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Chemical modifications of bile acids under high-intensity ultrasound or microwave irradiation

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Abstract

High-intensity ultrasound (HIU) and microwave (MW) irradiation, having emerged as effective promoters of organic reactions, were exploited for the synthesis of bile acids derivatives. Esterification, amidation, hydrolysis, oxidation, and reduction were investigated. Compared to conventional methods, both techniques proved much more efficient, increasing product yields and dramatically cutting down reaction times. Scaled-up studies are now under way.

Keywords: High-intensity ultrasound; Microwave; Synthesis; Cholic acid; Glycocholic acid; Taurocholic acid

1. Introduction

Bile acids (BA) have been the subject of numerous pharmacological studies. Their amphiphilic character generally increases cell membrane permeability. As they enhance the absorption of hydrophobic drugs, they can be used to improve the delivery of drugs that specifically target the liver [1]. Some BA derivatives are potent antibiotics against Gram-negative bacteria [2,3], and some dimeric BA have shown marked antifungal and antiproliferative activity in vitro [4]. Cosalanecholic adducts have been successfully tested as anti-HIV agents [5]. BA may be safely administered to patients suffering from altered cholesterol biosynthesis and metabolism [6]. Ursodeoxycholic acid (UDCA) and its hepatic metabolites, glyco- and tauroursodeoxycholic acids (GUDCA and TUDCA), are widely used to treat cholestatic liver disease [7] and to promote the dissolution of cholesterol gallstones. UDCA treatment reduces total and vesicular cholesterol, as well as viscosity, total amount of sedimentable fractions, and the formation of cholesterol crystals in gallbladder bile [8]. While pathological concentrations of the more hydrophobic BA will induce hepatocyte apoptosis (as in cholestatic disorders) [9,10], of great interest is the recent discovery that apoptosis is inhibited by UDCA and TUDCA in both hepatic and other cells [11,12]. Increased cell survival has been observed with hepatocytes subjected to a variety of toxic agents (including ethanol) as well as with other cell types and animal models of neurological disorders, including Alzheimer's, Hungtinton's and Parkinson's diseases [12–14]. Because of these findings, TUDCA has been proposed for the treatment of neurodegenerative diseases. The recent discovery of these pharmacological properties and potential therapeutic applications has rekindled the interest of the scientific community and of the pharmaceutical industry in synthesizing and testing new analogs.

Both high-intensity ultrasound (HIU) [15] and microwave (MW) [16] have emerged as effective promoters of organic reactions. These non-conventional techniques are being increasingly exploited not only in the laboratory, but also indus-

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trially to bring reactions to completion in minutes rather than hours or days. They also can induce reactions that would otherwise be very laborious and may bring out peculiar chemoselectivities, thus opening up new synthetic pathways. Although the application domains of HIU and MW do not overlap, they both have great potential for the development of green synthetic methods. It is widely documented that HIU and MW greatly speed-up chemical reactions in comparison with conventional conditions. For a discussion of underlying mechanisms, several fundamental monographs can be consulted [17,18].

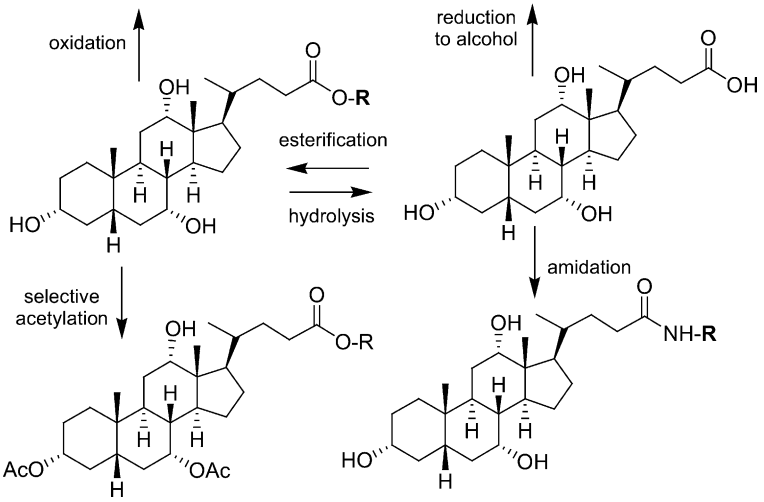
While MW irradiation has been widely used by Dayal et al. to promote specific chemical modifications of sterols and bile acids [19], such as hydrogenation [20], esterification and hydrolysis [21,22], we found only one very recent report of esterifications carried out under HIU [23]. The peculiar regioselectivity and dramatic reduction of reaction times that can be achieved by these methods prompted us to perform a more extensive study of esterification, amidation, hydrolysis, oxidation, and reduction of BA under HIU or MW irradiation (Scheme 1). We compared our results with those obtained under conventional conditions.

2. Experimental

BA used as starting materials and reference standards were obtained from PCA Spa (Basaluzzo, Italy). Other reagents and solvents were from Carlo Erba Reagenti and Acros Organics. Reactions were monitored by TLC on Fluka F_{254} (0.25 mm) plates, which were visualized by UV inspection or by spraying with molybdic acid and heating. Merck silica gel was used for column chromatography (CC). Melting points: Büchi SMP-20 (uncorrected); IR: Shimadzu FT-IR 8001 spectrophotometer; NMR: Bruker 300 Advance (300 and 75 MHz for ^{1}H and ^{13}C, respectively). For ^{1}H NMR, $CDCl_3$ was used as solvent, and C*H*Cl_3 at $\delta = 7.26$ was used as a reference. For ^{13}C NMR, $CDCl_3$ was the solvent, and *C*DCl_3 at $\delta = 77.0$ was used as a reference. Chemical shifts (δ) are given in ppm, and coupling constants (J) are given in Hz. Low-resolution mass spectra (LRMS): Finnigan-MAT TSQ70 in chemical ionization was performed with isobutane as the reactant gas. HPLC analysis: Thermo-Quest Spectra Series P200, Detectors UV/VIS Jasco 875-UV or a refractive index Gilson 133, integrator Millipore 740 Waters. The sonochemical apparatus used in the present work was designed for stringent reaction conditions [24]; it achieves optimal acoustic efficiency by rotating the reactor eccentrically around the horn axis and moving the probe alternatively up and down by a predetermined excursion and speed; frequency could be tuned between 17 and 40 kHz and power varied up to a maximum output of 200 W/cm^2. Sonochemical reactions were carried out in a PTFE flat-bottomed tube (diameter, 35 mm; thickness, 1 mm; volume, 40 mL) placed in the reactor bath that could be thermostatted down to −20 °C. MW-promoted reactions were carried out (with identical results) in two modified domestic ovens (Candy MSA 20 M and De Longhi MW 314). Temperature could be monitored with an infrared thermometer (MX2 Raytek) and also measured at the end of the reaction with a thermocouple thermometer. Among entries listed in Tables 1 and 2 , those marked with an asterisk were successively scaled up by a factor of 5 in a Milestone 1200 MW reactor. Products obtained with either kind of apparatus were identical, yields were comparable and reaction times agreed within 20%.

2.1. Esterification of cholic acid (general procedure)

(A) Cholic acid (200 mg, 0.49 mmol), *p*-toluenesulfonic acid (PTSA) or methanesulfonic acid (MSA) (0.147 mmol), the alcohol (2.45 mmol), excess anhydrous Na_2SO_4, and THF were added to the reaction vessel (a Teflon® tube for HIU, a pressure-resistant tube (pyrex) for MW, a round-bottomed flask for heating under reflux) and treated as



Scheme 1. Chemical modifications of cholic acid.