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ANTIINFLAMMATORY PRINCIPLES AND THREE NEW LABDANE–TYPE DITERPENES, HEDYCHILACTONES A, B, AND C, FROM THE RHIZOME OF *HEDYCHIUM CORONARIUM* KOENG†

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Abstract — Three new labdane–type diterpenes named hedychilactones A, B, and C were isolated from the methanolic extract of the fresh rhizome of *Hedychium coronarium* KOENG. Their structures were elucidated on the basis of chemical and physicochemical evidence, which included the application of the allylic benzoate rule. The methanolic extract and diterpene constituents were found to inhibit the increase of vascular permeability induced by acetic acid in mice and nitric oxide production in lipopolysaccharide-activated mouse peritoneal macrophages.

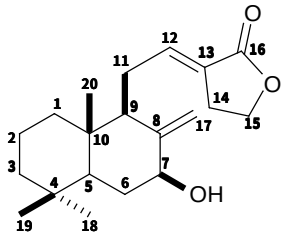
Hedychium coronarium KOENG (Zingiberaceae, 姜花 in Japanese) has been cultivated in Japan, China, India, and Southeast Asian countries. The rhizome of *H. coronarium*, which was known as a Chinese herbal medicine named "土羌活", has been used for the treatment of headache, sharp pain, and inflammation. As chemical constituents of this plant, several labdane-type diterpenes have been reported.[1-3] However, the pharmacological activity and bioactive constituents of this herbal medicine remained to be clarified.

In the course of our characterization studies on the bioactive constituents from Zingiberaceae herbal medicines,[4] we found that the methanolic extract from the fresh rhizome of *H. coronarium* showed the inhibitory activity on the increase of vascular permeability induced by acetic acid in mice. In this communication, we report the isolation and structure elucidation of three new labdane-type diterpene lactones named hedychilactones A (**1**), B (**2**), and C (**3**) from the methanolic extract together with the antiinflammatory activity of principal diterpene constituents, coronarin D (**4**) and its methyl ether (**5**), as well as the inhibitory activities of nine labdane-type diterpenes from this herbal medicine on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.

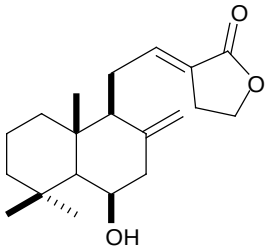
The methanolic extract from the fresh rhizome of *H. coronarium* cultivated in Japan, which inhibited the leakage of dye induced by acetic acid in mice after a single administration of 500 mg/kg dose, was partitioned into a mixture of ethyl acetate and water to furnish the ethyl acetate–soluble portion and water–soluble portion. The former portion was subjected to silica gel and ODS column chromatography and finally HPLC to furnish nine labdane–type diterpenes, hedychilactones A (**1**, 0.00021% from the fresh rhizome), B (**2**, 0.00023%), and C (**3**, 0.00045%), coronarins D (**4**,[1] 0.0083%), D methyl ether (**5**,[2] 0.0022%), and E (**6**,[3] 0.0011%), labda-8(17),13(14)-dien-15,16-olide (**7**,[5] 0.00006%), hedychenone (**8**,[6] 0.00090%) and 7-hydroxyhedychenone (**9**,[7] 0.00027%), and three farnesane–type sesquiterpenes, hedychiols A[8] (0.00084%), and B 8,9-diacetate[8] (0.000086%) and (+)-nerolidol[9] (0.0042%), and 5-hydroxy-3,7,4'-trimethoxyflavone[10] (0.00050%).

Hedychilactone A (**1**), colorless oil, $[\alpha]_D^{22}$ +12.3° (*c*=0.7, $CHCl_3$), $C_{20}H_{30}O_3$,[11] showed absorption bands at 3393, 1752, and 1676 cm^{-1} due to hydroxyl, lactone carbonyl, and olefin functions in the IR spectrum, while an absorption maximum due to the conjugated enone chromophore was observed at 226 (log ε 4.08) nm in the UV spectrum. In the electron impact (EI)-MS of **1**, molecular ion peak was observed at *m/z* 318 (M^+, 4) together with fragment ion peaks at *m/z* 300 ($M^+–H_2O$, 28) and 42 (base

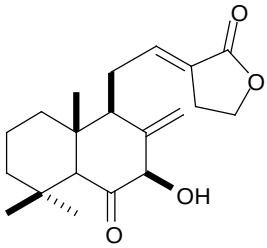
† Dedicated to Professor James P. Kutney, University of British Colombia, in cerebration of his 70th birthday



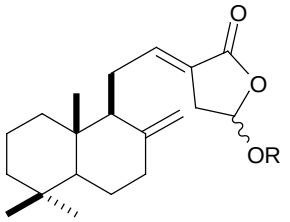
hedychilactone A (**1**)



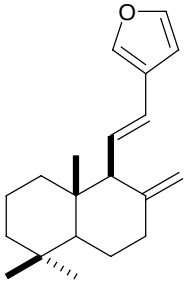
hedychilactone B (**2**)



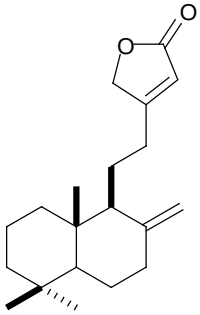
hedychilactone C (**3**)



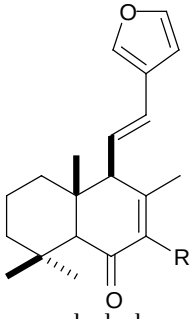
coronarin D (**4**)
: R=H
coronarin D methyl ether (**5**)
: R=CH_3



coronarin E (**6**)



labda-8(17),13(14)-dien-15,16-olide (**7**)



hedychenone (**8**)
: R=H
7-hydroxyhedychenone (**9**)
: R=OH

Chart 1

peak). The ^{1}H-12 and ^{13}C-NMR ($CDCl_3$, Table 1) spectra of **1** showed signals assignable to three methyls [δ 0.72, 0.83, 0.92 (all s, 20, 19 and 18-H_3)], an methylene bearing an oxygen function [δ 4.39 (2H, t-like, 15-H_2)], a methine bearing a hydroxyl group [δ 4.00 (dd-like, 7-H)], an *exo*-methylene [δ 4.58, 5.20 (both br s, 17-H_2)], and an olefin [δ 6.68 (m, 12-H)] together with six methylenes (1, 2, 3, 6, 11, 14-H_2), two methines (5, 9-H), and five quaternary carbons (4, 8, 10, 13, 16-C). The planar structure of **1** was constructed on the basis of various NMR experiments[13] as shown in Figure 1. Furthermore, the relative structure of **1** was characterized on the basis of the nuclear Overhauser effect spectroscopy (NOESY) experiment, in which the NOE correlations were observed between the following proton pairs of **1** (19-H_3 and 20-H_3; 5-H and 7-H, 9-H, 18-H_3). The above-mentioned evidence led us to confirm the skeleton of hedychilactone A (**1**) to be 7β-hydroxy-8(17),12-labdadien-16,15-olide.

Treatment of **1** with diisobutylalminium hydride (DIBAL) gave a reductant (**1a**).[14] In the NOESY experiment of **1a**, the NOE correlation of **1a** was observed between the 12-olefin proton [δ 5.48 (dd, *J*=6.4, 6.7 Hz, 12-H)] and 16-hydroxymethylene protons [δ 4.01 (2H, s, 16-H_2)] as shown in Figure 2, thus the geometry of **1a** was confirmed to be 12*E*-form. In addition, the CD spectrum of 7-*O*-*p*-bromobenzoate derivative (**1b**),[15] which was prepared by treatment of **1** with *p*-bromobenzoyl chloride in pyridine, showed positive Cotton effects at 245 nm ($\Delta\varepsilon$ +6.71) and 226 nm ($\Delta\varepsilon$ –2.25), indicating that the absolute configuration of the 7-position in **1b** was determined to be *S* by the application of the allylic benzoate rule.[16]

Hedychilactone B (**2**), colorless oil, $[\alpha]_D^{28}$ +10.6°(*c*=0.6, $CHCl_3$), $C_{20}H_{30}O_3$,[11] the positive-ion fast atom bonbardment (FAB)-MS: *m/z* 319 $(M+H)^+$, showed absorption bands at 3496, 1750, and 1674 cm^{-1} ascribable to hydroxyl, lactone carbonyl, and olefin functions in its IR spectrum. The UV spectrum of **2** showed absorption maximum at 227 nm (log ε 4.08) ascribable to a conjugated enone chromophore. The ^{1}H-17 and ^{13}C-NMR ($CDCl_3$, Table 1) spectra of **2**, which indicated the presence of the same functional groups as those of **1**, showed signals due to three methyls [δ 1.02, 1.05, 1.22 (all s, 18, 20 and 19-H_3)], a methylene bearing an oxygen function [δ 4.39 (2H, t-like, 15-H_2)], a methine

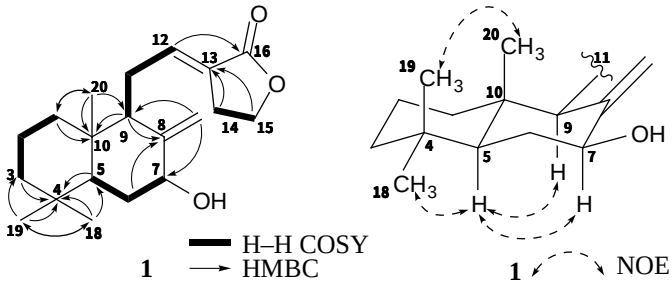


Figure 1

bearing a hydroxyl group [δ 4.40 (ddd, *J*=0.6, 7.0, 7.3 Hz, 6-H)], an *exo*-methylene [δ 4.67, 5.00 (both br s, 17-H_2)], and an olefin [δ 6.72 (m, 12-H)] together with six methylenes (1, 2, 3, 7, 11, 14-H_2), two methines (5, 9-H), and five quaternary carbons (4, 8, 10, 13, 16-C). As shown in Figure 3, the plane structure of **2** was elucidated on the basis of various NMR spectral data.[13] In addition, the relative stereostructure of **2** was elucidated by NOESY experiment, in which the NOE correlations were observed between the following proton pairs (19-H_3 and 20-H_3; 5-H and 6-H, 9-H, 18-H_3). Furthermore, treatment of **2** with DIBAL yielded a 6,15,16-triol derivative (**2a**),[18] whose NOESY experiment showed a NOE correlation between 12-olefin proton [δ 5.53 (dd, *J*=6.4, 6.4 Hz, 12-H)] and 16-hydroxymethyl proton [δ 4.03 (2H, s, 16-H_2)] pair. On the basis of this evidence, the geometric structure of **2** was determined to be shown.

Hedychilactone C (**3**), colorless oil, $[\alpha]_D^{23}$ +23.8°(*c*=0.7, $CHCl_3$), $C_{20}H_{28}O_4$,[11] EI-MS *m/z* 332 (M^+, 21), 314 (M^+–H_2O, 8) and 112 (base peak), showed an absorption maximum at 222 nm (log ε 3.92) suggestive of the conjugated enone chromophore in the UV spectrum. The IR spectrum of **3** showed absorption bands at 3492, 1750, 1717, and 1675 cm^{-1} ascribable to hydroxyl, lactone carbonyl, carbonyl, and olefin functions. The ^{1}H-[19] and ^{13}C-NMR ($CDCl_3$, Table 1) spectra of **3** showed signals assignable to three methyls [δ 0.67, 1.00, 1.26 (all s, 20, 18 and 19-H_3)], a methylene bearing an oxygen function [δ 4.42 (2H, t-like, 15-H_2)] and a methine bearing a hydroxyl group [δ 4.49 (br s, 7-H)], an *exo*-methylene [δ 4.67, 5.44 (both br s, 17-H_2)], and an olefin [δ 6.75 (m, 12-H)]. Those spectral data of **3** were similar to those of **1**, except for the presence of the signal due to the carbonyl group [δc 207.9 (6-C)]. Various NMR data[13] as shown in Figure 4 led us to confirm the skeleton of **3** being the 6-oxo-derivative of hedychilactone A (**1**). In the NOESY experiment on **3**, NOE correlations were observed between the following proton pairs (19-H_3 and 20-H_3; 5-H and 7-H, 9-H, 18-H_3). On the basis of this evidence, the stereostructure of **3** was determined.

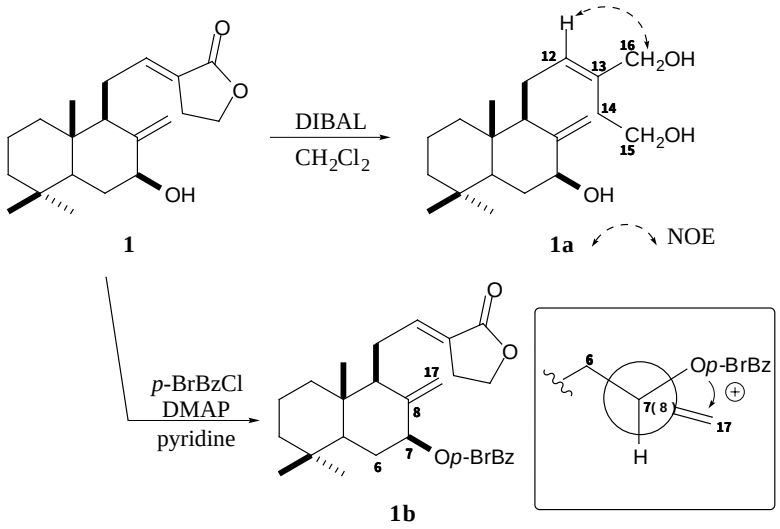


Figure 2

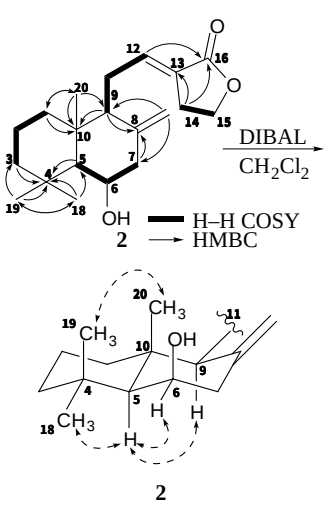


Figure 3

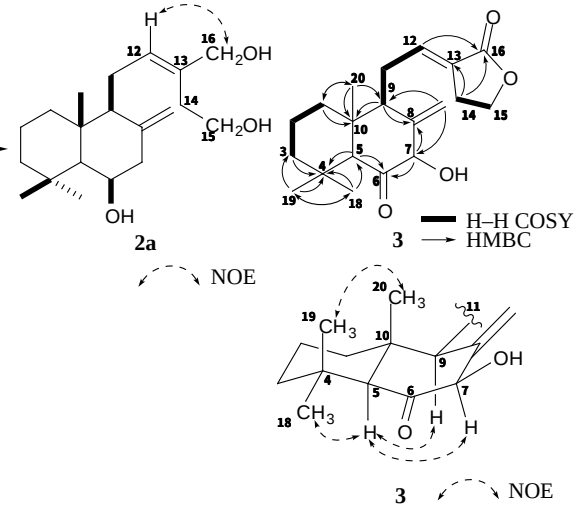


Figure 4

Table 1. ^{13}C-NMR Data for **1**—**3**

	1	**2**	**3**
C-1	39.1	42.2	38.8
C-2	19.3	19.5	18.8
C-3	41.8	43.8	42.2
C-4	33.5	34.4	32.7
C-5	53.0	57.3	64.1
C-6	33.5	69.1	207.9
C-7	73.6	47.3	79.9
C-8	150.1	143.8	145.7
C-9	54.4	56.8	53.7
C-10	39.2	40.6	42.4
C-11	25.2	25.4	25.4
C-12	141.5	141.6	140.2
C-13	124.9	124.9	125.7
C-14	25.3	25.2	25.3
C-15	65.3	65.3	65.3
C-16	171.2	171.2	171.1
C-17	104.3	111.2	107.7
C-18	33.5	33.6	32.5
C-19	21.6	23.6	21.9
C-20	14.4	17.1	15.6

Measured in $CDCl_3$ at 125 MHz.

In order to clarify the antiinflammatory activity of this herbal medicine, we examined the effects of principal diterpene constituents, coronarin D (**4**) and D methyl ether (**5**), on acetic acid-induced increase of vascular permeablility in mice.[20] As shown in Table 2, **4** and **5** (25—50 mg/kg, *p.o.*) dose-dependently inhibited the leakage of dye.

On the other hand, the inhibitory effects of constituents from *H. coronarium* against NO production were examined using lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.[4c,4f,21] Nine labdane-type diterpenes (**1**—**9**) were found to inhibit the overproduction of NO as shown in Table 3, especially, **4**, **7**, and **8** showed potent inhibitory activity.

The inhibitory effects of these compounds on acetic acid-induced increase of vascular permeablility and/or NO production in LPS-activated mouse peritoneal macrophages may be important evidence substantiating the traditional effects of this herbal medicine for the treatment of inflammation.

Table 2. Effects of MeOH ext., Coronarin D (**4**), and D methyl ether (**5**) from *H. coronarium* on Increase of Vascular Permeability Induced by Acetic Acid in Mice

	Dose (mg/kg, *p.o.*)	*N*	Amount of leaking dye (μg/animal)
Control	—	9	252.6±15.8
MeOH ext.	250	8	267.4±11.4
	500	7	195.1±14.9*
Control	—	11	223.9±11.7
Coronarin D (**4**)	12.5	7	188.0±15.8
	25	7	166.9±13.9*
	50	8	145.6±7.7**
Coronarin D methyl ether (**5**)	25	7	215.0±16.3
	50	7	166.0±16.4*
Aminopyrine	100	7	91.6±10.7**

Male ddY mice weighing 21—23 g were used. Two percent (w/v) Evans Blue solution in saline was injected intravenously (10 ml/kg) into the tail vein 55 min after the administration of a test compound. Five minutes later 1% (w/v) acetic acid solution in saline was injected intraperitoneally (10 mL/kg), and 20 min later the mice were sacrificed by cervical dislocation and the abdomen was immediately opened. After washing of the peritoneal cavity with 8 mL of saline, the washed solution was filtered through glass wool, and 0.1 mL of 1 M NaOH was added. The solution was filled up to 10 mL with saline, and the absorbance was measured at 620 nm. Vascular permeability was assessed by the amount of the dye which had leaked into the peritoneal cavity.

Each value represents the mean±S.E.M.

Significantly different from the control: *p<0.05, **p<0.01.

Table 3. Inhibitory Effects of Constituents from *H. coronarium* on NO Production in LPS–Activated Mouse Peritoneal Macrophages

	Inhibition (%)					
	0 μM	1 μM	3 μM	10 μM	30 μM	100 μM
Hedychilactone A (**1**)	0.0±0.9	5.2±2.2	4.4±4.9	27.2±3.2**	62.6±4.7**	70.6±1.3**
Hedychilactone B (**2**)	0.0±3.8	-4.2±8.2	10.4±1.5	18.9±2.1*	53.1±2.5**	69.2±1.5**[#]
Hedychilactone C (**3**)	0.0±4.5	-0.7±1.4	4.1±4.4	10.0±3.2	5.9±2.7	51.7±3.2**
Coronarin D (**4**)	0.0±1.2	-8.2±2.2	-8.8±1.7	23.2±6.6**	89.4±1.5**	101.9±0.2**[#]
Coronarin D methyl ether (**5**)	0.0±4.5	-1.4±1.6	4.1±3.5	16.9±1.9**	72.0±2.9**	98.6±1.1**[#]
Coronarin E (**6**)	0.0±1.1	-15.3±1.1	-2.7±3.7	9.2±3.9	47.3±2.8**	93.3±0.3**
Labda-8(17),13(14)-dien-15,16-olide (**7**)	0.0±5.3	-4.1±2.3	3.1±4.2	27.4±2.2**	95.4±1.0**	100.2±1.3**[#]
Hedychenone (**8**)	0.0±3.1	12.5±2.0*	30.5±4.7**	54.3±3.3**	92.2±0.6**	102.3±0.1*[#]
7-Hydroxyhedychenone (**9**)	0.0±3.1	3.3±3.8	3.1±1.1	6.0±3.8	34.6±4.2**	91.7±1.0**
L-NMMA	0.0±1.1	4.4±2.0	2.0±1.6	17.7±2.8**	52.3±1.5**	79.2±0.9**

Each value represents the mean±S.E.M. (*N*=4).

Significantly different from the control: *p<0.05, **p<0.01.

[#]Cytotoxic effect was observed.

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11 The molecular composition of the compound given with the chemical formula was determined by high-resolution EI-MS or FAB-MS.

12 **1**: ^{1}H-NMR ($CDCl_3$) δ : 0.72, 0.83, 0.92 (3H each, all s, 20, 19, and 18-H_3), 1.06 (1H, ddd, *J*=4.0, 12.5, 12.5 Hz, 1α-H), 1.18 (1H, dd, *J*=5.5, 11.9 Hz, 5-H), 1.21 (1H, m, 3α-H), 1.29 (1H, ddd, *J*=11.3, 11.9, 12.8 Hz, 6β-H), 1.45 (1H, br d, *J*=*ca*. 13 Hz, 3β-H), 1.54, 1.58 (1H each, both m, 2-H_2), 1.69 (1H, br d, *J*=*ca*. 13 Hz, 1β-H), 1.81 (1H, br d, *J*=*ca*. 11 Hz, 9-H), 2.11 (1H, ddd, *J*=2.4, 5.5, 12.8 Hz, 6α-H), 2.29, 2.40 (1H each, both m, 11-H_2), 2.88 (2H, m, 14-H_2), 4.00 (1H, dd-like, 7-H), 4.39 (2H, t-like, 15-H_2), 4.58, 5.20 (1H each, both br s, 17-H_2), 6.68 (1H, m, 12-H).

13 The ^{1}H- and ^{13}C-NMR spectra of new compounds (**1**—**3**) and their derivatives (**1a**, **1b**, and **2a**) were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.

14 **1a**: ^{1}H-NMR ($CDCl_3$) δ : 0.70, 0.82, 0.91 (3H each, all s, 20, 19, and 18-H_3), 1.03 (1H, ddd, *J*=4.0, 12.8, 12.8 Hz, 1α-H), 1.16 (1H, dd, *J*=2.4, 12.8 Hz, 5-H), 1.18 (1H, ddd, *J*=4.6, 13.1, 13.7 Hz, 3α-H), 1.28 (1H, m, 6β-H), 1.43 (1H, br d, *J*=*ca*. 14 Hz, 3β-H), 1.53, 1.57 (1H each, both m, 2-H_2), 1.64 (1H, br d, *J*=*ca*. 12 Hz, 9-H), 1.74 (1H, br d, *J*=*ca*. 13 Hz, 1β-H), 2.10 (1H, ddd, *J*=2.4, 5.5, 11.0 Hz, 6α-H), 2.13, 2.33 (1H each, both m, 11-H_2), 2.45 (2H, m, 14-H_2), 3.75 (2H, m, 15-H_2), 3.99 (1H, dd, *J*=5.5, 11.0 Hz, 7-H), 4.01 (2H, s, 16-H_2), 4.64, 5.18 (1H each, both br s, 17-H_2), 5.48 (1H, dd, *J*=6.4, 6.7 Hz, 12-H).

15 **1b**: ^{1}H-NMR ($CDCl_3$) δ : 0.80, 0.85, 0.92 (3H each, all s, 20, 19, and 18-H_3), 1.11 (1H, ddd, *J*=4.3, 12.5, 12.8 Hz, 1α-H), 1.21 (1H, ddd, *J*=4.3, 11.2, 13.1 Hz, 3α-H), 1.23 (1H, dd, *J*=4.3, 13.1 Hz, 5-H), 1.30 (1H, ddd, *J*=11.6, 13.1, 13.1 Hz, 6β-H), 1.47 (1H, br d, *J*=*ca*. 13 Hz, 3β-H), 1.51, 1.60 (1H each, both m, 2-H_2), 1.72 (1H, br d, *J*=*ca*. 13 Hz, 1β-H), 1.96 (1H, br d, *J*=*ca*. 11 Hz, 9-H), 2.18 (1H, m, 6α-H), 2.31, 2.44 (1H each, both m, 11-H_2), 2.88 (2H, m, 14-H_2), 4.38 (2H, t-like, 15-H_2), 4.60, 5.12 (1H each, both br s, 17-H_2), 5.39 (1H, dd, *J*=5.5, 11.6 Hz, 7-H), 6.70 (1H, m, 12-H), 7.60, 7.97 (2H each, both d, *J*=8.5 Hz, Ph).

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17 **2**: ^{1}H-NMR ($CDCl_3$) δ : 1.02, 1.05, 1.22 (3H each, all s, 18, 20 and 19-H_3), 1.11 (1H, d-like, 5-H), 1.12 (1H, ddd, *J*=3.7, 13.4, 13.7 Hz, 1α-H), 1.18 (1H, ddd, *J*=3.4, 13.4, 13.7 Hz, 3α-H), 1.39 (1H, br d, *J*=*ca*. 14 Hz, 3β-H), 1.52, 1.65 (1H each, both m, 2-H_2), 1.72 (1H, br d, *J*=*ca*. 14 Hz, 1β-H), 1.93 (1H, br s, *J*=*ca*. 11 Hz, 9-H), 2.28, 2.38 (1H each, both m, 11-H_2), 2.35 (2H, m, 7-H_2), 2.88 (2H, m, 14-H_2), 4.39 (2H, t-like, 15-H_2), 4.40 (1H, ddd, *J*=0.6, 7.0, 7.3 Hz, 6-H), 4.67, 5.00 (1H each, both br s, 17-H_2), 6.72 (1H, m, 12-H).

18 **2a**: ^{1}H-NMR ($CDCl_3$) δ : 1.01, 1.02, 1.21 (3H each, all s, 18, 20 and 19-H_3), 1.10 (1H, ddd, *J*=3.7, 13.0, 13.1 Hz, 1α-H), 1.10 (1H, d-like, 5-H), 1.18 (1H, ddd, *J*=3.7, 12.5, 13.0 Hz, 3α-H), 1.38 (1H, br d, *J=ca*. 13 Hz, 3β-H), 1.51, 1.63 (1H each, both m, 2-H_2), 1.76 (1H, br d, *J=ca*. 13 Hz, 1β-H), 1.77 (1H, br d, *J=ca*. 10 Hz, 9-H), 2.11, 2.33 (1H each, both m, 11-H_2), 2.35 (2H, br s, 7-H_2), 2.45 (2H, m, 14-H_2), 3.76 (2H, m, 15-H_2), 4.03 (2H, s, 16-H_2), 4.38 (1H, br s, 6-H), 4.73, 5.01 (1H each, both br s, 17-H_2), 5.53 (1H, dd, *J*=6.4, 6.4 Hz, 12-H).

19 **3**: ^{1}H-NMR ($CDCl_3$) δ : 0.67, 1.00, 1.26 (3H each, all s, 20, 18 and 19-H_3), 1.15 (1H, ddd, *J*=4.3, 13.1, 13.1 Hz, 3α-H), 1.28 (1H, m, 1α-H), 1.42 (1H, ddd, *J*=2.4, 2.4, 13.1 Hz, 3β-H), 1.61 (2H, m, 2-H_2), 1.80 (1H, ddd, *J*=3.1, 3.1, 12.5 Hz, 1β-H), 2.26 (1H, br s, 5-H), 2.36, 2.50 (1H each, both m, 11-H_2), 2.39 (1H, br d, *J=ca*. 11 Hz, 9-H), 2.91 (2H, m, 14-H_2), 4.42 (2H, t-like, 15-H_2), 4.49 (1H, br s, 7-H), 4.67, 5.44 (1H each, both br s, 17-H_2), 6.75 (1H, m, 12-H).

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