New Phloroglucinol Derivatives from *Hypericum papuanum*

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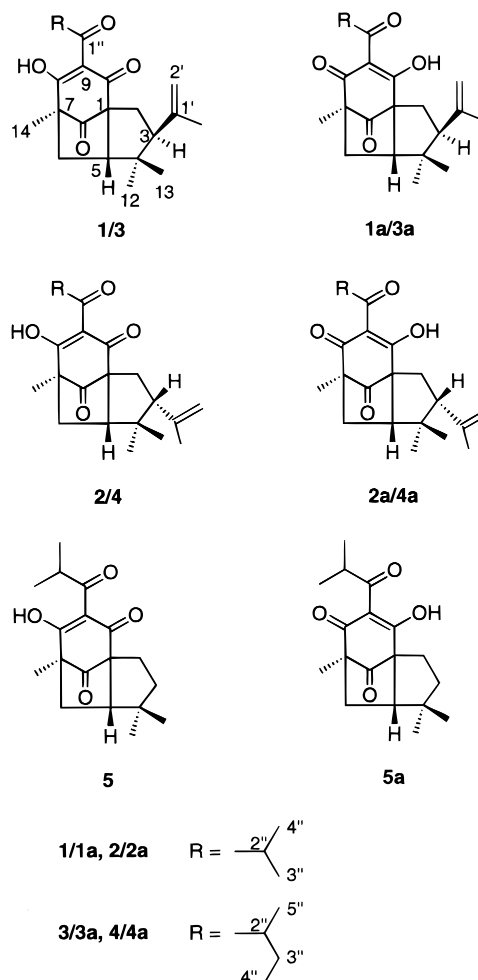
Bioactivity-guided fractionation of the petroleum ether extract of the aerial parts of *Hypericum papuanum* led to the isolation of five new tricyclic phloroglucinol derivatives. On the basis of extensive 1D and 2D NMR experiments as well as MS studies, their structures were elucidated as the C-3 epimers of 8-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**1**, **2**); the C-3 epimers of 8-hydroxy-4,4,7-trimethyl-9-(2-methylbutyryl)-3-(1-methylvinyl)-5β-H-tricyclo-[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**3**, **4**), and 8-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**5**), and their corresponding tautomers (**1a**, **2a**, **3a**, **4a**, **5a**). Compounds **1/1a**−**5/5a** were named ialibinones A−E, respectively. Compounds **1/1a**−**4/4a** showed antibacterial activity against *Bacillus cereus*, *Staphylococcus epidermidis*, and *Micrococcus luteus.*

The leaves of *Hypericum papuanum* Ridley (Guttiferae), a shrub or woody herb widespread in all mountainous regions of New Guinea,[1] are used in folk medicine for the treatment of sores.[2] In the genus *Hypericum* many phloroglucinol derivatives with antibiotic properties have been isolated, of which some are derivatives of the well-known hyperforin, isolated from *Hypericum perforatum* L., while others are phloroglucinol derivatives with filicinic acid moieties.[3−5] Bioactivity-guided fractionation of the petroleum ether extract of the aerial parts of *H. papuanum* has led to the isolation of five new phloroglucinol derivatives with tricyclic structures (**1/1a**−**5/5a**), four of which are active against *Bacillus cereus*, *Staphylococcus epidermidis*, and *Micrococcus luteus*. No reports concerning the isolation of similar tricyclic phloroglucinol derivatives from the family Guttiferae have appeared in the literature so far. However, structurally related semisynthetic transformation products of the hop constituents humulone and colupulone obtained by oxidation and isomerization have been described.[6,7] In addition, the isolation of aissatone from *Harrisonia abyssinica* Oliv. (Simaroubaceae) was published recently.[8]



Results and Discussion

The air-dried aerial parts of *H. papuanum* were extracted successively with petroleum ether, dichloromethane, methanol, and methanol−water mixtures. The petroleum ether extract showed antibacterial activity against *B. cereus*, *S. epidermidis*, and *M. luteus* and was therefore subjected to repeated vacuum−liquid chromatography (VLC) and HPLC, which led to the isolation of five new phloroglucinol derivatives (**1/1a**−**5/5a**). All five compounds were isolated as yellow oils. After being sprayed with vanillin−sulfuric acid reagent,[9] the substances gave turquoise spots by TLC.

The ^{1}H NMR spectrum of **1/1a** revealed the presence of 12 methyl groups, eight of which are tertiary (δ_H 0.85, 0.88, 0.99, 1.00, 1.35, 1.41, 1.78, 1.79, each s) and four secondary (δ_H 1.13, d, $J = 6.8$ Hz; 1.17, d, $J = 6.8$ Hz; 1.17, d, $J = 6.8$ Hz; 1.22, d, $J = 6.8$ Hz), as well as two septets indicative of methines in a 2-methylpropionyl side chain (δ_H 3.96, sept, $J = 6.8$ Hz; 4.03, sept, $J = 6.8$ Hz). Furthermore, four signals indicating terminal methylenes (δ_H 4.79, 4.81, 4.94, 4.95, each br s) could be detected in addition to a number of overlapping aliphatic signals in the range of 2.0 to 2.6 ppm. The unusually lowfield shifted signals (δ_H 18.43, 18.75, each br s) suggested the presence of hydroxyl protons that participate in rather strong hydrogen bonds (for ^{1}H NMR data, see Tables 1 and 2).

The ^{13}C NMR showed signals of 42 carbons, which could be sorted by DEPT experiments into 12 methyl, six meth-

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Table 1. ^{1}H NMR Spectral Data of the Preferred Tautomers **1**−**5** (δ ppm; m; *J* Hz)[a]

H	1	2	3	4	5
2α	2.19 (dd, 13.3, 5.9)	2.16 (t, 13.1)	2.19 (dd, 13.3, 5.9)	2.14[b] (t)	2.03[b] (m)
2β	2.51 (t, 13.1)	2.54 (dd, 13.5, 7.4)	2.50 (t, 13.3)	2.53 (dd, 13.0,7.0)	2.47[b] (m)
3	2.08 (dd, 12.8, 5.9)	2.44 (dd, 12.7, 7.2)	2.08 (dd, 12.6, 5.7)	2.44[b] (m)	1.65[b] (m)
					1.71[b] (m)
5	2.35 (t, 9.6)	2.30 (dd, 10.2, 5.8)	2.34 (t, 9.6, 9.4)	2.28 (dd, 10.4, 5.2)	2.22[b] (m)
6α	1.76 (dd, 13.1, 9.2)	1.88 (dd, 13.4, 5.8)	1.76[b] (m)	1.87 (dd, 13.1, 5.5)	1.80[b] (m)
6β	2.24 (dd, 13.1, 9.8)	2.23 (dd, 13.5, 10.3)	2.24[b] (m)	2.22[b](m)	2.22[b] (m)
12	1.00 (s)	0.60 (s)	1.00 (s)	0.59 (s)	0.81 (s)
13	0.85 (s)	0.97 (s)	0.84 (s)	0.98 (s)	0.98 (s)
14	1.41 (s)	1.39 (s)	1.41 (s)	1.39 (s)	1.39 (s)
2′	4.79 (br s)	4.79 (br s)	4.79 (br s)	4.80 (br s)	
	4.94 (br s)	4.98 (br s)	4.94 (br s)	4.97 (br s)	
3′	1.78 (s)	1.79 (s)	1.78 (s)	1.79 (s)	
2″	4.03 (sept, 6.8)	4.06 (sept, 6.8)	3.92 (m, 6.9)	3.93 (m, 6.6)	4.04 (sept, 6.8)
3″	1.17[b] (d, 6.8)	1.19 (d, 6.8)	1.46 (dd, 13.6, 7.3)	1.44 (m, 13.6, 7.3)	1.18 (d, 6.7)
			1.72 (dd, 13.7, 7.3)	1.72 (m, 13.5, 7.5)	
4″	1.17[b] (d, 6.8)	1.15 (d, 6.4)	0.94 (t, 7.4)	0.95[b](m)	1.15 (d, 6.9)
5″			1.15 (d, 6.8)	1.13 (d, 6.8)	
OH	18.75 (br s)	18.88[d](br s)	18.77 (br s)	18.85[c] (br s)	18.84 (br s)

[a] The chemical shifts of compounds **1/1a** and **2/2a** were determined at 600 MHz, in $CDCl_3$. Compounds **3/3a**−**5/5a** were determined at 500 MHz, in $CDCl_3$. [b] Signals overlapped. [c] Signal from ^{1}H 300 MHz at 300 K. [d] Signal from ^{1}H 500 MHz at 295 K.

Table 2. ^{1}H NMR Spectral Data of the Minor Tautomers **1a**−**5a** (δ ppm; m; *J* Hz)[a]

H	1a	2a	3a	4a	5a
2α	2.26[b] (dd, 6.2)	2.26[b] (m)	2.24[b] (m)	2.23[b] (m)	2.13[b] (m)
2β	2.54 (t, 13.1)	2.53 (dd, 12.7, 7.6)	2.54 (t, 13.6)	2.53 (dd, 13.0,7.0)	2.47[b] (m)
3	2.13[b]	2.50[b] (dd, 7.4)	2.14[b] (m)	2.48[b] (m)	1.65[b] (m)
					1.71[b] (m)
5	2.46 (t, 9.6, 8.9)	2.45 (dd, 10.3, 5.2)	2.45 (m)	2.44 (dd, 10.2, 5.3)	2.37 (dd, 10.4, 5.7)
6α	1.68 (dd, 13.3, 8.5)	1.79 (dd, 13.9, 5.3)	1.67 (dd, 13.4, 8.3)	1.76[b](m)	1.72 (dd, 13.8, 10.4)
6β	2.13 (dd, 13.2, 10.4)	2.11 (dd, 13.9, 10.4)	2.13[b] (m)	2.11[b](m)	2.10 (dd, 13.9, 5.9)
12	0.99 (s)	0.59 (s)	0.98 (s)	0.58 (s)	0.78 (s)
13	0.88 (s)	0.99 (s)	0.87 (s)	0.97 (s)	1.00 (s)
14	1.35 (s)	1.33 (s)	1.35 (s)	1.32 (s)	1.33 (s)
2′	4.81 (br s)	4.82 (br s)	4.81 (br s)	4.82 (br s)	
	4.95 (br s)	5.00 (br s)	4.95 (br s)	4.99 (br s)	
3′	1.79 (s)	1.81 (s)	1.79 (s)	1.80 (s)	
2″	3.96 (sept, 6.8)	4.04 (sept, 6.8)	3.82 (m, 6.9)	3.90 (m, 6.6)	4.02 (sept, 6.8)
3″	1.13 (d, 6.8)	1.19 (d, 6.7)	1.46 (dd, 13.6, 7.3)	1.44 (m, 13.6, 7.3)	1.20 (d, 6.6)
			1.72 (dd, 13.7, 7.3)	1.72 (m, 13.5, 7.5)	
4″	1.22 (d, 6.8)	1.15 (d, 6.2)	0.88 (t, 7.4)	0.90 (t, 8.7)	1.14 (d, 7.7)
5″			1.20 (d, 6.8)	1.17 (d, 6.6)	
OH	18.43 (br s)	18.80[d] (br s)	18.44 (br s)	18.79[c] (br s)	18.91 (br s)

[a] The chemical shifts of compounds **1/1a** and **2/2a** were determined at 600 MHz, in $CDCl_3$. Compounds **3/3a**−**5/5a** were determined at 500 MHz, in $CDCl_3$. [b] Signals overlapped. [c] Signal from ^{1}H 300 MHz at 300 K. [d] Signal from ^{1}H 500 MHz at 295 K.

ylene, six methine, and 18 quaternary carbons. Six of the quaternary carbons are ketone carbonyl groups (δ_C 191.0 s, 194.6 s, 206.1 s, 207.0 s, 207.6 s, 208.6 s) and two are substituted by enolic hydroxyl groups (δ_C 200.1 s, 201.6 s) (for ^{13}C NMR data, see Table 3). The molecular mass of 344 in combination with the ^{1}H and ^{13}C NMR spectra allowed the establishment of the molecular formula as $C_{21}H_{28}O_4$. However, doubled ^{1}H and ^{13}C NMR patterns in a ratio of approximately 1.8:1 (derived from the ^{1}H and ^{13}C NMR signal intensities) and only one pseudomolecular peak at *m*/*z* 345 $[M + H]^+$ in the positive FABMS allowed the conclusion that the compound appears in two isomeric forms, which was later shown to represent the two enol tautomers **1** and **1a** (see below) in solution ($CDCl_3$) in the ratio mentioned above, with **1** being the preferred tautomer. The HMBC and TOCSY experiments gave evidence for the presence of two enol tautomers by revealing two independent networks of correlations between the more intensive signals on one hand and the less intensive on the other. The assignment strategy presented below refers to the preferred tautomeric structure **1**. In the first step, the covalent connectivities of the new compounds were established by analysis of the DQF−COSY spectrum that revealed spin system A (H_3-3″, H_3-4″, H-2″) belonging to the methylpropionyl substituent, spin system B (H_2-6, H-5), and spin system C (H_2-2, H-3). Due to the high number of nonprotonated carbons, HSQC (^{1}H−^{13}C ^{1}J correlated 2D) and HMBC (^{1}H−^{13}C ^{n}J correlated 2D, $n > 1$) experiments were utilized extensively to complete the ^{1}H and ^{13}C NMR assignments. Spin systems B and C are linked through correlations to the dimethylated quaternary carbon C-4 as manifested by the HMBC correlations observed between C-3 and H-5, H_3-12, or H_3-13 and between C-4 and H_2-2, H-5, or H_2-6. Scalar couplings between C-2′ and H-3/H_3-3′ and between C-1′ and H-3/H_3-3′ confirmed the substitution of C-3 by a 1-methylvinyl group. HMBC correlations between C-1″ and H-2″, H-3″, and H-4″ established the position of the methylpropionyl side chain. The observed HMBC correlations of the hydroxyl proton to C-8, C-7, C-9, and C-2″ determined the position of the hydroxyl group at C-8. Interestingly, the correlation between the hydroxyl proton and C-2″ is propagated via a strong hydrogen bond between the hydroxyl proton at C-8 and the ketone carbonyl group at C-1″ (see Figure 1). Consideration of further HMBC connectivities (summarized in Figure 1), in conjunction with the conclusions drawn from the 1D NMR spectra, established the tricyclic structure of the preferred tautomer **1** as 8-hydroxy-4,4,7-trimethyl-9-(2-methylpro-

Table 3. ^{13}C NMR Spectral Data of Compounds **1/1a**−**5/5a**[a]

C	**1**	**1a**	**2**	**2a**	**3**	**3a**	**4**	**4a**	**5**	**5a**
1	72.1 s	67.9 s	72.4 s	68.3 s	72.4[b] s	68.0 s	72.5 s	68.4 s	75.9[b] s	71.3[b] s
2	25.7 t	24.9 t	24.9 t	24.2 t	25.8 t	25.0 t	24.9 t	24.2 t	20.6 t	19.8 t
3	54.5 d	54.8 d	58.5 d	58.7 d	54.5 d	54.8 d	58.4 d	58.7 d	43.2 t	43.2 t
4	42.8 s	43.4 s	44.9 s	45.2 s	42.8 s	43.5[b] s	44.9 s	45.2 s	41.8 s	42.2 s
5	55.4 d	57.7 d	55.8 d	58.2 d	55.5 d	57.7 d	55.7 d	58.1 d	54.7 d	57.2 d
6	33.4 t	33.4 t	32.7 t	31.1 t	34.4 t	33.5 t	32.7 t	31.2 t	33.3 t	31.7 t
7	61.4 s	65.1 s	62.1 s	66.2 s	61.5 s	65.1 s	62.2 s	66.2 s	62.3[b]s	66.5[b] s
8	201.6 s	194.6 s	201.7 s	193.6 s	201.8[b] s	194.8[b] s	201.9 s	193.7 s	201.7[b]s	193.9[b] s
9	109.6 s	109.4 s	108.0 s	108.0 s	110.3[b] s	110.0[b] s	108.4 s	108.4 s	108.4 s	108.1 s
10	191.0 s	200.1 s	191.0 s	200.3 s	191.3[b] s	200.3[b] s	191.1 s	200.3 s	191.2[b] s	200.2[b] s
11	206.1 s	207.1 s	206.7 s	207.2 s	206.1[b] s	207.2[b] s	206.8 s	207.4 s	206.6[b] s	207.3[b] s
12	24.4 q	24.4 q	16.9 q	16.5 q	24.4 q	24.4 q	16.9 q	16.4[c] q	22.3 q	21.6 q
13	25.8 q	25.7 q	27.2 q	27.2 q	25.7 q	25.8 q	27.2 q	27.2 q	28.2 q	28.1 q
14	12.4 q	13.1 q	12.4 q	13.1 q	12.4 q	13.1 q	12.4 q	13.2 q	12.4 q	13.2 q
1′	143.2 s	143.3 s	143.0 s	142.8 s	143.3[b] s	143.2[b] s	143.0 s	142.8 s		
2′	113.5 t	113.5 t	113.6 t	113.8 t	113.5 t	113.5 t	113.6 t	113.8 t		
3′	23.6 q	23.6 q	23.8 q	23.7 q	23.6 q	23.6 q	23.8 q	23.7 q		
1″	208.6 s	207.6 s	209.6 s	209.1 s	208.1[b] s	209.8[b] s	209.3 s	208.7 s	209.4[b] s	208.9[b] s
2″	34.8 d	34.3 d	35.0 d	34.7 d	41.1 d	40.7 d	41.3 d	41.0 d	34.9 d	34.6 d
3″	18.5 q	18.6 q	18.7 q	18.8 q	26.5 t	26.5 t	26.5 t	26.7 t	18.6 q	18.7 q
4″	19.0 q	19.2 q	18.9 q	18.9 q	11.8 q	11.8 q	11.8 q	11.7 q	19.0 q	18.9 q
5″					16.5 q	16.8 q	16.4[c] q	16.4[c] q		

[a] The chemical shifts of compounds **1/1a**−**4/4a** were determined at 75 MHz, in $CDCl_3$. Compound **5/5a** was determined at 125 MHz, in $CDCl_3$. Multiplicities were obtained from DEPT135/DEPT90 experiments. [b] Signals derived from HMBC experiments. [c] Signals overlapped.

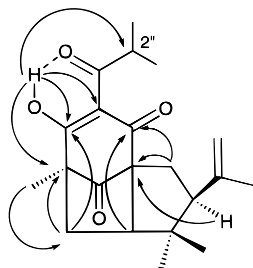

Figure 1. Key long-range (HMBC) correlations of **1**.

pionyl)-3β-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione. The tautomeric form **1a** was identified as 10-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3β-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-9-ene-8,11-dione using very similar arguments. The UV spectrum of **1/1a** showed the presence of a chromophore similar to the one that has been recorded previously for other compounds in this class with the same tricyclic system,[6,7] thereby confirming oxygen substituents at C-8, C-10, and C-11.

Compounds **2/2a**, obtained as a yellow oil, are almost identical to compounds **1/1a**, showing a pseudomolecular peak at m/z 345 $[M + H]^+$ in the positive FABMS, and the same number of protons and carbons as well as similar correlations in the ^{1}H−^{1}H COSY spectrum and in direct (HSQC) and long-range (HMBC) carbon−proton correlation experiments. It also exists in two enol tautomeric forms, which have both been characterized. The only difference from compounds **1/1a** is a remarkable upfield shift of C-12 in the ^{13}C NMR spectrum from δ 24.4 ppm (**1/1a**) to δ 16.9 ppm and to 16.5 ppm in compounds **2/2a**. Additionally, the ^{1}H NMR chemical shift of H_3-12 changed from δ 1.00/0.99 ppm to 0.60/0.59 ppm. Most likely, a difference in chemical shift for only a very few resonances is due to a change in the relative stereochemistry of the two compounds.

In a second step of the structure-elucidation procedure, the stereochemistry was established. Each of the compounds **1/1a** and **2/2a** has four chiral centers at C-1, C-3, C-5, and C-7. The relative stereochemistry at C-1 and C-7 is constrained by the fact that the bridge between C-1 and C-7 can only exist in the cis form.[7] Because of the rigid tricyclic ring system, the methyl group H_3-14 is fixed below the plane formed by the two five-membered rings. The determination of the relative stereochemistry at C-3 and C-5 was possible with NOESY experiments. In compounds **1/1a** and **2/2a** NOE cross-peaks between the hydroxyl proton at C-8 (**1/2**) and C-10 (**1a/2a**) and the proton at C-5 indicated the β position of H-5. In the tautomer **1a** the hydroxyl proton at C-10 showed a NOE to the more downfield-shifted proton of C-2, confirming the β position of this proton. This proton at C-2 also showed a strong NOE to the more upfield-shifted proton of C-2′ and to the methyl group H_3-3′, thus establishing the β position of the 1-methylvinyl group at C-3. The β position of the 1-methylvinyl substituent in **1** was confirmed because the hydroxyl group at C-8 in compound **1** showed a NOE to the more downfield-shifted proton of C-6, thereby establishing the β position of this proton. The methyl group H_3-12 displayed only a weak NOE to the β proton at C-6, but a strong NOE to the α proton of C-6. With H_3-12 being in the α position, the methyl group H_3-13 takes the β position, which was well supported by a strong NOE to H-5. Because the proton at C-3 showed a strong NOE only to H_3-12, H-3 must be in the α position and the 1-methylvinyl group in the β position. The NOEs used for the analysis of compound **1a** are summarized in Figure 2. In contrast, in compound **2a**, the α-positioned proton at C-2 showed a NOE to the more upfield-shifted proton at C-2′, thereby confirming the α position of the 1-methylvinyl group. The β-positioned

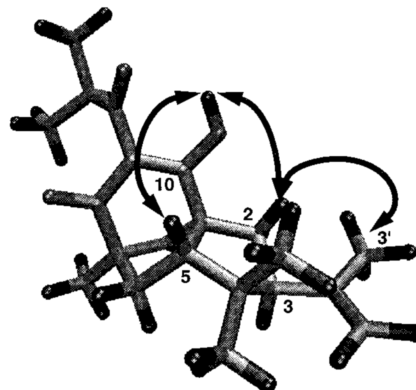

Figure 2. Selected NOE correlations of **1a**.

methyl group H_3-13 showed a strong NOE to H-5 and H-3, establishing H-3 as β. These observations were confirmed by NOE signals between the hydroxyl group at C-8 and the more downfield-shifted proton at C-6 in compound **2**. This β-positioned proton revealed no NOE to the methyl groups H_3-12 and H_3-13, whereas the α-positioned one showed a NOE to H_3-12; hence, C-12 is in the α position. These results led to the conclusion that the compounds **1/1a** and **2/2a** are the C-3 epimers of 8-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3-(1-methylvinyl)-5β-H-tricyclo-[5.3.1.0^{1,5}]undec-8-ene-10,11-dione and 10-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-9-ene-8,11-dione, with a 3β-(1-methylvinyl) group in compound **1/1a** and a 3α-(1-methylvinyl) group in compound **2/2a**.

Compounds **3/3a** and **4/4a**, both isolated separately as yellow oils, are similar to compounds **1/1a** and **2/2a**, each with a pseudomolecular peak at *m*/*z* 359 $[M + H]^+$ in the positive FABMS. The difference of only 14 atomic mass units compared to compounds **1/1a** and **2/2a** strongly indicated the presence of a further methylene group and established the molecular formula as $C_{22}H_{30}O_4$. A comparison of the corresponding 1D and 2D NMR experiments of **3/3a** and **4/4a** revealed chemical shifts and correlations in the tricyclic system identical to those found in **1/1a** and **2/2a**. However, COSY correlations between H-2″ and H_3-5″ or H_2-3″ and between H_2-3″ and H_3-4″ or H-2″ proved the replacement of the 2-methylpropionyl group at C-9 by a 2-methylbutyryl unit. Analysis of NOESY experiments performed in an analogous manner to substances **1/1a** and **2/2a** led to the conclusion that the two compounds **3/3a** and **4/4a** are C-3-epimers of 8-hydroxy-4,4,7-trimethyl-9-(2-methylbutyryl)-3-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]-undec-8-ene-10,11-dione and 10-hydroxy-4,4,7-trimethyl-9-(2-methylbutyryl)-3-(1-methylvinyl)-5β-H-tricyclo-[5.3.1.0^{1,5}]undec-9-ene-8,11-dione), with a 3β-(1-methylvinyl) group in compound **3/3a** and a 3α-(1-methylvinyl) group in compound **4/4a**.

A fifth substance, again isolated in the form of a yellow oil, was interpreted by comparing the ^{1}H and ^{13}C NMR chemical shifts with the corresponding data of compounds **1/1a** and **2/2a** and by considering the reduction of the molecular weight by 40 atomic mass units to *m*/*z* 304 (EIMS). This reduction indicated the loss of the 1-methylvinyl group at C-3, which was later confirmed by the 2D NMR (COSY, HSQC, HMBC) data. The NOESY experiment established the β-position of H-5. Again, **5/5a** exists in both tautomeric forms, 8-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,-11-dione (**5**) and 10-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-9-ene-8,11-dione (**5a**).

Because in all five compounds the same tautomeric form was preferred, it seems, therefore, that the neighboring five-membered ring system probably has a destabilizing effect on the enolic hydroxyl group compared to the methyl group (C-14).

The five isolates were evaluated for their antibacterial potential against *B. cereus*, *S. epidermidis*, and *M. luteus* (Table 4). Compounds **3/3a** and **4/4a** showed stronger activity than **1/1a** and **2/2a** against *B. cereus* and *S. epidermidis* but almost identical effectiveness against *M. luteus*. In contrast, compound **5/5a** lacked any antibacterial activity (minimum inhibitory concentration > 256 μg/mL and 128 μg/mL, respectively). Hence, we conclude that the 1-methylvinyl group at C-3 plays a crucial role for the antibacterial activity. No difference was observed between the antibacterial activities of the two corresponding epimers (**1/1a** vs **2/2a** and **3/3a** vs **4/4a**).

Table 4. Antibacterial Activities of Compounds **1/1a**−**5/5a**

	minimum inhibitory concentration (MIC) in broth (in μg/mL)		
compound	*B. cereus* (ATCC 10702)	*S. epidermidis* (ATCC 12228)	*M. luteus* (ATCC 9341)
1/1a	64	64	64
2/2a	64	128	64
3/3a	16	32	64
4/4a	16	32	64
5/5a	> 128	> 256	> 128
chloramphenicol	2	4	1

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a Perkin−Elmer 241 polarimeter using $CHCl_3$ as solvent. UV spectra were obtained in ethanol on a UVIKON 930 spectrophotometer. ^{13}C NMR spectra of **1/1a, 2/2a, 3/3a**, and **4/4a** were measured at 300 K on a Bruker AMX-300 spectrometer (operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C), 1D proton, ^{1}H−^{1}H COSY, ^{1}H−^{1}H TOCSY, 500-ms NOESY, HMBC, and HSQC experiments of compounds **1/1a** and **2/2a** at 295 K on a Bruker DRX-600 spectrometer (operating at 600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C). All other NMR spectra were recorded on a Bruker DRX-500 (operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C) at 295 K. The spectra were measured in $CDCl_3$ and referenced against residual C*H*Cl_3 in $CDCl_3$ (^{1}H 7.27 ppm) and *C*DCl_3 (^{13}C δ 77.0 ppm). EIMS were measured on a Hitachi−Perkin−Elmer−RMUGM mass spectrometer at 70 eV and positive mode FABMS on a ZAB 2-SEQ spectrometer, using 3-nitrobenzyl alcohol as matrix. HPLC separations were performed with a 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 5 ODS II, 5 μm, 250 × 8 mm). Si gel H & Y (Chromagel, sds), particle size 40−60 μm, and Si gel for column chromatography (Merck), particle size 15−40 μm, were used for VLC (columns 22 × 7 and 22 × 3 cm, respectively, vacuum by H_2O aspiration). Si gel 60 F_{254} precoated aluminum sheets (0.2 mm, Merck) and RP_{18} F_{254} precoated sheets (0.25 mm, Merck) were used for TLC controls. All solvents were of HPLC grade.

Plant Material. The aerial parts of *Hypericum papuanum* Ridley were collected north of Ialibu, Southern Highlands Province, Papua New Guinea, in September 1996. The plant was identified by Paul Katik, National Herbarium, Lae, Papua New Guinea, and Dr. M. M. J. van Baalgoy, 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. Air-dried and powdered aerial parts of *Hypericum papuanum* (2.2 kg) were extracted successively with petroleum ether, dichloromethane, and methanol, as well as 7:3 and 1:1 methanol−water mixtures, respectively, to afford 160 g of petroleum ether-soluble material after concentration under vacuum. A 46-g quantity of this extract was applied to VLC over Si gel (40−60 μm), as four separate portions (5 g, 11 g, 15 g, 15 g). Elution with hexane containing increasing amounts of ethyl acetate and final washing with methanol yielded 50 fractions of 180 mL each. Based on the TLC similarities, identical fractions were combined to give a total of 16 fractions. Altogether, 1.5 g of recombined VLC fraction 3 (eluted with hexane−ethyl acetate 98:2) was separated by VLC over Si gel (15−40 μm) using a step gradient from hexane to ethyl acetate and final washing with methanol. Based on TLC, the obtained 33 fractions of 100 mL each were combined to give 12 fractions. The bioactive fractions 10 and 11 (280 mg, eluted with hexane−ethyl acetate 50:50) were

chosen for further purification. Reversed-phase HPLC purification of the combined fractions 10 and 11 using acetonitrile−H_2O−trifluoroacetic acid (80:19.5:0.5) as eluent yielded **1/1a** (6.2 mg), **2/2a** (14.1 mg), **3/3a** (2.2 mg), **4/4a** (9.1 mg), and **5/5a** (2.8 mg), each as yellow oil.

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 using a modified procedure as described below.[10] Bacterial suspensions were obtained from overnight cultures in nutrient broth (Becton, Dickinson Co., 11479) cultivated at 37 °C and diluted to approximately 10^5 cells/mL in fresh medium. The isolated compounds were dissolved to 1 mg/mL in MeOH as stock solutions. The required amount of stock solution was pipetted into the wells at the first column of a 96-well tissue culture plate (Falcon) and dried. The sample was redissolved in 50 μL DMSO, 50 μL sterile nutrient broth, and 100 μL dilute culture suspension. Twofold dilutions were made in 100 μL volumes of dilute bacterial suspensions. The plates were kept in a moist atmosphere at 37 °C for 20 h. After incubation, 10 μL of 0.25% aqueous thiazolyl blue tetrazolium bromide was added in each well and reincubated for 4 h to detect living bacteria as violet turbid solutions. Chloramphenicol was used as a positive control. All pure compounds were tested within the range of 256−0.5 μg/mL.

Ialibinone A (1/1a): 8-Hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3β-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**1**) and 10-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3β-(1-methylvinyl)-5β-H-tricyclo-[5.3.1.0^{1,5}]undec-9-ene-8,11-dione (**1a**), respectively; yellow oil (6.2 mg); $[\alpha]^{20}_D$ −22° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (logϵ) 275 (4.13), 236 (sh) (3.78) nm; ^{1}H NMR data, see Tables 1 and 2; ^{13}C NMR spectral data, see Table 3; FABMS (positive) *m*/*z* 345.3 $[M + H]^+$; EIMS (CH_2Cl_2) *m*/*z* 344 $[M]^+$ (36), 301 $[M - C_3H_7]^+$ (8), 275 (8), 205 (48), 149 (15).

Ialibinone B (2/2a): 8-Hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3α-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**2**) and 10-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3α-(1-methylvinyl)-5β-H-tricyclo-[5.3.1.0^{1,5}]undec-9-ene-8,11-dione (**2a**), respectively; yellow oil (14.1 mg); $[\alpha]^{20}_D$ −91° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (logϵ) 273 (4.16), 234 (3.87) nm; ^{1}H NMR spectral data, see Tables 1 and 2; ^{13}C NMR spectral data, see Table 3; FABMS (positive) *m*/*z* 345.2 $[M + H]^+$; EIMS (CH_2Cl_2) *m*/*z* 344 $[M]^+$ (31), 301 $[M - C_3H_7]^+$ (24), 275 (21), 205 (100), 149 (34).

Ialibinone C (3/3a): 8-Hydroxy-4,4,7-trimethyl-9-(2-methylbutyryl)-3β-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**3**) and 10-hydroxy-4,4,7-trimethyl-9-(2-methylbutyryl)-3β-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-9-ene-8,11-dione (**3a**), respectively; yellow oil (2.2 mg); $[\alpha]^{20}_D$ −26° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (logϵ) 276 (4.05), 239 (sh) (3.77) nm; ^{1}H NMR spectral data, see Tables 1 and 2; ^{13}C NMR spectral data, see Table 3; FABMS (positive) *m*/*z* 359.2 $[M + H]^+$; EIMS (CH_2Cl_2) *m*/*z* 358 $[M]^+$ (36), 301 $[M - C_4H_9]^+$ (7), 289 (9), 205 (42), 149 (15).

Ialibinone D (4/4a): 8-Hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-3α-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**4**) and 10-hydroxy-4,4,7-trimethyl-9-(2-methylbutyryl)-3α-(1-methylvinyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-9-ene-8,11-dione (**4a**), respectively; yellow oil (9.1 mg); $[\alpha]^{20}_D$ −72° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (logϵ) 274 (4.07), 238 (sh) (3.80) nm; ^{1}H NMR spectral data, see Tables 1 and 2; ^{13}C NMR spectral data, see Table 3; FABMS (positive) *m*/*z* 359.2 $[M + H]^+$; EIMS (CH_2Cl_2) *m*/*z* 358 $[M]^+$ (15), 301 $[M - C_4H_9]^+$ (6), 289 (10), 205 (48), 149 (28).

Ialibinone E (5/5a): 8-Hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-8-ene-10,11-dione (**5**) and 10-hydroxy-4,4,7-trimethyl-9-(2-methylpropionyl)-5β-H-tricyclo[5.3.1.0^{1,5}]undec-9-ene-8,11-dione (**5a**), respectively; yellow oil (2.8 mg); $[\alpha]^{20}_D$ −33° (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (logϵ) 281 (3.77), 251 (3.75) nm; ^{1}H NMR spectral data, see Tables 1 and 2; ^{13}C NMR spectral data, see Table 3; EIMS (CH_2Cl_2) *m*/*z* 304 $[M]^+$ (54), 261 $[M - C_3H_7]^+$ (8), 235 (25), 205 (18), 149 (18).

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