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COLUTEQUINONE AND COLUTEHYDROQUINONE, ANTIFUNGAL ISOFLAVONOIDS FROM *COLUTEA ARBORESCENS*

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Key Word Index—*Colutea arborescens*; Leguminosae; antifungal activity; isoflavonoids; isoflavanquinones; 7,3′,4′-trimethoxyisoflavan-2′-5′-quinone; (3*R*)-colutequinone; 2′-5′-dihydroxy-7,3′,4′-trimethoxyisoflavan; (3*R*)-colutehydroquinone.

Abstract—Two new isoflavonoids have been isolated from the root bark of *Colutea arborescens* and identified as 7,3′,4′-trimethoxyisoflavan-2′,5′-quinone ((3*R*)-colutequinone) and the corresponding hydroquinone (2′,5′-dihydroxy-7,3′,4′-tri-methoxyisoflavan) ((3*R*)-colutehydroquinone). Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

Approximately 25 simple isoflavans, similar to **2**, are known, normally substituted with hydroxy/methoxy groups at the 7, 2′ and 4′ positions, less often at 8 and/or 3′ [1–4]. Rather fewer plant isoflavanquinones, having the same nucleus as **1**, are recorded, examples being claussequinone (the 7-hydroxy-4′-methoxy-derivative) [1, 5–7], mucroquinone (7-hydroxy-8,4′-dimethoxy-) [8, 9] astragaluquinone (3′-hydroxy-7,8-dimethoxy-) [4], pendulone (7-hydroxy-3′,4′-dimethoxy-) [5], amorphaquinone (7-hydroxy-8,3′,4′-trimethoxy-) [10], arbruquinone A (6,7,3′,4′-tetramethoxy-), abruquinone B (6,7,8,3′,4′-pentamethoxy-) and abruquinone C (6-hydroxy-7,8,3′,4′-tetramethoxy-) [11]. Both classes of compound are antimicrobial [4, 6, 12–14] and many isoflavans were first identified as phytoalexins [12–14]. Here a survey of antifungals has added a novel isoflavanhydroquinone and one more isoflavanquinone to the list of plant products.

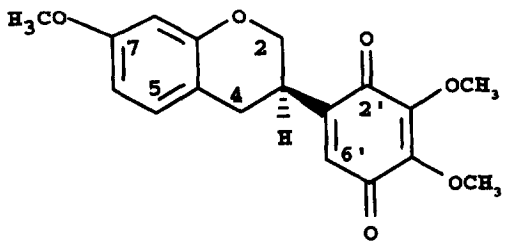
RESULTS AND DISCUSSION

Colutequinone (**1**) was treated as the primary compound because its stability in air allowed a full set of spectra to be obtained. High resolution-MS gave M^+ 330.11020 ±5 ppm, corresponding to $C_{18}H_{18}O_6$ (calc. *m/z* 330.11032). ^{13}C NMR resonances (Table 1) at δ 68.0, 30.7 and 28.8 were all characteristic of the core of an isoflavan, corresponding to C-2 (CH_2–O), C-3 (CH) and C-4 (CH_2) respectively [4, 15] and this spectrum also suggested the required 10 aromatic Cs (δ 101.5–157.3) and 2 CO groups (δ 184.5 and 184.0). ^1H NMR signals in both $CDCl_3$ (Table 2) and C_6D_6 were further analysed by individual decoupling experiments and a double quantum phase sensitive COSY: they were related to the ^{13}C resonances by a ^1H inverse detected ^1H–^{13}C short range coupled spectrum. The structure shown for **1** reconciles all these data: for example the small ($J = 1.2$) coupling constant linking the δ 6.37 (H-6′) and δ 3.44 (H-3) signals establishes the position of the one unsubstituted hydrogen on the B (quinone) ring. The structure is also consistent with the UV–VIS absorbance spectrum of **1**, which is closely comparable with those of 7-hydroxy-8,2′-dimethoxyisoflavan [9] and 6,8,2′,3′-tetramethoxyisoflavaquinone [11].

The ^1H NMR spectrum of of **2** (Table 2) was very similar to that of **1**, except for the presence of a broad singlet, integrating to 2H, at δ 5.50, suggesting two hydroxy groups. This was confirmed by acetylating **2** to **3** and showing (Table 2) that this generated two new singlets, at δ 2.33 and 2.35, corresponding to the signals from *two* acetyl groups. On standing in air at ambient temperature, a MeOH solution of **2** quantitatively converted to a new compound that co-chromatographed with **1** in solvents **a** and **b** and had an identical ^1H NMR spectrum. When **1** was reduced with aqueous sodium dithionite in $CHCl_3$, the product was established to be **2** by melting point, co-chromatography in solvents **a** and **b**, and by ^1H NMR.

The CD spectra of **1** and **3** correlated with that of (3*R*)-claussequinone [9], indicating the assigned configurations.

These experiments may explain why no isoflavan with an *o*- or *p*-dihydroxylated B ring has been previously reported: such substances tend to oxidize to the corresponding quinones during isolation. However, **2** appears to chromatograph in solvent **a** without decomposition: its antifungal zone does not streak forward on the plate. Chromatography of crude extracts, by showing the presence of both **1** and **2**, thus



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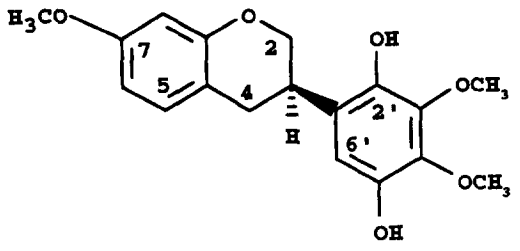

2

establishes that the quinone is a genuine natural product, not an artefact of the isolation method. The plant material showed healed lesions so **1** and **2** may not be constitutive compounds.

Colutea belongs to the subfamily Lotoideae (Leguminosae) which has yielded the bulk of the known isoflavans. However, the only such compound previously reported from *C. arborescens* is isomucronulatol (7,2′-dihydroxy-3′,4′-dimethoxyisoflavan): first believed to be a phytoalexin [1], it was later found that UV/$CuCl_2$ pretreatments do not increase its levels. Moreover, neither its isolation from unripe pods or its biosynthesis from labelled precursors in seedlings required such pretreatments [16].

EXPERIMENTAL

TLC was on commercial silica gel plates (Kieselgel 60, Merck Art. 5721), in one of the following solvents: **a**, MeC_6H_5–EtOAc–HOAc (25:3:1 developed ×3; **b**, $CHCl_3$–MeOH (50:1) developed ×2. R_{MNQ} values were measured relative to menadione (2-methyl-1,4-naphthoquinone). Detection was by inherent colour.

NMR; ^1H and ^{13}C experiments were normally

Table 1. ^{13}C NMR spectral data for **1** and **2** (150 MHz in $CHCl_3$)

Position	HMQC (1)	HMBC (1)	HMQC (2)
C-2	68.1 *t*	C-4, C-9, C-1′	69.5
C-3	30.7 *d*	C-2, C-4, C-10, C-1′, C-6′	32.0
C-4	28.8 *t*	C-3, C-5, C-9, C-10, C-1′	30.0
C-5	130.1 *d*	C-4, C-7, C-9	130.1
C-6	108.0 *d*	C-8, C-10	107.9
C-7	159.3 *d*		158.9
C-8	101.5 *d*	C-6, C-10	101.3
C-9	154.6 *s*		155.0
C-10	112.0 *s*		114.2
C-1′	146.5 *s*		122.2
C-2′	183.5 *s*		140.4
C-3′	145.0 *s*		137.9
C-4′	144.6 *s*		139.2
C-5′	184.0 *s*		142.1
C-6′	130.9 *d*	C-3, C-4′, C-2′	107.2
MeO on C-7	55.3 *q*	C-7	55.2
MeO on C-3′	61.3 *q*	C-3′	60.7
MeO on C-4′	61.2 *q*	C-4′	60.6

Multiplicities for **1** were determined by a DEPT experiment.

Table 2. ^{1}H NMR spectral data for **1**, **2** and **3** (600 MHz in $CDCl_3$)

H	1	2	3
2 eq.	4.26, *dd* (J = 10.5, 7.5 Hz)	4.34, *ddd* (J = 10.5, 4.1, 2 Hz)	4.27, *ddd* (J = 10.6, 3.0, 1.3 Hz)
2 ax.	4.06, *dd* (J = 10.5, 7.5 Hz)	4.05, *t* (J = 10.5 Hz)	3.92, *t* (J = 10.6 Hz)
3	3.44, *m*	3.56, *m* ($W_{1/2}$ = 14.7 Hz)	3.30, *m* ($W_{1/2}$ = 15 Hz)
4 eq.	3.05, *dd* (J = 15.3, 5.5 Hz)	2.97, *dd* (J = 16, 10.5 Hz)	2.90, *dd* (J = 16, 10.1 Hz)
4 ax.	2.72, *dd* (J = 15.3, 5.5 Hz)	2.91, *dd* (J = 16, 5.5 Hz)	2.86, *dd* (J = 16, 6.5 Hz)
5	6.95, *d* (J = 8.5 Hz)	6.99, *d* (J = 8.7 Hz)	6.97, *d* (J = 8.5 Hz)
6	6.49, *dd* (J = 8.5, 2.8 Hz)	6.48, *dd* (J = 8.7, 2.5 Hz)	6.49, *dd* (J = 8.5, 2.1 Hz)
8	6.38, *d* (J = 2.8 Hz)	6.43, *d* (J = 2.5 Hz)	6.43, *d* (J = 2.1 Hz)
6′	6.37, *d* (J = 1.2 Hz)	6.49, *s*	6.65, *s*
OMe on C-7	3.76, *s*	3.78, *s*	3.78, *s*
OMe on C-4′	4.02, *s*	3.94, *s*	3.88, *s*
OMe on C-5′	4.01, *s*	3.91, *s*	3.86, *s*
OH on C-2′ and C-5′		5.50, *bs*	
Ac on C-2′ and C-5′			2.33, *s* 2.35, *s*

Assignments of MeO groups based on HMBC for **1** and by comparison for **2** and **3**.

performed on a Brüker AMX600. Chemical shifts are shown in ppm relative to TMS at 0 ppm, using the solvent peaks at 7.27 ppm ($CDCl_3$) and 7.21 ppm (C_6D_6) for ^{1}H spectra and at 76.95 ppm ($CDCl_3$) for ^{13}C spectra, as the int. standards.

Isolation of **1** *and* **2**. *Colutea arborescens* L. was collected from the QMW campus during September, 1993 and stored at −20°. The material was authenticated by Dr D. Kircup, Royal Botanic Gardens, Kew and deposited there as voucher specimen PG. 1470.

Root bark (94.5 g) was treated with liquid N_2, ground to a fine powder and extracted with 3 × 500 ml 90% (v/v) aq. AR MeOH. This solvent and all others used prior to chromatography were made anoxic by preflushing with N_2 for 15 min. The pooled filtrates were evapd to 180 ml *in vacuo* at 40° (rotary film evaporator) and then extracted with 3 × 100 ml freshly distilled Et_2O. The ether phase was evapd as before, this time to dryness, and the res. redissolved in 40 ml EtOAc. Part of this concentrate (4 ml) was chromatographed on 18 20 × 20 cm TLC plates, each of which was developed 3× in solvent **a**. Zones were located by their activity against *Saccharomyces cerevisiae* using a bioautographic assay: while the bulk of each plate was stored under N_2, strips, previously dried *in vacuo*, were placed in contact with seeded malt extract agar [17] for 15 min at 22° and the cultures subsequently incubated for 24 hr at 25°. Then the pooled silica gel corresponding to each relevant zone was eluted with 10 ml AR acetone, yielding, after recrystallisation, 12.1 mg **1** and 3.6 mg **2**.

(3R)-*Colutequinone* (**1**). Fine orange needles, mp 76–80°, CH_2Cl_2/C_7H_{16}; R_{MNQ} (solvent), 0.76 (a), 0.99 (b), negative Gibbs reaction; UV–VIS λ_{max}^{EtOH} nm (log ε): 206 (4.71), 228 sh (4.22), 269 (4.12), 379 (3.35); ^{13}C NMR ($CDCl_3$): Table 1; ^{1}H NMR ($CDCl_3$): Table 2; (250 MHz, C_6D_6): δ 2.31 (1H, *dd*, J = 15, 7.5 Hz, H4 ax.), 2.56 (1H, *ddd*, J = 15, 5.8, 1.7 Hz, H4 eq.), 3.34 (3H, *s*, OMe on C7), 3.46 (1H, *m*, $J_{tot.}$ = 28 Hz, H-3), 3.60 (3H, *s*, OMe on C-5′), 3.61 (3H, *s*, OMe on C-4′), 3.68 (1H, *ddd*, J = 10, 3.7, 1.0 Hz, H2 ax.), 3.95 (1H, *ddd*, J = 10, 3.8, 1.7, H2 eq.), 6.14 (1H, *d*, J = 1.8 Hz, H6′), 6.60 (1H, *dd*, J = 8.0, 2.5 Hz, H6), 6.65, (1H, *d*, J = 2.5 Hz, H8), 6.78 (1H, *d*, J = 8.0 Hz, H5). EI-MS *m/z* (rel. int.): 330 (M^+; 100), 315 (15), 297 (10), 280 (17), 168 (12), 165 (9), 137 (22), 112 (27), 104 (24). CD: $[\theta]_{220}$ 2300, $[\theta]_{236}$0, $[\theta]_{258}$ −9900, $[\theta]_{272}$ −9900, $[\theta]_{272}$ 0, $[\theta]_{283}$ 4400, $[\theta]_{294}$ 1300, $[\theta]_{350}$ 0 (MeOH c. 0.0369 mg ml^{-1}): $[\theta]_{313}$ 1500, $[\theta]_{360}$ 132, $[\theta]_{413}$ 1400, $[\theta]_{452}$ 0, $[\theta]_{476}$ −600, $[\theta]_{553}$ 0 (MeOH c. 0.369 mg ml^{-1}).

(3R)-*Colutehydroquinone* (**2**). Yellow-tinged amorphous solid, mp 103–106 decomp., Me_2CO; R_{MNQ} (solvent), 0.66 (a), 0.77 (b), grey-brown Gibbs reaction; UV–VIS λ_{max}^{EtOH} nm (log ε): 207 (4.84), 284 sh (3.916), 289 (3.922); ^{13}C NMR ($CHCl_3$): Table 1; ^{1}H NMR ($CDCl_3$): Table 2.

(3R)-2′,5′-*Diacetyl-colutehydroquinone* (**3**). Shiny platelets, mp 166–7°, EtOH; ^{1}H NMR ($CDCl_3$): Table 2; UV-VIS λ_{max}^{EtOH} nm (log ε): 206 (4.85). 227 sh (4.27), 282 (3.62), 291 sh (3.48), 343 (3.27); CD: $[\theta]_{220}$ −4000, $[\theta]_{231}$ −8900, $[\theta]_{244}$ 0, $[\theta]_{256}$ 600, $[\theta]_{278}$ 2600, $[\theta]_{305}$ 0, $[\theta]_{360}$ 0 (MeOH c. 0.0350 mg ml^{-1}): $[\theta]_{300}$ 300, 100, $[\theta]_{400}$ 0, $[\theta]_{506}$ 0 (MeOH c. 0.350 mg ml^{-1}).

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