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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].

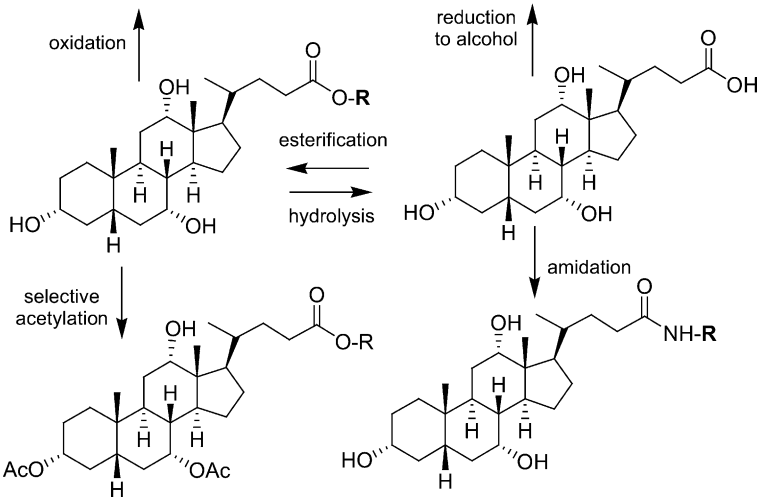
While MW irradiation has been widely used by Dayal et al. to promote specific chemical modifications of sterols and bile acids [19], such as hydrogenation [20], esterification and hydrolysis [21,22], we found only one very recent report of esterifications carried out under HIU [23]. The peculiar regioselectivity 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.

Table 1
Esterification of cholic acid

R—OH	HIU, 20.4 kHz, 25 °C, 400 W		MW[a], 400 W		Heating under reflux	
	Time (min)	Yield (%)	Time (min)	Yield (%)	Time (h)	Yield (%)
Methanol[b]	30	96	3	97	5	93
Ethanol[b]	40	94	3	95	5	92
t-Butanol[c]	40	60/89[b]	10	52/93[b]	6	41
Octanol[b,c]	60	93	10	93	5	87
Benzyl alcohol[c]	40	88	10	89	8	67
10-Undecenol[d]	60	91	12	93	6	49
2-Undecanol[d]	60	48/90[e]	14	57/91[e]	12	53
Octacos-10-enol[b,d]	80	86	20	90	24	60

[a] MW irradiation was interrupted every 3 min by a 1 min pause.
[b] Reaction scaled up by a factor of 5 in a Milestone 1200 MW reactor.
[c] Dissolved in dioxane.
[d] Dissolved in THF.
[e] Method B.

indicated in Table 1. Methyl and ethyl esters were prepared using the corresponding alcohols as solvents without THF.

(B) Cholic acid (200 mg, 0.49 mmol) was mixed in a mortar with dicyclohexylcarbodiimide (DCC) (202 mg, 0.98 mmol) and DMAP (0.049 mmol) and then added to the reaction vessel with the alcohol (1.47 mmol) and THF (3 ml).

2.1.1. *10-Undecenyl 7α,12α,3α-trihydroxy-5β-cholan-24-oate* **1**

The crude product was purified by column chromatography to yield **1** as a gum (see Table 1), TLC eluent $CHCl_3$/MeOH 9:1, R_f 0.46. IR 3400 (OH), 1736 (CO), 1716 (C–O), 1641, 1468, 1377, 1246, 1215 (C–O), 968, 912, 856; ^{1}H NMR 5.81 (m, ^{1}H, H-10′), 4.98 (ddt, ^{1}H, J = 6.7, 3.2 Hz, H-10′), 4.93 (dd, 2H, J = 20.0, 1.6 Hz, H-11′), 4.05 (t, 2H, J = 6.7 Hz, H-1′), 3.98 (brs, ^{1}H, H-12β), 3.85 (brs, ^{1}H, H-7β), 3.47 (m, ^{1}H, H-3β), 2.40–2.15 (m, 4H), 2.04 (q, 2H, J = 6.5 Hz, H-9′), 1.91–1.52 (m, 20H), 1.39–1.28 (m, 14H, H-2′-8′), 0.98 (d, 3H, J = 5.9 Hz, Me-21), 0.89 (s, 3H, Me-19), 0.68 (s, 3H, Me-18); ^{13}C NMR 68.8 (C1′), 68.9 (C7), 72.3 (C3), 73.5 (C12), 114.5 (C11′), 139.6 (C10’), 174.9 (C24). CIMS: 561 $(M + H)^+$, 543 $(M + H)^+$–H_2O, 525 $(M + H)^+$–$2H_2O$, 507 $(M + H)^+$–$3H_2O$.

2.1.2. *2-Undecyl 7α,12α,3α-trihydroxy-5β-cholan-24-oate* ***2***

The crude product was purified by column chromatography to yield **2** as a gum (see Table 1), TLC eluent $CHCl_3$/MeOH 9:1, R_f 0.48. IR 3400 (OH), 1736 (CO), 1716 (C–O), 1475, 1381, 1123, 1123 (C–O), 914, 844; ^{1}H NMR 4.89 (q, ^{1}H, J = 6.3 Hz, H-2′), 3.97 (brs, ^{1}H, H-12β), 3.85 (brs, ^{1}H, H-7β), 3.44 (m, ^{1}H, H-3β), 2.23 (m, 2H, H-23), 1.90–1.37 (m, 2^{1}H), 1.26 (brs, 16H, H-3′-10′), 1.19 (d, 3H, J = 6.2 Hz, H-1′), 0.99 (d, 3H, J = 5.8 Hz, Me-21), 0.88 (brs, 6H, Me-19, H-11′), 0.68 (s, 3H, Me-18); ^{13}C NMR 68.9 (C7), 71.2 (C2′), 72.3 (C3), 73.5 (C12), 174.4 (C24). CIMS: 563 $(M + H)^+$, 545 $(M + H)^+$–H_2O, 527 $(M + H)^+$–$2H_2O$, 509 $(M + H)^+$–$3H_2O$.

2.1.3. *Octacos-10-enyl 7α,12α,3α-trihydroxy-5β-cholan-24-oate* ***3***

The crude product was purified by column chromatography to yield **3** as a gum (see Table 1), TLC eluent $CHCl_3$/MeOH 9:1, R_f 0.68. IR 3390 (OH), 1738 (CO), 1715 (C–O), 1466, 1377, 1309, 1173, 1078, 1045, 982, 914, 858; ^{1}H NMR 5.44 (m, 2H, H-10′-11′), 4.15 (t, 2H, J = 6.7 Hz, H-1′), 4.06 (brs, ^{1}H, H-12β), 3.94 (brs, ^{1}H, H-7β), 3.54 (m, ^{1}H, H-3β), 2.50–2.30 (m, 4H), 2.12–1.60 (m, 24H), 1.37 (m,

Table 2
Hydrolysis of cholates

Cholate ester	HIU, 20.4 kHz, 25 °C, 400 W		MW, 300 W[a]		Heating under reflux	
	Time (min)	Yield (%)	Time (min)	Yield (%)	Time (h)	Yield (%)
Methyl cholate[b]	30	98	4	99	3	95
Ethyl cholate[b]	30	97	4	97	3	94
t-Butyl cholate	30	94	4	95	4	90
Benzyl cholate	40	95	4	96	4	74
Octyl cholate[b]	40	97	5	98	12	89
10-Undecenyl cholate	40	97	6	98	12	92
Octacos-10-enyl cholate	50	92	8	94	12	92

[a] MW irradiation was interrupted every 3 min by a 1 min pause.
[b] Reaction scaled up by a factor of 5 in a Milestone 1200 MW reactor.

Table 3
Amidation of cholic acid

R–NH_2	Method	MW[a], 400 W	
		Time (min)	Yield (%)
Benzylamine	A/C	4	88/94
Ethylglycine	C	8	91
Taurine	D	10	58

[a] MW irradiation was interrupted every 3 min by a 1 min pause.

44H, aliphatic chain), 1.08 (d, 3H, $J = 6.0$ Hz, Me-21), 0.99 (s, 3H, Me-19), 0.98 (t, 3H, $J = 6.5$ Hz, H-28'), 0.77 (s, 3H, Me-18); ^{13}C NMR 68.8 (C1′), 68.9 (C7), 72.3 (C3), 73.5 (C12), 130.2 (C10′), 130.3 (C11′), 174.9 (C24).

CIMS: 799 $(M + H)^+$, 781 $(M + H)^+–H_2O$, 763 $(M + H)^+–2H_2O$, 745 $(M + H)^+–3H_2O$.

2.2. Hydrolysis of cholates (general procedure)

The ester (250 mg) was dissolved in 0.05 M KOH in methanol/water (4:1) and treated as indicated in Table 2.

2.3. Amide formation under MW (general procedures)

(A) *Amidation of cholic acid*: Cholic acid (300 mg, 0.735 mmol) was mixed in a mortar with imidazole (50 mg, 0.735 mmol) and the amine (1.47 mmol; directly added to the tube when a liquid). The mixture was transferred to a pressure-resistant tube (pyrex) and irradiated with MW for the specified time (Table 3). The reaction mixture was extracted with dichloromethane.

(B) *Aminolysis of methyl cholate*: Potassium *t*-butoxide (165 mg, 1.47 mmol) was added to an intimate mixture of amine (0.735 mmol) and methyl cholate (300 mg, 0.735 mmol) placed in a pressure-resistant tube (pyrex) that was irradiated with MW for the specified time. The reaction mixture was extracted with dichloromethane.

(C) *Coupling with dicyclohexylcarbodiimide* (*DCC*): Cholic acid (200 mg, 0.49 mmol) was mixed in a mortar with DCC (202 mg, 0.98 mmol), DMAP (0.049 mmol), and the amine (2.45 mmol). The mixture was transferred to a pressure-resistant tube (pyrex) and irradiated with MW for the specified time.

(D) *Coupling with N′-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride*(*EDC*): Cholic acid (200 mg, 0.49 mmol), EDC (188 mg, 0.98 mmol), DMAP (0.049 mmol), and the amine (0.98 mmol) were dissolved in THF/water 1:1 (3 ml), transferred to a pressure-resistant tube, and irradiated with MW for the specified time.

2.4. 3,7-Selective acetylation of methyl cholate

Methyl cholate, acetic anhydride, DMAP (0.15%), and a base (see Table 4) 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 indicated in Table 4. DMAP concentration was crucial for selectivity. Dimethyl carbonate (DMC) was the most convenient solvent under all conditions and particularly suitable for the industrial scale.

2.5. Methyl cholate oxidation (general procedure)

The oxidant was added to a solution of methyl cholate in acetone/water, 4:1. The reactions were carried out in parallel under HIU and under stirring at room temperature (Table 5).

For the reaction under MW, the ester, the oxidant, and excess neutral alumina were intimately mixed and irradiated in a pressure-resistant tube (pyrex), as indicated in Table 5.

3. Results and discussion

We investigated several chemical modifications of BA under HIU or MW irradiation (Scheme 1), comparing results from these procedures with those obtained under conventional conditions. Many of these processes and some of the ensuing derivatives have industrial importance.

It is well known that ester formation and hydrolysis can be strongly promoted by HIU [15,25] and MW [16]. Dayal et al. described a straightforward preparation of BA methyl esters under MW [21]. Starting from cholic acid, we prepared in high yields and very short reaction times a series of cholates from other alcohols, including fatty alcohols like octacos-10-enol [26] that would otherwise require heating under reflux for some 24 h (Table 1). We indifferently used PTSA or MSA as catalyst because these mild acids do not promote ether formation. To trap the esterification water, we added anhydrous sodium sulphate, a cheaper alternative to molecu-

Table 4
3,7-Selective acetylation of methyl cholat

Reagents and solvent (cat. DMAP 0.15%)	HIU, 18.5 kHz, 450 W, 25 °C		MW, 350 W		Stirring, 20 °C	
	Time (min)	Yield (%)[a]	Time (min)	Yield (%)[a]	Time (h)	Yield (%)[a]
Neat Ac_2O	30	75	5	55	1	17
Ac_2O 15 eq, on neutral alumina	–	–	5	75	–	–
Ac_2O 3 eq, 0.5 eq Na_2CO_3, DMC	30	79	5	70	5	68
Ac_2O 3 eq, 3 eq $NaHCO_3$, DMC	30	75	4	64	12	70
Ac_2O 3 eq, 1 eq DBU, DMC	30	66	4	60	6	65
Ac_2O 3 eq, 1 eq DBU, DMAP 0.25%, DMC	15	74	3	67	5	72

[a] Methyl 3,7-diacetoxycholate.

Table 5
Oxidation of methyl cholate

Oxidant (slight excess)	Time (min)[a] (HIU, 18.5 kHz, 300 W, 25 °C)	Time (min)[a] (MW, 350 W on neutral Al_2O_3)	Time (h)[a] (Stirring, 20 °C)
NBS	20[b]	5[b]	48[b]
Jones	30	10[c]	2
$H_5IO_6 + CrO_3$ cat.	30	10	2
CAN, CH_3COOH	120	20	72
NaClO, 13–14%	60	15	24
PCC	120	15	72
$KMnO_4$	60	15	72
$K_2Cr_2O_7$	30[c]	10[c]	2

[a] Disappearance of starting material.
[b] Only 7-keto derivative with traces of diketones.
[c] Partial degradation.

lar sieves. Beside Fisher-type esterification, we found that the carbodiimide activation protocol (DCC/DMAP) worked very well under both HIU and MW (method B) [27]. Compared to the acid-catalyzed reaction (method A), the present reaction afforded excellent yields even of the *t*-butyl and 2-undecyl esters.

The reverse reactions (hydrolysis of cholates with 0.05 M KOH in methanol/water, 4:1) were also very fast, all going to completion in a few minutes (Table 2).

Several protocols have been devised for amide bond formation; in all cases, the carboxylic acid must be activated either by conversion of the carboxyl to a more reactive functional group (acyl halide, mixed anhydride, acyl azide, active ester) or by in situ activation with coupling reagents such as carbodiimides. Another approach is ester aminolysis, generally carried out under harsh conditions (prolonged heating in the presence of strong alkali).

A few biologically active amides of BA [28,29] were easily prepared under MW. The direct solvent-free amidation [30] of cholic acid, carried out by irradiating a finely powdered mixture of cholic acid, benzyl amine, and imidazole (catalyst) [31] (method A, Table 3) afforded the corresponding benzylamide in good yield. Under these conditions, the preparation of glycocholic (GCA) and taurocholic (TCA) acids using ethylglycine and taurine failed, affording only a feeble yield of GCA (8%). No better results were achieved when we turned to the aminolysis of methyl cholate by irradiating it in the presence of potassium *t*-butylate [32] and an amine; in this case, only cholic acid was recovered besides the starting material. A few minutes of irradiation of a solvent-free mixture of cholic acid, excess amine, DCC, and cat. DMAP (method C) gave ethyl glycocholate in good yield, but only a very modest amount of TCA. The same results were obtained when the coupling reaction was carried out in dimethylacetamide (DMA). Finally, TCA was obtained in good yield using a water-soluble carbodiimide (EDC) in THF/water (1:1), maintaining the temperature below 10 °C (method D).

Protecting hydroxyl groups of BA is a fundamental step in the synthesis of cholic acid derivatives [33]. Here, we report only two examples of esterification. Formylation went to completion in 20 min under HIU and in 2 min under MW, while it took no less than 3 h when carried out under simple stirring at 50 °C [34] (method A, Table 1).

3,7-Selective acetylation of methyl cholate, an important step in the industrial synthesis of chenodeoxycholic acid (CDCA) and UDCA, must spare the hydroxyl group in **12**, to be removed in a successive step. Selectivity did not improve under HIU and MW, although 3,7-diacetoxymethyl cholate was obtained in about 80% yield in minutes rather than hours as required by conventional methods (Table 4).

An industrial synthesis of UDCA involves the regioselective oxidation of the hydroxyl groups at C7 and C12 [35], which has been performed both chemically and biochemically [36]. Hydroxyls at different positions on the nucleus have different reactivities towards oxidizing agents, generally in the order C7 > C12 > C3. The 7-keto derivative has been obtained selectively with NBS [37], the 12-keto derivative formed by treatment with bromine in alkaline methanol [38], and 7α,12α-dihydroxy-3-oxo-5β-cholan-24-oic acid by oxidation with silver carbonate on Celite [39]. An indirect electrochemical oxidation of cholic acid with PbO_2 (platinum or carbon anodes) has also been proposed [40]. We found that oxidation of the three hydroxyl groups of methyl cholate with a series of common oxidants was much speeded up by HIU or MW (Table 5); with NBS, the observed selectivity was the same as under conventional conditions, and PCC also gave the 7-keto derivative selectively. Reaction times in Table 5 refer to the disappearance of the starting material; and the mono-, di- and triketones were obtained in varying amounts. Jones' reagent and potassium permanganate yielded the triketone very quickly, as did dichromate, albeit with partial degradation.

The oxidation of methyl 3,7-diacetoxycholate with sodium hypochlorite (7%) in the presence of catalytic amounts of Cr(VI) or with pure sodium hypochlorite (13–14%), both convenient processes for industrial application, proceeded very fast under HIU and MW. Turning to reduction, we found that selective conversion of triformylated cholic acid to the corresponding alcohol with borane/tetrahydrofuran complex, when carried out under MW or HIU, was complete in 20 min and 1 h, respectively [41].

In conclusion, a variety of straightforward chemical modifications of BA, carried out under HIU or MW, have been

presented. Compared to conventional methods, both techniques were much more efficient, increasing product yields and dramatically cutting down reaction times. Scale-up studies are under way. Because reservations should not be overlooked about the reproducibility of experiments carried out in domestic MW ovens, we compared results from this kind of apparatus (two different brands) with those obtained with a professional multi-mode system, and were pleased to find that the three series were in substantial agreement.

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