GAS CHROMATOGRAPHIC ANALYSIS OF METABOLITES OF TREES DAMAGED BY EXHALATIONS

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The composition of volatile organic compounds expired by the plant was investigated in dependence on the environment. Using two-step adsorption-concentration, hydrocarbons were preseparated from oxygen-containing compounds present in metabolites. In addition to gaseous samples water excreted by the plant was also analysed for the content of volatile organic compounds. Using gas chromatography differences were found in the content of unsaturated hydrocarbons, aldehydes and other substances in the metabolites of sick and healthy trees. The greatest differences in the composition of metabolites in dependence on the environment were found in Norway spruce (*Picea excelsa*), and the lowest for birch (*Betula verrucosa*).

Northern Bohemia is much attacked by exhalations due to large industrial agglomerations. In 1964 about 50 thousand hectares of wood were damaged by exhalations, of which 15 thousands were completely destroyed (of a total of 85 thousand hectares in the Ore Mountains[1]). The number of sources of noxious immissions is increasing and the amount of exhalates in the air is growing not only quantitatively, but also with respect to the number of noxious substances as well. This makes it difficult to choose newly cultivated growths, for which ever higher adaptability is required. A number of papers study organic compounds in the air[2]. Distinct differences are observed between exhalations in urban and rural regions[3,4]. The opinion has been expressed that in man an ethiologic dependence on the environment exists in the case of volatile organic substances present in metabolites[5]. From this point of view flora has not been investigated in greater detail. If pathological changes in the organism can be studied on the basis of volatile organic compounds in the expired air in man[6,7], similar studies in plants should also be of interest. The composition of human metabolites is not dependent on food, but primarily on the state of health of the observed persons[8]. Plants have a more complex metabolism than humans, it is true, but on the other hand the number of external factors is more limited.

Immissions act on vegetation mainly *via* the tracheae simultaneously with CO_2, during photosynthesis. An accumulation of toxic substances disturbs the proportionality in the content of substances and thus metabolic disturbances occur[9]. In the process of dissimilation various substances are formed as by-products, sometimes noxious, which the plant eliminates by respiration[10]. It was found that some plants eliminate hydrogen sulfide when the atmosphere contains sulfur dioxide (bioreduction of SO_2)[11]. Similarly, during anaerobic respiration an accumulation of ethanol, acetaldehyde *etc.* takes place and the plant dies[12]. It is also known that plants or their fruits produce gaseous organic compounds[13]. In addition to noxious gases present in industrial immissions, such as acetylene, hydrogen sulfide, ethylene. ammonia, carbon monoxide, and

combustion gases, peroxyalkyl nitrates ($RCOOONO_2$) which are formed by photosynthesis from olefins or aldehydes and nitrogen oxides and which are active components of smog[14] have also been recently investigated. Peroxyalkyl nitrates affect the decomposition of polysaccharides and they also block their formation by influencing enzymatic activity[15]. In addition to the damaging of the growth in woods by industrial exhalations (or just because of it) the trees are attacked by various pests. Healthy coniferous trees protect themselves by increased secretion of resin. In the case of sick trees (neighbourhood of Most) the secretion is relatively weak and the resin crumbles or even pulverizes. Evidently the trees suffer a deficiency of organic substances necessary for the formation of resins. The reason may consist either in a limited ability to form these substances, or — on the contrary — the plant itself liberates them when the metabolism is disturbed. The assumption that the plant exhales a number of organic substances according to its state of health motivated us to carry out this investigation.

The aim of our contribution is to draw attention to the possibility of a timely indication of undesirable changes in the vegetation, caused by immissions. Moreover, the determination of the degree of resistance of certain species of trees against certain noxious substances and the detection of the regions with a repeated occurrence of the same exhalates is quite realistic. The elaboration of the method of sample withdrawal and the concentration of organic substances before analysis also represents a part of this study.

EXPERIMENTAL

Samplings were carried out at the following localities: Litvínov, park near Koldom at the wood edge. Jesenice (in West Bohemia), the edge of wood near Ždár. The distance between both localities is about 80 km.

Sampling of Plant Metabolites

A polypropylene sack (in the form of a sleeve) of about 5 l volume was found best for keeping the investigated branch under gas-tight conditions. The sack was provided with a tube fitted with a closure for the withdrawal of the sample, which was done 24 h after insertion of the branch. For analysis a gas sample from the closed space and the condensed water were used. When the sample withdrawal was terminated, the sack was taken off the branch and it was no longer used. In the initial phases of the tests a blank control showed that the polypropylene sack does not set free any undesirable components into the gas phase or into the water. When tree metabolites were sampled (after putting on the sleeves (sacks)) the atmosphere of the given locality was also sampled.

Concentration of Organic Components

A two-step adsorption procedure for concentration was used. The gas was first sucked through Tenax GC (35—60 mesh, Applied Science Laboratories, USA) and thus polar components were adsorbed. In the second step adsorption of gaseous hydrocarbons on Porapak Q (50—80 mesh, Waters Assoc., USA) took place. Using this simple separation both polar and non-polar components were obtained which were adjusted for chromatography by thermal desorption and subsequent condensation by freezing.

Apparatus

Chromatographs of Hewlett Packard Co. model 5832, provided with FID, and of Carlo Erba Co., model 2 300, with ECD-NI[63] were used, both with a sensitivity in the $10^{-1}-10^{2}$ volume ppm range of concentrations. For the determination of hydrocarbons a stainless-steel column of 300 × × 0·2 cm (I.D.) dimensions was used. Carrier gas was helium flowing at a 25 ml/min flow rate. Column temperature was 50°C, detection with FID. Squalane (Carlo Erba, Italy), 25% on Chromosorb P, 80–100 mesh, and FFAP (Free Fatty Acid Phase, Supelco USA), 2% on Chromosorb G, 80–100 mesh, were used as stationary phases. The column with FFAP stationary phase was stainless steel, 250 × 0·2 cm I.D. Carrier gas helium, 25 ml/min flow, temperature 60°C, detection with FID. The polar substances desorbed from Tenax were determined in the same stainless steel column with FFAP at 65°C and 30 ml/min helium flow, using FID.

The qualitative determination of hydrocarbons was carried out on the basis of elution data. Elution data for C_1 to C_4 hydrocarbons were determined using standards (Union Carbide, USA). For the identification of unknown components their elution indices[16] were calculated and compared with the published data[17,18]. For the cheking of the identity of the substances determined a control was carried out on a polar column with FFAP. Quantitative analysis of individual hydrocarbons was carried out on the basis of absolute calibration of the standards in the required concentration, using an exponential diluter from Carlo Erba. Diluting gas was helium and the sample introduction was carried out with a gas loop.

A mixture of polar substances desorbed from Tenax is much more complex than the mixture of hydrocarbons desorbed from Porapak. The presence of esters, alcohols, aldehydes and so on may be assumed. In the given case the determination of elution indices of a large number of components on columns of different polarity is not clear. Therefore we restricted the analysis to the determination of elution indices of the peaks detected on the polar column with FFAP. Elution indices of about 50 standards were determined under identical conditions and individual substances were assigned on the basis of the similarity of data. In the case of alcohols and the carbonyl compounds the qualitative determination was completed by their selective chemisorption, using a McKeag[19] microreactor. The packing of the microreactor was boric acid with Chromosorb W, 60–80 mesh for alcohols (at 147°C), while for aldehydes and ketones benzidine on Chromosorb 60, 60–80 mesh, at 154°C was used. Semiquantitative evaluation of the analyzed sample was referred to the absolute calibration of 2-pentanone and 1-propanol. Model mixtures were prepared by volatilization of a known amount of standards in purified air in glass samplers, provided with a pricking septum and teflon seals. The sampling of the gas sample and the injection into the apparatus was carried out with a Hamilton gas syringe.

Chromatographic Analysis of Air and Gaseous Metabolites of Trees Using a Selective Electron Capture Detector (ECD)

When sampling the atmosphere and tree metabolites we also analysed the concentrate obtained on a chromatograph with ECD. In addition to the current record for atmosphere we also obtained additional distinct peaks. Considering the selectivity of EDC we assumed that the peaks might belong to peroxyalkyl nitrates. This assumption is also supported by the fact that olefins occur in the atmosphere of Litvínov and that peroxyalkyl nitrates are a current component of the polluted air of industrial areas[14]. For comparison the records of the rural atmosphere of the surroundings of Jesenice (*a*) and of a locality in Litvínov (*b*) are shown in Fig. 1. For calibration we used the findings of Stephens[20] who prepared the required concentration of peroxyalkyl nitrate photosynthetically from trace concentrations of nitrogen dioxide and the corresponding olefin in the air. Further work-up of the calibration gases prepared from nitrogen dioxide (Matheson Gas

Products, USA) ethylene and propylene (Union Carbide) was similar as in the analysis of atmosphere. The chromatographic spectrum of standards obtained was very similar to the analysed samples of the atmosphere. A concentrate from Tenax is obtained by thermal desorption (300°C) and peroxyalkyl nitrates are thermolabile and easily converted to nitrates. Hence it may be assumed that the components which were detected by ECD also belong to nitrates.

Chromatographic Analysis of Organic Components Dissolved in Water Eliminated by the Plant

For the purpose of this study the methods of direct chromatographic determination of organic components in water are not sufficiently sensitive. For the analysis of organic compounds expired by the plant together with water vapour the method of "head space" analysis is a good solution[21-23]. In order to achieve a distribution of the volatile components between the phases we used glass flasks of 10 ml volume. For the enrichment of the vapour phase sodium chloride was added (to achieve an approximately 20% concentration) and when the temperature rose to 90°C samples of 0·5 ml of the gas phase were withdrawn for injection into the chromatograph. During the chromatographic analysis the procedure was similar as in the case of oxygen-containing compounds obtained from Tenax. The use of the FID was motivated not only by the required sensitivity for organic compounds but by the sensitivity of FID to water as well.

Just as in the case of oxygen-containing compounds from Tenax, in the case of the components dissolved in water the qualitative detection was carried out merely on the basis of the similarity of elution values on one stationary phase, except for alcohols and oxo compounds which were also detected on the basis of their reaction with boric acid and benzidine. Semiquantitative analysis of substances dissolved in water was carried out by the method of absolute calibration of model solutions of 2-pentanone and 1-propanol.

RESULTS AND DISCUSSION

The first aim of our investigation was the elaboration of a method of preparation of samples for gas chromatographic analysis. In the first place we had to tackle the

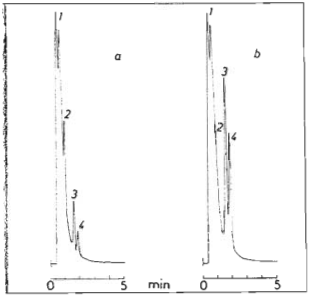

FIG. 1

Chromatogram of assumed peroxylakyl nitrates from the region affected by exhalation (Litvínov — *b* (about 40 ppb), and from the healthy environment (Jesenice) — *a* (about 7 ppb.) Pulse detector ECD — Ni 63. Column, 200 × 0·3 cm with Chromosorb, G, 3% FFAP. Column temperature 60°C, carrier gas purified nitrogen 60 ml/min. *1* Air, *2* unknown, *3* peroxyacyl nitrate (acetyl nitrate), *4* peroxypropionitrate (propionyl nitrate)

problem of undistorted sampling from living trees, *i.e.* if possible without disturbance of vegetation processes. For this reason we did not use the procedures common in the methods of determination of carbon dioxide consumption or oxygen elimination[24]. The collection of the metabolites in polypropylene sacks pulled over the branch and hermetically sealed was found most suitable.

A further step consisted in the concentration of organic compounds present in the gas phase in the sack. For this purpose adsorption procedures are recommended utilizing for example a carbonaceous molecular sieve (Carbosieve)[25], porous silica (Porasil)[26], organic porous polymers of the Chromosorb and Porapak[27] or Tenax GC (ref.[28]) type. Considering the broad spectrum of organic compounds present in our case, their adsorption onto one type of adsorbent and further determination is difficult. For this reason we elaborated a two-step concentration procedure. In the first step polar substances were retained on Tenax GC, and in the second the hydrocarbons which passed through unretained were adsorbed on Porapak Q. Further procedure consisted in thermal desorption of organic compounds from adsorbents and their subsequent condensation by freezing.

From the measurements performed it is evident that the atmosphere in regions with large industrial agglomerations is polluted, among other things by hydrocarbon immisions with prevailing content of olefins. Further contaminants are oxygenated compounds such as aldehydes and mainly peroxyalkyl nitrates the toxicity of which for plants was demonstrated[15]. The concentration of the atmospheric pollution varies according to the weather. All organic substances found in the atmosphere need not have unambiguous source in industrial exhalates, but – on the contrary – the plants themselves may be their source. For example, in September 1979, iso-

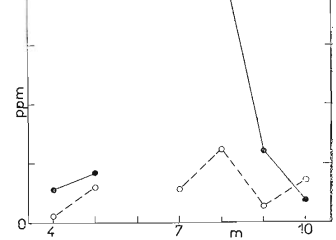

FIG. 2

Dependence of the concentration of unsaturated hydrocarbons in the atmosphere (———) and in the exhaled air of a sick spruce (*Picea excelsa*) (– – – –) from Litvínov in the season (April to October)

prene was detected in a slightly higher concentration in the atmosphere of Litvínov. Simultaneously an exceptionally high content of isoprene was found in gaseous metabolites of black poplar (*Populus nigra*). A similar effect may also be expected in the case of some oxygen-containing compounds. The source of a higher level of 2-propen-1-ol in the atmosphere of Litvínov in August may be due to the relatively high concentration of allyl alcohol in gaseous metabolites of sick Norway Spruce of the same date (Table I). This applies similarly for 1-propanol and 2-pentanone of which a higher concentration was also found in gaseous or liquid metabolites.

It is evident that the plants, *i.e.* trees, exhale a number of organic compounds. The analysis of the atmosphere closed in with the branch for 24 h shows that the content of some components is increased. During vegetation the exhalation of olefins is slighly higher in sick trees (in Litvínov) than in healthy ones (in Jesenice) – Table II. The increase of saturated hydrocarbons is minimum, with the exception of ethane. The difference in the concentration of olefins in metabolites of sick and healthy trees is gradually equalized during the vegetation period until it may achieve and opposite

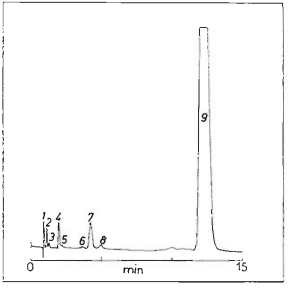

FIG. 3

Chromatogram of hydrocarbons exhaled by black poplar (*Populus nigra*) in September 1979. Separation on a 300 × 0·2 cm column with 25% of Squalane on Chromosorb P 80–100 mesh at 50°C and 25 ml/min of helium flow, FID. Components: *1* Methane, *2* ethylene, *3* ethane, *4* propene, *5* propane, *6* isobutane, *7* 1-butene (isobutene), *8* butane, *9* isoprene

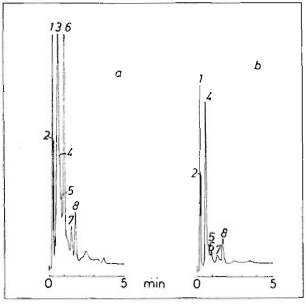

FIG. 4

Chromatogram of volatile substances dissolved in the eliminated water. *a* sick spruce (Litvínov), *b* healthy spure (Jesenice), April 1980. Column 250 × 0·2 cm with 2% FFAP on Chromosorb, W, 80–100 mesh at 65°C, helium 30 ml/min, FID. Components: *1* and *2* unknown, *3* 2-methylpropanal, *4* isopropyl formate, *5* 2-butanone, *6* 3-methyl-2-butanone, *7* 2-pentanone, *8* 1-propanol

ratio during the vegetation interval or shortly before it. It may also occur that the content of olefins in metabolites is deficient with respect to atmosphere (Fig. 2). The lowest of terpenes – isoprene – has a special position. Its concentration changes in the metabolites of trees are more distinct and take place earlier than in the case of other hydrocarbons. This is especially striking in the case of poplar when isoprene was one of the main exhaled organic components during the period of withering and yellowing of the leaves (Fig. 3).

TABLE I

Metabolites of a sick spruce (*Picea excelsa*) and the atmosphere in Litvínov – August 1979

Component	Atmosphere (vol. ppm)	Gaseous metabolites (vol. ppm)	Water (weight ppm)
2-Methylpropanal	0·01	0·27	1·22
Isopropyl formate	—	0·21	<0·01
2-Methyl-2-propanol	<0·01	0·16	2·46
3-Methyl-2-butanone	—	0·10	—
Ethanol	—	—	2·81
2-Pentanone	0·01	0·32	1·58
1-Propanol	0·09	0·73	2·12
2-Propen-1-ol	0·07	1·35	—
2-Ethyl-1-butanol	—	—	0·47

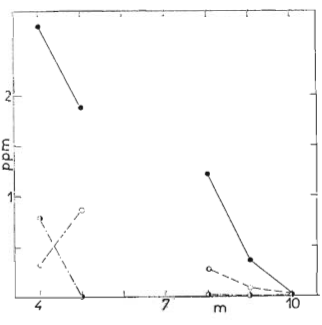

FIG. 5

Dependence of the concentration of 2-methylpropanal in the atmosphere (–·–·–) and the metabolites of a sick spruce in the season (April to October), gaseous metabolites (– – – –), eliminated water (———). Locality: Litvínov

A similar situation also exists in the case of organic oxygen-containing substances exhaled by the plant. In summer the gaseous metabolites of a sick spruce contain an increased content of aldehydes and allyl alcohol (Table I). In contrast to olefins these components of healthy trees do not appear over the whole vegetation period (Table III). Deciduous trees may be an exception. For example during the yellowing

TABLE II

Comparison of hydrocarbons exhaled by spruce (*Picea excelsa*) and birch (*Betula verrucosa*) from the region affected by exhalations (Litvínov) and from a healthy evironment (Jesenice), May 1980. Values in volume ppm

Component	Affected spruce Litvínov	Healthy spruce Jesenice	Birch	
			Litvínov	Jesenice
Methane	0·054	0·023	0·047	0·024
Ethylene	0·030	0·006	0·010	0·003
Ethane	0·016	0·003	0·004	0·001
Propylene	0·021	0·007	0·004	0·001
Propane	0·007	0·002	0·002	<0·001
Cyclopropane	0·001	<0·001	<0·001	<0·001
Isobutane	0·002	0·001	0·001	<0·001
1-Butene	0·032	0·020	0·009	0·001
n-Butane	0·003	0·001	0·003	<0·001
Isoprene	0·007	0·003	0·003	0·003

TABLE III

Comparison of the metabolites from a region affected by exhalations (Litvínov) with a healthy environment (Jesenice) observed in Norway spruce (*Picea excelsa*) and birch (*Betula verrucosa*) — May 1980. Values in volume ppm

Component	Affected spruce Litvínov	Healthy spruce Jesenice	Birch	
			Litvínov	Jesenice
2-Methylpropanal	0·86	<0·01	0·11	<0·01
Isopropyl formate	0·81	2·28	2·27	2·47
3-Methyl-2-butanone	0·60	0·84	0·75	0·99
2-Pentanone	0·19	0·20	0·32	0·63
1-Propanol	2·00	0·62	0·23	0·34
2-Ethyl-1-butanol	1·94	0·09	0·12	0·14

of the leaves of poplar its metabolites contain the mentioned components and in addition to this it probably also eliminates an unsaturated ketone – 2-methylpent-2-en--4-one (mesityl oxide) (Table IV).

As in the preceding cases of gaseous organic compounds exhaled by the plant the water deficit also contains a broad spectrum of compounds, probably oxygen-containing organic compounds. The composition of these metabolites, eliminated together with water, indicate an interesting circumstance. In the summer months and in relati-

TABLE IV

Metabolites of poplar (*Populus nigra*) at the period of yellowing leaves. Litvínov, September 1979

Component	Atmosphere vol. ppm	Gaseous metabolites vol. ppm	Water weight ppm
2-Methylpropanal	<0·01	<0·01	<0·01
Isopropyl formate	–	0·08	<0·01
n-Butyraldehyde	–	0·03	–
3-Methyl-2-butanone	–	0·40	1·72
2-Pentanone	0·02	0·08	–
1-Propanol	0·03	0·14	0·42
2-Propen-1-ol	–	0·09	–
Mesityl oxide	–	0·03	–

TABLE V

Substances dissolved in water from a relatively healthy prickly spruce (*Picea pungens*) and a block pine (*Pinus nigra*) from an affected region (Litvínov) – July 1979. Values in weight ppm

Component	Spruce	Pine
2-Methylpropanal	0·54	0·97
Isopropyl formate	0·03	0·01
2-Butanone	0·07	0·09
2-Methyl-2-butanone	0·17	0·19
2-Pentanone	<0·01	0·02
1-Propanol	5·06	9·85
2-Ethyl-1-butanol	0·13	~0·10

vely healthy trees even of various species a relative agreement in the composition of the components is observed (Table V). In contrast to this a distinctly sick spruce differs from a healthy one by a completely different concentration of volatile organic compounds in the water eliminated over the whole vegetation period (Fig. 4*a*,*b*). The comparison of oxygen-containing compounds in gaseous metabolites shows a similar picture. For example, already in the spring period a difference in the content of aldehydes is discernible in a sick tree as opposed to a healthy one (Table III).

In late autumn the qualitative and mainly the quantitative composition of organic compounds decreases, until it attains a minimum at the onset of hibernation. In Fig. 5 the changes of the content of 2-methylpropanal (isobutyraldehyde) in gaseous metabolites and in water of sick spruce (*Picea excelsa*) are shown in comparison with the atmosphere. This decrease in the elimination of organic substances was observed in all species of the investigated trees and for all metabolites analysed. The question arises whether in this state (lower concentrations of the components in the closed space around the branch, in comparison with the atmosphere) the plant is less or more sensitive to air pollution.

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