# Tannins and Related Compounds. XCV.[1] Isolation and Characterization of Helioscopinins and Helioscopins, Four New Hydrolyzable Tannins from *Euphorbia helioscopia* L. (1)

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**A chemical examination of polyphenols in *Euphorbia helioscopia* L. (Euphorbiaceae) has led to the isolation of four new hydrolyzable tannins named helioscopinins A (10) and B (9), and helioscopins A (11) and B (12), together with eight known tannins (1—8). On the basis of chemical and spectroscopic evidence, the structures of compounds 9—12 were established as 1,6-(*S*)-hexahydroxydiphenoyl-3-*O*-galloyl-β-D-glucose, 1,6-(*S*)-hexahydroxydiphenoyl-2,4-(*S*)-dehydrohexahydroxydiphenoyl-3-*O*-galloyl-β-D-glucose, 1,6-(*S*)-hexahydroxydiphenoyl-2,4-(*R*)-elaeocarpusinoyl-3-*O*-galloyl-β-D-glucose and 1,3,6-tri-*O*-galloyl-2,4-(*R*)-elaeocarpusinoyl-β-D-glucose, respectively.**

**Keywords** *Euphorbia helioscopia*; Euphorbiaceae; hydrolyzable tannin; helioscopinin A; helioscopinin B; helioscopin A; helioscopin B; hexahydroxydiphenic acid; dehydrohexahydroxydiphenic acid; elaeocarpusinic acid

Previous work has shown that hydrolyzable tannins in the plants of the family Euphorbiaceae have a great diversity of novel phenolcarboxylic acids, such as elaeocarpusinic acid,[2] putranjivaic acid,[3] 4-dehydrochebulic acid,[4] repandusinic acid,[4] mallotic acid,[4] *etc.*, probably all derived by oxidative metabolism of 3,3′,4,4′,5,5′-hexahydroxydiphenic acid. Thus, examination of tannins in the plants of this family is of particular significance in extending our knowledge of the metabolism of hydrolyzable tannins, especially of the component phenolcarboxylic acids. This paper presents the results of an examination of *Euphorbia helioscopia* L., including the isolation and characterization of four new hydrolyzable tannins named helioscopinins A (**10**) and B (**9**) and helioscopins A (**11**) and B (**12**) having a variety of phenolcarboxylic acid ester groups, together with eight known compounds (**1**—**8**).

Fresh whole plants of *E. helioscopia* were extracted with aqueous acetone. After concentration, the extract was subjected to a combination of chromatographies over Sephadex LH-20, MCI-gel CHP 20P, Fuji-gel ODS G-3 and Bondapak $C_{18}$/Porasil B to afford compounds **1**—**12**. Among them, compounds **1**—**8** were identified as corilagin (**1**),[2] punicafolin (**2**),[2] geraniin (**3**),[5] elaeocarpusin (**4**),[2] furosin (**5**),[5] terchebin (**6**),[5] mallotusinin (**7**)[5] and carpinusin (**8**)[6] by comparisons of their physical and spectral data with those of authentic samples.

Helioscopinin B (**9**) showed the $(M-H)^-$ ion peak at *m/z* 633 in the negative ion fast atom bombardment mass spectrum (FAB-MS). The proton nuclear magnetic resonance ($^1$H-NMR) spectrum of **9** suggested the presence of one galloyl ester group [δ 7.15 (2H, s)] and one 3,3′,4,4′,5,5′-hexahydroxydiphenoyl (HHDP) ester group [δ 6.76, 6.81 (each 1H, s)]. Hydrolysis of **9** with 5% sulfuric acid gave gallic acid, ellagic acid and D-glucose, while hydrolysis in hot water yielded ellagic acid and a hydrolysate (**9a**), which was characterized as 3-*O*-galloyl-D-glucose by $^1$H–$^1$H shift correlation spectroscopy (COSY).

In the $^1$H-NMR spectrum of **9**, three lowfield signals [(δ 4.64 (1H, t, $J$=10 Hz), 5.25 (1H, t, $J$=6 Hz) and 5.87 (1H, d, $J$=3 Hz)] were observed, and these could be assigned to glucose H-6, H-3 and H-1, respectively, by $^1$H–$^1$H COSY spectroscopy, thus indicating that the HHDP ester groups are located at the C-1 and C-6 positions of the glucopyranose moiety. Methylation of **9** with dimethyl sulfate and anhydrous potassium carbonate in dry acetone afforded the nonamethyl ether (**9b**). Subsequent alkaline hydrolysis of **9b**, followed by methylation with diazomethane, yielded methyl 3,4,5-trimethoxybenzoate (**9c**) and dimethyl 4,4′,5,5′,6,6′-hexamethoxydiphenoate (**9d**) whose sign of the specific optical rotation [−28.7° (acetone)] confirmed the *S*-configuration of the biphenyl bond.[7] Examination of the

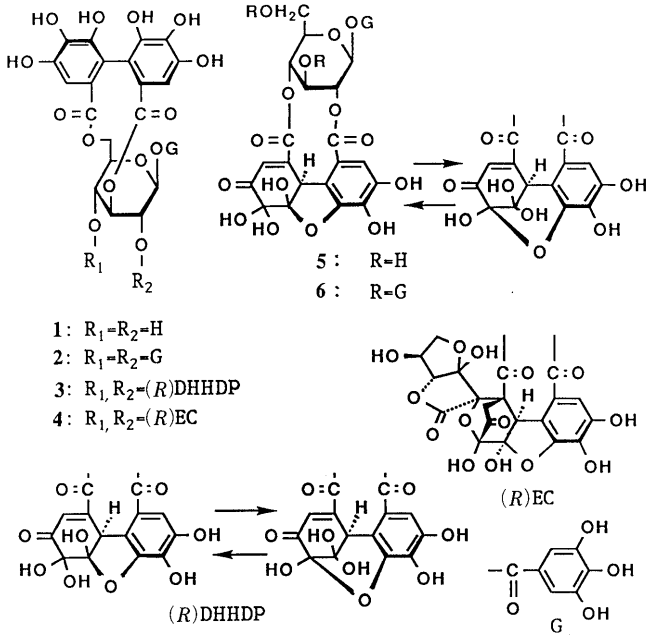


Chart 1

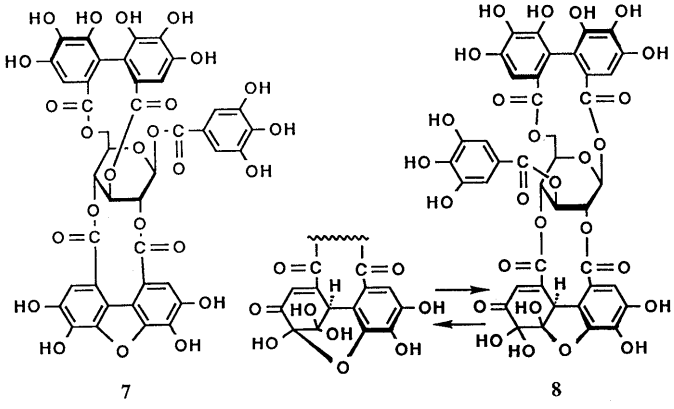


Chart 2

Dreiding model showed that bridging of the HHDP group over the C-1 and C-6 positions of the glucopyranose moiety is possible only in the case of the $\beta$-configuration at the anomeric center. Thus helioscopinin B was characterized as 1,6-(*S*)-HHDP-3-*O*-galloyl-$\beta$-D-glucose (**9**).

Helioscopinin A (**10**) showed the $(M-H)^-$ ion peak at *m/z* 951 in the negative FAB-MS. The presence of a galloyl ester group [$\delta$ 7.16 (2H, s)] and an HHDP ester group [$\delta$ 6.56, 6.70, 6.89 and 6.91 (2H in total, each s)] was indicated by $^1$H-NMR spectroscopy. The appearance of duplicated benzyl methine signals [($\delta$ 4.99 (d, $J$=2 Hz) and 5.24 (s), 1H in total] and olefinic proton signals [$\delta$ 6.27 (d, $J$=2 Hz) and 6.59 (s), 1H in total] was characteristic of the dehydrohexahydroxydiphenoyl (DHHDP) ester group existing as an equilibrium mixture of five- and six-membered hemiketal forms.[8] The presence of the DHHDP ester group was also supported by carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectral analysis, which showed pairs of signals due to the hydrated cyclohexenetrione moiety [$\delta$ 45.9, 51.7 (C-1), 148.9, 154.2 (C-2), 125.7, 129.1 (C-3), 192.2, 194.9 (C-4), 92.6, 96.2 (C-5), 92.4, 108.8 (C-6)].

Upon treatment with *o*-phenylenediamine in an acidic solution, **10** yielded a phenazine derivative (**10a**) [positive FAB-MS *m/z*: 1007 $(M+H)^+$]. Subsequent methylation of **10a** with ethereal diazomethane, followed by alkaline methanolysis, yielded methyl trimethoxybenzoate (**9c**), (*S*)-dimethyl hexamethoxydiphenoate (**9d**) and a phenazine dimethyl ester (**10c**). The negative sign of the optical rotation [$-34.5°$ (acetone)] of **10c** established the chirality of the DHHDP group to be in the *S*-series.[9]

The locations of acyl groups in the sugar moiety were determined as follows. Partial hydrolysis of the phenazine (**10a**) in hot water gave above-mentioned **9**, together with a phenazine bislactone (**10d**), thus showing that the DHHDP ester groups are located at the glucose C-2 and C-4 hydroxyl groups and the galloyl and HHDP groups at the C-3 position, and C-1 and C-6 positions, respectively.

The orientation of the DHHDP group was determined to be as shown in Chart 4 since $^1$H–$^{13}$C long-range COSY spectroscopy of the phenazine (**10a**) clearly showed the correlation between a one-proton aromatic singlet ($\delta$ 8.02), readily assignable to the phenazine C-3 proton, and a carboxyl carbon signal ($\delta$ 167.2) through a three-bond coupling, and this carboxyl carbon signal was also correlated with the glucose C-2 proton signal ($\delta$ 5.26) (Fig. 1).

Furthermore, treatement of **10** with triethylamine in acetonitrile[10] gave a product (**10e**) and brevifolin carboxylic acid (**10f**).[11] The $^1$H-NMR spectrum of **10e** showed signals due to a galloyl group [$\delta$ 7.18 (2H, s)] and an HHDP group [$\delta$ 6.86, 6.94 (each 1H, s)]. In addition, the observation of a one-proton aromatic singlet [$\delta$ 7.43 (1H, s)] and ABX-type aliphatic signals [$\delta$ 2.44 (1H, dd, $J$=3, 19 Hz), 3.12 (1H, dd, $J$=7, 19 Hz), 4.55 (1H, dd, $J$=3, 7 Hz)] suggested the existence of a brevifolin carboxyl group[11] in the molecule.

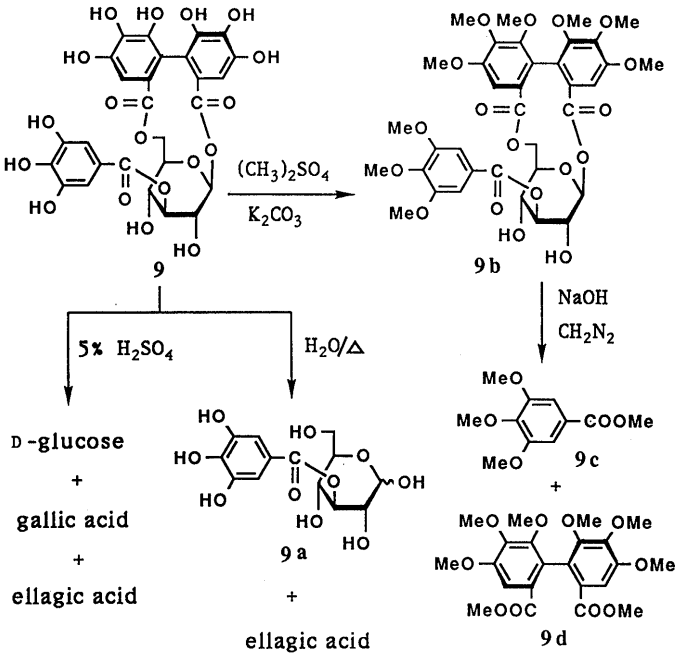


Chart 3

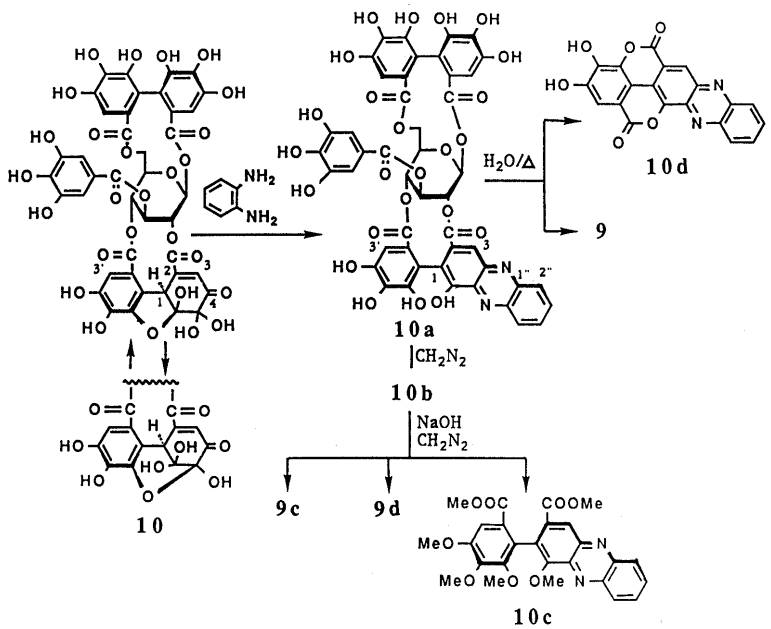


Chart 4

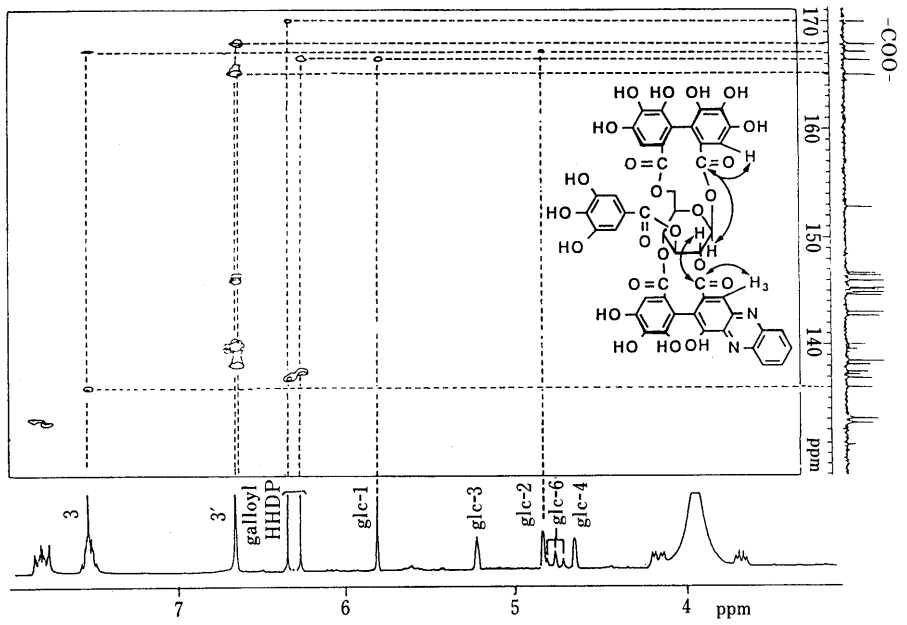


Fig. 1. The $^{1}H$–$^{13}C$ Long-Range COSY Spectrum of **10a** [in Acetone-$d_6$ + $D_2O$ ($J_{CH}$ = 10 Hz)]

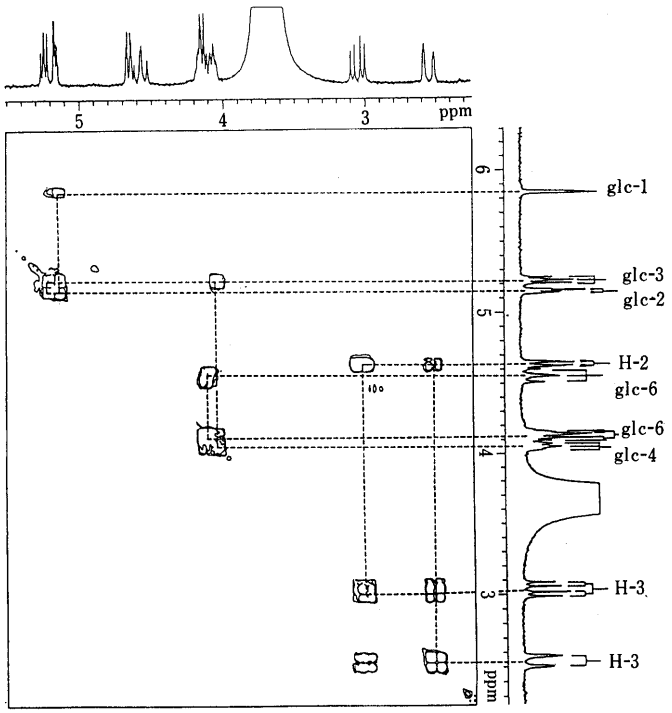


Fig. 2. The $^{1}H$–$^{1}H$ COSY Spectrum of **10e** (in Acetone-$d_6$ + $D_2O$)

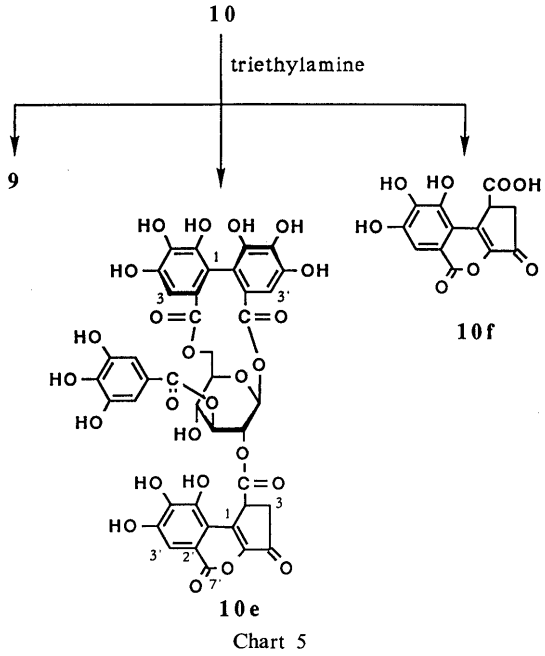


Chart 5

The $^{13}C$-NMR resonances [$\delta$ 193.6 (C-4), 161.7 (C-7′), 149.8 (C-5), 147.8 (C-1), 143.7 (C-2′), 140.4 (C-4′), 139.2 (C-3′), 116.0 (C-6′), 115.0 (C-1′), 41.5 (C-3), 37.7 (C-2)] were also consistent with the presence of the brevifolin carboxylic acid moiety. In the $^{1}H$-NMR spectrum of **10e**, a signal due to the glucose C-4 proton appeared at higher field ($\delta$ 4.12) than that of the parent compound (**10**) (Fig. 2). These observations indicated that the location of the brevifolin carboxyl ester group is at the C-2 hydroxyl group.

On the basis of these results, helioscopinin A was characterized as 1,6-(*S*)-HHDP-2,4-(*S*)-DHHDP-3-*O*-galloyl-$\beta$-D-glucose (**10**).

Helioscopin A (**11**) and helioscopin B (**12**) were unstable in aqueous solution, and gradually formed yellow-colored compounds whose *Rf* values on thin-layer chromatography (TLC) corresponded with those of compounds **8** and **6**, respectively. In the $^{1}H$-NMR spectrum of **11**, one of the characteristic features was the observation of a long-range coupling between one [$\delta$ 3.10 (1H, dd, $J$ = 2, 19 Hz)] of the methylene signals and a benzylic methine signal [$\delta$ 5.67 (1H, d, $J$ = 2 Hz)]. These observations were consistent with the presence of an elaeocarpusinoyl group.[2] Furthermore, the $^{13}C$-NMR spectrum of **11** exhibited signals [$\delta$ 38.0 (C-3), 50.0 (C-2), 52.0 (C-1), 74.4 (C-5″), 76.5 (C-6″), 80.9 (C-2″), 89.5 (C-4″), 96.3 (C-5), 109.0 (C-3″), 170.3 (C-1″), 198.6 (C-4)] whose chemical shifts were in good agreement with those of the elaeocarpusinoyl group. Further chemical evidence was obtained by alkaline methanolysis of the methyl ether (**11a**), which yielded (*R*)-dimethyl penta-

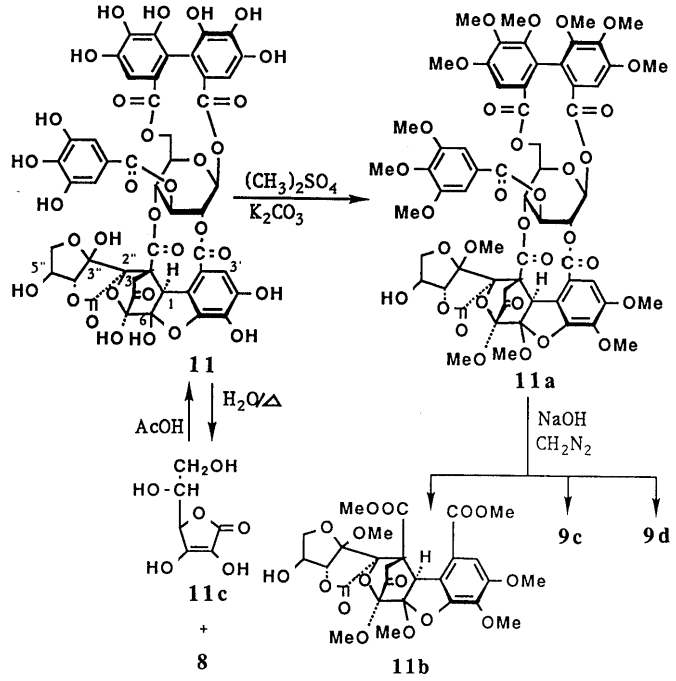


Chart 6

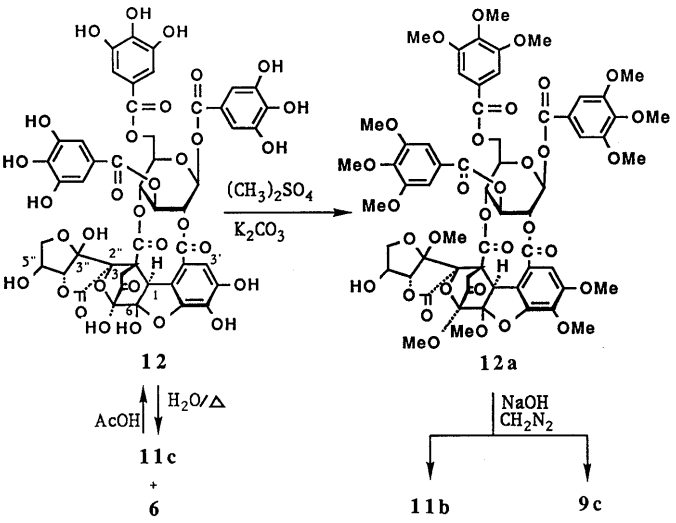


Chart 7

methoxyelaeocarpusinoate (**11b**), $[\alpha]_D$ +51.4° (chloroform)], together with **9c** and **9d**. On the other hand, heating of **11** in water liberated ascorbic acid (**11**) and a yellow compound, whose spectral data were found to be identical with those of carpinusin (**8**). Furthermore, **11** could readily be prepared by condensation of L-ascorbic acid (**11c**) and carpinusin (**8**) in 0.2 M acetic acid. On the basis of these results, helioscopin A was characterized as 1,6-(*S*)-HHDP-2,4-(*R*)-elaeocarpusinoyl-3-*O*-galloyl-*β*-D-glucose (**11**).

The $^1$H-NMR spectrum of **12** showed three two-proton singlets (*δ* 7.10, 7.23 and 7.25) due to galloyl groups and a one-proton singlet at *δ* 7.32 in the aromatic field. A long-range coupling of one [*δ* 3.14 (1H, dd, *J*=2, 19 Hz)] of the methylene proton signals and a benzylic methine signal [*δ* 5.72 (1H, d, *J*=2 Hz)] was analogous to that of **11**. The presence of the elaeocarpusinoyl group was further confirmed by methylation of **12**, followed by methanolysis, giving (*R*)-dimethyl pentamethoxyelaeocarpusinoate (**11b**). Compound **12** gave terchebin (**6**) and L-ascorbic acid (**11c**) on treatment in hot water, whereas condensation of terchebin (**6**) and L-ascorbic acid (**11c**) in 0.2 M acetic acid yielded **12**. On the basis of these findings, **12** was characterized as 1,3,6-tri-*O*-galloyl-2,4-(*R*)-elaeocarpusinoyl-*β*-D-glucose.

The occurrence of ellagitannins bearing the HHDP group at the 1,6-positions of the glucopyranose moiety is one of the characteristic feature of the tannins of this plant, since they have not been found in nature, except for a few cases.[8] Furthermore, helioscopinin A (**10**) is the first example of a tannin having the (*S*)-DHHDP group to be isolated from Euphorbiaceous plants. The coexistence of tannins [helioscopinin A (**10**) and carpinusin (**8**)] having (*R*)- and (*S*)-DHHDP groups is interesting from the viewpoint of the metabolism of ellagitannins, since it indicates that the oxidation of the HHDP group to the DHHDP group occurs highly regio- and stereo-specifically.

## 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. FAB-MS were taken with a JEOL JMS DX-300 instrument. $^1$H- and $^{13}$C-NMR spectra were recorded on JEOL FX-100 and JEOL GX-270 spectrometers, with tetramethylsilane as an internal standard, and chemical shifts are given in *δ* (ppm). Column chromatography was carried out with Sephadex LH-20 (25—100 *μ*, Pharmacia Fine Chemical Co., Ltd.) MCI-gel CHP 20P (75—150 *μ*, Mitsubishi Chemical Industry Co. Ltd.), Fuji-gel ODS G-3 (43—65 *μ*. Fuji-gel Hanbai Co., Ltd.) and Bondapak $C_{18}$/Porasil B (37—75 *μ*, Waters Associates Inc.). TLC was conducted on precoated Silica gel 60 $F_{254}$ plates (Merck) and precoated cellulose $F_{254}$ plates. Spots were visualized under ultraviolet (UV) and by spraying $FeCl_3$ (for phenolics) and dilute sulfuric acid followed by heating (for phenolics and sugars).

**Isolation of Tannins** The fresh whole plants (48.5 kg) of *Euphorbia helioscopia*, collected near Fukuoka City, Japan, were chopped into small pieces and extracted five times with 80% aqueous acetone at room temperature. The acetone was removed by evaporation under reduced pressure and the insolubles were removed by filtration. The filtrate was concentrated, and applied to a column of Sephadex LH-20. Elution with $H_2O$ containing increasing proportions of MeOH afforded five fractions; I (80 g), II (335 g), III (65 g), IV (20 g) and V (3 g). Fraction I was rechromatographed over MCI-gel CHP 20P with $H_2O$–MeOH (1 : 0—1 : 1, v/v), Sephadex LH-20 (EtOH) and Bondapak $C_{18}$/Porasil B with $H_2O$–MeOH (1 : 0—1 : 1, v/v) to give helioscopinin A (**10**) (18.5 g), corilagin (**1**) (660 mg) and helioscopinin B (**9**) (700 mg). Fraction II was repeatedly chromatographed over MCI-gel CHP 20P with $H_2O$–MeOH (1 : 0—2 : 3, v/v), Fuji-gel with $H_2O$–MeOH (1 : 0—1 : 1, v/v), Bondapak $C_{18}$/Porasil B with $H_2O$–MeOH (1 : 0—3 : 2, v/v) and Sephadex LH-20 with EtOH to give helioscopin A (**11**) (340 mg), carpinusin (**8**) (18 g), furosin (**5**) (520 mg) and elaeocarpusin (**4**) (640 mg). On similar chromatographies, fraction III gave geraniin (**3**) (69 g) and helioscopin B (**12**) (270 mg), while fraction IV yielded punicafolin (**2**) (120 mg) and terchebin (**6**) (3.6 g). Mallotusinin (**7**) (52 mg) was obtained from fraction V by similar chromatographic separation. Compounds **1**—**8** were identified by comparisons of their physical and spectral data with those of authentic samples.

**Helioscopinin B (9)** A white powder ($H_2O$), mp 219—220 °C, $[\alpha]_D$ +74.0° (*c*=0.3, acetone–$H_2O$). *Anal.* Calcd for $C_{27}H_{22}O_{18}\cdot 5H_2O$: C, 44.75; H, 4.54. Found: C, 44.78; H, 4.42. Negative FAB-MS *m/z*: 633 $(M-H)^-$. $^1$H-NMR (acetone-$d_6$+$D_2O$) ppm: 3.99 (1H, dd, *J*=3, 6 Hz, glc-2), 4.64 (1H, t, *J*=10 Hz, glc-6), 5.25 (1H, t, *J*=6 Hz, glc-3), 5.87 (1H, d, *J*=3 Hz, glc-1), 6.76, 6.81 (each 1H, s, HHDP-H), 7.15 (2H, s, galloyl-H). $^{13}$C-NMR (acetone-$d_6$+$D_2O$) ppm: 64.9 (glc-6), 67.8 (glc-4), 69.4 (glc-2), 73.3 (glc-3), 75.8 (glc-5), 95.8 (glc-1), 109.0, 109.5 (HHDP-3,3′), 110.4 (galloyl-2,6), 116.1, 116.8 (HHDP-1, 1′), 120.6 (galloyl-1), 124.7, 125.5 (HHDP-2, 2′), 136.7, 137.0 (HHDP-5, 5′), 139.3 (galloyl-4), 144.7, 145.0 (HHDP-4, 4′, 6, 6′), 145.8 (galloyl-3, 5), 166.9, 167.2, 169.4 (COO).

**Acid Hydrolysis of Helioscopinin B (9)** A solution of **9** (50 mg) in 5% $H_2SO_4$ (2 ml) was heated at 90 °C for 5 h. The insoluble material (9 mg) was collected by filtration and identified as ellagic acid by TLC and infrared (IR) comparisons with an authentic sample. The filtrate was neutraliz-

ed with $BaCO_3$ and was subjected to Sephadex LH-20 column chromatography with $H_2O$ to give D-glucose (7 mg) [*Rf* 0.39; cellulose, *n*-BuOH–pyridine–$H_2O$ (6 : 4 : 3)], Osazone: mp 209—210 °C, $[\alpha]_D$ −0.16° (*c* = 0.1, MeOH)]. Successive elution with EtOH yielded gallic acid (4 mg), which was identified by TLC and IR comparisons with an authentic sample.

**Partial Hydrolysis of Helioscopinin B (9)** A solution of **9** (30 mg) in $H_2O$–MeOH (1 : 1) (2 ml) was heated on a water bath (90 °C) for 4 h. After concentration, the reaction mixture was subjected to Sephadex LH-20 chromatography. Elution with 20% MeOH affforded 3-*O*-galloyl-D-glucose (**9a**) (4 mg) as a pale brown amorphous powder, $[\alpha]_D$ +37.0° (*c* = 0.4, acetone). Negative FAB-MS *m/z*: 331 $(M-H)^-$. $^1$H-NMR (acetone-$d_6$ + $D_2O$) ppm: 3.43 (t, *J* = 9 Hz, β-2-H), 4.69 (d, *J* = 9 Hz, β-1-H), 5.14 (t, *J* = 9 Hz, β-3-H), 5.22 (d, *J* = 4 Hz, α-1-H), 5.38 (t, *J* = 9 Hz, α-3-H), 7.16 (s, galloyl-H). Subsequent elution with EtOH gave ellagic acid (5 mg).

**Methylation of Helioscopin in Helioscopinin B (9)** A mixture of **9** (100 mg), dimethyl sulfate (1 ml) and anhydrous potassium carbonate (1.5 g) in dry acetone (20 ml) was heated under reflux (80 °C) for 3 h. After removal of inorganic salts by filtration, the filtrate was concentrated to a syrup, which was subjected to silica gel. Elution with benzene–acetone (9 : 1, v/v) gave the nonamethyl ether (**9b**) (85 mg) as an off-white amorphous powder, $[\alpha]_D$ +70.0° (*c* = 0.5, $CHCl_3$). *Anal.* Calcd for $C_{36}H_{40}O_{18}\cdot H_2O$: C, 54.27; H, 5.52. Found: C, 54.37; H, 5.74. Positive FAB-MS *m/z*: 761 $(M+H)^+$. $^1$H-NMR ($CDCl_3$) ppm: 3.67—3.95 (27H in total, $OCH_3$), 4.84 (1H, dd, *J* = 5, 9 Hz, glc-6), 5.42 (1H, br s, glc-3), 6.06 (1H, d, *J* = 4 Hz, glc-1), 6.28, 6.82 [(each 1H, s, hexamethoxydiphenoyl (HMDP)-H)], 7.19 (2H, s, trimethoxybenzoyl-H).

**Alkaline Hydrolysis of 9b, Followed by Diazomethane Methylation** A solution of **9b** (30 mg) in 5% aqueous sodium hydroxide (1 ml) and methanol (1 ml) was heated at 80 °C for 1 h. The mixture was acidified with 2 N HCl and extracted with ether. After washing with water, the ether layer was dried ($Na_2SO_4$) and concentrated to dryness. The residue was treated with ethereal diazomethane for 1 h, and the solution was concentrated to a syrup, which was subjected to silica gel chromatography. Elution with benzene–acetone (20 : 3, v/v) gave methyl 3,4,5-trimethoxybenzoate (**9c**) (5 mg) as a white powder (MeOH), mp 81 °C, and dimethyl 4,4′,5,5′,6,6′-hexamethoxydiphenoate (**9d**) (6 mg) as a yellow amorphous powder, $[\alpha]_D$ −28.7° (*c* = 0.8, acetone).

**Helioscopinin A (10)** A yellow crystalline powder ($H_2O$), mp 245—247 °C, $[\alpha]_D$ +101.4° (*c* = 0.3, acetone). *Anal.* Calcd for $C_{41}H_{28}O_{27}\cdot 6H_2O$: C, 46.55; H, 3.77. Found: C, 46.41; H, 3.77. Negative FAB-MS *m/z*: 951 $(M-H)^-$. $^1$H-NMR (acetone-$d_6$ + $D_2O$) ppm: 4.20 (1H, m, glc-6), 4.70 (1H, m, glc-5), 5.08—5.30 (3H in total, m, glc-2, 4, 6), 4.99 (d, *J* = 2 Hz, DHHDP-1), 5.24 (each s, DHHDP-1), 5.80, 5.92 (1H in total, each br s, glc-3), 6.27 (d, *J* = 2 Hz, DHHDP-3), 6.59 (br s, DHHDP-3), 6.56, 6.70, 6.89, 6.91 (2H in total, HHDP-H), 7.16 (2H, s, galloyl-H), 7.26 (1H, s, DHHDP-3′).

**Preparation of Phenazine (10a)** A mixture of **10** (300 mg), *o*-phenylenediamine (56 mg) and acetic acid (4 ml) in EtOH (16 ml) was left standing at room temperature for 10 h. The reaction mixture was diluted with water (5 ml) and evaporation of EtOH gave a precipitate (**10a**) (240 mg). **10a**: A yellow powder ($H_2O$), mp >300 °C, $[\alpha]_D$ +15.0° (*c* = 1.2, acetone–$H_2O$). *Anal.* Calcd for $C_{47}H_{30}O_{24}N_2\cdot 5H_2O$: C, 51.45; H, 3.65; N, 2.55. Found: C, 51.44; H, 3.79; N, 2.50. Positive FAB-MS *m/z*: 1007 $(M+H)^+$. $^1$H-NMR (acetone-$d_6$ + $D_2O$) ppm: 4.20 (1H, dd, *J* = 5, 11 Hz, glc-6), 4.45 (1H, d, *J* = 5 Hz, glc-5), 5.08 (1H, br s, glc-4), 5.15 (1H, t, *J* = 11 Hz, glc-6), 5.26 (1H, br s, glc-2), 5.76 (1H, br s, glc-3), 6.25 (1H, br s, glc-1), 6.72, 6.79 (each 1H, s, HHDP-H), 7.10 (1H, s, phenazine-3′), 7.11 (2H, s, galloyl-H), 8.02 (1H, s, phenazine-3), 8.03 (2H, m, phenazine-3″, 4″), 8.30 (2H, m, phenazine-2″, 5″). $^{13}$C-NMR (acetone-$d_6$ + $D_2O$) ppm: 61.6 (glc-3), 64.3 (glc-6), 67.8 (glc-2), 71.2 (glc-4), 73.3 (glc-5), 90.1 (glc-1), 108.1 (phenazine-3′), 108.5 (HHDP-3), 110.9 (HHDP-3′), 111.1 (galloyl-2,6), 114.6 (phenazine-1′), 116.1 (phenazine-1), 116.9 (HHDP-1), 117.5 (phenazine-3), 120.5 (galloyl-1), 123.6 (phenazine-2′), 124.9, 125.0 (HHDP-2, 2′), 129.6, 130.6 (phenazine-3″, 4″), 132.5, 132.9 (phenazine-2″, 5″), 136.7, 136.8 (phenazine-2, 5), 137.3 (HHDP-5), 137.9 (HHDP-5′), 138.4 (phenazine-5′), 139.8 (galloyl-4), 142.4, 142.8 (phenazine-1″, 6″), 144.9 (phenazine-4″), 145.0 (phenazine-6′), 145.1 (phenazine-4′), 144.7, 145.0, 145.1 (HHDP-4,4′,6,6′), 145.8 (galloyl-3,5), 152.7 (phenazine-6), 165.1, 166.5, 167.2, 167.9, 170.1 (COO).

**Partial Hydrolysis of Phenazine (10a)** A solution of **10a** (60 mg) in $H_2O$–MeOH (1 : 1) (4 ml) was heated on a water bath for 7 h. The resulting red precipitate (**10d**) (17 mg) was collected by filtration, and identified as phenazine bislactone (**10d**) by TLC and IR comparisons with an authentic sample. The filtrate was directly subjected to Sephadex LH-20 chromatography, and elution with 30% MeOH afforded **9** (32 mg).

**Methylation of Phenazine (10a)** A solution of **10a** (100 mg) in MeOH (2 ml) was methylated with ethereal diazomethane for 9 h at 0 °C. After removal of the solvent by evaporation, the residue was subjected to silica gel chromatography. Elution with benzene–acetone (8 : 1, v/v) gave the tridecamethyl ether (**10b**) (40 mg) as a yellow amorphous powder, $[\alpha]_D$ +0° (*c* = 0.4, acetone). Positive FAB-MS *m/z*: 1189 $(M+H)^+$. $^1$H-NMR ($CDCl_3$) ppm: 3.64—3.90 (39H in total, $OCH_3$), 5.56 (1H, br s, glc-1), 6.53, 6.85 (each 1H, s, HMDP-H), 7.23 (2H, s, trimethoxybenzoyl-H), 7.36 (1H, s, phenazine-3′), 7.90, 8.24 (each 2H, m, aromatic H), 8.75 (1H, s, phenazine-3).

**Methanolysis of 10b** **10b** (30 mg) was treated with 3% methanolic sodium methoxide (5 ml) in MeOH at room temperature for 20 h. The solution was acidified with 2 N HCl, concentrated and extracted with ether. The organic layer after concentration, was treated with ethereal diazomethane. After removal of the solvent by evaporation, the residue was chromatographed over silica gel. Elution with benzene–acetone (20 : 1, v/v) gave **9c** (3 mg), **9d** (4 mg) and the phenazine methylate (**10c**) (7 mg), $[\alpha]_D$ −34.5° (*c* = 0.5, acetone).

**Treatment of 10 with Triethylamine** A solution of **10** (300 mg) in acetonitrile (7 ml) was heated with triethylamine (0.5 ml) under reflux for 10 min. The solution was acidified with 2 N HCl. After concentration to about 3 ml, the solution was subjected to Sephadex LH-20 chromatography. Elution with 80% MeOH gave **9** (54 mg), **10e** (67 mg) and **10f** (2 mg). **10e**: A pale yellow amorphous powder, $[\alpha]_D$ +48.6° (acetone). Negative FAB-MS *m/z*: 907 $(M-H)^-$, $^1$H-NMR (acetone-$d_6$) ppm: 2.44 (1H, dd, *J* = 3, 19 Hz, brevifolin carboxyl (BC)-3), 3.12 (1H, dd, *J* = 7, 19 Hz, BC-3), 4.12 (1H, t, *J* = 6 Hz, glc-4), 4.57 (1H, dd, *J* = 3, 7 Hz, BC-2), 5.12 (1H, dd, *J* = 2, 6 Hz, glc-2), 5.20 (1H, dd, *J* = 4, 6 Hz, glc-3), 5.83 (1H, d, *J* = 2 Hz, glc-1), 6.86, 6.94 (each 1H, s, HHDP-H), 7.18 (2H, s, galloyl-H), 7.43 (1H, s, BC-3′). $^{13}$C-NMR (acetone-$d_6$) ppm: 37.7 (BC-2), 41.5 (BC-3), 64.0 (glc-6), 67.2, 72.8, 73.4, 74.8 (glc-2,3,4,5), 92.6 (glc-1), 109.5, 110.4 (BC-5′. HHDP-3, 3′), 110.0 (galloyl-2, 6), 115.0 (BC-1′), 116.0 116.8 (HHDP-1, 1′), 116.2 (BC-6′), 120.7 (galloyl-1), 124.5, 125.5 (HHDP-2, 2′), 137.0, 137.2 (HHDP-5, 5′), 139.2 (BC-3′, galloyl-4), 140.4 (BC-4′), 143.7 (BC-2′), 144.7, 145.0 (HHDP-4, 4′, 6, 6′), 145.9 (galloyl-3,5), 147.8 (BC-1), 149.8 (BC-5), 161.7 (BC-7′), 165.6, 166.0, 169.0, 172.3 (COO), 193.6 (BC-4).

**Helioscopin A (11)** A white powder ($H_2O$), mp 250—252 °C, $[\alpha]_D$ +33.5° (*c* = 0.4, acetone). *Anal.* Calcd for $C_{47}H_{34}O_{32}\cdot 8H_2O$: C, 44.97; H, 3.99. Found: C, 45.07; H, 3.81. Negative FAB-MS *m/z*: 1109 $[M-H]^-$. $^1$H-NMR (acetone-$d_6$ + $D_2O$) ppm: 2.15 (1H, d, *J* = 19 Hz, elaeocarpusinoyl (EC)-3), 3.10 (1H, dd, *J* = 2, 19 Hz, EC-3), 4.02 (1H, dd, *J* = 4, 10 Hz, glc-6), 4.05 (1H, d, *J* = 5 Hz, EC-6″), 4.25 (1H, dd, *J* = 5, 10 Hz, glc-6), 4.52 (1H, dd, *J* = 4, 5 Hz, EC-6″), 4.54 (1H, t, *J* = 4 Hz, glc-5), 4.61 (1H, d, *J* = 1 Hz, EC-4″), 4.87 (1H, d, *J* = 3 Hz, glc-3), 5.35 (1H, d, *J* = 1 Hz, glc-2), 5.51 (1H, s, EC-5″), 5.67 (1H, d, *J* = 2 Hz, EC-1), 6.21 (1H, s, glc-4), 6.69 (1H, br s, glc-1), 6.79, 6.87 (each 1H, s, HHDP-H), 7.14 (2H, s, galloyl-H), 7.29 (1H, s, EC-3′). $^{13}$C-NMR (acetone-$d_6$ + $D_2O$) ppm: 38.0 (EC-3), 50.0 (EC-2), 52.0 (EC-1), 59.0, 68.7, 69.7, 71.8 (glc-2, 3, 4, 5), 64.3 (glc-6), 74.4 (EC-5″), 76.5 (EC-6″), 80.9 (EC-2″), 89.5 (EC-4″), 90.5 (glc-1), 96.3 (EC-5), 108.6 (EC-6), 109.0 (EC-3″), 108.8, 109.9 (HHDP-3, 3′), 110.0 (galloyl-2,6), 114.5 (EC-3′), 116.2 (EC-1′), 115.8, 117.0 (HHDP-1, 1′), 118.7 (EC-2′), 119.8 (galloyl-1), 125.4, 125.5 (HHDP-2, 2′), 136.8 (EC-5′), 135.8, 137.3 (HHDP-5, 5′), 139.6 (galloyl-4), 144.7, 144.9, 145.2 (HHDP-4, 4′, 6, 6′), 146.0 (galloyl-3, 5), 147.4 (EC-4′), 148.6 (EC-6′), 164.6, 165.6, 166.0, 168.0, 170.0 (COO), 170.3 (EC-1″), 198.6 (EC-4).

**Methylation of 11** A mixture of helioscopinin A (**11**) (50 mg), dimethyl sulfate (0.5 ml) and anhydrous potassium carbonate (1 g) in dry acetone (15 ml) was heated under reflux (80 °C) for 2 h. The inorganic salts were filtered off, and the filtrate was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (7 : 1, v/v) afforded the tetradecamethyl ether (**11a**) (18 mg) as a pale yellow amorphous powder, $[\alpha]_D$ +57.3° (*c* = 0.3, $CHCl_3$). Positive FAB-MS *m/z*: 1207 $(M+H)^+$. $^1$H-NMR ($CDCl_3$) ppm: 2.20 (1H, dd, *J* = 2, 20 Hz, EC-3), 2.83 (1H, d, *J* = 20 Hz, EC-3), 3.38, 3.99 (42H in total, $OCH_3$), 4.42—4.51 (3H in total, EC-4″, 6″, glc-5), 4.61 (1H, br s, glc-3), 5.17 (1H, d, *J* = 13 Hz, glc-6), 5.35 (1H, br s, EC-5″), 5.49 (1H, br s, glc-2), 5.72 (1H, d, *J* = 2 Hz, EC-1), 6.28 (1H, s, glc-4), 6.85 (1H, br s, glc-1), 6.70, 7.01 (each 1H, s, HMDP-H), 7.21 (2H, s, trimethoxybenzoyl-H), 7.43 (1H, s, EC-3′).

**Methanolysis of 11a** A solution of **11a** (15 mg) in 3% methanolic sodium methoxide (2 ml) in MeOH was kept at room temperature for 28 h. The mixture was neutralized with Amberlite IR 120 B resin ($H^+$ form), and concentrated to dryness. The residue was dissolved in MeOH (1 ml) and treated with ethereal diazomethane (1 ml) for 1 h at room temperature.

After removal of the solvent by evaporation, the residue was chromatographed over silica gel. Elution with benzene–acetone (15 : 1, v/v) afforded **9c** (2 mg), **9d** (2 mg) and dimethyl pentamethoxyelaeocarpusinoate (**11b**) (3 mg), $[\alpha]_D$ +51.4° ($c$=0.2, $CHCl_3$).

**Hot Water Treatment of Helioscopinin A (11)** A solution of **11** (30 mg) in water (2 ml) was heated on a water bath (90 °C) for 1 h, and the reaction mixture was directly chromatographed over Sephadex LH-20. Elution with $H_2O$ gave L-ascorbic acid (**11c**) (3 mg), and successive elution with 30% methanol yielded **8** (11 mg).

**Preparation of Helioscopinin A (11)** A mixture of **8** (500 mg) and L-ascorbic acid (**11c**) (500 mg) in 0.2 M acetic acid (5 ml) was kept at 40 °C for 7 h. After concentration, the white precipitate (430 mg) formed was collected by filtration, and identified as **11** by physical and spectral comparisons.

**Helioscopin B (12)** A white powder ($H_2O$), mp>300 °C, $[\alpha]_D$ +45.7° ($c$=0.4, acetone). *Anal.* Calcd for $C_{47}H_{34}O_{33}\cdot 6H_2O$: C, 46.22; H, 3.77. Found: C, 46.36; H, 3.95. Negative FAB-MS $m/z$: 1111 $(M-H)^-$. $^1$H-NMR (acetone-$d_6$+$D_2O$) ppm: 2.26 (1H, d, $J$=19 Hz, EC-3), 3.14 (1H, dd, $J$=2, 19 Hz, EC-3), 4.00 (1H, dd, $J$=5, 10 Hz, glc-6), 4.35, 4.67 (4H in total, m, EC-4″, 5″, 6″), 5.04 (1H, br d, $J$=3 Hz, glc-3), 5.39 (1H, d, $J$=5 Hz, glc-2), 5.72 (1H, d, $J$=2 Hz, EC-1), 6.35 (1H, br d, $J$=3 Hz, glc-4), 6.68 (1H, d, $J$=5 Hz, glc-1), 7.10, 7.23, 7.25 (each 2H, s, galloyl-H), 7.32 (1H, s, EC-3′). $^{13}$C-NMR (acetone-$d_6$+$D_2O$) ppm: 38.3 (EC-3), 49.9 (EC-2), 52.2 (EC-1), 64.4 (glc-6), 64.9, 71.2, 75.2, 76.2 (glc-2, 3, 4, 5), 74.0 (EC-5″), 76.6 (EC-6″), 80.6 (EC-2″), 89.4 (EC-4″), 92.9 (glc-1), 96.3 (EC-5), 109.0 (EC-6, 3″), 109.4, 110.0, 110.3, (galloyl-2, 5), 113.9 (EC-3′), 116.6 (EC-1′), 119.2, 119.8, 120.3 (galloyl-1), 118.2 (EC-2′), 136.5 (EC-5′), 139.5, 139.8 (galloyl-4), 145.9, 146.1, 146.3 (galloyl-3), 147.6 (EC-4′), 148.7 (EC-6′), 165.0, 165.1, 166.8, 168.3 (COO), 171.3 (EC-1″), 198.3 (EC-4).

**Hot Water Treatment of Helioscopin B (12)** A solution of **12** (120 mg) in water (1 ml) was heated on a water bath (90 °C) for 1.5 h. The mixture was subjected to Sephadex LH-20 chromatography. Elution with $H_2O$ gave **11c** (2 mg). Subsequent elution with 60% MeOH yielded terchebin (**6**) (18 mg) as a yellow powder.

**Preparation of Helioscopin (12)** A mixture of **6** (200 mg) and L-ascorbic acid (**11b**) (200 mg) in 0.2 M acetic acid (2 ml) was left standing at 40 °C for 3 h. Work-up as described above yielded **12** (120 mg) as a white powder from $H_2O$.

**Methylation of Helioscopin (12)** A mixture of **12** (100 mg), dimethyl sulfate (1 ml) and anhydrous potassium carbonate (1.5 g) in dry acetone (15 ml) was heated under reflux (80 °C) for 2 h. The inorganic salts were filtered off, and the filtrate was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (8 : 1, v/v) afforded the tetradecamethyl ether (**12a**) (30 mg) as a yellow amorphous powder, $[\alpha]_D$ +64.0° ($c$=0.2, $CHCl_3$). Positive FAB-MS $m/z$: 1309 $(M+H)^+$. $^1$H-NMR ($CDCl_3$) ppm: 2.30 (1H, d, $J$=20 Hz, EC-3), 3.02 (1H, dd, $J$=2, 20 Hz, EC-3), 3.35—3.98 (42H in total, m, $OCH_3$), 4.09 (1H, m, glc-6), 4.20—4.77(6H in total, m, EC-4″, 5″, 6″, glc-5, 6), 4.97 (1H, d, $J$=5 Hz, glc-3), 5.57 (1H, d, $J$=5 Hz, glc-2), 5.72 (1H, d, $J$=2 Hz, EC-1), 6.39 (1H, d, $J$=5 Hz, glc-4), 6.82 (1H, d, $J$=5 Hz, glc-1), 7.19, 7.32, 7.39 (each 2H, s, trimethoxybenzoyl-H), 7.45 (1H, s, EC-3′).

**Alkaline Hydrolysis of 12a, Followed by Diazomethane Methylation** A solution of **12a** (20 mg) in 5% aqueous sodium hydroxide (1 ml) and methanol (1 ml) was heated at 80 °C for 1 h. The solution was acidified with 1 N HCl and extracted with ether. The ether layer was washed with water, dried over $Na_2SO_4$ and concentrated to dryness. The residue was treated with ethereal diazomethane for 1.5 h. After evaporation of the solvent and excess reagent, the residue was chromatographed over silica gel. Elution with benzene–acetone (23 : 2, v/v) yielded **9c** (6 mg). Subsequent elution with benzene–acetone (20 : 3, v/v) afforded **11b** (2 mg), $[\alpha]_D$ +50.9° ($c$=0.2, $CHCl_3$).

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