relative to that of the conventionally edible legumes. Potassium levels are generally high with corresponding low sodium content. The results with sodium were in accord with the contentions of Chamberlain (1955) that tropical crops carry subnormal concentrations of sodium, which is a reflection of the low sodium content of the soils. The species also appeared to be good sources of iron.

The results presented are mostly for raw seeds. As legumes, it should be expected that cooking or other forms of heat processing could affect their elemental composition if they contain unstable, highly soluble, or volatile compounds of the different elements. It should also be borne in mind that the potential of any feedstuff as a source of the major elements depends on the availability (as mentioned earlier) rather than the total content. There is evidence that certain plant constituents particularly decrease the availability of certain elements: zinc (O'Dell, 1969); iron (Sharpe et al., 1950), magnesium (Roberts and Yudkin, 1960), and calcium (Harrison and Mellanby, 1939). Legumes are particularly rich sources of phytates, which could reduce significantly the overall availability of the minerals found in them.

Futher investigations are needed in this direction to establish their real potentials as sources of divalent minerals.

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Registry No. Ca, 7440-70-2; Mg, 7439-95-4; Na, 7440-23-5; K, 7440-09-7; P, 7723-14-0; Zn, 7440-66-6; Cu, 7440-50-8; Fe, 7439-89-6; Mn, 7439-96-5.

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A Desert Plant from Egypt, *Anabasis setifera*: An Efficient Natural Factory of Carvacrol and Thymol

Mahmoud Abbas Saleh

The volatile oil from *Anabasis setifera* was found to consist of a highly pure mixture of two isomeric compounds, carvacrol (85%) and thymol (15%). No other compounds were detected in the oil by high-resolution gas chromatography–mass spectrometry. The selectivity and efficiency of the plant (volatile oil 2.5% of the fresh weight) in producing such isomers are remarkable.

In the course of our investigation of the chemistry of desert plants from Egypt, we encountered a fragrant undershrub growing widely in large colonies in the plains of the littoral zones of the western deserts, the Red Sea coastal region, and the Sinai. The plant is not attacked by insects or fungi and is not eaten by grazing animals. It was identified to be *Anabasis setifera* of the family Chenopodiaceae.

The volatile oil was obtained by steam distillation of the fresh aerial parts of the plant in 2.5% yield as described in the Experimental Section. The steam distillate had insecticidal activities toward cotton leaf worm (*Spodoptera littoralis*), housefly (*Musca domestica*), and rice weevil (*Stiophilus oryzae*). Analysis of the steam distillate by

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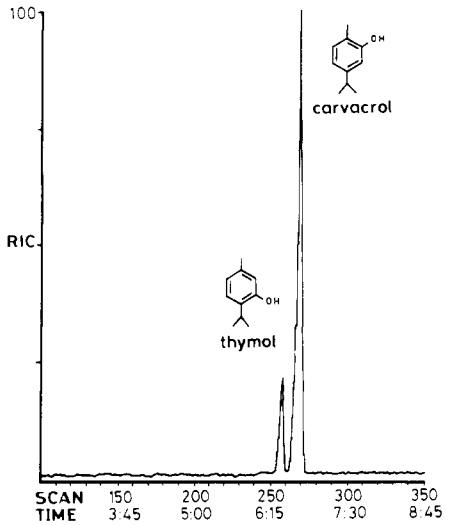

Figure 1. Reconstructed ion chromatogram of the steam distillate from *A. setifera*.

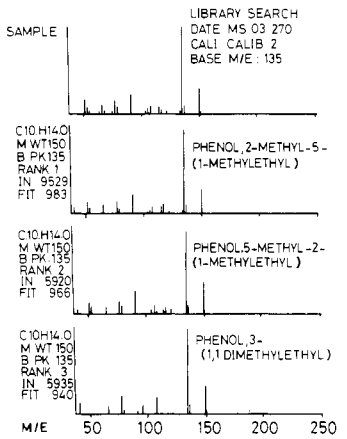

Figure 2. Mass spectra library search for the major compound.

fused silica capillary column gas chromatography–mass spectrometry (GC/MS) revealed only two compounds in the ratio of 85% to 15% as shown in Figure 1. Mass spectra of the two compounds were identical, indicating that they are closely related isomers. Comparison of the MS with the EPA/NIH/MSDC library and comparison of the GC retention times established that the major compound is 2-methyl-5-isopropylphenol (carvacrol) and the minor compound is 5-methyl-2-isopropylphenol (thymol) (Figure 2).

Carvacrol was separated from thymol by cooling and centrifuging the oil whereupon thymol separates in a solid form. NMR and IR spectra of the two purified isomers also confirmed their structures as carvacrol and thymol. Carvacrol is found in several volatile oils derived from various species of the labiatae and laminaceae families but usually in mixtures containing other volatile components such as cymene, pinene, terpinene, etc. (Broucke and Lemli, 1980; Delitala et al., 1981; Dev et al., 1982). Carvacrol and thymol are used as antiseptics and germicides in medicinal and oral preparations, in disinfectants, and in room sprays. They are also used in a local anesthetic for toothache, as an anthelemintic (Mehta, 1979; Viana et al., 1981), in scenting of soaps, in the preparations of artificial essential oils, and in chemical preparations (Broucke and Lemli, 1982).

The ability of the plant to produce high amounts of volatile oil (2.5% of its fresh weight), coupled with its high selectivity in producing carvacrol and thymol and the fact that the plant grows widely in heavy colonies in the Egyptian deserts without any agricultural practice, points to its potential as a valuable source of carvacrol and thymol from lands otherwise characterized by their marginal productivity. Furthermore, the high selectivity and efficiency of *A. setifera* in producing carvacrol and thymol may provide a model system for studying the biosynthesis of such aromatic compounds, determining how the plant synthesizes such high amounts of these secondary metabolites.

EXPERIMENTAL SECTION

Plants were collected during spring 1984 from the littoral zone of the western desert. The fresh aerial parts of the plant were cleaned and subjected to steam distillation (Saleh, 1984; Saleh, 1985). The aqueous steam distillate was extracted with ether in the usual manner and concentrated to give a light greenish, strong-smelling viscous oil in 2.5% yield. Bioassay for insecticidal activity was carried out on the steam distillate as follows. Susceptible strains of houseflies (*M. domestica* L.), rice weevils (*S. oryzae*), and fourth instar cotton leaf worm (*S. littoralis*) reared away from any insecticide contamination for several years were used. The houseflies were treated topically on the dorsum of the abdomen with an acetone solution of the test samples using 1 μL of acetone containing the crude steam distillate (Saleh and Casida, 1977). The *Spodoptera* larvae were treated topically on the dorsal surface of the thorax with 1 μL of acetone containing the steam distillate (Saleh et al., 1984). Each group of treated flies and larvae were placed in Petri dishes, held for 24 h at room temperature, and then mortality percentage and LD_{50} values were determined. Insecticidal activity to rice weevils (*S. oryzae*) was determined by film applications wherein a solution of the steam distillate in acetone was deposited on the bottoms of Petri dishes (Saleh, 1984). The solvent was allowed to evaporate. Then 25 rice weevils were placed in each Petri dish, covered, and kept at room temperature for 24 and 36 h before mortality and LD_{50} values were determined. Insecticidal activities were, LD_{50} = 0.25 mg/g to the housefly, 6.4 mg/g to the cotton worm, and 8.4 $\mu g/cm^2$ to the rice weevil.

Gas Chromatography–Mass Spectrometry (GC–MS). A Finnigan 4530 GC–MS data system equipped with a 30-m (0.25-mm i.d.) DBl fused silica capillary column was used for the analysis of the steam distillate. Helium was used as the carrier gas (40 cm/s). Chromatographic conditions were as follows: injection port and interface temperature, 250 °C; column temperature, 100 °C for 1 min and programmed to 250 °C at a rate of 5 °C/min. All spectra were recorded in the El mode at 70 eV with 1 scan/s. Infrared spectra were recorded using a Py-Unicam SP 1000 spectrophotometer as neat films. NMR spectra were recorded on a Varian EM-390 90 MHz NMR spectrometer in $CDCl_3$. IR and NMR spectra of the two compounds were identical with spectra for authentic carvacrol and thymol.

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Metabolism of the Persistent Plasticizer Chemical Bis(2-ethylhexyl) Phthalate in Cell Suspension Cultures of Wheat (*Triticum aestivum* L.). Discrepancy from the Intact Plant

Hans-Willi Krell[1] and Heinrich Sandermann, Jr.*[2]

Cell suspension cultures of wheat (*Triticum aestivum* L.) metabolized the persistent plasticizer chemical bis(2-ethylhexyl) phthalate (DEHP; 1 ppm) predominantly to β-D-glucosyl conjugates. After incubation for 48 h at 27 °C, 23% of the applied radioactively labeled chemical was recovered in the total polar metabolite fraction. Prior heat treatment or freeze-thawing of the wheat cells abolished conjugate formation and led to mono(2-ethylhexyl) phthalate (MEHP) as the predominant metabolite (up to 10% conversion). Direct feeding of MEHP to native wheat cells led to 93% conversion to polar metabolites, again consisting largely of β-D-glucosyl conjugates. This suggested that MEHP was a metabolic intermediate and that DEHP esterase activity was rate limiting in DEHP metabolism. The rate of cellular DEHP metabolism in fact agreed with the rate of the DEHP esterase reaction determined in crude cell-free extracts. Therefore, no significant permeation barrier between the intracellular enzyme and external DEHP appeared to exist in cell suspension cultures. In contrast, the DEHP esterase activity of intact leaves has previously been found to be inaccessible to external DEHP.

INTRODUCTION

The use of plant cell cultures offers a rapid, reproducible, and inexpensive way to study the metabolism of environmental chemicals without interference by abiotic or microbial degradation reactions (Mumma and Hamilton, 1979; Mumma and Davidonis, 1983; Sandermann et al., 1977, 1984). It is widely assumed that cultured plant cells can serve as a metabolic model of the intact plant. In fact, a considerable similarity between metabolism in cell cultures and in the intact plant has been observed for example for 2,4-D (Mumma and Hamilton, 1979; Bristol et al., 1977; Scheel and Sandermann, 1981), diphenamide (Davis et al., 1978), cisanilide (Frear and Swanson, 1975), fluorodifen (Locke and Baron, 1972), carbaryl (Locke et al., 1976), diclofopmethyl (Dusky et al., 1980, 1982), EL-494 (Abdelmonem and Mumma, 1982), fenvalerate (Davidonis and Mumma, 1984), fluoroimide (Ohori and Aizawa, 1983), baythroid (Preiss et al., 1984), 4-chloro-2-methylphenoxyacetic acid (Cole and Loughman, 1983), and pentachlorophenol (Scheel et al., 1984).

The extrapolation from cell cultures to the intact plant became, however, questionable when the metabolism of certain nonpolar chemicals that are known as ubiquitous persistent contaminants of natural vegetation was studied in plant cell suspension cultures. In spite of their known persistence, DDT (Scheel and Sandermann, 1977; Arjmand and Sandermann, 1985), DDE (Arjmand and Sandermann, 1985), benzo[*a*]pyrene (von der Trenck and Sandermann, 1978; Harms, 1983), hexachlorobenzene (Sandermann et al., 1984), and bis(2-ethylhexyl) phthalate (DEHP; Sandermann et al., 1984) were all found to be partially converted to polar metabolites. In the case of DEHP (Figure 1) a unique esterase catalyzing a cleavage of the plasticizer chemical has been discovered in cultured wheat cells. This enzyme has been purified to electrophoretic homogeneity and characterized (Krell and Sandermann, 1984). This esterase has also been purified 10-fold from intact wheat plants, where the enzyme was, however, shown to be inaccessible for external DEHP (Krell and Sandermann, 1985). The main purpose of the present study was to analyze the role of the esterase in DEHP metabolism by cultured wheat cells.

EXPERIMENTAL SECTION

Materials. [*Carboxyl*-^{14}C]-DEHP and [*carboxyl*-^{14}C]-MEHP were obtained and purified as previously described (Krell and Sandermann, 1984). [*Carboxyl*-^{14}C]-MEHP-β-D-glucosyl tetraacetate was prepared from [*carbonyl*-^{14}C]-MEHP and tetra-*O*-acetylglucosyl bromide by the procedure described for abscisic acid β-D-glucosyl tetraacetate (Neill et al., 1983). The product (retention time 24.4 min) was separated from unreacted MEHP (retention time 19.6 min) by reversed-phase HPLC (see below). Characterization was by chemical ionization mass spectroscopy (reagent gas ammonia) that showed in particular the molecular ion (+18) at m/z 626.

The cell suspension culture of wheat (*Triticum aestivum* L., var. Koga II) was maintained on B5 medium as previously described (Sandermann et al., 1984; Scheel et al., 1984).

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