The genus *Nectandra* is broadly distributed in Meso and South America which is grown in tropical regions and some members can reach 100 feet. 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 furopyran lignans. Magnoliaceae, Piperaceae and Lauraceae plants are well recognized as sources of neolignans, specially hydrobenzofuran and bicyclooctane-types, which have been found to be excellent platelet activating factor (PAF)-antagonists. 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 (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

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), 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. Following, the spectroscopic features for the new compounds 1—3 focusing to their structural elucidation are described.

**Fig. 1. The Structures of Compounds 1—10**
benzene ring (1610, 1505 cm\(^{-1}\)) functionalities. The \(^1\)H-NMR spectrum (Table 1) provides evidence of five methoxy groups ([\(\delta_C\) 3.88 (6H, s), 3.85 (3H, s)], and two methoxy groups attached to aliphatic carbons [\(\delta_C\) 3.45 (3H, s), 3.33 (3H, s)], an allyl group [\(\delta_C\) 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 [\(\delta_C\) 0.92 (3H, d, \(J=7.2\) Hz)], a symmetric 3,4,5-triaryloxyphenyl group [\(\delta_C\) 6.58 (2H, s)], an olefinic proton as part of a double bond conjugated with ketone system [\(\delta_C\) 6.90 (1H, s)], a carbinol proton [\(\delta_C\) 4.25 (1H, s)], a benzyl methine [\(\delta_C\) 2.44 (1H, d, \(J=7.7\) Hz)] and a methine proton [\(\delta_C\) 2.89—2.85 (1H, m)]. The \(^1\)C-NMR spectrum of I (Table 1) exhibited the presence of a carbonyl group (\(\delta_C\) 198.0), a 3,4,5-triaryloxyphenyl group (\(\delta_C\) 136.9 (\(\times 2\)), 105.2 (\(\times 2\)), 153.1 (\(\times 2\))), two carbinyl carbons (\(\delta_C\) 88.1, 88.8), a carbinol carbon (\(\delta_C\) 75.0), three methoxy groups on aromatic ring (\(\delta_C\) 56.4 (\(\times 2\)), 60.7), and two methoxy groups attached to aliphatic carbon (\(\delta_C\) 54.5, 53.7). Analysis of the \(^1\)H, \(^13\)C-NMR spectral data of I (Table 1) indicated that the molecule consisted of two \(C_3C_6\) moieties forming a bicyclo[3.2.1]octanoid core, 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 ([\(\delta_H\) 2.44 (1H, d, \(J=7.7\) Hz)] and H-8 ([\(\delta_H\) 2.89—2.85 (1H, m)]) with C-4 ([\(\delta_C\) 75.0]), and both H-7/H-8 ([\(\delta_H\) 3.08—3.03 (2H, m)]) of aliphatic group and H-6' ([\(\delta_H\) 6.90 (1H, s)]) with carboxyl carbon C-2 ([\(\delta_C\) 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/CH3-8 trans-relationship.\(^{8,9}\) Relative configuration was deduced through a nuclear Overhauser effect spectroscopy (NOE) 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 I put on view a positive Cotton effects ([\(\theta\)]\(^{259}\) +8799 and [\(\theta\)]\(^{259}\) +18740) which are consistent with reported data for related molecules\(^{9,10}\) with defined stereochemistry. By this means, the structure of I was determined to be \((7R,8S,4'R,3'S,5'S')-\Delta^8\)-4'-hydroxy-3,4,5,3',5'-pentamethoxy-2',3',4',5'-tetrahydro-2'-oxyo-7,3',8,5'-neo lignan (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, Caled for C\(_{23}\)H\(_{30}\)O\(_7\), 419.2070). Spectral comparison (IR, \(^1\)H-, \(^13\)C-NMR) (Table 1) indicated 2 is a 4’-epimer of I since the significant difference between them is the shifting to down field of both carbon C-4' (\(\delta_C\) 81.2) and its proton H-4' ([\(\delta_H\) 4.58 (1H, d, \(J=1.8\) Hz)]. Furthermore, other
two NMR spectral evidences explain this statement: 1) the occurrence of a W-coupling between carbilin proton H-4' (d, J = 1.8 Hz) and olefinic proton H-6' [δH 6.62 (1H, d, J = 1.9 Hz)] and 2) a key NOESY correlation between protons H-4' and H-8 [δH 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 C5-C6 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 268 nm ([α]268 +15235). Therefore the structure of 2 was established to be (7R,8S,4'S,3'S,5'S)-Δ2'-4'-hydroxy-3,4,5,3',5'-pentamethoxy-2',3',4',5'-tetrahydro-2'-oxo-7.3',8.5'-neolignan (necatamin B).

Compound 3 has a condensed formula as C12H12O3, which was assigned by HR-MS analysis ([M+H]+ m/z 421.2208, Calcd for C12H12O3, 421.2226). The 1H, 13C-NMR spectra of 3 have a similar signal pattern with that of 1 except for the presence of an additional carbilin carbon [δc 4.81 (1H, s); δc 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 signals of protons H-7 [δH 3.25 (1H, d, J = 7.3 Hz)] and H-8 [δH 2.88—2.84 (1H, m)] and carbilin carbon [δc 72.8]; and 2) between both signals H-7' [δH 3.11—3.07 (2H, mdd, J = 17.3, 7.0 Hz)] and olefinic H-6' [δH 5.71 (1H, s)] and carbilin carbon [δc 76.5], which was indicative of the position of the additional carbilin 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-1′-CH=CH-trans-re-lationship, and endo conformation of methyl group of 3 to be alike to that of 1 on the basis of its characteristic chemical shift (δc 0.93, H1-4,11) as well as the exo-hydroxy group at C-2' of 3 explains the relative protection (γ-effect) of C-4'.

The absolute configuration was determined since the CD spectrum exhibits positive Cotton effects at 318 and 257 nm ([α]318 +10035 and [α]257 +16778) and negative Cotton effects at 298 and 244 nm ([α]298 -8765 and [α]245 -15923) which are in agreement with reported data for related molecules. Accordingly, the structure of 3 was established to be (7R,8S,2'R,3'R,4'R,5'S)-Δ2'-4',4'-dihydroxy-3,4,5,3',5'-pentamethoxy-2',3',4',5'-tetrahydro-7.3',8.5'-neolignan (necatazamin C).

The structures of the known compounds were identified as (+)-lacinarin A 4, (+)-acuminatin B 5, denudatin B 6, lilifol B 7, macrophyllin B 8, denudanolide D 9 and kadsurenin C 10 respectively, by comparison on their NMR data with those reported in the literature.

PAF was discovered to be a lipid mediator of hyperresponsivity and inflammation. Studies have implicated PAF in such diseases as asthma, hypertension, cardiac anaphylaxis and arthritis as well as its clinical benefits on these cases. Compounds 1—10 were evaluated by inhibition of PAF-induced aggregation method on rabbit platelets. Of tested compounds, 1 was found to be the most potent PAF-antagonist, although the activity was slightly lower (IC50 1.4 μM) than positive control ginkgolide B, a known PAF-antagonist from Ginkgo biloba.

### Experimental

**General Experimental Procedures** Melting points were determined on a Fisher-Johns melting point apparatus without correction. Optical rotations were measured on a Polarronic-E-Schmidt-Hänisch polarimeter in CHCl3, CD spectra were registered on a Jasco J-720 spectrocolorimeter. IR spectra were recorded on a Thermo Nicolet 6700 spectrophotometer. 1H- and 13C-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 Zafiro” to 6h of Leticia city [coordinates: 4°37'7'S, 69°59'54'W], Department of Amazonas, Colombia, in August 2006. The plant material was identified by Biologist A. Jara Muñoz. A voucher specimen (number COL519188) 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: CHCl3; MeOH, 3:1:1) to afford eight fractions 1—8. Fraction 2 (11.2 g) was chromatographed by column chromatography (CC) on silica gel using 18% Me2CO in cyclohexane and fraction 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 on silica gel on 14% Me2CO in cyclohexane and following silica gel CC (19% CHCl3 and 4% Me2CO 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% CHCl3 and 7% Me2CO 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% Me2CO in n-hexane) to afford 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% EtOAc in n-hexane and 20% EtOAc and 7% MeOH in n-hexane to afford 4 and 5 respectively.

Nectazamin A (1): Yellowish oil; [α]252 +12.2 (c = 0.32, CHCl3); CD (c = 0.02, MeOH) [θ]257 +8799, [θ]298 +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 C23H32O7, 419.2075). Nectazamin B (2): Colourless oil; [α]252 +9.7 (c = 0.26, CHCl3); CD (c = 0.03, MeOH) [θ]257 +7438, [θ]298 +15235; IR (film) cm−1: 3345, 1667, 1615, 1488, 1176, 1099; 1H-NMR data are shown in Table 1. HR-MS m/z 419.2055 [M+H]+ (Calcd for C23H31O7, 419.2076). Nectazamin C (3): White powder; mp 177—179°C; [α]252 +7.5 (c = 0.03, CHCl3); CD (c = 0.04, MeOH) [θ]257 +10035, [θ]298 +8765, [θ]245 +16778, and [θ]245 +15923; NMR data are shown in Table 1. HR-MS m/z 421.2208 [M+H]+ (Calcd for C23H33O7, 421.2226).

### Inhibition of PAF-Induced Aggregation of Rabbit Platelets Assay

<table>
<thead>
<tr>
<th>Compounds</th>
<th>1</th>
<th>2</th>
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<th>4</th>
<th>5</th>
<th>6</th>
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<th>8</th>
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<th>10</th>
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<tbody>
<tr>
<td>IC50 (μM)a</td>
<td>1.4±0.72</td>
<td>1.7±0.53</td>
<td>3.8±1.8</td>
<td>N.D.</td>
<td>65.3±9.7</td>
<td>75.6±10.1</td>
<td>18.4±4.5</td>
<td>6.8±1.3</td>
<td>4.5±1.4</td>
<td>2.3±0.97</td>
<td>0.9±0.22</td>
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</table>

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