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NMR Assignments of Four Catechin Epimers

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Complete NMR assignments of four flavan-3-ols were isolated from green and black tea [(+)-catechin (**1**), (-)-catechin (**2**), (+)-*epi*-catechin (**3**) and (-)-*epi*-catechin (**4**)] in acetone-d_6 solvent have been achieved using ^{1}D and ^{2}D NMR spectroscopy.

Key Words: NMR, Flavan-3-ol, Catechins, Green and Black tea.

INTRODUCTION

Flavonoids have a diphenylpropane skeleton and widespread throughout the plant kingdom and as a result may be important in human diets[1]. Several milligrams to as much as 1 g of mixed flavonoids are consumed per day in the catechwestern diet[2]. They have several benefits to human health, including antioxidant activities, metal chelation[3,4] and antiproliferative[2], anticarcinogenic, antibacterial, antiinflammatory, antiallergic and antiviral effects[4]. They also have shown the ability to stimulate the immune system[4] and to prevent nitration of tyrosine[5]. Detailed knowledge of the flavonoid content of food and the development of analytical databases of values is essential in understanding the relationship of these dietary con stituents and human health[6]. The monomeric flavonoids common in food can be divided into five subclasses: anthocyanidins, catechins (flavan-3-ols), flavanones, flavones and flavonols[7]. Glycosylation[8] is often on C3 and less often on C5, C7 (4) and C4. The most common sugar is glucose, but other sugars are found, including rhamnose, galactose, xylose[4], rutinose and neohesperidose[9]. Several expidemiological studies have suggested that green tea consumption in humans affords protection against some types of cancer[10,11]. Green tea has been demonstrated to exert significant cancer preventive effects in numerous animal models of chemical carcinogenesis, including protection against aryl hydrocarbon induced cancers[12,13]. These protective effects are most often attributed to the unique flavonoids in green tea, commonly known as catechins. Theaflavins (TF)

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and thearubigins (TR, mistakenly presented as thearbigens in some literature) are present in black tea[14,15]. TF and TR are soluble in hot water and represent 20-30 % (w/w) of dry tea, from which about 45 % of the tea constituents can be infused into hot water[16]. Tea phenolic compounds, known as tea polyphenols[14], previously called tea tannins[17], have bene regarded as the quality parameters or indicators of tea[18-20]. In particular, theaflavins (TF) were used to assess the market value[21,22], clonal variations[23] and seasonal quality variations of black tea[24].

Over the last few decades a large number of catechins have been isolated and their structures characterized, mainly by the technique of NMR spectroscopy. However, the NMR data are widely scattered in the literature (particularly in the case of ^{1}H data) and are often found to be incomplete[25-27] or to contain incorrect assignments[28,29]. Therefore, in the course of the study of tea polyphenols, the task have been undertaken of obtaining a complete set of assigned NMR data for a representative set of the catechins found in green and black tea: (+)-catechin (**1**), (-)-catechin (**2**), (+)-*epi*-catechin (**3**) and (-)-*epi*-catechin (**4**).

EXPERIMENTAL

NMR spectra were measured on a Jeol LA500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] NMR spectrometer in acetone-d_6. The chemical shift values are reported in ppm (δ) units and the coupling constant (*J*) are in Hz. ^{1}H-^{1}H COSY, DEPT, HMQC, HMBC and NOEs NMR spectra were obtained with the usual pulse sequences. Optical rotatioins were obtained in MeOH at 20ºC on a Perkin-Elmer 341 polarimeter (Shelton, CT). Melting points were obtained on Buchi melting point apparatus and are uncorrected. IR spectra were obtained on a Jasco FT/IR 410 spectrometer. Low-resolution EI-MS were collected on a Quattro GC/MS spectrometer having a direct inlet system. High-resolution FAB-MS were collected on a Finnigan/Thermo Quest MAT 95XL spectrometer. TLC work was carried out using plates coated with silica gel 60 F254 (Merck Col.). Visualization of the TLC plates was carried out under UV at 254 and 366 nm and also by spraying ceric sulphate reagent with heating. Silica gel column chromatography was performed on Merck silica gel 60 (230-400 mesh). Sephadex LH-20 was used for the column chromatography (Pharmacia, 25-100 μm). All solvents were routinely distilled prior to use. Other chemicals were commercial grade without purification.

Instant green and black tea were purchased from local market in Taipei, Taiwan 106, Republic of China.

Extraction and isolation: The green tea (100 g) was extracted with (MeOH:H_2O, 1:1) three times, every time for 3 d. The extraction was concentrated under reducing pressure to give crude extract (6.1 g). The extract

was partitioned between *n*-hexane and 80 % MeOH to give the *n*-hexane (700 mg) The 80 % aq. MeOH was subsequently partitioned between EtOAc and H_2O to yield the H_2O extract (3.6 g) and the EtOAc extract (1.1 g). The EtOAc extract was subjected to silica gel CC and eluted with $CHCl_3$-MeOH (10:1-5:1) and $CHCl_3$-MeOH-H_2O (10:3:1) to give 7 fractions (frs A-G). Fraction E was chromatographed repeatedly on Sephadex LH-20 using MeOH-H_2O (4:1) to give 5 fractions. Fraction E4 was further purified by HPLC with MeOH-H_2O (1:1) to yield **1** (5.3 mg) and **4** (9.6 mg).

The black tea (250 g) was extracted with (MeOH:H_2O, 1:9) warming at 50ºC and stirring for 48 h. The extraction was concentrated under reducing pressure to give crude extract (22.7 g). The extract was partitioned between *n*-hexane and 80 % MeOH to give the *n*-hexane (2.2 g). The 8 % aq. MeOH was subsequently partitioned between EtOAc and H_2O to yield the H_2O extract (12.5 g) and the EtOAc extract (3.8 g). The EtOAc extract was subjected to silica gel CC and eluted with $CHCl_3$-EtOAc-MeOH-H_2O (2:8:2:1) and $CHCl_3$-MeOH-H_2O (10:3:1) to give 8 fractions (frs A-H). Fraction D was subjected to repeated chromatography on Sephadex LH-20 column (MeOH-H_2O, 7:3) followed by HPLC to afford **2** (6.3 mg) and **3** (5.7 mg).

(+)-Cetechin (1): Colourless solid: m.p. 175-187ºC; $[\alpha]_D^{25}$ = +14º (c = 0.4, Me_2CO); IR (KBr, ν_{max}, cm^{-1}): 3313, 1621, 1514, 1285, 1243, 1180, 1145, 1112, 1073; EIMS m/z (rel.int): $[M]^+$ 290 (27), 152 (40), 139 (100); ^1H and ^{13}C NMR data (Table-1).

(-)-Cetechin (2): Colourless powder: m.p. 180-188ºC; $[\alpha]_D^{25}$ = -57º (c = 0.5, Me_2CO); IR (KBr, ν_{max}, cm^{-1}): 3404, 1621, 1602, 1514, 1285, 1243, 1180, 1145, 1112; EIMS m/z (rel.int): $[M]^+$ 290 (37), 152 (40), 139 (100); ^1H and ^{13}C NMR data (Table-1).

(+)-*Epi*-cetechin (3): Colourless solid: m.p. 235-236ºC; $[\alpha]_D^{25}$ = +69º (c = 0.5, Me_2CO); IR (KBr, ν_{max}, cm^{-1}): 3404, 1621, 1602, 1514, 1285, 1243, 1180, 1145, 1112; EIMS m/z (rel.int): $[M]^+$ 290 (37), 152 (40), 139 (100); ^1H and ^{13}C NMR data (Table-1).

(-)-*Epi*-cetechin (4): Amorphous powder: m.p. 229-233ºC; $[\alpha]_D^{25}$ = -55º (c = 0.5, Me_2CO); IR (KBr, ν_{max}, cm^{-1}): 3404, 1621, 1602, 1514, 1285, 1243, 1180, 1145, 1112; EIMS m/z (rel.int): $[M]^+$ 290 (31), 152 (50), 139 (100); ^1H and ^{13}C NMR data (Table-1).

RESULTS AND DISCUSSION

The present work serves to give a set of unambiguous NMR assignments for a representative series of green and black tea polyphenols (Fig. 1). ^1H and ^{13}C NMR spectral data for compounds **1-4** are given in Table-1. Spectral data for the majority of the compounds shown in Fig. 1 have been reported previously in the literature[30]; however, assignments are generally

TABLE-1

^{1}H NMR (500 MHz) AND ^{13}C NMR (125 MHz) SPECTRAL DATA OF COMPOUNDS **1-4** IN ACETONE-d_6

Position	**1**		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	82.8	4.53 (d, 7.8)	82.8	4.55 (d, 7.5)	79.6	4.85 (s)	79.5	4.88 (s)
3	68.4	3.97 (ddd, 8.4, 7.8, 5.5)	68.3	3.98 (ddd, 8.0, 7.5, 5.5)	67.1	4.18 (br s)	67.0	4.21 (br s)
4axi	28.8	2.50 (dd, 16.1, 8.4)	28.8	2.52 (dd, 16.0, 8.0)	29.2	2.83 (br d, 17.0)	29.0	2.87 (br d, 16.5)
4eq	28.8	2.89 (dd, 16.1, 5.5)	28.8	2.91 (dd, 16.0, 5.5)	29.2	2.70 (br d, 17.0)	29.0	2.74 (br d, 16.5)
4a	100.7		100.6		100.0		100.0	
5	157.1		157.2		157.8		157.6	
6	96.2	6.00 (d, 2.3)	96.1	6.02 (d, 2.0)	96.3	5.99 (d, 1.8)	96.2	6.02 (d, 1.8)
7	157.6		157.7		157.8		157.6	
8	95.5	5.85 (d, 2.3)	95.3	5.87 (d, 2.0)	95.5	5.88 (d, 1.8)	95.7	5.92 (d, 1.8)
8a	156.7		156.9		157.4		157.2	
1´	132.3		131.8		132.5		132.3	
2´	115.3	6.88 (d, 1.9)	115.2	6.88 (d, 2.0)	115.5	7.01 (d, 1.8)	115.3	7.05 (d, 1.8)
3´	145.6		146.1		145.6		145.4	
4´	145.7		146.0		145.5		145.3	
5´	115.7	6.78 (d, 8.1)	115.7	6.77 (d, 8.0)	115.7	6.75 (d, 8.0)	115.5	6.79 (d, 8.0)
6´	120.1	6.73 (dd, 8.1, 1.9)	118.8	6.73 (dd, 8.1, 2.0)	119.6	6.80 (dd, 8.0, 1.8)	119.4	6.84 (dd, 8.0, 1.8)

Chemical shift as ppm.

Proton resonance multiplicity and coupling constants (*J* in Hz) are given in paretheses.

Assignments were assigned on the basis of DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC and NOEs spectra.

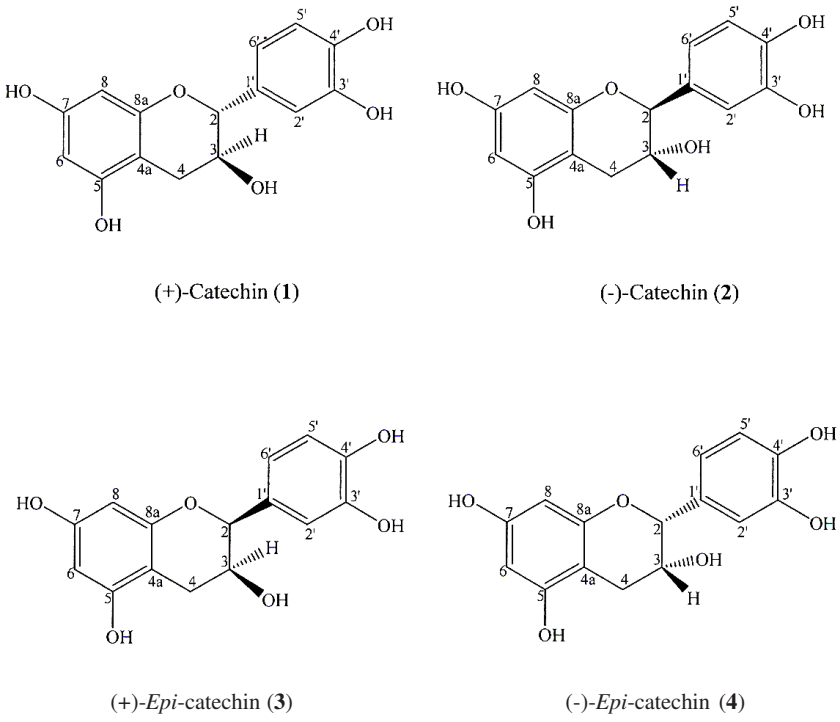

Fig. 1. Structure of (+)-catechin (**1**), (-)-catechin (**2**), (+)-*epi*-catechin (**3**) and (-)-*epi*-catechin (**4**)]

incomplete, particularly with respect to the C-5, C-7 and C-8a resonances. ^{1}H NMR data are more difficult to locate in the literature. It is also noted that unambiguous ^{1}H and ^{13}C NMR assignments have been published for **1** and **4** in DMSO-d_6, solvent[31]. However, it is well known that solvent differences can cause significant chemical shift changes; therefore, a complete ^{1}H and ^{13}C NMR assignment of **1-4** in acetone-d_6, solvent is included in present studies. ^{1}H and ^{13}C NMR assignments obtained in this work were achieved primarily by the means of proton carbon correlation methods specifically the HMQC experiment for direct correlations and the HMBC experiment for long-range correlations. These data are largely typical of the long-range correlation observed for flavan-3-ol molecules and assignments using these data are straightforward. However, in compounds with *cis* stereochemistry (*i.e.*, *epi*-catechin-like) the H-2 to C-8a correlation is weak. Consequently, C-8a, C-5 and C-7 could not be distinguished from each other and H-6 and H-8 could not be assigned. However, in all cases ambiguity in the assignment of H-6 and H-8 could be resolved by NOEs difference spectroscopy since H-8 is observed to exhibit an NOEs from H-2 and/or H-2′ in these compounds. This knowledge, combined with the

HMBC connectivities, allows a complete ^{13}C assignment to be made and all the protons to be assigned with the exception of H-4α and H-4β. NOE difference spectroscopy was used in order to distinguish between H-4α and H-4β, with the proton showing an NOEs from H-2 being assigned as H-4β, the quasi-axial proton. Therefore, a complete ^{1}H and ^{13}C NMR assignment was possible for compounds **1-4**. The data obtained in this work demonstrate that, in acetone-d_6, H-6 resonates at higher frequency that H-8 and that C-6 is at higher frequency than C-8. H-6 was also found to be at higher frequency that H-8 in both **1** and **4** in DMSO-d_6, solvent[31].

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