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ESTRAGOLE: AN ACUTE TOXIC PRINCIPLE FROM THE VOLATILE OIL OF THE LEAVES OF *CLAUSENA ANISATA*

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The toxicity of the volatile oil of the leaves of *Clausena anisata* Hook. f. (Rutaceae) to variegated grasshopper *Zonocerus variegatus* L. is reported. The plant species has a strong, aromatic odor compared by some to aniseed and by others to garlic (1). Its uses in some parts of Africa and the Phillipines had earlier been reviewed (1). One of the many uses is the burning of the dried plant to repel mosquitoes. Novak also demonstrated that the volatiles from the leaves of the plant had repellent activities against a tick, *Ixodes ricimus* (2).

Steam distillation of the fresh leaves yielded a strong, sweet-smelling, brownish-yellow oil. Acute toxicity tests on the oil showed that it was toxic to the third nymphal instar of the grasshopper. Chromatography led to the isolation of estragole (the major component of the oil) which was 1½ times more toxic than the crude oil. This represents the first report of the biological activity of this compound. Furthermore, its isolation as the major compound from the volatile oil of the leaves of this plant contradicts the earlier report of anethole as the major constituent (1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ir and uv spectra were obtained on Pye Unicam Sp3-300 and Sp8-400 spectrometers, respectively. The ^{1}H-nmr spectra were obtained on a Varian FT 80 A spectrometer (80 MHz) and the ^{13}C-nmr spectra were recorded on a Varian FT 80 A spectrometer (20 MHz). $CDCl_3$ was used as solvent with TMS as internal standard.

EXTRACTION AND ISOLATION OF ESTRAGOLE.—Collections were made from plants authenticated as *C. anisata* by Jaiyeola, Department of Botany, University of Ife, Unife, Nigeria. A voucher specimen is deposited in the Herbarium of the department.

Fresh leaves of *C. anisata* (0.2 kg collected from Unife environ in June 1986) were steam distilled for 2 h. The volatile oil (0.54 g; 1 ml) was separated from the condensed steam. The crude oil, which showed significant toxicity (LD_{50} 3780 mg/kg), was further purified by preparative tlc on Si gel $PF_{254+366}$ (hexane-EtOAc, 10:1). The fastest-moving component (Rf 0.78) was collected and extracted from the silica with $CHCl_3$. Concentration of the $CHCl_3$ extract left a colorless oil (70% of the total oil) that was identified as estragole [1-methoxy-4-(2′-propenyl) benzene] by comparison of its spectral data (uv, ir, ^{1}H nmr) with published data (3,4) and the ^{13}C-nmr data: 158.1 C-1, 137.9 C-2′, 132.1 C-4, 129.5 C-3 and 5, 115.4 C-2 and 6, 113.9 C-3′, 55.2−OCH_3, 39.4 C-1′.

BIOLOGICAL ASSAYS.—With the aid of a Hamilton 5-μl microsyringe, graded amounts (0.5-2.5 μl) of oil of *C. anisata* were applied topically to the ventral region of third nymphal instars of the variegated grasshopper *Z. variegatus*. The insects were collected from the field at first nymphal instar and were raised in the laboratory on the leaves of Cassava, *Manihot esculenta* (5). Inasmuch as each nymphal stadium of the grasshopper lasted 2-3 weeks, the insects were, thus, well-conditioned in the laboratory before the biological screening. Control insects were untreated. There were 10 insects for each concentration, and each treatment was replicated 3 times. Insects for each treatment were placed inside half-liter kilner jars with screen covers (6). Mortality data were corrected by Abbott's formula (7). The probit mortality against log concentration regression line was computed following methods employed by W.H.O. for malaria vector control (8).

After 24 h, LD_{50} for the crude oil was 3780 mg/kg while that of estragole was 2430 mg/kg. Toxic symptoms observed included restlessness among treated insects, tucking of heads ventrally by extending the dorsal cervix, and the antennae being continuously cleaned by the forelegs. Mortality occurred without kicking or jerking of legs.

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VOLATILES FROM THE ROOT OF *DIPLOTAENIA CACHRYDIFOLIA*, THE FIRST NATURAL SOURCE OF 6-CAMPHENONE

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A phytochemical survey of *Diplotaenia cachrydifolia* Boiss. (Umbelliferae) showed that the plant contained a volatile fraction in the root in addition to the essential oil of the fruit. The plant has also been found to contain the furanocoumarins xanthotoxin, bergapten, and isopimpinellin in the fruit and root (1), the dihydropyranocoumarins jatamansin (leaf, fruit, and root) and jatamansinol (root) (2), and the dihydrofuranocoumarins columbianetin and columbianadin (root) (3).

The plant grows at elevations of up to 2,600 m in the Kandavan Chalus Valley 100 km north of Tehran and also in other regions of Iran and in Turkey (4-7).

Only two species of *Diplotaenia* have been recorded to date. *Diplotaenia damavendica*, which occurs at elevations of up to 3,000 m around Lake Tar 120 km north of Tehran, is reported to contain xanthotoxin and angelicin, the latter causing photosensitization of the skin (8).

This is the first reported isolation and analysis of volatiles from the root of this species. 6-Camphenone (3,3-dimethyl-2-methylenebicyclo[2.2.1]-heptan-6-one) [**1**], the major constituent (43.0%), is the ketone of nojigiku alcohol (6-camphenol) and was first synthesized in 1974 (9). The oil also contained the biosynthetically related compounds 6-camphenol (nojigiku alcohol, 1.2%) and nojigiku acetate. These constituents have not previously been found in the volatile oil derived from the fruits of this species. Individual identification of the peaks is listed in Table 1.