(+)-Catechin: Benzoyl Protection of OH Groups and NMR Study of Products

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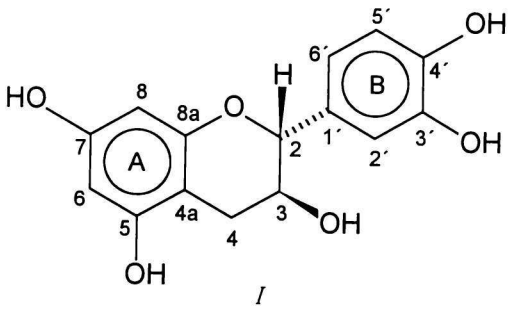
Several benzoyl esters of (+)-catechin [(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-benzo[*b*]-pyran-3,5,7-triol] have been synthesized by the dicyclohexylcarbodiimide promoted condensation of (+)-catechin hydroxy groups with benzoic acid. All the products were identified by MS, ^{1}H and ^{13}C NMR spectroscopy. The determination of the esterification position was carried out by long-range INEPT pulse sequence.

(+)-Catechin *I* [(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-benzo[*b*]pyran-3,5,7-triol] (Formula 1) and its 3-*O*-acyl derivatives show interesting activity against *Streptococcus mutans* [1, 2]. Because of this important biological activity the compounds have already been exploited in formulations of toothpastes and gargles.



I

Formula 1

Moreover, catechin derivatives possess anticholesteremic [3], hepatoprotective [4—6], antineoplastic [7] activities and they also found applications as enhancers of a sweet taste [8] and as cosmetic bases [9]. A few acetyl derivatives were prepared [10] by enzyme-mediated regioprotection-deprotection and determined by ^{13}C NMR spectra and by NOESY experiments.

We have investigated an opportunity to protect the hydroxy groups in catechin molecule by introducing benzoyl groups using dicyclohexylcarbodiimide (DCC) [11, 12] under catalysis of tertiary amines [13, 14]. The yield of such a reaction is usually decreased by the simultaneous formation of *N*-acylurea derivatives as by-products [15]. It has been found that the use of pyridine as a solvent promotes the formation of esters [12], while the addition of a catalytic amount of a strong acid [16] decreases the formation of *N*-acylurea compounds. In this paper we wish to describe the results obtained.

EXPERIMENTAL

All the products were identified by MS, ^{1}H and ^{13}C NMR spectroscopy. Mass spectra were measured on a Ribermag 10-10B (Nermag 5.A.). ^{1}H and ^{13}C NMR spectra were recorded on a Varian-Unity 400 instrument at 400 and 100 MHz, respectively. Signals were referenced to TMS as an internal standard or to the solvent signal. Assignment of the signals was based on long-range INEPT, COSY, and HETCOR experiments (direct and long-range). The experiments were performed using Varian pulse sequences (ineptlr, relayh, and hetcor, respectively) and are described in our last paper [17]. Column chromatography was performed with Merck Silicagel 60 (40—63 μm), TLC on Merck TLC Kieselgel 60 F_{254}. Commercially available (+)-catechin (Janssen) was used without further purification.

3′-*O*- and 4′-*O*-Benzoylcatechin (*II* and *III*)

To catechin (1.0 g; 3 mmol) dissolved in DMF (35 cm^3) benzene (35 cm^3) was added and then the mix-

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ture refluxed with the use of a Dean—Stark apparatus to remove a crystallinic water. Then PhCOOH (0.82 g; 6.7 mmol), 4-dimethylaminopyridine (DMAP) (0.08 g; 6.5 mmol), and DCC (2 g; 9.7 mmol) were added and the mixture refluxed for 5 h. The solvents were evaporated and the mixture lyophilized. The residue was dissolved in pyridine (15 cm^3) and poured into water (1 dm^3). Then the mixture was stirred for 4 h and filtered. The aqueous phase was twice extracted with ethyl acetate (100 cm^3), solvent evaporated and product lyophilized. The yielded solid was purified by column chromatography on silica gel using a mixture of hexane and acetone (1 1) as an eluent. The main fraction could be isolated and identified as a 1 1 mixture of *II* and *III*.

For $C_{22}H_{18}O_7$ (M_r = 394.11) mass spectrum (DCl, NH_3) *m/z* (I_r/%): 413 (4.5), 412 (13.6, M + NH_4^+), 396 (1.5), 395 (12.1, M + 1), 291 (24.2), 225 (100), 204 (33.3), 100 (90.9). ^{1}H NMR spectrum (acetone-d_6), δ: 2.48—2.59 (H_a-4 (*II*, *III*)), 2.84—3.02 (H_b-4 (*II*, *III*)), 3.96—4.06 (H-3 (*II*, *III*)), 4.59—4.69 (H-2 (*II*, *III*)), 5.85—6.01 (H-6, H-8 (*II*, *III*)), 6.95 (H-6′(*III*)), 6.98 (H-5′(*II*)), 7.06 (H-2′(*III*)), 7.12 (H-5′(*III*)), 7.19 (H-6′(*II*)), 7.21 (H-2′(*II*)), 7.51—7.57 (*m*-benzoyl), 7.64—7.69 (*p*-benzoyl), 8.12—8.16 (*o*-benzoyl). ^{13}C NMR spectrum (acetone-d_6), δ: 26.30 (C-4), 68.37 (C-3), 82.32 and 82.33 (C-2), 95.55 (C-8), 96.38 (C-6 (*III*)), 96.40 (C-6 (*II*)), 100.63 and 100.75 (C-4a), 116.89, 117.55, 119.66, 123.17, 123.68, 126.93, 129.49—130.85, 132.47, 134.32, 134.67, 139.40 (*II*), 139.64 (*III*), 149.62 (*II*,*III*), 165.13 (CO (C-3′(*II*))), 165.19 (CO (C-4′(*III*))).

3,3′,4′,5,7-Penta-*O*-benzoylcatechin (*IV*) and 3-*O*-Acetyl-3′,4′,5,7-tetra-*O*-benzoylcatechin (*V*)

Catechin (0.87 g; 3 mmol), PhCOOH (5.49 g; 45 mmol), and *p*-TSA (0.405 g) were dissolved in pyridine (40.5 cm^3). After addition of DCC (11.91 g; 58 mmol) the solution was stirred at room temperature for 92 h. Then acetic acid (4.5 cm^3) was added and the mixture was kept in a refrigerator overnight and then filtered. The formed crystals were washed with cold pyridine (45 cm^3) and dichloromethane (45 cm^3). Crashed ice (45 g) was added to the filtrate which was subsequently acidified with 40 cm^3 of 5 M-HCl. The organic phase was isolated and washed twice with 40 cm^3 of water, aqueous $NaHCO_3$ solution (40 cm^3), twice with water (40 cm^3), then evaporated and lyophilized yielding white crystals. ^{1}H NMR measurements showed nearly quantitative conversion to products *IV* and *V* (4 1). For the further purification it was dissolved in dichloromethane, filtered and evaporated. The residue (1.98 g) was separated on a column (130 g of silica gel, dichloromethane). Pentaester *IV* was isolated in the yield 0.87 g (36 %). Pure product *IV* was isolated in the yield necessary for determination (36 mg). Other portion of the product was isolated as a mixture of *IV* and *V*

3,3′,4′,5,7-Penta-*O*-benzoylcatechin (*IV*)

For $C_{50}H_{34}O_{11}$ (M_r = 810.21) mass spectrum (DCl, NH_3) *m/z* (I_r/%): 830 (3.7), 829 (12.3), 828 (14.5, M + NH_4^+), 766 (1.2), 244 (1.2), 227 (11.3), 139 (12.5), 122 (41.4), 105 (100). ^{1}H NMR spectrum (acetone-d_6), δ: 2.95 (1H, dd, H_a-4, J = 16.9 Hz, 6.2 Hz), 3.17 (1H, dd, H_b-4, J = 16.9 Hz, 5.0 Hz), 5.47 (1H, d, H-2, J = 6.0 Hz), 5.62 (1H, m, H-3), 6.92 (AB pattern centre, 2H, H-6, H-8, δ_A= 6.91, δ_B= 6.93, J = 2.2 Hz), 7.32—7.56 (18H, H-2′ H-5′, H-6′, *m*-benzoyl, *p*-benzoyl), 7.94—8.21 (10H, *o*-benzoyl). ^{1}H NMR spectrum ($CDCl_3$), δ: 2.95 (1H, dd, H_a-4, J = 16.9 Hz, 6.2 Hz), 3.13 (1H, dd, H_b-4, J = 16.9 Hz, 5.0 Hz), 5.53 (1H, d, H-2, J = 6.0 Hz), 5.58 (1H, m, H-3), 6.89 (AB pattern centre, 2H, H-6, H-8, δ_A= 6.89, δ_B = 6.90, J = 2.2 Hz), 7.29—7.63 (18H, H-2′, H-5′ H-6′, *m*-benzoyl, *p*-benzoyl), 7.81—8.08 (10H, *o*-benzoyl). ^{13}C NMR spectrum (acetone-d_6), δ: 25.32 (C-4), 69.93 (C-3), 78.77 (C-2), 108.74 and 110.16 (C-6, C-8), 111.91 (C-4a), 123.04 (C-2′), 124.65 (C-5′), 125.69 (C-6′), 129.27—134.71 (benzoyl), 137.51 (C-1′), 143.58 and 143.62 (C-3′, C-4′), 150.89 (C-5), 151.32 (C-7), 155.58 (C-8a), 164.34 (CO (C-4′)), 164.39 (CO (C-3′)), 164.63 and 165.07 (CO (C-5 and C-7)), 165.78 (CO (C-3)).

3-*O*-Acetyl-3′,4′,5,7-tetra-*O*-benzoylcatechin (*V*)

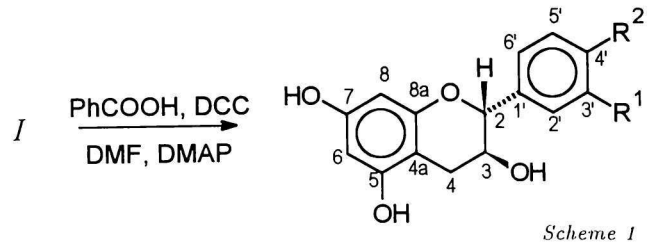
For $C_{45}H_{32}O_{11}$ (M_r = 748.19) mass spectrum (DCl, NH_3) *m/z* (I_r/%): 766 (3.8, M + NH_4^+), 244 (1.9), 139 (5.9), 122 (7.9), 106 (7.9), 105 (100). ^{1}H NMR spectrum (acetone-d_6), δ: 2.04 (3H, s, CH_3), 2.82 (1H, dd, H_a-4, J = 16.8 Hz, 6.4 Hz), 3.06 (1H, dd, H_b-4, J = 16.8 Hz, 5.2 Hz), 5.27 (1H, d, H-2, J = 6.4 Hz), 5.38 (1H, m, H-3), 6.91 (AB pattern centre, 2H, H-6, H-8, δ_A = 6.885, δ_B = 6.894, J = 2.3 Hz), 7.35—7.67 (15H, m, H-2′, H-5′, H-6′, *m*-benzoyl, *p*-benzoyl), 8.03—8.11 (8H, m, *o*-benzoyl). ^{13}C NMR spectrum (acetone-d_6), δ: 20.81 (CH_3), 24.97 (C-4), 68.78 (C-3), 78.61 (C-2), 108.61 and 109.97 (C-6 and C-8), 111.79 (C-4a), 122.88 (C-2′), 124.53 (C-5′), 125.44 (C-6′), 129.37—134.66 (benzoyl), 137.40 (C-1′), 143.47 (C-3′, C-4′), 150.78 (C-5)*, 151.21 (C-7)*, 155.44 (C-8a), 164.34 (CO (C-3′)), 164.41 (CO (C-4′)), 164.52 and 165.00 (CO (C-5 and C-7)), 169.97 (CO (C-3)).

RESULTS AND DISCUSSION

Preparation of Catechin Monobenzoyl Esters

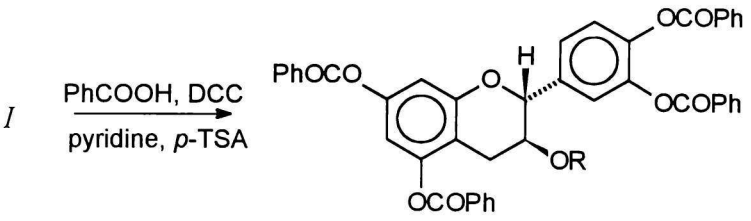
In order to protect hydroxy groups in catechin

* May be interchanged.



II R^1 = OCOPh R^2 = OH
III R^1 = OH R^2 = OCOPh

Scheme 1



IV R = COPh
V R = $COCH_3$

Scheme 2

molecule we have decided to start with a synthesis of catechin monoesters and so to recognize the most reactive catechin centres for this reaction. 3′-*O*-Monobenzoyl and 4′-*O*-monobenzoyl esters (*II* and *III*) in a mixture 1 1 were prepared when *I* was treated with two equivalents of PhCOOH (because of side *N*-benzoylurea derivative formation) in the presence of DCC and 4-dimethylaminopyridine (DMAP) in DMF after a final column chromatography separation (Scheme 1). However, the position of acylation is in a good agreement with the results of *Sweeny* and *Iacobucci* [18]. They have shown that the partial methylation of *I* with diazomethane gave also similar mixture of 3′-*O*- and 4′-*O*-monomethyl ethers.

The structures *II* and *III* in the mixture were determined by ^1H and ^{13}C NMR spectroscopy. For the signals assignment COSY and HETCOR pulse sequences were used. The position of the esterification was determined using long-range INEPT [19] pulse sequence. Irradiating the catechin proton in *ortho* position to the ester group gives a correlation with the carbon atom of the carboxylic group. Comparing long-range INEPT with long-range HETCOR, INEPT technique seems to give better response when small long-range coupling constants (about 1 Hz) were used ($^4J_{CH}$). Moreover, the application of this 1D sequence offers us an opportunity to optimize the long-range coupling constant in much shorter time than is necessary in 2D experiments.

Preparation of Catechin Pentaesters

Reaction of catechin (*I*) with 15 equivalents of PhCOOH and DCC in pyridine in the presence of a catalytic amount of *p*-TSA and the work-up procedure with acetic acid gave two main products *IV* and *V* (Scheme 2). After separation by column chromatography the products were identified as 3,3′,4′,5,7-penta-*O*-benzoylcatechin *IV* and 3-*O*-acetyl- 3′,4′,5,7-tetra-*O*-benzoylcatechin *V*

Also here the esterification positions were determined by long-range INEPT already described above. The chemical shifts of all the hydrogen signals in pentabenzoyl ester *IV* varied with the sample concentration and the type of the solvent used. In chloroform the signals of protons H-2′ H-5′ and H-6′ were overlapped with the hydrogen atom signals of benzoyl groups. But in acetone the separation of the signals H-2′ and H-5′ occurred that was necessary for an application of the selective pulse sequences. For an assignment of C-5 and C-7 carbon atoms the long-range INEPT pulse sequence was used again. When H-4 was irradiated, the correlation with C-5 was found only (no correlation with C-7). ^1H—^{13}C remote connectivities observed for the basic catechin skeleton are shown on Fig. 1.

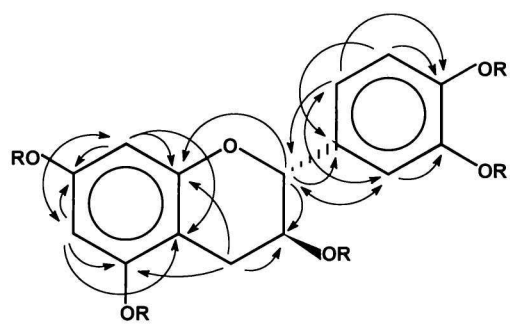


Fig. 1. Long-range ^1H—^{13}C interactions observed for the catechin skeleton of *IV* and *V* ($^1J_{C,H}$ = 140 Hz, $^nJ_{C,H}$ = 7.5 Hz).

Ester *V* had to be formed when 3′,4′,5,7-tetra-*O*-benzoylcatechin as another product in the reaction mixture got into contact with acetic acid during the work-up procedure. It could not be formed by a *trans*-esterification of *IV* as it was proved by attempted reaction of 3,3′,4′,5,7-penta-*O*-benzoylcatechin *IV* with acetic acid under the same reaction conditions. After the reaction we always isolated product *IV* only. This fact supported our findings that the position 3 is the most difficult for an esterification and its protection proceeds as the last and the most demanding step.

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