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Bioactive phenolic glycosides from *Amburana cearensis*

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Abstract

Coumarin (**1**) and two new phenolic glycosides have been isolated from the stem bark of *Amburana cearensis*, a tree reported in the Chacobo pharmacopoeia to be used against fever-causing diseases. The novel compounds were identified as: 4-(O-β-D-glucopyranosyl)-hydroxy-7-(3′,4′-dihydroxy-benzoyl)-benzyl alcohol (**2**) and 4-(O-β-D-glucopyranosyl)-hydroxy-7-(3′-methoxy-,4′-hydroxy-benzoyl)-benzyl alcohol (**3**). All three compounds were assayed in vitro for antimalarial, antiprotozoal, antifungal and antibacterial activities.

Keywords: *Amburana cearensis*; Leguminosae; Stem-bark; Phenolic glycosides; Coumarin; Biological activities

1. Introduction

Amburana cearensis A.C. Smith and *A. acreana* (Leguminosae) are trees of economic importance found in northeastern Bolivia and commonly used for their quality as fine and rare woods for the furniture industry (Killeen, Garcia, & Beck, 1993). Natives from the Acre state of Brazil prepare home cough and cold syrup with a mixture of barks of several plants including *Amburana cearensis*. Use of crushed seeds of this species for the treatment of toothache has also been reported (Kainer & Duryea, 1992). The Bolivian ethnic group Chacobo (Beni department), who lives in a region with endemic malaria, uses an aqueous decoction of the stem-bark of *A. cearensis* against fever symptoms. As a part of a program entitled: Environmental Conservation through Ethnobotanical and Ethnopharmacological Studies in Bolivia (Gimenez et al., 1996), aiming at the validation of the traditional medicinal knowledge owned by ethnic groups, we report our investigations on bioassay guided separations of extracts from the stem-bark of *A. cearensis*.

2. Results and discussion

Plant material, collected in the Beni department, was successively extracted with petrol, methylene chloride and ethyl acetate. Preliminary bioassays were performed on crude extracts and pure compounds on erythrocytic stages of *Plasmodium falciparum*. The methylene chloride extract showed a high antimalarial activity which led to the isolation of coumarin (**1**) with an IC_{50} of 9 μg/ml on a chloroquine sensitive strain. Although less active, the ethyl acetate extract was fractionated by vacuum liquid chromatography (VLC) on silica gel using methylene chloride–methanol mixtures of increasing eluting power. Two major phenolic glycosides with moderate biological activity: amburosides A (**2**) and B (**3**) were thus isolated and characterized by spectroscopic methods.

Coumarin was identified by its melting point and spectral data (Pouchert, 1983); it is a compound previously isolated from the seeds of *Amburana* species (Hegnauer & Hegnauer, 1994).

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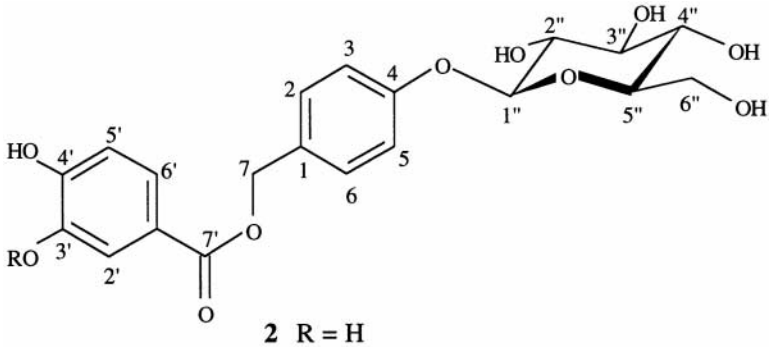

2 R = H

3 R = CH_3

Compound **2** showed in its positive ion FAB mass spectrum a quasi-molecular peak at *m/z* 429 $[M + Li]^+$ analyzed for $C_{20}H_{22}O_{10}Li$. ^1H and ^{13}C NMR analysis revealed signals for a glycosyl unit and two aromatic systems, one of which is a benzylic group (Table 1). A ^1J C–H correlation sequence allowed us to link the carbon signals to the directly bound protons as shown in Table 1. Remote ^2J and ^3J couplings were detected in a COLOC experiment. In the ^1H NMR spectrum, the observation of an AA'XX' system in the aromatic proton area, δ 7.37 (2H, br d, $J = 8.4$ Hz, H-2 and H-6) and δ 7.11 (2H, br d, $J = 8.4$ Hz, H-3 and H-5), was attributed to a *para*-disubstituted benzenic ring. The symmetry of the system was evidenced by the double intensity of the proton and carbon signals for the methines. The COLOC experiment showed ^2J and ^3J coupling of the AA'XX' system with a particularly deshielded carbon at δ 158.9 (s, C-4), allowing the identification of an oxygen substitution on this nucleus. The sixth aromatic and quaternary carbon of this ring was observed at δ 131.7 (C-1) and showed the same ^2J and ^3J couplings with the AA'XX' system and also with the benzylic methylene singlet at δ 5.22 ($\delta C = 67$). In the COLOC spec-

Table 1. NMR data for amburosides A and B (**2** and **3**) from *A. cearensis*

C	Amburoside A (**2**)		Amburoside B (**3**)	
	^1H NMR, δ (ppm[a])	^{13}C NMR, δ (ppm[a])	^1H NMR, δ (ppm[b])	^{13}C NMR, δ (ppm[b])
Benzoyl				
1′		122.6 s		121.7 s
2′	7.42 d (2.0)	117.4 d	7.53 d (1.9)	112.6 d
3′		146.1 s		147.4 s
4′		151.7 s		151.3 s
5′	6.80 d (8.5)	115.9 d	6.87 d (8.2)	114.9 d
6′	7.41 dd (8.5, 2.0)	123.7 d	7.59 dd (8.2, 1.9)	124.4 d
7′		168.1 s		167.1 s
CH_3O			3.90 s	56.1 q
Benzylic alcohol				
1		131.7 s		130.7 s
2, 6	7.37 d (8.4)	130.7 d	7.36 d (8.6)	130.0 d
3, 5	7.11 d (8.4)	117.7 d	7.08 d (8.6)	116.9 d
4		158.9 s		157.5 s
7	5.22 s	67.0 t	5.26 s	66.4 t
Glucose				
1″	4.92 d (7.4)	102.1 d	4.95 d (7.0)	101.0 d
2″	3.46 t (9.4)‡	74.8 d	3.56 t (9.4)‡	73.6 d
3″	3.48 t (9.4)‡	77.9 d	3.54 t (9.4)‡	76.7 d
4″	3.39 t (9.4)‡	71.3 d	3.52 t (9.4)‡	70.1 d
5″	3.44 m‡	78.0 d	3.44 m‡	76.5 d
6″	3.90 br d (11.7)	62.4 t	3.90 dd (12.3, 1.9)	61.8 t
	3.70 dd (11.7, 4.3)		3.77 dd (12.3, 4.4)	

^1H NMR 250 MHz, ^{13}C NMR 62.9 MHz, all chemical shifts from internal TMS, coupling constants (*J*) in Hz, carbon multiplicities determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3).

[a]In CD_3OD.

[b]In CD_3OD–$CDCl_3$, 1:9.

trum, the signal corresponding to the benzylic methylene exhibited correlations with the aromatic quaternary carbon at δ 131.7, with two equivalent methines at δ 130.7 (C-2 and C-6) and with a carbonyl at δ 168.1. The chemical shift of the methylene at δ 67 indicated that the position was directly substituted by an oxygen atom and therefore engaged in an ester function.

The second aromatic system was characterized by an ABX spin system whose analysis corresponded to a 1,2,4 substitution. This aromatic ring contained two quaternary oxygen-bearing carbons at δ 151.7 (s, C-4′) and δ 146.1 (s, C-3′) suggesting that substituents were two hydroxyl groups. By analysis of the ^2J and ^3J heteronuclear couplings observed in the COLOC experiment the structure of 3′,4′-dihydroxybenzoate ester was established.

The remaining signals were assigned to a hexose identified as glucose by comparison with literature data. It was assigned a β-D configuration according to the $J_{1,2}$ coupling of 7.4 Hz. The observation of a ^3J coupling cross-peak between the anomeric proton and C-4 of benzyl alcohol at δ 158.9 in the COLOC experiment allowed attachment of the glucose to position 4 of the benzyl alcohol. This fact was further confirmed by the observation of a NOE effect between aromatic protons H-3 and H-5 at δ 7.11 and the anomeric proton of glucose in the NOESY experiment. Compound 2 was thus assigned the structure of protocatechuoyl-3-deoxycalleryanin (Challice & Williams, 1968), a new compound for which the trivial name amburoside A is proposed.

Compound **3** displayed an $[M + Li]^+$ ion at m/z 443 in the FAB-mass spectrum suggesting a formula of $C_{21}H_{24}O_{10}Li$. The ^1H and ^{13}C NMR spectra revealed elements of structure identical to those of **2** plus signals for a supplementary methoxyl group at δ_H 3.90 and δ_C 56.1 (Table 1). The XHCORR and COLOC spectra allowed the complete assignment of the ^1H and ^{13}C NMR spectra. The methoxyl group was placed on C-3′ of the benzoyl moiety through the observation of a NOE effect between the singlet at δ 3.9 and the aromatic proton at δ 7.53 (1H, d, $J_{2,6} = 1.9$ Hz, H-2′) and of a $^3J_{C-H}$ coupling between the methoxyl protons and C-3′. Hence, structure of compound **3** was determined as the vanilloyl-3-deoxycalleryanin (Challice & Williams, 1968) to which the trivial name amburoside B has been given.

Amburosides A and B add to the small family of phenol glycosides, most representatives of which are found in the Flacourtiaceae (Ekabo, Farnsworth, Santisuk, & Reutrakul, 1993; Shaari & Waterman, 1995; Gibbons et al., 1995).

Coumarin and amburoside A were assayed for biological activity against a malarial parasite according to the 4-day suppressive method (Deharo et al., 1992) and showed 25 and 24% inhibition, respectively, of parasitaemia (*P. berghei*) at 50 mg/kg/day. Coumarin was also active on *Leishmania amazonensis*, *L. braziliensis* and *L. donovani* at 50 μg/ml. Antibacterial and antifungal tests were performed on the extracts and on the three pure compounds with *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Candida albicans* and *Epidermophiton floccosum*. While compounds **2** and **3** showed no activity, coumarin proved to be active at the 0.1 mg/ml level against *E. coli* and *S. flexneri* and at the 0.5 mg/ml level on *T. mentagrophytus*; toxicity on Kb cells showed an IC_{50} over 100 μg/ml.

To the best of our knowledge, this constitutes the first report on antimalarial, antileishmanial and Chagas disease activities for coumarin (Khalid, Farouk, Geary, & Jensen, 1986; Adesina, 1983) and may justify the use of *Amburana cearensis* as a medicinal plant in the Chacobo pharmacopeae. More elaborated coumarins have been reported to have interesting properties, for instance, a prenylated coumarin isolated from *Bacccharis pedunculata* showed antifungal activity against *Epidermophyton floccosum* (Rahalison et al., 1995) and antibiotics like novobiocin having a coumarin group in their structure, are known to interact with DNA gyrase, a bacterial topoisomerase (Gormley, Orphanides, Meyer, Cullis, & Maxwell, 1996).

3. Experimental

3.1. General

UV spectra were measured in MeOH. IR spectra were recorded in KBr. ^1H and ^{13}C NMR spectra were run at 250 and 62.9 MHz in CD_3OD or $CDCl_3$. Two dimensional NMR experiments were performed using standard Bruker microprograms.

3.2. Plant material

A. cearensis (Allemao) was collected and identified by Dr Sylvie Bergeron in the Alto Ivon region of the Beni department (Bolivia) in November 1993. A voucher specimen is deposited under SB 467 at the National Herbarium of the Universidad Mayor de San Andrés in La Paz.

3.3. Extraction and isolation of compounds

Powdered stem-bark (1 kg) was extracted with petrol (5 l), then with CH_2Cl_2 (1.5 l) and finally with EtOAc (1.5 l) in a soxhlet apparatus. The CH_2Cl_2 extract (4.3 g) was purified by chromatography on silica gel 60 H (300 g) under medium pressure, using CH_2Cl_2–MeOH

mixtures of increasing polarity as eluent. Pure coumarin (1.44 g, 0.14%) was obtained in frs 20–36 eluted with CH_2Cl_2. The EtOAc extract (1 g) was submitted to a VLC system on silica gel 60 H (283 g) using CH_2Cl_2–MeOH as eluent. Fr. 15 (91 mg) eluted with CH_2Cl_2–MeOH (17:3) was subjected to a silica gel prep. TLC and developed in CH_2Cl_2–MeOH (4:1) to isolate **3** (0.015 g; 0.0015%, R_f 0.62). Pure compounds were detected by irradiation under UV at λ 254 nm and recovered from adsorbent with MeOH. Fr. 17 eluted with CH_2Cl_2–MeOH (4:1) yielded **2** (0.23 g, 0.023%, R_f 0.47).

3.4. Amburoside A (**2**)

Pale yellow crystals; m.p. uncorr. 195–197°C; $[\alpha]_D$ −39° (*c* 0.76 MeOH); UV λ_{max}^{MeOH} nm (log ε): 220 nm (4.38); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340, 1680, 1609, 1510, 1280, 1240, 1080, 1040, 830 and 768; positive FAB-MS *m*/*z* (rel. int.): 429 $[M + Li]^+$ (41), 419 (16), 313 (60), 303 (18), 293 (29), 267 (100), 251 (22), 228 (13), 217 (38), 209 (82), 202 (87), 187 (70); ^1H and ^{13}C NMR: see Table 1.

3.5. Amburoside B (**3**)

White crystals; m.p. uncorr. 130–132°C; $[\alpha]_D$ −3° (*c* 0.48 MeOH); UV λ_{max}^{MeOH} nm (log ε): 220 nm (4.28); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1710, 1610, 1510, 1280, 1240, 1080, 1040, 840 and 770; positive FAB-MS *m*/*z* (rel. int.): 443 $[M + Li]^+$ (10), 353 (8), 339 (13), 285 (11), 233 (6), 221 (11), 207 (13), 197 (17), 181 (25), 171 (25), 159 (31), 151 (75), 135 (58), 121 (100); 459 $[M + Na]^+$ (11), 413 (7), 355 (6), 308 (9), 281 (22), 221 (32), 189 (13), 173 (19), 126 (67), 121 (100); ^1H and ^{13}C NMR: see Table 1.

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