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A CYTOTOXIC ISOFLAVONE FROM *CUDRANIA COCHINCHINENSIS*

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Key Word Index—*Cudrania cochinchinensis*; Moraceae; cudraisoflavone-A.

Abstract—A new isoflavone, cudraisoflavone-A was isolated from *Cudrania cochinchinensis* and was found to be cytotoxic to PS cells in culture. Its structure was elucidated on the basis of NMR, MS, UV and IR spectral data. In addition the known compounds, 3-*O*-methylorobol, dehydrocostus lactone, methyl linoleate, and sitosterol were also isolated and identified.

INTRODUCTION

The plant *Cudrania cochinchinensis* has been used in Chinese traditional medicine for the treatment of hepatitis [1]. Prior studies of this plant uncovered the agent cudranone [2]. We have investigated this plant in the course of a continuing search for tumour inhibitors from higher plant sources. Fractionation of the ethanol extract of the twigs and leaves, which was found to be cytotoxic and active in the P388 mouse leukemia system [2] led to isolation of a new isoflavone, (**1**) which was cytotoxic to PS cells in culture at a dose of $1 \times 10^{\circ}$ μg/ml. In addition the known compounds 3-*O*-methylorobol, dehydrocostus lactone, methyl linoleate and sitosterol were isolated and identified. Dehydrocostus lactone and methyl linoleate showed cytotoxicity at $0.9 \times 10^{\circ}$and $1.0 \times 10^{\circ}$ μg/ml against 9PS cells in culture [3].

RESULTS AND DISCUSSION

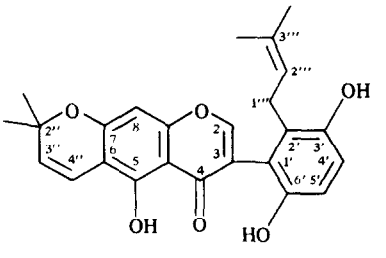
High resolution accurate mass measurement established a molecular formula of $C_{25}H_{24}O_6$ for cudraisoflavone-A (**1**). The IR spectrum of **1** indicated the presence of OH (3415 and 3240 cm^{-1}) and a chelated carbonyl group (1645 cm^{-1}). The downfield signal at δ12.87 confirmed the presence of an intramolecular hydrogen-bonded group at the C-5 position. The signal observed at δ7.87 is assigned to the C-2 proton of an isoflavone. The NMR spectrum also indicated the typical γ,γ-dimethylchromene ring signals at δ1.46 (6H, *s*), 5.63 (1H, *d*, $J=10$) and 6.73 (1H, *d*, $J=10$). the γ,γ-dimethylallyl group was indicated by signals at δ1.68 (3H, *s*), 1.80 (3H, *s*), 3.40 (2H, *d*, $J=8$) and 5.16 (1H, *t*, $J=8$). In order to distinguish between the linear or angular fusion of the pyran ring the NMR spectra of **1** and its triacetate were compared. An 0.24 ppm paramagnetic shift for H-4″ (*d*, $J=10$) and an 0.15 ppm diamagnetic shift for H-3″ (*d*, $J=10$) resulted from acetylation of the 5-OH group [4–6] supporting the linear fusion of the pyran framework. The singlet centered at δ7.03, assigned to the C-8 proton was shifted downfield to δ7.24 in the corresponding acetate. In the parent phenol **1**, the γ,γ-dimethylallyl group and the remaining two hydroxyl groups would be located on ring B. The presence of adjacent hydroxyl functions was excluded by comparing the MeOH and $AlCl_3$–HCl UV spectra (see Experimental). Thus three substitution patterns were possible for cudraisoflavone-A. The chemical shift of the two adjacent aromatic protons at δ6.83 (*d*, $J=8.4$) and 6.86 (*d*, $J=8.4$) is most consistent with the substitution pattern as shown in **1** and this was further confirmed by the NMR of the triacetate of **1** in which these aromatic protons appear as a *dd*, at δ7.34 and 7.38 ($J=8.4$). Therefore cudraisoflavone-A is **1**.

3-*O*-Methylorobol, dihydrocostus lactone, methyl linoleate and sitosterol were also isolated and identified by comparison to literature spectral data [7–13].

EXPERIMENTAL

Plant material. Twig and leaf of *Cudrania cochinchinensis* (Lown.) Kudo et Masam were obtained from Hong Kong in October 1982 and authenticated by the Economic Botany Laboratory Plant Genetic and Germplasm Institute (Building 265, Agricultural Research Center, East Beltsville, MD 20705).

General. Mps: uncorr. The UV spectra were obtained in EtOH or MeOH. The IR spectra were taken as KBr pellets. High resolution (470 MHz) ^{1}H NMR spectra were recorded in $CDCl_3$ (δ in ppm, J in Hz) using TMS as an int. standard. Low and high resolution mass spectra were measured on a Finnigan 4023 GC/MS with Incos 2000 data system and a Kratos MS50S respectively and recorded at 70 eV. Silica gel (230–400 mesh size) was used for flash CC and similar fractions were combined.



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Extraction and isolation. The dried, ground twig and leaf (3.42 kg) was exhaustively percolated with 95% EtOH. The extract was concd to dryness *in vacuo* at 40° and yielded the EtOH extract (196 g) which was then partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ fraction (90 g) was partitioned between hexane and MeOH. The MeOH fraction (38.4 g) was further partitioned between 40% aqueous MeOH and $CHCl_3$ and yielded the $CHCl_3$ extract (20 g) which exhibited antileukemic activity (3PS, 135/400, 115/200, 110/100, 107/50) in the P388 mouse leukemia system *in vivo* [3].

The $CHCl_3$ extract (20 g) was subjected to chromatography on a silica gel flash column with $CHCl_3$ and increasing concentrations of MeOH (5, 10, 20 and 50%) in $CHCl_3$, yielded 6 fractions (A–F).

Fraction A (0.62 g) was subjected to chromatography on a silica gel flash column eluted with hexane and increasing concentrations of EtOAc (1–50%) in hexane and combined into 6 fractions (G-L) on the basis of TLC. Fraction G (83 mg) was subjected to prep. TLC on silica gel with hexane–EtOAc (19:1) and yielded methyl linoleate (27 mg). Fraction I (100 mg) was purified by prep. TLC on silica gel with hexane–EtOAc (17:3) and yielded dehydrocostus lactone (58.6 mg). Fraction K (63.6 mg) was subjected to prep. TLC on silica gel with hexane–EtOAc (4:1) and yielded sitosterol (20.2 mg).

Fraction C (2.34 g) was subjected to chromatography on a silica gel flash column and eluted with $CHCl_3$ and increasing concentrations of MeOH (1, 3, 5, 10 and 50%) in $CHCl_3$ and yielded fractions M–O. Fraction N (1.69 g) was subjected to chromatography on a silica gel column and eluted with hexane–Et_2O and Et_2O–MeOH. This fractionation yielded fractions P-U which were combined based on TLC. Fraction R (316.7 mg) was subjected to prep. TLC on silica gel with hexane–Et_2O (1:3) and yielded **1** which was crystallized from $CHCl_3$ as yellow crystals (87.4 mg). Fraction T (113.2 mg) was crystallized from hexane–acetone and yielded 3′-*O*-methylorobol (31.2 mg).

Cudraisoflavone-A (**1**). Mp 167–170°; UV λ_{max}^{EtOH} nm (log ε) 202 (4.94), 224 (4.76), 290 (4.93), UV $\lambda_{max}^{EtOH+NaOAc}$ 217 (5.55), 290 (5.11), UV λ_{max}^{MeOH} 224 (4.68), 290 (4.85), UV $\lambda_{max}^{MeOH+AlCl_3}$ 227 (4.72), 308 (4.87), UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 229 (4.68), 307 (4.84); HRMS *m/z* 420.1577 (calcd for $C_{25}H_{24}O_6$ 420.1566), EIMS *m/z* 420 (M^+ 57%), 405 (100), 377 (15), 365 (10), 349 (3), 337 (4), 202 (3), 175 (2); 470 MHz ^1H NMR ($CDCl_3$) δ 1.46 (6H, *s*, 2″-2Me), 1.68 (3H, *s*, 3‴-Me, *trans*), 1.80 (3H, *s*, 3‴-Me, *cis*), 3.40 (2H, *d*, *J* = 8, 1‴-H), 5.16 (1H, *t*, *J* = 8, 2‴-H), 5.63 (1H, *d*, *J* = 10, 3″-H), 6.73 (1H, *d*, *J* = 10, 4″-H), 6.83 (1H, *d*, *J* = 8.4, 4′ or 5′-H), 6.86 (1H, *d*, *J* = 8.4, 5′ or 4′-H), 7.03 (1H, *s*, 8-H), 7.89 (1H, *s*, 2-H), 12.87 (1H, *s*, 5-OH). Triacetate of **1**: EIMS *m/z* 547 (M + 1, 6) 504 (58), 489 (100), 461 (15), 447 (31), 419 (18), 405 (27), 377 (5), 365 (4), 349 (4), 337 (3), 284 (18), 202 (3), 175 (3); 200 MHz ^1H NMR ($CDCl_3$) δ 1.48 (6H, *s*, 2″-2Me), 1.68 (3H, *s*, 3‴-Me, *trans*), 1.82 (3H, *s*, 3‴-Me, *cis*), 2.29 (6H, *s*, 2MeCO), 2.44 (3H, *s*, MeCO), 3.45 (2H, *d*, *J* = 8, 1‴-H), 5.18 (1H, *t*, *J* = 8, 2‴-H), 5.77 (1H, *d*, *J* = 10, 3″-H), 6.49 (1H, *d*, *J* = 10, 4″-H), 7.24 (1H, *s*, 8-H), 7.34 (1H, *d*, *J* = 8.4, 4′ or 5′-H), 7.38 (1H, *d*, *J* = 8.4, 5′ or 4′-H), 7.88 (1H, *s*, 2-H).

3′-O-*Methylorobol.* Mp. 217–219° (hexane–acetone), HRMS *m/z* 300.0673 (calcd for $C_{16}H_{12}O_6$ 300.0630), UV, IR, NMR were identical to literature data [7].

Dehydrocostus lactone. $[\alpha]_D^{20}$ −18.5° ($CHCl_3$; C 0.2); HRMS *m/z* 230.196 (calcd for $C_{15}H_{18}O_2$ 230.1302). **3** was identified by lit. comparison of UV, IR and NMR data [8, 9].

Methyl linoleate. HRMS *m/z* 294.2551 (calcd for $C_{19}H_{34}O_2$ 294.2550). This compound was identified as methyl linoleate by comparison of MS and NMR spectral data [10, 11].

Sitosterol. HRMS *m/z* 414.3840 (calcd for $C_{29}H_{50}O$ 414.3849). This compound was identified as sitosterol by lit. comparison of MS and NMR data [12, 13].

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