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ULTRASOUND IN CHEMICAL AND ENZYMATIC SYNTHESES

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SUMMARY

The effects of ultrasound were evaluated on the following reactions: preparation of β-hydroxyketones through the aluminium amalgam reductive opening of α,β-epoxy ketones; oxidation of homoallylic alcohols to enediones using the oxidant system tetra-*n*-propylammonium perruthenate / 4-methyl- morpholine N-oxide; oxidation of non-activated carbons performing the reaction of bromohydrins in the presence of (diacetoxyiodo) benzene (DIB) and iodine; hydrolysis of esters with Al_2O_3/KOH and enzyme-catalysed transesterifications. All the reactions were greatly improved by ultrasonic irradiation which made possible yields unavailable under classical conditions and also shorter reaction times.

INTRODUCTION

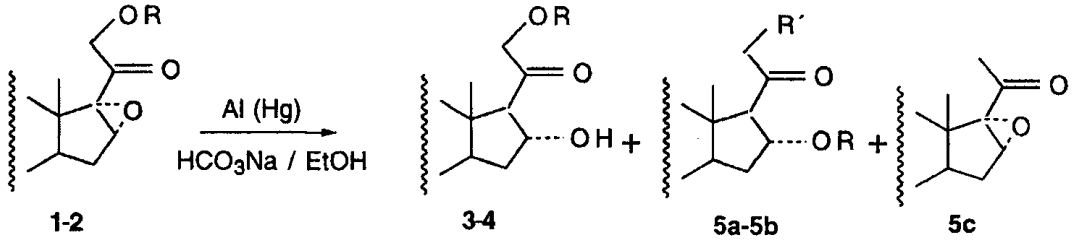
The term sonochemistry has been applied to refer the use of sound energy to affect chemical processes. The interest on this subject has expanded greatly over the last few years. There are a range of applications for the uses of ultrasound (20-40 KHz) in chemistry which includes organic synthesis, a target domain to the research in this field. The use of sonic waves in conjunction with whole cells or isolated enzymes to improve reactivity has been also deserving a growing attention and a few examples are already available in the literature.[1, 2]

Since 1989 our group has been performing some research in this field. Among the results that we have obtained up to now a few examples were selected to illustrate the synthetic possibilities offered by sonochemistry. It should be emphasized that almost all the sonochemical reactions reported herein, are steps of primordial importance in complex multistep synthetic sequences, leading to compounds which have relevant properties.

REDUCTIONS

Heterogeneous processes involving electron transfer in several steps have been object of numerous studies. The use of metals as reagents is an area of synthesis in which ultrasound has already made significant contributions. Reductive opening of α,β-epoxy ketones to give β-hydroxyketones using aluminium amalgam (Scheme 1) is a well known and useful reaction to synthesize natural products. Application of ultrasound offered a more expedient way of carring out these heterogeneous reductions.[3] Ultrasonic reactions were 4 times faster than the reactions conducted under magnetic stirring and the yields also suffered a marked enhancement. The method proved to be specially useful to accomplish the reduction of the substrates α´-oxygenated **1** (R=H) and **2** (R=Ac), allowing an improvement of the regioselectivity observed and, therefore, better yields of the corresponding β-hydroxyketones **3** and **4** were achieved (Scheme 1). The reaction times have to be carefully controlled, otherwise the amount of the unwanted by-products **5a-5c** suffer a dramatic increase.

Scheme 1



1-2 3-4 5a-5b 5c

Substrate[a]	R	Reaction Conditions[b]	Product (%)[c]	By-Product(s)[c]
1	H	20 min. / ↻	3 (23)	4% 5a (R=R'=H)
		20 min. /)))	3 (80)	14% 5a (R=R'=H)
2	Ac	60 min. / ↻	4 (40)	10% 5b (R=Ac, R'=OH) + 5c
		60 min. /)))	4 (63)	15% 5b (R=Ac, R'=OH) + 5c

a) Substrate: 0.15 mmoles; b) ultrasonic irradiation:))); magnetic stirring: ↻ c) Quantified by HPLC and identified by ^{1}H and ^{13}C nmr

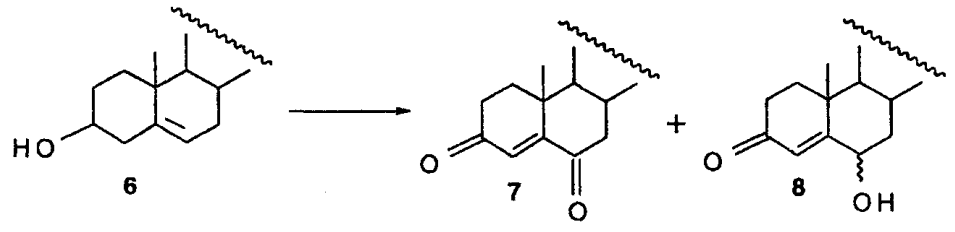
The use of a probe (immersion titanium horn, with variable acoustic potency up to 250 W, equipped with a flat tip, 12.7 mm diameter at the irradiating surface) was found to be more effective in promoting the reaction than an ultrasonic cleaning bath (40KHz). For reactions performed at 25°C an optimum acoustic intensity at 100 Wcm^{-2} was observed. Further increase of the ultrasonic intensity led to slower reactions. Evaluation of the temperature effects was also carried out. The results obtained suggest a mass transport dependence either for the reductions performed under magnetic stirring or upon the optimized ultrasonic conditions. As often observed for other reactions involving metals, a marked ultrasound effect was also visible on aluminium amalgam surface. Fractures of the protective oxide coating and erosion of the surface were observed by scanning electron microscopy thereby, increasing the surface area of the metal.

OXIDATIONS

Homoallylic Alcohols

A synthetically useful reaction which also benefits from the use of ultrasound is the oxidation of homoallylic alcohols **6** to the enediones **7** using the oxidant system tetra-*n*-propylammonium perruthenate / 4-methyl-morpholine N-oxide (Scheme 2).[4]

Scheme 2



6 7 8

Reaction Conditions[a]	Product 7 (%)	By-Products 8 (%)
↻	42	7
)))	82.5	6

a) Substrate: 1 mmole; TPAP / NMO: 0.1/3 mmoles; reaction time : 90 minutes; reaction temperature: 25°C (±2°C); ultrasonic irradiation:))); magnetic stirring: ↻

This is an example of a reaction which does not occur efficiently in the absence of ultrasonic irradiation. In fact, even when the experiment time was prolonged to 8 hours a significant amount of starting material remained unchanged. Under the influence of ultrasound (ultrasonic cleaning bath 225-450 W, 35 KHz) a full conversion occurred, affording higher yields of the isolated products in very short reaction times. Small amounts of hydroxy-enones **8**, mainly the β-hydroxy epimer, were also detected in the crudes of all the oxidations performed. These by-products were quantified by HPLC and identified by ^{1}H and ^{13}C nmr. The results presented in Scheme 2 refer to the silent and ultrasonic oxidation of cholesterol.

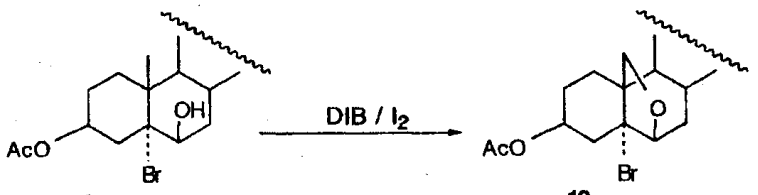
The method is quite versatile revealing a wide applicability to perform such conversion on steroidal homoallylic alcohols either of the pregnane, cholestane or androstane series.[4] Besides the convenient effects of ultrasound on yield and rate of the mentioned reaction with minimal side effects, this oxidation allows very mild reaction conditions. This is specially relevant for substrates containing labile funcionalities, namely acid sensitive groups as silyl enol ethers. Therefore, such sonochemical reaction provides a distinct alternative to other chemical processes since allows the efficient one-pot conversion of homoallylic alcohols to enediones.

Although the mechanism of this homogeneous oxidation is not completely understood, it is probable that yet again ultrasound is promoting single electron transfer processes.

Non-Activated Carbons

In connection with another project, the remote oxidation of non-activated carbons has been investigated performing the reaction of bromohydrins **9** in the presence of (diacetoxyiodo) benzene (DIB) and iodine (Scheme 3).[5] Through this process alkoxyl radicals strategically located to perform the intramolecular hydrogen abstration can be generated. Usually this type of functionalization on alcohols is promoted by photolyses. However, in these conditions (200W tungsten filament lamps) the bromohydrins failed to reach full conversion. Consequently an alternative energising method was demanded to make efficient this radical process which is typically a type I reaction according to the accepted classification of the ultrasonic reactions proposed by Luche.[1, 2] Therefore, replacement of light irradiation for ultrasound was immediatly considered. Thus, carring out the *in situ* sonolyses (ultrasonic cleaning bath Bandelin Sonorex Super RK 510H, 225-450 W, 35 KHz) of the reaction mixture containing bromohydrins **9**, the efficient synthesis of the corresponding cyclic ethers **10** was attained. The results presented in Scheme 3 refer to the reaction performed on the bromohydrin derivative of the androst-5-en-17-one using ultrasonic optimized conditions.

Scheme 3



9 **10**

Irradiation Conditions[a)]	Product (%)
light	77
)))	92

a) substrate:0.12 mmoles; solvent: cyclohexane/benzene (10:1); reaction time: 50 minutes; reaction temperature: 45°C; light irradiation: 200 w (tungsten lilament lamps); ultrasonic irradiation: ultrasonic bath 225-450 W, 35 KHz

This route has the advantage of simplicity, the products being produced in high yield and purity and under mild reaction conditions. Moreover, identical derivatives of the cholestane and the pregnane series are also readily accessible by the same ultrasonic approach.[6]

CLEAVAGE OF BASE—LABILE PROTECTING GROUPS

The overall strategy to the synthesis of polyfunctional molecules usually requires a correct choice of several protecting groups that must be cleavable under different conditions at an appropriate moment of the process. This approach can lead to a significant increase in the length of the synthesis with the inherent risk of unwanted losses of product. A more advantageous possibility is the use of protecting groups from the same class which can be removed in a single step. The successeful application of this alternative strategy in the synthesis of sensitive

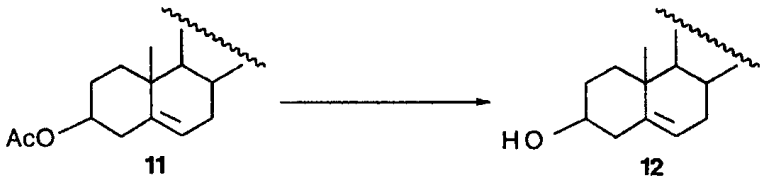
complex molecules,[7] led us to consider the study of convenient protection / deprotection sequences on polyhydroxylated steroids of the pregnane, androstane and cholestane series. In this report we present the preliminar results obtained when ultrasonic irradiation was applied to enhance the potentialities of a chemical and an enzymatic methodology to perform the deprotection of hydroxy groups. As acylation is one of the best-established OH protection methods, the polyhydroxylated substrates commercially available or prepared according to procedures already reported, were uniformly protected as acetates.

Hydrolysis of Esters with Al_2O_3 / KOH

Combined use of alumina and potassium hydroxyde has been known for a long time.[8] However, it has so far received little attention as a synthetic reagent potentially useful in the hydrolysis of esters. As part of our ongoing studies dealing with the synthesis and protection / deprotection of polyhydroxylated substrates we have reinvestigated this heterogeneous procedure.

Hydrolysis of steroidal substrates of type **11** (Scheme 4), carried out as previously described in the literature,[8] proceeded with very low conversion rates. The reaction time was extended to about 72h, however the hydrolysis were still far from reach total conversion. In order to improve these results, the reaction mixtures were submitted to ultrasonic irradiation using an immersion ultrasonic probe. Under these conditions, the reaction times were dramatically reduced (5-7hours) making the hydrolytic process really efficient. Toluene was found to be a suitable solvent for the sonochemical reaction affording good to excellent yields of the deprotected hydroxylated steroid **12**, after a straightforward work-up. The compatibility of this optimized hydrolytic procedure with labile functionalities is under investigation.

Scheme 4



11 **12**

Enzyme-Catalysed Transesterification

Enzymes can be advantageously employed to hydrolyze sensitive compounds, which would decompose extensively under conventional conditions for ester hydrolysis (i.e. strong acid or alkali).[9] Moreover, in the mid-1980s it was found with great surprise, that some enzymes, as *Candida Cylindracea* lipase, work efficiently in organic solvents containing a trace quantity of water.[10] The functional complexity of some synthetic intermediates led us to explore the potentialities of this mild biotransformation. Thus, on using *Candida Cylindracea* lipase in isopropyl ether as catalyst, the transesterification of a steroidal acetate analogue of type **11** (Scheme 4) with octanol was attempted. Under these conditions, the conversion rate into the corresponding hydroxy derivative was very low. In fact, after 120 hours the reaction remained incomplete. Ultrasonic irradiation has been also reported as an effective methodology to promote some enzymatic transformations.[1] Therefore, sonication was applied to the lipase-catalysed transesterification. Through such approach a quite remarkable improvement of this enzymatic reaction was achieved. In fact, using an ultrasonic cleaning bath (225-450 W, 35 KHz) and keeping the temperature at 38°C (± 2°C), ca. 66-70% conversion was achieved in a very short reaction time (10 hours). Further experiments will be undertaken to optimize the sonicating conditions and to apply this biotransformation to polyhydroxylated substrates.

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