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COMPARATIVE ANALYSIS OF VOLATILE ORGANIC COMPOUNDS FROM PLANTS BY GAS–LIQUID CHROMATOGRAPHY

Y. A. AKIMOV
Department of Plant Biochemistry, State Nikita Botanical Gardens, Yalta, Crimea 334267 (U.S.S.R.)

SUMMARY

The possibility of using a system of different variants of gas chromatography for studying the processes of the accumulation and release of volatile organic compounds by plants is discussed. The methods used allow amount and composition of volatile organic compounds to be determined both in air and in plant material. The results show good comparability and reproducibility.

INTRODUCTION

There is currently great interest in studying the properties of volatile organic compounds (VOCs) released by plants into the atmosphere. Antimicrobial activity has been reported[1], and also protective and attractive functions relating to insects[2] and animals[3], allelopathic properties[4] and the possibility of various effects on the human body[5].

Several attempts at the practical utilization of VOCs from plants have been made, *e.g.*, for air disinfection[6], as a means of controlling phytopathogenic microflora[7] and as a medical and prophylactic aid[8,9]. However, work in this field is fragmentary, mainly because of a poor knowledge of the processes of accumulation and release of VOCs by plants. Despite the fact that several chemical, biological and physical methods for determinating VOCs from plants have been proposed, none of them has been widely used[10].

Most data connected with the quantitative characteristics of VOCs from plants (their concentrations in air and emission levels from plants) have been obtained using various modifications of combustion methods[11,12]. The results are relative as they are expressed as amounts of carbon, carbon dioxide, turpentine, etc., which are difficult to compare.

There is little information on the composition of VOCs from plants. The most reliable data were obtained by gas chromatography–mass spectrometry[13], but such work is difficult because of the high equipment costs and the long duration of analysis.

In this work the possibility is discussed of using variants of gas–liquid chromatography for the qualitative and quantitative determination of volatile substances both in plant tissues and air.

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EXPERIMENTAL AND RESULTS

Methods of determining VOCs from plants in air

Plants emit small volumes of volatile compounds and their concentrations in air are relatively low, mainly below 1 mg/m^3 (ref. 14). Together with organic matter, plants emit large amounts of water vapour and it would therefore be expedient to sample VOCs from air above plants with preliminary concentration on polymeric sorbents of the Polysorb and Porapak type. The high specific surface area of such sorbents ensures complete sorption of a whole range of emitted organic compounds, and their inertness to water vapour allows its accumulation on the sorbent to be avoided. Data from the literature[15] show the possibility of concentrating substances of high volatility on these sorbents.

We have determined the retention parameters on Polysorb of monoterpenoid substances widely distributed among plants. The retention capacity of Polysorb increases in the following order: carbohydrates < alcohols < aldehydes < acetates < phenols. For all compounds with an amount of sorbent of 0.2 g and an air sample volume of 5 l, the factor of reserve, *i.e.*, the ratio of the retained volume to that of the air sample, is not less than 10. At a sorbent temperature of 180°C the desorption time of monoterpenoids does not exceed 20 min at a carrier gas flow-rate of 30 ml/min. In this manner all sorbed substasnces are completely extracted.

The analytical variant of concentrating VOCs from plants based on these data included pumping air through an ampoule containing 0.2 g of Polysorb at a flow-rate not higher than 0.5 l/min, with a volume of air up to 5 l/min. This sampling variant permits the determination of concentrations of VOCs from plants down to 0.001–0.01 mg/m^3 in air, which is adequate for various biological investigations.

When investigating VOCs from plants it is often necessary to determine air concentrations and emission values, *i.e.*, quantitative indices. To shorten the time required for such determinations, we used a variant of chromatographic analysis in which the total amount of VOCs sum is recorded as one peak. The ampoule containing the sample substances is connected to the chromatograph evaporator. In the thermostat, instead of a column, a capillary tube of dimensions 30 cm × 0.25 mm I.D. is installed. The substances desorbed at 180°C and with a flow-rate of carrier gas of 30 ml/min are not separated on passing through the capillary tube, and are recorded in one peak with an area that characterizes the amount of VOCs from plants.

A method of applying samples on to a sorbent with further desorption and analysis was used for calibration. The relative errror in five parallel tests did not exceed 8.5%. A significant difference in detector sensitivity was observed between carbohydrates and oxygen-containing compounds. Within these groups the differences did not exceed the test error.

To determine the composition of VOCs from plants, common chromatographic methods were used. To increase the efficiency of separation, the desorbed substances should be concentrated, for which low-temperature condensation is most conveniently used. This allows the spreading of the chromatographic peaks that occurs with direct input of vapour after desorption into the column to be avoided.

The concentrator for desorbed substances is a U-shaped tube of small diameter mounted between the evaporator and the column and cooled with liquid nitrogen.

When using a metallic capillary column, as a concentrator the initial part of the column can be used. After the desorption is completed, the concentrator is heated to room temperature and separation of VOCs is performed with temperature programming. Tests with essential oils applied to the sorbent before analysis and subsequently desorbed and separated by the above method showed the absence of qualitative changes in the compounds. The contents of the components differed within the limits of experimental error.

In the qualitative analysis of VOCs from plants, quantitative data can also be determined by using the overall chromatographic peak area. The error in the determination of VOCs does not exceed the error of the previous method.

Determination of VOCs in plant tissues

When comparing the accumulation and emission processes of VOCs plants, a rapid method for determining their content and composition in plants is needed. For this purpose we studied two variants of the direct chromatographic analysis of plant material.

In the first variant an autonomous heated syringe was used[16], in which the plant material sample is rapidly heated, the vapours subsequently being introduced into the chromatographic column. The device is convenient to use and allows the analysis of large samples (up to 1 g). In the second variant, the plant material is introduced into the evaporator by means of a special device mounted on the chromatograph evaporator[17], and vaporized substances in the flow of carrier gas enter the chromatographic column. This variant is limited in sample size (up to 0.2 g), but has less severe thermal effects on VOCs, as they are rapidly removed from the heating zone after emission from the plant tissues. Both devices give good reproducibility with regard to the qualitative and quantitative composition of VOCs.

Scheme for the comparative analysis of VOCs from plants

The proposed scheme includes the quantitative and qualitative determination of VOCs emitted by plants both in their habitats and in the laboratory at constant

TABLE I

CONTENT AND INTENSITY OF VOC EMISSION FROM *CEDRUS DEODARA*

Results are relative to the plant's natural weight.

Period of analysis	*Content in plant (%)*	*Essential oil content (%)*	*VOC emission under habitat conditions (mg per 100 g plant material)*	*VOC emission in laboratory (mg per 100 g plant material)*
January	1.00	0.15	0.27	0.98
April	2.84	0.12	0.55	1.06
May	2.74	0.15	1.09	2.73
July	1.97	0.14	1.21	1.67
October	1.06	0.17	0.96	1.05

TABLE II

COMPOSITION OF VOLATILE COMPOUNDS OF *CEDRUS DEODARA*

Parameter	*Sampling period*	*Light volatile compounds (%)*	*Terpenoids (%)*	*Content of constituents (%)*								
				α-Pinene	*Camphene*	*Sabinene*	*Myrcene*	*Δ^3-Carene*	*Limonene*	*β-Phellandrene*	*Terpinolene*	*Sum of sesquiterpenes*
VOC emitted under habitat conditions	January	0.4	99.6	45.8	1.7	15.2	33.3	0.8	2.6	0.1	–	–
	April	0.9	99.1	43.2	1.2	23.0	29.9	0.3	1.3	0.1	–	–
	May	8.4	91.6	26.5	1.4	18.6	17.3	2.7	3.7	1.2	–	–
	July	9.2	90.8	23.4	1.0	19.1	19.1	2.0	4.7	1.2	–	–
	October	7.1	92.9	30.9	1.5	23.1	20.6	2.5	3.0	0.5	–	–
VOC emitted in the laboratory	January	14.1	85.9	31.0	0.9	15.9	27.9	1.4	4.8	1.6	–	–
	April	5.5	94.5	33.2	1.2	26.0	23.1	0.4	10.2	1.5	–	–
	May	20.2	79.8	21.7	1.7	17.7	17.7	1.7	5.3	1.1	–	–
	July	18.8	81.2	21.2	1.9	17.9	19.5	1.6	6.2	1.6	–	–
	October	16.0	84.0	29.8	1.6	25.2	16.0	2.0	4.3	0.4	–	–
Essential oil	January	–	100.0	27.3	1.6	17.3	33.3	0.3	7.8	1.7	0.7	9.6
	April	–	100.0	29.9	0.5	23.0	27.6	0.2	4.4	0.3	0.6	12.7
	May	–	100.0	25.3	1.0	21.7	23.5	0.3	8.5	3.7	1.2	17.5
	July	–	100.0	20.2	2.0	21.9	27.0	0.8	9.0	0.5	0.9	16.2

temperature and humidity during prolonged periods of investigation, determination of VOCs contained in a plant and determination of hydrodistillation products of plant material.

The evaluation of the VOC content and composition in air above the plant under conditions of its habitat gives the characteristics of the emission process. The evaluation of the same data under constant (laboratory) conditions allows the influence of ecological factors and physiological conditions of a plant to be established, and also genotype characteristics of VOC emission process.

The evaluation of the VOC content and composition in plant tissues gives the characteristics of VOC accumulation. As direct chromatographic analysis is performed by rapid heating of the plant material, hydrolysis of bound forms of VOCs is excluded, and only free substances are evaluated. A comparison with data on the composition of emitted VOCs allows the ratio of their accumulation and emission processes to be established and mechanisms of the emission certain components and groups of compounds to be elucidated.

Hydrodistillation of plant material is accompanied by hydrolysis of bound forms of VOCs and allows their presence to be revealed. It also makes it possible to separate VOCs into hydrophilic and hydrophobic fractions and to accumulate material to identify individual VOCs. Analysis of hydrodistillation products is especially informative in the investigation of terpenoid-synthesizing plants.

The comparative analysis can be carried out by means of both successive and parallel sampling. With successive sampling first VOC in air are determined under conditions of the plant's habitat. Then the same plant material is analysed in laboratory, and subsequently direct chromatography and hydrodistillation of the remaining plant material are carried out.

Use of the scheme for the comparative analysis of VOC

As an example of the use of this scheme, results of studies of VOCs from *Cedrus deodara* under Crimean south coast conditions are given in Table I.

The data show that the emission of VOCs is not large, being not higher than 0.1% per hour. Terpenoids are not the main component of volatile compounds in plants, constituting not more than 17% of the total amount. The accumulation and emission of VOCs are subject to seasonal changes, and the course of these processes is different. The maximum accumulation of VOCs occurs during the active growth period and the emission maximum during the warmest period of the year. In the laboratory the VOC emission intensity is higher, which indicates temperature effects. At the same time, the character of the seasonal changes is retained, which can be interpreted as effects of the plant's physiological state.

In the composition of VOCs emitted by *C. deodara* (Table II) terpenoids predominate, their proportion in the plant's habitat being 90.8–99.6%. The influence of temperature manifests itself in an increased proportion of light VOCs of non-terpenic character. Sesquiterpenes are absent from all samples except the essential oil; they seem to be in the plant in a bound state and are not evaporated. Further, light VOCs of non-terpenic character are not found in the essential oil.

CONCLUSION

The results presented show clearly the possibilities of and information obtainable with the proposed scheme for analysis of VOCs of plants. The possibility of working in field, the use of stationary equipment for analysis, the comparability and reproducibility of the data obtained and the rapid acquisition of quantitative data are advantages of this scheme.

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