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## Note

# Gas–liquid chromatographic determination of stress-induced sesquiterpenes of the potato (*Solanum tuberosum*)

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Under the stress of bacterial and fungal infection, potato tuber tissue produces a number of sesquiterpenes that are not native to uninfected tissue[1–4]. These compounds are of current interest because of the suggested teratogenic effect on the offspring of mothers consuming potatoes infected with *Phytophthora infestans*[5] and the possibility that such compounds are involved in the resistance of the tuber and the potato plant to infection. Qualitative and quantitative analytical methods are needed for determining the conditions under which sesquiterpenes are formed in the potato tuber. Gas–liquid chromatography (GLC) has been used to separate and quantitate some of these compounds[6–8].

The purpose of this investigation was to establish GLC parameters suitable for the separation and quantitation of the four main sesquiterpenes produced in infected potato tuber tissue.

## EXPERIMENTAL

Phytuberin[9–11], katahdinone[12,13], rishitin[14] and lubimin[15–17] (Fig. 1) have been reported as the predominant sesquiterpenes produced in infected potato tissue. These compounds were isolated from potato tubers infected with *P. infestans* and were purified by combinations of vacuum steam distillation and column and thin-layer chromatographic techniques[7].

A standard mixture containing 0.5 $\mu$g/$\mu$l of phytuberin and 0.10 $\mu$g/$\mu$l of katahdinone, rishitin and lubimin in methanol was used to evaluate the separation capabilities of a number of GLC columns. GLC stationary phases with McReynolds' constants[8] (for seven compounds: benzene, 1-butanol, 2-pentane, nitropropane, pyridine, 2-methyl-2-pentanol and 2-octyne) ranging from 138 to 3227 were evaluated. Three types of column tubing were employed: stainless steel, glass and Teflon*. Column lengths varied from 1.8 to 3.0 m and inside diameters were 2.16, 2.00 and 1.58 mm for stainless steel, glass and Teflon, respectively. Three gas chromatographs equipped with flame-ionization detectors were used (Varian Series 1440 and 1520

* Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

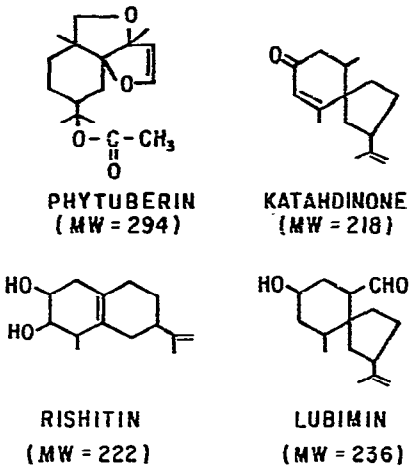


Fig. 1. Predominant sesquiterpenes produced in infected potato tubers.

and F & M Model 810). The flow-rates of hydrogen and air were 30 and 300 ml/min, respectively. The helium carrier gas flow-rate was adjusted to obtain the optimal separation on each column tested. The column temperature was programmed at 4°/min from an initial temperature of 115° to a maximum of 230°. The injection port and detector temperatures were 190° and 240°, respectively.

Quantification of the four main sesquiterpenes produced in infected potato tuber tissue was carried out on a glass column 1.8 m in length and 2.0 mm inside diameter, packed with 3% OV-225 on 100–120-mesh Gas-Chrom Q. The helium carrier gas flow-rate was 65 ml/min. A Varian Aerograph Series 1400 gas chromatograph operated under the conditions described above was used. Peak areas were measured by means of an Infotronics Digital Integration System, Model CRS-11HS, equipped with automatic baseline drift corrector. Standard curves for the quantitative determination were developed by use of a standard mixture of the four sesquiterpenes (phytuberin, katahdinone rishitin, and lubimin). At least three runs were made at each of three levels of concentration, 0.15, 0.30 and 0.45 $\mu$g per injection of each compound.

## RESULTS AND DISCUSSION

Of the three types of tubing investigated, glass and Teflon (stable to a maximal temperature of 250°) were satisfactory. Separations carried out in stainless-steel columns showed evidence of peak broadening due to thermal degradation, particularly for rishitin. Temperature programming was superior to isothermal operation of columns and provided adequate separation in a reasonable time of about 24 min on most of the experimental columns.

At a 3% loading of stationary phase, the four sesquiterpenes were completely separated on 1.8-m columns containing polyphenyl ether (OS-138-6 rings), ASI–50% methyl—25% phenyl—25% cyanopropylsilicone (OV-225), ASI–50% phenyl—50% cyanopropylsilicone, ethylene succinate–phenylsilicone copolymer (EGSP-Z), ethylene

succinate–methylsilicone copolymer (EGSS-Y) and ethylene succinate–cyanoethylsilicone copolymer (ECNSS-M). At a 10% loading of stationary phase, 50% trifluoropropyl–50% methylsilicone (QF-1 or OV-210) effected a separation of the four compounds on a 1.8-m column. Carbowax 20M effected a separation at 15% loading on a 1.8-m column. Other phases evaluated (Apiezon M, OV-1, OV-7, OV-17, OV-25, HI-EFF 8BP) did not provide complete separations under the conditions employed (up to 10% loading on a 3.0-m column). Of the columns tested, OV-225 gave the best separation and the sharpest peaks, was very stable and had a low bleed rate at the highest temperature. An example of a chromatogram with retention times and temperatures of the four major sesquiterpenes separated on OV-225 is presented in Fig. 2.

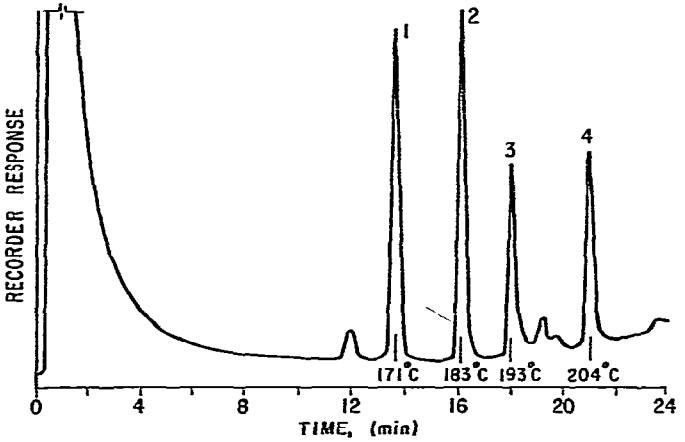


Fig. 2. Gas–liquid chromatogram of authentic sesquiterpenes: phytuberin (1), katahdinone (2), rishitin (3) and lubimin (4) (0.2 $\mu$g of each compound). Column packing: 3% OV-225 on Gas-Chrom Q (100–120 mesh) in a glass column, 1.8 m × 2 mm I.D., temperature programmed at 4°/min. Actual oven temperatures at times of elution shown on chromatogram. Carrier gas flow-rate: 65 ml/min.

As an aid in verifying the identity of the sesquiterpenes without resorting to mass spectral analysis of each sample, it is advantageous to obtain retention values on at least two different columns. The stationary phases should have as varied chemical compositions as possible, and it is desirable to use phases that alter the sequence of elution. The sequence of elution for all stationary phases that provided satisfactory resolution was phytuberin, katahdinone, rishitin, and lubimin, with one exception: on 50% trifluoropropyl–50% methylsilicone (QF-1 or OV-210), the sequence was rishitin, phytuberin, katahdinone and lubimin, making this phase particularly useful for verification of identities.

The GLC response factors and correction factors are given in Table I. The relative standard deviation for each compound was determined from 33 runs, 11 at each level of concentration over a period of 2 weeks. Under the conditions employed, the relative standard deviations that were obtained are given in Table II.

When a large number of potato tuber tissue samples must be analysed, it is desirable to avoid lengthy clean-up procedures and simply to extract freeze-dried samples with methylene chloride, concentrate the extract and inject an aliquot of

TABLE I

RELATIVE GAS–LIQUID CHROMATOGRAPHIC RECORDER RESPONSES FOR AUTHENTIC SESQUITERPENES

OV-225 column operated under stated conditions.

| *Compound* | *Response (A/W)** | *Correction factor (F)*** |
|---|---|---|
| Katahdinone | 631 | 1.00 |
| Phytuberin | 575 | 0.91 |
| Rishitin | 405 | 0.64 |
| Lubimin | 398 | 0.63 |

* $A$ = integrator counts/1000; $W$ = weight of compound in micrograms.

** $F$ for katahdinone assigned a value of 1.00: $F(A/W)_{\text{katahdinone}} = (A/W)_{\text{compound}}$.

TABLE II

STANDARD DEVIATIONS OBTAINED FROM REPLICATE GAS CHROMATOGRAPHIC ANALYSES OF AUTHENTIC SESQUITERPENES

OV-225 column operated under stated conditions.

| *Compound* | *Relative standard deviation (%)* | | |
|---|---|---|---|
| | *0.15 μg per injection* | *0.30 μg per injection* | *0.45 μg per injection* |
| Katahdinone | 6.2 | 6.1 | 2.2 |
| Phytuberin | 8.2 | 6.8 | 3.1 |
| Rishitin | 12.4 | 6.4 | 2.4 |
| Lubimin | 9.2 | 9.3 | 3.0 |

the concentrated extract into the gas chromatograph. Such crude extracts have been separated by GLC and the sesquiterpenes have been quantitated with success in this laboratory[7]. Fig. 3 is an example of a chromatogram of a crude extract of Kennebec variety potato slices infected with *Erwinia carotovora*. In this instance, all four compounds were produced, rishitin being the predominant one.

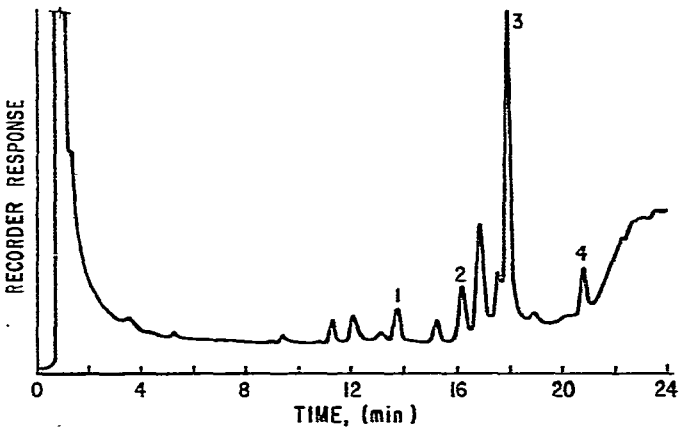


Fig. 3. Gas–liquid chromatogram of sesquiterpenes in a crude extract of Kennebec variety potato slices infected with *Erwinia carotovora*: phytuberin (1), katahdinone (2), rishitin (3) and lubimin (4). Column and operating conditions as in Fig. 2.

The GLC columns and operating conditions reported herein are being used routinely in this laboratory for the analysis of sesquiterpenes in potato tuber tissue that has been stressed and incubated under various conditions. These GLC parameters provide superior resolution and reduced analysis time compared with previously reported methods for these compounds.

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