Journal of Chromatography, 116 (1976) 468–471

CHROM. 8763

Note

Simple solid injection method for qualitative and quantitative estimation of essential oils

U. M. SENANAYAKE, R. A. EDWARDS and T. H. LEE

Department of Food Technology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033 (Australia)

(Received July 1st, 1975)

The direct gas chromatographic (GC) analysis of essential oils of plants has been described by several authors[1–8]. This technique offers advantages over liquid injection of solvent or steam-distilled extracts of the oils[9]; it may be applied to animal tissue[10] or to extracted volatile solids deposited on an inert material[11–13]. While pieces of plant tissue as small as 10 μg fresh weight[6] may be analysed, Von Rudloff[9] warns that the results, although they may be quantitative for the piece of tissue analysed, must be interpreted carefully as being representative of a larger tissue sample.

In most of the techniques described, the oils are volatilized from the tissue in a pre-heating attachment and then swept on to the GC column. A heated injection port that achieves volatilisation of oils from plant material has been described briefly by Henderson *et al.*[6], who placed the tissue sample (less than 1 mg fresh weight) in a proprietary glass liner tube that was then inserted into the injection port. Baerheim Svendsen and Karlsen[3] have described a procedure whereby 10–20 mg fresh plant material is placed into a basket which is inserted into the injection port by means of a magnet. No comparison, however, appears to have been made of such solid injection techniques against other methods of oil extraction such as steam distillation or solvent extraction.

This paper describes a direct loading technique which should be applicable to most gas chromatographs without instrument modification and compares results with those from solvent extraction techniques. The plant material is placed in a stainless-steel mesh basket which fits inside the injection port; the basket is of simple construction and the sample may be recovered for further analysis. We have been using the technique successfully in studies on the nature, biosynthesis, and site of synthesis of the major volatiles of *Cinnamomum zeylanicum*.

EXPERIMENTAL

Plant material

Root, bark, and leaf samples of *Cinnamomum zeylanicum* Nees were collected from the Sydney Royal Botanic Gardens and dried at room temperature. The dried tissue was cut into thin strips immediately before analysis.

Solid injection system and technique

The column was equilibrated at room temperature and a 5-cm section adjacent to the injection port was cooled with ice. The injector cap was removed and a stainless-steel mesh basket (2.5 × 0.3 cm) (Fig. 1) containing plant tissue (15–60 mg) was inserted into the injector port. A stainless-steel wire served to both insert and remove the basket. Following sample insertion, the injector cap was securely replaced and the volatiles vapourised from the tissue at 180° for 5 min. The fore-column was continually cooled with ice during this period which has been found sufficient for complete vapourisation of tissue volatiles as judged by solvent extraction studies on the residue. The injection cap was then removed and the basket withdrawn, using forceps, without the need to interrupt carrier gas flow. The injector cap was replaced, the ice condenser was removed, and the oven temperature was raised to 90° for 2 min, followed by programming at 2°/min to a final temperature of 240°.

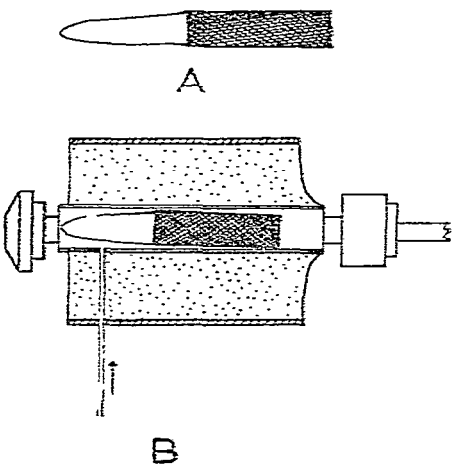

Fig. 1. Small stainless-steel mesh basket (A) which can be inserted and removed from the injection port (B) of a gas chromatograph.

The following operating conditions were used: gas chromatograph, Varian Aerograph 1520 equipped with Vidar 6300 digital integrator; detector, flame ionisation detector; column, 4.57 m × 3.2 mm I.D. stainless-steel coil packed with 10% Carbowax 20M on Chromosorb W AW DMCS, 100–120 mesh; flow-rate of carrier gas (nitrogen), 12 ml/min; flow-rates of hydrogen and air to detector, 25 and 250 ml/min, respectively.

RESULTS AND DISCUSSION

The most highly prized part of the cinnamon plant is the stem bark which becomes the cinnamon of commerce; the major volatiles of this tissue have been characterised through GC separation of the steam-distilled oil[14]. This method of oil recovery is comparable to that used in the commercial preparation of cinnamon bark oil, however, we have found the oil to be more completely extracted with a solvent

such as carbon disulphide[15]. Thus we have qualitatively examined the pattern of volatiles of dried stem bark as indicated by our solid injection technique (Fig. 2A) and by carbon disulphide extraction (Fig. 2B). Each volatile was then quantified by the peak area method as a percentage of the total peak area (Table I). The solid injection technique recovered significantly higher amounts of two of the major volatiles contained in this tissue, namely, β-caryophyllene and an unknown compound (peak 13). Comparable amounts of the other volatiles were recovered by both methods. Total volatiles recovered by the solid injection method was slightly higher than that by solvent extraction method judging by the peak areas. Both operations were carried out at the same attenuation.

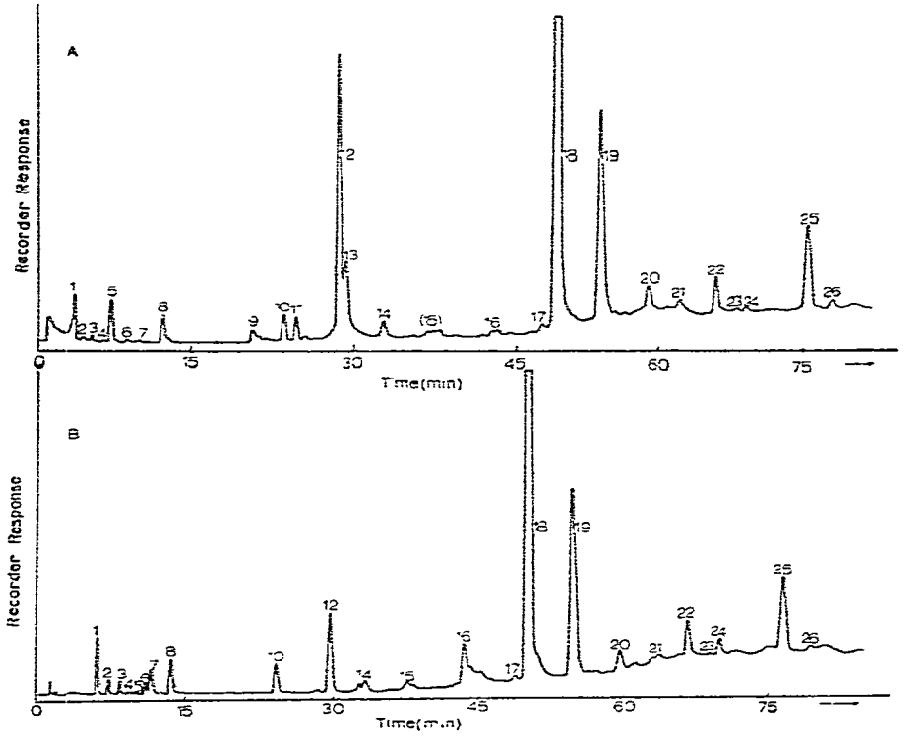

Fig. 2. GC pattern of the volatiles recovered from 60 mg of dried cinnamon stem bark using (A) solid injection and (B) carbon disulphide extraction.

The solid injection technique for determining the volatile pattern of thin pieces of plant tissues compares favourably, both qualitatively and quantitatively, with that of solvent extraction. The technique is extremely rapid and reproducible, works equally well on fresh and dried plant material, has given excellent results with other parts of the cinnamon plant such as root bark and leaves, and the residue is easily recovered for further analysis such as dry weight determination or radioactive counting.

TABLE I

QUANTITATIVE COMPARISON OF THE VOLATILES OF CINNAMON STEM BARK BY SOLID INJECTION TECHNIQUE AND SOLVENT EXTRACTION WITH CARBON DISULPHIDE

Each volatile is expressed as a percentage of the total peak area (means of duplicate analyses). Peak numbers refer to Fig. 2. ND = not detected.

Peak no.	*Compound or volatile*	*Solid injection*	*Solvent extraction with CS_2*
1	α-Pinene	1.0	1.3
2	Camphene	0.12	0.3
3	β-Pinene	0.18	0.25
4	α-Phelandrene	1.00	trace
5	α-Terpinene	1.00	trace
6	Limonene	trace	0.2
7	1:8-Cineole	trace	0.5
8	p-Cymene	0.8	0.8
9	Unknown	0.6	ND
10	Linalool	0.7	1.0
11	Unknown	0.6	ND
12	β-Caryophyllene	8.0	2.5
13	Unknown	2.6	ND
14	α-Terpineole	0.4	0.3
15	Cuminaldehyde	trace	trace
16	Safrole	0.5	2.0
17	Unknown	0.2	0.2
18	Cinnamic aldehyde	58.2	62.0
19	Eugenol	9.1	9.0
20	Cinnamyl alcohol	2.0	1.4
21	Unknown	—	—
22	Unknown	2.0	1.8
23	Unknown	trace	trace
24	Unknown	trace	1.6
25	Benzyl benzoate	3.7	4.0
26	Unknown	trace	trace

ACKNOWLEDGEMENTS

We acknowledge the assistance of Mr. Peter Hind, Royal Botanic Gardens, Sydney, the advice of Mr. W. R. Day, and the Australian Government for the award of a Colombo Plan Fellowship to U.M.S.

REFERENCES

1 C. Mathis and G. Ourisson, *Phytochemistry*, 3 (1964) 133.
2 E. von Rudloff, *J. Gas Chromatogr.*, 3 (1965) 390.
3 A. Baerheim Svendsen and J. Karlsen, *Planta Med.*, 14 (1966) 376.
4 E. P. Swan, *Forest Prod. J.*, 16 (1966) 51.
5 R. M. Harley and M. G. Bell, *Nature (London)*, 213 (1967) 1241.
6 W. Henderson, J. W. Hart, P. How and J. Judge, *Phytochemistry*, 9 (1970) 1219.
7 K. E. Rasmussen, S. Rasmussen and A. Baerheim Sevendsen, *J. Chromatogr.*, 66 (1972) 136.
8 J. Karlsen, *J. Chromatogr. Sci.*, 10 (1972) 642.
9 E. von Rudloff, *Recent Advan. Phytochem.*, 2 (1969) 127.
10 E. D. Morgan and L. J. Wadhams, *J. Chromatogr. Sci.*, 10 (1972) 528.
11 M. W. Ruchelman, *J. Gas Chromatogr.*, 4 (1966) 265.
12 A. O. Lurie and C. A. Villee, *J. Gas Chromatogr.*, 4 (1966) 760.
13 K. E. Rasmussen and J. Karlsen, *J. Chromatogr.*, 90 (1974) 285.
14 R. O. B. Wijesekera, A. L. Jayewardene and L. S. Rajapake, *J. Sci. Food Agr.*, 25 (1974) 1211.
15 U. M. Senanayake, R. A. Edwards and T. H. Lee, unpublished results.