PSILOSTACHYIN, A CYTOTOXIC CONSTITUENT OF *AMBROSIA ARTEMISIIFOLIA* L.*†

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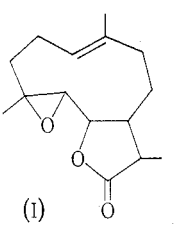
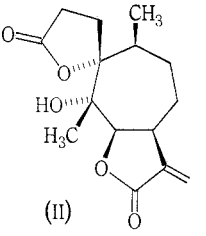
The screening of Australian plant species for tumour-inhibitory constituents has revealed weak but reproducible activity of extracts of *Ambrosia artemisiifolia* L. against cell cultures of human carcinoma of the nasopharynx (KB). Fractionation of the extracts has shown that the cytotoxic activity is associated with an ether-soluble fraction containing sesquiterpene lactones. Two of these lactones have been identified as dihydroparthenolide (I) and psilostachyin (II). Psilostachyin, recently



(I)



(II)

reported as a constituent of *Ambrosia psilostachya*,[1] is weakly active against KB and accounts for most of the activity of the plant extracts. Dihydroparthenolide has been reported previously as a reduction product of parthenolide, an isolate from *Chrysanthemum parthenium*[2] and *Michelia champaca*,[3] and is inactive against KB. Herz and Högenauer[4] have reported the occurrence of a related lactone, coronopilin, in *Ambrosia artemisiifolia*.

The cytotoxic activity of psilostachyin appears to be due to the α-methylene-γ-lactone grouping since other sesquiterpenes containing this grouping are known to be active against KB; e.g. gaillardin,[5] elephantin and elephantopin,[6] and euparotin

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acetate.[7] The activity of α,β-unsaturated lactones (cf. also steroid lactones,[8] calotropin,[9] and withaferin A[10]) may be associated with the possibility of addition of nucleophilic groupings to the polarized double bond.

Experimental

Initial Fractionation

Plants were collected at Beaudesert, Qld., in March 1966.

The initial fractionation of an ethanolic extract of dried whole plant (1·5 kg) was carried out according to the scheme in Figure 1. After removal of the ethanol, the residue was taken

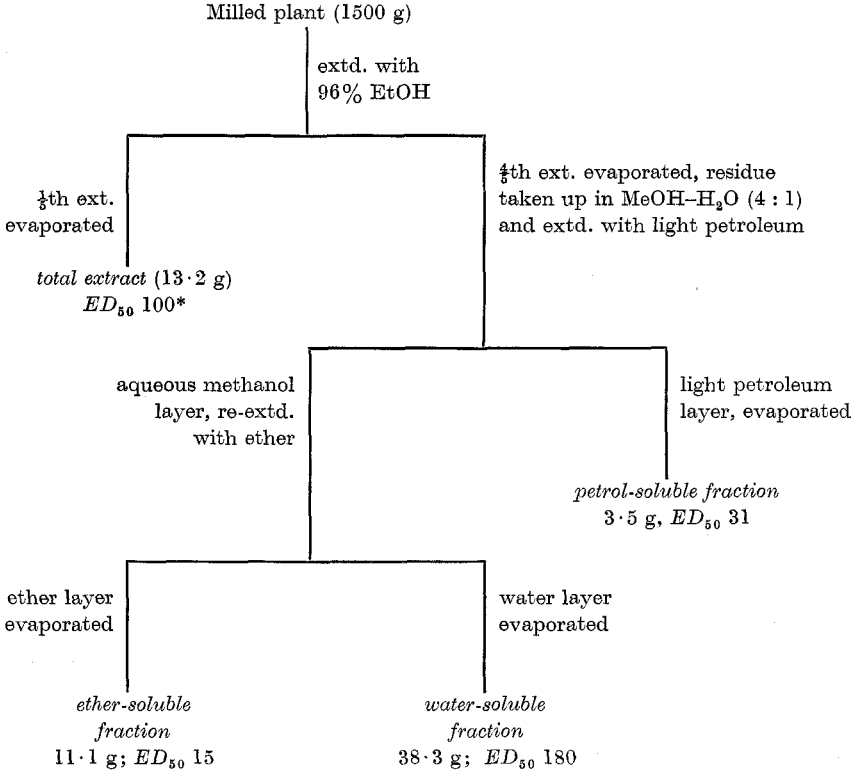

Fig. 1.—Preliminary fractionation of *Ambrosia artemisiifolia*.

up in methanol–water (4 : 1; 1500 ml) and extracted with light petroleum (b.p. 30–40°, 3 lots of 750 ml). The methanol–water layer was then re-extracted with ether (3 lots of 750 ml), concentrated until methanol was removed, and the residue finally freeze-dried. The weights and

* Previous total extracts showed ED_{50} values of 26, 11, 30, and 17.

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anti-KB activities (expressed as ED_{50}, the number of micrograms of material dissolved in 1 ml which inhibits growth of the cell culture to the extent of 50%) of the fractions (Fig. 1), show that the activity is mainly in the ether-soluble fraction.

The ether extract was chromatographed on silica gel columns (contained in Cellophane dialysis tubing) or thick layers with ether–chloroform (1 : 1) as developing solvent. Analysis of fractions was best accomplished by t.l.c., with layers of "Merck G" silica gel, development with ether–chloroform (1 : 4), and detection of spots by spraying with 50% H_2SO_4 and heating for a few minutes at 120°. Spot colours ranged through red, green, and black. A typical run, which indicated that several constituents of weak activity were present, was performed on a 60 by 1·3 cm column; development was continued until the fastest running solute approached the end of the column, and the column was then cut into six sections and eluted with acetone. The last three fractions were combined, rechromatographed on a thick layer, and again divided into three fractions. The weight and activities of the 6 fractions, in order from the top of the column were 80 mg, ED_{50} 28; 60 mg, ED_{50} 13; 125 mg, ED_{50} 6; 50 mg, ED_{50} 31; 50 mg, ED_{50} 50; 42 mg, $ED_{50} > 100$.

Isolation of Psilostachyin

Another portion of ether extract (5 g) was chromatographed similarly on a column of silica gel and two sections, 2 and 3, corresponding approximately to the preceding second and third sections, were separately eluted and rechromatographed on a thick layer. Section 2 was divided into three fractions on the basis of t.l.c.: 49 mg, ED_{50} 32; 39 mg, ED_{50} 28; and 52 mg, ED_{50} 5·4. Of these, the last was essentially one pure constituent which formed a dark red spot on thin-layer plates when sprayed with H_2SO_4 as described above. It was also present in the first two fractions. From methylene chloride–ether, it formed crystals, m.p. 211–215°. A high-resolution mass spectrum showed a molecular ion at *m/e* 280·131, thus establishing the empirical formula as $C_{15}H_{20}O_5$. The melting point and spectral properties were in agreement with those recently reported by Mabry *et al.*[1] for psilostachyin, m.p. 215°. There was no depression of melting point on admixture with an authentic sample kindly provided by Dr Mabry. Section 3 of the main column, eluted and rechromatographed by thick layer, gave five fractions as follows: 60 mg, $ED_{50}>100$; 30 mg, ED_{50} 24; 114 mg, ED_{50} 20; 65 mg, ED_{50} 19; 22 mg, ED_{50} 22. As judged by t.l.c., each of the four active fractions contained some psilostachyin.

Isolation of Dihydroparthenolide

Sections 5 and 6 of the main column gave dark green material (1·5 g) containing another constituent which gave a dark red spot with H_2SO_4. Isolated by the thick-layer technique, it crystallized from ether in long prisms, m.p. 137–138° (Found: C, 72·3; H, 8·8. Calc. for $C_{15}H_{22}O_3$: C, 72·0; H, 8·8). A mass spectrum confirmed the molecular weight of 250. Infrared and n.m.r. spectra coincided with those reported for dihydroparthenolide which also has m.p. 137°.[3]

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