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Regioselective Hydrolysis of Pentaacetyl Catechin and Epicatechin by Porcine Liver Esterase

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Abstract—3,5,7,3′,4′-Pentaacetyl catechin was selectively hydrolyzed to either 3,7,3′,4′-tetraacetyl catechin or 3-acetyl catechin depending upon the duration of hydrolysis. A similar result was also obtained in the epicatechin series.

The procyanidin oligomers have attracted considerable attention in recent years due to their diverse pharmacological properties[1] in addition to their continuing industrial use.[2] While developing[3] a general method for their synthesis in a stereo and region controlled manner,[4–6] we encountered a problem of deacetylation of per acetylated catechin or epicatechin or their oligomers by hydrolysis. Although deacetylation, especially of phenolic hydroxyl is a simple base mediated hydrolysis, the situation, in these systems, is much more complicated than expected. The stereochemical integrity at C-2 is lost[7] during the hydrolysis as shown in Scheme 1. Moreover for regiospecific coupling, one needs a differentially protected catechin or epicatechin to activate the C-6 or C-8 position. Herein, we report an enzymatic solution to both the problems. The method will help to synthesize higher oligomers with free phenolic hydroxyl groups in a regiocontrolled manner.

3,5,7,3′,4′-Pentaacetyl catechin (**1**), prepared by acetylation of (+) catechin with acetyl chloride and pyridine,[8] was treated with PLE (isolated as acetone powder from fresh pig liver in our laboratory)[9] in a mixture of acetone and phosphate buffer at pH 8.0. When the reaction was continued for 24 h, hydrolysis of all the phenolic acetates took place giving 3-acetyl catechin (**3**) as the only product. This was revealed by the appearance of a three proton singlet at δ 1.75 corresponding to the aliphatic acetyl methyl while the signals for all the methyls corresponding to the phenolic acetates appearing at around δ 2.2 disappeared. The hydrolysis was free from any epimerization at C-2 as confirmed by the appearance of only the signals for the expected product; no other extraneous peaks were seen in the NMR spectrum. Moreover **3** could be reacetylated back to the starting acetate **1**. Similar result was obtained in the epicatechin series (Scheme 2). The yield of the deacetylated product was almost quantitative (98%).

Having established the deprotection of all the phenolic hydroxyl groups without any epimerization and in high yields, we then turned our attention to the possibility of selective deacetylation of the phenolic hydroxyl groups if the hydrolysis was carried out for a short time. With this in mind, the reaction was allowed to proceed for one hour only. The product was isolated along with some unconverted starting material and the NMR showed that only one phenolic acetate group had been hydrolyzed. The yield of the mono deacetylated product, based on recovered starting material, was 70%. The upfield shift of C-6 and C-8 hydrogens indicated that the hydrolysis of either C-5 or C-7 acetate had taken place. There was no change in the chemical shift of the protons present in the aromatic ring C. To confirm exactly which acetate got hydrolyzed, the methyl ether **7** was prepared by treatment with diazomethane in ether for 36 h. The strong NOE (~5%) observed for only one aromatic hydrogen unambiguously pointed out that the C-5 acetate was hydrolyzed by PLE. If the hydrolysis had taken place at C-7, then the methyl ether should have shown NOE for both C-6 and C-8 hydrogens. The epicatechin penta acetate also behaved similarly; the C-5 hydrogen was hydrolyzed in preference to the other acetates as confirmed by NOE experiments.

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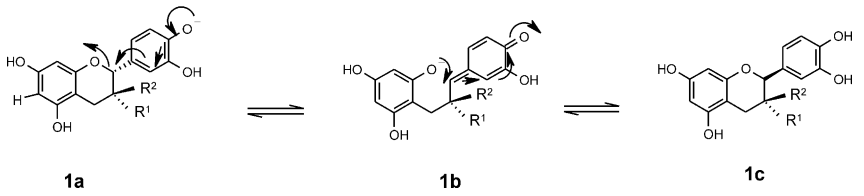
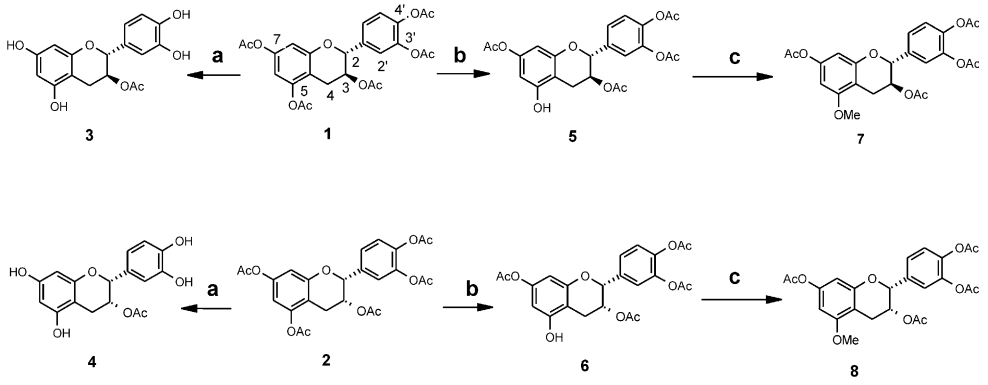


Scheme 1.



a PLE, acetone-phosphate buffer, pH 8.0, 24 h
b PLE, acetone phosphate buffer, pH 8.0, 1h
c CH_2N_2/ether/CH_2Cl_2

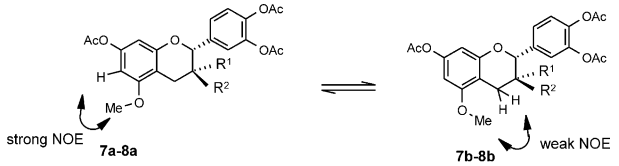
Scheme 2.

Additional evidence for the hydrolysis of C-5 acetate was obtained by the appearance of weak NOE for the C-4 hydrogens in both the series when the methyl signal was irradiated as shown in Scheme 3.

In conclusion, we have developed an enzymatic method for the deprotection of phenolic acetates in catechin or epicatechin series in regiospecific manner. The same method can also be applied for complete deprotection of phenolic acetates without any epimerization.

Selected Experimental Procedure and Spectral Data

Preparation of 3-acetyl (+) catechin (3). 3,5,7,3′,4′-Pentaacetyl catechin (**1**) (200 mg, 0.4 mmol) was dissolved in acetone (10 mL). Phosphate buffer (pH = 8.0, 20 mL) was added followed by PLE as crude acetone powder (100 mg) and the mixture was stirred at room temperature for 24 h. It was then filtered through Celite and the filtrate was extracted with ethyl acetate. The organic layer was dried, filtered and evaporated to leave the 3-acetyl catechin or as a brownish solid that is more than 90% pure. However, the material could be purified further by chromatography on silica gel using ethyl acetate as eluent. Yield: 130 mg (98%); δ_H(200 MHz, dMSO-d_6) 8.34 (1H, bs, Ar-OH), 8.13 (1H, bs, Ar-OH), 7.93 (2H, bs, Ar-OH), 6.96 (1H, s, H-2′), 6.75 (2H, s, H-5′, 6′), 6.01 (1H, d, J = 2.4 Hz, H-8), 5.92 (1H, d, J = 2.4 Hz, H-6), 5.32 (1H, m, H-3), 4.96 (1H, s, H-2), 2.93 (1H, dd, J = 4.6, 17.4 Hz, H-4), 2.73 (1H, J = 2.2, 17.4 Hz, H-4), 1.81 (3H, s, 3-OAc); mass (CI) 333 (MH^+), 291. 3-Acetyl epicatechin (**4**) was prepared in the same way in 97% yield starting from pentacetyl epicatechin (**2**).



7, R^1 = H, R^2 = OAc
8, R^1 = OAc, R^2 = H

Scheme 3.

Preparation of 3,7,3′,4′-tetraacetyl (+) catechin (5). Procedure as described for the preparation of **3**; the hydrolysis was allowed to proceed for 1 h; the compound **5** was isolated pure by column chromatography as brown solid; yield 70%; υ_{max} (KBr, cm^{-1}) 3422, 2929, 2859, 1751, 1614, 1505, 1434, 1375, 1213, 1118, 1033, 904, 831, 732, 611; δ_H (200 MHz, $CDCl_3$) 7.14–7.23 (3H, m, H-2′, 5′, 6′), 6.30 (1H, s, H-8), 6.20 (1H, s, H-6), 5.14 (1H, t, J = 5.8 Hz, H-3), 5.05 (1H, d, J = 6.2 Hz, H-2), 2.80–2.74 (2H, m, H-4), 2.27 (9H, s, 7, 2′, 3′-OAc), 1.99 (3H, s, 3-OAc); mass (CI) 459 (MH^+), 417, 279, 181.

Preparation of 3,7,3′,4′-tetraacetyl (−) epicatechin (6). Procedure as described for the preparation of **5**; the hydrolysis was allowed to proceed for 1 h; the compound **6** was isolated pure by column chromatography as brown solid; yield 70%; υ_{max} (KBr, cm^{-1}) 3436, 2926,

2859, 1765, 1615, 1505, 1434, 1379, 1213, 1118, 1050, 909, 827, 740, 616; δ_H (200 MHz, $CDCl_3$) 7.35–7.20 (3H, m, H-2′, 5′, 6′), 6.32 (1H, d, $J=4$ Hz, H-8), 6.20 (1H, d, $J=4$ Hz, H-6), 5.5 (1H, s, H-3), 5.06 (1H, s, H-2), 2.98 (2H, m, H-4), 2.29 (9H, s, 7, 2′, 3′-OAc), 1.91 (3H, s, 3-OAc); (CI) 459 (MH^+), 417, 375.

Preparation of 5-*O*-methyl-3,7,3′,4′-tetraacetyl (+) catechin (7). A solution of **5** in dry ether was treated with excess of ethereal diazomethane and was kept at room temperature for 36 h. Evaporation followed by chromatography of the residue afforded **7** as a gummy solid (60%); δ_H (200 MHz, $CDCl_3$) 7.27–7.14 (3H, m, H-2′, 5′, 6′-), 6.37 (1H, d, $J=2$ Hz, H-8), 6.23 (1H, d, $J=2$ Hz, H-6), 5.28 (1H, dd, $J=11.6$, 5.9 Hz, H-3), 5.11 (1H, d, $J=6.2$ Hz, H-2), 3.77 (3H, s, OMe), 2.86 (1H, dd, $J=17.2$, 5.2 Hz, H-4), 2.71 (1H, dd, $J=17.2$, 2.2 Hz, H-4), 2.29, 2.27 (9H, s, 7, 2′, 3′-OAc), 1.97 (3H, s, 3-OAc); mass (CI) 473 (MH^+), 431, 279.

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