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Tannins and Related Compounds. LXIII.[1] Isolation and Characterization of Mongolicains A and B, Novel Tannins from *Quercus* and *Castanopsis* Species

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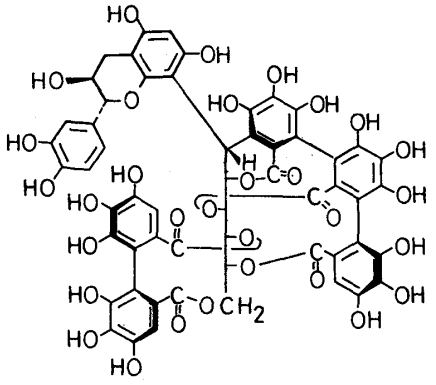
Continuing chemical examinations on tannins have led to the isolation of two new tannins, mongolicains A (**3**) and B (**4**), from five *Quercus* and one *Castanopsis* species. The structures of **3** and **4** have been characterized on the basis of chemical and spectroscopic data as novel flavano-ellagitannins, in which hydrolyzable tannin and flavan-3-ol moieties are connected through a carbon–carbon linkage. Their structural features suggest that **3** and **4** are biosynthetically formed by oxidation of accompanying acutissimins A (**1**) and B (**2**).

Keywords——*Quercus* sp.; *Castanopsis* sp.; Fagaceae; mongolicain A; mongolicain B; flavano-ellagitannin; *C*-glycosidic tannin; flavan-3-ol; oxidative metabolite; ^{1}H–^{13}C long-range shift correlation spectrum

Previously, we reported on the isolation and structural characterization of a series of novel tannins, stenophyllanins A, B and C,[2] acutissimins A (**1**) and B (**2**),[3] and stenophynins A and B,[4] which contain in each molecule a hydrolyzable tannin moiety and a flavan-3-ol (catechin) unit (one of the component units of condensed tannins) linked through a carbon–carbon bond. Furthermore, among tannin-containing plants so far investigated in our laboratory, Fagaceous plants such as *Quercus stenophylla* MAKINO (Japanese name: urajirogashi), *Q. mongolica* FISCHER *ex.* TURCZ. var. *grosseserrata* (BL.) REHD. *et* WILS. (mizunara), *Q. acutissima* CARRUTH. (kunugi), *Q. dentata* THUNB. (kashiwa), *Q. miyagii* KOIDZU. (okinawa-urajirogashi), and *Castanopsis cuspidata* var. *sieboldii* NAKAI (sudajii) were shown to be particularly rich sources of such tannins.[3] From all of these plant materials, we have now isolated two accompanying tannins with novel structures, mongolicains A (**3**) and B (**4**), which are considered to be oxidative metabolites derived from acutissimins A (**1**) and B (**2**), respectively. This paper presents a detailed account of the isolation and structural determination of these compounds.

The composition and contents of tannins differed remarkably in individual plants and even in the parts of the plant bodies. However, when the 80% aqueous acetone extracts were chromatographed on Sephadex LH-20 dextran gel with a solvent system of water–methanol–acetone,[5] mongolicains A (**3**) and B (**4**) were almost invariably eluted in the final fraction, except for the case when higher-molecular-weight gallotannins such as tetra- and pentagalloylglucoses were present in the extracts. The isolation of mongolicains A (**3**) and B (**4**) was successfully achieved by a combination of partition chromatographies over reverse-phase gels (MCI-gel CHP-20P, Fuji-gel ODS-G3 and Bondapak C_{18}/Porasil B) with water containing



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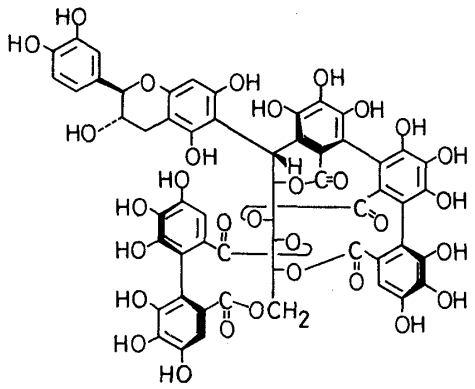

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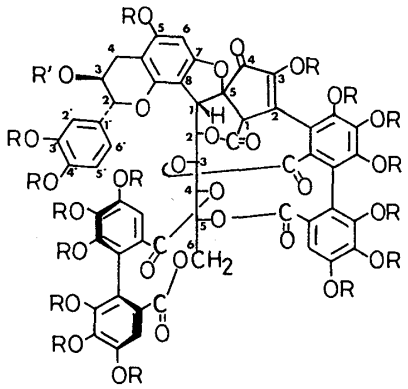
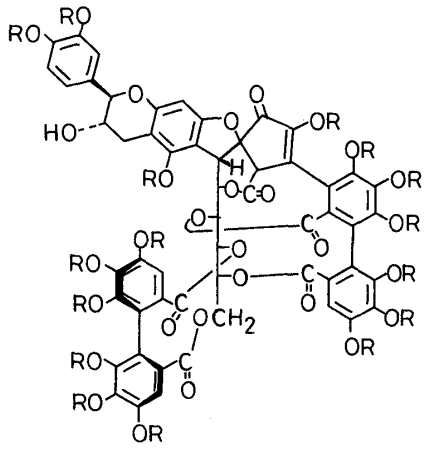


3 : R = R' = H
3a: R = Me , R' = H
3b: R = Me , R' = Ac



4 : R = H
4a: R = Me

increasing amounts of methanol.

Mongolicain A (**3**) showed, with the ferric chloride reagent, a dark blue coloration characteristic of a hydrolyzable tannin, and was also positive to the anisaldehyde–sulfuric acid test (reddish pink coloration),[5] suggesting the presence of a flavan-3-ol framework in the molecule.

The proton-nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** exhibited three aromatic one-proton singlets at δ 6.59, 7.08 and 6.65, the former two being attributable to the protons of a hexahydroxydiphenoyl ester group. Since in common ellagitannins, for example, those based on a D-glucopyranose core, the aromatic ring proton signals appear as a pair of singlets, the observation of the odd number of aromatic singlets suggested the presence of a *C*-glycosidic linkage in the molecule. This was also supported by the absence of an anomeric (hemiacetal or ketal) carbon resonance in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum.

The presence of a flavan-3-ol moiety was readily recognizable from the heterocyclic C-ring proton resonances (H-4: δ 2.50, dd, J = 17, 4 Hz; δ 2.85, dd, J = 17, 2 Hz; H-3: δ 4.57, m, H-2: δ 5.37, d, J = 3 Hz). The chemical shifts of these signals were consistent with those of a C-8 substituted catechin derivative,[2,6] although the coupling constants were somewhat different from those found in catechin (**5**), probably due to a conformational change caused by the steric interaction of the substituent. The appearance of aromatic ABX-type signals at δ 6.52

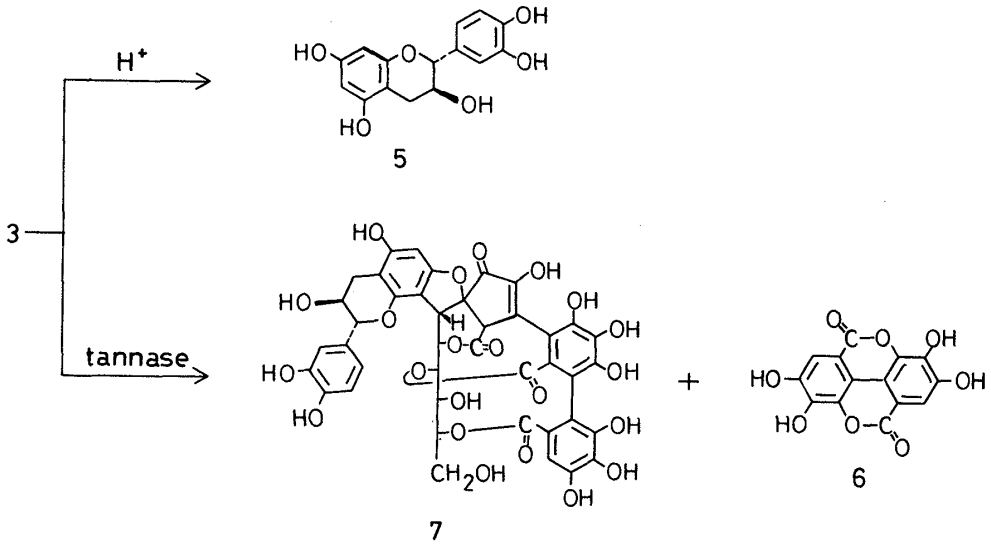


Chart 1

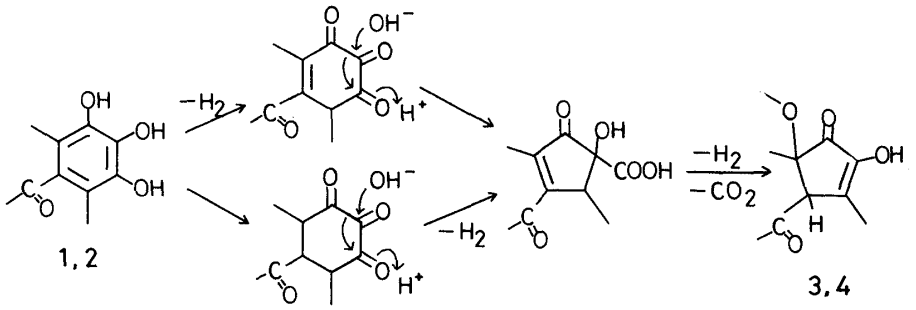


Chart 2. Possible Biosynthetic Pathways of Mongolicains (**3**, **4**) from Acutissimins (**1**, **2**)

(1H, dd, $J=2$, 8 Hz), 6.68 (1H, d, $J=8$ Hz) and 6.68 (1H, d, $J=2$ Hz), and a one-proton singlet at δ 6.16 arising from the B- and A-rings, respectively, were consistent with a 5,7,3′,4′-tetrahydroxy substitution system.[6] Confirmation of the structure of the flavan-3-ol moiety was achieved by acid-catalyzed degradation (20% acetic acid in ethanol) of **3**, which yielded catechin (**5**).[2,3]

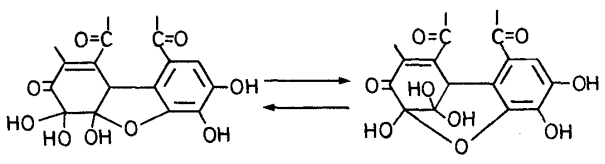
The chemical shifts and coupling patterns of protons in the polyalcohol moiety were closely correlated with those found in **1**,[3] indicating that this polyalcohol moiety has a similar substitution system and configurations. In particular, the appearance of the polyalcohol H-1 and H-2 signals as similar broad singlets at δ 4.23 and 5.80, respectively, established the configuration at the C-1 position to be the same as that of **1**. The configurations of the other polyalcohol carbons were confirmed unequivocally by oxidative degradation of **3** with ferric chloride,[2] which yielded glucose and arabinose. Furthermore, the substitution pattern in the polyalcohol moiety was established by enzymatic hydrolysis of **3** with tannase to liberate, together with ellagic acid (**6**), the hydrolysate (**7**) whose ^{1}H-NMR spectrum showed significant upfield shifts of only the H-4 and H-6 signals.

The most distinguishing features in the ^{13}C-NMR spectra between **3** and **1** were the observation (in **3**) of signals due to a carbonyl (δ 196.9), a tetra-substituted double bond (δ 148.9 and 140.1, each s), a quaternary carbon (δ 89.3, s) and a methine carbon (δ 50.3, d). Although an initial attempt to construct these functional groups readily recalled the presence of a cyclohexenone moiety,[7] since it commonly occurs as a part of the dehydrohexahydroxydi-

TABLE I. ^{13}C-NMR Spectral Data for Compounds **3**, **3a**, **8**, **9**, **4** and **4a** (δ-Values)[a]

	3	**3a**	**8**	**9**	**4**	**4a**
Glucose						
C-1	47.4	45.0	33.1	33.4	48.1	46.1
C-2	77.9	79.8	89.6	77.0	76.8	78.2
C-3	72.4	72.1	74.1	72.7	72.0	71.9
C-4	68.4	67.4	72.7	68.5	68.4	68.0
C-5	71.1	71.1	72.4	71.6	71.0	70.6
C-6	65.0	64.8	66.1	64.6	65.1	65.1
Catechin						
C-2	79.9	82.1	83.4	81.1	82.7	82.4
C-3	67.0	67.4	67.3	67.2	67.2	67.6
C-4	24.3	27.0	30.4	25.9	27.6	27.6
C-4a	101.0	102.6	101.7	100.2	96.5	97.6
C-5	158.6	159.5	155.9	155.6	157.5	157.4
C-6	90.6	86.8	96.7	96.9	104.5	104.8
C-7	160.1	160.2	155.9	155.8	159.5	158.7
C-8	103.6	104.3	102.0	104.1	97.2	93.6
C-8a	151.6	150.4	155.6	154.4	153.6	154.7
C-1′	130.6	129.2	130.0	131.1	131.3	129.7
C-2′	113.2	109.5	116.3	114.1	115.2	110.0
C-5′	116.1	111.3	116.3	116.3	115.9	111.3
C-6′	117.6	120.0	120.9	118.1	119.8	120.2
Five-membered ring						
C-1	50.3	48.4	130.8	43.8	50.7	48.7
C-2	140.1	140.4	34.6	50.9	140.1	141.0
C-3	148.9	148.5	79.0	86.2	149.2	148.4
C-4	196.9	194.8	77.9	104.5	196.8	194.8
C-5	89.3	89.3	140.6	47.6	89.3	89.1
HHDP[b]-C[c]	106.8	104.7	106.6	107.4	107.1	105.2
	107.8	107.4	110.6	107.9	109.0	105.9
Arom.-C[c]	107.8	105.9	109.6	107.7	108.1	105.6
–COO–	163.6	161.8	162.1	167.1	163.8	162.0
	166.7	164.7	162.8	167.8	167.0 (×2)	165.6
	167.3	165.9	167.6	169.4	169.0	165.9
	168.1	166.3	168.5	170.8	169.3	166.3
	169.4	167.7	170.0	200.6[d]		167.6

a) Measured in acetone-d_6+D_2O. *b*) HHDP represents hexahydroxydiphenoyl. *c*) Only carbons bearing a proton are described. *d*) Carbonyl group.



A

phenoyl ester group (A), a hemiacetal carbon signal could not be found in the spectrum, and also the observation of the intense $(M-H)^-$ peak at m/z 1175 in the negative fast atom bombardment mass spectrum (FAB-MS) of **3** was inconsistent with the expected structure. Furthermore, the measurement of the ^{1}H–^{13}C long-range shift correlation spectrum (Fig. 1)

TABLE II. ^{1}H-NMR Spectral Data for Compounds **3**, **4**, **8**, **9** and **13** (δ-Values)[a]

	3	**8**	**9**	**4**	**13**
Glucose					
H-1	4.23 (s)	4.34 (s)	3.82 (br d, J=2)	4.07 (s)	3.90 (d, J=2)
H-2	5.80 (br s)	4.79 (d, J=7)	5.33 (br d, J=2)	5.88 (br s)	5.30 (d, J=2)
H-3	5.25 (br d, J=8)	3.96 (dd, J=7, 2)	4.43 (d, J=7)	5.19 (br d, J=7)	4.49 (d, J=7)
H-4	5.62 (t, J=8)	5.02 (dd, J=7, 2)	5.29 (dd, J=7, 8)	5.59 (m)	5.32 (dd, J=7, 7)
H-5	5.54 (br d, J=8)	5.60 (br d, J=7)	5.40 (br dd, J=8, 2)	5.59 (m)	5.54 (br dd, J=7, 2)
H-6	4.06 (br d, J=12)	4.16 (br d, J=12)	3.91 (br d, J=13)	4.03 (br d, J=12)	—[b]
	4.54 (br d, J=12)	4.37 (br d, J=12)	4.63 (dd, J=13, 2)	4.72 (br d, J=12)	4.67 (dd, J=13, 2)
Catechin					
H-2	5.37 (d, J=3)	4.21 (d, J=9)	5.12 (d, J=7)	4.74 (d, J=8)	4.60 (d, J=8)
H-3	4.57 (m)	4.02 (ddd, J=6, 9, 10)	4.42 (m)	4.11 (m)	4.09 (ddd, J=6, 8, 9)
H-4	2.50 (dd, J=17, 4)	2.47 (dd, J=17, 10)	2.63 (dd, J=17, 5)	2.63 (dd, J=16, 8)	2.67 (dd, J=16, 9)
	2.85 (dd, J=17, 2)	3.03 (dd, J=17, 6)	2.74 (dd, J=17, 5)	2.89 (dd, J=16, 5)	3.02 (dd, J=16, 6)
H-6	6.16 (s)	6.08 (s)	6.20 (s)	—	—
H-8	—	—	—	6.22 (s)	6.14 (s)
H-2′	6.68 (d, J=2)	6.89 (d, J=2)	6.82 (d, J=2)	6.95 (d, J=2)	6.94 (d, J=2)
H-5′	6.68 (d, J=8)	6.94 (d, J=8)	6.78 (d, J=8)	6.84 (d, J=8)	6.85 (d, J=8)
H-6′	6.52 (dd, J=2, 8)	6.77 (dd, J=2, 8)	6.68 (dd, J=2, 8)	6.79 (dd, J=8, 2)	6.78 (dd, J=2, 8)
Five-membered ring					
H-1	4.38 (s)	—	4.35 (dd, J=10, 7)	4.40 (s)	4.04 (dd, J=7, 10)
H-2	—	2.93 (br)[9]	3.72 (d, J=10)	—	3.83 (d, J=10)
H-3	—	4.62 (t, J=6)	—	—	—
H-4	—	4.78 (d, J=6)	—	—	—
H-5	—	—	3.28 (dt, J=7, 2)	—	3.22 (dt, J=7, 2)
HHDP[c]-H	6.59 (s)	6.51 (s)	6.64 (s)	6.65 (s)	6.68 (s)
	7.08 (s)	6.95 (s)	6.88 (s)	6.96 (s)	6.70 (s)
Arom.-H	6.65 (s)	6.75 (s)	6.65 (s)	6.69 (s)	6.68 (s)

a) Measured at 270 MHz in acetone-d_6+D_2O. *b*) Overlapped with HOD signals. *c*) HHDP represents hexahydroxydiphenoyl.

of **3** clearly showed a relatively large three-bond coupling between the quaternary carbon signal at δ 89.3 and the polyalcohol H-2 signal at δ 5.80. The unusual lowfield shift of the signal at δ 89.3 may be interpreted in terms of the formation of an ether linkage with the catechin C-7 hydroxyl.[8] In fact, the hexadecamethyl ether (**3a**) [field-desorption mass spectrum (FD-MS) m/z: 1400 $(M)^+$], prepared by ordinary methylation with dimethyl sulfate and potassium carbonate in dry acetone, afforded, on acetylation even under drastic conditions, the monoacetate (**3b**) [FD-MS m/z: 1442 $(M)^+$], in which the acetyl group was shown by ^{1}H-NMR spectroscopy to be located at the catechin C-3 position. The occurrence of the ether linkage at the catechin C-7 position was further supported by the lowfield shift (δ 160.1) of the corresponding C-7 carbon signal and the upfield shift (δ 90.6) of the neighboring C-6 signal as compared with those of 8-substituted catechin derivatives.[6]

The ^{1}H–^{13}C long-range shift correlation spectrum (Fig. 1) clearly indicated the correlation of the carbonyl carbon at δ 196.9 with the polyalcohol H-1 signal through a three-bond coupling. Similarly, two- and three-bond long-range couplings of the methine proton signal at δ 4.38 were observed with the signals of the quaternary carbon (δ 89.3), two double bond carbons (δ 148.9 and 140.1) and carboxyl carbon (δ 168.1). In addition, since the polyalcohol H-2 signal was shown to be coupled with the above carboxyl carbon signal, the aliphatic carboxylic acid ester should be located at the C-2 position.

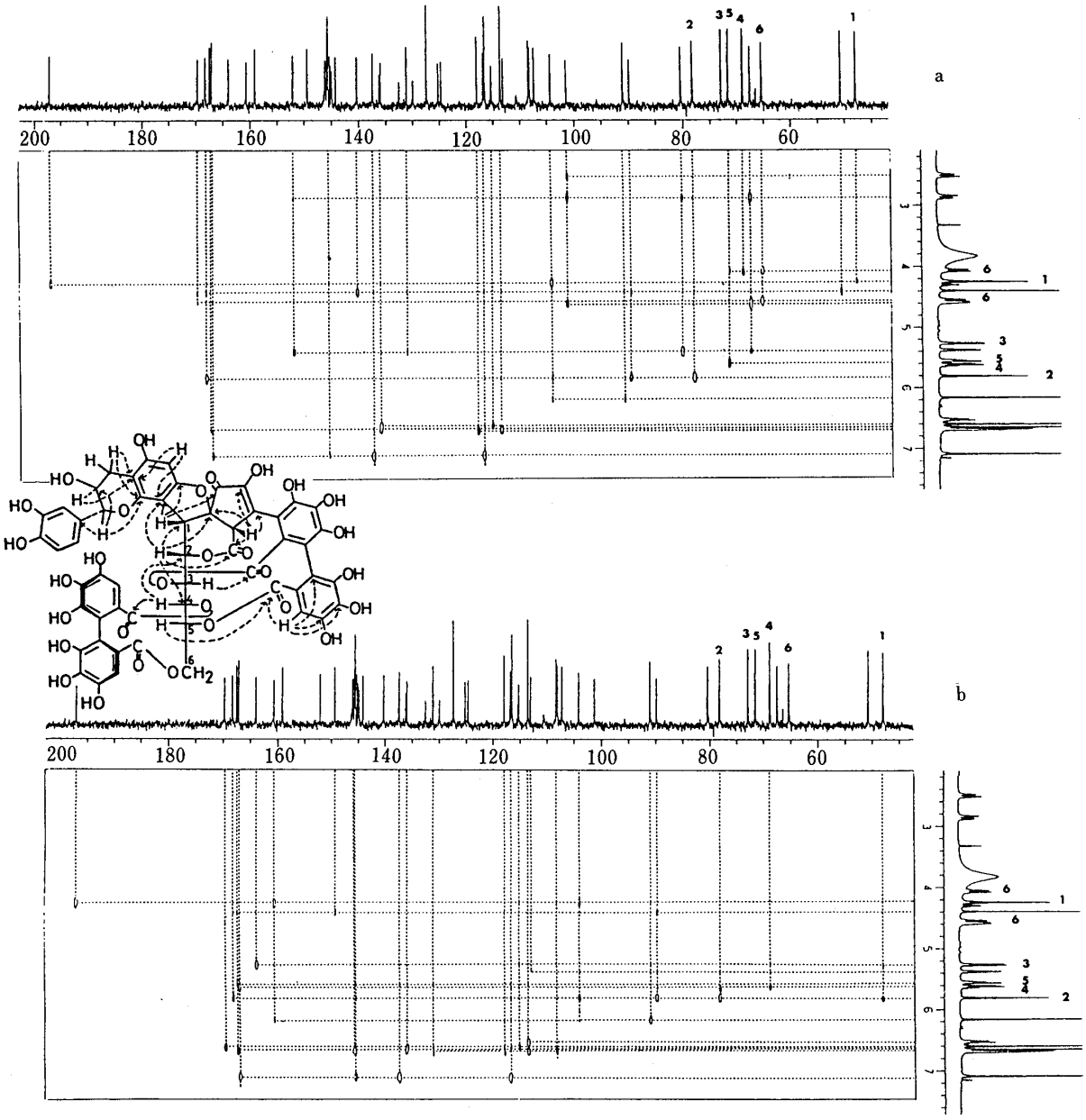
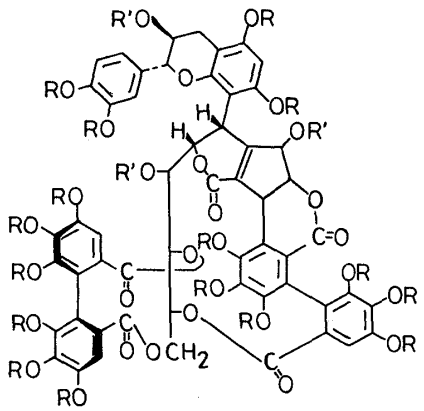


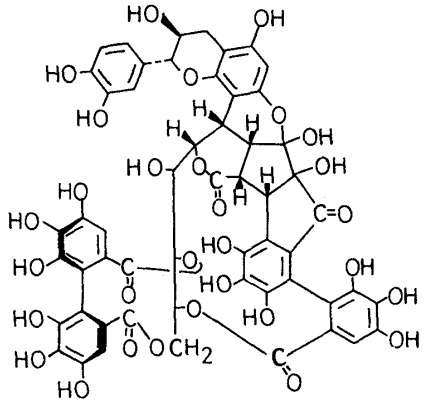
Fig. 1. ^{1}H–^{13}C Long-Range Shift Correlation Spectra of **3** in Acetone-d_6 + D_2O (a, J_{CH} = 10 Hz; b, J_{CH} = 5 Hz)

The presence of an enol function conjugated with the carbonyl group was deduced from the chemical shifts of the above-mentioned double bond signals and also from the appearance of an abnormally shifted methoxyl signal at δ 4.12 in the ^{1}H-NMR spectrum of the hexadecamethyl ether (**3a**). An attempt to hydrogenate the double bond in **3** with palladium on carbon was unsuccessful, but on platinum-catalyzed hydrogenation, **3** afforded two products (**8** and **9**) in 70 and 20% yields, respectively. The negative FAB-MS of these products showed the same prominent $(M-H)^-$ peak at *m/z* 1179, which is four mass units more than that of **3**. The ^{13}C-NMR spectrum of the major product (**8**) disclosed the absence of the carbonyl group and the oxygen-bearing quaternary carbon, and instead two methines each having an oxygen function newly appeared at δ 79.0 (d) and 77.9 (d). The chemical shifts of the signals arising from the catechin moiety, especially those from the A-ring, differed distinctly from those observed in **3**. Namely, the signal due to C-7 was shifted considerably

upfield (−4.2 ppm), while that of the neighboring C-6 appeared at lower field (+6.1 ppm) (Table I). Furthermore, the chemical shift values of the A-ring carbon signals in **8** were in good agreement with those of 8-substituted catechin derivatives.[6] These facts indicated the cleavage of an ether linkage between the catechin C-7 position and the cyclopentenone moiety. Further support for this ether cleavage was obtained by examination of the ^{1}H-NMR spectrum of the hexadecamethyl ether (**8a**) [FD-MS *m/z*: 1404 $(M)^+$] of **8**, which showed a complicated signal pattern, whereas the hexadecamethyl ether (**3a**) was amenable to first-order analysis. This unexpected signal complexity could be explained by conformational isomerism caused by the restricted rotation between the catechin moiety and the substituent.[2] This contrasts with the case of **3a** which has the rigid structure of the catechin moiety, forming an ether linkage.



8 : R=R'=H
8a: R=Me, R'=H
8b: R=Me, R'=Ac



9

The ^{1}H-NMR spectrum of **8** showed a moderate upfield shift (δ 3.96) of the polyalcohol H-3 signal, together with the presence of a new aliphatic AMX-type splitting pattern at δ 2.93 (br),[9] 4.62 (t, J=6 Hz) and 4.78 (d, J=6 Hz), among which the latter two were shown by ^{1}H–^{13}C shift correlation spectroscopy to be correlated with the above-mentioned oxygen-bearing methine carbons at δ79.0 and 77.9, respectively. These findings, coupled with the fact that the methyl ether (**8a**) resisted periodate oxidation, indicated that the carboxylic acid ester group originally located at the polyalcohol C-3 position had migrated to the hydroxyl in the cyclopentenol moiety, forming a six-membered lactone ring. This was further supported by formation of the triacetate (**8b**) on acetylation of **8a**. Consequently, taking into account the observation of the signals due to a tetra-substituted double bond (δ 140.6 and 130.8) and a methine carbon (δ 34.6) in the ^{13}C-NMR spectrum of **8**, it follows that the major reduction product can be formulated as **8**. It should be noted that further hydrogenation of the double bond in **8** at atmospheric pressure was unsuccessful.

The structure of the other product (**9**) was determined as follows. The ^{1}H-NMR spectrum of **9** exhibited a double-triplet signal at δ 3.28 (J=2, 7 Hz), which was confirmed by ^{1}H–^{1}H shift correlation spectroscopy to be coupled with the respective polyalcohol H-1 and H-2 signals at δ 3.82 (br d, J=2 Hz) and δ 5.33 (br d, J=2 Hz), together with a double-doublet signal at δ 4.35 (J=7, 10 Hz). Accordingly, the double-triplet could be assigned to the methine located next to the polyalcohol C-1 position. The long-range coupling between this signal and the H-2 signal was interpreted in terms of the so-called "W-letter rule," indicating that this methine proton possesses the same β-configuration as that of the C-2 proton. Since the above double-doublet signal at δ 4.35 was further coupled with the doublet at δ 3.72 with a J-value of

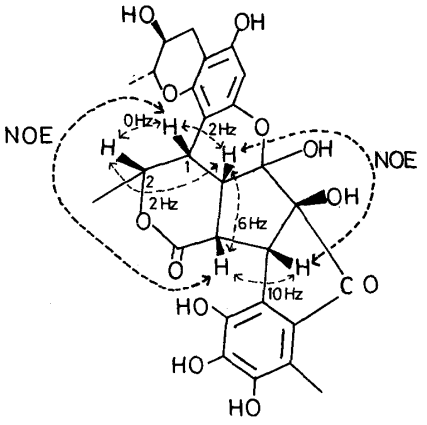
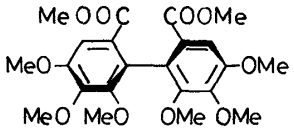


Fig. 2. Observed NOE and Coupling Constants in **9**

10 Hz, the sequence of these newly appearing methines was readily assignable. The configurations of these methine protons were determined to be all β by nuclear Overhauser effect (NOE) correlation spectroscopy, which clearly indicated the correlation between the polyalcohol H-1 signal and the signal at δ 4.35 and likewise between the signals at δ 3.28 and 3.72. The coupling constants of these methine signals were also consistent with the *cis* configurations.

The ^{13}C-NMR spectrum of **9** showed the presence of a carbonyl group (δ 200.6), a hemiacetal carbon (δ 104.5) and a quaternary carbon bearing an oxygen atom (δ 86.2). In addition, the outstanding feature in the spectrum was the observation of four carboxyl carbon signals (δ 167.1, 167.8, 169.4 and 170.8), *i.e.*, are one less than those found in **3** and other derivatives. The assignments of these carboxyl signals to the esters located at the polyalcohol C-5, C-4, C-6 and C-2, respectively, were achieved by observation of long-range couplings between the polyalcohol protons and the carboxyl carbons through the aid of ^{1}H–^{13}C long-range shift correlation spectroscopy. The absence of the C-3 carboxyl group, together with the appearance of the above carbonyl group, indicated the formation of a new carbon–carbon linkage as shown in the structure (**9**). On the basis of the above spectroscopic evidence, the minor product was concluded to be formulated as **9**.

The atropisomerism of the hexahydroxydiphenoyl ester group was determined in the following ways. Alkaline methanolysis of the hexadecamethyl ether (**3a**) with sodium methoxide in methanol yielded, among others, dimethyl hexamethoxydiphenoate (**10**). The specific optical rotation of **10** showed the negative sign [$-29.0°$ ($CHCl_3$)], thus establishing the chirality to be in the *S*-series.[10] On the other hand, the chirality of the remaining biphenyl bond positioned at the polyalcohol C-3 and C-5 could not be determined due to the lack of an appropriate model compound, although from the biosynthetic point of view, it was considered to have the *S*-configuration.[11]



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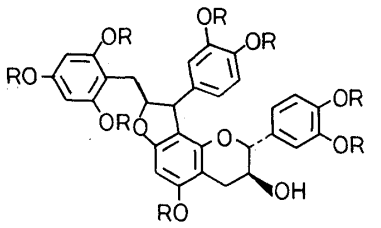
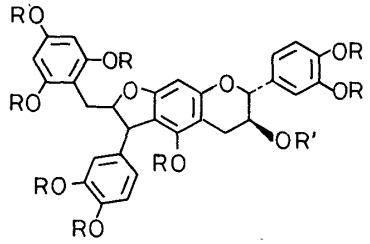
Finally, it should be noted that the chemical shifts of the catechin A-ring signals in **3** were compatible with those found in gambiriin B_1 (**11**).[6a] In particular, the chemical shifts of the most distinguishing C-6 signals were almost identical (**3**: δ 90.6; **11**: δ 90.3). On going from **3** to **3a**, the upfield shift of the C-6 signal was similarly observed in the gambiriin octamethyl ether (**11a**) (**3a**: δ 86.8; **11a**: δ 86.6).

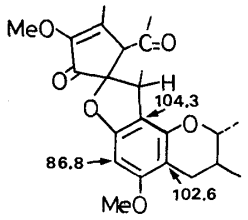
Based on the chemical and spectroscopic evidence described above, the structure of



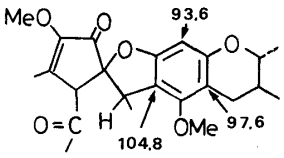
11: R=H
11a: R=Me



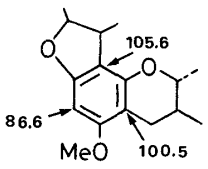
12: R=R'=H
12a: R=Me, R'=Ac



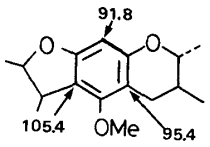
3a



4a



11a



12a

Fig. 3. ^{13}C-NMR Chemical Shifts of **3a**, **4a**, **11a** and **12a** (in $CDCl_3$)

mongolicain A was determined to be as represented by the formula **3**.

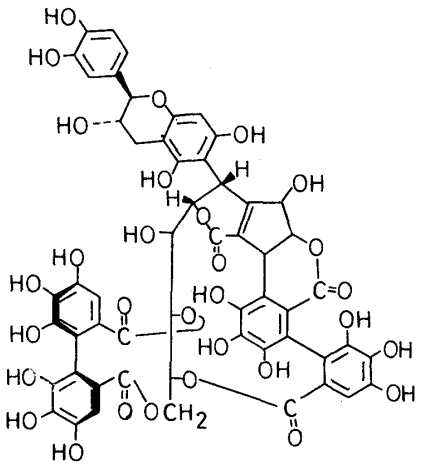
Mongolicain B (**4**) was shown to have the same molecular mass as **3** by the negative FAB-MS [*m/z* 1175 $(M-H)^-$]. The positive reaction of **4** in the anisaldehyde–sulfuric acid test suggested the presence of a flavan-3-ol moiety in the molecule. This was confirmed by the formation of catechin on treatment with acetic acid in ethanol.

The ^{13}C-NMR spectrum of **4** was quite similar to that of **3**, except for the chemical shifts of the catechin A-ring signals, suggesting that only the position of the substituent in the catechin A-ring is different. Comparison of the chemical shifts of the A-ring signals with those of the C-6 substituted catechin derivative, gambiriin B_3 (**12**),[6a] showed close similarities. In particular, the chemical shifts of the C-5 (δ 157.5), C-7 (δ 159.5) and C-8a (δ 153.6) in **4** agreed well with those of the corresponding carbons in **12** [C-5: δ 157.2; C-7: δ 159.6; C-8a: δ 153.6]. Furthermore, the ^{13}C-NMR spectrum of the hexadecamethyl ether (**4a**) of **4** exhibited C-4a (δ 97.6), C-6 (δ 104.8) and C-8 (δ 93.6) signals similar to those observed in the octamethyl diacetate (**12a**) of **12** (C-4a: δ 95.4; C-6: δ 105.4; C-8: δ 91.8).[12] The location of the substituent at the C-6 position was also confirmed by the ^{1}H-NMR chemical shifts of the catechin H-2 signal (δ 4.74) and the ABX-type B-ring signals (H-6′: δ 6.79, dd, J=2, 8 Hz; H-5′: δ 6.84, d, J=8 Hz; H-2′: δ 6.95, d, J=2 Hz), which were similar to those found in catechin (**5**), indicating that the substituent is located at a spatially remote position from these protons. Based on these spectral findings, the carbon–carbon and carbon–oxygen linkages were determined to be located at the C-6 and C-7 positions, respectively.

Similar catalytic hydrogenation of **4** with platinum oxide afforded, among many uncharacterized compounds, a product (**13**) [negative FAB-MS *m/z*: 1179 $(M-H)^-$], whose ^{1}H-NMR data (Table II) were consistent with the structure illustrated as **13**, showing coupling constants and a splitting pattern similar to those of **9**.

The configuration at the C-1 position and the substitution system in the polyalcohol moiety, as well as the structure of the cyclopentenone and polyalcohol moieties, were concluded to be the same as those of **3** on the basis of close similarities of their ^{1}H- and ^{13}C-NMR spectra and the formation of similar reaction products.

The almost identical circular dichroism (CD) spectra of **4a** and **3a** suggested that the



13

same chiralities exist in the molecules. Actually, the atropisomerism of the hexahydroxydiphenoyl ester group was confirmed to be in the *S*-series by methanolysis of **4a** to give (−)-dimethyl hexamethoxydiphenoate (**10**).[10] However, the chirality of the other biphenyl bond still remains to be solved, as in the case of **3**.

On the basis of these results, the structure of mongolicain B was concluded to be as shown by the formula **4**.

The structural features of mongolicains A (**3**) and B (**4**), as well as the fact that mongolicains almost invariably coexist with acutissimins in the Fagaceous plants, suggest that these tannins are derived biosynthetically from acutissimins *via* the pathways shown in Chart 2.

Experimental

All melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Infrared (IR), ultraviolet (UV) and CD spectra were obtained with JASCO DS 301, Hitachi 100-50 and JASCO J-20 spectrometers, respectively. FD- and FAB-MS were obtained with a JEOL JMS DX-300 instrument. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL PS-100, FX-100, GX-270 and GX-400 spectrometers, using tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Column chromatography was performed with Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemicals Co., Ltd.), MCI-gel CHP 20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.), Fuji-gel ODS-G3 (43—65 μ, Fuji Gel Hanbai Co., Ltd.) and Bondapak C_{18}/Porasil B (37—75 μ, Waters Associates, Inc.). Thin-layer chromatography (TLC) was carried out on precoated Kieselgel 60 F_{254} plates (Merck) and precoated cellulose plates (Funakoshi), using solvent systems of benzene–ethyl formate–formic acid (5 : 4 : 1, 2 : 7 : 1, 1 : 7 : 1 and 1 : 5 : 2) and 2% acetic acid, respectively, and spots were detected by UV illumination and by spraying 2% ethanolic ferric chloride, 10% sulfuric acid and anisaldehyde–sulfuric acid reagents. High-performance liquid chromatography (HPLC) was conducted with a Toyo Soda apparatus equipped with an SP-8700 solvent delivery system, a UV-8 model II spectrometer and a Nucleosil 5C_{18} column (4 mm i.d. × 300 mm). Analytical gas-liquid chromatography (GLC) was conducted with a Shimadzu GC 4BMPF instrument over 1.5% SE-30 with nitrogen as the carrier gas.

Isolation of Mongolicains A (3) and B (4)——Mongolicains A (**3**) and B (**4**) were isolated from the fresh barks of *Quercus stenophylla*, *Q. acutissima*, *Q. dentata*, *Q. mongolica* var. *grosseserata*, *Q. miyagii* and *Castanopsis cuspidata* var. *sieboldii*, the fresh leaves of *Q. mongolica* var. *grosseserata* and *Q. miyagii*, and the fresh acorn of *Q. mongolica* var. *grosseserata*. Two typical examples of the isolation procedures are described here.

a) From the Bark of *Q. acutissima*: The fresh bark (6.1 kg) was chopped into small pieces and extracted with 80% aqueous acetone at room temperature. The acetone was removed by evaporation under reduced pressure and the resulting precipitates were filtered off. The filtrate was, after concentration, subjected to Sephadex LH-20 chromatography. Elution with water containing increasing amounts of methanol and then with water–acetone (1 : 1) afforded three fractions. Fraction 1 consisted of simple phenolic glycosides and ellagitannins, while fraction 3 contained a mixture of gallotannins. Fraction 2 was rechromatographed over Sephadex LH-20, followed by Fuji-gel ODS-G3 chromatography, to give mongolicains A (**3**) (366 mg) and B (**4**) (185 mg).

b) From the Acorn of *Q. mongolica* var. *grosseserata*: The fresh acorns (12.3 kg) were crushed with a hammer and extracted with 80% aqueous acetone at room temperature. After concentration of the extract under reduced pressure, the resulting precipitates were removed by filtration. The aqueous solution was applied to a column of Sephadex LH-20, and elution with water containing increasing amounts of methanol yielded five fractions. Among them, the fourth fraction was rechromatographed over Sephadex LH-20 with 60% aqueous methanol to give a mixture of acutissimins and mongolicains. Repeated chromatography of the mixture over MCI-gel CHP 20P, Fuji-gel ODS-G3 and Bondapak C_{18}/Porasil B with water containing increasing proportions of methanol yielded mongolicains A (**3**) (*ca.* 1.0 g) and B (**4**) (308 mg).

Mongolicain A (3)——A pale brown amorphous powder, $[\alpha]_D^{18}$ −148.4° (c=0.86, MeOH). *Anal.* Calcd for $C_{55}H_{36}O_{30}\cdot 3H_2O$: C, 53.66; H, 3.44. Found: C, 53.72; H, 3.30. FAB-MS m/z: 1177 $(M+H)^+$. Negative FAB-MS m/z: 1175 $(M-H)^-$. CD ($c=2.3\times10^{-5}$, MeOH) $[\theta]^{23}$ (nm): −8100 (277), −32300 (260), −140000 (235).

Acid-Catalyzed Degradation of 3——**3** (10 mg) was heated under reflux for 5 d in ethanol (2.4 ml) containing acetic acid (0.6 ml). The solution was concentrated under reduced pressure to give a brown powder, which was applied to a small column of Sephadex LH-20. Elution with 80% aqueous methanol yielded a compound, which was positive to the anisaldehyde–sulfuric acid reagent. The identity of this compound with **5** was confirmed by TLC [Rf, 0.4; benzene–ethyl formate–formic acid (5:4:1)] and HPLC [t_R, 7.5 min; solvent, 19% CH_3CN/H_2O–25 mM $(COOH)_2$; flow rate, 0.75 ml/min].

Oxidative Degradation of 3 with Ferric Chloride——**3** (15 mg) was heated under reflux for 4 d with 10% aqueous ferric chloride (2 ml). The reaction mixture was neutralized with Amberlite MB-3, and the solution was passed through a column of Sep-pak. Elution with water yielded a sugar fraction, which was treated with bis(trimethylsilyl)acetamide (15 μl) in dry pyridine (2 drops). After standing at room temperature for 10 min, the products were analyzed by GLC, which showed peaks at t_R 7.0 and 11.6 min (flow rate, 40 ml/min; column temp., 150 °C) and t_R 5.3 min (flow rate, 40 ml/min; column temp., 130 °C). The former two peaks corresponded to the trimethylsilyl derivatives of glucose, and the latter to that of arabinose.

Enzymatic Hydrolysis of 3 with Tannase——**3** (83 mg) was shaken for 1 h with tannase in water at room temperature. The reaction mixture was concentrated under reduced pressure to dryness. The residue was treated with methanol, and the soluble portion was chromatographed over Sephadex LH-20. Elution with 80% aqueous methanol furnished ellagic acid (**6**) and the hydrolysate (**7**) (20 mg) as a pale brown amorphous powder, $[\alpha]_D^{27}$ −94.7° (c=0.39, MeOH). *Anal.* Calcd for $C_{41}H_{30}O_{22}\cdot 5H_2O$: C, 51.04; H, 4.17. Found: C, 51.01; H, 3.99. Negative FAB-MS m/z: 873 $(M-H)^-$. ^{1}H-NMR (100 MHz, acetone-d_6 + D_2O): 2.5—3.0 (2H, m, cat. H-4), 3.84 (1H, s, glc. H-1), 4.28 (1H, s, CH), 4.86 (1H, br s, glc. H-2), 4.90 (2H, m, glc. H-3 and cat. H-2), 5.10 (1H, m, glc. H-5), 6.12 (1H, s, cat. H-6), 6.60—6.92 (4H, m, arom. H). ^{13}C-NMR (25.05 MHz, acetone-d_6 + D_2O): 27.3 (cat. C-4), 48.6 (glc. C-1), 50.5 (CH), 62.0 (glc. C-6), 67.0 (cat. C-3), 67.9, 74.1, 74.6, 76.0 (glc. C-2, -3, -4 and -5), 81.8 (cat. C-2), 89.4 (C–O–), 90.3 (cat. C-6), 102.4 (cat. C-4a), 103.7 (cat. C-8), 108.1 (arom. H), 114.9 (cat. C-5′), 116.0 (cat. C-2′), 119.2 (cat. C-6′), 131.3 (cat. C-1′), 152.1 (cat. C-8a), 158.2 (cat. C-5), 159.7 (cat. C-7), 164.7, 167.9, 168.8 (–COO–), 197.3 (C=O). CD (c=0.9×10^{-5}, MeOH) $[\theta]^{23}$ (nm): −199000 (235), 0 (251), +35700 (259), 0 (269), −25500 (285), 0 (310). UV λ_{max}^{MeOH} nm (log ε): 270 (5.70), 230 sh (5.61).

Methylation of 3——A mixture of **3** (410 mg), dimethyl sulfate (2 ml) and anhydrous potassium carbonate (3.0 g) in dry acetone (20 ml) was refluxed for 3.5 h with stirring. After removal of the inorganic compounds by filtration, the filtrate was concentrated to dryness. The residue was applied to a column of silica gel, and elution with benzene–acetone (5:1) gave the hexadecamethyl ether (**3a**) (363 mg) as a white amorphous powder, $[\alpha]_D^{25}$ −213.2° (c=0.46, acetone). *Anal.* Calcd for $C_{71}H_{68}O_{30}\cdot 1/2H_2O$: C, 60.46; H, 4.93. Found: C, 60.24; H, 5.06. FD-MS m/z: 1400 (M^+). ^{1}H-NMR (100 MHz, $CDCl_3$): 2.4—3.1 (2H, m, cat. H-4), 4.42 (1H, s, CH), 4.66 (1H, d, J=8 Hz, glc. H-3), 4.74 (1H, br d, J=12 Hz, glc. H-6), 5.08 (1H, m, glc. H-5), 5.38 (1H, d, J=8 Hz, cat. H-2), 5.44 (2H, m, glc. H-2 and -4), 6.20 (1H, s, cat. H-6), 6.44—6.80 (6H, m, arom. H). ^{13}C-NMR (25.05 MHz, $CDCl_3$): 27.0 (cat. C-4), 64.8 (glc. C-6), 67.3 (cat. C-3), 82.1 (cat. C-2), 86.8 (cat. C-6), 89.3 (C–O–), 102.6 (cat. C-4a), 104.3 (cat. C-8), 104.7, 105.9, 107.4 (arom. C), 109.5, 111.3 (cat. C-2′ and -5′), 120.0 (cat. C-6′), 159.5, 160.2, 161.8 (cat. A-ring C), 164.7, 165.9, 166.3, 167.7 (–COO–), 194.8 (C=O).

Acetylation of 3a——**3a** (30 mg) was acetylated overnight with acetic anhydride (0.5 ml) and pyridine (0.5 ml) at room temperature. Usual work-up afforded the monoacetate (**3b**) as a white amorphous powder, $[\alpha]_D^{20}$ −208.3° (c=0.5, acetone). *Anal.* Calcd for $C_{73}H_{70}O_{31}$: C, 60.74; H, 4.89. Found: C, 60.27; H, 4.96. FD-MS m/z: 1442 $(M)^+$. ^{1}H-NMR (100 MHz, $CDCl_3$): 1.72 (3H, s, $COCH_3$), 2.60 (1H, dd, J=16, 8 Hz, cat. H-4), 3.06 (1H, dd, J=16, 6 Hz, cat. H-4), 3.61, 3.62, 3.67, 3.68, 3.72, 3.73, 3.80, 3.81, 3.88, 3.92, 3.96, 3.97, 4.00, 4.12 (48H in total, each s, OCH_3), 4.44 (1H, s, CH), 4.80 (1H, br d, J=12 Hz, glc. H-6), 4.88 (1H, d, J=8 Hz, glc. H-3), 5.00—5.52 (5H, m, glc. H-2, -4, -5, -6 and cat. H-2), 6.20 (1H, s, cat. H-6), 6.44—6.80 (6H, m, arom. H).

Hydrogenation of 3 with Platinum Oxide——**3** (500 mg) was hydrogenated with platinum oxide (100 mg) in ethanol (10 ml) under a hydrogen atmosphere for 1 h. After removal of the catalyst by filtration, the filtrate was chromatographed on Sephadex LH-20. Elution with 80% aqueous methanol yielded, among others, two major products, which were separately purified by Bondapak Porasil B chromatography with 20% aqueous methanol to give **8** (350 mg) and **9** (100 mg) as pale brown amorphous powders. **8**: $[\alpha]_D^{20}$ −31.6° (c=0.6, MeOH). *Anal.* Calcd for

$C_{55}H_{40}O_{30}\cdot 5H_2O$: C, 51.97; H, 3.96. Found: C, 51.51; H, 3.92. Negative FAB-MS *m/z*: 1179 $(M-H)^-$. **9**: $[\alpha]_D^{19}$ $-42.1°$ ($c=0.6$, MeOH). *Anal.* Calcd for $C_{55}H_{40}O_{30}\cdot 4H_2O$: C, 52.72; H, 3.86. Found: C, 52.41; H, 3.66. Negative FAB-MS *m/z*: 1179 $(M-H)^-$

Methylation of 8——A mixture of **8** (100 mg), dimethyl sulfate (0.5 ml) and anhydrous potassium carbonate (0.9 g) in dry acetone (5 ml) was refluxed for 3 h with stirring. Work-up as described in the case of **3** yielded the hexadecamethyl ether (**8a**) as a white amorphous powder, $[\alpha]_D^{20}$ $-69.0°$ ($c=0.7$, $CHCl_3$). *Anal.* Calcd for $C_{71}H_{72}O_{30}$: C, 60.68; H, 5.16. Found: C, 60.32; H, 5.46. FD-MS *m/z*: 1404 $(M)^+$. The ^{1}H-NMR spectrum of **8a** was complicated by conformational isomerism.

Acetylation of 8a——**8a** (30 mg) was acetylated in the same way as described above to give the triacetate (**8b**) as a white amorphous powder, $[\alpha]_D^{20}$ $-20.0°$ ($c=0.4$, $CHCl_3$). *Anal.* Calcd for $C_{77}H_{78}O_{33}$: C, 60.39; H, 5.13. Found: C, 59.96; H, 5.13. FD-MS *m/z*: 1530 $(M)^+$. The assignment of the ^{1}H-NMR spectrum could not be made owing to the complicated signal patterns caused by rotational isomerism.

Alkaline Methanolysis of 3a——**3a** (90 mg) was treated with 2% sodium methoxide in methanol at room temperature for 2 d. The reaction mixture was neutralized with Amberlite IRA-120B (H^+ form), and applied to a silica gel column. Elution with benzene–acetone (8 : 1) gave a colorless syrup (20 mg), $[\alpha]_D^{23}$ $-29.0°$ ($c=1.3$, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 2840 (CH), 1720 (–COO–), which was found to be identical with (*S*)-dimethyl hexamethoxydiphenoate (**10**) by physical and spectral comparisons.

Mongolicain B (4)——A pale brown amorphous powder, $[\alpha]_D^{18}$ -64.7 ($c=0.9$, MeOH). *Anal.* Calcd for $C_{55}H_{36}O_{30}\cdot 5H_2O$: C, 52.13; H, 3.66. Found: C, 51.92; H, 3.55. FAB-MS *m/z*: 1177 $(M+H)^+$. Negative FAB-MS *m/z*: 1175 $(M-H)^-$. CD ($c=1.4\times10^{-5}$, MeOH) $[\theta]^{23}$ (nm): -39600 (270), -8800 (253), -35200 (235), 0 (225).

Acid-Catalyzed Degradation of 4——**4** (10 mg) was treated with acetic acid (0.6 ml) in ethanol (2.4 ml) in the same way as described for **3**. Analysis of the products by TLC and HPLC showed the same results.

Enzymatic Hydrolysis of 4 with Tannase——**4** (32 mg) was shaken with tannase in water at room temperature for 2 h. The reaction mixture was worked up as described before to give ellagic acid (**6**) (10 mg) and the hydrolysate as a pale brown amorphous powder (11 mg), $[\alpha]_D^{29}$ $+4.3°$ ($c=0.7$, MeOH). *Anal.* Calcd for $C_{41}H_{30}O_{22}\cdot 8.5H_2O$: C, 47.91; H, 4.60. Found: C, 47.45; H, 3.99. Negative FAB-MS *m/z*: 873 $(M-H)^-$. ^{1}H-NMR (100 MHz, acetone-d_6+D_2O): 2.56 (1H, dd, $J=8$, 16 Hz, cat. H-4), 2.90 (1H, dd, $J=6$, 16 Hz, cat. H-4), 4.32 (1H, s, CH), 4.66 (1H, d, $J=8$ Hz, cat. H-2), 4.94—5.24 (3H, m, glc. H-2, -3 and -5), 6.18 (1H, s, cat. H-8), 6.7—7.0 (4H, m, arom. H). CD ($c=1.2\times10^{-5}$, MeOH) $[\theta]^{23}$ (nm): -195000 (235), 0 (250), $+58400$ (259). UV λ_{max}^{MeOH} nm (log ε): 268 (5.05), 230 (4.89).

Methylation of 4——A mixture of **4** (50 mg), dimethyl sulfate (0.3 ml) and anhydrous potassium carbonate (0.5 g) in dry acetone (5 ml) was refluxed for 3 h. The reaction products were purified by silica gel chromatography as described above to furnish the hexadecamethyl ether (**4a**) (25 mg) as a white amorphous powder, $[\alpha]_D^{20}$ $-114.0°$ ($c=0.8$, acetone). *Anal.* Calcd for $C_{71}H_{68}O_{30}\cdot 1/2H_2O$: C, 60.46; H, 4.93. Found: C, 60.43; H, 4.99. FD-MS *m/z*: 1400 $(M)^+$. ^{1}H-NMR (100 MHz, $CHCl_3$): 2.5—3.4 (2H, m, cat. H-4), 4.48 (1H, s, CH), 4.70 (1H, d, $J=8$ Hz, cat. H-2), 4.92 (1H, br d, $J=12$ Hz, H-6), 5.28 (1H, m, glc. H-3), 5.4—5.6 (3H, m, glc. H-2, -4 and -5), 6.16 (1H, s, cat. H-8), 6.64—7.12 (6H, m, arom. H). ^{13}C-NMR (25.05 MHz, $CDCl_3$): 27.6 (cat. C-4), 46.1 (glc. C-1), 48.7 (CH), 65.1 (glc. C-6), 67.6 (cat. C-3), 68.0, 70.6, 71.9, 78.2 (glc. C-2, -3, -4 and -5), 82.4 (cat. C-2), 89.1 (–C–O–), 93.6 (cat. C-8), 97.6 (cat. C-4a), 104.8 (cat. C-6), 105.2, 105.6, 105.9 (arom. H), 110.3, 111.3 (cat. C-2′ and -5′), 120.2 (cat. C-6′), 129.7 (cat. C-1′), 162.0, 165.6, 165.9, 166.3, 167.6 (–COO–), 194.8 (C=O).

Alkaline Methanolysis of 4a——**4a** (20 mg) was methanolyzed with 2% sodium methoxide in methanol at room temperature for 2 d. Similar work-up yielded (*S*)-dimethyl hexamethoxydiphenoate (**10**) as a sole isolable product.

Hydrogenation of 4 with Platinum Oxide——**4** (100 mg) was shaken with platinum oxide (80 mg) in ethanol for 10 h under a hydrogen atmosphere. The catalyst was removed by filtration, and the products were separated on Bondapak C_{18}/Porasil B with 30% aqueous methanol and then Sephadex LH-20 with 80% aqueous methanol to furnish **13** (50 mg) as a brown amorphous powder, $[\alpha]_D^{18}$ $-34.6°$ ($c=0.8$, MeOH). *Anal.* Calcd for $C_{55}H_{40}O_{30}\cdot 4H_2O$: C, 52.72; H, 3.86. Found: C, 52.91; H, 3.78. Negative FAB-MS *m/z*: 1179 $(M-H)^-$

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References and Notes

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