Novel, Complex Flavonoids from *Mallotus philippensis* (Kamala Tree)

by **Miyuki Furusawa**[a]), **Yoshimi Ido**[b]), **Toshiyuki Tanaka***[a]), **Tetsuro Ito**[a]), **Ken-ichi Nakaya**[a]), **Iliya Ibrahim**[c]), **Masayoshi Ohyama**[c]), **Munekazu Iinuma**[c]), **Yoshiaki Shirataka**[d]), and **Yoshikazu Takahashi**[e])

[a]) Gifu Prefectural Institute of Health & Environmental Sciences, 1-1 Naka-fudogaoka, Kakamigahara 504-0838, Japan (phone: +81-(0)583-80-2120; fax: +81-(0)583-71-5016; e-mail: yhy06063@nifty.ne.jp)

[b]) Gifu Prefectural Forest Science Research Institute, 1128-1 Sodai, Mino 501-3714, Japan

[c]) Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan

[d]) Faculty of Pharmaceutical Sciences, Josai Universiy, 1-1 Keyakidai, Sakaido, Saitama 350-0295, Japan

[e]) Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo, Japan

One new flavanone, 4′-hydroxyisorottlerin (**2**), and two new chalcone derivatives, kamalachalcones C (**3**) and D (**4**), were isolated from *Mallotus philippensis* (kamala tree). The largest compound (**4**; M_r 1098 g/mol) was shown to possess a unique, fused-ring system made of two hydroxy-chalcone units, giving rise to eight fused benzene/pyran units. From the same plant, the following six known compounds were also isolated: kamalachalcone A (**5**) and B (**6**), isoallorottlerin (**7**), isorottlerin (**8**), 5,7-dihydroxy-8-methyl-6-prenylflavanone (**9**); 6,6-dimethylpyrano(2″,3″:7,6)-5-hydroxy-8-methylflavanone (**10**), and rottlerin (**1**). The structures of the new compounds were confirmed by in-depth spectral analyses, including 2D-NMR techniques, and the full ^{13}C-NMR assignments of the known flavanones **1** and **7**–**10** are published for the first time.

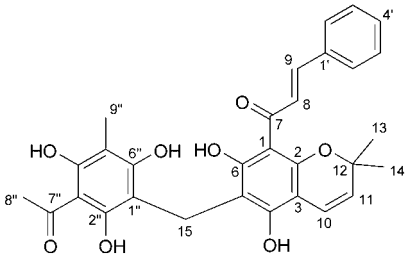
Introduction. – Granular hairs on the surface of the fruits of *Mallotus philippensis*[1]) MUELL (kamala tree) are covered with reddish exudates called 'kamala', which has been used both as a drug and as a dye. In previous works, the occurrence of many novel flavonoids has been reported from this plant [1–6], some of which contain isoprenyl and/or Me group(s), another being condensed with an acetophenone moiety *via* a CH_2 linkage, as is rottlerin (**1**). Some cognates have also been found in the pericarps of *Mallotus japonicus* [7][8], and they showed cytotoxic activity towards KB L-5178Y cell lines. In the case of rottlerin (**1**), an inhibitory effect towards protein kinase C was reported [9], and prenylated chalcones are known to inhibit nitric oxide (NO) production [6].

In a previous paper on *M. philippensis* [10], we reported novel natural products with a unique ring system formed by the dimerization of two chalcone units. In our continuing research of kamala, a further examination of the acetone soluble part of the extract has now resulted in the isolation of three new flavonoids: 4′-hydroxyisorottlerin (**2**), kamalachalcone C (**3**), and kamalachalcone D (**4**). These new compounds were isolated together with several known constituents: kamalachalcones A (**5**) and B (**6**) [10]; isoallorottlerin (**7**) [5]; isorottlerin (**8**) [5]; 5,7-dihydroxy-8-methyl-6-prenylflavanone (=6-*C*-prenyl-8-*C*-methylpinocembrin; **9**) [11]; 6,6-dimethylpyrano(2″,3″:7,6)-5-hydroxy-8-methylflavanone (**10**) [5], and rottlerin[2]) (**1**) [1]. In the present paper, we

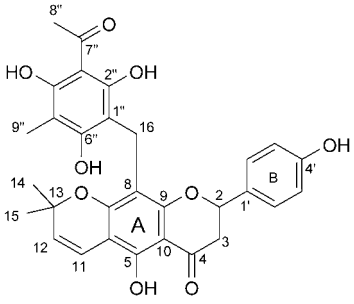
[1]) Also referred to as *M. philippinensis*

[2]) Also named *mallotoxin*.

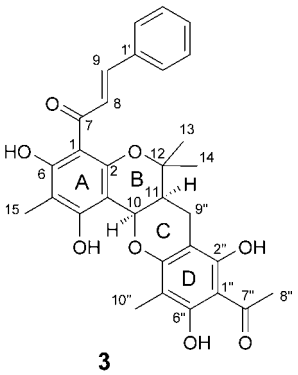
wish to report the isolation and structural elucidation of the novel compounds **2**–**4**, as well as the ^{13}C-NMR assignments of the above known flavanones.



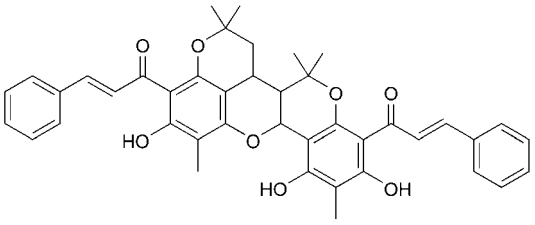
1



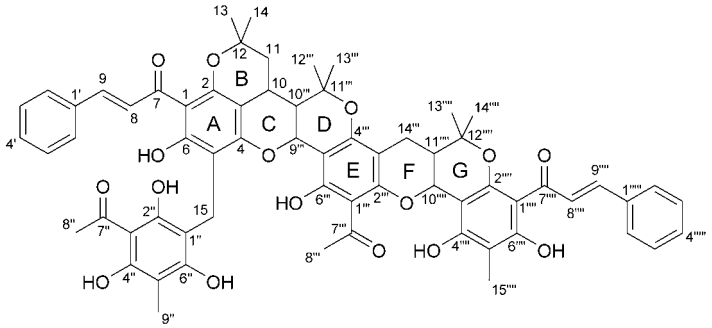
2



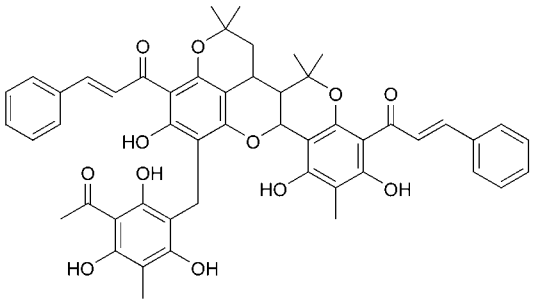
3



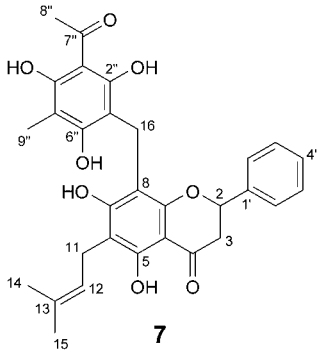
5



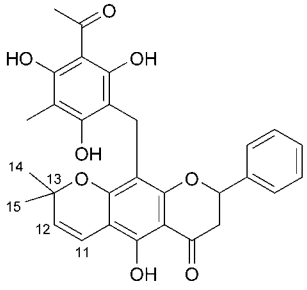
4



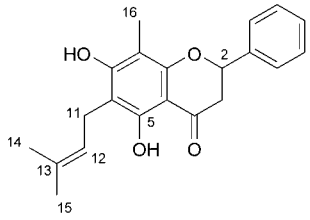
6



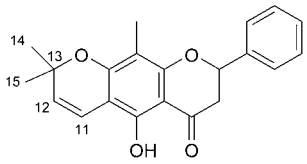
7



8



9



10

Results and Discussion[3]). – 4′-Hydroxyisorottlerin (**2**), a colorless, optically inactive solid ($[\alpha]_D = 0$ (MeOH)), tested positive towards $FeCl_3$ and showed the M^+ signal at *m*/*z* 532, corresponding to the molecular formula $C_{30}H_{28}O_9$ in the EI mass spectrum. In the ^{1}H-NMR spectrum (*Table 1*), three typical flavanone skeleton resonances were observed (δ(H) 5.58 (*dd*, *J* = 12, 3 Hz, H–C(2)); 2.75 (*dd*, *J* = 17, 3 Hz, 1 H of CH_2(3)); 3.37 (*dd*, *J* = 17, 12 Hz, 1 H of CH_2(3))). Moreover, the presence of a *para*-substituted benzene ring (δ(H) 6.91 (*d*, *J* = 8 Hz, H–C(3′,5′); 7.46 (*d*, *J* = 8 Hz, H–C(2′,6′))), a fused dimethyl-2*H*-pyrane moiety (δ(H) 1.43, 1.49 (2*s*, Me(14), Me(15)); 5.64 (*d*, *J* = 10 Hz, H–C(12)); 6.54 (*d*, *J* = 10 Hz, H–C(11)), a benzylic CH_2 group (δ(H) 3.68 (*s*, CH_2(16)), two additional Me groups (δ(H) 1.91, 2.48 (2*s*, Me(9″),

[3]) Arbitrary atom numberings were used for all compounds (see chemical formulae). For the systematic names of the new compounds, see the *Exper. Part*.

Me(8″)), and four OH groups (δ(H) 7.85, 8.82, 12.46, 12.94 (4*s*))) were also suggested by the ^{1}H-NMR spectrum. The ^{1}H- and ^{13}C-NMR spectral data (*Table 1*) indicated that an acetophenone moiety (as in rottlerin (**1**)) was attached *via* a benzylic CH_2 group to the *A*-ring of the flavanone part of **2**. An EI-MS signal at *m*/*z* 120 supported that the *B*-ring of the flavanone unit bears an OH group. In the COLOC spectrum (*Table 1*), significant correlations observed between C(6,10)/5-OH and C(6)/H–C(12) showed that the dimethyl-2*H*-pyrane unit was fused to a chromane unit at C(6) and C(7), and that the benzylic CH_2 group was attached to C(8). Detailed analysis of the COLOC spectrum also supported this type of substitution pattern, which was identical to that in isorottlerin (**1**). Therefore, compound **2** was identified as 4′-hydroxyisorottlerin [5], which corresponds to 10-(3-acetyl-2,4,6-trihydroxy-5-methylbenzyl)-2,6,7,8-tetrahydro-5-hydroxy-8-(4-hydroxyphenyl)-2,2-dimethylpyrano[3,2-*g*][1]benzopyran-6-one.

Table 1. *NMR Data of 4′-Hydroxyisorottlerin* (**2**)[3]). In (D_6)DMSO at 400 (^{1}H) and 100 MHz (^{13}C) resp.; δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)	COLOC
H–C(2)	5.58 (*dd*, *J* = 12, 3)	81.74	CH_2(3), H–C(2′,6′)
CH_2(3)	2.75 (*dd*, *J* = 17, 3)	43.08	H–C(2)
	3.37 (*dd*, *J* = 17, 12)		
C(4)		198.09	CH_2(3)
C(5)		157.85	5-OH
C(6)		103.57[a])	5-OH, H–C(12)
C(7)		158.70[b])	CH_2(16)
C(8)		107.14	CH_2(16)
C(9)		159.65[b])	CH_2(16)
C(10)		104.06[a])	5-OH
H–C(11)	6.54 (*d*, *J* = 10)	115.80	
H–C(12)	5.64 (*d*, *J* = 10)	127.52	Me(14), Me(15)
C(13)		81.23	Me(14), Me(15), H–C(11)
Me(14)	1.43 (*s*)	27.78	H–C(12)
Me(15)	1.49 (*s*)	27.94	H–C(12)
CH_2(16)	3.68 (*s*)	16.75	
C(1′)		129.11	H–C(2′,6′)
H–C(2′,6′)	7.46 (*d*, *J* = 8)	129.79	H–C(2)
H–C(3′,5′)	6.91 (*d*, *J* = 8)	116.37	
C(4′)		159.30	H–C(2′,6′)
C(1″)		104.75	CH_2(16)
C(2″)		158.17	CH_2(16)
C(3″)		105.90	Me(8″)
C(4″)		162.27	Me(9″)
C(5″)		103.89	Me(9″)
C(6″)		160.77	CH_2(16), Me(9″)
C(7″)		204.32	Me(8″)
Me(8″)	2.48 (*s*)	33.31	
Me(9″)	1.91 (*s*)	7.94	
OH	7.85 (br. *s*)		
OH	8.82 (br. *s*)		
OH	12.46 (br. *s*)		
5-OH	12.94 (br. *s*)		

[a]) [b]) Interchageable signals.

Kamalachalcone C (**3**), a pale yellow solid, was optically active ($[\alpha]_D = +18$ (acetone)), and tested positive towards $FeCl_3$. EI-MS Analysis showed the M^+ signal at m/z 530, corresponding to the molecular formula $C_{31}H_{30}O_8$ in the EI-MS. The ^{1}H-NMR spectrum (*Table 2*) exhibited the presence of a set of *trans*-olefinic H-atoms (δ(H) 7.65 ($d, J = 16$ Hz, H−C(9); 7.94 ($d, J = 16$ Hz, H−C(8))) and a mono-substituted benzene ring (δ(H) 7.68 (m, H−C(2′,6′)); 7.44 (m, H−C(3′,4′,5′))). The ^{13}C-NMR spectrum of **3** (*Table 2*) showed the presence of an α,β-unsaturated C=O group (δ(C)192.27). The above partial structures were connected by the HMBC correlations described in *Table 2*, which established the presence of a cinnamoyl group. Furthermore, the ^{1}H-NMR spectrum showed a strongly chelated OH group, as observed in kamalachalcones A (**5**) and B (**6**) [10]. Accordingly, it was suggested that **3** was a 2′-hydroxychalcone.

Table 2. *NMR Data of Kamalachalcone C* (**3**)[3]). In (D_6)DMSO at 500 (^{1}H) and 125 MHz (^{13}C), resp.; δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC
C(1)		100.09	
C(2)		154.32	
C(3)		104.43	
C(4)		164.66	
C(5)		103.00	
C(6)		161.65	
C(7)		192.27	
H−C(8)	7.94 ($d, J = 16$)	127.79	C(9), C(1′)
H−C(9)	7.65 ($d, J = 16$)	141.00	C(7), C(2′,6′)
H−C(10)	5.25 ($d, J = 4$)	65.85	C(2), C(3), C(6)
H−C(11)	2.52 ($dd, J = 9, 4$)	35.54	C(12)
C(12)		79.21	
Me(13)	1.20 (s)	21.41	C(12), C(14)
Me(14)	1.53 (s)	28.58	C(12), C(13)
Me(15)	2.00 (s)	7.86	C(4), C(5), C(6)
C(1′)		134.96	
H−C(2′,6′)	7.68 (m)	128.04	C(9), C(4′)
H−C(3′,5′)	7.44 (m)	129.08	C(1′)
H−C(4′)	7.44 (m)	130.24	C(2′,6′)
C(1″)		105.82	
C(2″)		158.62	
C(3″)		99.69	
C(4″)		158.58	
C(5″)		103.05	
C(6″)		157.74	
C(7″)		203.80	
Me(8″)	2.64 (s)	32.84	
CH_2(9″)	2.67 (br. $d, J = 18$)	18.37	C(10), C(11), C(12), C(3″), C(4″)
	2.92 ($dd, J = 18, 9$)		
Me(10″)	1.90 (s)	7.79	C(4″), C(5″), C(6″)
6-OH	9.84 (br. s)		C(1), C(5)
6″-OH	11.20 (br. s)		C(1″), (C5″)
2″-OH	11.99 (br. s)		C(1″), C(2″), C(3″)
4-OH	14.33 (s)		C(3), C(4), C(5)

The ^{1}H-NMR and ^{1}H,^{1}H-COSY spectrum of **3** (*Fig. 1*) indicated the presence of a CH−CH−CH_2 sequence (H−C(10)/H−C(11)/CH_2(9″)), two geminal Me groups (δ(H) 1.20, 1.53 (2*s*, Me(13), Me(14)); two Me groups attached to aromatic rings (δ(H) 1.90, 2.00 (2*s*, Me(10″), Me(15)), an acetyl Me group (δ(H) 2.64 (*s*, Me(8″))), and four phenolic OH groups (δ(H) 9.84, 11.20, 11.99, 14.33 (4*s*)). The HMBC correlations observed between H−C(10)/C(2), H−C(9″)/(C(12), and H−C(13,14)/C(12) allowed the expanded partial structure of **3** to be drawn as shown in *Fig. 1*. The positions of the two Me groups attached to aromatic rings, one acetyl Me group, and four OH groups, respectively, were also determined by HMBC analysis. Accordingly, a cinnamoyl group was established to be attached at C(1). Although no direct information about *B*- and *C*-ring heteroatoms, *i.e.*, the linkages at C(2), C(12), C(10), and C(4″), could be derived from the NMR spectra, when considering the molecular weight, the connectivity through O-atoms was deduced. The coupling constant of 4 Hz observed for H−C(10)/H−C(11) was the same as those for the structurally related, known compounds **5** and **6**. Thus, the relative configuration of H−C(10)/H−C(11) was suggested to be *cis*. Hence, the structure of kamalachalcone C (**3**) was identified as (2*E*)-1-[(6a*S**,12a*R**)-9-acetyl-6a,6,7,12a-tetrahydro-1,3,8,10-tetrahydroxy-2,6,6,11-tetramethyl[1]benzopyrano[4,3-*b*]-[1]benzopyran-4-yl]-3-phenylprop-2-en-1-one.

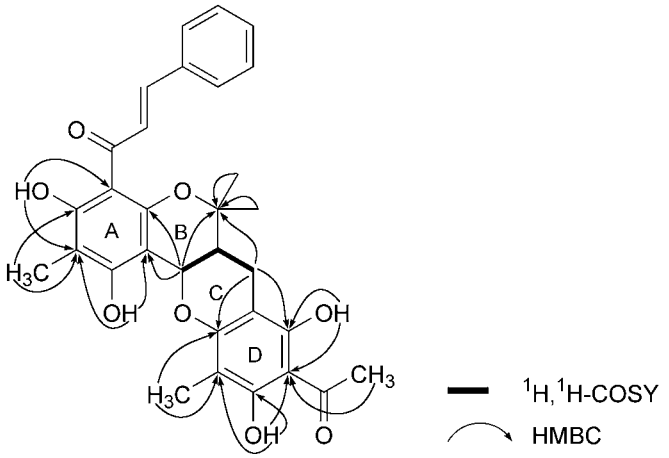


Fig. 1. *Selected ^{1}H,^{1}H-COSY and HMBC correlations for compound* **3**

Kamalachalcone D (**4**), a pale yellow solid, was optically active ($[\alpha]_D = +31$ ($CHCl_3$)), and tested positive towards $FeCl_3$. FAB-MS Analysis (negative mode) showed the $[M-H]^-$ signal at *m*/*z* 1097, corresponding to the molecular formula $C_{65}H_{62}O_{16}$. The ^{1}H-NMR (see *Exper. Part*) and ^{13}C-NMR spectra (*Table 3*) exhibited two sets of *trans*-olefinic resonances, two mono-substituted benzene rings, two strongly chelated OH groups (δ(H) 14.41, 15.81 (2 br. *s*)), and two α,β-unsaturated C=O groups (δ(C) 193.18, 193.57). This indicated that *two* 2′-hydroxychalcone units were present in **4**. The NMR spectra also showed the presence of an acetophenone unit connected to an aromatic ring *via* a CH_2 group (as in compounds **1**, **3**, and **6**). The ^{1}H-NMR and ^{1}H,^{1}H-COSY spectra further showed a CH−CH−CH−CH_2 sequence (H−C(9‴)/

Table 3. *^{13}C-NMR Data of Kamalachalcone D* (**4**)[3]). In $CDCl_3$ at 125 MHz; δ in ppm.

C-Atom	δ(C)	C-Atom	δ(C)
C(1)	107.25	C(1‴)	105.47
C(2)	155.70	C(2‴)	159.09
C(3)	102.80	C(3‴)	99.93
C(4)	156.57	C(4‴)	157.56
C(5)	109.17	C(5‴)	99.09
C(6)	161.31	C(6‴)	164.96
C(7)	193.57	C(7‴)	203.41
C(8)	126.79	C(8‴)	32.73
C(9)	143.78	C(9‴)	69.84
C(10)	25.26	C(10‴)	46.72
C(11)	42.12	C(11‴)	79.37
C(12)	78.65	C(12‴)	21.17
C(13)	27.36	C(13‴)	28.33
C(14)	30.39	C(1⁗)	105.94
C(15)	16.14	C(2⁗)	154.62
C(1′)	135.31	C(3⁗)	98.14
C(2′,6′)	128.17	C(4⁗)	159.51
C(3′,5′)	128.88	C(5⁗)	103.21
C(4′)	130.52	C(6⁗)	165.85
C(1″)	104.01	C(7⁗)	193.18
C(2″)	159.51	C(8⁗)	127.88
C(3″)	105.51	C(9⁗)	141.86
C(4″)	162.25	C(10⁗)	67.83
C(5″)	104.14	C(11⁗)	36.22
C(6″)	160.19	C(12⁗)	79.40
C(7″)	203.96	C(13⁗)	22.69
C(8″)	33.05	C(14⁗)	28.27
C(9″)	7.67	C(15⁗)	7.16
		C(1⁗)	135.61
		C(2⁗,6⁗)	128.44
		C(3⁗,5⁗)	129.06
		C(4⁗)	130.17

H−C(10‴)/H−C(10)/CH_2−(11)), a CH−CH−CH_2 sequence (H−C(10‴)/H−C(11⁗)/CH_2(14‴)), three pairs of geminal Me groups at positions 13/14, 12‴/13‴, and 13⁗/14⁗, respectively, an acetyl Me group (Me(8‴)), and a Me group (Me(15⁗) on an aromatic ring.

The two hydroxy chalcone partial structures in **4** were connected with the aid of HMBC analysis (*Fig. 2*), in same manner as described for **3**. The expanded partial structures were connected as drawn in *Fig. 2*; and the acetophenone unit was confirmed to be attached at C(5) of ring *A* by HMBC analysis. Similarly, the two cinnamoyl units were found to be attached to C(1) and C(1⁗) of the bis(hydroxy chalcone) skeleton. No clear information about the O-atom bridges in rings *B*–*D*, *F*, and *G* (C(2)/O/C(12), C(4)/O/C(9‴), C(4‴)/O/C(11‴), C(2‴)/O/C(10⁗), C(2⁗)/O/C(12⁗)) could be established from the NMR spectra. However, considering the molecular weight of the compound, it was evident that these connections had to be made *via* O-atoms.

The chemical shifts and coupling constants for H−C(9‴)/H−(10‴)/H−C(10)/CH_2(11) were very similar to those of kamalachalcone B (**6**), which has the same partial

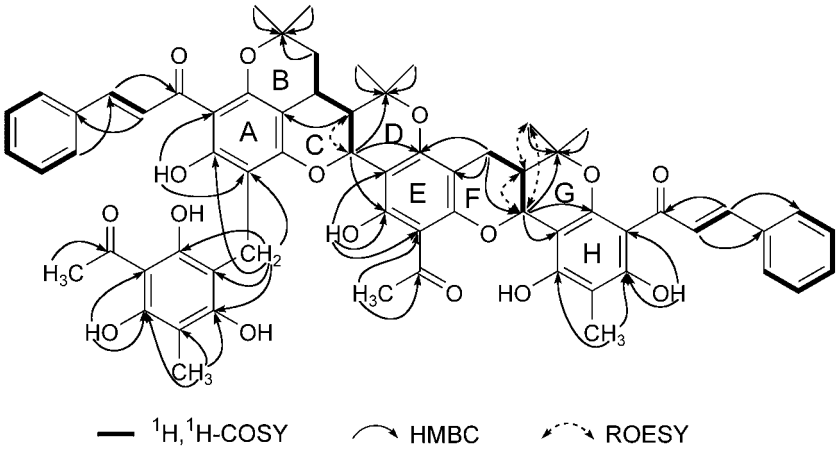


Fig. 2. *Selected ^{1}H,^{1}H-COSY, HMBC, and ROESY correlations for compound* **4**

structure. In the ROESY spectrum of **4** (*Fig. 2*), the correlation observed between H–C(9‴)/H–C(10‴) led to the relative configuration of the *C* and *D* rings, identical as in kamalachalcone B. Significant ROESY correlations observed between H–C(10⁗)/H–C(11⁗), H–C(10⁗)/H–C(14⁗), and H–C(11⁗)/ H–C(14⁗) revealed that H–C(10⁗) and H–C(11⁗) were *cis*-configured (rings *F*/*G*). Unfortunately, we were unable to determine the configuration of H–C(9‴)/H–C(10‴) (rings *C*/*D*).

The EI mass spectrum of **4** showed a characteristic signal at *m*/*z* 336 corresponding to fragment **A** (*Fig. 3*,*a*) [2]. Kamalachalcones A (**5**) and B (**6**), which incorporate the same partial structure, gave rise to the same EI-MS fragment. This information, thus, should be helpful in the future for the identification of similar natural products.

From a biogenetic point of view, the unique ring system of kamalachalcone D (**4**) may originate from condensation of rottlerin (**1**), the red-colored compound **A**, and the acetylated [1]benzopyran **B** (*Fig. 3*,*b*).

Although compounds **1** and **7**–**10** are known, full ^{13}C-NMR spectral data have not been published yet. We, thus, performed ^{13}C-NMR assignments by ^{1}H,^{13}C-COSY and COLOC spectra (see *Exper. Part*). Compounds **9** and **10** had previously been isolated as regioisomeric mixtures (inverted positions of prenyl and Me groups in **9**; different ring fusion in the cyclized variant **10**) [5]. In the present study, the two isomers were isolated in pure form. Compound **9** is a known bud constituent of *Platanus acerifolia* [11].

Some of the compounds isolated from kamala could not be subjected to biological assays due to their poor solubility in EtOH. For rottlerin (**1**) and kamalachalcone C (**3**), however, the scavenging activity (Sc_{50}) towards DPPH radicals[4]) [12][13] was determined as 5.6 and 44 μM, respectively.

---

[4]) DPPH is the trivial name for '1,1-diphenyl-2-picrylhydrazine', which corresponds to 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazine.

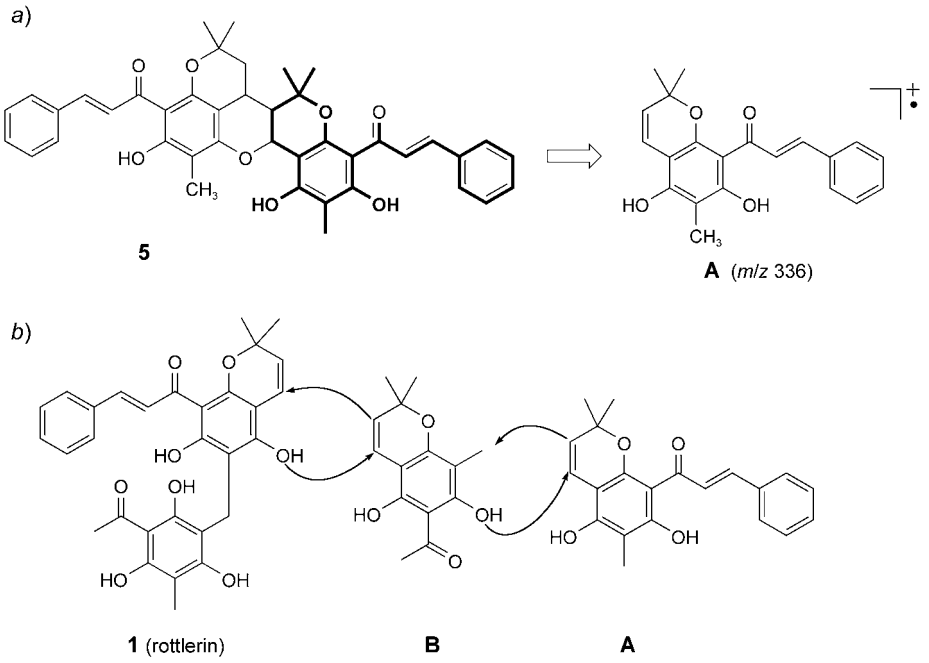


Fig. 3. a) *EI-MS Fragmentation of compound* **5** *giving rise to fragment* **A**, *which was also detected in the mass spectrum of* **4**; b) *plausible biogenesis of* **4** *by fusion of one molecule each of compounds* **1**, **A**, *and* **B**

Experimental Part

General. Column chromatography (CC): *Kieselgel 60* (70–230 mesh; *Merck*) or *Sephadex LH-20*. Prep. TLC: *Kieselgel* F_{254} (0.5 mm; *Merck*). UV Spectra: *Shimadzu UV-2200* spectrophotometer; λ_{max} in nm. Optical rotation: *JASCO P-1020* polarimeter. NMR Spectra: *JEOL EX-400* and *α-500*; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI- and FAB-MS: *JOEL JMX-DX-300*; in *m*/*z* (rel. %).

Plant Material, Extraction, and Isolation. Kamala (2 kg), purchased from *Caesar & Loretz GmbH* (*Caelo*; Germany), was successively extracted with acetone and MeOH. A part (100 g) of the acetone extract (125 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 10:1 → 1:1) to afford five main fractions (Fr.): *Fr. A* (eluted with $CHCl_3$/MeOH 10:1), *B* (8:1), *C* (5:1), *D* (4:1), and *E* (1:1). *Fr. A* was further purified by CC (SiO_2; $CHCl_3$/MeOH 20:1). The resulting flavonoid-containing fraction was further separated by CC (SiO_2 and *Sephadex LH 20* (MeOH)), followed by prep. TLC (hexane/acetone 3:1 and $CHCl_3$/MeOH 10:1), to afford **11** (54 mg), **9** (12 mg), and **10** (18 mg). *Fr. B* was further chromatographed (SiO_2; $CHCl_3$/MeOH 10:1) to afford **8** (10 mg). *Fr. C* contained a large amount of rottlerin (**1**), which was removed by crystallization. The resulting filtrate was then further purified by repeated CC (1. SiO_2, $CHCl_3$/MeOH 8:1; 2. *Sephadex LH 20*, MeOH), followed by prep. TLC (benzene/AcOEt 10:1), to afford pure **2** (15 mg), **3** (17 mg), **4** (7 mg), **5** (8 mg), **6** (3 mg), **7** (82 mg), and **8** (12 mg).

Rottlerin (**1**). Dark-reddish powder. ^{1}H-NMR (400 MHz, $CDCl_3$)[3]): 1.53 (2*s*, Me(14), Me(15)); 2.07 (*s*, Me(9″)); 2.71 (*s*, Me(8″)); 5.48 (*d*, *J* = 10, H–C(10)); 6.65 (*d*, *J* = 10, H–C(11)); 7.41 (*m*, H–C(3′,4′,5′)); 7.42 (*d*-like *m*, H–C(2′,6′)); 7.81 (*d*, *J* = 16, H–C(9)); 8.17 (*d*, *J* = 16, H–C(8)); 9.50 (br. *s*, 2 OH); 9.56 (br. *s*, OH); 16.45 (*s*, 6-OH). ^{13}C-NMR (100 MHz, $CDCl_3$)[3]): 8.05 (C(9″)); 15.88 (C(15)); 27.73 (C(13,14)); 32.62 (C(8″)); 77.89 (C(12)); 103.39 (C(3)); 103.50 (C(5″)); 104.99 (C(1″)); 105.07 (C(3″)); 105.18 (C(1)); 106.59 (C(5)); 116.92 (C(10)); 124.88 (C(11)); 126.49 (C(8)); 128.15 (C(2′,6′)); 128.15 (C(3′,5′)); 130.14 (C(4′)); 135.07 (C(1′)); 143.07 (C(9)); 155.09 (C(2)); 158.27 (C(4)); 158.27 (C(2″)); 159.20 (C(4″)); 159.86 (C(6″)); 162.57 (C(6)); 192.56 (C(7)); 204.07 (C(7″)).

4′-Hydroxyisorottlerin (=*10-(3-Acetyl-2,4,6-trihydroxy-5-methylbenzyl)-2,6,7,8-tetrahydro-5-hydroxy-8-(4-hydroxyphenyl)-2,2-dimethylpyrano[3,2-*g*][1]benzopyran-6-one* (**2**)). Colorless, amorphous powder. $[\alpha]_D = 0$ ($c = 0.1$, MeOH). UV/VIS (MeOH): 274, 349. ^{1}H- and ^{13}C-NMR[3]): see *Table 1*. EI-MS: 532 (35, M^+, $C_{30}H_{28}O_9^+$), 517 (11), 381 (11), 351 (28), 338 (39), 335 (16), 323 (100), 203 (88), 120 (13).

Kamalachalcone C (=*(2*E*)-1-[(6a*S**,12a*R**)-9-Acetyl-6a,6,7,12a-tetrahydro-1,3,8,10-tetrahydroxy-2,6,6,11-tetramethyl[1]benzopyrano[4,3-*b*][1]benzopyran-4-yl]-3-phenylprop-2-en-1-one* (**3**). Amorphous, pale-yellow powder. $[\alpha]_D = +18$ ($c = 0.07$, acetone). UV/VIS (MeOH): 296, 348. ^{1}H- and ^{13}C-NMR[3]): see *Table 2*. EI-MS: 530 (55, M^+, $C_{31}H_{30}O_8^+$), 515 (64), 411 (11), 336 (62), 321 (84), 231 (20), 217 (100), 179 (17).

Kamalachalcone D (=*(2*E*,2′*E*)-1,1′-[9-Acetyl-6-(3-acetyl-2,4,6-trihydroxy-5-methylbenzyl)-1,16a,17,19,19a,19b-hexahydro-5,8,11,13-tetrahydroxy-2,2,12,16,16,19,19-heptamethyl-2*H*,7a*H*,10a*H*,16*H*-[1]benzopyrano[4,3-*b*]pyrano[4″,3″,2″:4′,5′][1]benzopyrano[2′,3′:4,5]pyrano[2,3-*f*][1]benzopyrane-4,14-diyl]bis(3-phenylprop-2-en-1-one)*; **4**). Pale-yellow solid. $[\alpha]_D = +31$ ($c = 0.13$, $CHCl_3$). UV/VIS ($CHCl_3$): 241, 343. ^{1}H-NMR (500 MHz, $CDCl_3$)[3]): 1.34 (*s*, Me(12‴)); 1.45 (*s*, Me(13⁗)); 1.51 (*s*, Me(13)); 1.60 (*s*, Me(13‴)); 1.60 (*s*, Me(14)); 1.67 (*s*, Me(14⁗)); 1.95 (*dd*, $J = 13, 13$, H_α–C(11)); 1.98 (*s*, Me(9″)); 2.07 (*dd*, $J = 13, 4$, H_β–C(11)); 2.12 (*s*, Me(15⁗)); 2.19 (*dd*, $J = 4, 4$, H–C(10″)); 2.52 (*s*, Me(8″)); 2.52 (*m*, H–C(11⁗)); 2.60 (*m*, 1 H of CH_2(14‴)); 2.58 (*ddd*, $J = 13, 5, 5$, H–C(10)); 2.69 (*s*, Me(8‴)); 3.02 (*dd*, $J = 16, 10$, 1 H of CH_2(14‴)); 3.92, 3.83 (2*d*, $J = 16$, CH_2(15)); 4.95 (*d*, $J = 4$, H–C(9‴)); 5.44 (*d*, $J = 4$, H–C(10‴)); 7.40–7.43 (*m*, H–C(3′,3⁗,4′,4⁗,5′,5⁗); 7.61 (*m*, H–C(2′,2⁗,6′,6⁗)); 7.77 (*d*, $J = 16$, H–C(9‴)); 7.86 (*d*, $J = 16$, H–C(9)); 7.97 (*d*, $J = 16$, H–8‴); 8.06 (*d*, $J = 16$, H–C(8)); 8.52, 8.40, (2 br. *s*, 2″- and 6″-OH); 13.87 (*s*, 4″-OH); 14.41 (*s*, 6⁗-OH); 14.69 (*s*, 6‴-OH); 15.81 (*s*, 6-OH); 4⁗–OH not observed. ^{13}C-NMR[3]): see *Table 3*. EI-MS (70 eV, M^+ not obs.): 573 (3), 568 (5), 516 (15), 501 (8), 336 (48), 321 (100), 307 (28), 217 (86), 167 (36). FAB-MS (neg.): 1097 ($[M-H]^-$, $C_{65}H_{61}O_{16}^-$).

Isoallorottlerin (**7**). Colorless, amorphous solid. ^{1}H-NMR (400 MHz, (D_6)DMSO)[3]): 1.62 (br. *s*, Me(15)); 1.71 (br. *s*, Me(14)); 1.94 (*s*, Me(9″)); 2.53 (*s*, Me(8″)); 2.80 (*dd*, $J = 17, 3$, 1 H of CH_2(3)); 3.16 (*dd*, $J = 17, 13$, 1 H of CH_2(3)); 3.22 (*d*, $J = 7$, CH_2(11)); 3.75 (*s*, CH_2(16)); 5.11 (*t*-like *m*, H–C(12)); 5.54 (*dd*, $J = 13, 3$, H–C(2)); 7.50 (*m*, H–C(2′,3′,4′,5′,6′)); 12.45 (*s*, 5-OH). ^{13}C-NMR (100 MHz, (D_6)DMSO)[3]): 8.72 (C(8″)); 16.60 (C(16)); 17.70 (C(14)); 21.61 (C(11)); 25.14 (C(15)); 32.72 (C(8″)); 42.26 (C(3)); 78.84 (C(2)); 101.94 (C(10)); 102.87 (C(5″)); 105.43 (C(3″)); 105.59 (C(8)); 106.01 (C(1″)); 108.14 (C(6)); 122.66 (C(12)); 126.51 (C(2′,6′)); 128.36 (C(3′,5′)); 130.41 (C(4′)); 130.41 (C(13)); 140.87 (C(1′)); 157.35 (C(9)); 158.45 (C(2″)); 158.83 (C(5)); 158.99 (C(4″)); 160.00 (C(6″)); 161.76 (C(7)); 196.61 (C(4)); 203.34 (C(7″)).

Isorottlerin (**8**). Colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$)[3]): 1.58, 1.60 (2*s*, Me(14), Me(15)); 1.98 (*s*, Me(9″)); 2.50 (*s*, Me(8″)); 2.85 (*dd*, $J = 17, 3$, 1 H of CH_2(3)); 3.30 (*dd*, $J = 17, 13$, 1 H of CH_2(3)); 3.63 (br. *s*, CH_2(16)); 5.53 (*dd*, $J = 13, 3$, CH_2(2)); 5.69 (*d*, $J = 10$, H–C(12)); 6.67 (*d*, $J = 10$, H–C(11)); 7.52 (*m*, H–C(2′,3′,4′,5′,6′)); 7.66 (*s*, 6″-OH); 7.83 (*s*, 2″-OH); 12.29 (*s*, 5-OH); 13.62 (*s*, 4″-OH). ^{13}C-NMR (100 MHz, $CDCl_3$)[3]): 7.54 (C(9″)); 15.56 (C(16)); 27.81 (C(14)); 27.87 (C15)); 33.13 (C(8″)); 42.91 (C(3)); 81.46 (C(13)); 81.61 (C(2)); 102.97 (C(8)); 103.08 (C(10)); 104.1 (C(5″)); 105.40 (C(3″)); 106.22 (C(6,1″)); 115.59 (C(11)); 126.41 (C(2′,6′)); 126.41 (C(12)); 128.20 C((3′,5′)); 130.00 (C(4′)); 136.14 (C(1′)); 152.49 (C(9)); 156.13 (C(2″)); 157.87 (C(7)); 159.44 (C(6″)); 157.49 (C(5)); 162.46 (C(4″)); 195.80 (C(4)); 203.70 (C(7″)).

5,7-Dihydroxy-8-methyl-6-prenylflavanone (**9**). Colorless solid. ^{1}H-NMR (400 MHz, (D_6)DMSO)[3]): 1.64 (br. *s*, Me(15)); 1.75 (br. *s*, Me(14)); 1.97 (*s*, Me(16)); 2.82 (*dd*, $J = 17, 3$, 1 H of H–C(3)); 3.09 (*dd*, $J = 17, 13$, 1 H of H–C(3)); 3.31 (br. *d*, $J = 7$, CH_2(11)); 5.12 (*t*-like *m*, H–C(12)); 5.51 (*dd*, $J = 13, 3$, H–C(2)); 7.37–7.46 (*m*, H–C(3′,4′,5′)); 7.56 (br. *d*, $J = 7$, H–C(2′,6′)); 8.44 (br. *s*, 7-OH); 12.40 (s, 5-OH). ^{13}C-NMR (100 MHz, (D_6)DMSO)[3]): 7.51 (C(16)); 17.91 (C(14)); 21.81 (C(11)); 25.92 (C(15)); 43.61 (C(3)); 79.39 (C(2)); 108.81 (C(6)); 103.24 (C(10)); 103.33 (C(8)); 123.44 (C(12)); 131.71 (C(13)); 158.59 (C(9)); 159.94 (C(5)); 162.59 (C(7)); 197.22 (C(4)).

6″,6″-Dimethylpyrano(2″,3″:7,6)-5-hydroxy-8-methylflavanone (**10**). Colorless solid. ^{1}H-NMR (400 MHz, (D_6)DMSO)[3]): 1.45, 1.46 (2*s*, Me(14), Me(15)); 1.97 (*s*, Me(16)); 2.86 (*dd*, $J = 17, 3$, 1 H of CH_2(3)); 3.15 (*dd*, $J = 17, 13$, 1 H of CH_2(3)); 5.60 (*dd*, $J = 13, 3$, CH_2(3)); 5.62 (*d*, $J = 10$, H–C(12)); 6.59 (*d*, $J = 10$, H–C(11)); 7.40 (*m*, H–C(4′)); 7.42 (*t*-like *m*, H–C(3′,5′)); 7.60 (*d*-like *m*, H–C(2′,6′)); 12.41 (*s*, 5-OH). ^{13}C-NMR (100 MHz, (D_6)DMSO)[3]): 7.53 (C(16)); 28.41 (C(14,15)); 43.51 (C(3)); 78.80 (C(13)); 79.62 (C(2)); 103.14(C(6); 103.22 (C(10)); 104.69 (C(8)); 116.04 (C(11)); 126.98 (C(12)); 127.02 (C(2′,6′)); 129.27 (C(4′)); 129.47 (C(3′,5′)); 140.1 (C(1′)); 157.08 (C(5)); 160.34 (C(7)); 160.48 (C(9)); 195.48 (C(4)).

Biological Assay. The DPPH[4]) radical scavenging activities were determined according to literature procedures. For rottlerin (**1**) and kamalachalcone C (**3**), scavenging activity values (Sc_{50}) of 5.6 and 44 μM, were found, resp.

REFERENCES

[1] A. McGookin, F. P. Reed, A. Robertson, *J. Chem. Soc.*, **1937**, 748.
[2] L. Crombie, C. L. Green, B. Tuck, D. A. Whiting, *J. Chem. Soc. (C)* **1968**, 2625.
[3] M. Lounasmaa, C.-J. Widen, C.-M. Tiff, A. Huhtikangas, *Planta Med.* **1975**, *28*, 16.
[4] C.-J. Widen, S. Puri, *Planta Med.* **1980**, *40*, 284.
[5] V. K. Ahluwalia N. D. Sharma, B. Mittal, S. Gupta, *Indian J. Chem.*, *Sect. B* **1988**, *27*, 238.
[6] A. Daikonya, S. Katsuki, S. Kitanaka, *Chem. Pharm. Bull*. **2004**, *52*, 1326.
[7] M. Arisawa, A. Fujita, R. Suzuki, T. Hayashi, N. Morita, N. Kawano, S. Koshimura, *J. Nat. Prod.* **1985**, *48*, 455.
[8] M. Arisawa, A. Fujita, M. Saga, T. Hayashi, N. Morita, N. Kawano, S. Konoshima, *J. Nat. Prod*. **1985**, *49*, 298.
[9] M. Gschwendt, H. Muller, K. Kielbasa, R. Zang, W. Kittstein, G. Rincke, F. Marks, *Biochem. Biophys. Res. Commun.* **1994**, *199*, 93.
[10] T. Tanaka, T. Ito, M. Iinuma, Y. Takahashi, H. Naganawa, *Phytochemistry* **1998**, *48*, 1423.
[11] M. Kaouadji, P. Ravanel, A.-M. Mariotte, *J. Nat. Prod.* **1986**, *49*, 153.
[12] T. Morikawa, F. Xu, H. Matsuda, M. Yoshikawa, *Heterocycles* **2002**, *57*, 1983.
[13] M. Yoshikawa, E. Harada, A. Miki, K. Tsukamoto, S.-Q. Liang, J. Yamahara, N. Murakami, *Yakugaku Zasshi* **1994**, *114*, 129.

Received January 10, 2005