104. Studies on the Enzymatic Acylation of Quinic Acid, Shikimic Acid, and Their Derivatives in Organic Solvents

by **Bruno Danieli*** and **Paolo De Bellis**

Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, Centro C.N.R. di Studio delle Sostanze Organiche Naturali, Via Venezian 21, I-20133 Milano

and **Laura Barzaghi, Giacomo Carrea, Gianluca Ottolina,** and **Sergio Riva***

Istituto di Chimica degli Ormoni, C.N.R., Via Mario Bianco 9, I-20131 Milano

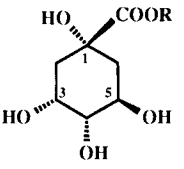
(10.III.92)

Quinic acid (**1a**), shikimic acid (**2**), and their derivatives were acylated in organic solvents by several lipases and by the protease subtilisin *Carlsberg*. The most satisfactory results were obtained with methyl (or benzyl) quinate (**7a** (or **8a**)) and lipase from *Chromobacterium viscosum* adsorbed on *Celite*, which showed an overwhelming preference towards the acylation of OH–C(4). Under optimized conditions, the synthetically useful 4-*O*-acetylquinate **8d** was isolated in *ca.* 90% yield. On the other hand, acylation of methyl shikimate (**10a**) showed no regioselectivity with any of the enzymes tested. A possible rationale for the different behavior of *Chromobacterium viscosum* lipase towards **7a** and **10a** is given, comparing the conformations of these two molecules, as deduced from ^{1}H-NMR and molecular-mechanics calculation.

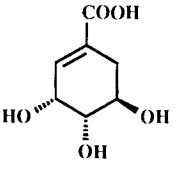
Introduction. – (–)-Quinic acid (**1a**) and (–)-shikimic acid (**2**) are important secondary metabolites widely spread in higher plants [1] [2], which are receiving growing attention as useful chiral building blocks [3] and as optically active synthetic precursors for natural compounds [4] and pseudosugars [5]. In particular, quinic acid was extensively used for the multistep synthesis of a large variety of compounds, among which the glyoxalase I inhibitor COTC [4a], mycosporins [4b], palintantin [4c], and D-*myo*-inositol 1,4,5-triphosphate [4d] are worth of mention.

Utilization of **1a** in synthesis takes advantage of the selective manipulation of the functional groups on the cyclohexane ring, which allows the introduction of a variety of appendages or new functions. While the two groups at C(1) may be regarded as a masked carbonyl group, few well-established methodologies are known to differentiate among the three secondary OH groups. More specifically, while OH–C(5) can be easily protected *via* acid-catalyzed lactonization to quinolactone **4a** [6]; only two processes are known to discriminate between OH–C(3) and OH–C(4). In the first one, catalytic oxidation of the axial OH–C(3) of **1a** with a Pt/O_2 system gave the 3,3-*O*-didehydroquinic acid (**3**) in *ca.* 50% isolated yield [7]. In the second one, better results were obtained by the treatment of the protected quinide **5** (obtained by acid-catalyzed acetalization of **1a** with benzaldehyde) with *N*-bromosuccinimide to give the bromo-benzoate **6** [4b] [8].

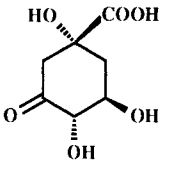
With this in mind, we reasoned that new methods able to selectively modify quinic acid's OH groups might offer new opportunities to the synthetic elaboration of this compound. Therefore, we decided to include quinic acid and some of its derivatives, as well as shikimic acid and related compounds, in our research program, directed towards the



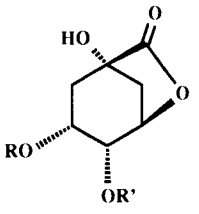
1a R = H
b R = Bu_4N



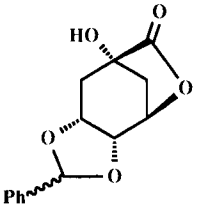
2



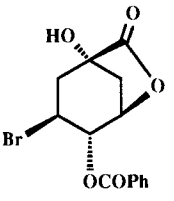
3



4a R = R′ = H
b R = Ac, R′ = H
c R = H, R′ = Ac



5

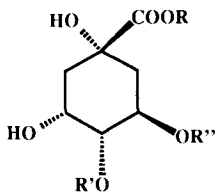


6

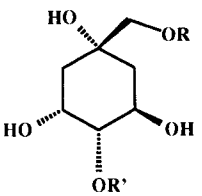
enzymatic modification of sugars and polyhydroxylated natural compounds in organic solvents [9], with the hope that the specific interactions between enzymes and substrates would result in discrimination of their various OH groups. We now report on the results of our study.

Results and Discussion. – The general methodology developed by *Klibanov* [10] for enzymatic transesterifications in organic solvents was applied to **1a**. Because of the high polarity of **1a**, the reactions were performed in pyridine. After two days of stirring in the presence of the standard ester trifluoroethyl butanoate, there was no conversion, neither with the protease subtilisin nor with porcine pancreatic lipase. Since it is well documented that only a few proteases and lipases are catalytically active in pyridine [11], we titrated **1a** with 1.5M aq. Bu_4NOH and then lyophilized the soln. to give salt **1b**. Due to the nature of its cation, **1b** was also soluble in more hydrophobic solvents, however, still no conversion was observed after prolonged stirring with a variety of enzymes (see *Exper. Part*).

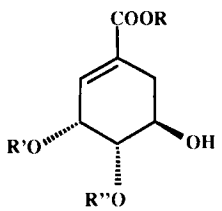
Methyl quinate (**7a**) [12] was subjected to the action of several lipases and of the protease subtilisin under different conditions. Among lipases, the one from *Chromobacterium viscosum* (*Ch.v.*) gave the most satisfactory results in terms of conversion and



7a R = Me, R′ = R″ = H
b R = Me, R′ = PrCO, R″ = H
8a R = Bz, R′ = R″ = H
b R = Bz, R′ = PrCO, R″ = H
c R = Bz, R′ = H, R″ = PrCO
d R = Bz, R′ = Ac, R″ = H



9a R = R′ = H
b R = Ac, R′ = H
c R = R′ = Ac



10a R = Me, R′ = R″ = H
b R = Me, R′ = Ac, R″ = H
c R = Me, R′ = H, R″ = Ac

selectivity (see *Table*) and, therefore, the reaction was scaled up to isolate and characterize the product. Thus, 4-*O*-butanoyl derivative **7b** was obtained after chromatography in 61% yield. The site of the acylation was unambiguously established by ^{1}H-NMR analysis and by the comparison with the data for the parent **7a**.

The ^{1}H-NMR of **7a** in (D_6)DMSO exhibited a *ddd* at 3.89 ppm for H–C(3) with the apparent coupling constants $J(3,2ax) = 7.8$, $J(3,2eq) = 3.5$, and $J(3,4) = 3.2$ Hz. H–C(4) resonated at 3.34 ppm as a *dd* with $J(4,5) = 7.5$ Hz, and H–C(5) was found at 3.76 ppm as a *dt* due to the similarity of the vicinal couplings to $CH_2(6)$ ($J = 6.5$ Hz). These data are in substantial agreement with those reported by *Meier* and *Tamm* [13] for quinic acid in CD_3OD. However, due to the different solvent used by us, the diastereoisotopic $CH_2(6)$ (instead of $CH_2(2)$ as in quinic acid) appeared as a 2 H *d* at 1.82 ppm, with an apparent splitting of 3.8 Hz due to the fortuitous coincidence of their chemical shifts, whereas the protons of $CH_2(2)$ (instead of $CH_2(6)$) were the *AB* part of an *ABX* system at 1.70 and 1.95 ppm, with $J_{gem} = 13$ Hz and vicinal coupling constants to H–C(3) of 7.8 and 3.5 Hz, respectively. Acylation at OH–C(4) in **7b** was proved by the 1.3 ppm downfield shift of H–C(4), which appeared at 4.63 ppm ($J(3,4) = 3.2$, $J(4,5) = 7.5$ Hz), while H–C(3) and H–C(5) were almost unaffected at 4.02 and 3.88 ppm.

The interesting result obtained with *Ch.v.* lipase allowing for the first time the selective esterification of OH–C(4) without affecting either OH–C(3) or OH–C(5) prompted us to investigate its synthetic potential. Thus, we enzymatically acylated the benzyl ester **8a** under the same conditions, which gave a 64% isolated yield of the corresponding 4-*O*-butanoyl derivative **8b**, as confirmed by ^{1}H-NMR (see *Exper. Part*). The reaction was optimized using *Ch.v.* lipase adsorbed on *Celite* [9f] and vinyl acetate as solvent and acylation agent. Under these conditions, the conversion of **8a** was complete after 1 h at 45°, and 4-*O*-acetyl derivative **8d** was isolated in 87–90% yield.

The protease subtilisin behaved differently from the lipases. Regioselectivity was not as good as with *Ch.v.* lipase, the esterification of **8a** giving rise to the three mono-*O*-acyl isomers in a 9:16:3 ratio. A preparative-scale reaction was carried out with **8a** and 2,2,2-trifluoroethyl butanoate in the presence of subtilisin. The product with the fastest R_f (second in abundance) was identical to **8b** as shown by direct comparison. The major product was the 5-*O*-butanoyl isomer **8c**, and the third compound, present in traces, could not be isolated in pure form.

In the ^{1}H-NMR of **8c**, H–C(5) appeared at 5.02 ppm as a *td*, having very similar couplings with H–C(4) and H_{ax}–C(6) ($J = 7$ Hz) and a smaller coupling with H_{eq}–C(6) ($J = 5$ Hz); H–C(4) was at 3.55 ppm as *dd* ($J = 3$ Hz), whereas H–C(3) resonated at 3.93 ppm as a *dt* with $J(2ax,3) = 5$ and $J(2eq,3) = 3$ Hz.

Two other derivatives of quinic acid, the pentol **9a** [14] and the quinide **4a** [9], were also studied. In polyol **9a**, *Ch.v.* lipase acylated first the more accessible primary OH group, forming in pyridine the mono-acetate **9b** in *ca.* 50% yield (CH_2(OH)–C(1) at 3.82 and 3.90 ppm (*AB*, $J = 12.5$ Hz)). Further acylation in vinyl acetate gave diester **9c**, with the second AcO group at C(4) (H–C(4) of **9c** at 4.52 ppm instead of 3.25 ppm (**9b**)).

Enzyme-mediated esterification of lactone **4a** was much slower (*ca.* 50% conversion after 24 h at 45°) and nonspecific, giving a mixture of two mono-acetates in comparable amounts. Moreover, although the two esters had different R_f's in TLC, we were unable to separate them, probably because of their interconversion during column chromatography. ^{1}H-NMR of a fraction enriched in one of the two products (*ca.* 77:23 ratio) showed two sets of signals, corresponding to 3-*O*-acetylquinolactone **4b** and 4-*O*-acetylquinolactone **4c** (see *Exper. Part*).

Finally, screening of methyl shikimate (**10a**) under experimental conditions similar to those reported in the *Table* showed that *Ch.v.* lipase was the best catalyst in this case too. GC analysis indicated a 69% conversion to a mixture of two products (60% isolated

yield) which were not separable by flash chromatography, due to their close running properties under a variety of elution conditions. ^{1}H-NMR analysis showed that two mono-esters were present in 2.5:1 ratio, and we identified them as 3-*O*-acetyl derivative **10b** and 4-*O*-acetyl derivative **10c** by selective decoupling experiments.

The ^{1}H-NMR spectrum showed two sets of related signals attributed to each of the two isomers **10b** and **10c**. The most intense set showed the olefinic H–C(2) at 6.78 ppm as a *dd*, with a vicinal coupling to H–C(3) (J = 4.5 Hz) and a small allylic coupling ($J \approx 2$ Hz) to the quasiaxial H–C(6) at 2.25 ppm. After irradiation, H–C(3) was found at 5.58 ppm as a *td* ($J(2,3) = J(3,4) = 4.5$ and $J(3,6ax) \approx 1$ Hz). H–C(4) appeared at 3.78 ppm as a *dd* ($J(4,5) = 9$ Hz) and H–C(5) at 4.01 as a *dd* ($J(5,6ax) = 9$ and $J(5,6eq) = 5.5$ Hz). Finally, the CH(6) group showed a geminal coupling (J = 19 Hz), and H_{eq}–C(6) appeared as a *dd* at 2.95 ppm, without additional long-range couplings. The low-field resonance of H–C(3), compared to the 4.26 ppm in the parent methyl shikimate (**10a**; in (D_6)DMSO at 70°, see below) indicated that esterification yielding the major isomer **10b** had occurred at OH–C(3). The large value of $J(4,5)$ and $J(5,6ax)$ (9 Hz) suggested that H–C(5), H–C(4), and H_{ax}–C(6) were in an almost *trans-diaxial* orientation. Accordingly, H_{eq}–C(6) exhibited no allylic and homoallylic couplings to H–C(2) and H–C(3). The set of signals due to the less abundant isomer **10c** consisted of a *dt* at 6.85 ppm for the olefinic proton, with a vicinal coupling constant of 4.5 Hz and two additional small long-range couplings ($\approx$ 1 Hz) to H–C(2) and H–C(3). Among the oxymethine protons, the most downfield signal appeared at 4.93 ppm as a *dd*. This signal was attributed to H–C(4) because irradition of it disclosed H–C(3) at 4.64 ppm as a broad *td* ($J(2,3) \approx J(3,4) \approx 4.5$ Hz and a small long-range coupling ($J \approx 1$ Hz)) and H–C(5) at 4.22 ppm as a *ddd* ($J(4,5) = 8.8$, $J(5,6ax) = 5.5$, and $J(5,6eq) = 4.5$ Hz; H_{ax}–C(6) and H_{eq}–C(6) at 2.81 and 2.32 ppm, resp.). This compound was, therefore, identified as the methyl 4-*O*-acetylshikimate (**10c**). A careful examination of the ^{1}H-NMR spectrum revealed an impurity with clean signals at 4.42 and 5.18 ppm, suggesting the presence of traces of the 5-*O*-acetyl derivative.

The results showed a marked difference between 'good' substrates, such as **7a**, **8a**, and **9a**, which fit the active site of *Ch.v.* lipase correctly, affording an almost quantitative conversion with excellent regioselectivity, and 'poor' ones, such as **4a** and **10a**, which suffer a lower conversion with scarce regioselectivity. In an attempt to rationalize these results, we considered the preferred conformation of methyl quinate (**7a**) and methyl shikimate (**10a**).

According to ^{1}H-NMR data [13], methyl quinate (**7a**) exists preferentially in a chair conformation (**A**), with OH–C(4), OH–C(5), and the COOMe group equatorially oriented and OH–C(3) axially oriented. This was confirmed by molecular-mechanics calculations. Using the INSIGHT and DISCOVER software, we constructed two shaped conformations for **7a**: the one suggested by ^{1}H-NMR data (**A**) and the inverted one (**B**), in which OH–C(4), OH–C(5), and COOMe are axial (see *Fig. 1*). Energy minimizations

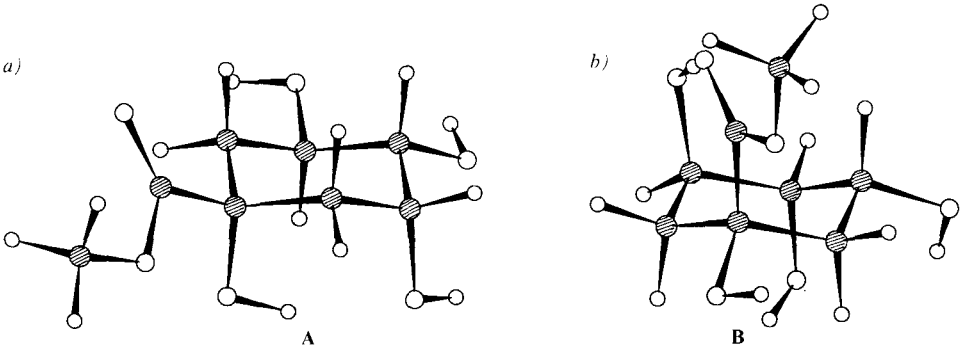


Fig. 1. a) *Calculated global-energy-minimum conformer* **A** *and* b) *local-energy-minimum conformer* **B** *of* **7a**. Empty circles are O- and H-atoms, hatched circles represent C-atoms.

indicated that conformer **A** represented a global minimum in various solvents. In fact, the energy differences with respect to the local minimum represented by **B** indicated that **A** was 2.31 kcal/mol more stable *in vacuo*, 2.62 kcal/mol in AcOEt (ε 6.02), 2.41 kcal/mol in acetone (ε 20.56), and 2.31 kcal/mol in DMSO (ε 45.43). Therefore, **B** is expected to make a negligible contribution to the conformation of **7a** in solution.

It was reported [13] that methyl shikimate (**10a**) prefers a half-chair conformation in H_2O or MeOH solution, with OH–C(4) and OH–C(5) in an equatorial orientiation and OH–C(3) in a quasiaxial one. The ^{1}H-NMR spectrum (600 MHz, (D_6)DMSO) of **10a**, recorded at room temperature, showed a series of broad signals that made the interpretation of the splitting patterns impossible; at 70°, most signals were sharp and split, and their analysis casts some doubts on the proposed conformation [13] of **10a**, at least in the solvent used by us ((D_6)DMSO).

The ^{1}H-NMR (600 MHz, (D_6)DMSO, 70°) of **10a** showed H–C(2) and H–C(3) still as br. *s* at 6.65 ($w_{1/2}$ = 4 Hz) and 4.26 ppm ($w_{1/2}$ = 10 Hz). The *dd* at 3.58 ppm was assigned to H–C(4) (J(4,5) = 6.4 Hz, J(3,4) = 4.2 Hz). H–C(5) appeared as a *dt* because of the similar coupling (J = 4.5 Hz) to both H_{ax}- and H_{eq}–C(6). These last two protons resonated at 2.08 and 2.50 ppm as a *dddd*; in addition to the large geminal coupling (J = 18.0 Hz) and to the vicinal coupling to H–C(5) (J = 4.5 Hz), both showed couplings arising from long-range interactions with H–C(2) ($J \approx$ 1.6 and 2.3 Hz, resp.) and H–C(3) ($J \approx$ 1 and 2 Hz). These data, specifically the vicinal coupling-constant values displayed by H–C(5) and the existence of comparable allylic and homoallylic couplings of H–C(2) and H–C(3) to both H_{ax}–C(6) and H_{eq}–C(6), are not consistent with the proposed half chair [13].

Molecular-mechanics calculations, starting from the two opposite half-chair geometries **C** and **D** of **10a**, showed that the two corresponding minima differ by only 0.7 kcal/mol *in vacuo*, 0.46 kcal/mol in AcOEt, 0.59 kcal/mol in acetone, and 0.63 kcal/mol in DMSO (see *Fig. 2*). In these two arrangements, the COOMe has its carbonyl group turned away from H–C(2), and the torsion angles towards the olefinic double bond are *ca.* 20 and 28°, respectively. The relatively small differences obtained in these calculations suggest that there are conformational equilibria in which **C** and **D** are present in comparable amounts. Although the determination of the preferred conformation in solution is only a prerequisite for the evaluation of substrate recognition by enzymes [14], the conformational interconversion of methyl shikimate (**10a**) could be responsible for the loss of selectivity displayed by *Ch.v.* lipase, whose active site could have been approached by different conformers of this compound with similar stability. On the other hand, the extremely high selectivity of this lipase with methyl quinate (**7a**) might be due to the existence of only one stable conformer.

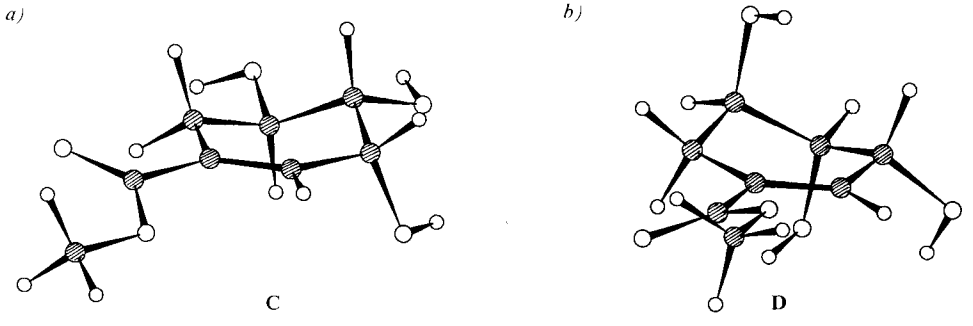


Fig. 2. *Energy-minimized conformers* a) **C** *and* b) **D** *of* **10a**. See also *Fig. 1*.

A better understanding of the above results will be given by structural information on the active site of *Chromobacterium viscosum* lipase, presently not available, and by examination of the conformation assumed by these substrates in approaching the acyl-enzyme intermediate.

In conclusion, we have shown that the lipase from *Chromobacterium viscosum* easily and efficiently esterifies OH–C(4) of methyl quinate (**7a**) and benzyl quinate (**8a**). These new 4-*O*-acyl derivatives might be useful chirons for natural-product synthesis.

Experimental Part

1. *General.* For lipase sources, see *Table*. Subtilisin was obtained from *Sigma* (protease type VIII). (–)-Quinic acid (**1a**) and (–)-shikimic acid (**2**) were purchased from *Aldrich*. Methyl quinate (**7a**), methyl shikimate (**10a**), benzyl quinate (**8a**), quino-1,5-lactone (**4a**), and 1α,3α,4α,5β-tetrahydroxycyclohexane-1β-methanol (**9a**) were obtained following literature procedures (see above) and showed ^{1}H-NMR, ^{13}C-NMR, and FAB-MS data in accordance with the proposed structures. Reactions were monitored by GC and TLC. GC: *25 HP1* capillary silica-gel column, coated with methylsilicone gum (*Hewlett-Packard*); reaction mixtures were transformed with 1,1,1,3,3,3-hexamethyldisilazane [16] prior to analysis. TLC: precoated silica gel *60* F_{254} plates (*Merck*); detection with conc. H_2SO_4, followed by heating. FC = flash chromatography. ^{1}H- and ^{13}C-NMR spectra: *Bruker-AC-300* (^{1}H at 300 and ^{13}C at 50.2 MHz) and *Bruker-AM-600* instrument (^{1}H at 600 MHz). FAB-MS: *VG-70-70-EQ-HF* instrument equipped with its own source, using Xe as gas and glycerol as matrix.

2. *Immobilization of* Ch.v. *Lipase. Ch.v.* lipase (3 g) was mixed accurately with *Hyflo Super Cell* (10 g, *Fluka*). Then, 0.1M K_3PO_4 buffer (pH 7; 10 ml) was added, the mixture shaken vigorously and dried for 24 h at 0.02 mbar. The H_2O content, determined by the optimized *Fisher* method, was 2% (*w*/*w*).

3. *Molecular-mechanics calculations* were performed with the software package INSIGHT and DISCOVER from *Biosym Inc.*, Munich, using an *IRIS-4D-70-GT* workstation from *Silicon Graphics*.

4. *Attempted Enzymatic Acylation of Tetrabutylammonium Quinate* (**1b**). The soln. of 1 g of **1a** in 10 ml of H_2O was titrated to pH 7 with $(Bu_4N)OH$ (40% in H_2O) and then freeze-dried to give **1b** as an amorphous solid. At 45°,

Table. *Enzymatic Acylation of Methyl Quinate* (**7a**)[a])

Enzyme	% Conversion[b])	Products ratio[c])
Chromobacterium viscosum lipase[d])	79[e])	75:1:3
	83[f])	80:0.5:2.5
Pseudomonas lipase[g])	27[e])	27:0:0
	21[f])	21:0:0
Humicola lanuginisa lipase[g])	41[e])	34:1:6
	18[f])	15:0:3
Porcine pancreatic lipase[h])	42[e])	42:0:0
	27[f])	27:0:0
Candida cylindracea lipase[h])	9[e])	3:5:1

[a]) Conditions: **7a**, 65 μmol; 2,2,2-trifluoroethyl butanoate, 10 equiv.; solvent, 1 ml; lipase, 100 mg (subtilisin, 20 mg); shaking at 250 rpm for 24 h at 45°.

[b]) Determined by GC analysis (see *Exper. Part*).

[c]) The first value refers to the least retained isomer (identified as 4-*O*-acetyl derivative, see text), the third one to the most retained isomer.

[d]) *Finnsugar Biochemicals Inc.*

[e]) Solvent: toluene/pyridine 5:1.

[f]) Solvent: acetone/pyridine 3:1.

[g]) *Amano Pharmaceuticals Ltd.*

[h]) *Sigma Chemical Co.*

1 ml of 0.1M **1b** in anh. vinyl acetate/DMF 20:1 was shaken in the presence of subtilisin (20 mg) for 48 h. No reaction was observed (TLC and GC). The same negative result was obtained using 100 mg of the crude lipases described in the *Table*.

5. *Methyl 4-*O*-Butanoylquinate* (= *Methyl 4α-(Butanoyloxy)-1α,3α,5β-trihydroxycyclohexane-1β-carboxylate*; **7b**). To a soln. of 200 mg of **7a** in 10 ml of acetone/pyridine 3:1 containing 1.5 ml of 2,2,2-trifluoroethyl butanoate (10 equiv.), 1 g of crude *Ch.v.* lipase and 1 g of molecular sieves were added. The suspension was shaken at 250 rpm and 45° for 18 h. The enzyme was filtered off, the solvent evaporated, and the crude residue purified by FC (CH_2Cl_2/MeOH/H_2O 90:6:0.5): 169 mg (61%) of **7b**. Oil. R_f (CH_2Cl_2/MeOH/H_2O 90:6:0.5) 0.29. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.63 (*dd*, J = 7.8, 2.2, H–C(4)); 4.03 (*td*, J = 3.3, 2.2, H–C(3)); 3.89 (*td*, J = 7.8, 4, H–C(5)); 3.60 (*s*, MeO); 2.30 (*t*, J = 8), 1.54 (*m*, J = 8), 0.87 (*t*, J = 8, PrCO). FAB-MS: 277 (79, $[M + H]^+$), 259 (100), 189 (34), 171 (90), 151 (66).

6. *Benzyl 4-*O*-Butanoylquinate* (**8b**). As described for **7b**, with 100 mg of benzyl quinate (**8a**): 80 mg (64%) of **8b**. Oil. R_f (hexane/AcOEt 7:3) 0.35. ^{1}H-NMR (300 MHz, (D_6)DMSO): 7.40 (Ph); 5.10 (PhCH_2); 4.66 (*dd*, J = 7.8, 2.2, H–C(4)); 4.03 (*dt*, J = 3.3, 2.2, H–C(3)); 3.90 (*dt*, J = 7.8, 4, H–C(5)); 2.06 (*t*), 1.11 (*m*), 0.98 (*t*, PrCO). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 173.2 (*C*OOCH$_2$Ph); 75.7 (C(4)); 73.8 (C(1)); 65.5 (C(5) or C(3)); 64.2 (C(3) or C(5)); 39.9 (C(6)); 37.7 (C(2)); 136.2, 128.4, 127.9, 127.6, 65.8 (PhCH_2); 172.7, 35.7, 18.0, 13.4 (PrCO). FAB-MS: 353 (63, $[M + H]^+$), 335 (54), 263 (7), 245 (15), 181 (30), 91 (100).

7. *Benzyl 4-*O*-Acetylquinate* (= *Benzyl 4α-Acetoxy-1α,3α,5β-trihydroxycyclohexane-1β-carboxylate*; **8d**). To a soln. of 200 mg of **8a** in 7 ml of vinyl acetate, 160 mg of *Ch.v.* lipase adsorbed on *Celite* (540 mg) were added. The suspension was stirred at 45° and 250 rpm for 4 h. After this time, the enzyme was filtered off, the solvent evaporated and the residue purified by FC (AcOEt) giving 210 mg (90%) of **8d**. Oil. R_f (hexane/AcOEt, 7:3) 0.21. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.63 (*dd*, J = 7.5, 3, H–C(4)); 4.05 (*dt*, J = 3, 1.8, H–C(3)); 3.93 (*dt*, J = 7.5, 4, H–C(5)); 2.05 (*s*, Ac). FAB-MS: 325 (20, $[M + H]^+$), 307 (27), 217 (6), 181 (14), 91 (100).

8. *Benzyl 5-*O*-Butanoylquinate* (= *Benzyl 5β-(Butanoyloxy)-1α,3α,4α-trihydroxycyclohexane-1β-carboxylate*; **8c**). To a soln. of 200 mg of **8a** in 10 ml of acetone/pyridine 3:1 containing 2 ml of 2,2,2-trifluoroethyl butanoate, subtilisin (200 mg) was added. The suspension was stirred at 55° for 3 days. TLC and GC: three different mono-esters, ratio 9:16:3. Usual workup and FC gave **8b** (see above), R_f (hexane/AcOEt 7:3) 0.35 and the more abundant isomer **8c**. Oil. R_f (hexane/AcOEt 7:3) 0.31. ^{1}H-NMR (300 MHz, (D_6)DMSO, 80°): 5.07 (*dt*, J = 6, 5, H–C(5)); 3.93 (*dt*, J = 6, 3, H–C(3)); 3.55 (*dd*, J = 6, 3, H–C(4)); 2.05 (*t*), 1.55 (*m*), 0.88 (*t*, PrCO). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 172.9 (*C*OOCH$_2$Ph): 73.4 (C(1)); 70.8 (C(4)); 69.5 (C(5)); 67.1 (C(3)); 36.9 (C(2)); 35.4 (C(6)); 136.0, 128.5, 128.0, 127.5, 65.8 (PhCH_2); 172.0, 35.4, 17.8, 13.3 (PrCO). FAB-MS: 353 (51, $[M + H]^+$), 335 (6), 263 (3), 245 (4), 181 (8), 91 (100).

9. *3-*O*- and 4-*O*-Acetylquino-1,5-lactone* (= *3-*exo*-Acetoxy-1,4-*exo*-dihydroxy- and 4-*exo*-Acetoxy-1,3-*exo*-dihydroxy-6-oxabicyclo[3.2.1]octan-7-one*; **4b** and **4c**, resp.). To a soln. of 200 mg of lactone **4a** in 24 ml of acetone/pyridine 2:1 containing 2.1 ml (20 equiv.) of vinyl acetate, *Ch.v.* lipase adsorbed on *Celite* (1.1 g total weight) was added, and the suspension was stirred at 45° for 2 days. After that time, the conversion was almost 50% (2 products). Usual workup followed by FC (AcOEt/hexane 6:4) gave **4b**/**4c** 77:23. R_f (AcOEt/MeOH/H_2O 9:1:0.5): 0.69 (**4b**), 0.62 (**4c**). ^{1}H-NMR (**4b**/**4c** 77:23; 300 MHz (D_6)DMSO): **4b**: 6.12 (*s*, OH–C(1)); 5.62 (*d*, J = 5, OH–C(4)); 4.69 (*t*, J = 5, H–C(5)); 4.61 (*ddd*, J = 11.25, 7.2, 5, H–C(3)); 4.11 (*q*, J = 5, H–C(4)); 2.05 (*s*, Me); **4c**: 6.08 (*s*, OH–C(1)); 5.18 (*d*, J = 6.25, OH–C(3)); 5.08 (*t*, J = 5, H–C(4)); 4.77 (*t*, J = 5, H–C(5)); 3.74 (*dddd*, J = 11.25, 7.2, 6.25, 5, H–C(3)); 2.09 (*s*, Ac).

10. *Acylation of Pentol* **9a**. 10.1. To a soln. of 200 mg of **9a** in 10 ml of anh. pyridine containing 1 ml of vinyl acetate, *Ch.v.* lipase adsorbed on *Celite* (1 g) was added and the suspension shaken overnight at 45°. Usual workup and purification by FC ($CHCl_3$/MeOH 8:2) gave 120 mg (51%) of *1α,3α,4α,5β-tetrahydroxycyclohexane-1β-methyl acetate* (**9b**). R_f (CH_2Cl_2/MeOH 8:2) 0.45. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.92 (*m*, H–C(3), H–C(5)); 3.90 (*d*, J = 12.5), 3.82 (*d*, J = 12.5, CH_2OAc); 3.25 (*dd*, J = 9, 3, H–C(4)); 2.03 (*s*, Ac). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 74.8 (C(4)); 72.0 (C(1)); 70.2 (*C*H$_2$OAc); 69.1 (C(3)); 66.9 (C(5)); 40.1 (C(6)); 36.5 (C(2)); 170.6, 20.8 (Ac).

10.2. To a soln. of 120 mg of **9b** in 7 ml of vinyl acetate containing 3 ml of pyridine, *Ch.v.* lipase adsorbed on *Celite* (1 g) was added and the suspension shaken overnight at 45°. Usual workup gave 95 mg (66%) of *4α-acetoxy-1α,3α,5β-trihydroxycyclohexane-1β-methyl acetate* (**9c**). R_f (CH_2Cl_2/MeOH 8:2) 0.57. ^{1}H-NMR (200 MHz, (D_6)DMSO): 4.52 (*dd*, J = 9, 3, H–C(4)); 3.98 (*m*, H–C(3), H–C(5)); 3.93 (*d*, J = 12.5), 3.82 (*d*, J = 12.5, CH_2OAc); 2.05 (*s*, Ac). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 77.6 (C(4)); 71.7 (C(1)); 70.0 (*C*H$_2$OAc); 66.6 (C(3));

63.6 (C(5)); 40.4 (C(6)); 36.6 (C(2)); 170.3, 21.1, 20.7 (Ac). FAB-MS: 263 (26, $[M + H]^+$), 245 (100), 227 (13), 203 (8), 185 (24), 167 (14), 143 (15), 125 (71), 107 (58).

11. *Methyl 3-*O*- and 4-*O*-Acetylshikimate* (= *Methyl 3α-Acetoxy-4α,5β-dihydroxy-* and *4α-Acetoxy-3α,5β-dihydroxycyclohex-1-ene-1-carboxylate*; **10b** and **10c**, resp.). To a soln. of 200 mg of methyl shikimate (**10a**) in 10 ml of vinyl acetate, *Ch.v.* lipase on *Celite* (1 g) was added. The suspension was shaken for 24 h at 45° and 250 rpm. Usual workup and purification (AcOEt/hexane 7:3) gave **10b**/**10c** 2.5:1 (153 mg, 60%). Oil. R_f (hexane/AcOEt 7:3) 0.29. ^{1}H-NMR (300 MHz, $CDCl_3$): **10b**: 6.75 (*d*, $J = 3$, H–C(2)); 5.58 (*t*, $J = 3$, H–C(3)); 4.02 (*dt*, $J = 9, 5$, H–C(5)); 3.79 (*dd*, $J = 9, 3$, H–C(4)); 3.75 (*s*, MeO); 2.13 (*s*, Ac); **10c**: 6.83 (br. *s*, H–C(2)); 4.93 (*dd*, $J = 9, 3$, H–C(4)); 4.64 (br. *s*, H–C(3)); 4.23 (*dt*, $J = 9, 5$, H–C(5)); 3.75 (*s*, MeO); 2.13 (*s*, Ac).

This work was financially supported by C.N.R., Target Project 'Biotechnology and Bioinstrumentation'.

REFERENCES

[1] a) E. Haslam, 'Shikimic Acid Metabolites', in 'Comprehensive Organic Chemistry', Eds. D. Barton and D. W. Ollis, Pergamon Press, London, 1979, Vol. 5, p. 1167; b) P. Manitto, 'Biosynthesis of Natural Products', Ellis Horwood Ltd., Chichester, 1981, Chapt. 7.

[2] a) U. Waiss, J. M. Edwards, 'The Biosynthesis of Aromatic Compounds', Wiley, New York, 1980; b) E. Haslam, 'The Shikimate Pathway', Wiley, New York, 1974; c) B. Ganem, *Tetrahedron* **1978,** *34,* 3353.

[3] a) A. V. Rama Rao, T. K. Chakraborty, D. Sankaranayanan, A. V. Purandare, *Tetrahedron Lett.* **1991,** *32,* 547; b) S. Hanessian, P. Beaulieu, D. Dube', *ibid.* **1986,** *27,* 5071; c) D. Desmaele, S. Tanier, *ibid.* **1985,** *26,* 4941; d) D. H. R. Barton, S. D. Gero, C. D. Maycock, *J. Chem. Soc., Chem. Commun.* **1980,** 1089; e) J. E. Audia, L. Boisvert, A. D. Patten, A. Villabolos, S. J. Danishefsky, *J. Org. Chem.* **1989,** *54,* 3738; f) J. C. Barriere, J. Cleophax, S. D. Gero, M. Vulihorgne, *Helv. Chim. Acta* **1983,** *66,* 296.

[4] a) T. K. M. Shing, Y. Tang, *Tetrahedron* **1990,** *46,* 6575; b) J. D. White, J. M. Cammack, K. Sakuma, *J. Am. Chem. Soc.* **1989,** *111,* 8970; c) S. Hanessian, J. Sakito, D. Dhanoa, L. Baptistella, *Tetrahedron* **1989,** *45,* 6623; d) J. R. Falck, P. Yadagiri, *J. Org. Chem.* **1989,** *54,* 5851; e) B. M. Trost, A. G. Romero, *ibid.* **1986,** *51,* 2332; f) H. B. Wood, B. Ganem, *J. Am. Chem. Soc.* **1990,** *112,* 8907.

[5] T. K. M. Shing, Y. Tang, *Tetrahedron* **1991,** *47,* 4571.

[6] J. Wolinsky, R. Novak, R. Vasileff, *J. Org. Chem.* **1964,** *29,* 3596.

[7] E. Haslam, M. J. Turner, O. Sargent, R. S. Thompson, *J. Chem. Soc. (C)* **1971,** 1489.

[8] D. A. McGowan, G. A. Berchtold, *J. Am. Chem. Soc.* **1982,** *104,* 7036.

[9] a) G. Carrea, S. Riva, F. Secundo, B. Danieli, *J. Chem. Soc., Perkin Trans. 1* **1989,** 1057; b) S. Riva, J. Chopineau, A. P. G. Kieboom, A. M. Klibanov, *J. Am. Chem. Soc.* **1988,** *110,* 584; c) B. Danieli, P. DeBellis, G. Carrea, S. Riva, *Gazz. Chim. Ital.* **1991,** *121,* 123; d) B. Danieli, P. DeBellis, G. Carrea, S. Riva, *Helv. Chim. Acta* **1990,** *73,* 1837; e) B. Danieli, P. DeBellis, G. Carrea, S. Riva, *Heterocycles* **1989,** *29,* 2061; f) G. Ottolina, G. Carrea, S. Riva, *J. Org. Chem.* **1990,** *55,* 2366; g) S. Riva, R. Bovara, G. Ottolina, F. Secundo, G. Carrea, *ibid.* **1989,** *54,* 3161; h) S. Riva, A. M. Klibanov, *J. Am. Chem. Soc.* **1988,** *110,* 3291.

[10] a) A. M. Klibanov, *Acc. Chem. Res.* **1990,** *23,* 114; b) A. M. Klibanov, *Trends Biochem. Sci.* **1989,** *14,* 141; c) A. M. Klibanov, *Chemtech* **1986,** *16,* 354.

[11] a) A. Zaks, A. M. Klibanov, *J. Biol. Chem.* **1988,** *263,* 3194; b) A. Zaks, A. M. Klibanov, *Proc. Natl. Acad. Sci. U.S.A.* **1985,** *82,* 3192.

[12] J. D. Elliott, M. Hetmanski, R. J. Stoodley, M. N. Palfreyman, *J. Chem. Soc., Perkin Trans. 1* **1981,** 1782.

[13] R. M. Meier, Ch. Tamm, *Helv. Chim. Acta* **1991,** *74,* 807.

[14] D. Mercier, J. Leboul, J. Cleophax, S. D. Gero, *Carbohydr. Res.* **1971,** *20,* 299.

[15] S. Castellino, G. C. Leo, R. D. Sammons, J. A. Sikorski, *J. Org. Chem.* **1991,** *56,* 5176.

[16] C. C. Sweeley, R. Bentley, M. Mikita, W. W. Wells, *J. Am. Chem. Soc.* **1963,** *85,* 2497.