# Medicinal Flowers. XXIV.[1] Chemical Structures and Hepatoprotective Effects of Constituents from Flowers of *Hedychium coronarium*

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**The 80% aqueous acetone extract from the flowers of *Hedychium coronarium* was found to show a protective effect on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes. On the other hand, two new labdane-type diterpene glycosides, coronalactosides I (1) and II (2), and a new labdane-type trinorditerpene, coronadiene (3), were isolated together with 8 known compounds from the extracts, which were obtained with chloroform and 80% aqueous acetone from the flowers of *H. coronarium*. The structures of new constituents were elucidated on the basis of chemical and physicochemical evidence. In addition, the principal constituents, coronaririn C and 15-hydroxylabda-8(17),11,13-trien-16,15-olide, displayed hepatoprotective effects, which were stronger than that of the hepatoprotective agent, silybin.**

**Key words** coronalactoside; *Hedychium coronarium*; medicinal flower; Zingiberaceae; labdane-type diterpene; hepatoprotective effect

*Hedychium coronarium* KOEN. (Zingiberaceae) is widely cultivated in Japan, India, South China, and Southeast Asian countries. The rhizomes of *H. coronarium* have been used for the treatment of headache, contusion inflammation, and sharp pain due to rheumatism in Chinese traditional medicine. Previously, we reported that the extract from the rhizomes of *H. coronarium* showed inhibitory effects on increase in vascular permeability and nitric oxide production and on the release of $\beta$-hexosaminidase from RBL-2H3 cells and the structures of various farnesane-type sesquiterpenes and labdane-type diterpene were characterized.[2,3] However, the chemical constituents as well as the pharmacological properties of the flowers of *H. coronarium* have not been characterized. In the course of our serial studies on the bioactive constituents of medicinal flowers,[1,4—15] the 80% aqueous acetone extract from the flowers of *H. coronarium* was found to show a protective effect on D-galactosamine (D-GalN)-induced cytotoxicity in primary cultured mouse hepatocytes. In addition, we have isolated two new labdane-type diterpene glycosides, coronalactosides I (**1**) and II (**2**), and a new labdane-type trinorditerpene, coronadiene (**3**), together with 8 known compounds including labdane-type diterpene derivatives from the extracts, which were obtained with chloroform and 80% aqueous acetone from the flowers of *H. coronarium*. Furthermore, we examined the protective effects of principal constituents on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes. In this paper, we describe the isolation and structure elucidation of the new constituents (**1**—**3**) and the hepatoprotective effects of principal constituents from the flowers of *H. coronarium*.

The fresh flowers of *H. coronarium* were extracted with chloroform under room temperature [the yield (0.46%) was obtained from the fresh flowers]. Subsequently, the residue was extracted with 80% aqueous acetone under room temperature, followed by 80% aqueous acetone under reflux (0.75% and 0.43% were obtained, respectively). The 80% aqueous acetone extract obtained under room temperature was partitioned into an EtOAc–$H_2O$ mixture to furnish an EtOAc-soluble fraction (0.074%) and aqueous layer. The aqueous layer was further extracted with *n*-BuOH to give *n*-BuOH- and $H_2O$-soluble fractions (0.10% and 0.56%, respectively). In the same procedure, the EtOAc-, *n*-BuOH- and $H_2O$-soluble fractions were given from the 80% aqueous acetone extract obtained under reflux (0.18%, 0.044%, and 0.21%, respectively). The chloroform extract (0.46%), EtOAc-soluble fraction given from the 80% aqueous acetone extract obtained under room temperature, and *n*-BuOH-soluble fraction given from the 80% aqueous acetone extract obtained under reflux were subjected to normal-phase and reversed-phase column chromatographies, and finally HPLC to give coronalactosides I (**1**, 0.00029% from the flowers) and II (**2**, 0.00014%) and coronadiene (**3**, 0.00008%) together with 8 known compounds, (*E*)-labda-8(17),12-diene-15,16-dial (**4**, 0.00051%),[16] coronarins B (**5**, 0.00015%),[16] C (**6**, 0.00019%),[16] and D (**7**, 0.00029%),[16] 15-hydroxylabda-8(17),11,13-trien-16,15-olide [*E*] (**8**, 0.00015%),[17] 16-formyllabda-8(17),12-dien-15,11-olide (**9**, 0.00053%),[16] kaempferol 3-*O*-(2″-$\alpha$-L-rhamnopyranosyl)-$\beta$-D-glucuronopyranoside (**10**, 0.0043%),[18] and ferulic acid (0.00065%).[19]

Coronalactoside I (**1**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{28}$ −47.2° in MeOH). The IR spectrum of **1** showed absorption bands at 1752 and 1655 $cm^{-1}$ ascribable to lactone carbonyl and olefin functions and broad bands at 3450 and 1050 $cm^{-1}$ suggestive of an oligoglycoside structure. In the positive-ion fast atom bombardment (positive-ion FAB)-MS of **1**, a quasimolecular ion peak was observed at *m/z* 663 $(M+Na)^+$, while the negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 639 $(M-H)^-$ together with a fragment ion peak at *m/z* 493 $(M-C_6H_{11}O_4)^-$, which were derived by cleavage of the glycoside linkage of the terminal hexose part. The molecular formula, $C_{32}H_{48}O_{13}$, was determined by high-resolution (HR)-MS measurement of the quasimolecular ion peak $(M+Na)^+$. Acid hydrolysis of **1** with 1.0 M aqueous hydrochloric acid (HCl) liberated D-glucose and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector.[20]

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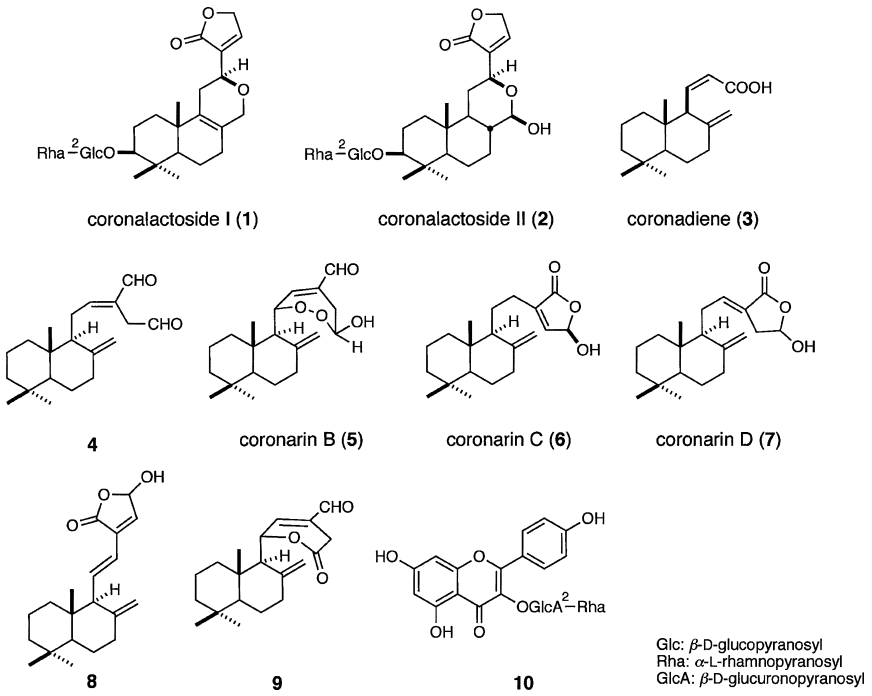


Chart 1. Structures of New Constituents from the Flowers of *H. coronarium*

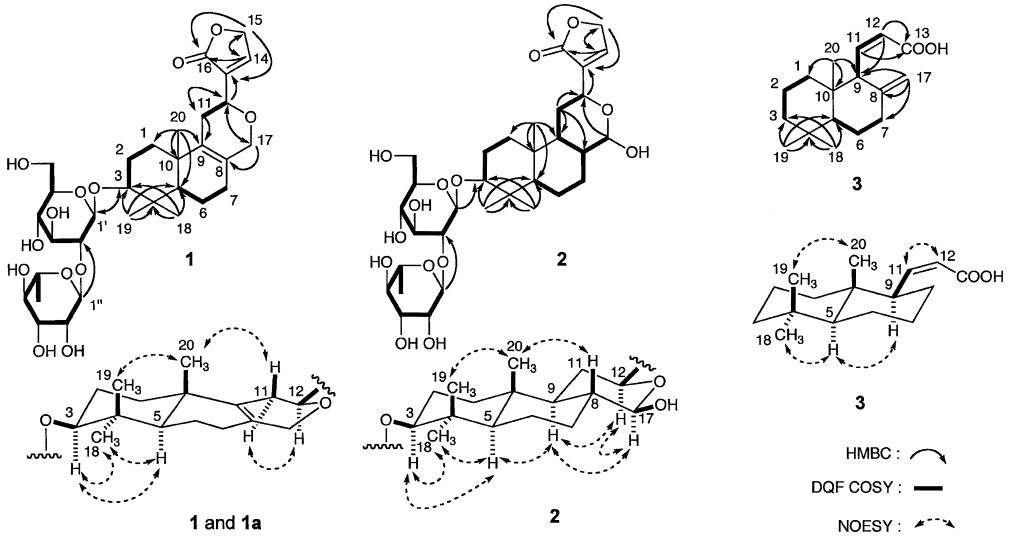


Fig. 1. Selected HMBC and NOE Correlations

Enzymatic hydrolysis of **1** with naringinase afforded a new aglycon termed coronalactone (**1a**), of which the molecular formula, $C_{20}H_{28}O_4$, was determined from a molecular ion peak [*m/z* 332 $(M)^+$] and by HR-EI (electron ionization)-MS measurement. The $^1$H- ($CDCl_3$) and $^{13}$C-NMR (Table 1) spectra of **1a**, which were assigned by various NMR experiments,[21] showed signals assignable to three methyls [$\delta$ 0.83, 1.00, 1.03 (3H each, all s, $H_3$-19, 20, 18)], two methylenes bearing an oxygen function [$\delta$ 3.99, 4.09 (1H each, both d, $J$=15.1 Hz, H-17), 4.84 (2H, d, $J$=1.4 Hz, H-15)], two methines bearing an oxygen function [$\delta$ 3.27 (1H, dd, $J$=3.4, 11.7 Hz, H-3), 4.38 (1H, m, H-12)], a tetra-substituted double bond [$\delta_C$ 125.1 (C-8) and 134.7 (C-9)], and a carbonyl carbon [$\delta_C$ 175.1 (C-16)]. As shown in Fig. 1, the double quantum filter correlation spectroscopy (DQF COSY) experiment on **1a** indicated the presence of partial structures written in bold lines. In a heteronuclear multiple-bond correlation (HMBC) experiment, long-range correlations were observed between the following protons and carbons: $H_2$-7 and C-8; H-12 and C-13, 17; H-14 and C-15, 16; $H_2$-15 and C-13, 14, 16; $H_2$-17 and C-8, 9, 12; $H_3$-18, 19 and C-3, 4, 5; $H_3$-20 and C-1, 5, 9, 10. On the basis of these evidence, the planar structure of **1a** was characterized. The $^1$H-NMR ($CD_3OD$) and $^{13}$C-NMR (Table 1) spectra[21] of **1** showed signals assignable to a coronalactone moiety together with a $\beta$-D-glucopyranosyl part [$\delta$ 4.41 (1H, d, $J$=7.6 Hz, H-1′)], and an $\alpha$-L-rhamnopyranosyl part [$\delta$ 5.38 (1H, s like, H-1″)]. The proton and carbon signals due to the glycoside moieties in the $^1$H- and $^{13}$C-NMR spectra of **1** were similar to those of floralginsenoside M.[9] Furthermore, the position of the glycoside linkages were determined by a HMBC experiment, which showed long-range correlations between the following

Table 1. $^{13}$C-NMR (125 MHz) Data for **1**—**3**, and **1a**

| Carbon | **1**[a)] | **1a**[b)] | **2**[a)] | **2**[c)] | **3**[b)] | Carbon | **1**[a)] | **2**[a)] | **2**[c)] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 36.7 | 35.2 | 38.4 | 37.4 | 40.8 | 1′ | 105.7 | 105.7 | 105.5 |
| 2 | 27.8 | 27.6 | 27.5 | 27.0 | 19.0 | 2′ | 78.9 | 79.5 | 80.0 |
| 3 | 90.0 | 78.7 | 90.1 | 88.7 | 42.1 | 3′ | 79.6 | 78.9 | 78.4 |
| 4 | 40.5 | 39.0 | 40.4 | 39.6 | 33.6 | 4′ | 72.2 | 72.2 | 72.1 |
| 5 | 52.9 | 50.8 | 56.0 | 54.7 | 54.4 | 5′ | 77.6 | 77.6 | 77.7 |
| 6 | 19.0 | 17.9 | 21.5 | 20.7 | 23.2 | 6′ | 62.8 | 62.8 | 62.8 |
| 7 | 28.2 | 27.4 | 29.6 | 29.2 | 36.6 | 1″ | 101.9 | 101.9 | 101.6 |
| 8 | 126.4 | 125.1 | 42.6 | 42.0 | 148.3 | 2″ | 72.1 | 72.1 | 72.5 |
| 9 | 136.0 | 134.7 | 53.0 | 51.9 | 60.6 | 3″ | 72.1 | 72.1 | 72.5 |
| 10 | 37.8 | 36.9 | 36.8 | 35.8 | 39.3 | 4″ | 74.0 | 74.0 | 74.2 |
| 11 | 27.7 | 26.9 | 30.6 | 29.8 | 150.7 | 5″ | 70.0 | 70.0 | 69.6 |
| 12 | 70.3 | 69.6 | 71.5 | 70.6 | 122.9 | 6″ | 18.0 | 18.0 | 18.7 |
| 13 | 135.1 | 135.5 | 135.4 | 135.4 | 170.5 | | | | |
| 14 | 148.9 | 145.1 | 148.7 | 146.5 | | | | | |
| 15 | 72.4 | 70.6 | 72.5 | 71.1 | | | | | |
| 16 | 175.1 | 172.4 | 174.9 | 173.0 | | | | | |
| 17 | 69.3 | 68.8 | 101.9 | 101.7 | 108.8 | | | | |
| 18 | 28.4 | 28.0 | 28.5 | 28.1 | 33.5 | | | | |
| 19 | 17.1 | 15.4 | 17.2 | 17.1 | 21.9 | | | | |
| 20 | 20.6 | 20.1 | 14.7 | 14.3 | 15.0 | | | | |

*a*) Measured in methanol-$d_4$; *b*) measured in $CDCl_3$; *c*) measured in pyridine-$d_5$.

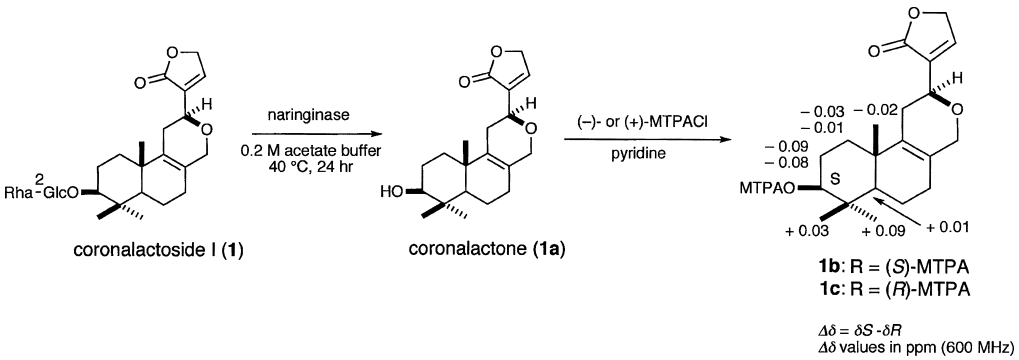


Fig. 2.

protons and carbons: H-3 and C-1′; H-1′ and C-3; H-1″ and H-2′. The relative stereostructure of **1** and **1a** were characterized by difference nuclear Overhauser enhancement spectroscopy (difference NOESY) experiment, which showed NOE correlation between the following proton pairs: H-3$\alpha$ and H-5$\alpha$, $H_3$-18; H-5$\alpha$ and $H_3$-18; H-11$\alpha$ and H-12$\alpha$; H-11$\beta$ and $H_3$-20; $H_3$-19 and $H_3$-20 (Fig. 1). Finally, the absolute configuration of 3-position in **1a** was characterized by the application of the modified Mosher's method. Namely, the (*S*)-MTPA ester (**1b**) was derived from **1a** upon reaction with (−)-2-methoxy-2-trifluoromethylphenylacetyl chloride [(−)-MTPACl] in pyridine. In addition, the (*R*)-MTPA ester (**1c**) was also obtained from **1a** using (−)-MTPACl in pyridine. As shown in Fig. 2, the signals due to protons attached to the 1, 2 and 20-positions in the (*S*)-MTPA ester (**1b**) were observed at higher fields compared with those of the (*R*)-MTPA ester (**1c**) [$\Delta\delta$: negative], while the signals due to protons on the 5, 18 and 19-positions in **1b** were observed at lower fields compared with those of **1c** [$\Delta\delta$: positive]. Thus, the absolute configuration at the 3-position in **1a** was determined to be *S*. Consequently, the absolute stereostructure of coronalactoside I (**1**) was determined as shown.

Coronalactoside II (**2**), obtained as a white powder with negative optical rotation ($[\alpha]_D^{28}$ −38.5° in MeOH), showed absorption bands at 3450, 1750 and 1050 $cm^{-1}$ assignable to hydroxyl, lactone carbonyl, and ether functions in the IR spectrum. The positive-ion and negative-ion FAB-MS of **2** exhibited quasimolecular ion peaks at *m/z* 681 $(M+Na)^-$ and *m/z* 657 $(M-H)^-$, respectively. In addition, the fragment ion peak was observed at *m/z* 511 $(M-C_6H_{11}O_4)^-$ in negative-ion FAB-MS. The HR-MS analysis revealed the molecular formula of **2** to be $C_{32}H_{50}O_{14}$. The acid hydrolysis of **2** liberated D-glucose and L-rhamnose, which were identified by HPLC analysis.[20] The $^1$H-NMR ($CD_3OD$) and $^{13}$C-NMR (Table 1) spectra[21] of **2** showed signals assignable to a diterpene moiety {three methyls [$\delta$ 0.87, 0.89, 1.05 (3H each, all s, $H_3$-20, 19, 18)], a methylene bearing an oxygen function [$\delta$ 4.90 (2H, s like, H-15)], three methines bearing an oxygen function [$\delta$ 3.19 (1H, dd, *J*=4.1, 11.7 Hz, H-3), 4.22 (1H, d like, *J*=9.6 Hz, H-12), 4.34 (1H, d, *J*=9.6 Hz, H-17)], and a carbonyl carbon [$\delta_C$ 174.9 (C-16)]}, a $\beta$-D-glucopyranosyl part [$\delta$ 4.41 (1H, d, *J*=7.6 Hz, H-1′)], and an $\alpha$-L-rhamnopyranosyl part [$\delta$ 5.37 (1H, s like, H-1″)]}. The proton and carbon signals due to the 3-*O*-glycoside moiety in the $^1$H- and $^{13}$C-NMR spectra of **2** were superimposable on those of floralginsenoside M[9] and coronalactoside I (**1**), while the proton and carbon signals assignable to the diterpene part of **2** resembled those of **1a**, except for the pyran moiety. The

Table 2. Effects of the 80% Aqueous Acetone Extract and Constituents from the Flowers of *H. coronarium* on D-GalN-Induced Cytotoxicity in Primary Cultured Mouse Hepatocytes[a)]

| Treatment | Inhibition (%) | | | |
|---|---|---|---|---|
| Conc. ($\mu$g/ml): | 0 | 3 | 10 | 30 |
| 80% Aqueous acetone extract | 0.0±2.8 | 13.3±3.0 | 22.9±0.6** | 40.1±2.6** |
| Conc. ($\mu$M): | 0 | 3 | 10 | 30 |
| **4** | 0.0±3.2 | 11.4±3.2 | 26.8±2.5** | — |
| Coronarin B (**5**) | 0.0±7.8 | 29.8±5.3 | 66.4±9.9* | — |
| Coronarin C (**6**) | 0.0±1.4 | 38.4±5.1** | 84.8±4.0** | 106.3±6.6** |
| Coronarin D (**7**) | 0.0±6.4 | 15.0±5.9 | 43.9±4.8** | 49.8±7.5** |
| **8** | 0.0±4.0 | 42.3±4.4** | 104.3±3.3** | 132.7±13.8** |
| **9** | 0.0±2.1 | 8.7±3.1 | 20.2±7.0** | 49.4±4.0** |
| **10** | 0.0±8.2 | 12.9±5.7 | 22.1±6.8 | 39.0±4.9** |
| Ferulic acid | 0.0±6.5 | 8.4±8.8 | 21.9±4.2 | 49.8±6.0 |
| Silybin[b,27)] | 0.0±0.3 | 4.8±1.1 | 7.7±0.7 | 45.2±8.8** |

*a*) Each value represents the mean±S.E.M. ($n$=4). Significantly different from the control, $*p<0.05$, $**p<0.01$. (—): Cytotoxicity. *b*) Commercial silybin (Funakoshi Co., Ltd., Tokyo, Japan) was used as a reference compound.

structure of **2** was characterized by means of DQF COSY and HMBC experiments (Fig. 1). That is, the DQF COSY data of **2** indicated the presence of the partial structures written in bold lines and long-range correlations in the HMBC experiment were observed between the following proton and carbon: $H_2$-11 and C-8, 9; H-12 and C-13; H-14 and C-15, 16; $H_2$-15 and C-13, 14, 16; H-17 and C-12; $H_3$-18, 19 and C-3, 4, 5; $H_3$-20 and C-1, 5, 9, 10; H-1′ and C-3; H-1″ and C′-2. The relative stereostructure of the diterpene part in **2** was characterized by difference nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs: H-3$\alpha$ and H-5$\alpha$, $H_3$-18; H-5$\alpha$ and H-9$\alpha$, $H_3$-18; H-8$\beta$ and $H_3$-20; H-9$\alpha$ and H-12$\alpha$, 17$\alpha$; H-12$\alpha$ and H-17$\alpha$; $H_3$-19 and $H_3$-20. On the basis of this evidence, the structure of coronalactoside II (**2**) was determined as shown.

Coronadiene (**3**), obtained as colorless oil with a positive optical rotation ($[\alpha]_D^{23}$ +15.2° in $CHCl_3$), showed absorption bands at 1697 and 1655 $cm^{-1}$ assignable to carboxyl and olefin functions in the IR spectrum, respectively. In the EI-MS of **3**, a molecular ion peak was observed at *m/z* 262 $(M)^+$. HR-EI-MS analysis revealed the molecular formula of **3** to be $C_{17}H_{26}O_2$. The $^1$H-NMR ($CDCl_3$) and $^{13}$C-NMR (Table 1) spectra[17)] of **3** showed the presence of three methyls [$\delta$ 0.84, 0.89, 0.90 (3H each, all s, $H_3$-19, 20, 18)], an *exo*-methylene [$\delta$ 4.42, 4.79 (1H each, both s like, $H_2$-17)], a disubstituted olefin [$\delta$ 5.83 (1H, d, $J$=8.6 Hz, H-12), 7.16 (1H, d, $J$=5.6, 8.6 Hz, H-11)], and a carboxyl carbon [$\delta_C$ 170.5 (C-13)]. The proton and carbon signals of **3** in the $^1$H- and $^{13}$C-NMR spectra were similar to those of (*E*)-14,15,16-trinorlabda-8(17),11-dien-13-oic acid.[22)] Furthermore, the planer structure of **3** was characterized by means of DQF COSY and HMBC experiments (Fig. 1). The geometry of the double bond in **3** was determined to be *cis* by the coupling constant (8.6 Hz) between two olefinic protons (H-11 and H-12). NOESY experiments on **3** showed correlations between the following proton pairs: H-5$\alpha$ and H-9$\alpha$, $H_3$-18; H-11 and H-12; $H_3$-19 and $H_3$-20, so that the relative stereochemistry of **3** could be characterized. Consequently, the structure of coronadiene was determined to be (*Z*)-14,15,16-trinorlabda-8(17),11-dien-13-oic acid (**3**).

Recently, we have reported the isolation and structure elucidation of several constituents with hepatoprotective effects from natural traditional medicine, *Camellia sinensis*,[23)] *Sedum sarmentosum*,[24,25)] and *Rhodiola sachalinensis*,[20)] and *Piper chaba*.[26)] Since the 80% aqueous acetone extract obtained under room temperature from the flowers of *H. coronarium* was found to show protective effects on D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes [inhibition: 40.1% (30 $\mu$g/ml)], the activities of the principal constituents **4**—**9** were examined. As shown in Table 2, labdane-type diterpene, coronarins C (**6**), and D (**7**), 15-hydroxylabda-8(17),11,13-trien-16,15-olide (**8**), and 16-formyllabda-8(17),12-dien-15,11-olide (**9**), showed the hepatoprotective effects. Particularly, the effects of coronarin C (**6**) and 15-hydroxylabda-8(17),11,13-trien-16,15-olide (**8**) were stronger than that of the hepatoprotective agent, silybin.[27)] These results suggested that the hydroxy-butenolide moiety was important to exerting the potent effect.

## Experimental

**General Experimental Procedures** The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l$=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and HR-EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-FAB-MS, JEOL JMS-SX 102A mass spectrometer; $^1$H-NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; $^{13}$C-NMR spectra, JEOL EX-270 (68 MHz) JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector; and HPLC column, YMC-Pack ODS-A (YMC, Inc., 250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60$F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 $F_{254S}$ (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 $WF_{254S}$ (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous $H_2SO_4$ followed by heating.

**Plant Material** The fresh flowers of *H. coronarium* were collected on Tanegashima Island, Kagoshima Prefecture, Japan in 2007 and identified by one of authors (M. Y.).

**Extraction and Isolation** The fresh flowers of *H. coronarium* (247 g) were finely minced and extracted 3 times with 80% aqueous acetone under

room temperature for 24 h. Evaporation of the solvent under reduced pressure provided an extract (2.6 g, 1.1% from the fresh flowers), which was used for bioassay. On the other hand, the fresh flowers of *H. coronarium* (7.2 kg) were finely minced and extracted 3 times with chloroform under room temperature for 24 h. The residue was extracted 3 times with 80% aqueous acetone under room temperature for 24 h. Furthermore, the residue was extracted 3 times with 80% aqueous acetone under reflux for 3 h. The chloroform extract (33.0 g, 0.46%), 80% aqueous acetone extract (53.7 g, 0.75%) obtained under room temperature, and 80% aqueous acetone extract (31.2 g, 0.43%) obtained reflux were provided by evaporation of the solvent under reduced pressure, respectively.

The chloroform extract (33.0 g) was subjected to ordinary-phase silica gel column chromatography [1.0 kg, *n*-hexane→*n*-hexane–EtOAc (20 : 1→10 : 1→3 : 1→1 : 1)→$CHCl_3$–MeOH (10 : 1→5 : 1)→MeOH] to give seven fractions [Fr. 1, Fr. 2, Fr. 3 (1.05 g), Fr. 4 (1.56 g), Fr. 5 (4.33 g), Fr. 6 (5.09 g), and Fr. 7]. Fraction 3 (1.05 g) was subjected to reversed-phase silica gel column chromatography [32 g, MeOH–$H_2O$ (80 : 20→90 : 10, v/v)→MeOH] to give five fractions [Fr. 3-1, Fr. 3-2 (163 mg), Fr. 3-3, Fr. 3-4, and Fr. 3-5]. Fraction 3-2 (163 mg) was further purified by HPLC [$CH_3CN$–$H_2O$ (80 : 20, v/v)] to give (*E*)-labda-8(17),12-diene-15,16-dial (**4**, 37 mg). Fraction 4 (1.56 g) was subjected to reversed-phase silica gel column chromatography [45 g, $CH_3CN$–$H_2O$ (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give five fractions [Fr. 4-1, Fr. 4-2 (51 mg), Fr. 4-3, Fr. 4-4, and Fr. 4-5]. Fraction 4-2 (51 mg) was further purified by HPLC [$CH_3CN$–$H_2O$ (80 : 20, v/v)] to give coronarin B (**5**, 11 mg). Fraction 5 (4.33 g) was subjected to reversed-phase silica gel column chromatography [130 g, $CH_3CN$–$H_2O$ (60 : 40→80 : 20, v/v)→ MeOH] to give five fractions [Fr. 5-1, Fr. 5-2, Fr. 5-3 (91 mg), Fr. 5-4, and Fr. 5-5]. Fraction 5-3 (91 mg) was further purified by HPLC [$CH_3CN$–$H_2O$ (80 : 20, v/v)] to give 16-formyllabda-8(17),12-dien-15,11-olide (**9**, 39 mg). Fraction 6 (5.09 g) was subjected to reversed-phase silica gel column chromatography [180 g, $CH_3CN$–$H_2O$ (60 : 40→80 : 20, v/v)→ MeOH] to give six fractions [Fr. 6-1 (530 mg), Fr. 6-2, Fr. 6-3, Fr. 6-4, Fr. 6-5, and Fr. 6-6]. Fraction 6-1 (530 mg) was further purified by HPLC [$CH_3CN$–$H_2O$ (20 : 80, v/v)] to give ferulic acid (47 mg).

The 80% aqueous acetone extract (53.7 g) obtained under room temperature was partitioned into an EtOAc–$H_2O$ (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (5.3 g, 0.074%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (7.3 g, 0.10%) and an $H_2O$-soluble fraction (41.0 g, 0.57%). The *n*-BuOH-soluble fraction (7.3 g) was subjected to reversed-phase silica gel column chromatography [220 g, MeOH–$H_2O$ (10 : 90→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give six fractions [Fr. 1, Fr. 2, Fr. 3 [=kaempferol 3-*O*-(2″-$\alpha$-L-rhamnopyranosyl)-$\beta$-D-glucuronopyranoside (**10**, 310 mg)], Fr. 4 (711 mg), Fr. 5 (290 mg), and Fr. 6]. Fraction 4 (711 mg) was further purified by HPLC [MeOH–$H_2O$ (50 : 50, v/v)] to give coronalactoside II (**2**, 9.8 mg). Fraction 5 (290 mg) was further purified by HPLC [MeOH–$H_2O$ (60 : 40, v/v)] to give coronalactoside I (**1**, 21 mg).

The 80% aqueous acetone extract (31.2 g) obtained under reflux was partitioned into an EtOAc–$H_2O$ (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (12.9 g, 0.18%) and an aqueous phase. The aqueous phase was further extracted with *n*-BuOH to give an *n*-BuOH-soluble fraction (3.2 g, 0.044%) and an $H_2O$-soluble fraction (15.0 g, 0.21%). The EtOAc-soluble fraction (12.9 g) was subjected to ordinary-phase silica gel column chromatography [390 g, *n*-hexane→*n*-hexane–EtOAc (20 : 1→10 : 1→3 : 1→1 : 1)→$CHCl_3$–MeOH (5 : 1→1 : 1)→MeOH] to give seven fractions [Fr. 1, Fr. 2, Fr. 3, Fr. 4 (2.34 g), Fr. 5, Fr. 6, and Fr. 7]. Fraction 4 (2.34 g) was subjected to reversed-phase silica gel column chromatography [390 g, MeOH–$H_2O$ (30 : 70→50 : 50→70 : 30→90 : 10, v/v)→MeOH] to give six fractions [Fr. 4-1, Fr. 4-2, Fr. 4-3 (104 mg), Fr. 4-4, Fr. 4-5, Fr. 4-6]. Fraction 4-3 (104 mg) was further purified by HPLC [$CH_3CN$–$H_2O$ (70 : 30, v/v)] to give coronadiene (**3**, 5.8 mg), coronarin C (**6**, 14 mg), coronarin D (**7**, 21 mg), and 15-hydroxylabda-8(17),11,13-trien-16,15-olide (**8**, 11 mg).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, $^1$H-NMR, $^{13}$C-NMR, and MS) with reported values.

Coronalactoside I (**1**): A white powder; $[\alpha]_D^{28}$ −47.2° (*c*=1.32, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 208 (4.04) nm; IR (KBr) $\nu_{max}$ 3450, 2962, 1752, 1655, 1050, 841 cm$^{-1}$; $^1$H-NMR ($CD_3OD$, 600 MHz) $\delta$: 0.91, 1.03, 1.09 (3H each, all s, $H_3$-19, 20, 18), 1.24 (1H, m, H-5), 1.90 (1H, m, $H_2$-7), 2.02, 2.41 (1H each, both m, H-11), 3.20 (1H, d like, *J*=11.6 Hz, H-3), 3.92, 4.05 (1H each, both d, *J*=15.1 Hz, $H_2$-17), 4.33 (1H, m, H-12), 4.41 (1H, d, *J*=7.6 Hz, H-1′), 4.87 (2H, s like, $H_2$-15), 5.38 (1H, s like, H-1″), 7.54 (1H, s like, H-14); $^{13}$C-NMR data see Table 1; positive-ion FAB-MS *m/z* 663 $[M+Na]^+$; negative-ion FAB-MS *m/z* 639 $[M-H]^-$, 493 $[M-C_6H_{11}O_4]^-$; HR-FAB-MS *m/z* 663.2988 (Calcd for $C_{32}H_{48}O_{13}$ $[M+Na]^+$, 663.2993).

Coronalactoside II (**2**): A white powder; $[\alpha]_D^{28}$ −38.5° (*c*=0.25, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 208 (4.54) nm; IR (KBr) $\nu_{max}$ 3450, 2960, 1750, 1050, 841 cm$^{-1}$; $^1$H-NMR ($CD_3OD$, 600 MHz) $\delta$: 0.87, 0.89, 1.05 (3H each, all s, $H_3$-20, 19, 18), 0.93 (1H, d like, *J*=12.3 Hz, H-5), 1.30 (1H, m, H-8), 3.19 (1H, dd, *J*=4.1, 11.7 Hz, H-3), 4.22 (1H, d like, *J*=9.6 Hz, H-12), 4.34 (1H, d, *J*=9.6 Hz, H-17), 4.41 (1H, d, *J*=7.6 Hz, H-1′), 4.90 (2H, s like, H-15), 5.37 (1H, s like, H-1″), 7.56 (1H, s like, H-14); $^1$H-NMR (pyridine-$d_5$, 600 MHz) $\delta$: 0.74, 1.18, 1.26 (3H each, all s, $H_3$-20, 19, 18), 0.80 (1H, d like, *J*=12.4 Hz, H-5), 1.08 (1H, m, H-9), 1.64 (1H, m, H-8), 2.00, 2.12 (1H each, both m, $H_2$-11), 3.38 (1H, dd, *J*=3.5, 11.6 Hz, H-3), 4.51 (1H, d like, *J*=11.0 Hz, H-12), 4.80 (1H, d, *J*=7.6 Hz, H-17), 4.83 (2H, s like, H-15), 4.97 (1H, d, *J*=7.6 Hz, H-1′), 6.61 (1H, s like, H-1″), 7.61 (1H, s like, H-14); $^{13}$C-NMR data see Table 1; positive-ion FAB-MS *m/z* 681 $[M+Na]^+$; negative-ion FAB-MS *m/z* 657 $[M-H]^-$, 511 $[M-C_6H_{11}O_4]^-$; HR-FAB-MS: *m/z* 681.3105 (Calcd for $C_{32}H_{50}O_{14}$ $[M+Na]^+$, 681.3098).

Coronadiene (**3**): Colorless oil; $[\alpha]_D^{23}$ +15.2° (*c*=0.97, $CHCl_3$); IR (KBr) $\nu_{max}$ 2926, 1697, 1655, 1283, 893 cm$^{-1}$; $^1$H-NMR ($CD_3OD$, 600 MHz) $\delta$: 0.84, 0.89, 0.90 (3H each, all s, $H_3$-19, 20, 18), 1.09 (1H, m, H-5), 1.08, 1.44 (1H each, both m, $H_2$-1), 1.20, 1.44 (1H each, both m, $H_2$-3), 1.35, 1.73 (1H each, both m, $H_2$-6), 1.41, 1.54 (1H each, both m, $H_2$-2), 2.09, 2.44 (1H each, both m, $H_2$-7), 2.51 (1H, d, *J*=5.6 Hz, H-9), 4.42, 4.79 (1H each, both s like, H-17), 5.83 (1H, d, *J*=8.6 Hz, H-12), 7.16 (1H, d, *J*=5.6, 8.6 Hz, H-11); $^{13}$C-NMR data see Table 1; EI-MS *m/z* 262 $[M]^+$; HR-EI-MS: *m/z* 262.1930 (Calcd for $C_{17}H_{26}O_2$ $[M+Na]^+$, 262.1933).

**Acid Hydrolysis of 1 and 2** Solution of **1** and **2** (each 1.0 mg) in 1 M HCl (2.0 ml) were each heated under reflux for 3 h. After cooling, each reaction mixture was neutralized with Amberlite IRA-400 ($OH^-$ form) and filtrated, and the solution was partitioned with EtOAc to give two layers. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC $NH_2$-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, $CH_3CN$–$H_2O$ (85 : 15, v/v); flow rate 0.8 ml/min; column temperature, room temperature. Identifications of L-rhamnose and D-glucose present in the aqueous layer were carried out by comparison of their retention times and optical rotations with those of authentic samples. $t_R$: L-rhamnose, 9.6 min (negative optical rotation); D-glucose, 13.5 min (positive optical rotation).

**Enzymatic Hydrolysis of 1 with Naringinase** A solution of **1** (8.9 mg, 0.014 mmol) in 0.1 M acetate buffer (pH 3.8, 1.0 ml) was treated with narignginase (45 mg, Sigma Chemical Co.), and the solution was stirred at 40 °C for 24 h. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by reversed-phase silica gel column chromatography [1 g, MeOH–$H_2O$ (30 : 70→50 : 50→70 : 30→90 : 10, v/v)→MeOH] to coronalactonol I (**1a**, 2.8 mg, 61%).

Coronalactonol I (**1a**): Colorless oil; $[\alpha]_D^{28}$ −12.9° (*c*=2.50, $CHCl_3$); IR (KBr) $\nu_{max}$ 3450, 1746, 1655 cm$^{-1}$; $^1$H-NMR ($CDCl_3$, 600 MHz) $\delta$: 0.83, 1.00, 1.03 (3H each, all s, $H_3$-19, 20, 18), 1.19 (1H, m, H-5), 1.33, 1.75 (1H each, both m, $H_2$-1), 1.54, 1.78 (1H each, both m, $H_2$-6), 1.63, 1.90 (1H each, both m, $H_2$-7), 1.63, 1.74 (1H each, both m, $H_2$-2), 1.96, 2.47 (1H each, both m, $H_2$-11), 3.27 (1H, dd, *J*=3.4, 11.7 Hz, H-3), 3.99, 4.09 (1H each, both d, *J*=15.1 Hz, $H_2$-17), 4.38 (1H, m, H-12), 4.84 (2H, d, *J*=1.4 Hz, $H_2$-15), 7.40 (1H, t, *J*=1.4 Hz, H-14)]; $^{13}$C-NMR data see Table 1; EI-MS *m/z* 332 $[M]^+$; HR-EI-MS: *m/z* 332.1992 (Calcd for $C_{20}H_{28}O_4$ $[M+Na]^+$, 332.1987).

**Preparation of the (*S*)- and (*R*)-MTPA Esters, 1b and 1c, with MTPA-Cl** Solution of **1a** (1.6 mg, 0.0048 mmol) in pyridine (1.0 ml) was treated with (−)-MTPA-Cl (0.01 ml, 0.048 mmol), and the mixture was stirred at room temperature for 3 h. Removal of the solvent from the reaction mixture under reduced pressure furnished a residue, which was purified by reversed-phase silica gel column chromatography [1 g, MeOH–$H_2O$ (70 : 30→80 : 20, v/v)→MeOH] to give (*S*)-MTPA ester derivative (**1b**, 0.8 mg, 30%). Using a similar procedure, (*R*)-MTPA ester derivative (**1c**, 1.0 mg, 44%) was obtained from **1a** (1.0 mg, 0.0042 mmol).

(*S*)-MTPA Ester **1b**: $^1$H-NMR ($CDCl_3$, 600 MHz) $\delta$: 0.86, 0.95, 1.01 (3H each, all s, $H_3$-19, 18, 20), 1.31 (1H, m, H-5), 1.41, 1.76 (1H each, both m, $H_2$-1), 1.65, 1.86 (1H each, both m, $H_2$-2), 4.73 (1H, dd, *J*=4.1, 12.4 Hz, H-3), 3.96, 4.11 (1H each, both d, *J*=15.8 Hz, $H_2$-17), 4.84 (2H, s like, H-15), 4.38 (1H, m, H-12), 7.39 (1H, s like, H-14)]; $^{13}$C-NMR ($CDCl_3$, 150 MHz) $\delta$: 16.5 (C-19), 17.8 (C-6), 20.1 (C-20), 23.6 (C-2), 26.8 (C-11), 27.2 (C-7), 28.1 (C-18), 34.8 (C-1), 36.7 (C-10), 37.9 (C-4), 50.8 (C-5), 68.7 (C-17), 69.5 (C-12), 70.5 (C-15), 83.8 (C-3), 125.3 (C-8), 134.2 (C-9), 135.4 (C-13), 145.2 (C-14), 172.3 (C-16).

(*R*)-MTPA Ester **1c**: $^1$H-NMR ($CDCl_3$, 600 MHz) $\delta$: 0.83, 0.86, 1.03 (3H each, all s, $H_3$-19, 18, 20), 1.30 (1H, m, H-5), 1.42, 1.79 (1H each, both m,

$H_2$-1), 1.74, 1.94 (1H each, both m, $H_2$-2), 4.75 (1H, dd, $J$=4.1, 12.4 Hz, H-3), 3.96, 4.11 (1H each, both d, $J$=15.8 Hz, $H_2$-17), 4.83 (2H, s like, H-15), 4.38 (1H, m, H-12), 7.39 (1H, s like, H-14)]; $^{13}$C-NMR ($CDCl_3$, 150 MHz) $\delta$: 16.3 (C-19), 17.8 (C-6), 20.1 (C-20), 23.9 (C-2), 26.8 (C-11), 27.2 (C-7), 27.7 (C-18), 34.8 (C-1), 36.8 (C-10), 38.0 (C-4), 50.8 (C-5), 68.7 (C-17), 69.5 (C-12), 70.5 (C-15), 83.6 (C-3), 125.4 (C-8), 134.2 (C-9), 135.4 (C-13), 145.2 (C-14), 172.4 (C-16).

**Protective Effect on Cytotoxicity Induced by D-GalN in Primary Cultured Mouse Hepatocytes** The hepatoprotective effects of the constituents were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay using primary cultured mouse hepatocytes. Hepatocytes were isolated from male ddY mice (30—35 g) by collagenase perfusion method.[28,29] The cell suspension at $4\times10^4$ cells in 100 $\mu$l William's E medium containing fetal calf serum (10%), penicillin G (100 units/ml), and streptomycin (100 $\mu$g/ml) was inoculated in a 96-well microplate, and precultured for 4 h at 37 °C under a 5% $CO_2$ atmosphere. The fresh medium (100 $\mu$l) containing D-GalN (2 mM) and a test sample were added and the hepatocytes were cultured for 44 h. The medium was exchanged with 100 $\mu$l of the fresh medium, and 10 $\mu$l of MTT (5 mg/ml in phosphate buffered saline) solution was added to the medium. After 4 h culture, the medium was removed, 100 $\mu$l of isopropanol containing 0.04 M HCl was then added to dissolve the formazan produced in the cells. The optical density (O.D.) of the formazan solution was measured by microplate reader at 562 nm (reference: 660 nm). Inhibition (%) was obtained by following formula.

$$\text{inhibition (\%)}=[(\text{O.D.}_{(\text{sample})}-\text{O.D.}_{(\text{control})})/(\text{O.D.}_{(\text{normal})}-\text{O.D.}_{(\text{control})})]\times100$$

**Statistics** Values were expressed as means±S.E.M. For statistical analysis, one-way analysis of variance followed by Dunnett's test was used. Probability ($p$) values less than 0.05 were considered significant.

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