Phytochemistry, 1967, Vol. 6, pp. 1271 to 1280. Pergamon Press Ltd. Printed in England

NEPETALACTONE AND EPINEPETALACTONE FROM *NEPETA CATARIA* L.* †

F. E. REGNIER‡, E. J. EISENBRAUN§ and G. R. WALLER

Departments of Chemistry and Biochemistry, Oklahoma State University,
Stillwater, Oklahoma, U.S.A.

(*Received* 1 *February* 1967)

Abstract—An analytical gas chromatographic technique for the separation of the catnip lactones—nepetalactone and epinepetalactone—and their degradation products, the dimethyl nepetalinates, is reported. *Nepeta cataria* L. plants grown in Nebraska, Michigan and Oklahoma produce predominantly nepetalactone. The nepetalactone:epinepetalactone ratio in individual plants varies from 99·9 to 0·1 to 3–1; however, variations in these ratios were not observed in selected parts of the plant. Epimerization during isolation and analysis was shown not to occur. With the techniques described, a single leaf can be analyzed to determine the ratio of catnip lactones in a given plant. Mass spectral data for the nepetalactones and dimethyl α-nepetalinate are reported.

INTRODUCTION

IT HAS become increasingly evident that the methylcyclopentane monoterpenoids are widespread in nature with the isolation of many compounds containing this carbon skeleton. Nepetalactone (Fig. 1; Ia) from *Nepeta cataria* L., a typical example of this series, was the first to be isolated[1] and chemically characterized.[2,3] Extensive investigations of these monoterpenoids of plant and animal origin have shown that most stereochemical variations occur at the 4 and 7a positions in the carbon skeleton.** The similarity in stereochemistry of these compounds at positions 4a and 7 suggests the existence of a common biosynthetic pathway.

The structure[2,3] and stereochemistry[3] of nepetalactone (Ia), the feline attractant of oil of catnip, have been established in earlier studies. There is substantial evidence[3,4,5] that a stereoisomer of Ia is also present in commercial catnip oil. It has been established[5] that this isomer, for which we propose the name epinepetalactone (Ib), is the 7a epimer of Ia. Bates and Sigel[4] partially separated Ia and Ib by gas chromatography and estimated that a sample of

---

* (*a*) Taken in part from the Ph.D. thesis of F. E. Regnier, Oklahoma State University, 1965, (*b*) Abstracts of papers, 150th Meeting of the American Chemical Society, section C, Atlantic City, New Jersey, September, 1965, p. 166, and the Fourth International Symposium on The Chemistry of Natural Products, Stockholm, Sweden, June, 1966, p. 170.

† Supported in part by Research Grants GM-11144 from the National Institutes of Health, United States Public Health Service and GB-3482 from the National Science Foundation.

‡ NIH Trainee Fellow, 1963–1965.

§ To whom correspondence should be sent.

** We thank Dr. Kurt L. Loening, Director of Nomenclature, Chemical Abstracts, for advice in selecting the numbering system for the methylcyclopentane monoterpenoids. See *The Ring Index*, p. 179, item 1377.

[1] S. M. McELVAIN, R. D. BRIGHT and P. R. JOHNSON, *J. Am. Chem. Soc.* **63**, 1558 (1941).

[2] J. MEINWALD, *J. Am. Chem. Soc.* **76**, 4571 (1954).

[3] R. B. BATES, E. J. EISENBRAUN and S. M. McELVAIN, *J. Am. Chem. Soc.* **80**, 3420 (1958).

[4] R. B. BATES and C. W. SIGEL, *Experentia* **19**, 564 (1963).

[5] T. SAKAN, S. ISOE, S. B. HYEON, R. KATSUMURA, T. MAEDA, J. WOLINSKY, D. DICKERSON, M. SLABAUGH and D. NELSON, *Tetrahedron Letters* 409 (1965).

commercial catnip oil contained 75% Ia and 25% Ib. In our studies essential oil isolated from *N. cataria* L. plants from greenhouse stock contained from 70 to 99·9% Ia and 0·1 to 30% Ib.

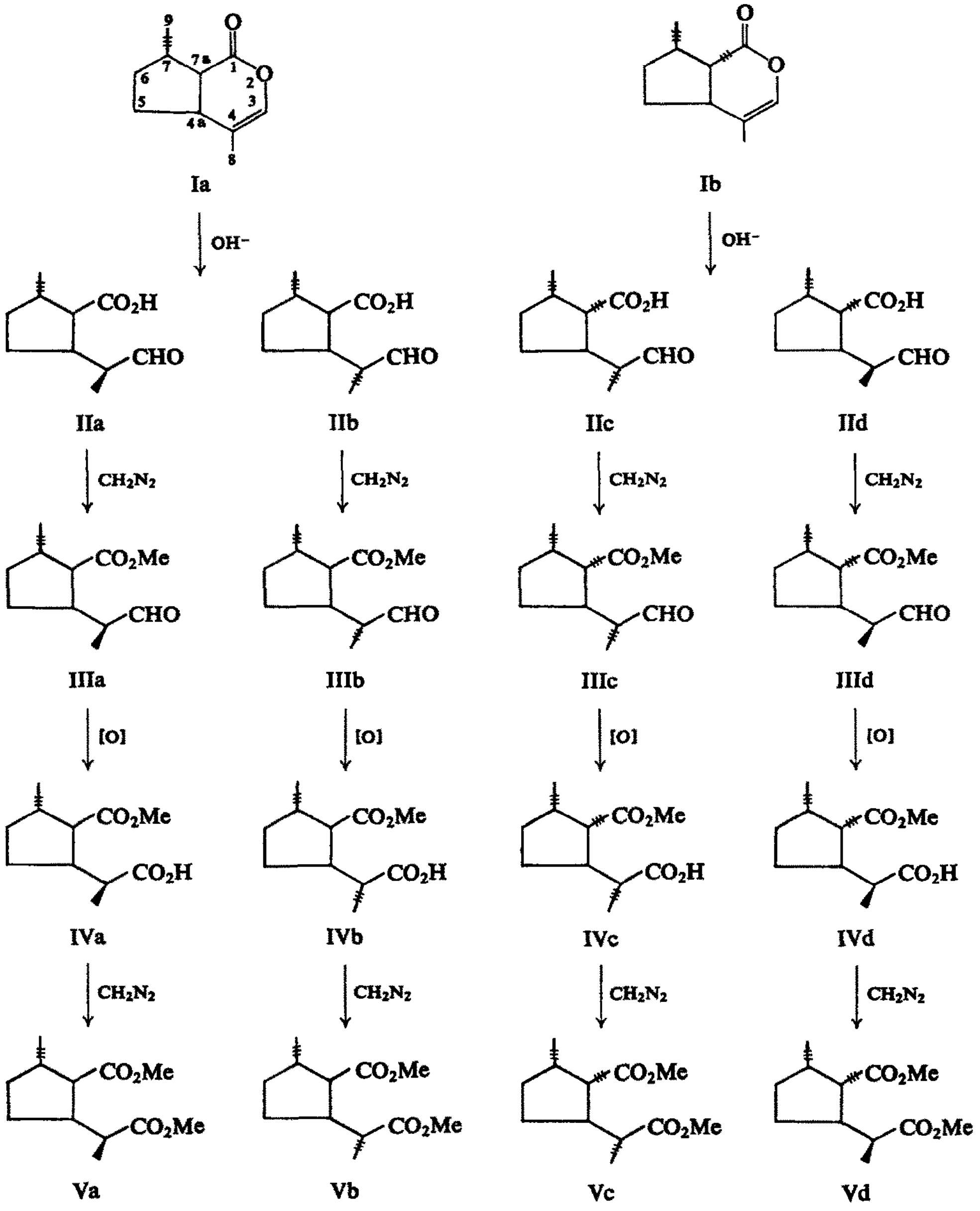

Fig. 1. Chemical degradation of nepetalactone (Ia) and epinepetalactone (Ib) to the corresponding dimethyl nepetalinates (nepetalactone yields the α- and δ-nepetalinates (Vb and Va) and epinepetalactone yields the β- and γ-nepetalinates (Vd and Vc)).

It was considered important to obtain a better understanding of the variations in Ia–Ib ratios in plants as an aid in studies on the biosynthesis of Ia[6] and on determining the

---

[6] F. E. Regnier, G. R. Waller, E. J. Eisenbraun and H. Auda. *Phytochem.* In press.

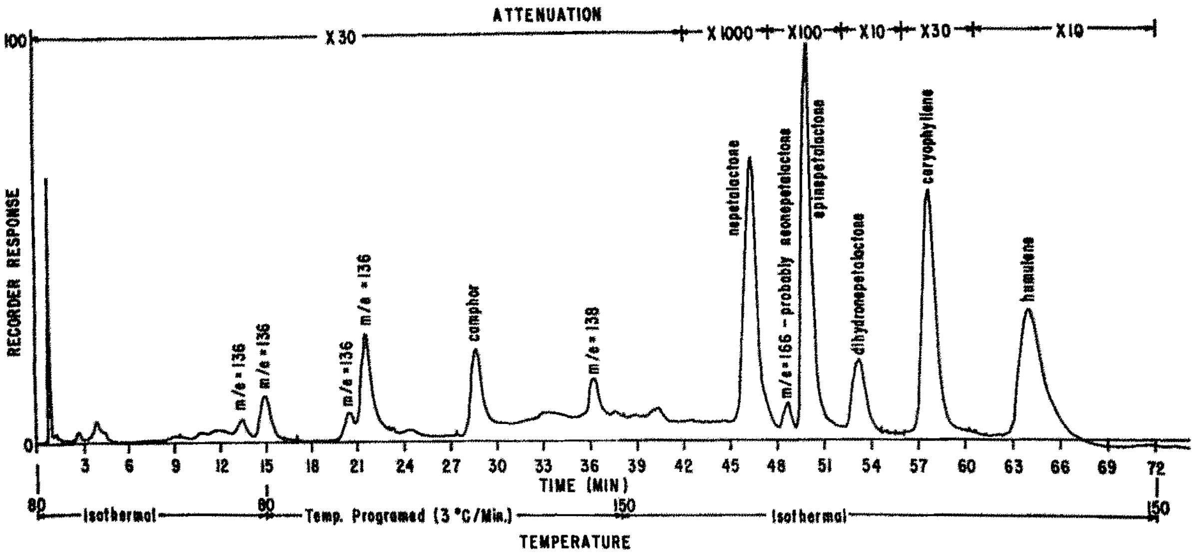

FIG. 2. GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF *Nepeta cataria* OIL. 0·05 μl OF THE OIL WAS USED. THE COLUMN WAS 45 M × 0·5 MM, APIEZON L-COATED AND KEPT AT 150°. THE HYDROGEN FLAME DETECTOR WAS KEPT AT 200°. THE HELIUM PRESSURE WAS KEPT AT 25 LB/IN^2.

composition of the essential oils from various *Nepeta* species.[7] It was also necessary to determine if the isomers were interconverted during isolation and analysis—a possibility recognized by other investigators.[8, 9]

This paper reports an analytical technique for the gas chromatographic separation of the catnip lactones and their degradation products, the dimethyl nepetalinates (Va–Vd). Mass spectral data obtained from nepetalactones (Ia and Ib) and dimethyl nepetalinates (Va–Vd) are presented and discussed. Ratios of Ia to Ib in different *N. cataria* plants and parts of plants are also reported.

RESULTS

The essential oil samples were analyzed by two different techniques: (1) direct gas–liquid chromatography of mixtures containing Ia and Ib and (2) chemical degradation of Ia and Ib to the corresponding dimethyl nepetalinates (Fig. 1, Va–Vd). By comparing the quantitative results from these two techniques, it was possible to establish that epimerization of Ia and Ib did not occur during analysis.

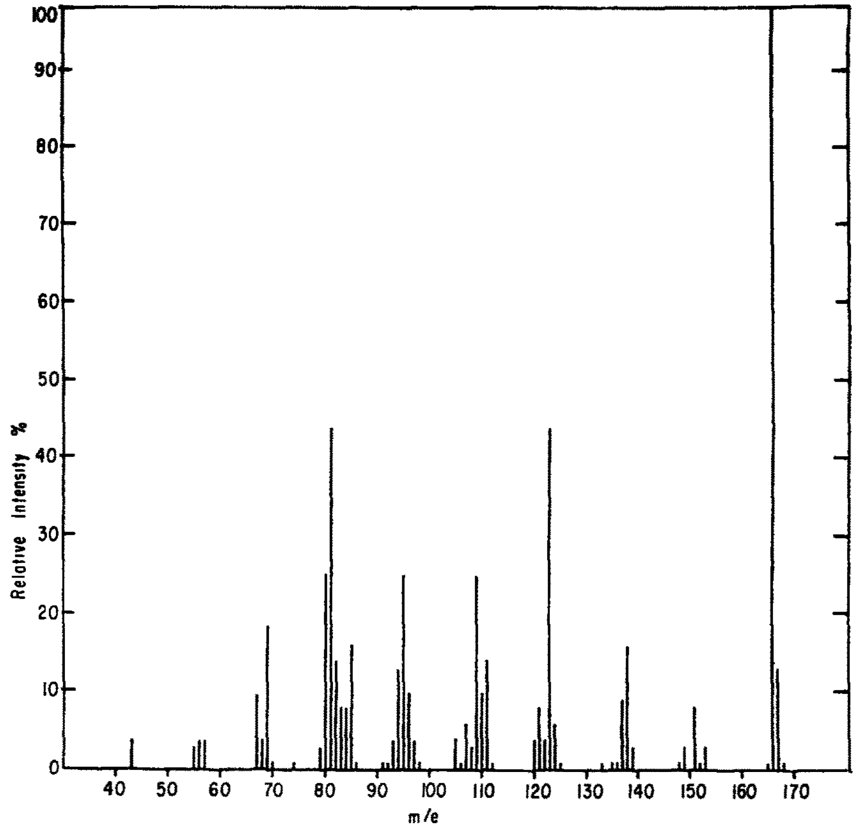

FIG. 3. MASS SPECTRUM OF NEPETALACTONE (Ia).

---

[7] F. E. REGNIER, C. R. WALLER and E. J. EISENBRAUN, *Phytochem.* 6, 1281 (1967).

[8] G. W. K. CAVILL, Personal communication.

[9] S. M. MCELVAIN and E. J. EISENBRAUN, *J. Am. Chem. Soc.* 77, 1599 (1955).

Complete separation (see Fig. 2) of Ia and Ib in essential oil samples was achieved on a 45 m × 0·5 mm open tubular column interior-coated with Apiezon L. These two compounds usually constituted 80–95 per cent of the total steam-volatile compounds in *Nepeta cataria.* Mass spectral data for Ia and Ib were obtained with a combination mass spectrometer–gas chromatograph. The parent ion (m/e = 166) was the most abundant ion in the spectra of Ia and Ib. Intense ions were also found at m/e = 138 (M^+-28), 123 (M^+-43), 109, 95 and 81 in the spectra of these compounds. Metastable peaks were observed at m* = 137·6, 114·7, 109·6, 100·3, 89·6, 72·9, 63·9 and 47·6. As expected, only very small variations in ion intensity were noted in the spectra of Ia and Ib. The mass spectrum of Ia is shown in Fig. 3.

The chemical degradation of Ia and Ib is shown in Fig. 1. Alkaline hydrolysis of each of the enol-lactones Ia and Ib results in the generation of a new asymmetric center at position 4 with the formation of the corresponding aldehydo acids IIa and IIb from Ia, and IIc and IId from Ib. Esterification of IIa–IId with diazomethane followed by air oxidation and further esterification with diazomethane produced the dimethyl nepetalinates Va–Vd. In this degradation Ia yields dimethyl-α-nepetalinate (Vb) and dimethyl δ-nepetalinate (Va) while Ib yields dimethyl β-nepetalinate (Vd) and dimethyl γ-nepetalinate (Vc).[10] Epimerization was not found to occur during alkaline hydrolysis of Ia and Ib. Gas chromatographic separation of the dimethyl nepetalinates (Va–Vd) was achieved on a 120 m × 0·5 mm open tubular column coated with LAC 886. The order of elution from this column was Va, Vb, Vc and Vd, as is shown in Fig. 4.

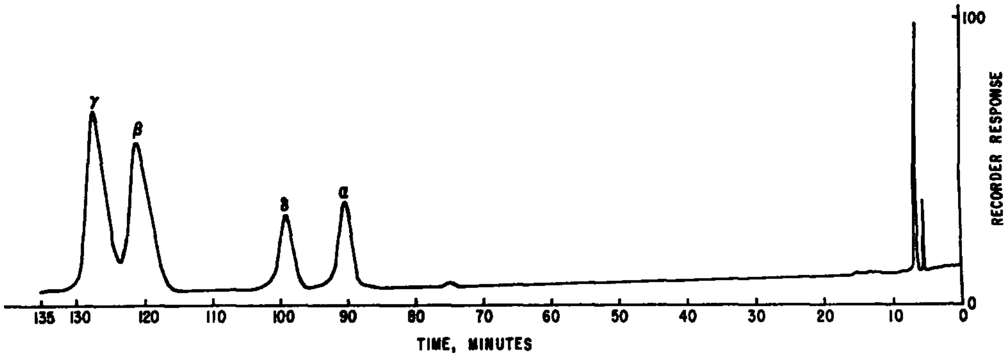

FIG. 4. GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF THE DIMETHYL NEPETALINATES. 0·05 μl OF THE MIXTURE WAS USED. THE COLUMN WAS 120 M × 0·5 MM, COATED WITH LAC 886, AND KEPT AT 162°. THE HYDROGEN FLAME DETECTOR WAS KEPT AT 200°. THE HELIUM PRESSURE WAS KEPT AT 25 LB/IN^2.

The mass spectrum of dimethyl α-nepetalinate (Vb) is shown in Fig. 5. The β-, γ- and δ-isomers (Vb–Vd) have nearly the same spectra, differing only by small variations in ion intensity. The parent ion of Vb (m/e = 228) was not very intense and the base peak was at m/e = 81. Intense ions were found at m/e = 197 (M^+-31), 168 (M^+-60), 141, 109 and 81. Metastable peaks were observed at m* = 145·1, 104·2, 84·5, 70·8, 60·7 and 47·2.

It was considered that prolonged steam distillation in the presence of metals or salts might cause epimerization of Ia to Ib. Accordingly, a mixture of Ia and Ib in the ratio 99 : 1 was heated with 10 volumes of water at reflux temperature (98·7°) for several hours to simulate steam distillation conditions. Distillation for 2 hr in the presence of Zn, ZnO, Al, Al_2O_3, Fe and FeO did not convert Ia to Ib.

---

[10] E. J. EISENBRAUN, A. BRIGHT and H. H. APPEL, *Chem. Ind.* (*London*) 1242 (1962).

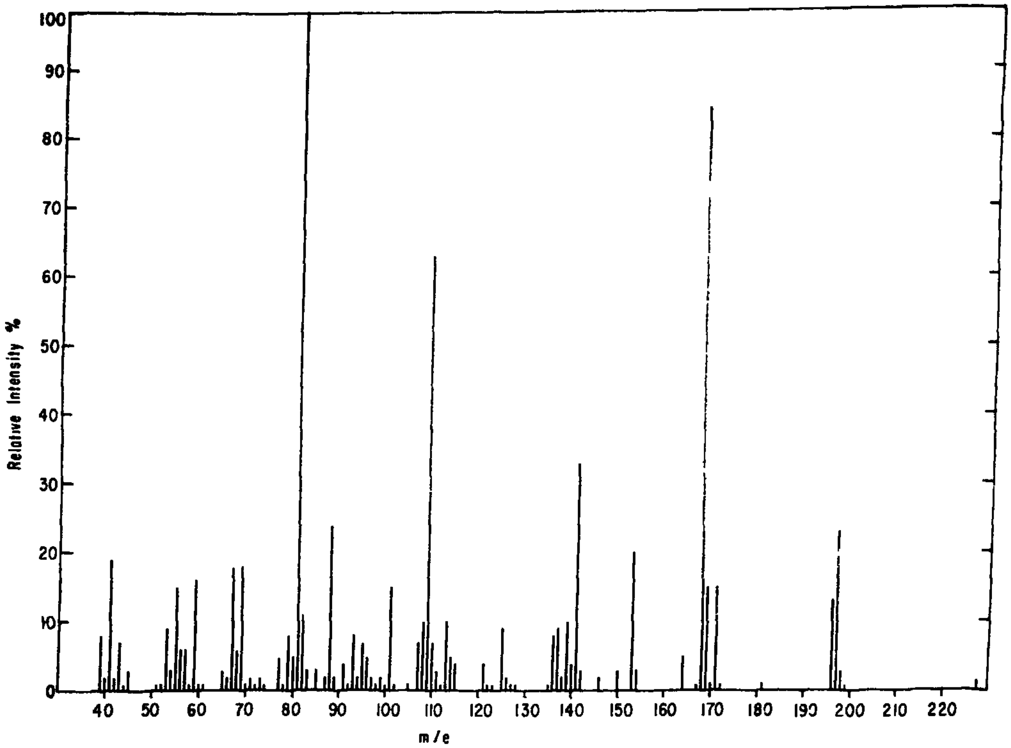

Fig. 5. Mass spectrum of dimethyl α-nepetalinate.

Table 1. Relative concentration of nepetalactone and epinepetalactone in *Netepa cataria* oils

Sample	Nepetalactone[a] (%)	Epinepetalactone[a] (%)	Dimethyl nepetalinates[b] α (%)	δ (%)	β (%)	γ (%)
Nebraska oil	60	40	23	35	22	20
Michigan oil	99	1	64	36	trace	trace
Fritzsche[c] oil	60	40	—	—	—	—
Nepetalactone[d] from nepetalic acid	99	1	68	32	trace	trace
Nepetalactone from extraction of *N. cataria*[e]	99·6	0·4	73	27	trace	trace
	99·9	0·1	—	—	—	—
	70·2	29·8	—	—	—	—

[a] Estimates by direct gas chromatographic analysis on 45 m × 0·5 mm stainless steel open tubular column coated on the inside with Apiezon L and operated at 150°.

[b] The dimethyl nepetalinates were obtained from chemical degradation of Ia and Ib and were separated by chromatography on a 120 m × 0·5 mm stainless steel open tubular column coated on the inside with LAC 886 and operated at 178°.

[c] Obtained from Fritzsche Brothers, New York, New York.

[d] Nepetalactone was obtained by the pyrolysis of nepetalic acid.[1]

[e] Nepetalactone was extracted from the leaves by macerating the tissue in diethyl ether. The ether solution was analyzed directly.

The results in Table 1 show that the ratios of Ia and Ib obtained from chemical degradation studies and direct GLC analysis are in close agreement. Ia formed by the pyrolysis of nepetalic acid (IIb) contained only traces of Ib. The ratio of Ia–Ib in Nebraska oil was found to be 60:40 by direct GLC analysis whereas degradation to the dimethyl nepetalinates (Va–Vd) gave a ratio of 58:42. Oil of catnip obtained from Fritzsche Bros. also contained large amounts (40%) of Ib; however, this sample was not degraded to Va–Vd. All the other samples analysed by both techniques were found to contain only traces of I b. The Michigan oil and the oil isolated from selected plants grown in the local greenhouse contained over 99% Ia relative to Ib. Essential oil samples isolated by either steam distillation or ether extraction from stems, leaves or flowers of the greenhouse stock showed the same Ia–Ib ratios as essential oil from the whole plants. The ratio varies from 99·9:0·1 to 70:30 in plants randomly selected from the greenhouse stock (last line of Table 1). However, the majority of the plants analyzed have a Ia–Ib ratio greater than 97:1. It was possible to analyze a small part of one leaf, about 2 cm square, by extracting with ethyl ether and direct analysis of the ether extracts by GLC on the Apiezon L open tubular column.

DISCUSSION

The close agreement of the data from the two analytical methods (Table 1) permits several conclusions. It is conceivable that lactones might be epimerized by contact of their vapors with metals. However, it is evident from these data that there is no thermal interconversion of the diastereoisomers Ia and Ib in the inlet of the gas chromatograph. It was also concluded that strong base (10–20% KOH) does not epimerize Ia prior to, during, or after ring opening to the salt of the aldehydo acid. Evidently the base-catalyzed ring cleavage of the enol-lactones Ia and Ib was sufficiently rapid to exclude epimerization at the asymmetric center adjacent to the carbonyl group. Once ring cleavage was achieved, base-catalyzed epimerization at the position adjacent to the carboxylic acid anion would not be expected.

The gas chromatographic resolution of Ia and Ib (Fig. 2) and the dimethyl nepetalinates (Va–Vd) (Fig. 4) is of value in the study of the stereochemistry of the methylcyclopentane monoterpenoids. The nepetalinic acids were the first members of this family of monoterpenoids to have their stereochemistry established[11] and as a consequence have been the primary references for stereochemical assignments of most of the other methylcyclopentane monoterpenoids. A comparison of the GLC curves of the dimethyl nepetalinates with those of the corresponding derivatives from methylcyclopentane monoterpenoids of unknown stereochemistry will greatly facilitate future stereochemical assignments.

The mass spectral analyses of nepetalactone (Ia) and epinepetalactone (Ib) with the rapid-scan combination mass spectrometer–gas chromatograph produce spectra that vary only slightly in ion intensities (observed in six runs). In both compounds the parent ion ($m/e = 166$) is the most abundant ion in the spectrum. It is generally accepted that the most facile ionization in carbonyl compounds is at the carbonyl oxygen.[12] This leads to subsequent cleavage of the bonds adjacent to the carbonyl group. Alpha-cleavage in lactones occurs more at the carbon–carbon bond than at the carbon–oxygen bond.[13] Initial cleavage of the carbon–carbon bond adjacent to the carbonyl group followed by cleavages at other positions in the molecule could account for some of the fragments observed in the spectrum of Ia and Ib. The expulsion of CO and CO_2 from lactones[12] and esters[14] following electron impact is well documented.

---

[11] E. J. Eisenbraun and S. M. McElvain, *J. Am. Chem. Soc.* **77**, 3383 (1955).

[12] I. Kanomata, *Bull. Chem. Soc. Japan* **34**, 1864 (1961).

[13] L. Friedman and F. A. Long, *J. Am. Chem. Soc.* **75**, 2823 (1953).

[14] L. Friedman and A. P. Wolf, *J. Am. Chem. Soc.* **80**, 2424 (1958).

Ions at m/e = 138 (M^+-28)[*] and 122 (M^+-44) in the spectrum of Ia and Ib are probably the result of such eliminations; however, the latter ion is of very low intensity. The ion at m/e = 123 (M^+-43) may be formed by two routes: (*a*) could be the result of a two-step decomposition; first the transition of $M^+ \rightarrow 151^+ + CH_3$ ($m^* = 137{\cdot}5$)[†] and then the transition of $151^+ \rightarrow 123 + C_2H_2$ ($m^* = 100{\cdot}3$): (*b*) m/e = 138^+ ion losses a methyl group (probably the lactone ring methyl) and this transition is confirmed by $m^* = 109{\cdot}6$. The ions m/e = 123 can fragment via two patterns: (*a*) $123^+ \rightarrow 105^+ + 18$ (H_2O) ($m^* = 89{\cdot}6$) and (*b*) $123^+ \rightarrow 95 + 28$ (CO) ($m^* = 72{\cdot}9$). The formation of the ion at m/e = 109 could arise by several transitions: (*a*) $M^+ \rightarrow 109^+ + 57$, (*b*) $138^+ \rightarrow 109^+ + 29$ or (*c*) $123^+ \rightarrow 109^+ + 14$. Metastable ions to support the formation of m/e = 109 were not observed. The ion at m/e = 81 probably results from an α-cleavage followed by further fragmentation leading to loss of carbons 3, 4 and 8 of Ia (Fig. 1) with retention of the positive charge by the methylcyclopentane ring. This transition is $138^+ \rightarrow 81^+ + 57$ and was confirmed by appearance of $m^* = 47{\cdot}6$. It may also occur by other decomposition pathways.

The mass spectra of the dimethyl nepetalinates differ from those of the nepetalactones but, as might be expected, exhibit an intense ion at m/e = 81 which is characteristic of the methylcyclopentane ring. The first major fragment formed by the dimethyl nepetalinates upon electron impact is m/e = 197 (M^+-31) which corresponds to the loss of the —OCH_3 group. Such cleavage was first reported by Ryhage and Stenhagen[15, 16] to occur in the methyl esters of fatty acids and since has been recognized as a general reaction of esters upon electron impact. The second most intense peak, m/e = 168 (M^+-60), corresponds to the loss of acetic acid from the parent ion. This is confirmed by the presence of $m^* = 124{\cdot}3$; however, this transition may occur in two steps as follows: (*a*) $228 \rightarrow 227^+ + 1$ (H) and (*b*) $227^+ \rightarrow 168^+ + 59$ (CH_3COO). The acetic acid originates from one of the carboxyl groups but it is not possible to tell which one. A hydrogen migration occurs in Ia (Fig. 1), most likely one of the tertiary hydrogen atoms attached to either carbon 4 or 7a. The decomposition of α-methyl esters[16] to give m/e = 88 instead of m/e = 74 occurs in this compound as is evidenced by the presence of both m/e = 141 and m/e = 88. The peak at m/e = 109 corresponds to the loss M^+-(60+59) and to the transition $141^+ \rightarrow 109^+ + 32$ (CH_3OH), $m^* = 84{\cdot}3$ and probably consists of two types of ions. The loss of 60 mass units provides m/e = 168 and the subsequent loss of 59 mass units, which probably represents an acetate radical, is the most likely path ($m^* = 70{\cdot}7$; $168^+ \rightarrow 109^+ + 59$). The methylcyclopentane ring fragment, m/e = 81, is formed by the loss of 28 (—CH_2CH_2) units from ion m/e = 109 (confirmed by $m^* = 60{\cdot}3$) or by the loss of 60 mass units from ion m/e = 141. High resolution mass spectrometry is needed to confirm many of these postulates.

From the results in Table 1 and the data obtained from prolonged steam distillation of Ia, it may be concluded that neither the isolation technique nor the method of analysis caused extensive epimerization of Ia–Ib in the commercial catnip oils studied previously[3, 4, 5] or the sample of oil obtained from the Nebraska catnip plants. The fact that the ratio of Ib–Ia was uniformly low throughout the parts of most of the local greenhouse plants eliminates the possibility that one part of the plant produces high concentrations of Ib. Since the plant materials obtained from Nebraska and local greenhouse stock have all been classified as

---

[*] Confirmed by the metastable ion $m^* = 114{\cdot}7$.

[†] m^* refers to metastable ions.

[15] R. Ryhage and E. Stenhagen, *Arkiv Kemi* **13**, 523 (1959).

[16] R. Ryhage and E. Stenhagen, In *Mass Spectrometry of Organic Ions* (Edited by F. McLafferty), p. 399. Academic Press, New York.

Nepeta cataria, it is suggested that the large variations observed among the samples are due to genetic variation within the species. This idea is supported by the fact that plants were occasionally found in the greenhouse stock which had a Ia–Ib ratio of approximately 3:1 (Table 1). The higher concentration of Ib in commercial catnip oil may be due to: (*a*) contamination of *N. cataria* with other species, such as *N. mussini*, which is known to contain large concentrations of Ib,[7] during harvest, (*b*) hybridization occurring where different species grow together, or (*c*) genetic variability within the species. Since the origin of the commercial oil is not known, no definite conclusion can be drawn regarding this sample.

The observation that the ratio of Ia–Ib is constant throughout the various parts of the plant made it possible to determine this ratio in the whole plant by analyzing only one small section of a leaf by gas chromatography. These findings are of considerable value in biosynthesis studies.[6] A plant may be selected for the biosynthesis of nepetalactone or both nepetalactone and epinepetalactone by the analysis of a part of a single leaf.

EXPERIMENTAL

Plant Material

The plants were grown from seeds and cuttings in the greenhouse of the Department of Horticulture of Oklahoma State University. Additional oil of catnip was obtained from catnip plants grown near Fairbury, Nebraska, and East Lansing, Michigan; these samples are referred to as Nebraska oil and Michigan oil respectively.

Isolation of the Essential Oil

The plants were subjected to steam distillation for 1·5 hr, using an all-glass system. The distillate was saturated with sodium chloride and extracted with ethyl ether. The ether solution was dried over anhydrous magnesium sulfate, filtered and the essential oil concentrated by distilling at atmospheric pressure. The essential oil was stored at −15° until used.

Gas–Liquid Chromatography

Gas–liquid chromatography was performed on a Barber Coleman Model 5000 gas chromatograph equipped with a hydrogen flame detector. Stainless steel capillary columns were used, one 45 m × 0·5 mm coated on the inside with Apiezon L and the other 120 m × 0·5 mm coated on the inside with LAC 886. Nepetalactone (Ia) and epinepetalactone (Ib) were gas chromatographed on the Apiezon L column at 150°. The dimethyl nepetalinates (Va–Vd) were gas chromatographed on the LAC 886 column at 178°. Peak areas were measured by planimetry and converted to amounts of materials by comparison with standard curves obtained with pure compounds. Nepetalactone (Ia), nepetalic acid (IIb), and nepetalinic acids (Va) with known absolute configuration and stereochemistry were available from earlier studies[9] and were also freshly prepared. Apiezon L and LAC 886 were purchased from Applied Science Labs., State College, Pennsylvania.

Conversion of Ia and Ib to the Dimethyl Nepetalinates.

Approximately 0·1 ml of crude catnip oil was treated with 1 ml of 10% NaOH and stirred for 1 hr at room temperature. The neutral components of the oil were removed by three successive extractions with equal volumes of ethyl ether. After adjustment to pH 2 with 2 N HCl, the solution was extracted again with ethyl ether and the crude nepetalic acid was esterified with diazomethane. Air oxidation for 48 hr at room temperature yielded the half ester of nepetalinic acid. The samples were prepared for gas chromatography by esterifying a second time with diazomethane. This scheme is shown in Fig. 1.

Mass Spectrometry.

A prototype[17] of the LKB-9000 Mass Spectrometer–Gas Chromatograph was used to obtain mass spectra of the gas chromatographic eluates. The mass range 12–200 was scanned in 2·25 sec so that concentration changes during the course of a scan were minimized. Spectra were taken at 20 eV.

[17] G. R. Waller, *Proc. Oklahoma Acad. Sci.* **47**, In press (1967).

Preparation of Diazomethane Using EXR-101*

In a 500-ml flask, a solution of 24 g of NaOH in 20 ml of water, 60 ml of Carbitol (diethylene glycol monoethyl ether), and 150 ml of ether were cooled to 0°, and then 7·1 g of EXR-101 was added at one time. Magnetic stirring was started and the reaction mixture was warmed slowly. The evolution of CH_2N_2 became apparent at 15° to 20°. In the 30–40° range, the CH_2N_2 and ether distilled and condensed to a bright yellow solution. The reaction was assumed complete when the yellow color in the reaction flask and in the distilling ether disappeared. During this reaction, the receiver should be cooled with dry ice (without acetone) and ether should be added from the dropping funnel whenever the amount of ether in the reaction flask becomes small. By this method, an ether solution containing 2 g of CH_2N_2 was obtained from 7·1 g of EXR-101.

This ether solution was used to esterify the acids by adding it dropwise until a permanent yellow color was obtained and nitrogen was no longer evolved during addition.

Acknowledgement—We wish to thank Professor U. T. Waterfall of the Botany Department for making the plant classifications and Dr Earl D. Mitchell for helpful discussions.

* Product of E. I. du Pont de Nemours and Co., Penns Grove, New Jersey; EXR-101 contains 70 per cent active ingredient, N,N′-dinitroso-N,N′-dimethylterephthalamide, and 30 per cent mineral oil.