theoretical investigations allow the knowledge base of chemically and biologically important dihydropyridine to expand, unencumbered by the practical confines imposed by experimental methodologies.

Acknowledgment. We are indebted to M. J. S. Dewar for his many useful discussions on this project. In addition, the expert editorial skills of Joan Martignago are thankfully appreciated.

Synthesis of Ester Derivatives of Chloramphenicol by Lipase-Catalyzed Transesterification in Organic Solvents

Gianluca Ottolina, Giacomo Carrea,* and Sergio Riva

Istituto di Chimica degli Ormoni, CNR, Via Mario Bianco 9, 20131 Milano, Italy

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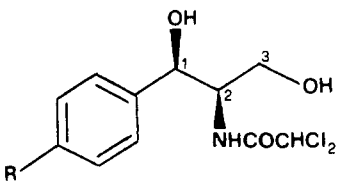
Regioselective esterification of chloramphenicol (1) and its synthetic analogue thiamphenicol (2) has been achieved by the action of lipase in acetone and several methyl carboxylates. Aliphatic and aromatic esters of different sizes and natures have been introduced selectively on the primary hydroxyl group of these molecules by modification of the reaction conditions (e.g., temperature, solvent, and lipase source).

Introduction

Chloramphenicol (1) is a natural antibiotic with a fairly wide spectrum of antimicrobial activity.[1] It inhibits protein synthesis in bacteria and, to a lesser extent, in eukaryotic cells by binding to the 50S ribosomal subunit, thus preventing the access of aminoacyl tRNA to the ribosome. Chloramphenicol is widely employed in veterinary medicine against infections of the urinary tract and other bacterial diseases. On the other hand, human therapy with 1 is limited to those infections (typhoid fever, bacterial meningitis) in which the benefits of the drug outweigh the risks of potential toxicity (1 can cause serious and fatal blood dyscrasias). Chloramphenicol may be administered



1 : R = NO_2

2 : R = CH_3SO_2

orally, usually as the water-insoluble 3-*O*-palmitate, or intravenously as the inactive 3-*O*-succinate ester. Both these derivatives are rapidly hydrolyzed in vivo to the biologically active drug.[1,2] Since the preparation of several other esters of 1 has been reported in the patent literature,[3] we focused on exploiting enzymatic acylation of chloramphenicol as a model for the production of useful simple drug derivatives. Here we report our results for the synthesis of various 3-*O*-esters of 1 by the action of lipase in organic solvents.

Results and Discussion

The standard production method for fatty acid 3-*O*-esters of chloramphenicol (*threo*-(1*R*,2*R*)-1-(4-nitrophenyl)-2-(dichloroacetamido)-1,3-propanediol (1)) involves the reaction of 1 with a suitable acyl anhydride or acyl chloride in the presence of a tertiary amine.[3] More recently, chloramphenicol esters have been obtained through biological catalysis. An initial paper[4] reported the isolation of a mixture of acyl derivatives of 1 upon incubation with spores, washed mycelium, or whole cultures of *Streptomyces griseus*. A second approach[5] took advantage of the catalytic action of a specific enzyme (chloramphenicol acetyltransferase from *Streptococcus faecalis*) to achieve the synthesis of 3-*O*-acetyl-1. A serious drawback of this last method is the requirement for a stoichiometric amount of the expensive cofactor acetyl coenzyme A.

Looking for a different and more effective enzymatic approach, we noticed that the inert 3-*O*-palmitate ester of 1, used in the pharmaceutical preparation to circumvent the bitter taste of 1, is hydrolyzed to the free, biologically active chloramphenicol by intestinal and pancreatic lipase.[1] We decided to take advantage of this class of enzymes by reversing their hydrolytic activity. It is well-known that lipases, when suspended in a suitable organic solvent, can catalyze esterification or transesterification reactions,[6]

Table I. Percentage Acylation (%) in the Transesterification Reactions between Chloramphenicol and Various Methyl Carboxylates[a]

	methyl carboxylate				
lipase	acetate	propionate	butanoate	hexanoate	octanoate
pancreatic[b]	45.0	57.5	72.2	47.4	50.7
P[c]	76.9	76.6	85.1	74.0	73.2
CE-5[d]	53.4	70.6	71.1	72.8	65.8
Ch.v.[e]	83.0	83.6	85.6	81.8	78.5
G[f]	82.6	83.0	83.6	81.1	81.6

[a] Conditions: 50 mM chloramphenicol and 100 mg of lipase "straight from the bottle" in 1 mL of anhydrous methyl carboxylate were shaken at 250 rpm for 96 h at 45 °C. Conversion estimated by HPLC: Partisil 10 column (Whatman) eluted with hexane–propanol, 9:1; flow rate, 1 mL min^{-1}; readings were made at 300 nm. [b] Sigma Chemical Co. [c] Lipase from *Pseudomonas fluorescens* (Amano Pharm. Ltd.). [d] Lipase from *Humicola langinosa* (Amano Pharm. Ltd.). [e] Lipase from *C.v.* (Finnsugar Biochem. Inc.). [f] Lipase from *P. cyclopium* (Amano Pharm. Ltd.).

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Table II. Initial Rates and Percentage Acylation in the Reaction between 1 and Methyl Butanoate Catalyzed by *Chromobacterium viscosum* (*Ch.v.*) Lipase[a]

Ch.v. lipase[b]	molecular sieves	*T*, °C	init rates,[c] μmol h^{-1} mg^{-1} lipase	conversn,[d] %
B (100 mg)		20	0.018	60.1
B (100 mg)		45	0.049	84.6
B (100 mg)		60	0.103	89.6
B (100 mg)		75	0.149	92.6
B (100 mg)	50 mg	45	0.035	94.8
B (100 mg)	100 mg	45	0.034	97.8
L (5 mg)		45	7.100	90.2
L (5 mg)		60	13.250	94.0
L (5 mg)	100 mg	45	3.500	98.9
L (5 mg)	100 mg	60	10.875	99.2

[a] Chloramphenicol (50 mM), 1 mL of anhydrous methyl butanoate, shaken at 250 rpm. [b] B: "straight from the bottle". L: lyophilized. [c] Estimated by HPLC (see Table I). [d] After 18 h.

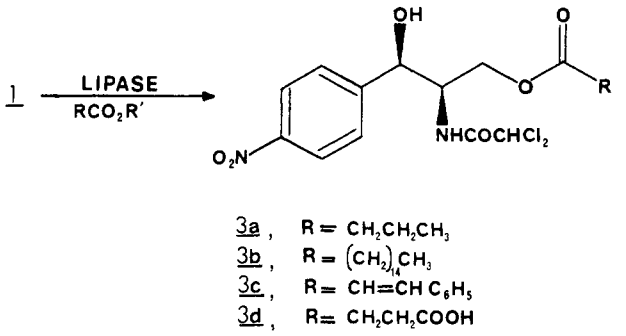
usually with excellent regioselectivity for the primary OH in polyhydroxylated molecules.[7]

Since 1 is quite soluble in short-chain fatty acid methyl esters, we first checked the activities of several lipases in these media. As pointed out by Klibanov,[7a] methyl carboxylates act as both solvents and acylating agents. Of about 20 lipases tested, the five reported in Table I gave the most satisfactory results. After 4 days of shaking at 45 °C, lipase from *Chromobacterium viscosum* (*Ch.v.*) produced the greatest acylation with methyl butanoate, so this enzyme and ester were chosen for more detailed experiments. Table II shows the initial rates and the percentage acylation (after 18 h) for the reaction between 1 and methyl butanoate catalyzed by *Ch.v.* lipase under different conditions. These data provide interesting information about the effects of temperature, lyophilization, and addition of molecular sieves on the kinetics and the equilibrium of this transesterification reaction. Upon increasing the temperature (lines 1–4, 7, 8), we observed increases in both the initial rate and the yield of the 3-*O*-butanoyl ester. The high activity at 75 °C is in agreement with the reported high thermostability of these enzymes in organic solvents.[8] Lyophilization of the lipase (previously dissolved in water, with or without pH adjustment of the solution to 7) had a dramatic effect on the initial rate (more than a 100-fold increase) and a pronounced effect on the equilibrium (compare lines 2 and 3 with lines 7 and 8). The action of molecular sieves on the percentage acylation, which became almost 100% both with the lipase "straight from the bottle" and with the lyophilized one, is surprising. This marked effect can only be explained on the basis of molecular sieves adsorbing the MeOH that is released in the reaction

$$1 + \text{PrCOOMe} \rightleftharpoons \text{1-OCOPr} + \text{MeOH} \quad (1)$$

The optimized conditions (i.e., 50 mM 1, 10 mg/mL lyophilized lipase, 100 mg/mL molecular sieves, 45 °C) were subsequently utilized for a larger scale reaction. After 7 h, 1.6 g of 1 in 50 mL of anhydrous methyl butanoate was converted to a single product (95% yield by HPLC), which was recovered by filtering the enzyme, evaporating the solvent, and purifying by crystallization (compound **3a**, see Scheme I). ^{1}H NMR spectroscopy confirmed the regioselective acylation at the primary OH (downfield shift of the signal due to the methylene at C-3).

Scheme I



3a, R = $CH_2CH_2CH_3$
3b, R = $(CH_2)_{14}CH_3$
3c, R = $CH{=}CHC_6H_5$
3d, R = CH_2CH_2COOH

Table III. Percentage Acylation (%) of the Transesterification Reaction between Chloramphenicol and Trifluoroethyl Butanoate in Various Solvents[a]

	solvent			
lipase[b]	acetone	dioxane	acetonitrile	*tert*-amyl alcohol
pancreatic	21.1	15.4	44.7	35.6
P	78.0	74.7	80.1	65.7
CE-5	41.6	24.6	49.9	30.9
Ch.v.	96.8	64.6	59.9	79.3
G	61.8	60.9	47.3	49.4

[a] Chloramphenicol (50 mM), 150 mM TFE butanoate, and 100 mg of lipase "straight from the bottle" in 1 mL of anhydrous solvent were shaken at 250 rpm for 72 h at 45 °C. Conversions estimated by HPLC (see footnote *a* to Table I). No appreciable conversion was detected without enzyme. [b] Lipase sources are indicated in footnotes *b–f* to Table I.

Table IV. Percentage Acylation (%) in the Transesterification Reactions between Chloramphenicol and Various Trifluoroethyl Esters in Anhydrous Acetone[a]

	ester		
lipase[b]	TFE laurate	TFE palmitate	TFE cinnamate
pancreatic	10.6	9.7	30.2
P	76.2	57.1	80.4
CE-5	47.7	40.7	24.5
Ch.v.	44.4	40.5	99.2
G	84.8	87.2	72.7

[a] Conditions: 50 mM chloramphenicol; 150 mM TFE esters; 100 mg of lipase "straight from the bottle"; 1 mL of anhydrous acetone; 45 °C; shaken at 250 rpm for 72 h. Conversions estimated by HPLC (see footnote *a* to Table I). No appreciable conversions were detected without enzyme. [b] Lipase sources are indicated in footnotes *b–f* to Table I.

The same reaction protocol could not be used with long-chain fatty acid methyl esters ($>C_8$), because of the insolubility of 1. As a consequence, we searched for a suitable solvent for the reaction between 1 and fatty acid activated esters:

$$1 + \text{RCOOCH}_2\text{CF}_3 \rightleftharpoons \text{1-OCOR} + \text{CF}_3\text{CH}_2\text{OH} \quad (2)$$

As a model, the reaction with trifluoroethyl (TFE) butanoate was studied first. The results, reported in Table III, clearly indicate that acetone is the solvent of choice, combining the good solubility of our substrate with high enzymatic activity. Using this solvent, we investigated the esterification with fatty acid derivatives TFE laurate and TFE palmitate. Preliminary tests indicated that lipase G (from *Penicillium cyclopium*) was the best catalyst (first two columns in Table IV). Lyophilization of the lipase

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from a water solution adjusted to pH 5, optimum for enzyme activity, did not influence the initial rate of the reaction between 1 and TFE palmitate (0.47 μmol h^{-1} mg^{-1} lipase versus 0.40 μmol h^{-1} mg^{-1} lipase "straight from the bottle") but improved the degree of acylation. The lack of effect of enzyme prelyophilization on reaction rates, contrary to what was observed with *Ch.v.* lipase, indicates that the optimal operation conditions are greatly different for different enzymes. Thus, a general procedure suitable for all lipases will be difficult to develop. The reaction was subsequently scaled up to 800 mg of **1**. The acylation was 98% after 24 h (single peak by HPLC and single spot by TLC), and a simple workup provided 3-*O*-palmitoylchloramphenicol (**3b**). In a further study, TFE cinnamate was chosen as an interesting model for the introduction of aromatic acyl groups. Lipase from *Ch.v.* was again the best enzyme (third column in Table IV). Initial rate determinations confirmed the dramatic effect of lyophilization on the activity of this enzyme (about a 35-fold increase). High conversion (94%) of 1 was observed after 48 h in a preparative-scale experiment. The usual reaction workup and crystallization provided pure 3-*O*-cinnamoylchloramphenicol (**3c**) (66% yield). The enzyme was recovered and lyophilized again at pH 7 to check its residual activity. In another preparative-scale reaction, this "used" lipase transformed 95% of 1 after 48 h.

Finally, 3-*O*-succinylchloramphenicol (**3d**) was selected as a target for our enzymatic syntheses. Cyclic anydrides have previously been employed in lipase-catalyzed acylation,[9] so we studied the reaction between 1 and succinic anhydride. Because of the high chemical reactivity of this anhydride and the ready accessibility of the primary 3-OH, our first aim was to eliminate any spontaneous chemical reaction. After several attempts, we found that spontaneous acylation was negligible in dioxane at 20 °C. Among the different lipases tested, lipase G was best (almost 100% conversion after 20 h). The reaction was scaled up (see Experimental Section for details), and after 20 h, 97% of 1 was transformed into **3d** (64% isolated yield).

We also examined the enzymatic acylation of the antibiotic thiamphenicol (**2**), a synthetic structural analogue of 1. When the protocol described above was used, acylation of **2** with TFE palmitate catalyzed by lipase G provided 3-*O*-palmitoylthiamphenicol, isolated in 83% yield after the usual workup (96% conversion by HPLC analyses of the crude reaction mixture). As expected, modification of the aromatic part of the molecule did not influence enzyme regioselectivity.

Conclusions

We have demonstrated that lipase-catalyzed acylations can be a simple and effective way to obtain regioselective esterification of chloramphenicol and its structural analogue thiamphenicol. Acyl groups of different sizes and natures can be introduced in this way. Some of them (palmitate and succinate) are of interest for pharmaceutical preparations of these and other drugs. Work is in progress to see whether the stereochemistry of the chiral centers of chloramphenicol (namely, C-1 and C-2) influences the observed enzymatic regioselectivity.[10]

Experimental Section

Materials. The sources of the lipases are indicated in footnotes *b–f* to Table I. Chloramphenicol and thiamphenicol were obtained from Sigma, while the various methyl carboxylates were from Aldrich. Trifluoroethyl butanoate, trifluoroethyl laurate, trifluoroethyl palmitate, and trifluoroethyl cinnamate were prepared as described elsewhere.[11] All other chemicals used in this work were purchased from commercial suppliers. Acetone, dioxane, acetonitrile, *tert*-amyl alcohol and methyl carboxylates (analytical grade) were used without further purification beyond drying by shaking with 3-Å molecular sieves (Merck).

General Methods. Enzymatic transesterifications of 1 were followed by HPLC (JASCO 880/PU pump; JASCO 870 UV/VS detector) using a silica gel column (Partisil 10, Whatman) eluted with hexane–propanol, 9:1, flow rate, 1 mL min^{-1}; readings were made at 300 nm (in the case of **2**, the eluting system was hexane–propanol, 75:25, and readings were made at 260 nm). An Erbasil NH_2-amino 5-μm column (Carlo Erba) eluted with a 15-min linear gradient from 90% to 20% acetonitrile in water (containing 5 mM $AcONH_4$, pH 6.4) was employed to follow the reactions with succinic anhydride.

Melting points are uncorrected and were determined in open-ended capillaries. Optical rotations were measured at 589 nm at 25 °C. ^{1}H NMR spectra were obtained at 270 MHz in deuterated acetone containing D_2O, with Me_4Si as internal standard.

In addition to HPLC, the purity of the products was also investigated by TLC with precoated silica gel 60 F_{254} plates (Merck) eluted with the appropriate mixtures of AcOEt–hexane (a mixture CH_3CN–CH_3COOH, 100:0.1, was used in the reaction with succinic anhydride).

***threo*-(1*R*,2*R*)-1-(4-Nitrophenyl)-2-(dichloroacetamido)-1,3-propanediol 3-Butanoate (3a).** The experimental procedure for the synthesis of **3a** is described in the text. Mp: 123–4 °C from aqueous acetone (lit.[12] mp 115 °C). [α]: +24° (c = 5 in EtOH). ^{1}H NMR: δ 8.19 (2 H, d, J = 8.7 Hz) and 7.74 (2 H, d, J = 8.7 Hz), aromatic protons; 6.33 (1 H, s, CCl_2H); 5.23 (1 H, d, J = 2.9 Hz, H-1); 4.48 (1 H, ddd, J = 2.9, 5.2, and 8.2 Hz, H-2); 4.41 (1 H, dd, J = 5.2, and 10.9 Hz) and 4.27 (1 H, dd, J_1 = 8.2 Hz, J_2 = 10.9 Hz), CH_2-3; 2.34 (2 H, t), 1.61 (2 H, m), and 0.94 (3 H, t), aliphatic chain. Anal. Calcd for $C_{15}H_{18}Cl_2N_2O_6$: C, 45.82; H, 4.61; N, 7.13. Found: C, 46.04; H, 4.47; N, 7.08.

***threo*-(1*R*,2*R*)-1-(4-Nitrophenyl)-2-(dichloroacetamido)-1,3-propanediol 3-Palmitate (3b).** Lipase from *P. cyclopium* (lipase G) prelyophilized from pH 5 (500 mg) was added to 25 mL of anhydrous acetone containing 2.5 mmol (800 mg) of 1 and 7.5 mmol of trifluoroethyl palmitate, and the suspension was shaken at 250 rpm and 45 °C. After 24 h, the enzyme was removed by filtration, the solvent evaporated, and the crude residue washed with 3 × 10 mL of petroleum ether to eliminate residual TFE palmitate. The product was crystallized from aqueous acetone. Mp: 87 °C (*Merck Index* 90 °C). [α]: +23.5° (c = 1.5 in EtOH). ^{1}H NMR: selected data δ 5.25 (1 H, d, J = 2.9 Hz, H-1); 4.49 (1 H, ddd, J = 2.9, 5.2, and 8.2 Hz, H-2); 4.38 (1 H, dd, J = 5.2 and 10.9 Hz) and 4.26 (1 H, dd, J = 8.2 and 10.9 Hz), CH_2-3; 2.31 (2 H, t), 1.61 (2 H, m), 1.31 (24 H, m), and 0.91 (3 H, t), aliphatic chain. Anal. Calcd for $C_{27}H_{42}Cl_2N_2O_6$: C, 57.75; H, 7.54; N, 4.99. Found: C, 59.39; H, 7.74; N, 4.61.

***threo*-(1*R*,2*R*)-1-(4-Nitrophenyl)-2-(dichloroacetamido)-1,3-propanediol 3-Cinnamate (3c).** Chloramphenicol (800 mg) was dissolved in 25 mL of anhydrous acetone containing 2 molar equiv of TFE cinnamate. *Ch.v.* lipase prelyophilized from pH 7 (1 g) was added, and the suspension was shaken at 250 rpm and 45 °C for 48 h. The enzyme was filtered, and the solvent was evaporated. The crude residue was washed with 3 × 10 mL of petroleum ether to eliminate residual TFE cinnamate and crystallized from aqueous acetone. Mp: 122–3 °C (lit.[3b] mp 122–3 °C). [α]: +48.7° (c = 5 in EtOH). ^{1}H NMR: selected data δ 5.34 (1 H, d, H-1); 4.57 (1 H, ddd, H-2); 4.51 (1 H, dd) and 4.38 (1 H, dd), CH_2-3; 7.66 (2 H, m), 7.44 (3 H, m), 7.70 (1 H, d, J = 16.0 Hz), and 6.54 (1 H, d, J = 16.0 Hz), cinnamoyl moiety. Anal. Calcd for $C_{20}H_{19}Cl_2N_2O_6$: C, 52.87; H, 4.22; N, 6.17. Found: C, 52.24; H, 4.09; N, 5.98.

***threo*-(1*R*,2*R*)-1-(4-Nitrophenyl)-2-(dichloroacetamido)-1,3-propanediol 3-Succinate (3d).** Lipase G prelyo-

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philized from pH 5 (1 g) was added to 25 mL of anhydrous dioxane containing 2.5 mmol of 1 and 5 mmol of succinic anhydride. The suspension was shaken at 250 rpm and 20 °C. After 24 h, the enzyme was removed by filtration and the solvent evaporated. The crude residue was dissolved in 40 mL of AcOEt and extracted with 5% $NaHCO_3$. The aqueous solution was acidified to pH 4.5 and extracted with AcOEt. Anhydrification of the organic layer and evaporation of the solvent furnished crude **3d**, which was crystallized from benzene. Mp: 126–8 °C (lit.[13] mp 127 °C). [α]: +12.8° (*c* = 5 in EtOH). ^1H NMR: selected data δ 5.32 (1 H, d, H-1); 4.55 (1 H, ddd, H-2); 4.50 (1 H, dd) and 4.39 (1 H, dd), CH_2-3; 2.53 (4 H, s), succinyl moiety. Anal. Calcd for $C_{15}H_{16}Cl_2N_2O_8$: C, 42.57; H, 3.81; N, 6.62. Found: C, 42.81; H, 3.65; N, 6.42.

***threo*-(1*R*,2*R*)-1-(4-Methylsulfonyl)-2-(dichloroacetamido)-1,3-propanediol 3-Palmitate.** Thiamphenicol (880 mg) was dissolved in 75 mL of anhydrous acetone containing 3 molar equiv of TFE palmitate. Lipase G prelyophilized from pH 5 (500 mg) was added, and the suspension was shaken at 250 rpm and 45 °C for 48 h. Following the previously described workup, 3-*O*-palmitoylthiamphenicol was obtained in 83% yield after crystallization from aqueous acetone. Mp: 101–3 °C. [α]: −20.8° (*c* = 2.5 in AcOEt). ^1H NMR: δ 7.80 (2 H, d, *J* = 8.5 Hz) and 7.60 (2 H, d, *J* = 8.5 Hz), aromatic protons; 6.23 (1 H, s, CCl_2H); 5.07 (1 H, d, *J* = 2.8 Hz, H-1); 4.35 (1 H, ddd, J_1 = 2.8 Hz, J_2 = 5.3 Hz, J_3 = 8.2 Hz, H-2); 4.27 (1 H, dd, J_1 = 5.3 Hz, J_2 = 10.9 Hz) and 4.12 (1 H, dd, J_1 = 8.2 Hz, J_2 = 10.9 Hz), CH_2-3; 3.03 (3 H, s, CH_3SO_2); 2.20 (2 H, t), 1.20 (26 H, m), and 0.76 (3 H, t), aliphatic chain. Anal. Calcd for $C_{28}H_{45}Cl_2NO_6S$: C, 56.56; H, 7.63; N, 2.36. Found: C, 57.30; H, 7.81; N, 2.20.

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Registry No. 1, 56-75-7; 2, 15318-45-3; **3a**, 59005-99-1; **3b**, 530-43-8; **3c**, 14399-14-5; **3d**, 3544-94-3; TFE butanoate, 371-27-7; TFE laurate, 70253-78-0; TFE palmitate, 119596-14-4; TFE cinnamate, 23094-31-7; lipase, 9001-62-1; methyl acetate, 79-20-9; methyl propionate, 554-12-1; methyl butanoate, 623-42-7; methyl hexanoate, 106-70-7; methyl octanoate, 111-11-5; succinic anhydride, 108-30-5; *threo*-(1*R*,2*R*)-1-[4-(methylsulfonyl)phenyl]-2-(dichloroacetamido)-1,3-propanediol 3-palmitate, 21478-01-3.

Conversion of Sandaracopimaric Acid into an Androstane Analogue Steroid

A. Abad,* C. Agulló, M. Arnó,* L. R. Domingo, and R. J. Zaragozá

Departamento de Quimica Orgánica, Universidad de Valencia, Dr. Moliner 50, 46100 Burjasot, Valencia, Spain

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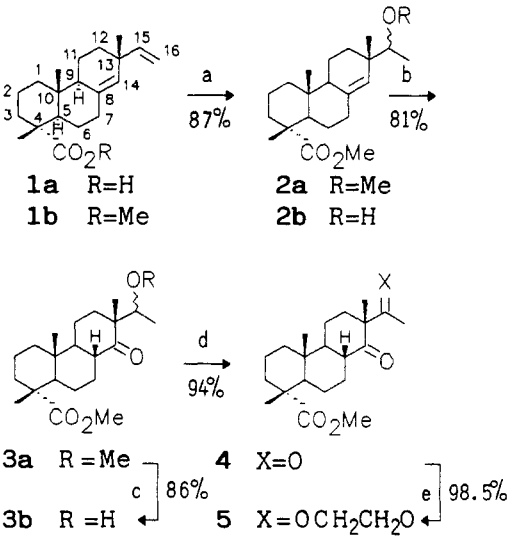
The conversion of the diterpenoid resin acid, sandaracopimaric acid **1a**, into a tetracyclic system of steroid is described. Key steps for the construction of the D ring are reductive nucleophilic acylation of ketone **5** and intramolecular aldol condensation of keto aldehyde **10**. Various unsuccessful attempted methods for achieving the one-carbon homologation of the C-14 carbonyl group of **5** are described.

The use of podocarpic, abietic, and dehydroabietic resin acids as starting materials for the synthesis of steroids has been investigated by several groups.[1] In recent years the conversion of tricyclic diterpenes with pimarane or isopimarane skeleton into such compounds has also been reported,[2] but hitherto none of these syntheses has resulted in the preparation of a steroidal structure possessing the trans-anti-trans arrangement of BCD rings of androstane steroids. In this paper, we describe the conversion of the readily available[3] sandaracopimaric acid **1a** into an androstane analogue steroid, utilizing a new approach for the construction of the D ring which involves as key steps reductive nucleophilic acylation of ketone **5** and intramolecular aldol condensation of keto aldehyde **10**, as illustrated by the reaction sequence shown in Schemes I–IV.

Scheme I[a]



[a] (a) (i) $Hg(OAc)_2$, MeOH; (ii) $NaBH_4$, NaOH, dioxane; (b) (i) BH_3, THF; (ii) H_2O_2, KOH, H_2O–dioxane; (iii) Jones reagent, acetone; (iv) NaOMe, MeOH; (c) NaI, TMSCl; (d) Jones reagent, acetone; (e) $HO(CH_2)_2OH$, PTSA, benzene.

Treatment of the methyl ester **1b** with mercuric acetate in methanol (Scheme I) followed by treatment with sodium borohydride afforded the methyl ether **2a** as a C-15[4] ep-

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