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NEW ALKALOIDS FROM *CONCHOCARPUS GAUDICHAUDIANUS*.

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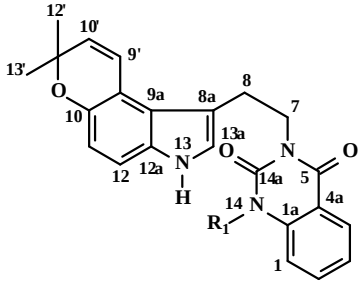
Abstract – The methanol extract from stems of *Conchocarpus gaudichaudianus* yielded four new alkaloids: 3-(2-(7,7-dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl)quinazoline-2,4(*1H,3H*)-dione (**1**), 3-(2-(7,7-dimethyl-3,7-dihydropyrano-[3,2-*e*]indol-1-yl)ethyl)-1-hydroxyquinazoline-2,4(1*H,3H*)-dione (**2**), 3-(2-(7,7-dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl-1-methylquinazoline-2,4(*1H,3H*)-dione (**3**), (*E*)-3-(6,7-dihydroxy-3,7-dimethyloct-2-enyl)-4-methoxy-1-methylquinolin-2(1*H*)-one (**6**), in addition to the known alkaloids *N-trans*-coumaroyltyramine and *N-trans*-feruloyltyramine. The structures of these compounds were identified by IR, UV, ^{1}H and ^{13}C NMR spectroscopy, gHSQC and gHMBC spectral analysis and comparison of data from the literature.

Conchocarpus gaudichaudianus, a tree, is used by the aboriginal natives in northern Brazil.[1] Of the 45 species in the genus *Conchocarpus*, only *C. heterophyllus, C. inopinatus* and *C. paniculata* have been investigated by spectroscopic analysis. Extracts from leaves of *C. heterophyllus* and the dichloromethane fraction of *C. inopinatus* show in vitro trypanocidal activity[2,3] and *C. paniculata* exhibits molluscicidal activity.[4]

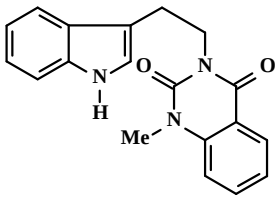
Indolopyridoquinazoline type alkaloids, like rutaecarpine isolated from *Evodia rutaecarpa*, shows a variety of pharmacological effects including anti-inflammatory[5] antithrombotic and vasorelaxant effects.[6]

We report studies on the stems of *C. gaudichaudianus*, which afforded three new indoloquinazolone alkaloids (1, 2 and 3) and one 2-quinolone alkaloid (6).

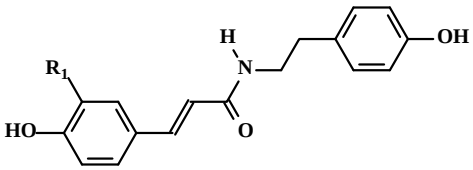
The methanol extract from stems of *Conchocarpus gaudichaudianus* yielded six alkaloids 1 - 6, including the known *N*-trans-coumaroyltyramine (4) and *N*-trans-feruloyltyramine (5).



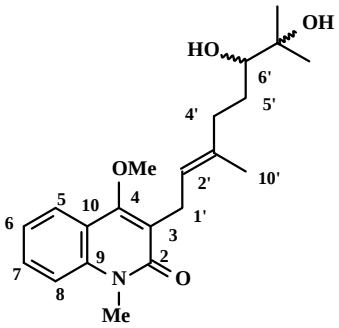
1: R_1 = H
2: R_1 = OH
3: R_1 = Me



3a



4: R_1 = H
5: R_1 = OMe



6

Figure 1. Compounds from *Conchocarpus gaudichaudianus*

The molecular formula of compound (**1**) was established to be $C_{23}H_{21}N_3O_3$ by analysis of its HR-MS (ESI) [*m/z*: 410.1519 $(M+Na)^+$] and NMR data (Table 1). IR spectrum showed absorption of a tertiary amide function at 1660 cm^{-1}. The UV results (see Experimental) were also in agreement with a highly aromatic conjugation system. The ^1H NMR spectrum (300 MHz) of **1** showed two symmetrical triplets at δ 4.13 and δ 3.02, corresponding to protons H-7 and H-8, an NH indolic group at δ 10.44, and six aromatic hydrogens at δ 6.56 (d, *J* = 8.7 Hz, H-11); 7.08 (d, *J* = 8.7 Hz, H-12) and at δ 7.19 (d, *J* = 8.1 Hz, H-1); 7.65 (td, *J* = 8.1 and 1.3 Hz, H-2); 7.21 (t, *J* = 8.1 Hz, H-3); 7.95 (dd, *J* = 8.1 and 1.3 Hz, H-4). The presence of a 2,2-dimethylchromene ring was evidenced by a methyl singlet at δ 1.35 integrating for six hydrogens and two doublets H-9' and H-10' at δ 5.72 (d, *J* = 9.8 Hz, H-10') and 7.28 (d, *J* = 9.8 Hz, H-9').

Full assignments of the hydrogens and protonated carbons of alkaloid **1** were attained by ^1H-^1H gCOSY and ^1H-^{13}C heteronuclear correlated 2D NMR spectra (gHMQC). Also, by a hydrogen detected multiple bond ^1H-^{13}C correlation spectrum (gHMBC) all of the quaternary carbon signals were assigned (Table 1). Based on the above data and by comparison with data from goshuyuamide-II[8] (**3a**) structure **1** was

assigned to 3-(2-(7,7-dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl)-1-hydroxyquinazoline-2,4(1*H*,*3H*)-dione.

Compound (**2**) was obtained as a yellow oil. The UV spectrum of **2** showed it to be related to **1**. In the IR spectrum, the absorption at 3397 cm^{-1} indicated the presence of a hydroxyl group. Evidence for a hydroxyl at N-14 was given by the mass spectrum, which exhibited a fragment at *m/z* 226 [*m/z* 404-178 ($C_{15}H_{16}NO$)] and 179 [*m/z* 404-225($C_8H_7N_2O_3$)] and by correlation for a HN-13 at δ10.80 d, *J*=2.40 Hz with H13a obtained by ^1H-^1H gCOSY. The new alkaloid was therefore identified as 3-(2-(7,7-dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl) -1-hydroxyquinazoline-2,4(*1H,3H*)-dione .

Compound **3** differed from **1** by the presence of a methyl group at N-14. The correlation for methyl protons with C-14a and C-1a, which indicated the location of the methyl group shown by the gHSQC and gHMBC experiments and the signal at δ10.76 *d*, *J*=2.40 Hz, HN-13 and obtained by ^1H-^1H gCOSY. Thus, the structure of the new alkaloid was characterized as 3-(2-(7,7-dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl) -1-methylquinazoline-2,4(*1H,3H*)-dione.

The known N-*trans*-coumaroyltyramine (**4**) and *N-trans*-feruloyltyramine (**5**) were also isolated and identified by ^1H and ^{13}C NMR, mass spectrometry, and comparison of published spectral data.[9,10,11]

Compound **6** was obtained as an amorphous yellow solid. The $[\alpha]_D$ 0° obtained indicated that this compound is a raceme mixture. Its UV and IR spectra showed the characteristic absorption of a 2-quinolones[9]. The ESI-MSMS mass spectrum indicated the $[M+H]^+$ at *m/z* 360 (base peak), and the characteristic fragment ion of the 4-methoxy-1-methyl-2-quinolone alkaloids the $[M+H]^+$ at *m/z* 203.[12] The ^1H NMR spectrum revealed the presence of two signals at δ 3.94 s and 3.69 s due to methoxyl and *N*-methyl groups (Table 2), four aromatics at δ 7.84, (dd, *J*=7.8 and 1.5 Hz), 7.27, (td, *J*=7.8 and 0.9 Hz), 7.55, (td, *J*=7.8 and 1.5 Hz) and 7.38, (dd, *J*=7.8 and 0.9 Hz). There were two methyl singlets at δ 1.07, thus placing an isopropyl group at the end of the side chain, and one signal at δ 1.80, br s, H-10'. The observed signals for a hydroxyl-tertiary carbon at δ 72.8 and three methyl singlets at δ_H 1.07 (s, 6H), 1.80 (brs, 3H), a signal at δ 5.30 (td, *J*=6.6 and 1.1 Hz), 3.37 (d, *J*=6.6 Hz), in the ^{13}C (Table 2) and ^1H NMR, and complete assignment of chemical shifts for all carbons, confirmed the presence of a 3',7'-dimethyl-6',7'dihydroxy-2'-octenyl chain. Using the prenylquinoline alkaloid[11] as a model, the structure of this new alkaloid was established as (*E*)-3-(6,7-dihydroxy-3,7-dimethyloct-2-enyl)-4-methoxy-1-methylquinolin-2(1*H*)-one.

EXPERIMENTAL

General

The NMR spectra were obtained in BRUKER DRX400 (9.4 T) and VARIAN GEMINI300 (7.05 T) spectrophotometers, using TMS as the internal standard. For gHMBC the coupling constants were optimized for 4, 6, 8 and 12 Hz. IR: film NaCl plates; High-resolution ESI-MS were recorded on a Micromass Q-Tof mass spectrometer, ESI-MSMS: low resolution on a triple quadrupole were recorded on a Micromass Quattro LC instrument equipped with a "Z-spray" ion source, and EI-MS on a CG/EM-SHIMADZU QP 2000 A. CC: silica gel 60 (70-230 and 230-400 mesh); TLC: silica gel plates F_{254} (0.25 mm in thickness).

Plant Material

The aerial parts of *Conchocarpus gaudichaudianus* subsp. *bahiensis* were collected in Itacaré, Bahia, Brazil, in February 1993. The specimen was identified by Dr. Jose Pirani from the Department of Botany of the University of São Paulo, Brazil, and the voucher herbarium specimen was deposited in this University.

Extraction and Isolation

The stems (200 g) from *Conchocarpus gaudichaudianus* were exhaustively extracted with MeOH at rt, and the extract was evaporated *in vacuo* to yield 3.8 g. The extract was submitted to vacuum chromatography over a silica gel support and eluted with *n*-hexane, *n*-hexane-$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$-EtOAc (1:1), EtOAc, and MeOH, giving 11 fractions. Fraction 8 (480 mg) was chromatographed over a gel filtration Sephadex LH-20 column using MeOH and MeOH-$CHCl_3$ (1:1) successively to give **1** (1.0 mg), **2** (4.8 mg) and **3** (1.2 mg). Fraction 9 (520 mg) was twice chromatographed (Sephadex LH-20 MeOH and MeOH-$CHCl_3$ (1:1); then silica gel, hexane-CH_2Cl_2-EtOAc-MeOH gradient) to give compounds **1** (2.8 mg), **4** (5.3 mg) and **5** (6.8 mg). Fraction 2 (1.82 g) was chromatographed on silica gel columns (hexane-CH_2Cl_2-EtOAc-MeOH gradient solvents) to yield Fr 89 (540 mg), and was purified by Sephadex LH-20 eluting with MeOH and MeOH-$CHCl_3$ (1:1) respectively to yield the majority compound **6** (340 mg).

3-(2-(7,7-Dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl)quinazoline-2,4(*1H,3H*)-dione (1).

Amorphous yellow solid; [UV MeOH λ_{max} nm (log ε)]: 242 (3.97), 312 (3.78). IRν_{max} ($CHCl_3$) cm^{-1}: 3341, 1660,1454 and 964. ^{1}H NMR and ^{13}C NMR: see Table 1. ESI-MSMS, *m/z* (rel. int.): 386 $[M-H]^-$ (20), 161(100). HR-MS (ESI) $[M+Na]^+$ Found *m/z*: 410.1519 (calc. for $C_{23}H_{21}N_3O_3Na$: 410.1481).

Table 1. NMR Data for compounds **1-3** in DMSO-d_6 and **7** in piridine-d_5

	1			**2**		**3**		**3**[e]
position	δ_H[a] (*J* in Hz)	δ_C[c]	gHMBC	δ_H[b] (*J* in Hz)	δ_C[d]	δ_H[a] (*J* in Hz)	δ_C[d]	δ_C[d]
1	7.19 d (8.1)	115.6	H-3	7.22 d (8.1)	115.2	7.45 d (8.1)	114.7	114.2
2	7.65 td (8.1,1.3)	135.5	H-4	7.66 t (8.1)	134.9	7.78 td (8.1, 1.8)	135.4	137.7
3	7.21 t (8.1)	122.9	H-1	7.22 t (8.1)	122.4	7.31 t (8.1)	122.9	122.7
4	7.95 dd (8.1, 1.3)	127.9	H-2	7.96 d (8.1)	127.4	8.05 dd (8.1, 1.5)	127.9	128.6
4a		114.3	H-1; H-3		114.2		114.7	116.0
5		162.6	H-4; H-7		161.9		161.3	161.7
7	4.13 t (8.1)	41.7	H-8	4.14 t (8.1)	41.5	4.18 t (7.8)	42.6	42.9
8	3.02 t (8.1)	25.4	H-7	3.02 t (8.1)	25.3	3.02 t (7.8)	25.2	25.5
8a		111.2	H-8; H-13a		110.6		110.9	112.5
9a		122.9	H-12; H-13a		122.4		122.9	
9		112.5	H-11; H-10’		112.5		112.0	119.3
10		146.2	H-11		145.5		145.9	119.5
11	6.56 d (8.7)	112.1		6.57 d (8.5)	111.6	6.56 d (8.7)	111.0	121.9
12	7.08 d (8.7)	112.5		7.09 d (8.5)	111.6	7.08 d (8.7)	111.7	112.0
12a		132.9	H-13a; H-11; H-12		132.3		132.5	140.9
13a	7.13 s	125.4	H-8	7.15 d (2.4)	124.8	7.15 d (2.4)	125.0	123.5
14a		150.7	H-7		150.1		150.6	161.7
1a		139.9	H-2; H-4		139.4		140.6	150.9
9'	7.28 d (9.8)	120.6		7.28 d (9,8)	120.2	7.26 d (9.6)	120.3	
10'	5.72 d (9.8)	129.9	H-12’; H-13’	5.69 d (9.8)	129.3	5.69 d (9.6)	129.5	
11'		74.7	H-9’; H-12’; H-10’; H-13’		74.1		74.4	
12'	1.35 s	27.2	H-13’	1.35 s	27.1	1.34 s	27.1	
13'	1.35 s	27.5	H-12’	1.35 s	27.1	1.34 s	27.1	
13-N-H	10.44 s			10.80 d (2.4)		10.76 d (2.4)		
14-N-H	11.43 s							
14-N-Me						3.55 s	30.8	30.5
14-OH				11.53 s				

[a]Recorded at 300 MHz. [b]Recorded at 400 MHz. [c]Recorded at 75.5 MHz. [d]Recorded at 100 MHz. [e]Literature data, see ref. 8

3-(2-(7,7-Dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl)-1-hydroxyquinazoline-2,4(1*H*,3*H*)-dione (2).

Amorphous yellow solid. [UV MeOH λ_{max} nm (log ε)]: 242 (4.02), 312 (3.78). IRν_{max} ($CHCl_3$) cm^{-1}: 3397, 1645, 1450, and 982. ^1H NMR and ^{13}C NMR: see Table 1. ESI-MSMS, *m/z* (rel. int.): 404 $[M+H]^+$ (100), 389 (8), 336 (4), 226 (19), 179 (8). HR-MS (ESI) $[M-H]^-$ at *m/z*: 402.1431 (calc. for $C_{23}H_{20}N_3O_4$ 402.1453).

Table 2. NMR Data for compound **6** in $CDCl_3$

position	δ_H[a]	(*J* in Hz)	δ_C[c]	gHMBC
2			164.2	N-CH_3
3			123.7	H-1’
4			161.2	H-1’
5	7.84 dd (7.8, 1.5)		124.2	H-6; H-7
6	7.27 td (7.8, 0.9)		122.8	
7	7.55 td (7.8, 1.5)		131.3	H-5
8	7.38 dd (7.8, 0.9)		115.4	H-6
9			140.4	N-CH_3; H-8; H-7; H-5
10			118.5	H-5; H-6
1’	3.37 d (6.6)		24.7	H-2’
2’	5.30 td (6.6, 1.1)		122.7	H-4’B, H-10’
3’			136.7	H-10’; H-1’; H-4’B
4’-HA	1.96-2.07 m		37.7	H-2’; H-5’B; H-6’
4’-HB	2.20-2.29 m			
5’-HA	1.72-1.62 m		30.1	H-4’B
5’-HB	1.42-1.26 m			
6’	3.22 dd (10.4, 1.6)		78.5	H-8’; H-9’
7’			72.8	H-8’; H-9’
8’	1.07 s		25.8	H-9’
9’	1.07 s		25.2	H-6’; H-8’
10’	1.80 brs		16.5	H-2’
N-CH_3	3.69 s		30.4	
-OCH_3	3.94 s		62.3	

[a]Recorded at 300 MHz. [c]Recorded at 75 MHz.

3-(2-(7,7-Dimethyl-3,7-dihydropyrano[3,2-*e*]indol-1-yl)ethyl)-1-methylquinazoline-2,4(*1H*,*3H*)-dione (3).

Amorphous yellow solid. [UV MeOH λ_{max} nm (log ε)]: 244 (4.10), 313 (3.92). IRν_{max} ($CHCl_3$) cm^{-1}: 3367, 2923, 1663, 1466 and 1022. ^{1}H NMR and ^{13}C NMR: see Table 1. ESI-MSMS, *m/z* (rel. int.): 400 $[M-H]^-$ (20), 175(100).

(E)-3-(6,7-Dihydroxy-3,7-dimethyloct-2-enyl)-4-methoxy-1-methylquinolin-2(1*H*)-one (6).

Amorphous yellow solid. $[\alpha]_D$ 0° ($CHCl_3$, *c* 0.01) [UV MeOH λ_{max} nm (log ε)]: 244 (4.21), 327 (3.83). IRν_{max} ($CHCl_3$) cm^{-1}: 3425, 2929, 1634, 1103 and 756. ^{1}H NMR and ^{13}C NMR: see Table 2. ESI-MSMS, *m/z* (rel. int.): 360 $[M+H]^+$ (100), 341 (20), 203 (65). HR-MS (ESI) $[M-H]^-$ at *m/z*: 358.2032 (calc. for $C_{21}H_{28}NO_4$ 358.2019);

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