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CYTOTOXIC AND ANTIBACTERIAL DIHYDROCHALCONES FROM *PIPER ADUNCUM*

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ABSTRACT.—Bioactivity-guided fractionation of a CH_2Cl_2 extract from the leaves of *Piper aduncum* afforded three new dihydrochalcones, piperaduncins A [**3**], B [**4**], and C [**5**], as well as two known dihydrochalcones, 2′,6′-dihydroxy-4′-methoxydihydrochalcone [**1**] and 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone [**2**] (asebogenin), together with sakuranetin, anodendroic acid methyl ester, and the carotenoid lutein. The structures of the isolates were elucidated by spectroscopic methods, mainly 1D- and 2D nmr spectroscopy. The proposed stereochemistry for compound **4** was deduced by NOESY spectroscopy and the corresponding energy minimum was established by molecular modelling calculations and translated into a 3D structure.

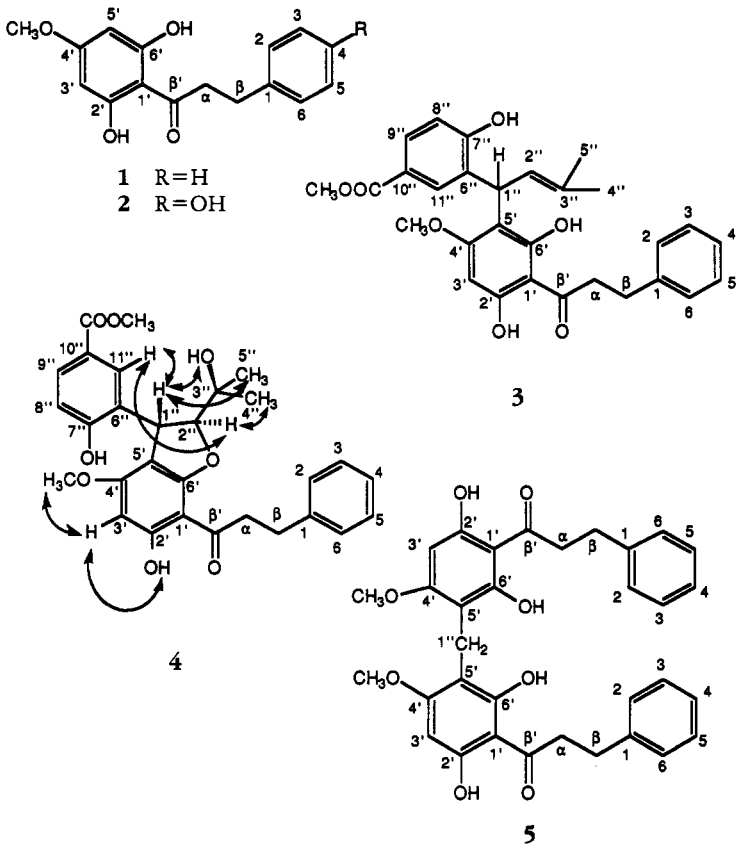
The leaves of *Piper aduncum* L. (Piperaceae), a slender tree, were collected in Papua New Guinea (PNG) as part of a research program aimed at the isolation of biologically active metabolites from plants that are employed in the traditional medicine of PNG. Villagers from the coastal areas of the Morobe Province of PNG use the fresh leaves of *P. aduncum* as an antiseptic. Crushed fresh leaves are applied either directly to wounds or uncrushed as bandages (1,2). For the treatment of diarrhea, a decoction of the leaves is used in Peru (3). Previous investigations conducted with the essential oil and the petroleum ether extract of this species have demonstrated it to contain monoterpenes, phenylpropene and benzoic acid derivatives as well as dihydrochalcones (3–6).

Biological screening of the crude CH_2Cl_2 extract of the leaves from *P. aduncum* indicated it to have significant antibacterial activity towards *Bacillus subtilis, Micrococcus luteus*, and *Escherichia coli* as well as being cytotoxic towards KB nasopharyngal carcinoma cells. Bioactivity-guided fractionation, employing KB cells as well as bioautographic tlc assays, led to the isolation and characterization of three new dihydrochalcones [**3–5**], as well as five known natural products.

RESULTS AND DISCUSSION

The dried leaves of *P. aduncum* were extracted successively with petroleum ether, CH_2Cl_2, EtOAc, and MeOH. Chromatographic separations, over Si gel, of the bioactive CH_2Cl_2 extract, using vlc and mplc, with further purification of the most active fractions using hplc, led to the isolation of eight compounds.

Two known dihydrochalcones, **1** and **2**, were identified as 2′,6′-dihydroxy-4′-methoxydihydrochalcone and 2′,6′,4-trihydroxy-4′-methoxydihydrochalcone (asebogenin), and a flavanone was identified as sakuranetin (racemic). Ms, ^{1}H-nmr, and ^{13}C-nmr spectral data analysis and comparison with reported data of authentic samples (6–9) were used. The presence of dihydrochalcones and flavanones in *Piper* spp. has been reported (6,10); this is, however, the first report of asebogenin [**2**] and sakuranetin in the genus *Piper*. (+)-Anodendroic acid methyl ester, previously reported from *Eriodictyon*



sessilifolium (Hydrophyllaceae) and as a synthetic product, was identified by comparison with published physical and spectroscopic data (11,12).

Piperaduncin A [**3**] was obtained as a pale yellow amorphous powder. The fabms of **3** gave an $[M+H]^+$ peak at *m/z* 491, consistent with the molecular formula $C_{29}H_{30}O_7$. Its ir spectrum contained absorptions due to OH (3350 cm^{-1}), conjugated ester carbonyl (1710 cm^{-1}), conjugated carbonyl (1685 cm^{-1}), and aryl (1590 cm^{-1}) moieties. The uv spectrum exhibited maxima at 265, 283, and 340 nm, and, upon addition of $AlCl_3$, the 283 nm absorption underwent a bathochromic shift of 18 nm, indicating the presence of a chelated phenolic OH group. In the ^{1}H-nmr spectrum of **3** (Table 1), signals characteristic of a 2′,6′-dihydroxy-4′-methoxydihydrochalcone, as seen for compound **1**, were observed at δ 7.32–7.18 (5H, m), 3.00 (2H, t), 3.37 (2H, br t), 5.61 (1H, s) and 3.45 (3H, s). The ^{13}C-nmr spectrum of **3** (Table 2), also contained resonances consistent with the presence of a dihydrochalcone skeleton similar to that of **1**, except for the resonance of C-5′ which was at δ 109.7 (s), suggesting **3** to be a 2′,6′-dihydroxy-4′-methoxydihydrochalcone substituted at C-5′.

The ^{1}H-nmr spectrum of **3** further contained resonances for three coupled aromatic protons (δ 6.85, d, J=8.5 Hz, 1H; 8.09 d, J=1.9 Hz, 1H; 7.78, dd, 1H; J=8.5 Hz and 1.9 Hz), which implied the presence of a 1,3,4-substituted benzene ring, an aryl Me ester function (δ 3.88, s), also evident in the ^{13}C-nmr spectrum (52.0, q), and a set of four resonances, a methine proton (δ 5.38, d, 1H), an olefinic proton (δ 6.01, br d, 1H), and two Me groups (δ 1.60, s, 3H, δ 1.76, s, 3H) for an isoprene moiety (C-1″–C-5″), connectivity within the latter group being established from the results of a DQF-COSY experiment. Correlation cross-peaks were seen for the methine proton at δ 5.38 and the

TABLE 1. ^{1}H-Nmr Spectral Data of Dihydrochalcones **1** and **3–5** (300 MHz, $CDCl_3$, δ ppm, *J* Hz).

Proton(s)	Compound			
	1	**3**	**4**	**5**[a]
H-2–H-5 . . .	7.13–7.32 m	7.18–7.32 m	7.13–7.30 m	7.20–7.32 m
H-α	3.40 t α,β (7.3)	3.37 br t α,β (7.8)	3.25–3.44 m	3.43 t α,β (7.8)
H-β	3.02 t β,α (7.3)	3.00 t β,α (7.8)	3.05 t β,α (7.8)	3.01 t β,α (7.8)
H-3′	5.93 s	5.61 s	6.09 s	6.13 s
H-5′				
H-1″		5.38 d 1′,2′ (8.2)	4.72 d 1′,2′ (4.4)	3.73 s
H-2″		6.01 br d 2′,1′ (8.2)	4.47 d 2′,1′ (4.4)	
H-4″		1.76 s	1.40 s	
H-5″		1.60 s	1.20 s	
H-8″		6.85 d 8″,9″ (8.5)	6.94 d 8″,9″ (8.4)	
H-9″		7.78 dd 9″,8″ (8.5) 9″,11″ (1.9)	7.86 dd 9″,8″ (8.5) 9″,11″ (2.2)	
H-11″		8.09 d 11″,9″ (1.9)	7.41 d 11″,9″ (2.2)	
OCH_3	3.79 s	3.45 s	3.69 s	4.00 s
$COOCH_3$ . . .		3.88 s	3.81 s	
OH (C-2′) . .	8.54 br s		13.49 s	8.73 s
OH (C-3″) . .			1.56 br s	

[a]Each value represents 2 proton signals, except H-1″.

TABLE 2. ^{13}C-Nmr Data of Dihydrochalcones **1** and **3–5** (75.5 MHz, $CDCl_3$, δ ppm).

Carbon	Compound			
	1	**3**	**4**	**5**
C-1	141.6 (s)	141.5 (s)	141.2 (s)	141.7 (s)×2
C-2, 6	128.5 (d)×2	128.5 (d)×2	128.4 (d)×2	128.5 (d)×4
C-4	126.0 (d)	126.0 (d)	126.1 (d)	125.9 (d)×2
C-3, 5	128.4 (d)×2	128.4 (d)×2	128.3 (d)×2	128.4 (d)×4
C-α	45.6 (t)	45.8 (t)	44.0 (t)	45.9 (t)×2
C-β	30.5 (t)	30.5 (t)	30.1 (t)	30.6 (t)×2
C-β′	204.5 (s)	205.4 (s)	202.9 (s)	205.6 (s)×2
C-1′	104.8 (s)	104.6 (s)	105.1 (s)	104.4 (s)×2
C-2′	165.6 (s)	160.4 (s)	162.0[a] (s)	165.3[c] (s)×2
C-3′	94.5 (d)	92.1 (d)	93.4 (d)	106.4 (s)×2
C-4′	163.2 (s)	163.3 (s)	162.4[a] (s)	161.0[c] (s)×2
C-5′	94.5 (d)	109.7 (s)	101.9 (s)	92.6 (d)×2
C-6′	165.6 (s)	160.4 (s)	166.9[b] (s)	158.6[c] (s)×2
C-1″	—	32.6 (d)	39.8 (d)	15.6 (t)
C-2″	—	122.6 (d)	96.9 (d)	—
C-3″	—	135.5 (s)	73.6 (s)	—
C-4″	—	25.9 (q)	28.3 (q)	—
C-5″	—	18.2 (q)	22.4 (q)	—
C-6″	—	129.0 (s)	127.8 (s)	—
C-7″	—	158.7 (s)	158.1 (s)	—
C-8″	—	116.1 (d)	117.4 (d)	—
C-9″	—	129.4 (d)	130.5 (d)	—
C-10″	—	121.7 (s)	122.5 (s)	—
C-11″	—	131.4 (d)	129.6 (d)	—
$COOCH_3$	—	167.8 (s)	167.2[b] (s)	—
$COOCH_3$	—	52.0 (q)	51.8 (q)	—
OCH_3	55.5 (q)	55.2 (q)	55.8 (q)	56.5 (q)

[a–c]Interchangeable.

olefinic proton at δ 6.01, which in turn showed cross-peaks to the Me protons with resonances at δ 1.60 and at δ 1.76, thus establishing the connectivity network for a γ,γ-dimethylallyl moiety. Consideration of the molecular formula of all delineated partial structures ($C_{29}H_{29}O_6$) and the ^{13}C-nmr spectral data revealed the outstanding fragment to be a phenolic OH group (HO-C-7″).

The partial structures (Figure 1) 2′,6′-dihydroxy-4′-methoxydihydrochalcone (A), 1,3,4-trisubstituted aryl moiety (B), γ,γ-dimethylallyl (C), aryl Me ester (D), and phenolic OH (E) were linked together from the results of a HMBC experiment made with **3**. This 2D spectrum revealed the connectivities via carbon spin couplings with the protons two- and three-bonds distant. From the HMBC spectrum of **3** (Table 3), diagnostic long-range correlations were observed from H-1″ to C-4′, C-5′, C-6′, C-2″, C-3″, C-6″, C-7″, and C-11″. These data thus enabled the connectivities between fragments A, B, and C to be established. The substitution pattern of fragment B was also resolved from the results of the long-range correlation, with diagnostic correlations being from both H-9″ and H-11″ to the carbonyl group of the Me ester function ($COOCH_3$).

The results of a 2D NOESY experiment also supported the previous finding, with diagnostic nOe cross-peaks being from H-1″ to H-11″ and from the aryl Me ester function to H-9″. These findings were also consistent with the proton resonance for the C-4′ MeO group of **3** appearing at relatively higher field than that of **1**, due to the anisotropic effect of the 1,3,4-trisubstituted aryl moiety (partial structure B). Piperaduncin

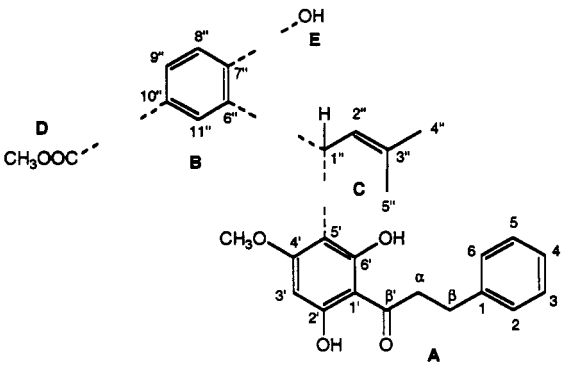


FIGURE 1. Partial Structures of **3**.

A (**3**) is methyl 3-{1-[2,4-dihydroxy-6-methoxy-3-(3-phenylpropanoyl)-phenyl]-3-methyl-2-butenyl}-4-hydroxybenzoate.

Piperaduncin B [**4**] was obtained as a yellow oil, of a molecular formula consistent with $C_{29}H_{30}O_8$, as determined by mass spectrometry. All of the spectral data of **4** indicated it to be closely related to **3**. In particular, the ^{1}H-nmr spectrum showed **4** to have a 5′-substituted 2′,6′-dihydroxy-4′-methoxydihydrochalcone, a 1,3,4-substituted benzene ring, and an aryl Me ester, as seen in **3**. The ^{1}H-nmr spectrum further disclosed the presence of a hydrogen-bonded OH group (δ 13.49), an aliphatic OH (δ 1.56, br s, exchangeable), two methine protons as an AB system at δ 4.72 and 4.47 ($J_{A,B}$=4.4 Hz), as well as two Me groups attached to an oxygen-bearing carbon at δ 1.40 (3H, s) and at δ 1.20 (3H, s) [-$C(CH_3)_2OH$]. In the HMQC nmr spectrum of **4** the two methine proton signals (δ 4.72 and 4.47) were seen to correlate with ^{13}C-nmr resonances at 39.8 ppm and 96.9 ppm, respectively. This confirmed that one of them (δ 4.47) was

TABLE 3. Long Range Correlations for **3**.[a]

Proton(s)	Long-range correlations
H_2-α	C-1, C-β
H_2-β	C-1, C-2, C-6, C-α
H-1″	C-4′, C-5′, C-6′, C-2″, C-3″, C-6″, C-7″, C-11″
H-2″	C-4″, C-5″
H_3-4″	C-2″, C-3″, C-5″
H_3-5″	C-2″, C-3″, C-4″
H-8″	C-7″, C-9″, C-10″
H-9″	C-7″, C-11″, $COOCH_3$
H-11″	C-1″, C-7″, C-9″, $COOCH_3$
OCH_3	C-4′
$COOCH_3$	$COOCH_3$

[a]Delays optimized for $J_{C,H}$=8.7 Hz.

associated with an oxygen-bearing carbon (δ 96.9), while the other (δ 4.72) was associated with a carbon (δ 39.8) adjacent to two aryl moieties, as for C-1″ in **3**.

In the ^{13}C-nmr spectrum (Table 2) of **4** the resonances for the $\Delta^{2'',3''}$ double bond (δ 122.6, d and δ 135.5, s) seen in **3** were absent. Instead were two resonances associated with carbons bearing oxygen (δ 73.6, s, δ 96.9, d). The chemical shift of C-2″ (δ 96.9) indicated it to be part of a 2,3-dihydrobenzo[b]furan moiety, as observed in (+)-anodendroic acid (11), while the quaternary carbon with a resonance at 73.6 ppm was indicative of the quaternary carbon in a -$C(Me)_2OH$ moiety. Based on the above spectroscopic evidence, together with the structural features of **3**, the basic framework for **4** was, thus, deduced. Unambiguous confirmation of the structure and stereochemistry was obtained from the results of a 2D NOESY experiment (see the structural formula of **3** for a summary of the diagnostic nOe interactions).

These experimental results were translated into a 3D structure utilizing SYBYL molecular modelling software as seen in Figure 2. For details see the Experimental section. The exploration of the torsion angle space for 7″-6″-1″-5′ resulted in two energy minima centered around 166° and 315°, respectively (Figure 3). As a result of the steric interactions between the C-7″ OH group and the hydrogen at C-2″ or the MeO at C-4′, both minima are separated by a rotation barrier, which cannot be overcome at room temperature. A restricted rotation about the C-1″, C-6″ carbon-carbon bond for a torsion angle region of approximately 80° for both the energy minima is likely. The experimentally derived nOe minimum conformation agrees perfectly with the population of conformations out of the second minimum, centered around the 315° torsion angle (Figure 2). Piperaduncin B [**4**] was deduced to be methyl 4-hydroxy-3-[2,3-dihydro-2-(1-hydroxy-1-methyl-ethyl)-6-hydroxy-4-methoxy-7-(3-phenylpropanoyl)-benzo[b]furan-3-yl] benzoate.

Piperaduncin C [**5**] appeared as virtually identical to compound **1**, spectroscopically (ir, ^{1}H-nmr, ^{13}C-nmr and eims). The different tlc behavior of **5** and **1**, as well as some inconsistencies in the nmr data of **5**, however, made a more detailed investigation necessary. The fabms of **5** displayed a $[M+H]^+$ ion at *m/z* 557, consistent with the molecular formula of $C_{33}H_{32}O_8$. Its ^{1}H-nmr spectrum consisted of signals for a 2′,6′-dihydroxy-4′-methoxydihydrochalcone, δ 7.32–7.20 (5H, m), 3.01 (2H, t), 3.43 (2H, br t), 6.13 (1H, s) and 4.00 (3H, s), and one additional resonance at δ 3.73 (s), as well as one exchangeable resonance at δ 8.73 (br s). The ^{13}C-nmr spectrum of **5** contained only 17 resonances, suggesting that a number of these represented more than one carbon. The results obtained from recording the HMQC spectrum of **5** indicated the proton signal

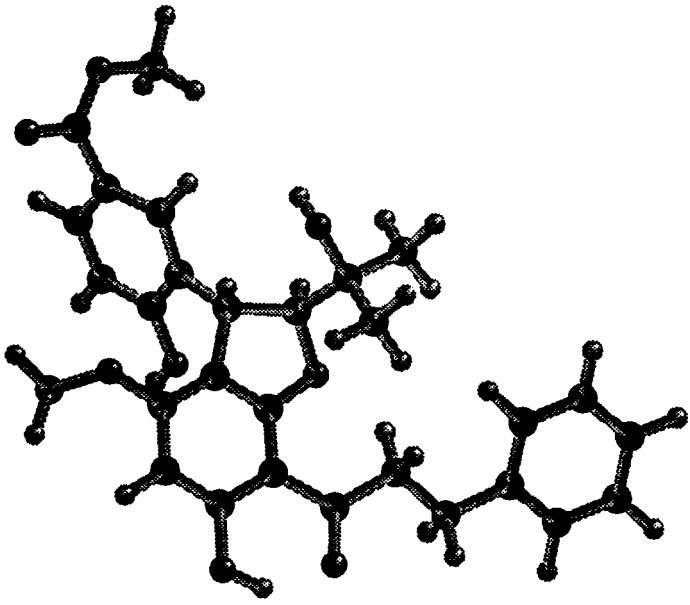


FIGURE 2. Ball-and-stick representation of **4** for one of the possible energy minimum conformations selected from the population of the energy minimum around 315° torsion angle.

at δ 3.73 (integrating as 1 proton) to correlate with the δ 15.6 (t) ^{13}C-nmr resonance. This result clearly indicated all ^{1}H-nmr and ^{13}C-nmr resonances, except that for C-1″, to be doubled. Based upon this deduction, it was evident that **5** was a symmetrical dimer of **1**, connected via the C-1″ methylene group, with the point of connection on the dihydrochalcones being at the equivalent C-5′ atoms, as shown in **5**. The above findings were confirmed from the results of a 2D-NOESY experiment performed with **5**, with key nOe observations being from both the equivalent H-3′ atoms to both the equivalent MeO groups at C-4′ and to both the equivalent OH groups at C-2′. Piperaduncin C [**5**] is *bis*-[α,β-dihydro-2′,6′-dihydroxy-4′-methoxy-chalcone-5′-yl]methane.

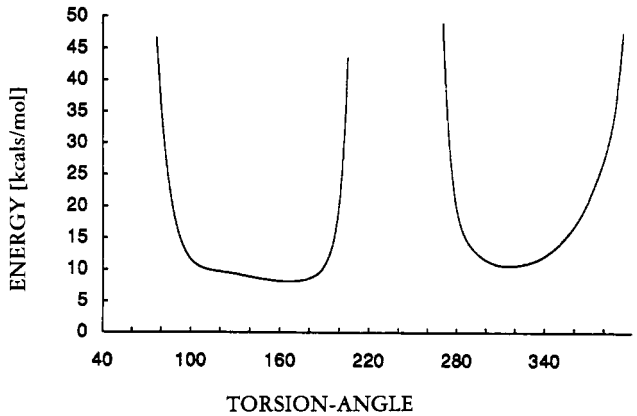


FIGURE 3. Energy plot of the SEARCH results. Two minima were found, the right one corresponds to the experimental derived geometry. Energy values obtained from force field calculations and are not absolute energy values.

The cytotoxic activity towards KB nasopharyngal carcinoma cells, antibacterial potential against three bacteria (*B. subtilis, M. luteus,* and *E. coli*), and molluscicidal effects of all isolates were evaluated (Table 4). Sakuranetin, **3**, and **4** were found to be moderately cytotoxic towards the KB cells, with the other isolates being inactive (ED_{50} >10 μg/ml). The previously reported cytotoxicity for sakuranetin, in the KB cell assay at an ED_{50} of 11 μg/ml (13), is consistent with the findings reported here. All isolates were antibacterial towards *B. subtilis* and *M. luteus*, with compounds **1**, **2**, and **4** being most potent. Sakuranetin was found to be antibacterial against *B. subtilis* and *Staphylococcus aureus*, consistent with previous findings (14), while **2** showed molluscicidal activity at a concentration of 12 ppm.

TABLE 4. Biological Activities of Compounds Isolated.[a]

Compound	Antimicrobial (mininum growth inhibition concentration in μg on TLC) activity			Cytotoxicity for KB cells (ED_{50} μg/ml)	Molluscicidal activity (LC_{100} ppm)
	E. coli	*M. luteus*	*B. subtilis*		
Sakuranetin	>5	2.0	0.5	10	>30
Anodendroic ... acid methyl ester	>5	>5	3.2	>10	—
1	>5	0.3	0.3	>10	>30
2	>5	0.6	0.1	>10	12
3	>5	1.6	1.6	2.3	—
4	>5	0.4	0.2	4.7	—
5	>5	3.0	1.5	>10	—
Chloramphenicol	0.05	0.05	0.02	—	—
Podophyllotoxin	—	—	—	0.0003	—

[a]For details see Experimental.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps (uncorrected) were determined on a Büchi SMP 20 mp apparatus. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. Ir spectra were obtained with a Perkin-Elmer 781 spectrometer. Uv spectra were recorded in MeOH using a Perkin-Elmer Lambda 3 spectrophotometer. Eims spectra were taken on a Hitachi-Perkin-Elmer-RMUGM mass spectrometer at 70 eV. Fabms were recorded on a ZAB 2-SEQ spectrometer in the positive mode using 3-NOBA as matrix. ^{1}H-Nmr, ^{13}C-nmr, HMQC, 2D NOESY and ^{1}H-^{1}H-COSY nmr spectra were measured employing a Bruker AMX-300 instrument operating at a basic frequency of 300 MHz, using the solvent as internal standard ($CDCl_3$ at δ 7.26 and δ 77.0). The HMBC experiments were conducted on a Bruker AMX-500 instrument operating at a basic frequency of 500 MHz. Si gel (Merck) for vacuum liquid chromatography (vlc) (column 6.5×20 cm, vacuum by H_2O aspiration) had a particle size of 40–63 μm. Medium pressure lc (mplc) separations were carried out using a Büchi 681 pump, a LKB Ultrorac fraction collector, and a Büchi mplc column (46 cm×1.5 cm i.d.) including a precolumn. The column was dry packed with tlc Si gel HF 254, particle size 15 μM (Merck). The mobile phase used was optimized using over-pressure layer chromatography (oplc) and the PRISMA model (15). Hplc separations were performed with a Waters 590 pump and Perkin Elmer LC 55 spectrophotometer for detection (column Spherisorb S5 ODS II, 5 μm, 250×16 mm, Knauer).

MOLECULAR MODELLING.—Structures were built in SYBYL (Tripolis Associates, Inc., St. Louis, MO.) from a fragment library and subsequently energy minimized using the TRIPOS force field (16). Exploration of the torsion angle space was performed using the SEARCH module (17). The increment of the torsion angle was set to one degree with the Van der Waals radii scaling factor being 0.8 and the charges have been calculated by the Gasteiger-Huckel method within SYBYL. The resulting number of conformations was 190. All calculations and structure display were done on an ESV 10 workstation.

PLANT MATERIAL.—Leaves of *P. aduncum* were collected near Gawam village, Morobe Province of PNG, during September 1988 (18). Herbarium specimens are deposited at the Herbarium (ZT, 11819) ETH, Zürich, Switzerland, as well as at UPNG Herbarium, Port Moresby, PNG, and at National Herbarium in Lae, PNG.

ANTIMICROBIAL ASSAYS.—All chromatographic fractions were monitored for bioactivity by bioautographic assays (19) using a modified procedure. Test organisms were *B. subtilis* (ATCC 6633), *M. luteus* (ATCC 9341), and *E. coli* (ATCC 25922). Bacterial suspensions were obtained from overnight cultures in BBL nutrient broth (Becton & Dickinson Co. 11479) cultivated at 37°. After complete removal of the solvent, a developed plate of the fractions was dipped in a Desaga staining tube, containing a suspension of test bacteria. Plates inoculated with *E. coli* were kept in a moist atmosphere at 37° for 6 h, and plates inoculated with *B. subtilis* and *M. luteus* for 20 h. After incubation plates were sprayed with 0.5% iodonitrotetrazolium chloride in H_2O. Plates inoculated with *E. coli* were kept in a moist atmosphere at room temperature for 15 h, and plates inoculated with *B. subtilis* and *M. luteus* were kept in a moist atmosphere at 37° for 4 h to reveal living bacteria as pink colonies. Antibacterial compounds were detected as white zones of inhibition. Chloramphenicol dissolved in CH_2Cl_2 was used as a positive control and all pure compounds were tested within the range of 0.1–5 μg dissolved in CH_2Cl_2 and applied as a spot (3 mm) with a 5-μl micro pipette (Camag). The extracts and fractions were tested at 100 μg.

CYTOTOXICITY TESTING.—Cytotoxic potential was assessed using cultured KB (human nasopharyngal carcinoma) cells essentially by the method of Swanson *et al.* (20). The KB cells were maintained in Basal Medium Eagle (BME) containing 10% heat-inactivated fetal bovine serum. The cells were cultured at 37° in a humidified atmosphere of 5% CO_2 in air. Samples dissolved in DMSO were added to the cultured cells (at log growth-phase, final DMSO concentration 0.5%). Five concentrations of test materials were employed. The cells were incubated under the same conditions for 72 h. The quantity of the cells in each tube was evaluated by protein determination and expressed as a percentage relative to controls treated only with solvent (DMSO), after correcting for the cell number at the start of the experiment. All assays were performed in duplicate, and the dose that inhibited cell growth by 50% (ED_{50}) was calculated using semilogarithmic plots from the average data.

MOLLUSCICIDAL ASSAYS.—The screening for molluscicidal potential was carried out as previously described by Hostettmann and co-workers (21). The test organism was *Biomphalaria glabrata*. The samples were dissolved in 100 μl of DMSO and then diluted to 100 ml with distilled H_2O. The snails were observed after 24 h and considered dead when no heart beat could be detected on microscopic investigation.

EXTRACTION AND ISOLATION.—Air-dried and powdered leaves (1.55 kg) were successively percolated with petroleum ether, CH_2Cl_2, EtOAc, and MeOH at room temperature. The dried CH_2Cl_2 extract (74 g) was found to exhibit antimicrobial activity and activity toward KB cells (ED_{50}=12 μg/ml). The isolation process was guided throughout by the results of the KB cytotoxicity and bioautographic tlc assays.

A portion of the bioactive CH_2Cl_2 extract (15 g) was loaded onto a vacuum column and eluted with hexane containing increasing portions of EtOAc to yield 20 fractions each of 150 ml. The fractions were combined, based on tlc similarities, to yield eight combined fractions, which were then assayed for antimicrobial and cytotoxic activities. Fractions 4 to 8 showed antibacterial activity and fraction 7 was also cytotoxic (ED_{50} <20 μg/ml).

Vlc fraction 7 (930 mg) was further fractionated by mplc and eluted with CH_2Cl_2-Me_2CO-EtOH-hexane (12:4:2:82) to yield 200 fractions (15 ml). The fractions were combined into eight pools based on tlc and bioactivity. Mplc pool 3 (62 mg, ED_{50} <20 μg/ml) was further purified by hplc [solvent system MeOH-H_2O (70:30)] to furnish **3** (14.3 mg, ED_{50}=2.3 μg/ml). Mplc pool 5 (70 mg, ED_{50} <20 μg/ml) was further purified by hplc [solvent system MeOH-H_2O (75:25)] to furnish **4** (5.2 mg, ED_{50}=4.7 μg/ml). Mplc pool 4 (53 mg, ED_{50} >20 μg/ml) was dissolved in MeOH, giving a pale yellow precipitate, compound **5** (5.1 mg). Mplc pool 6 (130 mg, ED_{50} ≈20 μg/ml), consisting of one major compound, was dissolved in $CHCl_3$, giving **2** as a yellowish precipitate (102.1 mg, ED_{50} >10 μg/ml), and mplc pool 7 (ED_{50} >20 μg/ml) turned out to be pure lutein (40.7 mg).

Vlc fraction 4 (1030 mg), possessing antibacterial activity, was further purified by mplc and eluted with CH_2Cl_2-Me_2CO-hexane (17:5:78) to yield 130 fractions (15 ml). The fractions were combined into 12 pools based on tlc and bioactivity. Mplc pool 5 (15 mg) was further purified by hplc [solvent system MeOH-H_2O (65:35)] to furnish (+)-anodendroic acid methyl ester (3.8 mg).

Vlc fraction 5 (2061 mg), possessing antibacterial activity, was dissolved in CH_2Cl_2 giving a yellowish precipitate, **1** (813.9 mg).

Vlc fraction 6 (510 mg), possessing antibacterial activity, was further purified by mplc and eluted with CH_2Cl_2-Me_2CO-EtOH-hexane (15:3:3:79) to yield 100 fractions (15 ml). The fractions were combined into 6 pools based on tlc and bioactivity. Mplc pool 3 (130 mg) was further purified by hplc [solvent system MeOH-H_2O (7:3)] to furnish sakuranetin (8.5 mg) and **1** (26.3 mg).

Piperaduncin A [**3**].—Isolated as pale yellow amorphous powder (14.3 mg, 0.005%): $[\alpha]^{20}D$ −3.1° (MeOH, *c*=0.64); uv (MeOH) λ max (log ε) 265 sh (3.96), 283 (4.04), 340 sh nm (3.19); (+$AlCl_3$) 311, 380; ir (KBr) ν max 3350 (OH), 1710 (C=O), 1685, 1620, 1590, 1420, 1290, 1215 cm^{-1}; fabms *m/z*:

$[M+H]^+$ 491 (30); eims *m/z* $[M]^+$ 490 (1), 489 (2), 473 (1), 272 (20), 218 (10), 203 (100), 167 (61), 159 (10), 144 (17), 140 (11), 115 (14), 91 (15); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

Piperaduncin B [**4**].—Obtained as oil (5.2 mg, 0.002%); $[\alpha]^{20}D$ −15° (MeOH, *c*=0.10); uv (MeOH) λ max (log ε) 264 sh (4.08), 282 (4.17), 335 sh nm (3.39); (+$AlCl_3$) 305, 380; ir (film) ν max 3420 (OH), 1710 (C=O), 1695, 1630, 1600, 1430, 1280, 1200 cm^{-1}; eims *m/z* $[M]^+$ 506 (30), 488 $[M-H_2O]^+$ (9), 473 (100), 448 (12), 343 (8), 316 (6), 311 (8), 167 (8), 149 (6), 105 (7), 91 (18); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

Piperaduncin C [**5**].—Isolated as yellowish amorphous powder (5.1 mg, 0.002%); uv (MeOH) λ max (log ε) 288 (4.25), 330 sh nm (3.27); (+$AlCl_3$) 310; ir (KBr) ν max 3350 (OH), 1620, 1590, 1410, 1290, 1140 cm^{-1}; fabms *m/z* $[M+H]^+$ 557 (12), 285 (100); eims *m/z* 272 (1), 167 (97), 153 (8), 140 (8), 124 (8), 111 (12), 104 (21), 95 (22), 91 (100), 79 (32), 77 (56), 91 (15); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

2′,6′-Dihydroxy-4′-methoxydihydrochalcone [**1**].—840.2 mg, 0.27%, spectroscopic and chemical data are identical with previously reported data (6). ^1H nmr, see Table 1; ^{13}C nmr, see Table 2.

2′,6′,4-Trihydroxy-4′-methoxydihydrochalcone [**2**].—102.1 mg, 0.039%, spectroscopic and chemical data are identical with previously reported data (7,8).

(±)-Sakuranetin.—8.5 mg, 0.003%, spectroscopic and chemical data agree with previously reported data (8,9).

Anodendroic acid methyl ester.—3.8 mg, 0.001%, $[\alpha]^{20}D$ +58.2° (*c*=0.38, MeOH); spectroscopic and chemical data are identical with previously reported data (11,12).

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