Full Paper

Long-Chain 3-Acyl-4-hydroxycoumarins: Structure and Antibacterial Activity*

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By reacting 4-hydroxycoumarin with long-chain acyl chlorides, a number of new 3-acyl derivatives were prepared and the relationship was studied between their structures and activities against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, and *Propionibacterium acnes* ATCC 11827. Antibacterial activity was associated with enolic tautomers of a tricarbonylmethane group bearing a lipophilic side chain (undec-10-enoyl, undec-10-ynoyl, palmitoyl or octadec-9-enoyl). A newly synthesized 2-acyl derivative of 2*H*-indene-1,3-dione containing the same tricarbonylmethane motif showed a comparable activity.

Keywords: 4-Hydroxycoumarin / 3-Acyl-4-hydroxycoumarins / 2H-Indene-1,3-dione / Antibacterial

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Introduction

Many coumarin derivatives are biologically active [2, 3]. Much research has been focused on the inhibition of bacterial growth by naturally occurring coumarins (xanthotoxin, herniarin, umbelliferone, and scopoletin) and on the antifungal activity of umbelliferone, scopoletin, and coumarin itself [4 – 7]. Some coumarin derivatives (novobiocin and analogs) have proven very active as antibiotics [8, 9]. Among synthetic derivatives, several antibacterial 3-acyl- [10 – 14] and 3-carbamoyl-4-hydroxycoumarins [15, 16] have been described. The simplest one that proved moderately active, 3-acetyl-4-hydroxycoumarin [12], exists in its crystalline form, as shown in a X-ray diffraction study, predominantly as tautomer **A** (Scheme 1) with an intramolecular H-bond [17, 18].

In a search for more active compounds, we prepared a series of 3-acyl-4-hydroxycoumarins, **1a – d** and tested their activity on *Staphylococcus aureus* ATCC 6538, *Propionibacterium acnes* ATCC 11827, and *Staphylococcus epidermidis* ATCC 12228. Results shed light on the relative importance for antibacterial action of the 4-hydroxycoumarin nucleus, the tricarbonylmethane motif and the side-chain length.

Results and discussion

Chemistry

We began by reacting under sonochemical conditions [19] 4-hydroxycoumarin with several long-chain acyl chlorides (Scheme 1) to prepare a new series of 3-acyl-4-hydroxycoumarins (Scheme 2). The same reaction, when carried out on 4-hydroxyquinolin-2-(1*H*)-one, afforded the 4-*O*-acyl derivative (2-oxo-1,2-dihydroquinolin-4-yl-undec-10-enoate, **2a**) exclusively. In microbiological tests carried out on **2a** and on a few previously synthesized 3-alkyl-4-hydroxycoumarins and 4-alkyloxycoumarins [20] none of these compounds inhibited bacterial growth to an appreciable extent. We therefore concluded that anti-

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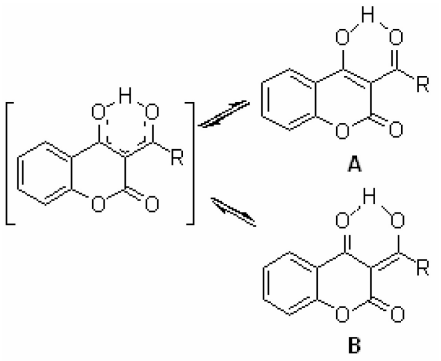
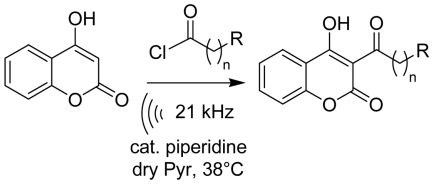


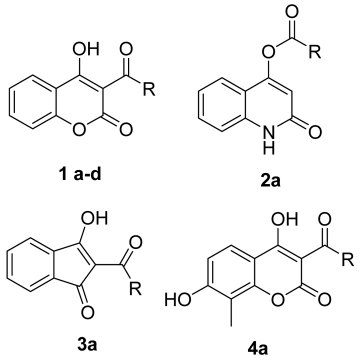
Scheme 1. Tautomers of 3-acyl-4-hydroxycoumarin.



Scheme 2. General procedure for the preparation of 3-acyl-4-hydroxycoumarins.

bacterial activity required the presence of a tricarbonylmethane motif associated with a lipophilic side chain (C_{10} or longer). Provided these crucial features were presumed that replacing the 4-hydroxycoumarin with the 2*H*-indene-1,3-dione nucleus **3a** had no effect on the antimicrobial activity. We prepared a large number of acyl derivatives of which only the most active are described below (**1a**–**d**), namely those bearing an undec-10-enoyl- (**1a**) [21], undec-10-ynoyl- (**1b**), palmitoyl- (**1c**), or (*Z*)-octadec-9-enoyl- (**1d**) chain (Fig. 1).

We also prepared 4,7-dihydroxy-8-methyl-3-undec-10-enoyl-2*H*-chromen-2-one **4a** starting from 2,4-dihydroxy-3-methylacetophenone [22]. This was selectively protected (Scheme 3) in position 4 with 2-methoxyethoxymethyl chloride (MEM chloride) to yield an ether that was stable under the harsh conditions (NaH in toluene under reflux) required for cyclization with diethyl carbonate. After acylation with undec-10-enoyl chloride, the



1 a-d **2a** **3a** **4a**

a R = $-(CH_2)_8-CH=CH_2$
b R = $-(CH_2)_8-C\equiv CH$
c R = $-(CH_2)_{14}-CH_3$
d R = $-(CH_2)_7-CH=CH-(CH_2)_7-CH_3$

Figure 1. List of compounds synthesized and tested.

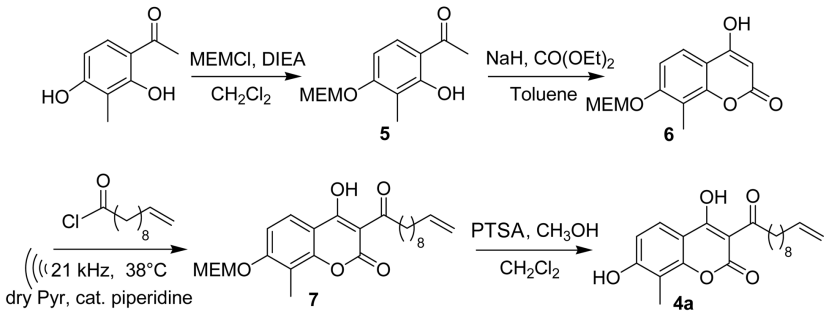
MEM ether was removed under acidic conditions with PTSA in CH_2Cl_2/MeOH.

Antimicrobial activity

As shown in Table 1, most active against *Staphylococcus aureus* ATCC 6538 were coumarins **1a**, **1b**, and the 1*H*-inden-1-one derivative **3a**; all of them bearing a terminal unsaturated bond on a C_{11} side chain. Acute toxicities of compounds **1a**, **1b**, and **3a** were very low (all mice survived a single dose of 2 g/kg without any marked symptoms of intolerance). The minimum inhibitory concentrations (MIC) of all compounds tested fell between 3.6 and 25.6 μg/mL (Table 1). The three most active compounds, **1a**, **1b**, and **3a**, were tested on *Propionibacterium acnes* ATCC 11827 and *Staphylococcus epidermidis* ATCC 12228 (Table 2), confirming that the activities of **1a** and **3a** were close to each other, while **1b** was less active.

Table 1. Minimum inhibitory concentration (MIC) (mg/mL) on *Staphylococcus aureus* ATCC 6538.

Compound	1a	1b	1c	1d	3a	4a
MIC [mg/mL]	3.6	6.2	25.0	25.6	6. 8	22.6



Scheme 3. Synthesis of 4,7-dihydroxy-8-methyl-3-undec-10-enoyl-2*H*-chromen-2-one **4a**.

Table 2. Antimicrobial activity (MIC) of compounds **1a**, **1b**.

Organism	MIC [μg/mL]	
	1a	**1b**
Propionibacterium acnes ATCC 11827 (1.0×10^3 c.f.u./mL)	0.6	0.6
Staphylococcus epidermidis ATCC 12228 (1.6×10^3 c.f.u./mL)	1.2	1.8
Staphylococcus aureus ATCC 6538 (1.5×10^3 c.f.u./mL)	3.6	6.2

Conclusion

We obtained in good yields a number of new 3-acyl derivatives of 4-hydroxycoumarin **1a**–**d** and 4,7-dihydroxy-8-methylcoumarin **4a** that we found to be endowed with a marked antibacterial activity. This required the presence of a tricarbonylmethane motif associated with a lipophilic side chain (C_{10} or longer, best if bearing a terminal ethylene bond). Provided these crucial features were preserved, replacing 4-hydroxycoumarin with 2*H*-indene-1,3-dione **3a** hardly affected the antibacterial activity.

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Experimental

Chemistry

General methods

Anhydrous conditions were achieved (when indicated) by flame-drying flasks and other equipment. Reactions were monitored by TLC on Alugram Sil – Macherey-Nagel F254 (0.25 mm) plates (Macherey-Nagel, Düren, Germany), which were visualized by UV inspection and stained with a 5% $KMnO_4$ solution or by heating after a spray of 5% H_2SO_4 in ethanol. Macherey-Nagel silica gel was used for column chromatography. A Waters microPorasil column 7.8 – 300 (Waters Milford, MA, USA) was used for semi-preparative HPLC, a Gilson 133 refractive index refractometer (Gilson SA, Villiers-le-Bel, France) serving for peak detection. Melting points were obtained on a Büchi SMP-20 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. ^{1}H-NMR (300 MHz) spectra were recorded on a Bruker 300 Advance spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) at 25°C. CIMS were performed on a Finnigan-MAT TSQ70 (Thermo Electron Corporation, Bremen, Germany) with isobutane as reactant gas. Unless otherwise noted, commercially available reagents and solvents were used without further purification. Some synthetic steps were carried out under high-intensity ultrasound using a new sonochemical reactor developed in the authors' laboratory [22].

General procedure for the preparation of acyl chlorides

In a 50 mL two-necked, round-bottomed flask equipped with a magnetic stirrer, a dropping funnel and a condenser, long chain carboxylic acid (45 mmol) and anhydrous CH_2Cl_2 (0.8 mL/mmol) were mixed. $SOCl_2$ (1.5 equivalent mol) was added dropwise to the stirred mixture, which was subsequently heated under reflux for 10 h. The product was recovered by evaporating the solvent and the excess of $SOCl_2$ under vacuum.

General procedure for the acylation

The reaction was carried out under nitrogen atmosphere in the above mentioned sonochemical reactor that was thermostated by four Peltier modules. To the PTFE reaction vessel, 4-hydroxycoumarin, 4,7-dihydroxy-8-methylcoumarin or 2*H*-indene-1,3-dione, a catalytic amount of piperidine, and anhydrous pyridine (1 mL/mmol) were added. After the solution was cooled to 0°C, acyl chloride (1.6 equivalent mol) was slowly added through a septum. The mixture was sonicated (21 kHz, 40 W/cm^2) for about 1.5 h under nitrogen at 38°C, and monitored by TLC. Work-up: the reacted mixture was diluted with EtOAc and washed with 5% HCl and $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated to dryness.

4-Hydroxy-3-undec-10-ynoyl-2H-chromen-2-one ***1b***

Compound **1b** was obtained as white powder; mp. 109°C. IR (KBr) ν cm^{-1} 1724, 1618, 1553, 760; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.07 (1H, dd, *J* = 7.9 Hz, 1.5 Hz, H-5), 7.70 (1H, td, *J* = 7.9 Hz, 1.5 Hz, H-7), 7.37 – 7.27 (2H, m, H-8 and H-6), 3.20 (2H, t, *J* = 7.2 Hz, 2′-CH_2), 2.13 (2H, td, *J* = 6.9 Hz, 2.2 Hz, 9′-CH_2), 1.95 – 1.30 (13H, m, aliph. chain). CIMS: 327 $[M+H]^+$. R_f = 0.45 (hexane/EtOAc (4 : 1)).

4-Hydroxy-3-palmitoyl-2H-chromen-2-one ***1c*** *[11]*

Compound **1c** was obtained as white powder; mp. 103 – 104°C. IR (KBr) ν cm^{-1} 1716, 1606, 1472, 1229, 899; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.08 (1H, dd, *J* = 7.6 Hz, 1.5 Hz, H-5), 7.69 (1H, td, *J* = 7.6 Hz, 1.5 Hz, H-7), 7.37 – 7.26 (2H, m, H-8 and H-6), 3.20 (2H, t, *J* = 7.3 Hz, 2′-CH_2), 1.69 (2H, quintet, *J* = 7.1 Hz, 3′-CH_2), 1.42 – 1.16 (24H, m, aliph. chain), 0.88 (3H, t, *J* = 6.9 Hz, 16′-CH_3). CIMS: 401 $[M+H]^+$. R_f = 0.6 (hexane/EtOAc (4 : 1)).

4-Hydroxy-3-[(9Z)-octadec-9-enoyl]-2H-chromen-2-one ***1d***

Compound **1d** was obtained as white powder; mp. 196 – 197°C. IR (KBr) ν cm^{-1} 1713, 1607, 1549, 1468, 768; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.0 (1H, dd, *J* = 7.8 Hz, 1.5 Hz, H-5), 7.70 (1H, td, *J* = 7.8 Hz, 1.5 Hz, H-7), 7.27 – 7.37 (2H, m, H-8 and H-6), 5.35 (2H, m, H-9′ and H-10′), 3.20 (2H, t, *J* = 7.2 Hz, 2′-CH_2), 2.01 (4H, m, 8′-CH_2 and 11′-CH_2), 1.73 (2H, quintet, *J* = 6.7 Hz, 3′-CH_2), 1.51 – 1.13 (20H, m, aliph. chain), 0.88 (3H, t, *J* = 6.9 Hz, 18′-CH_3). CIMS: 427 $[M+H]^+$. R_f = 0.65 (hexane/EtOAc (4 : 1)).

3-Hydroxy-2-undec-10-enoyl-2H-indene-1,3-dione ***3a***

Compound **3a** was obtained as yellow oil. IR (KBr) ν cm^{-1} 2359, 1709, 1655, 1610, 883; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.86 – 7.82 (2H, m, H-7, H-4), 7.76 – 7.67 (2H, m, H-5, H-6), 5.87 – 5.74 (1H, m, H-10′), 5.02 – 4.91 (2H, m, H-11′), 2.97 (2H, t, *J* = 7.4 Hz, 2′-CH_2), 2.12 (2H, m, 9′-CH_2), 1.62 (2H, quintet, 3′-CH_2), 1.48 – 1.11 (10H, m, aliph. chain). CIMS: 313 $[M+H]^+$. R_f = 0.6 (hexane/EtOAc (4 : 1)).

1-{2-Hydroxy-4-[(2-methoxyethoxy)methoxy]-3-methylphenyl}ethanone ***5***

Compound **5** was obtained as white powder; mp. 57 – 59°C. IR (KBr) ν cm^{-1} 1634, 1499, 1422, 1381, 1333, 1260, 1073, 1101; ^{1}H-

NMR ($CDCl_3$, 300 MHz): δ 12.81 (1H, s, H-2), 7.59 (1H, d, *J* = 9.00 Hz, H-6), 6.72 (1H, d, *J* = 9.0 Hz, H-5), 5.37 (2H, s, H-1′), 3.85 (2H, t, *J* = 4.5 Hz, 3′-CH_2), 3.57 (2H, *J* = 4.5 Hz, 4′-CH_2), 3.39 (3H, s, 6′-OCH_3), 2.59 (3H, s, 2″-CH_3), 2.13 (3H, s, 3-CH_3). CIMS: 255 $[M+H]^+$. R_f = 0.65 (hexane/EtOAc (3 : 2)).

4-Hydroxy-7-[(2-methoxyethoxy)methoxy]-8-methyl-2H-chromen-2-one ***6***

To a magnetically stirred suspension of 0.355 g of NaH (8.12 mmol, 2 equivalent/mol) in 15 mL anhydrous toluene 1.03 g (4.06 mmol, 1 equivalent/mol) of compound **5** were added, followed by slowly dropping in 1.23 mL (10.16 mmol, 2.5 equivalent/mol) of diethylcarbonate. The mixture was refluxed for 12 h and the reaction was monitored by TLC, eluent $CHCl_3$/MeOH (9 : 1). Work-up: the reacted mixture was transferred to a separatory funnel and extracted with H_2O. When the aqueous phase was acidified with 2N H_2SO_4, a voluminous precipitate was obtained. The solid was collected on a Büchner funnel, washed with cold H_2O, and dried by heating at a temperature no higher than 60°C, yielding 0.76 g of **6** (yield 67%).

Compound **6**: white powder, mp. 132 – 134°C. IR (KBr) ν cm^{-1} 1709, 1608, 1555, 1466, 1252, 1196, 972, 762; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.78 (1H, brs, 4-OH), 7.62 (1H, d, *J* = 8.8 Hz, H-5), 7.09 (1H, d, *J* = 8.8 Hz, H-6), 5.76 (1H, s, H-3), 5.40 (2H, s, 1′-CH_2), 3.90 (2H, t, *J* = 4.4 Hz, 3′-CH_2), 3.64 (3H, t, *J* = 4.4 Hz, 4′-CH_2), 3.42 (3H, s, 6′-OCH_3), 2.28 (3H, s, 8-CH_3). CIMS: 281 $[M+H]^+$. R_f = 0.71($CHCl_3$/MeOH (9 : 1)).

4-Hydroxy-7-[(2-methoxyethoxy)methoxy]-8-methyl-3-undec-10-enoyl-2H-chromen-2-one ***7***

Compound **7** was obtained as white powder; mp. 65 – 67°C. IR (KBr) ν cm^{-1} 1725, 1613, 1559, 1458, 1248, 1078, 988; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.89 (1H, d, *J* = 8.9 Hz, H-5), 7.15 (1H, d, *J* = 8.9 Hz, H-6), 5.85 (1H, m, H-10″), 5.43 (2H, s, 1′-CH_2), 5.03 – 4.92 (2H, m, 11″-CH_2), 3.86 (2H, t, *J* = 4.35 Hz, 3′-CH_2), 3.57 (2H, t, *J* = 4.35 Hz, 4′-CH_2), 3.38 (3H, s, 6′-CH_3), 3.18 (2H, t, *J* = 7.3 Hz, 2″-CH_2), 2.30 (3H, s, 8-CH_3), 2.12 (2H, m, 9″-CH_2), 1.75 (2H, quintet, *J*= 7.0 Hz, 3″-CH_2), 1.57 – 1.20 (10H, m, aliph. chain). CIMS: 447 $[M+H]^+$. R_f = 0.57 (hexane/EtOAc (3 : 2)).

4,7-Dihydroxy-8-methyl-3-undec-10-enoyl-2H-chromen-2-one ***4a***

Compound **4a** was obtained as white powder; mp. 161 – 163°C. IR (KBr) ν cm^{-1} 3480, 2922, 1688, 1618, 1568, 1090; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9,8 (2H, brs, 2 × OH), 7.83 (1H, d, *J* = 8.3 Hz, H-5), 6.81 (1H, d, *J* = 8.3 Hz, H-6), 5.80 (1H, m, H-10′), 5.02 – 4.91 (2H, m, 11′-CH_2), 3.17 (2H, t, *J* = 7.3 Hz, 2′-CH_2), 2.31 (3H, s, 8-CH_3), 2.04 (2H, m, 9′-CH_2), 1.72 (2H, quintet, *J* = 7.1 Hz, 3′-CH_2) 1.57 – 1.27 (10H, m, aliph. chain). CIMS: 359 $[M+H]^+$. R_f = 0.64 (hexane/EtOAc (3 : 2)).

Antimicrobial activity

Compounds to be tested were dissolved in acetone (10 mg/mL) and diluted with warm culture medium. Mueller-Hinton Broth containing 1% Tween 20 was used for tests with *Staphylococcus aureus* and *Staphylococcus epidermidis*; incubations were carried out aerobically at 36 ± 1°C. Actinomyces Broth containing 1% Tween 20 was used for *Propionibacterium acnes* that was incubated anaerobically at 36 ± 1°C. Duplicate blanks were incubated alongside culture aliquots containing scalar dilution of each tricarbonyl derivative. After 1 – 4 days of incubation, bacterial growth was estimated by turbidimetry or by plating on agar medium.

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