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Cyclopentene dialdehydes from *Tabebuia impetiginosa*

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Abstract

The isolation of two cyclopentene dialdehydes, 2-formyl-5-(4′-methoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde and 2-formyl-5-(3′,4′-dimethoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde, from the bark of *Tabebuia impetiginosa* is reported. The structures were established by analysis of spectroscopic data. These compounds showed anti-inflammatory activity.

Keywords: *Tabebuia impetiginosa*; Bignoniaceae; Cyclopentene dialdehyde; Anti-inflammatory activity

1. Introduction

The stem bark of the South American tree *Tabebuia impetiginosa* Mart. *ex* DC (Bignoniaceae), which is a source of furanonaphthoquinones, has been used in North and South America for many years as an anticancer, antifungal, antibacterial, and anti-inflammatory drug (Abbott, Hartwell, Leiter, Perdue & Schepartz, 1967; Hartwell, 1968; Zani, de Oliveira & de Oliveria, 1991). An investigation of the constituents of *T. impetiginosa* led us to isolate two new cyclopentene dialdehydes together with known furanonaphthoquinones and benzoic acid and benzaldehyde derivatives (Oliveira, Raslan, de Oliveira & Maia, 1993; Wagner, Kreher, Lotter, Hamburger & Cordell, 1989).

We used the nitro blue tetrazolium chloride (NBT) reduction system to determine the anti-inflammatory effects of two cyclopentene dialdehydes in activated human granular white blood cells (WBC, including neutrophils) (Christman, Holden & Blackwell, 1995). This paper describes the structure elucidation and anti-inflammatory activity of the new compounds.

2. Results

The methanol extract of *T. impetiginosa* was dissolved in water and sequentially partitioned with *n*-hexane, chloroform, dichloroethane, and ethyl acetate. Silica gel column chromatography of the *n*-hexane and chloroform soluble layers yielded β-sitosterol, stigmasterol, 4′-methoxybenzyl 4-methoxybenzoate, 9-hydroxy-3-methylnaphtho[2,3-*b*]pyran-2,5,10-trione, (-)-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin, and seven known furanonaphthoquinones. The residue from the dichloroethane soluble layer was chromatographed on silica gel to afford compounds **1** and **2**.

Compound **1**, $C_{17}H_{18}O_5$, showed IR absorptions due to an ester (1707 cm^{-1}), a conjugated aldehyde (1664 cm^{-1}), simple aldehyde (1712 cm^{-1}), and an aromatic ring (1512, 1607 cm^{-1}). Its ^{1}H-NMR spectrum indicated the presence of a para-substituted aromatic ring on the basis of chemical shift values of the AA′BB′ system at δ 6.90 (*d*, J = 9 Hz) and δ 7.95 (*d*, J = 9 Hz). A methoxy signal was observed at δ 3.85 (*s*), along with two aldehyde protons at δ 9.79 (*t*, J = 1.5 Hz) and δ 10.00 (*s*), and a methyl group at δ 2.22 (*s*). The multiplet signal at δ 2.70 and double double doublet at δ 2.87 (J = 17, 5.5, 1.5 Hz) were assigned to the methylene protons adjacent to the simple alde-

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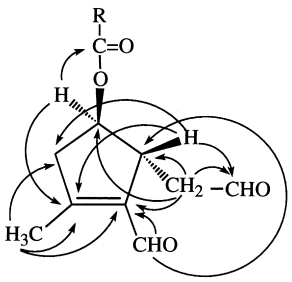
Table 1
^{13}C-NMR chemical shifts of compounds **1** and **2** (in $CDCl_3$)

C	1	2
Me	14.36	14.38
1	45.95	45.95
2	137.29	137.26
3	160.60	160.56
4	45.88	45.95
5	76.87	77.10
CH_2CHO	44.96	44.98
CH_2CHO	200.87	200.80
CHO	187.44	187.45
Acyl 1′	122.23	122.32
2′	131.67	112.01
3′	113.64	148.68
4′	163.56	153.24
5′	113.64	110.22
6′	131.67	123.70
C=O	165.98	166.09
OMe	55.45	56.05
OMe		56.05

hyde (CH_2CHO), and the doublet-like signal at δ 5.17 (J = 7 Hz) is ascribable to H-5. The ^{13}C-NMR spectrum showed 17 carbon signals consisting of one methyl carbon (δ 14.36), one methoxy carbon (δ 55.45), two methylene carbons (δ 44.96 and 45.88), six methine carbons, four quaternary carbons, one carbonyl carbon (δ 165.98) and two aldehyde carbons (δ 187.44 and 200.87) (Table 1). NOEs were observed between H-1 at δ 3.57 and H-6 at δ 2.87, and between H-5 at δ 5.17 and H-6 at δ 2.70, showing that these protons are in a *cis* relationship with each other. The structure of **1** was determined by analysis of ^{1}H-, ^{13}C-NMR, HMQC, HMBC, NOESY (Fig. 1), and IR spectroscopic data as 2-formyl-5-(4′-methoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde.

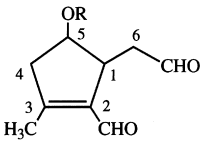
Compound **2**, $C_{18}H_{20}O_6$, showed ester, conjugated aldehyde, and aromatic ring absorptions in its IR spectrum. The ^{1}H-NMR spectrum indicated the presence of a 3′,4′-dimethoxybenzoyl moiety. Other spectral data were similar to those of **1**. Thus, **2** was regarded as 2-formyl-5-(3′,4′-dimethoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde. Compounds **1** and **2** would be the hydrolysis products of the naturally-occurring iridoid glucoside (**3**) (Nakano, Maruyama, Murakami, Takaishi & Tomimatsu, 1993) (Davini, Iavarone & Trogolo, 1987; Watanabe, Takada, Matsuo & Nishimura, 1995; Drewes, Horn, Connolly & Bredenkamp, 1998).



1 R = 4'-methoxyphenyl
2 R = 3',4'-dimethoxyphenyl

Fig. 1. ^{13}C-^{1}H long range correlations in the HMBC spectrum.

Fig. 2 shows the inhibition curves of NBT reduction in the 12-*o*-tetradecanoylphorbol-13-acetate (TPA)-activated PMN by the samples. Compounds **1** and **2** showed potent anti-inflammatory activity. The 50 and 100% inhibitory concentrations (IC_{50} and IC_{100}) of **1** were 0.8 and 3.0 μg/ml, respectively. Compound **2** showed 1.05 μg/ml for IC_{50} and 4.0 μg/ml for IC_{100}.



R
1 R = 4'-methoxybenzoyl
2 R = 3',4'-dimethoxybenzoyl

3. Experimental

3.1. NMR

500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR, $CDCl_3$, TMS as int. standard; CC: silica gel (Mallinckrodt, AR) in amounts equivalent to 50 times of the extracts; PTLC: silica gel (Merck, $60F_{254}$; thickness, 0.5 mm); pre-packed column size B (Merck, LiChroprep Si 60).

3.2. Isolation

The dried chips of the bark of *Tabebuia impetiginosa* (17 kg) (obtained from Santosflora, Sao Pâulo, Brazil) were extracted with hot MeOH. The MeOH extract was concentrated under reduced pressure; the residue (704 g) was dissolved in water and sequentially partitioned with *n*-hexane (residue 41.1 g), $CHCl_3$ (137.3 g), $C_2H_4Cl_2$ (35.1 g), and EtOAc (110.9 g). The $C_2H_4Cl_2$-soluble portion (4 g) was chromatographed on silica gel (pre-packed column) using solvent systems of $CHCl_3$ (fraction 1: 500 ml, residue 48 mg; fr. 2: 500 ml, 103 mg; fr. 3: 1000 ml, 55 mg), $CHCl_3$–MeOH (fr. 4: 1% MeOH 2000 ml, 176 mg; fr. 5: 3% MeOH 2000

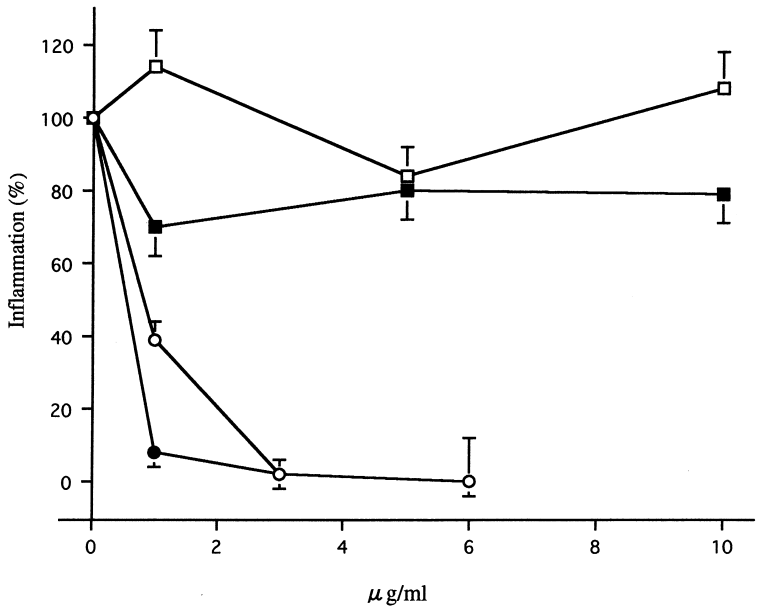


Fig. 2. Anti-inflammatory activities of **1** and **2** in the TPA-activated human PMN in comparison with alkylated benzoic acids. ○ **1**; ● **2**; □ 4-methoxybenzoic acid; ■ 3,4-dimethoxybenzoic acid.

ml, 127 mg). Fr. 2 was subjected to prepared TLC on silica gel using $CHCl_3$–MeOH–acetone (120:2:1) to give the mixture of **1** and **2** (15 mg), a mixture of 5- and 8-hydroxy-2-(hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione (12 mg), 4-methoxy-, and 3,4-dimethoxybenzoic acid. The mixture of **1** and **2** was subjected to prepared TLC with C_6H_6–Et_2O (2:3) to afford **1** (*Rf* = 0.48) and **2** (*Rf* = 0.38) (2:1).

*3.3. 2-Formyl-5-(4′-methoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde (**1**)*

Oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1712, 1707, 1664, 1607, 1512; ^{1}H-NMR ($CDCl_3$) δ:2.22 (3H, *s*, OMe), 2.70 (2H, *m*, H-4, C$\underline{H}_2$CHO), 2.87 (1H, *ddd*, *J* = 17, 5.5 and 1.5 Hz, C$\underline{H}_2$CHO), 3.25 (1H, *dd*, *J* = 20 and 7 Hz, H-4), 3.57 (1H, *t*-like, *J* = 5.5 Hz, H-1), 3.85 (3H, *s*, OMe), 5.17 (1H, *d*-like, *J* = 7 Hz, H-5), 6.90 (2H, *d*, *J* = 9 Hz, H-3′, 5′), 7.95 (2H, *d*, *J* = 9 Hz, H-2′,6′), 9.79 (1H, *t*, *J* = 1.5 Hz, $CH_2C\underline{H}O$), 10.00 (1H, *s*, 2-CHO); MS *m*/*z*: 302.1140 (M^+, calculated for $C_{17}H_{18}O_5$, 302.1153).

*3.4. 2-Formyl-5-(3′,4′-dimethoxybenzoyloxy)-3-methyl-2-cyclopentene-1-acetaldehyde (**2**)*

Oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1714, 1706, 1664, 1602, 1515; ^{1}H-NMR ($CDCl_3$) δ:2.23 (3H, *s*, Me), 2.71 (2H, *m*, H-4, C$\underline{H}_2$CHO), 2.96 (1H, *ddd*, *J* = 18.5, 5, and 1.5 Hz, C$\underline{H}_2$CHO), 3.27 (1H, *dd*, *J* = 19.5 and 6.5 Hz, H-4), 3.58 (1H, *t*-like, *J* = 5 Hz, H-1), 3.82, 3.91 (3H × 2, *s*, OMe), 5.18 (1H, *dt*, *J* = 6.5 and 2 Hz, H-5), 6.89 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.50 (1H, *d*, *J* = 2 Hz, H-2′), 7.63 (1H, *dd*, *J* = 8.5 and 2 Hz, H-6′), 9.80 (1H, *t*, *J* = 1.5 Hz, $CH_2C\underline{H}O$), 10.00 (1H, *s*, 2-CHO); MS *m*/*z*: 332.1240 (M^+, calculated for $C_{18}H_{20}O_6$, 332.1258).

3.5. Anti-inflammatory assay with human WBC

Compounds **1** and **2** were tested. 4-methoxybenzoic acid and 3,4-dimethoxybenzoic acid that were also obtained from the $C_2H_4Cl_2$-soluble portion of *T. impetiginosa*, were used as controls.

Venous blood from healthy donors was harvested and the granular WBC fractions were isolated by the use of a Ficoll–Hypaque solution. The cells were suspended in an ice-cold phosphate buffered saline (PBS). The NBT medium consisted of 25 μg/ml NBT, 9 μM $CaCl_2$, 5 μM $MgCl_2$, 0.1% glucose and 10 mM NaN_3 in PBS (Hirai, Moriguchi & Wang, 1991). The assay was performed by combining 1×10^4 WBC with 50 ng/ml TPA in an NBT medium for 20 min at 37°C. The diformazan pigments formed were extracted with DMSO, and absorbance at 560 nm was measured in a spectrophotometer. The inhibition rate was estimated by the modified method of Evans (Rice-Evans, Diplock & Symons, 1991).

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