AFINIDAD

REVISTA DE QUÍMICA TEÓRICA Y APLICADA
EDITADA POR LA ASOCIACIÓN DE QUÍMICOS E INGENIEROS
DEL INSTITUTO QUÍMICO DE SARRIÁ

Kinetics of the Thermal Decomposition Reaction of Ascaridole in Solution

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RESUMEN

Existen diferencias entre los valores de algunas propiedades físicas de ascaridol natural aislado del vegetal denominado vulgarmente «Paico» (*Chenopodium ambrosioides* L., Chenopodiaceae) y la sustancia sintética obtenida mediante la reacción de fotooxigenación de α-terpineno. Es más, el uso de decocciones de dicha planta, utilizadas como medicina antihelmíntica en los humanos, ha despertado un debate científico debido a las propiedades tóxicas atribuídas a su principio activo (ascaridol), el cual sería responsable de la muy conocida acción beneficiosa. En este trabajo se ha realizado la preparación de esa sustancia por el método de Schenck, pero trabajando a 0 ºC con alcohol isopropílico como solvente y efectuando su separación y purificación a temperatura ambiente por cromatografía en columna preparativa, a fin de prevenir su degradación térmica. De esta manera se obtuvieron excelentes rendimientos de ascaridol (ca. 99% GC-FID), de un alto grado de pureza (> 98% GC; RP-HPLC; ^{1}H y ^{13}C RMN). Además, se informan aquí los parámetros de activación de la reacción de descomposición térmica de ascaridol en solución de n-hexano, alcohol isopropílico y metanol, en el ámbito de temperaturas de 120ºC a 170ºC. Es evidente también un efecto de solvente en la homólisis unimolecular de esa sustancia.

Palabras clave: **Endoperóxidos. Ascaridol. Estabilidad térmica. Cinética de reacción. Sustancias antimaláricas.**

SUMMARY

Discrepancies exist between the values of some physical properties of ascaridole isolated from the vegetal named «Paico» (*Chenopodium ambrosioides* L., Chenopodiaceae) and the synthetic substance obtained by the photoxygenation reaction of α-terpinene. Moreover, the use of decoctions of the above mentioned plant as anthelmintic medicine in humans arouse a scientific debate because of toxic properties attributed to its main active drug (ascaridole), which would be responsible of the well-known beneficial action. This lead to perform here the preparation of that substance by Schenck's method, but working at 0 ºC with isopropyl alcohol as solvent and doing its separation and purification at room temperature by preparative column chromatography to prevent its thermal degradation. Thus, excellent yields of ascaridole were obtained (ca. 99 % GC-FID) of high degree of purity (> 98% GC; RP-HPLC; ^{1}H and ^{13}C NMR). Furthermore, the kinetics of the thermal stability of ascaridole in solution is now advanced, reporting the activation parameters values for their decomposition reactions in *n*-hexane, isopropyl alcohol and methanol, in the 120º-170 ºC temperature range. A solvent effect on the unimolecular homolysis of that substance is also evident.

Key words: **Endoperoxides. Ascaridole. Thermal Stability. Reaction Kinetics. Antimalarial Substances.**

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RESUM

Hi ha diferències entre els valors d'algunes propietats físiques de l'ascaridol natural, aïllat del vegetal dit vulgarment «Paico» (*Chenopodium ambrosioides* L., Chenopodiaceae), i la substància sintètica obtinguda mitjançant la reacció de fotooxigenació d'α-terpinè. Encara més, l'ús de decoccions de l'esmentada planta, emprades com a medicina antihelmíntica en humans, ha despertat un debat científic, donades les propietats tòxiques atribuïdes al seu principi actiu (ascaridol), que seria el responsable de la molt coneguda acció beneficiosa. En aquest treball, s'ha realitzat la preparació d'aquesta substància pel mètode de Schenck, però treballant a 0 ºC amb alcohol isopropílic com a dissolvent i efectuant la seva separació i purificació a temperatura ambient mitjançant cromatografia de columna preparativa, per tal de prevenir la seva degradació tèrmica. D'aquesta manera, s'obtenen excel.lents rendiments d'ascaridol (*ca*. 99% GC-FID), d'un alt grau de puresa (> 98% GC; RP-HPLC; ^{1}H i ^{13}C RMN). A més, es presenten els paràmetres d'activació de la reacció de descomposició tèrmica de l'ascaridol en dissolució de *n*-hexà, alcohol isopropílic i metanol, en el marge de temperatures de 120ºC a 170 ºC. Així mateix, és evident un efecte del dissolvent en l'homòlisi unimolecular d'aquesta substància.

Mots clau: **Endoperòxids. Ascaridol. Estabilitat tèrmica. Cinètica de reacció. Substàncies contra la malària.**

INTRODUCTION

The synthesis, properties and kinetics of the thermal stability in solvents of some substituted cyclic peroxides (1,2,4-trioxanes, 1,2,4,5-tetroxanes and endoperoxides), continue to be relevant mainly for their potential therapeutic action as antimalarial agents. In principle, one can think that as these molecules are peroxides, they should be thermally unstable, considering the reduced strength of their peroxydic bonds. On the contrary, most of these compounds resist heating to relatively high temperatures, either in solid state as in certain solvents. Nevertheless, their thermal decompositions is very sensitive to the type of the solvent used or metallic ions eventually present in the solution[1]. Artemisinin, as an example, is a natural sesquiterpenic lactone belonging to the family of the 1,2,4-trioxanes, that can be heated to 190 ºC in solid state before the molecule breaks down; on the other hand, this substance in benzene, methanol or ethanol solution is thermally much less stable[2], being also catalyzed its decomposition by the presence of ferrous ions[3].

Ascaridole (**I**) is a monoterpenic endoperoxide (1-methyl-4-(1-methyethyl-2,3-dioxa-bicyclic [2.2.2] oct-5-ene, 1,4-epidioxi-*p*-menthane or 1,4-peroxide-*p*-ment-2-ene, with molecular formula $C_{10}H_{16}O_2$ and CAS number 512-85-6, which constitutes the main component (60-80%) of the volatile oil of *Chenopodium ambrosioides* L., Chenopodiaceae. Initially **I** was isolated by steam distillation independently by Nelson and Wallach[4,5] from the vegetal commonly denominated «Paico» (foot of goose, Epazote, Aritasou, Herb of Santa María or Wormseed, names assigned according to the countries where it is grown as a weed). Their infusions and prepared decoctions with different liquids are still continued applying for the popular treatment of several parasitic affections, although discrepancies exist on its therapeutic effectiveness and grade of toxicity.

After almost 40 years without being continued the investigations on that substance, Beckett *et alt.*[6] isolated with excellent yields the active principle of «Paico» using column chromatography on silica gel, identified as ascaridole and further employed as an analytical standard. These authors as well reported the main physical properties of that substance ($n^{D}_{20\,°C}$ 1.4733; boiling point, 37°-38 ºC / 0.15 Torr); 39°-40 ºC / 0.2 Torr; density$_{20\,°C}$ 1.0113; freezing point 3.3 ºC), values that almost are coincident with those presented later on in the Merck Index[7].

In this work the Schenck´s method[8] to prepare ascaridole had been verified but performing the photoxidation reaction of α-terpinene in isopropyl alcohol as solvent and at much lower temperature. Furthermore, it was changed the methodology used for the purification of the crude reaction product, diminishing significantly the formation of hydroperoxydic by-products, which were more recently identified[9]. This procedure allowed to achieve with excellent yields a substance appropriate to determine the kinetic parameters of the thermal decomposition reactions of **I** in different solvents and, eventually to perform toxicological essays, because it is free of the hydroperoxydic contaminants already detected in the oil of *Chenopodium ambrosioides L*.

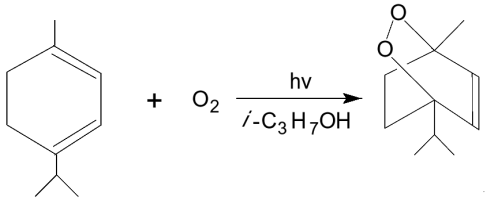
EXPERIMENTAL

Materials

Solvents of analytical grade were employed, carefully purified by distillation, including the removal of metal-ionic traces by treatment with the sodium salt of ethylenediaminotetracetic acid (Na_2-EDTA). This procedure precludes the catalytic decomposition of **I** through an ET mechanism of reaction[10], which also may take place with other cyclic peroxides in solution[1].

Preparation of ascaridole

In a typical experiment 5 g (37 mmoles) of α-terpinene (Sigma-Aldrich Co., Catalog Nº T-225, 89% w/w, GC) dissolved in 200 mL of isopropyl alcohol (Baker, p.a.), added of 0.1 g of methylene blue (Fluka, Purum) as photosensibilizant agent, were placed in a Pyrex beaker (500 mL of capacity) surrounded by an ice-NaCl bath (at ca. 0 ºC) in which, with mechanical agitation an oxygen stream was bubbled, exposing the mixture to intense day-light radiation in successive periods (in total ranging 2 - 48 h) during the different runs performed.



(1)

The progress of the preparation of **I** (eq. 1) was monitored every 40 minutes by TLC analysis on commercial aluminum-sheets covered with silica gel (Merck, 60 A type) and eluting either with an ethyl acetate (5% v/v) / *n*-hexane or ethyl acetate (7% v/v) / toluene, mixtures. During the course of the experiences an intense brown stain (TLC, R_f = 0.43-0.50) was detected by the vanillin-sulfuric acid developer[11], accompanied by other 3 tiny spots (R_f = 0.38, 0.21 and 0.13, respectively), which corresponded to relatively much smaller concentrations of confirmed peroxydic substances. Once an increment of the intensity of the main stain was not observed, the reacting solution was alternatively processed, as following:

a) After reduction of the initial volume by evaporation of the solvent at reduced pressure (from 25 ºC / 2 Torr to a Pyrex trap immersed at cal. -190 ºC in liquid air, the residue was processed in a chromatographic preparative column (4 cm i.d. x 50 cm length) filled with Silicagel 60 (Merck, 230-400 ASTM mesh) previously activated by heating at 120 ºC. The eluted diethyl ether fraction (ca. 200 mL) was further evaporated at reduced pressure (Rotavapor, 25 ºC / 100 Torr), finally obtaining a pale yellow and rather volatile viscous residue identified as crude ascaridole.

b) The solution was successively extracted with petroleum ether (30º- 65 ºC boiling fraction), *n*-hexane and dichloromethane (this solvent with added water drops to achieve the best separation of the organic layer) and further treatment with anhydrous Na_2SO_4. Finally the pale yellow liquid was evaporated at reduced pressure and room temperature, obtaining a clear oily residue of crude ascaridole.

The impurities detected by monitoring the crude reaction product showed characteristic acidities (pH ca 5.8) after spot tests performed on the TLC plates. Then, the crude ascaridole was further treated at room temperature successively with an aqueous $NaHCO_3$ solution and with anhydrous Na_2SO_4. Thus the mixture of isomeric hydroperoxydes already reported by Matush[9] were effectively removed (TLC and GC analysis). Alternatively, those impurities were also removed by preparative column chromatography on Silicagel using a petroleum ether (30º- 65 ºC boiling range) / 1 % isopropyl alcohol mixture as eluant.
In both of the above procedures more than 4 g (> 98 % w/w yield) of a clear and viscous liquid was identified as ascaridole (> 99 % w/w, GC, freezing point ca. 3 ºC, lit. 3.3 ºC[6], ^1H and ^{13}C NMR[12]. The GC analysis of **I** in *n*-hexane solution showed a RT ca. 13.8 min in a fused silica capillary column (0.25 i.d. x 30 m length) with polymethylsiloxane as stationary bonded phase (0.25 µm film thickness), programmed from 50 ºC to 180 ºC using nitrogen as carrier gas (1 mL / min flow rate) and the injector port at 90 ºC in a 8000 model Perkin Elmer Series 2 gas chromatograph with FID detection.

Kinetic Methods

Closed in an extreme Pyrex tubes (8 cm length, 4 mm i.d.) loaded with ca. 0.3 mL of the **I** stock solution (ca. 0.02 M, GC) in the corresponding solvents, were degassified in the vacuum line (at -190 °C) and then sealed with a flame torch. The ampoules were introduced in a thermostatic silicone oil bath (± 0.1 °C), removing one by one at fixed reaction times and stopping **I** decomposition by further immersion in an ice-bath. The determination of the concentrations of **I** remainder in the solutions were generally carried out by capillary GC analysis although a RP-HPLC technique was also employed in some runs to check the already obtained kinetics results.

Reaction Product Analysis

Solutions of **I** (ca. 0.02 M) in the selected solvents were loaded (2 mL) as indicated above and introduced in the thermostatic silicone oil bath in the 130º-160 ºC temperature range by periods of ca. 2 hours. The main organic products observed (GC, RT in min) in the **I** thermolysis were *p*-cymene (ca. 7.3) and 1,2-3,4-bis epoxy-1-methylethyl-4-methyl cyclohexane (isoascaridole, ca. 13.0), both identified by comparison of their chromatographic retention data with that of authentic samples or by examining the quadrupolar MS spectrum of the corresponding GC peaks.
Those findings correspond with results reported[13] in the thermolysis of neat ascaridole at 140 ºC.

Calculation Methods

The reaction rate constant values (k_{exp}) of the thermal decomposition of **I** were calculated using a first-order kinetic law, evaluating the adjustment to a lineal regression for minimum squares. The corresponding activation parameters of the initial unimolecular reactions were obtained applying the Arrhenius equation method and the error limits with a computational procedure[14].

RESULTS AND DISCUSSION

The thermal decomposition of **I** investigated in different solvents follows pseudo-first order kinetic laws until ca. 80 % conversions (Figure 1).

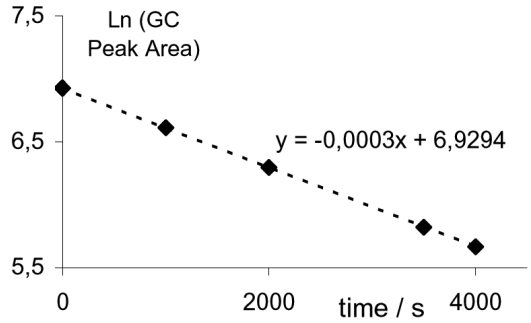
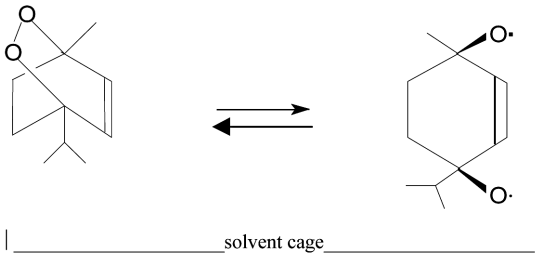


Figure 1. Typical First-order Kinetic Plot for the Thermolysis of **I** (0.02 M) at 140 ºC in *i*-propyl alcohol Solution.

The initial concentrations of **I** in the kinetic experiments performed in *n*-hexane, *i*-propyl alcohol and methanol solution are sufficiently low (ca. 0.02 M). This condition allow to conclude that induced decomposition processes in the **I** thermolysis are non-significant. Then, it is reasonable to suppose that the observed rate constants values (k_{exp}, Table I) actually correspond to the unimolecular decomposition reactions of that molecule.
Consequently, in the reaction cage of **I** thermolysis the formation of a non-saturated 1,6-dioxy-diradical by cleavage of its peroxydic bond can be reasonably postulated (eq. 2).



(2)

Hence, the temperature effect on the rate determining step of the decomposition reactions of **I** (eq. 2) in the 120.0º-170.0 ºC temperature range and in the different solvents investigated, can be represented (Figure 2) by the following Arrhenius equations.

$$\ln k\ (s^{-1}) = (28.2 \pm 0.1) - (29293 \pm 500)\ /RT \text{ (in } n\text{-hexane)}$$

$$\ln k\ (s^{-1}) = (26.3 \pm 0.2) - (28235 \pm 600)\ /RT \text{ (in } i\text{-propyl alcohol)}$$

$$\ln k\ (s^{-1}) = (8.7 \pm 0.4) - (13383 \pm 700)\ /RT \text{ (in methanol)}$$

TABLE I

First-Order Rate Constant Values for the Thermal Decomposition. Reactions of I (0.02 M) in *n*-hexane, *i*-propyl alcohol and Methanol Solutions.

Temp K	Reaction Solvent	k_{exp} x 10^3 s^{-1}
393.2	*n*-hexane	0.086
408.2	*n*-hexane	0.34
413.2	*n*-hexane	0.54
428.2	*n*-hexane	1.80
443.2	*n*-hexane	6.00
393.2	*i*-propanol	0.050
408.2	*i*-propanol	0.20
413.2	*i*-propanol	0.31
433.2	*i*-propanol	1.00
443.2	*i*-propanol	3.00
393.2	Methanol	0.20
408.2	Methanol	0.40
413.2	Methanol	0.50
431.2	Methanol	0.94
443.2	Methanol	1.32

The linearity of these plots ($R^2 \geq 0.9928$) over a relatively large temperature range supports that the calculated activation parameters for I reactions (Table II) belong to single processes.

TABLE II

Activation Parameters Values calculated at 140 ºC for the Unimolecular Thermolysis of I in Solution

Reaction Solvent	$\Delta H^{\#}$ kcal mol^{-1}	$\Delta S^{\#}$ cal mol^{-1} K^{-1}	$\Delta G^{\#}$ kcal mol^{-1}
n-hexane	28.5 ± 0.5	–3.2 ± 1.0	30.6 ± 0.5[a]
i-propanol	27.4 ± 0.6	–6.9 ± 3.0	31.1 ± 0.6[a]
Methanol	12.6 ± 0.7	–41.9 ± 8.0	30.7 ± 0.7[b]

Note: [a] analysis of I performed by GC (FID), in a «megabore» column (530 µm i.d., 10 m length) at 100 ºC, injector port at 90 ºC; RT ascaridole ca. 11 min; [b] analysis of I performed by RP-HPLC, with C18 as stationary phase in a 10 cm Spherisorb column with MeOH (75%)-water mixture as eluant at 25 ºC, RT ca. 4.3 min , using a differential refraction index as detector.

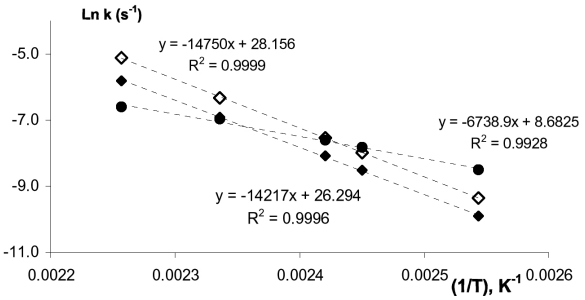
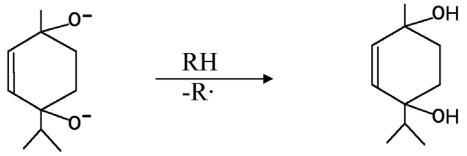


Figure 2. Arrhenius Equation Plots for the Unimolecular Thermolysis of I in Solvents. Symbols: ◇, *n*-hexane; ◆, *i*-propyl alcohol; •, methanol.

The activation enthalpy obtained in the hydrocarbon solvent correspond to the already estimated BDE value (29 kcal mol^{-1}) for the I molecule in the gas phase[10]. These figures are significantly lower than the value of 37 kcal mol^{-1} accepted for the O-O bond rupture in most of the acyclic and cyclic peroxide molecules[15, 16]. The differences are expected because molecular strain and repulsive forces due to eclipsing interaction of the lone pairs on the oxygen atoms of I molecule. However, in the more polar environment of reaction as the alcohol solvents the corresponding values for the thermolysis of I (Table II) reflect a significant solvent effect on the unimolecular reactions considered. On the other hand, the entropy of activation values observed in all the solvents investigated are in keeping with the above contention, rendering for the three reactions nearly similar energy of activation values (Table II).

The postulated initial diradical (eq. 2) is probably an unstable specie, that it would originate the reaction products observed (see **EXPERIMENTAL**). Hence, the other main stages of the thermolysis of I can be represented by the following equations **(Scheme)**.



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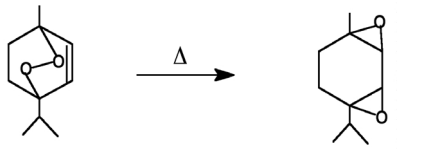

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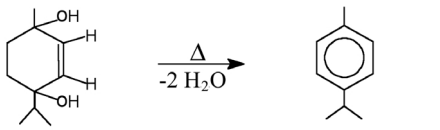

(5)

The formation of the isomeric diepoxide (isoascaridole, eq. 4), a secondary product observed, represents the thermal rearrangement reaction of I molecule which had been reported to occur heating this substance in inert solvents[17] or in the presence of ruthenium (II) catalysts[18].

CONCLUSIONS

The ascaridole obtained by photoxydation of α-terpinene in isopropyl alcohol solution at ca. 0 ºC with intense solar radiation and further isolation from a Silicagel chromatographic preparative column, represents a product of excellent quality, appropriate for kinetic studies and other applications, which do not exhibit other peroxydic substances, effectively detected in the natural ascaridole isolated from «Paico». The activation parameters values for the unimolecular homolysis of ascaridole in the solvents investigated are comparable with those observed for the rupture of peroxydic bonds of more complex organic molecules. Furthermore, it is significant a kinetic solvent effect on the reactions investigated.

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