217. Chemo-enzymatic Synthesis of 6″-*O*-(3-Arylprop-2-enoyl) Derivatives of the Flavonol Glucoside Isoquercitrin

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A chemo-enzymatic approach to some 6″-*O*-(3-arylprop-2-enoyl) derivatives of the flavonol glucoside isoquercitrin (**2a**) was explored to overcome the inability to directly introduce these acyl moieties by an enzyme-catalyzed reaction of **2a** with the corresponding activated esters. This new approach was based on the regioselective introduction of a methyl malonate residue at the CH_2OH of the sugar moiety by catalysis with the protease subtilisin (→ **22a**). The mixed diester **22a** was then subjected to chemoselective hydrolysis of the methoxycarbonyl function by another enzyme, biophine esterase (→ **23**). Finally, the malonic monoester **23** was reacted in a *Knoevenagel*-type condensation with benzaldehyde, 4-hydroxybenzaldehyde, or 4-hydroxy-3-methoxybenzaldehyde to afford the target 6″-*O*-(3-arylprop-2-enoyl) isoquercitrins **2b–d**.

Introduction. – Flavonoid glycosides are an important group of natural compounds widely distributed in the plant kingdom, in which they are often present acylated with aliphatic and aromatic acids, mainly acetic acid, malonic acid, *p*-coumaric acid (= 3-(4-hydroxyphenyl)prop-2-enoic acid), and ferulic acid (= 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) at specific OH's of their sugar moieties [1]. The aromatic esters are quite widespread, and many of them possess interesting properties. To take a few examples, the *p*-coumarate of some kaempferol and quercetine disaccharide monoglycosides are active components (together with ginkolides) of the very popular medicinal extract of the leaves of *Ginko biloba*, which is used to increase peripheral and cerebral blood flow [2]. Furthermore, the 7-[6″-O-(*p*-coumaroyl)-β-D-glucopyranosides] of kaempferol and isorhamnetine have recently been shown to be potent tyrosinase inhibitors [3], and, finally, complex *p*-coumarates of anthocyanins were shown to play a fundamental role in determining the colour and colour variations of flowers [4].

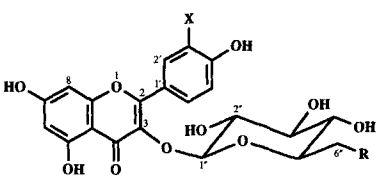
In nature, the formation of these esters is the last step in the biosynthetic pathway, and it is catalyzed by different acyltransferases [5]. These enzymes show relative flexibility towards the acyl groups but strict selectivity for the substrate to be esterified. In laboratory syntheses, acyltransferases are not very convenient as they require stoichiometric amounts of the corresponding acyl-coenzyme A. On the other hand, chemical acylation of glycosides with excess of alkanoyl chlorides, anhydrides, or activated esters leads invariably to a mixture of the possible mono-, di-, and polyesters, while chemical synthesis of individual esters usually requires complex multistep procedures. *E.g.*, tiliroside (**1**), which seems to have the widest distribution among the flavonol glycosides hitherto found

[1], was the first compound of this type to be synthesized through a sequence of delicate protective and deprotective reactions [6].

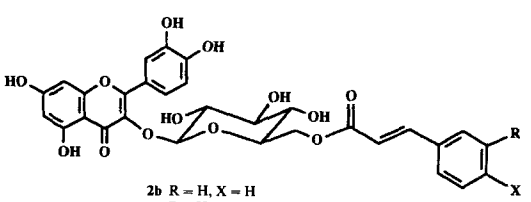
Exploiting *Klibanov*'s pioneering findings on the catalytic activity of enzymes in organic solvents [7], we recently showed that the esterification of flavonol monoglycosides derived from mono- and disaccharides with 2,2,2-trifluoroethyl butanoate by the proteolytic enzyme subtilisin in anhydrous pyridine takes place with a high degree of regioselectivity for the OH of the sugar moieties [8]. These successful results suggested to us to apply this methodology to the more challenging introduction of the different arylpropenoate groups, in order to have access to the cinnamates (= 3-phenylprop-2-enoate), *p*-coumarates, and ferulates of these glycosides. We report here the results of our efforts in the phenylpropenoation of a representative flavonoid glycoside, namely isoquercitrin (= 3-*O*-(β-D-glucopyranosyl)quercetin (= 2-(3,4-dihydroxyphenyl)-3-(β-D-glucopyranosyloxy)-5,7-dihydroxy-4*H*-1-benzopyran-4-one; **2a**). Although we were unable to introduce the phenylpropenoate groups directly, we were able to achieve our synthetic goal through a versatile approach based on a suitable combination of enzymatic and chemical reactions and on the use of different enzymes to perform the crucial steps in the synthetic scheme.

Results and Discussion. – Following our general protocol for the enzymatic butanoylation of natural glycosides [8], isoquercitrin (**2a**) was dissovled in pyridine containing the activated ester 2,2,2-trifluoroethyl cinnamate (**3a**) and the enzyme subtilisin added. In analogy with the butanoylation, we expected to obtain the corresponding 6″-*O*-cinnamoyl-isoquercitrin (**2b**), but, disappointingly, **2a** was completely unreactive, after even a prolonged reaction time and in the presence of a large excess of enzyme and activated ester.

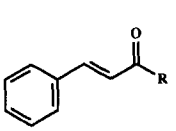
In a first attempt to overcome this negative result, we thought to take advantage of the proteolytic nature of subtilisin and, more specifically, of the fact that the peptide bond between phenylalanine and another amino acid is one of the best substrates for this enzyme in both hydrolysis and synthesis [9]. In addition, it was reported [10] that subtilisin catalyzes the acylation of sugars with an activated ester of *N*-protected phenyl-



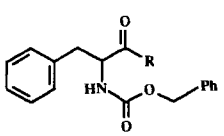
1 X = H, R = PhCH=CHCOO
2a X = OH, R = OH



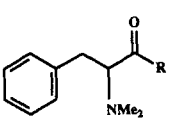
2b R = H, X = H
c R = H, X = OH
d R = MeO, X = OH



3a R = CF_3CH_2O
b R = $Me(CH_2)_3O$



4a R = $ClCH_2CH_2O$
b R = $Me(CH_2)_3O$



5 R = $ClCH_2CH_2O$

alanine. Therefore, we envisaged a convenient route to the target 6″-*O*-arylpropenoyl-isoquercitrins **2b–d** based on the enzymatic transfer of a phenylalanine residue to **2a** followed by the transformation of the amino-acid moiety into the desired esters through a series of known chemical reactions [11]. For this, we synthesized the 2-chloroethyl esters **4a** and **5** of *N*-[(benzyloxy)carbonyl]-L-phenylalanine and of *N*,*N*-dimethyl-L-phenylalanine, respectively. However, neither **4a** nor **5** showed any appreciable reactivity with **2a** in the presence of subtilisin (*Table 1*). A plausible reason for this behaviour emerged from a comparison with the facile enzymatic esterification of BuOH with **3a** and **4a**. The butyl esters **3b** and **4b**, respectively, were produced in good yields, indicating that the activated esters **3a** and **4a** interacted with the enzyme to form the corresponding acyl-enzyme intermediates[1]) (*Table 1*). However, the bulkiness of the acyl residue encumbers the active site, preventing the approach by large molecules such as **2a** (similar results were obtained in the acylation of sucrose [13]).

Table 1. *Acylation of Isoquercitrin (***2a***) and BuOH with the Activated Esters* **3a**, **4a** *and* **5**[a])

	2a	BuOH	**2a**	BuOH	**2a**	BuOH
Ester	**3a**	**3a**	**4a**	**4a**	**5**	**5**
Conversion [%][b])	–[c])	66	–[c])	20	–[c])	–[c])

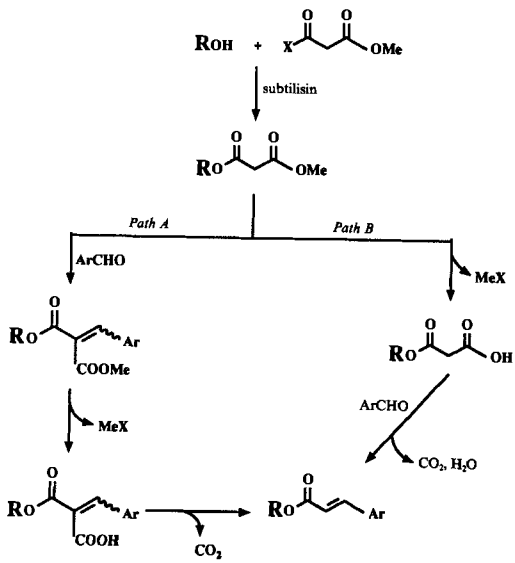
[a]) Conditions: **2a**, 0.06 mmol; BuOH, 0.1 mmol; esters **3a**, **4a**, or **5**, 3 equiv.; subtilisin, 20 mg; pyridine, 1 ml; 45°.
[b]) Determined by HPLC (**2a**) or GC (BuOH) after 48 h.
[c]) No conversion detectable.

The results led us to conclude that the arylpropenoates of **2a** could be obtained only through a combined approach based on the enzymatic introduction of a small acyl group suitable for transformation into the target 3-arylpropenoates **2b–d** by chemical reactions. As depicted in *Scheme 1*, a strategy based on the *Knoevenagel* condensation was chosen. The substrate ROH could be first transformed into a methyl malonate with the assistance of subtilisin. Subsequent chemical elaboration could be either condensation with an aromatic aldehyde (to form a (phenylmethylidene)malonate) followed by selective hydrolysis of the COOMe group and decarboxylation (*Path A*), or preliminary hydrolysis of the COOMe group followed by condensation and concomitant decarboxylation (*Path B*).

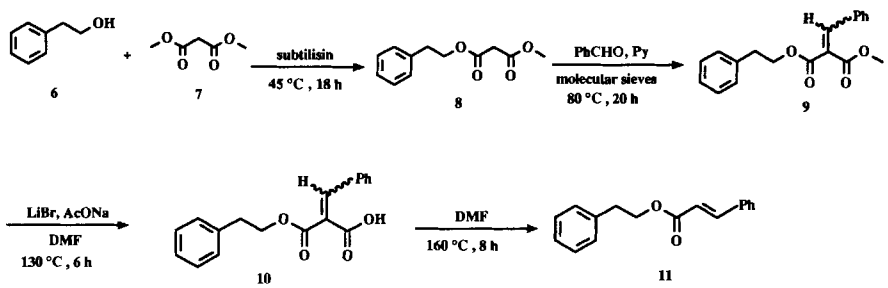
To determine the feasibility of this scheme, the investigation was first carried out with the simplest malonate derivative, *i.e.*, dimethyl malonate (**7**), as the acylating agent, and 2-phenylethanol (**6**) as the substrate (*Scheme 2*). Subtilisin was added to a solution of **6** in **7**, and the suspension stirred overnight at 45°. Methyl 2-phenylethyl malonate (**8**) was obtained in almost quantitative yield, and its condensation with benzaldehyde (according to *Path A*) in the presence of molecular sieves gave the methyl 2-phenylethyl esters **9** of (phenylmethylidene)malonic acid as a mixture of (*E*)- and (*Z*)-isomers (determined by ^{1}H-NMR; see *Exper. Part*). Selective hydrolysis of the COOMe function at 130° by an excess of LiBr in DMF containing 1 equiv. of AcONa as methyl scavenger [14] gave the 2-phenylethyl ester **10**, which, after heating in DMF at 160°, was transformed into the

[1]) *N*-[(*E*)-cinnamoyl]imidazole is currently employed as a reagent for the titration of proteases active sites [12].

Scheme 1



Scheme 2

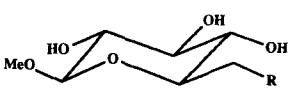


2-phenylethyl ester **11** of (*E*)-cinnamic acid (32% overall yield). No traces of the (*Z*)-cinnamate analogue were found on careful inspection of the ^{1}H-NMR spectra.

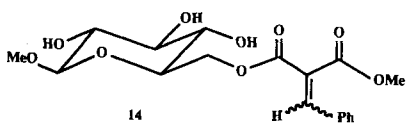
Our synthetic scheme was subsequently checked on methyl β-D-glucopyranoside (**12**), which is a model more similar to our flavonoid glycosides than 2-phenylethanol (**6**). Due to the lesser solubility of **12**, the enzymatic acylation was performed with pyridine as cosolvent (20% pyridine in dimethyl malonate (**7**)) and methyl 6-*O*-[2-(methoxycarbonyl)acetyl]-β-D-glucopyranoside (**13**) was obtained in good yield. Diester **13** was then modified according to *Path A*. Condensation with benzaldehyde was successful and yielded the mixed (phenylmethylidene)malonate **14** ((*E*)/(*Z*)-mixture). Any attempts at further elaboration following the procedure described above did not give the desidered methyl 6-*O*-cinnamoyl-β-D-glucopyranoside **17**; hydrolytic conditions were too harsh

and non-selective, giving a complex mixture of products in which only the starting sugar **12** could be identified. Similar efforts to hydrolyze chemically the COOMe function of **13** with LiBr/AcONa in DMF according to *Path B* led again to the starting sugar **12**.

Since the chemoselective cleavage of the COOMe group in **13** or **14** was mandatory to achieve our goal, different milder hydrolytic conditions were tried, including the use of biological systems. Among the different protocols, only pig-liver esterase, a well-known enzyme widely employed for the hydrolysis of methyl esters [15], completely hydrolyzed **13**. However, selectivity was not high, because both ester functions were affected, yielding a 35:65 mixture of the starting **12** and of the monoester methyl 6-*O*-(2-carboxyacetyl)-*β*-D-glucopyranoside (**15**), which could be isolated after chromatography. In analogy with other natural and synthetic monoesters of malonic acid [16], **15** underwent a loss of carbonic anhydride on heating, to give methyl 6-*O*-acetyl-*β*-D-glucopyranoside (**16**). Subsequent elaboration of **15** into methyl 6-*O*-[(*E*)-cinnamoyl]-*β*-D-glucopyranoside (**17**) was easily accomplished by *Path B*, by heating overnight a pyridine solution of **15** with an excess of benzaldehyde in the presence of molecular sieves. Ester **17** was obtained as the sole stereoisomer in 24% overall yield (from methyl *β*-D-glucopyranoside). The above reported enzymatic hydrolysis and condensation with benzaldehyde were consolidated in a single operation, and **17** was obtained in comparable yield after chromatographic separation from **12**[2]).



12 R = OH
13 R = $(MeOOC)CH_2COO$
15 R = $(HOOC)CH_2COO$
16 R = AcO
17 R = PhCH=CHCOO
18 R = $C_9H_{19}CH$=CHCOO



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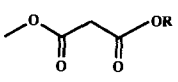
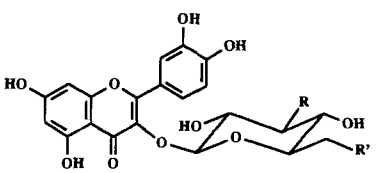


7 R = Me
19 R = $ClCH_2CH_2$
20 R = CCl_3CH_2
21 R = CF_3CH_2



22a R = OH, R′ = $(MeOOC)CH_2COO$
b R = $(MeOOC)CH_2COO$, R′ = OH
c R = R′ = $(MeOOC)CH_2COO$
23 R = OH, R′ = $(HOOC)CH_2COO$

Structurally diagnostic signals in the ^{1}H-NMR spectrum of **13** were a downfield-shifted *AB* portion of an *ABX* system at 4.33 ppm (J = 11.5 and 1.5 Hz) and 4.14 ppm (J = 11.5 and 6 Hz), due to the diastereoisotopic protons of the acylated primary alcoholic function at C(6), and 2 *s* at 3.53 (2 H) and 3.64 ppm (3 H), due to the CH_2COOMe group. In the ^{13}C-NMR spectrum, the $(MeOOC)CH_2COO$ moiety was indicated by 2*s* at 167.0 and 166.6 ppm for

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[2]) Several other aldehydes were also employed. *E.g.*, with decanal, the corresponding methyl 6-*O*-(dodec-2-enoyl)-*β*-D-glucopyranoside (**18**) was obtained with similar overall yield after FC purification (AcOEt/MeOH 10:0.3). ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.37 (*s*, MeO); 4.09 (*d*, J = 7.5, H–C(1)); 4.13 (*dd*, J = 11.5, 5, H_a–C(6)); 4.35 (*d*, J = 11.5, H_b–C(6)); 5.90 (*d*, J = 16), 6.90 (*dt*, J = 16, 8, (*E*)-CH=CH). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 103.7 (C(1)); 76.0 (C(3)); 73.6 (C(5)); 73.1 (C(2)); 69.8 (C(4)); 63.3 (C(6)); 56.0 (MeO); acyl moiety: 165.9, 150.3, 120.6, 31.4, 31.2, 28.4, 27.4, 22.0, 13.9.

the 2 C=O groups, and by the signals at 52.3 (*q*) and 40.9 (*t*) ppm. Acylation of the primary OH was further confirmed by the downfield shift of C(6) and the upfield shift of C(5) compared to the starting sugar (64.6 and 73.3 ppm *vs.* 61.8 and 76.3 ppm, resp.). Compared to **13**, the ^{13}C-NMR spectrum of **15** lacked only the *q* at 52.3 ppm (MeO): The negative FAB-MS displayed an intense $[M - H]^-$ ion at *m/z* 279, accompanied by a base peak at *m/z* 235, due to the loss of CO_2. The presence of the (*E*)-cinnamoyl moiety of **17** was clearly indicated by the 2 *d* at 6.69 and 7.68 ppm ($J = 16$ Hz) in the ^{1}H-NMR spectrum and at 118.0 and 144.5 ppm in the ^{13}C-NMR spectrum (see *Exper. Part*).

Acylation of the target isoquercitrin (**2a**) with dimethyl malonate (**7**), was then undertaken and, because of the polar nature of this compound, pyridine had to be used as solvent. However, subtilisin-catalyzed acylation with **7** (up to 30 equiv.) was unsuccessful, showing a very low conversion, even after prolonged reaction. Thus, we synthesized the 2-chloroethyl methyl malonate (**19**), methyl 2,2,2-trichloroethyl malonate (**20**), and methyl 2,2,2-trifluoroethyl malonate (**21**). The trichloro- and trifluoroethyl esters **20** and **21** were too reactive, giving uncatalyzed aspecific chemical acylations of **2**, which the monochloroethyl ester **19** did not. Besides, the latter proved to be a good substrate for subtilisin.

The lyophilized enzyme and molecular sieves were added to a solution of isoquercitrin (**2a**) in anhydrous pyridine containing 30 equiv. of **19**, and the suspension was stirred at 55° for seven days. HPLC analysis showed a 68% conversion to three products (9:1:1 ratio), which were isolated by flash chromatography and identified as 6″-*O*-[2-(methoxycarbonyl)acetyl]isoquercitrin (**22a**), 3″-*O*-[2-(methoxycarbonyl)acetyl]isoquercitrin (**22b**), and 3″,6″-di-*O*-[2-(methoxycarbonyl)acetyl]isoquercitrin (**22c**), respectively. Subsequent attempts to manipulate **22a** met the same difficulties as encountered in the methyl β-D-glucopyranoside series. Specifically, chemical hydrolysis of the COOMe group were unsuccessful, and pig-liver-esterase-catalyzed hydrolysis gave a 3:7 mixture of isoquercitrin (**2a**) and a new product, presumably the desired acid **23**. Since we were confident that the biocatalytic approach should offer better results, we screened several other hydrolases, and, to our gratification, found that biophine esterase was the catalyst of choice (*Table 2*). HPLC Analysis of the crude extract obtained from the hydrolytic solution revealed that the starting **22a** had disappeared, and a 9:1 mixture of the same new product obtained by pig-liver-esterase hydrolysis and **2a** had formed. An aliquot of the crude reaction mixture was purified, leading to isolation of the new compound,

Table 2. *Enzymatic Hydrolysis of 6″-O-[2-(Methoxycarbonyl)acetyl]isoquercitrin* (**22a**)[a])

Enzyme	Conversion [%]	**2a**/**23**[b])
Subtilisin	97	94.5: 2.5
Chymotrypsin	98	97 : 1
Candida cylindracea lipase	13	13 : 0
Pseudomonas fluorescens lipase	8	8 : 0
Chromobacterium viscosum lipase	12	12 : 0
Porcine pancreatic lipase	5	5 : 0
Pig-liver esterase	92	33 :59
Horse-liver esterase	41	23 :18
Biophine esterase	49	4 :45

[a]) Conditions: **22a** (5 mg) dissolved in DMF (50 µl); enzymes, 2 mg (lipases, 10 mg); phosphate buffer 20 mM (pH 7), 0.5 ml; room temperature.

[b]) Determined by HPLC after 24 h.

identified as 6″-*O*-(2-carboxyacetyl)isoquercitrin (**23**). This molecule is *per se* a natural product [17] and, to our knowledge, was never obtained synthetically before. Finally, crude **23** was heated in pyridine with different aromatic aldehydes and, in this way, the target 3-arylpropenoyl derivatives of isoquercitrin, specifically the 6″-*O*-cinnamoyl, 6″-*O*-(*p*-coumaroyl), and 6″-*O*-feruloyl derivatives **2b–d**, were isolated in 20–22% yield from **22a**.

Negative FAB-MS established that only one $(MeOOC)CH_2COO$ residue was present in **22a** and **22b** ($[M - H]^-$ ion at m/z 563). Compared to the parent **2a**, the ^{1}H-NMR spectrum of **22a** showed the characteristic shift of CH_2(6″) (*AB* portion of an *ABX* system at 4.25 (br. *d*, J = 11.5 Hz) and 4.03 (*dd*, J = 11.5 and 3 Hz) ppm). Furthermore, ^{13}C-NMR showed a downfield shift of C(6″) ($\Delta\delta$ = +2.8 ppm) and an upfield shift of C(5″) ($\Delta\delta$ = –3.0 ppm). On the other hand, a downfield-shifted *t* at 4.89 ppm was present in the ^{1}H-NMR of **22b**, and its ^{13}C-NMR spectrum showed a downfield-shifted C(3″) ($\Delta\delta$ = +0.8 ppm) and an upfield-shifted C(2″) ($\Delta\delta$ = –2.0 ppm) and C(4″) ($\Delta\delta$ = –2.4 ppm), indicating that esterification had occurred at C(3″). Negative FAB-MS identified **22c** as a diacyl derivative of **2a** ($[M - H]^-$ ion at m/z 663). ^{1}H- and ^{13}C-NMR of **22c**, being combinations of the spectra of **22a** and **22b**, unambiguously identified it. Our spectroscopic data of **23** were in excellent agreement with those reported [17]. Compared to **22a**, the NMR spectra of **23** lacked the signal of the MeO group, namely the *q*. at 52.0 ppm in the ^{13}C-NMR spectrum and the *s* at 3.56 ppm in the ^{1}H-NMR. In addition, the structure was confirmed by negative FAB-MS ($[M - H]^-$ ion at m/z 549 and base peak at m/z 301). Negative FAB-MS were in agreement with the structures of **2b–d**, showing $[M - H]^-$ ions at m/z 593 for **2b**, 609 for **2c**, and 639 for **2d**. ^{1}H- and ^{13}C-NMR spectra were again diagnostic for the identification of the acylation positions, for the nature of ester moieties, and for the configurational purities (only (*E*)-isomer **2b–d** (for details, see *Exper. Part*)).

Conclusion. – To circumvent our inability to obtain the 6″-*O*-cinnamoylisoquercitrin (**2b**) by direct acylation of isoquercitrin (**2a**) with 2,2,2-trifluoroethyl cinnamate in the presence of subtilisin, we developed a synthetic route based on a suitable combination of enzymatic and chemical reactions. In a first enzymatic step, subtilisin was used to regioselectively introduce methyl malonate at the 6″-OH of **2a** (→ **22a**). Then another enzyme, biophine esterase, was employed for the selective hydrolysis of the COOMe function of the mixed diester **22a** (→ **23**). In a chemical step, different aldehydes were reacted with the activated methylene group of the malonic monoester **23** in a *Knoevenagel* type reaction to obtain the desired 6″-*O*-cinnamoyl-6″-*O*-(*p*-coumaroyl)-, and 6″-*O*-feruloylisoquercitrins **2b–d**.

The previously reported aptitude of subtilisin to acylate other flavonol monoglycosides derived from mono- and disaccharides [8] suggests that this approach can be extended to the synthesis of aromatic esters of these compounds, to obtain, *e.g.*, the above mentioned tiliroside and tyrosinase inhibitors.

We thank the *C.N.R.*, Rome, 'Target Project on Biotechnology and Bioinstrumentation' for financial support of this work.

Experimental Part

1. *General.* Isoquercitrin (**2a**) was purchased from *Extrasynthese*. Subtilisin (*EC 3.4.21.14*, protease from *B. licheniformis*), chymotrypsin (*EC 3.4.21.1*, protease from bovine pancreas), esterase (*EC 3.1.1.1*, carboxyl esterase from pig liver), lipase (*EC 3.1.1.3*) from *Candida cylindracea* and porcine pancreas were from *Sigma*. Lipase from *Chromobacterium viscosum* was from *Finnsugar*. Lipase from *Pseudomonas fluorescens* and esterase from horse liver were from *Amano*. Biophine esterase was a generous gift of IBIS. Enzymatic transesterifications and hydrolyses in the isoquercitrin (**2a**) series were followed by TLC and HPLC. Enzymatic esterifications and chemical manipulations in the 2-phenylethyl alcohol (**6**) and methyl β-D-glucopyranoside (**12**) series were followed by TLC and GC; reaction mixtures were transformed with 1,1,1,3,3,3-hexamethyldisilazane prior to analysis. TLC: precoated silica gel *60* F_{254} plates from *Merck*. HPLC: *JASCO 880/PV* pump, *JASCO 870 UV/VS* detector; *Erbasil* 10 μm C_{18}/*M* column (250 mm × 4.6 mm); 15-min linear gradient from 20 to 40% MeCN in H_2O (containing 0.1% CF_3COOH); flow rate 1 ml/min, readings at 254 nm. GC: *25 HP1* capillary silica-gel column, coated with methylsilicone gum (*Hewlett-Packard*). NMR Spectra: *Bruker-AC-300* (^1H at *300* and ^{13}C at *50.2* MHz). FAB-MS: *VG-70-70-EQ-HF* instrument equipped with its own source, using Xe as gas and glycerol matrix. FC = flash chromatography.

2. *Aromatic Activated Esters.* The 3,3,3-trifluoroethyl (*E*)-3-phenylprop-2-enoate (**3a**) was prepared as described [13]. The 2-chloroethyl *N*-[(benzyloxy)carbonyl]-L-phenylalaninate (**4a**) and 2-chloroethyl *N*,*N*-dimethyl-L-phenylalaninate (**5**) were prepared by heating the corresponding acid and 2-chloroethanol in the presence of TsOH [9a]. Spectroscopic and anal. data: in agreement with the proposed structures.

3. *Activated Esters of Malonic Acid.* The following procedure for the preparation of **19** is representative: To a cooled soln. of 2-chloroethanol (21 ml, 1.1 equiv.), Et_3N (31 ml, 1.1 equiv.), and a catalytic amount of 4-(dimethylamino)pyridine in CH_2Cl_2 was added dropwise methyl (chloroformyl)acetate (20 ml; *Aldrich*). The soln. was left at r.t. for *ca.* 3 h. The solid $(Et_4N)Cl$ was filtered off, the soln. washed with H_2O and 5% $NaHCO_3$ soln., the org. layer dried (Na_2SO_4), the solvent evaporated and the crude residue distilled under vacuum.

2-Chloroethyl Methyl Propanedioate (**19**): B.p. 128°/18 Torr. ^1H-NMR (300 MHz, $CDCl_3$): 3.44 (*s*, CH_2); 3.68 (*t*, CH_2O); 3.73 (*s*, MeO); 4.40 (*t*, CH_2Cl).

Methyl 2,2,2-Trichloroethyl Propanedioate (**20**): B.p. 141°/18 Torr., ^1H-NMR (300 MHz, $CDCl_3$): 3.65 (*s*, CH_2); 3.74 (*s*, MeO); 4.75 (*s*, CH_2CCl_3).

Methyl 2,2,2-Trifluoroethyl Propanedioate (**21**): B.p. 63°/18 Torr. ^1H-NMR (300 MHz, $CDCl_3$): 3.48 (*s*, CH_2); 3.75 (*s*, MeO); 4.55 (*q*, CH_2CF_3).

4. *2-Phenylethyl 3-Phenylprop-2-enoate* (**11**). To a soln. of 2-phenylethyl alcohol (**6**; 1 g) in dimethyl malonate (**7**; 40 ml) subtilisin (400 mg; lyophilized from pH 7.8 [13]) was added and the soln. shaken at 50° and 250 rpm. After 18 h, 98% of **6** was acylated. The enzyme was removed by filtration and the solvent evaporated. Crude **8** was dissolved in pyridine (10 ml) containing benzaldehyde (1.4 ml; 3 equiv.) and piperidine (250 μl). Following addition of molecular sieves, the soln. was heated at 80° until complete conversion of **8** in **9** (*ca.* 2 h). Molecular sieves were filtered off, pyridine evaporated, and the crude **9** redissolved in DMF (50 ml). LiBr (2.8 g, 10 equiv.) and AcONa (260 mg, 1 equiv.) were added, and the mixture was heated at 130° for 6 h (TLC monitoring (AcOEt/MeOH/H_2O 10:0.5:0.5)). The soln. was poured in H_2O, acidified to pH 3, and extracted with AcOEt. The solvent was evaporated and the crude **10** dissolved in DMF (10 ml) and refluxed for 8 h. DMF was evaporated and the residue purified by FC (silica gel, hexane/AcOEt 9:1): 415 mg of **11** (32% overall yield).

Methyl 2-Phenylethyl Propanedioate (**8**): ^1H-NMR (300 MHz, $CDCl_3$): 2.95 (*t*, $J = 6.7$, PhCH_2); 3.35 (*s*, CH_2CO); 3.70 (*s*, MeO); 4.36 (*t*, $J = 6.7$, CH_2O).

Methyl 2-Phenylethyl 2-(Phenylmethylidene)propanedioate (**9**): ^1H-NMR (300 MHz, $CDCl_3$): 2.95 (*t*, $J = 6.7$, PhCH_2); 3.70 (*s*, MeO); 4.36 (*t*, $J = 6.7$, CH_2O); 7.70, 7.80 (*2s*, CH=C).

2-Phenylethyl Hydrogen 2-(Phenylmethylidene)propanedioate (**10**): ^1H-NMR (300 MHz, $CDCl_3$): 2.95 (*t*, $J = 6.7$, PhCH_2); 4.36 (*t*, $J = 6.7$, CH_2O); 7.70, 7.80 (*2s*, CH=C).

Ester **11**: ^1H-NMR (300 MHz, $CDCl_3$): 3.0 (*t*, $J = 6.7$, PhCH_2); 4.40 (*t*, $J = 6.7$, CH_2O); 6.37 (*d*, $J = 16$), 7.65 (*d*, $J = 16$, CH=CH).

5. *Methyl 6-O-[(E)-3-Phenylprop-2-enoyl]-β-D-glucopyranoside* (**17**). 5.1. *Methyl 6-O-[2-(Methoxycarbonyl)acetyl]-β-D-glucopyranoside* (**13**). To a soln. of methyl β-D-glucopyranoside (**12**; 500 mg) in dimethyl malonate (**7**)/pyridine 4:1 (25 ml), lyophilized subtilisin (500 mg) was added and the suspension stirred overnight at 45°. Usual workup and purification by FC (AcOEt/MeOH/H_2O 9:0.5:0.2) gave pure **13** (460 mg, 69%). ^1H-NMR (300 MHz, (D_6)DMSO): 3.34 (*s*, MeO); 3.53 (*s*, $COCH_2CO$); 3.64 (*s*, MeO); 4.07 (*d*, $J = 7.5$, H–C(1)); 4.14 (*dd*,

J = 11.5, 5, H_a–C(6)); 4.33 (br. *d*, J = 11.5, H_b–C(6)). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 103.8 (C(1)); 76.3 (C(3)); 73.4, 73.3 (C(5), C(2)); 69.9 C(4)); 64.6 (C(6)); 55.9 (MeO); (MeOOC)CH_2COO moiety: 167.0, 166.6, 52.2, 40.9. FAB-MS: 295 (20, $[M + H]^+$), 263 (100), 245 (82), 227 (18).

5.2. *Methyl 6-*O*-[(*E*/*Z*)-2-(Methoxycarbonyl)-3-phenylprop-2-enoyl]-β-*D*-glucopyranoside* (**14**). To a soln. of **13** (400 mg) in pyridine (10 ml) containing benzaldehyde (280 µl, 2 equiv.) and piperidine (200 µl), molecular sieves (500 mg), were added. The mixture was stirred at 50° for 10 h, the solvent evaporated, and the crude residue purified by FC: 225 mg of **14**. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.38 (*s*, MeO); 3.82 (*s*, MeO); 4.07, 4.11 (2*s*, H–C(1)); 4.25, 4.28 (2*dd*, J = 11.5, 5, H_a–C(6)); 4.66, 4.51 (2*d*, J = 11.5, H_b–C(6)); 7.77, 7.78 (2*s*, PhC*H*=C). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 103.9, 103.7 (C(1)); 76.3 (C(3)); 73.4, 73.2 (C(5), C(2)); 70.0 (C(4)); 65.1, 64.8 (C(6)); 55.8 (MeO); PhCH=C(COOMe)COO moiety: 166.5 and 165.9, 164.0 and 163.3, 141.9, 125.5 and 125.2, 52.8. FAB-MS: 381 (100, $[M - H]^-$), 205 (30), 193 (18), 161 (87), 147 (21).

Attempts to hydrolyze the COOMe group of **14** and to decarboxylate according to the above described procedure gave back the starting sugar **12** together with unidentified by-products.

5.3. *Methyl 6-*O*-(2-Carboxyacetyl)-β-*D*-glucopyranoside* (**15**). A soln. of **13** (200 mg) in MeOH (2 ml) was added to 10 mM phosphate buffer pH 7 (18 ml) containing 200 units of pig-liver esterase. The mixture, whose pH was maintained at a constant value by addition of 50 mM NaOH with an automatic titrator, was left at r.t. for 24 h. (TLC (AcOEt/MeOH/H_2O 8:3:2): complete conversion of **13** to 2 more polar products in a 65:35 ratio). The H_2O solvent was evaporated with a mechanic vacuum pump and the residue purified by FC (AcOEt/MeOH/H_2O 8:3:2): 98 mg of **15**. ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 103.8 (C(1)); 76.7 (C(3)); 73.7 (C(5)); 73.3 (C(2)); 70.1 (C(4)); 63.7 (C(6)); 56.0 (MeO); (HOOC)CH_2COO moiety: 170.0, 169.5, 45.0. FAB-MS: 279 (87, $[M - H]^-$), 235 (100), 193 (85).

5.4. *Ester* **17**. The crude hydrolyzed residue obtained according to *5.3* was redissolved in pyridine (5 ml) containing 3 equiv. of benzaldehyde and a catalytic amount of piperidine. Molecular sieves were added, and the soln. was stirred at 50° overnight. Usual workup and FC (AcOEt/MeOH/H_2O 9:3:0.1) gave 67 mg of **17**. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.40 (*s*, MeO); 4.10 (*d*, J = 7.5, H–C(1)); 4.22 (*dd*, J = 11.5, 5, H_a–C(6)); 4.45 (br. *d*, J = 11.5, H_b–C(6)); 6.69, 7.68 (2*d*, J = 16), 7.45 (*m*, 3 H), 7.75 (*m*, 2 H, PhCH=CH moiety). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 103.9 (C(1)); 76.5 (C(3)); 73.8 (C(5)); 73.3 (C(2)); 70.2 (C(4)); 63.9 (C(6)); 56.0 (MeO); PhCH=CHCOO moiety: 166.2, 144.5, 134.0, 128.9, 128.3, 118.0. FAB-MS: 323 (21, $[M - H]^-$), 193 (15), 147 (100).

6. *Methyl 6-*O*-Acetyl-β-*D*-glucopyranoside* (**16**). A soln. of **15** (5 mg) in (D_6)DMSO (0.5 ml) in a NMR tube was heated at 95° for 1 h. ^{1}H-NMR: 2.01 (*s*, Ac); 3.70 (*s*, MeO); 4.07 (*d*, J = 7.5, H–C(1)); 4.15 (*dd*, J = 11.5, 5, H_a–C(6)); 4.33 (br. *d*, J = 11.5, H_b–C(6)).

7. *Enzymatic Esterification of* **2a** *with* **19**. To a soln. of **2** (500 mg) in pyridine (25 ml) containing **19** (30 equiv.), subtilisin (400 mg) was added and the suspension shaken at 45° for 7 days. TLC (AcOEt/MeOH/H_2O 10:1:0.3) and HPLC showed a 65% conversion to **22a–c**. Usual workup and FC (AcOEt/MeOH/H_2O 10:1:0.3) gave **22a** (273 mg, 45%), **22b** (30 mg, 5%), and **22c** (41 mg).

*2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-{6-*O*-[2-(methoxycarbonyl)acetyl]-β-*D*-glucopyranosyloxy}-4*H*-1-benzopyran-4-one* (**22a**). ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.56 (*s*, MeO); 4.03 (*dd*, J = 11.5, 3, H_a–C(6″)); 4.25 (*d*, J = 11.5, H_b–C(6″)); 5.40 (*d*, J = 7.5, H–C(1″)); 6.22 (*d*, J = 2.5, H–C(8)); 6.42 (*d*, J = 2.5, H–C(6)), 6.86 (*d*, J = 9, H–C(5′)); 7.54 (*d*, J = 9, H–C(6′)); 7.56 (*s*, H–C(2′)). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 177.4 (C(4)); 164.2 (C(7)); 161.2 (C(5)); 156.4 (C(2), C(8a)); 148.6 (C(4′)); 144.8 (C(3′)); 133.1 (C(3)); 121.6 (C(6′)); 121.1 (C(1′)); 116.2 (C(5′)); 115.2 (C(2′)), 103.9 (C(4a)); 100.9 (C(1″)); 98.8 (C(6)); 93.6 (C(8)); 76.2 (C(3)); 73.9 (C(5″), C(2″)); 69.6 (C(4″)); 63.9 (C(6″)); (MeOOC)CH_2COO moiety: 166.9, 166.1, 52.0, 40.8. FAB-MS: 563 (85, $[M - H]^-$), 463 (100), 419 (30), 386 (40), 301 (97).

*2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-{3-*O*-[2-(methoxycarbonyl)acetyl]-β-*D*-glucopyranosyloxy}-4*H*-1-benzopyran-4-one* (**22b**). ^{1}H-NMR (300 MHz (D_6)DMSO): 3.69 (*s*, MeO); 4.89 (*t*, J = 7.5, H–C(3″)); 5.61 (*d*, J = 7.5, H–C(1″)); 6.22 (*d*, J = 2.5, H–C(8)); 6.44 (*d*, J = 2.5, H–C(6)); 6.88 (*d*, J = 9, H–C(5′)); 7.58 (*d*, J = 9, H–C(6′)); 7.61 (*s*, H–C(2′)). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 177.3 (C(4)); 164.3 (C(7)); 161.2 (C(5)); 156.3 (C(2), C(8a)); 149.0 (C(4′)); 144.8 (C(3′)); 133.1 (C(3)); 121.6 (C(6′)); 121.0 (C(1′)); 116.2 (C(5′)); 115.3 (C(23′)); 104.0 (C(4a)); 100.4 (C(1″)); 98.7 (C(6)); 93.6 (C(8)); 78.8 (C(3″)); 77.2 (C(5″)); 71.8 (C(2″)); 67.6 (C(4″)); 60.5 (C(6″)); (MeOOC)CH_2COO moiety: 166.9; 166.1, 52.1, 40.8. FAB-MS: 563 (35, $[M - H]^-$), 463 (79), 301 (100).

*3-{3,6-Bis-*O*-[2-(methoxycarbonyl)acetyl]-β-*D*-glucopyranosyloxy}-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one* (**22c**). ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.57 (*s*, MeO); 3.69 (*s*, MeO); 4.09 (*dd*, J = 11.5, 2.5, H_a–C(6″)); 4.23 (*d*, J = 11.5, H_b–C(6″)); 4.91 (*t*, J = 7.5, H–C(3″)); 5.55 (*d*, J = 7.5, H–C(1″)); 6.22 (*d*,

J = 2.5, H–C(8)); 6.43 (*d*, J = 2.5, H–C(6)); 6.87 (*d*, J = 9, H–C(5′)); 7.53 (*d*, J = 9, H–C(6′)); 7.57 (*s*, H–C(2′)). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 177.2 (C(4)); 164.3 (C(7)); 161.2 (C(5)); 156.6 (C(2)); 156.4 (C(8a)); 148.6 (C(4′)); 144.8 (C(3′)); 132.8 (C(3)); 121.5 (C(6′)); 120.9 (C(1′)); 116.2 (C(5′)); 115.3 (C(2′)); 103.9 (C(4a)); 100.5 (C(1″)); 98.8 (C(6)); 93.6 (C(8)); 78.2 (C(3″)); 73.5 (C(5″)); 71.6 (C(2″)); 67.3 (C(4″)); 63.3 (C(6″)); (MeOOC)CH_2COO moieties: 166.9, 166.1, 165.9, 52.0, 40.8. FAB-MS: 663 (15, $[M - H]^-$), 631 (5), 563 (10), 531 (8), 505 (4), 464 (20), 301 (100).

8. *3-[6-*O*-(2-Carboxyacetyl)-β-*D*-glucopyranosyloxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one* (**23**). A soln. of **22a** (100 mg) in DMF (1 ml) was added to 10 mM phosphate buffer pH 7 (25 ml) containing biofine esterase (100 mg). The pH was maintained at 7 by automatic addition of 50 mM NaOH in a pH-stat. After 2 days, the conversion to **23**/**2a** 9:1 was complete. Purification prep. reversed-phase by HPLC (0.1% CF_3COOH/MeCN 8:2) gave **23**. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.12 (*s*, $COCH_2CO$); 4.01 (*dd*, J = 11.5, 2, H_a–C(6″)); 4.23 (*d*, J = 11.5, H_b–C(6″)); 5.41 (*d*, J = 7.5, H–C(1″)); 6.21 (*d*, J = 2, H–C(8)); 6.42 (*d*, J = 2, H–C(6)); 6.86 (*d*, J = 9, H–C(5′)); 7.52 (*d*, J = 9, H–C(6′)); 7.54 (*s*, H–C(2′)). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 177.3 (C(4)), 163.9 (C(7)); 160.9 (C(5)); 156.6 (C(2)); 156.4 (C(8a)); 148.3 (C(4′)); 144.6 (C(3′)); 133.2 (C(3)); 121.6 (C(6′)); 121.1 (C(1′); 116.1 (C(5′)); 115.1 (C(2′)); 103.9 (C(4a)); 100.9 (C(1″)); 98.6 (C(6)), 93.6 (C(8)); 75.9 (C(3″)); 73.8 (C(5″), (C(2″)); 69.4 (C(4″)); 63.6 (C(6″)); (HOOC)CH_2COO moiety: 167.7, 166.5, 40.6. FAB-MS: 549 (27, $[M - H]^-$), 301 (100).

9. *2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-{6-*O*-[(*E*)-3-phenylprop-2-enoyl]-β-*D*-glucopyranosyloxy}-4*H*-1-benzopyran-4-one* (**2b**). Crude **23** (obtained from 100 mg of **22a** as described above) was dissolved in anh. pyridine (3 ml) containing benzaldehyde (60 µl, 3 equiv.) and piperidine (20 µl). Molecular sieves were added, and the mixture was heated at 60° for 2.5 h. Usual workup and purification by FC (AcOEt/MeOH/AcOH 10:1:0.5) gave 32 mg of **2b**). ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.09 (*dd*, J = 11.5, 6, H_a–C(6″)); 4.30 (*d*, J = 11.5, H_b–C(6″)); 5.45 (*d*, J = 6.3, H–C(1″)); 6.09 (*d*, J = 2.5, H–C(8)); 6.53 (*d*, J = 2.5, H–C(6)); 6.84 (*d*, J = 7.5, H–C(5′)); 7.50 (*dd*, J = 7.5, 1.5, H–C(6′)); 7.53 (*d*, J = 1.5, H–C(2′)); ester moiety: 6.25 (*d*, J = 15); 7.36 (*d*, J = 15), 7.40 (*m*). ^{13}C-NMR (50.2 MHz, (D_6)DMSO): 177.4 (C(4)); 164.2 (C(7)); 161.2 (C(5)); 156.3 (C(2), (C(8a)); 148.6 (C(4′)); 144.9 (C(3′)); 133.1 (C(3)); 121.5 (C(6′)); 121.1 (C(1′)); 116.2 (C(5′)); 115.2 (C(2′)); 103.8 (C(4a)); 100.7 (C(1″)); 98.8 (C(6)); 93.5 (C(8)); 76.3 (C(3″)); 74.3 (C(5″)); 74.0 (C(2″)); 70.0 (C(4″)); 63.6 (C(6″)); ester moiety: 165.8, 144.4, 133.8, 130.4, 128.9, 128.2, 117.5. FAB-MS: 593 (100, $[M - H]^-$), 463 (42), 389 (69), 301 (80).

10. *2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-{6-*O*-[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]-β-*D*-glucopyranosyloxy}-4*H*-1-benzopyran-4-one* (**2c**). Similarly (see *Exper. 9*), from **23** and 4-hydroxybenzaldehyde (67 mg, 3 equiv.): 25 mg of **2c**. ^{1}H-NMR (300 MHz, CD_3COCD_3): 4.15 (*dd*, J = 12, 6, H_a–C(6″)); 4.33 (*d*, J = 12, H_b–C(6″)); 5.37 (*d*, J = 7.5, H–C(1″)); 6.20 (*d*, J = 2, H–C(8)); 6.44 (*d*, J = 2, H–C(6)); 6.86 (*d*, J = 8.5, H–C(5′)); 7.62 (*dd*, J = 8.5, 1.5, H–C(6′)); 7.75 (*d*, J = 1.5, H–C(2′)); ester moiety: 6.13 (*d*, J = 16), 6.83 (*d*, J = 8.5), 7.38 (*d*, J = 8.5); 7.40 (*d*, J = 16). FAB-MS: 609 (100, $[M - H]^-$), 463 (16), 301 (34).

11. *2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3-{6-*O*-[(*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]-β-*D*-glucopyranosyloxy}-4*H*-1-benzopyran-4-one* (**2d**). Similarly (see *Exper. 9*), from **23** and 4-hydroxy-3-methoxybenzaldehyde (75 mg, 3 equiv.): 35 mg of **2d**. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.09 (*dd*, J = 11, 7.5, H_a–C(6″)); 4.39 (*d*, J = 11, H_b–C(6″)); 5.50 (*d*, J = 7.5, H–C(1″)); 6.13 (*d*, J = 1.5, H–C(8)); 6.38 (*d*, J = 1.5, H–C(6)); 6.83 (*d*, J = 9, H–C(5′)); 7.54 (*dd*, J = 9, 1.5, H–C(6′)); 7.56 (*d*, J = 1.5, H–C(2′)); ester moiety: 3.82 (*s*), 6.25 (*d*, J = 16), 6.80 (*d*, J = 9), 6.92 (br. *d*, J = 9), 7.21 (br. *s*), 7.37 (*d*, J = 16). ^{13}C-NMR (300 MHz, (D_6)DMSO): 177.5 (C(4)); 164.1 (C(7)); 161.0 (C(5)); 156.4 (C(2), C(8a)); 149.3 (C(4′)); 144.8 (C(3′)); 133.3 (C(3)); 121.7 (C(6′)); 121.2 (C(1′)); 116.2 (C(5′)); 115.3 (C(2′)); 104.0 (C(4a)); 100.9 (C(1″)); 98.7 (C(6)); 93.7 (C(8)); 76.2 (C(3″)); 74.5 (C(5″)); 74.0 (C(2″)); 69.9 (C(4″)); 63.2 (C(6″)); ester moiety: 166.5, 148.4, 148.0, 145.1, 125.6, 123.1, 115.6, 114.1, 111.0, 55.8. FAB-MS: 639 (60, $[M - H]^-$), 505 (20), 463 (16), 301 (100).

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