Four New Lignans with a Bicyclo[3.3.1]nonadienemethanol Skeleton from *Cunninghamia lanceolata*

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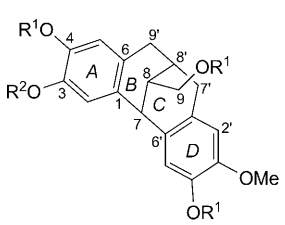
Four unique bicyclo[3.3.1]nonadienemethanol lignans, designated lanceolatanins A (**1**), B (**2**), C (**3**), and D (**4**), along with one previously known compound, isolariciresinol (**5**), were isolated from the MeOH extracts of the heartwood of *Cunninghamia lanceolata.* Their structures were elucidated by application of various spectroscopic methods, including 1D- and 2D-NMR techniques, to their acetylated derivatives **1a**, **2a**, **3a**, and **4a**. Their possible biosynthetic formations are also discussed.

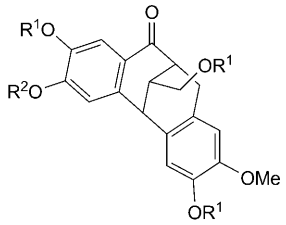
Introduction. – The genus *Cunninghamia* (Taxodiaceae) is an indigenous Chinese tree having two species, *C. lanceolata* and *C. konishii*, in eastern Asia [1]. *C. lanceolata* is cultivated in Taiwan and is used as a traditional Chinese medicine for the treatment of stranguaria, arthritis, and hernia [2]. The chemical constituents of its leaves, roots, and wood have been investigated previously and have been found to contain a series of flavonoids [3][4], diterpenoids [5–8], lignans [9], and essential oils [10][11]. To date, less than 25 secondary metabolites have been isolated from this species. The heartwood of *C. lanceolata* has not been fully investigated. Thus, we herein report the isolation and structural characterization of four new bicyclo[3.3.1]nonadienemethanol lignans from *C. lanceolata*, and also that of one previously known compound, isolariciresinol (**5**). Their structures were elucidated by application of various spectroscopic methods, including 1D- and 2D-NMR techniques, to their acetylated derivatives **1a**, **2a**, **3a**, and **4a** (*Fig. 1*), and their possible biosynthetic formations were discussed.

Results and Discussion. – A mixture of four new bicyclo[3.3.1]nonadienemethanol lignans, lanceolatanins A–D (**1**–**4**), were isolated from the MeOH extract of the heartwood of *C. lanceolata* by column chromatography (CC). Compounds **1**–**4** in the crude mixture were difficult to isolate and purify. Therefore, they were purified after acetylation to produce four pure acetylated derivatives, peracetyllanceolatanins A (**1a**), B (**2a**), C (**3a**), and D (**4a**).

Triacetyllanceolatanin A (**1a**) was assigned a molecular formula $C_{26}H_{28}O_8$ (M^+ at m/z 468.1787) from the HR-EI-MS, with 13 degrees of unsaturation. The ^{13}C-NMR (*Table 1*) and DEPT spectra of **1a** displayed 26 resonances, containing three Ac C=O groups. This was supported by an IR spectrum, which showed absorption bands at 1772



	R^1	R^2
1	H	Me
1a	Ac	Me
2	H	H
2a	Ac	Ac



	R^1	R^2
3	H	Me
3a	Ac	Me
4	H	H
4a	Ac	Ac

Fig. 1. *Structures of Compounds* **1**–**4**[1]) *isolated from* Cunninghamia lanceolata

and 1739 cm^{-1}, indicating the existence of AcOAr and AcOR C=O groups, respectively. Also, 12 aromatic C-atoms were present including four aromatic CH (δ(C) 111.4 and 123.1 in ring *A* and δ(C) 112.8 and 121.9 in ring *D*) and eight

Table 1. *^{13}C-NMR Spectroscopic Data of Compounds* **1a**–**4a**[1])[a]). δ in ppm.

	1a	**2a**	**3a**	**4a**
C(1)	137.8 (*s*)	132.6 (*s*)	123.3 (*s*)	128.4 (*s*)
C(2)	111.4 (*d*)	121.8 (*d*)	109.4 (*d*)	121.6 (*d*)
C(3)	149.2 (*s*)	139.9 (*s*)	155.8 (*s*)	148.2 (*s*)
C(4)	139.7 (*s*)	139.9 (*s*)	149.7 (*s*)	146.3 (*s*)
C(5)	123.1 (*d*)	123.2 (*d*)	122.6 (*d*)	123.3 (*d*)
C(6)	126.0 (*s*)	139.4 (*s*)	138.8 (*s*)	141.0 (*s*)
C(7)	41.2 (*d*)	40.3 (*d*)	41.5 (*d*)	40.8 (*d*)
C(8)	37.2 (*d*)	36.8 (*d*)	38.1 (*d*)	37.8 (*d*)
C(9)	65.6 (*t*)	65.3 (*t*)	63.9 (*t*)	63.7 (*t*)
C(1′)	132.1 (*s*)	131.8 (*s*)	130.3 (*s*)	131.0 (*s*)
C(2′)	112.8 (*d*)	114.6 (*d*)	113.1 (*d*)	113.0 (*d*)
C(3′)	148.9 (*s*)	148.9 (*s*)	150.2 (*s*)	150.3 (*s*)
C(4′)	138.0 (*s*)	137.6 (*s*)	138.2 (*s*)	138.4 (*s*)
C(5′)	121.9 (*d*)	121.9 (*d*)	122.7 (*d*)	122.8 (*d*)
C(6′)	130.6 (*s*)	130.1 (*s*)	128.0 (*s*)	127.7 (*s*)
C(7′)	33.1 (*t*)	32.8 (*t*)	27.6 (*t*)	27.6 (*t*)
C(8′)	26.7 (*d*)	26.5 (*d*)	41.7 (*d*)	41.8 (*d*)
C(9′)	37.6 (*t*)	37.8 (*t*)	198.8 (*s*)	198.9 (*s*)
*Me*O–C(3)	56.1 (*q*)		56.2 (*q*)	
*Ac*O–C(3)		20.6 (*q*), 168.4 (*s*)		20.6 (*q*), 168.0 (*s*)
*Ac*O–C(4)	20.7 (*q*), 169.0 (*s*)	20.5 (*q*), 168.3 (*s*)	20.5 (*q*), 168.6 (*s*)	20.5 (*q*), 167.5 (*s*)
*Me*O–C(3′)	55.9 (*q*)	55.8 (*q*)	55.9 (*q*)	55.9 (*q*)
*Ac*O–C(4′)	20.7 (*q*), 169.3 (*s*)	20.6 (*q*), 168.9 (*s*)	20.7 (*q*), 168.9 (*s*)	20.7 (*q*), 168.8 (*s*)
*Ac*O–C(9)	20.9 (*q*), 171.1 (*s*)	20.8 (*q*), 170.9 (*s*)	20.8 (*q*), 170.9 (*s*)	20.8 (*q*), 170.8 (*s*)

[a]) Multiplicity was determined using DEPT experiments (*s* quaternary; *d* CH; *t* CH_2; *q* Me).

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

quaternary C-atoms, together with two MeO signals (δ(C) 56.1 and 55.9). The remaining six C-atom resonances comprise aliphatic CH_2 (δ(C) 33.1 and 37.6), one CH_2OH (δ(C) 65.6), and three CH groups (δ(C) 26.7, 37.2, and 41.2). On the basis of the above analysis, 11 of the 13 degrees of unsaturation were associated with three C=O groups and two aromatic rings (*A* and *D*), and the remaining two degrees of unsaturation were considered to be accounted for by a bicyclic system (*B* and *C*). The ^{1}H-NMR data (*Table 2*) showed the characteristics of two 1,2,4,5-tetrasubstituted aromatic rings from the chemical shifts at δ(H) 6.68 (*s*, H–C(5)), 6.86 (*s*, H–C(5′)), 6.58 (*s*, H–C(2′)), and 6.70 (*s*, H–C(2)). Two phenylpropane units, elucidated as $PhCH_2CHCH_2$ and $PhCHCHCH_2O$, were evident from the analysis of the 2D-NMR spectra (HMQC, HMBC, and ^{1}H,^{1}H-COSY), suggesting that **1a** is a lignan. The ^{1}H,^{1}H-COSY plot showed ^{1}H,^{1}H coupling between H–C(8) and H–C(7) and H_a–C(9), and between H–C(8′) and H_β–C(7′) and H_β–C(9′) (*Fig. 2*). In the HMBC spectrum, three-bond correlations were found between H–C(5) and C(9′), H–C(5′) and C(7), and H–C(2) and C(7) (*Fig. 2*). The relative configuration of **1a** was determined by analysis of its ^{1}H-NMR chemical shifts and 2D-NOESY correlations. In the ^{1}H-NMR spectrum of **1a**, H–C(7) (δ(H) 3.76) is deshielded relative to H–C(8′) (δ(H) 2.54), indicating that H–C(7) should be equatorially orientated causing the anisotropic deshielding effects of the two aromatic rings *A* and *D*. In the NOESY plot, the signal of H–C(7) showed mutual correlations with those of H–C(8), H–C(2), and H–C(5′), also confirming H–C(7) to be equatorially oriented. In addition, mutual correlations between H_β–C(7′) (δ(H) 3.12) and CH_2(9) (δ(H) 3.86 and 4.10) confirmed the position of CH_2(9) on the right-hand side of the tricycle (*Fig. 3*). Thus, the structure of

Table 2. *^{1}H-NMR Spectroscopic Data of Compounds* **1a**–**4a**[1])[a]). δ in ppm, *J* in Hz.

	1a	**2a**	**3a**	**4a**
H–C(2)	6.70 (*s*)	6.96 (*s*)	6.83 (*s*)	7.24 (*s*)
H–C(5)	6.68 (*s*)	6.84 (*s*)	7.61 (*s*)	7.75 (*s*)
H–C(7)	3.76 (br. *s*)	3.86 (br. *s*)	3.84 (br. *s*)	3.89 (br. *s*)
H–C(8)	2.41 (*dd*, *J* = 7.6, 6.8)	2.40 (*dd*, *J* = 7.6, 6.8)	2.94 (*dd*, *J* = 7.6, 6.8)	2.97 (*dd*, *J* = 7.6, 6.8)
CH_2(9)	4.10 (*dd*, *J* = 11.2, 6.8), 3.86 (*dd*, *J* = 11.2, 7.6)	4.11 (*dd*, *J* = 11.2, 6.8), 3.86 (*dd*, *J* = 11.2, 7.6)	4.13 (*dd*, *J* = 10.8, 6.8), 4.03 (*dd*, *J* = 10.8, 7.6)	4.15 (*dd*, *J* = 10.8, 6.8), 3.86 (*dd*, *J* = 10.8, 7.6)
H–C(2′)	6.58 (*s*)	6.58 (*s*)	6.58 (*s*)	6.58 (*s*)
H–C(5′)	6.86 (*s*)	6.81 (*s*)	6.89 (*s*)	6.85 (*s*)
CH_2(7′)	3.12 (*dd*, *J* = 18.0, 7.2), 2.60 (*d*, *J* = 18.0)	3.12 (*dd*, *J* = 18.0, 7.2), 2.61 (*d*, *J* = 18.0)	3.14 (*dd*, *J* = 17.6, 8.0), 2.87 (*d*, *J* = 17.6)	3.16 (*dd*, *J* = 17.6, 7.2), 2.87 (*d*, *J* = 17.6)
H–C(8′)	2.54 (*t*, *J* = 7.2)	2.55 (*t*, *J* = 7.2)	3.05 (*d*, *J* = 8.0)	3.07 (*d*, *J* = 7.2)
CH_2(9′)	3.22 (*dd*, *J* = 17.6, 7.2), 2.68 (*d*, *J* = 17.6)	3.26 (*dd*, *J* = 17.6, 7.2), 2.75 (*d*, *J* = 17.6)		
AcO–C(3)		2.24 (*s*)		2.25 (*s*)
MeO–C(3)	3.76 (s)		3.90 (*s*)	
AcO–C(4)	2.23 (*s*)	2.20 (*s*)	2.25 (*s*)	2.24 (*s*)
MeO–C(3′)	3.72 (*s*)	3.73 (*s*)	3.72 (*s*)	3.72 (*s*)
AcO–C(4′)	2.25 (*s*)	2.25 (*s*)	2.26 (*s*)	2.28 (*s*)
AcO–C(9)	2.03 (*s*)	2.02 (*s*)	2.03 (*s*)	2.04 (*s*)

[a]) Multiplicities were determined by a ^{1}H,^{1}H-COSY experiment.

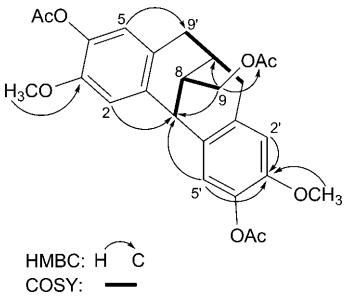


Fig. 2. *Significant HMBCs and* $^1H,^1H$*-COSY correlations of* **1a**[1])

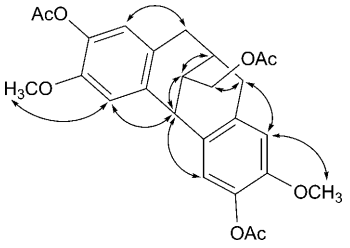


Fig. 3. $^1H,^1H$*-NOESY correlations of* **1a**[1])

triacetyllanceolatanin A (**1a**) was determined, as shown by the formula, to be a unique bicyclo[3.3.1]nonadienemethanol lignan.

Tetraacetylanceolatanin B (**2a**) was assigned the molecular formula $C_{27}H_{28}O_9$ by HR-EI-MS (M^+ at m/z 496.1731) and by the ^{13}C-NMR data (*Table 1*). It had 28 mass units more than **1a**. As anticipated, **2a** had one Ac group more and one Me group less than **1a**. The ^{1}H-NMR spectrum of **2a** indicated four aromatic H-atoms (δ(H) 6.96 (*s*, H−C(2)) and 6.84 (*s*, H−C(5)) for ring *A* and δ(H) 6.81 (*s*, H−C(5′)) and 6.58 (*s*, H−C(2′)) for ring *D*) and two C_3 units of consecutive H-atoms, at δ(H) 3.86 (br. *s*, H−C(7)), 2.40 (*dd*, $J = 7.6$, 6.8 Hz, H−C(8)), 4.11 (*dd*, $J = 11.2$, 6.8 Hz, H_a−C(9)), and 3.86 (*dd*, $J = 11.2$, 7.6 Hz, H_b−C(9)), and at δ(H) 3.12 (*dd*, $J = 18.0$, 7.2 Hz, H_β−C(7′)), 2.61 (*d*, $J = 18.0$ Hz, H_α−C(7′)), 2.55 (*t*, $J = 7.2$ Hz, H−C(8′)), 3.26 (*dd*, $J = 17.6$, 7.2 Hz, H_β−C(9′)), and 2.75 (*d*, $J = 17.6$ Hz, H_α−C(9′)), which were similar to **1a** (*Table 2*). Analysis of the 2D-NMR spectra (HMQC, HMBC, and ^{1}H,^{1}H-COSY) showed that the AcO groups were attached to C(3), C(4), C(4′), and C(9), and the MeO group was attached to C(3′). Thus, tetraacetyllanceolatanin B was unambiguously allocated structure **2a**.

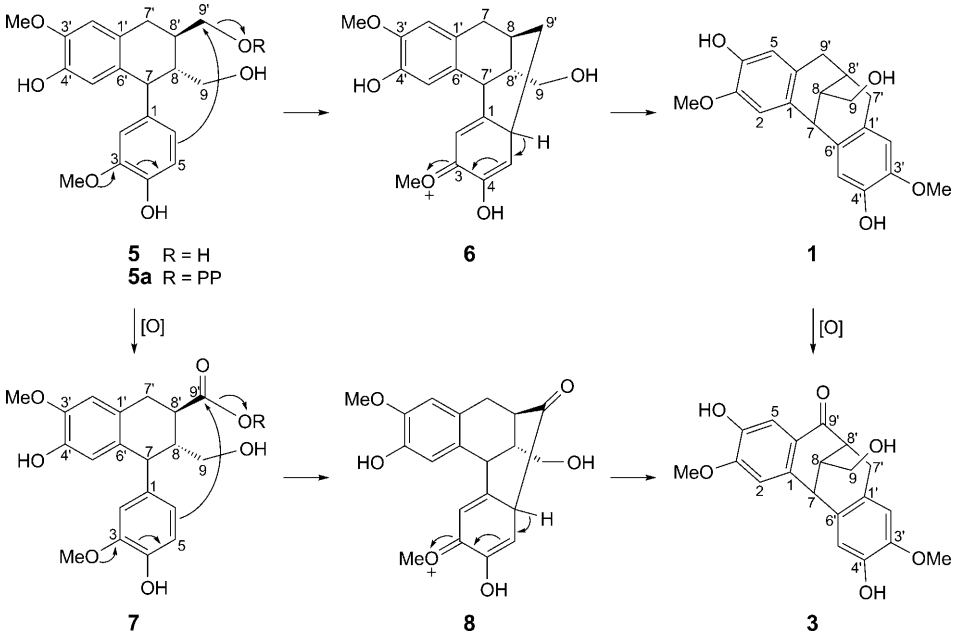
The HR-EI-MS of triacetyllanceolatanin C (**3a**) (m/z 482.1587 (M^+)) showed that it has the molecular formula $C_{26}H_{26}O_9$ with 14 degrees of unsaturation. The IR spectrum of **3a** was indicative of the functionalities ROAc (1739 cm^{-1}), ArOAc (1765 cm^{-1}), a conjugated ketone (1672 cm^{-1}), and aromatic rings (1606 and 1500 cm^{-1}). The MS of **3a** showed that it had 14 mass units more than **1a**, which suggested the presence of an additional oxo group. The NMR data of **1a** and **3a** were also closely related, which indicated the presence of a central bicyclo[3.3.1]nonadienemethanol lignan moiety in **3a** (*Tables 1* and *2*). Careful inspection of the ^{1}H-NMR spectrum of **3a** indicated chemical shifts similar to those of **1a**, except that the signals of CH_2(9′) had disappeared. The UV absorption (λ_{max} 235 and 279 nm) and C=O resonance (δ(C)

198.8) confirmed the conjugated aromatic ketone system. Moreover, the downfield chemical shift of H–C(5) (aromatic CH) resonating at δ(H) 7.61 suggested an *ortho* location of the C=O group at C(9′). Analysis of the HMBC spectra showed correlations from *C*(9′)=O to H–C(5) (δ(H) 7.61) and CH_2(7′) (δ(H) 3.14 and 2.87). The MeO group was predicted to be at C(3) because of the NOESY correlations H–C(2) (δ(H) 6.83)/MeO–C(3) and H–C(7) (δ(H) 3.84). Also the NOESY correlations H–C(2′) (δ(H) 6.58)/MeO–C(3′) and H_β–C(7′), and H_β–C(7′)/H–C(8′) and H–C(9) were observed. This evidence suggested that the C=O group was located at C(9′). Hence, triacetyllanceolatanin C was elucidated as structure **3a**.

The HR-EI-MS analysis (M^+ at *m*/*z* 510.1528) determined the molecular composition of tetraacetyllanceolatanin D (**4a**) to be $C_{27}H_{26}O_{10}$ and thus to have 15 degrees of unsaturation. Its UV spectrum showed absorption maxima at λ_{max} 235, 260, and 280 nm. The ^{1}H- (*Table 2*) and ^{13}C-NMR (*Table 1*) spectra of **4a** were also similar to those of **3a**, except that they featured an Ac group instead of a Me group. The location of the AcO group at C(3) was determined on the basis of the observed HMBCs. Hence, the structure of tetraacetyllanceolatanin D was elucidated as **4a**.

A bicyclo[3.3.1]nonadienemethanol-type lignan was first isolated from *L. tulipifera* [12]. This is the second report on such lignans, *i.e.*, the unique lignans obtained from *C. lanceolata* as established by adequate spectroscopic evidence. The biosynthetic pathways to **1** and **3** can be proposed to start with **5a**, the pyrophosphate (PP) of the concomitantly isolated isolariciresinol (**5**) (*Scheme*). The aromatic ring serves as a nucleophile, which attacks the pyrophosphate moiety to form intermediate **6**. After

Scheme. *Proposed Biosynthetic Formations for* **1** *and* **3**[1])



rearrangement, compound **1** is produced. Compound **3** could be derived from **1** by oxidation. Another pathway may be correlated to compound **7**, which is an oxidative product of **5a**. After nucleophilic cyclization, intermediate **8** is formed, which subsequently rearranges to give **3**. The biosynthetic pathways will be investigated in a future study.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; 70–230 mesh; *Merck*). HPLC: *Hitachi-L-7000* series; *Merck-LiChrospher-Si* (5 μm; 10 × 250 mm) semiprep. column. Optical rotations: *Jasco-DIP-180* digital polarimeter. UV Spectra: *Shimadzu-UV-1601PC* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Magna-550* spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *Varian-Unity-Plus-400* spectrometer; at 400 (^1H) and 100 MHz (^{13}C); δ in ppm, J in Hz; Me_4Si as internal standard; $CDCl_3$ solns.; 2D-NMR by standard pulse sequences. EI-MS: *Finnigan-TSQ-700* spectrometer. HR-EI-MS: *Jeol-JMS-HX300* spectrometer; in *m/z* (rel. %).

Plant Material. The heartwood of *C. lanceolata*, about 7 years old, was collected from Nantou County, Taiwan, in June 1998, and was identified by Mr. *Chii-Cheng Liao*, a technician of the Department of Botany, National Taiwan University. A voucher specimen (No. 19980615) was deposited with the Department of Chemistry, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The air-dried heartwood of *C. lanceolata* (7.1 kg) was extracted for 7 d at r.t. with MeOH (80 l). This procedure was repeated two more times. The collected MeOH extracts were evaporated to give 315.2 g of a crude residue. The residue was extracted with AcOEt/H_2O 1:1 (*v/v*; 3 ×), and the AcOEt extracts were concentrated to give an oil mixture (121.6 g). This oil was mixed with SiO_2 (300 g) and then purified by CC (SiO_2, hexane/AcOEt 5:3, then hexane/AcOEt 5:7 and AcOEt): *Fractions I–III*. *Fr. I* was further purified by CC (SiO_2, hexane/AcOEt 2:1): **5** (9 mg). *Fr. II* was difficult to purify by CC, and the crude mixture (63 mg) was treated with Ac_2O (4 ml) in pyridine (4 ml) at 40° overnight. The mixture was then poured into cold H_2O (30 ml) and stirred for 1 h. The crude mixture was extracted with AcOEt (30 ml × 2), and the combined AcOEt layers were washed with 1N HCl, 3% aq. $NaHCO_3$ soln., and brine. The org. layer was concentrated and the residue purified by CC (SiO_2, hexane/AcOEt 3:1) and then HPLC (hexane/AcOEt 4.2:1): pure **1a** (7 mg), **2a** (21 mg), **3a** (16 mg), and **4a** (20 mg).

Triacteyllanceolatanin A (=*13-[(Acetyloxy)methyl]-5,6,7,12-tetrahydro-3,10-dimethoxy-6,12-methanodibenzo[*a,d*]cyclooctene-2,9-diyl Diacetate*; **1a**): Colorless oil. $[\alpha]_D^{25} = +22.5$ ($c = 0.6$, $CHCl_3$). IR (neat): 2932, 1772, 1739, 1679, 1606, 1500, 1195, 1029, 910. ^1H- and ^{13}C-NMR: *Tables 2* and *1*. EI-MS: 468 (20, M^+), 426 (55), 384 (100), 294 (20), 187 (38). HR-EI-MS: 468.1787 (M^+, $C_{26}H_{28}O_8^+$; calc. 468.1784).

Tetraacetyllanceolatanin B (=*13-[(Acetyloxy)methyl]-5,6,7,12-tetrahydro-9-methoxy-6,12-methanodibenzo[*a,d*]cyclooctene-2,3,10-triyl Triacetate*; **2a**): Colorless oil. $[\alpha]_D^{25} = +23.2$ ($c = 0.6$, $CHCl_3$). IR (neat): 2912, 1772, 1732, 1613, 1500, 1202, 1096, 910. ^1H- and ^{13}C-NMR: *Tables 2* and *1*. EI-MS: 496 (20, M^+), 454 (90), 412 (70), 370 (100), 310 (10), 187 (18). HR-EI-MS: 496.1731 (M^+, $C_{27}H_{28}O_9^+$; calc. 496.1733).

Triacetyllanceolatanin C (=*3,10-Bis(acetyloxy)-13-[(acetyloxy)methyl]-7,12-dihydro-2,9-dimethoxy-6,12-methanodibenzo[*a,d*]cycloocten-5(6*H*)-one*; **3a**): Colorless oil. $[\alpha]_D^{25} = +25.5$ ($c = 0.5$, $CHCl_3$). UV (MeOH): 235 (4.21), 279 (4.0). IR (neat): 2912, 1765, 1739, 1672, 1606, 1500, 1261, 1023. ^1H- and ^{13}C-NMR: *Tables 2* and *1*. EI-MS: 482 (10, M^+), 440 (18), 398 (35), 324 (10), 178 (15), 101 (100). HR-EI-MS: 482.1587 (M^+, $C_{26}H_{26}O_9^+$; calc. 482.1577).

Tetraacetyllanceolatanin D (=*2,3,10-Tris(acetyloxy)-13-[(acetyloxy)methyl]-7,12-dihydro-9-methoxy-6,12-methanodibenzo[*a,d*]cycloocten-5(6*H*)-one*; **4a**): Colorless oil. $[\alpha]_D^{25} = +24.6$ ($c = 0.7$, $CHCl_3$). IR (neat): 2932, 1763, 1732, 1679, 1606, 1500, 1195, 1029, 910. UV (MeOH): 235 (4.18), 260 (4.00), 280

(3.69). ^{1}H- and ^{13}C-NMR: *Tables 2* and *1*. EI-MS: 510 (1, M^+), 468 (7), 101 (85), 83 (15), 59 (100). HR-EI-MS: 510.1528 (M^+, $C_{27}H_{26}O_{10}^+$; calc. 510.1526).

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