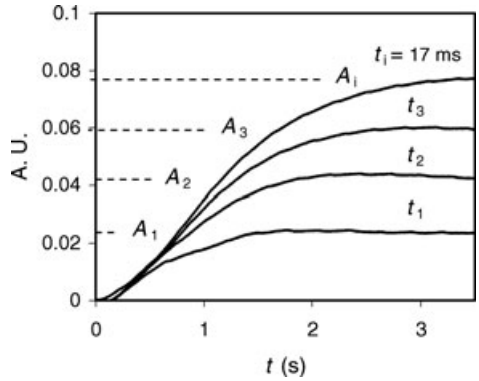


Figure 2 AAL–NBP adduct formation. Absorbance values $A_1, A_2, A_3, \ldots$ (arbitrary units) were measured when the plateau was reached, $t_1, t_2, t_3, \ldots$ being the times at which the alkylation was stopped by injecting 12.5 μL of Et_3N onto mixtures of 50 μL of lactone and 50 μL of NBP. Time on abscissas corresponds to the development of the blue color. $[NBP]_0 = 3 \times 10^{-4}$ M; $[AAL]_0 = 0.1$ M; $T = 27.5°C$.

RESULTS AND DISCUSSION

Experiments performed to measure the influence of the AAL concentration revealed the reaction to be first-order with respect to this reagent

$$\frac{d[AD]}{dt} = k_{alk}[AAL][NBP] = k_1[NBP] \qquad (1)$$

where [AD] represents the concentration of the AAL–NBP adduct, and $k_1 = k_{alk}$ [AAL] is the pseudo-first-order rate constant.

Figure 3 shows the fitting of the absorbance A_1, A_2, A_3, ... values ($\lambda = 583$ nm) and those of the t_1, t_2, t_3, ... times to the integrated form of the rate

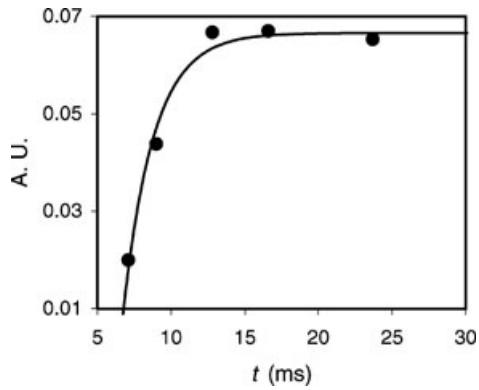


Figure 3 Variation in absorbance (arbitrary units) with time. Fitting of the results to Eq. (2). $[NBP]_0 = 3 \times 10^{-4}$ M; $[AAL]_0 = 0.1$ M; $T = 27.5°C$.

Table I Alkylation Rate Constant as a Function of Temperature for α-Angelicalactone in 7:3 Water/Dioxane Medium

T (°C)	$10^{-3}\ k_{alk}$ ($M^{-1}\ s^{-1}$)[a]
20.0	4.9 ± 0.1
22.5	5.5 ± 0.1
25.0	6.0 ± 0.1
27.5	6.3 ± 0.1
30.0	7.2 ± 0.1
32.0	7.9 ± 0.1

[a] As k_{alk} in Eq. (1). Rate constant values are given with their standard errors.

equation (1)

$$A_t = A_\infty \exp(-k_1 t) \qquad (2)$$

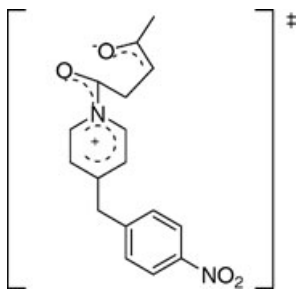
A_t and A_∞ being absorbance at t and infinite times, respectively.

Table I gives the values of the alkylation rate constant (as k_{alk} in Eq. (1)) at different temperatures. Table II shows the values of the activation parameters. The activation parameters determined previously for BPL and BBL are also included.

The results in Tables I and II show the extremely high reactivity of AAL to NBP as compared with that of lactones such as BPL and BBL [7]. As can be seen, both $\Delta H^{\#}$ and $\Delta S^{\#}$ contribute in the same direction to give a much smaller $\Delta G^{\#}$ value for AAL than for BPL and BBL. As a consequence, the formation of the lactone–NBP adduct is about 900,000-fold faster with AAL than with β-propiolactone, the most effective carcinogenic lactone [4].

The substantial gain of entropy must be caused by the lactone ring being open in the transition state. The strain relief as well as the electronic delocalization with NBP (Scheme 2) implies $\Delta H^{\#}$ loss, as was indeed observed (Table II).

It may be concluded that the formation of the AAL–NBP adduct is an entropy-strain-catalyzed reaction caused by an early lactone ring cleavage.



Scheme 2

Table II Activation Parameters for NBP Alkylation by α-Angelicalactone in 7:3 Water/Dioxane Medium

Lactone	$\Delta H^{\#}$ (kJ mol^{-1})[a]	$-\Delta S^{\#}$ (J K^{-1} mol^{-1})[a]	$\Delta G^{\#}$ (35°C) (kJ mol^{-1})
α-Angelicalactone[b]	26 ± 2	86 ± 6	52 ± 2
β-Propiolactone[c]	41 ± 2	148 ± 6	87 ± 2
β-Butyrolactone[c]	47 ± 2	148 ± 6	93 ± 2

[a] Values are given with their standard deviations.
[b] This work.
[c] Values are taken from [7].

The kinetic results are consistent with the antimutagenic capacity of AAL: (a) since an intact lactone ring is necessary for carcinogenesis [16], early cleavage of AAL ring blocks its potential mutagenic ability; and (b) because former results suggest that one of the mechanisms of inhibition of carcinogen-induced neoplasia by AAL is through the inhibition of DNA adduct formation [9], the very fast formation of AAL-adducts with nucleophilic substrates could be one of those inhibiting mechanisms.

We thank the referees for their valuable comments.

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