Glutathione-Mediated Conversion of the Ellagitannin Geraniin into Chebulagic Acid

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Received July 17, 1995; accepted August 22, 1995

Geraniin (1), a widely distributed ellagitannin having a dehydrohexahydroxydiphenoyl (DHHDP) ester moiety, was converted into chebulagic acid (2), an ellagitannin having a chebuloyl ester moiety, which was reported to be a potent inhibitor of DNA topoisomerases. This was achieved by addition of the thiol group of glutathione to the six-membered acetal ring form of the DHHDP moiety (1a) with concomitant hydrolytic ring cleavage and subsequent reductive desulfurization with Raney nickel. The concurrent addition of the thiol to the five-membered acetal ring form of the DHHDP group (1b) generated the product 14 and its precursor 13, which are structurally related to naturally occurring ellagitannins isolated from Euphorbiaceous plants. Thus, the rearrangements observed in these reactions may be relevant to the metabolism of DHHDP esters in plants.

Key words tannin; glutathione; geraniin; chebulagic acid; thiol; dehydrohexahydroxydiphenic acid

The hydrolyzable tannins are a large group of plant polyphenols biosynthetically derived from multiple galloyl esters with a polyalcohol core (usually D-glucose). Interest aroused by their structural diversity and various biological activities, including ecological significance as anti-herbivores, has prompted many scientists in various fields to study these widespread plant metabolites.[1,2] Among the hydrolyzable tannins, the most intriguing group is the ellagitannin subfamily, members of which contain hexahydroxydiphenoyl[3] (HHDP) ester and its derivatives.[4] Although biosynthesis of these tannins begins with simple C–C or C–O oxidative coupling of galloyl esters attached to glucose,[2] various combinations of couplings both within a galloylglucose and between different galloylglucose units generate structural diversity of these metabolites.[4] Furthermore, there is a distinctive class of ellagitannins, represented by geraniin (**1**)[5] and chebulagic acid (**2**),[6] which are assumed to be generated by dehydrogenation and hydrolytic cleavage of one of the aromatic rings of the HHDP group.[4] Recently many ellagitannins of this class (see Chart 1) have been isolated from various plant sources, particularly from Euphorbiaceous plants.[7] Their acyl groups spanning the oxygens at C-2 and C-4 of glucopyranose, which adopts $^1C_4$ or a related skew-boat conformation, also seem to be biosynthesized from the HHDP group, probably *via* its oxidative metabolite, the dehydrohexahydroxydiphenoyl (DHHDP) group.[8] Repandusinic acid A (**3**),[7b,9] mallotusinin (**4**),[7a,9] elaeocarpusin (**5**)[7a,c,10] and phyllanthusiin D (**6**)[7d,f,11] have already been demonstrated to be chemically derived from geraniin (**1**). The biogenesis of the chebuloyl group which spans O(2) and O(4) of glucose in chebulagic acid (**2**), however, remains obscure. Since its oxidation state is the same as that of the HHDP group, the chebuloyl group has been thought to be directly derived from the HHDP group by hydrolytic cleavage of its aromatic ring.[8b,12] However, the co-occurrence of tannins having a chebuloyl group with those having a DHHDP group at the same positions on the glucose core in some Euphorbiaceous,[7c,e,f] Combretaceous,[6d] and Betulaceous[13] plants may imply that the chebuloyl group is reductively biosynthesized from the DHHDP group.[6d]

It has been pointed out that covalent bond formation is likely between tannins (or oxidized metabolites) and thiol groups of proteins and other compounds in living tissues.[14] In the course of studies on the chemical properties of DHHDP esters, we have previously found that geraniin quantitatively forms a dihydro-1,4-thiazoine adduct with L-cysteine methyl ester under physiological conditions.[15] These results suggested that similar addition of thiol groups to the DHHDP ester might occur in plants or in the alimentary canal of herbivores, and led us to study further the reaction between the DHHDP group and naturally occurring thiol compounds, such as glutathione (GSH). This paper deals with the reaction of geraniin with some thiol compounds, and describes the first conversion of geraniin into chebulagic acid, which has been reported to be a potent inhibitor of mammalian DNA topoisomerases I[16] and II.[17]

Reaction of Geraniin and Glutathione, and Synthesis of Chebulagic Acid and Neochebulagic Acid Geraniin (**1**) was selected as the substrate of the reaction because this tannin is available in sufficient amounts from plants of the Euphorbiaceae,[7] Elaeocarpaceae,[10a] Geraniaceae,[5] and Betulaceae.[13] The reaction was started by addition of GSH to a solution of **1** in phosphate buffer at pH 7.0, and the mixture was left to stand at room temperature for 8 h. At this stage, HPLC analysis of the reaction mixture showed the presence of the two major products **10** and **13**; however, **13** is unstable and gradually changed into **14** (and partly its methyl ester **14a** in the presence of methanol) during the separation procedure. Occurrence of this conversion in the chromatography column hampered the purification of the products; hence, the reaction mixture was acidified prior to separation in order to convert **13** into **14** in advance. Subsequent column chromatography on highly porous polymer polystyrene gel (MCI-gel CHP 20P) gave two major products **10** (30%) and **14** (29%), together with **11** (8%), **14a** (10%) and corilagin (**8**, 16%)[6a,7] (Chart 1).

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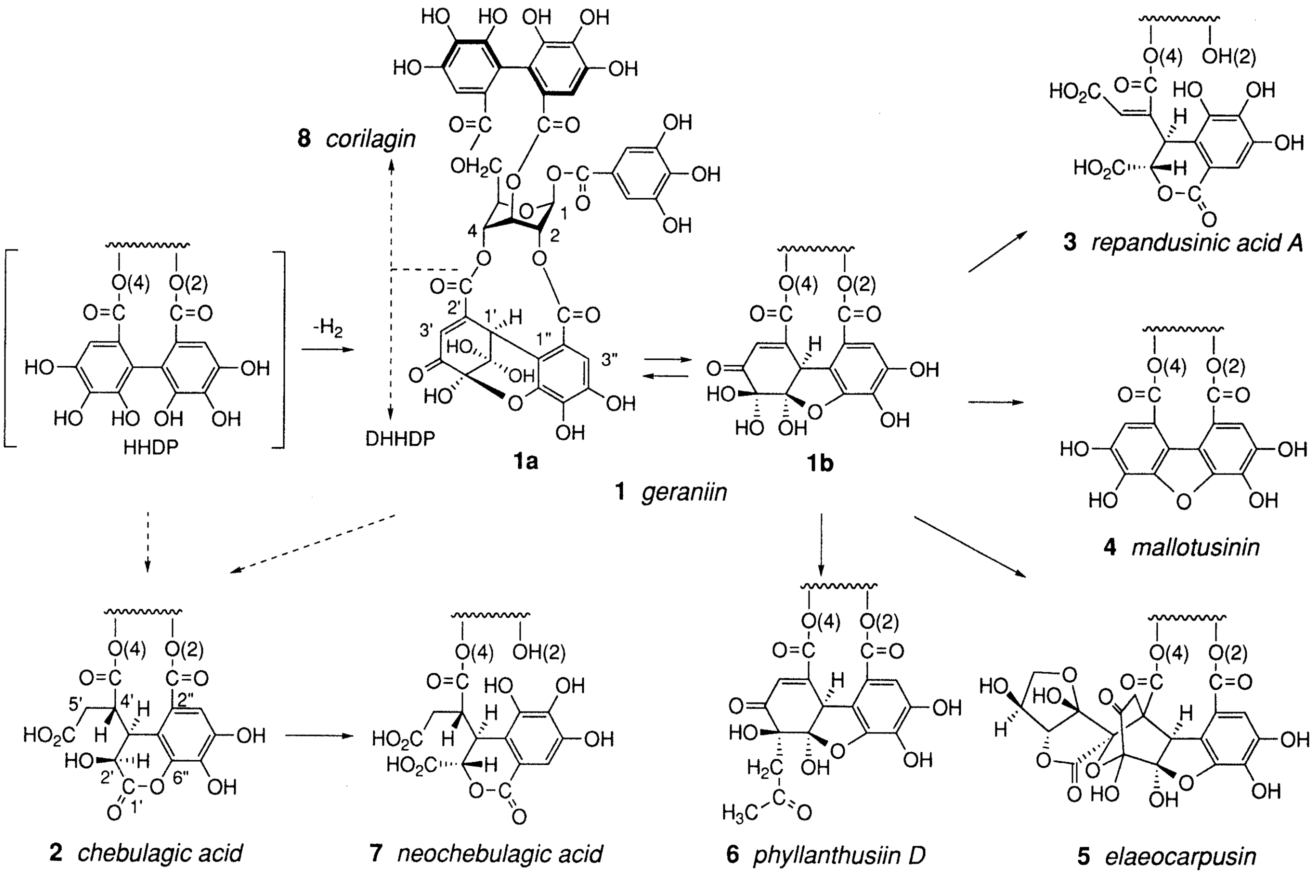


Chart 1

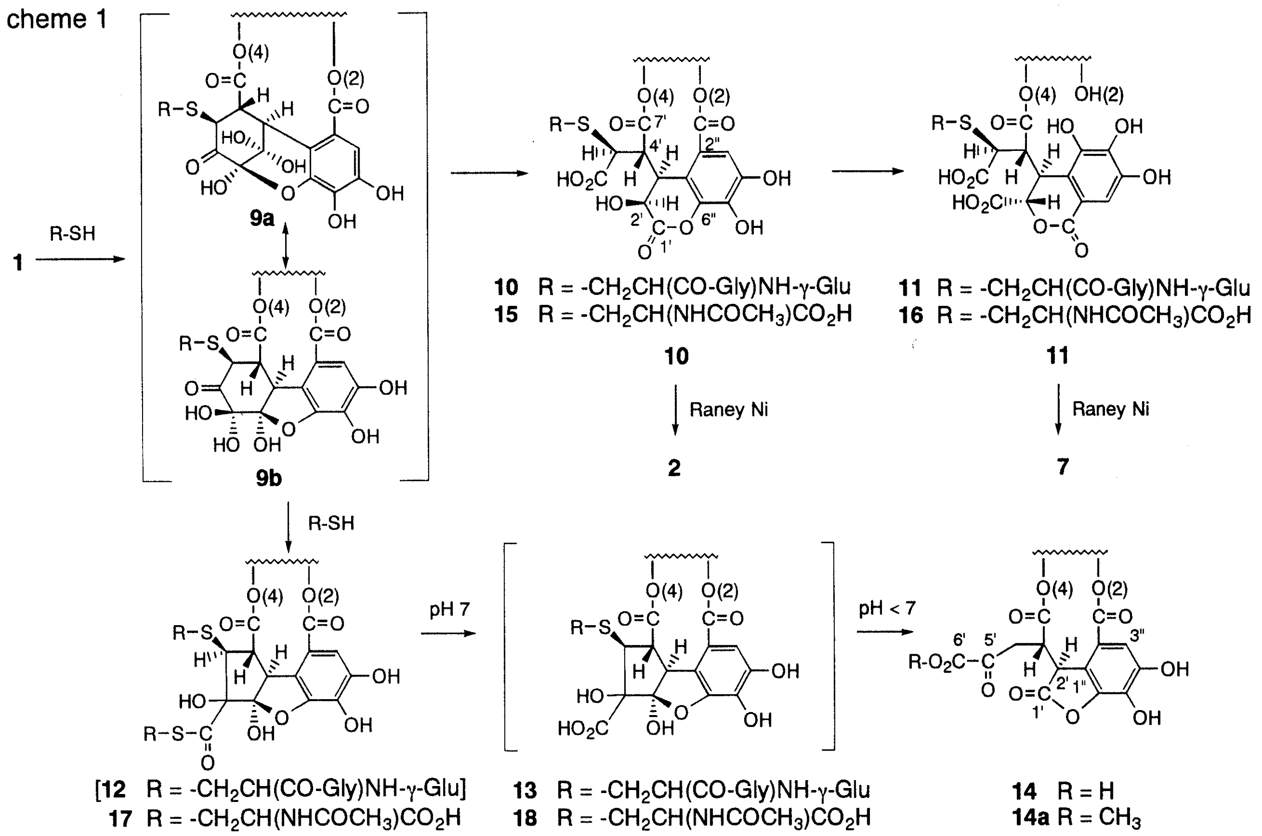


Chart 2

The negative and positive fast ion bombardment (FAB) mass spectra of **10** (*m/z* 1258 $[M-H]^-$ and *m/z* 1260 $[M+H]^+$, respectively) revealed the molecular weight (1259), which was consistent with the sum of the molecular weights of **1** and GSH. In the ^1H- and ^{13}C-NMR spectra, the signals arising from the GSH residue and 1-*O*-galloyl-

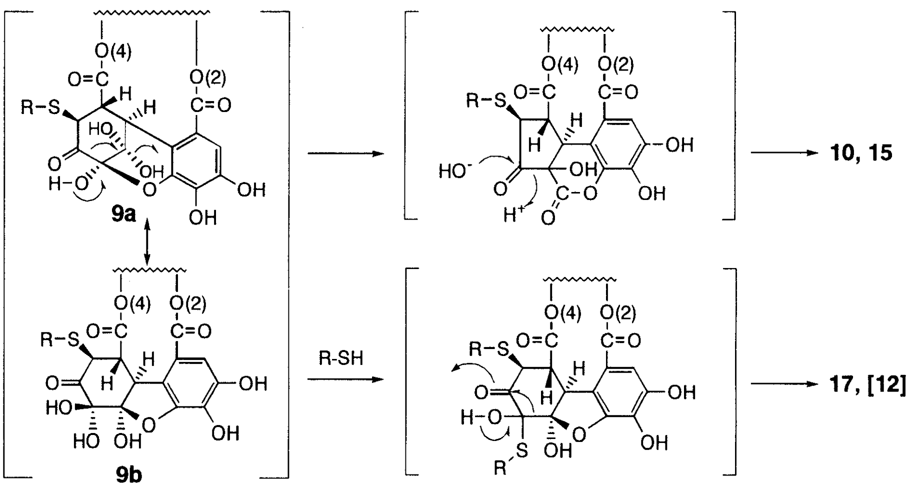


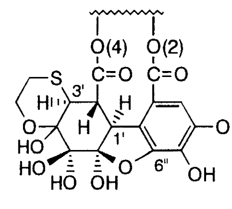
Chart 3

3,6-(R)-HHDP-D-glucopyranose moiety were similar to those of **1** and GSH, respectively, except for the low-field shift of the carbon signal (δ 38.0) due to the methylene of the cysteine residue compared with that of GSH (δ 25.5), indicating that GSH residue was attached to the tannin moiety through an S–C bond. The presence of the 5-substituted chebuloyl moiety in **10** was deduced from the appearance of the carbon signals due to four methine [δ 40.6 (C-3′), 44.6 (C-4′), 49.8 (C-5′), 67.1 (C-2′)] and two carboxyl groups [δ 170.1 (C-1′), 175.0 (C-6′)] which were related to the chebuloyl moiety of **2**. The corresponding methine proton signals [δ 5.25 (br d, $J=7$ Hz, 3′-H), 4.94 (d, $J=7$ Hz, 2′-H), 4.42 (br s, 4′-H), 3.14 (d, $J=2$ Hz, 5′-H)] were also similar to those of the chebuloyl group [δ 5.12 (dd, $J=1$, 7 Hz, 3′-H), 4.92 (d, $J=7$ Hz, 2′-H)], except for the appearance of the methine proton signal (5′-H) where the sulfur atom of GSH was attached. The structure of **10** was supported by heteronuclear multiple bond connectivity (HMBC) spectroscopy (8 Hz), which showed ^{1}H–^{13}C long-range couplings between 2′-H and C-1′, between 3′-H and C-6″ (δ 141.0), between 4′-H and C-1″ (δ 117.7), and between 5′-H and C-7′ (δ 172.2). From these spectral data, **10** was determined to be the glutathione conjugate of chebulagic acid (**2**). This product is considered to be generated by *anti*-Markownikoff addition of the thiol radical to the double bond of the DHHDP group accompanied with a concomitant benzylic acid-type rearrangement of **9a** and hydrolytic ring cleavage (Chart 3).

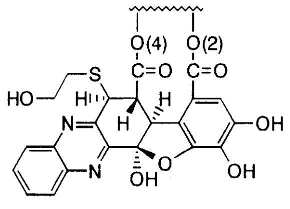
The ^{1}H-NMR spectrum of the minor product **11** was related to that of **10**, showing the presence of a chebuloyl moiety linked with the GSH residue; however, the large upfield shift of the 2-H signal of glucose ($\Delta\delta$ −1.38) and the coupling constants ($J_{2',3'}=1$ Hz, $J_{3',4'}=8$ Hz) of the signals due to the chebuloyl group were analogous with those of neochebulagic acid (**7**).[6a] Furthermore, heating of an aqueous solution of **10** afforded **11**, confirming that the product **11** is the glutathione conjugate of neochebulagic acid.

Subsequent desulfurization of **10** with Raney nickel afforded chebulagic acid (**2**) (28%). Similarly, **11** yielded neochebulagic acid (**7**) (40%). Thus, the first conversion of a tannin having DHHDP esters into one having the chebuloyl group without protection of functional groups was successfully achieved.

The configurations of C-5′ of the chebuloyl group of **10** and **11** were deduced from the following experiment. The reaction of **1** with 2-mercaptoethanol in acetate buffer at pH 4 yielded an adduct **19** as a major product, the molecular weight [negative FAB-MS m/z 1029 $(M-H)^-$] of which was consistent with the sum of those of **1** and 2-mercaptoethanol. The ^{13}C-NMR spectrum of **19** showed three methine (δ 53.5, 51.5, 42.3) and three acetal carbon signals (δ 93.3, 94.4, 109.7) of the acyl group, suggesting the addition of the thiol to the double bond of the DHHDP group. Taking the molecular weight into account, the appearance of the three acetal carbon signals indicated the presence of a bicyclic structure which comprised the hydrated cyclohexanetrione ring and the oxathiane ring. Furthermore, the chemical shifts of one of the acetal carbons (δ 109.7) and the oxygen-bearing aromatic carbon (δ 147.2) were in accord with those of the five-membered acetal ring form of the DHHDP group (**1b**, δ 108.9, 147.3, respectively),[5] indicating the presence of a similar dihydrofuran ring structure in **19**. In the ^{1}H-NMR spectrum, the large coupling constants ($J_{1',2'}=$ 10 Hz, $J_{2',3'}=12$ Hz) of methine protons of the hydrated cyclohexanetrione ring suggested that these protons are oriented in all-*trans*-axial configuration. This was further supported by the observation of similar J values ($J_{1',2'}=$ 11 Hz, $J_{2',3'}=12$ Hz) of the corresponding methine signals in the spectrum of the quinoxaline derivative **19a** obtained by treatment of **19** with o-phenylenediamine: the appearance of the carbon signals at δ 106.3 (C-5′) and 147.4 (C-6″) in the ^{13}C-NMR spectrum indicated that **19a** also has a dihydrofuran ring system similar to that of **19**. Because the cyclohexene ring of **19a** is most likely to take



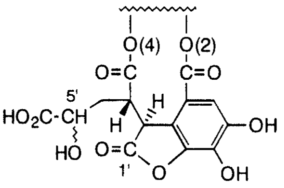
19



19a

a half-chair form, the large coupling constant between the methine proton signals confirmed the *trans*-axial orientation of these three methine protons in **19a**. This also indicated that the addition of 2-mercaptoethanol to the DHHDP group occurred from the β side in a *cis* mode. On the assumption that the addition of GSH to DHHDP group occurred in a similar manner (Chart 2), the configurations of C-5′ of **10** and **11** were deduced to be *S*.

The Mechanism of Generation of Product 14 The other major product **14** obtained by the reaction of **1** with GSH (Chart 2) was shown to have the same molecular weight as that of **1** by observation of the $(M-H)^-$ peak at *m/z* 951 in the negative FAB-MS. The infrared absorption at $1800\,cm^{-1}$ suggested the presence of an unsaturated γ-lactone ring in **14**. The ^{1}H-NMR spectrum showed aliphatic ABMX-type signals due to the partial structure $-CH-CH-CH_2-$ [δ 5.02 (d, $J=3$ Hz, 2′-H) 3.76 (ddd, $J=2$, 3, 11 Hz, 3′-H), 2.90 (dd, $J=11$, 19 Hz, 4′-H), 2.37 (dd, $J=2$, 19 Hz, 4′-H)]. Furthermore, the carbon signal due to a ketone appeared at δ 195.5. Reduction of this ketone with sodium cyanoborohydride yielded an alcohol **14c**. In the ^{1}H- and ^{13}C-NMR spectra of **14c**, the methine proton of the secondary alcohol (5′-H) was coupled with the vicinal methylene protons (4′-H), and the carboxyl carbon signal originally seen at δ 163.4 in **14** was shifted to lower field (δ 175.3), confirming the position of the carbonyl group in **14**. Thus, the structure of this product was determined to be as shown in formula **14**.[18)]



14c

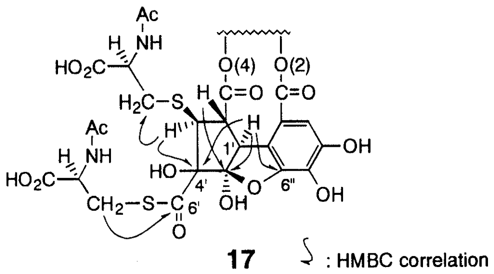


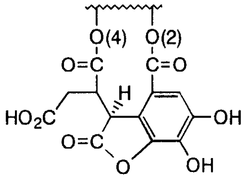
Chart 4

The mechanism of the formation of **14** from **1** was revealed by the following experiments: as compared to the reaction with GSH, the reaction of **1** with *N*-acetyl-L-cysteine in phosphate buffer (pH 6.5) proceeded slowly to give five products, **14** (6%), **14a** (2%), **15** (20%), **16** (7%) and **17** (10%) along with recovery of **1** (38%). The ^{1}H- and ^{13}C-NMR spectra of **15** and **16** were analogous to those of **10** and **11**, respectively, except for the appearance of the *N*-acetylcysteine moiety in place of the GSH moiety. The products **15** and **16** were hence determined to be *N*-acetyl-L-cysteine congeners of **10** and **11**, respectively. This result indicated that reaction of **1** with *N*-acetyl-L-cysteine proceeded through a mechanism similar to that with GSH. The product **17** showed the $[M-H]^-$ peak at *m/z* 1259 in the negative FAB-MS, indicating that this product has two *N*-acetyl-L-cysteine moieties in the molecule. The ^{1}H-NMR spectrum showed AMX-type signals due to the neighboring three methine protons [δ 4.33 (d, $J=8$ Hz, 1′-H), 3.80 (dd, $J=8$, 14 Hz, 2′-H), 3.59 (d, $J=14$ Hz, 3′-H)]. In the HMBC spectrum (8 Hz), the correlation between methine signal at δ 4.33 (1′-H) and aromatic carbon signal at δ 147.8 (C-6″) indicated that this methine is located at the benzylic position (C-1′) (Chart 4). This proton signal (1′-H) was also correlated with the acetal carbon (C-5′, δ 116.0) and quaternary carbon (C-4′, δ 89.3). Furthermore, the correlation between this quaternary carbon and the 3′-H signal at δ 3.59 revealed the presence of the hydrated cyclopentanone ring in **17**. The locations of two cysteine moieties were derived from the following observation: first, the 3′-H signal (δ 3.59) was correlated with the methylene carbon (δ 35.5) of one of the cysteine moieties indicating that the sulfur atom of cysteine is attached to the C-3′ position. Next, the long-range coupling between the methylene protons [δ 3.08 (dd, $J=10$ and 14 Hz), 3.76 (dd, $J=5$ and 14 Hz)] of the other cysteine moiety and carbonyl carbon resonating at δ 200.7 (C-6′), which is connected to the above-mentioned quaternary carbon (C-4′), indicated that this cysteine is attached through a thioester linkage. Although the stereochemistry of the quaternary carbon has not yet been determined, the configurations of the three methine carbons of the cyclopentanone ring were deduced to be similar to those of **19** from their large coupling constants [$J_{1',2'}=8$ Hz, $J_{2',3'}=14$ Hz], and by analogy with **19**. On the basis of these spectral data, the structure of the product **17** was concluded to be as shown in Chart 2. This compound is considered to be formed by a benzylic acid-type rearrangement of the thioacetal of **9b** (Chart 3). The product **17** was rather stable in acidic solution (pH 1, at room temperature for 12 h); however, under a neutral condition (pH 7.0, room temperature, 12 h) **17** was gradually converted to **18** (Chart 2). Although **18** could not be isolated owing to its instability, HPLC analysis showed that **18** was changed into **14** almost quantitatively when the solution was acidified again to pH 2. The intermediate **18** was considered to be the carboxylic acid generated by hydrolysis of the thioester, and hence, the mechanism of the reaction of **1** with thiol compounds affording **14** can be summarized as shown in Chart 2. The adduct of GSH to the DHHDP group (**9a**, **9b**, Chart 2) was detected on HPLC analysis as a broad peak at the initial stage of the reaction, and disappeared as the reaction proceeded. The unstable intermediate **13**, which could only be detected by HPLC analysis before acidification, seems to be the GSH congener of **18**, and a minor peak probably arising from the intermediate **12** was also observed. Formation of the methyl ester **14a**, obtained along with **14**, can be accounted for by methanolysis of the thioesters of **17** and **12** in aqueous methanol used as the chromatographic solvent.

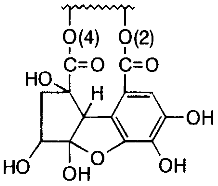
Conclusion

The reactions of geraniin (**1**) with three thiol compounds, glutathione, 2-mercaptoethanol and *N*-acetyl-L-cysteine, were examined and the first conversion of the DHHDP ester to chebuloyl ester was achieved. These reactions begin with the *cis*-addition of a thiol radical to the double bond from the *β*-side. It is probable that in addition to the reversible complex formation intrinsic to tannins with other molecules such as proteins by hydrophobic and hydrogen bonding, irreversible covalent bond formation between ellagitannins having a DHHDP group and thiol groups of other molecules occurs in plants or in regions where the cell structure is degraded by the battle against fungi, bacteria and other predators.19)

We do not intend to suggest the possibility of participation of thiol compounds or thiol enzyme in the biosynthesis of the chebuloyl group; however, the rearrangements of the hydrated cyclohexenetrione ring of the DHHDP group observed in this experiment might well be related to the metabolism of ellagitannins in plants. Though compound **14** has not yet been isolated from any plant source, this compound is considered to be a biosynthetic precursor of euphormisin M_2 (**20**), which was isolated from *Euphorbia humifusa* together with geraniin (**1**).7e) Furthermore, the structural similarity between **17** and phyllanthusiin C (**21**), which also coexists with **1** in *Phyllanthus flexuosus*,7f) suggests that **21** is also biosynthesized *via* **1**.



20 *euphormisin M_2*



21 *phyllanthusiin C*

Experimental

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were obtained with Varian Unity plus 500, Varian Gemini 300, and JEOL GX-270 spectrometers operating at 500, 300 and 270 MHz for ^{1}H, and 125, 75 and 67.8 MHz for ^{13}C, respectively; chemical shifts are reported in parts per million on the δ scale from internal tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate, and coupling constants are in hertz. Negative and positive FAB-MS were recorded on a JEOL JMS DX-303 spectrometer with glycerol as a matrix. HPLC analysis was performed on a Tosoh apparatus equipped with a CCPM solvent delivery system, UV-8 model II spectrometer (280 nm) and a Cosmosil $5C_{18}$-AR (Nacalai Tesque Inc.) column (4.6 mm i.d. × 250 mm) (mobile phase, acetonitrile–50 mM phosphoric acid, gradient elution from 15 to 35% acetonitrile for 40 min; flow rate, 0.8 ml/min). Column chromatographies were performed with MCI-gel CHP 20P (75—150 μm, Mitsubishi Kasei Co.) and Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co. Ltd.) eluting with water containing increasing proportions of methanol. Thin layer chromatographies were performed on precoated Silica gel $60F_{254}$ plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (1 : 7 : 1, v/v) and precoated cellulose F_{254} plates (0.1 mm thick, Merck) with 2% acetic acid, and spots were detected by ultraviolet (UV) illumination and by spraying 2% ethanolic ferric chloride reagent. Glutathione and *N*-acetyl-L-cysteine were purchased from Nacalai Tesque and used without further purification.

Reaction of Geraniin (1) with GSH To a solution of 2.0 g (2.1 mmol) of **1** in 500 ml of 0.1 M potassium–sodium phosphate buffer (pH 7.0) was added 774 mg (2.5 mmol) of GSH, and the mixture was stirred at room temperature (22—27 °C). After 2 h, HPLC analysis of the reaction mixture showed three major peaks arising from the intermediate **9a** and **b** (t_R 8.6 min), **1** (t_R 12.3 min) and **10** (t_R 13.1 min), together with a small peak due to the intermediate **12** (t_R 17.0 min). After 8 h, the peaks due to **1** and **9a** and **b** had disappeared and two major peaks corresponding to **10** and the intermediate **13** (t_R 13.4 min), along with minor peaks due to **8** (t_R 10.2 min), **11** (t_R 6.5 min) and intermediate **12**, were observed; however, the peak corresponding to **14** (t_R 28.7 min) was not observed. At this stage, the reaction mixture was acidified (pH 2) with 5 M HCl at 5 °C, and directly applied to a column of MCI-gel CHP 20P (30 mm i.d. × 350 mm) with water. The column was first eluted with 500 ml of water to wash out inorganic salts and then eluted with a mixture of water and methanol [5% stepwise increase of concentration of methanol (200 ml of each concentration) from 10% to 70%] to give **11** (211 mg, 0.17 mmol, 8%), **8** (209 mg, 0.33 mmol, 16%), **10** (779 mg, 0.63 mmol, 30%), **14** (576 mg, 0.61 mmol, 29%) and **14a** (198 mg, 0.20 mmol, 10%).

For **10**: A white amorphous powder, $[\alpha]_D^{21}$ −65.7° (c=0.3, methanol–H_2O, 1 : 1, v/v). ^{1}H-NMR (500 MHz, acetone-d_6+D_2O) δ: 7.53 [1H, s, chebuloyl(che) 3″-H], 7.17 (2H, s, galloyl-H), 7.08, 6.68 (each 1H, s, HHDP-H), 6.51 (1H, s, glc 1-H), 5.60 (1H, br s, glc 3-H), 5.56 (1H, br s, glc 2-H), 5.41 (1H, d, J=3 Hz, glc 4-H), 5.25 (1H, br d, J=7 Hz, che 3′-H), 4.94 (1H, d, J=7 Hz, che 2′-H), 4.84 (2H, m, glc 5-H, 6-H), 4.51 (1H, t, J=5 Hz, Cys 2-H), 4.42 (1H, br s, che 4′-H), 4.37 (1H, dd, J=9, 6 Hz, glc 6-H), 3.90 (3H, br s, Gly 2-H_2, Glu 2-H), 3.14 (1H, d, J=2 Hz, che 5′-H), 2.52—2.66 (4H, m, Cys 3-H_2, Glu 4-H_2), 2.20 (2H, m, Glu 3-H_2). ^{13}C-NMR (125 MHz, acetone-d_6+D_2O) δ: 175.0 [chebuloyl(che)-6′], 174.4 (Glu-5), 173.2 (Glu-1), 172.8 (Gly-1), 172.2 (che-7′), 172.0 (Cys-1), 170.1 (che-1′), 168.8, 166.7 (HHDP-7,7′), 165.6 (che-7″), 165.3 (galloyl-7), 146.4 (che-4″), 145.9 (galloyl-3,5), 145.5, 145.1, 145.0, 144.6 (HHDP-4,4′,6,6′), 141.0 (che-6″), 140.0 (galloyl-4), 139.6 (che-5″), 137.7, 136.2 (HHDP-5,5′), 125.4, 124.2 (HHDP-2,2′), 119.8, 119.7 (che-2″, galloyl-1), 117.7 (che-1″), 117.0 (che-3″), 117.2, 115.3 (HHDP-1,1′), 110.6 (galloyl-2,6), 110.4, 107.7 (HHDP-3,3′), 91.3 (glc-1), 72.9 (glc-5), 69.9 (glc-2) , 67.1 (che-2′), 65.6 (glc-4), 63.7 (glc-6), 62.7 (glc-3), 54.3 (Glu-2), 54.0 (Cys-2), 49.8 (che-5′), 44.6 (che-4′), 41.6 (Gly-2), 40.6 (che-3′), 38.0 (Cys-3), 32.1 (Glu-4), 26.7 (Glu-3). Negative FAB-MS (glycerol) m/z: 1258 $(M-H)^-$, positive FAB-MS m/z: 1260 $(M+H)^+$. *Anal.* Calcd for $C_{51}H_{45}N_3O_{33}S\cdot 3H_2O$: C, 46.62; H, 3.91; N, 3.20. Found: C, 46.52; H, 4.02; N, 3.15. Assignments of the NMR signals were achieved with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), HSQC and HMBC spectra.

For **11**: A white amorphous powder, $[\alpha]_D^{21}$ −68.9° (c=0.7, methanol). ^{1}H-NMR (500 MHz, D_2O) δ: 7.15 (1H, s, che 3″-H), 6.96 (2H, s, galloyl-H), 6.85, 6.63 (each 1H, s, HHDP-H), 6.29 (1H, s, glc 1-H), 5.61 (1H, d, J=3 Hz, glc 4-H), 5.21 (1H, d, J=1 Hz, che 2′-H), 4.89 (1H, t, J=11 Hz, glc 6-H), 4.82 (1H, br s, glc 3-H), 4.72 (overlapped with HOD signal, glc 5-H), 4.63 (1H, dd, J=8, 5 Hz, Cys 5-H), 4.27 (1H, dd, J=11, 8 Hz, glc 6-H), 4.21 (1H, dd, J=8, 1 Hz, che 3′-H), 4.18 (1H, s, glc 2-H), 4.00 (1H, d, J=18 Hz, Gly 2-H), 3.95 (1H, d, J=18 Hz, Gly 2-H), 3.93 (1H, t, J=6 Hz, Glu 2-H), 3.90 (1H, d, J=11 Hz, che 5′-H), 3.62 (1H, dd, J=11, 8 Hz, che 4′-H), 3.20 (1H, dd, J=14, 5 Hz, Cys 3-H), 3.02 (1H, dd, J=14, 8 Hz, Cys 3-H), 2.52 (m, 2H, Glu 4-H_2), 2.18 (2H, m, Glu 3-H_2). ^{13}C-NMR (125 MHz, D_2O) δ: 174.6 (Glu-5), 173.3 (che-1′), 173.1, 172.9, 172.8 (che-6, Glu-1, Gly-1), 172.1 (Cys-1), 171.4 (che-7′), 169.2, 167.3 (HHDP-7,7′), 166.4 (che-7″), 166.0 (galloyl-7), 145.3 (che-4″), 144.6 (galloyl-3,5), 144.5, 144.8, 143.8, 143.7 (HHDP-4,4′,6,6′), 142.7 (che-6″), 139.9 (che-5″), 139.0 (galloyl-4), 136.6, 135.8 (HHDP-5,5′), 124.8, 123.6 (HHDP-2,2′), 119.3 (galloyl-1), 116.5 (che-1″), 115.7 (che-2″), 115.5, 114.7 (HHDP-1,1′), 110.6 (galloyl-2,6), 109.9 (che-3″), 110.2, 108.1 (HHDP-3,3′), 94.4 (glc-1), 78.1 (che-2′), 71.7 (glc-5), 67.7 (glc-3), 65.3 (glc-2), 63.5 (glc-6), 63.2 (glc-4), 53.3 (Glu-2), 53.0 (Cys-2), 49.9 (che-4′), 46.0 (che-5′), 41.3 (Gly-2), 36.2 (che-3′), 32.9 (Cys-3), 31.2 (Glu-4), 25.7 (Glu-3). *Anal.* Calcd for $C_{51}H_{45}N_3O_{33}S\cdot 5H_2O$: C, 45.37; H, 4.11; N, 3.11. Found: C, 45.38; H, 4.00; N, 3.24. Assignments of the NMR signals were achieved with the aid of ^{1}H-^{1}H COSY, HSQC and HMBC spectra.

For **14**: A white amorphous powder, $[\alpha]_D^{21}$ −80.8° (c=1.1, methanol). ^{1}H-NMR (300 MHz, acetone-d_6+D_2O) δ: 7.33 [1H, s, 2,4-acyl group (acyl) 3″-H], 7.15 (2H, s, galloyl-H), 7.04, 6.68 (each 1H, s, HHDP-H), 6.44 (1H, s, glc 1-H), 5.94 (1H, br s, glc 3-H), 5.41 (1H, br s, glc 2-H), 5.31 (1H, br d, J=3 Hz, glc 4-H), 5.03 (1H, d, J=3 Hz, acyl 2′-H), 4.94 (1H, br t, J=9 Hz, glc 5-H), 4.79 (1H, t, J=10 Hz, glc 6-H), 4.44 (1H, dd, J=10, 8 Hz, glc 6-H), 3.75 (1H, ddd, J=12, 3, 2.5 Hz, acyl 3′-H), 2.93 (1H, dd, J=19, 12 Hz, acyl 4′-H), 2.37 (1H, dd, J=19, 2.5 Hz, acyl 4′-H). ^{13}C-NMR (67.8 MHz, acetone-d_6+D_2O) δ: 195.5 (acyl-5′), 176.2 (acyl-1′), 171.3 (acyl-7′), 169.0, 166.6, 165.5, 164.5 (HHDP-7,7′, acyl-7″, galloyl-7), 163.4 (acyl-6′), 147.9 (acyl-6″), 145.9 (galloyl-3,5), 145.5, 145.2, 144.9, 144.7 (HHDP-4,4′,6,6′), 143.1 (acyl-4″), 140.1 (galloyl-4), 137.8, 136.3 (2C) (acyl-5″, HHDP-5,5″), 125.3, 124.1 (HHDP-2,2′), 119.7 (galloyl-1), 117.4, 117.2, 115.3, 114.5 (acyl-1″,2″,3″, HHDP-1,1′), 110.6 (galloyl-2,6), 110.4, 107.7 (HHDP-3,3′), 92.0 (glc-1), 73.4 (glc-5), 70.1 (glc-2), 67.1 (glc-4), 64.0 (glc-6), 61.2 (glc-3), 47.7, 43.9 (acyl-2′,3′), 35.2 (acyl-4′). IR (KBr) 3250, 1800, 1720, 1600 cm^{-1}. Negative FAB-MS *m/z*: 952 $(\mathrm{M-H})^-$, 879 $(\mathrm{M-COCO_2H})^-$. *Anal.* Calcd for $\mathrm{C_{41}H_{28}O_{27}\cdot H_2O}$: C, 50.73, H, 3.11. Found: C, 50.82; H, 3.33.

For **14a**: A white amorphous powder; ^{1}H-NMR (300 MHz, acetone-d_6) δ: 7.32 (1H, s, acyl 3″-H), 7.16 (2H, s, galloyl-H), 7.05, 6.68 (each 1H, s, HHDP-H), 6.43 (1H, s, glc 1-H), 5.95 (1H, br s, glc 3-H), 5.40 (1H, br s, glc 2-H), 5.28 (1H, d, $J=3$ Hz, glc 4-H), 5.04 (1H, d, $J=3$ Hz, acyl 2′-H), 4.92 (1H, br t, $J=9$ Hz, glc 5-H), 4.79 (1H, t, $J=11$ Hz, glc 6-H), 4.44 (1H, dd, $J=11$, 8 Hz, glc 6-H), 3.32 (1H, ddd, $J=12$, 3, 2.5 Hz, acyl 3′-H), 3.74 (3H, s, $\mathrm{CH_3}$), 2.94 (1H, dd, $J=19$, 12 Hz, acyl 4′-H), 2.38 (1H, dd, $J=19$, 2.5 Hz, acyl 4′-H). ^{13}C-NMR (75 MHz, acetone-$d_6+\mathrm{D_2O}$) δ: 193.0 (acyl-5′), 175.6 (acyl-1′), 171.3 (acyl-7′), 168.3, 166.3, 164.8, 164.3 (acyl-7″, HHDP-7,7′, galloyl-7), 161.4 (acyl-6′), 147.7 (acyl-6″), 146.0 (galloyl-3,5), 145.5, 145.3, 145.1, 144.6 (HHDP-4,4′,6,6′), 143.2 (acyl-4″), 139.7 (galloyl-4), 137.8, 136.6, 136.1 (acyl-5″, HHDP-5,5′), 125.7, 124.5 (HHDP-2,2′), 120.3 (galloyl-1), 118.2, 117.8, 117.1, 115.1, 114.5 (acyl-1″,2″,3″, HHDP-1,1′), 110.8 (galloyl-2,6), 110.3, 108.1 (HHDP-3,3′), 91.8 (glc-1), 73.5 (glc-5), 70.1 (glc-2), 67.3 (glc-4), 63.9 (glc-6), 61.3 (glc-3), 53.2 ($\mathrm{CH_3}$), 47.7, 44.4 (acyl-2′,3′), 34.9 (acyl-4′).

Conversion of 10 into 11 A solution of 17 mg of **10** in 5 ml of water was heated at 90 °C for 2 h, and subjected to column chromatography over Sephadex LH-20 (20 mm i.d. × 200 mm) with a mixture of methanol–water (4 : 1, v/v) to give **11** (7.6 mg) and **8** (2.2 mg).

Synthesis of Chebulagic Acid (2) A solution of 100 mg (0.079 mmol) of **10** in 7 ml of ethanol–water (7 : 3, v/v) containing 3% acetic acid was stirred with freshly prepared Raney nickel (W-4) at 27 °C for 4 h. Before filtration of the mixture, the catalyst was completely dissolved with 0.5 M HCl in order to increase the recovery of the products which were adsorbed on the catalyst. The filtrate was directly applied to a column of MCI-gel CHP 20P (20 mm i.d. × 150 mm) with water containing increasing proportions of methanol to yield chebulagic acid (**2**) (21 mg, 0.022 mmol, 28%), together with recovery of **10** (29 mg, 29%). **2** was obtained as a white amorphous powder, $[\alpha]_D^{21}$ $-50.6°$ ($c=0.8$, ethanol). ^{1}H-NMR (300 MHz, acetone-$d_6+\mathrm{D_2O}$) δ: 7.52 (1H, s, che 3″-H), 7.18 (2H, s, galloyl-H), 6.68, 6.53 (each 1H, s, HHDP-H), 6.53 (1H, s, glc 1-H), 5.92 (1H, br s, glc 3-H), 5.52 (1H, br s, glc 2-H), 5.25 (1H, d, $J=3$ Hz, glc 4-H), 5.10 (1H, dd, $J=7$, 1.5 Hz, che 3′-H), 4.91 (1H, d, $J=7$ Hz, che 2′-H), 4.86 (1H, br t, $J=9$ Hz, glc 5-H), 4.75 (1H, d, $J=10$ Hz, glc 6-H), 4.44 (1H, dd, $J=11$, 8 Hz, glc 6-H), 3.84 (1H, m, che 4′-H), 2.18 (2H, m, che 5′-$\mathrm{H_2}$).

Synthesis of Neochebulagic Acid (7) A solution of 50 mg (0.04 mmol) of **11** in 5 ml of ethanol–water (3 : 2, v/v) containing 3% acetic acid was stirred with freshly prepared Raney nickel (W-4) at 80° for 30 min and at 27 °C for 3 h. The resulting mixture was worked up in a manner similar to that described for **10**, and separated by column chromatography on MCI-gel CHP 20P (20 mm i.d. × 150 mm). Elution of the column with 25 to 40% methanol yielded **7** (15.4 mg, 0.016 mmol, 40%), together with recovery of **11** (8.1 mg, 16%). **7** was obtained as a white amorphous powder, $[\alpha]_D^{21}$ $-26.3°$ ($c=0.8$, acetone). ^{1}H-NMR (300 MHz, acetone-$d_6+\mathrm{D_2O}$) δ: 7.17 (2H, s, galloyl-H), 7.12 (1H, s, che 3″-H), 6.90 (1H, s, HHDP-H), 6.75 (1H, s, HHDP-H), 6.27 (1H, d, $J=4$ Hz, glc 1-H), 5.76 (1H, d, $J=3$ Hz, glc 4-H), 5.51 (1H, d, $J=1$ Hz, che 2′-H), 4.97 (1H, br d, $J=3$ Hz, glc 3-H), 4.69 (1H, dd, $J=10$, 8 Hz, glc 6-H), 4.62 (1H, br t, $J=7$ Hz, glc 5-H), 4.31 (1H, dd, $J=10$, 7 Hz, glc 6-H), 4.26 (1H, br d, $J=4$ Hz, glc 2-H), 3.98 (1H, dd, $J=8$, 1 Hz, che 3′-H), 3.31 (1H, m, che 4′-H), 2.96 (1H, dd, $J=17$, 11 Hz, che 5′-H), 2.43 (1H, dd, $J=17$, 4 Hz, che 5′-H).

Reduction of 14 A solution of 200 mg (0.21 mmol) of **14** in 5 ml of methanol–water (1 : 1, v/v) containing 3% acetic acid was treated with 200 mg of $\mathrm{NaBH_3CN}$ and the mixture was stirred at 25 °C for 1 h, then treated with 3 ml of acetone and concentrated. Separation of the products by column chromatography over MCI-gel CHP 20P (30 mm i.d. × 250 mm) with a mixture of water and methanol gave a mixture (2 : 1) of diastereomers **14c** as a white amorphous powder (66 mg, 33%): $[\alpha]_D^{21}$ $-79.3°$ ($c=0.7$, methanol). Negative FAB-MS *m/z*: 953 $(\mathrm{M-H})^-$. *Anal.* Calcd for $\mathrm{C_{41}H_{30}O_{27}\cdot 5H_2O}$: C, 47.14; H, 3.86. Found: C, 47.37; H, 3.86. For the major diastereomer: ^{1}H-NMR (300 MHz, acetone-$d_6+\mathrm{D_2O}$) δ: 7.29 (1H, s, acyl 3″-H), 7.15 (2H, s, galloyl-H), 7.05, 6.67 (each 1H, s, HHDP-H), 6.43 (1H, s, glc 1-H), 6.08 (1H, br s, glc 3-H), 5.38 (1H, br s, glc 2-H), 5.23 (1H, d, $J=4$ Hz, glc 4-H), 4.94 (1H, d, $J=3$ Hz, acyl 2′-H), 4.85 (1H, m, glc 5-H, 6-H), 4.41 (1H, dd, $J=10$, 7 Hz, glc 6-H), 4.10 (1H, dd, $J=8$, 6 Hz, acyl 5′-H), 3.57 (1H, ddd, $J=10$, 3, 2.5 Hz, acyl 3′-H), 1.76 (1H, ddd, $J=14$, 10, 6 Hz, acyl 4′-H), 1.26 (1H, ddd, $J=14$, 8, 2.5 Hz, acyl 4′-H). ^{13}C-NMR (75 MHz, acetone-$d_6+\mathrm{D_2O}$) δ: 175.7 (acyl-1′), 175.3 (acyl-6′), 171.8, 168.6, 166.5, 165.0, 164.4 (acyl-7′,7′, galloyl-7, HHDP-7,7′), 147.6 (acyl-6″), 145.9 (galloyl-3,5), 145.8, 145.4, 145.0, 144.6 (HHDP-4,4′,6,6′), 143.1 (acyl-4″), 139.8 (galloyl-4), 137.7, 136.4, 136.0 (acyl-5″, HHDP-5,5′), 125.6, 124.5 (HHDP-2,2′), 120.1 (galloyl-1), 117.8 (2C), 117.1, 115.2, 114.1 (acyl-1″,2″,3″, HHDP-1,1′), 110.6 (galloyl-2,6), 110.3, 107.6 (HHDP-3,3′), 91.6 (glc-1), 73.5 (glc-5), 70.0 (glc-2), 68.6 (acyl-5′), 67.0 (glc-4), 64.0 (glc-6), 61.2 (glc-3), 48.8, 44.2 (acyl-2′,3′), 31.0 (acyl-4′).

Reaction of Geraniin (1) with 2-Mercaptoethanol A solution of 1.0 g (1.1 mmol) of geraniin (**1**) and 15 g (192 mmol) of 2-mercaptoethanol in 150 ml of methanol–0.1 M acetate buffer (pH 4.0) (1 : 2, v/v) was stirred at 20 °C for 36 h. The reaction mixture was directly applied to a column of MCI-gel CHP 20P (30 mm i.d. × 300 mm) with water containing increasing proportions of methanol to yield **19** (608 mg, 0.59 mmol, 54%) as a white powder from water, mp 235—240 °C (dec.), $[\alpha]_D^{21}$ $-72.4°$ ($c=0.5$, methanol). ^{1}H-NMR (300 MHz, acetone-d_6) δ: 7.18 (2H, s, galloyl-H), 7.14 (1H, s, acyl 3″-H), 7.07, 6.63 (each 1H, s, HHDP-H), 6.48 (1H, s, glc 1-H), 5.86 (1H, br s, glc 3-H), 5.47 (1H, br s, glc 2-H), 5.21 (1H, d, $J=3.5$ Hz, glc 4-H), 4.91 (1H, t, $J=10$ Hz, glc 6-H), 4.83 (1H, br t, $J=8$ Hz, glc 5-H), 4.34 (1H, dd, $J=10$, 7 Hz, glc 6-H), 4.23 (1H, d, $J=11$ Hz, acyl 1′-H), 4.21 (1H, br t, $J=11$ Hz, $\mathrm{SCH_2C\underline{H}_2O}$), 3.84 (1H, dd, $J=11$, 2 Hz, $\mathrm{SCH_2C\underline{H}_2O}$), 3.81 (1H, d, $J=12.5$ Hz, acyl 3′-H), 2.97 (1H, ddd, $J=13.5$, 11, 2 Hz, $\mathrm{SC\underline{H}_2CH_2O}$), 2.37 (1H, br d, $J=13.5$ Hz, $\mathrm{SC\underline{H}_2CH_2O}$), 2.25 (1H, dd, $J=12.5$, 11 Hz, acyl 2′-H). ^{13}C-NMR (75 MHz, acetone-d_6) δ: 169.7, 168.5, 166.3, 165.0, 164.8 (COO), 147.2 (acyl-6″), 146.0 (galloyl-3,5), 145.5, 145.3, 145.1, 144.6 (2C) (acyl-4″, HHDP-4,4′,6,6′), 139.8 (galloyl-4), 137.9, 137.4, 136.4 (acyl-5″, HHDP-5,5′), 125.9, 125.8, 124.6 (acyl-2″, HHDP-2,2′), 120.3 (galloyl-1), 117.0, 116.7, 115.0 (acyl-1″, HHDP-1,1′), 111.8 (acyl-3″), 110.8 (galloyl-2,6), 110.5, 107.6 (HHDP-3,3′), 109.7 (acyl-6′), 94.4, 93.3 (acyl-3′,4′), 91.9 (glc-1), 73.3 (glc-5), 69.4 (glc-2), 66.3 (glc-4), 63.9 (glc-6), 61.7 (glc-3), 60.7 ($\mathrm{CH_2O}$), 53.5, 51.5, 42.3 (acyl-1′,2′,3′), 27.9 ($\mathrm{CH_2S}$). Negative FAB-MS *m/z*: 1029 $(\mathrm{M-H})^-$. *Anal.* Calcd for $\mathrm{C_{43}H_{32}O_{28}S\cdot 5H_2O}$: C, 46.16; H, 3.78. Found: C, 46.37; H, 3.85.

Synthesis of the Quinoxaline Derivative (19a) A solution of 57 mg (0.055 mmol) of **19** in 3 ml of ethanol containing 10% acetic acid was treated with 15 mg of *o*-phenylenediamine at 20 °C for 15 h. After concentration, the products were separated by column chromatography on Sephadex LH-20 (20 mm i.d. × 200 mm) with ethanol, followed by purification by MCI-gel CHP 20P chromatography to give the quinoxaline derivative **19a** (16 mg, 0.015 mmol, 27%) as a yellow amorphous powder. ^{1}H-NMR (300 MHz, acetone-$d_6+\mathrm{D_2O}$) δ: 8.20, 7.97 (each 2H, m, acyl-2‴,3‴,4‴,5‴), 7.29 (1H, s, acyl 3″-H), 7.19 (2H, s, galloyl-H), 7.11, 6.73 (each 1H, s, HHDP-H), 6.58 (1H, d, $J=1.5$ Hz, glc 1-H), 6.05 (1H, br s, glc 3-H), 5.56 (1H, d, $J=1.5$ Hz, glc 2-H), 5.34 (1H, d, $J=3.5$ Hz, glc 4-H), 5.09 (1H, d, $J=13$ Hz, acyl 3′-H), 4.99 (1H, br t, $J=8$ Hz, glc 5-H), 4.70 (1H, dd, $J=11$, 9 Hz, glc 6-H), 5.63 (1H, d, $J=12$ Hz, acyl 1′-H), 4.49 (1H, dd, $J=11$, 8 Hz, glc 6-H), 8.81 (2H, br t, $J=6$ Hz, $\mathrm{SCH_2C\underline{H}_2OH}$), 3.20 (overlapped with HOD signal, $\mathrm{SC\underline{H}_2CH_2OH}$), 2.95 (1H, dt, $J=14$, 6 Hz, $\mathrm{SC\underline{H}_2CH_2OH}$), 2.81 (1H, dd, $J=13$, 12 Hz, acyl 2′-H). ^{13}C-NMR (75 MHz, acetone-$d_6+\mathrm{D_2O}$) δ: 166.9 (2C), 168.6, 166.4, 165.1 (COO), 151.4, 148.9 (acyl-4′,5′), 147.4, 146.6 (acyl-4″,6″), 146.0 (galloyl-3,5), 145.4, 145.1, 145.0, 144.7 (HHDP-4,4′,6,6′), 143.2, 142.2 (acyl-1‴,2‴), 139.8 (galloyl-1), 137.5, 137.3, 136.5 (acyl-5″, HHDP-5,5′), 132.2, 131.6 (acyl-2‴,5‴), 129.8, 129.7 (acyl-3‴,4‴), 125.3, 124.8, 123.4 (acyl-2″, HHDP-2,2′), 120.2 (galloyl-1), 117.5, 117.0, 115.3 (acyl-1″, HHDP-1,1′), 112.6 (acyl-3″), 110.5 (galloyl-2,6), 110.1, 108.3 (HHDP-3,3′), 106.3 (acyl-6′), 92.2 (glc-1), 74.2 (glc-5), 71.2 (glc-2), 67.3 (glc-4), 64.3 (glc-6), 62.4 (glc-3), 61.5 ($\mathrm{CH_2OH}$), 54.9, 52.3, 47.6 (acyl-1′,2′,3′), 36.6 ($\mathrm{CH_2S}$). Negative FAB-MS *m/z*: 1083 $(\mathrm{M-H})^-$. *Anal.* Calcd for $\mathrm{C_{49}H_{36}N_2O_{25}S\cdot 7/2H_2O}$: C, 51.27; H, 3.78; N, 2.44. Found: C, 51.25; H, 4.12; N, 2.31.

Reaction of Geraniin (1) with *N*-Acetyl-L-cysteine A solution of 2.0 g (2.1 mmol) of geraniin (**1**) and 411 mg (2.5 mmol) of *N*-acetyl-L-cysteine in 500 ml of 0.1 M potassium–sodium phosphate buffer (pH 6.5) was stirred at 25 °C for 8 h. The reaction mixture was acidified with 2 M HCl to pH 2, and subjected to column chromatography over MCI-gel CHP 20P (30 mm i.d. × 300 mm) with water containing increasing proportions of methanol to yield **16** (154 mg, 0.14 mmol, 7%), **15** (460 mg, 0.41 mmol, 20%), **17** (279 mg, 0.22 mmol, 10%), **14** (113 mg, 0.12 mmol, 6%) and **14a** (41 mg, 0.04 mmol, 2%), together with recovery of **1** (755 mg, 38%).

For **15**: A white powder from water, mp 215—228 °C (dec.), $[\alpha]_D^{21}$ $-74.6°$ ($c=0.9$, methanol). ^{1}H-NMR (300 MHz, acetone-d_6+D_2O) δ: 7.54 (1H, d, $J=8$ Hz, NH), 7.51 (1H, s, che 3″-H), 7.18 (2H, s, galloyl-H), 7.10, 6.66 (each 1H, s, HHDP-H), 6.50 (1H, s, glc 1-H), 5.70 (1H, br s, glc 3-H), 5.53 (1H, d, $J=1$ Hz, glc 2-H), 5.35 (1H, d, $J=3.5$ Hz, glc 4-H), 5.24 (1H, dd, $J=7$, 1.5 Hz, che 3′-H), 4.98 (1H, d, $J=7$ Hz, che 2′-H), 4.89 (1H, t, $J=10$ Hz, glc 6-H), 4.79 (1H, br dd, $J=10$, 7 Hz, glc 5-H), 4.48 (1H, m, Cys 2-H), 4.34 (1H, dd, $J=10$, 8 Hz, glc 6-H), 4.34 (1H, br s, che 4′-H), 3.19 (1H, d, $J=2$ Hz, che 5′-H), 2.72—2.60 (2H, m, Cys 3-H_2), 1.93 (3H, s, $COCH_3$). ^{13}C-NMR (75 MHz, acetone-d_6+D_2O) δ: 173.7, 171.9, 171.6, 171.3, 169.8, 168.6, 166.2, 165.5, 164.9 (COO), 146.7 (che-6″), 146.1 (galloyl-3,5), 145.5, 145.2, 145.0, 144.7 (HHDP-4,4′,6,6′), 141.5, 139.9, 139.5, 137.8, 136.6 (che-4″,5″, galloyl-4, HHDP-5,5′), 125.7, 124.8 (HHDP-2,2′), 120.4, 120.2, 117.9, 117.2, 117.1, 115.3 (che-1″,2″,3″, galloyl-1, HHDP-1,1′), 110.8 (galloyl-2,6), 110.5, 107.9 (HHDP-3,3′), 91.3 (glc-1), 73.2 (glc-5), 70.1 (glc-2), 67.3 (che-2′), 65.9 (glc-4), 63.9 (glc-6), 62.6 (glc-3), 53.2 (Cys-2), 48.5 (che-4′), 44.2 (che-5′), 40.9 (che-3′), 38.2 (Cys-3), 22.7 (CH_3). Negative FAB-MS *m*/*z*: 1114 $(M-H)^-$. *Anal.* Calcd for $C_{46}H_{37}NO_{30}S \cdot 6H_2O$: C, 45.14; H, 4.04; N, 1.14. Found: C, 45.42; H, 3.91; N, 1.00.

For **16**: A white amorphous powder, $[\alpha]_D^{21}$ $-35.5°$ ($c=0.8$, methanol). ^{1}H-NMR (300 MHz, acetone-d_6+D_2O) δ: 7.16 (2H, s, galloyl-H), 7.06 (1H, s, che 3″-H), 6.90, 6.80 (each 1H, s, HHDP-H), 6.35 (1H, d, $J=4$ Hz, glc 1-H), 5.82 (1H, d, $J=3$ Hz, glc 4-H), 5.26 (1H, s, che 2′-H), 5.14 (1H, br s, glc 3-H), 4.80—4.65 (3H, m, glc 5-H, 6-H_2), 4.31 (1H, dd, $J=7$, 5 Hz, Cys 2-H), 4.25 (1H, d, $J=4$ Hz, glc 2-H), 4.23 (1H, d, $J=8$ Hz, che 3′-H), 3.95 (1H, d, $J=11$ Hz, che 5′-H), 3.65 (1H, dd, $J=11$, 8 Hz, che 4′-H), 3.22 (1H, dd, $J=13$, 5 Hz, Cys 3-H), 3.08 (1H, dd, $J=13$, 7 Hz, Cys 3-H), 1.91 (3H, s, $COCH_3$). ^{13}C-NMR (75 MHz, acetone-d_6+D_2O) δ: 172.3, 172.1, 171.5, 170.8, 168.7, 167.1, 165.6, 164.2 (COO), 146.2 (che-6″), 145.9 (galloyl-3,5), 145.3, 144.9 (3C), 143.7 (che-4‴ HHDP-4,4′,6,6′), 139.5, 139.2, 137.2, 136.7 (che-5″, galloyl-4, HHDP-5,5′), 125.3 (2C) (HHDP-2,2′), 120.5 (galloyl-1), 117.4, 116.7, 115.5 (2C) (che-1″,2″, HHDP-1, 1′), 110.6 (galloyl-2,6), 109.9, 109.4, 108.6 (che-3″, HHDP-3,3′), 94.7 (glc-1), 77.6 (che-2′), 74.1 (glc-5), 71.1 (glc-3), 69.9 (glc-2), 65.3, 64.5 (glc-4,6), 52.5 (Cys-2), 50.9 (che-4)′, 46.3 (che-5′), 37.0 (che-3′), 34.4 (Cys-3), 22.6 (CH_3). Negative FAB-MS *m*/*z*: 1132 $(M-H)^-$.

For **17**: A white amorphous powder, $[\alpha]_D^{21}$ $-23.6°$ ($c=0.7$, methanol). ^{1}H-NMR (500 MHz, acetone-d_6) δ: 7.86 [1H, d, $J=9$ Hz, Cys-B(cysteine connected to thioester) NH], 7.47 [1H, d, $J=8$ Hz, Cys-A(cysteine attached to C-3′) NH], 7.25 (1H, s, acyl 3″-H), 7.21 (2H, s, galloyl-H), 7.03, 6.64 (each 1H, s, HHDP-H), 6.54 (1H, d, $J=5$ Hz, glc 1-H), 5.88 (1H, d, $J=4$ Hz, glc 3-H), 5.49 (1H, d, $J=5$ Hz, glc 2-H), 5.26 (1H, d, $J=4$ Hz, glc 4-H), 5.08 (1H, ddd, $J=9$, 8, 5 Hz, Cys-B 2-H), 4.87 (1H, dd, $J=8$, 5 Hz, glc 5-H), 4.70 (1H, dd, $J=12$, 8 Hz, glc 6-H), 4.55 (1H, ddd, $J=8$, 8, 4 Hz, Cys-A 2-H), 4.33 (1H, d, $J=8$ Hz, acyl 1′-H), 4.20 (1H, dd, $J=12$, 5 Hz, glc 6-H), 3.80 (1H, dd, $J=14$, 8 Hz, acyl 2′-H), 3.76 (1H, dd, $J=14$, 5 Hz, Cys-B 3-H), 3.59 (1H, d, $J=14$ Hz, acyl 3′-H), 3.10 (1H, dd, $J=14$, 4 Hz, Cys-A 3H), 3.08 (1H, dd, $J=14$, 10 Hz, Cys-B 3-H), 2.89 (1H, dd, $J=14$, 8 Hz, Cys-A 3-H), 2.03 (3H, s, Cys-B $COCH_3$), 1.91 (3H, s, Cys-A $COCH_3$). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 200.7 (acyl-6′), 172.3 (Cys-B $\underline{CO}CH_3$), 172.2, 171.8 (Cys-A-1, Cys-B-1), 171.4 (Cys-A $\underline{CO}CH_3$), 170.0 (acyl-7′), 168.4 (HHDP-7′), 166.2 (HHDP-7), 165.3 (acyl-7″), 165.1 (galloyl-7), 147.8 (acyl-6″), 147.4 (acyl-4″), 146.1 (galloyl-3,5), 145.3, 145.1, 145.0, 144.9 (HHDP-4,4′,6,6′), 139.8 (galloyl-4), 137.4, 136.3 (HHDP-5,5′), 135.7 (acyl-5″), 125.4, 124.6 (HHDP-2,2′), 119.9 (galloyl-1), 117.1 (acyl-2″), 116.6 (acyl-1″), 116.4 (HHDP-1), 116.0 (acyl-5′), 114.7 (HHDP-1′), 113.7 (acyl-3″), 110.6 (galloyl-2,6), 109.6, 108.4 (HHDP-3,3′), 92.0 (glc-1), 89.3 (acyl-4′), 76.3 (glc-5), 74.6 (glc-2), 68.3 (glc-4), 64.9 (glc-6), 64.4 (glc-3), 57.0 (acyl-1′), 53.6 (Cys-A-2), 52.7 (acyl-2), 52.6 (acyl-3′), 50.7 (Cys-B-2), 35.5 (Cys A-3), 31.4 (Cys-B-3), 22.7, 22.6 (CH_3). Negative FAB-MS *m*/*z*: 1259 $(M-H)^-$, 1096 $(M-H-\text{acetylcysteine})^-$. *Anal.* Calcd for $C_{51}H_{44}N_2O_{32}S_2 \cdot 3/2H_2O$: C, 47.56; H, 3.68; N, 2.17. Found: C, 47.53; H, 3.94; N, 2.05. Assignments of the NMR signals were achieved with the aid of ^{1}H–^{1}H COSY, HSQC and HMBC spectra.

Conversion of 17 into 14 A solution of 100 mg (0.079 mmol) of **17** in 5 ml of 0.1 M potassium–sodium phosphate buffer (pH 7.0) was kept at 25 °C for 15 h. HPLC analysis of the reaction mixture showed that **17** (t_R 17.7 min) was almost completely changed into the intermediate **18** (t_R 11.7 min). Separation of the mixture by column chromatography on MCI-gel CHP 20P (20 mm i.d. × 200 mm) with water containing increasing proportions of methanol gave **14** (60 mg, 0.063 mmol, 80%).

Acknowledgment We thank Mr. K. Inada and Mr. N. Yamaguchi (Nagasaki University) for NMR and MS measurements, respectively. This work was supported by a Grant-in-Aid for Scientific Research (No. 05671753) from the Ministry of Education, Science and Culture of Japan, and by a Yamamura Yuichi Memorial WAKAN-YAKU Research Grant.

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