

Table I. Comparison of % TNC in Cotton Stems by the Authors' and Smith's Methods

Sample no.	Methods	
	Authors'	Smith's
1	11.3	12.1
2	8.2	8.2
3	11.5	12.1
4	8.4	8.6
5	11.3	12.0
6	8.0	8.2
7	11.8	12.5
8	8.5	9.4
9	12.0	12.1
10	8.6	8.5
	Av ^a	10.37

^a A standard statistical comparison of the averages based on the "t" test showed no significant differences ($P < 0.01$).

with ten replications showed a high degree of accuracy. Ten replicate analyses of cotton plant samples with 10% to 15% TNC gave standard deviations of 0.28 and 0.14, respectively. The same degree of accuracy was obtained when analyzing different parts of cotton plants and lyophilized cactus pads (*Opuntia*, s.p.) harvested at different seasons of the year. These materials ranged from 10% to 70% TNC. The authors' technique can be conducted for about one-tenth the cost of other methods in use. A complete analysis can be performed in 24 h elapsed time, while other techniques require 3 to 4 days. It should also be noted that a minimum of glassware is required. This represents a saving of initial cost and routine glassware-washing time. It is especially noteworthy that the detergent action of the Teles' reagent allows effective cleansing of the Folin tubes by simple rinsing four times with distilled water. In addition, it also allows the use of a flow-through curvette. The reduced number of operations make the technique more accurate and less tedious. The reagents used, in addition to being less expensive, can be prepared with distilled water with no need for deionizers or other water retreatment. The substitution of dinitrosalicylic acid (Summer and Somer, 1949) for Teles' reagent gave similar results. One technician easily analyzed an average of 96 samples in 24-h elapsed time.

In case the laboratory is equipped with only an ordinary centrifuge (maximum speed ca. 1500 rpm), the use of 4 mL

of a 2% solution of neutral lead acetate (only the neutral salt) in water instead of 4 mL of distilled water before centrifugation also gives a clear supernatant. This amount of lead salt does not interfere with the analysis as was demonstrated in the course of the experiment with comparative analyses. The lead precipitation also would allow aliquot filtration, but the authors avoided this operation since it increases time and cost.

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LITERATURE CITED

- Burriss, J. S., Brown, R. H., Blaser, R. E., *Crop Sci.* **7**, 22 (1967).
 Greub, L. J., Wedin, W. F., *Crop Sci.* **9**, 595 (1969).
 Heinze, P. H., Murneek, A. E., *Mo., Agric. Exp. Stn. Res. Bull.* **314** (1940).
 Smith, D., *Wis., Agric. Exp. Stn. Res. Rep.* **41** (1969).
 Smith, D., Paulsen, G. M., Raguse, C. A., *Plant Physiol.* **39**, 960 (1964).
 Sumner, J. B., Somers, G. F., "Laboratory Experiments in Biological Chemistry", Academic Press, New York, N.Y., 1949, p 38.
 Teles, F. F. F., Ph.D. Dissertation, The University of Arizona, Tucson, 1977.
 Teles, F. F. F., Young, C. K., Stull, J. W., *J. Dairy Sci.* **61**, in press (1978).
 Weinman, H., *Plant Physiol.* **22**, 279 (1947).
 Yemm, E. W., Willis, A. J., *Biochem. J.* **57**, 508 (1954).

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Anise-Like Flavor of *Croton* aff. *zehntneri* Pax et Hoffm.

Estragole, the major constituent of the essential oil of *Croton* aff. *zehntneri*, family Euphorbiaceae, occurs together with minor quantities of camphor, anethole, isoborneol, caryophyllene, γ -elemene, safrol, methylisoeugenol, *n*-heptadecane, and *n*-eicosane. These compounds are being reported for the first time in the genus *Croton*. Fifteen predominant volatile constituents of the oil were analyzed by GC-MS and identified. The oil has a typical pleasant odor reminiscent of anise (*Pimpinella anisum* L.)

The genus *Croton* is largely disperse in the semiarid lands of Brazilian Northeast and includes many species that produce essential oils with pleasant odors.

This report deals with the examination of the volatile constituents of *Croton* aff. *zehntneri* (canelinha) family Euphorbiaceae collected in the state of Ceará. The in-

fusion of the leaves is used by local people as a beverage in place of coffee and as a remedy for stomachache and insomnia.

EXPERIMENTAL SECTION

Analytical GLC was performed on a FID instrument

Table I. Volatile Constituents of *Croton aff. zehntneri*

Compound ^a	Leaves, %	Stems, %	Wood, %	Identification
α -Pinene		t		MS, GLC
β -Pinene		t		MS, GLC
Myrcene	1	t	3	MS, IR, GLC
1,8-Cineole		2	2	MS, IR, GLC
Camphor		t		MS, GLC
Isoborneol		t		MS, GLC
Estragole	61	56	9	MS, RMP, GLC
<i>trans</i> -Anethole		t		MS, GLC
Methyleugenol	5	34	34	MS, RMP, IR, GLC
Methylisoeugenol		t	26	MS, GLC
Safrole	7			MS, IR, GLC
Caryophyllene	2	5	8	MS, GLC
γ -Elemene	1	1	2	MS, GLC
<i>n</i> -Heptadecane ^b		t		MS
<i>n</i> -Eicosane ^b		t		MS
Unidentified	24		16	

^a Authentic samples of α -pinene, β -pinene, and caryophyllene were supplied by Dragoco S. A. (Sao Paulo, Brasil); isoborneol, 1,8-cineol, and *trans*-anethole from Farbeje (New York); myrcene, camphor, estragole, methyleugenol, methylisoeugenol, safrole, and γ -elemene from the author's collection. ^b Comparison with reference spectrum (Stenhagen et al., 1974). No authentic samples available.

equipped with a stainless steel column (3 mm \times 1.5 m) packed with 1.5% OV-101 on Chromosorb W programmed from 50–250 °C, at a rate of 4 °C/min, using N₂ as the carrier gas.

Preparative GLC was carried out using a modified FID chromatograph (splitter 100:1) with a 9 mm \times 6.5 m aluminum column packed with 20% OV-210 on Chromosorb W.

GC-MS separation was carried out with a glass column (3 mm \times 1.5 m) packed with 3% OV-1 on Chromosorb W coupled to a quadrupole mass spectrometer operated at 70 eV.

Infrared spectra were obtained using liquid films.

NMR spectra were run in CCl₄, using a 60 MHz instrument and TMS as internal standard. Double resonance experiments were realized with the same above conditions at room temperature.

Extraction of the Oils. The plant was collected in Araripe-Ceará and transported to the laboratory in an air-conditioned vehicle. Finely ground material was steam distilled in a modified steam generator (Craveiro et al., 1976); the oils were dried with anhydrous Na₂SO₄ and sealed in all-glass vials under N₂.

RESULTS AND DISCUSSION

The essential oils were obtained by steam distillation of the leaves, stems, and wood in yields of 0.7, 2.5, and 0.2%, respectively. The chemical composition of the above oils is described in Table I.

The separations were performed initially with a GLC analytical column, followed by analysis with a GC-MS system. Whenever possible, preparative GLC was used to allow NMR and IR confirmations. Previous work in the essential oils of the Euphorbiaceae family, genus *Croton* (Guenther, 1950; Bracho and Crowley, 1966; Craveiro et al., 1976) do not make any reference to the present species which is native to the Brazilian Northeast.

The presence of estragole, camphor, anethole, isoborneol, caryophyllene, γ -elemene, safrole, methylisoeugenol, *n*-heptadecane, and *n*-eicosane is related for the first time in the genus as far as we know. Estragole imparts to the oil the characteristic flavor similar to anise seed oil. Methyleugenol, a potent anesthetic (Carlini et al., 1976), should be responsible for the pharmacological activity of the plant infusions.

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LITERATURE CITED

- Bracho, R., Crowley, J. K., *Phytochemistry* 5, 921 (1966).
 Carlini, E. A., Sell, A. B., *Pharmacology* 14, 367 (1976).
 Craveiro, A. A., Alencar, J. W., Matos, F. J. A., Andrade, C. H. S., *Cienc. Cult. (Sao Paul.)*, Suppl. 28, 180 (1976).
 Craveiro, A. A., Alencar, J. W., Matos, F. J. A., *J. Chem. Educ.* 53, 652 (1976).
 Guenther, E., "The Essential Oils", Vol. VI, Robert E. Krieger Publishing Co., Huntington, 1950, p 169.
 Stenhagen, E., Abrahamson, S., "Registry of Mass Spectral Data", McLafferty, F. W., Ed., Wiley, New York, N.Y., 1974.

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