Optical Isomers of α-Copaene Derived from Several Plant Sources

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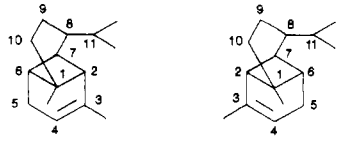
α-Copaene, a compound reported to be attractive to the Mediterranean fruit fly [*Ceratitis capitata* (Wiedemann)], can exist in two optically active forms. It has now been reisolated in pure form from commercial copaiba [*Copaifera officinalis* (Jacq.) L.] root oil and found to be levorotatory, whereas that isolated from commercial angelica (*Angelica archangelica* L.) seed oil proved to be dextrorotatory. The isolation procedures used in this study resulted in improved yields of α-copaene from copaiba oil over those published previously.

The Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann), is a highly destructive pest of citrus and fruit crops, nuts, and flowers in Hawaii, the Mediterranean countries of Europe, and the Middle East. Despite rigid quarantines, a constant hazard exists that this pest may be introduced into the continental United States due to increased tourist travel and world trade. Indeed, incipient infestations of the insect have been detected on a number of occasions since 1929 in Florida and California by deploying thousands of traps baited with the synthetic attractant trimedlure (Beroza et al., 1961) as part of a cooperative survey operation by federal, state, and county regulatory agencies (Shorey, 1981). Although these outbreaks have usually been quickly eradicated following early detection, the discovery and development of new and improved survey attractants for the fly have high priority on the list of needs by the USDA Animal and Plant Health Inspection Service (APHIS).

Steiner et al. (1957) reported that the essential oil of the roots and, especially, the seeds of *Angelica archangelica* L., an umbellifer occurring naturally in various parts of Europe but especially cultivated in Belgium and France, was quite attractive to male medflies, and this was confirmed by evaluation in an olfactometer (Beroza and Green, 1963) and in outdoor field traps. Fornasiero et al. (1969) isolated from the seed oil two compounds, α-copaene (I) and α-ylangene (II) (a stereoisomer of I), by a combination of fractional distillation, liquid chromatography, and gas chromatography. These compounds proved to be equally attractive to male medflies when tested in the laboratory (Guiotto et al., 1972), but attractancy to α-ylangene apparently has not been confirmed in the field.



Since α-copaene may possibly possess different optical activity under natural conditions and the levorotatory form has previously been found to occur in the essential oils of copaiba [*Copaifera officinalis* (Jacq.) L.], ylang-ylang [*Cananga odorata* (Lam.) Hook. f. & Thoms.], *Chloranthus sp.*, and *Cedrela toona* Roxb. (Büchi et al., 1963; De Mayo et al., 1965; Gaydou et al., 1986), we sought to determine the optical activity of the α-copaene present in angelica oil following its isolation in pure form from this oil and from copaiba oil.

EXPERIMENTAL SECTION

Materials and Methods. Angelica (*A. archangelica* L.) seed oil (FCC PIN 03900) and copaiba oil (FCC Extra) were obtained from Fritzsche Dodge & Olcott Inc., New York, NY. All solvents used were reagent grade, except for hexane, which was free of UV-absorbing materials (Burdick & Jackson Laboratories, Muskegon, MI), and ether, which was anhydrous.

Column chromatographic separations were carried out on Bio-Sil HA silicic acid (-325 mesh; Bio-Rad Laboratories, Richmond, CA) alone or impregnated with 10% or 20% $AgNO_3$, packed in hexane, prewashed with hexane, and eluted with this solvent.

Fractionation of the essential oils was monitored on a Hewlett-Packard Model 5880A GC instrument equipped with a capillary injection system and flame ionization detector. Fractions were compared on the gas chromatograph to pure α-copaene prepared by HPLC of an impure sample (provided by Professor Buchi). A 12 m × 0.2 mm (i.d.) dimethylsilicone (surface deactivated with Carbowax 20M) flexible fused-silica capillary column was used for the analyses. Temperatures employed were as follows: injection port, 200 °C; column, 105 °C. The sample injected was 1 μL of hexane containing 1.25 μg of test material.

Capillary GC–MS was carried out on a Finnigan Model 4500 mass spectrometer fitted with a fused silica column (30 m × 0.32 mm (i.d.)) coated with DB-1 bonded dimethylpolysiloxane (0.1-μm film thickness). The column was held at 75 °C for the first 2 min and then temperature-programmed at 2 °C/min to 250 °C and held at this point. Column inlet pressure was 15 psi of He.

^1H and ^{13}C NMR spectra were obtained with a Nicolet QE-300 spectrometer using Me_4Si as an internal standard in $CDCl_3$. IR spectra were determined as KBr disks on a Beckman Model 18-A spectrophotometer.

Fractional Distillation of the Essential Oils. Angelica seed oil was distilled at 12 mm pressure through a Vigreux column (28 × 2 cm), collecting fractions boiling at (A) 45–62 °C, (B) 65 °C, and (C) 100–145 °C. Copaiba oil was distilled at 0.3 mm pressure through the same size column, collecting fractions boiling at (D) 68–78 °C and (E) 80–85 °C.

Isolation of α-Copaene from Angelica Oil. Distillates A–C obtained from 741 g of oil consisted of 409 g of colorless oil (n^{25}_D 1.4808), 151 g of pale yellow oil (n^{25}_D 1.4819), and 130 g of yellow oil (n^{25}_D 1.4910), respectively. Only distillate C contained α-copaene; it was divided into two

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equal portions, and each portion was chromatographed on a column (48 × 8 cm) of silicic acid, eluting successively with 2-L portions of hexane, hexane–ether (95:5), hexane–ether (90:10), and hexane–ether (75:25). Similar eluates from both columns were combined and freed of solvent at ambient temperature (20 mm pressure) to give 99 g of pale yellow oil (n^{25}_D 1.4974), 21 g of mobile yellow liquid (n^{25}_D 1.4843), 5 g of mobile yellow liquid (n^{25}_D 1.4903), and 3.2 g of yellow oil (n^{25}_D 1.4946), respectively. The hexane eluate, which showed a typical hydrocarbon IR spectrum, was chromatographed batchwise (10-g each) 15 times on successive columns (47 × 3 cm) of silicic acid impregnated with 10% $AgNO_3$, eluting with hexane to yield 2.7 g of a colorless oil whose gas chromatogram indicated a 3.3:96.7 mixture of two components emerging from the column in 3.70 and 3.79 min, respectively. Complete separation of these components was achieved only after batchwise (300-mg each) chromatography on nine columns (36 × 2 cm) of silicic acid impregnated with 20% $AgNO_3$. After 10 successive passes per column a total of 200 mg of the slightly more volatile component (C-1) and 2.5 g of the second component (C-2) were obtained as colorless oils.

Isolation of α-Copaene from Copaiba Oil. Distillation fractions D and E obtained from 45 g of oil consisted of 20 g of colorless oil (n^{25}_D 1.4937) and 20.4 g of colorless oil (n^{25}_D 1.4950), respectively. GLC spectra revealed the presence of α-copaene in fraction D only; this was chromatographed in two equal portions on columns (42 × 3.5 cm) of silicic acid, eluting each with 2 L of hexane. Removal of solvent from the combined eluate under reduced pressure gave 19.4 g of colorless oil (n^{25}_D 1.4890), which was chromatographed on two successive columns of silicic acid (54 × 3.5 cm each) using hexane (2 L) as elution solvent and collecting the eluate in 100-mL fractions. GLC showed pure α-copaene only in fractions 5 and 6, giving a total α-copaene yield of 2.7 g (6% of the commercial oil) as a colorless oil, n^{25}_D 1.4897, $[\alpha]^{23}_D$ −6.3° (*c* 1.20, $CHCl_3$), which is identical with (−)-α-copaene reported by De Mayo et al. (1965).

RESULTS AND DISCUSSION

Comparison of the GLC (Kovats index 1538.5), IR, and GC–MS of component C-1 from angelica oil with those of an authentic specimen of α-ylangene showed the compounds to be identical (Motl et al., 1965; Heathcock et al., 1967; Coscia, 1984).

Component C-2 from angelica seed oil was a colorless liquid, n^{25}_D 1.4900, showing dextrorotation: $[\alpha]^{23}_D$ +6.4° (*c* 1.20, $CHCl_3$); GLC Kovats index 1551.3; IR_{max} 3029, 1665, 786 cm^{-1}; MS *m/z* (rel intens) 204 (20), 161 (96), 133 (11), 119 (100), 105 (93), 93 (48), 77 (20), 55 (21); ^1H NMR, multiplet at δ 4.77 (1 H, C=CH), doublet at δ 8.33 (3 H, C=CCH_3), doublet at δ 9.17 [6 H, $CH(CH_3)_2$], singlet at δ 9.22 (3 H, CCH_3); ^{13}C NMR δ 19.40, 19.78, 20.09, 21.91, 23.22, 30.18, 32.37, 36.38, 37.06, 39.53, 44.44, 44.89, 54.39, 116.24, 143.99.

These data are identical with those reported for the GLC (Coscia, 1984) IR (Kapadia et al., 1965; Wenninger et al., 1967; Fornasiero et al., 1969), MS (Hunter and Brogden, 1964), and ^1H NMR (De Mayo et al., 1965; Fornasiero et al., 1969) of α-copaene. Both samples of α-copaene were shown unequivocally by GLC, MS, and ^{13}C NMR to be completely free of contaminants.

The isolation of (+)-α-copaene from angelica seed oil was somewhat unexpected in view of the fact that only levorotatory α-copaene has previously been isolated from the essential oils of chloranthus, copaiba, ylang-ylang, and *C. toona*. However, comparable precedent exists in the case of natural α-ylangene (Kikuchi et al., 1982; Ohta and Hirose, 1969).

The occurrence of α-copaene of undetermined stereochemistry has recently been reported in leaves of common wheat (*Triticum aestivum* L.) (0.6–3.0%) (Buttery et al., 1985), leaves of cotton (*Gossypium hirsutum* L.) (1.5%) (Elzen et al., 1985), leaves of *Siparuna guianensis* (Aubl.) (0.76%) (Antonio et al., 1984), *Lippia nodiflora* (L.) Greene (8.4%) (Elakovich and Stevens, 1985), commercial oils of sage (Salvia *spp.*) (0.14%) and juniper berry (*Juniperus communis* L.) (0.1%) (Formacek and Kubeczka, 1982), pineapple fruits (Berger et al., 1983), and the essential oil of the above-ground portions of *Heracleum dissectum* Ledeb. (0.34%) (Papageorgiou et al., 1985). Yields of (−)-α-copaene reported from other sources were 9% from chloranthus oil and 8.8% from oil of *C. toona* (Büchi et al., 1963; De Mayo et al., 1965) (copaiba oil in our hands yielded 6% of the levo form). Comparative yields of (+)-α-copaene obtained from commercial angelica seed oil were 0.16% (Fornasiero et al., 1969) and 0.34% (Formacek and Kubeczka, 1982; our research).

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Registry No. (+)-α-Copaene, 14912-44-8; (−)-α-copaene, 3856-25-5.

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Isolation and HPLC Determination of Methyllycaconitine in a Species of Low Larkspur (*Delphinium nuttallianum*)

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The neurotoxic diterpenoid alkaloid methyllycaconitine was isolated from *Delphinium nuttallianum*, and a method was developed utilizing ion-pair HPLC to determine its concentration in freeze-dried plant samples.

Larkspurs are responsible for severe livestock losses on North American rangeland (Cronin and Nielsen, 1978). One of several low-growing larkspurs in Western Canada and the United States, *Delphinium nuttallianum* Pritz., occurs from southwestern British Columbia to northern California and eastward to Colorado (Hitchcock and Cronquist, 1964). It is widely distributed on rangelands in the interior of British Columbia, where its habitat varies from sagebrush desert to upper grassland. Appearing early in spring, often before most of the grasses, *D. nuttallianum* can be palatable but poisonous to cattle (Looman et al., 1985). Methyllycaconitine was recently established as the toxic principle in a related species of low larkspur, *Delphinium bicolor* Nutt., which occurs in southeastern British Columbia and Alberta (Kulanthaivel et al., 1986). This alkaloid is a potent neuromuscular blocking agent (Benn and Jacyno, 1983; Nation et al., 1982), and recently it was shown to be a naturally occurring insecticide (Jennings et al., 1986). Since clinical signs of poisoning are similar with both species of *Delphinium*, we suspected that similar alkaloids could also be present in *D. nuttallianum* and accordingly undertook an investigation for such compounds. The absence of information on the quantitative determination of *Delphinium* alkaloids prompted us to develop an efficient method for their extraction and analysis by HPLC. The method was then applied to field samples of *D. nuttallianum*, some of which were collected from rangeland sites where cattle had been poisoned by low larkspur. Heretofore, larkspur toxicity has been mainly estimated by rat or mouse bioassays (Olsen, 1977, 1983).

EXPERIMENTAL SECTION

Plant Material. Aerial portions of *D. nuttallianum* were collected during May 1985, from the Research Station in Kamloops, BC, and from seven rangeland sites located within a 20-km radius of Kamloops. Voucher specimens were deposited in the herbarium at the Provincial Museum in Victoria, BC. The fresh material was frozen, freeze-dried, and ground to pass a 2-mm screen.

Large-Scale Isolation of Methyllycaconitine. Freeze-dried *D. nuttallianum* (960 g) was extracted by repeated maceration (Waring Blendor) in 95% ethanol (3 × 4 L). The extracts were concentrated to a dark green gum that was partitioned between 0.1 M aqueous H_2SO_4 (100 mL) and $CHCl_3$ (300 mL). The $CHCl_3$ extract was then extracted with more 0.1 M H_2SO_4 (3 × 100 mL), and the combined aqueous layers were back-washed with $CHCl_3$ (2 × 100 mL). After addition of ice, the aqueous solution was brought to pH 4.5–5.0 (external indicator paper) with saturated aqueous Na_2CO_3 and extracted with $CHCl_3$ (3 × 100 mL). This extraction was repeated at pH 8.0–8.5 and 11 (each time with 3 × 100 mL of $CHCl_3$). The $CHCl_3$ extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure to yield the crude bases. These were then redissolved in $CHCl_3$ and extracted into 0.2 M aqueous H_2SO_4 (3 × 50 mL). The combined aqueous extracts were back-washed with $CHCl_3$, basified, as before, to pH 5.0, 8.0, and 11 with Na_2CO_3, and extracted at each of these points with $CHCl_3$ (3 × 50 mL). Removal of solvents from these extracts yielded the alkaloids as off-white foams (1.0, 1.2, and 0.1 g, respectively; i.e., 2.3 g or about 0.2% of the dry weight of the plant). TLC analysis (silica gel 60; $CHCl_3$–methanol, 5:1 or 8:1, v/v) suggested that the pH 5 and 8 fractions were very similar with a single major component while the pH 11 fraction yielded more polar alkaloids.

A portion of the pH 5 mixed bases (100 mg) was fractionated by centrifugally accelerated radial TLC (Chromatotron) (1-mm silica gel 60 F254), the plate being developed with $CHCl_3$ and then $CHCl_3$–methanol (8:1). A UV-absorbing band was rapidly eluted and one fraction (40 mg) appeared to be homogeneous by TLC (system as before). The ^1H and ^{13}C NMR spectra of this material were in accord with this being methyllycaconitine (Figure 1); i.e. it was in agreement with the spectra reported for authentic specimens of this alkaloid (Pelletier et al., 1984). The MS (DCI-probe, MS-80) revealed a highest mass ion at m/z 682 as required for this alkaloid.

Quantitative Determination. A freeze-dried and ground sample of *D. nuttallianum* (1 g) was extracted with

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