Coluteol and Colutequinone B, More Antifungal Isoflavonoids from *Colutea arborescens*

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The new antifungal compounds, coluteol (3′,5′-dihydroxy-7,2′,4′-trimethoxyisoflavan) and colutequinone B (7,4′,6′-trimethoxyisoflavan-2′,5′-quinone) have been isolated from the root bark of *Colutea arborescens* (common bladder senna) and identified by a combination of ^{1}H- and ^{13}C-NMR techniques.

Approximately 25 simple isoflavans, structurally related to **1**, are known as plant products together with nine isoflavanquinones, similar to **2**.[1−4] All are substituted with hydroxy/methoxy groups, most commonly at positions 7, 2′, and 4′, less often at 6, 8, and 3′. Both classes of compounds are antimicrobial,[4−8] and several were first identified as phytoalexins.[6−8] This paper reports the isolation of two more antifungal isoflavonoids, one of them a quinone from *Colutea arborescens* L. var. *arborescens* (Leguminosae); neither has been previously recorded as a natural product.

Coluteol (**1**) was obtained as a pale brown resin. The FABMS of **1** gave an [M + 1] peak at *m/z* 333, consistent with the molecular formula, $C_{18}H_{20}O_6$. ^{13}C-NMR resonances (Table 2) at δ 70.2, 31.44, and 31.42 were all characteristic of an isoflavan, corresponding to C-2 (CH_2O), C-3 (CH), and C-4 (CH_2), respectively,[4,8] and this spectrum also indicated the required 12 aromatic carbons (δ 101.3−159.0). ^{1}H-NMR signals (Table 1) were further analyzed by a long-range COSY; they were related to the ^{13}C resonances by a ^{1}H inverse-detected ^{1}H−^{13}C short-range-coupled spectrum (HMQC) (Table 2). The structure shown for **1** reconciles all these data; for example, correlation cross peaks were seen between δ 6.99 (H-5) and δ 2.87 (H-4eq), establishing the juxtaposition of the heterocyclic ring C next to the aryl A ring; fainter cross peaks(<0.3 Hz) between δ 6.50 (H-6), 6.48 (H-8), and 3.78 (OMe on C-7) confirmed the A ring's methoxylation pattern; another small coupling linked δ 6.28 (H-6′) to δ 3.50 (H-3), establishing the location of the one unsubstituted position on the B ring.

The order of the substitution on the B ring was initially decided by an HMBC experiment. Here the delays were set at 10 Hz, the optimal value for detection of ^3J (C,H) connectivities across aromatic systems.[10] From the HMBC spectrum of **1** (Table 2), the diagnostic long-range correlations were observed for H-3 to C-2′ and C-6′ as well as H-6′ connectivities to C-2′ and C-4′; for methoxyl groups at δ 3.71 and 3.76 to C-2′ and C-4′ positions, respectively. So, by default, the C-3′ and C-5′ carbons must bear OH.

The results of a 2D NOESY experiment supported these findings, with diagnostic NOE cross peaks from the C-2′ methoxyl to H-3 and H-4eq; from H-6′ to H-3 and heterocyclic axial protons; from the OH signal to the B ring methoxyls. As expected the methoxyl carbons of the B ring have resonances in the range 59−63 ppm, which is diagnostic of sterically hindered methoxy groups with substituents in both ortho positions; this contrasts with the range of 55−56.5 ppm found for methoxyls with one unsubstituted ortho position or more, like that of the A ring.[11,12]

(3*R*)-Isoflavans exhibit a positive Cotton effect in the region 270−300 nm, while (3*S*)-isoflavans have a negative one.[13,14] Coluteol gave a positive Cotton effect, indicating the (3*R*)-alternative. Thus coluteol (**1**) is (3*R*)-(3,5-dihydroxy-2,4-dimethoxyphenyl)-2,3-dihydro-7-methoxy-4H-1-benzopyran.

Coultequinone B (**2**) was isolated as an orange amorphous powder. The FABMS of **2** gave an [M + 1] peak at *m/z* 331, consistent with a molecular formula of

Table 1. ^{1}H NMR Assignments of Coluteol (**1**) and Colutequinone B (**2**)[a]

protons	compound **1**	compound **2**
H-2eq	4.28 ddd: 2eq, 2ax (10.5); 2eq, 3 (3.7); 2eq, 4eq (1.8)	4.43 ddd: 2eq, 2ax (10.9); 2eq, 3 (10.9); 2eq, 4eq (0.3)
H-2ax	3.98[c]	4.14 ddd[b]: 2ax, 2eq (10.9); 2ax, 3 (2.7); 2ax, 4ax (2.7)
H-3	3.51 m	3.60 m
H-4eq	2.87 ddd: 4eq, 4ax (16.2); 4eq, 3 (5.4); 4eq, 2eq (1.8)	3.12 ddd: 4eq, 4ax (15.5); 4eq, 3 (13.6); 4eq, 2eq (0.3)
H-4ax	2.92 dd: 4ax, 4eq (16.2); 4ax, 3 (11.9)	2.66 ddd: 4ax, 4eq (15.5); 4ax, 3 (5.3); 4ax, 2ax (2.7)
H-5	6.92 br d: 5, 6 (8.4)	6.92 d: 5, 6 (8.2)
H-6	6.50 dd: 6, 5 (8.4); 6, 8 (2.4)	6.46 dd: 6, 5 (8.2); 6, 8 (2.3)
H-8	6.48 d: 8, 6 (2.4)	6.40 d: 8, 6 (2.3)
H-6′	6.27 d: 6′, 3 (0.6)	
H-3′		5.86 s
OMe on C-7	3.78 s	3.76 s
OH on C-5′	5.51 br s	
OMe on C-4′	3.96 s	3.80 s
OH on C-3′	5.51 br s	
OMe on C-6′	3.79 s	
OMe on C-6′		3.97 s

[a] Measurements made in $CDCl_3$ at 600 MHz are given in the following order: Chemical shifts in ppm (δ), signal multiplicity: interacting nuclei (coupling constant *J*, Hz). [b] More accurately, a doublet of a pseudo triplet. [c] Further details were obscured by the 3.96-ppm singlet.

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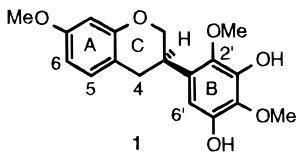
Table 2. ^{13}C NMR Assignments of Coluteol (**1**) and Colutequinone B (**2**)[a]

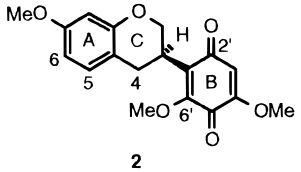
	compound **1**		compound **2**	
carbons	HMQC[c]	HMBC correlations[d]	HMQC	HMBC correlations
C-2	70.2 t	C-4, C-9	67.5 t	C-3, C-4
C-3[b]	31.4 d	C-2, C-4, C-1′, C-2′, C-6′	30.8 d	C-2, C-4, C-10, C-2′, C-6′
C-4[b]	31.4 t	C-2, C-3, C-5, C-9, C-10	28.6 t	C-2, C-5, C-9
C-5	130.1 d	C-4, C-7, C-9	129.6 d	C-4, C-7, C-9
C-6	107.2 d	C-7, C-8, C-10	107.0 d	C-7, C-8, C-10
C-7	159.0 s		158.8 s	
C-8	101.3 d	C-6, C-9, C-10	101.2 d	C-6, C-7, C-9, C-10
C-9	154.8 s		154.8 s	
C-10	114.1 s		113.5 s	
C-1′	129.6 s		130.7 s	
C-2′	139.2 s		186.4 s	
C-3′	142.0 s		106.9 d	C-1′, C-4′, C-5′
C-4′	133.6 s		157.1 s	
C-5′	145.7 s		177.9 s	
C-6∀	104.0 d	C-3, C-2′, C-4′, C-5′	155.2 s	
OMe on C-7	55.2 q	C-7	55.1 q	C-7
OMe on C-2′	61.9 q	C-2′		
OMe on C-4′	60.8 q	C-4′	56.3 q	C-4′
OMe on C-6′			61.7 q	C-6′

[a] HMQC measurements in $CDCl_3$ at 150 MHz are given in ppm (δ). Multiplicities were determined by a DEPT experiment. [b] For compound **1** these values were obtained from DEPT 135 (C-3) and DEPT 90 (C-4) experiments: the ^{13}C broad-band decoupled spectrum showed only one intense peak at 31.4 ppm. [c] Delays optimized for $J = 130$ Hz. [d] Delays optimized for $J = 10$ Hz.

$C_{18}H_{18}O_6$. The ^{13}C-NMR spectrum of **2** (Table 2) was very similar to that of **1**, except that two of the resonances, at δ 186.4 and 177.9, were much further downfield, corresponding to the two CO groups. This time the relative positions of the H atoms were established by individual decoupling experiments in C_6D_6; the single hydrogen on the B ring at δ 5.40 showed no detectable coupling with H-3 so must be more remote from it than in **1**. Further NMR experiments, as before, gave details of the B ring: three-bond correlations from the HMBC of **2** were observed for H-3 to C-2′ and C-6′; for H-3′ to C-1′ and C-5′; affirming NOE cross peaks were observed from the methoxyl on C-6′ to the C-4′ methoxyl and to H-3, and from H-3′ to the C-4′ methoxyl.



1



2

Interestingly, the C-5′ carbonyl resonance gave an atypical chemical shift of 177.9 ppm as compared to a reported shift range of δ 186.5−181.7 for carbonyls in other isoflavanquinones.[5,15] The latter carbonyl groups all had only one free ortho oxysubstituted position. The shift value given by the less deshielded carbonyl on **2** can be considered as diagnostic for di-ortho methoxy-substituted benzoquinone carbonyls.

Table 3. Antifungal Activities of Isolates[a]

compound	minimum inhibitory concentration (μg mL^{-1})[b]
coluteol	<100
colutequinone A	<50
colutequinone B	<50
nystatin	<5

[a] Against *Saccharomyces cerevisiae*.[15] [b] Final concentrations tested were 0.5, 1, 5, 25, 50, 100, and 200 μg mL^{-1}.

The CD spectrum of **2** correlated with that of (3*R*)-claussequinone and colutequinone A.[5,15] Thus, colutequinone B (**2**) is (3*R*)-2-(3,4-dihydro-7-methoxy-2*H*-1-benzopyran-3-yl)-3,5-dimethoxy-2,5-cyclohexadiene-1,4-dione. MICs for **1** and **2** against *S. cerevisiae* are shown in Table 3; these values are similar to that recorded for claussequinone against *Candida albicans*.[5]

Colutea belongs to the subfamily Lotoideae, which has provided the bulk of the known isoflavans. *C. arborescens* itself, has previously yielded isomucronulatol (7,2′-dihydroxy-3′,4′-dimethoxyisoflavan);[1] colutehydroquinone (2′,5′-dihydroxy-7,3′,4′-trimethoxyisoflavan), a structural isomer of **1**; and colutequinone A (7,3′,4′-trimethoxyisoflavan-2′,5′-quinone), isomeric with **2**.[15] All three are antifungal, though isomucronulatol is unlikely to be a phytoalexin as was first believed.[1]

Experimental Section

General Experimental Procedures. Melting points were determined with an Electrothermal 1A 9000 (digital series) apparatus and are uncorrected. R_{MNQ} values were measured relative to menadione (2-methyl-1,4-naphthoquinone). CD spectra were measured with a JASCO J600 spectropolarimeter in MeOH flushed with N_2. UV spectra were obtained in MeOH using a Hitachi U-2000 spectrometer. FABMS were recorded with a VG ZAB-SE4F spectrometer in the positive mode using 3-NOBA as matrix. ^{1}H-NMR, homonuclear COSY,[16] ^{13}C-NMR, DEPT 90, DEPT 135,[17] phase-sensitive 2D NOESY, and HMBC experiments were conducted on a Bruker AMX-600 instrument operating at 600.13 MHz, using the solvent as internal standard ($CDCl_3$ giving signals at δ 7.27 and 76.95). The NOESYs were obtained with 0.9 s mixing times,[18] HMQC was optimized for $J = 130$ Hz[19] and HMBC for $J = 10$ Hz.[20] ^{1}H NMR in C_6H_6 and associated individual decoupling experiments employed a Bruker AMX-250 spectrometer at 250 MHz, again using the solvent as internal standard at δ 7.21. TLC was carried out on commercial Si gel plates (Kieselgel 60, Merck Art. 5721). Nystatin (N-9767), 5010 units mg^{-1}, was obtained from Sigma, Poole.

Plant Material. Roots of *C. arborescens* were collected on the Queen Mary and Westfield College campus during September 1993, washed with distilled H_2O, and stored at −20 °C. The plant material was authenticated by Dr. D. Kircup, Royal Botanic Gardens, Kew, and deposited there as voucher specimen PG.1470.

Bioassays. Bioautography for antifungal compounds was performed as previously reported.[15] MICs of pure compounds were determined against *Saccharomyces*

cerevisiae (IMI 14119) in a microdilution assay using malt extract broth (Oxoid, CM57). This was inoculated with a culture (previously grown for 16 h at 25 °C) to give a cell concentration of 5×10^4 mL^{-1}. Titers (200 μL) were mixed with 8 μL of test compound dissolved in 1:1 v/v, EtOH−Na_2HPO_4/NaH_2PO_4 buffer, 0.1M, pH 7.4, and shaken for 16 h at 25 °C followed by hemocytometer cell counts. The yeast was purchased from the International Mycological Institute.

Extraction and Isolation of the Isoflavonoids. Root bark scrapings (94.5 g) were treated with liquid N_2, ground to a fine powder, and extracted with 3 × 500 mL 90% (v/v) aqueous MeOH. The pooled filtrates were evaporated to 180 mL *in vacuo* at 40 °C and then extracted with 3 × 100 mL freshly distilled Et_2O. The ether phase was evaporated as before, this time to dryness, and the residue redissolved in 40 mL of EtOAc. Part of the concentrate (4 mL) was chromatographed with MeC_6H_5−EtOAc−HOAc (25:3:1, developed × 3) on Si gel plates. Zones were located with the bioautographic assay, using a strip from each plate, while the remainders were stored under N_2. The Si gel corresponding to each relevant zone was pooled and eluted with Me_2CO, yielding 6.7 mg crude **1** and, after recrystallization, 6.9 mg **2**. Compound **1** was further purified in CH_2Cl_2−$CHCl_3$ (1:1, developed × 3) on Si gel plates and eluted as before to yield 4.1-mg residue.

***(R)*-Coluteol [1]**: pale brown resin; R_{MNQ} (solvent a) 0.50 (MeC_6H_6−EtOAc−HOAc, 23:3:1, × 3); R_{NNQ} (solvent b), 0.26 (CH_2Cl_2−$CHCl_3$, 1:1, × 3); brown Gibbs reaction; UV (MeOH) λ_{max} (log ϵ) 207 (4.59), 219 sh (4.13), 282 (3.63); CD: $[\theta]_{221}$ 0, $[\theta]_{232}$ −2805, $[\theta]_{244}$ −218, $[\theta]_{363}$ 0, $[\theta]_{377}$ 825, $[\theta]_{386}$ 528, $[\theta]_{337}$ 0, $[\theta]_{360}$ 0 (MeOH 0.035 mg mL^{-1}); FABMS *m/z* $[M + 1]^+$ 333 (30); ^{1}H NMR in $CDCl_3$, Table 1; ^{13}C NMR in $CDCl_3$, Table 2.

Colutequinone B [2]: yellow-orange amorphous powder; mp 113−117 °C; R_{MNQ} (solvent a) 0.75; R_{MNQ} (solvent b) 0.74; negative Gibbs reaction; UV (MeOH) λ_{max} (log ϵ) 207 (4.72), 220 sh (4.23), 282 (4.12); CD $[\theta]_{222}$ 2640, $[\theta]_{228}$ 0, $[\theta]_{235}$ −3135, $[\theta]_{244}$ −1287, $[\theta]_{250}$ −1056, $[\theta]_{259}$ −1320, $[\theta]_{272}$ 0, $[\theta]_{283}$ 957, $[\theta]_{292}$ 238, $[\theta]_{306}$ 924, $[\theta]_{336}$ 0, $[\theta]_{360}$ 0 (MeOH 0.035 mg mL^{-1}); FABMS *m/z* $[M + 1]^+$ 331 (87); ^{1}H NMR in $CDCl_3$, Table 1; ^{1}H NMR in C_6D_6, δ 4.59 (dd, J = 10.0, 10.5 Hz, H-2 eq), δ 4.25 (ddd, J = 10.0, 3.0, 0.5 Hz, H-2 ax), δ 3.85 (m, H-3), δ 3.21 (ddd, J = 15.0, 12,0, <0.25 Hz, H-4 eq), δ 2.63 (ddd, J = 15.0, 5.0, 3.0 Hz, H-4 ax), δ 6.99 (d, J = 8.8 Hz, H-5), δ 6.65 (dd, J = 8,8, 2.5 Hz, H-6), δ 6.79 (d, J = 2.5 Hz, H-8), δ 5.40 (s, H-3′), δ 2.87, 3.41, 3.47 (s, MeO groups on C-7, C-4′ and C-6′); ^{13}C NMR in $CDCl_3$, Table 2.

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