

ISOLATION AND IDENTIFICATION OF AN INSECT REPELLENT COMPOUND FROM *SAUSSUREA LAPPA* C.B. CLARKE

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The insect repellent activity of the rhizomes of *Saussurea lappa* C.B. Clarke was found to be due to dehydrocostus lactone.

INTRODUCTION

There are a number of plant materials which have been used in the past for keeping insects away from human beings, food materials and woollens[1]. Although these plant materials have been in use for years, their properties have not been documented. Malik & Naqvi[2] evaluated some indigenous plants of Pakistan and reported that *Saussurea lappa* C.B. Clarke exhibited repellent activity against red flour beetle, *Tribolium castaneum* Hbst. The essential oil of *S. lappa* is used in perfumery and in medicine as tonic, carminative stimulant and in controlling bronchial asthma[3]. The petroleum ether extract of the roots is reported to be nontoxic against house flies[4]. The present studies describe the isolation and identification of insect-repellent active principle from rhizomes of *S. lappa*.

Experimental Procedures

Insect Culture. The culture of red flour beetle, *Tribolium castaneum* Hbst. was maintained on whole wheat flour with 5% yeast at a controlled temperature of $29 \pm 1^\circ\text{C}$ and humidity 55% R.H.

Plant Material and Extraction. The dried rhizomes of *S. lappa* were supplied by the Forest Department of Azad Kashmir. The rhizomes were grounded and extracted in a 1:4 mixture of petroleum ether (B.P. $60-80^\circ\text{C}$) and acetone in Soxhlet extractor, the solvent evaporated under reduced pressure and crude extract dried over anhydrous sodium sulphate and tested for insect repellence, at 1% concentration in acetone. Chromatographic procedures were used for isolation of the active principle from the crude extract.

Biotests. The repellency of crude extract and chromatographic fractions was evaluated against *T. castaneum* by the method of repellency or attractancy described by McDonald, *et al.* [5] and Malik & Naqvi [2].

Isolation of Repellent Compound. The crude extract of *Saussurea* was chromatographed on preparative layer of silica gel GF₂₅₄ in hexane-ethyl acetate (9:1) developed twice upto 15 cm each time. The plates were viewed under

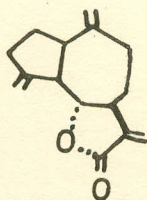
U.V. light and part of the plate sprayed with warm 50% H₂SO₄. Four areas of the plate were marked as (i) Fraction 1: from 0-1 cm, brownish colour. (ii), Fraction 2: from 1-4.5 cm, fluorescent under U.V. light (254 nm), no colour with H₂SO₄. (iii) Fraction 3: reddish brown with H₂SO₄ and (iv) Fraction 4: from 5.5 cm to the solvent front, brownish with H₂SO₄. Unsprayed areas of the plate corresponding to the spots were eluted with acetone and tested for repellent activity. It was observed that maximum activity was exhibited by fraction 3, which was chromatographed on a silica gel column (Silica gel 60, 70-230 mesh ASTM) eluted successively with hexane, and increasing polarities of hexane:ethyl acetate and ethyl acetate alone. The active fraction was eluted with hexane: ethyl acetate (95:5), which was further purified on PLC in subsequent development in hexane-ethyl acetate 9:1 and in benzene-ethyl acetate (95:5). The pure active compound gave class V activity and single spot on Kiesel gel 60F₂₅₄ (E. Merck) plates having R_F value of 0.25 in hexane-diethylether (2:1), 0.57 in benzene-methanol (95:5) and 0.44 in benzene-diethyl ether (4:1).

For larger amounts, the rhizomes were extracted in acetone only. After evaporation of acetone under reduced pressure, crude extract was partitioned between hexane and 95% methanol. To methanol layer, 20% water and equal amount of CCl₄ were added; the CCl₄ layer contained repellent compound. After evaporation of CCl₄, to the residue was added a small amount of petroleum ether and kept in a freezer. After about 3-4 weeks white crystals appeared which were purified by crystallization in hexane.

Identification of Repellent Compound

The U.V. spectrum (Beckman sp25) of the repellent compound showed max λ (hex) 220 m μ . An IR spectrum in CCl₄ (Beckman 4210) showed absorptions at 3080 (m, exocyclic methylene group), 2960(s) 2865(m), 2930(s), 1775(S, 5 membered lactone), 1638 (w, vinyl diene) 1450 (w) and medium peaks at 1302, 1255, 1215, 1166, 1142, 1120, 938 and 696, cm⁻¹. The absence of a peak at 1380 in-

licated absence of methyl group. The mass spectrum showed a molecular ion M^+ at m/e 230 and other abundant peaks at m/e 91 (base peak), 53, 79, 117, 132 and 65. The molecular formula assigned was thus $C_{15}H_{18}O_2$. These spectral characters were compared with the published data[6-9] on compounds isolated from *S. lappa*, and repellent compound was identified a sesquiterpene lactone as dehydrocostus lectone.



The melting point after crystallization in hot methanol was $59.5^{\circ}C$ (reported 61-61.5[7] 60.5[8]).

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