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ISOLATION, NMR STUDIES, AND BIOLOGICAL ACTIVITIES OF ONOPORDOPICRIN FROM *CENTAUREA SONCHIFOLIA*

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ABSTRACT.—A sesquiterpene lactone, onopordopicrin [**1**], has been isolated from *Centaurea sonchifolia*. Its structure was established by 2D nmr (^{1}H-^{1}H and ^{13}C-^{1}H correlations), and the conformation in $CHCl_3$ was examined by nOe studies. Cytotoxic, antibacterial, and antifungal activities are reported.

Onopordopicrin [**1**] is known to be major constituent of the genus *Onopordone* (1–4), with occasional appearance in some species of other genera (5). Previous investigation of the aerial parts of *Centaurea sonchifolia* L. from the Canary Islands resulted in the isolation of acetylartemisiifolin (6) and artemisiifolin (7). Investigation of the aerial parts of *C. sonchifolia* from locations in southwestern Greece, however, resulted in the isolation of onopordopicrin, which was found to be the principal constituent of the lactone mixture. The structure was determined by ^{13}C-^{1}H correlation experiments, COSY, DEPT, and other 2D nmr evidence. The conformation in $CDCl_3$ solution was studied by nOe enhancement spectroscopy. This lactone shows cytotoxic activity against the KB cell line (a human epidermoid carcinoma of the nasopharynx). It also inhibits the growth of *Staphylococcus aureus*.

Table 1 reports the 200 MHz ^{1}H-nmr spectral assignments of onopordopicrin [**1**] in $CDCl_3$, including assignments of all proton resonances, ^{1}H chemical shifts, multiplicities, and proton-proton correlations derived from a COSY experiment. Table 2 reports the ^{13}C spectrum of onopordopicrin in $CDCl_3$ including assignments of all carbon resonances, ^{13}C chemical shifts, multiplicities as derived from DEPT experiments, and C-H correlations as derived from a C-H heteronuclear correlation experiment.

NOe enhancement experiments (8) of **1** (Table 3) indicated the α position of

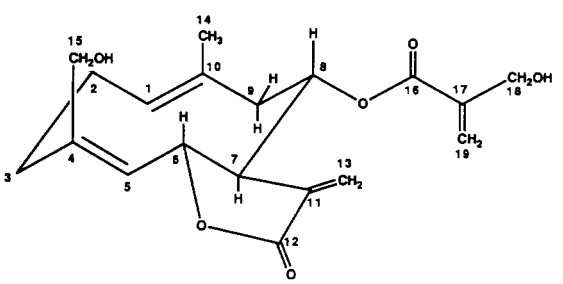

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TABLE 1. ^{1}H-nmr Data of Onopordopicrin [1] (200 MHz, in $CDCl_3$, TMS as internal standard).

Proton	δ (ppm)	multiplicity	COSY
H-1	5.02	dd (br)	2
H-2	2.24	m	1
H-2′	2.00	m	1
H-3α	2.24	m	3β, 2β
H-3β	2.62	m	3α, 2, 2′
H-5	4.85	d	15, 15′, 6
H-6β	5.14	dd	5, 7α
H-7α	3.11	m	6, 13′
H-8β	5.19	dd (br)	9α
H-9α	2.62	m	8β
H-9β	2.00	m	—
H-13	6.29	d	7α
H-13′	5.81	d	7α
H-14	1.51	s (br)	9α
H-15	4.23	d	5
H-15′	4.07	d	5
H-18	4.31	s (br)	19, 19′, 18′
H-19	6.30	s (br)	19′, 18, 18′
H-19′	6.00	s (br)	18, 18′, 19

H-1, H-5, and H-7. Thus, saturation of H-7 at δ 3.11 ppm resulted in enhancement of H-5 at δ 4.85 ppm (10%). Similarly, saturation of H-5 resulted in

TABLE 2. ^{13}C-nmr Data of Onopordopicrin [1] (200 MHz, $CDCl_3$, TMS as internal standard).

Carbon	δ (ppm)	multiplicity	Proton correlation
C-1	129.67	d	5.02
C-2	26.04	t	2.24/2.00
C-3	34.49	t	2.24/2.62
C-4	144.50	s	—
C-5	127.86	d	4.85
C-6	77.28	d	5.14
C-7	52.83	d	3.11
C-8	72.85	d	5.19
C-9	48.80	t	2.62/2.00
C-10	139.60	s	—
C-11	132.10	s	—
C-12	165.05	s	—
C-13	125.56	t	6.29/5.81
C-14	16.55	q	1.51
C-15	60.48	t	4.23/4.07
C-16	170.49	s	—
C-17	135.24	s	—
C-18	60.91	t	4.31
C-19	125.67	t	6.00/6.30

TABLE 3. NOe Difference Spectral Data of Onopordopicrin [1].

Saturation	Observed nOe
Me-14	H-6, H-8
H-2′	H-2, H-5
H-9	H-9′
H-3α	H-3β, H-5
H-2	Me-14, H-1
H-9α	Me-14, H-9β, H-1
H-5	H-1, H-6, H-7
H-1	H-5, H-6, Me-14
H-8	H-15, H-6, Me-14
H-13′	H-19, H-13, H-19′
H-19′	H-19, H-13′
H-19	H-19′, H-13

enhancement of H-7 and H-1 at δ 3.11 and δ 5.02 ppm, supporting the α position assignment of H-1, H-5, and H-7. NOe experiments also indicated proximity and β location of H-6 and H-8. Thus, saturation of the axial Me-14 resonance at δ 1.51 ppm resulted in enhancement of H-6 and H-8 at δ 5.14 ppm and δ 5.19 ppm, respectively. Similarly, saturation of H-8 at δ 5.19 ppm produced enhancement of the Me-14 and H-6 resonances at δ_1 1.51 ppm and δ_2 5.14 ppm, respectively. No enhancement was observed between H-6 and H-7, thus supporting a *trans*-fused lactone ring structure.

A strong nOe was observed between H-13 and H-13′ (at δ_1 6.29 ppm and δ_2 5.81 ppm) with H-19 and H-19′ at δ_1 6.30 ppm and δ_2 6.00 ppm, indicating close proximity of the two double bonds (C-11–C-13 and C-17–C-19). Thus, irradiation at H-13 resulted in enhancement of H-19 and H-19′. Similar enhancement was observed for H-19 and H-19′ upon irradiation of H-13′. The close proximity of the two methylene groups, CH_2-19 and CH_2-13, and of the two double bonds, C-11–C-13 and C-17–C-19, may be the result of π-π interactions. This interaction is reminiscent of aromatic ring stacking observed in biological systems (9, 10), and may be important for bioactivity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—1D and 2D ^{1}H-nmr spectra were recorded on a Varian XL-200 instrument at 200 MHz in $CDCl_3$, and ^{13}C-nmr spectra were recorded at 50 MHz in $CDCl_3$. The COSY spectrum was recorded using standard Varian software (Vs 6.2, Rev. B) with spectral widths of 1500 Hz in both frequency dimensions. Sixteen transients were recorded for each of 256 t_1 increments, with a recycle delay time of 3 sec per transient for a total experiment time of 4 h. One order of zero-filling was applied in t_1 prior to Fourier transformation to produce a digital resolution of 0.015 ppm. Chemical shifts are reported in δ units (ppm) relative to TMS as internal standard. Ir spectra were recorded with a Perkin-Elmer 457 instrument, while ms were obtained with a gc-ms Finnigan 4021 EI-Cl-INCOS system at 70 eV. Purity of onopordopicrin was checked also by gc analysis performed on a Varian 6000 instrument equipped with a flame ionization detector.

PLANT MATERIAL.—The plant material was collected in Kalogria beach, near Patras, Greece, in March 1988, and identified by Professor Th. Georgiadis, Department of Plant Biology, Division of Plant Taxonomy, University of Patras. A voucher specimen, No. 3710, is deposited at the Herbarium of the University of Patras.

ISOLATION OF THE LACTONE.—Dried and ground aerial plant material (500 g) of *C. sonchifolia* was extracted with three 2-liter portions of MeOH. The MeOH solution was evaporated and concentrated to 100 ml H_2O (1000 ml) was then added, followed by addition of lead acetate (10 g) dissolved in 100 ml of H_2O. The mixture stayed at room temperature for 30 min and then was filtered. The filtrate was extracted with $CHCl_3$ (1000 ml), and the $CHCl_3$ solution was dried over $MgSO_4$ and evaporated to give the lactone fraction as a crude syrup (10 g).

A portion (500 mg) of the lactone fraction was chromatographed on three preparative tlc plates (20 × 20 cm, 2 mm) to afford the major lactone in oily form (177 mg). The lactone was extracted from the preparative Si gel plate with 200 ml of $CHCl_3$-MeOH (9:1) and was found to be homogeneous on tlc systems of $CHCl_3$-MeOH (9:1) and $CHCl_3$-Me_2CO (3:2).

The oily lactone was subjected to gc-ms analysis, which confirmed its purity. It was, furthermore, subjected to 2D nmr and distortionless enhancement by polarization transfer (DEPT) experiments (Table 1–3). The lactone was crystallized from $CHCl_3/Et_2O$ and identified by comparison with authentic onopordopicrin: mp 55–58°; $[\alpha]^{25}D + 16.2°$ ($c = 0.5$, MeOH). The eims, ir, and ^{1}H- and ^{13}C-nmr spectra were also identical with those of the reference: ms *m/z* (%) 85 (100), 91 (34), 119 (52), 147 (22), 215 (8), 228 (5), 246 (3); ir (KBr) 3400 (hydroxyl), 2920 (methylene), 1750 (γ lactone), 1700 cm^{-1} (ester carbonyl unsaturated).

BIOLOGICAL ACTIVITIES.—In vitro cytotoxic activity of onopordopicrin, using the human epidermoid carcinoma cell line KB (ATCC CCL 17), was determined by methods previously described (11,12). The ED_{50} value was 0.85 μg/ml. Cytotoxicity and antimicrobial activity of α-methylene-γ-lactone-bearing sesquiterpene lactones has been earlier reported (13–15).

Antibacterial activity experiments were conducted applying the disk diffusion assay (16). Onopordopicrin was found to possess antibacterial activity against *St. aureus* (ATCC 25923) but not against other Gram-positive and Gram-negative bacteria that were tested.

No antifungal activity at 250 μg/disk was observed against the test organisms, *Candida albicans* (strain A-26, Eli Lilly), *Trichophyton mentagrophytes* (strain A-23, Eli Lilly), and *Saccharomyces cerevisiae* (strain M25, generously provided by Dr. Bruce Adams, Department of Microbiology, University of Hawaii).

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