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Tannins and Related Compounds. XV.[1] A New Class of Dimeric Flavan-3-ol Gallates, Theasinensins A and B, and Proanthocyanidin Gallates from Green Tea Leaf. (1)

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Along with four dimeric proanthocyanidin gallates, *viz.* prodelphinidin B-2 3′-*O*-gallate (IV) and procyanidins B-2 3,3′-di-*O*-gallate (V), B-2 3′-*O*-gallate (VI) and B-4 3′-*O*-gallate (VII), two novel dimeric flavan-3-ol gallates (VIII and IX) named theasinensins A and B, in which two flavan units are linked at the B-ring, have been isolated from fresh green tea leaves, and their structures have been established on the basis of spectroscopic evidence in conjunction with enzymatic hydrolyses with tannase. Three new monomeric acylated flavan-3-ols have also been isolated, and their structures were similarly characterized as (−)-epigallocatechin 3-*O*-*p*-coumaroate (I), (−)-epigallocatechin 3,3′-di-*O*-gallate (II) and (−)-epigallocatechin 3,4′-di-*O*-gallate (III). In addition, the occurrence of the known (−)-epicatechin 3-*O*-gallate (X), (−)-epigallocatechin 3-*O*-gallate (XI), (+)-catechin (XII), (−)-epicatechin (XIII), (−)-epigallocatechin (XIV) and (−)-epicatechin 3-*O*-(3-*O*-methyl)-gallate (XV) in green tea leaves was confirmed.

Keywords——green tea leaf; *Thea sinensis*; Theaceae; tannin; theasinensin; proanthocyanidin gallate; flavan-3-ol *p*-coumaroate; flavan-3-ol gallate

It has been reported that up to 40% of the dry matter of green tea leaf is composed of polyphenols[2] and that flavan-3-ol derivatives, especially those with a galloyl group, are the major constituents of the polyphenolic fraction.[3] Apart from small quantities of gallic acid and theogallin (5-*O*-galloyl quinic acid),[4] four flavan-3-ol gallates, namely, (−)-epicatechin 3-*O*-gallate, (−)-epigallocatechin 3-*O*-gallate, (−)-epicatechin 3,5-di-*O*-gallate and (−)-epigallocatechin 3,5-di-*O*-gallate, have been isolated from tea leaf.[3,5] In addition, flavan-3-ol derivatives occurring in tea leaf include (+)-catechin, (−)-epicatechin, (+)-gallocatechin and (−)-epigallocatechin.[3,5] Recently, the isolation of 3-*O*-methyl gallates of (−)-epicatechin and (−)-epigallocatechin from tea leaf was reported,[6] and these compounds may be of considerable importance from the viewpoints of gallic acid biosynthesis and/or metabolism. The occurrence of a variety of these flavan-3-ols in green tea leaf implies the co-existence of proanthocyanidins (condensed tannins) which possess higher ability to combine with proteins and hence give rise to an astringent taste. It is of interest, therefore, to have some knowledge of the structures and compositions of higher-molecular-weight polyphenols in tea leaf.

This paper deals with the isolation and structural characterization of four dimeric proanthocyanidin gallates (IV, V, VI, and VII) and two novel dimeric flavan-3-ol gallates linked at the B-ring, named theasinensins A and B (VIII and IX), together with (−)-epigallocatechin *p*-coumaroate (I) and (−)-epigallocatechin digallates (II and III).

The isolation of compounds I—IX from the ethyl acetate-soluble fraction of the aqueous acetone extracts of fresh green tea leaf was successfully achieved by a combination of Sephadex LH-20 and high porosity polystyrene gel (Diaion HP-20)[7,8] chromatography (Chart 1). (−)-Epicatechin 3-*O*-gallate (X),[9] (−)-epigallocatechin 3-*O*-gallate (XI),[10] (+)-

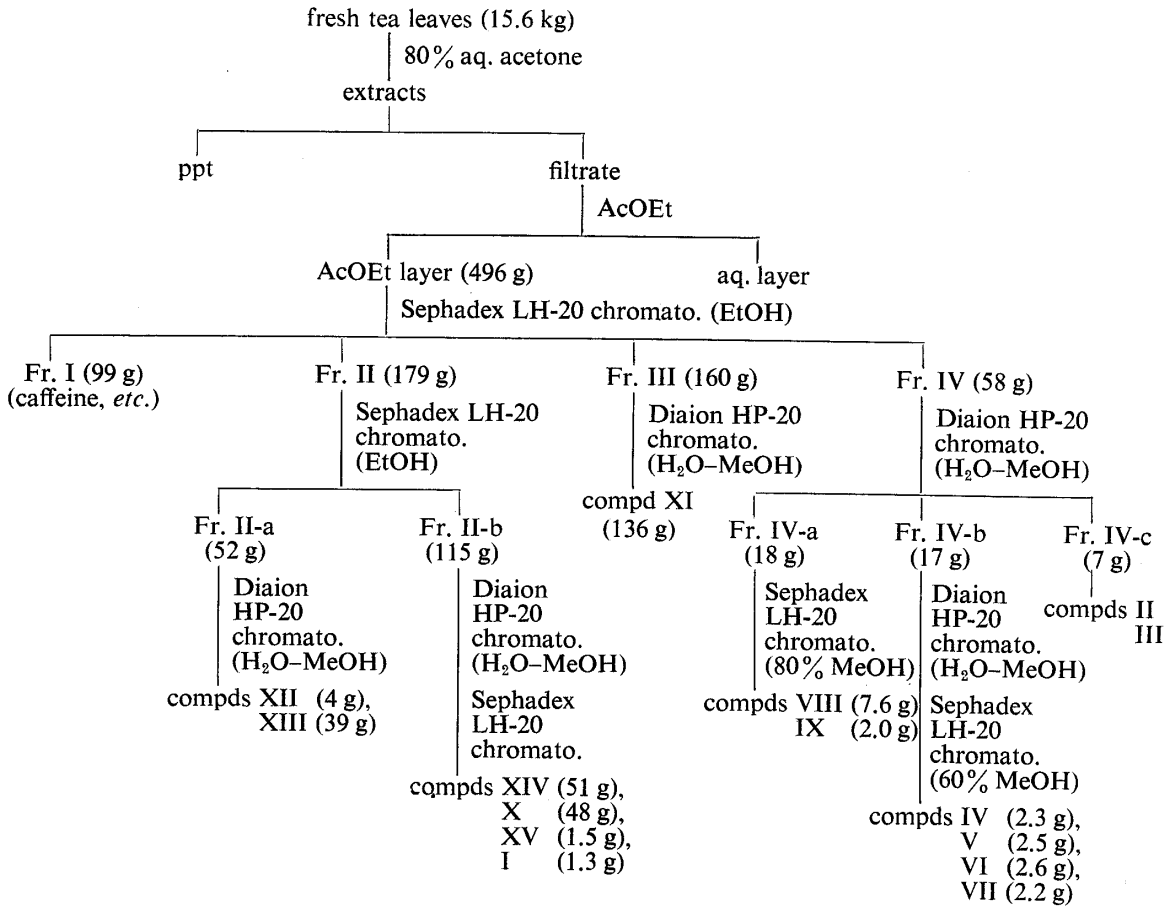


Chart 1

catechin (XII),[9] (−)-epicatechin (XIII)[9] and (−)-epigallocatechin (XIV)[10] were identified by comparison with authentic samples. In addition, (−)-epicatechin 3-*O*-(3-*O*-methyl)-gallate (XV) was obtained, and this was identified by comparison of the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectral data with those described in the literature.[6]

Compound I, an off-white amorphous powder, $[\alpha]_D$ −158.3° (acetone), $C_{24}H_{20}O_9 \cdot 1^1/_2H_2O$, is positive to the ferric chloride and anisaldehyde-sulfuric acid reagents (blue and orange colorations, respectively). The ^{1}H-NMR spectrum (Table I) shows the presence of a flavan skeleton with a 3,5,7,3′,4′,5′-hexasubstitution pattern. The appearance of two *trans*-olefinic and A_2B_2-type aromatic proton signals suggests the occurrence of a *p*-coumaroyl group in I. The downfield shift of the flavan C_3 proton, analogous to that found in (−)-epigallocatechin 3-*O*-gallate (XI), is indicative of the location of an acyl group at this position. Methylation of I with dimethyl sulfate and potassium carbonate in dry acetone yielded a hexamethyl ether (Ia), the electron-impact mass spectrum (EI-MS) of which exhibits the molecular ion peak at *m/z* 536, along with significant peaks at *m/z* 358 and 161 derived from a pentamethoxyflavinium ion and *p*-methoxycinnamoyl cation, respectively. On methanolysis with sodium methoxide in methanol, Ia afforded (−)-epigallocatechin pentamethyl ether (Ib) and methyl *p*-methoxycinnamate, thus establishing the structure of I as (−)-epigallocatechin 3-*O*-*p*-coumaroate.

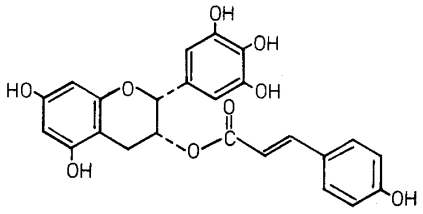
Compounds II and III were obtained as an inseparable mixture. The ^{1}H-NMR spectrum (Table I) shows, in addition to the presence of two galloyl groups in both compounds, flavan C-ring proton signals almost identical with those of I and XI. Enzymatic hydrolysis of the mixture with tannase furnished (−)-epigallocatechin (XIV) and gallic acid. These facts indicated that II and III are (−)-epigallocatechin digallates and that one galloyl residue

TABLE I. ^{1}H-NMR Spectral Data for Monomeric Flavan-3-ols[a)]

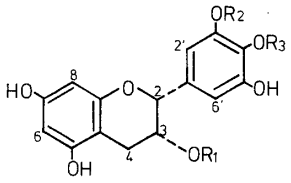
	I	II	III	X	XI	XII	XIII	XIV	XV
C-Ring									
C_2–H	5.04 (s)	5.16, 5.18 (each s)		5.14 (s)	5.08 (s)	4.57 (d, J=8 Hz)	4.88 (s)	4.81 (s)	5.18 (s)
C_3–H	5.48 (m)	5.60 (m)	5.60 (m)	5.55 (m)	5.56 (m)	3.99 (m)	4.22 (m)	4.18 (m)	5.54 (m)
C_4–H	2.72—3.17 (m)	2.80—3.23 (m)		2.79—3.21 (m)	2.78—3.22 (m)	2.52 (dd, J=8, 16 Hz), 2.92 (dd, J=6, 16 Hz)	2.56—3.04 (m)	2.59—3.00 (m)	2.83—3.24 (m)
A-Ring									
C_6–H	6.04 (s)	6.02—6.12 (m)		6.06 (s)	6.08 (s)	5.86 (d, J=2 Hz)	5.92 (d, J=2 Hz)	5.91 (d, J=2 Hz)	6.07 (s)
C_8–H	6.04 (s)			6.06 (s)	6.08 (s)	6.02 (d, J=2 Hz)	6.02 (d, J=2 Hz)	6.01 (d, J=2 Hz)	6.07 (s)
B-Ring									
$C_{2',6'}$–H	6.61 (s)	6.91, 7.00 (each d, J=2 Hz)	6.74 (s)	6.65—7.08 (m)	6.64 (s)	6.75—6.96 (m)	5.96—7.16 (m)	6.57 (s)	6.73—7.11 (m)
$C_{5'}$–H	—	—	—		—			—	
Galloyl	—	7.04, 7.24 (each s)		7.02 (s)	7.04 (s)	—	—	—	—
3-*O*-Methyl-galloyl									
$C_{2'',6''}$–H	—	—	—	—	—	—	—	—	7.06, 7.16 (each d, J=2 Hz)
OCH_3	—	—	—	—	—	—	—	—	3.80 (s)
p-Coumaroyl									
–CH=CH–	6.22, 7.49 (each d, J=16 Hz)	—	—	—	—	—	—	—	—
$C_{2'',3''}$–H	6.84, 7.48 (each d, J=8 Hz)	—	—	—	—	—	—	—	—

a) Spectra were run in acetone-d_6 at 100 MHz. s, singlet; d, doublet; m, multiplet.

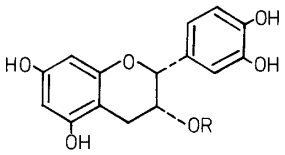
should be located at the C_3-position in both cases. Comparison of the ^{1}H-NMR spectrum with that of XI suggested that the second galloyl group is present at the $C_{3'}$–H and $C_{4'}$-position, respectively; the chemical shifts of the two *meta*-coupled signals arising from the A-ring closely resemble each other, while a singlet at δ 6.74 due to $C_{2'}$ and $C_{6'}$ protons, as well as *meta*-coupled $C_{2'}$ and $C_{6'}$ proton signals at δ 6.91 and 7.00 (d, J=2 Hz) showing an unsymmetrical pattern of the B-ring, are shifted downfield as compared with those of XI. On the basis of these results, the structures of II and III are assigned as (−)-epigallocatechin 3,3′-di-*O*-gallate and (−)-epigallocatechin 3,4′-di-*O*-gallate. These compounds may be present in green tea leaf as a mixture in a ratio of *ca.* 1 : 1 as judged from the integrated intensities of the respective galloyl and B-ring proton signals in the ^{1}H-NMR spectrum.



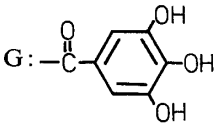
I



II: R_1=R_2=G, R_3=H
III: R_1=R_3=G, R_2=H
XI: R_1=G, R_2=R_3=H
XIV: R_1=R_2=R_3=H



X: R=G
XIII: R=H



It should be noted that methylation of these compounds with dimethyl sulfate and potassium carbonate in dry acetone afforded a mixture of the 3,4′- and 3,3′-disubstituted epigallocatechin decamethylates in a ratio of *ca.* 1 : 7,[11] indicating that migration of the galloyl group from the $C_{4'}$ to $C_{3'}$-hydroxyl occurred partially during methylation.[12]

Compounds IV, $[\alpha]_D$ −52.7° (acetone) and V, $[\alpha]_D$ −95.2° (acetone), obtained as pale brown amorphous powders, were concluded to be proanthocyanidins from their chromatographic properties and the characteristic orange colors with the anisaldehyde reagent. Furthermore, the intense blue colorations with the ferric chloride reagent suggest the presence of galloyl group(s). The ^{1}H-NMR spectra of IV and V show the occurrence of two flavan-3-ol units with one galloyl group in IV and two galloyl groups in V (Table II). These compounds were finally shown by comparisons of their physical and ^{1}H-NMR data to be identical with prodelphinidin B-2 3′-*O*-gallate and procyanidin B-2 3,3′-di-*O*-gallate isolated previously from the bark of *Myrica rubra*[10] and rhubarb,[9] respectively.

Compound VI, a pale brown amorphous powder, $[\alpha]_D$ −45.8° (acetone), $C_{37}H_{30}O_{16}\cdot 1^1/_2H_2O$, gave a ^{1}H-NMR spectrum (Table II) analogous to that of IV except for aromatic proton signals arising from the B,B′-rings. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum (Table III) closely resembles that of procyanidin B-2 (XVI),[9] and shows the presence of an additional galloyl group. The downfield shift of the $C_{3'}$ carbon resonance and the upfield shifts of the neighboring $C_{2'}$ and $C_{4'}$ resonances as compared with those of XVI, as well as the close analogy of the aliphatic proton signals with those of IV, clearly indicated that the galloyl residue is located at the $C_{3'}$-position. From these findings, the structure of VI was concluded to be procyanidin B-2 3′-*O*-gallate. This compound was previously isolated as its acetate from grapes of the Gamay Beaujolais and Siebel varieties.[13]

Compound VII, a pale brown amorphous powder, $[\alpha]_D$ −255.7° (acetone), $C_{37}H_{30}O_{16}\cdot 2H_2O$, possesses chromatographic properties similar to those of IV and VI. The ^{1}H-NMR spectrum, measured at room temperature, was not amenable to first-order analysis owing to conformational isomerism caused by a steric interaction between the upper and lower

TABLE II. ^{1}H-NMR Spectral Data for Proanthocyanidins and Teasinensins[a]

	IV	V	VI	VIII	VIIIb	IX
C_2–H	5.20 (s)	5.67 (s)	5.21 (s)	4.80 (s)	4.63 (s)	4.78 (s)
C_3–H	4.02 (m)	5.63 (m)	4.04 (m)	5.35 (m)	4.04 (m)	5.30 (m)
C_4–H	4.88 (m)	4.88 (m)	4.86 (m)	2.43—3.02 (m)	2.25—2.81 (m)	2.42—3.01 (m)
$C_{2'}$–H	5.20 (s)	5.00 (s)	5.25 (s)			4.62 (s)
$C_{3'}$–H	5.56 (m)	5.63 (m)	5.58 (m)			4.07 (m)
$C_{4'}$–H	2.88—3.22 (m)	2.78—3.24 (m)	2.80—3.24 (m)			2.42—3.01 (m)
B,B′-Ring	6.52, 6.74 (s) (s)	6.57—6.96 (m)	6.65—7.16 (m)	6.88 (s)	6.85 (s)	6.82, 6.89 (s) (s)
Galloyl	7.11 (s)	7.00, 7.08 (s)	7.08 (s)	7.00 (s)		6.96 (s)

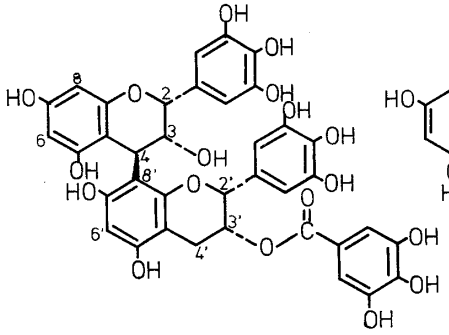
a) Spectra were run in acetone-d_6 at 100 MHz. s, singlet; m, multiplet.

TABLE III. ^{13}C-NMR Spectral Data for Tea Leaf Polyphenols[a]

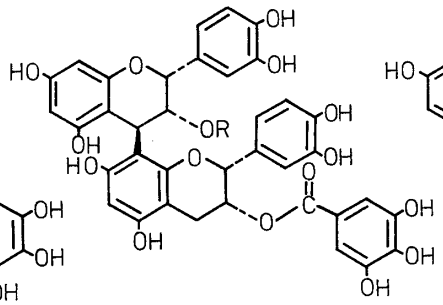
	I	X	XI	XIII	XIV	XV	IV	V	VI	VIII	VIIIb	IX
C_2	77.8	77.8	78.0	79.3	79.2	77.9	77.0	75.5	77.8	76.1	77.1	76.1
C_3	69.3	69.7	69.9	66.9	66.9	70.1	73.0	74.8	72.9	68.9	64.9	68.6
C_4	26.5	26.4	26.4	28.4	28.7	26.3	36.3	33.6	36.5	26.8	28.7	26.8
C_{4a}	98.7	98.5	98.7	99.7	99.8	98.6	101.4	101.9	101.5	98.3	99.2	98.5
C_6	95.5	95.3	95.5	95.7	96.2	95.4	95.7	95.3	95.6	95.5	95.5	95.7[b]
C_8	96.4	96.3	96.4	96.2	96.2	96.4	96.4	95.9	96.2	96.4	96.1	96.5[b]
$C_{2'}$							78.0	77.8	76.9			77.2
$C_{3'}$							69.0	69.1	69.3			64.9
$C_{4'}$							26.6	—[c]	26.6			28.6
$C_{6'}$							97.2	96.9	97.1			95.9[b]
$C_{8'}$							107.8	106.8	107.7			96.1[b]
–COO–	167.5	166.3	166.7			166.6	166.2	166.2 166.5	166.7	167.0		166.7
–CH=CH–	114.9 131.0											

a) Spectra were run in acetone-d_6+D_2O at 25.05 MHz.
b) Assignments may be reversed.
c) This signal overlapped with solvent signals.

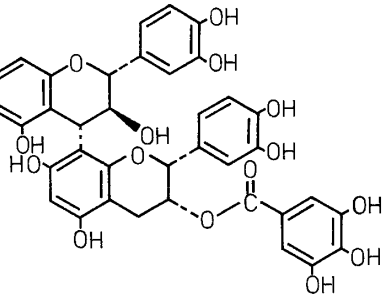
flavan units. This sort of isomerism is known[14,15] to be observable only in the cases of procyanidins B-3 and B-4, where a catechin unit (C_2, C_3: *trans*) is located in the upper half and is bonded, with α-configuration, to the lower unit. The occurrence of one galloyl group in the molecule was deduced from the ^{13}C-NMR resonances at δ 111.2, 138.8, 145.6 and 166.1. When hydrolyzed with tannase, VII yielded gallic acid and an amorphous hydrolysate (VIIa), the latter being identified as procyanidin B-4[15,16] from the ^{1}H- and ^{13}C-NMR spectra. The galloyl group was determined to be at the $C_{3'}$-position by the downfield shift of the $C_{3'}$ carbon signal (δ 69.6) in the ^{13}C-NMR spectrum of VII as compared with that of VIIa (δ 66.6). On the basis of these results, VII was characterized as procyanidin B-4 3′-*O*-gallate.



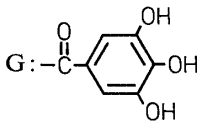
IV



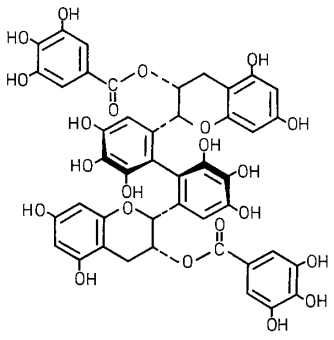
V: R=G
VI: R=H



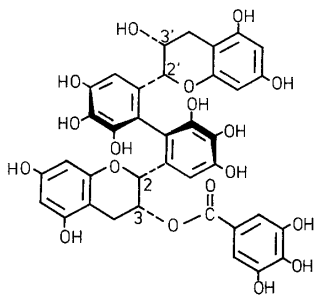
VII



Compound VIII, a pale brown amorphous powder, $[\alpha]_D$ −226.8° (acetone), $C_{44}H_{34}O_{22} \cdot 1^1/_2H_2O$, is structurally correlated with XI, as revealed by ^{1}H- and ^{13}C-NMR comparisons (Tables I—III). A clear distinction in these ^{1}H-NMR spectra is the observation in VIII of a seemingly one-proton singlet at δ6.88 ascribable to flavan B-ring protons. The ^{13}C-NMR spectra of VIII and XI are virtually indistinguishable except for the signals due to $C_{2'}$ and $C_{6'}$. Methylation of VIII in the same way as described for I, II and III gave a hexadecamethyl ether (VIIIa), whose field-desorption mass spectrum(FD-MS) shows a molecular ion peak at *m/z* 1138 as the base peak, indicating that VIII has a dimeric nature. From these observations VIII is presumed to be 2′,2′-linked bis-3-*O*-galloyl-(−)-epigallocatechin



VIII



IX

which possesses a symmetrical pattern and a biphenyl function in the molecule. Further support for the assignment of this structure was provided by ^{1}H- and ^{13}C-NMR analyses of a desgalloylated product (VIIIb) obtained by tannase hydrolysis of VIII; the symmetrical nature of the molecule was also confirmed by these analyses, and the spectra showed signal patterns closely related to those of XIV except for the B-ring signals. The appearance of a doublet signal at δ 108.0 and a singlet at δ 111.9 for $C_{6'}$ and $C_{2'}$, respectively, in the off-resonance ^{13}C-NMR spectrum indicated the presence of a substituent with a carbon–carbon linkage at the $C_{6'}$-position. The facts that the optical rotation of VIII (*vide ante*) is fairly close to that of XI (−180.7°), and that epigallocatechin exists as its (−)-enantiomer in green tea leaf, imply that VIII possesses the same absolute configuration at the C_2-

and C_3-positions as (−)-epigallocatechin.

The chirality of the biphenyl moiety was determined to be *S* on the basis of analysis of the circular dichroism (CD) spectrum of VIII, which exhibits a negative Cotton effect at 220 nm and two positive effects at 244 and 265 nm analogous to those found in gomisin A, a dibenzocyclooctadiene lignan isolated from *Schisandra chinensis*.[17] From these chemical and spectroscopic results, the structure of VIII was concluded to be 2′,2′-linked bis-3-*O*-galloyl-(−)-epigallocatechin with the *S*-biphenyl configuration.

Compound IX, a pale brown amorphous powder, $[\alpha]_D$ −147.2° (acetone), $C_{37}H_{30}O_{18} \cdot {}^1/_2H_2O$, is related to VIII, containing two flavan-3-ol units with a 3,5,7,3′,4′,5′-hexahydroxy substitution pattern, and one galloyl group in the molecule, as shown by the ^{1}H- and ^{13}C-NMR measurements (Tables II and III). In the ^{1}H-NMR spectrum a deshielded C_3 hydrogen signal at δ 5.30 is indicative of the occurrence of the galloyl group at this position. Upon tannase hydrolysis, IX yielded gallic acid and a desgalloylated compound, and the latter was shown to be identical with VIIIb by $[\alpha]_D$ and ^{1}H-NMR comparison, thus permitting the assignment of the structure IX for this compound.

This is the first report of the isolation of proanthocyanidins from green tea leaf, and it is considered that these compounds may make important contributions to the taste of tea infusions due to their strong astringency. Theasinensins A and B represent a new class of dimeric flavan-3-ols in that two flavan units are coupled at the B-ring, forming a biphenyl function.

Further chemical examination of other minor polyphenolic components in green tea leaf is under way, and in parallel with this, several sorts of biological tests of the compounds isolated in this study are in progress.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Infrared spectra were obtained with a JASCO DS-301 spectrometer. EI- and FD-MS were taken with JEOL JMS D-300 and JMS DX-300 instruments using a direct inlet system. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL PS-100 and JEOL FX-100 spectrometers, respectively, with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). CD data were obtained with a JASCO J-20 machine. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), Diaion HP-20 (75—150 μ, Mitsubishi Chemical Industries Ltd.) and Kieselgel 60 (70—230 mesh, Merck). Thin-layer chromatography was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm, Merck) using (A) benzene–ethyl formate–formic acid (1:7:1, v/v), (B) benzene–acetone–acetic acid (5:4:1, v/v) and benzene–acetone (9:1, 5:1, 3:1, v/v) as solvent systems, and spots were located by ultraviolet illumination, and with $FeCl_3$, 10% H_2SO_4 and anisaldehyde–H_2SO_4 spray reagents.

Isolation of Compounds I—XV——Fresh green tea leaves (15.6 kg), harvested at Yame City, Fukuoka Prefecture (August 1982), were extracted four times with 80% aqueous acetone at room temperature. The acetone was removed by evaporation under reduced pressure (*ca.* 40 °C), and the resulting aqueous solution afforded dark green precipitates consisting mainly of chlorophylls, which were removed by filtration. The filtrate was extracted with seven successive equal volumes of ethyl acetate. The ethyl acetate layer was concentrated to dryness (*ca.* 40 °C) to yield a brown solid (496 g). Chromatography of this solid on Sephadex LH-20 using EtOH as an eluent gave four fractions; Fr. I (99 g), II (179 g), III (160 g) and IV (58 g).

Fr. I was negative to the $FeCl_3$ reagent, and yielded, on crystallization from MeOH, caffeine as colorless needles (*ca.* 20 g).

Fr. II was rechromatographed over Sephadex LH-20 with EtOH to give two further fractions; Fr. II-a (52 g) and Fr. II-b (115 g). Diaion HP-20 chromatography of Fr. II-a using H_2O with an increasing amount of MeOH (0—40%) furnished (+)-catechin (XII) (4 g) and (−)-epicatechin (XIII) (39 g). Fr. II-b was similarly chromatographed over Diaion HP-20, followed by repeated Sephadex LH-20 chromatography using a variety of solvent systems, *viz.* 80% aqueous MeOH, 60% aqueous MeOH and EtOH, to give (−)-epigallocatechin (XIV) (51 g), (−)-epicatechin 3-*O*-gallate (X) (48 g), (−)-epicatechin 3-*O*-(3-*O*-methyl)-gallate (XV) (1.5 g) and compound I (1.3 g).

Fr. III was dissolved in H_2O, and the solution was passed through a column of Diaion HP-20, pre-swollen in a mixture of H_2O–MeOH (7:3, v/v). Elution with the same solvent, followed by crystallization from H_2O afforded (−)-epigallocatechin 3-*O*-gallate (XI) (146 g).

Fr. IV was further divided by Diaion HP-20 chromatography using H_2O–MeOH (4:1—2:3, v/v) into three fractions; Fr. IV-a (18 g), IV-b (17 g) and IV-c (7 g). Chromatography of Fr. IV-a on Sephadex LH-20 using 80% aqueous MeOH yielded compounds VIII (7.6 g) and IX (2.0 g). Fr. IV-b, containing a complicated mixture of dimeric proanthocyanidins, was repeatedly chromatographed over Diaion HP-20 (H_2O–MeOH) and Sephadex LH-20 (EtOH and 60% aqueous MeOH) to give compounds IV (2.3 g), V (2.5 g), VI (2.6 g) and VII (2.2 g). Fr. IV-c contained compounds II and III, and several attempts to separate these compounds were unsuccessful.

Compound I (I)——An off-white amorphous powder, $[\alpha]_D^{22}$ −158.3° (c=0.6, acetone). *Anal.* Calcd for $C_{24}H_{20}O_9 \cdot 1^1/_2H_2O$: C, 60.12; H, 4.93. Found: C, 59.67; H, 4.75. ^{1}H- and ^{13}C-NMR: Tables I and II.

Methylation of I——A mixture of I (40 mg), dimethyl sulfate (0.7 ml) and anhydrous potassium carbonate (1.0 g) in dry acetone (8 ml) was refluxed for 1.5 h with stirring. After removal of inorganic salts by filtration, the solution was concentrated to a syrup, which was chromatographed over silica gel using benzene–acetone (47:3, v/v) to furnish the hexamethyl ether (Ia) as a white amorphous powder (22 mg), $[\alpha]_D^{19}$ −90.8° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{32}O_9$: C, 67.15; H, 6.01. Found: C, 67.66; H, 6.26. ^{1}H-NMR ($CDCl_3$): 2.96—3.04 (2H, m, C_4–H), 3.80—3.84 (18H, 6×OCH_3), 5.02 (1H, s, C_2–H), 5.60 (1H, m, C_3–H), 6.10 (1H, d, J=2 Hz, C_6–H), 6.21 (1H, d, J=16 Hz, olefinic H), 6.22 (1H, d, J=2 Hz, C_8–H), 6.70 (2H, s, $C_{2',6'}$–H), 6.83 (2H, d, J=8 Hz, $C_{3'',5''}$–H), 7.36 (2H, d, J=8 Hz, $C_{2'',6''}$–H), 7.51 (1H, d, J=16 Hz, olefinic H). EI-MS m/z (rel. int.): 536 [M^+] (10), 358 (100), 161 (18).

Methanolysis of Ia——A mixture of Ia (20 mg) and sodium methoxide (30 mg) in MeOH (3 ml) was refluxed for 45 min. Neutralization with Dowex 50WX (H^+ form) and evaporation of the solvent gave a syrup, which was chromatographed on a silica gel column using benzene–acetone (19:1, v/v) to yield (−)-epigallocatechin pentamethyl ether (7.7 mg) as colorless needles (MeOH), mp 167—168 °C, $[\alpha]_D^{22}$ −71.4° (c=0.58, acetone), and methyl p-methoxycinnamate as colorless needles (2.2 mg), mp 84—85 °C. These compounds were identified by thin-layer cochromatography and mixed melting point dedermination with authentic samples.

Hydrolysis of II and III with Tannase——An aqueous solution of II or III (30 mg) was shaken with tannase at room temperature for 1 h, and the solution was concentrated to dryness under reduced pressure. The residue was treated with MeOH, and the MeOH solubles were applied to a Sephadex LH-20 column. Elution with EtOH afforded gallic acid (6 mg) and (−)-epigallocatechin (XIV) (11 mg), mp 218—220 °C, $[\alpha]_D^{20}$ −42.3° (c=0.76, acetone).

Methylation of II and III——A mixture of II and III (50 mg), dimethyl sulfate (0.7 ml) and anhydrous potassium carbonate (1.0 g) in dry acetone (5 ml) was refluxed for 45 min with stirring. The reaction mixture was worked up as described above to yield a mixture of the decamethyl ethers, as a white amorphous powder. *Anal.* Calcd for $C_{39}H_{42}O_{15}$: C, 62.39; H, 5.64. Found: C, 62.64; H, 5.65. ^{1}H-NMR ($CDCl_3$): 2.96—3.16 (2H, m, C_4–H), 3.68—3.90 (OCH_3), 5.15 (1H, s, C_2–H), 5.66 (1H, m, C_3–H), 6.11 (1H, d, J=2 Hz, C_6–H), 6.24 (1H, d, J=2 Hz, C_8–H), 6.78 ($^1/_4$H, s, $C_{2',6'}$–H), 6.89, 7.00 ($^7/_4$H in total, each d, J=2 Hz, $C_{2',6'}$–H), 7.16, 7.41 (each 2H, s, galloyl H).

Compounds IV—VII——These compounds were obtained as pale brown amorphous powders, IV, $[\alpha]_D^{20}$ −52.7° (c=0.62, acetone); V, $[\alpha]_D^{22}$ −95.2° (c=1.1, acetone); VI, $[\alpha]_D^{19}$ −45.8° (c=0.72, acetone). *Anal.* Calcd for $C_{37}H_{30}O_{16} \cdot 1^1/_2H_2O$: C, 58.65; H, 4.39. Found: C, 58.85; H, 4.77. VII, $[\alpha]_D^{22}$ −255.7° (c=0.56, acetone).

Hydrolysis of VII with Tannase——An aqueous solution of VII (18 mg) was treated with tannase at room temperature for 30 min. Work-up as before gave a mixture of hydrolysates, which was separated by Sephadex LH-20 column chromatography using EtOH to yield gallic acid (3 mg) and procyanidin B-4 (3.8 mg).[15,16]

Compound VIII——A pale brown amorphous powder, $[\alpha]_D^{15}$ −226.8° (c=0.73, acetone). *Anal.* Calcd for $C_{44}H_{34}O_{22} \cdot 1^1/_2H_2O$: C, 56.51; H, 4.35. Found: C, 56.28; H, 4.44. ^{1}H- and ^{13}C-NMR: Tables II and III. CD (c=0.029, MeOH) $[\theta]^{20}$ (nm) −464000 (220), 0 (239), +40900 (244), 0 (253), −20500 (265).

Methylation of VIII——A mixture of VIII (150 mg), dimethyl sulfate (1.2 ml) and potassium carbonate (1.5 g) in dry acetone was refluxed for 2.5 h. Treatment of the reaction mixture as before yielded the hexadecamethyl ether (VIIIa) as a white amorphous powder (118 mg), $[\alpha]_D^{20}$ −235.4° (c=1.05, $CHCl_3$). *Anal.* Calcd for $C_{60}H_{60}O_{22}$: C, 63.26; H, 5.84. Found: C, 63.58; H, 5.87. FD-MS m/z: 1138 [M^+]. ^{1}H-NMR ($CDCl_3$): 2.24—3.12 (4H, m, C_4–H), 3.52—3.81 (OCH_3), 4.71 (2H, s, C_2–H), 5.76 (2H, br s, C_3–H), 6.04 (4H, s, $C_{6,8}$–H), 7.06 (2H, s, $C_{6'}$–H), 7.24 (4H, s, galloyl H). CD (c=0.0077, MeOH) $[\theta]^{20}$ (nm): +362000 (208), 0 (214), −362000 (222).

Hydrolysis of VIII with Tannase——An aqueous solution of VIII (50 mg) was shaken with tannase at room temperature for 1 h. The reaction mixture was treated in the same way as described above to yield gallic acid (12 mg) and a hydrolysate (VIIIb) (28 mg) as a pale brown amorphous powder, $[\alpha]_D^{18}$ −20.4° (c=0.75, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{14} \cdot 1^1/_2H_2O$: C, 56.51; H, 4.59. Found: C, 56.64; H, 5.19. ^{1}H-NMR (acetone-d_6): 2.24—2.85 (4H, m, C_4–H), 4.04 (2H, br s, C_3–H), 4.63 (2H, s, C_2–H), 5.82 (2H, d, J=2 Hz, C_6–H), 5.94 (2H, d, J=2 Hz, C_8–H), 6.86 (2H, s, $C_{6'}$–H). ^{13}C-NMR (acetone-d_6+D_2O): 28.7 (C_4), 64.9 (C_3), 77.1 (C_2), 95.5, 96.1 ($C_{6,8}$), 99.2 (C_{4a}), 108.0 ($C_{6'}$), 111.9 ($C_{2'}$), 129.1 ($C_{1'}$), 133.0 ($C_{4'}$), 144.9, 145.8 ($C_{3',5'}$), 157.0, 157.1, 157.4 ($C_{5,7,9}$). CD (c=0.0058, MeOH) $[\theta]^{20}$ (nm): −549000 (220), 0 (237), +305000 (247).

Compound IX——A pale brown amorphous powder, $[\alpha]_D^{21}$ −147.2° (c=0.5, acetone). *Anal.* Calcd for $C_{37}H_{30}O_{18} \cdot {}^1/_2H_2O$: C, 57.26; H, 4.66. Found: C, 57.00; H, 4.64. ^{1}H- and ^{13}C-NMR: Tables II and III.

Hydrolysis of IX with Tannase——An aqueous solution of IX (20 mg) was treated with tannase at room temperature for 1 h. Work-up as before yielded gallic acid (3 mg) and a hydrolysate (6.7 mg), the latter being identified as VIIIb by comparison of the $[\alpha]_D$ and ^{1}H-NMR spectrum.

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