REACTION OF DEHYDROELLAGITANNINS WITH L-CYSTEINE METHYL ESTER[1]

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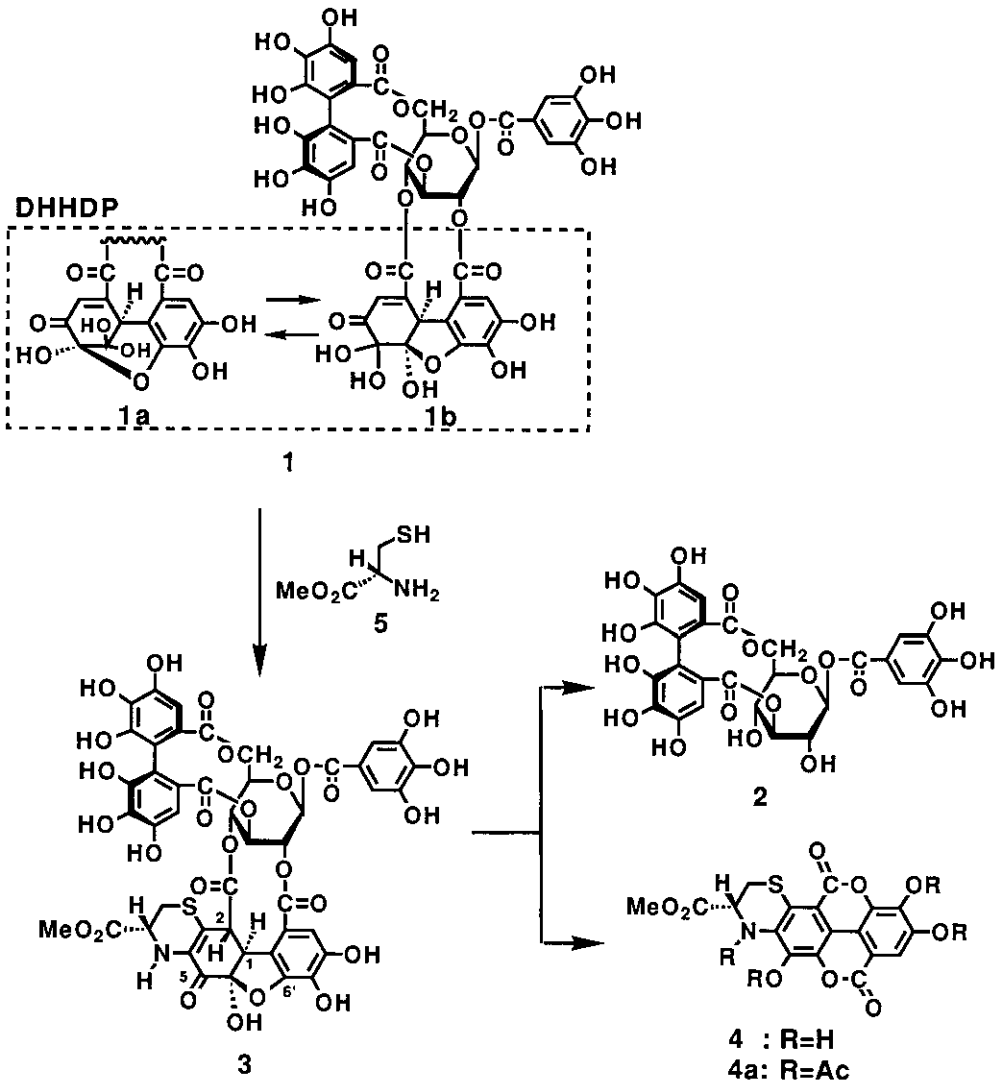
Abstract---Reaction of dehydroellagitannins (e.g. **1**) with L-cysteine methyl ester (**5**) at room temperature yielded the condensation products (e.g. **3** and **4**), together with a partial hydrolysate (e.g. **2**), while heating the mixture at 80°C afforded **4** and the hydrolysate(**2**) in fairly good yields. In addition, reduction of a dehydrohexahydroxydiphenoyl ester group to a hexahydroxy-diphenoyl group with thiols is also described.

Dehydroellagitannins, a group of hydrolyzable tannins possessing dehydrohexahydroxydiphenoyl (DHHDP) ester group(s) in the molecule, are widely distributed in the plants of Euphorbiaceae,[2] Geraniaceae,[3] Elaeocarpaceae,[4] Combretaceae,[5] Punicaceae,[6] etc., and occur in most cases as major metabolites. They have a unique hydrated cyclohexenetrione ring and usually exist in solutions as an equilibrium mixture of five- and six-membered hemiketal forms (**1a** and **1b**). In a previous paper, we reported that these dehydroellagitannins react with various amines and inorganic bases to yield a variety of novel degradation products.[7] In continuing our studies on the reaction of the dehydroellagitannins, we have found that the thiol-containing amino acid ester, L-cysteine methyl ester (**5**), readily condenses with the DHHDP esters occurring as a mixture of five- and six-membered forms, but reaction with a dehydroellagitannin having a rigid hemiketal structure does not give rise to a condensation product and instead affords unexpectedly a reduction product under a much stronger condition. This paper describes the results of these experiments.

First, we attempted the reaction by employing as a representative of dehydroellagitannins, geraniin (**1**),[2,3] which occurs in many plants as a major metabolite, and is available in sufficient amounts. Treatment of **1** with L-cysteine methyl ester (**5**) in aqueous methanol afforded a product (**3**), together with many uncharacterized compounds. To minimize the formation of these by-products, we changed pH of the reaction mixture by addition of sodium



acetate, acetic acid and ammonium formate, and found that reaction at pH 6 (adjusted with ammonium formate) gave a satisfactory result, whereas many decomposition products were formed in the acidic (acetic acid) and alkaline (sodium acetate) media. Reaction of **1** at room temperature yielded three major products (**2**, **3**, and **4**) among which **2** was identified as corilagin by comparisons of its spectral data with those of an authentic sample.[2]

The yellow product (**3**) exhibited the $[M-H]^-$ ion peak at m/z 1050 in the negative FAB ms, the larger molecular weight suggesting that **3** was formed by condensation of **5** and **1**. The ^1H nmr spectrum revealed the absence of the equilibrium forms, and showed signals arising from a fully acylated glucopyranose core, the chemical shifts and the coupling patterns being similar to those of **1**. The signals due to the cysteine methyl ester moiety were observed at δ 3.20 (1H, dd, J=4, 13 Hz, CH_2), 3.34 (1H, dd, J=5, 13 Hz, CH_2), 3.77 (3H, s, CH_3), 4.64 (1H, ddd, J=4, 4, 5 Hz, CH) and 5.72 (1H, d, J=4 Hz, NH, exchangeable with D_2O). The absence of an olefinic proton signal originally observed in the spectrum of **1**, and instead the appearance of a vicinal coupling between benzylmethine (δ 4.63, d, J=10 Hz, H-1) and a methine proton (δ 3.31, d, J=10 Hz, H-2) suggested the occurrence of an anti-Markownikoff-type addition of the thiol group to the olefine bond of the DHHDP group.[8] In addition, the large coupling constant (J=10 Hz) between these signals indicated that the benzylmethine and the methine proton are mutually *trans*-oriented. In the ^{13}C nmr spectrum, the chemical shifts of the hemiketal carbon signal at δ 102.7 and the aromatic carbon signal at δ 147.3 due to C-6' were similar to those of **1b** (δ 108.9 and 147.3) rather than **1a** (δ 96.3 and 143.4), suggesting the presence of a five-membered hemiketal ring. Furthermore, the observation of a signal due to an α,β-unsaturated carbonyl carbon at δ 180.5 (assignable to C-5) suggested that the amine group is substituted at the C-4 position. On the basis of these findings, the structure of this product was concluded to be as represented by the formula **3**.

The product (**4**) gave a greenish brown color with the ferric chloride reagent. The negative FAB ms exhibited the $[M-H]^-$ ion peak at m/z 416, suggesting **4** to be formed by condensation of the DHHDP ester and **5**, followed by hydrolysis. The ^1H nmr spectrum of **4** showed signals due to the cystaine methyl ester moiety [δ 3.17 (2H, br d, J=4 Hz, CH_2), 3.68 (3H, s, CO_2CH_3), 4.67 (1H, br s, CH) and 6.46 (1H, br d, J=4 Hz, NH, exchangeable with D_2O)], while the ^{13}C nmr spectrum

exhibited, besides the cysteine methyl ester signals, the presence of two aromatic rings and two unsaturated lactone carbonyl carbons (δ 158.5 and 158.9), indicating that **4** possesses a bislactone structure similar to that of ellagic acid (δ 158.7). The structure of **4** was further confirmed by acetylation of **4**, which afforded the tetraacetate (**4a**) (FD ms: m/z 585 M^+), the ^1H nmr spectrum showing signals due to three O-acetyl [δ 2.40 and 2.47 (x2)] and one N-acetyl (δ 2.08) group. Furthermore, upon hydrolysis in hot water with sodium acetate, **3** yielded **4**, together with **2**. Based on these physico-chemical evidence, the structure of this product was established to be as represented by the formula **4**.

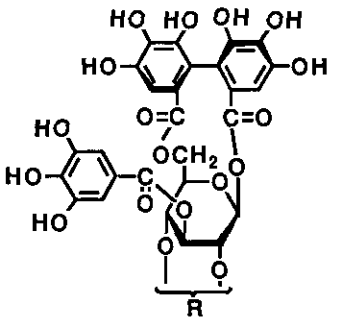
Similarly, condensation of L-cysteine methyl ester with other dehydroellagitannins gave the desired products. On the other hand, reaction at higher temperature (80°C) caused hydrolysis to afford **4** and a partial hydrolysate in fairly good yields (Table).

Table Reaction of Dehydroellagitannins with L-Cysteine Methyl Ester (at 80°C)

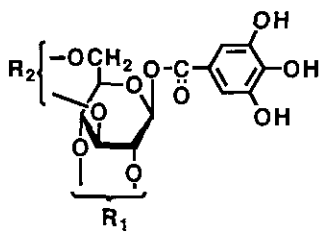
Starting material	Yield*(mol %) of **4**	Hydrolysates and Reduction Product	Yield*(mol %)
geraniin (**1**)	95	corilagin (**2**)	77
helioscopinin A (**6**)[2b]	91	helioscopinin B (**7**)[2b]	62
mallotusinic acid (**8**)[2a]	97	mallotinic acid (**9**)[2a]	56
furosin (**10**)[2a]	60	1-O-galloyl-β-D-glucose (**11**)	74
carpinusin (**12**)[1]	87	helioscopinin B (**7**)	62
trapain (**13**)	27	1,2,3-tri-O-galloyl-β-D-glucose (**14**)	27
		eugeniin (**15**)	67

* Isolated yield.

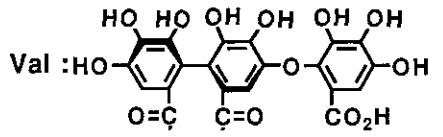
Although these methods were found to be highly applicable to the structural elucidation of dehydroellagitannins, particularly to the selective cleavage of the DHHDP ester group, we have encountered the case where the reaction did not proceed smoothly. When trapain (**13**)[9] was treated with **5** at room temperature, no reaction occurred. However, heating the mixture at 80°C yielded, together with small quantities of **4** and a partial hydrolysate (**14**), the reduction product as a major product which was identified as eugeniin (**15**).[10] This reduction was

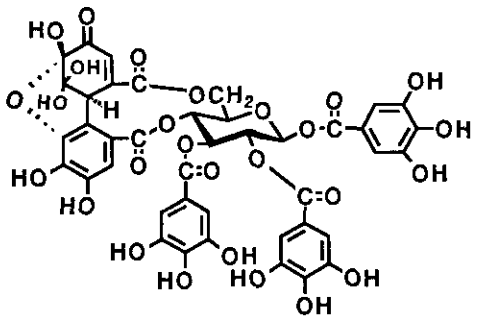


6 : R=(*R*)-DHHDP
7 : R=H, H
12: R=(*S*)-DHHDP



8 : R_1=(*R*)-DHHDP, R_2=Val
9 : R_1=H, H, R_2=Val
10 : R_1=(*R*)-DHHDP, R_2=H, H
11 : R_1=R_2=H, H
16 : R_1=R_2=(*R*)-HHDP





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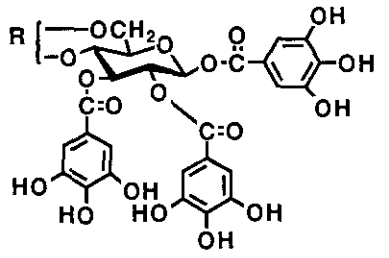

14 : R=H, H
15 : R=(*S*)-HHDP

considered to be caused by the action of the thiol group, which is capable of undergoing oxidation. In fact, when thioglycolic acid was used instead of cysteine methyl ester, similar reduction took place in the case of geraniin (**1**), yielding 1-O-galloyl-2,3;4,6-bis-(R)-hexahydroxydiphenoyl(HHDP)-β-D-glucose (**16**).[7] The reason why only in the case of **12**, the reduction occurred is not clear, but it is probably due to the existence of **12** as a rigid six-membered hemiketal ring system which prevents the formation of the carbonyl group at the C-5 position.

The fact that the dehydroellagitannins readily react with L-cysteine methyl ester implies that similar condensation reaction occurs with proteins having thiol group(s) in the living bodies, and besides the physico-chemical interactions such as hydrogen and hydrophobic bondings, the formation of covalent bonds between the DHHDP group and the thiol group(s) in the protein moieties possibly plays also an important role in their complexation reaction and also the inhibition of enzymes.

It should be noted finally that when **5** was added to the aqueous solution of the extracts of Geranium thunbergii,[3] Punica granatum,[6] Elaeocarpus sylvestris var. ellipticus[4] and Euphorbia hirta,[2c] which contain dehydroellagitannins, yellow precipitates of the condensation products were formed in a few minutes. On the other hand, the extracts of Cornus officinalis[11] and Camellia sinensis,[12] which do not contain dehydroellagitannins, did not afford such precipitates. The reaction with **5** therefore may become a useful tool for the detection of dehydroellagitannins.

EXPERIMENTAL

Melting points were determined with a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. FD ms were taken with a JEOL JMS DX-300 instrument equipped with a JMA 3500 data system and FAB ms with a JEOL JMS-HX100/JMA 3500 data system using DMSO-glycerol as a matrix. ^{1}H and ^{13}C *nmr spectra were recorded on JEOL FX-100 and GX-270 spectrometers using* TMS *as an internal* standard. Column chromatography was carried out with Sephadex LH-20 (25-100μm, Pharmacia Fine Chemical Co., Ltd.) and MCI-gel CHP-20P (75-150μm, Mitsubishi Chemical Industries, Ltd.). Thin-layer chromatography (tlc) was conducted on precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck) with a solvent system of benzene-ethyl formate-formic acid (1:7:1) and precoated cellulose F_{254} plates (0.1 mm, Merck) with 2% acetic acid, and spots were visualized by ultraviolet illumination and by spraying the ferric chloride reagent.

Reaction of geraniin (**1**) with L-cysteine methyl ester (**5**).

A solution of **1** (1.0 g, 1.05 mmol), **5**(360 mg, 2.7 mmol) and ammonium formate (150 mg, 2.4 mmol) in 25% aqueous methanol (20 ml) was stirred at room temperature for 12 h. The resulting yellow precipitates of **4** were collected by filtration and the filtrate was directly subjected to Sephadex LH-20 column chromatography with 80 % methanol to yield corilagin (**2**)(218 mg, 33 %) and **3** (542 mg, 52 %). Further elution with methanol afforded **4**, which was combined with the above precipitates and recrystalized from methanol to give **4** (89 mg, 20 %).

Product 3

A yellow powder (H_2O), mp 242°C(decomp.), $[\alpha]_D^{23}$-144.4° (c=1.0, MeOH), Anal. Calcd for $C_{45}H_{33}NO_{27}S$ 1/2H_2O: C, 50.09; H, 3.36; N, 1.30. Found: C, 50.67; H, 2.83; N, 1.40. Negative FAB ms: m/z 1050 $(M-H)^-$. ^{1}H-Nmr (acetone-d_6, 270 MHz):δ 3.20 (1H, dd, J=4, 13 Hz, CH_2), 3.31 (1H, d, J=10 Hz, H-2), 3.34 (1H, dd, J=5, 13 Hz, CH_2), 3.77 (3H, s, $COOCH_3$), 4.32 (1H, dd, J=8, 11 Hz, glc-6), 4.63 (1H, d, J=10 Hz, H-1), 4.64 (1H, ddd, J=4, 4, 5 Hz, CH), 4.74 (1H, br t, J=10 Hz, glc-5), 4.93 (1H, t, J=11 Hz, glc-6), 5.30 (1H, d-like, J=4 Hz, glc-4), 5.52 (1H, br s, glc-2), 5.72 (1H, d, J=4 Hz, NH), 5.86 (1H, br s, glc-3), 6.52 (1H, s, glc-1), 6.64, 7.09 (each 1H, s, HHDP-H), 7.17 (2H, s, galloyl-H), 7.21 (1H, s, H-3'). ^{13}C-Nmr (acetone-d_6, 25.05 MHz): δ 28.1 (CH_2), 52.1 (C-1), 53.0 (x2)(CH, OCH_3), 53.8 (C-2), 61.8 (glc-4), 63.9 (glc-6), 66.8 (glc-3), 69.4 (glc-2), 73.2 (glc-5), 91.8 (glc-1), 102.7 (C-6), 107.5, 110.6 (HHDP-3,3'), 110.7 (galloyl-2, 6), 112.2 (C-3'), 115.0, 116.7, 116.9 (C-1', HHDP-1, 1'), 120.2, 120.6, (C-2'. galloyl-1), 124.4(x2), 125.8(C-3, HHDP-2, 2"), 132.0 (C-4), 136.2, 137.4, 137.7, 139.6 (C-5', galloyl-4, HHDP-5, 5'), 144.6, 144.9, 145.1, 145.3, 145.8, 146.5, 147.3 (C-4', 6' galloyl-4, 6, HHDP-4, 4', 6, 6'), 164.5, 164.9, 166.3, 168.4, 168.8, 171.5 (COO), 180.5 (C-5).

Product 4

A yellow powder (MeOH), mp 254°C, $[\alpha]_D^{20}$-103.3° (c=0.5, pyridine), Anal. Calcd for $C_{18}H_{11}NO_9S$: C, 50.71; H, 2.84; N, 3.29. Found: C, 50.46; H, 2.69; N, 3.55. Negative FAB ms: m/z 416 $(M-H)^-$. Ir ν_{max}^{KBr}cm^{-1}: 3400, 1720, 1620, 1595, 1580, 1495. ^{1}H Nmr (DMSO-d_6, 100 MHz): δ 3.17 (2H, br d, J=4 Hz, CH_2), 3.68 (3H, s, $COOCH_3$), 4.67 (1H, br s, CH), 6.46 (1H, br d, J=4 Hz, NH), 7.43 (1H, s, H-3'). ^{13}C Nmr (DMSO-d_6, 25.05 MHz): δ 26.3 (CH_2), 51.4 (CH), 52.3 (OCH_3), 104.1, 107.2, 110.0, 110.5, 112.5, 114.9 (C-1, 2, 3, 1', 2', 3'), 132.8, 135.7, 136.1, 139.1, 147.5 (C-4, 5, 6, 4', 5', 6'), 158.5, 158.9 (δ-lactone), 170.9 (COO).

Hydrolysis of 3

A solution of **3** (30 mg, 0.03 mmol) and sodium acetate (30 mg) in 50% aqueous methanol (2 ml) was heated at 80°C for 1 h. After concentration under reduced pressure, the resulting precipitates formed (8 mg, 66 %)(identified as **4** by co-tlc and comparison of the ir spectrum) were collected by filtration, and the filtrate was applied to an MCI-gel CHP-20P column with water containing increasing amounts of methanol to give **2**(13 mg, 71 %)

Acetylation of 4

4 (20 mg, 0.05 mmol) was acetylated at room temperature with acetic anhydride(0.2 ml) and pyridine (0.2 ml) for 20 h. The mixture was poured into ice water, and resulting precipitates were crystallized from methanol to give a white powder (6 mg, 21 %). mp 244°C. FD ms: m/z 585 (M^+). Ir ν_{max}^{KBr} cm^{-1}:2950, 1795, 1750, 1700. ^{1}H Nmr ($CDCl_3$, 100 MHz): δ 2.08 (3H, s, N-Ac), 2.40 (3H, s, O-Ac), 2.47 (6H, s, O-Ac x2), 3.18, 3.52 (each 1H, dd, J=7, 13 Hz, CH_2), 3.64 (3H, s, $COOCH_3$), 5.86 (1H, t, J=7 Hz, CH), 8.07 (1H, s, H-3').

General procedures for reaction of dehydroellagitannins with L-cysteine methyl ester (**5**) at elevated temperature.

A solution of each dehydroellagitannin (100 mg, 0.09-0.15 mmol), ammonium formate (50 mg, 0.8 mmol) and **5** (50 mg, 0.37 mmol) in 50% aqueous acetonitrile (2 ml) was heated at 80°C for 2-

5 h. After concentration, the precipitates of **4** were collected by filtration. The filtrate was subjected to Sephadex LH-20 column chromatography with 80% methanol to yield hydrolysates: **2** (51.2 mg, 77 %) and **4** (41.7 mg, 95 %) from **1** (0.11 mmol); helioscopinin B (**7**)(41 mg, 62 %) and **4** (40 mg, 91 %) from helioscopinin A (**6**)(0.11 mmol); mallotinic acid (**9**)(40.4 mg, 56 %) and **4** (36 mg, 97 %) form mallotusinic acid (**8**)(0.09 mmol); 1-O-galloyl-β-D-glucose (**11**)(38 mg, 74 %) and **4** (38 mg, 60 %) from furosin (**10**)(0.15 mmol); **6** (41 mg, 62 %) and **4** (38 mg, 87 %) from carpinusin (**12**)(0.11 mmol).

Reaction of trapain (**13**) with L-cysteine methyl ester (**5**)

A solution of **13**(50 mg, 0.05 mmol), ammonium formate (25 mg, 0.4 mmol) and **5** (50 mg, 0.37 mmol) in 50% aqueous acetonitrile (2 ml) was heated at 80°C for 5 h. The reaction mixture was worked up in a manner similar to that described above to yield **4** (6 mg, 27 %), 1,2,3-tri-O-galloyl-β-D-glucose (**14**)(9 mg, 27 %) and eugeniin (**15**)(33 mg, 67 %).

Reaction of geraniin (**1**) with thioglycolic acid

A solution of **1** (1.0 g 1.05 mmol) and thioglycolic acid (1.0 ml, 14 mmol) in 90% aqueous ethanol (20 ml) was heated at 60°C for 9 h. The reaction mixture was concentrated and subjected to Sephadex LH-20 column chromatography with 80% methanol to yield 1-O-galloyl-2,4;3,6-bis-(R)-HHDP-β-D-glucose (**16**)(334 mg, 36 %).

Detection of dehydroellagitannins in the plant extract

The fresh leaves (10 g) of Geranium thunbergii, Punica granatum, Elaeocarpus sylvestris var. ellipticus and Euphorbia hirta, which are known to contain dehydroellagitannins, and Cornus officinalis and Camellia sinensis, which do not contain dehydroellagitannins, were extracted at room temperature with 80% aqueous acetone (50 ml) for 24 h. After concentration under reduced pressure, the insoluble materials formed were filtered off. To the filtrate (5 ml), a solution of **5** (100 mg) and ammonium formate (100 mg) in water (2 ml) was added at room temperature. After few minutes, yellow precipitates were observed in the extracts of G. thunbergii, P. granatum, E. silvestris var. ellipticus and E. hirta. The precipitates formed in the extracts of G. thunbergii and E. sylvestris var. ellipticus were collected by filtration, and examined by tlc. The chromatogram showed a major spot whose Rf value was the same as that of **3**. On the other hand, the extracts of C. officinalis and C. sinensis did not afford such precipitates.

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