New 3-Methyoxyflavones, an Iridoid Lactone and a Flavonol from *Duroia hirsuta*

Rita Aquino,*,† Nunziatina De Tommasi,† Medardo Tapia,‡ Maria Rosaria Lauro,† and Luca Rastrelli†

Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università di Salerno, Piazza V. Emanuele 9, 84084, Penta di Fisciano (SA), Italy, and Centro Tecnologico de Recursos Amazonicos de la Organizacion de Pueblos Indigenos "Fatima" of Pastaza, Ecuador

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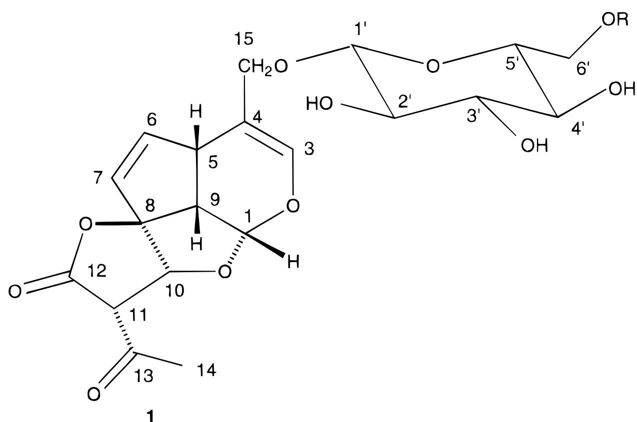
Investigation of the roots of *Duroia hirsuta* from Ecuador yielded the iridoid lactone duroin (**1**) together with 3,7,3′,5′-tetramethoxy-4′-hydroxyflavone (**2**), 3,7,3′-trimethoxy-4′,5′-dihydroxyflavone (**3**), and 7,3′,5′-trimethoxy-3,4′-dihydroxyflavone (**4**) as novel constituents.

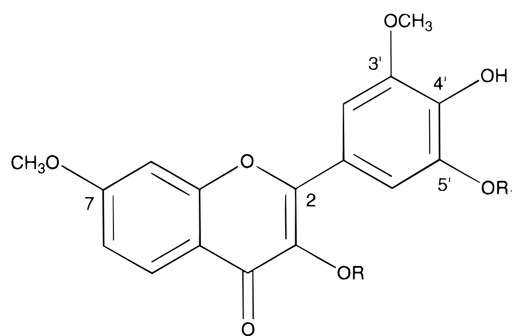
Duroia hirsuta (Poepping & Endl) K. Schum (Rubiaceae) grown in the tropical forest possesses allelopathic properties, which inhibit growth of other plants in the same habitat. Where a group of 20 or more individuals of *D. hirsuta* grows, a zone lacking of vegetation is formed in the usually dense tropical forest. This open zone is called "Devil's Garden" in Colombia and Ecuador.[1] Previous field studies showed that soil samples from beneath *D. hirsuta* are able to inhibit germination and growth of lettuce seeds. Bioactivity-directed fractionation of the root extracts led to the isolation of plumericin, a tetracyclic iridoid with a characteristic spirolactone ring at C-8, which was effective in inhibiting lettuce radicle elongation.[2]

Owing to the importance of allelochemicals in inhibiting plant germination and growth and potentially in controlling infesting weeds, we have undertaken a systematic investigation of secondary constituents of *D. hirsuta*. Particular emphasis was placed on iridoids and phenolic constituents that are implicated as causative agents in higher-plant allelophatic interactions.[3] In this paper, we report the isolation and structure elucidation of a new highly functionalized tetracyclic iridoid lactone, duroin (**1**), structurally related to plumericin, two new flavonol-3-O-methyl ethers (**2** and **3**), and a new flavonol (**4**) from the roots of *D. hirsuta*.



Results and Discussion

Column chromatography and HPLC of an extract (MeOH) of the roots of *D. hirsuta* yielded four new compounds **1**−**4**.



Shikimic acid, rosmarinic acid, and quinic acid were also isolated, and the structures were established by comparison of their NMR data with literature.[4−6]

Compound **1** had molecular formula $C_{20}H_{24}O_{11}$ based on FABMS and ^{13}C and DEPT ^{13}C NMR analyses. FABMS (negative ion mode) showed a quasimolecular anion $[M - H]^-$ at *m*/*z* 439. An examination of its NMR data (Table 1) and a comparison with the literature suggested that compound **1** was a derivative of plumericin,[7] such as a Δ^6-iridoid with a five-membered lactone ring at C-8 and with a −$COCH_3$ moiety at C-11.[7−9] In the ^1H NMR spectrum of **1**, peaks characteristic of a plumericin derivative due to the proton involved in an acetal linkage at C-1 (δ 5.70), olefinic protons at C-6 and C-7 (δ 6.48 and 5.75, respectively), and three methynes at C-5, C-9, and C-10 (δ 2.42, 3.62, and 5.00, respectively) were observed. The major differences were in the chemical shift of H-3 (δ 6.48), in the presence of methyne (H-11, δ 3.21) and methylene (H-15, δ 4.12, 2H, d, $J = 12$ Hz) signals, and the absence of an additional sp^2 signal ascribable to H-13 in plumericin.

Each proton was assigned by means of $^1H-^1H$ DQF−COSY, which showed connectivities (H-1/H-9, H-9/H-5, H-5/H-6, H-6/H-7) within the five spin system containing the H-1 resonance, typical of a proton of an iridoid involved in an acetal linkage. The $^1H-^1H$ COSY spectrum also established connectivities between the hydrogen resonating at δ 5.00 (br d, $J = 1.5$ Hz) and at δ 3.21 (br d, $J = 1.5$ Hz) corresponding to a fragment −OCHCH− at C-10 and C-11. The ^{13}C NMR spectrum, analyzed with the aid of DEPT, showed 20 carbon signals, six of which were assigned to a

* To whom correspondence should be addressed. Phone: 0039 89 968936. Fax: 0039 89 968910. E-mail: luca@pluto.farmacia.unisa.it.
† Università di Salerno.
‡ Centro Tecnologico de Recursos Amazonicos.

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Table 1. ^{1}H and ^{13}C NMR Chemical Shifts for Compound **1**[a]

position	1	
	δ_C	δ_H
1	104.4	5.70 (d, 5.8)
3	151.4	6.48 (br s)
4	109.4	
5	31.5	2.42 (dd, 2.0, 10.0)
6	126.1	6.48 (dd, 5.5, 2.0)
7	151.4	5.75 (d, 5.5)
8	109.0	
9	49.1	3.62 (dd, 10.0, 5.8)
10	82.8	5.00 (d, 1.5)
11	64.3	3.21 (d, 1.5)
12	177.0	
13	180.5	
14	29.9	2.03 (s)
15	65.9	4.12 (d, 12.0)
1′	104.4	4.27 (d, 8.0)
2′	75.2	3.30 (dd, 8.0, 9.5)
3′	78.3	3.42 (t, 9.5)
4′	71.9	3.34 (t, 9.5)
5′	78.2	3.28 (m)
6′	62.3	3.72 (dd, 2.0, 3.0); 3.88 (dd, 12.0, 5.0)

[a] Chemical shift values (in CD_3OD) are in ppm from TMS and *J* values in Hz are presented in parentheses. All signals were assigned by ^{1}H−^{1}H DQF−COSY and ^{1}H−^{13}C HSQC studies.

β-D-glucopyranosyl moiety[10] and 14 to the iridoid moiety. Carbon resonances (Table 1) were assigned with the aid of a ^{1}H−^{13}C HSQC spectrum. Thus, the signals from C-1 to C-9 were typical of an iridoid skeleton, while signals from C-8 to C-12 demonstrated the presence of a five-membered *γ*-lactone [*δ* 177.0 (C-12), 64.3 (C-11), 82.8 (C-10), 109.0 (C-8)]. The signals ascribable to C-13 and C-14 showed the presence of a C-11 side chain (carbonyl at *δ* 180.5 for C-13; Me linked to a carbonyl δ_H 2.03 and δ_C 29.9 for C-14 position). The attachment of the five-membered lactone ring at C-8 and C-1 was indicated by the marked downfield shifts of C-1 (*δ* 104.4), typical of a carbon involved in an acetal linkage, and of C-10 (82.8, CH), characteristic of a substituted carbinol group; both were similar to those observed in plumericin.[7] Signals for a carboxyl or a carbomethoxy function at C-4, seen in the NMR spectra of plumericin derivatives,[7−9] was absent in **1**, but a methylene signal was present (δ_H 4.12 and δ_C 65.9, CH_2). Evidence for this $-CH_2OR$ group at C-4 was derived from the H-3 and H-5 signals, which were shifted upfield by over 1.0 ppm in **1** with respect to plumericin.

Further evidence was provided by C-3, which exhibited an upfield shift (from *δ* 154.9 in plumericin to 151.4 in **1**), and by C-4, showing a downfield shift (from *δ* 104.5 in plumericin to 109.4 in **1**). Similar shifts were observed in asperulosidol[11] and all the valeriana iridoids,[12] which have a $-CH_2OH$ group at C-4 when compared with asperuloside and iridoids, which possess a −COOH group at C-4.[13]

The position of the sugar moiety was confirmed using an NOEDs experiment. Irradiation of the signal at *δ* 4.27 (H-1′ of the glucopyranosyl moiety) gave an NOE with the signals at *δ* 4.12 ($-CH_2OR$). Thus, the glucopyranosyl moiety must be linked at C-15. The stereochemistry was established by analysis of coupling constants, which were the same as in allamandicin.[8] The *J* between H-5/H-6 (2.0 Hz), H-6/H-7 (5.5 Hz), and H-5/H-9 (10.0 Hz) indicated that the configuration at C-5 and C-9 was cis as in plumericin and allamandicin.[7−9] Since the *J* between H-10/H-11 was of 1.5 Hz, the orientation of H-11 must be *β* and the −COMe moiety at C-11 had the α-orientation as well as the −CHOHMe moiety in allamandicin. Thus, the structure of compound **1** is as shown.

Compound **2** had molecular formula $C_{19}H_{18}O_7$ as determined by FABMS and ^{13}C and DEPT analyses. The ^{1}H NMR spectrum exhibited signals at *δ* 6.83, 7.57 (d, $J = 8$ Hz), and 7.23, suggesting only a substituent at C-7 for ring A.[14,15] A signal integrating for two meta-coupled protons at *δ* 7.29 was in agreement with a 3′,4′,5′-trisubstitution pattern for ring B. Two signals at *δ* 3.94 and 3.92 indicated three methoxyl groups linked to the aromatic rings, while the signal at *δ* 3.84 suggested a methoxyl linked at C-3.[16] Their location at C-7, C-3′, C-5′, and C-3, respectively, was established on the basis of the ^{13}C NMR spectrum. The signals of C-2 (*δ* 158.1), C-3 (*δ* 139.9), and C-4 (*δ* 180.1) as well as the methoxyl (*δ* 58.1) suggested that the methoxy group was at C-3.[15] Attachment of a −OMe at C-7 was deduced from the resonance of C-7 (*δ* 163.7) shifted downfield by 1.3 ppm and of C-6 (*δ* 111.7) and C-8 (*δ* 98.8) shifted upfield by ca. 3.0 and 3.6 ppm with respect to unmethoxylated models.[14] The location of the other two methoxy groups at C-3′ and C-5′ was by similarity to the 3′,5′-methoxy-4′-hydroxy models.[16] Thus, compound **2** was determined to be 3,7,3′,5′-tetramethoxy-4′-hydroxyflavone.

Compound **3** had molecular formula $C_{18}H_{16}O_7$ as determined by FABMS, ^{13}C, and DEPT analyses. Signals at *δ* 3.94 and 56.8 in the ^{1}H and ^{13}C NMR spectra, corresponding to the methoxy group at C-5′ in **2**, were not present in **3**. Another difference in **3** was observed for C-6′ which shifted downfield by 4.7 ppm in comparison to **2**. Therefore, **3** is 3,7,3′-trimethoxy-4′,5′-dihydroxyflavone.

The molecular formula ($C_{18}H_{16}O_7$) of **4** was determined by FABMS, ^{13}C, and DEPT NMR analyses. The C-3 methoxy signal in **2** was not present in **4**. The signals of C-3 and C-4 shifted upfield by 6.5 and 1.9 ppm, respectively, in comparison to **2**, indicating that **4** is 7,3′,5′-trimethoxy-3,4′-dihydroxyflavone.

Experimental Section

General Experimental Procedures. FABMS, in the negative-ion mode, were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of 2−6 kV energy in a Kratos MS 902 spectrometer. Optical rotations were measured on a Perkin-elmer 141 polarimeter using a sodium lamp operating at 589 nm in 0.1% w/v solutions in MeOH. IR spectra were performed with a Bruker IFS-48 spectrophotometer. UV spectra were obtained from a Beckman DU 670 spectrophotometer. A Bruker DRX-600 spectrometer, operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C, using the UXNMR software package, was used for NMR experiments in CD_3OD. The DEPT experiments were performed using a transfer pulse of 135° to obtain positive signals for CH and CH_3 and negative ones for CH_2. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. ^{1}H−^{1}H DQF−COSY[17] and ^{1}H−^{13}C HSQC[18] experiments were obtained using the conventional pulse sequences as described in the literature. NOE experiments were performed using the spectral subtraction technique (NOEDS). The sample for NOE measurements was degassed by bubbling argon through the solution for 40 min in advance. HPLC separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector, a U6K injector, using a Waters *μ*-Bondapak C-18 column.

Plant Material. The roots of *D. hirsuta* were collected in the "Centro Tecnologico de Recursos Amazonicos de la Organizacion de Pueblos Indigenos − Fatima" of Pastaza, Ecuador, July 1994, and identified by Dr. Carlos Donoso of Escuela Politecnica del Chimborazo (ESPOCH), Riobamba, Ecuador. A voucher sample is deposited at the herbarium of the ESPOCH

Table 2. NMR Spectral Data for Compounds **2**–**4** (in CD_3OD)[a]

position	**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	158.1		158.3		158.2	
3	139.9		139.8		133.4	
4	180.1		180.0		178.1	
5	125.0	7.57 (d, 8)	125.1	7.57 (d, 8)	124.9	7.56 (d, 8)
6	111.7	6.83 (dd, 8, 2)	111.7	6.85 (dd, 8, 2)	111.5	6.85 (dd, 8, 2)
7	163.7		163.2		163.5	
8	98.8	7.23 (d, 2)	98.8	7.26 (d, 2)	98.7	7.25 (d, 2)
9	159.2		159.1		159.3	
10	116.0		114.0		116.1	
1′	121.0		120.2		120.9	
2′	105.0	7.29 (d, 2)	104.9	7.59 (d, 2)	105.0	7.27 (d, 2)
3′	146.9		148.0		146.8	
4′	136.1		138.1		136.0	
5′	146.9		145.8		146.8	
6′	105.1	7.29 (d, 2)	109.8	7.40 (d, 2)	105.1	7.27 (d, 2)
OMe at C-3	58.1	3.84 (s)	58.2	3.84 (s)		
OMe at C-7	57.0	3.92 (s)	57.1	3.91 (s)	57.0	3.90 (s)
OMe at C-3′	56.8	3.94 (s)	56.8	3.95 (s)	56.8	3.94 (s)
OMe at C-5′	56.8	3.94 (s)			56.8	3.94 (s)

[a] Chemical shift values are in ppm from TMS and *J* values in Hz are presented in parentheses. Carbon multiplicities were determined using DEPT experiments.

Extraction and Isolation. Powdered, dried roots (300 g) were extracted with petroleum ether and $CHCl_3$ in a Soxhlet apparatus and extracted successively at room temp. with $CHCl_3$–MeOH (9:1) and MeOH to afford 2.1, 4.5, 3.2, and 10.5 g, respectively. The MeOH extract was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH portion (3.6 g), which was chromatographed on a Sephadex LH-20 column using MeOH as an eluent. The fractions obtained were combined according to TLC (silica gel, *n*-BuOH–HOAc–H_2O 60:15:25) to give five main fractions I–V. Fraction III (200 mg) was submitted to reversed-phase HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.5 mL/min) using MeOH–H_2O (50:50) as an eluent to yield pure compounds **1** (6.2 mg, $t_R = 16$ min) and **5** (16.4 mg, $t_R = 5.3$ min). Fraction IV (350 mg) containing the flavonol mixture was separated by HPLC using MeOH–H_2O (60:40) to yield pure compounds **2** (17.7 mg, $t_R = 20$ min), **3** (20.8 mg, $t_R = 14$ min), and **4** (25.5 mg, $t_R = 10$ min). Fraction V, with the same HPLC procedure, provided pure compounds **6** (7.2 mg, $t_R = 13$ min) and **7** (6.1 mg, $t_R = 16$ min).

Compound 1: $C_{20}H_{24}O_{11}$; mp 215–221 °C; $[\alpha]^{25}_D = -95.6°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 244 (3.12), 212 (4.43); IR (KBr) 3420, 3096, 1745, 1680 cm^{-1}; FABMS, negative mode, *m*/*z* 439 $[M - H]^-$, 277 $[(M - H) - 162]^-$, 261 $[(M - H) - 178]^-$. For ^1H and ^{13}C NMR data, see Table 1.

Compound 2: $C_{19}H_{18}O_7$; mp 220–227 °C; $[\alpha]^{25}_D = +132°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 256 (4.03), 3.16 (4.32); FABMS, negative mode, *m*/*z* 357 $[M - H]^-$. For ^1H and ^{13}C NMR data, see Table 2.

Compound 3: $C_{18}H_{16}O_7$; mp 230–238 °C; $[\alpha]^{25}_D = +154°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 259 (3.98), 313 (4.14); FABMS, negative mode, *m*/*z* 343 $[M - H]^-$. For ^1H and ^{13}C NMR data, see Table 2.

Compound 4: $C_{18}H_{16}O_7$; mp 232–239 °C; $[\alpha]^{25}_D = +158°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 258 (3.76), 314 (4.21); FABMS, negative mode, *m*/*z* 343 $[M - H]^-$. For ^1H and ^{13}C NMR data, see Table 2.

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