95. New Monoterpene-Substituted Dihydrochalcones from *Piper aduncum*

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Five new unusual monoterpene-substituted dihydrochalcones, the adunctins A–E ((1″*S*)-1-{2′-hydroxy-4′-methoxy-6′-[4″-methyl-1″-(1‴-methylethyl)cyclohex-3″-en-1″-yloxy]phenyl}-3-phenylpropan-1-one (**1**), (5a*R**, 8*R**,9a*R**)-3-phenyl-1-[5′a,8′,9′,9′a-tetrahydro-3′-hydroxy-1′-methoxy-8′-(1″-methylethyl)-5′a-methyldibenzo-[*b*,*d*]furan-4′-yl]propan-1-one (**2**), (2′*R**,4″*S**)-1-{6′-hydroxy-4′-methoxy-4″-(1‴-methylethyl)spiro[benzo[*b*]-furan-2′(3′*H*),1″-cyclohex-2″-en]-7′-yl}-3-phenylpropan-1-one (**3**), (2′*R**,4″*R**)-1-{6′-hydroxy-4′-methoxy-4″-(1‴-methylethyl)spiro[benzo[*b*]furan-2′(3′*H*),1″-cyclohex-2″-en]-7′-yl}-3-phenylpropan-1-one (**4**), and (5′a*R**,6′*S**, 9′*R**,9′a*S**)-1-[5′a,6′,7′,8′,9′a-hexahydro-3′,6′-dihydroxy-1′-methoxy-6′-methyl-9′-(1″-methylethyl)dibenzo[*b*,*d*]-furan-4′-yl]-3-phenylpropan-1-one (**5**)) were isolated from the leaves of *Piper aduncum* (Piperaceae) by preparative liquid chromatography. In addition, (–)-methyllindaretin (**6**), *trans*-phytol, and α-tocopherol (= vitamin E) were also isolated and identified. The structures were elucidated by spectroscopic methods, including 1D- and 2D-NMR spectroscopy as well as single-crystal X-ray diffraction analysis. The antibacterial and cytotoxic potentials of the isolates were also investigated.

1. Introduction. – As part of a research program aimed at the phytochemical investigation of plants employed in the traditional medicine of Papua New Guinea (PNG), we have investigated the leaves of *Piper aduncum* L. (Piperaceae). *P. aduncum*, a monoecious shrub or slender tree, is distributed throughout a large part of tropical America and was naturalized in many places in Malesia, including PNG [1]. *P. aduncum* is used by traditional healers in the Finschhafen area of PNG for the treatment of fresh wounds. Fresh leaves are crushed and applied directly to the wound [1]. For the treatment of diarrhoea, a decoction of the leaves is used in Peru [2]. The leaves are also used against dysentery and as haemostatic in Colombia [3].

The genus *Piper* has been continuously investigated since *Oestred* isolated piperine, the pungent principle of *P. nigrum* in 1819 [4]. Chemical and biological investigations of *P. aduncum* were also undertaken with species from Fiji [5], Panama [6], Peru [2], Colombia [3] [7] [8], and Jamaica [9]. The secondary metabolites found were predominantly monoterpenes and phenylpropene, benzoic-acid, and dihydrochalcone (= 1,3-diphenylpropan-1-one) derivatives.

The crude petroleum-ether extract of the leaves from *P. aduncum* showed, in *in-vitro* biological screening, significant antibacterial activity against *Bacillus subtilis*, *Micrococcus luteus*, and *Escherichia coli*. We report here on the isolation and characterization of minor dihydrochalcones from *P. aduncum*. Emphasis is placed on the structure elucida-

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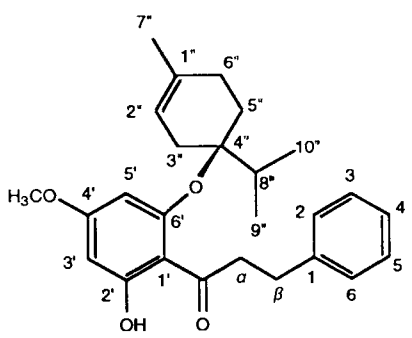
tion of the five new unusual monoterpene-substituted dihydrochalcones **1–5** which we call adunctins A–E.

2. Results and Discussion. – Fractionation of the light-petroleum-ether extract, obtained from the air-dried leaves of *P. aduncum*, by a combination of forced-flow column-chromatographic methods over silica gel and *RP-18* material, led to the isolation of compounds **1–6**, *trans*-phytol, and α-tocopherol (see *Exper. Part*).



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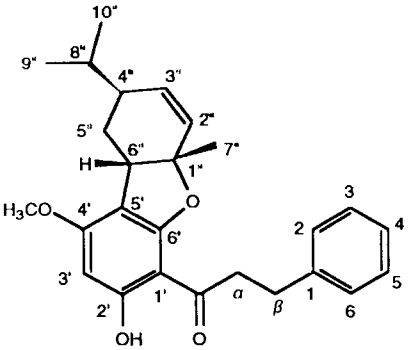

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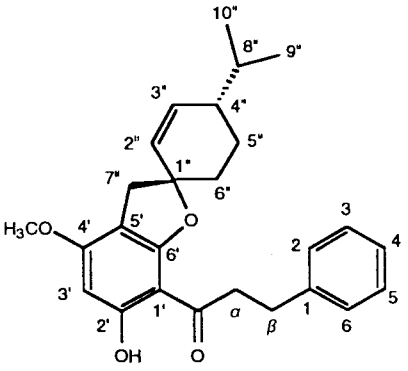

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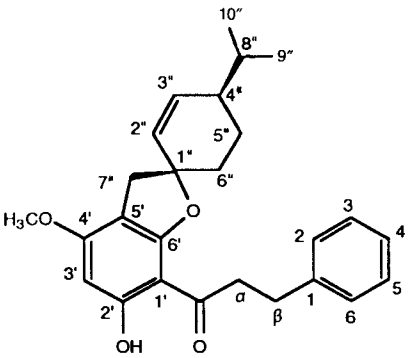

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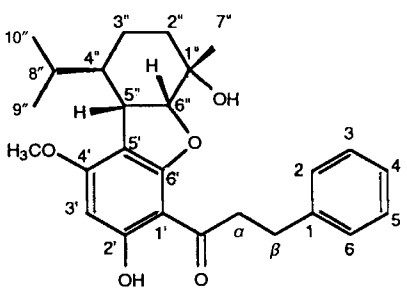

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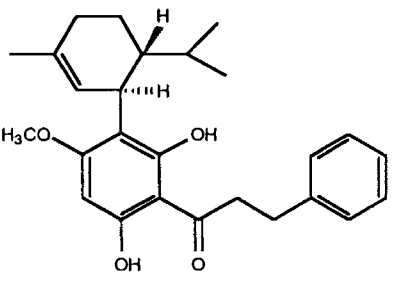

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[2]) Chalcone- and monocyclic-terpene numbering is given in the *Formulae* and used in *Tables* and discussions.

Table 1. *^{1}H-NMR Data* ($CDCl_3$, 300 MHz) *of Adunctins A–E* (**1–5**). Chemical shifts δ in ppm, J in Hz.

H-Atom	**1**[a])	**2**	**3**	**4**	**5**
Dihydrochalcone					
H–C(2) to H–C(6)	7.20 (br. *m*)	7.24 (br. *m*)	7.24 (br. *m*)	7.24 (br. *m*)	7.24 (br. *m*)
H_a–C(α)	3.46 (*m*)	3.28 (*t*, *J* = 7.8	3.32 (*m*)	3.28 (*t*, *J* = 6.9)	3.38 (*m*)
H_b–C(α)	3.35 (*m*)				
CH_2(β)	3.10 (*t*, *J* = 7.9)	2.98 (*t*, *J* = 7.8)	3.00 (*m*)	2.99 (*t*, *J* = 7.8)	3.02 (*t*, *J* = 7.6)
H–C(3′)	6.20 (*d*, *J* = 2.0)	5.99 (*s*)	6.00 (*s*)	5.99 (*s*)	6.04 (*s*)
H–C(5′)	6.28 (*d*, *J* = 2.0)	–	–	–	–
OH	14.99 (*s*)	13.37 (*s*)	13.38 (*s*)	13.40 (*s*)	13.23 (*s*)
MeO	3.20 (*s*)	3.82 (*s*)	3.83 (*s*)	3.82 (*s*)	3.81 (*s*)
Monoterpene					
H_a–C(2″)	4.86 (br. *s*)	5.61 (*dd*, *J* = 10.2, 2.1)	5.79 (*d*, *J* = 9.9)	5.85 (br. *d*, *J* = 10.3)	2.09 (br. *m*)
H_b–C(2″)	–	–	–	–	1.59 (br. *m*)
H_a–C(3″)	1.93 (br. *d*, *J* = 16.0)	5.87 (*dd*, *J* = 10.2, 2.1)	5.86 (*d*, *J* = 9.9)	5.76 (br. *d*, *J* = 10.3)	1.33 (*m*)
H_b–C(3″)	1.82 (*m*)	–	–	–	
H–C(4″)	–	1.89 (br. *m*)	1.96 (*m*)	2.07 (*m*)	1.08 (*m*)
H_a–C(5″)	2.12 (*dd*, *J* = 12.0, 4.7)	2.27 (*dt*, *J* = 13.2, 4.8)	1.62 (br. *m*)	1.85 (*m*)	3.07 (*dd*, *J* = 11.0, 5.4)
H_b–C(5″)	1.40 (*m*)	1.60 (br. *m*)		1.47 (*m*)	–
H_a–C(6″)	1.82 (*m*)	3.41 (*t*, *J* = 4.8)	2.20 (*m*)	1.96 (*m*)	4.50 (*d*, *J* = 5.4)
H_b–C(6″)	1.54 (*m*)	–	1.62 (br. *m*)		–
Me(7″)[b])	1.48 (br. *s*)	1.58 (*s*)	2.95, 2.87 (*d*, *J* = 15.3)	2.93 (*s*)	1.43 (*s*)
H–C(8″)	2.57 (*sept.*, *J* = 7.0)	1.60 (br. *m*)	1.62 (br. *m*)	1.66 (*m*)	1.83 (*m*)
Me–C(9″)	0.73 (*d*, *J* = 7.0)	0.91 (*d*, *J* = 6.8)	0.87 (*d*, *J* = 7.0)	0.93 (*d*, *J* = 7.1)	0.85 (*d*, *J* = 6.8)
Me–C(10″)	0.57 (*d*, *J* = 7.0)	0.89 (*d*, *J* = 6.8)	0.80 (*d*, *J* = 7.0)	0.90 (*d*, *J* = 7.1)	0.83 (*d*, *J* = 6.8)

[a]) Recorded in C_6D_6.
[b]) CH_2(7″) for **3** and **4**.

Adunctin A (**1**) was obtained as a colourless oil with molecular weight of 408 (FAB-MS: 431 ($[M + Na]^+$)), consistent with the molecular formula $C_{26}H_{32}O_4$. Extensive spectral analyses of **1** established the structure to be (1″*S*)-1-{2′-hydroxy-4′-methoxy-6′-[4″-methyl-1″-(1‴-methylethyl)cyclohex-3″-en-1″-yloxy]phenyl}-3-phenylpropan-1-one, a new cyclized-monoterpene-substituted dihydrochalcone. The configuration at C(4″)[2]) was established as shown on the basis of the positive optical rotation (+17°) of adunctin A (**1**), which suggests that it is related to (+)-(*S*)-terpinen-4-ol [10][2]).

The IR spectrum of **1** showed absorptions due to conjugated C=O (1620 cm^{-1}) and aryl (1590 cm^{-1}) moieties. The absorption for the C=O moiety appeared at a low wave number, due to the formation of an intermolecular H-bond. The UV spectrum exhibited maxima at 226, 288, and 325 nm and upon addition of $AlCl_3$, the 288-nm absorption underwent a bathochromic shift of 24 nm, indicating the presence of a chelated phenolic OH group. In the ^{1}H-NMR spectrum of **1** (*Table 1*), signals characteristic of a 2′,6′-dihydroxy-4′-methoxydihydrochalcone derivative were observed at δ 7.20 (br. *m*, 5 H), 3.46 (*m*, 2 H), 3.35 (*m*, 1 H), 3.10 (*t*, 2 H), 6.28 (*d*, 1 H), 6.20 (*d*, 1 H), and 3.20 (*s*, 3 H) [9]. The ^{13}C-NMR spectrum of **1** (*Table 2*) also contained resonances consistent with the presence of a substituted dihydrochalcone, besides additional ten resonances at δ 134.3 and 87.3 (2*s*), 119.0 and 32.8 (2*d*), 32.1, 29.6, and 28.0 (3*t*), and 23.7, 18.7, and 17.2 (3*q*). The latter, together with the molecular formula, suggested **1** to have a monocyclic-monoterpene substituent. Using DQF-COSY and HMQC (J = 136 Hz) spectra of **1** the structure of this monoterpene unit could be established. Thus, ^{1}H-NMR signals characteristic of an isolated i-Pr moiety (δ 2.57, 0.73, 0.57 ($Me_2CH(8'')$)) and an allylic Me group (δ 1.48 (Me(7″))) with long-range coupling

Table 2. *^{13}C-NMR Data* ($CDCl_3$, 75.5 MHz) *of Adunctins A–E* (**1–5**). Chemical shifts δ in ppm.

C-Atom	**1**[a])	**2**	**3**	**4**	**5**
Dihydrochalcone					
C(1)	143.3 (*s*)	141.6 (*s*)	141.4 (*s*)	141.5 (*s*)	141.2 (*s*)
C(2), C(6)	129.2 (*d*)	128.4 (*d*)	128.3 (*d*)	128.3 (*d*)	128.3 (*d*)
C(4)	126.6 (*d*)	125.9 (*d*)	125.8 (*d*)	125.9 (*d*)	126.0 (*d*)
C(3), C(5)	129.6 (*d*)	128.4 (*d*)	128.3 (*d*)	128.5 (*d*)	128.4 (*d*)
C(α)	46.8 (*t*)	44.3 (*t*)	44.1 (*t*)	44.3 (*t*)	43.7 (*t*)
C(β)	31.5 (*t*)	30.9 (*t*)	30.4 (*t*)	31.0 (*t*)	30.1 (*t*)
C=O	205.7 (*s*)	203.7 (*s*)	203.4 (*s*)	203.6 (*s*)	203.2 (*s*)
C(1′)	108.2 (*s*)	102.4 (*s*)	102.0 (*s*)	102.0 (*s*)	102.7 (*s*)
C(2′)	169.9 (*s*)	165.9 (*s*)	166.0 (*s*)	166.2 (*s*)	165.3 (*s*)
C(3′)	94.2 (*d*)	92.1 (*d*)	91.8 (*d*)	91.9 (*d*)	92.8 (*d*)
C(4′)	166.2 (*s*)	162.7 (*s*)	161.8 (*s*)	161.8 (*s*)	161.8 (*s*)
C(5′)	96.2 (*d*)	107.8 (*s*)	104.4 (*s*)	104.4 (*s*)	112.8 (*s*)
C(6′)	160.6 (*s*)	161.6 (*s*)	161.1 (*s*)	161.3 (*s*)	161.6 (*s*)
MeO	55.7 (*q*)	55.5 (*q*)	55.5 (*q*)	55.6 (*q*)	55.5 (*q*)
Monoterpene					
C(1″)	134.3 (*s*)	89.0 (*s*)	88.1 (*s*)	90.3 (*s*)	80.9 (*s*)
C(2″)	119.0 (*d*)	129.2 (*d*)	129.2 (*d*)	130.6 (*d*)	31.8 (*t*)
C(3″)	32.1 (*t*)	135.3 (*d*)	136.0 (*d*)	133.2 (*d*)	17.1 (*t*)
C(4″)	87.3 (*s*)	37.9 (*d*)	41.7 (*d*)	41.3 (*d*)	46.3 (*d*)
C(5″)	29.6 (*t*)	26.0 (*t*)	22.0 (*t*)	22.8 (*t*)	39.8 (*d*)
C(6″)	28.0 (*t*)	44.1 (*d*)	35.5 (*t*)	34.8 (*t*)	87.6 (*d*)
C(7″)	23.7 (*q*)	26.4 (*q*)	38.5 (*t*)	38.5 (*t*)	22.0 (*q*)
C(8″)	32.8 (*d*)	31.3 (*d*)	31.7 (*d*)	31.7 (*d*)	27.1 (*d*)
C(9″)	17.2 (*q*)	19.8 (*q*)	19.0 (*q*)	19.2 (*q*)	15.4 (*q*)
C(10″)	18.7 (*q*)	19.6 (*q*)	19.5 (*q*)	19.6 (*q*)	21.7 (*q*)

[a]) Recorded in C_6D_6.

to H–C(2″) (δ 4.86), confirming its location at C(1″)=C(2″), were detected. H–C(2″) showed further couplings to H_a–C(3″) (δ 1.93) and H_b–C(3″) (δ 1.82). Further, two intercoupled CH_2 groups (δ 2.12, 1.40, 1.82, 1.54 (CH_2(5″), CH_2(6″))) were also present. These data, together with the *s* at δ(^{13}C) 87.3 for a quaternary O-substituted C-atom, were consistent with a terpinen-4-yloxy substituent. This deduction was supported by the FAB-MS of **1**, which contained significant fragment ions at *m/z* 273 ($[M + H - C_{10}H_{16}]^+$) and 137 ($[C_{10}H_{17}]^+$), indicating the presence of a *p*-menthenyl fragment. Further confirmation of the proposed structure resulted from a 2D-NOESY experiment with **1**. The following key NOE's were observed: H–C(3′)/MeO–C(4′) and OH–C(2′), Me(7″)/H_a–C(6″), and H–C(5′)/H–C(8″) and H_a–C(5″).

Adunctin B (**2**) was obtained as white needles with molecular formula of $C_{26}H_{30}O_4$, as determined by mass spectrometry. Its structure was deduced similarly to that of **1**. Adunctin B is (5a*R**,8*R**,9a*R**)-3-phenyl-1-[5′a,8′,9′,9′a-tetrahydro-3′-hydroxy-1′-methoxy-8′-(1″-methylethyl)-5′a-methyldibenzo[*b*,*d*]furan-4′-yl]propan-1-one. Final confirmation of the proposed structure and its relative configuration were deduced from a single-crystal X-ray analysis[3]).

All spectral data of **2** pointed to a structure closely related to that of **1**, with an additional ring present, however. In particular, the ^{1}H- and ^{13}C-NMR data were consistent with a 2′,6′-dihydroxy-4′-methoxydihydrochalcone derivative, with the *H*–C(5′) signal missing and the C(5′) signal being a *s* at δ 107.8, suggesting substitution at this position. The ^{13}C-NMR spectrum of **2** contained an additional ten resonances different to those found for **1** (see *Table 2*), indicating the presence of a different cyclic monoterpene substituent. The structure of the latter was established from the combined results of DQF-COSY and HMQC (J = 136 Hz) experiments. Thus, correlation cross-peaks were observed for H–C(2″) (δ 5.61)/H–C(3″) (δ 5.87) and H–C(4″) (δ 1.89), for H–C(3″)/H–C(4″), for H–C(4″)/H_a–C(5″) (δ 2.27) and H–C(8″) (δ 1.60), for H_a–C(5″)/H–C(6″) (δ 3.41), and for H–C(8″)/Me(9″) (δ 0.91) and Me(10″) (δ 0.89). Two connectivities between the monoterpene and dihydrochalcone moieties of **2** were suggested by its molecular formula, one being similar to that of **1** (*cf.* δ(C(1″)) 89.0 for **2** with δ (C(4″) 87.3 for **1**). The chemical shifts of C(6″) (δ 44.1) and *H*–C(6″) (δ 3.41) of **2** were indicative of a CH group adjacent to an aryl moiety, as seen in methyllindaretin [11]. Thus, C(6″) was directly connected to C(5′) of the dihydrochalcone moiety. 2D-NOESY Experiments confirmed the structure of **2**. Diagnostic NOE cross-peaks were observed for Me(10″)/H–C(3″) and H–C(4″) and for Me(7″)/H–C(2″) and H–C(6″), thus confirming the location of the i-Pr and the Me(7″) group, besides NOE's for H–C(3′)/MeO–C(4′) and OH–C(2′), confirming the substitution pattern of the dihydrochalcone moiety. The significant cross-peak observed for Me(7″)/H–C(6″) also established the relative configuration at C(1″) and C(6″), Me(7″) and H–C(6″) being *cis* to each other.

Adunctin C (**3**) was obtained as yellow prisms. The ^{13}C-NMR and DEPT spectra indicated the presence of 26 C-atoms. Their multiplicities, together with the molecular weight of 406, established the molecular formula $C_{26}H_{30}O_4$. All spectral data of **3** indicated it to be also a 5′-monoterpene-substituted 2′,6′-dihydroxy-4′-methoxydihydrochalcone derivative. Extensive NMR experiments, including HMBC and 2D-NOESY, allowed the identification of **3** as (2′*R**,4″*S**)-1-{6′-hydroxy-4′-methoxy-4″-(1‴-methylethyl)spiro-[benzo[*b*]furan-2′(3′*H*),1″-cyclohex-2″-en]-7′-yl}-3-phenylpropan-1-one. To establish the relative configuration of **3**, the crystalline material was subjected to single-crystal X-ray analysis; its preliminary data[3]) confirmed the overall connectivity deduced by NMR analysis and established the relative configuration at C(1″) and C(4″).

The ^{1}H- and ^{13}C-NMR data showed **3** to be a 2′,6′-dihydroxy-4′-methoxydihydrochalcone derivative, substituted at C(5′) with an unsaturated cyclic monoterpene moiety (*Tables 1* and *2*). The structure of the monoterpene unit was derived from the combined results of DQF-COSY, HMQC (J = 136 Hz), and HMBC (J = 10 Hz) experiments. Due to the overlapping ^{1}H-NMR resonances (CH_2(5″), H_b–C(6″), and H–C(8″) at δ 1.62), a complete connectivity network could not be established from the DQF-COSY experiment alone. However, the latter revealed connectivities for H–C(2″) (δ 5.79)/H–C(3″) (δ 5.86) and for H–C(3″)/H–C(4″) (δ 1.96).

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[3]) Refined X-ray crystallographic data will be published elsewhere.

Table 3. *HMBC* (^{1}H, 500 MHz, $CDCl_3$) *for Adunctin C* (**3**)[a])

Proton(s)	Long-range correlations	Proton(s)	Long-range correlations
H–C(2) to H–C(6)	C(1) to C(6), C(β)	H–C(3″)	C(1″), C(4″), C(5″), C(8″)
$CH_2(\alpha)$	C(β), C=O, C(1)	CH_2(6″)	C(4″)
$CH_2(\beta)$	C(α), C=O, C(1)	Me(9″)	C(4″), C(8″), C(10″)
H–C(3′)	C(1′), C(2′), C(4′), C(5′)	Me(10″)	C(4″), C(8″), C(9″)
CH_2(7″)	C(4′), C(5′), C(6′), C(1″), C(2″), C(6″)	MeO	C(4′)
H–C(2″)	C(1″), C(4″), C(6″)	OH	C(1′), C(2′), C(3′)

[a]) Delays optimized for J(C,H) = 10 Hz.

Further, an isolated CH_2 group (CH_2(7″), δ 2.95 and 2.87) and an i-Pr group (Me(9″) and Me(10″), δ 0.87 and 0.80) both showed a cross-peak to the overlapping resonance at δ 1.62 (H–C(8″)). The HMBC spectrum of **3** allowed to establish the connectivity of the monoterpene and dihydrochalcone moieties and also the overall structure (*Table 3*). Cross-peaks diagnostic for the monoterpene moiety were observed for *H*–C(3″)/C(1″), C(4″), C(5″), and C(8″), for C*H*$_3$(9″)/C(4″), C(8″), and C(10″), and for C*H*$_2$(7″)/C(4′), C(5′), C(6′), C(1″), C(2″), and C(6″). The long-range correlations from C*H*$_3$O–C(4′) to C(4′) and from the chelated O*H*$_2$–C(2′) to C(1′), C(2′), and C(3′) confirmed the structure of the 2′,6′-dihydroxy-4′-methoxydihydrochalcone moiety.

Adunctin D (**4**) was obtained as a yellow amorphous powder, with the same molecular formula ($C_{26}H_{30}O_4$) as adunctin C (**3**). Extensive spectral studies confirmed **4** to be (2′*R**,4″*R**)-1-{6′-hydroxy-4′-methoxy-4″-(1‴-methylethyl)spiro[benzo[*b*]furan-2′-(3′*H*),1″-cyclohex-2″-en]-7′-yl}-3-phenylpropan-1-one, a diastereoisomer of **3**.

As **2** and **3**, **4** had a 2′,6′-dihydroxy-4′-methoxydihydrochalcone skeleton substituted at C(5′) by a cyclic monoterpene moiety. The constitution of the latter was the same as in **3**, as deduced from DQF-COSY and HMQC (J = 136 Hz) experiments in the same manner as for **2** (no overlapping resonances in the ^{1}H-NMR). The different optical rotation as well as the significant difference in the ^{1}H- and ^{13}C-NMR resonances for the monoterpene moiety (*Tables 1* and *2*), however, suggested **3** and **4** to be diastereoisomer. This was confirmed by a 2D-NOESY experiment which revealed diagnostic NOE interactions (see *Exper. Part*) and established the relative configuration at C(1″) and C(4″), the i-Pr and CH_2(7″) group being *cis* to each other.

Adunctin E (**5**) was obtained as a white amorphous powder of the molecular formula $C_{26}H_{32}O_5$. Spectral analysis identified **5** as (5′a*R**,6′*S**,9′*R**,9′a*S**)-1-[5′a,6′,7′,8′,9′,9′a-hexahydro-3′,6′-dihydroxy-1′-methoxy-6′-methyl-9′-(1″-methylethyl)dibenzo[*b*,*d*]furan-4′-yl]-3-phenylpropan-1-one, the monoterpene substituent being a fully saturated tertiary alcohol.

The nature of the monoterpene substituent was established by DQF-COSY and HMQC (J = 136 Hz) experiments. Thus, in the DQF-COSY spectrum, correlation cross-peaks were seen for H–C(6″) (δ 4.50)/H–C(5″) (δ 3.07), for H–C(5″)/H–C(4″) (δ 1.08), for H–C(4″)/CH_2(3″) (δ 1.33) and H–C(8″) (δ 1.83), for CH_2(3″)/H_a–C(2″) (δ 2.09) and H_b–C(2″) (δ 1.59), and for H–C(8″)/Me(9″) (δ 0.85) and Me(10″) (δ 0.83). In the HMQC spectrum of **5** the chemical shifts of *H*–C(5″) (δ 3.07) and C(5″) (δ 39.8) were typical for a CH group adjacent to an aryl moiety (*cf.* CH(6″) of **2**), thus C(5″) was directly connected to C(5′) of the dihydrochalcone moiety. *H*–C(6″) (δ 4.50) was associated to a C-atom (C(6″), δ 87.6) bearing an O-atom and being part of a relatively strained system. Confirmation of the structure and of the relative configuration of **5** was obtained from the 2D-NOESY experiment (see *Exper. Part* for diagnostic NOE interactions). From the coupling constant J(4″,5″) = 11 Hz, the relationship between H–C(4″) and H–C(5″) was deduced to be *trans* axial.

(–)-Methyllindaretin (**6**) was identified by comparison with published physical and spectroscopic data of a previously reported compound from *Lindera umbellata* L. (Lauraceae) [11]. The isolate from *Piper aduncum* was identical with the latter, save for the sign of optical rotation, indicating **6** to be the optical antipode and hence (–)-methyllindaretin.

For all isolates, the cytotoxic activity towards KB nasopharyngal carcinoma cells and antibacterial potential against the bacteria *Bacillus subtilis*, *Micrococcus luteus*, and *Escherichia coli* were evaluated. (−)-Methyllindaretin (**6**) was found to be cytotoxic towards KB nasopharyngal carcinoma cells with an ED_{50} of 6.1 μg/ml, while all other isolates were inactive at 10 μg/ml. Adunctins B, C, and D (**2**–**4**) and (−)-methyllindaretin (**6**) showed antibacterial effects towards *M. luteus* at concentrations of 3.5, 2.4, 2.9, and 2.5 μg, respectively, in a bioautographic assay, all other isolates exhibited no activity at 5 μg. Further, no significant activity (< 5 μg) could be detected for any of the isolates towards *B. subtilis* and *E. coli*.

This is the first report of dihydrochalcones substituted by a cyclized monoterpene in the family of Piperaceae. Linear geranyl side chains are known in several genera [12]. Unsaturated *p*-methane derivatives of dihydrochalcone (obtained by formal cyclization of geranyl derivatives) were only reported from *Lindera umbellata* L. (Lauraceae) [11], *i.e.* (+)-methyllindaretin and (+)-lindaretin.

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Experimental Part

General. TLC: silica gel *60* F_{254} precoated Al sheets (*Merck*). Vacuum liquid chromatography (VLC): *RP-18* 40–63 μm (*Baker*), silica gel 40–63 μm (*Merck*), column 20 × 6.5 cm (i.d.). Medium-pressure liquid chromatography (MPLC): *Büchi 681* system (*Büchi*); column *A* (80 cm × 3.6 cm (i.d.)) and column *B* (45 cm × 2.6 cm (i.d.)) dry packed with TLC silica gel HF_{254} 15 μm (*Merck*), mobile phase optimized employing over pressure layer chromatography (OPLC) and the PRISMA model [13]. HPLC: *Waters-590* pump, *Perkin-Elmer-LC-55* spectrophotometer; column *Spherisorb S5 ODS II* (5 μm, 250 × 15 mm, *Knauer*). $[\alpha]_D$: *Perkin-Elmer-141* polarimeter. UV Spectra (λ_{max}): *Perkin-Elmer-Lambda-3* spectrophotometer; *p.a.* grade MeOH (*Romil*). IR Spectra (cm^{-1}): *Perkin-Elmer-781* spectrometer; KBr pellets. ^1H- and ^{13}C-NMR Spectra (δ [ppm], *J* [Hz]): at 300 (^1H) and at 75.5 MHz (^{13}C) using *Bruker AMX-300*, and at 500 (^1H) and at 125 MHz (^{13}C) using *Bruker AMX-500* for HMBC experiments [14]. EI-MS: *Hitachi-Perkin-Elmer-RMUGM* mass spectrometer, 70 eV. FAB-MS (positive-ion mode): *ZAB-2-SEQ* spectrometer in 3-nitrobenzyl alcohol (3-NOBA).

Plant Material. Leaves of *Piper aduncum* were collected near Gawam village, Morobe Province of PNG, during September, 1988 [15]. Herbarium specimens are deposited at the Herbarium ZT, ETH, Zürich, Switzerland, at the UPNG Herbarium, Port Moresby, PNG, and at the National Herbarium, Lae, PNG.

Antimicrobial Assay. All chromatographic fractions were monitored for bioactivity using bioautographic assays as described previously [16]. Test organisms were *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), and *Escherichia coli* (ATCC 25922).

Cycotoxicity Testing. Cytotoxic potential was assessed using cultured KB (human nasopharyngal carcinoma) cells by the method of *Swanson et al.* [17].

Extraction and Isolation. Air-dried and powdered plant material (1.55 kg) was percolated with 9 l of light petroleum ether at r.t. The resulting extract was evaporated: 91.0 g of an oily residue. The extract was divided into five parts, and each part was subjected to VLC (*RP18*, step gradient MeOH/H_2O 6:4, 8:2, and 10:0) to give three fractions *A*, *B*, and *C*. Fraction *C* was subjected to VLC (silica gel, hexane/AcOEt gradient 1:0→1:1). Eluted material was collected in twenty 100-ml fractions and monitored by TLC, identical fractions were combined to give six fractions *C1*–*C6*. Fraction *C2* was further fractionated by MPLC (column *A*, EtOH/CH_2Cl_2/*t*-BuOMe/hexane 0.3:0.3:0.4:99). Collected fractions were combined (TLC analysis) to give seven fractions *C2.1*–*C2.7*. Fraction *C2.4* was further purified by HPLC (*RP-18*, MeOH/H_2O 4.1): **1** (21.7 mg, 0.0015%) and **2** (4.8 mg, 0.0003%). Fraction *C2.5* was purified by MPLC (column *B*, toluene/AcOEt/hexane 9:1:10) to give four fractions *C2.5.1*–

C2.5.4. Fraction *C2.5.2* was further purified by HPLC (*RP18*, MeOH/H_2O 4:1): **3** (18.3 mg, 0.0012%) and **4** (5.9 mg, 0.0004%). Fraction *C2.7* was further purified by HPLC (*RP18*, MeOH/H_2O 9:1): **6** (25.3 mg) and α-tocopherol (35.0 mg, 0.0023%). Fraction *C3* was subjected to VLC (silica gel, toluene/AcOEt 97:3) to give 5 fractions *C3.1–C3.5*. Fraction *C3.1* was further purified by HPLC (*RP18*, MeOH/H_2O 4:1): **6** (91.0 mg, 0.0061%), **5** (7.3 mg, 0.0005%), and *trans*-phytol (16.9 mg, 0.0011%).

Adunctin A (**1**): Clear oil. $[\alpha]_D^{20} = +17.0$ (MeOH, $c = 0.85$). UV (MeOH): 226 (sh, 4.23), 288 (4.22), 325 (3.59). UV (MeOH, $AlCl_3$): 312, 330. IR (KBr): 1620 (C=O), 1590 (C=C), 1420, 1200, 1160, 1090. EI-MS: 273 (30), 272 (24), 167 (52), 140 (28), 137 (57), 136 (58), 121 (32), 95 (32). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (3-NOBA): 431 ($[M + Na]^+$), 273 ($[M + H - 136]^+$), 137 ($[M + H - 272]^+$).

Adunctin B (**2**): White needles from hexane. M.p. 63°. $[\alpha]_D^{20} = +36.1$ (MeOH, $c = 0.44$). UV (MeOH): 228 (sh, 4.26), 285 (4.39), 340 (3.60). UV (MeOH, $AlCl_3$): 310, 400. IR (KBr): 3400 (br., OH), 1630 (C=O), 1595 (C=C), 1200, 1150, 1060. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 406 (36, M^+), 391 (24), 363 (24), 272 (81), 255 (19), 179 (18), 167 (89), 140 (72), 105 (46), 91 (100).

Adunctin C (**3**): Yellow prisms from hexane. M.p. 78°. $[\alpha]_D^{20} = -71.4$ (MeOH, $c = 0.73$). UV (MeOH): 230 (sh, 4.42), 285 (4.73), 340 (3.42). UV (MeOH, $AlCl_3$): 310, 400. IR (KBr): 3400 (br., OH), 1640 (C=O), 1600 (C=C), 1220, 1150. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 406 (100, M^+), 364 (43), 363 (92, $[M - C_3H_7]^+$), 285 (59), 267 (37), 179 (54), 149 (39), 105 (45), 91 (99).

Adunctin D (**4**): Yellow amorphous powder. $[\alpha]_D^{20} = +31.0$ (MeOH, $c = 0.52$). UV (MeOH): 228 (sh, 4.19), 285 (4.46), 340 (3.52). UV (MeOH, $AlCl_3$): 310, 400. IR (KBr): 3400 (br., OH), 1635 (C=O), 1600 (C=C), 1200, 1140. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. NOE: H–C(3′)/MeO–C(4′); MeO–C(4′)/2 H–C(7″); 2 H–C(7″)/H–C(5″); H–C(5″)/Me(9″) and Me(10″). EI-MS: 406 (100, M^+), 363 (97, $[M - C_3H_7]^+$), 285 (50), 267 (25), 179 (32), 153 (23), 105 (25), 91 (75).

Adunctin E (**5**): White amorphous powder. $[\alpha]_D^{20} = +16.3$ (MeOH, $c = 0.65$). UV (MeOH): 230 (sh, 4.21), 283 (4.28), 340 (3.47). UV (MeOH, $AlCl_3$): 309, 400. IR (KBr): 3400 (br., OH), 1630 (C=O), 1600 (C=C), 1370, 1215, 1205, 1130, 1080. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. NOE: H–C(3′)/MeO–C(4′); H–C(5″)/H–C(6″), H–C(8″), Me(9″) and Me(10″); H–C(6″)/Me(7″). EI-MS: 424 (1, M^+), 339 (5), 279 (6), 167 (13), 149 (81), 97 (33).

(−)-Methyllindaretin (**6**): $[\alpha]_D^{20} = -40.1$ ($CHCl_3$, $c = 0.1$; [11]: for (+)-methyllindaretin, $[\alpha]_D^{20} = +41.0$). All other physical and chemical properties as previously described [11].

α-Tocopherol and trans*-Phytol*: Physical and chemical properties identical to those previously reported [18] [19].

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