Further Prenylated Bi- and Tricyclic Phloroglucinol Derivatives from *Hypericum papuanum*

by **Karin Winkelmann**[a]), **Jörg Heilmann**[a]), **Oliver Zerbe**[a]), **Topul Rali**[b]), and **Otto Sticher***[a])

[a]) Department of Applied BioSciences, Institute of Pharmaceutical Sciences, Swiss Federal Institute of Technology (ETH) Zürich, Winterthurerstrasse 190, CH-8057 Zürich

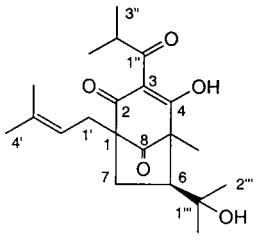
[b]) *PNG Biodiversity Research PTY Ltd.,* Port Moresby, Papua New Guinea

From the petroleum-ether extract of the dried aerial parts of *Hypericum papuanum*, three new prenylated tricyclic and four new bicyclic acylphloroglucinol derivatives were isolated by bioactivity-guided fractionation. The structures of the bicyclic compounds enaimeone A, B, and C (**1**/**1a**, **2**/**2a**, and **3**/**3a**, resp.) were elucidated as *rel*-(1*R*,5*R*,6*S*)-4-hydroxy-6-(1-hydroxy-1-methylethyl)-5-methyl-1-(3-methylbut-2-enyl)-3-(2-methylpropanoyl)-bicyclo[3.2.1]oct-3-ene-2,8-dione (**1**/**1a**), *rel*-(1*R*,5*R*,6*R*)-4-hydroxy-6-(1-hydroxy-1-methylethyl)-5-methyl-1-(3-methylbut-2-enyl)-3-(2-methylpropanoyl)bicyclo[3.2.1]oct-3-ene-2,8-dione (**2**/**2a**), *rel*-(1*R*,5*R*,6*R*)-4-hydroxy-6-(1-hydroxy-1-methylethyl)-5-methyl-3-(2-methylbutanoyl)-1-(3-methylbut-2-enyl)bicyclo[3.2.1]oct-3-ene-2,8-dione (**3**/**3a**). The tricyclic isolates 8-hydroxy-3β-(1-hydroxy-1-methylethyl)-4,4,7-trimethyl-9-(2-methylpropanoyl)-5βH-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**4**), 8-hydroxy-3α-(1-hydroxy-1-methylethyl)-4,4,7-trimethyl-9-(2-methylpropanoyl)-5βH-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**5**), and 8-hydroxy-3α-(1-hydroxy-1-methylethyl)-4,4,7-trimethyl-9-(2-methylbutanoyl)-5βH-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**6**), and their corresponding tautomers **4a**, **5a**, and **6a**, were named 1′-hydroxyialibinones A, B, and D, respectively. Oxidative decomposition of furonewguinone A (= 2,3,3a,5-tetrahydro-3a-hydroxy-2-(1-hydroxy-1-methylethyl)-5-methyl-5-(3-methylbut-2-enyl)-7-(2-methylpropanoyl)-benzofuran-4,6-dione; **7**) led to furonewguinone B (= 3,3a,7,7a-tetrahydro-3a,6,7a-trihydroxy-2-(1-hydroxy-1-methylethyl)-7-methyl-7-(3-methylbut-2-enyl)-5-(2-methylpropanoyl)benzofuran-4(2*H*)-one; **8**/**8a**). Structure elucidation was based on extensive 1D and 2D NMR studies, as well as on data derived from mass spectrometry. Furthermore, the cytotoxicity towards KB nasopharyngeal carcinoma cells and the antibacterial activity were determined.

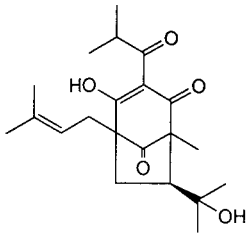
1. Introduction. – In the traditional medicine of Papua New Guinea, the leaves of *Hypericum papuanum* RIDLEY (vernacular name: enaime) are used to treat sores [1]. *Leach et al.* [2] reported about the antibacterial activity of a *Soxhlet* acetone extract of the leaves of *H. papuanum* (Guttiferae) against the *Gram*-positive bacterium *S. aureus.* In our continuing search for biologically active metabolites derived from plants that are employed in the traditional medicine of Papua New Guinea, we have previously isolated 13 acylphloroglucinol derivatives (ialibinones A–E, papuaforins A–E, hyperguinones A and B, and hyperpapuanone) from the petroleum-ether extract of this plant [3][4]. Some of them displayed antibacterial (*Bacillus cereus*, *Staphylococcus epidermidis*, and *Micrococcus luteus*) and cytotoxic activity (KB cells). Further study of the more polar fractions of the petroleum-ether extract has now led to the isolation of seven new phloroglucinol derivatives. The bicyclic ring system of **1**/**1a**, **2**/**2a**, and **3**/**3a** forms part of the tricyclic skeleton of the ialibinones. The tricyclic compounds **4**/**4a**, **5**/**5a**, and **6**/**6a** are derivatives of the already mentioned ialibinones A, B, and D. Compounds **7** and **8**/**8a** have a benzofuran skeleton. In addition, the antibacterial and cytotoxic activities of the isolates were evaluated.

2. Results and Discussion. – Compounds **1**/**1a** were isolated as viscous oil. Its DEI-MS displayed a molecular ion at *m/z* 362 (M^+), whereas the HR-MALDI-MS revealed a $[M+Na]^+$ at *m/z* 385.1983 (calc. 385.1991). These data are consistent with the molecular formula $C_{21}H_{30}O_5$ and, therefore, indicated seven degrees of unsaturation. However, doubled ^{1}H- and ^{13}C-NMR signals in $CDCl_3$ (*Tables 1* and *2*) in a ratio of approximately 1.7:1 and the absence of further molecular-ion peaks in the MS indicated that the compound is a mixture **1**/**1a** of two enol tautomers, with **1** being the predominant one. The spectral data, including HMBC (heteronuclear multiple-bond correlation) and DQF-COSY (double-quantum filtered correlation spectroscopy) results allow to establish the structure of the preferred tautomer **1** as 4-hydroxy-6-(1-hydroxy-1-methylethyl)-5-methyl-1-(3-methylbut-2-enyl)-3-(2-methylpropanoyl)bicyclo[3.2.1]oct-3-ene-2,8-dione. The very unusual HMBC connectivity between the proton OH–C(4) and H–C(2″) can only be explained by through-bond scalar coupling *via* the H-bond to C(1″)=*O*. The tautomeric form **1a** was identified by means of very similar arguments, and the different position of the enolic OH group could be determined unambiguously by HMBC connectivities. The following assignment strategy refers to the major tautomer.

By means of ^{13}C-NMR and DEPT experiments, the C-atoms of **1** were sorted into seven Me, two CH_2, three CH, and nine C. The signals of the three carbonyl atoms C(2), C(8), and C(1″) at δ(C) 192.8, 205.8, and 208.4, and a quaternary atom C(4) at δ(C) 200.3, substituted by an enolic OH group (δ(H) 18.89 (*s*)), are characteristic signals for tautomeric acylphloroglucinol derivatives. The extremely lowfield-shifted ^{1}H-NMR signal at 18.89 ppm is common for OH protons which participate in strong H-bonds. The most likely H-bond acceptor is C(1′)=*O*. Signals for CH_2(1′), H–C(2′), C(3′), Me(4′), Me–C(3′) at δ(C) 25.5, 118.9, 135.2, 26.0, and 18.0 indicate a prenyl residue. This group is attached to C(1) as confirmed by HMBC (^{13}C,^{1}H ^nJ correlated 2D, $n>1$) cross-peaks between C(1), C(2), C(7), and C(8) and CH_2(1′). Considering the structural elements described above and five degrees of unsaturation, compound **1** needs to be bicyclic. The spin system comprising H–C(6)

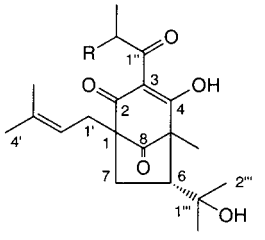


1 enaimeone A

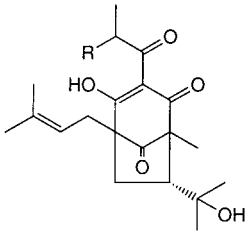


1a

Main HMBCs of **1**:
C(1)/CH_2(1′), CH_2(7)
C(2)/CH_2(1′), CH_2(7)
C(3)/OH–CH(4)
C(4)/OH–CH(4), H–C(6)
C(5)/OH–C(4), CH_3–C(5), H–C(6)
C(6)/CH_3–C(5), CH_3(2‴), CH_3–C(1‴)
C(7)/H–C(6), CH_2(1′)
C(8)/CH_3–C(5), CH_2(1′), CH_2(7)
C(2′)/CH_2(1′), CH_3(4′), CH_3–C(3′)
C(3′)/CH_3(4′), CH_3–C(3′)
C(1″)/H–C(2″), CH_3(3″), CH_3–C(2″), OH–C(4)
C(2″)/OH–C(4)
C(1‴)/CH_2(7)



2/3 enaimeone B/C



2a/3a

2/2a R = Me

3/3a R = Et

Table 1. *^{13}C- and ^{1}H-NMR Data of the Major Tautomers* **1**–**3**[a]). δ in ppm, J in Hz.

	1		**2**		**3**[f])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	65.6 (*s*[b]))		65.6 (*s*[b]))		65.6 (*s*[b]))	
C(2)	192.8 (*s*[b]))		193.3 (*s*[b]))		193.3 (*s*[b]))	
C(3)	111.2 (*s*)		107.4 (*s*)		107.7, 107.9 (2*s*[b]))	
C(4)	200.3 (*s*)		201.7 (*s*[b]))		201.7 (*s*[b]))	
C(5)	62.5 (*s*[b]))		62.7 (*s*)		62.9 (*s*[b]))	
H–C(6)	53.8 (*d*)	2.09 (*m*[e]))	50.0 (*d*)	2.28 (*dd*, *J* = 5.4, 10.0)	50.0 (*d*)	2.27 (*dd*, *J* = 5.5, 9.7)
CH_2(7)	29.3 (*t*)	2.09 (*m*[e])) 2.13 (*m*[e]))	31.3 (*t*)	2.04 (*m*, 2 H)[e])	31.2, 31.3 (2*t*)	1.98–2.09 (*m*[e]))
C(8)	205.8 (*s*[b]))		207.1 (*s*[b]))		207.2 (*s*[b]))	
Me–C(5)	14.4 (*q*)	1.55 (*s*)	11.5 (*q*)	1.57 (*s*)	11.46, 11.49 (2*q*)	1.566, 1.571 (2*s*)
CH_2(1′)	25.5 (*t*)	2.52 (*m*[e])) 2.69 (*dd*, *J* = 8.4, 15.5)	25.6 (*t*)	2.51 (*m*) 2.69 (*dd*, *J* = 8.9, 15.0)	25.6 (*t*)	2.51 (*m*) 2.68 (*dd*, *J* = 9.0, 14.7)
H–C(2′)	118.9 (*d*)	5.11 (*m*)	119.7 (*d*)	5.21 (*m*)	119.7 (*d*)	5.21 (*m*[e]))
C(3′)	135.2 (*s*)		135.2 (*s*)		135.2 (*s*[b]))	
Me(4′)	26.0 (*q*)	1.73 (br. *s*)	25.9 (*q*)	1.73 (br. *s*)	25.9 (*q*)	1.73 (br. *s*)
Me–C(3′)	18.0 (*q*)	1.69 (br. *s*)	18.0 (*q*)	1.68 (br. *s*)	18.0 (*q*)	1.68 (br. *s*)
C(1″)	208.4 (*s*[b]))		209.8 (*s*[b]))		209.3 (*s*[b]))	
H–C(2″)	34.8 (*d*)	4.01 (*sept.*, *J* = 6.8)	35.0 (*d*)	4.01 (*sept.*, *J* = 6.8)	41.2, 41.3 (2*d*)	3.82–3.95 (*m*[e]))
Me(3″) or CH_2(3″)	18.7 (*q*[c]))	1.13–1.16 (*m*[e]))	18.6 (*q*)	1.17 (*d*, *J* = 6.8)	26.5 (*t*)	1.70–1.77 (*m*[e])) 1.38–1.46 (*m*[e]))
Me–C(2″)	18.8 (*q*[c]))	1.13–1.16 (*m*[e]))	19.0 (*q*)	1.16 (*d*, *J* = 6.8)	11.7, 11.8 (2*q*)	0.88–1.00 (*m*[e]))
Me(4″)					16.1, 16.5 (2*q*)	1.14/1.15 (*d*, *J* = 6.9)
C(1‴)	72.0 (*s*[d]))		74.2 (*s*)		74.2 (*s*)	
Me(2‴)	29.3 (*q*)	1.45 (*s*)	26.4 (*q*)	1.21 (*s*)	26.4 (*q*)	1.20 (br. *s*)
Me–C(1‴)	29.4 (*q*)	1.17 (*s*)	29.5 (*q*)	1.23 (*s*)	29.3 (*q*)	1.23 (*s*)
OH		18.89 (*s*)		18.80 (*s*)		18.80, 18.83 (2*s*)

[a]) Spectra measured at 500 (^{1}H) or 75 MHz (^{13}C), 295 K, in $CDCl_3$. [b]) Signals derived from HMBC experiments. [c]) [d]) Values may be interchanged between minor and major tautomer. [e]) Multiplicity and/or coupling constant not determined due to overlapping signals. [f]) Some signals are broadened or even doubled because of epimerization at C(2″).

and CH_2(7), confirmed by DQF-COSY (^{1}H,^{1}H *J*-correlated 2D) cross-peaks, is part of the five-membered ring. Its position is unambiguously determined by many HMBC cross-peaks. Additionally, the five-membered ring is substituted at C(6) by a 1-hydroxy-1-methylethyl group (δ(C) 72.0 (C(1‴)), 29.3 (C(2‴)), and 29.4 (Me–C(1‴)). The acyl side chain at C(3) is identified as a 2-methylpropanoyl moiety.

The tautomer mixture **2**/**2a** is almost identical to **1**/**1a**, possessing an identical molecular formula (deduced from the $[M + Na]^+$ ion at *m/z* 385.1982 in HR-MALDI) and similar correlations in the DQF-COSY, HSQC, and HMBC plots. The minor chemical-shift differences for some resonances are due to a change in the relative configuration of the two compounds (*Tables 1* and *2*). The exact determination of the relative configuration is based upon the following arguments: the rigid bicyclic ring

Table 2. *^{13}C- and ^{1}H-NMR Data of the Minor Tautomers* **1a**–**3a**[a]). δ in ppm, J in Hz.

	1a		**2a**		**3a**[f])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	61.2 (s[b]))		61.3 (s)		61.4 (s[b]))	
C(2)	201.2 (s[b]))		202.1 (s[b]))		202.2 (s[b]))	
C(3)	111.3 (s[b]))		107.4 (s)		107.8 (s[b]))	
C(4)	193.8 (s[b]))		193.6 (s[b]))		193.8 (s[b]))	
C(5)	67.1 (s[b]))		66.7 (s[b]))		66.8 (s[b]))	
H–C(6)	52.3 (d)	2.00 (t, J = 9.4)	48.7 (d)	2.16 (m[e]))	48.66, 48.70 (2d)	2.18 (m[e]))
CH_2(7)	31.1 (t)	2.24 (br. d, J = 10.1, 2 H)	33.4 (t)	2.15 (m, 2 H)[e])	33.3, 33.4 (2t)	2.10–2.18 (m, 2 H)[e])
C(8)	206.3 (s[b]))		207.4 (s[b]))		207.5 (s[b]))	
Me–C(5)	15.4 (q)	1.50 (s)	12.0 (q)	1.52 (s)	12.0 (q)	1.51, 1.52 (2s)
CH_2(1′)	24.9 (t)	2.57 (m[e])) 2.74 (dd, J = 8.3, 15.4)	25.0 (t)	2.56 (m) 2.73 (dd, J = 8.8, 14.9)	25.0 (t)	2.56 (m) 2.73 (dd, J = 7.0, 15.0)
H–C(2′)	118.4 (d)	5.16 (m)	118.9 (d)	5.24 (m)	119.0 (d)	5.24 (m[e]))
C(3′)	135.6 (s)		135.5 (s)		135.5 (s[b]))	
Me(4′)	26.0 (q)	1.74 (br. s)	25.9 (q)	1.74 (br. s)	25.9 (q)	1.74 (br. s)
Me–C(3′)	18.0 (q)	1.70 (br. s)	18.0 (q)	1.70 (br. s)	18.0 (q)	1.70 (br. s)
C(1″)	208.3 (s)		209.3 (s[b]))		208.9 (s[b]))	
H–C(2″)	34.7 (d)	3.99 ($sept$, J = 6.7)	34.8 (d)	3.99 ($sept$, J = 6.8)	41.0, 41.1 (2d)	3.82–3.95 (m[e]))
Me(3″) or CH_2(3″)	18.7 (q[c]))	1.13–1.16 (m[e]))	18.7 (q)	1.14 (d, J = 6.4)	26.6 (t)	1.70–1.77 (m[e])) 1.38–1.46 (m[e]))
Me–C(2″)	18.8 (q[c]))	1.13–1.16 (m[e]))	18.9 (q)	1.19 (d, J = 6.8)	11.7, 11.8 (2q)	0.88–1.00 (m[e]))
Me(5″)					16.0, 16.4 (2q)	1.12, 1.17 (2d, J = 6.9)
C(1‴)	72.1 (s[d]))		73.9 (s)		73.9 (s[b]))	
Me(2‴)	29.3 (q)	1.45 (s)	27.1 (q)	1.22 (s)	26.8 (q)	1.21 (br. s)
Me–C(1‴)	29.5 (q)	1.15 (s)	29.5 (q)	1.23 (s)	29.3 (q)	1.23 (br. s)
OH		18.74 (s)		18.75 (s)		18.74, 18.77 (2s)

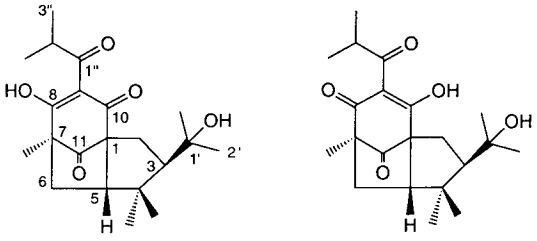
[a]) Spectra measured at 500 (^{1}H) or 75 MHz (^{13}C), 295 K, in $CDCl_3$. [b]) Signals derived from HMBC experiments. [c]) [d]) Values may be interchanged between minor and major tautomer. [e]) Multiplicity and/or coupling constant not determined due to overlapping signals. [f]) Some signals are broadened or even doubled because of epimerization at C(2″).

system determines the relative configuration at the chirality centres C(1) and C(5). The carbonyl bridge between C(1) and C(5) constrains the substituents at C(1) and C(5) to be *cis* and, hence, Me–C(5) is positioned below the plane of the five-membered ring. NOESY Experiments allow the determination of the relative configuration at the third chiral centre C(6). In **1**/**1a**, NOE cross-peaks between Me–C(5) and H–C(6) confirm the *endo* position of H–C(6). No corresponding NOE signal is observed in **2**/**2a**, and, hence, H–C(6) most probably is in *exo* position. Thus, **1**/**1a** and **2**/**2a** are epimers at C(6), with a 6-*exo*-(1-hydroxy-1-methylethyl) group in the case of **1**/**1a** and a 6-*endo*-(1-hydroxy-1-methylethyl) group in the case of **2**/**2a**.

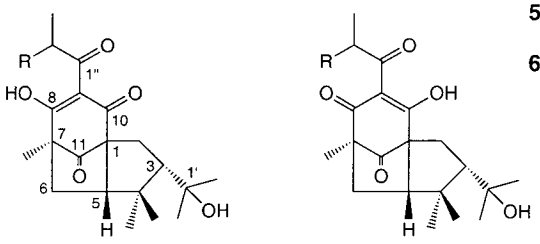
A third substance and its tautomer, *i.e.*, **3** and **3a**, respectively, are identified by comparing the ^{1}H- and ^{13}C-NMR chemical shifts with the corresponding data from **1**/**1a**

and **2**/**2a** (*Tables 1* and *2*). An increase of the molecular weight by 14 atomic mass units to give rise to M^+ at *m/z* 376 (DEI-MS) and to $[M+K]^+$ at *m/z* 415.2090 (HR-MALDI) suggests the replacement of the 2-methylpropanoyl group at C(3) by a 2-methylbutanoyl group. Further data obtained from HSQC (heteronuclear single-quantum coherence), HMBC, DQF-COSY, and NOESY experiments allow the identification of **3** as 4-hydroxy-6-*endo*-(1-hydroxy-1-methylethyl)-5-methyl-3-(2-methylbutanoyl)-1-(3-methylbut-2-enyl)bicyclo[3.2.1]oct-3-ene-2,8-dione. In addition, some of the signals in the ^{1}H- and ^{13}C-NMR spectra are broadened or even doubled. These signal doubling and chemical-shift differences are most probably caused by epimerization. However, it was not possible to determine which of the chiral C-atoms caused the epimerization by interpretation of the NMR data. But the fact that only isolates with a 2-methylbutanoyl unit at C(3), and not the ones with a 2-methylpropanoyl side chain show epimerization, strongly suggests that the introduction of the chirality centre C(2″) is responsible. Furthermore, for OH–C(4), four clearly separated ^{1}H-NMR signals at δ(H) 18.83 and 18.80 (**3**), and δ(H) 18.77 and 18.74 (**3a**) in a ratio of *ca.* 1.6 : 1.7 : 1.0 : 1.0 are displayed for the tautomer mixture **3**/**3a**. These facts suggest that the compound exists not only in two tautomeric forms in a ratio of *ca.* 1.6 : 1 (**3**/**3a**), but is also an inseparable mixture of epimers at C(2″) in a ratio of *ca.* 1 : 1.

The tautomer mixture **4**/**4a** shows a molecular ion M^+ at *m/z* 362 (DEI-MS). This molecular mass in combination with ^{1}H- and ^{13}C-NMR spectra (including DEPT135/90) (*Tables 3* and *4*) establish the molecular formula as $C_{21}H_{30}O_5$. In contrast to all other isolated compounds, the HR-MALDI-MS displays no $[M+Na]^+$ peak (see *Exper. Part*). Once again, doubled ^{1}H- and ^{13}C-NMR patterns in a ratio of *ca.* 2 : 1, and the absence of further molecular-ion peaks in the MS establish the presence of two enol tautomers, with **4** being the predominant one. Interpretation of the 1D and 2D spectra



4 1'-hydroxyialibinone A **4a**



5/5a R = Me

6/6a R = Et

5/6 1'-hydroxyialibinone B/D **5a/6a**

Table 3. *^{13}C- and ^{1}H-NMR Data of the Major Tautomers* **4**–**6**[a]). δ in ppm, J in Hz.

	4		**5**		**6**[d])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	71.4 (*s*)		71.4 (*s*)		71.5 (*s*)	
CH_2(2)	23.6 (*t*)	2.27 (*dd*, J = 6.4, 13.5)	22.7 (*t*)	2.13 (*t*, J = 13.1)	22.7 (*t*)	2.13 (br. *t*, J = 13.0)
		2.46 (*dd*, J = 11.3, 13.5)		2.63 (*dd*, J = 7.5, 13.4)		2.63 (*dd*, J = 7.4, 13.3)
H–C(3)	57.9 (*d*)	1.55 (*dd*, J = 6.4, 11.3)	60.9 (*d*)	1.85 (*dd*, J = 7.5, 12.8)	60.8, 60.9 (2*d*)	1.86 (*m*[c]))
C(4)	43.4 (*s*)		44.8 (*s*)		44.8 (*s*)	
H–C(5)	57.0 (*d*)	2.35 (br. *t*, J = 9.1, 9.7)	56.3 (*d*)	2.23 (*m*[c]))	56.3, 56.4 (2*d*)	2.23 (*m*[c]))
CH_2(6)	34.2 (*t*)	1.75 (*dd*, J = 8.9, 13.2)	32.0 (*t*)	1.89 (*dd*, J = 4.8, 13.3)	32.1 (*t*)	1.89 (*m*[c]))
		2.21 (*dd*, J = 9.9, 13.2)		2.18 (*m*[c]))		2.18 (*t*, J = 13.0)
C(7)	61.4 (*s*)		61.6 (*s*)		61.7 (*s*)	
C(8)	201.7 (*s*)		201.9 (*s*)		202.0 (*s*[e]))	
C(9)	109.4 (*s*)		107.9 (*s*)		108.3 (*s*[e]))	
C(10)	191.5 (*s*)		191.3 (*s*)		191.3 (*s*[e]))	
C(11)	206.3 (*s*)		206.9 (*s*)		207.0 (*s*[e]))	
Me_α–C(4)	26.5 (*q*)	1.08 (*s*)	17.2 (*q*)	0.83 (*s*)	17.2 (*q*)	0.83 (*s*)
Me_β–C(4)	26.6 (*q*)	1.10 (*s*)	29.0 (*q*)	1.08 (*s*)	29.0 (*q*)	1.076, 1.081 (2*s*)
Me–C(7)	12.3 (*q*)	1.40 (*s*)	12.3 (*q*)	1.39 (*s*)	12.3 (*q*)	1.385, 1.389 (2*s*)
C(1′)	73.1 (*s*)		72.8 (*s*)		72.9 (*s*)	
Me(2′)	30.6 (*q*)	1.34 (*s*[b]))	30.3 (*q*)	1.36 (*s*)	30.3 (*q*)	1.37 (*s*)
Me–C(1′)	30.9 (*q*)	1.34 (*s*[b]))	31.3 (*q*)	1.34 (*s*)	31.3 (*q*)	1.34 (*s*)
C(1″)	208.7 (*s*)		209.6 (*s*)		209.2, 209.3 (2*s*[e]))	
H–C(2″)	34.9 (*d*)	4.02 (*sept.*, J = 6.8)	35.0 (*d*)	4.05 (*sept.*, J = 6.8)	41.2, 41.3 (2*d*)	3.94 (*m*, J = 6.8)[c])
Me(3″) or CH_2(3″)	18.5 (*q*)	1.159 (*d*, J = 6.8)	18.6 (*q*)	1.19 (*d*, J = 6.8)	26.3 (*t*)	1.46 (*m*[c]))
						1.76 (*m*[c]))
Me–C(2″)	19.0 (*q*)	1.156 (*d*, J = 6.8)	19.0 (*q*)	1.15 (*d*, J = 6.8)	11.8 (*q*)	0.95 (*t*, J = 7.4)
Me(4″)					16.2, 16.6 (2*q*)	1.132, 1.174 (2*d*, J = 6.8)
OH		18.80 (*s*)		18.86 (*s*)		18.89, 18.86 (2*s*)

[a]) Spectra measured at 500 (^{1}H) or 75 MHz (^{13}C), 295 K, in $CDCl_3$. [b]) Values may be interchanged between minor and major tautomer. [c]) Multiplicity and/or coupling constant not determined due to overlapping signals. [d]) Some signals are broadened or even doubled because of epimerization at C(2″). [e]) Signals derived from HMBC experiments.

of this compound and comparison with the spectral data of the previously isolated ialibinones A–E show unambiguously that **4**/**4a** is a derivative of ialibinone A. The only difference is the replacement of the 1-methylvinyl group at C(3) with a 1-hydroxy-1-methylethyl group.

A HSQC (^{13}C,^{1}H ^1J correlated 2D) experiment with **4** was utilized to assign the protons to their attached C-atoms. The substituent at C(3) is identified by signals for a quaternary C(1′) at δ(C) 73.1 and two tertiary Me groups Me(2′) and Me–C(1′) at δ(C) 30.6 and 30.9 ppm. The chemical-shift value of the quaternary C(1′) is explained by an OH group located at C(1′). The position of this 1-hydroxy-1-methylethyl group at C(3) is

Table 4. *^{13}C- and ^{1}H-NMR Spectral Data of the Minor Tautomers* **4a**–**6a**[a]). δ in ppm, J in Hz.

	4a		**5a**		**6a**[c])	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
C(1)	67.1 (*s*)		67.4 (*s*)		67.5 (*s*[d]))	
CH_2(2)	22.8 (*t*)	2.32 (*dd*, *J* = 6.3, 13.5)	22.0 (*t*)	2.25 (*t*, *J* = 13.5)	22.1 (*t*)	2.24, 2.25 (2 br. *t*, *J* = 13.6)
		2.49 (*dd*, *J* = 11.4, 13.5)		2.61 (*dd*, *J* = 7.9, 13.7)		2.62 (*dd*, *J* = 7.5, 13.6)
H–C(3)	57.8 (*d*)	1.56 (*dd*, *J* = 6.3, 11.4)	61.0 (*d*)	1.92 (*dd*, *J* = 7.8, 12.7)	61.0 (*d*)	1.92 (*dd*, *J* = 7.7, 12.7)
C(4)	43.6 (*s*)		45.0 (*s*)		45.0 (*s*)	
H–C(5)	59.4 (*d*)	2.42 (*dd*, *J* = 8.5, 10.0)	58.5 (*d*)	2.38 (*dd*, *J* = 4.6, 10.4)	58.5 (*d*)	2.37 (*m*[e]))
CH_2(6)	33.4 (*t*)	1.66 (*dd*, *J* = 8.5, 13.3)	30.4 (*t*)	1.79 (*dd*, *J* = 4.6, 14.2)	30.5 (*t*)	1.78 (*m*[e]))
		2.10 (*dd*, *J* = 10.1, 13.3)		2.06 (*dd*, *J* = 10.4, 14.2)		2.06 (*dd*, *J* = 10.5, 14.3)
C(7)	65.1 (*s*)		65.7 (*s*)		65.7 (*s*[d]))	
C(8)	194.5 (*s*)		193.5 (*s*)		193.7 (*s*[d]))	
C(9)	109.3 (*s*)		107.8 (*s*)		108.3 (*s*[d]))	
C(10)	200.4 (*s*)		200.2 (*s*)		200.3 (*s*[d]))	
C(11)	206.8 (*s*)		207.4 (*s*)		207.5 (*s*[d]))	
Me_α–C(4)	26.5 (*q*)	1.07 (*s*)	16.7 (*q*)	0.81 (*s*)	16.7 (*q*)	0.81 (*s*)
Me_β–C(4)	26.6 (*q*)	1.13 (*s*)	29.0 (*q*)	1.09 (*s*)	29.0 (*q*)	1.09 (br. *s*)
Me–C(7)	13.1 (*q*)	1.34 (*s*)	13.0 (*q*)	1.32 (*s*)	13.0 (*q*)	1.325, 1.323 (2*s*)
C(1′)	73.0 (*s*)		72.8 (*s*)		72.9 (*s*)	
Me(2′)	30.6 (*q*)	1.35 (*s*[b]))	30.3 (*q*)	1.38 (*s*)	30.3 (*q*)	1.38 (*s*)
Me–C(1′)	30.9 (*q*)	1.35 (*s*[b]))	31.2 (*q*)	1.35 (*s*)	31.2 (*q*)	1.35 (*s*)
C(1″)	207.5 (*s*)		209.1 (*s*)		208.7 (*s*[d]))	
H–C(2″)	34.2 (*d*)	3.94 (*sept.*, *J* = 6.8)	34.7 (*d*)	4.03 (*sept.*, *J* = 6.8)	41.0 (*d*)	3.91 (*m*[e]))
Me(3″) or CH_2(3″)	18.6 (*q*)	1.13 (*d*, *J* = 6.8)	18.8 (*q*)	1.19 (*d*, *J* = 6.8)	26.6 (*t*)	1.41 (*m*[e]))
						1.72 (*m*[e]))
Me–C(2″)	19.2 (*q*)	1.21 (*d*, *J* = 6.8)	18.8 (*q*)	1.14 (*d*, *J* = 6.8)	11.7 (*q*)	0.903, 0.907 (2*t*, *J* = 7.4)
Me(4″)					16.2, 16.3 (2*q*)	1.128, 1.168 (2*d*, *J* = 6.8)
OH		18.42 (*s*)		18.81 (*s*)		18.83, 18.00 (2*s*)

[a]) Spectra measured at 500 (^{1}H) or 75 MHz (^{13}C), 295 K, in $CDCl_3$. [b]) Values may be interchanged between minor and major tautomer. [c]) Some signals are broadened or even doubled because of epimerization at C(2″). [d]) Signals derived from HMBC experiments. [e]) Multiplicity and/or coupling constant not determined due to overlapping signals.

verified by a HMBC experiment, showing correlations between C(3) and Me(2′) and Me–C(1′), on the one hand, and between C(1′) and H–C(3), on the other hand. Similar arguments were used for the structure elucidation of the minor tautomer **4a**.

The spectral data of the isolated viscous oils **5**/**5a** and **6**/**6a** are very similar to those of **4**/**4a**. Their FAB-MS (positive mode) shows pseudomolecular-ion peaks $[M+H]^+$ at *m/z* 363 (**5**/**5a**) and 377 (**6**/**6a**). HR-MALDI displays $[M+Na]^+$ ions at *m/z* 385.1982

(calc. 385.1991) and *m/z* 399.2137 (calc. 399.2148), respectively, which are in agreement with the molecular formulae $C_{21}H_{30}O_5$ and $C_{22}H_{32}O_5$. Again, interpretation of the NMR data (*Tables 3* and *4*) and comparison with the ialibinones reveal their close relationship in the covalent structure. The data allow the conclusion that **4**/**4a** and **5**/**5a** are the C(3) epimers of 8-hydroxy-3-(1-hydroxy-1-methylethyl)-4,4,7-trimethyl-9-(2-methylpropanoyl)-5βH-tricyclo[5.3.1.$0^{1,5}$]undec-8-ene-10,11-dione, with a 3β-(1-hydroxy-1-methylethyl) group in **4**/**4a** and a 3α-(1-hydroxy-1-methylethyl) group in **5**/**5a**. Considering the close relationship to the ialibinones A and B, the new metabolites are named 1′-hydroxyialibinones A (**4**/**4a**) and B (**5**/**5a**), respectively.

The tautomer mixture **5**/**5a** has the same molecular formula $C_{21}H_{30}O_5$ as **4**/**4a**, as established by spectral data from FAB-MS and ^{1}H- and ^{13}C-NMR and DEPT135 experiments. Moreover, **5**/**5a** displays similar HSQC, HMBC, and DQF-COSY cross-peak patterns suggesting an almost identical covalent structure. The only noticeable difference is a significant upfield shift of Me_α–C(4) in the ^{13}C-NMR spectrum from δ(C) 26.5/26.5 ppm (**4**/**4a**) to 17.2/16.7 ppm (**5**/**5a**). A similar effect is observed for the ^{1}H-NMR shifts of Me_α–C(4) changing from δ(H) 1.08/1.07 (**4**/**4a**) to 0.83/0.81 (**5**/**5a**). A correlation between the position of the substituent at C(3) and the shift of the NMR signals of Me_α–C(4) has already been shown for the ialibinones. Substitution in α position (ialibinones B and D) led to a remarkable upfield shift in comparison to the β-substituted ialibinones A and C.

In analogy to the ialibinones B and D, the DQF-COSY and HMBC data readily reveal that the 2-methylpropanoyl group at C(9) is replaced by a 2-methylbutanoyl unit in compounds **6**/**6a**. This finding is further supported by a difference of 14 atomic mass units for **6**/**6a** compared to compound **5**/**5a**. As already described for **3**/**3a**, some of the signals in the ^{1}H- and ^{13}C-NMR spectra are broadened or even doubled, which in all probability is caused by epimerization at C(2″). This doubling effect is particularly remarkable in the signals of OH–C(8): for the tautomer mixture **6**/**6a**, four clearly separated ^{1}H-NMR signals at δ(H) 18.89 and 18.86 (**6**) and δ(H) 18.83 and 18.80 (**6a**) in a ratio of 1.3 : 2.4 : 1.9 : 1.0 are displayed. These data suggest that the compound exists not only in two tautomeric forms in a ratio of *ca.* 1.3 : 1 (**6**/**6a**), but forms again an inseparable mixture of epimers at C(2″) in a ratio of *ca.* 1 : 2. Except for the replacement of the 1-methylvinyl group at C(3) by a 1-hydroxy-1-methylethyl group, compound **6**/**6a** is identical to ialibinone D, and, therefore, we suggest the name 1′-hydroxyialibinone D.

We could safely exclude that the three 1′-hydroxyialibinones are artefacts of isolation procedures, because the previously (without further precaution) isolated ialibinones A–E showed no degradation, even after several months of storage at 4° and occasional exposure to room temperature and air. Until now, the stability of the 1′-hydroxyialibinones seems to be comparable to the stability of the ialibinones.

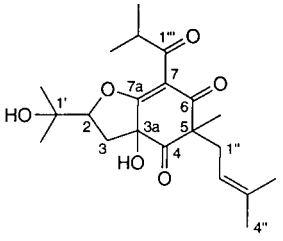
Finally, the most polar component **7** was isolated by reversed-phase HPLC. Within a few days, the isolate decomposed in $CDCl_3$ quantitatively to compound **8** and its tautomer **8a**. However, it was possible to measure a ^{1}H-NMR of pure **7**, as well as ^{1}H, ^{13}C, DQF-COSY, HSQC, and HMBC experiments of only partially decomposed **7**. Furthermore, all 1D and 2D NMR experiments were repeated after total conversion from **7** to **8**/**8a**. This allowed – once the structure **8**/**8a** was determined – the assignment of the NMR signals of the mixture to the corresponding compounds and thus to establish the constitution of **7**.

The molecular formula of **8**/**8a**, $C_{21}H_{32}O_7$, was derived from FAB- and HR-MALDI-MS, as well as from ^{1}H- and ^{13}C-NMR spectroscopy (*Table 5*), including

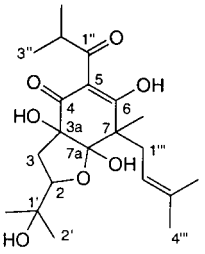
HMBC experiments. Comparison of the NMR data of an oxidation product of hyperforin [5] and the benzoylphloroglucinol derivative sampsonione L [6] with **8** confirmed the proposed structure. The tautomer mixture **8**/**8a** has the four chiral centres C(2), C(3a), C(7), and C(7a); however, it was not possible to determine the relative configuration at these positions by a NOESY experiment. This was due to the low proton density and the lack of suitable nonexchangeable protons.

^{1}H-and ^{13}C-NMR Data of **8** indicate the presence of a prenyl residue: δ(C) 29.3 (CH_2(1‴)), 121.0 (H–C(2‴)), 135.2 (C(3‴)), 26.2 (Me(4‴)), and 18.0 (*Me*–C(3‴)). Furthermore, signals common for a 2-methylpropanoyl side chain (δ(C) 207.0 (C(1″)), 34.5 (H–C(2″)), 19.2 (Me(3″)), and 19.3 (Me–C(2″)), a keto group (δ(C) 195.5 (C(4))), and a quaternary C(6) (δ(C) 204.9) substituted by an enol OH group can be identified. Signals for another Me group, *Me*–C(7) (δ(C) 21.4), and four additional quaternary C-atoms (C(7), C(3a), C(7a), and C(5) at δ(C) 51.3, 83.2, 102.2, and 106.6, resp.) are identified. These data exhibit enough characteristic features to recognize that **8** presents a tautomeric acylphloroglucinol derivative substituted with a prenyl group. Due to the high number of quaternary C-atoms, HMBCs are the only valuable tool to connect these basic fragments and to establish the covalent structure. Likewise, the position of the OH groups at C(3a), C(6), and C(7a) is determined by HMBCs between the OH proton and the adjacent C-atoms.

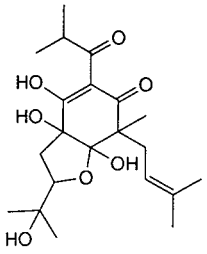
In addition to the partial structure for **8** described above, the presence of a H–C(2) at δ(C) 82.9 showing COSY cross-peaks with CH_2(3) is identified. HMBCs between C(2) and Me(2′)/Me–C(1′), between C(1′) and H–C(2)/CH_2(3), as well as between C(1′) and Me(2′)/Me–C(1′) confirm the substitution of C(2) by the dimethylated C(1′). Further HMBCs allow the determination of the position of CH_2(3) at C(3a). Considering the chemical shifts and the molecular formula obtained by FAB- and HR-MALDI-MS, C(2) and C(1′) are O-substituted, one with a free OH group, the other most probably being part of an ether bridge to C(7a), participating either in a five- or six-membered ring. An HMBC between C(7a) and H – C(2) strongly indicates an ether bridge between C(2) and C(7a), thereby forming a tetrahydrofuran ring. Consequently, C(1′) has to be substituted by the free OH group. The signal of this fourth free OH function is too broad to show any HMBC cross-peaks. Hence, the position of this OH group is verified by measurement of the deuterium-isotope shift.



7 furonewguinone A



8 furonewguinone B



8a

Main HMBCs of **8**:
C(2)/CH_3(2′), CH_3–C(1′)
C(3)/H–C(2)
C(3a)/CH_2(3), OH–C(3a), OH–C(7a)
C(4)/CH_2(3), OH–C(3a)
C(5)/OH–C(6)
C(6)/OH–C(6), CH_3–C(7), CH_2(1‴)
C(7)/CH_3–C(7), CH_2(1‴), OH–C(7a)
C(7a)/H–C(2), CH_2(3), OH–C(3a), CH_3–C(7), CH_2(1‴), OH–C(7a)
C(1′)/CH_3(2′), CH_3–C(1′), H–C(2), CH_2(3)
C(1″)/H–C(2″), CH_3(3″), CH_3–C(2″), OH–C(6)
C(2″)/CH_3(3″), CH_3–C(2″), OH–C(6)
CH_3–C(7)/CH_2(1‴)
C(2‴)/CH_3(4‴), CH_3–C(3‴)
C(3‴)/CH_2(1‴), CH_3(4‴), CH_3–C(3‴)

Table 5. *^{13}C- and ^{1}H-NMR Data of* **7** *and* **8**/**8a**[a]). δ in ppm, J in Hz.

	7		**8**		**8a**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
H–C(2)	92.3 (*d*)	4.81 (*t*, *J* = 7.8)	82.9 (*d*)	3.61 (*dd*, *J* = 7.3, 9.7)	82.2 (*d*)	3.65 (*dd*, *J* = 6.6, 10.2)
CH_2(3)	32.7 (*t*)	2.30 (*d*, *J* = 7.8, 2 H)	38.4 (*t*)	2.38 (*dd*, *J* = 7.3, 13.1) 2.48 (*dd*, *J* = 9.7, 13.1)	36.7 (*t*)	2.44 (*dd*, *J* = 10.2, 12.8) 2.71 (*dd*, *J* = 6.6, 12.8)
C(3a)	80.0 (*s*[b]))		83.2 (*s*)		80.3 (*s*)	
C(4)	198.4 (*s*[b]) [c]))		195.5 (*s*)		190.8 (*s*)	
C(5)	57.9 (*s*)		106.6 (*s*)		107.9 (*s*[b]))	
C(6)	204.7 (*s*[b]) [c]))		204.9 (*s*)		196.7 (*s*[b]))	
C(7)	– (*s*[d]))		51.3 (*s*)		52.4 (*s*)	
C(7a)	173.0 (*s*[b]))		102.2 (*s*)		102.2 (*s*)	
Me–C(5) or Me–C(7)	24.5 (*q*)	1.50 (*s*)	21.4 (*q*)	1.26 (*s*)	19.7 (*q*)	1.34 (*s*)
C(1′)	70.6 (*s*[b]))		70.3 (*s*)		70.3 (*s*)	
Me(2′)	23.8 (*q*)	1.20 (*s*)	24.3 (*q*)	1.04 (*s*)	24.5 (*q*)	1.06 (*s*)
Me–C(1′)	26.5 (*q*)	1.35 (*s*)	27.3 (*q*)	1.24 (*s*)	27.3 (*q*)	1.24 (br. *s*)
CH_2(1″) or C(1″)	37.4 (*t*)	2.45 (*dd*, *J* = 8.4, 13.7) 2.67 (*dd*, *J* = 7.0, 13.7)	207.0 (*s*[b]))		211.3 (*s*)	
H–C(2″)	118.2 (*d*)	4.73 (br. *t*, *J* = 8.2, 7.0)	34.5 (*d*)	3.75 (*sept.*, *J* = 6.8)	36.1 (*d*)	3.90 (*sept.*, *J* = 6.8)
C(3″) or Me(3″)	135.8 (*s*[b]))		19.2 (*q*)	1.14 (*d*, *J* = 6.8)	18.9 (*q*)	1.10 (*d*, *J* = 6.8)
Me(4″) or Me–C(2″)	25.9 (*q*)	1.56 (br. *s*)	19.3 (*q*)	1.25 (*d*, *J* = 6.8)	18.9 (*q*)	1.20 (*d*, *J* = 6.8)
Me–C(3″)	17.7 (*q*)	1.54 (br. *s*)				
C(1‴) or CH_2(1‴)	204.9 (*s*)		29.3 (*t*)	2.62 (br. *dd*, *J* = 6.0, 14.8) 2.78 (*dd*, *J* = 9.4, 14.8)	29.9 (*t*)	2.49 (*m*[e])) 2.86 (*dd*, *J* = 10.8, 14.8)
H–C(2‴)	40.2 (*d*)	3.04 (*sept.*, *J* = 6.9)	121.0 (*d*)	5.52 (*m*[e]))	121.3 (*d*)	5.54 (*m*[e]))
Me(3‴) or C(3‴)	17.6 (*q*)	1.13 (*d*, *J* = 6.9)	135.2 (*s*[b]))		137.4 (*s*[b]))	
Me–C(2‴) or Me(4‴)	18.0 (*q*)	1.07 (*d*, *J* = 6.9)	26.2 (*q*)	1.76 (br. *s*)	26.2 (*q*)	1.79 (br. *s*)
Me–C(3‴)			18.0 (*q*)	1.74 (br. *s*)	18.3 (*q*)	1.79 (br. *s*)
OH–C(3a)				3.65 (*s*)		3.31 (*s*)
OH–C(4)				–		18.32 (*s*)
OH–C(6)				18.68 (*s*)		–
OH–C(7a)				4.59 (*s*)		4.95 (*s*)

[a]) Spectra measured at 300 (^{1}H of **7**), 500 (^{1}H of **8**/**8a**), or 75 MHz (^{13}C), 295 K, in $CDCl_3$. [b]) Signals derived from HMBC experiments. [c]) Values may be interchanged. [d]) Chemical shift not determined due to low amount (no signal observed in ^{13}C experiment) and no long-range correlations, which would allow an indirect determination. [e]) Multiplicity and/or coupling constant not determined due to overlapping signals.

Addition of a drop of D_2O to the $CDCl_3$ solution induces a substantial upfield shift (-0.1 ppm) of C(1′), compared to the values obtained in pure $CDCl_3$, thereby confirming the substitution of C(1′) with a free OH group.

The structure of **7** was proposed based upon the results of **8/8a**. The ^{1}H-NMR spectrum of **7** shows no signals in the region of 18 to 20 ppm, and the ^{1}H- and ^{13}C-NMR spectra contain no doubled peaks (*Table 5*). These facts strongly suggest that the keto-enol equilibrium of the β-dicarbonyl system is blocked by formation of an enol ether. Apart from the described difference, the ^{1}H- and ^{13}C-NMR spectra of **7** are highly similar to those of **8/8a**. COSY and HMBC experiments clearly define that C(5) is flanked by two keto groups and is still substituted by a Me and a prenyl side chain, thus the remaining oxidized prenyl residue at C(3a) forms a furan ring with an O-atom at C(7a). Although all spectral data confirm that the structure of **7** is indeed correct, rapid decomposition precluded a detailed analysis by 2D NMR and MS. However, the similarity of the compounds **7** and **8/8a** indicates that **8** is formed from **7** by a oxidative rearrangement reaction and, hence, **7** presents a likely precursor of **8**.

The isolates **1/1a** to **6/6a** and the decomposition product **8/8a** were tested for their antibacterial potential against *B. cereus*, *M. luteus*, and *S. epidermidis*, as well as for their cytotoxic potential against a KB cell line (*Table 6*). The 1′-hydroxyialibinones **4/4a**, **5/5a**, and **6/6a**, and the other tested compounds show identical or slightly reduced antibacterial activity compared with the ialibinones [3]. The cytotoxic activity is rather weak, compared with other phloroglucinol derivatives previously isolated from *H. papuanum* [4]. The ialibinones A, B, and D displayed IC_{50} values of 8.0 ± 2.1, 7.3 ± 1.9, and 6.6 ± 1.9 μg/ml, respectively, compared to 25.3 ± 1.4, >40, and 32.5 ± 3.2 μg/ml of the corresponding 1′-hydroxyialibinones **4/4a**, **5/5a**, and **6/6a**, respectively. Hence, the hydroxylation of the ialibinones reduces the cytotoxicity remarkably.

Together with the stronger antibacterial activity of the previously isolated compounds, the activities reported here suggest that the aerial parts of this plant may have a beneficial effect on sores and, therefore, justifies their traditional use as a remedy for wounds.

Table 6. *Biological Activities of the Isolated Compounds*

	Minimum inhibition concentration (*MIC*) [μg/ml] in broth			Cytotoxicity against KB cells (ATCC CCL 17) (IC_{50} [μg/ml])
	B. cereus (ATCC 10702)	*S. epidermidis* (ATCC 12228)	*M. luteus* (ATCC 9341)	
1/1a	128	64	64	20.7 ± 3.5
2/2a	64	64	64	17.9 ± 0.3
3/3a	64	32	32	12.4 ± 0.75
4/4a	128	64	64	25.3 ± 1.4
5/5a	128	128	64	>40
6/6a	128	64	64	32.5 ± 3.2
8/8a	–[a])	128	128	>40
Chloramphenicol	2	4	2	
Podophyllotoxin				0.006 ± 0.0003

[a]) No difference to blind test.

Experimental Part

General. All solvents were HPLC grade. High-speed counter-current chromatography (HSCCC): *Kromaton II* from *S.E.A.B. Company* (F-Villejuif) with an anal. (75 ml) or prep. column (total volume 1000 ml), connected to a cooling unit (model *SK 3390*, *RITTAL-Werk*, D-Herborn), a manometric module *807*, and a high-pressure pump (model *305*) from *Gilson* (F-Villiers-le-Bel). HPLC: *Merck-Hitachi L6200A-Intelligent* pump connected to a *Rheodyne-7125* injector, a *Merck-Hitachi L-4250* UV/VIS detector, a *Merck D-2500* chromato-integrator, and a *Knauer* HPLC column (*Spherisorb S5 ODS II*, 5 μm, 250 × 16 mm). Open column chromatography (open CC): column 100 × 4.5 cm; silica gel (*Merck*), particle size 63–200 μm. TLC: silica gel *60* F_{254} precoated aluminium sheets (0.2 mm; *Merck*) and *RP-18-*F_{254} precoated sheets (0.25 mm; *Merck*) for TLC controls. Optical rotations: *Perkin-Elmer 241* polarimeter; MeOH soln. UV Spectra: *Uvikon 930* spectrophotometer; MeOH soln. ^{1}H-NMR, ^{1}H,^{1}H-COSY, 500-ms NOESY, and ^{13}C,^{1}H-HMBC/HSQC experiments: *Bruker DRX-500*; at 295 K and 500.13 (^{1}H) or 125.77 MHz (^{13}C); for ^{13}C-NMR and the 2D spectra (COSY, HSQC, and HMBC) of **7**, *Bruker AMX-300* spectrometer at 295 K and 300.13 (^{1}H) or 75.47 MHz (^{13}C); $CDCl_3$ soln. referenced against residual non-deuterated solvent C*H*Cl_3 (δ(H) 7.27) and C*D*Cl_3 (δ(C) 77.0). DEI-MS: micromass *Tribrid* double-focusing mass spectrometer; 70 eV. FAB-MS (positive mode): *VG-ZAB-2SEQ* spectrometer; 3-nitrobenzyl alcohol (3-NOBA) as matrix. HR-MALDI-MS: *IonSpec-Ultima-FTMS* spectrometer; 2,5-dihydroxybenzoic acid (DHB) as matrix.

Plant Material. The aerial parts of *Hypericum papuanum* RIDLEY (Guttiferae) were collected north of Ialibu, Southern Highlands Province, Papua New Guinea, during September 1996. The plant was identified by *Paul Katik*, National Herbarium, Lae, PNG, and Dr. *M. M. J. van Balgooy*, Rijksherbarium, Leiden, The Netherlands. A voucher specimen is deposited in the Rijksherbarium (Leiden, The Netherlands) with the identification number ETH 96/34 27-09-96.

Extraction and Isolation. The detailed procedure for the extraction of the plant and the preliminary fractionation of the petroleum-ether extract with VLC has been reported previously [3]. An aliquot (1.83 g) of VLC fraction *5* (7.63 g, eluted with hexane/AcOEt 9:1 and 8:2) was further separated by HSCCC (prep. column; hexane/abs. EtOH/AcOEt/H_2O 83:67:33:17 (*v*/*v*) with the lower phase being the mobile phase (modified after [7]), rotation speed 450 rpm, flow rate 4 ml/min, column temp. 20°, flow direction from centre to periphery of the column; displaced amount of stationary phase 260 ml). Based on the TLC similarities, various fractions were combined to give 8 fractions at all. TLC analysis (acylphloroglucinol derivatives give turquoise to grey-blue spots on TLC, after being sprayed with the vanillin/sulfuric acid reagent) indicated *Fr. 3* (43.6 mg), *4* (27.1 mg), *5* (141.0 mg) and *7* (127 mg) to be of further interest. Consequently, these four fractions were further purified by reversed-phase HPLC (MeCN/H_2O/CF_3COOH 70:30:0.5, flow 7.5 ml/min): **4**/**4a** (2.8 mg) and **5**/**5a** (2.1 mg) from *Fr. 3*, **4**/**4a** (1.6 mg), **5**/**5a** (3.7 mg), and **2**/**2a** (2.6 mg) from *Fr. 4*, **5**/**5a** (3.5 mg), **6**/**6a** (5.9 mg), and **3**/**3a** (4.3 mg) from *Fr. 5*, and finally **1**/**1a** (5.8 mg) from *Fr. 7*. All were obtained as colorless or slightly yellowish oils.

The more polar VLC *Fr. 10* (2.45 g) was subjected to open CC (gradient hexane/AcOEt 9:1 → AcOEt (100%)): 18 fractions. The antibacterial active *Fr. 8* (299 mg) was then applied in three separate portions (59 mg, 2 × 120 mg) to HSCCC (anal. column, conditions as mentioned above, except that due to the smaller volume of the anal. column, flow rate of 1 ml/min and displaced amount of stationary phase 30 ml). Identical fractions were combined to give 4 fractions. Compound **7** (10.1 mg), a colorless oil, was isolated from *Fr. 3* by reversed-phase HPLC (MeCN/H_2O 5:5 (0–10 min), and 6:4 (10–25 min), flow 7 ml/min). Within a few days, the isolate decomposed in $CDCl_3$ soln. quantitatively to **8**/**8a**.

Cytotoxicity Study. The cytotoxicity of the phloroglucinol derivatives was determined by means of a KB cell line (ATCC CCL 17) as described by *Ankli et al.* [8]. The test was performed at least in triplicate.

Antibacterial Assays. The test organisms were *Bacillus cereus* (ATCC 10702, *Gram*-positive), *Staphylococcus epidermidis* (ATCC 12228, *Gram*-positive), and *Micrococcus luteus* (ATCC 9341, *Gram*-positive). Antibacterial assays were carried out by the doubling dilution method with a modified procedure as published previously [3]. All compounds were tested, except the decomposed isolate **7**. Chloramphenicol was used as a positive control.

Enaimeone A (= rel-*(1*R,*5*R,*6*S)-*4-Hydroxy-6-(1-hydroxy-1-methylethyl)-5-methyl-1-(3-methylbut-2-enyl)-3-(2-methylpropanoyl)bicyclo[3.2.1]oct-3-ene-2,8-dione*; **1**/**1a**). Yellow oil. $[\alpha]_D^{20} = +27.8$ ($c = 0.10$, MeOH). UV (MeOH): 274 (4.1), 203 (3.9). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. DEI-MS (pos.): 362 (12, M^+), 344 (4, $M - H_2O]^+$, 235 (44), 205 (18), 165 (24). HR-MALDI-MS (pos.): 385.1983 ($[M + Na]^+$; calc. 385.1991).

Enaimeone B (= rel-(*1*R,*5*R,*6*R)-4-*Hydroxy-6-(1-hydroxy-1-methylethyl)-5-methyl-1-(3-methylbut-2-enyl)-3-(2-methylpropanoyl)bicyclo[3.2.1]oct-3-ene-2,8-dione*; **2**/**2a**). Colorless oil. $[\alpha]_D^{20} = +29.4$ ($c = 0.10$,

MeOH). UV (MeOH): 274 (4.1), 203 (3.9). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 363 (100, $[M+H]^+$). HR-MALDI-MS (pos.): 385.1982 $[M+Na]^+$ (calc. 385.1991).

Enaimeone C (= rel-*(1*R,*5*R,*6*R)*-4-Hydroxy-6-(1-hydroxy-1-methylethyl)-5-methyl-3-(2-methylbutanoyl)-1-(3-methylbut-2-enyl)bicyclo[3.2.1]oct-3-ene-2,8-dione*; **3**/**3a**). Yellow oil. $[\alpha]_D^{20} = +32.9$ ($c = 0.10$, MeOH). UV (MeOH): 274 (4.0), 203 (4.0). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. DEI-MS (pos.): 376 (6, M^+), 358 (5, $[M-H_2O]^+$), 249 (100), 205 (15), 165 (28). HR-MALDI-MS (pos.): 415.2090 ($[M+K]^+$; calc. 415.1887).

1′-Hydroxyialibinone A (= *8-Hydroxy-3β-(1-hydroxy-1-methylethyl)-4,4,7-trimethyl-9-(2-methylpropanoyl)-5β*H*-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione* = rel-*(2*R,*3a*S,*7*S,*8a*S*)-1,2,3,7,8,8a-Hexahydro-6-hydroxy-2-(1-hydroxy-1-methylethyl)-1,1,7-trimethyl-5-(2-methylpropanoyl)-4*H*-3a,7-methanoazulene-4,9-dione*; **4**/**4a**). Yellow oil. $[\alpha]_D^{20} = +3.7$ ($c = 0.10$, MeOH). UV (MeOH): 274 (4.1), 203 (3.8). ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. DEI-MS (pos.): 362 (4, M^+), 344 (37, $[M-H_2O]^+$), 275 (10), 205 (62), 149 (16). HR-MALDI-MS (pos.): no M^+ observable.

1′-Hydroxyialibinone B (= *8-Hydroxy-3α-(1-hydroxy-1-methylethyl)-4,4,7-trimethyl-9-(2-methylpropanoyl)-5β*H*-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione* = rel-*(2*R,*3a*R,*7*R,*8a*R*)-1,2,3,7,8,8a-Hexahydro-6-hydroxy-2-(1-hydroxy-1-methylethyl)-1,7,7-trimethyl-5-(2-methylpropanoyl)-4*H*-3a,7-methanoazulene-4,9-dione*; **5**/**5a**). Yellow oil. $[\alpha]_D^{20} = -35.7$ ($c = 0.10$, MeOH). UV (MeOH): 272 (4.1), 204 (3.8). ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. FAB-MS (pos.): 363 (35, $[M+H]^+$), 345 (100, $[M-OH]^+$). HR-MALDI-MS (pos.): 385.1982 ($[M+Na]^+$; calc. 385.1991).

1′-Hydroxyialibinone D (= *8-Hydroxy-3α-(1-hydroxy-1-methylethyl)-4,4,7-trimethyl-9-(2-methylbutanoyl)-5β*H*-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione* = rel-*(2*R,*3a*R,*7*R,*8a*R*)-1,2,3,7,8,8a-Hexahydro-6-hydroxy-2-(1-hydroxy-1-methylethyl)-1,1,7-trimethyl-5-(2-methylbutanoyl)-4*H*-3a,7-methanoazulene-4,9-dione*; **6**/**6a**). Yellow oil. $[\alpha]_D^{20} = -30.3°$ ($c = 0.10$, MeOH). UV (MeOH): 272 (4.1), 203 (3.9). ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. FAB-MS (pos.): 377 (32, $[M+H]^+$), 359 (100, $[M-OH]^+$); HR-MALDI-MS (pos.) 399.2137 ($[M+Na]^+$; calc. 399.2148).

Furonewguinone A (= *2,3,3a,5-Tetrahydro-3a-hydroxy-2-(1-hydroxy-1-methylethyl)-5-methyl-5-(3-methylbut-2-enyl)-7-(2-methylpropanoyl)benzofuran-4,6-dione*; **7**). Yellow oil. ^{1}H- and ^{13}C-NMR: *Table 5*. Further physical and spectroscopic data not determined due to instability.

Furonewguinone B (= *3,3a,7,7a-Tetrahydro-3a,6,7a-Trihydroxy-2-(1-hydroxy-1-methylethyl)-7-methyl-7-(3-methylbut-2-enyl)-5-(2-methylpropanoyl)benzofuran-4(2*H*)-one*; **8**/**8a**). Yellow oil (decomposition product of **7**). $[\alpha]_D^{20} = +15.6$ ($c = 0.10$, MeOH). UV (MeOH): 278 (4.2), 203 (3.9). ^{1}H- and ^{13}C-NMR: *Table 5*. FAB-MS (pos.): 397 (12, $[M+H]^+$), 379 (100, $[M-OH]^+$). HR-MALDI-MS (pos.) 419.2038 ($[M+Na]^+$; calc. 419.2046).

Special thanks go to Mr. *M. Wasescha* (Institute of Pharmaceutical Sciences, ETH-Zurich) for performing the KB-cell cytotoxicity assays. We thank *P. Katik* (National Herbarium, Lae, Papua New Guinea) and Dr. *M. M. J. van Balgooy* (Rijksherbarium, Leiden, The Netherlands) for identification of the plant material. Thanks are also due to Dr. *E. Zass* (ETH-Zurich, Chemistry Department) for performing literature searches as well as Mr. *O. Greter*, Mr. *R. Häfliger*, and Dr. *W. Amrein* (Mass Spectral Service of the Laboratory of Organic Chemistry, ETH Zurich) for recording the mass spectra. This work was supported by the *Swiss National Science Foundation*.

REFERENCES

[1] D. Holdsworth, E. Lacanienta, *Quart. J. Crude Drug Res.* **1981**, *19*, 141.
[2] A. J. Leach, D. N. Leach, G. J. Leach, *Science in New Guinea* **1988**, *14*, 1.
[3] K. Winkelmann, J. Heilmann, O. Zerbe, T. Rali, O. Sticher, *J. Nat. Prod.* **2000**, *63*, 104.
[4] K. Winkelmann, J. Heilmann, O. Zerbe, T. Rali, O. Sticher, *J. Nat. Prod.* **2001**, *64*, 701.
[5] S. Trifunovic, V. Vajs, S. Macura, N. Juranic, Z. Djarmati, R. Jankov, S. Milosavljevic, *Phytochemistry* **1998**, *49*, 1305; H. C. J. Orth, H. Hauer, C. A. J. Erdelmeier, P. C. Schmidt, *Pharmazie* **1999**, *54*, 76; L. Verotta, G. Appendino, E. Belloro, J. Jakupovic, E. Bombardelli, *J. Nat. Prod.* **1999**, *62*, 770.
[6] L. H. Hu, K. Y. Sim, *Tetrahedron* **2000**, *56*, 1379.
[7] L. A. Decosterd, H. Stoeckli Evans, J. C. Chapuis, J. D. Msonthi, B. Sordat, K. Hostettmann, *Helv. Chim. Acta* **1989**, *72*, 464.
[8] A. Ankli, J. Heilmann, M. Heinrich, O. Sticher, *Phytochemistry* **2000**, *54*, 531.

Received April 14, 2001