Acylated Flavonol Glycosides from Leaves of *Stenochlaena palustris*

Hongmei Liu,[‡] Jimmy Orjala,[†,‡] Otto Sticher,*[,‡] and Topul Rali[§]

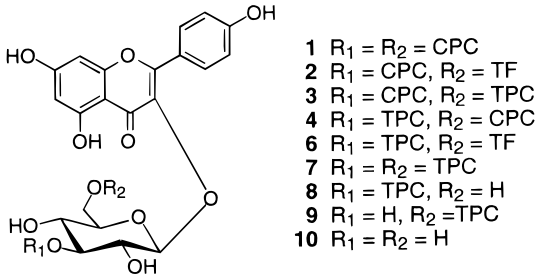
Department of Pharmacy, Swiss Federal Institute of Technology (ETH) Zurich, CH-8057 Zürich, Switzerland, and PNG Biodiversity PTY Ltd., Port Moresby, Papua New Guinea

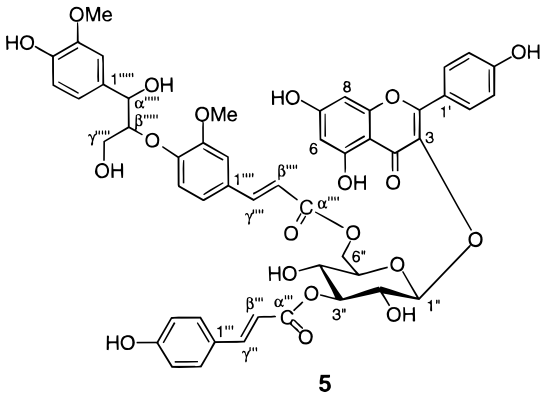
Received May 4, 1998

From the leaves of *Stenochlaena palustris* five new *O*-acylated flavonol glycosides, stenopalustrosides A−E (**1**−**5**), have been isolated along with five known compounds, kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)−(6″-*O*-*E*-feruloyl)-*β*-D-glucopyranoside (**6**), kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside (**7**), kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside (**8**), kaempferol 3-*O*-(6″-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside (**9**); and kaempferol 3-*O*-*β*-D-glucopyranoside (**10**). The structures of the isolates were elucidated by spectroscopic methods, mainly 1D and 2D NMR. Compounds **1**−**4** showed significant antibacterial activities against Gram-positive strains. The structural difference between the isolated antibacterial and nonantibacterial compounds is discussed.

Stenochlaena palustris (Burm.) Bedd. (Pteridaceae) is a fern trailing over the ground or scrambling high up trees. It is endemic to a large part of tropical areas from southern and northern India through Malaysia to Polynesia and Australia.[1] The tender leaves of *S. palustris* are used as a contraceptive by the local people in the central district province of Papua New Guinea (PNG) and in the Nicobar Islands.[2,3] A search for alkaloid-containing plants in New Guinea found the leaves of *S. palustris* to be alkaloid-negative.[4] No other chemical studies on this species have been reported.

In our study on medicinal plants from PNG, we have investigated the leaves of *S. palustris* collected in the central district, near Port Moresby. The current report describes the isolation and structure elucidation of five new *O*-acylated flavonol glycosides, namely stenopalustrosides A−E (**1**−**5**), along with five known compounds, kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-(6″-*O*-*E*-feruloyl)-*β*-D-glucopyranoside (**6**), kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside (**7**), kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside (**8**), kaempferol 3-*O*-(6″-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside (tiliroside) (**9**), and kaempferol 3-*O*-*β*-D-glucopyranoside (**10**). The occurrence of acylated flavonol glycosides in ferns has not previously been described. We further report the antibacterial activities of compounds **1**−**4** against *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Micrococcus luteus*. The complete ^{13}C NMR spectral data for the known compounds **6**−**8** are also presented for the first time.





5

Results and Discussion

The air-dried leaves of *S. palustris* were extracted in turn with MeOH and 70% aqueous MeOH at room temperature. The MeOH extract was partitioned between *n*-hexane and 90% aqueous MeOH. The alcoholic phase was further partitioned between $CHCl_3$ and 60% aqueous MeOH. The residue of the $CHCl_3$ phase showed pronounced antibacterial activities against *B. cereus*, *S. epidermidis*, *S. aureus*, and *M. luteus*, and was therefore subjected to VLC, MPLC, preparative TLC, and HPLC, which led to the isolation of nine *O*-acylated flavonol glycosides (**1**-**9**) and kaempferol 3-*O*-*β*-D-glucopyranoside (**10**).

Stenopalustroside A (**1**) was isolated as a yellow amorphous powder. After being sprayed with natural products−polyethylene glycol reagent (NP−PEG),[5] it gave a yellow spot with intense fluorescence in UV −366 nm on TLC. The ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) showed the presence of multiple aromatic systems and a sugar moiety

* To whom correspondence should be addressed. Tel.: +41 1 635 6050. Fax: +41 1 635 6882. E-mail: sticher@pharma.ethz.ch.
† Present address: AgraQuest Inc., 1105 Kennedy Place, Davis, CA 95616, USA.
‡ Department of Pharmacy.
§ PNG Biodiversity PTY Ltd.

10.1021/np980179f CCC: $18.00
Published on Web 11/12/1998

Table 1. ^{1}H NMR Spectral Data of **1**−**5**

			3/**4** mixture		
H	**1**	**2**	**3**	**4**	**5**[e]
6	6.20 (d, 2.1)	6.12 (d, 2.0)	6.14 (d, 2.0)	6.20 (d, 2.0)	6.08 (d, 1.9)
8	6.32 (d, 2.1)	6.29 (brs)	6.33 (d, 2.0)[a]	6.32 (d, 2.0)[a]	6.24 (brs)
2′	7.96 (d, 8.9)	7.99 (d, 8.9)	7.99 (d, 8.7)[b]	7.97 (d, 8.7)[b]	7.98 (d, 8.6)
3′	6.84 (d, 8.9)	6.84 (d, 8.9)	6.84 (d, 8.7)	6.84 (d, 8.7)	6.85 (d, 8.6)
5′	6.84 (d, 8.9)	6.84 (d, 8.9)	6.84 (d, 8.7)	6.84 (d, 8.7)	6.85 (d, 8.6)
6′	7.96 (d, 8.9)	7.99 (d, 8.9)	7.99 (d, 8.7)[c]	7.97 (d, 8.7)[c]	7.98 (d, 8.6)
1″	5.32 (d, 7.9)	5.38 (d, 7.9)	5.37 (d, 7.9)[d]	5.33 (d, 7.9)[d]	5.43 (d, 7.8)/5.41 (d, 7.8)
2″	3.61 (dd, 9.3, 7.9)	3.63 (dd, 9.3, 7.9)	3.66 (m)	3.66 (m)	3.68 (dd, 9.1, 7.8)
3″	5.09 (t, 9.3)	5.12 (t, 9.3)	5.13 (t, 9.3)	5.11 (t, 9.1)	5.14 (t, 9.1)
4″	3.47 (t, 9.3)	3.51 (m)	3.52 (m)	3.52 (m)	3.56 (m)
5″	3.52 (m)	3.58 (m)	3.59 (m)	3.48 (m)	3.61 (m)
6″	4.22 (2H, m)	4.28 (2H, m)	4.32 (dd, 11.8, 2.0) 4.22 (m)	4.22 (2H, m)	4.31 (m, 2H)
β‴	5.88 (d, 12.8)	5.88 (d, 12.8)	5.88 (d, 12.8)	6.43 (d, 15.9)	6.43 (d, 15.9)
γ‴	6.89 (d, 12.8)	6.90 (d, 12.8)	6.90 (d, 12.8)	7.69 (d, 15.9)	7.70 (d, 15.9)
2‴	7.68 (d, 8.6)	7.68 (d, 8.7)	7.68 (d, 8.7)	7.49 (d, 8.6)	7.49 (d, 8.6)
3‴	6.75 (d, 8.6)	6.76 (d, 8.7)	6.76 (d, 8.7)	6.80 (d, 8.6)	6.82 (d, 8.6)
5‴	6.75 (d, 8.6)	6.76 (d, 8.7)	6.76 (d, 8.7)	6.80 (d, 8.6)	6.82 (d, 8.6)
6‴	7.68 (d, 8.6)	7.68 (d, 8.7)	7.68 (d, 8.7)	7.49 (d, 8.6)	7.49 (d, 8.6)
β⁗	5.53 (d, 12.8)	6.13 (d, 15.9)	6.09 (d, 15.9)	5.55 (d, 12.8)	6.16 (d, 15.9)/6.14 (d, 15.9)
γ⁗	6.72 (d, 12.8)	7.41 (d, 15.9)	7.41 (d, 15.9)	6.73 (d, 12.8)	7.39 (d, 15.9)/7.38 (d, 15.9)
2⁗	7.50 (d, 8.6)	7.07 (brs)	7.32 (d, 8.7)	7.50 (d, 8.6)	7.08 (brs)
3⁗	6.69 (d, 8.6)		6.82 (d, 8.7)	6.71 (d, 8.6)	
5⁗	6.69 (d, 8.6)	6.81 (d, 8.3)	6.82 (d, 8.7)	6.71 (d, 8.6)	7.06 (d, 8.9)
6⁗	7.50 (d, 8.6)	6.93 (d, 8.3)	7.32 (d, 8.7)	7.50 (d, 8.6)	6.94 (d, 8.9)
3⁗- OMe		3.90 (s)			3.90 (s)/3.83 (s)
α⁗⁗					4.92 (d, 5.8)/[f]
β⁗⁗					4.56 (m)/4.50 (m)
γ⁗⁗					3.87 (m, 2H)/3.78 (m, 2H)
2⁗⁗					7.06 (d, 1.3)
5⁗⁗					6.78 (d, 8.2)
6⁗⁗					6.88 (d, 8.2)
3⁗⁗- OMe					3.84 (s)

[a−d] The signals with the same descriptor are interchangeable. [e] A mixture of regioisomers and diastereoisomers. Most of the corresponding protons have the identical resonances. [f] Overlapped with solvent signals.

in the molecule. The positive FABMS of **1**, showing the molecular peaks at *m*/*z* 741 $[M + H]^+$ and 763 $[M + Na]^+$, and the ^{13}C NMR spectrum, as well as the DEPT experiments, which sorted 39 carbons into one methylene, 23 methines, and 15 quaternary carbons, allowed the establishment of the molecular formula as $C_{39}H_{32}O_{15}$. The ^{1}H NMR resonances of two coupled doublets at δ 6.20 and 6.32 with a small coupling constant (J = 2.1 Hz), which correlated to carbons at δ 100.1 and 94.9 in the HMQC spectrum, were characteristic of two meta-related H-6 and H-8 protons of ring A of a flavonoid derivative.[6] Their chemical shifts further indicated a 5,7-dihydroxy substitution pattern of ring A.[6] Ring B was assigned as a 1,4-substituted benzene ring (δ_H 7.96, d, 2H, J = 8.9 Hz; 6.84, d, 2H, J = 8.9 Hz) from the results of a HMBC experiment. These data together indicated that the aglycon moiety was kaempferol. The sugar functionality was identified as a β-glucopyranose by the ^{1}H and ^{13}C NMR spectral data. The HMBC experiment displayed a long-range correlation between C-3 (δ 135.1) and the anomeric proton (δ 5.32, d, J = 7.9 Hz), revealing the site of glycosidation to be 3-OH of kaempferol. Two additional 1,4-substituted aromatic rings [(δ_H 7.68, d, 2H, J = 8.6 Hz; 6.75, d, 2H, J = 8.6 Hz) and (δ_H 7.50, d, 2H, J = 8.6 Hz; 6.69, d, 2H, J = 8.6 Hz)], together with two pairs of double-bond proton doublets (J = 12.8 Hz) at δ 6.89, 5.88, 6.72, and 5.53, as well as two ester carbonyl carbons at δ 167.9 and 167.7, suggested the presence of two *cis*-*p*-coumaroyl moieties. These findings were confirmed by the positive FABMS, which gave fragment ions at *m*/*z* 601 $[M + Na - 162]^+$, formed by elimination of one coumaric acid moiety from the molecule, and at *m*/*z* 287 $[M + H - 454]^+$, due to the loss of the acylated glucose. The linkages of the acyl coumaroyl substituents to 3″-OH and to 6″-OH were established by the significant downfield shifts of H-3″ (δ 5.09) and H-6″ (δ 4.22, 2H) of the glucose. The HMBC spectrum, in which the ester carbonyl carbon at δ 167.9 (C-α‴) was correlated to H-3″ and the other at δ 167.7 (C-α⁗) to H-6″, further substantiated these findings. Thus, the structure of compound **1** was determined as kaempferol 3-*O*-(3″,6″-di-*O*-*Z*-*p*-coumaroyl)-β-glucopyranoside, namely stenopalustroside A.

Compound **2** was obtained as a yellow amorphous powder. It also showed a positive reaction with NP−PEG on TLC. The ^{13}C NMR and DEPT spectra displayed 40 carbons as one methyl, one methylene, 22 methines, and 16 quaternary carbons. The positive FABMS spectrum of **2** gave a $[M + 2H]^+$ peak at *m*/*z* 772 and a $[M + H + Na]^+$ peak at *m*/*z* 794, consistent with the molecular formula $C_{40}H_{34}O_{16}$. These data indicated that **2** has an additional methoxyl group when compared to compound **1**. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) revealed the aglycon moiety to be, again, kaempferol and the sugar residue to be β-glucopyranose. It also showed the presence of one *cis*-*p*-coumaroyl moiety, but the second coumaroyl moiety was absent. Instead, the ^{1}H NMR spectrum of **2** contained two coupled trans double-bond protons (δ 7.41, d, J = 15.9 Hz; 6.13, d, J = 15.9 Hz) and three coupled aromatic protons (δ 7.07, brs; 6.81, d, J = 8.3; 6.39, d, J = 8.3), which were assigned to a 1,3,4-substituted benzene ring. The presence of a *trans*-feruloyl moiety was evident by the connectivities observed in the HMBC spectrum from the methoxyl group to C-3⁗ and from the trans double-bond to the 1,3,4-substituted aromatic ring as well as to an ester carbonyl. The site of glycosidation was established at the 3-OH position of kaempferol, as demonstrated by the HMBC

Table 2. ^{13}C NMR Spectral Data of **1**−**8**

			3/**4** mixture					
C	**1**	**2**	**3**	**4**	**5**[d]	**6**	**7**	**8**
2	159.4	159.4	159.3	159.3	159.2	159.3	159.3	159.0
3	135.1	135.1	135.1	135.1	135.0	135.1	135.1	135.4
4	179.3	179.3	179.3	179.3	179.3	179.3	179.3	179.4
5	163.1	162.6	163.0[a]	163.1[a]	162.9	163.0	163.0	163.1
6	100.1	100.0	100.0	100.0	100.0	100.0	100.0	100.2
7	166.4	166.0	166.0	166.0	165.8	166.0	166.0	166.7
8	94.9	94.8	94.9	94.9	94.8	94.8	94.8	94.9
9	158.5	158.5	158.4	158.4	158.3	158.4	158.4	158.6
10	105.6	105.6	105.6	105.6	105.6	105.6	105.6	105.6
1′	122.7	122.7	122.7	122.7	122.7	122.7	122.7	122.8
2′	132.2	132.2	132.2	132.2	132.2	132.2	132.2	132.3
3′	116.1	116.1	116.1	116.1	116.1	116.1	116.1	116.2
4′	161.5	161.6	161.5	161.5	161.5	161.6	161.6	161.6
5′	116.1	116.1	116.1	116.1	116.1	116.1	116.1	116.2
6′	132.2	132.2	132.2	132.2	132.2	132.2	132.2	132.3
1″	103.8	103.7	103.8	103.8	103.5	103.7	103.7	104.0
2″	74.0	74.0	74.1	74.1	74.1	74.1	74.1	74.1
3″	78.3	78.3	78.3	78.7	78.7	78.7	78.7	78.9
4″	70.0	70.1	70.0[b]	70.1[b]	70.3	70.2	70.2	69.6
5″	75.5	75.8	75.7[c]	75.4[c]	75.7	75.8	75.7	78.3
6″	63.8	64.0	64.0	63.7	64.1	64.0	64.1	62.3
α‴	167.9	168.0	167.9	169.0	168.9	169.0	169.0	169.0
β‴	116.8	116.8	116.8	115.4	115.4	115.4	115.4	115.5
γ‴	144.9	144.9	144.9	146.7	146.7	146.7	146.7	146.7
1‴	127.6	127.7	127.6	127.3	127.3	127.3	127.3	127.3
2‴	133.7	133.7	133.7	131.2	131.2	131.2	131.2	131.2
3‴	115.8	115.8	115.8	116.8	116.8	116.8	116.8	116.8
4‴	160.0	160.0	159.9	161.2	161.3	161.3	161.2	161.2
5‴	115.8	115.8	115.8	116.8	116.8	116.8	116.8	116.8
6‴	133.7	133.7	133.7	131.2	131.2	131.2	131.2	131.2
α⁗	167.7	168.7	168.7	167.7	168.4	168.7	168.7	
β⁗	116.1	114.9	114.7	116.2	116.3	115.0	114.7	
γ⁗	145.5	146.9	146.6	145.4	146.3	146.9	146.6	
1⁗	127.6	127.7	127.1	127.6	129.6/129.5	127.6	127.1	
2⁗	133.7	111.6	131.2	133.7	112.2/112.0	111.6	131.2	
3⁗	115.8	149.3	116.8	115.8	151.7/151.6	149.3	116.8	
4⁗	160.0	150.6	161.2	159.9	152.0	150.6	161.2	
5⁗	115.8	116.4	116.8	115.8	117.5	116.4	116.8	
6⁗	133.7	124.3	131.2	133.7	123.7/123.6	124.2	131.2	
3⁗- OMe		56.4			56.6[e]	56.4		
α⁗′					74.1			
β⁗′					86.2/85.5			
γ⁗′					62.4/62.1			
1⁗′					133.7			
2⁗′					111.8			
3⁗′					148.9			
4⁗′					147.3			
5⁗′					115.9/115.8			
6⁗′					121.1/120.8			
3⁗′- OMe					56.4[e]			

[a−c,e] The signals with the same descriptor are interchangeable. [d] A mixture of two regioisomers and diastereoisomers. Most of the corresponding carbons have the identical resonances.

spectrum. The acylated *cis*-*p*-coumaroyl and *trans*-feruloyl were linked to 3″-OH and 6″-OH, respectively, as indicated by the downfield shifts of H-3″ (δ 5.12) and H-6″ (δ 4.28, 2H), and as confirmed by the HMBC experiment. The structure of stenopalustroside B (**2**) was therefore identified as kaempferol 3-*O*-(3″-*O*-*Z*-*p*-coumaroyl)-(6″-*O*-*E*-feruloyl)-*β*-glucopyranoside.

Stenopalustroside C (**3**) and stenopalustroside D (**4**) were isolated as an inseparable mixture. The ^{1}H and ^{13}C NMR spectra contained all the resonance signals that had been detected in the ^{1}H and ^{13}C NMR spectra of both compound **1** and the known compound **7**, kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside. The positive FABMS gave quasi-molecular peaks at *m*/*z* 742 $[M + 2H]^+$ and 764 $[M + H + Na]^+$, similar to compounds **1** and **7**. Moreover, the same aglycon (kaempferol), the same sugar moiety (*β*-glucopyranose), and the glycosidation at 3-OH, as well as the acylation at 3″-OH and 6″-OH, could be identified with the help of the ^{1}H, ^{13}C NMR, DQF−COSY, HMQC, and HMBC spectra. Four 1,4-substituted aromatic rings (Tables 1 and 2) as well as two cis double-bonds (J = 12.8) and two trans double-bonds (J = 15.9), together with four ester carbonyl signals at δ_C 169.0, 168.7, 167.9, and 167.7, revealed the presence of two *cis*-*p*-coumaroyl and two *trans*-*p*-coumaroyl moieties, as evident in the HMBC spectrum. Further studies on the long-range correlations, as displayed in the HMBC experiment, indicated that the two *cis*-*p*-coumaroyl groups were linked to 3″-OH and 6″-OH, respectively. The experiment also showed that the same was true for the two *trans*-*p*-coumaroyl groups. These results, in combination with the isolation of stenopalustroside A (**1**) and kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl)-*β*-D-glucopyranoside (**7**), strongly suggested a mixture of two regioisomers, kaempferol 3-*O*-(3″-*O*-*Z*-*p*-coumaroyl)-(6″-*O*-*E*-*p*-coumaroyl)-*β*-glucopyranoside (**3**) and kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-(6″-*O*-*Z*-*p*-coumaroyl)-*β*-glucopy-

ranoside (**4**). The intensity of each proton or each carbon indicated the composition of the two molecules to be ca. 1:1. This mixture was successfully separated from the analogues, compounds **1** and **7**, by MPLC on Si gel with CH_2Cl_2−EtOAc−MeOH (5:3:0.2) as mobile phase, but all the efforts to separate these two structures from each other failed.

Compound **5**, an amorphous yellow powder, was considered to be a flavonoid on the basis of its positive reaction to NP−PEG on TLC. The ^{1}H and ^{13}C NMR spectra of **5** showed it also to contain kaempferol as the aglycon moiety and one β-glucopyranose residue. The ^{1}H NMR spectrum pattern of **5** was very similar to that of kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-(6″-*O*-*E*-feruloyl)-β-D-glucopyranoside (**6**),[7] except the presence of some additional resonance signals (Table 1). With the help of the ^{13}C NMR (Table 2), DEPT, HMQC, and DQF−COSY spectra, these additional signals were identified as arising from a 1,3,4-substituted aromatic ring and a quasi-glycerol moiety. The HMBC spectrum of **5** showed correlations from H-α‴″ (δ 4.92, d, J = 5.8 Hz) of glycerol moiety to C-1‴″ (δ 133.7), C-2‴″ (δ 111.8), and C-6‴″ (δ 120.8), as well as from H-β‴″ (δ 4.50, m) to C-4‴″ (δ 152.0). Furthermore, a correlation between the methoxyl group at δ_H 3.84 (s, 3H) and C-3‴″ (δ 148.9) was observed. These observations indicated the quasi-glycerol moiety to be connected via a C−C bond to a 3-hydroxy-4-methoxyphenyl group and via an ether link to the acylated *trans*-feruloyl group. The HMBC experiment also supported the glycosidation to be at 3-OH, the acylation of trans-*p*-coumaroyl group at 3″-OH, and the acylation of the etherified *trans*-feruloyl group at 6″-OH. The structure given in formula **5** is the only logical conclusion that could account for the observed NMR data. This structure was further confirmed by the positive FABMS spectrum, in which molecular peaks at *m*/*z* 967 $[M + H]^+$ and 989 $[M + Na]^+$, corresponding to the molecular formula $C_{50}H_{46}O_{20}$, a fragment peak at *m*/*z* 595 $[M + H - 372]^+$ due to the loss of the etherified feruloyl moiety, were observed. Further two signals at *m*/*z* 413 $[M + H - 554]^+$ and 287 $[M + H - 680]^+$, referring to the dehydrated kaempferol glucoside unit and the aglycon unit, respectively, were detected. Thus, compound **5** was found to possess the structure of kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-[6″-*O*-*E*-{4-*O*-[1-(4-hydroxy-3-methoxyphenyl)-1,3-dihydroxy-isopropyl]-feruloyl}]-β-glucopyranoside, and was designated as stenopalustroside E.

Consideration of the intensity of proton and carbon resonances of compound **5**, combined with the observation that several NMR signals (Tables 1 and 2) belonging to the quasi-glycerol moiety and two 1,3,4-substituted aromatic systems significantly split, indicated the presence of a mixture in the sample. An example that has the functional group −CH(OH)CH(O−)CH(O−) similar to compound **5** is present in flavonolignans, for example, silybin and dehydrosilybin,[8] isosilybin,[9] and rhodiolin,[10] as well as in the xanthonolignan kielcorin,[11] all of which are mixtures of enantiomers or diastereoisomers. This phenomenon has been explained by a free radical oxidative coupling of dihydroxy grouping in ortho-position of a phenyl ring with coniferyl alcohol in the plant, which could give rise to a mixture of regioisomers.[9] Accordingly, we propose that stenopalustroside E (**5**) is a mixture of two regioisomers and diastereoisomers with different configurations at C-α‴″ and/or C-β‴″. It may be supposed that the coupling of the coniferyl alcohol moiety with the hydroxyl of the feruloyl moiety of the acylated kaempferol glycoside and with H_2O present in the plant resulted in the formation of structure **5** and the presence of two isomers. The absolute and the relative configurations at C-α‴″ and C-β‴″ remain to be determined for either of the isomers.

The identification of three known *O*-acylated flavonol glycosides, kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-(6″-*O*-*E* feruloyl)-β-D-glucopyranoside (**6**), kaempferol 3-*O*-(3″,6″-di-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside (**7**), and kaempferol 3-*O*-(3″-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside (**8**), were carried out by spectral analysis (^{1}H, ^{13}C NMR, DQF−COSY, HMQC, HMBC, and FABMS) and the comparison of ^{1}H NMR spectra with those published.[7,12,13] They have all been previously isolated only from the needles of *Picea obovata* Ledeb. (Pinaceae).[7,12,13] Compound **9** was identified as kaempferol 3-*O*-(6″-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside; compound **10**, as kaempferol 3-*O*-β-D-glucopyranoside, by MS, ^{1}H NMR, and ^{13}C NMR data analysis and comparison with the reported data of authentic samples.[14,15]

It is noteworthy that four of the isolated compounds contain *cis*-*p*-coumaroyl moieties in the molecules. Stenopalustroside A (**1**), which has two *cis*-*p*-coumaroyl moieties in the structure, was isolated as a minor component. Stenopalustroside B (**2**), stenopalustroside C (**3**), and stenopalustroside D (**4**), each of which has one *cis*-*p*-coumaroyl functionality and one *trans*-*p*-coumaroyl or one *trans*-feruloyl group, were found in significantly higher amounts in the extract. Turner et al.[16] have examined the role of light in the isomerization of (*E*)-form cinnamic acid derivatives of plant cell walls to (*Z*)-form and have concluded that the isomerization reaction is light-mediated in both live and dead plant tissue. The examination performed by Rataboul et al.[17] suggested the involvement of other mechanisms, for example, an enzymatic (isomerase) conversion, in trans to cis isomerization of *o*-coumaric acid glucoside in *Melitolus alba* mesophyll cell protoplasts, since trans to cis conversion did occur under conditions of darkness. In the case of acylated flavonol glycosides, several cis-derivatives have also been isolated from different plant sources.[18−22] These observations indicated that compounds **1**−**4** very probably existed in the original plant. Upon the isolation of compounds **1**−**9**, the isomerization of trans- to cis-derivatives and *vice versa* was not observed during storage at 4 °C.

All the isolates were evaluated for their antibacterial potential against *B. cereus*, *S. epidermidis*, *S. aureus*, and *M. luteus*. Four compounds were found to be active against all the test organisms (Table 3). Stenopalustroside A (**1**) even showed a lower MIC (minimum inhibition concentration, 2 μg/mL) against *S. epidermidis* than chloramphenicol (MIC = 4 μg/mL), which was used as a positive control. A very interesting observation is that all four antibacterial compounds (**1**−**4**) have one or two *cis*-*p*-coumaroyl groups in their molecules; in contrast, the other five structurally similar, but nonantibacterial compounds (**5**−**9**) (MIC > 256 μg/mL) contain only *trans*-*p*-coumaroyl or *trans*-feruloyl moieties. From these results, it seems that the configuration of the double-bond of coumaroyl-substituted flavonol glycosides could play a role in the antibacterial activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured in MeOH on a Perkin−Elmer model 241 polarimeter. UV spectra were obtained on a Kontron-Uvikon 930 spectrophotometer, using MeOH as solvent. IR spectra were recorded in KBr pellets on a Perkin−Elmer 2000 FT infrared spectrophotometer. FABMS were obtained in the positive mode on a ZAB 2-SEQ spectrometer, using 3-NOBA as matrix. All NMR spectra were recorded in CD_3OD on a

Table 3. Antibacterial Activities of **1**−**4**

compound	minimum inhibition concentration (MIC) in broth (in μg/mL)			
	B. cereus (ATCC 10702)	*S. epidermidis* (ATCC 12228)	*S. aureus* (ATCC 25923)	*M. luteus* (ATCC 9341)
1	4	2	16	8
2	8	64	32	16
3/**4**[a]	16	8	64	16
chloramphenicol	2	4	4	2

[a] A mixture of ca. 1:1 composition. The present information indicated that compounds **3** and **4** both contributed to the antibacterial activity.

Bruker AMX-300 spectrometer, operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C. The residual CH_3OH resonances at δ_H 3.31 and δ_C 49.0 were used as internal references. Si gel (particle size 15 μm, Merck) and RP-18 material (particle size 15−35 μm, Baker) were used for VLC. MPLC was carried out using Büchi 681 pump and 45 × 3.5 cm Büchi MPLC column packed with Si gel (particle size 15 μm, Merck). HPLC separation was performed on a Knauer Spherisorb S5 ODS II column (250 × 16 mm, particle size 5 μm, Merck) with a Merck−Hitachi L-6200 Intelligent pump and Merck−Hitachi L-4000 UV detector.

Plant Material. The leaves of *S. palustris* were collected near Port Moresby, central district province, Papua New Guinea, in March 1991. The plant was identified by Dr. P. Hovenkamp, University of Leiden, The Netherlands, where a voucher specimen with the identification number ETH 91/11 27-03-91 was deposited.

Extraction and Isolation. Air-dried and powdered leaves of *S. palustris* (1.52 kg) were percolated successively with MeOH and 70% aqueous MeOH at room temperature. The MeOH extract was concentrated under vacuum and partitioned between *n*-hexane and 90% aqueous MeOH. The alcoholic phase was further partitioned between $CHCl_3$ and 60% aqueous MeOH. The residue (19.4 g) of the $CHCl_3$ phase was subjected to VLC (Si gel) using a step gradient of MeOH−$CHCl_3$ to give 14 fractions (C1−C14). Fraction C8 (1190 mg) was again separated by VLC using increasing amounts of MeOH in CH_2Cl_2−EtOAc (1:1) as eluent to yield 7 subfractions (C8.1−C8.7). Subfraction C8.2 (700 mg) was further fractionated by MPLC (Si gel) with CH_2Cl_2−EtOAc−MeOH (5:3:0.2) as the mobile phase, giving compound **1** (3.9 mg); a mixture (52 mg) containing compounds **2**, **3**, and **4**; and a mixture (24 mg) of compounds **6** and **7**. Of the first mixture 10 mg yielded 3 mg of **2** and the mixture (6 mg) of **3** and **4**, 10 mg of the second one gave 3.8 mg of **6** and 5.2 mg of **7**, both using preparative TLC developed twice with $CHCl_3$−MeOH (7:1) as eluent. Fraction C9 (2930 mg) was worked up as fraction C8 and gave nine subfractions (C9.1−C9.9). Subfraction C9.5 (125 mg) was further separated by HPLC on RP-18 with MeOH−H_2O (7:3) as mobile phase to furnish compound **8** (7.7 mg). Subfraction C9.6 (148 mg) containing compound **5** was subjected to VLC on RP-18 using a step gradient of H_2O−ACN. The final purification of **5** (5 mg) was achieved by HPLC on RP-18 eluted with MeOH−H_2O (7:3). Fraction C13 (1200 mg) was fractionated by VLC on Si gel, eluted with gradients of EtOAc−MeOH−H_2O. The subfraction (126 mg) containing **9** and **10** was then introduced to VLC on RP-18 using H_2O with increasing amounts of ACN. Compound **9** (2.5 mg) and yellow needle crystals of **10** (crystals in MeOH) were yielded from the corresponding fractions.

Antibacterial Activity. The test organisms were *B. cereus* (ATCC 10702), *S. epidermidis* (ATCC 12228), *S. aureus* (ATCC 25923), and *M. luteus* (ATCC 9341). Antibacterial assays were carried out by the doubling dilutions method[20] using a modified procedure. Bacterial suspensions were obtained from overnight cultures in BBL nutrient broth (Becton & Dickinson Co. 11479) cultivated at 37 °C and diluted to ca. 10^5 cells/mL in fresh medium. The isolates were dissolved in MeOH to 1 mg/mL as stock solutions. The required amount of stock solutions was pipetted into the wells at the first column of a 96-well tissue culture plate (Falcon) and dried. The sample was redissolved in 25 μL DMSO, 75 μL sterile BBL 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 methylthiazolyltetrazolium chloride 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 ppm.

Stenopalustroside A (1): $[\alpha]^{20}_D$ −26° (c 0.19, MeOH); FABMS (positive) *m*/*z* 763 $[M + Na]^+$, 741 $[M + H]^+$, 601 $[M + Na - 162]^+$, 287 $[M + H - 454]^+$; UV (MeOH) λ_{max} 269, 315 nm; IR (KBr) ν_{max} 3445, 1698, 1652, 1604, 1511, 1359, 1260, 1169, 839 cm^{-1}; ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2.

Stenopalustroside B (2): $[\alpha]^{20}_D$ −18° (*c* 0.20, MeOH); FABMS (positive) *m*/*z* 794 $[M + H + Na]^+$, 772 $[M+2H]^+$, 397 $[M + H - 374]^+$, 287 $[M + H - 484]^+$; UV (MeOH) λ_{max} 268, 314 nm; IR (KBr) ν_{max} 3444, 1679, 1654, 1605, 1514, 1361, 1278, 1179, 841 cm^{-1}; ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2.

Stenopalustroside C (3) and Stenopalustroside D (4): ca. 1:1 mixture; FABMS (positive) *m*/*z* 764 $[M + H + Na]^+$, 742 $[M+2H]^+$, 287 $[M + H - 454]^+$; ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2.

Stenopalustroside E (5): $[\alpha]^{20}_D$ −69° (c 0.47, MeOH); FABMS (positive) *m*/*z* 989 $[M + Na]^+$, 967 $[M + H]^+$, 595 $[M + H - 372]^+$, 413 $[M + H - 554]^+$, 287 $[M + H - 680]^+$; ^{1}H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2.

Acknowledgment. This work was supported by the Swiss National Science Foundation. We thank Dr. B. Baumgartner (ETH, Zurich) and Dr. P. Hovenkamp (University of Leiden) for collection and identification of plant material. Thanks are also due to Dr. O. Zerbe (ETH, Zurich) for assisting in the NMR measurement, Mr. R. Häfliger and Mr. O. Greter (ETH, Zurich) for recording the mass spectra, Dr. E. Zass (ETH, Zurich) for performing literature searches, and Dr. R. Joshi for providing systematic names for new compounds.

References and Notes

(1) Holttum, R. E. *A Revised Flora of Malaya*; Government Printing Office: Singapore, 1968; Vol. 2, p 412.
(2) Wolff-Eggert, R. *Über Heilpflanzen von Papua-Neuguinea*. Ph.D. Thesis, Friedrich-Alexander-Universität, Erlangen-Nürnberg, 1977; p 142.
(3) Dagar, H. S. *J. Econ. Tax Bot*. **1989**, 13, 395−397.
(4) Hertley, T. G.; Dunstone, E. A.; Fitzgerald, J. S.; Johns, S. R.; Lamberton, J. A. *J. Nat. Prod*. **1973**, 36, 217−319.
(5) Wagner, H.; Bladt, S. *Plant Drug Analysis*: *A Thin Layer Chromatography Atlas*; Springer: Berlin, 1996; p 362.
(6) Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer: Berlin, 1970; p 261.
(7) Zapesochnaya, G. G.; Ivanova, S. Z.; Scheichenko, V. I.; Tyukavkina, N. A.; Medvedeva, S. A. *Khim. Prir. Soedin*. **1978**, 570−576.
(8) Hänsel, R.; Schulz, J.; Pelter, A.; Rimpler, H.; Rizk, A. F.; *Tetrahedron Lett*. **1969**, 4417−4420.
(9) Arnone, A.; Merlini, L.; Zanarotti, A. *J. Chem. Soc., Chem. Commun*. **1979**, 696−697.
(10) Zapesochnaya, G. G.; Kurkin, V. A. *Khim. Prir. Soedin*. **1983**, 23−32.
(11) Nielson, H.; Arends, P. *Phytochemistry* **1978**, 17, 2040−2041.
(12) Zapesochnaya, G. G.; Stepanov, A. N.; Petrow, A. A.; Ivanova, S. Z. *Khim. Prir. Soedin*. **1983**, 582−589.
(13) Zapesochnaya, G. G.; Ivanova, S. Z.; Scheichenko, V. I.; Tyukavkina, N. A.; Medvedeva, S. A. *Khim. Prir. Soedin*. **1980**, 186−190.
(14) Kaouadji, M. *Phytochemistry* **1990**, 29, 2295−2297.

(15) Merfort, I.; Wendisch, D. *Planta Med*. **1987**, 53, 434−437.
(16) Turner, L. B.; Harvey, I. M.; Mcallan, A. B. *Phytochemistry* **1993**, 33, 791−796.
(17) Rataboul, P.; Alibert, G.; Boller, T.; Boudet, A. M. *Biochim. Biophys. Acta* **1985**, *816*, 25−36.
(18) Asen, S.; Griesbach, R. J.; Norris, K. H.; Leonhardt, B. A. *Phytochemistry* **1986**, 25, 2509−2513.
(19) Brasseur, T.; Angenot, L. *Phytochemistry* **1987**, 26, 3331−3334.
(20) Brasseur, T.; Angenot, L. *Phytochemistry* **1988**, 27, 1487−1490.
(21) Chiale, C. A.; Cabrera, J. L.; Juliani, H. R. *Phytochemistry* **1991**, 30, 1042−1043.
(22) Romussi, G.; Bignardi, G. *Arch. Pharm*. **1991**, 324, 519−524.
(23) Frost, J. A. In *Methods in Practical Laboratory Bacteriology*; Chart, H., Ed.; CRC: Boca Raton, FL, 1994; pp 73−82.

NP980179F