extracts led to the separation of ten compounds (**3.1-3.10**). Compounds **3.4**, **3.6** and **3.7** were methylated using diazomethane in diethylether to obtain their methoxy derivatives (**3.4A, 3.4B, 3.6A, 3.6B** and **3.7B**). The structure elucidations were determined by spectroscopic methods.

Structure Elucidation of Compound 3.1

Compound **3.1** was obtained as colorless oil (3.2 mg). The HR-FABMS (negative mode) showed the $[M-H]^-$ ion at *m/z* 225.14837 corresponding to $C_{13}H_{21}O_3$. The IR spectrum showed the presence of a hydroxyl (ν_{max} 3406 cm^{-1}), a carbonyl (ν_{max} 1713 cm^{-1}) and an ether (ν_{max} 1127, 1039 cm^{-1}) groups. The ^{13}C-NMR spectrum indicated thirteen carbons including three methyl, five methylene, two methine and three quaternary carbons among which one is a keto carbonyl (δ_C 207.8). The ^{1}H-NMR spectrum showed three methyl singlet signals at δ_H 2.15, 1.23 and 0.98 in addition to the presence of a less shielded CH_3CO signal at δ_H 2.15 and a carbinolic proton signal at δ_H 4.06. The ^{1}H-^{1}H COSY spectrum indicated correlations between H-8 (δ_H 2.54)/ H_2-7 (δ_H 1.75 and 1.58); H_2-7/H-6 (δ_H 1.28) as well as correlations between H-3 (δ_H 4.06)/ H_2-2 (δ_H 1.67 and 1.41) and H_2-4 (δ_H 1.88 and 1.41). Attachments of C-4 to C-5 and C-2 to C-1 were revealed from the ^{1}H-^{13}C correlations between H-2/ C-1 (δ_C 43.0), C-3 (δ_C 66.2), C-4 (δ_C 41.5), C-6 (δ_C 53.6), C-12 (δ_C 21.6) and H-4/C-2 (δ_C 39.8), C-3, C-5 (δ_C 83.4), C-6, C-11 (δ_C 25.4), respectively. Connection between C-7 to C-6 was evident from the long range ^{1}H-^{13}C correlations particularly between H-6 (δ_H 1.28)/ C-2, C-4, C-5, C-7 (δ_C 18.5), C-8 (δ_C 43.0), C-11 and C-12. Placement of a keto group at C-9 (δ_C 207.8) was also obtained from the ^{1}H-^{13}C correlations especially between H_2-7 and H_3-10 (δ_H 2.15)/ C-9. The key ^3J ^{1}H-^{13}C correlations particularly between H_2-13 (δ_H 3.60 and 3.42) /C-5 in conjunction with the absence of long range ^{1}H-^{13}C correlation between H_3-12 (δ_H 0.98)/C-5 indicated an ether linkage between C(5)-O-C(13). Compound **3.1** was identified as tanarifuranonol. Full assignments of the ^{1}H and ^{13}C chemical shifts were established from the HMQC and HMBC correlations (Table 3.1). The relative configuration of **3.1** was revealed from the NOESY spectrum (Figure 3.1) which indicated that the 3-OH group and the ether linkage are both in β–axial arrangements.

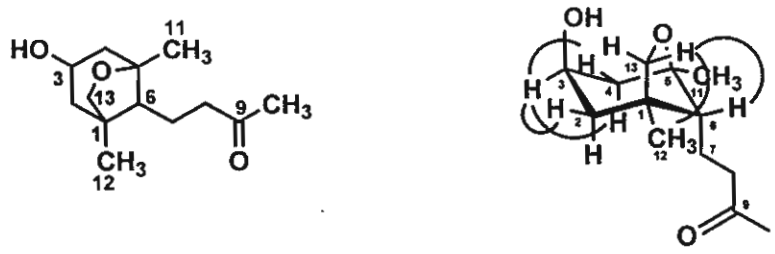
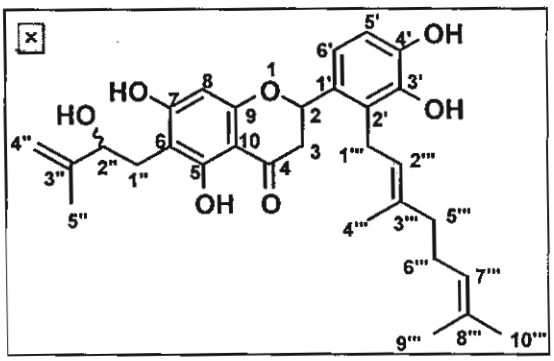


Figure 3.1 Structure and NOE correlations of **3.1**

Structure Elucidation of Compound 3.2

Compound **3.2** was isolated as yellow amorphous solid. The HR-FABMS (positive mode) which exhibited an $[M+H]^+$ ion at *m/z* 509.25421 corresponded to molecular formula $C_{30}H_{36}O_7$ The IR spectrum exhibited an absorption band of a hydroxyl group at ν_{max} 3430 cm^{-1} and a carbonyl group at ν_{max} 1635 cm^{-1}. The ^{13}C-NMR spectrum of compound 3.**2** showed 30 carbon signals comprising four methyl, six methylene including one vinylic carbon, seven methine and thirteen quaternary carbons including one carbonyl (δ_C 196.3). The ^{1}H- NMR spectrum of compound 3.**2** in $CDCl_3$ (Table 1) exhibited the presence of nonequivalent methylene protons at δ_H 2.72 (1H, dd, J = 17.2 and 2.4 Hz) and 3.10 (1H, dd, J = 17.2 and 13.2 Hz) and a double doublet signal at δ_H 5.48 (1H, dd, J = 13.1 and 2.6 Hz, H-2) commonly found in a flavanone nucleus. The low field signal at δ_H 12.46 (1H, s) indicated a C-5-OH proton intramolecularly H-bonded to C-4 carbonyl oxygen atom. Aromatic proton signals at δ_H 6.95 (1H, d, J = 8.4 Hz) and 6.82 (1H, d, J = 8.4 Hz) indicated the two ortho-coupled protons of the tetrasubstituted aromatic ring. The geranyl group signals were implied from the ^{1}H-^{1}H–COSY spectrum. Signal at δ_H 3.46 (H-1‴) not only correlated with H-2‴ (δ_H 5.19) but also showed long-range ^{1}H-^{1}H correlations with the methyl group signal (H_3-4‴, δ_H 1.76) and the methylene proton signals at δ_H 2.08 (H_2-5‴). The H_2-5‴ also showed correlations with H_3-4‴ and H_2-6‴ (δ_H 2.06). The olefinic proton at δ_H 5.03 (H-7‴) correlated not only with H-6‴ but also with the two methyl group signals at δ_H 1.66 (H_3-9‴) and 1.57 (H_3-10‴). The ^{1}H-^{1}H correlations of the oxymethine proton at δ_H 4.34 (H-2″) with the non-equivalent methylene proton signals at δ_H 3.10 and 2.76 (H_2-1″), a vinylic protons signal at δ_H 4.98 and 4.88 (H_2-4″) as well as a methyl proton signal at δ_H 1.84 (H_3-5″) in the ^{1}H-^{1}H COSY spectrum indicated the presence of a 2-hydroxy-3-methyl-but-3-enyl group.

The long-range ^{1}H-^{13}C correlations particularly between H-2/C-2′ and H-1‴/C-2′ and C-2‴ indicated the attachment of a geranyl group to ring B at C-2′. The ^{3}J ^{1}H-^{13}C correlations between H-1″/C-5, C-6 and C-7 also indicated the bonding of the 2-hydroxy-3-methyl-but-3-enyl group to ring A at C-6. The use of ^{1}H-^{1}H COSY, HMQC, HMBC experiments led to the identification of compound **3.2** as tetrahydroxytanariflavanone A. Full assignment of the ^{1}H and ^{13}C chemical shifts are as shown in Tables 3.2-3.3. Stereochemistries at C-2 and C-2″ position are at this stage not studied due to scarcity of the material.

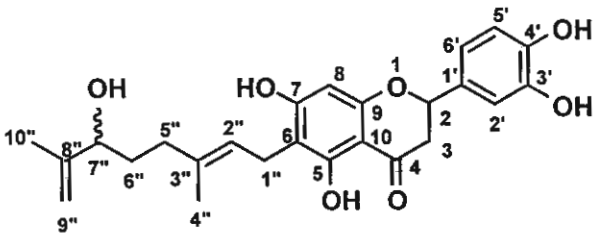


3.2

Structure Elucidation of Compound 3.3

Compound **3.3** was obtained as yellow amorphous solid. Its molecular formula $C_{25}H_{28}O_7$ was obtained from the HR-FAB-MS (positive mode) which exhibited a $[M+H]^{+}$ ion at *m/z* 441.19210. The FT-IR spectrum showed absorption band at ν_{max} 3382 and 1641 cm^{-1} indicating the presence of a hydroxyl and a carbonyl groups, respectively. The ^{13}C-NMR spectrum of compound **3.3** showed 25 carbon signals comprising two methyl, five methylene, seven methine and eleven quaternary carbons including one carbonyl (δ_C 196.0). The ^{1}H-NMR spectrum of compound **3.3** exhibited the presence of C-3 methylene protons at δ_H 2.66 (1H, dd, J =17.2 and 2.8 Hz) and 2.97 (1H, dd, J = 12.8 and 17.1 Hz) as well as the double doublet at δ_H 5.16 (1H, dd, J = 12.8 and 2.8 Hz, H-2) commonly observed in a flavanone nucleus as found in **3.2**. The presence of a 6-hydroxy-3,7-dimethyl-octa-2,7-dienyl group was revealed from the continuous ^{1}H-^{1}H COSY correlations between H-1″(δ_H 3.20)/H-2″ (δ_H 5.20), H_3-4″ (δ_H 1.72) and H-5″ (δ_H 1.96); H-5″/H-2″, H_3-4″ and H_2-6″ (δ_H 1.58) as well as H-7″ (δ_H 3.94) /H_2-6″, H-9″ (δ_H 4.81 and 4.74) and H_3-10″ (δ_H1.63). The presence of a pair of AB proton doublets at δ_H 6.79 (d, 8.1) and 6.73 (d, 8.1) as well as a broad singlet signal

at δ_H 6.862 indicated a tri-substituted aromatic ring. The long range ^1H-^{13}C correlations in the HMBC spectrum particularly between H-1″/C-5, C-6, C-7, C-2″, C-3″ indicated the attachment of the 6-hydroxy-3, 7-dimethyl-octa-2,7-dienyl group at C-6 of ring A. Compound **3.3** could therefore be identified as tetrahydroxytanariflavanone B. Full assignment of all the ^1H and ^{13}C chemical shifts (Tables 3.2-3.3) were obtained by using ^1H-^1H COSY, HMQC, HMBC correlations. Stereochemistries at C-2 and C-7″ position are also at this stage not studied due to scarcity of the material.



3.3

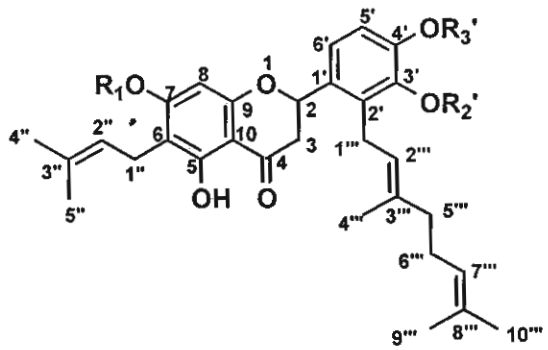
Structure Elucidation of Compound 3.4

Compound **3.4** was isolated as yellow amorphous solid. Its molecular formula $C_{30}H_{36}O_6$ was obtained from the MS spectrum which exhibited an $[M]^+$ peak at m/z 492. The IR spectrum exhibited an absorption band of a hydroxyl group (O-H) at ν_{max}3522 cm^{-1} and a carbonyl group at ν_{max} 1645 cm^{-1}. The ^{13}C-NMR spectrum of compound **3.4** showed 30 carbon signals. Analyses of ^{13}C-NMR spectra of this compound (Table 3.1) suggested the presence of five methyl carbons, five methylene carbons, seven methine carbons, thirteen quaternary carbons including a carbonyl carbon (δ_C 196.49). The ^1H- NMR spectrum of compound **3.4** in $CDCl_3$ (Table 3.1) exhibited the presence of non equivalent methylene proton at δ 2.76 (1H, dd, J=2.8 and 17.1 Hz, H_{eq}-3) and 3.13 (1H, dd, J= 13.2 and 17.1 Hz, H_{ax}-3) and a double doublet at δ 5.51 (1H, dd, J= 2.7 and 13.4 Hz, H-2) commonly found in a flavanone nucleus. The low field signal at δ 12.1 (1H, s) indicated a C-5-OH proton intramolecularly H-bonded to C-4 carbonyl oxygen atom. Aromatic proton signals at δ 6.85 (1H, d, J= 8.4 Hz) and 6.99 (1H, d, J= 8.4 Hz) indicated the two ortho-coupled protons probably of the tetrasubstituted ring B of the flavanone. The geranyl and prenyl group signals were implied from the ^1H-^1H –COSY spectrum. Signal at δ3.46 (H-1‴) correlates with H-2‴ (δ5.18) which in turn showed long-range ^1H-^1H correlations with the methyl group signal (H-4‴, δ1.78). The methylene

proton signals at δ2.08 (H-5‴) showed correlations with H-4‴ and H-6‴ (δ 2.08). The olefinic proton at δ 5.04 (H-7‴) correlated not only with H-6‴ but also with the two methyl group signals at δ 1.67 (H-9‴) and 1.59 (H-10‴). The olefinic proton signal at δ5.25 (H-2″) correlated with H-1″ (δ 3.36) and the two singlet signals at δ 1.82 (H-4″) and 1.76 (H-5″).

The long-range ^{1}H-^{13}C correlations particularly between H-2/C-2′ and H-1‴/C-2′ and C-2‴ indicated that the geranyl group is bonded to ring B at C-2′. Correlations between H-1″/C-5, C-6 and C-7 also indicated that the γ,γ-dimethylallyl group is bonded to ring A at C-6. The use of several 2D-NMR techniques including HMQC, HMBC experiments led to the identification of compound **3.4** as nymphaeol C. This compound was reported to be previously obtained from *Hernandia nymphaefolia* (presl) Kubitzki by Yakushijin *et al.* in 1980 [3.25] and later in coincidence with our work from *M. tanarius*. [3.24] Full assignment of the ^{1}H and ^{13}C chemical shifts were as shown in Table 3.4.

The dimethoxy and trimethoxy derivatives of compound 3.4A and 3.4B were obtained by reacting compound **3.4** with diazomethane. The spectral data of compound 3.**4A** and 3.4B were almost identical to compound 3.4 except for the presence of additional OMe signals in the ^{1}H and ^{13}C spectra. All ^{1}H and ^{13}C chemical shifts of compounds 3.4A and 3.4B were also fully assigned using the HMQC and HMBC correlations (Tables 3.5 and 3.6, respectively).



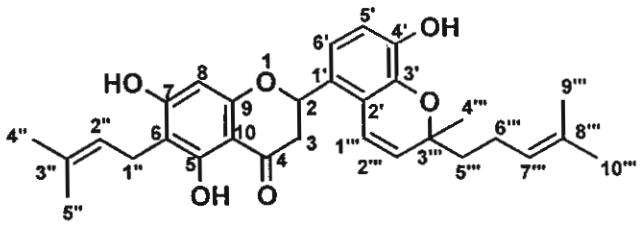
3.4, $R_1 = R_2 = R_3 = H$
3.4A, $R_1 = R_3 = Me$
3.4B, $R_1 = R_2 = R_3 = Me$

Structure Elucidation of Compound 3.5

Compound **3.5** was isolated as yellow oil. Its molecular formula $C_{30}H_{34}O_6$ was obtained from the high resolution FAB-MS which exhibited an $[M]^+$ peak at m/z 490. The IR spectrum exhibited an absorption band of a hydroxyl group at ν_{max} 3269 cm^{-1}. The presence of C = O and C = C stretching of carbonyl and aromatic group were revealed from the absorption bands at ν_{max} 1635 and 1496 cm^{-1}, respectively. The ^{13}C spectrum of compound **3.5** showed 30 carbon signals comprising five methyl carbons, four methylene carbons, eight methine carbons, thirteen quaternary carbons including one carbonyl (δ 196.28). The ^1H-NMR spectrum of compound **3.5** in $CDCl_3$ (Table 3.7) exhibited the presence of the non equivalent signals methylene proton signals at δ 2.73 and 3.11 (1H, dd, J =2.9 and 17.2 Hz, 1H, dd, J = 13.3 and 17.1 Hz, H- 3) as well as the double doublets at δ 5.49 (1H, dd, J = 2.8 and 13.2 Hz, H-2) indicating the flavanone nucleus. Two doublet signals at δ 6.84 and 6.89 (J = 8.4 Hz) were assigned to the two ortho-coupled aromatic protons. The presence of a low field signal at δ 12.1 (1H, s) indicated a C-5-OH proton intramolecularly H-bonded to C-4 carbonyl oxygen atom commonly found in the flavonoid nucleus. The ^1H-^1H COSY spectrum showing correlations between the olefinic protons at δ 5.23 (1H, br t, J = 7.0 Hz, H-2″), the less shielded allylic protons at δ 3.34 (2H, br d, J = 7.1 Hz, H-1″) as well as the two methyl groups signal at δ 1.80 (s), 1.74 (s) suggested the presence of a γ,γ- dimethylallyl group.

The presence of three methyl group protons at δ 1.40 (s), 1.65 (s) and 1.56 (s), two sets of methylene proton multiplets at δ 2.09 (m) and 2.40 (m) and the presence of the vicinally coupled protons at δ 5.66 (d, J = 10.2 Hz, H-2‴) and 6.59 (d, J = 10.2 Hz, H-1‴) indicated that an oxygen atom at C-3′ must have cyclized onto C-3‴ to form a pyrane ring. Further evidence of the pyrane ring next to ring B was obtained from the long range ^1H -^{13}C correlations between H-1‴ / C-1′ and C-3′ as well as between H-4‴ / C-2‴ and C-3‴. Full assignment of all the ^1H and ^{13}C chemical shifts (Table 3.7) could be further obtained by using the HMQC, HMBC correlations.

These evidences led to the identification of compound **3.5** as tanari-flavanone B. This compound was recently obtained from *M. tanarius* by Tseng *et al.* in 2001 in coincidence with our investigation.[3.24]



3.5

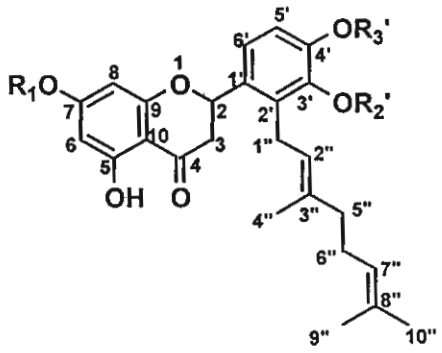
Structure Elucidation of Compound 3.6

Compound **3.6** was obtained as yellow amorphous solid. Its molecular formula $C_{25}H_{28}O_6$ was obtained from the high resolution FAB-MS (negative mode) which exhibited an $[M-H]^-$ peak at m/z 423.18091.

The FT IR spectrum showed absorption bands at ν_{max} 3382 and 1641cm^{-1} indicating the presence of a hydroxyl and carbonyl groups, respectively. The ^{13}C-NMR spectrum of compound 3.6 showed 25 carbon signals. Analyses of ^{13}C-NMR spectra of this compound (Table 3.8) suggested the presence of three methyl carbons, four methylene carbon, seven methine carbons and eleven quaternary carbons including one carbonyl carbon (δ 196.77). The ^{1}H-NMR spectrum of compound **3.6** in $CDCl_3$ (see Table 3.6) exhibited the presence of methylene proton at δ 2.70 (1H, dd, J =2.5 and 17.2 Hz, H_{eq}-3) and 3.08 (1H, dd, J = 13.4 and 17.2 Hz, H_{ax}- 3), as well as the double doublet at δ 5.49 (1H, dd, J = 2.5 and 13.3 Hz, H-2), commonly observed in a flavanone nucleus. Two doublet signals at δ 6.78 (J = 8.37 Hz) and 6.91 (J = 8.41 Hz) were assigned to two ortho coupled aromatic protons of the tetrasubstituted aromatic ring. The two meta-coupled doublet at δ 5.98 (J = 1.93 Hz), 5.94 (J = 1.95 Hz) were also presented. The presence of a low field signal at δ 12.1 (1H, s) indicated a C-5-OH proton intramolecularly H-bonded to C-4 carbonyl oxygen atom commonly found in the flavonoid nucleus. The two olefinic proton at δ 5.11 (1H, t, J = 6.3 Hz) and 4.99 (1H, t, J = 6.6 Hz), allylic protons at δ 3.46 together with two sets of two protons signals at δ 2.01 (2H, m) and 2.04 (2H, m), in addition to three methyl group signals at δ 1.72 (s), 1.63 (s) and 1.55 (s) suggested the presence of a geranyl group bonded to an aromatic ring. Full assignment of all the ^{1}H and ^{13}C chemical shifts (Table 3.8) could be obtained by using HMQC, HMBC correlations.

Based on the data described above compound **3.6** was identified to be nymphaeol B. This compound was reported to be previously obtained from *Hernandia nymphaefolia* (presl) Kubitzki by Yakushijin *et al.* in 1980. [3.25]

The dimethoxy and trimethoxy derivatives of compound 3.6 (compounds **3.6A** and 3.6B, respectively) were obtained by reacting compound 3.6 with diazomethane. The spectral data of the dimethoxy and trimethoxy derivatives of compound 3.6 were almost identical to compound **3.6** except for the presence of the extra methyl group signals of the methoxy groups. The use of HMQC, HMBC correlations as well as NOESY spectral data led to the assignments of all the ^{1}H and ^{13}C chemical shifts of compounds 3.6A and **3.6B** (Tables 3.9 and 3.10, respectively).



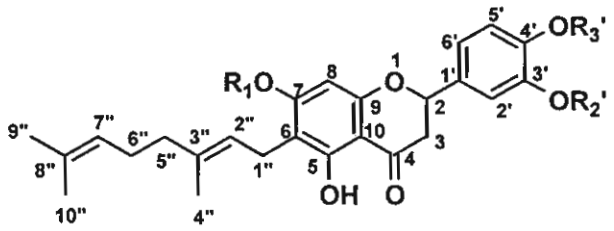
3.6, $R_1 = R_2 = R_3 = H$
3.6A, $R_1 = R_3 = Me$, $R_2 = H$
3.6B, $R_1 = R_2 = R_3 = Me$

Structure Elucidation of Compound 3.7

Compound **3.7** was obtained as yellow solid. The ^{13}C-NMR spectrum of compound **3.7** showed 25 carbon signals comprising three methyl carbons, four methylene carbon, seven methine carbons and eleven quaternary carbons including one carbonyl carbon (δ 196.1). The ^{1}H-NMR spectrum of compound **3.7** in $CDCl_3$ (see Table 3.11) exhibited the presence of methylene proton at δ 2.77 (1H, dd, J =3.1 and 17.0 Hz, H_{eq}-3) and 3.02 (1H, dd, J = 12.8 and 17.1 Hz, H_{ax}-3), as well as the double doublet at δ 5.16 (1H, d, J = 12.7 Hz, H-2), commonly observed in a flavanone nucleus. Two doublet signals at δ 6.74 (J = 7.8 Hz) and 6.79 (J = 7.6 Hz) and a singlet signal at 6.87 were assigned to three aromatic protons of the

trisubstituted aromatic ring. The presence of a low field signal at δ 12.2 (1H, s) indicated a C-5-OH proton intramolecularly H-bonded to C-4 carbonyl oxygen atom commonly found in the flavonoid nucleus. The two olefinic proton at δ 5.18 (1H, t, J = 6.8 Hz) and 5.02 (1H, t, J = 6.9 Hz), allylic protons at δ 3.23 together with two sets of two protons signals at δ 1.93 (2H, m) and 2.01 (2H, m), in addition to three methyl group signals at δ 1.73 (s), 1.60 (s) and 1.53 (s) suggested the presence of a geranyl group bonded to an aromatic ring. The HMBC correlations particularly between H-1″/ C-5, C-6, C-7 and C-2″ indicated the attachment of the geranyl group to C-6 of A ring. Full assignment of all the ^{1}H and ^{13}C chemical shifts (Table 3.11) could be obtained by using HMQC, HMBC correlations.

Based on the data described above compound **3.7** was identified to be nymphaeol A. This compound was reported to be previously obtained from *Hernandia nymphaefolia* (presl) Kubitzki by Yakushijin *et al.* in 1980. [3.25]



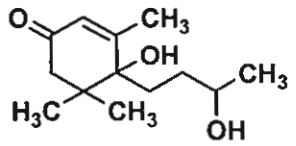
3.7, $R_1 = R_2 = R_3 = H$
3.7A, $R_1 = R_3 = Me$, $R_2 = H$
3.7B, $R_1 = R_2 = R_3 = Me$

The dimethoxy and trimethoxy derivatives of compound 3.7 (compounds **3.7A** and **3.7B**) were obtained by reacting compound 3.7 with diazomethane. Compound **3.7A** was co-eluted with part of compound **3.7B** during chromatographic separation and was not obtained in pure state. The spectral data of the trimethoxy derivative (**3.7B**) was almost identical to compound **3.7** except for the presence of the extra methyl group signals of the methoxy groups. The use of HMQC, HMBC correlations as well as NOESY spectral data led to the assignments of all the ^{1}H and ^{13}C chemical shifts of compound **3.7B** (Tables 3.11-3.12).

Structure Elucidation of Compound 3.8

Compound **3.8** was obtained as colorless oil (10.2 mg). The ^{13}C-NMR spectrum indicated thirteen carbons comprising four methyl carbons, three methylene carbons, two methine carbons and four quaternary carbons including one carbonyl carbon. The ^{1}H NMR spectrum showed the presence of an α-proton of the β-substituted-α,β-carbonyl group at δ 5.82 (H-4). The two sets of doublets at δ 2.47 and 2.21 could be assigned to the non equivalent methylene protons bonded to a carbonyl group (H-2).

The ^{1}H-^{1}H COSY spectrum indicated correlations between H-9 / H-10 and H-8 as well as correlations between H-8/H-7. The ^{1}H-^{1}H long-range correlation between H-4/ H-13 (δ2.03) was also observed indicating that the β-substituted group of an α,β-unsaturated carbonyl is a methyl group (H-13). The long-range ^{1}H-^{13}C correlations particularly between H-4 / C-1, C-6, C-13 and between H-2 / C-1, C-3, C-4, C-6, C-11 and C-12 indicated structure of compound 3.**8**. Further assignment of ^{1}H and ^{13}C chemical shifts was as shown in Table 3.13. Compound 3.**8** could therefore be identified as 7,8-dihydrovomifoliol which had been previously isolated from *Prunus persicanectarina*.

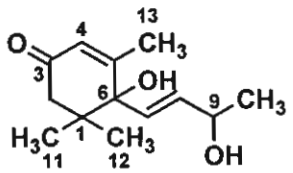


3.8

Structure Elucidation of Compound 3.9

Compound **3.9** was obtained as colorless oil. The ^{13}C-NMR spectrum indicated thirteen carbons comprising four methyl carbons, one methylene carbons, four methine of which three are olefinic and one oxymethine carbons in addition to four quaternary carbons including one carbonyl and one oxygenated quaternary. The ^{1}H NMR spectrum is rather similar to that of **3.8** which showed the presence of an α-proton of the β-substituted-α,β-carbonyl group at δ 5.91 (H-4). The two sets of doublets at δ2.44 and 2.24 could be assigned to the non equivalent methylene protons bonded to a carbonyl group (H-2). The double doublet signal at δ5.85 (J = 15.6, 5.1 Hz) showed ^{1}H-^{1}H correlations to doublet signal at δ5.78 and also to signal at δ

4.40 indicating that the additional double bond is at C-7(8). Compound 3.9 could therefore be identified as vomifoliol which had been previously isolated from *Prunus persicanectarina*. Further assignment of ^{1}H and ^{13}C chemical shifts was as shown in Table 3.14.]



3.9

Structure Elucidation of Compound 3.10

Compound **3.10** was obtained as colorless oil (15.1 mg). The IR spectrum showed the presence of a hydroxyl (3417 cm^{-1}), keto carbonyl (1714 cm^{-1}) and an ether (1227, 1126 cm^{-1}) functions. The ^{13}C-NMR spectrum indicated thirteen carbons comprising three methyl carbons, five methylene carbons, two methine carbons and three quaternary carbons including one carbonyl carbon of a keto group (δ 209.42). The ^{1}H-NMR spectrum showed three methyl group signals at δ 1.30 (s), 1.22 (d, J = 6.18) and 1.06 (s). The ^{1}H-^{1}H COSY spectrum indicated correlations between signals at δ 3.81 (H-9)/ H-10 (δ 1.22) and H-8 (δ 1.64 and 1.58); H-8 also correlate with signals at δ 1.62 and 1.35 (H-7) and H-7 correlates with H-6 (δ 1.64). The non-equivalent methylene protons (H-12 at δ 3.61 and 3.55; H-2 at δ 2.36 and 2.21) were revealed from ^{1}H-^{1}H COSY and HMQC spectra.

The ^{1}H-^{13}C correlations between H-4 / C-3, C-5, C-6, C-13 ; H-2 / C-1, C-3, C-4, C-6, C-11, C-12 and particularly between H-12 / C-1, C-2, C-5, C-6 and C-11 as well as H-6 / C-1, C-11, C-12, C-13 led to the assignments of the structure of compound **3.10**.is identical to annuionone E (Table 3.15) which had been isolated previously from *Helianthus annuus* but the structure was incorrectly assigned until recently.[3.26]

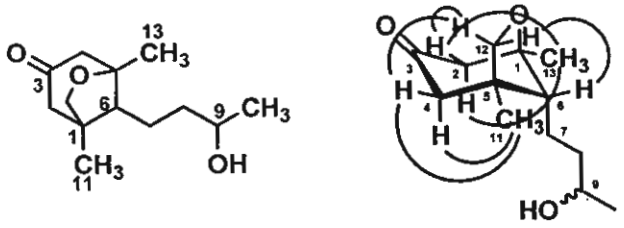


Fig. 3.2 Structure and NOE correlations of **3.10**

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. EI-MS and HR-FABMS spectra were recorded on a Finnigan MAT 90 instrument. ^{1}H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference.

Plant Material and Extract Preparation. The leaves of *M.tanarius* (Euphorbiaceae) used in this study were collected from Trang Province, during April 2001. A voucher specimen (SSMT/2001) is kept at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University.

Isolation of compounds. The *n*-hexane extract of the leave (143.18 g) was subjected to silica gel column chromatography with gradient of *n*-hexane/EtOAc 95:5 to $CHCl_3$ /MeOH 30:70 to obtain eleven fractions. Fraction 10 was column chromatographed (silica gel, *n*-hexane: $CHCl_3$ 70:30 to $CHCl_3$: MeOH 50:50) to give ten subfractions (subfrs. 10.1 to 10.10). Subfraction 10.9 was further separated by reversed phase colomn chromatography (RP-C-18 silica gel, H_2O: MeOH 20:80 to 0:100) to give six subfractions (subfrs. 10.9.1 to 10.9. 6). Subfraction 10.9.1 was purified using reversed phase silica gel (H_2O: MeOH 20:80 to 0:100) to give subfractions 10.9.1.1 to 10.9.1.6. Subfraction 10.9.1.3 was chromatographed (silica gel, *n*-hexane: $CHCl_3$ 90:10 to $CHCl_3$: MeOH 20:80 to give nine subfractions. Subfrs. 10.9.1.3.7 contained compound **10** (17.6 mg) and subfraction 10.9.1.3.8 contained compound **8** (10.2 mg). Subfraction 10.5 was purified using silica gel column chromatography (*n*-hexane: $CHCl_3$

10:90 to $CHCl_3$: MeOH 50: 50) and yielded six subfractions (subfrs. 10.5.1 to 10.5.6). Subracation 10.5.4 contained compound **4** (372.7 mg) Further purification of subfraction 10.5.5 (2×, silica gel, *n*-hexane: $CHCl_3$ 10:90 to $CHCl_3$: MeOH 50:50 then *n*-hexane: $CHCl_3$ 20:80 to $CHCl_3$: MeOH 80:20) gave compound **5** (2.7 mg).

The chloroform extract (122.3 g) was fractionated using silica gel column chromatography with gradient of *n*-hexane: $CHCl_3$ 10:90 to $CHCl_3$: MeOH 20:80) yielded thirteen fractions. Fraction 10 after reversed phase column chromatography (RP-C-18 silica gel, H_2O: MeOH 30:70 to 0:100) gave additional amount of compound **4** (500.5 mg). Fraction 12 was purified using silica gel column chromatography ($CHCl_3$: MeOH 99.5:0.5 to 50:50) yielded six subfractions (subfrs. 12.1 to 12.6). Subfraction 12.2 was column chromatographed using reversed silica gel (H_2O: MeOH 30:70 to 0:100) to give seven subfractions (subfrs.12.2.1 to 12.2 7). Subfraction 12.2.2 was further purified (silica gel, *n*-hexane: EtOAc 95:5 to 70:30) to give four subfractions (subfrs. 12.2.2.1 to 12.2 .2.4). Subfraction 12.2.2.1 contained compound **6** (53.7 mg) and subfraction 12.2.2.4 after column chromatography (silica gel, $CHCl_3$: MeOH 99:1) gave compound **3** (5.3 mg). Subfraction 12.5 was rechromatographed (RP-C-18, silica gel, H_2O: MeOH 30:70 to 0:100) then silica gel column chromatography ($CHCl_3$: MeOH 99:1 to 80:20) gave ten subfractions. Subfraction 12.5.5 contained compound **7** (17.4 mg). Fraction 11 was column chromatographed (silica gel, *n*-hexane: CH_2Cl_2 20:80 to CH_2Cl_2: MeOH 70:30) to yield fourteen subfractions (subfrs 11.1 to 11.14). Subfraction 11.8 was further purified using silica gel column chromatography (*n*-hexane: CH_2Cl_2 10:90 to CH_2Cl_2: MeOH 70:30) to obtain twelve subfractions (subfrs. 11.8.1 to 11.8.12). Subfraction 11.8.9 after column chromatography (silica gel, CH_2Cl_2: MeOH 100:0 to 70:30) gave additional quantity of compound **4** (10.1 mg). Subfraction 11.8.12 was carefully purified using reversed phase column chromatography (RP-C-18 silica gel, H_2O: MeOH 50:50 to 0:100) to give fifteen subfractions. Subfraction 11.8.12.11 contained compound **2** (5.6 mg). Fraction 13 was fractionated using silica gel column chromatography (CH_2Cl_2: MeOH 100:0 to 70:30) to yield fifteen subfractions. Subfraction 13.15 was further purified (silica gel, $CHCl_3$) and the moderately polar fraction (subfr. 13.15.10) was fractionated using reversed phase column chromatography (RP-C-18 silica gel, H_2O: MeOH 40: 60 to 0:100). The most polar fraction after further column chromatography (2×, silica gel, CH_2Cl_2: MeOH 98:2 then CH_2Cl_2: MeOH 99:1) yielded compound **1** (4.5 mg) and compound **9** (21.1 mg).

Tanarifuranonol (3.1): $[\alpha]_D$ 13.2895° (*c* 0.0760 w/v %, $CHCl_3$) IR (KBr) ν_{max} 3406, 2931, 2870, 1768, 1713, 1455, 1434, 1378, 1265, 1168,

1127, 1039, 1013, 824, 723, 642, 538 cm^{-1} ; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.1; HR-FABMS (negative mode) $[M-H]^-$225.14837 (calcd for $C_{13}H_{21}O_3$, 225.14907)

Tetrahydroxytanariflavanone A, 3',4',5,7-tetrahydroxy-2'-(geranyl)-6-(2-hydroxy-3-methylbut-3-enyl)-flavanone (3.2): $[\alpha]_D$ -3.0566° (*c* 0.2650 w/v %, MeOH) IR (KBr) ν_{max} 3430, 2922, 1635 (br), 1455, 1340, 1294, 1158, 1095, 1005, 902, 817, 758, 550 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Tables 3.2 and 3.3; HR-FABMS (positive mode) $[M+H]^+$ 509.25421 (calcd for $C_{30}H_{37}O_7$, 509.25393)

Tetrahydroxytanariflavanone B, 3',4',5,7-tetrahydroxy-6-(6-hydroxy-3,7-dimethylocta-2',7'-dienyl)-flavanone (3.3): $[\alpha]_D$ -12.6809° (*c* 0.2350 w/v %, MeOH) IR (KBr) ν_{max} 2919, 1738, 1635, 1455, 1338, 1296, 1159, 1085, 1019, 815, 777, 453 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Tables 3.2 and 3.3; HR-FABMS (positive mode) $[M+H]^+$ 441.19210 (calcd for $C_{25}H_{29}O_7$, 441.19135)

Nymphaeol C (**3.4**): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.4

4', 7-Dimethoxy-nymphaeol C (**3.4A**): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.5

3', 4', 7-Trimethoxy-nymphaeol C (**3.4B**): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.6.

Tanariflavanone B (**3.5**): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.7.

Nymphaeol B (**3.6**): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.8

4', 7-Dimethoxy-nymphaeol B (**3.6**A): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.9.

3', 4', 7-Trimethoxy-nymphaeol B (**3.6B**): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.10.

Nymphaeol A (3.7): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Tables 3.11 and 3.12.

3', 4', 7-Trimethoxy-nymphaeol A (3.7B): ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Tables 3.11 and 3.12.

7, 8-Dihydrovomifoliol **(3.8)** ^{1}H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.13.

Vomifoliol **(3.9)**: ^{1}H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.14

Annuionone E **(3.10)**: ^{1}H and ^{13}C NMR data (measured in $CDCl_3$) see Table 3.15.

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Table 3.1 ^{1}H and ^{13}C NMR data and HMBC correlations of **3.1**.

Position	δ_H	δ_C	HMBC
1		43.0 (s)	
2	1.67 (m), 1.41 (m)	39.8 (t)	C-1, C-3, C-4, C-6, C-12
3	4.06 (dddd, 17.1, 13.5, 10.4, 6.7)	66.2 (d)	
4	1.88 (dd, 13.2, 6.7); 1.41 (m)	41.5 (t)	C-2, C-3, C-5, C-6, C-11
5		83.4 (s)	
6	1.28 (t, 6.3)	53.6 (d)	C-2, C-4, C-5, C-7, C-8, C-11, C-12
7	1.75 (m), 1.58 (m)	18.5 (t)	C-1, C-5, C-6, C-8, C-9
8	2.54 (t, 7.8)	43.0 (t)	C-6, C-7, C-9
9		207.8 (s)	
10	2.15 (s)	30.0 (q)	C-8, C-9
11	1.23 (s)	25.4 (q)	C-3, C-4, C-5, C-6
12	0.98 (s)	21.6 (q)	C-1, C-2, C-3, C-6, C-13
13	3.60 (d,7.7); 3.42 (dd, 7.7, 2.3)	77.2 (t)	C-1, C-2, C-4, C-5, C-6, C-12

Coupling constants are listed in parentheses in Hertz; Multiplicities were assigned from DEPT experiments.

Table 3.2 ^{1}H and ^{13}C NMR spectral data of **3.2** and **3.3**.

Position	δ_H **3.2**	δ_C **3.2**	Position	δ_H **3.3**	δ_C **3.3**
2	5.48 (dd, 13.1, 2.6)	76.4 (d)	2	5.155 (dd, 12.8, 2.8)	78.9 (d)
3	3.10 (dd, 17.2, 13.2), 2.72 (dd, 17.2, 2.4)	42.6 (t)	3	2.967 (dd, 17.1, 12.8), 2.653 (dd, 17.2, 2.8)	43.1 (t)
4		196.3 (s)	4		196.0 (s)
5	OH-5, 12.46 (s)	161.9 (s)	5	OH-5, 12.18 (s)	164.1 (s)
6		106.0 (s)	6		108.2 (s)
7		161.7 (s)	7		160.9 (s)
8	6.07 (s)	96.7 (d)	8	5.91 (s)	95.0 (d)
9		165.6 (s)	9		161.0 (s)
10		102.6 (s)	10		102.5 (s)
1′		128.5 (s)	1′		130.7 (s)
2′		126.3 (s)	2′	6.862 (br s)	113.3 (d)
3′	5.48	142.5 (s)	3′		144.5 (s)
4′		144.9 (s)	4′		144.9 (s)
5′	6.82 (d, 8.4)	113.0 (d)	5′	6.788 (d, 8.1)	115.2 (d)
6′	6.95 (d, 8.4)	119.0 (d)	6′	6.725 (d, 8.1)	118.6 (d)
1″	3.10 (m), 2.76 (m)	28.0 (t)	1″	3.197 (d, 6.8)	20.9 (t)
2″	4.34 (d, 7.8)	77.6 (d)	2″	5.195 (t, 6.8)	122.9 (d)
3″		146.7 (s)	3″		135.6 (s)
4″	4.98, 4.88	110.5 (t)	4″	1.716 (s)	15.8 (q)
5″	1.84 (s)	18.6 (q)	5″	1.963 (t, 6.7)	35.8 (t)
1‴	3.46(br d, 6.8)	25.5 (t)	6″	1.582 (dd, 6.7, 6.3)	32.8 (t)
2‴	5.19 (t, 6.4)	121.3 (d)	7″	3.941 (t, 6.2)	75.7 (d)
3‴		139.2 (s)	8″		47.2 (s)
4‴	1.76 (s)	16.3 (q)	9″	4.810 (br s), 4.735 (br s)	110.8 (t)
5‴	2.08 (m)	39.6 (t)	10″	1.628 (s)	17.5 (q)
6‴	2.06 (m)	26.3 (t)			
7‴	5.03 (obs t, 6.8)	123.7 (d)			
8‴		132.3 (s)			
9‴	1.66 (s)	25.7 (q)			
10‴	1.57 (s)	17.7 (q)			

[a]Coupling contants are listed in parentheses in Hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.3 HMBC correlations of **3.2** and **3.3**.

Position	HMBC **3.2**	Position	HMBC **3.3**
2	C-1′, C-2′, C-6′	2	C-3, C-1′, C-2′, C-6′
3	C-2, C-4, C-9	3	C-2, C-4, C-10, C-1′
5	OH-5/ C-5, C-6, C-10	5	
8	C-6, C-7, C-9, C-10	8	C-4, C-6, C-10
2′		2′	C-2, C-3′, C-4′, C-6′
3′		3′	
5′	C-1′, C-3′, C-4′	5′	C-1′, C-3′
6′	C-2′, C-4′	6′	C-2, C-2′, C-4′
1″	C-5, C-6, C-7, C-2″, C-3″	1″	C-5, C-6, C-7, C-2″, C-3″
2″	C-6, C-1″, C-4″, C-5″	2″	C-6, C-1″, C-4″, C-5″
4″	C-2″, C-3″, C-5″	4″	C-2″, C-5″
5″	C-2″, C-3″, C-4″	5″	C-2″, C-4″, C-6″, C-7″
1‴	C-1′, C-3′, C-2‴, C-3‴	6″	C-5″, C-7″
2‴	C-1‴, C-4‴, C-5‴	7″	C-5″, C-6″, C-8″, C-9″, C-10″
3‴		8″	
4‴	C-2‴, C-3‴, C-5‴	9″	C-7″, C-8″, C-10″
5‴	C-2‴, C-4‴, C-6‴, C-7‴	10″	C-7″, C-8″, C-9″
6‴	C-3‴, C-5‴		
7‴			
8‴			
9‴	C-7‴, C-8‴, C-10‴		
10‴	C-7‴, C-8‴, C-9‴		

[a]Coupling contants are listed in parentheses in Hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.4 ^{1}H and ^{13}C spectral data and HMBC correlation of **3.4**

Position	δ_H[a]	δ_C[b]	HMBC
2	5.51 (dd, 13.4, 2.7)	76.35 (d)	C-4, C-1′, C-2′, C-6′
3	3.13 (dd, 17.1, 13.2)	42.60 (t)	C-2, C-4, C-10, C-2′
	2.76 (dd, 17.1, 2.8)		
4	-	196.49 (s)	
5	-	161.28 (s)	
6	-	107.13(s)	
7	-	161.19 (s)	
8	5.97 (s)	95.46 (d)	C-4, C-6, C-7, C-9, C-10
9	-	163.70 (s)	
10	-	102.85 (s)	
1′	-	128.41 (s)	
2′	-	126.35(s)	
3′	-	142.50 (s)	
4′	-	144.72 (s)	
5′	6.85 (d, 8.4)	112.98 (d)	C-1′, C-2′, C-3′
6′	6.99 (d, 8.4)	118.87 (d)	C-2, C-2′, C-4′
1″	3.36 (br d, 7.0)	21.11 (t)	C-5, C-6, C-7, C-3″
2″	5.25 (br t, 7.2)	121.48 (d)	C-6, C-1″, C-4″, C-5″
3″	-	135.22 (s)	
4″	1.82 (s)	17.69 (q)	C-2″, C-3″, C-5″
5″	1.76 (s)	25.66 (q)	C-2″, C-3″, C-4″
1‴	3.46 (br d, 6.7)	25.35 (t)	C-2′, C-3′, C-2‴, C-3‴, C-4‴, C-5‴, C-6‴
2‴	5.18 (br t, 6.3)	121.32 (d)	C-2′, C-1‴, C-4‴, C-5‴
3‴	-	138.80 (s)	
4‴	1.78 (s)	16.25 (q)	C-3‴, C-5‴
5‴	2.08 (m)	39.56 (t)	C-3‴, C-4‴, C-6‴, C-7‴
6‴	2.08 (m)	26.30 (t)	C-3‴, C-5‴, C-7‴
7‴	5.04 (br t, 6.5)	123.70 (d)	C-5‴, C-6‴, C-10‴
8‴	-	132.17 (s)	
9‴	1.67 (s)	25.79 (q)	C-7‴, C-8‴, C-10‴
10‴	1.59 (s)	17.84 (q)	C-7‴, C- 8‴
5-*OH*	12.1 (s)		
3′-*OH*	6.23 (s)		
7-*OMe*	6.23 (s)		
4′-*OMe*	6.23 (s)		

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.5 ^{1}H and ^{13}C spectral data and HMBC correlation of **3.4A**

Position	δ_H[a]	δ_C[b]	HMBC
2	5.56 (dd, 13.4, 2.7)	76.20 (d)	C-3, C-1′, C-2′, C-6′
3	3.06 (dd, 17.1, 13.4) 2.70 (dd, 17.1, 2.7)	43.03 (t)	C-2, C-4, C-10, C-1′
4	-	196.42 (s)	
5	-	160.28 (s)	
6	-	109.91 (s)	
7	-	165.39 (s)	
8	6.03 (s)	90.99 (d)	C-4, C-6, C-7, C-9, C-10, C-1″
9	-	161.69 (s)	
10	-	102.84 (s)	
1′	-	129.92(s)	
2′	-	125.89 (s)	
3′	-	143.65 (s)	
4′	-	146.58 (s)	
5′	6.80 (d, 8.5)	108.49 (d)	C-1′, C-2′, C-3′, C-4′, C-6′
6′	7.04 (d, 8.5)	117.48 (d)	C-2, C-2′, C-3′, C-4′, C-5′, C-1‴
1″	3.24 (d, 7.0)	21.04 (t)	C-5, C-6, C-7, C-2″, C-3″, C-4″, C- 5″
2″	5.17 (t, 7.1)	122.32 (d)	C-1″, C-4″, C-5″
3″	-	131.60 (s)	
4″	1.75 (s)	17.69 (q)	C-2″, C-3″, C-5″
5″	1.66 (s)	25.77 (q)	C-2″, C-3″, C-4″
1‴	3.45 (d, 6.5)	24.54 (t)	C-1′, C-2′, C-3′, C-3‴, C-4‴, C-5‴, C-6‴
2‴	5.09 (t, 6.1)	122.07 (d)	C-2′, C-1‴, C-4‴, C-5‴
3‴	-	135.97 (s)	
4‴	1.67 (s)	16.39 (q)	C-2‴, C-3‴, C-5‴
5‴	1.94 (m)	39.65 (t)	C-2‴, C-3‴, C-4‴, C-6‴
6‴	2.00 (m)	26.66 (t)	C-5‴, C-7‴, C-8‴
7‴	5.02 (t, 7.1)	124.18 (d)	C-5‴, C-6‴, C-9‴, C-10‴
8‴	-	131.45 (s)	
9‴	1.61 (s)	17.61 (q)	C-7‴, C-8‴, C-10‴
10‴	1.53 (s)	25.58 (q)	C-7‴, C- 8‴, C- 9‴
5-*OH*	12.10 (s)	-	C-5, C-6, C-10
3′-*OH*	5.79 (s)	-	C-2′, C-3′, C-4′
7-*OMe*	3.79 (s)	55.78 (q)	C-7, C-8
4′-*OMe*	3.89 (s)	56.02 (q)	C-4′

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.6 ^{1}H and ^{13}C spectral data and HMBC correlation of compound **3.4B**

Position	δ_H[a]	δ_C[b]	HMBC
2	5.50 (dd, 13.5, 2.7)	76.17 (d)	C-4, C-1′, C-2′, C-6′
3	2.69 (dd, 17.1, 2.7)	43.02 (t)	C-2, C-4, C-10, C-1′
	3.07 (dd, 17.1, 13.5)		
4	-	196.35 (s)	
5	-	160.29 (s)	
6	-	109.95 (s)	
7	-	165.39 (s)	
8	6.04 (s)	90.96 (d)	C-4, C-6, C-7, C-9, C-10
9	-	161.68 (s)	
10	-	102.81 (s)	
1′	-	129.57 (s)	
2′	-	134.29 (s)	
3′	-	147.20 (s)	
4′	-	153.11 (s)	
5′	6.87 (d, 8.6)	110.37 (d)	C-1′, C-2′, C-3′, C-4′
6′	7.28 (d, 8.6)	122.14 (d)	C-2, C-2′, C-3′, C-4′
1″	3.24 (d, 7.0)	21.04 (t)	C-5, C-6, C-7, C-2″, C-3″, C- 5″
2″	5.19 (t, 5.8)	122.30 (d)	C-6, C-1″, C-4″, C-5″
3″	-	131.51 (s)	
4″	1.75 (s)	17.73 (q)	C-2″, C-3″, C-5″
5″	1.66 (s)	25.81 (q)	C-2″, C-3″, C-4″
1‴	3.46 (d, 6.2)	24.89 (t)	C-1′, C-2′, C-3′, C-2‴, C-3‴
2‴	5.04 (t, 6.0)	122.72 (d)	C-2′, C-1‴, C-4‴, C-5‴
3‴	-	135.86 (s)	
4‴	1.67 (s)	16.37 (q)	C-2‴, C-3‴, C-5‴
5‴	1.95 (m)	39.61 (t)	C-2‴, C-3‴, C-4‴, C-6‴, C-7‴
6‴	1.99 (m)	26.60 (t)	C-3‴, C-5‴, C-7‴
7‴	5.01 (t, 6.9)	124.06 (d)	C-6‴, C-9‴, C-10‴
8‴	-	131.61 (s)	
9‴	1.60 (s)	25.61 (q)	C-7‴, C-8‴, C-10‴
10‴	1.53 (s)	17.64 (q)	C-7‴, C- 8‴, C- 9‴
7-*OMe*	3.79 (s)	60.66 (q)	C-7,
3′-*OMe*	3.79 (s)	55.78 (q)	C-3′,
4′-*OMe*	3.89 (s)	55.78 (q)	C-4′,
5-*OH*	12.10 (s)		C-5, C-6, C-7, C-10

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.7 ^{1}H and ^{13}C spectral data and HMBC correlations of **3.5**

Position	δ_H[a]	δ_C[b]	HMBC
2	5.49 (dd, 13.2, 2.8)	75.94 (d)	C-1′
3	3.11 (dd, 7.1, 13.3)	42.51 (t)	C-2, C-4, C-1′
	2.73 (dd, 17.2, 2.9)		
4	-	196.28 (s)	
5	-	161.29 (s)	
6	-	107.00 (s)	
7	-	163.69 (s)	
8	5.97 (s)	95.53 (d)	C-5, C-6, C-7, C-9, C-10
9	-	161.08 (s)	
10	-	102.94 (s)	
1′	-	124.83 (s)	
2′	-	118.96 (s)	
3′	-	139.68 (s)	
4′	-	145.10 (s)	
5′	6.84 (d, 8.4)	114.51 (d)	C-1′, C-2′, C-3′, C-4′, C-6′
6′	6.89 (d, 8.4)	118.73 (d)	C-2, C-1′, C-2′, C-4′, C-1″
1″	3.34 (br d, 7.1)	21.12 (t)	C-5, C-6, C-7, C-2″, C-3″
2″	5.23 (br t, 7.0)	121.38 (d)	C-1″, C-4″, C-5″
3″	-	135.64 (s)	
4″	1.80 (s)	17.86 (q)	C-2″, C-3″, C-5″
5″	1.74 (s)	25.81 (q)	C-2″, C-3″, C-4″
1‴	6.59 (d, 10.2)	118.73 (d)	C-1′, C-2′, C-3′, C-6′, C-3‴
2‴	5.66 (d, 10.2)	130.87 (d)	C-2′, C-1‴, C-3‴
3‴	-	79.02 (s)	
4‴	1.40 (s)	26.04 (q)	C-2‴, C-3‴, C-5‴
5‴	2.09 (m)	46.75 (t)	C-7‴
6‴	2.40 (m)	22.77 (t)	C-10‴
7‴	5.07 (br t, 6.6)	123.69 (d)	C-5‴, C-6‴, C-9‴, C-10‴
8‴	-	132.09 (s)	
9‴	1.65 (s)	25.63 (q)	C-7‴, C-8‴, C-10‴
10‴	1.56 (s)	17.64 (q)	C-6‴, C-7‴, C-8‴, C-9‴

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.8 ^{1}H and ^{13}C spectral data and HMBC correlations of **3.6**

Position	δ_H[a]	δ_C[b]	HMBC
2	5.49 (dd, 13.3, 2.5)	76.27 (d)	C-3, C-4, C-9, C-1′, C-2′, C-6′
3	2.70 (dd, 17.2, 2.5)	42.34 (t)	C-2, C-4, C-10, C-1′
	3.08 (dd, 17.2, 13.4)		
4	-	196.77 (s)	
5	-	164.10 (s)	
6	5.98 (d, 1.93)	96.72 (d)	C-5, C-7, C-8, C-10
7	-	165.42 (s)	
8	5.94 (d, 1.95)	95.59 (d)	C-4, C-6, C-7, C-9, C-10
9	-	163.46 (s)	
10	-	102.89 (s)	
1′	-	128.33 (s)	
2′	-	126.55 (s)	
3′	-	142.54 (s)	
4′	-	144.57 (s)	
5′	6.78 (d, 8.37)	113.16 (d)	C-1′, C-3′, C-4′, C-6′
6′	6.91 (d, 8.41)	118.74 (d)	C-2, C-3′, C-4′, C-5′, C-1″
1″	3.46 (br d)	25.24 (t)	C-1′, C-2′, C-3′, C-2″, C-3″, C-4″, C-5″, C-6″
2″	5.11 (t, 6.3)	121.27(d)	C-2′, C-1″, C-4″, C-5″
3″	-	138.55 (s)	
4″	1.72 (s)	16.25 (q)	C-2″, C-3″
5″	2.01 (m)	39.54 (t)	C-2″, C-4″, C-6″, C-7″
6″	2.04 (m)	26.34 (t)	C-3″, C-4″, C-5″, C-7″, C-8″
7″	4.99 (t, 6.6)	123.63 (d)	C-5″, C-9″, C-10″
8″	-	132.17 (s)	
9″	1.63 (s)	25.65 (q)	C-5″, C-6″, C-7″, C-8″, C-10″
10″	1.55 (s)	17.67 (q)	C-5″, C-6″, C-7″, C-8″, C-9″
5-*OH*	12.05 (s)		
7-*OH*	7.56		
3′-*OH*	5.69 (br s)		C-3′, C-4′
4′-*OH*	6.28 (br s)		

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.9 ^{1}H and ^{13}C spectral data and HMBC correlations of **3.6A**

Position	δ_H [a]	δ_C [b]	HMBC
2	5.53 (dd, 13.3, 2.7)	76.22 (d)	C-4, C-1′, C-2′, C-6′
3	3.08 (dd, 17.1, 13.3)	42.88 (t)	C-2, C-4, C-10, C-1′
	2.71 (dd, 17.1, 2.7)		
4	-	196.30 (s)	
5	-	164.15 (s)	
6	6.05 (d, 2.3)	94.98 (d)	C-2, C-5, C-7, C-8, C-10
7	5.02 (t, 6.7)	167.87 (s)	
8	6.01 (d, 2.3)	94.14 (d)	C-6, C-7, C-9, C-10
9	-	163.18 (s)	
10		102.60 (s)	
1′	-	129.75 (s)	
2′	-	125.92 (s)	
3′	-	143.68 (s)	
4′	-	146.40 (s)	
5′	6.82 (d, 8.5)	108.47 (d)	C-1′, C-2′, C-3′, C-4′
6′	7.05 (d, 8.5)	117.52 (d)	C-2′, C-3′, C-4′
1″	3.46 (br d, 6.7)	24.54 (t)	C-1′, C-3′, C-2″, C-3″, C-4″, C-5″
2″	5.09 (t, 6.1)	122.13 (d)	C-2′, C-1″, C-4″, C-5″
3″	-	135.94 (s)	
4″	1.67 (s)	16.24 (q)	C-2″, C-3″, C-5″
5″	2.00 (m)	39.64 (t)	C-2″, C-3″, C-4″, C-6″, C-7″
6″	1.94 (m)	26.64 (t)	C-5″, C-7″, C-8″
7″	5.02 (t, 6.7)	124.08 (d)	C-6″, C-9″, C-10″
8″	-	131.30 (s)	
9″	1.61 (s)	25.63 (q)	C-7″, C-8″
10″	1.54 (s)	17.66 (q)	C-7″, C-8″, C-9
5-*OH*	12.08 (s)		C-5, C-6, C-7, C-9, C-10
3′-*OH*	5.79 (s)		C-2′, C-3′, C-4′
7-*OMe*	3.78 (s)	55.59 (q)	C-7
4′-*OMe*	3.89 (s)	56.00 (q)	C-4′

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.10 ^{1}H and ^{13}C spectral data and HMBC correlations of **3.6B**

Position	δ_H[a]	δ_C[b]	HMBC
2	5.51 (dd, 13.4, 2.7)	76.09 (d)	C-1′
3	3.09 (dd, 17.2, 13.4)	42.90 (t)	C-2, C-4, C-1′
	2.71 (dd, 17.2, 2.8)		
4	-	196.20 (s)	
5	-	164.00 (s)	
6	6.06 (d, 2.3)	95.02 (d)	C-5, C-8
7	-	167.80 (s)	
8	6.01 (d, 2.3)	94.14 (d)	C-6, C-7, C-9, C-10
9	-	163.10 (s)	
10		102.80 (s)	
1′	-	129.20 (s)	
2′	-	134.20 (s)	
3′	-	146.80 (s)	
4′	-	153.20 (s)	
5′	6.87 (d, 8.7)	110.28 (d)	C-1′, C-3′, C-4′
6′	7.25 (d, 8.7)	122.14 (d)	C-2, C-2′, C-4′
1″	3.46 (ob t, 5.5)	24.90 (t)	C-2′, C-3′, C-2″, C-3″
2″	5.04 (t, 6.0)	122.73 (d)	C-4″, C-5″
3″	-	135.70 (s)	
4″	1.66 (s)	16.30 (q)	C-2″, C-3″, C-5″
5″	1.95(m)	39.61 (t)	C-6″
6″	1.99 (m)	26.58 (t)	C-5″
7″	5.01 (ob t, 6.0)	124.07 (d)	C-5″
8″	-	131.40 (s)	
9″	1.60 (s)	25.50 (q)	C-7″, C-8″, C-10″
10″	1.53 (s)	17.60 (q)	C-7″, C-8″, C-9″
7-*OMe*	3.78 (s)	60.71 (q)	C-7
3′-*OMe*	3.79 (s)	55.64 (q)	C-3′
4′-*OMe*	3.87 (s)	55.74 (q)	C-4′
5-*OH*	12.06 (s)		C-5, C-6, C-10

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

Table 3.11 ^{1}H and ^{13}C NMR of compounds **3.7** and **3.7B**

Position	δ_H[a] **3.7**	δ_H[a] **3.7B**	. δ_C[b] **3.7**	δ_C[b] **3,7B**
2	5.163 (d, 12.7)	5.324 (dd, 13.2, 3.3)	78.8 (d)	79.4 (d)
3	2.662 (dd, 17.0, 2.2)	3.079 (dd,) 2.756 (dd,)	43.1 (t)	43.5 (t)
4			196.1 (s)	195.9 (s)
5	5-OH, 12,20 (s)	5-OH, 12.04 (s)	160.8 (s)	160.4 (s)
6			108.2 (s)	103.0
7			164.2 (s)	165.6 (s)
8	5.902 (s)	6.068 (s)	95.0 (d)	90.9 (d)
9			161.1 (s)	161.4 (s)
10			10.5 (s)	110.3 (s)
1'			130.6 (s)	131.1 (s)
2'	6.873 (s)	6.972 (d, 1.6)	113.3 (d)	118.9 (d)
3'			144.9 (s)	149.6 (s)
4'			144.5 (s)	149.7 (s)
5'	6.735 (d, 7.8)	6.885 (d, 8.7)	118.6 (d)	111.6 (d)
6'	6.790 (br d, 7.6)	6.965 (d, 8.7, 1.6)	115.1 (d)	109.8 (d)
1"	3.230 (d, 6.9)	3.248 (d, 7.0)	21.0 (t)	21.0 (t)
2"	5.179 (t, 6.8)	5.152 (t, 7.0)	121.8 (d)	122.3 (d)
3"			136.6 (s)	135.1 (s)
4"	1.726 (s)	1.744 (s)	16.0 (q)	16.1 (q)
5"	1.932 (m)	1.940	39.7 (t)	39.8 (t)
6"	2.008 (m)	2.027	26.6 (t)	26.8 (t)
7"	5.018 (t, 6.9)	5.045 (t, 5.6)	124.2 (d)	124.6 (d)
8"			131.4 (s)	131.2 (s)
9"	1.602 (s)	1.631 (s)	25.5 (q)	17.6 (q)
10"	1.527, (s)	1.559 (s)	17.5 (q)	25.6 (q)
7-*OMe*		3.81 (s)		55.8 (q)
3'-*OMe*		3.90 (s)		56.1 (q)*
4'-*OMe*		3.88 (s)		56.1 (q)*

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.
* overlapped signal

Table 3.12 HMBC correlations of compounds **3.7** and **3.7B**

Position	HMBC **3.7**	HMBC **3.7B**
2	C-4, 1', 5'	C-4, 1', 2', 6'
3	C-2, 4, 10, 1'	C-2, 4, 1'
4		
5		
6		C-2',
7		
8	C-4, 5, 6, 7, 10	C-4, 6, 7, 9; 10, 1"
9		
10		
1'		
2'	C-2, 3', 5'	C-2, 1', 3', 4', 6'
3'		
4'		
5'	C-3'	C-1',3', 4'
6'	C-2, 1', 2', 4'	C-2, 1', 3', 4'
1"	C-5, 6, 7, 2", 3", 4", 5"	C-5, 7, 10, 2", 3", 5"
2"	C-6, 1", 2", 4", 5"	C-1", 4", 5"
3"		
4"	C-6?, 2", 3", 5"	C-3", 5"
5"	C-2", 4", 6", 7"	C-3", 4", 6"
6"	C-3", 5", 7", 8"	C-3", 5"
7"	C-5", 9", 10"	C-9", 10"
8"		
9"	C-7", 8", 10"	C-10"
10"	C-7", 8", 9"	C-9"
5-OH	C-5, 6, 7, 10	C-4, 5, 6, 7, 10

Table 3.13 ^{1}H and ^{13}C spectral data and HMBC correlations of **3.8**

Position	δ_H[a]	δ_C[b]	HMBC
1	-	41.70 (s)	
2	2.47 (d, 18.1)	50.01 (t)	C-1, C-3, C-4, C-6, C-11,
	2.21 (d, 18.1)		C-12
3	-	197.93 (s)	
4	5.82 (br s)	126.03 (d)	C-2, C-6, C-13
5	-	168.09 (s)	
6	-	77.77 (s)	
7	1.97 (m)	34.69 (t)	C-1, C-5, C-6, C-8, C-9
	1.80 (m)		
8	1.61 (m)	33.52 (t)	C-6, C-7, C-9, C-10
	1.50 (m)		
9	3.75 (m)	68.64 (d)	C-7
10	1.20 (d, 6.2)	24.10 (q)	C-8, C-9
11	1.03 (s)	23.86 (q)	C-1, C-2, C-3, C-6, C-12
12	1.07 (s)	23.66 (q)	C-1, C-2, C-3, C-6, C-11
13	2.03 (br s)	21.55 (q)	C-4, C-5

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments

Table 3.14 ^{1}H and ^{13}C spectral data and HMBC correlations of compound **3.9**

Position	δ_H[a]	δ_C[b]	HMBC
1	-	41.13 (s)	
2	2.44 (d, 17.1)	49.64 (t)	C-1, C-3, C-4, C-6, C-11,
	2.24 (d, 17.1)		C-12
3	-	198.21 (s)	
4	5.91 (t, 1.1)	126.79 (d)	C-2, C-6, C-13
5	-	163.09 (s)	
6	-	79.50 (s)	
7	5.78 (d, 15.7)	128.98 (d)	C-5, C-6, C-8, C-9
8	5.85 (dd, 15.6, 5.1)	135.67 (d)	C-6, C-7, C-9, C-10
9	4.40 (dq, 6.3, 5.9)	67.99 (d)	C-7, C-8, C-10
10	1.30 (d, 6.4)	23.67 (q)	C-8, C-9
11	1.08 (s)	22.86 (q)	C-1, C-2, C-6, C-12
12	1.02 (s)	23.99 (q)	C-1, C-2, C-3, C-6, C-11
13	1.90 (d, 1.3)	18.92 (q)	C-3, C-4, C-5, C-6

[a]Coupling contants are listed in parentheses in hertz.

Table 3.15 ^{1}H and ^{13}C spectral data and HMBC correlations of **3.10**

Position	δ_H[a]	δ_C[b]	HMBC
1	-	43.42 (s)	
2	2.36 (d, 17.7) 2.21 (d, 17.7)	48.71 (t)	C-1, C-3, C-4, C-6, C-11, C-12
3	-	209.42 (s)	
4	2.35 (br s)	49.45 (t)	C-3, C-5, C-6, C-13
5	-	83.51 (s)	
6	1.64 (m)	53.70 (d)	C-1, C-2, C-4, C-5, C-7, C-8, C-11, C-12, C-13
7	1.62 (m) 1.35 (m)	21.45 (t)	C-1, C-5, C-6, C-8, C-9
8	1.64 (m) 1.58 (m)	38.62 (t)	C-6, C-7, C-9, C-10
9	3.81 (dd q, 6.09, 6.16, 6.1)	68.08 (d)	C-7, C-8, C-10
10	1.22 (d, 6.18)	23.82 (q)	C-8, C-9
11	1.30 (s)	20.81 (q)	C-1, C-2, C-3, C-6, C-12
12	3.61 (d, 8.0) 3.55 (dd, 8.0, 2.8)	78.37 (t)	C-1, C-2, C-5, C-6, C-11
13	1.06 (s)	24.88 (q)	C-3, C-4, C-5, C-6

[a]Coupling contants are listed in parentheses in hertz.
[b]Multiplicities were assigned from DEPT experiments.

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A novel 29-*nor*-3,4-*seco*-friedelane triterpene and a new guaiane sesquiterpene from the roots of *Phyllanthus oxyphyllus*

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Abstract—An unusual 29-*nor*-3,4-*seco*-friedelan-4(23),20(30)-dien-3-oic acid and a new 5-hydroxy-6,9-epoxyguaiane have been isolated along with other rare terpenes and lignans from *Phyllanthus oxyphyllus* roots. Their structures were elucidated from spectroscopic data. The radical scavenging properties of some of these compounds were evaluated. *seco*-Isolariciresinol showed strong antioxidant activity (IC_{50} 0.017±0.001 mM).

1. Introduction

A large number of *Phyllanthus* species are found throughout most of the tropical and subtropical part of the world and several of which have been widely used in traditional medicines. A considerable number of species have been investigated and some recent studies revealed various bioactive constituents such as alkaloids,[1–3] lignans,[4–6] terpenes,[7–9] flavonoids[10] and tannins.[11,12]

In our ongoing search for bioactive compounds from the Euphorbiaceae we have studied *Phyllanthus oxyphyllus* Miq. (symnonymous name, *P. frondosus* Wall.ex Muell. Arg.), known in Thai as 'yaai chuung laan'. This plant is a shrubby tree which grows to about 1–3 m in height. A hot water decoction of its leaves is used to wash new-born babies as an anti-infective and also as drink to relieve fever, diaphoretic and gonorrhea.[13]

2. Results and discussion

The dichloromethane extract of the roots which caused 100% death to the *Artemia salina* (brine shrimps) nauplii at 100 ppm concentration[14] and exhibited radical scavenging properties towards the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical in a TLC autographic assay was chosen for further purification. Repeated column chromatography of the extract resulted the isolation of a novel 29-*nor*-3,4-*seco*-friedelane triterpene (**1**), a new guaiane sesquiterpene (**2**), from which a mono-acetate derivative (**3**) was prepared, in addition to eight known compounds. The known compounds were identified as lupeol (**4**), β-sitosterol (**5**), 3,12-dihydroxy-cleistantha-8,11,13,15-tetraene, spruceanol (**6**),[15,17] 2,3,12-trihydroxy-cleistantha-8,11,13,15-tetraene, cleistanthol (**7**),[16,17] 2,3-bis-(4′-hydroxy-3′-methoxybenzyl)-butane-1,4-diol, *seco*-isolariciresinol (**8**),[18] 2,6-di-(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo-[3,3,0]-octane, pinoresinol (**9**),[19] 3-oxofriedelan-29-oic acid, polpunonic acid (**10**)[20] and stigmast-4,5-en-3-one (**11**) by comparison of their physical and spectroscopic data with those of the related compounds previously reported.

Compound **1** was obtained as a colourless sticky liquid. The FT-IR spectrum showed the presence of a carboxyl group at ν_{max} 3065 and 1707 cm^{-1} as well as C=C stretching at ν_{max} 1647 cm^{-1}. The EI-MS spectrum gave the molecular ion peak at *m/z* 426 corresponding to $C_{29}H_{46}O_2$. The ^{13}C NMR and DEPT spectra indicated twenty nine carbons comprising five methyl carbons, thirteen methylene carbons of which two were olefinic, four methine carbons and seven quaternary carbons, including one carboxyl and one olefinic quaternary. The presence of a vinylic double bond was revealed from the ^1H chemical shifts at δ_H 5.60 (dd, J=17.4, 10.8 Hz, H-4) and a pair of double doublets at δ_H 4.91 (dd, J=10.7, 1.1 Hz), 4.88 (dd, J=17.3, 1.1 Hz) in addition to the ^{13}C shifts at δ_C 150.9 (d), 111.0 (t). An exocyclic double bond was also implied from the ^1H chemical shift at δ_H 4.57

Keywords: *Phyllanthus oxyphyllus*; Euphorbiaceae; friedelane; guaiane; cleistanthane; lignans; antioxidant.
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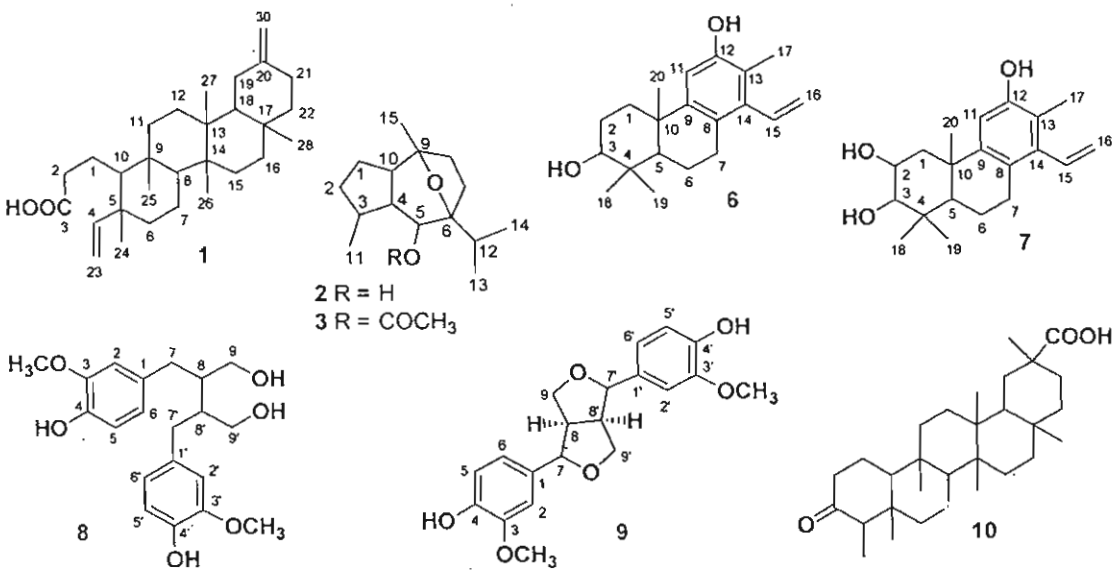

(m, $w_{1/2}$=8.5 Hz) and ^{13}C chemical shifts at δ_C 149.3 (s) and 107.3 (t).

The presence of the key ^3J $^1H-^{13}C$ correlations between H-8/C-10, C-11, C-25 and C-26; as well as H-10/C-1, C-2, C-8, C-24 and C-25 demonstrated the connectivities that would be encountered only in the moiety shown in boldface of the friedelane skeleton (Fig. 1). The presence of a carboxyl group at C-3 which also implied the rupture of a bond joining C-3 and C-4 was deduced from the ^3J $^1H-^{13}C$ correlations between H-1 and H-2/C-3 as well as H-10/C-1, C-2 and C-24. Correlations particularly between H_3-24/C-4, C-6 and C-10 together with correlations between H-4/C-5, C-6, C-10 and C-24 indicated a vinylic double bond at C-4(23). An exocyclic double bond at C-20(30) was implied from the correlations between H-18 (δ_H 1.51)/C-20 and C-28 as well as the correlations between H-30 (δ_H 4.57)/C-19, C-20 and C-21. Full assignment of the ^1H and ^{13}C chemical shifts (Table 1) was based on the HMQC and HMBC spectra. The relative stereochemistry of **1** (Fig. 2) was deduced from the NOESY spectrum. Compound **1** was concluded to be 29-*nor*-3,4-*seco*-friedelan-4(23),20(30)-dien-3-oic acid.

Compound **2** was obtained as a colourless liquid. The FT-IR spectrum revealed the presence of a hydroxyl group (ν_{max} 3430 cm^{-1}) and a cyclic ether (ν_{max} 1261, 939 and 888 cm^{-1}). The HREIMS showed the molecular ion at *m/z* 238.1929 corresponding to $C_{15}H_{26}O_2$. The ^{13}C NMR spectrum showed fifteen carbon signals comprising four methyl, four methylene, five methine and two quaternary carbons. The presence of one oxymethine group and two oxygenated quaternary carbons were deduced from the ^1H and ^{13}C chemical shifts at δ_H 3.69 and δ_C 71.8 (d), 83.0 (s) and 86.4 (s). The guaiane sesquiterpene skeleton and location of oxygenated carbons at C-5, C-6 and C-9 were revealed from the existence of an isopropyl group (δ_H 1.01

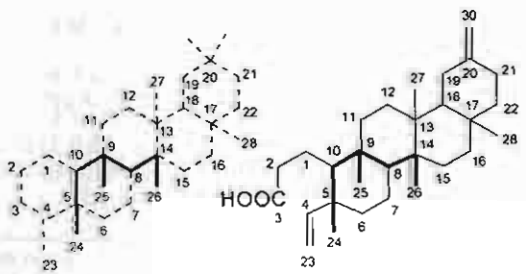

Figure 1.

Table 1. ^1H and ^{13}C NMR spectral data of compound **1** (in $CDCl_3$)

Position	δ_H	δ_C	HMBC
1	1.46	21.2 (t)	C-2, 3, 10
2	2.33	37.1 (t)	C-1, 3, 10
3		179.2 (s)	
4	5.60 (dd, 17.4, 10.8)	150.9 (d)	C-5, 6, 10, 24
5		42.0 (s)	
6	1.44 (α-H), 1.32	41.4 (t)	C-5, 7, 8, 24
7	1.39, 1.30	17.7 (t)	C-5, 6
8	1.29	49.5 (d)	C-7, 10, 11, 15, 25, 26
9		38.7 (s)	
10	0.91	58.4 (d)	C-1, 2, 4, 8, 24, 25
11	1.43, 1.34 α-H	34.9 (t)	C-8, 10, 12, 13, 25
12	1.48, 1.33	28.8 (t)	C-9, 11, 13, 27
13		40.6 (s)	
14		39.5 (s)	
15	1.35, 1.24 α-H	28.3 (t)	C-13, 16, 22, 26
16	1.74, 1.26 α-H	36.1 (t)	C-15, 17, 18, 22, 28
17		31.3 (s)	
18	1.51	45.4 (d)	C-12, 13, 14, 17, 19, 20, 22, 27, 28
19	2.37, 2.27	29.8 (t)	C-13, 17, 18, 20, 21, 30
20		149.3 (s)	
21	2.31, 2.16 (dd, 13.1, 5.0)	30.8 (t)	C-17, 19, 20, 30
22	2.02 (α-H, dt, 13.6, 5.1), 1.10	38.2 (t)	C-16, 17, 20, 21, 28
23	4.91, (dd, 10.7, 1.1), 4.88 (dd, 17.3, 1.1)	111.0 (t)	C-4, 5, 10
24	0.95 (s)	18.1 (q)	C-4, 5, 6, 10, 23
25	0.89 (s)	19.3 (q)	C-8, 9, 10, 11
26	0.83 (s)	15.1 (q)	C-8, 13, 14, 15
27	1.02 (s)	18.1 (q)	C-12, 13, 14, 18
28	1.09 (s)	31.4 (q)	C-16, 17, 18, 22
30	4.57 (m, $w_{1/2}$=8.5)	107.3 (t)	C-19, 20, 21

Assignments were based on COSY, HMQC and HMBC experiments. Coupling constants are listed in parentheses in Hertz.

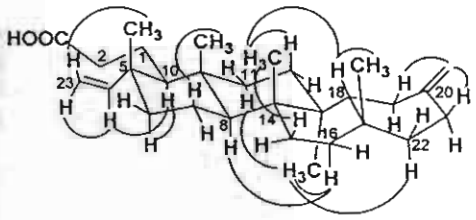

Figure 2. The NOE interactions of 1.

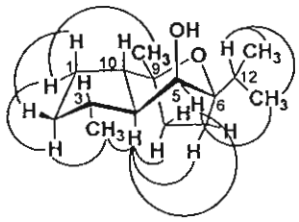

Figure 3. The NOE interactions of 2.

and 1.02, each d, H-13 and H-14 and δ_H 1.94, H-12) together with the $^1H-^1H$ COSY correlations between H-11/H-3; H-3/H-4; H-4/H-10 and H-4/H-5 as well as the long range $^1H-^{13}C$ correlations between H-4/C-5, C-6, C-9, C-10, C-11; H-5/C-3, C-6, C-12; H-10/C-3, C-4, C-9, C-15; H-13/C-6, C-12, C-14 and H-14/C-6, C-12, C-13. Upon acetylation (using acetic anhydride, DMAP and pyridine), the H-5 signal of the acetylated product (3) was observed at the less shielded position (δ_H 5.11) indicating the presence of a free hydroxyl group in 2 at C-5. The C–O–C bridge between C-6 and C-9 could thus be proposed. The ^1H and ^{13}C NMR chemical shifts were fully assigned (Table 2) by the use of HMQC and HMBC spectral data. The relative stereochemistry of 2 (Fig. 3) was obtained from the NOESY spectrum. Compound 2 was established as 5-hydroxy-6,9-epoxyguaiane.

Due to the scarcity of the pure compounds, the brine shrimp toxicity test of the pure compounds was thus not carried out. The observed toxicity in the crude dichloromethane extract is believed to be due partly to the presence of the cytotoxic constituents, such as compounds **6**[15] and **10**.[20] Only compounds **6–8** were evaluated for their antioxidant properties using the DPPH stable radical.[21] Compound **8** showed an IC_{50} 0.017±0.001 mM which is about two times stronger than the standard antioxidant, 2,6-di-(*tert*-butyl)-4-methylphenol (BHT), IC_{50} 0.031±0.001 mM. Compounds **6** and **7** also exhibited rather significant antioxidant properties (IC_{50} 0.124±0.022 and 0.285±0.038 mM, respectively).

3. Experimental

3.1. General

The specific rotations were measured by a Jasco DIP 1020 polarimeter. The IR spectra were obtained on a Perkin–Elmer 1760x FT-IR spectrophotometer. EI-MS and HR-EIMS spectra were recorded on a Finnigan MAT 90 instrument. ^1H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference.

3.2. Plant material

The roots of *Phyllanthus oxyphyllus* Miq. (Euphorbiaceae) were collected from Hin Phoeng Village, Ampur Klongtom, in Krabi Province in July 1997. The botanical identification was achieved through comparison with voucher specimens No. SN 237290-237299 in the herbarium collections of the Sirindhorn Museum, Botanical Section, Department of

Table 2. ^1H and ^{13}C NMR spectral data of compounds 2 and 3 (in $CDCl_3$)

Position	Compound 2			Compound 3		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	1.59 (β-H, m), 0.99 (α-H, m)	25.9 (t)[a]	C-2, 4, 9	1.58 (m); 1.07 (ddd, 11.6, 7.5, 4.1)	24.9 (t)	C-3, 9, 10
2	1.97 (β-H, ddd, 13.0, 11.1, 2.3), 1.16 (α-H, dddd 11.2, 8.4, 5.1, 2.7)	31.6 (t)	C-1, 3, 11	1.91 (m), 1.20 (m)	31.2 (t)	C-1, 3, 4, 11
3	2.28 (β-H, obs. dddq 15.3, 13.0, 7.2, 2.6)	30.5 (d)	C-1, 10, 11	2.04 (br q, 6.9)	31.2 (d)	C-1, 10, 11
4	1.38 (α-H, m)[a]	48.7 (d)	C-1, 2, 3, 5, 6, 9, 10, 11	1.50 (m)	47.5 (d)	C-3, 5, 10, 11
5	3.69 (β-H, d, 10.4)	71.8 (d)	C-3, 4, 6, 7, 12	5.11 (d, 10.3)	72.7 (d)	5-O*C*OCH$_3$, C-4, 6, 7
6		86.4 (s)		–	85.8 (s)	
7	1.78 (ddd, 11.6, 7.2, 2.4), 1.66 (m)	29.2 (t)	C-5, 6, 8, 12	1.74 (m)	30.3 (t)	C-5, 6, 8, 9, 12
8	1.71 (m), 1.38 (m)[a]	31.7 (t)	C-6, 7, 9, 10	1.72 (m)[a]; 1.41 (ddd, 12.4, 6.9, 3.5)	31.5 (t)	C-6, 7, 9, 10, 15
9	–	83.0 (s)	–	–	83.1 (s)	–
10	1.61 (β-H, m)	49.4 (d)	C-1, 2, 3, 4, 8, 9, 15	1.71 (m)[a]	48.9 (d)	C-1, 5, 8, 9, 15
11	0.88 (d, 7.1)	17.0 (q)	C-2, 3, 4	0.87 (d, 7.1)	17.1 (q)	C-3, 4
12	1.94 (qq, 6.9, 6.7)	32.5 (d)	C-5, 6, 7, 13, 14	1.76 (m)	33.3 (d)	C-6, 7, 13, 14
13	1.02 (d, 7.1)[b]	17.5 (q)[b]	C-6, 12, 14	0.96 (d, 6.9)[b]	17.5 (q)[b]	C-6, 12, 14
14	1.01 (d, 6.8)[b]	18.4 (q)[b]	C-6, 12, 13	0.91 (d, 7.1)[b]	18.2 (q)[b]	C-6, 12, 13
15	1.22 (β, s)	24.3 (q)	C-8, 9, 10	1.23 (s)	24.4 (q)	C-8, 10
CH$_3$CO				2.00 (s)	21.4 (q)	5-O*C*OCH$_3$
CH$_3$*CO*					169.9 (s)	

[a] Overlapped signals.
[b] Chemical shifts in the same column may be interchangeable.

Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10903. A voucher specimen (SSPO/1997) is kept at the Chemistry Department, Faculty of Science, Ramkhamhaeng University.

3.3. Extraction and isolation

The dried roots of *Phyllanthus oxyphyllus* were milled to obtain 4.2 kg of powder. The pulverized roots were extracted successively with *n*-hexane, dichloromethane and methanol using a Soxhlet extraction apparatus. Extracts were filtered and concentrated to remove solvent under reduced pressure on a rotary evaporator to yield the pale yellow sludge of *n*-hexane (25.8 g, 0.6% dry wt), reddish brown sludge of dichloromethane (21.2 g, 0.5% dry wt) and reddish brown sludge of methanol (80.8 g, 1.9% dry wt) extracts.

The dichloromethane extract of the roots (21.2 g) was subjected to a silica gel column chromatography with gradient of *n*-hexane/$CHCl_3$ 20:80 to $CHCl_3$/MeOH 50:50 to obtain five fractions. Fraction 3 was subjected to column chromatography (silica gel, *n*-hexane/CH_2Cl_2 80:20 to CH_2Cl_2/EtOAc 10:90) to give 11 subfractions (subfrs. 3.1–3.11). Subfraction 3.5 was purified by recrystallization to give compound **4** (5.3 mg, 1.26×10^{-4}% based on dry wt). Fraction 4 was column chromatographed (silica gel, $CHCl_3$ to $CHCl_3$/MeOH 50:50) to yield six subfractions (subfrs. 4.1–4.6). Subfraction 4.5 was further purified by column chromatography (silica gel, *n*-hexane/CH_2Cl_2 80:20 to CH_2Cl_2/MeOH 50:50) to give 7 subfractions (subfrs. 4.5.1–4.5.7). Subfraction 4.5.5 was subjected to additional column chromatography (2×, silica gel, *n*-hexane/$CHCl_3$ 75:25 to $CHCl_3$/EtOAc 50:50 then $CHCl_3$) to yield subfraction 4.5.5.5 which contained compound **5** (5.5 mg, 1.31×10^{-4}%). Subfraction 4.5.7 was further purified using column chromatography (silica gel, CH_2Cl_2/EtOAc 98:2 to EtOAc/MeOH 50:50) to obtain 18 subfractions (subfrs. 4.5.7.1–4.5.7.18). Additional column chromatography of subfraction 4.5.7.10 (2×, silica gel, *n*-hexane/CH_2Cl_2 to CH_2Cl_2/MeOH 50:50 then *n*-hexane/CH_2Cl_2 10:90 to CH_2Cl_2/MeOH 50:50) yielded compound **6** (13.6 mg, 3.24×10^{-4}%). Column chromatography of subfraction 4.5.7.15 (silica gel, *n*-hexane/EtOAc 80:20 to EtOAc/MeOH 90:10) yielded compounds **7** (11.1 mg, 2.64×10^{-4}%) and **8** (3.5 mg, 8.33×10^{-5}%). Subfraction 4.5.7.11 was further column chromatographed (silica gel, *n*-hexane/EtOAc 85:15 to EtOAc/MeOH 50:50) to obtain 11 fractions. The most polar fraction was purified using HPLC (RP-C-18, MeOH/H_2O 25:75, 0.5 ml/min) to yield compound **9** (R_t=4.0 min, 0.9 mg, 2.14×10^{-5}%). The moderately polar fraction was purified by recrystallization to yield compound **10** (24.6 mg, 5.86×10^{-4}%). Subfraction 4.5.5.10 was purified by column chromatography (silica gel, *n*-hexane/CH_2Cl_2 50:50) to yield further nine subfractions. The moderately polar subfraction was column chromatographed (2×, silica gel, *n*-hexane/EtOAc 95:5 then reversed phase RP C-18, MeOH) to yield compound **2** (24.3 mg, 5.79×10^{-4}%) and compound **11** (13.4 mg, 3.19×10^{-4}%). Subfraction 4.5.7 was column chromatographed (EtOAc/CH_2Cl_2 2:98 to EtOAc/MeOH 50:50) to yield 18 subfractions. Subfraction 4.5.7.5 contained compound **1** (31.2 mg, 7.43×10^{-4}%).

3.3.1. 29-*nor*-3,4-*seco*-Friedelan-4(23),20(30)-dien-3-oic acid (1). Colourless sticky liquid; R_f=0.22 [silica gel, *n*-hexane/EtOAc 9:1]; $[\alpha]_D^{26}=-58.44$ (*c* 0.180, $CHCl_3$); IR (film) ν_{max}: 3065 (br), 2994, 2930, 2868, 2676, 1707, 1647, 1541, 1458, 1412, 1388, 1287, 1219, 1140, 1052, 1004, 911, 882, 759, 674, 610, 471 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 1; EI-MS (70 eV) *m/z* (rel. int.): 426 (M^+, 17), 411 (11), 301 (8), 284 (11), 269 (15), 257 (21), 227 (11), 215 (17), 201 (23), 189 (84), 173 (35), 159 (36), 145 (44), 133 (49), 119 (61), 105 (76), 99 (100), 77 (77); HR-EIMS *m/z* 426.3534 $[M]^+$ (calcd for $C_{29}H_{46}O_2$, 426.3498).

3.3.2. 5-Hydroxy-6,9-epoxyguaiane (2). Colourless liquid; R_f=0.25 [silica gel, *n*-hexane/CH_2Cl_2 2:8]; $[\alpha]_D^{26}=-40.18$ (*c* 0.225, $CHCl_3$); IR (film) ν_{max}: 3430, 2955, 1708, 1470, 1379, 1308, 1261, 1105, 1064, 1006, 939, 888, 787, 592 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 2; EI-MS (70 eV) *m/z* (rel. int.): 239 (M^++1, 100), 238 (42), 222 (16), 221 (95), 203 (25), 149 (18), 127 (10), 109 (9); HR-EIMS *m/z*238.1929 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.3.3. 5-*O*-Acetyl-6,9-epoxyguaiane (3). Colourless sticky liquid, R_f=0.45 [silica gel, *n*-hexane/CH_2Cl_2 2:8]; $[\alpha]_D^{26}=-30.53$ (*c* 0.095, $CHCl_3$); ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 2; EI-MS (70 eV) *m/z* (rel. int.): 281 (M^++1, 60), 280 (30), 222 (15), 221 (100), 203 (23), 177 (21), 149 (29),134 (39), 121 (16), 119 (49), 109 (28), 107 (28), 105 (16), 93 (22); HR-EIMS *m/z* 280.20345 $[M]^+$ (calcd for $C_{17}H_{28}O_3$, 280.20385).

3.4. Bioassay

Compounds **6–8** were tested for radical scavenging properties using DPPH.[21] 50 μL of a solution containing the compound to be tested was added to 5 ml of a 0.004% methanolic solution of DPPH. Absorbance at 517 nm was determined after 30 min at 37°C, and the percent of activity was calculated. IC_{50} is the mean±standard deviation of three assays.

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Bioactive acetylated oleanane triterpene pentosides from the leaves of *Phyllanthus polyphyllus*

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In our ongoing search for biologically active compounds from *Phyllanthus* plants of the Euphorbiaceae which are known to contain varieties of biologically active natural products such as alkaloids,[1-2] lignans,[3-4] terpenes,[5-7] flavonoids[8] and tannins[9] we chose *Phyllanthus polyphyllus* Willd. *var siamensis*, known in Thai as "Sieo Yai". This plant is a deciduous shrub or small tree which grows up to 6-12 m. in height. There have been no reports on medicinal usage, chemical constituents

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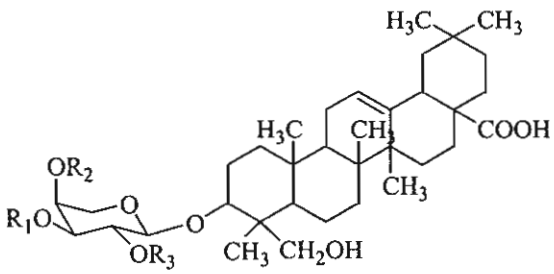
and bioactivity of *P. polyphyllus*. The use of brine shrimp lethality test as an in-house bioassay-guided method for screening plant extracts for cytotoxic constituents, showed that the dichloromethane extract of the leaves of this plant caused 93.3 and 50% death of the brine shrimp nauplii at 250 and 100 ppm concentrations, respectively. The dichloromethane extract was therefore chosen for further investigation. Systematic fractionation of the dichloromethane extract led to the separation of two new acetylated arabinosyl olea-12-en-28-oic acid derivatives (**1-2**) together with seventeen known compounds. The structures of the isolated compounds were elucidated by spectroscopic method. The known compounds were identified as 2'-O-acetyl-3-O-α-L-arabinosyl-23-hydroxy-olea-12-en-28-oic acid (**3**),[10] seco-isolariciresinol (**4**),[11] scopoletin (**5**),[12] meridinol (**6**),[13] dihydrocubebin (**7**),[14] menisdaurilide (**8**),[15] aquilegiolide (**9**),[16] 2,3-dihydromenisdaurilide (**10**),[15] 2,3-dihydroaquilegiolide (**11**),[15] blumenol B (**12**),[17] boscialin (**13**),[18] 7-megastigmen3,6,9-triol (**14**),[19] 3,23-dihydroxyolean-12-en-28-oic acid, hederagenin (**15**),[20] 3-O- α-L-arabinosyl oleanolic acid (**16**),[21] mixture of 3'-O-acetyl-3-O-α-L-arabinosyl oleanolic acid and 4'-O-acetyl-3-O-α-L-arabinosyl oleanolic acid (**17**),[21] β-sitosteryl glucopyranoside (**18**) and 3-O-α-L-arabinosyl hederagenin (**19**)[20] by comparison with the previously reported data.

Compound **1** was obtained as colorless crystalline solid. The FT-IR spectrum showed the presence of a carboxyl group at ν_{max} 3446 and 1717 cm^{-1} as well as C=C stretching at ν_{max} 1653 cm^{-1} .The HR-FABMS revealed the $[M+H]^+$ ion peak at *m/z* 645.40078 corresponding to $C_{37}H_{57}O_9$ .The ^{13}C NMR spectrum revealed the presence of thirty seven carbon signals comprising seven methyl, twelve methylene, nine methine and nine quaternary carbon signals including two carbonyl carbons. The ^1H and ^{13}C spectra exhibiting a trisubstituted olefinic group (δ_H 5.25 and δ_C 143.8 s and 122.4 d) and an oxymethine group [δ_H 3.58; δ_C 84.9 (d)] in addition to a supplementary hydroxymethylene group at δ_H 3.6 and 3.39 and δ_C 66.4 (t) indicated the 3, 23-dihydroxy-olean-12-en skeleton. The presence of an OH group at C-23 was further confirmed by the NOE interaction between H-3/H_2-23 in the NOESY spectrum. The ^{13}C signal at δ_C 182.4 which revealed ^3J correlations particularly with the proton signal

at δ_H 2.79 (H-18) confirmed the placement of the carboxyl group at C-28.The connection of an aglycone and the sugar unit between C-3 and C-1' was evidenced from the long-range ^1H-^{13}C correlations particularly between ^1H signal at δ_H 3.58 (m, H-3) and the acetal ^{13}C signal at δ_C 104.8 (d, C-1'). The presence of an acetyl group [δ_H 2.15 (s) and δ_C 21.0 9 (q) and 171.1 (s)], a doublet of doublet signal at δ_H 4.75 (J=9.9, 3.2 Hz) in addition to the ^1H-^1H COSY spectrum indicating cross-peak between H-1'(δ_H 4.33)/H-2'(δ_H 3.76) which in turn further correlated with the less shielded signal at δ_H 4.75 (H-3') indicated the connection of an O-acetyl group at C-3' of the sugar residue. The ^1H and ^{13}C NMR signals of the sugar residue was closely resembled to those of arabinopyranose.[22] Further ^1H and ^{13}C chemical shifts assignment was secured from the ^1H-^1H COSY, HMQC and HMBC correlations (Tables 1 and 2). Compound **1** was concluded to be 3'-O-acetyl-3-O-α-L-arabinosyl-23-hydroxy-olean-12-en-28-oic acid



1, R_1=$COCH_3$, R_2, R_3=H

2, R_2=$COCH_3$, R_1, R_3=H

3, R_3=$COCH_3$, R_1, R_2=H

Compound **2** was obtained as colorless powder. The FT-IR spectrum showed absorption bands of a carboxyl and an olefinic groups at ν_{max} 3436, 1729 and 1696 cm^{-1}, respectively, The ^1H and ^{13}C NMR spectra showed similar patterns of signal as those of **1.** The presence of one acetate methyl singlet at δ_H 2.07 and a low field oxymethine proton resonated as broad singlet at δ_H 4.98 indicated that the acetyl group is at different position from that found in **1**. The ^1H-^1H COSY and HMBC spectra indicated the attachment of the acetoxyl group at C-4'. Further assignment of ^1H and ^{13}C chemical shifts were

achieved with the use of the ^{1}H-^{1}H COSY, HMQC and HMBC correlation data (Tables 1 and 2). Compound **2** was identified as 4'-O-acetyl-3-O-α-L-arabinosyl-23-hydroxy-olea-12-en-28-oic acid.

Compound **3** was isolated as colorless solid. The FT-IR spectrum showed absorption bands of a carboxyl and an olefinic groups (ν_{max} 3418, 1731 and 1693 cm^{-1}, respectively). The ^{1}H and ^{13}C NMR spectra also showed similar patterns of signals as those of compounds **1** and **2**. The acetate methyl protons and a less shielded oxymethine proton signal resonated at δ_H 2.08 and 4.87 respectively. The ^{1}H-^{1}H COSY and HMBC spectra indicated the placement of the acetyl group at 2'-position of the arabinopyranose unit. Compound **3** was concluded to be 2'-O-acetyl-3-O-α-L-arabinosyl-23-hydroxy-olea-12-en-28-oic acid. This compound was claimed to be isolated firstly from *Patrinia scabiosaefolia* but no detailed ^{1}H and ^{13}C spectral data were however reported.[10] The use of HMQC and HMBC experiments allowed full assignment of the ^{1}H and ^{13}C resonances of **3** as showed in Tables 1 and 2.

Biological Activity. The isolates and chemical transformation products obtained, except for compounds **5**, **6**, **9**, **13**, **14** and **18,** were evaluated for their cytotoxic activity against three human cancer cell lines including human mouth carcinoma (KB), breast cancer (BC) and small cell lung cancer (NCI-H187) as well as their anti-malarial activity against *Plasmodium falciparum* (K1 strain), anti-mycobacterial (anti-TB) activity against *Mycobacterium tuberculosis* H37Ra and antifungal against *Candida albicans*. The active compounds were also tested for toxicity using vero cell. Compounds **8**, **10**, **11**, **12** and **15** were found to be inactive against all tests. Compounds **4** and **7** were inactive in the cytotoxic and anti-malarial assays but showed weak anti-TB activity with MIC values of 200 and 100 μg/ml, respectively (Table 3). Compounds **1**, **3**, **16** and **19** showed weak whereas **2** showed moderate anti-fungal activity. Compounds **1**, **2**, **3**, **16**, **19** and their derivatives **16A** and **19A** showed strong to weak cytotoxic activities against KB, BC and NCI H187 cell lines. It is interesting to note that the acetate derivatives of **16** and **19** (**16A** and **19A**) lost their potency in the anti TB and anti-fungal assays in comparison to the natural occurring compounds **16** and **19**. Whereas in the cytotoxicity assays the acetate derivatives showed either comparable or superior potency to those of the parent compounds,

This study indicated the presence of an arabinosyl moiety in the hederagenin glycosides to be crucial for a cytotoxic activity.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. EI-MS and HR-FABMS spectra were recorded on a Finnigan MAT 90 instrument. ^{1}H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference.

Plant Material and Extract Preparation. The leaves of *P. polyphyllus* (Euphorbiaceae) were collected from Ban Talad Village, Tare District, Ampur Uthumpornpisai, Srisagate Province in June, 1999. Botanical identification was kindly made by Mr. Winai Somprasong, the Sirindhorn Museum, Botanical Section, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok 10903. A voucher specimen (SSPP/1999) is kept at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University. The dried leaves of *P. polyphyllus* were milled to obtain 12.56 kg of powder. The pulverized leaves were extracted successively with *n*-hexane, dichloromethane in a Soxhlet extraction apparatus. After extraction with dichloromethane, the marc was soaked in methanol 15 days. The extracts were filtered and concentrated to remove solvent under reduced pressure on a rotary evaporator to obtain dark green sludge of *n*-hexane (472.33 g), dark green sludge of dichloromethane (83 g), and reddish brown sludge of methanol (824 g).

Isolation of compounds. The dichloromethane extract of the leaves of *P .polyphyllus* (83 g) was subjected to silica gel column chromatography with gradient of n-hexane/CH_2Cl_2 20:80 to CH_2Cl_2/MeOH 100:0 to 0:100 to obtain nineteen fractions. Fraction 10 was column chromatographed (silica gel, n-hexane/EtOAc 90:10 to EtOAc/MeOH 1:100) to obtain twenty-six subfractions (subfrs.10.1 to 10.26). Subfraction 10.17 was further separated by column chromatography (silica gel, CH_2Cl_2/MeOH 99.5:0.5 to 80:20) to yield seventeen subfractions (subfrs. 10.17.1 to 10.17.17). Subfraction 10.17.12 was further purified by column chromatography (silica gel, CH_2Cl_2/MeOH 100:0

to 90:10) to yield compound **4** (9.4 mg). Subfraction 10.20 was column chromatographed (silica gel, CH_2Cl_2/MeOH 100:0 to 90:10) to yield sixteen subfractions (subfrs. 10.20.1 to 10.20.16). Subfraction 10.20.9 was further purified using reversed phase column chromatography (RP-C-18, H_2O/MeOH 70:30 to 0:100) to give compound **5** (6.2 mg). Fraction 11 was subjected to column chromatography (silica gel, n-hexane:CH_2Cl_2 10:90 to CH_2Cl_2/MeOH 30:70) to yield twenty six subfractions (subfrs. 11.1 to 11.26). Subfraction 11.14 was rechromatographed (silica gel, CH_2Cl_2/MeOH 99.5:0.5 to 50:50) and yielded twelve subfractions (subfrs. 11.14.1 to 11.14.12). Subfraction 11.14.4 was further purified by column chromatography (silica gel, n-hexane:EtOAc 85:15 to 50:50) to yield eleven subfractions (subfrs. 11.14.4.1 to 11.14.4.11). Subfraction 11.14.4.9 after column chromatography using reversed phase RP-C-18, H_2O/MeOH 40:60 to 0:100) gave compound **6** (15 mg). Subfraction 11.19 was purified by column chromatography (silica gel, n-hexane/EtOAc 50:50 to 0:100) to obtain fourteen subfractions (subfrs. 11.19.1 to 11.19.14). Subfraction 11.19.8 was column chromatographed (silica gel, n-hexane/EtOAc 80:20 to 75:25) to obtain eight subfractions (subfrs. 11.19.8.1 to 11.19.8.8). Subfraction 11.19.8.8 was further purified by column chromatography (RP-C-18, H_2O/MeOH 50:50 to 0:100) to yield compound **7** (8.8 mg). Subfraction 11.19.14 contained mixture of compounds **8** and **9** which was further separated by column chromatography (2×, silica gel, n-hexane:EtOAc 60:40 to 0:100 then CH_2Cl_2:MeOH 100:0 to 99:1) to yield compound **8** (120.2 mg) and compound **9** (17.4 mg). Subfraction 11.22.11 was column chromatographed (silica gel, CH_2Cl_2:MeOH 98:2 to 50:50) to yield nineteen subfractions (subfrs. 11.22.11.1 to 11.22.11.19). Subfraction 11.22.11.10 was purified (silica gel, CH_2Cl_2:MeOH 98:2 to 80:20) to obtain 12 subfracions (subfrs. 11.22.11.10.1 to 11.22.11.10.12). Subfraction 11.22.11.10.7 contained compound **15** (39.2 mg). Fraction 12 was column chromatographed. (silica gel, CH_2Cl_2:MeOH 98:2 to 50:50) to yield seventeen subfractions (subfrs. 12.1 to 12.17). Subfraction 12.9 was further chromatographed (silica gel, n-hexane:EtOAc 70:30 to 0:100) to get fourteen subfractions (subfrs. 12.9.1 to 12.9.14). Subfraction 12.9.8 was purified using reversed phase column chromatography to yield ten subfractions (subfrs. 12.9.8.1 to 12.9.8.10). Subfraction 12.9.8.3 further purified (RP-C-18, H_2O:MeOH 100:0 to 30:70) to yield three subfractions. The first fraction of which

after column chromatography (silica gel, n-hexane:EtOAc 60:40 to 40:60) yielded compounds **13** (4.8 mg) and **12** (3.5 mg). Subfraction 12.9.9 was purified by reversed phase column chromatography (H_2O:MeOH 100:0 to 40:60) and yielded eight subfractions. Subfraction 12.9.9.6 contained pure compound **17** (14.8 mg). Fraction 15 after repeated column chromatography yielded compound **18** (3.5 mg). Column chromatography of subfraction 12 using CH_2Cl_2:MeOH 98:2 to 50:50 as mobile phase gave seventeen subfractions (subfrs.12.1-12.17). Subfraction 12.11 after column chromatographed (silica gel, n-hexane:EtOAc 70:30 to 0:100 then EtOAc:MeOH 100:0 to 65:35) gave thirteen subfractions (subfrs. 12.11.1-12.11.13). Subfraction 12.11.10 gave six subfractions (subfrs.12.11.10.1-12.11.10.6) after RP-C-18 column chromatography (MeOH:H_2O 10:90 to 100:0). Further column chromatography of subfraction 12.11.10.6 (2x, silica gel, n-hexane:EtOAc 60:40 to 50:50 then silica gel, n-hexane:EtOAc 50:50) gave compounds **10** (163.8 mg) and **11** (260.2 mg). Fraction 13 was column chromatographed (silica gel, n-hexane:EtOAc 50:50) to yield nine subfractions (subfrs. 13.1-13.9). Subfraction 13.4 was further chromatographed (silica gel, n-hexane:EtOAc 65:35) to obtain ten subfractions (subfrs. 13.4.1-13.4.10). Subfraction 13.4.6 contained compound **14** (63.9 mg). Subfraction 13.5 was further chromatographed (silica gel, CH_2Cl_2:MeOH 96:4) to yield five subfractions (subfrs. 13.5.1-13.5.5). Subfraction 13.5.2 was purified using silica gel column chromatography, n-hexane:EtOAc 20:80 to give compound **16** (26.3 mg). Subfraction 13.5.4 was column chromatographed (2×, silica gel, n-hexane:EtOAc 40:60 then CH_2Cl_2: MeOH 97:3) to give compound **19** (10.2 mg). Subfraction 13.4.8 was subjected to column chromatography (silica gel, CH_2Cl_2:MeOH 94:4) to give five subfractions (subfrs.13.4.8.1-13.4.8.5). Subfraction 13.4.8.2 was column chromatographed (reversed phase C-18, MeOH:H_2O 80:20) to give three subfractions (subfrs. 13.4.8.2.1-13.4.2.3). Subfraction 13.4.8.2.1 was further chromatographed (silica gel, CH_2Cl_2:MeOH 96:4) to yield three subfractions (subfrs. 13.4.8.2.1.1-13.4.8.2.1.3). The less polar subfraction 13.4.8.2.1.1 contained compound **1** (20.6 mg). The most polar subfraction 13.4.8.2.1.3 gave compound **2** (30.2 mg). The moderately polar subfraction 13.4.8.2.1.2 after further column chromatographed (silica gel, n-

hexane:EtOAc 30:70) yielded compound **3** (5.5 mg). Compounds **16** and **19** were acetylated using Ac_2O in pyridine to obtain tri-acetate (**16A**) and tetra-acetate (**19A**) derivatives, respectively.

Bioassays. Antimalarial activity was evaluated against *Plasmodium falciparum* (K1 multidrug-resistant strain) cultured continuously according to Trager and Jensen.[23] Quantitative determination of antimaralial activity in vitro was achieved by mean of the microculture radioisotope technique based on Dejardin et al described method.[24] The standard drugs, rifempicin, kanamycin and isoniazide, used as positive control for the antimycobacterial activity showed minimum inhibitory concentration (MIC) of 0.0023, 2.5 and 0.1 μg/ml, respectively. The cytotoxicity assay were was performed using the colorimetric method as that of Skehan and co-workers.[25] The reference compound ellipticine exhibited activities against breast cancer and nasopharyngeal carcinoma cell lines with an IC_{50} of 0.26±0.08 and 0.36±0.07 μg/ml, respectively. *Candida albicans* (ATCC 90028) used for antifungal activity test was grown on potato dextrose agar (PDA) plate at 30°C for 3 days. Three to five single colonies were then suspended in RPMI1640 and cultured in a shaking flask until cell density reaches 2 x10^6 CFU/ml. One hundred µl of the culture was added to each well of 96-well plate containing 100 µl of test sample and incubated at 37°C for 4 hrs. Fifty µl of XTT/PMS mixture solution (mix 1 mg/ml of XTT in RPMI 1640 with 1.53 mg/ml of PMS in PBS at the ratio of 1:6) was added to each well and incubated at 37°C for an additional 4 hrs. Subsequently absorbance at 450 nm was determined using the multilabel counter Victor^{3}V. Orange color developed in each well indicates growth of *C. albicans* while inhibitory effects of the sample result in no change of color. Amphotericin B and DMSO were used as a positive and a negative control, respectively.

3′-O-Acetyl-3-O-α-L-arabinopyranosyl hederagenin (1): mp= 192-194° C, $[\alpha]_D$ 32.400° (*c* 0.125 w/v %, $CHCl_3$); IR (KBr) ν_{max} 3446, 2942, 1717, 1697, 1653, 1457, 1375, 1251, 1092, 757 cm^{-1}; ^{1}H and ^{13}C NMR data (measured in $CDCl_3$) see Tables 1 and 2; HR-FABMS 645.40077 (calcd for $C_{37}H_{57}O_9$, 645.40026)

4′-O-Acetyl-3-O-α-L-arabinopyranosyl hederagenin (2): mp= 272-274° C, $[\alpha]_D$ 70.570° (*c* 0.190 w/v %, MeOH) IR (KBr) ν_{max} 3436, 2943, 1729, 1696, 1456, 1376, 1252, 1092, 758, 647, 603, 454 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Tables 1 and 2; HR-FABMS 645.40023 (calcd for $C_{37}H_{57}O_9$, 645.40026)

2′-O-Acetyl-3-O-α-L-arabinopyranosyl hederagenin (3): $[\alpha]_D$ 28.881° (*c* 0.045 w/v %, $CHCl_3$) IR (KBr) ν_{max} 3418, 2926, 2856, 2651, 1731, 1693, 1455, 1365, 1305, 1269, 1239, 1168, 1137, 1053, 1006, 954, 932, 870, 824, 757, 646 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Tables 1 and 2; HR-FABMS 645.40020 (calcd for $C_{37}H_{57}O_9$, 645.40026)

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Table 1. ^1H NMR spectral data of compounds **1-3**.

Position	**1**	**2**	**3**	HMBC of **1**
1	0.92, 1.59	0.87, 1.55	0.92, 1.56	C-10, C-25
2	1.09, 1.78	1.78	1.67	
3	3.58	3.54	3.63 (dd, 11.6, 4.8)	C-23, C-24, C-1′
5	1.04	1.0	1.13	C-4, C-6, C-10, C-23, C-25
6	1.31, 1.44	1.28, 1.42	n.o	

7	1.24, 1.42	1.22, 1.43	1.25, 1.44	
9	1.50	1.52	1.54	C-1, C-5, C-8, C-10, C-14, C-18, C-25, C-26
11	1.84	1.82	1.83	
12	5.25 (br s)	5.20 (br s)	5.23 (br s)	C-9, C-11, C-14, C-18, C-27
15	1.05, 1.63	0.99	1.03, 1.67	C-27
16	1.57, 1.90	1.54, 1.88	1.93	C-28
18	2.79 (br d, 12.6)	2.76 (dd, 12.3, 3.0)	2.78 (br d, 13.6)	C-12, C-13, C-14, C-16, C-17, C-28
19	1.06, 1.56	1.08, 1.55	1.10, 1.57	C-18, C-20, C-29, C-30
21	1.16, 1.30	1.12, 1.27	1.16, 1.31	C-17, C-20, C-30
22	1.53 , 1.72	1.51, 1.69	1.53, 1.73	C-16, C-17, C-21, C-28
23	0.76 (s)	0.72 (s)	0.61 (s)	C-3, C-4, C-5, C-24
24	3.39 (d, 11.2) 3.6	3.31 (d, 11.3) 3.53	3.34	C-3, C-5, C-23
25	0.94 (s)	0.89 (s)	0.91 (s)	C-1, C-5, C-9, C-10
26	0.73 (s)	0.70 (s)	0.73 (s)	C-7, C-8, C-9, C-14
27	1.10 (s)	1.07 (s)	1.08 (s)	C-8, C-13, C-14, C-15
29	0.88 (s)	0.83 (s)	0.86 (s)	C-19, C-20, C-21, C-30
30	0.90 (s)	0.86 (s)	0.89 (s)	C-19, C-20, C-21, C-29
1'	4.33 (d, 7.49)	4.31 (d, 7.0)	4.57(br d, 4.8)	C-3, C-3′
2'	3.76 (dd, 9.8, 7.6)	3.56 (dd, 9.5, 6.9)	4.87 (dd, 6.9, 5.0)	C-1′, C-3′
3'	4.75 (dd, 9.9, 3.2)	3.62 (dd, 9.4, 3.3)	3.70	C-2′, C-3′-*CO*CH_3
4'	3.99 (br s)	4.98 (br s)	3.84	C-1, C-2′
5'	3.6 , 3.95 (dd, 12.9, 2.1)	3.51, 3.92 (dd, 13.4, 2.3)	3.54, 3.95	C-1′, C-3′, C-4′
CO*CH*$_3$	3', 2.15 (s)	4', 2.07 (s)	2', 2.08 (s)	C-3′, C-3′-*CO*CH_3

Assignments were based on COSY, HMQC and HMBC experiments, coupling constants are listed in parentheses in Hertz. Only the HMBC correlations of **1** is illustrated.

Table 2. ^{13}C NMR spectral data of compounds **1-3**

Position	**1**	**2**	**3**
1	38.1 t	38.0 t	38.1 t
2	24.8 t	24.7 t	25.0 t
3	84.9 d	85.2 d	82.5 d
4	42.5 s	42.4 s	42.6 s
5	48.1 d	48.0 d	47.0 d
6	18.0 t	18.0 t	17.8 t
7	32.4 t	32.3 t	32.5 t
8	39.3 s	39.2 s	39.2 s
9	47.6 d	47.6 d	47.6 d
10	36.8 s	36.7 s	36.5 s
11	23.4 t	23.4 t	23.4 t
12	122.4 d	122.2 d	122.2 d
13	143.8 s	143.8 s	143.8 s
14	41.6 s	41.6 s	41.7 s
15	27.7 t	27.6 t	27.7 t
16	22.9 t	22.9 t	23.0 t
17	46.4 s	46.4 s	46.3 s
18	41.0 d	41.0 d	41.2 d
19	45.9 t	45.9 t	46.0 t
20	30.63 s	30.6 s	30.6 s
21	33.8 t	33.8 t	33.8 t
22	32.4 t	32.4 t	32.5 t
23	12.5 q	12.4 q	12.8 q
24	66.4 t	66.3 t	64.4 t

25	16.0 q	15.9 q	15.8 q
26	17.2 q	16.9 q	16.8 q
27	25.9 q	25.8 q	25.8 q
28	182.4 s	181.6 s	180 4 s
29	33.0 q	33.0 q	33.0 q
30	23.6 q	23.5 q	23.5 q
1'	104.8 d	104.4 d	100.6 d
2'	69.8 d	72.0 d	72.1 d
3'	75.5 d	71.7 d	70.5 d
4'	67.1 d	70.7 d	66.4 d
5'	66.2 t	63.8 t	62.8 t
CO*CH*$_3$	3', 21.0 q	4', 21.0 q	2', 20.9 q
*CO*CH$_3$	171.1 s	171.3 s	170.8 s

Multiplicities were obtained from DEPT experiments

Table 3 Biological activities of the isolates and chemical transformed products

Com pounds	Anti-KB[a]	Anti-BC[a]	Anti-NCI H187[a]	Vero cell[a]	Anti-TB[b]	Anti-fungal[c]	Anti-malarial[d]
1	inac	m, 6.06	w, 11.33	13.5	nd	w, 24.88	nd
2	w, 15.44	S, 3.63	m, 8.65	10.7	50	m, 17.45	nd
3	nd	nd	17.13	nd	inac	w, 43.37	nd
4	inac	inac	inac	nd	200	nd	inac
7	inac	inac	inac	nd	100	nd	inac
16	m, 5.10	S, 1.65	S, 3.17	2.8	50	w, 24.29	nd
19	inac	m, 7.49	w, 13.16	nd	50	w,	inac

						21.78	
16A	inac	S, 3.28	m, 5.58	3.2	200	ina	inac
19A	S, 3.68	S, 3.61	S, 2.23	nd	200	ina	inac

nd=not determined because the amount of the available compounds were insufficient; inac= inactive; s, m and w represent strongly, moderately and weakly active, respectively; [a] IC_{50} in μg/ml, inactive at 20 μg/ml; [b]MIC in μg/ml, inactive at 200 μg/ml; [c] inactive at 50 μg/ml; [d] inactive at 10 μg/ml

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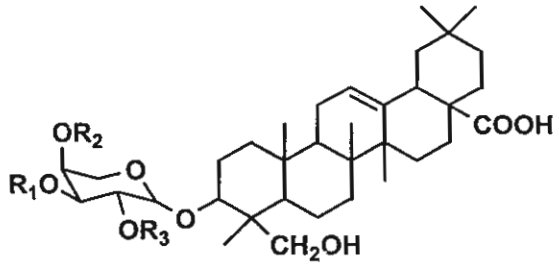
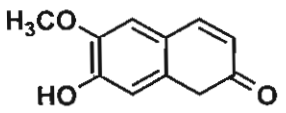
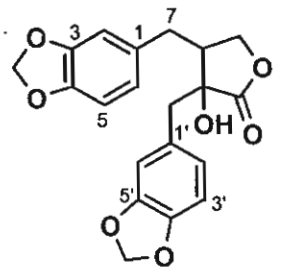
Figure 1 Structures of the isolates





5

	R_1	R_2	R_3
1	$COCH_3$	H	H
2	H	$COCH_3$	H
3	H	H	$COCH_3$
19	H	H	H



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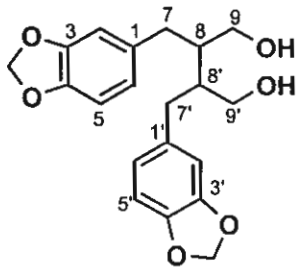

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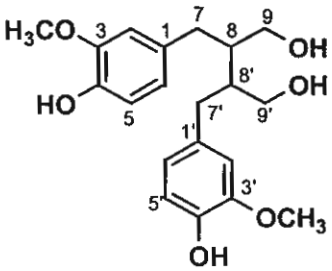

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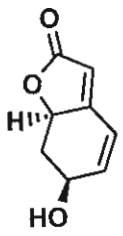

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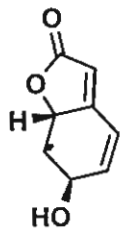

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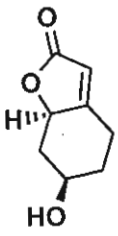

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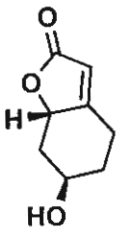

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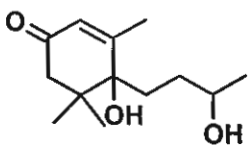

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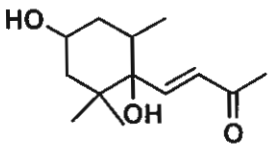

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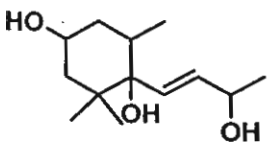

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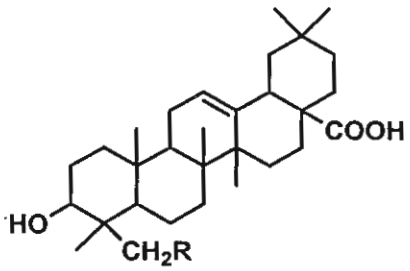
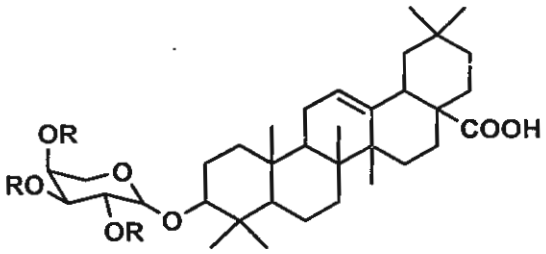


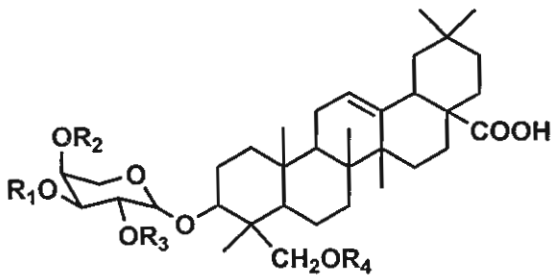
15, R = OH

20, R = H



16, R = H

16A, R = $COCH_3$



19, $R_1 = R_2 = R_3 = R_4 = H$

19A, $R_1 = R_2 = R_3 = R_4 = COCH_3$

Biological activities and new constituents of the leaves of *Macaranga tanarius*

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ABSTRACT From the leaves of *Macaranga tanarius* three new constituents, tanarifuranonol, tetrahydroxytanariflavanone A and tetrahydroxytanariflavanone B together with seven known compounds were isolated and identified. Some isolates and the chemically transformed products were found to possess cytotoxic, antifungal, anti-TB, antioxidation activities as well as inhibitory activity against cycloxygenase-2 assay system.

MANUSCRIPT TEXT *Macaranga tanarius* (Linn.) Muell. Arg.(synonymous name *M. tomentosa* Bl.) is known in Thailand as "Mek". A decoction of the root of this plant is drunk as an antipyretic and also as an anti-tussive. The pounded dried root is used as an emetic agent whereas the pounded fresh leaves used to cover wound as an anti-inflammatory. The crude extract of the leaves showed antibiotic property against *Staphylococcus*.[1] In our on going search for biologically active compounds from the Euphorbiaceae plants particularly the *Macaranga* species,[2] we have studied *M. tanarius*. Some recent investigations on several *Macaranga* species included *M. conifera*,[3] *M. denticulata*,[2] *M. indica*,[4] *M. peltata*,[5] *M. pleiostemona*,[6] *M. triloba*,[7] *M. schweinfurthii*,[8] as well as *M. tanarius*.[9-12] Although there have been reports on chemical constituents of *M. tanarius*, from the *n*-hexane and chloroform extracts of the leaves of this plant which exhibited immediate antioxidation properties towards the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical in a TLC autographic assay we isolated three new constituents (**1-3**) together with seven known compounds (**4-10**). The known compounds, nymphaeol C (**4**),[13, 14] tanariflavanone B (**5**),[13] nymphaeol B (**6**),[14] nymphaeol A (**7**),[14] 7, 8-dihydrovomifoliol, blumenol B (**8**),[13, 15] vomifoliol, blumenol A (**9**)[13, 15] and annuionone E (**10**)[16] were elucidated by spectroscopic methods with comparison to the previously reported data. The isolates and some chemically transformed products were evaluated for their biological activities and some of which showed cytotoxic, antifungal, anti-TB, antioxidation activities as well as inhibitory activity against cycloxygenase-2 assay system.

Compound **1** was obtained as colorless oil (3.2 mg). The HR-FABMS (negative mode) showed the $[M-H]^-$ ion at *m/z* 225.14837 corresponding to $C_{13}H_{21}O_3$. The IR spectrum showed the presence of a hydroxyl (ν_{max} 3406 cm^{-1}), a carbonyl (ν_{max} 1713 cm^{-1}) and an ether (ν_{max} 1127, 1039 cm^{-1}) groups. The ^{13}C-NMR spectrum indicated thirteen carbons including three methyl, five methylene, two methine and

three quaternary carbons among which one is a keto carbonyl (δ_C 207.8). The ^{1}H-NMR spectrum showed three methyl singlet signals at δ_H 1.23 and 0.98 including a less shielded CH_3CO signal at δ_H 2.15 in addition to the presence of a carbinolic proton signal at δ_H 4.06. The ^{1}H-^{1}H COSY spectrum indicated correlations between H-8 (δ_H 2.54)/ H_2-7 (δ_H 1.75 and 1.58); H_2-7/H-6 (δ_H 1.28) as well as correlations between H-3 (δ_H 4.06)/ H_2-2 (δ_H 1.67 and 1.41) and H_2-4 (δ_H 1.88 and 1.41). Attachments of C-4 to C-5 and C-2 to C-1 were revealed from the ^{1}H-^{13}C correlations between H-2/ C-1 (δ_C 43.0), C-3 (δ_C 66.2), C-4 (δ_C 41.5), C-6 (δ_C 53.6), C-12 (δ_C 21.6) and H-4/C-2 (δ_C 39.8), C-3, C-5 (δ_C 83.4), C-6, C-11 (δ_C 25.4), respectively. Connection between C-7 to C-6 was evident from the long range ^{1}H-^{13}C correlations particularly between H-6 (δ_H 1.28)/ C-2, C-4, C-5, C-7 (δ_C 18.5), C-8 (δ_C 43.0), C-11 and C-12. Placement of a keto group at C-9 (δ_C 207.8) was also obtained from the ^{1}H-^{13}C correlations especially between H_2-7 and H_3-10 (δ_H 2.15)/ C-9. The key ^3J ^{1}H-^{13}C correlations particularly between H_2-13 (δ_H 3.60 and 3.42) /C-5 in conjunction with the absence of long range ^{1}H-^{13}C correlation between H_3-12 (δ_H 0.98)/C-5 indicated an ether linkage between C(5)-O-C(13). Compound **1** was identified as tanarifuranonol. Full assignments of the ^{1}H and ^{13}C chemical shifts were established from the HMQC and HMBC correlations (Table 1). The relative configuration of **1** was revealed from the NOESY spectrum (Figure 1) which indicated that the 3-OH group and the ether linkage are both in β–axial arrangements.

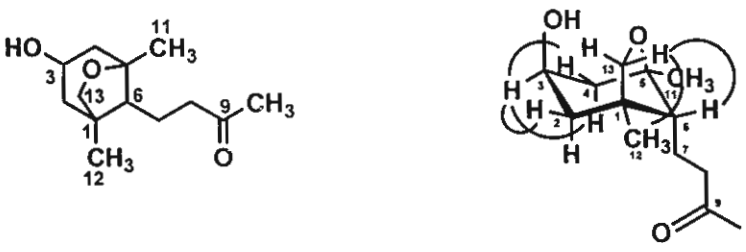
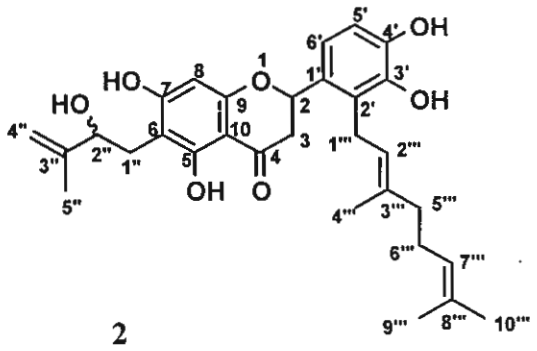


Figure 1 Structure and NOE correlations of **1**

Compound **2** was isolated as yellow amorphous solid. The HR-FABMS (positive mode) which exhibited an $[M+H]^+$ ion at *m/z* 509.25421 corresponded to molecular formula $C_{30}H_{36}O_7$ The IR spectrum exhibited an absorption band of a hydroxyl group at ν_{max} 3430 cm^{-1} and a carbonyl group at ν_{max} 1635 cm^{-1}. The ^{13}C-NMR spectrum of compound **2** showed 30 carbon signals comprising four methyl, six methylene including one vinylic carbon, seven methine and thirteen quaternary carbons including one carbonyl (δ_C 196.3). The ^{1}H- NMR spectrum of compound **2** in $CDCl_3$ (Table 1) exhibited the presence of nonequivalent methylene protons at δ_H 2.72 (1H, dd, J = 17.2 and 2.4 Hz) and 3.10 (1H, dd, J = 17.2 and 13.2 Hz) and a double doublet signal at δ_H 5.48 (1H, dd, J = 13.1 and 2.6 Hz, H-2) commonly found in a flavanone nucleus. The low field signal at δ_H 12.46 (1H, s) indicated a C-5-OH proton intramolecularly H-bonded to C-4 carbonyl oxygen atom. Aromatic proton signals at δ_H 6.95 (1H, d, J = 8.4 Hz) and 6.82 (1H, d, J = 8.4 Hz) indicated the two ortho-coupled protons of the tetrasubstituted aromatic ring. The geranyl group signals were implied from the ^{1}H-^{1}H–COSY spectrum. Signal at δ_H 3.46 (H-1‴) not only correlated with H-2‴ (δ_H 5.19) but also showed long-range ^{1}H-^{1}H correlations with the methyl group signal (H_3-4‴, δ_H 1.76) and the methylene proton signals at δ_H 2.08 (H_2-5‴). The H_2-5‴ also showed correlations with H_3-4‴ and H_2-6‴ (δ_H 2.06). The olefinic proton at δ_H 5.03 (H-7‴) correlated not only with H-6‴ but also with the two methyl group signals at δ_H 1.66 (H_3-9‴) and 1.57 (H_3-10‴). The ^{1}H-^{1}H correlations of the oxymethine proton at δ_H 4.34 (H-2″) with the non-equivalent methylene proton signals at δ_H 3.10 and 2.76 (H_2-1″), a vinylic protons signal at δ_H 4.98 and 4.88 (H_2-4″) as well as a methyl proton signal at δ_H 1.84 (H_3-5″) in the ^{1}H-^{1}H COSY spectrum indicated the presence of a 2-hydroxy-3-methyl-but-3-enyl group.

The long-range ^{1}H-^{13}C correlations particularly between H-2/C-2′ and H-1‴/C-1′, C-3′ and C-2‴ indicated the attachment of a geranyl group to ring B at C-2′. The ^3J ^{1}H-^{13}C correlations between H-1″/C-5, C-6 and C-7 also indicated the bonding of the 2-hydroxy-3-methyl-but-3-enyl group to ring A at C-6. The use of ^{1}H-^{1}H COSY, HMQC, HMBC experiments led to the identification of compound **2** as

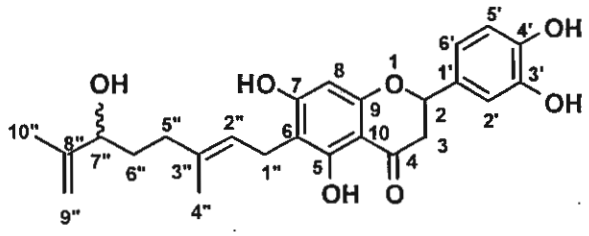
tetrahydroxytanariflavanone A. Full assignment of the ^{1}H and ^{13}C chemical shifts are as shown in Table 1. Stereochemistries at C-2 and C-2″ position are at this stage not studied due to scarcity of the material.



2

Compound **3** was obtained as yellow amorphous solid. Its molecular formula $C_{25}H_{28}O_7$ was obtained from the HR-FAB-MS (positive mode) which exhibited a $[M+H]^+$ ion at *m/z* 441.19210. The FT-IR spectrum showed absorption band at ν_{max} 3382 and 1641 cm^{-1} indicating the presence of a hydroxyl and a carbonyl groups, respectively. The ^{13}C-NMR spectrum of compound **3** showed 25 carbon signals comprising two methyl, five methylene, seven methine and eleven quaternary carbons including one carbonyl (δ_C 196.0). The ^{1}H-NMR spectrum of compound **3** exhibited the presence of C-3 methylene protons at δ_H 2.66 (1H, dd, J =17.2 and 2.8 Hz) and 2.97 (1H, dd, J = 12.8 and 17.1 Hz) as well as the double doublet at δ_H 5.16 (1H, dd, J = 12.8 and 2.8 Hz, H-2) commonly observed in a flavanone nucleus as found in **2**. The presence of a 6-hydroxy-3,7-dimethyl-octa-2,7-dienyl group was revealed from the continuous ^{1}H-^{1}H COSY correlations between H-1″(δ_H 3.20)/H-2″ (δ_H 5.20), H_3-4″ (δ_H 1.72) and H-5″ (δ_H 1.96); H-5″/H-2″, H_3-4″ and H_2-6″ (δ_H 1.58) as well as H-7″ (δ_H 3.94) /H_2-6″, H-9″ (δ_H 4.81 and 4.74) and H_3-10″ (δ_H1.63). The presence of a pair of AB proton doublets at δ_H 6.79 (d, 8.1) and 6.73 (d, 8.1) as well as a broad singlet signal at δ_H 6.862 indicated a tri-substituted aromatic ring. The long range ^{1}H-^{13}C correlations in the HMBC spectrum particularly between H-1″/C-5, C-6, C-7, C-2″, C-3″ indicated the attachment of the 6-hydroxy-3, 7-dimethyl-octa-2,7-dienyl group at C-6 of ring A. Compound **3** could

therefore be identified as tetrahydroxytanariflavanone B. Full assignment of all the ^{1}H and ^{13}C chemical shifts (Table 2) were obtained by using ^{1}H-^{1}H COSY, HMQC, HMBC correlations. Stereochemistries at C-2 and C-7" position are also at this stage not studied due to scarcity of the material.



3

The dimethoxy and trimethoxy derivatives of compounds **4**, **6** and **7** were obtained after reacting compounds **4**, **6** and **7** with diazomethane and further purified using column chromatography. The dimethoxy derivative of **7** (**7A**) was co-eluted with part of **7B** during chromatographic separation and was not obtained in pure state. The spectral data of these derivatives were almost identical to the parent compounds except for the presence of additional OMe signals in the ^{1}H and ^{13}C spectra. All ^{1}H and ^{13}C chemical shifts of these compounds were also fully assigned using the ^{1}H-^{1}H COSY, HMQC and HMBC correlations (Tables 4 to 11, see Supporting Information). It is interesting to note that the rate of methylation using diazomethane generating from diazald/ KOH at the phenolic OH groups in compounds **4**, **6** and **7** were found to be at 4'-OH ~ 7-OH > 3'-OH >> 5-OH. No sign of any product arising from reaction at C-5-OH group was obtained which give further clear evidence for the strong hydrogen bonding between 5-OH group and C-4 carbonyl oxygen.

Biological activities. All of the isolated compounds and some of the methylated products except for compounds **1** and **10** were tested for an anti-inflammatory activity as well as cytotoxic activity using human mouth carcinoma (KB), breast cancer (BC) and small cell lung cancer (NCI-H187) cell lines (Table 3). Only compound **6** showed inhibitory effect to COX-2 cell assay system with IC_{50} of 3.7 μg/ml. The *in vitro* inhibitory potency diminished significantly after replacing hydroxyl groups at C-3', 4'

and 7 with methoxy groups as in **6A** and **6B**. Compounds **2**, **6**, **6A**, **6B**, **7B**, **8** and **9** were inactive with all cytotoxic assays, whereas **7** showed strong cytotoxic activity against all cell lines. Compound **3** was active with KB and BC while **4** active with BC and NCI H-187 and compounds **4A**, **4B** and **5** active selectively with only NCI H187 cell line. Only compounds **6** was submitted for anti-TB assay and found to be moderately active with MIC of 50 μg/ml. Compounds **3**, **7** and **7B** were tested for anti-fungal activity but only **7** was found to moderately inhibit growth of *Candida albicans* with IC_{50} of 11.36 μg/ml. Compounds **4**, **6**, and **7** were tested for anti-oxidation property with DPPH stable radical and found to have comparable radical scavenging property with IC_{50} of 0.015 ±0.002, 0.013 ±0.002 and 0.014 ±0.001 mM, respectively all of which were stronger than 2,6-di-(*tert*-butyl)-4-methylphenol (BHT) which showed IC_{50} of 0.030 ±0.001 mM. The two compounds **3** and **7** which were also submitted for anti malarial test showed no activity.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. EI-MS and HR-FABMS spectra were recorded on a Finnigan MAT 90 instrument. ^{1}H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz spectrometer with the solvent signal as internal reference.

Plant Material and Extract Preparation. The leaves of *M.tanarius* (Euphorbiaceae) used in this study were collected from Trang Province, during April 2001. A voucher specimen (SSMT/2001) is kept at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University.

Isolation of Compounds. The *n*-hexane extract of the leave (143.18 g) was subjected to silica gel column chromatography with gradient of *n*-hexane/EtOAc 95:5 to $CHCl_3$ /MeOH 30:70 to obtain eleven fractions. Fraction 10 was column chromatographed (silica gel, *n*-hexane: $CHCl_3$ 70:30 to $CHCl_3$: MeOH 50:50) to give ten subfractions (subfrs. 10.1 to 10.10). Subfraction 10.9 was further separated by reversed phase colomn chromatography (RP-C-18 silica gel, H_2O: MeOH 20:80 to 0:100) to give six

subfractions (subfrs. 10.9.1 to 10.9. 6). Subfraction 10.9.1 was purified using reversed phase silica gel (H_2O: MeOH 20:80 to 0:100) to give subfractions 10.9.1.1 to 10.9.1.6. Subfraction 10.9.1.3 was chromatographed (silica gel, *n*-hexane: $CHCl_3$ 90:10 to $CHCl_3$: MeOH 20:80 to give nine subfractions. Subfrs. 10.9.1.3.7 contained compound **10** (17.6 mg) and subfraction 10.9.1.3.8 contained compound **8** (10.2 mg). Subfraction 1 0.5 was p urified using silica gel column chromatography (*n*-hexane: C HCl_3 10:90 to $CHCl_3$: MeOH 50: 50) and yielded six subfractions (subfrs. 10.5.1 to 10.5.6). Subracation 10.5.4 contained compound **4** (372.7 mg) Further purification of subfraction 10.5.5 (2×, silica gel, *n*-hexane: $CHCl_3$ 10:90 to $CHCl_3$: MeOH 50:50 then *n*-hexane: $CHCl_3$ 20:80 to $CHCl_3$: MeOH 80:20) gave compound **5** (2.7 mg).

The chloroform extract (122.3 g) was fractionated using silica gel column chromatography with gradient of *n*-hexane: $CHCl_3$ 10:90 to $CHCl_3$: MeOH 20:80) yielded thirteen fractions. Fraction 10 after reversed phase column chromatography (RP-C-18 silica gel, H_2O: MeOH 30:70 to 0:100) gave additional amount of compound **4** (500.5 mg). Fraction 12 was purified using silica gel column chromatography ($CHCl_3$: MeOH 99.5:0.5 to 50:50) yielded six subfractions (subfrs. 12.1 to 12.6). Subfraction 12.2 was column chromatographed using reversed silica gel (H_2O: MeOH 30:70 to 0:100) to give seven subfractions (subfrs.12.2.1 to 12.2 7). Subfraction 12.2.2 was further purified (silica gel, *n*-hexane: EtOAc 95:5 to 70:30) to give four subfractions (subfrs. 12.2.2.1 to 12.2 .2.4). Subfraction 12.2.2.1 contained compound **6** (53.7 mg) and subfraction 12.2.2.4 after column chromatography (silica gel, $CHCl_3$: MeOH 99:1) gave compound **3** (5.3 mg). Subfraction 12.5 was rechromatographed (RP-C-18, silica gel, H_2O: MeOH 30:70 to 0:100) then silica gel column chromatography ($CHCl_3$: MeOH 99:1 to 80:20) gave ten subfractions. Subfraction 12.5.5 contained compound **7** (17.4 mg). Fraction 11 was column chromatographed (silica gel, *n*-hexane: CH_2Cl_2 20:80 to CH_2Cl_2: MeOH 70:30) to yield fourteen subfractions (subfrs 11.1 to 11.14). Subfraction 11.8 was further purified using silica gel column chromatography (*n*-hexane: CH_2Cl_2 10:90 to CH_2Cl_2: MeOH 70:30) to obtain twelve subfractions (subfrs. 11.8.1 to 11.8.12). Subfraction 11.8.9 after column chromatography (silica gel, CH_2Cl_2: MeOH 100:0 to 70:30) gave additional quantity of compound **4** (10.1 mg). Subfraction 11.8.12

was carefully purified using reversed phase column chromatography (RP-C-18 silica gel, H_2O: MeOH 50:50 to 0:100) to give fifteen subfractions. Subfraction 11.8.12.11 contained compound **2** (5.6 mg). Fraction 13 was fractionated using silica gel column chromatography (CH_2Cl_2: MeOH 100:0 to 70:30) to yield fifteen subfractions. Subfraction 13.15 was further purified (silica gel, $CHCl_3$) and the moderately polar fraction (subfr. 13.15.10) was fractionated using reversed phase column chromatography (RP-C-18 silica gel, H_2O: MeOH 40: 60 to 0:100). The most polar fraction after further column chromatography (2×, silica gel, CH_2Cl_2: MeOH 98:2 then CH_2Cl_2: MeOH 99:1) yielded compound **1** (4.5 mg) and compound **9** (21.1 mg).

Tanarifuranonol (1): $[\alpha]_D$ 13.2895° (*c* 0.0760 w/v %, $CHCl_3$) IR (KBr) ν_{max} 3406, 2931, 2870, 1768, 1713, 1455, 1434, 1378, 1265, 1168, 1127, 1039, 1013, 824, 723, 642, 538 cm^{-1} ; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 1; HR-FABMS (negative mode) $[M-H]^-$225.14837 (calcd for $C_{13}H_{21}O_3$, 225.14907)

Tetrahydroxytanariflavanone A, 3′,4′,5,7-tetrahydroxy-2′-(geranyl)-6-(2-hydroxy-3-methylbut-3-enyl)-flavanone (2): $[\alpha]_D$ -3.0566° (*c* 0.2650 w/v %, MeOH) IR (KBr) ν_{max} 3430, 2922, 1635 (br), 1455, 1340, 1294, 1158, 1095, 1005, 902, 817, 758, 550 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 2, HMBC correlations: H-2/ C-1′, C-2′, C-6′; H-3/ C-2, C-4, C-9; H-8/ C-6, C-7, C-9, C-10; H-5′/ C-1′, C-3′, C-4′; H-6′/ C-2′, C-4′; H-1″/ C-5, C-6, C-7, C-2″, C-3″; H-2″/ C-6, C-1″, C-4″, C-5″; H-4″/ C-2″, C-3″, C-5″; H-5″/ C-2″, C-3″, C-4″; H-1‴/ C-1′, C-3′, C-2‴, C-3‴; H-2‴/ C-1‴, C-4‴, C-5‴; H-4‴/ C-2‴, C-3‴, C-5‴; H-5‴/ C-2‴, C-4‴, C-6‴, C-7‴; H-6‴/ C-3‴, C-5‴; H-9‴/ C-7‴, C-8‴, C-10‴; H-10‴/ C-7‴, C-8‴, C-9‴; 5-OH/ C-5, C-6, C-10; HR-FABMS (positive mode) $[M+H]^+$ 509.25421 (calcd for $C_{30}H_{37}O_7$, 509.25393)

Tetrahydroxytanariflavanone B, 3′,4′,5,7-tetrahydroxy-6-(6-hydroxy-3,7-dimethylocta-2′,7′-dienyl)-flavanone (3): $[\alpha]_D$ -12.6809° (*c* 0.2350 w/v %, MeOH) IR (KBr) ν_{max} 2919, 1738, 1635, 1455, 1338, 1296, 1159, 1085, 1019, 815, 777, 453 cm^{-1}; ^1H and ^{13}C NMR data (measured in $CDCl_3$) see Table 2, HMBC correlations: H-2/ C-3, C-1′, C-2′, C-6′; H-3/ C-2, C-4, C-10, C-1′; H-8/ C-4, C-6, C-10;

H-2′/ C-2, C-3′, C-4′, C-6′; H-5′/ C-1′, C-3′; H-6′/ C-2, C-2′, C-4′; H-1″/ C-5, C-6, C-7, C-2″, C-3″; H-2″/ C-6, C-1″, C-4″, C-5″; H-4″/ C-2″, C-5″; H-5″/ C-2″, C-4″, C-6″, C-7″; H-6″/ C-5″, C-7″; H-7″/ C-5″, C-6″, C-8″, C-9″, C-10″; H-9″/ C-7″, C-8″, C-10″; H-10″/ C-7″, C-8″, C-9″; HR-FABMS (positive mode) $[M+H]^+$ 441.19210 (calcd for $C_{25}H_{29}O_7$, 441.19135)

Bioassays. Anti-malarial activity was evaluated against *Plasmodium falciparum* (K1 multidrug-resistant strain) cultured continuously according to Trager and Jensen.[17] Quantitative determination of anti-malarial activity *in vitro* was achieved by mean of the microculture radioisotope technique based on Desjardins et al described method.[17] The anti-mycobacterial activity (anti-TB) assay was performed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[18] The cytotoxicity assays were performed using the colorimetric method as described by Skehan and co-workers.[19, 20] Antifungal test was performed against *Candida albicans* (ATCC 90028) using tetrazolium/formazan assay method.[20, 21] Anti-inflammation (COX-2) assay was performed by analyzing for PGE_2 production in the presence of test samples as described previously by Kirtikara and co-workers.[22] Anti-oxidation assay for radical scavenging property of test compounds was undertaken using DPPH stable radical.[23] Details are presented in the Supporting Information section.

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SUPPORTING INFORMATION PARAGRAPH The tabulated ^{1}H, ^{13}C NMR spectral data and HMBC correlations of **4**, **4A**, **4B**, **6**, **6A**, **6B**, **7** and **7B** (Tables 4 to 11); Brief bioassay methods and Structures of the isolates and chemically transformed products.

Table 1. ^{1}H and ^{13}C NMR spectral data and HMBC correlations of **1**.

Position	δ_H	δ_C	HMBC
1		43.0 (s)	
2	1.67 (m),	39.8 (t)	C-1, C-3, C-4, C-6,
	1.41 (m)		C-12
3	4.06 (dddd, 17.1, 13.5, 10.4, 6.7)	66.2 (d)	
4	1.88 (dd, 13.2, 6.7); 1.41 (m)	41.5 (t)	C-2, C-3, C-5, C-6, C-11
5		83.4 (s)	
6	1.28 (t, 6.3)	53.6 (d)	C-2, C-4, C-5, C-7, C-8, C-11, C-12
7	1.75 (m),	18.5 (t)	C-1, C-5, C-6, C-8,
	1.58 (m)		C-9
8	2.54 (t, 7.8)	43.0 (t)	C-6, C-7, C-9
9		207.8 (s)	
10	2.15 (s)	30.0 (q)	C-8, C-9
11	1.23 (s)	25.4 (q)	C-3, C-4, C-5, C-6
12	0.98 (s)	21.6 (q)	C-1, C-2, C-3, C-6, C-13
13	3.60 (d,7.7);	77.2 (t)	C-1, C-2, C-4, C-5,
	3.42 (dd, 7.7, 2.3)		C-6, C-12

Coupling constants are listed in parentheses in Hertz;

Multiplicities were assigned from DEPT experiments.

Table 2. ^{1}H and ^{13}C NMR spectral data of **2** and **3**.

Position	δ_H **2**	δ_C **2**	Position	δ_H **3**	δ_C **3**
2	5.48 (dd, 13.1, 2.6)	76.4 (d)	2	5.16 (dd, 12.8, 2.8)	78.9 (d)
3	3.10 (dd, 17.2, 13.2), 2.72 (dd, 17.2, 2.4)	42.6 (t)	3	2.97 (dd, 17.1, 12.8), 2.65 (dd, 17.2, 2.8)	43.1 (t)
4		196.3 (s)	4		196.0 (s)
5	OH-5, 12.46 (s)	161.9 (s)	5	OH-5, 12.18 (s)	164.1 (s)
6		106.0 (s)	6		108.2 (s)
7		161.7 (s)	7		160.9 (s)
8	6.07 (s)	96.7 (d)	8	5.91 (s)	95.0 (d)
9		165.6 (s)	9		161.0 (s)
10		102.6 (s)	10		102.5 (s)
1′		128.5 (s)	1′		130.7 (s)
2′		126.3 (s)	2′	6.86 (br s)	113.3 (d)
3′	5.48	142.5 (s)	3′		144.5 (s)
4′		144.9 (s)	4′		144.9 (s)
5′	6.82 (d, 8.4)	113.0 (d)	5′	6.79 (d, 8.1)	115.2 (d)
6′	6.95 (d, 8.4)	119.0 (d)	6′	6.73 (d, 8.1)	118.6 (d)
1″	3.10 (m), 2.76 (m)	28.0 (t)	1″	3.20 (d, 6.8)	20.9 (t)
2″	4.34 (d, 7.8)	77.6 (d)	2″	5.20 (t, 6.8)	122.9 (d)
3″		146.7 (s)	3″		135.6 (s)
4″	4.98, 4.88	110.5 (t)	4″	1.72 (s)	15.8 (q)
5″	1.84 (s)	18.6 (q)	5″	1.96 (t, 6.7)	35.8 (t)
1‴	3.46(br d, 6.8)	25.5 (t)	6″	1.58 (dd, 6.7, 6.3)	32.8 (t)
2‴	5.19 (t, 6.4)	121.3 (d)	7″	3.94 (t, 6.2)	75.7 (d)

3‴		139.2 (s)	8″		147.2 (s)
4‴	1.76 (s)	16.3 (q)	9″	4.81 (br s), 4.74 (br s)	110.8 (t)
5‴	2.08 (m)	39.6 (t)	10″	1.63 (s)	17.5 (q)
6‴	2.06 (m)	26.3 (t)			
7‴	5.03 (obs t, 6.8)	123.7 (d)			
8‴		132.3 (s)			
9‴	1.66 (s)	25.7 (q)			
10‴	1.57 (s)	17.7 (q)			

[a]Coupling contants are listed in parentheses in Hertz.

[b]Multiplicities were assigned from DEPT experiments.

Table 3. Inhibitory activities of some isolates and chemically transformed products against cyclooxygenase-2, KB, BC and NCI-H187 cell lines.

compounds	COX-2[a]	KB[b]	BC[b]	NCI-H187[b]
2	inact	inact	inact	Inact
3	nd	w, 11.71	m, 6.52	inact
4	inact	inact	w, 10.12	w, 10.45
4A	inact	inact	inact	m, 7.30
4B	inact	inact	inact	w, 13.98
5	inact	inact	inact	m, 8.67
6	3.7	inact	inact	inact
6A	inact	inact	inact	inact
6B	inact	inact	inact	inact
7	inact	s, 4.56	s, 2.70	s, 1.46
7B	nd	inact	inact	inact
8	inact	inact	inact	inact
9	inact	inact	inact	inact

nd, inact, s, m and w stand for not determined, inactive, strongly active, moderately active and weakly active, respectively. [a] Aspirin was used as positive control drug, with IC_{50} of 13.6 μg/ml; [b] inactive at 20 μg/ml; With vero cell line, compounds **3** and **7** showed IC_{50} values of 19.5 and 3.8 μg/ml, respectively.

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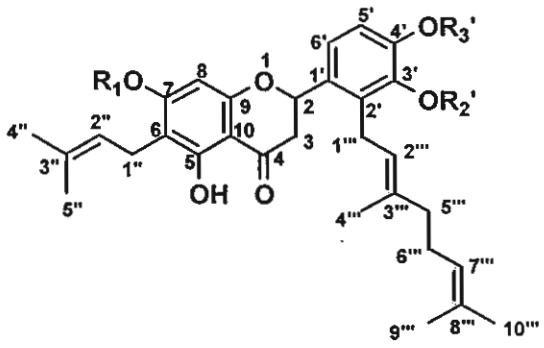
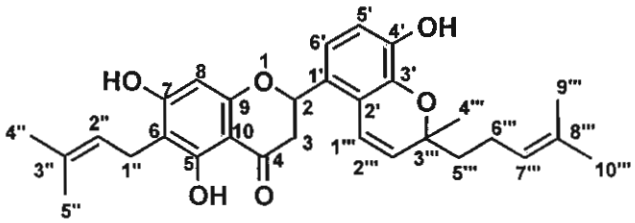
Figure 1 Structures of the isolates and chemically transformed products.



4, $R_1 = R_2 = R_3 = H$
4A, $R_1 = R_3 = Me$, $R_2 = H$
4B, $R_1 = R_2 = R_3 = Me$



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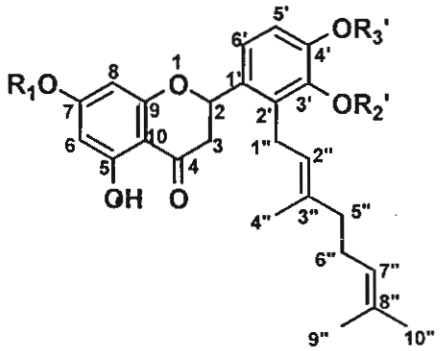
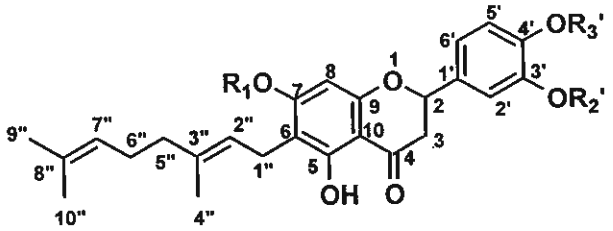
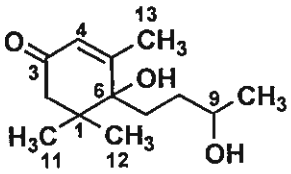


6, $R_1 = R_2 = R_3 = H$
6A, $R_1 = R_3 = Me$, $R_2 = H$
6B, $R_1 = R_2 = R_3 = Me$



7, $R_1 = R_2 = R_3 = H$
7A, $R_1 = R_3 = Me$, $R_2 = H$
7B, $R_1 = R_2 = R_3 = Me$



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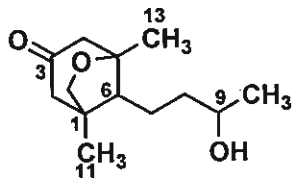

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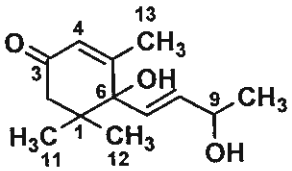

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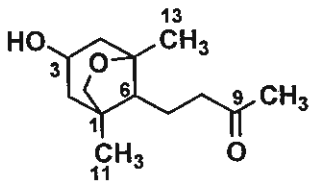

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