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J. Nat. Prod., **1993**, 56 (10), 1718-1722• DOI: 10.1021/np50100a009 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 10, pp. 1718-1722, October 1993

TWO NEW CYTOTOXIC CHALCONES FROM *CALYTHROPSIS AUREA*

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ABSTRACT.—The crude extract of *Calythropsis aurea* (Myrtaceae) produced a pattern of differential cytotoxicity in the NCI 60 cell line assay which was similar to those of known tubulin-interactive compounds. Cytotoxicity-guided fractionation led to the isolation of two new chalcones, calythropsin [**1**] and dihydrocalythropsin [**2**], which were responsible for the activity. Calythropsin was demonstrated to have a weak effect on mitosis, and presumably also on tubulin polymerization.

We have been investigating the use of the NCI in vitro primary screen (1–3) for discovery of novel, tubulin-interactive cytotoxic chemotypes from natural sources. Through application of a pattern-matching algorithm (2) to data from the NCI 60-cell line human-disease-oriented tumor screening panel (1), we have found that compounds with similar mechanisms of action or biochemical targets give rise to similar patterns of differential cytotoxicity in the assay. Thus, when compounds which are known to act through a tubulin-dependent mechanism are tested, the resulting profiles of differential cytotoxicity share considerable similarities as to which cell lines are relatively more sensitive or resistant to this class of agents.

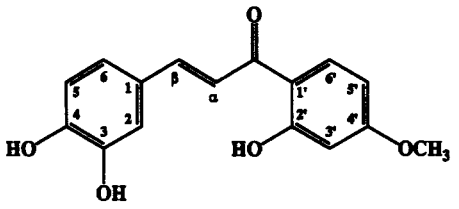
The differential cytotoxicity fingerprints of several well-studied tubulin-interactive "standard" compounds (e.g., vincristine, vinblastine, taxol, dolastatin 10, maytansine, rhizoxin) were used to probe the NCI extracts database containing data from approximately 6000 extracts screened as of September 1990. Based upon the relatively high correlations of the screening profiles with those of the standards, a number of extracts were selected for fractionation. We have recently reported (3) the isolation of the tubulin-interactive cytotoxic compound centaureidin from one of those extracts.

The crude organic extract of *Calythropsis aurea* C.A. Gardner (Myrtaceae) selected for the present study showed very modest but reproducible correlations (e.g., 0.45–0.56; data not shown) to the tubulin-active seed patterns. This monotypic species from Western Australia had not previously been studied chemically or pharmacologically, while the only report on a related genus, *Calytrix*, described essential oils containing β-triketones (4).

RESULTS AND DISCUSSION

The organic extract of the roots of *C. aurea* was fractionated by solvent-solvent partitioning to yield an active $CHCl_3$ fraction. Sephadex LH-20 gel permeation in CH_2Cl_2/MeOH and reversed-phase hplc on C_{18} media gave a cytotoxic fraction. Cyano-bonded phase hplc, using hexane/EtOAc, was then used to purify compound **1**.

Hreims established a molecular formula of $C_{16}H_{14}O_5$. The yellow color and ^{1}H-nmr data indicated a conjugated aromatic system. One methoxyl and an unsaturated ketone



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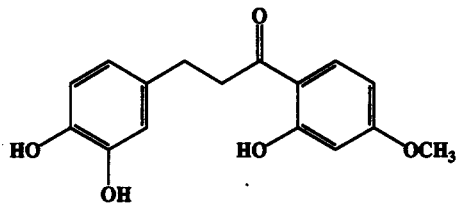

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(188 ppm) were apparent in the ^{13}C nmr. The use of COSY, HMQC, HMBC, and difference nOe experiments (Table 1, Figure 1), permitted the assignment of the structure of **1**. The proton scalar couplings and COSY pointed to a pair of 1,2,4-trisubstituted aromatic rings. HMQC data were used to assign carbon resonances to their attached protons. HMBC correlations from the carbonyl to proton signals at 7.28, 7.37, and 7.50 ppm were important in determining the structure (Figure 1). Further HMBC correlations to the 7.50 ppm signal from the carbon resonances at 160 and 162 ppm were seen, and the MeO proton resonance (3.83 ppm) correlation to 160 ppm fixed its point of attachment. The remaining 2- and 3-bond HMBC correlations were consistent with the substitution pattern shown and no other. The 15.8 Hz coupling of the α and β olefinic protons indicated a trans configuration of the double bond. This previously unreported compound has been assigned the trivial name calythropsin.

Dihydrocalythropsin [**2**] was isolated in a similar fashion from the $CHCl_3$ partition fraction. The similarity in structure to **1**, with the exception of the reduced double bond, was readily apparent from the hreims formula of $C_{16}H_{16}O_5$, a MeO carbon, a ketone at 201 ppm in the ^{13}C nmr, and a pair of 2H triplets at 2.60 and 2.98 ppm in the ^{1}H nmr. COSY, HMQC, and HMBC were also used in this case to determine the structure; however, overlap of several ^{13}C- and ^{1}H-nmr resonances in DMSO-d_6 required a solvent change to $CDCl_3/CD_3OD$ to resolve those signals sufficiently for unambiguous assignments to be made (Table 2).

Testing of calythropsin in the NCI 60-cell line panel yielded the mean response parameter values of GI_{50} (50% net growth inhibition, relative to controls) 0.66 μM, TGI (net cytostasis or total growth inhibition) 19.7 μM, and LC_{50} (50% net cell killing) >50 μM. Dose-response curves showed a typical cytostatic plateau which we have observed for other tubulin-active compounds. Pattern analyses using calythropsin [**1**] screening data as the seed against the standard agent database (1) revealed only weak, if at all meaningful, correlations of 0.36–0.57 (data not shown) to representative tubulin-interactive standards (vide supra). Dihydrocalythropsin [**2**] showed a similar screening profile but was approximately tenfold less potent than **1** in the 60-cell line panel. However, it occurred in considerably greater abundance in the extracts.

Calythropsin was evaluated in an in vitro tubulin polymerization assay (5), where it showed no detectable activity. This negative result is consistent with an extensive

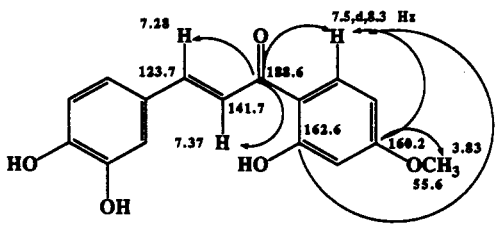

FIGURE 1. Key HMBC correlations establishing the structure of **1**.

TABLE 1. Nmr Data for Calythropsin [1] (500 MHz ^{1}H, 125 MHz ^{13}C).

Position	^{1}H[a] (mult, J in Hz)	^{13}C	HMBC to carbon no.
C=O	—	188.66	—
α	7.28 (d, 15.8)	123.73	1, 3, 4
β	7.37 (d, 15.8)	141.72	1, 2, 5, 9
1	—	126.44	—
2	7.07 (d, 2.0)	114.38	3, 6, 7, 9
3	—	145.58	—
4	—	148.20	—
5	6.76 (d, 8.2)	115.82	4, 6, 7
6	6.97 (dd, 8.2, 2.0)	121.63	3, 5, 7
1′	—	120.22	—
2′	—	162.55	—
3′	6.49 (d, 1.9)	99.25	1′, 2′, 4′, 5′
4′	—	160.37	—
5′	6.44 (dd, 8.3, 1.9)	107.88	1′, 3′
6′	7.50 (d, 8.3)	132.23	1, 2′, 4′
4′-OMe	3.83 (s)	55.65	4′

[a]In DMSO-d_6.

examination of compounds suggested by the Compare algorithm to be tubulin inhibitory. In that study (6), in vitro activity against tubulin was generally observed only when correlation coefficients to standard antimitotic drugs were ≥0.6. However, a further cytotoxicity experiment using L1210 cells showed a cytotoxic dose (IC_{50}) of 7 μM for calythropsin [1]. In the same experiment, mitotic figures were detected in the drug-treated cells at a significantly higher level than in untreated controls. Thus, calythropsin would appear to have a weak effect on tubulin polymerization at doses which are also cytotoxic. The weak antimitotic activity of calythropsin is consistent with the report of Edwards *et al.* (7), who studied structure-activity relationships in a series of synthetic

TABLE 2. Nmr Data for Dihydrocalythropsin [2] (500 MHz ^{1}H, 125 MHz ^{13}C).

Position	^{1}H[a] (mult, J in Hz)	^{13}C shift	HMBC to carbon no.	^{1}H[b]	^{13}C
C=O	—	201.54	—	—	198.04
α	2.98 (t, 7.8)	45.85	1, 3, 4	3.03	45.19
β	2.60 (t, 7.8)	30.55	1, 2, 4, 5, 9	2.65	29.55
1	—	134.07	—	—	132[c]
2	6.48 (d, 2.0)	115.66	4, 6, 9	6.57	115[d]
3	—	144.78	—	—	144.89
4	—	142.98	—	—	143.12
5	6.51 (d, 7.9)	115.45	4, 6, 7, 9	6.57	115[d]
6	6.34 (dd, 2.0, 7.9)	120.06	3, 5, 7	6.39	118[e]
1′	—	119.71	—	—	118[e]
2′	—	163.51	—	—	162.95
3′	6.21 (m)	99.13	1′, 5′	6.46	99.03
4′	—	161.87	—	—	160.90
5′	6.23 (m)	108.22	1′, 3′	6.39	107.84
6′	7.44 (d, 9.0)	133.02	1, 2′, 4′	7.55	132[c]
3-OH	—	—	—	8.57[c]	—
4-OH	—	—	—	8.69[c]	—
2′-OH	—	—	—	10.27	—
4′-OMe	3.63 (s)	55.52	4′	3.80	55.54

[a]In $CDCl_3$-MeOH-d_4 (2:1).
[b]In DMSO-d_6.
[c–e]Resonances with matching letters may have assignments reversed.

chalcones, some of which were quite potent inhibitors of tubulin function. Those chalcones were mostly substituted by functionalities not normally found in naturally occurring chalcones.

In summary, calythropsin is a novel, but only weakly cytotoxic, chalcone with similarly weak or minimal effects on tubulin function or mitosis. Nonetheless, it may serve as a useful template in comparison to previously synthesized chalcones (7) for additional structure-activity studies.

EXPERIMENTAL

PLANT MATERIAL.—The roots of *C. aurea* were collected in Western Australia approximately 70 km north of Geraldton between the old and new coastal highways in a cut-over *Banksia* woodland at an elevation of 900 ft. in September 1981. Dried plant material (381 g) was extracted by the standard NCI method (8) to yield 8.23 g of crude organic extract (2.2% dry wt). The plant was identified by R. Spjut (SPJ-7041), and a voucher is deposited in the Smithsonian Institution.

ISOLATION.—Crude extract (4.38 g) was partitioned between 90% aqueous MeOH and hexane. The MeOH solution was adjusted to 80% MeOH and extracted with CCl_4. The aqueous MeOH phase was adjusted to 60% MeOH and extracted with $CHCl_3$ to give 1.72 g of $CHCl_3$ fraction. Gel permeation of the $CHCl_3$ fraction on a 6×70 cm column of Sephadex LH-20 in CH_2Cl_2-MeOH (1:1) yielded an active fraction of 179.2 mg. Reversed-phase hplc of this material on a C_{18} column (Rainin Dynamax 8μ, 4.1×25 cm), eluting with 25% MeCN, yielded 14.7 mg of semipure chalcone, which was further purified by normal phase hplc (Rainin Dynamax Cyano 8μ, 2.1×25 cm) with hexane-EtOAc (13:7) to yield 9.6 mg of calythropsin [**1**].

From 771 mg of the $CHCl_3$ fraction, compound **2** was isolated by Sephadex LH-20 permeation [CH_2Cl_2-MeOH (1:1)] to give 62.6 mg of an active fraction. Si gel flash chromatography ($CHCl_3$/MeOH, 15-step gradient from 49:1 to 100% MeOH, 100 ml each step) of this fraction yielded 10.8 mg of dihydrocalythropsin [**2**].

Calythropsin [**1**].—Uv (EtOH) 365 nm (log ε 4.22) 246 (sh, 3.98), 204 (4.54) (EtOH+NaOH) 415, 329, 215; eims *m/z* 286 (56), 258 (13), 176 (32), 151 (40), 149 (100), 136 (20); hreims 286.0840 (calcd for $C_{16}H_{14}O_5$, 286.0841); ^{1}H nmr and ^{13}C nmr see Table 1.

Dihydrocalythropsin [**2**].—Uv (EtOH) 302 nm (log ε 3.88) 271 (3.99), 206 (4.44) (EtOH+NaOH) 331, 250, 215; eims *m/z* 288 (31), 270 (2), 166 (8), 151 (100), 136 (16); hreims 288.0957 (calcd for $C_{16}H_{16}O_5$, 288.0997); ^{1}H nmr and ^{13}C nmr see Table 2.

BIOLOGICAL TESTING.—Two cell lines (OVCAR-3, A549) selected from the NCI screening panel were used in a two-day XTT tetrozolium cytotoxicity assay (9) to guide fractionation. The 60-cell in vitro human tumor screening panel testing was performed as previously described (1). The COMPARE algorithm (2) was used to evaluate the similarity of patterns of differential response.

MITOTIC INDEX.—Cytotoxicity studies with L1210 cells measured growth after 24 h, mitotic index after 16 h (6).

TUBULIN POLYMERIZATION.—Turbidimetric measurement of tubulin polymerization was performed as described previously (5). Reaction mixtures contained 1.0 mg/ml tubulin, 1.0 M monosodium glutamate (pH 6.6), 1 mM $MgCl_2$, 4% (v/v) DMSO, and 0.4 mM GTP. All components except GTP (volume 240 μl) were preincubated for 15 min at 37°. Reaction mixtures were chilled on ice, GTP was added in 10 μl aliquots, and the reaction mixture was transferred to 0° cuvettes. Baselines were established, and at time zero the temperature was raised to 37° over 75 sec. IC_{50} values were based on 50% inhibition of extent of reaction after 20 min incubation. A minimum of three experiments per compound were performed.

ACKNOWLEDGMENTS

The authors thank Anne Monks and Dominic Scudiero for antitumor screening assays, Thomas G. McCloud for plant extraction, and Richard Spjut for the plant collection and identification.

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Received 4 February 1993