CENTAUREPENSIN: A CYTOTOXIC CONSTITUENT OF *CENTAUREA SOLSTITIALIS* AND *C. REPENS* (ASTERACEAE)[1]

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Centaurea solstitialis L., the "yellow star thistle" (Asteraceae), has been shown by previous investigators to contain alkaloids (1), cyanogenic glycosides (2), sesquiterpene lactones (3–5), and a taraxane-type triterpene (6) showing activity against P-388 lymphoid leukemia in mice. Previous researchers have reported (7–10) the presence in *C. repens* L.[3], "Russian knapweed", of a hydrocarbon, a hydrocarbon ester, a triterpene, and sesquiterpene lactones of the guaianolide type. Our observation of 9KB (human nasopharynx carcinoma) *in vitro* cytotoxic activity in the alcoholic extracts of each of these plants prompted the isolation and characterization of the active component.

After the active ethanolic extract of *C. repens* was partitioned between chloroform-water and the chloroform extract was partitioned between 90% aqueous methanol and petroleum ether, the methanol solubles were purified by lead acetate precipitation. The supernatant (alcohol-soluble) fraction, when concentrated, dissolved in chloroform, and chromatographed on silica gel, yielded pure Substance A which possessed cytotoxic activity (ED_{50} 1.7 μg/ml) (11). Proton magnetic resonance spectra indicated that Substance A was an α-methylene-sesquiterpene lactone, and the large M+2 peak in the ms (M:M+2 of 1:0.75) suggested the unusual presence of chlorine.

Simultaneously, the active ethanolic extract of *C. solstitialis*, after purification by treatment with lead acetate, was partitioned between chloroform and water. Cytotoxic activity was found in the chloroform fraction; further partitioning between petroleum ether and methanol resulted in concentration of the activity in the methanol fraction. Chromatography through Sephadex LH-20 and then through silica gel afforded a pure cytotoxic compound (ED_{50} 1.2 μg/ml) which was recrystallized until it had a constant melting point. This compound proved to be identical to substance A by elemental analysis, ir, and pmr.

Elemental analysis of Substance A indicated a molecular formula of $C_{21}H_{24}Cl_2O_7$, which corresponds to that of the known compound centaurepensin (chlorophyssopifolin A[1]) previously isolated from *C. hyssopifolia* (12), *C. repens* (10), *C. solstitialis* (5), and other *Centaurea* species (see 5).

Comparison of the pmr spectrum of substance A with the expected and

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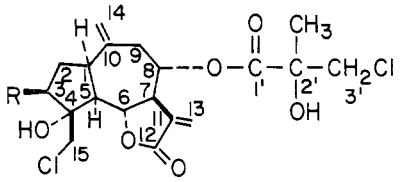
[3]Synonyms for *C. repens* L. include *C. picris* Pallas ex Willd., *Acroptilon repens* (L.) DC., and *A. picris* (Pallas ex Willd.) C. A. May.

actual spectra of centaurepensin showed close agreement. An analysis of the cmr spectra (table 1) of Substance A [1] and the synthesized 3-oxo derivative [2] confirmed the structure. The carbon signals were assigned with the help of reported cmr spectra of similar compounds and other sesquiterpene lactones (13–15). The upfield shifts observed in the spectrum of the ketone [2] relative to the alcohol [1] for C–4 (83.747 to 79.083), C–5 (58.001 to 54.421), C–6 (76.540 to 74.685), and C–15 (49.676 to 46.017) allowed these signals to be differentiated and assigned. The presence of a triplet at 50.938 corresponding to C–3' indicated a chloromethylene group in the ester side chain. Also the upfield shift seen for C–10 (143.762 to 141.514) and for C–6 (as previously noted), positions γ to the carbonyl, verified the assignment of the secondary hydroxyl to C–3 and not C–2. These observations are consistent with the revised structure of centaurepensin [1] (16). This unusual chlorosesquiterpenoid may now be added to the growing list of cytotoxic α-methylene lactones isolated from natural sources (17, 18).

TABLE 1. CMR spectral data (chemical shifts in ppm).

Assignment	Alcohol (1)	Ketone (2)
C–1'	172.2	172.3
C–12	168.5	168.6
C–10	143.8	141.5
C–11	137.9	137.0
C–13	120.9	122.4
C–14	116.7	118.7
C–4	83.7	79.1
C–6	76.5	74.7
C–3	75.1	211.1
C–8	74.4	74.7
C–2'	74.2	74.4
C–5	58.0	54.4
C–3'	50.9	51.0
C–15	49.7	46.0
C–7	47.2	46.7
C–1	45.6	39.8
C–9	39.0	38.8
C–2	34.1	36.9
C–4'	23.6	23.8



(1) R = H, OH (centaurepensin)

(2) R = O (3-oxo-centaurepensin)

EXPERIMENTAL[4]

PLANT MATERIAL OF *C. repens*.—The plant material which was collected in May 1974 in Garmsar, 80 km east of Tehran, was identified by Dr. A. Ghahreman, Faculty of Science, University of Tehran. A voucher specimen (No. APC97) is in the Dept. of Pharmacognosy, School of Pharmacy, University of Tehran. The air-dried roots, stems, leaves and flowers were milled to a coarse powder.

EXTRACTION OF *C. repens*.—Extraction of one kg of the ground plant material in a Soxhlet apparatus with 8 liters of 95% ethanol yielded a tar (9.5%). After the tar was partitioned between chloroform and water, the resulting chloroform extract (5.3%) was partioned between 90% aqueous methanol and petroleum ether (30–60°). The ethanol solubles, obtained from treatment of the 90% methanol extract (1.8%) with 2.5% lead acetate in 50% aqueous ethanol, were concentrated and extracted with chloroform. Column chromatography of the chloroform extract (0.53%) on silica gel G with chloroform followed by chloroform-methanol gradient as eluting solvents yielded Substance A (0.11%): mp 217–218.5° [lit. mp 217–219 (19)]; found: C, 52.79; H, 5.75; Cl 16.24; O, 25.62; calc. for $C_{19}H_{24}Cl_2O_7$: C, 52.39; H, 5.56; Cl, 16.31; O, 25.74; uv λ max (MeOH) nm: 210 (log ϵ=4.7); ir ν max (KBr) cm^{-1}: 3510, 3440, 2940, 1735, 1655, 1630; pmr (100 MHz, $(CD_3)_2CO$) δ: 4.12 (dd, J=10, 2, C-3H), 5.01 (t, J=9, C-6H), 5.29 (ddd, J=10, 5, 1, C-8H), 6.05 and 5.67 (d, J=3, C-13H_2), 5.13 and 4.96 (d, J=2, C-14H_2), 4.27 and 3.84 (d, J=11.5, C-15H_2), 3.96 and 3.76 (d, J=11, C-3'H_2),

[4]All mps are uncorr. The nmr spectra were recorded on either a Jeol 100 MHz, Varian 60 MHz, or Varian 80 MHz spectrometer with TMS as an internal standard. The ir spectra were measured in KBr on a Beckman-33 unit. The low resolution ms were measured on a Dupont spectrometer via chemical ionization (cims) with isobutane. The 9KB cytotoxicity assays were performed at the Cell Culture Laboratory, Purdue Cancer Center.

1.56 (s, C-2'Me) (essentially identical to that in reference centaurepensin, *1*); cmr (100 MHz, DMSO-d_6): see table 1; cims *m/e*: 435 (MH^+), 385, 367, 279 ($M-C_4H_8ClO_3-H_2O$, 100%), 261.

PLANT MATERIAL OF *C. solstitialis*.—Air-dried whole plant material (roots, stems, leaves, flowers, and fruits) collected in 1976 in California was supplied by the Medicinal Plant Resources Laboratory, USDA, Beltsville, Maryland, through which voucher specimens are preserved (B-612580, PR-46467). The material was pulverized in a Fitzpatrick mill.

EXTRACTION OF *C. solstitialis*.—Extraction of the powdered plant material with 95% ethanol (10 liters/kg) via percolation gave a green tar (9.4%). After lead acetate precipitation (19), the ethanol extracts yielded a brown solid (0.64%). After chloroform-water partitioning, the chloroform-soluble solids (0.39%) were then partitioned between pertroleum ether and 90% aqueous methanol to yield 0.29% of methanol extract. Column chromatography through Sephadex LH-20 with chloroform followed by trituration with ethyl ether yielded a white solid (0.025%) which was further purified by column chromatography through silica gel eluted with 3% methanol in chloroform. Crystallization from ethyl acetate/hexane afforded Substance A (0.005%): found: C, 53.57; H, 5.65; Cl, 16.30, O, 24.68; cims MH^-=435; ir ν max (KBr) cm^{-1}: 3510, 3440, 2940, 1735, 1655, 1630; pmr identical to that for authentic centaurepensin (**1**).

SARRET OXIDATION OF SUBSTANCE A (CENTAUREPENSIN).—Substance A (67 mg) was combined with dry methylene chloride (4 ml) and treated with 2 x 5 ml Sarrett's reagent (19), and the mixture was stirred for 30 min at room temp. The reaction mixture was diluted with water, acidified, and extracted with chloroform. The chloroform extract was washed, dried, and concentrated. Column chromatography of the residue on silica gel and elution with ethyl acetate-benzene (1:3) gave solid *2* (30%): cims *m/e*: 433 (MH^-), 415, 397, 383, 379, 361, 295, 277 ($M-C_4H_8ClO_3-H_2O$, 100%), 259; ir ν max (KBr) cm^{-1}: 3450, 1765, 1740, 1725, 1660, 1645; pmr (60 MHz, $(CD_3)_2CO$) δ: 4.67 (dd, J=10, 8, C-6), 5.20 (dd, J=10, 6, C-8), 6.20 and 5.95 (d, J=3, C-13H_2), 5.40 and 5.30 (bs, C-14H_2), 3.97 (s, C-15H_2), 3.97 and 3.86 (s, C-3'H_2) 1.56 (s, C-2'Me); cmr (100 JHz, DMSO-d^1): see table 1.

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