VOLATILE CONSTITUENTS OF BRAZILIAN EUPHORBIACEAE. GENUS *CROTON*[1]

A. A. Craveiro, A. S. Rodrigues, C. H. S. Andrade, F. J. A. Matos,
J. W. Alencar and M. I. L. Machado

Universidade Federal do Ceará, Departamento de Química Orgânica e Inorgânica,
60.000 Fortaleza, Ceará, Brazil

Abstract.—The analysis of essential oils in several species of the Euphorbiaceae family was conducted as part of an intensive program of chemical investigation of odoriferous flora of northeastern Brazil. From 24 different *Croton* species studied, we have selected nine to report on in this work: *C. sonderianus*, *C. essequiboensis*, *C. argyrophylloides*, *C. affinis mucronifolius*, *C. rhamnifolius*, *C. nepetaefolius*, *C. jacobinensis*, *C. micans* and *C. zehntneri*; in the latter species at least two varieties were detected as far as the chemical composition is concerned. The isolated oils were analyzed by gc/ms with open tubular glass column coupled to a data processing system. Library search programs for identification of essential oils constituents were developed; algorithms were used for computation of chromatographic retention times (Kovat's indexes) and simplified mass spectra (ms). Confirmations were made by comparison of full ms or other spectroscopic data with that of standard samples whenever available. Library search programs using mass spectral data and Kovat's indexes were found to be an efficient method for identification of chemical constituents in essential oil analysis.

Before 1975 very little information was available about odoriferous flora of northeastern Brazil. At the present time over two hundred essential oils from different plants are under pharmacological and chemical screening as well as botanical investigation.

In the Euphorbiaceae family, the genus *Croton*, due to high incidence and species dispersion, contains by far the largest number of essential oil producing plants in the Brazilian northeast.

This communication describes library search programs based on gas chromatography (gc) and mass spectrometric data developed for essential oil analysis and its application to the investigation of ten samples extracted from Brazilian Euphorbiaceae genus *Croton*; *C. argyrophylloides* Muell. Arg. (1); *C. essequiboensis* Kl; *C. jacobinensis* Baill; *C. micans* Muell. Arg.; *C. sonderianus* Muell. Arg. (2); *C. nepetaefolius* Baill. (3); *C. rhamnifolius* H.B.K.; *C. zehntneri* var *anethole* Pax et (4) Hoff.; and *C. zehntneri* var *eugenol* Pax et Hoff.

Among several works (5–11) dealing with retention index coupled to mass spectral data in library search programs, only one is concerned with identifications of lower terpenoids and sesquiterpenoids and uses *n*-heptyl acetate (=1.000) as internal standard to calculate retention times (10).

After creating a library of 200 entries and developing a library search program based on mass spectral data for identification of components in essential oils (12), we found it necessary to improve the program with the inclusion of retention indexes.

To minimize the standard deviation arising from employing a sole substance as internal standard, we decided to employ a retention index, namely, Kovat's Index (13) obtained by use of a mixture of the essential oil with eight *n*-alkanes (C_8, C_9, C_{10}, C_{11}, C_{12}, C_{14}, C_{16} and C_{18}) as internal standards. This approach greatly improved the identification, especially for those compounds with very similar fragmentation patterns.

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DISCUSSION

Kovat's indexes calculations by mass chromatography.—To calculate Kovat's indexes, eight *n*-alkanes were mixed in convenient proportions to the essential oil to be analyzed. The mixture was then injected in a gc/ms equipped with an open tubular glass column and a data processing system. For comparision purposes, separated runs of *n*-alkanes and the essential oil were made.

Reconstructed mass chromatograms of the *n*-alkanes were obtained by use of the ion *m/z* 85 (11). The computer reconstructed gc of the essential oil was obtained by mass chromatography with ion *m/z* 91, which was always present in terpenes, sesquiterpenes, their oxygenated derivatives, and aromatic compounds commonly found in the essential oils (12). The relative intensity of ion *m/z* 91 in these substances never fell below 30% of the base peak.

Mass chromatography of the ions *m/z* 85 and *m/z* 91 made it possible to obtain, with a single injection of the essential oil plus *n*-alkanes, information usually obtained with more than two injections, such as:

a) the computer plot of the total ion current of the mixture (see fig. 1 as an example). This information is important for calculating Kovat's indexes.

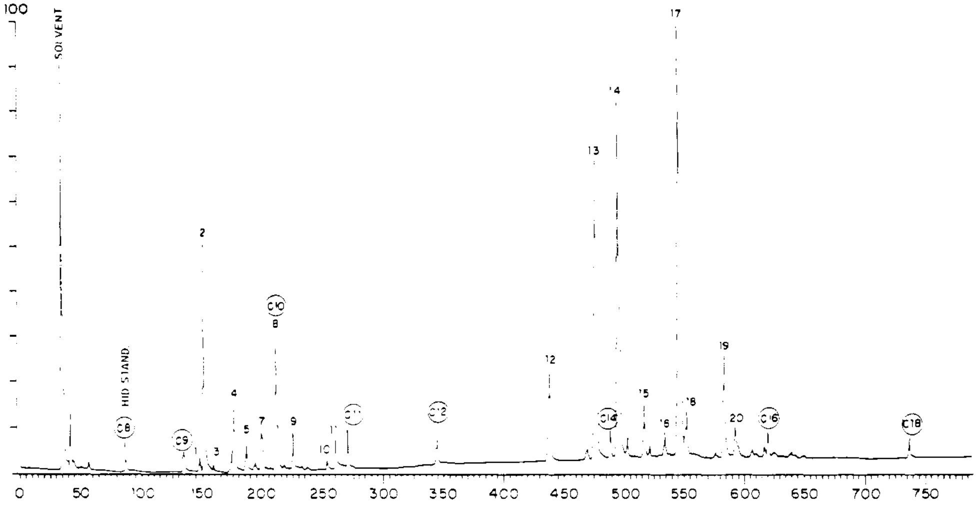

FIG. 1. Total ion chromatogram of the essential oil of *Croton micans* plus *n*-alkanes.

b) computer reconstructed *n*-hydrocarbons standards chromatogram where only the peaks having *m/z* 85 are plotted. (see fig. 2).

c) essential oil computer reconstructed chromatogram where only the peaks having the ion *m/z* 91 are plotted (see fig. 3). This ion reproduces a clear qualitative plot of the essential oil when compared with the total ion current of the same oil.

The peak superposition, if present, is easily detected by matching the total ion chromatogram against reconstructed chromatograms of ions *m/z* 85 and *m/z* 91.

Computer programs.—Essential oil analysis of the species related here were performed with the aid of three computer programs AREA 1, KOVT 16, and BSKO 10 written in BASIC language.

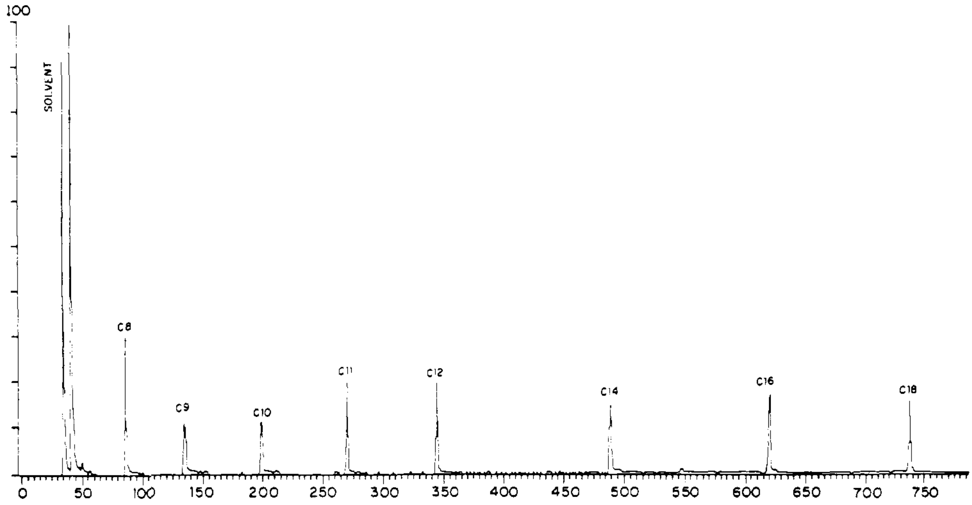

FIG. 2. Reconstructed chromatogram from the mixture shown in fig. 1 monitoring the ion m/z 85 (n-alkane peaks).

AREA 1.—Uses the scans at the top and bottom of each peak for calculating the peak area.

KOVT 16.—Uses the expression {1} for calculating Kovat's indexes.

$$KI = 100\,(C_{n-1}) + (C_n - C_{n-1})\,\frac{N_n - P_{n-1}}{P_n - P_{n-1}} \qquad \{1\}$$

where C_n and C_{n-1} = carbon numbers hydrocarbons standards which bracket the unknown peak in retention time.

N_n = scan number of unknown, searched by the ion m/z 91

P_n and P_{n-1} = scan numbers of n and n-1 hydrocarbons standards, searched by the ion m/z 85.

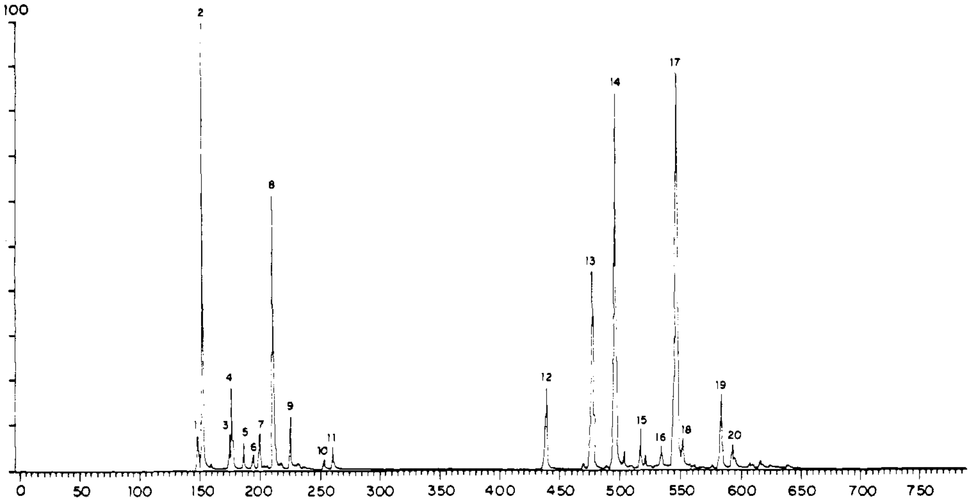

FIG. 3. Reconstructed chromatogram from mixture shown in fig. 1 monitoring the ion m/z 91 (essential oil peaks).

BSKO 10.—A library search program for automatic identification of unknowns based on mass spectral data using visually interpretative peaks (VIP) (14) and Kovat's indexes (13).

It uses the expression {2} for calculating an Identity Grade (IG), matching the unknown against each library entry. It also uses the arbitrary factor (IG+1)/2 for raising IG if the Kovat's index of the selected entry is within the range ±3 of the calculated Kovat's index for the unknown. The factor IG/2 is used for decreasing IG if the range is larger than ±3.

$$IG = \frac{1}{2N}\left(C + \sum_{N=1}^{N} \frac{I_1}{I_2}\right) \qquad \{2\}.$$

where

IG = Identity grade (=0 for complete mismatching and =1 for complete Identity), which indicates how similar the mass spectrum of each library entry (reduced to VIP) is when matched against the mass spectrum of the unknown.

N = Number of VIPs in each matched library entry.

C = Number of VIPs with same *m/z* in both the unknown and the library entry.

I_1 and I_2 = lower and higher intensity peak in each pair being compared.

TABLE 1. Identification of peak no. 9 in Chromatogram 3 by a library search program using only mass spectral data.

Entry	Compound	IG
04	α-pinene	0.84
05	car-3-ene	0.83
09	γ-terpinene	0.81
23	triciclene	0.80
17	bornilene	0.74

The use of this program is illustrated by the identification of compound no. 9 (fig. 3) in the essential oil *Croton micans*.

This compound was found by visual analysis to be car-3-ene. This identification was also achieved by BSKO 10 program but not by a program using only mass spectral data where α-pinene had the highest similarity (see tables 1 and 2).

The inspection of the α-pinene (fig. 4) and car-3-ene (fig. 5) mass spectra showed only minor quantitative differences, although they had distinct Kovat's indexes (927±3 and 1040±3, respectively). This made it easy for the program

TABLE 2. Identification of peak no 9 in Chromatogram 3 by BSKO 10 program.

Entry	Compound	IG
05	car-3-ene	0.91
04	α-pinene	0.42
09	γ-terpinene	0.41
23	triciclene	0.40
17	bornilene	0.17

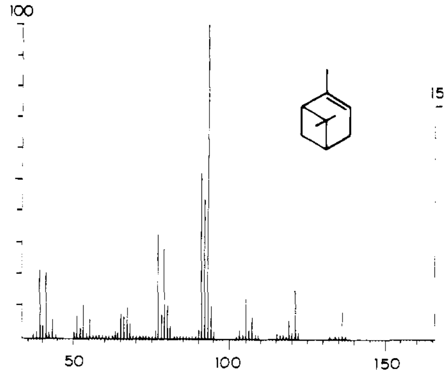

FIG. 4. Mass spectrum of α-pinene.

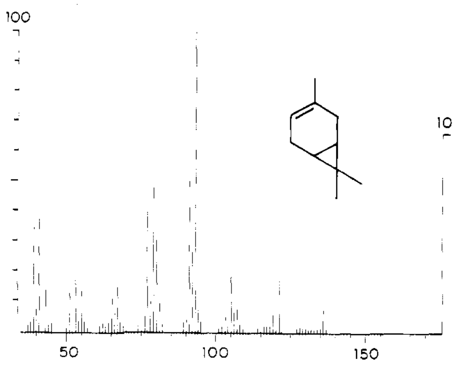

FIG. 5. Mass spectrum of car-3-ene.

BSKO 10 to find the right answer by matching Kovat's indexes of the unknown against the Kovat's indexes of selected entries.

Essential oils.—Essential oils found in table 3 were analyzed by the method described. Confirmations of the identified compounds were made by visual matching of each mass spectrum against standards of a certified collection (15), and by the use of other spectrometric methods (nmr) and authentic samples, if available. The final results are summarized in table 4.

TABLE 3. Essential oils from Euphorbiaceae species genus *Croton*: voucher, collection sites and times, part studied and yields.

File[1]	*Croton* species	Voucher[2]	Collection sites (Brazil)	When Collected	Part studied	yield[4]
F-001	*C. argyrophylloides*	3088	Viçosa-Ceará[4]	Feb. 77	Leaves	1.0
F-010	*C. essequiboensis*	3084	Taperoaba-Ceará[5]	Feb. 77	Leaves	0.3
F-130	*C. jacobinensis*	3156	Cocal-Piauí[6]	Apr. 77	Leaves	0.5
F-224	*C. micans*	3767	Jacobina-Bahia[7]	Feb. 78	Leaves	0.5
F-011	*C. aff. mucronifolius*	3092	Viçosa-Ceará[4]	Feb. 77	Leaves	2.2
F-092	*C. nepetaefolius*	3185	Taperoaba-Ceará[5]	Mar. 77	Leaves	0.03
F-247	*C. rhamnifolius*	2813	Pedra Branca-Ceará[8]	Jun 77 and Apr. 78	Leaves	0.4
F-015	*C. sonderianus*	3085	Taperoaba-Sobral-Ceará[4]	Feb. 77	Leaves	0.3
F-414	*C. zehntneri* var *anethole*	5728	Tianguá-Ceará[9]	Mar. 79	Leaves	1.7
F-025	*C. zehntneri* var *eugenal*	3116	Quixadá-Ceará[10]	Mar. 77	Leaves	1.0

[1] File number in the Dep. de Química Orgânica—Universidade Federal do Ceará—Brazil.
[2] Registry number in the Herbarium at Depart. de Biologia—Univ. Fed. Ceará—Brazil.
[3] Voucher is from material when first collected (June, 1976); analysis is from recollected plant material (April, 1978).
[4] Lat. 3°23'S long. 4°6'W; [5] Lat. 4°4'S long. 4°6' W; [6] Lat. 3°29'S Long. 41°34'W; [7] Lat. 11°13'S Long. 40°30'W; [8] Lat. 5°26'S long 39°44'W; [9] Lat. 3°44'S long. 40°59'W; [10] Lat. 4°58'S long. 30°0'W.

EXPERIMENTAL

PLANT COLLECTION.—The plants were collected at the locations mentioned in table 3. Botanical identifications were made by Prof. Afrânio G. Fernandes, Universidade Federal do Ceará, Brazil, and Prof. G. L. Webster University of California, USA. Voucher specimens

TABLE 4. Chemical composition of essential oils from Euphorbiaceae species, genus *Croton*.[e]

Compound*	VOUCHER**										Confirmation
	3088	3084	3156	3767	3092	3185	2813	3085	5728	3116	
MONOTERPENOIDS											
car-3-ene	—	—	—	—	1.6	—	0.6	0.7	—	—	—
α-phellandrene	—	—	—	1.1	1.2	—	9.9	2.9	—	—	a
myrcene	—	—	—	1.3	—	—	—	1.5	1.1	—	a, b.
α-pinene	27.4	18.4	—	7.9	1.2	1.5	—	6.6	2.7	—	a, b
β-pinene	—	14.1	—	2.3	—	1.5	—	—	—	—	a. b
sabinene	41.1*	—	—	0.6	—	3.0	6.5	1.5	—	—	a, b
γ-terpinene	—	—	—	—	2.0	—	—	—	—	—	a, c
α-terpinolene	—	—	1.0	—	—	—	—	2.1	—	—	—
α-thujene	—	—	—	0.8	—	—	—	0.7	—	—	—
p-cymene	—	—	—	—	32.4	—	20.3*	14.3	—	2.9	—
borneol	—	—	—	—	1.6	—	—	—	—	—	—
camphor	—	—	—	—	6.2	—	—	—	—	—	a, b, c
1.8-cineole	—	—	—	—	2.7	37.5	11.0	—	—	—	a, b, c
geranial	—	—	—	—	—	—	—	—	—	23.1	a
linalool	—	—	—	—	—	—	2.8	—	—	0.5	a
neral	—	—	—	—	—	—	—	—	—	13.7	a
α-terpineol	—	—	—	—	—	4.0	1.6	—	—	—	a
terpinen-4-ol	—	—	—	—	—	—	1.1	—	—	—	—
ascaridole	—	—	—	—	1.2	—	—	—	—	—	a
iso-ascaridole	—	—	—	—	28.9	—	—	—	—	—	a
TOTAL	68.50	32.50	1.00	14.00	79.00	47.50	53.80	30.30	3.80	40.20	—
PHENYLPROPANOIDS											
t-anethole	—	1.2	—	—	—	—	—	—	32.3	—	a, b, c
estragole	—	0.6	—	—	—	—	—	—	52.4*	0.5	a, b
eugenol	—	—	—	—	—	—	—	—	—	47.2*	a, b, c
methyl eugenol	—	—	—	—	—	0.5	—	—	—	—	a
n-propil-catechol	—	—	—	—	—	1.0	—	—	—	—	—
elemicin	—	—	—	—	—	4.0	—	—	—	—	a, b
TOTAL	—	1.80	—	—	—	5.50	—	—	84.70	47.70	—
SESQUITERPENOIDS											
aromadendrene	—	—	0.5	—	—	—	3.3	3.2	—	—	—
α-bergamoptene	—	—	—	—	—	—	—	—	—	6.1	—
γ-cadinene	—	—	—	—	—	—	6.3	2.2	—	—	—
β-caryophyllene	4.5	19.6	4.0	19.0*	—	23.0	5.3	24.4*	1.1	1.7	a, b, c
α-copaene	—	0.6	—	—	—	—	0.8	—	—	—	a
α-cubebene	—	0.4	—	—	—	—	1.1	—	—	—	a
B-elemene	2.3	1.2	28.0	14.2	—	1.0	—	3.3	—	—	—
γ-elemene	7.6	—	33.8	6.8	—	12.0	3.6	—	5.9	—	a
δ-elemene	—	—	2.8	5.2	—	4.5	—	—	—	—	a, b
α-farnesene	—	—	2.3	—	—	—	—	—	—	—	—
β-farnesene	—	—	0.5	—	—	—	—	—	—	0.9	—
germacrene B	—	—	—	17.1	—	—	—	—	—	—	—
β-guayene	—	—	—	—	—	—	—	—	—	0.9	—
α-humulene	1.4	5.2	2.4	2.1	—	2.0	2.4	2.7	—	—	a, b
γ-muurolene	—	—	—	—	—	—	—	—	1.6	—	—
TOTAL	15.80	27.00	74.30	64.40	—	42.50	22.80	35.80	8.60	9.60	—
n-identified	15.70	38.70[d]	24.70	21.60	21.00	4.50	23.40	33.90	2.90	2.50	—

*Authentic samples of α-pinene, β-pinene, γ-terpinene, β-caryophyllene, myrcene were from Dragoco S.A. (São Paulo, Brazil); borneol, 1.8-cineole, anethole, from Farbejé (N. York, USA); p-cymene, eugenol and elemicin from author's collection.

**Voucher numbers, deposited in Dep. de Biologia Univ. Fed. do Ceará—Brazil: 3088 *Croton argyrophylloides*; 3084 *C. essequiboensis*; 3156 *C. jacobinensis*; 3767 *C. micans*; 3092 *C. affinis mucronifolius*; 3185 *C. nepetaefolius*; 2813 *C. rhamnifolius*; 3085 *C. sonderianus*; 5728 *C. zehntneri* var *anethole*; 3116 *C. zehntneri* var *eugenol*.

a. Kovat's index; b. Kovat's index and nmr; c. Kovat's index, nmr and authentic sample.

[d] Major constituent (30.2%) among not identified. [e] Values are expressed in per cent.

were deposited in the herbarium of the Departamento de Biologia Universidade Federal do Ceará under the numbers specified in table 3.

EXTRACTION.—Essential oil extraction was carried out by steam distillation of finely ground plant material (16). The yields (volume/volume) are reported in the text. Each essential oil, after separation, was dried with anhydrous sodium sulfate, filtered, and sealed in glass vials under nitrogen atmosphere.

ANALYSIS.—The fresh oils were analyzed on a FINNIGAN 3300 quadrupole mass spectrometer coupled to a gas chromatograph with an open tubular glass column (30m x 0.25mm id) with SP 2100 as the stationary phase with helium as the carrier gas. On line mass chromatography computations were made on a 6115 Finnigan data processing system with 32 K configurations with the aid of 3 megabite disk, magnetic tapes and the library search programs described here (17).

The nmr data were obtained in a Varian EM 360 and Varian XL 100 nmr instruments.

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17. Program listings are available on request.