STUDIES ON THE CHEMICAL CONSTITUENTS OF THE FOOD PLANTS OF THE INSECTS, 1. TERPENIC CONSTITUENTS OF *ARISTOLOCHIA DEBILIS*, *HETEROTROPA* SPP., AND *CRATAEVA RELIGIOSA*

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Recent years have witnessed tremendous strides in the establishment of the influence of natural odoriferous compounds, especially terpenes, on the behavior of the anthropods (1-5). During our studies along these lines, curiously enough, we observed the *Byasa alcinous* Klug., *Luehdorfia japonica* Leeck, and *Hebomoia glaucippe* L. normally are attracted to *Aristolochia dibilis* Sieb. et Zucc. (Aristolochiaceae), *Heterotropa* spp. (Aristolochiaceae) (6-7), and *Crataeva religiosa* Forst. (Capparaceae). This prompted us to examine the volatile components of the five food plants of some butterflies.

EXPERIMENTAL

PLANT MATERIALS.—The fresh plants (200-1000 g) of *A. debilis* and *C. religiosa* were collected from Hiroshima and Kagoshima Prefectures in Japan, and the essential oils were isolated by steam distillation. The fresh, whole plants (50-100 g) of three species of *Heterotropa* were collected from Kyoto, Wakayama, and Nagano Prefectures, and the essential oils were obtained by both steam distillation and solvent extraction with EtOAc. The essential oils were analyzed by means of gc and gcms (8-10). Voucher specimens are available for inspection at the Herbarium of Faculty of Integrated Arts and Sciences, Hiroshima University.

GAS CHROMATOGRAPHY.—Quantitative gc analysis were carried out on GC-350 (Gasukuro Kogyo) with a 0.28 mm×30 m glass capillary column coated with OV-101. The column temperature was programmed from 100° to 250° at a rate of 2°/min. Ms spectra were measured with JMS-D100 Mass Spectrometer coupled to a JGC 20KP Gas Chromatograph equipped with a 3 mm×1 m glass column containing OV-101 on 100-200 mesh Chromosorb WAW. Helium was the carrier gas (15 ml/min), and the column temperature was programmed from 50° to 200° at a rate of 5°/min. Mass spectra were obtained at 25eV.

Constituents of the essential oils were as follows: *A. debilis* (identified by Dr. T. Seki, from Hiroshima Prefecture, food plant of *B. alcinous*); leaves and stems 2-hexenal, 19.1; α-pinene, 3.5; camphene, trace; β-pinene, 3.8; myrcene, 10.3; limonene, 7.3; *trans*-β-ocimene, 1.0; *p*-cymene, trace; 1,8-cineole, 0.4; camphor, 1.7; borneol, 1.3; bornyl acetate, 0.8; 3,4,5-trimethoxytoluene, 0.4; caryophyllene, 39.3; α-humulene, 6.0; β-elemene, 3.0; calamenene, 1.3; unknown, 0.4%; roots α-pinene, 5.0; camphene, 15.6; β-pinene, 1.5; 1,8-cineole, 3.6; camphor, 6.6; borneol, 0.7; α-cubebene, 1.5; α-guaiene, 1.5; β-chamigrene, 12.6; δ-cadinene, 11.1; γ-cadinene, 4.8; α-santalene, 2.2; δ-guaiene, 1.5; calamenene, 0.7; aromadendrene, 1.5%.

Heterotropa takaoi F. Maekawa (from Kyoto, food plant of *L. japonica*); leaves, α-pinene, 0.6; camphene, 0.1; β-pinene, 3.2; *p*-cymene, 1.1; Δ^3-carene, 1.6; limonene, 0.3; 1,8-cineole, 0.4; linalool, 1.2; safrole, 4.4; β-farnesene, 3.6; myristicin, 46.7; *n*-pentadecane, 13.6; elemicin, 22.4%; roots α-pinene, 17.9; camphene, 10.2; β-pinene, 16.2; *p*-cymene, 0.8; *trans*-β-ocimene, 0.6; limonene, 5.1; borneol, 6.1; safrole, 0.4; methyl eugenol, 3.3; γ-elemene, 0.7; myristicin, 2.5; *n*-pentadecane, 2.6; elemicin, 28.0; 1-allyl-2,3,4,5-tetramethoxybenzene, 0.4%.

Heterotropa takaoi var. *hisauchii* F. Maekawa (from Wakayama Prefecture, food plant of *L. japonica*); leaves myrcene, 1.6; safrole, 68.4; methyl eugenol, 2.0; caryophyllene, 2.4; *trans*-β-ocimene, 5.2; *n*-pentadecene, 8.2; *n*-pentadecane, 10.4; elemicin, 1.8%; roots α-pinene, 2.1; camphene, 1.4; β-pinene, 2.1; 1,8-cineole, 1.0; camphor, 0.7; borneol, 3.6; eugenol, 0.7; 3,4,5-trimethoxytoluene, 5.8; *n*-pentadecane, 2.9; elemicin and its isomers, 76.7%.

Heterotropa fauriei var. *nakaii* F. Maekawa (from Nagano Prefecture, food plant of *L. japonica*); leaves α-pinene, 7.6; camphene, 3.4; β-pinene, 17.9; *p*-cymene, 2.6; Δ^3-carene, 3.1; limonene, 2.2; 1,8-cineole, 2.5; linalool, 3.6; safrole, 0.2; β-farnesene, 0.6; α-bergamotene, 3.3; caryophyllene, 3.1; myristicin, 6.1; *n*-pentadecane, 51.6; elemicin, 2.2%; roots α-pinene, 19.9; camphene, 12.5; β-pinene, 16.3; *p*-cymene, 1.4; *trans*-β-ocimene, 3.4; limonene, 5.0; borneol, 8.1; *n*-pentadecane, 4.4; elemicin, 23.5; 1-allyl-2,3,4,5-tetramethoxybenzene, 1.5; unknown, 2.2%.

C. religiosa Forst. (from Kagoshima Prefecture, food plant of *H. glaucippe*); leaves 2-hexenal, 50.3; 3-hexen-1-ol, 40.2; *p*-cymene, 0.5; limonene, 0.6; linalool, 0.6; α-ionone, 0.2; β-ionone, 0.2%.

LITERATURE CITED

1. J. Rudinsky and M.O. Zethner, *Canad. Entomol.*, **99**, 911 (1967).
2. M. Beroza, "Chemicals Controlling Insect Behavior," Academic Press, New York, 1970, pp. 21-40.
3. G.V. Dethier, in: "Chemical Ecology," Ed. by F. Sondheimer and T.B. Simone, Academic Press, New York, 1970, pp. 83-102.
4. L.M. Schoonhoven, in: "Chemical Mediators between Plants and Phytophagous Insects in Semiochemicals," Ed. by D.A. Norlund, R.L. Jones, and W.J. Lewis, John Wiley & Sons, New York, 1978, pp. 31-50.
5. J.H. Visser and A.K. Minks, "Insect-Plant Relationships," Centre for Agricultural Publishing and Documentation, Wageningen, 1982, pp. 95-122.
6. N. Hayashi, M. Sholichin, T. Sakao, Y. Yamamura, and H. Komae, *Biochem. Syst. Ecol.*, **8**, 109 (1980).
7. N. Hayashi, M. Masuoka, K. Maeshima, T. Sakao, M. Tabata, and H. Komae, *Agric. Biol. Chem.*, **47**, 1397 (1983).
8. Y. Hirose, *Shitsuryo Bunseki*, **15**, 162 (1967).
9. Y. Yukawa and S. Ito, "Spectral Atlas of Terpenes and the Related Compounds," Hirokawa, Tokyo, 1973.
10. Y. Masada, "Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry," Hirokawa, Tokyo, 1975.

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PYRROLIZIDINE ALKALOIDS FROM *SENECIO BRASILIENSIS* POPULATIONS

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Senecio brasiliensis (Spreng.) Less. (Compositae) has been associated with cattle poisoning in countries bordering the Rio de La Plata (1,2). We report here the pyrrolizidine alkaloids of three *S. brasiliensis* populations growing on rangelands in Paraguay. The pyrrolizidine alkaloid content and composition based on dried material and variation in different organs and collection sites are summarized in Table 1.

EXPERIMENTAL

PLANT MATERIAL.—Collections were made on November 10, 1985, along Ruta 2 in Department Alto Paraná, Paraguay. Voucher specimens have been filed with the Smithsonian Institution (US), Washington, DC (Schmeda 742-744).

EXTRACTION AND ISOLATION.—Freshly collected samples were separated into flowering tops, leaves, stems, and roots and extracted with 95% EtOH at room temperature for 48 h. After filtration, the plant material was dried, ground, and re-extracted twice with 95% EtOH at room temperature. The combined extracts were evaporated under reduced pressure and processed by standard procedures (3-5). The free base and *N*-oxide pyrrolizidine-alkaloid content were determined for each sample by the ^{1}H-nmr method (5) using a Varian EM 390 spectrometer. Small amounts of each plant part were air-dried in the shade to determine the ratio of fresh to dry weight.

All crude alkaloid bases and *N*-oxides (ca. 4 g) were chromatographed on Si gel with $CHCl_3$/EtOH gradient with increasing amounts of EtOH, affording after tlc (Si gel; $CHCl_3$-EtOH, 85:15; Dragendorff reagent) and recrystallization, integerrimine, retrorsine, and its 20-21 *E*-isomer. Compounds were identified by ^{1}H-nmr (6), ms, mp (7), and authentic sample comparisons.

Details of the identification are available upon request to the senior author.