A New Biphenyl from *Clusia melchiorii* and a New Tocotrienol from *C. obdeltifolia*

Josanaide S. R. Teixeira,a Luciana de M. Moreira,a Maria L. da S. Guedesb and Frederico G. Cruz*a

aInstituto de Química and bInstituto de Biologia, Universidade Federal da Bahia, 40170-290 Salvador-BA, Brazil

O extrato diclorometânico do tronco de *Clusia melchiorii*, após fracionamento e purificação por cromatografia em gel de sílica, produziu um novo bifenilo, 2,2-dimetil-5-hidroxi-7-fenilcromeno, ao lado de outros compostos conhecidos, 2,2-dimetil-5,10-diidro-2H-benzo[g]cromeno-5,10-diona (xiloidona), ácido betulínico, friedelina, friedelinol, eufol, sitostenona, estigmastenona e uma mistura de β-sitosterol e stigmasterol. O extrato hexânico do tronco de *Clusia obdeltifolia* depois de fracionado em gel de sílica produziu um novo álcool δ-tocotrienílico, 2Z,6E,10E-13-(6-hidroxi-2,8-dimetil-3,4-diidro-2H,2-cromenil)-2,6,10-trimetil-2,6,10-tridecatrien-1-ol, ao lado dos ácidos 2E e 2Z-δ-tocotrienóico, betulínico e betulônico, betulinaldeído, glutinol, friedelina, sitostenona e uma mistura de β-sitosterol e estigmasterol. As estruturas dos compostos isolados foram determinadas através de seus dados espectroscópicos.

Chromatographic purification of the dichloromethane extract of *Clusia melchiorii* trunk leading to the isolation of a new biphenyl, 2,2-dimethyl-5-hydroxy-7-phenylchromene, along with the known compounds 2,2-dimethyl-5,10-dihydro-2H-benzo[g]chromene-5,10-dione (xiloidone), betulonic acid, friedelin, friedelinol, euphol, sitostenone, stigmasterone and a mixture of β-sitosterol and stigmasterol. The hexane extract of *Clusia obdeltifolia* trunk yielded, after chromatographic fractionation, a new δ-tocotrienolic alcohol 2Z, 6E, 10E-13-(6-hydroxy-2,8-dimethyl-3,4-dihydro-2H,2-cromenyl)-2,6,10-trimetil-2,6,10-tridecatrien-1-ol, along with 2Z and 2E-δ-tocotrienolic acids, betulonic acid, betulonic acid, betulinaldehyde, glutinol, friedelina, sitostenona and a mixture of β-sitosterol and stigmasterol. Their structures were determined from spectral data and comparison with data from previously reported compounds.

Keywords: *Clusia obdeltifolia*, *Clusia melchiorii*, Clusiaceae, biphenyl, tocotrienol, triterpene, xiloidone

Introduction

The genus *Clusia* belongs to Clusiaceae and comprises about 250 species that occur in tropical and subtropical regions of South and Central America. *Clusia* species have been used as purgative, antirheumatic, for stomach problems and to heal wounds. The literature indicated that species of this genus produce polyprenylated benzophenones,1,2 triterpenes and sterols,3,4 flavonoid glycosides5 and not so often tocotrienols,6 biphenyls7,8 and quinones.9

Biphenyls in Clusiaceae are rare and the literature related the isolation of only eight of them in this family, two from *Pentaphalangium solomonse*,10 five from *C. paralicola*,7,8 and one from *Kielmeyera coriacea*.11 The presence of these compounds in plants was associated to a phytoalexin response.12

Tocopherols and tocotrienols are lipid soluble molecules that differ in the degree of saturation of prenyl side chain. They are compounds of vitamin E group and are essential to maintain membrane integrity. These compounds protect the plant from oxygen toxicity. Tocopherols, especially α-tocopherol, are found in fruits, roots, tubers, cotyledons, stems, leaves, and flowers of higher plants, while tocotrienols are found principally in seeds and specialized cells like latex tuber.13 From our knowledge, the presence of tocotrienols in Clusiaceae is described just to *Kielmeyera lathrophyton*,14 *K. reticulata*,15 *Garcinia kola*,16 *Tovomitopsis psychotriifolia*17 and *Clusia grandiflora*.6

Results and Discussion

The dichloromethane extract of *C. melchiorii* yielded, after repeated silica gel column chromatography, one new biphenyl, 2,2-dimethyl-5-hydroxy-
7-phenylchromene, 1. In addition were identified one 
naphthoquinone, four triterpenes and four sterols. The 
structure of 1 was determined by NMR and MS 
experiments. Its molecular formula was deduced to be 
C_{17}H_{16}O_{2} based in the M⁺ ion peak at m/z 252 in the 
EIMS spectrum and in the ¹H and ¹³C NMR data (Table 
1). The ¹H NMR presented two multiplets at δ 7.53 
and δ 7.36 integrating to 2H and 3H, respectively. These 
results combined with the presence of the ion m/z 77 in 
the EIMS spectrum indicated the presence of a phenyl 
group. Two broad singlets at δ 6.56 and δ 6.68 
suggested the presence of two meta-coupled aromatic 
protons. Two doublets at δ 6.65 and δ 5.61 (J 9.9 Hz) 
integrating to 1H beside the singlet at δ 1.45 integrating 
to 6H, indicated the presence of a dimethylpyrane 
moiety. The ¹³C NMR data confirmed the presence of 
a phenyl and a dimethylpyrane groups in the molecule 
and also indicated the presence of other six aromatic 
carbons being two oxygenated and two methine. 

COLOC cross peaks indicated a 1,3-relation between 
the methine carbons. Data from COLOC and NOEDIF 
experiments were not conclusive about the position of 
the dimethylpyrane ring. With these data two structures 
were proposed: a biphenyl with the 2,2-dimethylpyrane 
ring at C-3 and C-4 and one hydroxyl group at C-5 and 
another with the 2,2-dimethylpyrane ring at C-2 and 
C-3. In order to deduce the correct location of the 
dimethylpyrane ring, compound 1 was submitted to 
methylation with diazomethane yielding 2. The irradiation of methoxyl hydrogens of 2 enhanced the 
signal of H-4' allowing to locate unambiguously the 
dimethylpyrane ring at C-3 and C-4 and not at C-2 and 
C-3 (Figure 1). Compound 2 had been previously 
isolated from Moureira fluviatilis along with its isomer 
with the dimethylpyrane ring at C-2 and C-3. The 
authors differentiated the two isomers using an 
argument based only in the analysis of ¹H chemical 
shifts differences for the meta-coupled aromatic 
hydrogens. However, the comparison of the NMR data 
of 2 with those of compounds obtained from M. 
fluviatilis demonstrated that the data of the two isomers 
were changed. Recently we have reported the isolation of 
five polyoptenylated benzophenones from the trunk of 
C. obdeltifolia. A re-examination of the hexane 
extract of the trunk from same plant provides, after 
repeated silica gel column chromatography, one new 
δ-tocotrienolic acid, 2Z, 6E, 10E-13-(6-hydroxy-2,8-
dimethyl-3,4-dihydro-2H,2-chromenyl)-2,6,10-
trimethyl-2,6,10-tridecatrien-1-ol, 3, along with the 
known 2Z and 2E-δ-tocotrienolic acids, five 
triterpenes and three sterols.

The molecular formula of compound 3, C_{27}H_{46}O_{3}, was 
determined by EIMS and by ¹H and ¹³C NMR data. The IR 
data were consistent with a phenol moiety. In the ¹H NMR 
spectrum, a pair of doublets at δ 6.48 and δ 6.38 (J 2.8 Hz) 
of meta-coupled aromatic protons, defined a tetra-
substitution pattern for the aromatic ring. A singlet 
integrating to 3H at δ 2.12 suggested the presence of a 
benzylic methyl group. The presence of a prenyl side chain 
was deduced from the observation of three triplets of olefinic 
protons at δ 5.08, δ 5.12 and δ 5.27 and three singlets of 
allylic methyl groups at δ 1.57, δ 1.58 and δ 1.78. Another 
singlet at δ 4.11 integrating to 2H suggested the presence 
of a primary allylic alcohol. The analysis of hydrogen 
broadband decoupled (HBBD) and DEPT 135⁰ ¹³C NMR 
spectra indicated the presence of five methyl groups, nine 
methylene carbons, five methine carbons and eight non-
hydrogenated carbons. The ¹³C chemical shifts were 
coherent with a benzopyrane moiety containing one 
hydroxyl and one methyl in a 1,3-substitution pattern and 
prenyl side chain with three trisubstituted double bonds. 
These results were corroborated by fragments at m/z 177 
[C_{11}H_{13}O_{2}]⁺, 58%, 3a, originated from cleavage of bond 
between C-2 and C-1', at m/z 137 [C_{8}H_{9}O_{2}]⁺, 99%, 3b, 
produced by retro Diels-Alder rearrangement involving a 
hydrogen transfer to the oxygen, which likely rearranges 
to the more stable tropylium ion and at m/z 136 [C_{12}H_{14}O_{3}]⁺, 
22%, 3c, originated from a retro Diels-Alder rearrangement 
of benzopyrane moiety without hydrogen transfer (Figure 
1). The structural formula of 3 (Figure 1) was confirmed 
by comparison of its spectroscopic data with spectroscopic 
data of the product obtained by reduction of 2Z-
tocotrienolic acid with LiAlH₄ in ethyl ether and with data 
of the 2E isomer.

\[ \begin{align*} 
\text{Figure 1. Structures of compounds 1 (showing selected COLOC cross} 
\text{peaks), 2 (showing nOe between H-4' and OMe) and 3 (showing} 
\text{the main EIMS fragments, 3a, 3b and 3c).} 
\end{align*} \]
Experimental

General procedures

IR spectra were obtained with a JASCO FT-IR spectrophotometer. A VARIAN Gemini 300 spectrometer, operating at 300 MHz for $^1$H and 75 MHz for $^{13}$C in CDCl$_3$, with TMS as internal standard were used to obtain NMR data. EIMS was obtained with direct probe insertion at 70 eV in an HP MSD 5973 apparatus.

Plant material

*Clusia melchiorii* Gleason was collected in the vicinity of Mucugê at Parque Nacional da Chapada Diamantina, Bahia, Brazil, in October 1999. *Clusia obdeltifolia* Bittrich was collected in an area near Palmeiras at Parque Nacional da Chapada Diamantina, Bahia, Brazil, in April 1996. Voucher specimens number ALCB-038358 and ALCB-035997, respectively, have been deposited at Alexandre Leal Costa Herbarium, Instituto de Biologia, Universidade Federal da Bahia, Salvador, Brazil.

Extraction and isolation

The dried powdered trunk (6 kg) of *C. melchiorii* was extracted with dichloromethane at room temperature. The extract (55.3 g) was submitted to repeated column chromatography on silica gel eluted with mixtures of hexane-EtOAc (0-100%) leading to the isolation of a new biphenyl, 1 (18.2 mg), along with the known compounds: 2,2-dimethyl-5,10-dihydro-2$^H$-benzo[g]chromene-5,10-dione (xyloidone) (10.3 mg), betulinic acid (349.6 mg), betulinic acid (93.4 mg), betulinic aldehyde (24.6 mg), glutinol (83.9 mg), friedelin (47.3 mg), sitostenone (127.3 mg), stigmasterol (179.1 mg), and a mixture of $\beta$-sitosterol and stigmasterol (50.2 mg). Compound 1 (8.4 mg) was further methylated with ethereal diazomethane prepared from Diazald (Aldrich Chemical Co.) in the usual procedure to yield 2 (8.9 mg).

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Table 1. NMR data from compounds 1 and 3 ($^1$H (300 MHz) and $^{13}$C (75 MHz), CDCl$_3$, $\delta$ (ppm))

The dried powdered trunk (4400 g) of *C. obdeltifolia* was extracted with hexane. Evaporation of solvent under reduced pressure yielded 39.2 g of extract. This extract was submitted to chromatography on silica gel column using hexane-EtOAc (0-100%) in order to give 19 fractions. Fraction 6 (6.8 g) was submitted to column chromatography on silica gel eluted with mixtures of hexane and EtOAc. Some fractions of this column non-studied early were purified by repeated column chromatography leading to the isolation of known compounds betulinic acid (77.4 mg), betulinic acid (93.4 mg), betulinic aldehyde (24.6 mg), glutinol (83.9 mg), friedelin (47.3 mg), sitostenone (32.8 mg) and a mixture of $\beta$-sitosterol and stigmasterol (50.2 mg). Fraction 7 (1.68 g) was submitted to column chroma-
tography on silica gel eluted with mixtures of hexane and acetone leading to the isolation of a new δ-tocotrienilic alcohol, 3 (20.1 mg), along with the known compounds 2Z-δ-tocotrienoloic acid (1360.5 mg) and 2E-δ-tocotrienoloic acid (151.6 mg). A

cis-δ-Tocotrienoloic acid (108 mg) was further reduced with LiAlH4 in ethyl ether in the usual procedure to yield 3 (52 mg).

2Z, 6E, 10E-13-(6-hydroxy-2,8-dimethyl-3,4-dihydro-2H,2-chromenyl)-2,6,10-trimethyl-2,6,10-tridecatrien-1-ol, 3. Pale yellow oil, C27H40O3, 1H NMR and 13C NMR in Table 1. EIMS (70 eV) m/z 412 [M]+ (35), 192 (15), 189 (26), 177 (58), 137 (99), 136(22), 121(37), 105 (100), 69 (62).

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References


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