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Synthesis of antibacterial isocoumarins: Synthesis and antibacterial activity of 3-alkylisocoumarins and (*dl*)-3-alkyl-3,4-dihydroisocoumarins

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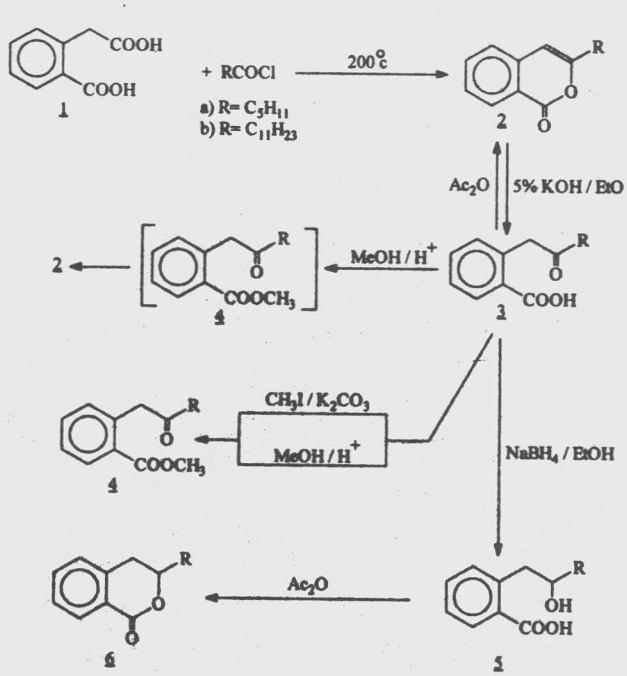
3-Pentyl- and 3-undecylisocoumarins **2a,b** have been conveniently prepared in high yields by direct condensation of acyl chlorides with homophthalic acid. Alkaline hydrolysis of **2a,b** yields the corresponding ketoacids **3a,b**. The latter are reconverted to **2a,b** either by treatment with acetic anhydride or with slightly acidified methanol. Treatment of **3a,b** with methyl iodide or dry methanol in the presence of a catalytic amount of sulfuric acid affords the methyl keto-esters **4a,b**. (*dl*)-3,4-Dihydroisocoumarins **6a,b** are obtained by reduction of **3a,b** to the racemic hydroxyacids **5a,b** followed by cyclodehydration using acetic anhydride. The isocoumarins **2a,b** and dihydroisocoumarins **6a,b** have been examined *in vitro* for their antibacterial activity. Compounds **2a** and **6b** show significant activity comparable to the standard antibiotics.

Isocoumarins and 3,4-dihydroisocoumarins are the metabolites of a wide variety of fungi, bacteria, insects and higher plants[1,2] and exhibit a wide spectrum of biological activities such as antifungal, antihypertensive, antirheumatic and anticoagulant. However, there are only a few reports of the antibacterial action of isocoumarins like isocoumarinylpenicillin derivatives. In this article we report that simple 3-alkylisocoumarins and dihydroisocoumarins are quite effective against the pathogenic Gram positive and Gram negative bacteria.

3-Pentyl- and 3-undecyl-isocoumarins were prepared by the method of Nakajima *et al.*[3,4] involving the direct condensation of hexanoyl and dodecanoyl chlorides with homophthalic acid. The isocoumarins showed characteristic 1H singlet at δ 6.22 for C_4-H and the lactonic carbonyl absorptions at 1720 and 1715 cm^{-1}. Alkaline hydrolysis afforded the keto acids **3a,b**. In the ^1H NMR of these compounds 2H singlets at δ 4.02 and 4.03 and 1H exchangeable broad singlets at δ10.2 and 11.3 were observed. The carbonyl absorptions were observed at 1720 and 1680 cm^{-1}. Isocoumarins **2a,b** were obtained by refluxing the keto-acids **3a,b** with acetic anhydride. Following an already reported procedure[7], compounds **3a,b** were refluxed with dry acidic methanol for 8 hr to furnish the keto-esters **4a,b** as indicated by TLC. It may be pointed out that the work-up of this reaction mixture involved the use of sodium bicarbonate which might have hydrolyzed the keto-esters **4a,b** to the corresponding keto-acids **3a,b** which under the



work-up conditions were ocnverted to the lactones **2a,b** identical in all respect with those prepared earlier. Methylation of **3a,b** with excess of methyl iodide or with acidic dry methanol under reflux for 8 hr without using sodium bicarbonate in the work-up also yielded the methyl keto-esters **4a,b** which showed carbonyl absorption at 1720 cm^{-1} and a 3H singlet at δ 3.82. It is presumed that sodium borohydride reduction of **3a,b** afforded the corresponding hydroxyacids **5a,b** (not isolated) which under the direct influence of acetic anhydride yielded the racemic dihydroisocoumarins **6a,b**. The latter showed carbonyl absorption at 1720 cm^{-1} and the typical *ABX* pattern of C_3-H and C_4-H protons in ^1H PMR spectra. Thus, each of the C-4 protons showed a double doublet (δ 2.86-2.89 and 2.92-2.97 respectively). The protons of methylene groups adjacent to either side of the chiral center exhibited diastereotopic effect.

Antibacterial activity

The isocoumarins **2a,b** and dihydroisocoumarins **6a,b** were tested *in vitro* for their antibacterial activity against pathogenic Gram positive and Gram negative bacteria and the results are summarized in Table I. 3-Pentylisocoumarin **2a** showed activity against two Gram negative bacteria, *Staphylococcus aureus* and *Corynebacterium diphtheriae* comparable to the standard antibiotics Ampicillin and Amoxicillin. 3-Pentyldihydroisocoumarin **6a** and 3-undecyldihydroisocoumarin **6b** were found active against three Gram positive, *S. aureus, C. diphtheriae* and *Bacillus cesus*, and two Gram negative bacteria, *Escherichia coli* and *Salmonella typhi* in accordance with the general trend that the 3,4-dihydroisocoumarins are more potent than the corresponding isocoumarins.

Experimental Section

General. Melting points were determined using a MELTEMP MP-D apparatus and are uncorrected. IR spectra were recorded on a Hitachi 270-50 infrared spectrophotometer as KBr discs or as neat liquids. ^1H NMR (500 MHz) spectra were recorded in $CDCl_3$ on a Bruker AM-500 spectrometer using TMS as internal standard and EIMS on a MAT-112-S machine.

3-Pentylisocoumarin 2a. A mixture of homophthalic acid (2.0 g, 0.011 mole) and hexanoyl chloride (6.28 g, 0.0466 mole) was heated on an oil-bath at 200°C for 3 hr and then refluxed for 1 hr with methanol (20 mL) to convert the excess hexanoyl chloride into ester. The residue obtained after concentration was chromatographed over silica gel column using pet. ether (60-80°) as eluant affording **2a** as a yellow oil (1.5 g, 0.007 mole, 63%); IR (neat): 1720, 1665, 1615, 1590 cm^{-1}; ^1H NMR: δ 0.88 (3H, t, *J*=7.2 Hz, H-5′), 1.32 (4H, m, H-3′,4′), 1.68 (2H, p, *J*=7.4, 10.3 Hz, H-2′), 2.49 (2H, t, *J*=7.6 Hz, H-1′), 6.22 (1H, s, H-4), 7.32-7.33 (1H, dd, *J*=1.15, 7.8 Hz, H-5), 7.39-7.42 (1H, ddd, *J*=1.15, 7.5, 8.2 Hz, H-7), 7.62-7.65 (1H, ddd, *J*=1.35, 7.4, 8.7 Hz, H-6), 8.20-8.22 (1H, dd, *J*=1.25, 8.0 Hz, H-8); MS (70 eV): m/z 216 (100%) [M^+], 188 (5.48), 159 (4.43), 118 (44.37), 57 (78.56) [$C_{14}H_{16}O_2$: Calcd 216.1150; Found 216.1130 (MS)].

2-(2-Oxoheptyl)benzoic acid 3a. A solution of 3-pentylisocoumarin **2a** (0.5 g, 0.0024 mole) in etha-

Table I—Antibacterial activities of isocoumarins **2a,b** and 3,4-dihydroisocoumarins **6a,b**

Compd	Zone of inhibition (mm) 200 μg/100 mL				
	Staphylococcus aureus	*Corynebacterium diphtheriae*	*Bacillus cesus*	*Escherichia coli*	*Salmonella tryphi*
2a	9.0	2.0	—	—	—
2b	—	—	—	—	—
6a	—	—	—	—	—
6b	9.0	7.5	8.5	5.5	8.0
Ampicillin	12.0	—	8.5	15.0	11.0
Amoxicillin	10.0	10.5	9.0	16.0	14.0
Cfuroxime	—	15.5	*	18.5	8.0

— =Not screened; *=No inhibition of growth was observed at 100 μg/mL concentration.

nol (20 mL) and potassium hydroxide (5%, 40 mL) was refluxed for 4 hr. After cooling the reaction mixture, ethanol was removed under reduced pressure. Cold water (30 mL) was then added and the mixture acidified with dil hydrochloric acid and extracted with dichloromethane (2×15 mL). The extract was dried (Na_2SO_4), and the solvent removed on a rotary evaporator to give **3a** as a yellow oil (0.4 g, 0.0017 mole, 74%); IR (neat): 1720, 1680, 1605 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.9 (3H, t, *J*=7.0 Hz, H-7′), 1.32 (4H, m, H-5′,6′), 1.62 (2H, p, *J*=7.36, 14.73 Hz, H-4′), 2.47 (2H, t, *J*=7.38 Hz, H-3′), 4.02 (2H, s, H-1′), 7.18-7.20 (1H, dd, *J*=1.15, 7.8 Hz, H-3), 7.35-7.39 (1H, ddd, *J*=1.1, 7.7, 8.7 Hz, H-5), 7.50-7.54 (1H, ddd, *J*=1.4, 7.5, 8.9 Hz, H-4), 8.11-8.13 (1H, dd, *J*=1.32, 7.8 Hz, H-6), 10.2 (1H, br.s, D_2O exchangeable, COOH); MS (70 eV): m/z 234 (10.65%) [M^+], 216 (14.8) [M^+–H_2O], 163 (2.43), 135 (15.59), 118 (100), 99 (59.69), 71 (66.18) [$C_{14}H_{18}O_3$: Calcd 234.1243; Found 234.1243 (MS)].

3-Pentylisocoumarin 2a. Method A. Compound **3a** (30 mg, 0.13 mmole) was refluxed with acetic anhydride (0.7 mL, 6.8 mmole) for 12 hr. After cooling, the reaction mixture was poured into ice-water (20 mL) and extracted with ethyl acetate (2×5 mL). The extracts were combined, washed with sodium bicarbonate (2×5 mL, 5%) and water (2×10 mL), and the organic layer was dried with (Na_2SO_4) and concentrated to give **2a** as an oil (25 mg, 0.12 mmole, 89%); R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of the already synthesized **2a**.

Method B. A solution of **3a** (150 mg, 0.64 mmole) in dry methanol (100 mL) and conc. sulfuric acid (as a catalyst) was refluxed for 8 hr. The reaction mixture was cooled, neutralized with solid sodium bicarbonate and filtered. The filtrate was rotatory evaporated to afford a yellow oil which was purified by preparative thin layer chromatography to furnish **2a** (120 mg, 0.56 mmole, 89%) as a bright yellow oil.

Methyl 2-(2-oxoheptyl)benzoate 4a. Method A. A mixture of **3a** (150 mg, 0.64 mmole), methyl iodide in excess and anhydrous potassium carbonate (1.0 g) in dry acetone (10 mL) was heated under reflux for 1 hr. The reaction mixture was filtered while hot, the cake washed with warm dry acetone (10 mL) and the solvent evaporated *in vacuo* leaving **4a** as a yellow oil (140 mg, 0.58 mmole, 90%); R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of **4a** synthesized by Method B.

Method B. A solution of **3a** (150 mg, 0.64 mmole) in dry methanol (100 mL) and conc. sulfuric acid (two drops) was refluxed for 8 hr. Water (50 mL) was then added, methanol removed under reduced pressure and the reaction mixture extracted with ether (2×20 mL). The combined extract was dried (Na_2SO_4), the solvent removed under reduced pressure, and the residue purified by preparative thin layer chromatography to furnish **4a** as a yellow oil (91 mg, 0.37 mmole, 57%); IR (neat): 1720, 1685 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, *J*=7.2 Hz, H-7′), 1.34 (4H, m, H-5′,6′), 1.69 (2H, p, *J*=7.4, 15 Hz, H-4′), 2.50 (2H, t, *J*=7.85 Hz, H-3′), 3.82 (3H, s, OMe), 4.06 (2H, s, H-1′), 7.32-7.34 (1H, dd, *J*=3.2, 7.05 Hz, H-3), 7.41-7.44 (1H, ddd, *J*=1.15, 7.5, 8.2 Hz, H-5), 7.66-7.63 (1H, ddd, *J*=1.35, 3.8, 7.75 Hz, H-4), 8.24-8.22 (1H, ddd, *J*=0.65, 1.25, 8Hz, H-6); MS (70 eV): m/z 248 (5.10%) [M^+], 217 (3.39), 216 (100), 192 (8.32), 118 (16.3) [$C_{15}H_{20}O_3$: Calcd 248.1412; Found 248.1420 (MS)].

(*dl*)-3,4-Dihydro-3-pentylisocoumarin 6a. Compound **3a** (150 mg, 0.64 mmole) was heated under reflux with sodium borohydride (0.15 g) in abs. ethanol (15 mL) for 4 hr. Ethanol was then rotatory evaporated and the residue diluted with cold water and acidified with dil. sulfuric acid to give a precipitate which was extracted with ethyl acetate (2×10 mL). The solvent was evaporated to leave **5a** as an oil (0.12 g). This crude compound was dissolved in acetic anhydride (1 mL) and heated under reflux for 2 hr. The reaction mixture was then cooled, and water (10 mL) was added. The oil which separated on stirring was extracted with dichloromethane (2×5 mL). The extracts were combined, treated with sodium bicarbonate (2×5 mL, 5%), washed with water, dried over sodium sulfate (anhydrous) and filtered. The filtrate was stripped off solvent on a rotary evaporator to leave **6a** as an oil (100 mg, 0.46 mmole, 71%); IR (neat):

1720, 1615 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88(3H, t, *J*=7.05 Hz, H-5′), 1.3 (4H, m, H-3′,4′), 1.5 (2H, p, *J*=7.2, 14.3 Hz, H-2′), 1.66-1.72 (1H, m, H-1′), 1.82-1.89 (1H, m, H-1′), 2.86-2.89 (AB pattern, 1H, d, d, J_{vic}=3.6, J_{gem}=16.25 Hz, H-4), 2.92-2.97 (*AB* pattern, 1H, d, d, J_{vic}=11.0, J_{gem}=16.2 Hz, H-4), 4.52-4.47 (1H, m, H-3), 7.20-7.22 (1H, dd, *J*=1.1, 7.55 Hz, H-5), 7.32-7.37 (1H, dd, *J*=5.75, 13.3 Hz, H-7), 7.48-7.51 (1H, ddd, *J*=1.4, 7.55, 8.95 Hz, H-6), 8.05-8.07 (1H, dd, *J*=1.25, 7.8 Hz, H-8); MS (70 eV): m/z 218 (5.32%) [M^+], 217 (31.47), 146 (76.49), 118 (100), 91 (81.18) [$C_{14}H_{18}O_2$: Calcd 218.1307; Found 218.1317 (MS)].

3-Undecylisocoumarin 2b. A mixture of homophthalic acid (2.0 g, 0.011 mole) and dodecanoyl chloride (16.2 g, 0.047 mole) was heated in an oil-bath at 200°C for 3 hr. The residue was purified by column chromatography over silica gel using pet. ether (60-80°) as eluant to afford **2b** (2.8 g, 9.3 mmole, 87%) as a semi-so9lid; IR (neat): 1715, 1660, 1610 cm^{-1}, ^1H NMR ($CDCl_3$): δ 0.86 (3H, t, *J*=7.05 Hz, H-11′), 1.24-1.37 (16H, m, H-3′-10′), 1.57-1.63 (2H, p, *J*=7.4, 14.75 Hz, H-2′), 2.49-2.52 (2H, t, *J*=7.75 Hz, H-1′), 6.23 (1H, s, H-4), 7.32-7.34 (1H, dd, *J*=1.15, 7.6 Hz, H-5), 7.41-7.44 (1H, ddd, *J*=1.1, 7.65, 8.75 Hz, H-7), 7.63-7.66 (1H, ddd, *J*=1.35, 7.7, 9.1 Hz, H-6), 8.22-8.24 (1H, dd, *J*=1.2, 7.95 Hz, H-8); MS (70 eV): m/z 300 (14.43%) [M^+], 299 (64.51), 183 (3.56), 159 (58.39), 141 (1.46), 145 (1.92), 117 (100) [$C_{20}H_{28}O_2$: Calcd 300.2089; Found: 300.2104 (MS)].

2-(2-Oxotridecyl)benzoic acid 3b. A suspension of **2b** (2.4 g, 8.0 mmole in ethanol (135 mL) and potassium hydroxide (5%, 270 mL) was refluxed for 4 hr. After cooling, the reaction mixture was stripped off ethanol under reduced pressure, cold water (60 mL) added and the mixture acidified with dil. hydrochloric acid. The reaction mixture was then extracted with dichloromethane (2×40 mL), dried (Na_2SO_4) and the solvent rotatory evaporated to give a yellow oil which solidified on standing. The crude solid was recrystalized from ethyl acetate and pet. ether (60-80°) to afford **3b** (1.9 g, 6.0 mmole, 74.7%), m.p. 40°; IR (KBr): 1720, 1685, 1610 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (3H, t, *J*=6.53 Hz, H-13′), 1.1-1.3 (16H, m, H-5′-12′), 1.59-1.66 (2H, p, *J*=7.44, 14.52 Hz, H-4′), 2.31-2.35 (2H, t, *J*=7.48 Hz, H-3′), 4.03 (2H, s, H-1′), 7.17-7.19 (1H, dd, *J*=1.22, 7.56 Hz, H-3), 7.34-7.37 (1H, ddd, *J*=1.19, 8.0, 9.27 Hz, H-5), 7.46-7.52 (1H, ddd, *J*=1.22, 7.58, 8.8 Hz, H-4), 8.09-8.12 (1H, dd, *J*=1.2, 7.8 Hz, H-6), 11.35 (1H, s, D_2O exchanged, COOH); MS (70 eV): m/z 318 (11.5%) [M^+], 300 (29.5) [M^+-H_2O), 191 (23.5), 163 (3.4) [$C_{20}H_{30}O_3$: Calcd 318.2195, Found 318.2145 (MS)].

3-Undecylisocoumarin 2b. Method A. Compound **3b** (200 mg, 0.63 mmole) was refluxed with acetic anhydride (2 mL) for 12 hr. After cooling, the reaction mixture was poured into ice-water and extracted with ethyl acetate (2×20 mL). The extracts were combined and washed with aqueous sodium bicarbonate (2×10 mL, 5%), water (15 mL), dried (Na_2SO_4) and concentrated to give **2b** (160 mg, 0.53 mmole, 84.6%) as a semi-solid; R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of **2b** synthesized above.

Method B. A solution of **3b** (100 mg, 0.31 mmole) in dry methanol (75 mL) and conc. sulfuric acid (as a catalyst) was refluxed for 8 hr. The reaction mixture was neutralized with solid sodium bicarbonate, filtered and methanol rotatory evaporated to afford **2b** (80 mg, 0.27 mmol, 92%) as a semi-solid.

Methyl 2-(2-oxotridecyl)benzoate 4b. Method A. Compound **3b** (300 mg, 0.94 mmole), methyl iodide in excess and anhydrous potassium carbonate (2.0 g) in dry acetone (20 mL) were heated under reflux for 2 hr. The reaction mixture was filtered while hot. The cake was washed with warm dry acetone (10 mL) and the solvent evaporated *in vacuo* leaving **4b** as an oil (280 mg, 0.84 mol, 89%); R_f value, mass, high resolution mass, IR and ^1H NMR spectral data were in good agreement with those of **4b** synthesized by Method B.

Method B. A solution of **3b** (150 mg, 0.47 mmole) in dry methanol (100 mL) and conc. sulfuric acid (two drops) was refluxed for 8 hr. Water (50 mL) was then added, methanol removed under reduced pressure, and the reaction mixture extracted with ether (2×20 mL). The extracts were combined,

dried (Na_2SO_4) and the solvent was removed under reduced pressure. The compound was purified by preparative layer chromatography to give **4b** as a yellow oil (109 mg, 0.33 mmole, 70%); IR (neat): 1720, 1685 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, *J*=6.49 Hz, H-13′), 1.23-1.36 (16H, m, H-5′-12′), 1.54-1.62 (2H, p, *J*=7.17, 14.59 Hz, H-4′), 2.25-2.30 (2H, t, *J*=7.37 Hz, H-3′), 3.82 (3H, s, OMe), 4.06 (2H, s, H-1′), 7.13-7.16 (1H, dd, *J*=0.89, 7.56 Hz, H-3), 7.28-7.33 (1H, ddd, *J*=1.38, 7.66, 9.06 Hz, H-5), 7.41-7.47 (1H, ddd, *J*=1.50, 7.49, 9.00 Hz, H-4), 7.96-7.99 (1H, dd, *J*=1.27, 7.79 Hz, H-6); MS (70 eV): m/z 332 (0.92%) [M^+], 301 (2.52), 300 (100), 192 (31.25), 183 (38.65), 149 (6.58), 118 (16.29), 59 (6.13) [$C_{21}H_{32}O_3$: Calcd 332.2351; Found 332.2360 (MS)].

(*dl*)-3, 4-Dihydro-3-undecylisocoumarin 6b. Compound **3b** (1.0 g, 3.0 mmole) was heated under reflux with sodium borohydride (1.0 g) in absolute ethanol (75 mL) for 4 hr. Ethanol was rotatory evaporated, cold water (225 mL) added, and the reaction mixture acidified with dil. sulfuric acid and extracted with ethyl acetate (2×50 mL). The extract was dried over (Na_2SO_4) and solvent evaporated to leave **5a** as yellow oil (0.8 g). The crude oil was dissolved in acetic anhydride (5 mL) and heated under reflux for 2 hr. The reaction mixture was cooled, water (50 mL) added, stirred and extracted with dichloromethane (2×30 mL). The extracts were washed with sodium bicarbonate (2×20 mL, 5%), then with water (20 mL), dried (Na_2SO_4) and rotatory evaporated to leave **6b** as an oil (0.7 g, 2.3 mmole, 77%); IR (neat): 1720, 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (3H, t, *J*=6.9 Hz, H-11′), 1.22-1.33 (16H, m, H-3′-10′), 1.58-1.64 (2H, p, *J*=7.5, 14.7 Hz, H-2′), 1.66-1.73 (1H, m, H-1′), 1.83-1.90 (1H, m, H-1′), 2.86-2.87 (*AB* pattern, 1H, d, d, J_{vic}=3.45, J_{gem}=16.25 Hz, H-4), 2.93-2.98 (*AB* pattern, 1H, 1.4, 7.6 Hz, H-5), 7.35-7.38 (1H, ddd, *J*=1.46, 7.6, 9.0 Hz, H-7), 7.49-7.52 (1H, ddd, *J*=1.4, 7.55, 8.90 Hz, H-6), 8.07-8.08 (1H, dd, *J*=1.2, 7.75 Hz, H-8); MS (70 eV): m/z 302 (11%) [M^+], 301 (48.56), 156 (3.0), 146 (96.0), 117 (100), 90 (34.0) [$C_{20}H_{30}O_2$: Calcd 302.2246; Found 302.2236 (MS)].

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