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PHYTOCHEMICAL AND BIOLOGICAL SCREENING OF SAUDI MEDICINAL PLANTS, PART 6.[1] ISOLATION OF 2α-HYDROXY-ALANTOLACTONE THE ANTILEUKEMIC PRINCIPLE OF *FRANCOEURIA CRISPA*

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The genus *Francoeuria* (Compositae) is represented in Saudi Arabia by one species prevalent in the Central region of the Kingdom, *Francoeuria crispa* (Forssk.) Cas. (1), which is known locally as *gethgath*. This plant, recognized in folk medicine, is currently used by people of rural areas to treat inflammation. In the form of an infusion, it is applied locally to the affected area, and also is used as a locust repellent.[2] Based on these observations, research was initiated on *F. crispa*, resulting in this report on the first phytochemical and biological study of this plant.

RESULTS AND DISCUSSION

Following a published procedure (2), the phytochemical screening of the plant indicated the presence of volatile oils, anthraquinones, flavonoids, coumarins, sterols, and/or tannins and triterpenes.

A major component (**1**) of the petroleum ether extract was eluted from a silica column with a mixture of C_6H_6-EtOH and purified by recrystallization from petroleum ether. High resolution electron impact mass spectra showed an M^+-H_2O peak of 230.1308 ($C_{15}H_{20}O_3$-H_2O). Uv absorption at λ max (EtOH) 210-212 nm (ε, 8200) and ir absorption at 1740 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone. The 470 MHz ^{1}H-nmr spectra of **1** further supported this structural feature and several homonuclear decoupling experiments were done to assign the structure of **1**. All chemical shifts and coupling constants are listed in Table 1.

Comparison of the recorded ^{1}H-nmr spectra to published data indicated that **1** was the eudesmanolide, 2α-hydroxyalantolactone (3). Since published ^{1}H-nmr spectra were recorded at 270 MHz, the higher field 470 MHz spectra afforded some refinements in the assignments of proton chemical shifts and coupling constants of **1**. Previously unreported shifts for H-3α and H-3β were assigned to δ 1.46 and 1.89 ppm, respectively. The H-9α and H-1α chemical shifts were found to be δ 1.56 and 1.07 ppm, respectively. These latter values differed from those reported in the literature by 0.4 ppm and 0.2 ppm, respectively. The 50 MHz ^{13}C-nmr spectra of **1** were obtained and chemical shifts for all carbons are reported in Table 2. These assignments were based on single-frequency decoupling experiments and fully support the proposed structure.

Compound **1** was isolated as white needles melting at 104°, while the previous isolation of 2α-hydroxyalantolactone yielded an oil (3). The optical rotation of **1** was also different from the published values presumably due to a difference in the state of purity. Based on

[1]For Part 5, See: J.S. Mossa, M.A. Al-Yahya, I.A. Al-Meshal, and M. Tariq, "Phytochemical and Biological Screening of Saudi Medicinal Plants," *Fitoterpia*, **54** (4), 147 (1983).

[2]This information was gathered by one of us (M.A. Al-Yahya) on field trips to the Central Region of Saudi Arabia.

TABLE 1. 470 MHz ^{1}H-nmr Data of 2α-Hydroxyalantolactone (**1**), 11,13-Dihydro-2β-(**3**), and 11,13-Dihydro-2α-hydroxyalantolactone (**4**) in $CDCl_3$.

Proton	Chemical Shift in ppm (J in Hz)		
	Compound 1	Compound 3	Compound 4
1α	1.07 dd ($J_{1\alpha,1\beta}$=11.7; $J_{1\alpha,2\beta}$=11.7)	1.56 dd ($J_{1\alpha,1\beta}$=13.7; $J_{1\alpha,2\alpha}$=6.2)	1.07 dd ($J_{1\alpha,1\beta}$=11.7; $J_{1\alpha,2\beta}$=11.3)
1β	1.93 ddd ($J_{1\alpha,1\beta}$=11.7; $J_{1\beta,2\beta}$=3.9; $J_{1\beta,3\beta}$=2.5)	1.72 dd ($J_{1\alpha,1\beta}$=13.7; $J_{1\beta,2\alpha}$=6.2)	1.90 ddd ($J_{1\alpha,1\beta}$=11.7; $J_{1\beta,2\beta}$=3.7; $J_{1\beta,3\beta}$=2.1)
2α	1.58 s (-OH)	3.96 dddd ($J_{1\alpha,2\alpha}$=6.2; $J_{1\beta,2\alpha}$=6.2; $J_{2\alpha,3\alpha}$=6.1; $J_{2\alpha,3\beta}$=6.2)	———
2β	4.20 dddd ($J_{1\alpha,2\beta}$=11.7; $J_{1\beta,2\beta}$=3.9; $J_{2\beta,3\alpha}$=11.3; $J_{2\beta,3\beta}$=4.1)	———	4.19 dddd ($J_{1\alpha,2\beta}$=11.3; $J_{1\beta,2\beta}$=3.7; $J_{2\beta,3\alpha}$=11.7; $J_{2\beta,3}$=3.7)
3α	1.46 ddd ($J_{2\beta,3\alpha}$=11.3; $J_{3\alpha,3\beta}$=11.9; $J_{3\alpha,4}$=6.1)	1.58 dddd ($J_{2\alpha,3\alpha}$=6.1; $J_{3\alpha,3\beta}$=13.8; $J_{3\alpha,4}$=7.4)	1.46 ddd ($J_{2\beta,3\alpha}$=11.7; $J_{3\alpha,3\beta}$=11.9; $J_{3\alpha,4}$=2.3)
3β	1.89 dddd ($J_{1\beta,3\beta}$=2.5; $J_{2\beta,3\beta}$=4.1; $J_{3\alpha,3\beta}$=11.9)	1.95 dddd ($J_{2\alpha,3\beta}$=6.2; $J_{3\alpha,3\beta}$=13.8; $J_{3\beta,4}$=7.4)	1.94 dddd ($J_{1\beta,3\beta}$=2.1; $J_{2\beta,3\beta}$=3.7; $J_{3\alpha,3\beta}$=11.9; $J_{3\beta,4}$=2.3)
4	2.62 qdd ($J_{3\alpha,4}$=6.1; $J_{3\beta,4}$=2.1; $J_{4,15}$=7.7)	2.54 qdd ($J_{3\alpha,4}$=7.4; $J_{3\beta,4}$=7.4; $J_{4,15}$=7.5)	2.66 qdd ($J_{3\alpha,4}$=2.3; $J_{3\beta,4}$=2.3; $J_{4,15}$=7.7)
6	5.23 d ($J_{6,7\alpha}$=4.2)	5.24 d ($J_{6,7\alpha}$=3.5)	5.23 d ($J_{6,7\alpha}$=3.3)
7α	3.60 dddd ($J_{6,7\alpha}$=4.2; $J_{7\alpha,8\alpha}$=6.5; $J_{7\alpha,13}$=1.9; $J_{7\alpha,13'}$=1.6)	3.07 ddd ($J_{6,7\alpha}$=3.5; $J_{7\alpha,8\alpha}$=6.7; $J_{7\alpha,11\alpha}$=7.2)	3.06 ddd ($J_{6,7\alpha}$=3.3; $J_{7\alpha,8\alpha}$=5.8; $J_{7\alpha,11\alpha}$=7.2)
8α	4.82 ddd ($J_{7\alpha,8\alpha}$=6.5; $J_{8\alpha,9\alpha}$=3.1; $J_{8\alpha,9\beta}$=3.2)	4.72 ddd ($J_{7\alpha,8\alpha}$=6.7; $J_{8\alpha,9\alpha}$=2.7; $J_{8\alpha,9\beta}$=3.0)	4.71 ddd ($J_{7\alpha,8\alpha}$=5.8; $J_{8\alpha,9\alpha}$=2.5; $J_{8\alpha,9\beta}$=3.2)
9α	1.56 dd ($J_{8\alpha,9\alpha}$=3.1; $J_{9\alpha,9\beta}$=14.9)	1.52 dd ($J_{8\alpha,9\alpha}$=2.7; $J_{9\alpha,9\beta}$=11.7)	1.54 dd ($J_{8\alpha,9\alpha}$=2.5; $J_{9\alpha,9\beta}$=14.7)
9β	2.17 dd ($J_{8\alpha,9\beta}$=3.2; $J_{9\alpha,9\beta}$=14.9)	2.18 dd ($J_{8\alpha,9\beta}$=3.0; $J_{9\alpha,9\beta}$=11.7)	2.17 dd ($J_{8\alpha,9\beta}$=3.2; $J_{9\alpha,9\beta}$=11.7)
11α	———	2.88 m ($J_{7\alpha,11\alpha}$=7.2; $J_{11\alpha,13}$=7.3)	2.89 m ($J_{7\alpha,11\alpha}$=7.2; $J_{11\alpha,13}$=7.3)
13	6.22 d ($J_{7\alpha,13}$=1.9)	1.22 d ($J_{11\alpha,13}$=7.3) (1.05 d)[a]	1.22 d ($J_{11\alpha,13}$=7.3) (1.01 d)[a]
13′	5.65 d ($J_{7\alpha,13'}$=1.6)	———	———
14	1.22 s (-CH_3)	1.39 s (-CH_3)	1.26 s (-CH_3)
15	1.13 d ($J_{4,15}$=7.7)	1.26 d ($J_{4,15}$=7.5)	1.16 d ($J_{4,15}$=7.7)

[a]These upfield chemical shifts for the 13-methyl of **3** and **4** were obtained with C_6D_6 as the solvent.

TABLE 2. 50 MHz ^{13}C-nmr Data for 2α-Hydroxyalantolactone (**1**)

Carbon	Chemical Shift (ppm)	Carbon	Chemical Shift (ppm)
C-1	50.33	C-9	42.40
C-2	63.24	C-10	33.61
C-3	41.78	C-11	139.35
C-4	38.53	C-12	170.26
C-5	146.75	C-13	122.12
C-6	119.72	C-14	29.61
C-7	39.53	C-15	23.39
C-8	75.66		

these observations, a chemical study was undertaken to rule out the possibility that **1** was a closely related isomer of 2α-hydroxyalantolactone. Oxidation of **1** yielded a product **2** with physical properties, optical rotation, and nmr data identical to published values for the ketone, 2-oxoalantolactone (**2**), which had been prepared by oxidation of **1** (3). This interrelationship supported the structure and stereochemistry of **1** at all centers except C-2. The configuration of

the 2-hydroxy group in **1** was further defined by analysis of the nmr spectra of the epimeric alcohols 11α,13-dihydro-2β-hydroxyalantolactone (**3**) and 11α,13-dihydro-2α-hydroxyalantolactone (**4**). Stereoselective reduction of **2** with sodium borohydride produced the 11α,13-dihydro, 2-β hydroxy derivative **3** (4). The 470 MHz ^{1}H-nmr spectra of both compounds **3** and **4** are reported in Table 1, and the data allowed the assignment of stereochemistry at the 2 and 11 positions. Inspection of the 2α-proton splitting pattern in **3** showed four couplings to protons at C-1 and C-3 of 6.2 Hz. The magnitude of these coupling constants clearly indicates that the proton at C-2 must be equatorial. Analysis of the 2β-proton splittings in **4** yielded coupling constants of 11.3 Hz and 3.7 Hz to protons at C-1 and 11.7 Hz and 3.7 Hz to protons at C-3. The magnitudes of these coupling terms indicate axial interaction between the 2β-proton and the 1 and 3 protons. Comparison of the data to the ^{1}H-nmr spectra for **1** which had coupling constants $J_{1,2}=11.7$ Hz, $J_{1,2}=3.9$ Hz, $J_{2,3}=11.3$ Hz, and $J_{2,3}=4.1$ Hz consistent with an equatorial hydroxyl at C-2, confirmed **1** to be 2α-hydroxyalantolactone. In addition, examination of the solvent dependent changes in ^{1}H-chemical shifts for the 13-methyl of **3** and **4** and the magnitude of the $J_{7,11}$ coupling constants provided sufficient evidence to designate the configuration at C-11. Upfield shifts of 0.17 ppm in **3** and 0.21 ppm in **4** were observed for the 13-methyl on changing solvents from $CDCl_3$ to C_6D_6. This shift is indicative of a pseudo-equatorial methyl group in a *cis*-fused-γ-lactone (5). The coupling pattern and the coupling constant ($J_{7,11}=7.2$ Hz) is also consistent with that reported for pseudo-axial protons in *cis*-fused-γ-lactone of the eudesmanolide group (5).

Compound **1** was cytotoxic (2×10^{-1} μg/ml in 9KB; 2 μg/ml in 9 PS) and showed borderline activity against the murine P-388 lymphocytic leukemia (T/C 123% at 75 mg/kg). An antimicrobial screen against five microorganisms [*Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC 10662), *Salmonella* sp. (College of Pharmacy. King Saud University), and *Candida albicans*(NCTC 3153)] showed slight ac-

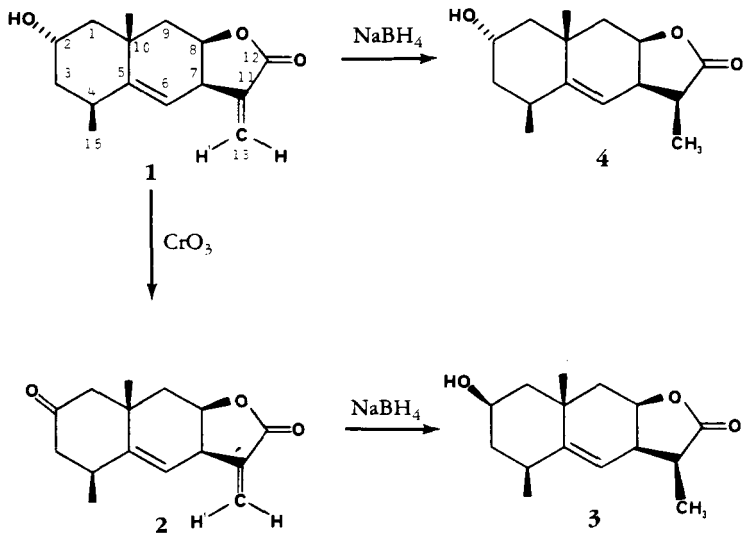

FIGURE 1. Structure of 2α-Hydroxyalantolactone (**1**) and Chemical Conversion to 2-Oxoalantolactone (**2**) and 11α,13-Dihydro-2β-hydroxy-(**3**) and 11α,13-Dihydro-2α-hydroxy-(**4**) alantolactones.

tivity against *S. aureus* only, with a minimum inhibitory concentration of 6.25×10^{-1} mg/ml.

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.—The plant was collected at the flowering stage from Al-Quaseem (Saudi Arabia); its identity was confirmed by Professor A.M. Migahid, College of Science, King Saud University, and a voucher specimen was deposited in the herbarium of the Medicinal Plants Unit, Research Center at King Saud University in Riyadh.

EXTRACTION PROCEDURE AND ISOLATION OF 2α-HYDROXYALANTOLACTONE (**1**).—The dried aerial parts of the plant (1 kg) were powdered and exhaustively extracted with petroleum ether (60-80°) in a Soxhlet apparatus. The extract (18 g) was concentrated and refrigerated for several days, and the deposit that formed was redissolved in petroleum ether and fractionated on a 500-g silica gel (S) column (70-230 mesh size) using C_6H_6-EtOH (19:1). The first 600 ml of eluate yielded some minor components followed by the major compound (15 g, 1.5% w/w) which eluted in the following 1600 ml.

The purified compound was tested against the 9KB, 9PS (in vitro), and 3PS (in vivo) systems in accordance with established protocols (6).

PHYSICAL CONSTANTS OF 2α-HYDROXY ANTOLACTONE (**1**).—This compound crystallized as white needles mp 104° (uncorr.) from petroleum ether; ir (KBr) ν max 3360 (br), 2900, 1740, 1640 cm^{-1}; uv (EtOH) λ max 210-212 (ϵ, 8200) nm; ms *m/z* M^+-H_2O) 230.1308 (calcd $C_{15}H_{20}O_3$, M^+-H_2O 230.1307), 215 (14%), 121 (100%); [α] (λ, nm) +156.3° (589), +163.9° (578), +189.0° (546), +353.8 (436) (c=1.0 in $CHCl_3$).

OXIDATION OF 2α-HYDROXYALANTOLACTONE TO 2-OXOALANTOLACTONE (**2**).—This was carried out by adding 100 mg of chromic anhydride to 50 mg of the compound in 5 ml of pyridine. The mixture was allowed to stir overnight at room temperature. The ketone (6.7 mg) was eluted from a small silica column (10 g) with $CHCl_3$ and recrystallized from Et_2O mp 151-153° (lit. 152-153°); [α] (λ, nm) +301.2° (589), +315.6° (578), +366° (546), +703° (436) (c=0.25 in $CHCl_3$) identical with the published literature values (3).

REDUCTION OF **2** WITH SODIUM BOROHYDRIDE.—The reaction was initiated by addition of excess $NaBH_4$ to a solution of **2** (21.5 mg) in ice-cold MeOH. After 30 min, 20 ml of H_2O was added, and the mixture was acidified with 1 N HCl and extracted with $CHCl_3$. The organic solvent was removed under vacuum, and 13.1 mg of the pure compound **3** was obtained by gradient $CHCl_3$-MeOH elution on the chromatotron; ir (KBr) ν max 3230, 1750, 1190 cm^{-1}; ms *m/z* 250 (M^+, 1%), 232 (M^+-H_2O, 1.5%), 217 (3%), 159 (6%), 40 (100%); [α] (λ, nm) −17.0° (589), −18.1° (578), −20.6° (546), −42.0° (436) (c=0.66 in $CHCl_3$).

PREPARATION OF **4**, THE BOROHYDRIDE REDUCTION PRODUCT OF **1**.—Compound **4** was prepared in a manner identical to that of **3** from 30 mg of starting material giving 14.6 mg of product; ir (KBr) ν max 3230, 1730, 1450, 1370, 1190 cm^{-1}; ms *m/z* 232 (M^+-H_2O, 2%), 217 (0.6%), 159 (4%), 42 (100%); [α] (λ, nm) −41.6° (589), −43.3° (578), −49.9° (546), −90.2° (436) (c=0.68 in $CHCl_3$).

ANTIMICROBIAL ACTIVITY.—Strains of *S. aureus*, *E. coli*, *C. albicans*, *P. vulgaris*, and *P. aeruginosa* from the National Collection of Type Cultures (NCTC) London, England, and a laboratory strain of *Salmonella* sp. were used. MIC was measured using the tube dilution method. The compound was tested in concentrations of 5, 2.5, 1.25, 0.62, 0.31 and 0.155 mg/ml. All dilutions were prepared in antibiotic assay broth No. 1 (7) with one drop of 10^{-2} dilution of an overnight broth culture of the test organism. All inoculated dilutions and a blank of broth and inoculum were incubated overnight at 37° before recording the minimum inhibitory concentration.

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