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Epimers of labdane diterpenes from the rhizomes of *Hedychium coronarium* J. Koenig

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ABSTRACT: The phytochemical study of the rhizomes from *Hedychium coronarium* resulted in the isolation of benzoyl eugenol (**1**), along with the labdane diterpenes isocoronarin D (**2**) and ethoxy coronarin D (**3**), obtained as epimeric mixtures at C-14 and C-15, respectively. Structures were determined based on spectroscopic data, mainly 1D and 2D NMR.

Keywords: *Hedychium coronarium*, epimers, isocoronarin D, ethoxy coronarin D, benzoyl eugenol.

INTRODUCTION

Hedychium coronarium J. Koenig (Zingiberaceae) is a rhizomatous herb cultivated in India, Southeast Asia countries, China, Japan and Brazil (Matsuda et al., 2002). It is popularly used in Brazil to treat pains, wounds, infections and rheumatism (Pio Corrêa, 1974; Ribeiro et al., 1988). Previous phytochemical work on this species resulted in the isolation of several labdane diterpenes, some of them possessing cytotoxic and anti-inflammatory activities (Matsuda et al., 2002; Itokawa et al., 1988a,b; Singh et al., 1991; Nakatani et al., 1994).

The dichloromethane extract from the rhizomes of *H. coronarium* produced 86% inhibition of 5-lipoxygenase, at a concentration of 19 µg/ml, indicating a potential anti-asthma activity (Braga et al., 1999). Based on this finding, the species was selected for phytochemical study. Herein is described the isolation of benzoyl eugenol (**1**) along with the C-14 epimers of the labdane diterpene isocoronarin D (**2a, 2b**) and the C-15 epimers of the ethoxyl derivative from coronarin D (**3a, 3b**). Both diterpenes have been previously described for the species; however, their occurrence as epimeric mixtures is here reported for the first time.

MATERIAL AND METHODS

General procedures

^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY and HMQC spectra were recorded on a Bruker DRX-400 spectrometer (^{1}H 400 MHz and ^{13}C 100 MHz) using TMS as internal standard for both nuclei. Samples were dissolved in $CDCl_3$ for analysis. Chemical shifts (δ) are given in *ppm* and *J* couplings in Hertz (Hz). Semi preparative HPLC was carried out on a Shimadzu system (Japan) composed of pump LC-8A, UV-VIS detector SPD-GAV, controller system SCL-8A and integrator C-R4A, at room temperature. A semi-preparative silica gel column (Shim-pack prep-Sil 250 × 20 mm, i.d., Shimadzu, Japan) was employed throughout the work.

Plant material

Hedychium coronarium was collected in February 2002, at UFMG campus, in Minas Gerais state, Brazil. The species was identified by Dr. J. A. Lombardi, from the Departamento de Botânica, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Brazil, where a voucher specimen is deposited (BHCB 68447).

Extraction and isolation

The dried rhizomes of *H. coronarium* (707 g) were percolated with 96% EtOH at room temperature. The solvent was removed under reduced pressure affording 84.29 g of residue, that was successively extracted with solvents of increasing polarity to yield the *n*-hexane (33.65 g), dichloromethane (25.55 g) and methanol (15.30 g) fractions. A portion of the DCM fraction (20.55 g) was chromatographed on a silica gel column (70-230 mesh, Merck), eluting with *n*-hexane-ethyl acetate (95:5-1:1) gradient. The fraction eluted with *n*-hexane-ethyl acetate (9:1) afforded **1** (48.8 mg), whereas the *n*-hexane-ethyl

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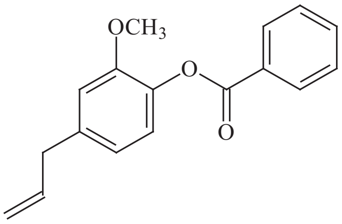
Table 1. ^{13}C NMR spectral data of compounds **2a**/**b** and 3**a**/**b** (100 MHz in CDCl$_3$ solution)

C	*2a*	*2b*	*3a*	*3b*
1	39.6	39.8	39.2	39.3
2	19.3	19.3	19.3	19.3
3	42.0	42.0	42.0	42.0
4	33.6	33.6	33.6	33.6
5	55.4	55.5	55.3	55.4
6	24.2	24.2	24.1	24.1
7	37.9	37.9	37.8	37.8
8	148.0	148.2	147.8	148.1
9	56.5	56.5	56.1	56.1
10	39.6	39.8	39.4	39.4
11	24.8	25.2	25.4	25.4
12	149.7	149.9	142.9	143.0
13	127.8	127.9	124.1	124.1
14	66.3	66.6	32.9	32.9
15	74.2	74.2	101.0	101.0
16	169.9	170.0	169.8	169.8
17	107.5	107.9	107.3	107.7
18	33.6	33.6	33.6	33.6
19	21.7	21.7	21.7	21.7
20	14.4	14.5	14.3	14.4
21			65.1	65.1
22			15.0	15.0



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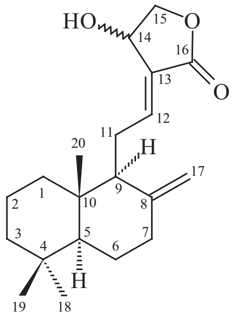

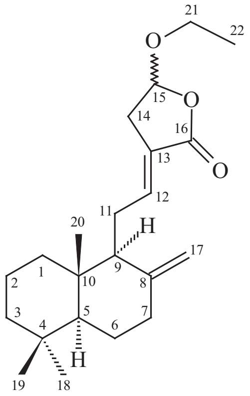

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acetate (85:15) eluent (4.184 g) was further submitted to VLC on silica gel and various fractions were taken using DCM and EtOAc mixtures. The fraction eluted with DCM afforded **3** (311.2 mg) and the one obtained with DCM:EtOAc (98:2) yielded **2** (5.8 mg), after purification by semi preparative HPLC, employing isocratic elution of *n*-hexane-dichloromethane (6:4), at a flow rate of 5 ml/min.

Synthesis of benzoyl eugenol

Table 2. ^{1}H NMR spectral data of compounds **2a**/**b** and 3**a**/**b** (400 MHz in CDCl$_3$ solution)

H		*2a*	*2b*	*3a*	*3b*
1	*ax*	1.15 (ddd 4.0, 12.0, 12.0 Hz)	1.13 (ddd 4.0, 12.0, 12.0 Hz)		
	eq	1.55 (m)	1.55 (m)		
2	*ax*	1.55 (m)	1.55 (m)		
	eq	1.50 (m)	1.50 (m)		
3	*ax*	1.20 (ddd 4.2, 13.4, 13.4 Hz)	1.20 (m)		
	eq	1.42 (m)	1.42 (m)		
5		1.13 (dd 2.5, 12.8 Hz)	1.13 (dd 2.5, 12.8 Hz)	1.11 (m)	1.15 (m)
6	*ax*	1.74 (m)	1.74 (m)		
	eq	1.34 (ddd 4.2, 12.8, 12.9 Hz)	1.34 (ddd 4.2, 12.8, 12.9 Hz)		
7	*ax*	2.02 (ddd 4.9, 12.8, 13.1 Hz)	2.02 (m)		
	eq	2.41 (ddd 2.1, 4.2, 13.1 Hz)	2.40 (ddd 2.1, 4.2, 13.1 Hz)		
9		1.86 (br. d 10.0 Hz)	1.95 (br. d 12.8 Hz)	1.86 (dt 4.8, 9.7 Hz)	1.99 (t 9.7 Hz)
11		2.52 (m)	2.52 (m)	2.21 (m)	2.67 (m)
		2.53 (m)	2.57 (m)	2.33 (m)	2.72 (m)
12		6.98 (m)	6.98 (m)	6.73 (m)	6.73 (m)
14		5.04 (d 6.0 Hz)	5.06 (d 6.0 Hz)	2.70 (m)	3.00 (m)
				2.70 (m)	3.00 (m)
15		4.26 (dd 2.0, 10.4 Hz)	4.24 (dd 2.0, 10.4 Hz)	5.56 (t 2.4 Hz)	5.57 (t 2.4 Hz)
		4.45 (dd 6.0, 10.4 Hz)	4.44 (dd 6.0, 10.4 Hz)		
17		4.56 (d 1.2 Hz)	4.36 (d 1.2 Hz)	4.35 (s)	4.41 (s)
		4.88 (d 1.2 Hz)	4.83 (d 1.2 Hz)	4.81 (s)	4.83 (s)
18		0.89 (s)	0.89 (s)	0.89 (s)	0.89 (s)
19		0.82 (s)	0.82 (s)	0.82 (s)	0.82 (s)
20		0.74 (s)	0.74 (s)	0.72 (s)	0.72 (s)
21				3.63 (m)	3.92 (m)
				3.63 (m)	3.92 (m)
22				1.24 (t 7.0 Hz)	1.25 (t 7.0 Hz)

An aliquot of eugenol (1 ml) was dissolved in 5% NaOH aqueous solution (30 ml) and treated with benzoyl chloride (2 ml). The reaction mixture was stirred in an ice bath for 60 min and the formed precipitate was vacuum filtered. It was thoroughly washed with cold water, yielding 670 mg of benzoyl eugenol. ^{1}H and ^{13}C NMR data were recorded for this sample and compared with those obtained for **1**.

RESULTS AND DISCUSSION

The chromatographic separation of the dichloromethane fraction from the ethanol extract of *H. coronarium* rhizomes on a silica gel column afforded three compounds. Compound **1**, a white amorphous solid, showed a molecular formula of $C_{17}H_{16}O_3$, determined from the quasimolecular peak at *m/z* 269.1204 $(M + H)^+$ on positive-ion high resolution FABMS. The IR spectrum of **1** showed a strong absorption band at 1740 cm^{-1}, characteristic of aryl esters. The ^{13}C NMR and DEPT spectra gave signals for a total of seventeen carbons, including one methoxyl, two methylenes, nine methynes and five quaternary carbons.

The ^{1}H NMR spectrum of **1** showed signals of a non substituted (δ 7.49 2H, *d*, *J* 7.5 Hz; δ 7.62, 1H, *t*, *J* 7.4 Hz; δ 8.21, *d*, 2H, *J* 7.4 Hz) and a 1,2,4-trisubstituted phenyl group (δ 6.81 1H, *d*, *J* 7.9 Hz; δ 6.83 1H, *s*; δ 7.06 1H, *d*, *J* 7.9 Hz). The signal appearing as a doublet at δ 2.39 (2H, 6.7 Hz) was assigned to a methylene flanked by an aryl group and the multiplet at δ 5.12 was credited to another methylene group. Further, the methyne at δ 5.99 appeared as a multiplet, indicating its coupling with both methylenes, which was confirmed by the connections depicted in the COSY spectrum, indicating an allyl group. The assignment of the carbon resonances was accomplished by a HMQC experiment.

Based on these data the structure of **1** was assigned as benzoyl eugenol and confirmed by direct comparison with synthetic sample prepared from the reaction of eugenol with benzoyl choride (see Experimental). Eugenol has been previously identified as a constituent of the essential oil from the leaves of *H. coronarium* (Hagga; El-Shamy, 1977). The occurrence of its benzoyl derivative, however, has not been reported so far from any natural source.

Compound **2**, isolated as white needles, mp 179-182°C, showed IR absorption bands characteristic of a hydroxyl group (3400 cm^{-1}), an α,β-unsaturated γ-lactone ring (1720 cm^{-1}) and an *exo*-methylene (3100, 1680, 980 and 850 cm^{-1}). FABMS spectrum of **2** showed a molecular ion (M^+) peak at *m/z* 318, in addition to a base peak at *m/z* 301 ($MH^+–H_2O$). The ^1H NMR spectrum of **2** showed characteristic signals of labdane type-diterpenoids, *viz*. three methyl groups (δ 0.74, 0.82 and 0.89, 3H each) and *exo*-methylene hydrogenes (δ 4.88, 4.83, 4.56 and 4.36).

Comparison of physical data obtained for **2** with literature records for labdane diterpenes indicated the previously isolated isocoronarin D (Singh et al., 1991; Nakatani et al., 1994). However, its ^1H and ^{13}C NMR spectra presented some resonances as pairs of signals, with nearly identical values of chemical shifts, suggesting that **2** was a mixture of compounds structurally related. This was specially evidenced by the signals attributed to H-14 (δ 5.04, *d*, *J* 6.0 Hz and δ 5.06, *d*, *J* 6.0 Hz), H-15 (δ 4.26, *dd*, *J* 2.0, 10.4 Hz; δ 4.45, *dd*, *J* 6.0, 10.4 Hz and δ 4.24, *dd*, *J* 2.0, 10.4 Hz, δ 4.44, *dd*, *J* 6.0,10.4 Hz) and H-17 (δ 4.88, *d*, *J* 1.2 Hz; δ 4.56, *d*, *J* 1.2 Hz and δ 4.83, *d*, *J* 1.2 Hz; δ 4.36, *d*, *J* 1.2 Hz), that indicated an epimeric mixture of **2** at C-14. Similarly, it was also possible to identify duplicated signals in the ^{13}C NMR spectrum, especially for the carbons of the lactone ring (Table 1).

The complete ^1H and ^{13}C assignment for the epimers **2a/b** (Tables 1 and 2) was obtained from HMQC and ^1H,^1H-COSY experiments. The presence of the epimers in the ratio 1:1.5 in the mixture allowed the unambiguous identification of the signals from each isomer. The coupling of C-15 methylene hydrogens with H-14 showed identical values for **2a** (*J* 2.0, 10.4 Hz) and **2b** (*J* 2.0, 10.4 Hz), which is in accordance with the proposed structures for the epimers **2a/b**.

The relative stereochemistry at C-14 was previously established for isocoronarin D based on an X-ray study, which defined the β-position for the hydroxyl group (Singh et al., 1991). In the present work, the relative stereochemistry at C-14 for the mixture **2a**/**b** could not be determined by NMR data. However, considering the data previously reported for isocoronarin D, we can presume the predominance of the β-isomer in the mixture, by comparison with the chemical shifts reported for this single isomer (Singh et al., 1991).

The isolation of isocoronarin D was first reported by Singh et al. from the rhizomes of *H. coronarium* collected in Nepal. According to the authors, the oxygenation at C-14 rather than C-15 is unusual in labdane diterpenes and might arise from secondary oxidation of a 14,15 double bond (Singh et al., 1991). Isocoronarin D, also reported as the single OH-C14 β-isomer, was further isolated from the rhizomes of the same species collected in the Philippines (Nakatani et al., 1994). Noteworthy is the isolation of the single OH-C14 α-isomer of isocoronarin D from another Zingiberaceae species (*Alpinia calcarata*), named calcaratarin D (Kong et al., 2000). The isolation of a C-14 epimeric mixture of **2** collected in Brazil suggests that the secondary oxidation of the 14,15 double bond might be influenced by environmental factors.

Compound **3**, a colorless oil, showed IR bands of a hydroxyl group (3400 cm^{-1}), an α,β-unsaturated γ-lactone ring (1760 cm^{-1}) and an *exo*-methylene (3100, 1680, 940 and 880 cm^{-1}). The molecular formula $C_{22}H_{34}O_3$ was determined from the molecular ion peak at *m/z* 347 (M^+) on FABMS, along with the base ion peak at *m/z* 301 ($M^+–C_2H_5OH$). ^1H NMR spectrum evidenced the labdane nature of **3**, which revealed three signals of methyl groups (δ 0.72, 0.82 and 0.89, 3H each) and *exo*-methylene hydrogens (δ 4.39, 4.81, and 4.82). Another feature of this spectrum was the presence of two ethoxyl groups, displayed by the two sets of triplets (δ 1.24 *J* 7.0 Hz and δ 1.25 *J* 7.0 Hz, 3H each) and multiplets (δ 3.63 and δ 3.92, 2H each). Similarly to **2**, the ^1H and ^{13}C spectra of **3** presented duplicated signals, with similar values of chemical shifts, suggesting an epimeric mixture.

Comparison of the spectral data of **3** with others labdane diterpenes allowed its identification as an epimeric mixture of the ethoxyl derivative from coronarin D. The use of HMQC and homonuclear COSY spectroscopy allowed the assignment of ^1H and ^{13}C spectra for the epimeric mixture **3a/b** (Tables 1 and 2). This compound has been previously reported as a single isomer from the rhizomes of *H. coronarium* and was regarded as an artefact of the extraction process with ethanol (Singh et al., 1991). Data reported by the authors also suggest the presence of an epimeric mixture, which was not identified due to the incorrect assignment of the two sets of multiplets at δ 3.60 and δ 3.85 to each one of the methylene hydrogens of the ethoxyl group. However, no other duplicated signal was reported by Singh et al. for the ^1H and ^{13}C NMR spectra of **3**. Further evidence that **3** is an artefact produced by ethanol percolation of the plant material is furnished by the isolation of coronarin D methyl ether from an extract of *H. coronarium* rhizomes prepared by reflux with methanol (Matsuda et al., 2002).

It is worth mentioning that the isolation of isocoronarin D (**2**) and of coronarin D ethoxyl derivative (**3**) has been previously reported (Singh et al., 1991; Nakatani et al., 1994). However, the identification of those diterpenes as epimeric mixtures is here described for the first time. The occurrence of epimers of labdane diterpenes at C-15 and C-16 positions has been reported; none of them, however, from a *Hedychium* species (Kong et al., 2000; Malakov et al., 1985; Zani et al., 2000). Hence, this is also the first report on the occurrence of such epimers in the genus.

The compounds here reported might support the traditional use of *H. coronarium* for the treatment of inflammatory processes, since several structure-related labdane diterpenes, isolated from the same species, showed *in vitro* inhibitory effects on increase in vascular permeability and nitric oxide production (Matsuda et al., 2002).

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