PERGAMON

Phytochemistry 55 (2000) 837–841

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Anthrone and oxanthrone *C*,*O*-diglycosides from *Picramnia teapensis*

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Received 6 January 2000; received in revised form 21 April 2000

In honour of Professor Otto R. Gottlieb's 80th birthday

Abstract

Two *C*,*O*-diglycosylated compounds, the anthrone picramnioside F, and the oxanthrone mayoside C, were isolated from the stem bark of *Picramnia teapensis*, along with the previously reported anthraquinones, 1-*O*-β-D- and 8-*O*-β-D-glucopyranosyl emodin. The compounds were separated by recycling-HPLC, and their structures were determined on the basis of spectroscopic analysis. CD measurements were used to establish the absolute configuration of the anthrone and oxanthrone. The antifungal activity of 1-*O*-β-D- and 8-*O*-β-D-glucopyranosyl emodin against *Leucoagaricus gongilophorus* was shown to be similar to that of the lignan sesamin.

Keywords: *Picramnia teapensis*; Simaroubaceae; Anthraquinone glycosides; Anthrone glycoside; Oxanthrone glycoside; Picramnioside; Mayoside; Antifungal activity

1. Introduction

In a previous paper (Rodríguez-Gamboa et al., 1999) we reported the isolation and characterization of two *C*-glycosylated anthrones (picramniosides D, E), and two *C*-glycosylated oxanthrones (mayoside and mayoside B), as well as emodin and umbelliferone, from the stem bark of *Picramnia teapensis* Tul., a tree found in Central America. Further investigations afforded, in minor quantities, two new *C*,*O*-diglycosylated compounds: an anthrone, picramnioside F (**1**), and an oxathrone, mayoside C (**2**), along with the known anthraquinone glycosides, 1-*O*-β-D-glucopyranosyl emodin (**3**) and 8-*O*-β-D-glucopyranosyl emodin (**4**). The antifungal activity of the isolated compounds was determined by comparison with sesamin, a lignan active against *Leucoagaricus gongilophorus* (Pagnocca et al., 1996). This report deals with the isolation and structure elucidation of compounds **1** and **2**, and the antifungal activity of the isolated compounds.

2. Results and discussion

The EtOAc extract from the bark of *P. teapensis* afforded, in minor quantities, a mixture of compounds **1**–**4**, which was separated by recycling-HPLC on a Shimadzu polymeric packing column. The anthraquinones **3** and **4** were identified on the basis of spectroscopic data and by comparison with published data (Rawat et al., 1989; Coskun et al., 1990; Inoue et al., 1992).

Compound **1** was obtained as a yellow amorphous solid. The PI-ESIMS spectrum exhibited pseudo-molecular ions at *m*/*z* 671 ($[M+1]^+$), 693 ($[M+Na]^+$) and 709 ($[M+K]^+$), with the base peak at *m*/*z* 509 ($[M+1-C_6H_{10}O_5]^+$). The corresponding molecular formula, $C_{33}H_{34}O_{15}$, was consistent with the ^1H and ^{13}C NMR spectroscopic data.

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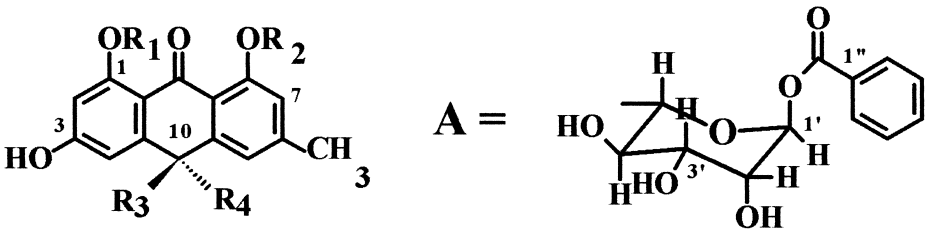
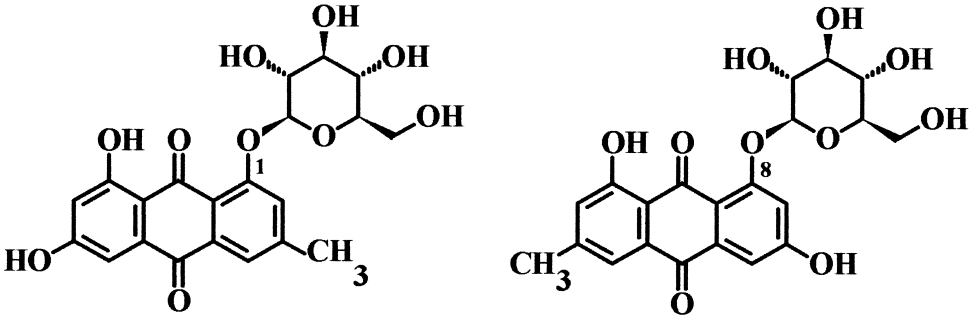
0031-9422/00/$ - see front matter
PII: S0031-9422(00)00323-X



1 $R_1 = H$, $R_2 = Glucose$, $R_3 = H$, $R_4 = A$

2 $R_1 = Glucose$, $R_2 = H$, $R_3 = A$, $R_4 = OH$



3 **4**

All of the spectroscopic characteristics of **1** were coincident with those of anthrones which we have previously reported (Rodríguez-Gamboa et al., 1999), showing UV absorptions at λ_{max} 228, 274 and 340 nm, and IR absorptions typical of hydroxyl (3387 cm^{-1}), ester carbonyl (1735 cm^{-1}) and chelated carbonyl (1639 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed two groups of signals in the aromatic region, one at δ 7.4–7.8 (5H), typical of a benzoyl group, and two pairs of *meta*-coupled protons from an emodin anthrone moiety. The methyl protons at δ 1.84 (3H) and a doublet at δ 4.48, characteristic of H-10 of an anthrone (Rodríguez-Gamboa et al., 1999), were also observed. The spectrum showed signals for two anomeric protons at δ 5.82 and 4.97, and a complex group of signals at δ 3.40–3.95, indicating sugar moieties.

The presence of 33 carbons was evident from the ^{13}C NMR spectrum (Table 1). The C-10 signal of the anthrone was observed at δ 44.8 and the anomeric carbons at δ 95.8 and δ 105.4. Signals in the region of δ 62–81 indicated the presence of two sugar moieties, one of them being lyxose, previously characterized in mayoside, uveoside and saroside (Hernández-Medel et al., 1996, 1998, 1999). In a previous paper (Rodríguez-Gamboa et al., 1999) the sugar name of picramniosides D–E as well as of mayoside and mayoside B was cited as xylose, but the structures and the spectroscopic data are in agreement with lyxose (Hernández-Medel et al., 1996, 1998, 1999). The previous name was based on Solís et al. (1995) which identified the sugar of picramniosides A–C as xylose. The signals of the sugar unit bonded at C-8-*O* were typical of glucose (Manitto et al., 1993). DEPT experiments confirmed only one CH_2 signal at δ 61.5, corresponding to C-6 of the glucose unit.

Complete assignment of the ^1H and ^{13}C NMR spectroscopic signals (Table 1) was established on the basis of one-bond and long-range 2D-NMR experiments (HMQC, HMBC, COSY and NOESY). In particular, diagnostic correlations were as follows: (i) ^3J coupling between the ester carbonyl (δ 164.7) and H-1′, H-2″ and H-6″, indicating the linkage of the benzoyl group to C-1′ of the lyxose sugar; and (ii) ^3J coupling between H-10 of the anthrone and C-5′ (δ 81.4), confirming the position of the aglycone at C-5′ of lyxose. Furthermore, another seven correlations with C-10 were observed (Table 2), and a ^3J correlation of the anomeric

Table 1
^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) chemical shifts for compounds **1** and **2** (methanol-d_4)

	Compound **1**		Compound **2**[a]	
C	δ C (ppm)	δ H (ppm)	δ C (ppm)	δ H (ppm)
1	164.8	–	162.0	–
1a	114.4	–	n.o.[b]	–
2	102.6	6.23 *d* (2.3)	107.6	6.74 *d* (2.4)
3	165.6	–	n.o.	–
4	109.6	6.54 *d* (2.3)	108.7	6.97 *d* (2.4)
4a	143.0	–	n.o.	–
5	124.4	6.82 *s*	119.2	7.18 *br. d* (1)
5a	147.4	–	145.0	–
6	147.0	–	147.0	–
7	120.8	7.09 *s*	118.1	6.73 *br. d* (1)
8	159.6	–	n.o.	–
8a	122.8	–	n.o.	–
9	190.6	–	n.o.	–
10	44.8	4.48 *d* (2.3)	76.0	–
11	21.4	1.84 *s br*	22.1	2.42 *s*
1′	95.8	5.82 *s*	95.4	5.80 *d* (1.2)
2′	71.5	3.75–3.95 *m*	70.4[c]	3.76–3.80 *m*
3′	74.0	3.75–3.95 *m*	73.4	3.76–3.80 *m*
4′	68.5	3.75–3.95 *m*	70.0[c]	3.58–3.66 *m*
5′	81.4	3.98 *dd* (12.1, 2.3)	80.1	3.55–3.64 *m*
C=O	164.8	–	n.o.	–
1″	130.6	–	n.o.	–
2″ and 6″	130.6	7.79 *dm* (7.9)	130.4	7.80 *dm* (8.0)
3″ and 5″	129.8	7.48 *tm* (7.4)	129.7	7.47 *tm* (7.4)
4″	134.6	7.63 *tt* (7.4, 1.30)	134.4	7.61 *tm* (7.4)
1‴	105.4	4.97 *d* (7.5)	105.1	4.97 *d* (7.5)
2‴	75.1	3.44–3.53 *m*	74.9	3.56–3.63 *m*
3‴	77.6	3.44–3.53 *m*	77.4	3.57–3.61 *m*
4‴	71.1	3.75–3.95 *m*	71.1	3.43–3.50 *m*
5‴	78.8	3.44–3.53 *m*	78.6	3.43–3.50 *m*
6‴	62.8	3.75–3.95 *m*	62.5	3.97 *m*; 3.78 *m*

[a] ^{13}C values based on HSQC and HMBC experiments.
[b] Not observed.
[c] May be exchangeable.

Table 2
^{1}H-^{13}C (HMBC) and ^{1}H-^{1}H (NOESY) correlations for compounds **1** and **2**

	1		**2**	
H	H	C	H	C
2	–	1[a]		4
4	10, sugar moiety[a]	1a, 2, 10	sugar moiety	2
5	10, sugar moiety[a]	8a, 10, 11		7, 10, 11
7	1‴, 11	5, 8a, 11		11
10	sugar[a], 4, 5	4, 5, 6, 1a, 4a, 5a, 8a, 5′	–	
11	5, 7	5, 7		5, 6, 7
1′	lyxose	3′, 5′, C=O	lyxose	sugar moiety[a]
1‴	7, glucose	8	2 or 7, glucose	1
2″ and/or 6″	sugar, 3″ or 5″	C=O, 3″ or 5″, 4″	sugar moiety	4″

[a] Most probably lyxose.

proton of the glucose unit (H-1, δ 4.97) and C-8, and the spatial correlation with H-7 in the NOESY experiment reinforced the position of the *O*-glucose unit at C-8.

The magnitude of the coupling constant between H-5′ and H-10 (2.3 Hz) indicated an *eq–eq* conformation for these protons. The configuration of the lyxose at the anthrone moiety must be β, based on comparison with other anthrone glycosides (Solís et al., 1995; Hernández-Medel et al., 1998; Rodríguez-Gamboa et al., 1999). No correlation was observed between H-1′ and H-2′ ($J=0$ Hz) suggesting an *eq–eq* conformation for these protons. Likewise, the *J* value of H-5‴ (12 Hz) indicated an *ax–ax* conformation for H-5‴ and H-4‴ in the most stable glucopyranosyl moiety.

Further observations helped to establish the configuration at C-10. The spectral data of ring A of **1** (^{1}H and ^{13}C NMR) were almost identical to those of picramnioside E (Rodríguez-Gamboa et al., 1999). In addition, the following expected differences in ring B were observed: (i) deshielding of H-7, C-1a and C-7, and (ii) shielding of C-5 and C-8 due to *O*-glucosylation at C-8.

Compound **2** was isolated as a yellow amorphous solid. The PI-ESIMS spectrum of **2** gave pseudo-molecular ions at *m/z* 709 ($[M+Na]^+$) and 725 ($[M+K]^+$). The NI-ESIMS spectrum gave a molecular ion at 685.3 ($[M-H]^-$), which was consistent with the molecular formula $C_{33}H_{34}O_{16}$. The daughter ions, from an MS–MS experiment, indicated the sequential loss of benzoic acid (*m/z* 563), lyxose (*m/z* 432), CH_2OH (*m/z* 401), and the rest of the glucose unit, leading to the fragment corresponding to emodin (269).

As observed previously for the mayosides (Hernández-Medel et al., 1996, 1999; Rodríguez-Gamboa et al., 1999), the ^{1}H NMR spectrum of **2** showed the same pattern described for **1**, except for the absence of the H-10 signal (Table 1). Particularly, ring B of **2** had almost identical ^{1}H and ^{13}C NMR spectroscopic data as those of mayoside B (Rodríguez-Gamboa et al., 1999). *O*-Glyco-sylation at C-1 was suggested by the deshielding of C-2 and H-2 from compound **2** at ring A.

Assignment of NMR signals was made on the basis of an HSQC experiment, and by comparison with published data (Rodríguez-Gamboa et al., 1999). Although HMBC and NOESY experiments did not afford all of the expected information, some correlations were detected and they are listed at Table 2. In particular, the correlation of H-1‴ with C-1 (δ 162) helped to confirm the position of the glucose at the anthraquinone moiety. In the NOESY experiment, the almost identical chemical shifts of the H-2 (6.74) and H-7 (6.73) signals, made it impossible to distinguish which proton (H-2 or H-7) had a spatial interaction with H-1‴.

Circular dichroism confirmed the absolute configuration at C-10 of **1** and **2** as *S* and *R*, respectively. The CD data of

Table 3
Growth inhibition activity of sesamin, mayoside B, picramnioside E, **3**, **4**, emodin and umbelliferone against *Leucoagaricus gongilophorus*

Compounds	Minimum active concentration tested (μg ml^{-1})	Inhibition %
Sesamin	70	80
Mayoside B	104	80
Picramnioside E	103	0
3	20	70
3	10	70
4	28	70
4	14	70
Umbelliferone	130	70
Umbelliferone	70	20
Umbelliferone	40	0
Emodin	111	40
Emodin	70	20
Emodin	40	0
Umbelliferone:emodin 1:1	87	20
Umbelliferone:emodin 2:3	49	20
Umbelliferone:emodin 1:5	76	0

1 and **2** presented the same Cotton effects as picramnioside B and E (Solís et al., 1995; Rodríguez-Gamboa et al., 1999) and saroside (Hernández-Medel et al., 1999), namely a negative Cotton effect near 270 and 315 nm, and a positive Cotton effect near 350 nm.

The compounds mayoside B, picramnioside E, **3**, **4**, emodin and umbelliferone were tested in the growth inhibition assay of *Leucoagaricus gongilophorus*, using the methodology described by Pagnocca et al. (1996). The results are shown in Table 3. Compounds **3** and **4** presented appreciable activity, being more active than sesamin, a lignan common in *Sesamum indicum*. Emodin and umbelliferone were also active, and their mixture seem to increase the growth inhibition of the fungi (Table 3). The other compounds were not assayed due to lack of material.

3. Experimental

3.1. General

Mp: uncorr.; IR: BOMEM-FT-IR; UV: Hewlett Packard 8452A; ^{1}H NMR, COSY, NOESY, HMQC, HSQC, HMBC and ^{13}C NMR, in methanol-d_4, containing TMS as int. standard: Bruker DRX-400; ESIMS: Micromass Quattro LC, HRMS: Autospec-Micromass EBE.

3.2. Plant material

The bark of *P. teapensis* was collected in Costa Rica, in the region of San José de la Montaña, September, 1990. A voucher specimen (CR194274) was deposited in the herbarium of the Universidad Nacional de Heredia, Costa Rica.

3.3. Isolation of constituents

Dried and powdered bark of *P. teapensis* (615 g) was macerated with EtOH–H_2O (4:1, v/v). The extract was filtered and concentrated under vacuum, and 50% of it (10.3 g) was partitioned between H_2O and $CHCl_3$. The aq. layer was extracted sequentially with EtOAc and then *n*-BuOH. VLC fractionation of the EtOAc extract (668 mg) on silica gel was carried out, eluting with CH_2Cl_2-MeOH mixtures of increasing polarity, to yield 80 fractions, each of 50 ml. These fractions were divided into 13 groups on the basis of analytical TLC. Fraction ten was subjected to HPLC separation on a polymeric packing column (Shimadzu, Asahipak GS-310 P, 21.5 cm ID×50.0 cm l) using MeOH for elution (Flow rate: 7 ml min^{-1} UV detector at 254 nm), to afford compound **1** (16 mg, 0.005% w/w) and 40 mg (0.013% w/w) of a mixture of 1-*O*-β- (**3**) and 8-*O*-β-glucopyranosyl emodin (**4**). Further purification of **3** (20 mg, 0.007% w/w) and **4** (18 mg, 0.006 w/w) was carried out by HPLC (two cycles of 40 min in the recycling mode, using the same conditions previously described). Fraction 11 was submitted to HPLC separation under the same conditions (6.5 ml min^{-1}, 7 cycles) to afford pure compound **2** (1.2 mg, 0.0004% w/w).

3.4. Picramnioside F (*1*)

Yellow powder, m.p. 195°C (decomposition); HRMS: found 670.1894 $[M]^+$., requires 670.1897; $[\alpha]_D^{25}$ (MeOH, *c* 3.7):−18.9; UV λ_{max} (MeOH) nm (ε):340 (12920), 274 (12280), 228 (31020); IR (KBr) ν_{max} (cm^{-1}): 3387, 2916, 2841, 1735, 1639, 1456, 1378, 1259, 1056, 753; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data: Table 1; PI-ESIMS *m*/*z* (rel. int.%): 709 (7) $[M+K]^+$, 693 (30) $[M+Na]^+$, 671 (12) $[M+1]^+$, 509 (100) $[M+1\text{-}C_6H_{10}O_5]^+$; CD (MeOH, *c* 82.0 μM) λ ($\Delta\varepsilon^{max}$): 348 (+0.8), 315 (−0.5), 269 (−2.3).

3.5. Mayoside C (*2*)

Yellow powder, m.p. 179°C (decomposition); HRMS: found 686.1851 $[M]^+$, requires 686.1846; $[\alpha]_D^{25}$ (MeOH, *c* 4.0):−12.5; UV λ_{max} (MeOH) nm (ε): 350 (12000), 276 (9990), 226 (29640); IR (KBr) ν_{max} (cm^{-1}): 3452, 2935, 2839, 1726, 1635, 1485, 1270, 1043; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectral data: Table 1; PI-ESIMS *m*/*z* (rel. int.%): 725.6 (17) $[M+K]^+$, 709.6 (100) $[M+Na]^+$; NI-ESIMS-MS (rel. int.%): 685.3 (100) $[M\text{-}H]^-$, 563.5 (10) $[M\text{-}H\text{-}C_6H_5COOH]^-$, 432.1 (5), 401.2 (5), 269 (5), 121 (5) $[C_6H_5COO]$-; CD (MeOH, ca 70.0 μM) λ ($\Delta\varepsilon^{max}$): 354 (+2.4), 312 (−1.9), 270 (−5.8).

3.6. *Leucoagaricus gongilophorus* growth inhibition assay

The assay was performed as described by Pagnocca et al. (1996).

Acknowledgements

The authors are grateful to Fundação de Amparo á Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Coordenação de Aperfeiçãoamento de Pessoal de Ensino Superior (CAPES) and PRONEX/MCT-FINEP (Financiadora de Estudos e Projetos) for financial support, as well as to Dr Otaciro Rangel Nascimento, from the Instituto de Física de São Carlos, USP, for recording the CD data.

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