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

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Five new prenylated tricyclic and three new bicyclic acylphloroglucinol derivatives have been isolated by bioactivity-guided fractionation of the petroleum ether extract of the dried aerial parts of *Hypericum papuanum*. The tricyclic compounds (**1**−**5**) were named papuaforins A−E. The bicyclic compounds were isolated together with their corresponding tautomers and were named hyperguinones A and B (**6/6a**, **7/7a**) and hyperpapuanone (**8/8a**), respectively. Their structures were elucidated on the basis of extensive 1D and 2D NMR experiments, as well as mass spectrometry. Furthermore, the cytotoxicity toward KB nasopharyngeal carcinoma cells and the antibacterial activity of the isolated compounds were determined.

In the course of our systematic phytochemical and biological studies of plants that are employed in the traditional medicine of Papua New Guinea, we recently reported the isolation of five new tricyclic phloroglucinol derivatives with antibacterial activity from *Hypericum papuanum* Ridley (Guttiferae).[1] In folk medicine the leaves of this shrub or woody herb, which is found in all mountainous regions of New Guinea,[2] are used for the treatment of sores.[3] Continuing our bioactivity-guided studies, five new tricyclic (**1**−**5**) and three new bicyclic compounds (**6**−**8**) were isolated from the petroleum ether extract of the dried aerial parts of this plant. The present paper describes the isolation, structure elucidation, and biological activity of these prenylated acylphloroglucinol derivatives.

Results and Discussion

After extraction of the air-dried aerial parts of *H. papuanum* with petroleum ether, dichloromethane, methanol, and methanol−water mixtures, the antibacterial active petroleum ether extract was subjected to bioassay-guided (antibacterial activity) fractionation by repeated vacuum-liquid chromatography (VLC). Final purification was made by semipreparative reversed-phase HPLC (RP-HPLC). All compounds were obtained as yellowish or red oils.

The molecular formula, $C_{26}H_{36}O_4$ as derived from HR-MALDIMS and ^{1}H and ^{13}C NMR experiments, indicated nine degrees of unsaturation for the structural isomers papuaforin A (**1**) and papuaforin B (**2**). Considering the fact that the carbon spectra contained only three carbonyl atoms and three double bonds, the compounds must be tricyclic in order to satisfy the molecular formula. The ^{1}H and ^{13}C NMR spectra of **1** contained signals for seven tertiary (δ_H 1.03, 1.25, 1.29, 1.40, 1.50, 1.56, 1.66, each s) and two secondary methyl groups (δ_H 1.04, d, J not determined due to signal overlap; 1.13, d, J = 6.5 Hz). Additionally two methylene (δ_H 1.39, t, J = 12.9 Hz; 1.96, dd, J = 3.7, 12.9 Hz; 1.68, m; 2.14, m), five methine (δ_H 1.50, m; 2.11, m; 4.97, bt, J = 6.4 Hz; 5.37, d, J = 10.0 Hz; 6.49, d, J = 10.0 Hz; the last three were part of double bonds), and 10 quaternary carbon atoms were observed.

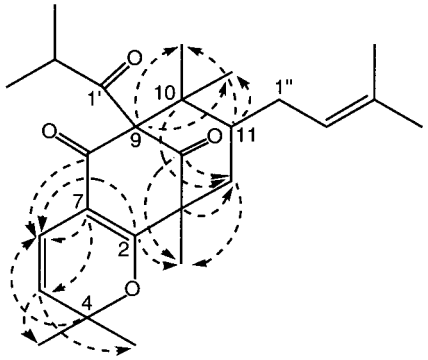

Figure 1. Key long-range C → H (HMBC) correlations of **1**.

Two quaternary carbons were assigned as part of double bonds (δ_C 114.0, C-7; 133.3, C-3″), whereas the resonances at δ_C 188.6 (C-8), 207.0 (C-13), and 209.1 (C-1′) corresponded to carbonyl functions, with one substituted to form an enol ether (δ_C 170.8, C-2). Two of the remaining four aliphatic quaternary carbons were shifted downfield either due to oxygen substitution (δ_C 81.9, C-4) or because of the deshielding effect of three neighboring carbonyl groups (δ_C 83.4, C-9). A HSQC experiment was utilized to assign the protons to their attached carbons, and DQF-COSY cross-peaks revealed the existence of three spin systems (A, B, C). Due to the large number of nonprotonated carbons, a linkage between the three spin systems was only possible by extensive HMBC analyses. The combined interpretation of 2D spectra showed that spin system A (H-2′, H_3-3′, H_3-4′) and the carbonyl function C-1′ (δ_C 209.1) formed the isobutyryl substituent. Spin system B (H-5, H-6) was part of the 2,2-dimethyl-2*H*-pyran ring evident from HMBC correlations between C-2 and H-6, C-4 and H-5/H-6/H_3-15/H_3-16, C-5 and H_3-15/H_3-16, C-7 and H-5, and C-8 and H-6. The third spin system C (H_2-12, H-11, H_2-1″, H-2″) belonged to a six-membered ring substituted by a 3-methylbut-2-enyl side chain. These assignments are confirmed by HMBC correlations between C-1 and H_2-12 and C-13 and H_2-12, on one hand, and between the dimethylated quaternary carbon C-10 and H-11 as well as between C-2″ and the geminal methyl groups H_3-4″ and H_3-5″, on the other hand. Further HMBC connectivities (summarized in Figure 1) established the tricyclic structure.

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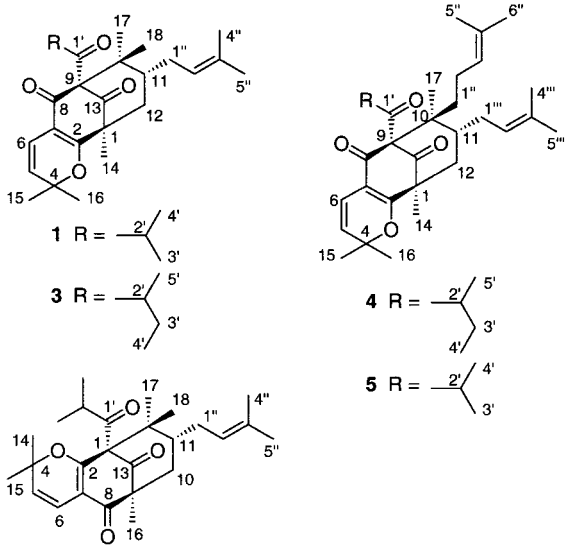
The relative stereochemistry of compound **1** was determined by a NOESY experiment and molecular modeling based on the coordinates of the crystal structure from a closely related compound. Therein, the different substituents of **1** were introduced by making appropriate changes to the coordinates of the *p*-bromobenzoate ester of hyperforin.[4] In the latter, the saturated six-membered ring moiety displayed a chair configuration with the 3-methylbut-2-enyl and the 4-methylpent-3-enyl side chains occupying equatorial positions. After subsequent energy minimization of the model of **1** the six-membered ring remained in a chairlike configuration. The rigid structure of the bicyclic ring system (C-1, C-2, and C-7 to C-13) required the substituents at C-1 and C-9 to be equatorial. The equatorial proton on C-12 appeared as a doublet of doublets with 3.7 and 12.9 Hz couplings, whereas the axial proton was observed as an apparent triplet with a 12.9 Hz coupling, which is only consistent with a diaxial arrangement of H-12ax and H-11. Therefore, the 3-methylbut-2-enyl side chain was placed in an equatorial orientation. This assignment is consistent with the stereochemistry of previously isolated compounds of this class.[5,6] Additionally, H_3-17 showed a NOE to the axial proton at C-12, a fact that confirmed the axial position of this methyl group. Furthermore, the second methyl group at C-10, which was assigned with an equatorial orientation, exhibited a strong NOE correlation to the proton at C-11 (see Figure 2). Analogous considerations were applied to the determination of the relative stereochemistry of compounds **2**−**5**.



The only difference between the two structural isomers **1** and **2** is the different constitution of the 2,2-dimethyl-2*H*-pyran ring. In compound **1** the quaternary carbon C-2 (δ_C 170.8) showed HMBC connectivities to H-6 and H_3-14, and the carbonyl carbon C-8 (δ_C 188.6) to H-6. However, in compound **2**, C-2 (δ_C 167.3) showed only a correlation to H-6, whereas the carbonyl carbon C-8 (δ_C 191.9) displayed HMBC cross-peaks to H-6 and H_3-16. Although pure **2** isomerized to a mixture of both **1** and **2**, with **1** being the strongly preferred isomer, it was sufficiently stable to determine the structure unambiguously as shown.

A difference of 14 atomic mass units between **1** and **3** was derived from DEIMS, establishing together with ^{13}C NMR data the molecular formula as $C_{27}H_{38}O_4$. A comparison of the 1D and 2D NMR spectra of **3** with those of **1** showed that the structural differences are restricted to the

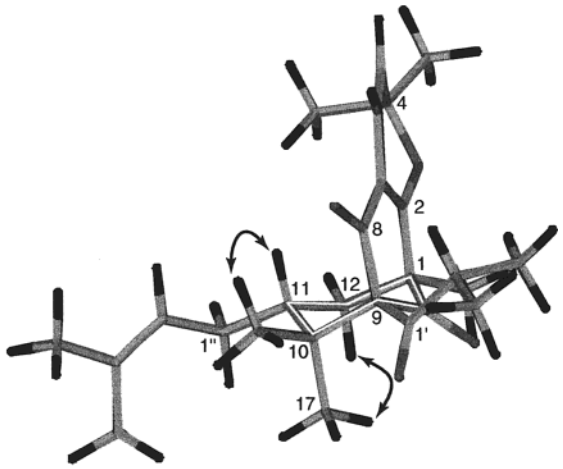


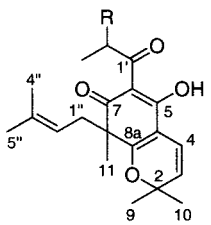
Figure 2. Selected NOE correlations of **1**.

acyl side chain. DQF-COSY correlations between H_3-4′ and H_2-3′, H_2-3′ and H-2′, and H-2′ and H_3-5′ as well as HMBC connectivities between C-1′ and H_3-5′, C-2′ and H_2-3′/H_3-4′/H_3-5′, and C-3′ and H_3-4′/H_3-5′ proved the replacement of the isobutyryl substituent in **1** by a 2-methylbutyryl side chain in **3**.

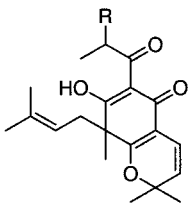
A molecular ion at *m*/*z* 494, obtained by positive DEI-MS in combination with the ^1H and ^{13}C NMR spectra, allowed the establishment of the molecular formula as $C_{32}H_{46}O_4$ for compound **4**, which was isolated as a yellowish oil. Comparison with the ^1H and ^{13}C NMR spectra of **3** showed close similarities between **3** and **4**. The additional ^{13}C (δ_C 36.5, 25.1, 124.7, 131.1, 25.7, and 17.7) and ^1H (δ_H 1.36, m; 1.97, m; 1.92, m; 2.16, m; 5.06, m; 1.60, bs; 1.64, bs) NMR resonances strongly indicated the presence of a 4-methylpent-3-enyl side chain in **4**. HMBC correlations between C-10 and H_2-1″, C-17 and H_2-1″, and C-9 and H_2-1″ showed that one of the former two methyl groups at C-10 in **3** was now replaced by this side chain. The axial position of the methyl group at C-10 was confirmed by a NOE correlation to the axial proton at C-12, so therefore the 4-methylpent-3-enyl group was assigned as equatorial.

Detailed analysis of the 1D and 2D NMR spectra of the isolate **5** in comparison to the spectra of the compounds **1** and **4** revealed that the only difference between **4** and **5** was the replacement of the 2-methylbutyryl side chain in **4** by an isobutyryl moiety in **5**.

The chemical structures of papuaforins A−E (**1**−**5**) were similar to the structure of the well-known hyperforin, isolated from *Hypericum perforatum* L.[4,7] The additional 2,2-dimethyl-2*H*-pyran ring is most probably formed by cyclization of a 3-methylbut-2-enyl side chain with an enolic hydroxyl group. To our knowledge the isolation of a similar tricyclic system has been reported only twice previously, as prenylated benzophenone derivatives from *Clusia plukenetii*[6] and Cuban propolis.[5] The ^1H and ^{13}C NMR data of the isolates **1**−**5** are summarized in Tables 1 and 2.



6 R = H
7 R = CH_3



6a R = H
7a R = CH_3

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

H	1	2	3	4	5
5	5.37 d (10.0)	5.40 d (10.0)	5.38 d (10.0)	5.37 d (10.0)	5.37 d (10.0)
6	6.49 d (10.0)	6.51 d (10.0)	6.48 d (10.0)	6.48 d (10.0)	6.48 d (10.0)
10 ax		1.33 m[b]			
10 eq		1.99 dd (4.0, 13.7)			
11	1.50 m[b]	1.66 m[b]	1.51 m[b]	1.62 m[b]	1.64 m[b]
12 ax	1.39 t (12.9)		1.39 m[b]	1.40 bt (13.0)	1.41 m[b]
12 eq	1.96 dd (3.7, 12.9)		1.95 dd (13.7, 4.1)	1.93 dd (4.2, 13.6)	1.94 m[b]
14	1.29 s	1.55 s	1.29 s	1.28 s	1.28 s
15	1.50 s	1.50 s	1.51 s	1.50 s	1.50 s
16	1.40 s	1.25 s	1.40 s	1.40 s	1.40 s
17	1.03 s	1.06 s	1.03 s	1.03 s	1.03 s
18	1.25 s	1.36 s	1.25 s		
2′	2.11 m[b]	2.40 sept (6.6)	1.85 m	1.87 m	2.12 m[b]
3′	1.13 d (6.5)	1.16 d (6.6)	1.31 m	1.32 m[b]	1.14 d (6.5)
			1.70 m	1.76 m[b]	
4′	1.04 d[d]	1.09 d (6.6)	0.79 t (7.5)	0.80 t (7.5)	1.06 d (6.5)
5′			1.13 d (6.5)	1.13 d (6.5)	
1″	1.68 m[b]	1.65 m[b]	1.67 m	1.36 m[b]	1.36 m[b]
	2.14 m[b]	2.14 m[b]	2.13 m	1.97 m[b]	1.97 m[b]
2″	4.97 bt (6.4)	4.96 m[b]	4.97 t (7.3)	1.92 m[b]	1.93 m[b]
				2.16 m[b]	2.15 m[b]
3″				5.06 m	5.06 m
4″	1.56 bs	1.55 s	1.56 bs		
5″	1.66 bs	1.67 s	1.65 bs	1.60 bs	1.60 bs
6″				1.64 bs	1.64 bs[c]
1‴				1.76 m[b]	1.76 m[b]
				2.14 m[b]	2.14 m[b]
2‴				4.97 m	4.97 m
4‴				1.57 bs	1.57 bs
5‴				1.66 bs	1.67 bs[c]

[a] Spectra measured at 500 MHz, 295 K, in $CDCl_3$. [b] Multiplicity not determined due to overlapping signals. [c] Values interchangeable. [d] Coupling constant not determined due to signal overlap.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**5**[a]

C	1	2	3	4	5
1	52.6 s	75.8 s	52.6 s	52.4 s	52.4 s
2	170.8 s	167.3[b] s	170.7 s	170.8 s	170.9 s
4	81.9 s	83.7[b] s	81.8 s	81.8 s	81.8 s
5	123.9 d	124.1 d	124.0 d	123.9 d	123.9 d
6	115.8 d	115.9 d	115.8 d	115.8 d	115.7 d
7	114.0 s	115.2[b] s	114.0 s	114.1 s	114.0 s
8	188.6 s	191.9[b] s	188.7 s	188.7 s	188.7 s
9	83.4 s	61.1[b] s	83.4 s	84.3 s	84.4[b] s
10	46.4 s	42.5 t	46.4 s	48.4 s	48.3 s
11	43.3 d	43.7 d	43.3 d	43.9 d	43.8 d
12	40.3 t	46.3 s	40.3 t	39.8 t	39.9 t
13	207.0 s	206.6[b] s	207.0 s	207.0 s	207.0 s
14	15.3 q	29.0 q	15.3 q	15.2 q	15.2 q
15	28.2 q	29.4 q	28.2 q	28.3 q	28.2 q
16	28.3 q	16.2 q	28.3 q	28.2 q	28.3 q
17	15.8 q	16.8 q	15.8 q	13.4 q	13.4 q
18	22.8 q	24.0 q	22.8 q		
1′	209.1 s	209.1[b] s	208.7 s	209.2 s	209.7 s
2′	42.3 d	40.8 d	49.0 d	48.9 d	42.3 d
3′	20.5 q	21.3 q	27.5 t	27.4 t	20.5 q
4′	21.6 q	21.5 q	11.6 q	11.6 q	21.5 q
5′			16.7 q	16.6 q	
1″	26.5 t	27.4 t	26.5 t	36.5 t	36.5 t
2″	122.6 d	122.3 d	122.6 d	25.1 t	25.0 t
3″	133.3 s	133.4[b] s	133.3 s	124.7 d	124.7 d
4″	17.8 q	17.8 q	17.8 q	131.1 s	131.1 s
5″	25.7 q	25.8 q	25.7 q	17.7 q	17.7 q
6″				25.7 q	25.7 q
1‴				27.0 t	27.0 t
2‴				122.6 d	122.6 d
3‴				133.3 s	133.3 s
4‴				17.9 q	17.9 q
5‴				25.7 q	25.7 q

[a] Spectra measured at 75 MHz, 295 K, in $CDCl_3$ (δ ppm). Multiplicities were obtained from DEPT135/DEPT90 experiments. [b] Signals derived from HMBC experiments.

The ^{1}H and ^{13}C NMR spectra obtained for **6/6a** and **7/7a** were almost identical, and their mass spectra displayed only a difference of 14 atomic mass units. A detailed analysis of the ^{1}H and ^{13}C NMR spectral data of **6/6a** revealed the presence of two components in a ratio of approximately 2.5:1 (in $CDCl_3$). Moreover, there were two low-field signals (δ_H 18.43, s; 18.97, s) from hydrogen-bonded hydroxyl protons, indicating that these components represent the two enol tautomers **6** and **6a**, with **6** being the preferred form. The previously isolated tricyclic acylphloroglucinol derivatives ialibinones A−E showed the existence of comparable tautomeric structures.[1] The molecular formula, $C_{20}H_{26}O_4$ as derived from DEIMS and ^{13}C NMR, indicated eight degrees of unsaturation. The compound has to be bicyclic considering the NMR data of the major tautomer, which showed signals for two ketone carbonyls (δ_C 195.8, C-7; 204.4, C-1′), one enolic carbon (δ_C 185.7, C-5), a quaternary carbon ascribed to an enol ether (δ_C 173.0, C-8a), an isopentenyl side chain (δ_C 37.7, 118.4, 134.8, 25.8, 18.0), and a further double bond between C-3 and C-4. Extensive use of HSQC, COSY, and HMBC experiments allowed the complete assignment of all signals to the major and the minor tautomers. The key HMBC connectivities are displayed in Figure 3. The acyl side chain was identified as a propionyl moiety.

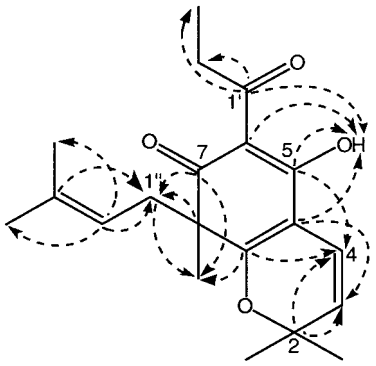


Figure 3. Selected long-range C → H (HMBC) connectivities of **6**.

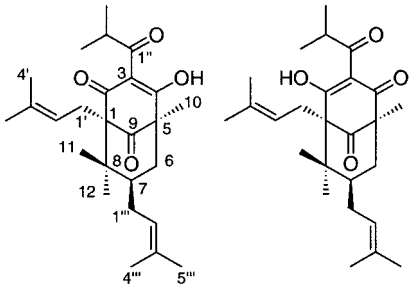
In compound **7/7a** the propionyl group is replaced by an isobutyryl side chain as confirmed by 1D and 2D NMR data

Table 3. ^{1}H NMR Data of **6/6a** and **7/7a** (δ ppm; m; *J* Hz)[a]

H	6	6a	7	7a
3	5.35 d (10.1)	5.32 d (10.0)	5.35 d (10.1)	5.32 d (10.0)
4	6.46 d (10.1)	6.53 d (10.0)	6.47 d (10.1)	6.54 d (10.0)
9	1.44 s	1.40 s	1.44[b] s	1.40[b] s
10	1.45 s	1.42 s	1.45[b] s	1.42[b] s
11	1.36 s	1.49 s	1.36 s	1.49 s
2′	3.05 q (7.3) 3.04 q (7.3)	3.22 q (7.3) 3.21 q (7.3)	3.99 sept (6.8)	4.24 sept (6.8)
3′	1.16 t (7.3)	1.18 t (7.3)	1.15 d (6.8)	1.17 d (6.8)
4′			1.13 d (6.8)	1.17 d (6.8)
1″	2.48 dd (7.5, 13.9) 2.67 dd (7.1, 14.4)	2.61 dd (7.3, 14.1) 2.70 m[c]	2.47 dd (7.3, 13.9) 2.67 dd (7.5, 13.8)	2.59 dd (7.3, 13.5) 2.69 m[c]
2″	4.78 m[c]	4.75 m[c]	4.80 m[c]	4.76 m[c]
4″	1.57 s	1.56 s	1.57 s	1.55 s
5″	1.58 s	1.57 s	1.58 s	1.57 s
OH	18.97 s	18.43 s	19.14 s	18.61 s

[a] Spectra measured at 500 MHz, 295 K, in $CDCl_3$. [b] Values are interchangeable. [c] Multiplicity not determined due to overlapping signals.

and by the 14 atomic mass units increase of the molecular weight. With the exception of signals due to the acyl side chain, the ^{1}H and ^{13}C NMR chemical shifts closely resembled those of compound **6/6a** (Tables 3 and 4). The two tautomeric forms occurred in a ratio of about 3:1. Similar bicyclic structures with different substitution pattern have been isolated previously from various *Clusia* species.[8,9]



8 **8a**

The eighth compound, hyperpapuanone (**8**/**8a**), was again obtained as a yellowish oil. Similarly to the previously described compounds **6** and **7**, doubling of the ^{1}H and ^{13}C NMR signals due to two tautomeric isomers in a ratio of approximately 4:3 was observed. The following assignment strategy refers to the preferred tautomeric structure **8**. DEIMS and ^{13}C NMR indicated a molecular formula of $C_{26}H_{38}O_4$ (molecular ion at *m*/*z* 414). Two vinylic protons, four vinylic methyl groups, and four allylic protons apparent in the ^{1}H NMR spectrum (Table 5) indicated the presence of two isopent-2-enyl groups. The ^{13}C NMR spectrum exhibited signals for two nonconjugated ketones (δ_C 208.7, C-9; 207.5, C-1″), an enolized 2,4-diketone (δ_C 114.5, C-3; 195.1, C-2; 199.6, C-4), and two quaternary carbons (δ_C 56.1, C-5; 68.9, C-1) typical for acylphloroglucinol derivatives. The bicyclo[3.3.1]non-3-ene ring system was finally completed by ^{13}C NMR signals for a quaternary (δ_C 48.1, C-8) carbon, a methine (δ_C 45.9, C-7) carbon, and a methylene (δ_C 41.0, C-6) carbon. HMBC correlations between C-1/C-2/C-9 and H_2-1′ on one hand, and C-1‴ and H_2-6 on the other hand, established that the isopentenyl groups were substituted at C-1 and C-7. Selected HMBC correlations are shown in Figure 4.

Determination of the relative stereochemistry of **8** was based on data obtained from the NOESY spectrum. Because signals significantly overlapped in the major tautomer, the stereochemistry has been established from NOE

Table 4. ^{13}C NMR Data for Compounds **6/6a** and **7/7a**[a]

C	6	6a	7	7a
2	81.0 s	79.3[c] s	80.9 s	79.3[c] s
3	123.4 d	123.6 d	123.4 d	123.6 d
4	114.7[c] d	116.0 d	114.8[c] d	116.1 d
4a	104.3 s	109.5[c] s	104.5 s	109.5[c] s
5	185.7 s	181.2[c] s	186.2 s	181.0[c] s
6	106.3[c] s	110.2[c] s	105.6[c] s	109.4[c] s
7	195.8 s	196.7[c] s	195.6 s	197.8[c] s
8	52.3 s	47.4[c] s	52.4 s	47.7[c] s
8a	173.0 s	165.6 s	172.8 s	165.4 s
9	28.3 q	28.0 q	28.3[b] q	28.0[b] q
10	28.6 q	28.5 q	28.6[b] q	28.5[b] q
11	23.7 q	23.8 q	23.5 q	23.4 q
1′	204.4 s	206.9 s	207.6 s	209.8 s
2′	33.4 t	34.2 t	35.4 d	36.0 d
3′	8.9 q	8.7 q	18.8 q	18.8 q
4′			18.9 q	19.2 q
1″	37.7 t	36.5 t	37.7 t	36.8 t
2″	118.4 d	117.7 d	118.4 d	117.7 d
3″	134.8 s	135.5[c] s	134.7 s	135.5[c] s
4″	18.0 q	18.0 q	18.0 q	17.9 q
5″	25.8 q	25.8 q	25.8 q	25.7 q

[a] Spectra measured at 75 MHz, 295 K, in $CDCl_3$ (δ ppm). [b] Values are interchangeable. [c] Chemical shifts derived from HMBC experiments.

Table 5. ^{1}H and ^{13}C NMR Data of **8/8a**[a]

	8 δ_H (ppm) (m; *J* Hz)	8a δ_H (ppm) (m; *J* Hz)	8 δ_C (ppm)	8a δ_C (ppm)
1			68.9 s	66.0 s
2			195.1 s	200.9 s
3			114.5 s	113.6 s
4			199.6 s	194.1 s
5			56.1 s	60.8 s
6	2.06 m[b]	1.96 dd (6.3, 14.3)	41.0 t	42.8 t
	2.26 dd (2.0, 14.5)	2.19 dd (4.6, 14.4)		
7	1.43 m[b]	1.41 m[b]	45.9 d	46.3 d
8			48.1 s	48.7 s
9			208.7 s	208.4 s
10	1.34 s	1.27 s	17.2 q	17.0 q
11	1.24 s	1.24 s	22.4 q	22.3 q
12	0.99 s	1.03 s	26.9 q	26.7 q
1′	2.47 m	2.63 m (2H)	26.5 t	26.3 t
	2.71 dd (8.6, 13.5)			
2′	4.77 m	4.67 m	119.6 d	118.7 d
3′			134.3 s	134.7 s
4′	1.68 s	1.67 s	18.1 q	18.1 q
5′	1.56 bs	1.56 bs	25.9 q	25.9 q
1″			207.5 s	208.3 s
2″	3.88 sept (6.8)	3.97 sept (6.8)	35.0 d	35.5 d
3″	1.21 d (6.8)	1.18 d (6.8)	18.5 q	18.5 q
4″	1.07 d (6.8)	1.12 d (6.8)	18.9 q	19.2 q
1‴	2.05 m[b]	1.85 m 2.11 m[b]	28.9 t	29.3 t
2‴	4.83 m	4.88 m	123.9 d	123.6 d
3‴			133.1 s	133.1 s
4‴	1.42 bs	1.48 bs	17.6 q	17.8 q
5‴	1.67 s	1.66 s	25.8 q	25.7 q
OH	18.60 s	18.96 s		

[a] Spectra measured at 500 MHz (^{1}H) or 75 MHz (^{13}C), 295 K, in $CDCl_3$. [b] Multiplicity not determined due to overlapping signals.

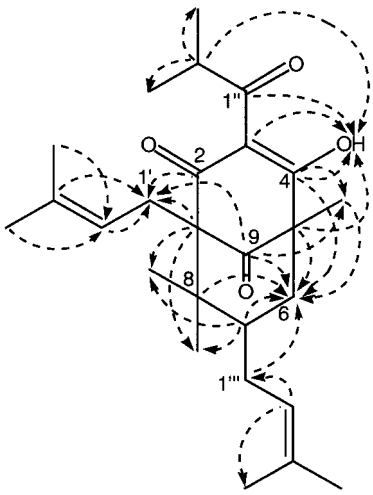


Figure 4. HMBC (C → H) correlations of **8**.

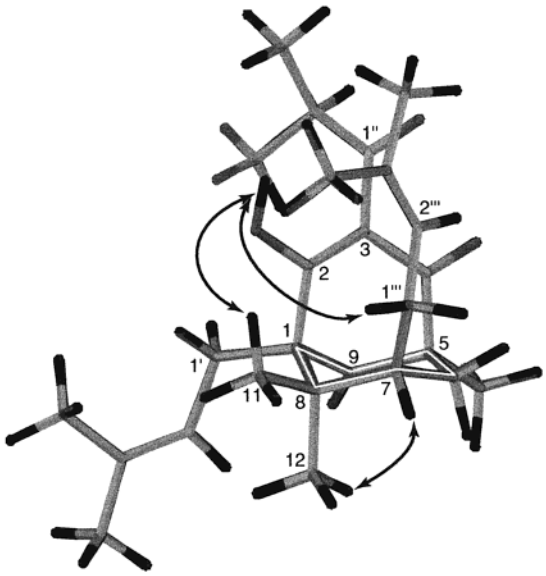


Figure 5. Key NOE cross-peaks of **8a**.

correlations of the minor isomer. The rigid bicyclic ring system required the isopentenyl group at C-1 and the methyl group at C-5 to be in the equatorial position. Weak NOE cross-peaks between the hydroxyl proton and the methylene protons at C-1‴ confirmed the β orientation of the isopentenyl side chain at C-7. The β stereochemistry of C-11 was established by an NOE correlation between the hydroxyl proton and H_3-11. This fact was well supported by a strong NOE interaction between the methyl group H_3-12 and the α-orientated proton H-7 (Figure 5). 3-Benzoyl derivatives with similar bicyclic structures have previously been isolated from the genera *Clusia*, *Garcinia*, and *Symphonia* (Guttiferae), of which some show HIV-inhibitory and cytotoxic activities.[9−12] However, to our knowledge we report here for the first time the isolation of this skeleton with nonbenzoylic substitution at C-3.

Cytotoxicity toward the KB cell line (ATCC CCL 17; human nasopharyngeal carcinoma) and antibacterial potential against three microorganisms (*Micrococcus luteus*, *Staphylococcus epidermidis*, and *Bacillus cereus*) were evaluated. The results are displayed in the Experimental Section. Compounds **1**, **3**, **4**, **5**, and **8** were found to be only moderately cytotoxic, and hyperguinone B (**7**) was even less active. However, the IC_{50} values were very similar among the compounds, and conclusions concerning structure−activity relationships for these molecules are not possible from the present results. The antibacterial effect of the isolates was either rather weak or in the range of the negative control (solvent) test, except for compound **8**, which showed moderately potent antibacterial activity against all of the three tested bacteria. Accordingly, the antimicrobial activities of these metabolites, together with those of the previously isolated antibacterial ialibinones A−D,[1] are supportive of the traditional use of *H. papuanum* in Papua New Guinea for wound healing.

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter with methanol as solvent. UV spectra were obtained in methanol on a UVIKON 930 spectrophotometer. ^{13}C NMR spectra were measured at 295 K on a Bruker AMX-300 spectrometer (operating at 300.13 MHz for ^1H and 75.47 MHz for ^{13}C), and ^1H, [^1H, ^1H]-COSY, 500 ms NOESY, [^{13}C, ^1H]-HMBC/HSQC experiments at 295 K on a Bruker DRX-500 (operating at 500.13 MHz for ^1H and 125.77 MHz for ^{13}C). The spectra were measured in $CDCl_3$ and referenced against residual nondeuterated solvent $CHCl_3$ (^1H δ 7.27 ppm) and $CDCl_3$ (^{13}C δ 77.0 ppm). DEI mass spectra were measured on a micromass TRIBRID double-focusing mass spectrometer at 70 eV. 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 S5 ODS II, 5 μm, 250 × 16 mm). Silica gel A.C.C., particle size 40−60 μm (Chromagel), and silica gel for column chromatography, particle size 15−40 μm (Merck), were used for VLC (columns 22 × 7 and 22 × 3 cm, respectively). Silica 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 (Guttiferae) were collected north of Ialibu, Southern Highlands Province, Papua New Guinea (PNG), in 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. Air-dried and powdered aerial parts of *Hypericum papuanum* (2.2 kg) were extracted successively with petroleum ether, dichloromethane, methanol, and 7:3 and 1:1 methanol−water mixtures, to afford 160 g of petroleum ether-soluble material after removal of solvent under vacuum. A 46 g quantity of this extract was applied to VLC over Si gel (40−60 μm) in 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. Identical fractions, as identified by comparable TLC R_f values, 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 (100%) to ethyl acetate (100%) and final washing with methanol. Based on TLC, the obtained 33 fractions of 100 mL each were combined to give 12 fractions. The antibacterially active fractions 7, 8, and 9 (288 mg, eluted with hexane−ethyl acetate 99.3:0.7, 99:1, and 95:5, respectively) were combined for further purification. Reversed-phase HPLC purification of the combined fraction using a step gradient from acetonitrile−H_2O, 9:1 to 17:3, yielded **1** (6.1 mg), **2** (1.5 mg), **3** (2.7 mg), **4** (4.4 mg), **5** (3.1 mg), **6/6a** (1.4 mg), **7/7a** (5.3 mg), and **8/8a** (3.5 mg), each as a yellow or slightly reddish oil.

Cytotoxicity Study. The cytotoxicity of the compounds was determined using a KB cell line (ATCC CCL 17; human nasopharyngeal carcinoma) as described by Ankli et al.[13] The test was performed at least in triplicate. Considering that the quantity of the isolated **6/6a** was very low, and the fact that

the structurally closely related compound **7/7a** showed only weak activity, the test was not performed with **6/6a**. Due to limited stability, **2** was also excluded from the cytotoxicity study. The IC_{50} values were 7.5 ± 0.47 μg/mL (**1**), 4.9 ± 0.59 μg/mL (**3**), 6.6 ± 1.2 μg/mL (**4**), 5.6 ± 0.57 μg/mL (**5**), 13.0 ± 1.5 μg/mL (**7**/**7a**), and 3.2 ± 1.1 μg/mL (**8**/**8a**). Podophyllotoxin was used as a positive control (IC_{50} 0.006 ± 0.0003 μg/mL).

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 published previously.[1,14] All pure compounds were tested, except the unstable isolate **2**. Chloramphenicol was used as a positive control showing minimum inhibition concentration (MIC) of 2 μg/mL (*B. cereus*, *M. luteus*) and 4 μg/mL (*S. epidermidis*). The MICs in broth against *B. cereus* were 8 μg/mL (**8**/**8a**), 32 μg/mL (**6**/**6a**, **7**/**7a**), 64 μg/mL (**1**, **3**, and **5**), and 128 μg/mL (**4**). Against *S. epidermidis* the MICs were 8 μg/mL (**8**/**8a**) and 32 μg/mL (**5**, **6**/**6a**); compounds **1**, **3**, **4**, and **7**/**7a** showed no difference from the negative control. Against *M. luteus* compounds **8**/**8a** and **3** showed MIC values of 16 and 32 μg/mL, respectively. For the other compounds no difference from the negative control was observed.

Papuaforin A (9-isobutyryl-1,4,4,10,10-pentamethyl-11α-(3-methylbut-2-enyl)-3-oxatricyclo[7.3.1.0^{2,7}]trideca-2(7),5-diene-8,13-dione, 1): yellow oil (6.1 mg); $[\alpha]^{25}_D$ +13° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.22), 262 (3.91), 324 (sh) (3.67) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR spectral data, see Table 2; DEIMS (CH_2Cl_2) *m*/*z* 412 $[M]^+$ (3), 301 (6), 275 (5), 261 (9), 233 (13), 69 (18); HRMALDIMS (pos. mode) *m*/*z* 413.2686 $[M + H]^+$ (calcd 413.2692).

Papuaforin B (1-isobutyryl-4,4,9,12,12-pentamethyl-11α-(3-methylbut-2-enyl)-3-oxatricyclo[7.3.1.0^{2,7}]trideca-2(7),5-diene-8,13-dione, 2): pale yellow oil (1.5 mg); ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; further physical and spectroscopic data not determined due to instability.

Papuaforin C (1,4,4,10,10-pentamethyl-11α-(3-methylbut-2-enyl)-9-(2-methylbutyryl)-3-oxatricyclo[7.3.1.0^{2,7}]-trideca-2(7),5-diene-8,13-dione, 3): pale yellow oil (2.7 mg); $[\alpha]^{25}_D$ +23° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.16), 261 (3.89), 327 (sh) (3.55) nm; ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; DEIMS (CH_2Cl_2) *m*/*z* 426 $[M]^+$ (3), 315 (5), 289 (5), 275 (9), 233 (13), 69 (16); HRMALDIMS (pos. mode) *m*/*z* 449.2668 $[M + Na]^+$ (calcd 449.2668).

Papuaforin D (1,4,4,10α-tetramethyl-11α-(3-methylbut-2-enyl)-9-(2-methylbutyryl)-10β-(4-methylpent-3-enyl)-3-oxatricyclo[7.3.1.0^{2,7}]trideca-2(7),5-diene-8,13-dione, 4): pale yellow oil (4.4 mg); $[\alpha]^{25}_D$ +64° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.23), 262 (3.85), 323 (sh) (3.54) nm; ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; DEIMS (CH_2Cl_2) *m*/*z* 494 $[M]^+$ (6), 425 $[M - C_5H_9]^+$ (5), 291 (77), 289 (17), 275 (100), 233 (28), 69 (73); HRMALDIMS (pos. mode) *m*/*z* 517.3294 $[M + Na]^+$ (calcd 517.3294).

Papuaforin E (9-Isobutyryl-1,4,4,10α-tetramethyl-11α-(3-methylbut-2-enyl)-10β-(4-methylpent-3-enyl)-3-oxatricyclo[7.3.1.0^{2,7}]trideca-2(7),5-diene-8,13-dione, 5): pale yellow oil (3.1 mg); $[\alpha]^{25}_D$ +41° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.21), 267 (3.84) nm; ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; DEIMS (CH_2Cl_2) *m*/*z* 480 $[M]^+$ (<1), 277 (8), 233 (5), 69 (18); HRMALDIMS (pos. mode) *m*/*z* 503.3140 $[M + Na]^+$ (calcd 503.3137).

Hyperguinone A (5-hydroxy-2,2,8-trimethyl-8-(3-methylbut-2-enyl)-6-propionyl-2,8-dihydro-1-benzopyran-7-one, 6/6a): yellow oil (1.4 mg); $[\alpha]^{25}_D$ +8° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.08), 270 (3.95), 352 (sh) (3.41) nm; ^{1}H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 4; DEIMS (CH_2Cl_2) *m*/*z* 330 $[M]^+$ (<1), 262 (6), 247 (20), 135 (3), 69 (15); HRMALDIMS (pos.), no molecular ion peak observable.

Hyperguinone B (5-hydroxy-6-isobutyryl-2,2,8-trimethyl-8-(3-methylbut-2-enyl)-2,8-dihydro-1-benzopyran-7-one, 7/7a): reddish oil (5.3 mg); $[\alpha]^{25}_D$ +28° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.17), 270 (4.06), 352 (sh) (3.60) nm; ^{1}H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 4; DEIMS (CH_2Cl_2) *m*/*z* 344 $[M]^+$ (1), 276 (4), 261 (13), 149 (3), 69 (7); HRMALDIMS (pos.), no molecular ion peak observable.

Hyperpapuanone (4-hydroxy-3-isobutyryl-5,8,8-trimethyl-1-(3-methylbut-2-enyl)-7β-(3-methylbut-2-enyl)-bicyclo[3.3.1]non-3-ene-2,9-dione, 8/8a): pale yellow oil (3.5 mg); $[\alpha]^{20}_D$ +15° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.12), 281 (3.99) nm; ^{1}H NMR and ^{13}C NMR spectral data, see Table 5; DEIMS (CH_2Cl_2) *m*/*z* 414 $[M]^+$ (5), 399 (4), 277 (11), 261 (6), 235 (4), 69 (13); HRMALDIMS (pos.), no molecular ion peak observable.

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