Three New 7.3′,8.5′-Connected Bicyclo[3.2.1]octanoids and Other Neolignans from Leaves of *Nectandra amazonum* Nees. (Lauraceae)

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Three new 7.3′,8.5′-connected (macrophyllin-type) bicyclo[3.2.1]octanoid neolignans (nectamazins A—C, 1—3) were isolated from leaves of *Nectandra amazonum* Nees., along with seven known neolignans (4—10). The structures of 1—3 were characterized by spectroscopic methods (1D, 2D NMR) and the absolute configuration was assigned on the basis of circular dichroism (CD) spectra supported by nuclear Overhauser effect spectroscopy (NOESY) correlations. The new compounds showed inhibition activity against platelet activating factor (PAF)-induced aggregation of rabbit platelets.

Key words *Nectandra amazonum*; Lauraceae; macrophyllin-type bicyclo[3.2.1]octanoid; neolignan

The genus *Nectandra* is broadly distributed in Meso and South America which is grown in tropical regions and some members can reach 100 feet.[1)] Its wood is used in furniture, as it preserves well, and easily develops a smooth finish, because of that, in some regions of the world, species of the genus *Nectandra* are also called "Sweetwood." *Nectandra amazonum*, an evergreen tree (formerly *N. ambigua* Meissn.), is a native plant from Amazonian region, as well as in Colombian Amazonia, where is known as "Jigua" or "Canelo" (in Brazil the common name is "Louro") whose use is focused in timber purposes. So far, only one report about chemical constituents from *N. amazonum* had been performed which yielded two furofuran lignans.[2)] Magnoliaceae, Piperaceae and Lauraceae plants are well recognized as sources of neolignans, specially hydrobenzofuran and bicyclooctane-types,[3—5)] which have been found to be excellent platelet activating factor (PAF)-antagonists.[6)] As part of our research on the chemistry of neolignans on Lauraceae species, herein it's described as a phytochemical exploration carried out on leaves of *N. amazonum* affording three new macrophyllin-type bicyclo[3.2.1]octanoid neolignans **1**—**3** (named as nectamazins A—C), along with seven known neolignans (**4**—**10**). The structures of the new compounds were determined by spectroscopic methods such as ^{1}H-, ^{13}C-NMR, 2D NMR and circular dichorism (CD), while the known compounds were established by spectral comparison with data provided by existing literature whose structures are given in Fig. 1. Inhibition of PAF-induced aggregation of rabbit platelets were tested with all purified compounds (**1**—**10**) which exhibited moderate to good inhibitory properties.

Results and Discussion

Starting from the EtOH extract from the leaves of *N. amazonum* yielded three new neolignans (**1**—**3**), along with other constituents of known structure (**4**—**10**), which were isolated by a combination of repeated conventional column chromatography on either sephadex LH-20 or silica gel, using as eluent different solvent mixtures. Bicyclo[3.2.1]octanoid neolignans are further divided into the guianin-type (or 7.1′,8.3′-connected) and the macrophyllin-type (or 7.3′,8.5′-connected),[7)] and compounds **1**—**3**, **8** and **10** has the latter core mentioned above. Although guianin-types are the most known bicyclooctanoids, and only a few species from Piperaceae, Magnoliaceae and Lauraceae have exhibited the occurrence of the macrophyllin-types, other species of *Nectandra* have shown the presence of this type of neolignans.[7)] Following, the spectroscopic features for the new compounds **1**—**3** focusing to their structural elucidation are described.

Compound **1** was obtained as yellowish oil, whose molecular formula of $C_{23}H_{30}O_7$ was determined on the basis of HR-MS-ESI ($[M+H]^+$ *m/z* 419.2054, Calcd for $C_{23}H_{31}O_7$, 419.2070). The IR spectrum indicates the presence of hydroxyl (3421 cm^{-1}), conjugated ketone (1655 cm^{-1}), and

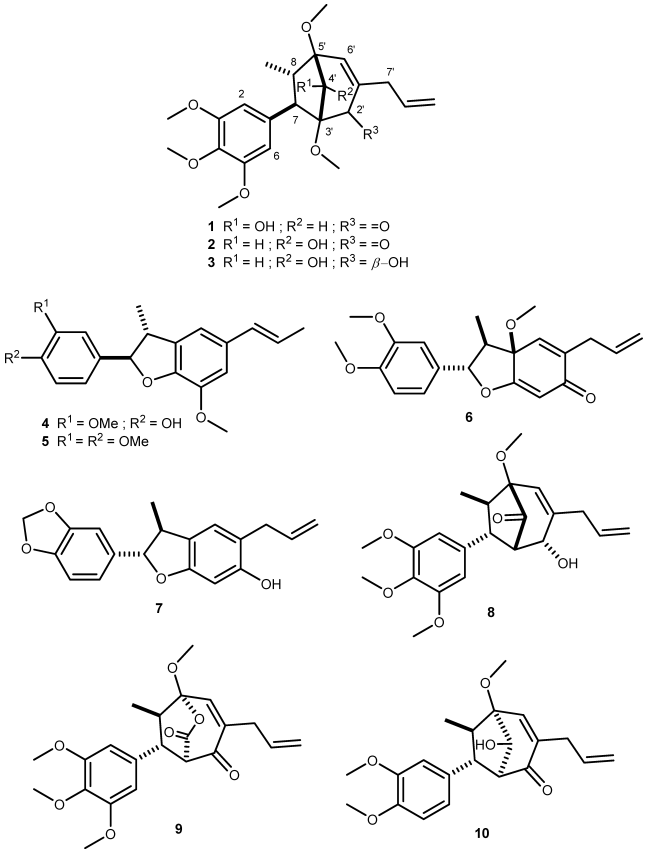

Fig. 1. The Structures of Compounds **1**—**10**

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benzene ring (1610, 1505 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (Table 1) provides evidence of five methoxy groups (three methoxy groups attached to aromatic carbons [δ_H 3.88 (6H, s); 3.85 (3H, s)], and two methoxy groups attached to aliphatic carbons [δ_H 3.45 (3H, s), 3.33 (3H, s)]), an allyl group [δ_H 5.16—5.09 (2H, m), 5.91—5.84 (1H, ddt, J=16.5, 9.7, 6.8 Hz), 3.08—3.03 (2H, m)], a methyl group [δ_H 0.92 (3H, d, J=7.2 Hz)], a symmetric 3,4,5-trioxyphenyl group [δ_H 6.58 (2H, s)], an olefinic proton as part of a double bond conjugated with ketone system [δ_H 6.90 (1H, s)], a carbinol proton [δ_H 4.25 (1H, s)], a benzylic methine [δ_H 2.44 (1H, d, J=7.7 Hz)] and a methine proton [δ_H 2.89—2.85 (1H, m)]. The ^{13}C-NMR spectrum of **1** (Table 1) exhibited the presence of a carbonyl group (δ_C 198.0), a 3,4,5-trioxyphenyl group [δ_C 136.9 (×2), 105.2 (×2), 153.1 (×2)], two carbinyl carbons (δ_C 88.1, 88.8), a carbinol carbon (δ_C 75.0), three methoxy groups on aromatic ring (δ_C 56.4 (×2), 60.7) and two methoxy groups attached to aliphatic carbon (δ_C 54.5, 53.7). Analysis of the ^{1}H-, ^{13}C-NMR spectral data of **1** (Table 1) indicated that the molecule consisted of two C_6C_3 moieties forming a bicyclo[3.2.1]octanoid core,[8] which was confirmed by heteronuclear multiple bond connectivity (HMBC) spectral evidences. The HMBC spectrum showed a long-range correlation between both methine protons H-7 [δ_H 2.44 (1H, d, J=7.7 Hz)] and H-8 [δ_H 2.89—2.85 (1H, m)] with C-4′ (δ_C 75.0), and both H_2-7′ [δ_H 3.08—3.03 (2H, m)] of allyl group and H-6′ [δ_H 6.90 (1H, s)] with carbonyl carbon C-2′ (δ_C 198.0), which established the connectivity of two C_6C_3 moieties. The coupling constant of benzylic methine H-7 (J=7.7 Hz) indicated an Ar-7/CH_3-8 *trans*-relationship.[8,9] Relative configuration was deduced through a nuclear Overhauser effect spectroscopy (NOESY) experiment: a key NOE correlation between methyl group signal H-9 and olefinic proton of the enone moiety H-6′ indicated the *endo* conformation of methyl group; and a key NOE correlation between H-4′ and H-6′ was a sign of the relative configuration of hydroxy group toward aryl group (Fig. 2). Absolute configuration was defined by the CD measurements of **1** put on view a positive Cotton effects ([θ]$_{327}$ +8799 and [θ]$_{259}$ +18740) which are consistent with reported data for related molecules[9,10] with defined stereochemistry. By this means, the structure of **1** was determined to be (7*R*,8*S*,4′*R*,3′*S*,5′*S*)-$\Delta^{8'}$-4′-hydroxy-3,4,5,3′,5′-pentamethoxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan (nectamazin A).

Interestingly, compound **2** has also the condensed formula $C_{23}H_{30}O_7$ assigned by HR-MS-ESI analysis ([M+H]$^+$ *m/z* 419.2055, Calcd for $C_{23}H_{31}O_7$, 419.2070). Spectral comparison (IR, ^{1}H-, ^{13}C-NMR) (Table 1) indicated **2** is a 4′-*epimer* of **1** since the significant difference between them is the shifting to down field of both carbon C-4′ (δ_C 81.2) and its proton H-4′ [δ_H 4.58 (1H, d, J=1.8 Hz)]. Furthermore, other

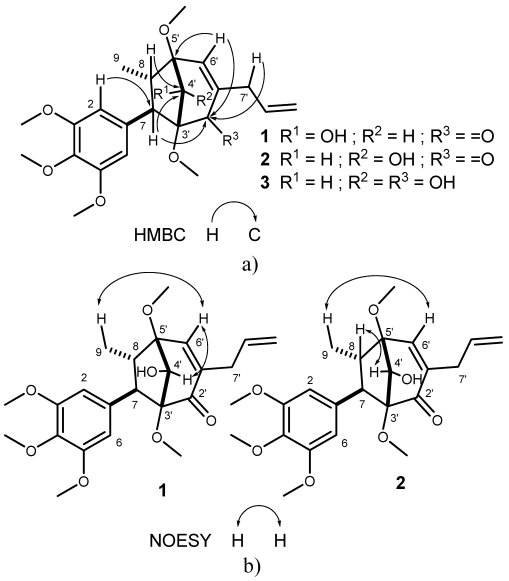

Fig. 2. (a) Key HMBC Correlations of Compounds **1**—**3** and (b) Key NOESY Correlations of Compounds **2** and **3**

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts (δ) of Compounds **1**—**3** (400 MHz ^{1}H, 100 MHz ^{13}C, $CDCl_3$)

No.	**1**		**2**		**3**	
	δ_C	δ_H (∫, m, J Hz)	δ_C	δ_H (∫, m, J Hz)	δ_C	δ_H (∫, m, J Hz)
1, 4	136.9		136.5		140.2	
2, 6	105.2	6.58 (2H, s)	104.8	6.62 (2H, s)	104.7	6.60 (2H, s)
3, 5	153.1		153.8		154.1	
7	56.5	2.44 (1H, d, J=7.7)	53.8	3.01 (1H, d, J=7.5)	50.1	3.25 (1H, d, J=7.3)
8	47.6	2.89—2.85 (1H, m)	46.6	2.45—2.40 (1H, m)	48.1	2.88—2.84 (1H, m)
9	13.6	0.92 (3H, d, J=7.2)	13.2	0.95 (3H, d, J=7.0)	14.0	0.93 (3H, d, J=6.9)
1′	139.9		140.2		140.6	
2′	198.0		196.7		76.5	4.81 (1H, s)
3′	88.1		87.5		88.5	
4′	75.0	4.25 (1H, s)	81.2	4.58 (1H, d, J=1.8)	72.8	4.15 (1H, s)
5′	88.8		89.2		89.0	
6′	148.4	6.90 (1H, s)	149.0	6.55 (1H, d, J=1.9)	126.9	5.71 (1H, s)
7′	32.8	3.08—3.03 (2H, m)	32.7	3.09—3.05 (2H, m)	37.1	3.11—3.07 (2H, mdd, J=17.3, 7.0)
8′	134.4	5.91—5.84 (1H, ddt, J=16.5, 9.7, 6.8)	134.1	5.89—5.83 (1H, ddt, J=16.0, 10.4, 6.8)	135.0	5.96—5.87 (1H, ddt, J=17.1, 9.5, 7.1)
9′	118.0	5.16—5.09 (2H, m)	118.1	5.10—5.05 (2H, m)	117.8	5.6—5.12 (2H, m)
OME-3,5	56.3	3.88 (6H, s)	56.4	3.88 (6H, s)	56.1	3.85 (6H, s)
OME-4	60.7	3.85 (3H, s)	61.0	3.86 (3H, s)	60.9	3.80 (3H, s)
OME-5′	54.5	3.45 (3H, s)	54.0	3.48 (3H, s)	54.0	3.55 (3H, s)
OME-3′	53.7	3.33 (3H, s)	53.9	3.35 (3H, s)	54.3	3.32 (3H, s)

Table 2. PAF-Antagonistic Activity of Compounds **1**—**10**

	Compounds										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**C**[b]
IC_{50} (μM)[a]	1.4±0.72	1.7±0.53	3.8±1.8	N.D.	65.3±9.7	75.6±10.1	18.4±4.5	6.8±1.3	4.5±1.4	2.3±0.97	0.9±0.22

a) The data were expressed as means 95% confidence intervals of 4 rabbits. *b*) Ginkgolide B (BN52021) as positive control. N.D.: not detected.

two NMR spectral evidences explain this statement: 1) the occurrence of a *W*-coupling between carbinol proton H-4′ (d, J=1.8 Hz) and olefinic proton H-6′ [δ_H 6.62 (1H, d, J=1.9 Hz)] and 2) a key NOESY correlation between protons H-4′ and H-8 [δ_H 2.45—2.40 (1H, m)]. HMBC and NOESY correlations (Fig. 2) for compound **2** were also similar with those of **1** confirming both the origin of two C_3C_6 units and the *endo*-methyl group. The absolute configuration of **2** was defined to be identical with that of **1** because the CD spectrum shows positive Cotton effects at 337 and 268 nm ($[\theta]_{337}$ +7438 and $[\theta]_{268}$ +15235). Therefore the structure of **2** was established to be (7*R*,8*S*,4′*S*,3′*S*,5′*S*)-$\Delta^{8'}$-4′-hydroxy-3,4,5,3′,5′-pentamethoxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan (nectamazin B).

Compound **3** has a condensed formula as $C_{23}H_{32}O_7$ which was assigned by HR-MS analysis ($[M+H]^+$ *m/z* 421.2208, Calcd for $C_{23}H_{33}O_7$, 421.2226). The ^{1}H-, ^{13}C-NMR spectra of **3** have a similar signal pattern with that of **1** except for the presence of an additional carbinol carbon [δ_H 4.81 (1H, s); δ_C 76.5] as a replacement for the carbonyl carbon, explaining the absence of a respective IR band. In addition, the olefinic proton is shifted to high field which is a feature of a non-conjugated olefin. HMBC spectrum of **3** has long-range C–H correlations as follows: 1) between both signal of protons H-7 [δ_H 3.25 (1H, d, J=7.3 Hz)] and H-8 [δ_H 2.88—2.84 (1H, m)] and carbinol carbon [δ_C 72.8]; and 2) between both signals H_2-7′ [δ_H 3.11—3.07 (2H, mdd, J=17.3, 7.0 Hz)] and olefinic H-6′ [δ_H 5.71 (1H, s)] and carbinol carbon [δ_C 76.5], which was indicative of the position of the additional carbinol carbon at C-2′ of **3**, in substitution of a carbonyl carbon. The constant coupling of the proton H-7 (d, J=7.3 Hz) also allowed to establish an aryl-7/CH_3-8 *trans*-relationship, and *endo* conformation of methyl group of **3** to be alike to that of **1** on the basis of its characteristic chemical shift (δ_H 0.93, H_3-9),[11] as well as the *exo*-hydroxy group at C-2′ of **3** explains the relative protection (γ-effect) of C-4′.[12] The absolute configuration was determined since the CD spectrum exhibits positive Cotton effects at 318 and 257 nm ($[\theta]_{318}$ +10035 and $[\theta]_{257}$ +16778) and negative Cotton effects at 298 and 244 nm ($[\theta]_{298}$ −8765 and $[\theta]_{245}$ −15923) which are in agreement with reported data for related molecules.[12] Accordingly, the structure of **3** was established to be (7*R*,8*S*,2′*R*,3′*R*,4′*R*,5′*S*)-$\Delta^{8'}$-2′,4′-dihydroxy-3,4,5,3′,5′-pentamethoxy-2′,3′,4′,5′-tetrahydro-7.3′,8.5′-neolignan (nectamazin C).

The structures of the known compounds were identified as (+)-licarin A **4**,[13,14] (+)-acuminatin **5**,[15] denudatin B **6**,[16] liliflol B **7**,[10] macrophyllin B **8**,[7] denudanolide D **9**[17] and kadsurenin C **10**[8] respectively, by comparison on their NMR data with those reported in the literature.

PAF was discovered to be a lipid mediator of hypersensitivity and inflammation.[18] Studies have implicated PAF in such diseases as asthma, hypertension, cardiac anaphylaxis and arthritis as well as its clinical benefits on these cases.[19,20] Compounds **1**—**10** were evaluated by inhibition of PAF-induced aggregation method on rabbit platelets.[21] Of tested compounds, **1** was found to be the most potent PAF-antagonist, although the activity was slightly lower (IC_{50} 1.4 μM) than positive control ginkgolide B, a known PAF-antagonist from *Ginkgo biloba*.[22]

Experimental

General Experimental Procedures Melting points were determined on a Fisher-Johns melting point apparatus without correction. Optical rotations were measured on a Polartronic-E Schmidt-Hänsch polarimeter in $CHCl_3$. CD spectra were registered on a Jasco J-720 spectropolarimeter. IR spectra were recorded on a Thermo Nicolet 6700 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 spectrometer, using tetramethyl silane (TMS) as internal shift reference. HR-MS were determined on a Shimadzu LCMS-IT-TOF mass spectrometer system with electrospray ionization (ESI) in positive mode. Column chromatography (CC) was carried out with silica gel (70—230, 230—400 mesh, Merck).

Plant Material The whole plant of *N. amazonum* was collected near the biological station "El Zafire" to 6 h of Leticia city [coordinates: 4°3′7″S, 69°59′54″W], Department of Amazonas, Colombia, in August 2006. The plant material was identified by Biologyst A. Jara Muñoz. A voucher specimen (number COL519818) was deposited at Herbario Nacional Colombiano, Universidad Nacional de Colombia.

Extraction and Isolation Air-dried leaves of *N. amazonum* (250 g) were macerated with ethanol at 18 °C. The ethanolic extract was evaporated by drying (98.2 g), from which 30 g were fractionated by column chromatography on sephadex LH-20 (*n*-hexane : $CHCl_3$: MeOH, 3 : 1 : 1) to afford eight fractions 1—8. Fraction 2 (1.12 g) was chromatographed by column chromatography (CC) on silica gel using 18% Me_2CO in cyclohexane and fraction 2.2 was then washed affording **3** (19.0 mg) as amorphous solid. The resulting organic phase was subjected to silica gel CC using 35% toluene in AcOEt to give **9** (12.3 mg). Fraction 3 was chromatographed by CC on silica gel using 14% Me_2CO in cyclohexane and following silica gel CC (19% $CHCl_3$ and 4% Me_2CO in cyclohexane) affording **8** (9.5 mg) and **10** (8.8 mg). Compounds **1** (26.3 mg) and **2** (9.8 mg) were obtained from fraction 4 by means of silica gel CC using 27% AcOEt in cyclohexane following CC on silica gel using 19% $CHCl_3$ and 7% Me_2CO in cyclohexane. Fraction 5 was chromatographed by silica gel CC (20% AcOEt in *n*-hexane) obtaining 6 fractions 5.1—5.6. Fraction 5.2 was purified by CC on silica gel (15% AcOEt and 5% Me_2CO in *n*-hexane) to afford **6** (11.3 mg) and fraction 5.6 was subjected to silica gel CC (28% $CHCl_3$ and 3% MeOH in toluene) to give **7** (25.3 mg). Finally, compounds **4** (12.5 mg) and **5** (18.2 mg) were obtained from fraction 6 through silica gel CC (18% AcOEt in cyclohexane) following two CC on silica gel using 13% Et_2O in *n*-hexane and 20% Et_2O and 7% MeOH in *n*-hexane to afford **4** and **5** respectively.

Nectamazin A (**1**): Yellowish oil; $[\alpha]_D^{25}$ +12.2 (c=0.32, $CHCl_3$); CD (c=0.02, MeOH) $[\theta]_{327}$ +8799, $[\theta]_{259}$ +18740; IR (film) cm^{-1}: 3421, 1655, 1610, 1505, 1124; NMR data are shown in Table 1. HR-MS *m/z* 419.2054 $[M+H]^+$ (Calcd for $C_{23}H_{31}O_7$, 419.2070).

Nectamazin B (**2**): Colourless oil; $[\alpha]_D^{25}$ +9.7 (c=0.26, $CHCl_3$); CD (c=0.03, MeOH) $[\theta]_{337}$ +7438, $[\theta]_{268}$ +15235; IR (film) cm^{-1}: 3435, 1667, 1615, 1488, 1176, 1099; NMR data are shown in Table 1. HR-MS *m/z* 419.2055 $[M+H]^+$ (Calcd for $C_{23}H_{31}O_7$, 419.2070).

Nectamazin C (**3**): White powder; mp 177—179 °C; $[\alpha]_D^{25}$ +7.5 (c=0.03, $CHCl_3$); CD (c=0.04, MeOH) $[\theta]_{318}$ +10035, $[\theta]_{298}$ −8765, $[\theta]_{257}$ +16778, and $[\theta]_{245}$ −15923; NMR data are shown in Table 1. HR-MS *m/z* 421.2208 $[M+H]^+$ (Calcd for $C_{23}H_{33}O_7$, 421.2226).

Inhibition of PAF-Induced Aggregation of Rabbit Platelets Assay

Details of inhibition of PAF-induced aggregation assay procedure were described in the literature.[19] Briefly, convenient platelet rich plasma (PRP) suspensions from rabbit's blood were stirred at 800 rpm and maintained at 37 °C. Samples of PRP were preincubated for 5 min at 37 °C with tested compounds in dimethyl sulfoxide (DMSO). Aggregation was induced by the addition of 10 μl diluted PAF. The final PAF concentration was 1 ng/ml for PRP. In order to eliminate the effect of the solvent on the aggregation, the final concentration of DMSO was fixed at 0.5%, and did not affect the aggregation measured. Inhibition of platelet aggregation *versus* a solvent control was calculated in percent. Half-maximal inhibition concentrations (IC_{50}) were determined by non-linear regression analysis using the software GraphPad prism 5.00 (GraphPad software, San Diego, CA, U.S.A.).

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