SYNTHESIS OF MONOMETHYL AND DIMETHYL DERIVATIVES OF EPICATECHIN GALLATE (ECg) AND THEIR PHOTO-SENSITIVITY

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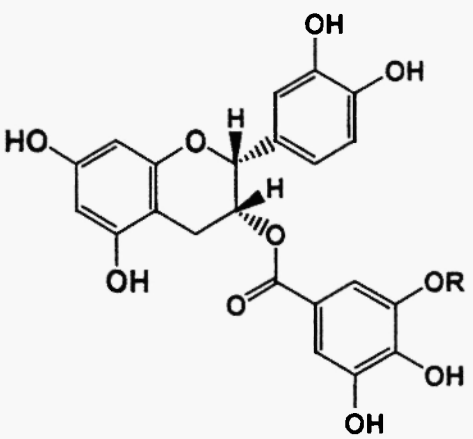
Abstract: Epicatechin gallate(ECg) 1, one of the major tea catechins, was methylated with CH_2N_2 to give monomethyl derivatives 2a~c and dimethyl derivatives 3ab and their stabilities under exposure to visible light were examined.

Four major catechins (1,2) and many minor components including their methylated derivatives such as 2b (3) had been isolated from tea leaves, *Thea sinensis L.*, and their many different biological activities (4) had also been reported recently. We started a study of their structure-activity relationships and a role of the methylated minor components in the plant. In this paper, we report first synthesis of 2b from Epicatechin gallate (ECg) 1 by methylation with CH_2N_2 and photo-sensitivity of the monomethyl derivatives.



1 ECg : R=H
2b R=CH_3

Starting material : ECg 1 was isolated from crude tea catechin mixture (5) by a modified method using open column of ODS silica gel in large scale (~5g). Since methylation of 1 at 25℃ gave a mixture of polymethylated products, the reaction conditions at low temp. were examined. Finally, a cooled methanol solution of 100mg ECg 1 at -50℃ was mixed with a cooled ether solution of CH_2N_2 at -50℃ and kept at -50℃, and reaction was monitored with HPLC [Namsil ODS-9 (6), 4.6mm ϕ × 250mm]. After 3hr, a content of 1 was less than 50% and excess CH_2N_2 was quenched by addition of acetic acid. Rough separation of the reaction mixture with prep. HPLC gave 1 (32mg), monomethyl derivatives 2a~c (38mg, 2a/2b/2c≒4:2:1) and dimethyl derivatives 3ab (27mg, 3a/3b≒2:1).

Although three kinds of monomethyl derivatives [2a, 2b, and 2c] were very similar with one another, each component was isolated by combination of HPLC using normal phase silica gel (6) and reverse phase ODS silica gel (6). Their structures 2a~c were determined by comparison of their ^{1}H-nmr spectra to be 4"-O-methyl-, 3"-O-methyl- and 3'-O-methyl- derivatives 2a[22mg] (7), 2b[9mg] (8) and 2c[6mg] (9), respectively. ^{1}H-nmr spectrum, retention time in HPLC etc. of 2b were identical with those of natural 3"-O-methyl ECg (2) obtained from crude tea catechin (5). Two dimethyl derivatives were also isolated from the mixture of 3ab and the structures were determined to be 3",4"-O-dimethyl-, 3",5"-O-dimethyl- derivatives 3a[2mg](10) and 3b[1mg] (11), respectively. Consequently, a order of the reactivities of the phenolic OH of ECg with CH_2N_2 was 4">3">3' as shown in the figure.

Photo-sensibility of ECg 1 and it's monomethyl derivatives 2a~c were studied by exposure of their solution (1/2a/2b/2c≒1:1:1:1, 20ppm in H_2O) to the visible light (at 5cm from 100W silica lamp). As shown in the table, it is clear that 2a, 2b and 2c, was moderately stabilized by methylation at B-ring (3'-posotion) or gallate part (4" and 3" -position). Therefore, it was indicated that phenolic OH not only in the B-ring but also in the gallate part played an important role for the photo-oxidation of ECg.

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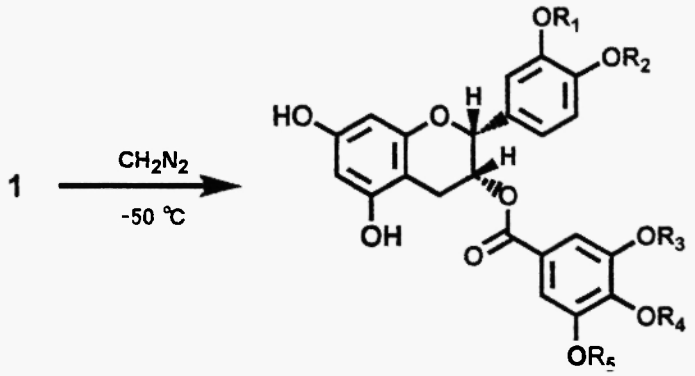

(**2a**) : R_4=CH_3, R_1=R_2=R_3=R_5=H (**3a**) : R_3=R_4=CH_3, R_1=R_2=R_5=H
(**2b**) : R_3=CH_3, R_1=R_2=R_4=R_5=H (**3b**) : R_3=R_5=CH_3, R_1=R_2=R_4=H
(**2c**) : R_1=CH_3, R_2=R_3=R_4=R_5=H

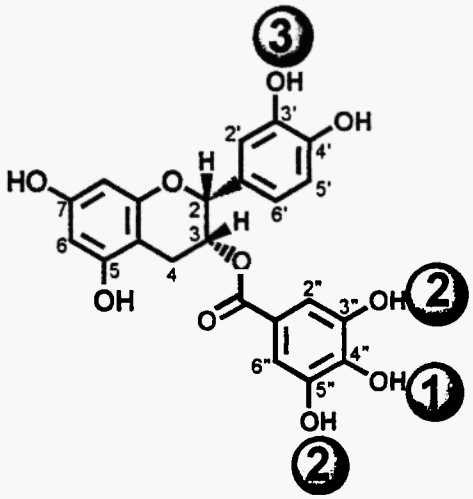

Fig. Reactivity of ECg **1** with CH_2N_2

Table. Photo-sensibility of ECg **1** and it's *O*-methyl derivatives **2a-c**

Time (hr)	Recovery(%)			
	1	**2a**	**2b**	**2c**
0	100	100	100	100
1	76	88	87	87
2	72	81	80	83
4	59	76	73	75

sample: 1/2a/2b/2c≒1:1:1:1, condition: 2ml of 20ppm in H_2O solution in 10ml erlenmeyerflask, light source: 100W silica lamp, distance: 5cm from light source, reaction temp.: 20 ℃

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(6) Those silica gel and ODS silica gel were prepared and packed into stainless column for HPLC in our laboratory.

(7) 2a: ^{1}H-nmr(CD_3OD, 500MHz) δ (ppm) 2.90(1H, dd, J=17.1 & 2.1, H-4), 3.00(1H, dd, J=17.1 & 4.7, H-4), 3.84(3H, s, OCH_3-4"), 5.04(1H, br.s, H-2), 5.54(1H, br.d, d=1.6, H-3), 5.98(1H, d, J=2.5, H-6), 5.99(1H, d J=2.3, H-8), 6.72(1H, d, J=8.2, H-5'), 6.82(1H, dd, J=8.2 & 2.1, H-6'), 6.95(1H, s, H-2'), 6.95(1H, s, H-2" & H-6").

(8) 2b: ^{1}H-nmr(CD_3OD, 500MHz) δ (ppm) 2.94(1H, dd, =17.4 & 2.6, H-4), 3.02(1H, dd,J=17.4 & 4.5, H-4), 3.84(3H, s, OCH_3-3"), 5.06(1H, br.s, H-2), 5.52(1H, br.s, H-3), 5.98(1H, d, J=2.3, H-6), 5.98(1H, d, J=2.3, H-8), 6.72(1H, d, J=8.2, H-5'), 6.82(1H, dd, J=8.0 & 1.8, H-6'), 6.97(1H, d, J=1.8, H-6"), 7.03(1H, d, J=1.8 H-2"), 7.10(1H, d, J=1.8, H-2').

(9) 2c: ^{1}H-nmr(CD_3OD, 400MHz) δ (ppm) 2.89(1H, br.d, J=16.1, H-4), 3.01(1H, dd, J=16.1 & 4.8, H-4), 3.61(3H, s, OCH_3-3'), 5.06(1H, br.s, H-2), 5.53(1H, br.s, H-3), 5.97(1H, d, J =2.2, H-6), 5.98(1H, d, J=2.6, H-8), 6.74(1H, d, J=8.2, H-5'), 6.86(1H, dd, J=8.2 &1.8, H-6'), 7.01(2H, s, H-2" & H-6"), 7.08(1H, d, J =1.8, H-2').

(10) 3a: ^{1}H-nmr(CD_3OD, 500MHz) δ (ppm) 2.94(1H, br.d, J=19.1, H-4), 3.02(1H, dd, J=19.1 & 4.6, H-4), 3.82(3H, s, OCH_3-3"), 3.83(3H, s, OCH_3-4"), 5.07(1H, br.s, H-2), 5.53(1H, br.s, H-3), 5.98(1H, d, J=2.3, H-6), 6.01(1H, d, J=2.3, H-8), 6.73(1H, d, J=8.4, H-5'), 6.82(1H, dd, J=8.4 & 2.1, H-6'), 6.97(1H, d, J=2.1, H-2'), 7.01(1H, d, J=2.0, H=6"), 7.08(1H, d, J=2.0, H-2").

(11) 3b: ^{1}H-nmr(CD_3OD, 400MHz) δ (ppm) 3.02(2H, m, H-4), 3.82(3H, s, OCH_3-3"), 3.84(3H, s, OCH_3-5"), 5.10(1H, br.s, H-2), 5.51(1H, br.s, H-3), 5.99(1H, d, J=2.2, H-6), 6.03(1H, d, J=2.2, H-8), 6.74(1H, d, J=8.2, H-5'), 6.82(1H, dd, J=8.2 & 1.8, H-6'), 7.00(1H, d, J=1.8, H-2'), 7.15(2H, s, H-2"& H-6").

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