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# POTENTIAL CANCER CHEMOPREVENTIVE AND CYTOTOXIC AGENTS FROM *PULICARIA CRISPA*

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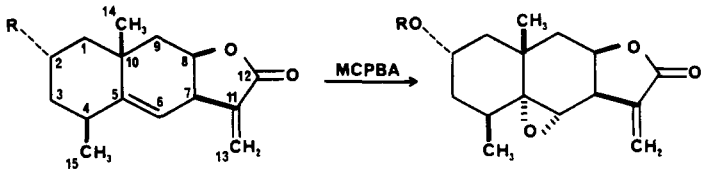
*Pulicaria crispa* (syn. *Francoeuria crispa* Forssk., Cas) is a member of the Compositae prevalent in central Saudi Arabia (1). This plant, which is commonly known as "gethgath," is used locally to treat inflammation and as an insect repellent (2). This species is under study as part of a systematic examination of medicinal plants of Saudi Arabia.

Prior work yielded the cytotoxic sesquiterpene lactone 2α-hydroxyalantolactone [**1**] as a major component of the petroleum ether extract of the dried aerial parts (3). Further investigation of the 95% EtOH extract of the defatted material yielded a new sesquiterpene lactone epoxide **2** that was determined to be structurally related to compound **1**. High resolution eims showed an $M^+$ at 264.1366 corresponding to a molecular formula of $C_{15}H_{20}O_4$. Because compound **1** had a formula of $C_{15}H_{20}O_3$, it followed that **2** was a closely related epoxide. This was confirmed by analysis of the ir, uv, $^1$H-nmr, and $^{13}$C-nmr data of **2**. The presence of the α,β-unsaturated-γ-lactone was confirmed by the ir absorption at 1760 $cm^{-1}$ and the typical uv [λ max (EtOH) 212.5 nm, ε = 9800]. Examination of the 470 MHz $^1$H-nmr spectra of **2** supported this assignment. Using homonuclear decoupling experiments it was possible to assign all of the protons in the $^1$H nmr and propose that **2** was the 5,6-epoxy derivative of **1**. The 50 MHz $^{13}$C-nmr spectra of **2** were also obtained and fully supported the structure (see Experimental section).

In order to confirm the structure of **2** and assign its stereochemistry, the parent compound **1** was epoxidized with *m*-chloroperbenzoic acid (MCPBA) in $CH_2Cl_2$. Kitagawa *et al.* (4) have established that MCPBA generates exclusively 5,6-α-epoxyalantolactone from the parent alantolactone **3**. Reaction of **1** with MCPBA at 25° for 1 h gave a major oxidation product which was purified by centrifugal radial tlc and then compared to **2**. The product was identical by tlc, ms, and $^1$H nmr to the natural product, thus confirming our structural assignment.

Compound **2** was cytotoxic to KB cells in culture ($ED_{50}$ = 0.4 μg/ml) (5). The parent compound **1** was active at the same level ($ED_{50}$ = 0.33 μg/ml). The acetate of **2**, compound **4**, was more cytotoxic, showing an $ED_{50}$ = 0.05 μg/ml. .

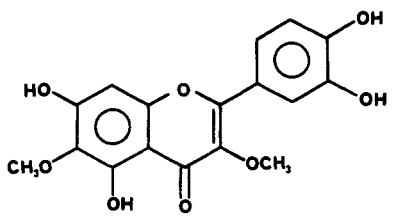
Within the last few years the concept




**1** R=OH
**3** R=H

**2** R=H
**4** R=Ac

of the prevention of cancer through dietary modification has become a widely accepted goal. One of the compounds isolated during this study was active in a screen developed in our laboratory to detect potential inhibitors of carcinogenesis (6,7). This compound exhibited color reactions and uv absorptions typical of a flavonoid (8,9) and had mp, uv, ms, and $^1$H-nmr data identical to the known compound axillarin [**5**] (10,11). $^{13}$C-nmr data is presented for the first time for this compound and fully support the structure for **5**.




**5**

Flavonoids as a group have produced compounds which possess anticarcinogenic activity (12,13). The synthetic flavonoid 7,8-benzoflavone inhibits the initiation of mouse skin tumors by 7,12-DMBA (14). Using the assay for benzo(*a*)pyrene metabolism in cultured hamster embryo cells (6), we have found that axillarin [**5**] is active at 25 μg/ml medium and decreased the metabolism of benzo(*a*)pyrene by an average of 61.3% over DMSO controls. Further experiments are underway to determine the potential of **5** as a chemopreventive agent in animal models.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir spectra were recorded in KBr on a Beckman-33 unit. Uv spectra were obtained on a Perkin-Elmer (Coleman 124) double-beam spectrophotometer. Low- and high-resolution mass spectra were measured on a Finnegan 4023 gc/ms with Incos 2000 data system and a Kratos MS50S, respectively. High resolution (470 MHz) $^1$H-nmr spectra and homo decoupling were recorded at the Purdue University Biological Magnetic Resonance Laboratory on a Nicolet NTC-470 NMR Spectrometer, and the 50.3 MHz $^{13}$C-nmr spectra were recorded on a Varian XL-200 Spectrometer fully decoupled and gated decoupled. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter.

PLANT MATERIALS.—Details of the collection and identification of the plant have been presented previously (3).

EXTRACTION PROCEDURE AND ISOLATION.—The defatted plant material (5 kg) was extracted with 95% EtOH, and the extract was then chromatographed on a Si gel (Merck) column using a $CHCl_3$/EtOH gradient. Compound **2** was eluted as a major component with 2% EtOH in $CHCl_3$. Compound **5**, which was eluted in MeOH from the original Si gel column, was purified by repeated gel filtration in Sephadex LH-20 with MeOH as a solvent.

PHYSICAL CONSTANTS OF COMPOUND **2**.—White amorphous powder, mp 198–200° (dec) from EtOH; ir ($CDCl_3$) $\nu$ max cm$^{-1}$ 3500, 2920, 1760; uv (EtOH) λ max 212.5 nm (ε = 9800); hreims *m/z* 264.1366 (calcd $C_{15}H_{20}O_4$ for $M^+$ = 264.1362), eims *m/z* (rel. int.) 264 (7), 220 (20), 149 (15), 135 (16), 131 (15), 121 (57), 107 (100); [α] nm +96.6° (589), +98.3° (578), +110.2° (546), +194.9° (436) (*c* = 0.059 in $CHCl_3$); $^1$H nmr ($CDCl_3$) ppm 1.32 (dd, $J_{1\alpha,1\beta}$ = 11.7 Hz, $J_{1\alpha,2\beta}$ = 11.7 Hz), 1.83 (ddd, $J_{1\alpha,1\beta}$ = 11.7 Hz, $J_{1\beta,2\beta}$ = 4.1 Hz, $J_{1\beta,3\beta}$ = 2.2 Hz), 1.34 (s), 4.14 (dddd, $J_{1\alpha,2\beta}$ = 11.7 Hz, $J_{1\beta,2\beta}$ = 4.1 Hz, $J_{2\beta,3\alpha}$ = 12 Hz, $J_{2\beta,3\beta}$ = 4.1 Hz), 1.74 (ddd, $J_{2\beta,3\beta}$ = 12 Hz, $J_{3\alpha,3\beta}$ = 12 Hz, $J_{3\alpha,4}$ = 5.9 Hz), 1.87 (ddd, $J_{1\beta,3\beta}$ = 2.2 Hz, $J_{2\beta,3\beta}$ = 4.1 Hz, $J_{3\alpha,3\beta}$ = 12 Hz), 1.53 (qdd, $J_{3\alpha,4\alpha}$ = 5.9 Hz, $J_{3\beta,4\alpha}$ = 1.6 Hz, $J_{4\alpha,15}$ = 7.9 Hz), 3.01 (d, $J_{6\beta,7\alpha}$ = 2.5 Hz), 3.69 (dddd, $J_{6\beta,7\alpha}$ = 2.5 Hz, $J_{7\alpha,8\alpha}$ = 8.8 Hz, $J_{7\alpha,13}$ = 2.8 Hz, $J_{7\alpha,13'}$ = 2.5 Hz), 4.67 (ddd, $J_{7\alpha,8\alpha}$ = 8.8 Hz, $J_{8\alpha,9\alpha}$ = 1.8 Hz, $J_{8\alpha,9\beta}$ = 4.5 Hz), 1.65 (dd, $J_{8\alpha,9\alpha}$ = 1.8 Hz, $J_{9\alpha,9\beta}$ = 15 Hz), 1.90 (dd, $J_{8\alpha,9\beta}$ = 4.4 Hz, $J_{9\alpha,9\beta}$ = 15 Hz), 6.40 (d, $J_{7\alpha,13\beta}$ = 2.8 Hz), 5.76 (d, $J_{7\alpha,13\alpha}$ = 2.5 Hz), 1.14 (s, 14-Me), 1.08 (d, $J_{4\alpha,15}$ = 7.9 Hz); $^{13}$C nmr ($CDCl_3$) δ 46.6 (C-1), 63.1 (C-2), 39.0 (C-3 or C-9), 38.0 (C-4), 66.2 (C-5), 60.8 (C-6), 37.0 (C-7), 74.5 (C-8), 39.3 (C-9 or C-3), 33.6 (C-10), 136.3 (C-11), 169.8 (C-12), 123.9 (C-13), 24.9 (C-14), 18.7 (C-15).

SYNTHESIS OF **2** FROM **1**.—A solution of MCPBA (10 mg) in $CH_2Cl_2$ (5 ml) was added dropwise to a solution (10 mg) of 2α-hydroxyalantolactone [**1**] in $CH_2Cl_2$ (5 ml). The mixture was kept stirring at room temperature for 1 h, then treated with 10% aqueous $Na_2SO_3$. The $CH_2Cl_2$ extract was then successively washed with 5% $NaHCO_3$ and $H_2O$, filtered through anhydrous $Na_2SO_4$, and evaporated to dryness using a rotary evaporator. The product was then purified by a chromatotron using a gradient of

$CHCl_3$/MeOH, yielding 4 mg of **2**. The $^1$H-nmr and mass spectra were identical to those of the natural epoxide **2**. The natural epoxide **2** is, therefore, 2α-hydroxy,5α-6α-epoxyalantolactone.

PHYSICAL CONSTANTS OF **4**.—Compound **4** was prepared by the reaction of **2** with $Ac_2O$ in pyridine, mp 198–200° ($CHCl_3$). Uv λ max (EtOH) 210 nm (ε = 8900), ir (KBr) ν max $cm^{-1}$ 3000, , 2955, 1755, 1740, 1603, 1240, 1025, 640; cims ($CH_4$) *m/z* (rel. int.) $[M+1]^+$ 307 (15.4), 247 (79.3), 229 (100), 219 (28.3), 211 (11.3), 201 (14.0), 183 (21.9); $^1$H nmr ($CDCl_3$) ppm 1.42 (dd, $J_{1\alpha,1\beta}$ = 11.9 Hz, $J_{1\alpha,2\beta}$ = 11.9 Hz), 1.85 (ddd, $J_{1\alpha,1\beta}$ = 11.9 Hz, $J_{1\beta,2\beta}$ = 5.2 Hz, $J_{1\beta,3\beta}$ = 2.6 Hz), 5.21 (dddd, $J_{1\alpha,2\beta}$ = 11.9 Hz, $J_{1\beta,2\beta}$ = 5.2 Hz, $J_{2\beta,3\alpha}$ = 11.4 Hz, $J_{2\beta,3\beta}$ = 5.3 Hz), 1.85 (m, H3 +3), 1.57 (ddd, $J_{4\alpha,15}$ = 7.9 Hz, $J_{4\alpha,3\beta}$ = 8.6 Hz, $J_{4\alpha,3\alpha}$ = 2.5 Hz), 3.01 (d, $J_{6\beta,7\alpha}$ = 2.6 Hz), 3.69 (dddd, $J_{7\alpha,8\alpha}$ = 8.8 Hz, $J_{7\alpha,6\beta}$ = 2.6 Hz, $J_{7\alpha,13'}$ = 2.6 Hz), 4.65 (ddd, $J_{8\alpha,9\alpha}$ = 1.8 Hz, $J_{8\alpha,9\beta}$ = 4.5 Hz, $J_{8\alpha,7\alpha}$ = 8.8 Hz), 1.65 (dd, $J_{9\alpha,9\beta}$ = 15.0 Hz, $J_{9\alpha,8\alpha}$ = 1.8 Hz), 1.90 (dd, $J_{9\alpha,9\beta}$ = 15.0 Hz, $J_{9\beta,8\alpha}$ = 4.5 Hz), 5.78 (d, $J_{13,13'}$ = 2.7 Hz), 6.42 (d, $J_{13',13}$ = 2.7 Hz), 1.19 (s, 14-Me), 1.12 ($J_{15,4\alpha}$ = 7.9 Hz), 2.01 (s, 2-Ac).

PHYSICAL CONSTANTS OF AXILLARIN [**5**].—Uv, ir, ms, and $^1$H nmr are identical to literature values (10,11). $^{13}$C nmr (DMSO-$d_6$) δ 178.1 (C-4), 157.3 (C-7), 155.4 (C-2), 152.4 (C-9) 151.5 (C-5), 1487 (C-4′), 145.2 (C-3′), 137.3 (C-3), 131.1 (C-6), 120.8 (C-1′), 120.6 (C-6′), 115.7 (C-5′), 115.4 (C-2)′, 104.5 (C-10), 93.9 (C-8), 60.0 (3-OMe), 59.7 (6-OMe).

CELL CULTURE ASSAY.—Hamster embryo cell cultures were prepared and grown as described previously (15). Tertiary cultures were plated in 60-mm plastic dishes (Falcon) ($7 \times 10^5$ cells), and 2 to 4 h later the test compound was added at 10-fold dilutions from 500 μg/ml medium to 0.05 μg/ml for 24 h. Cultures were examined microscopically for inhibition of cell growth, and the highest noninhibitory dose was selected for metabolism studies.

Tertiary hamster embryo cell cultures ($7 \times 10^5$ cells per 25-$cm^2$ flask, three flasks per group) were plated in 10 ml medium containing 10% calf serum and refed with 10 ml fresh serum containing medium after 5 to 8 h. After 72 h, cultures were treated with the test compound in DMSO or with DMSO as a control, and 30 min later [$^3$H] benzo(*a*)pyrene (1 μg/ml, specific radioactivity 2.5 Ci/mmol) was added. Twenty-four h later medium was removed and stored at −20°. Aliquots (0.2 ml) were extracted by a $CHCl_3$/MeOH/$H_2O$ procedure (16). The radio activity in the organic and aqueous MeOH phases was measured by liquid scintillation counting of 0.1-ml aliquots. Butylated hydroxyanisole, a known inhibitor of carcinogenesis and benzo(*a*)pyrene metabolism (17), was used to treat a positive control group in all assays at a concentration of 50 μg/ml medium.

ACKNOWLEDGMENTS

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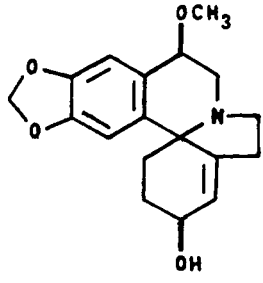
## ERRATUM

Amrik Singh Chawla has requested the following correction for the paper entitled "Alkaloidal Constituents of *Erythrina crista-galli* Flowers," *J. Nat. Prod.*, **50**, 1146 (1987).

The corrected structure of 11-methoxy-erythratine [**2**] is as follows:

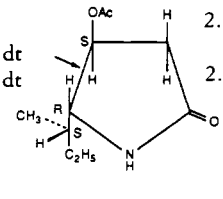



## ERRATUM

Kenneth L. Rinehart has requested the following correction for the paper entitled "Didemnins and Tunichlorin: Novel Natural Products from the Marine Tunicate *Tridemnum solidum*," *J. Nat. Prod.*, **51**, 1 (1988).

On page 13 in Scheme 6, the two left-hand lines of chemical shift data should be interchanged and Scheme 6 should appear as follows:

| *J*, Hz | δ |
|---|---|
| 7.1, 2.0 | 5.11 dt |
| 5.2, 1.8 | 3.51 dt |




| δ | *J*, Hz |
|---|---|
| 2.30 dd | 18.4, 2.6 |
| 2.75 dd | 18.4, 7.0 |