Phytochemistry, 1972, Vol. 11, pp. 3377 to 3381. Pergamon Press. Printed in England.

VOLATILE CONSTITUENTS OF *ZINGIBER OFFICINALE**

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(*Revised Received* 20 *May* 1972. *Accepted* 1 *June* 1972)

Key Word Index—*Zingiber officinale*; Zingiberaceae: ginger; essential oils; terpenes; sulphides; aldehydes and ketones.

Abstract—Low-boiling constituents from the rhizome of *Zingiber officinale* Roscoe were examined by GLC. The following compounds were identified: *n*-heptane, *n*-octane, *n*-nonane, acetaldehyde, propionaldehyde, *n*-butyraldehyde, isovaleraldehyde, acetone, *n*-propanol, *n*-nonanol, diethyl sulfide, ethyl isopropyl sulfide, methyl allyl sulfide, methyl and ethyl acetates, α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, β-phellandrene and 1,8-cineole.

INTRODUCTION

GINGER, *Zingiber officinale* Roscoe is utilized for spice and perfume manufacture.[1-6] Though the plant is known to be native to tropical Asia, the chief producing countries at present are China, India, Japan, Sierra Leone and Jamaica. Although terpene constituents of plants have been much studied, other low-boiling constituents have hardly been investigated. In continuation of previous papers on the low-boiling constituents of *Geranium* species[7] and *Cryptotaenia japonica* Hassk,[8] the present investigation was undertaken on *Zingiber officinale* to clarify the nature of the perfume.

RESULTS AND DISCUSSION

The rhizome of large-type ginger was steam-distilled and the distillate was collected in water, ice, and dry ice traps. Since it had been shown previously, that GLC of the head space vapour of the stored tuber is more suitable than that of the essential oil itself for examining low-boiling constituents, the head space vapour of the dry ice-cooled trap was first gas-chromatographed on Carbowax 1500. The peak assignments were carried out by comparing the retention times (R_t) with authentic samples, and the results are listed in the third column of Table 1, in which mixed components are marked with an asterisk.

* This work was presented at the 14th Symposium on the Chemistry of Terpenes, Essential Oils and Aromatics of Chemical Society of Japan, in Kitami (September 1970).

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TABLE 1. IDENTIFICATION OF LOW-BOILING COMPOUNDS STEAM DISTILLED FROM *Zingiber officinale* ROSCOE

Authentic compounds	Relative concn (%)†	GC of head space vapour from dry ice-cooled trap	2,4-DNPH TLC	2,4-DNPH GC	3,5-DNB PC	3,5-DNB GC	GC of mercuric chloride complex	PC of hydroxamic-acid	GC–MS
n-Heptane		1							
n-Octane		3							
n-Nonane		6*							
Acetaldehyde		2		1					
Propionaldehyde		4		2					
n-Butyraldehyde		6*		4					
Isovaleraldehyde		8		5					
Acetone		5*		3					
Methyl isobutyl ketone				6					
Glyoxal									
Methylglyoxal	2								
n-Propanol		16*				1*			
sec-Butanol						1*			
tert-Butanol		10							
n-Nonanol						2			
Diethyl sulfide		7*					1		
Ethyl isopropyl sulfide		9					2		
Methyl allyl sulfide		11*					3		
Methyl acetate		5*							
Ethyl acetate		7*							
Ethyl propionate		11*							
Tricyclene	3	12							
α-Pinene	25	13							2
Camphene	41	15							3
β-Pinene	4	16*							4
Sabinene	1·5	17							5
Δ^3-Carene	0·5	18							
Myrcene	7	20							6
Limonene	2	21							7
β-Phellandrene	6	22							8
1,8-Cineole	7	23							9

* Nonseparable peak; numbers indicate order of separation by GLC.
† This was calculated from peak areas in the gas chromatogram of the head space vapour.

In order to obtain further support for the above assignment, 2,4-dinitrophenylhydrazone (2,4-DNPH), 3,5-dinitrobenzoate (3,5-DNB), mercuric chloride complex, and hydroxamic acid derivatives were prepared by treating the condensate in the water-cooled trap, and they were analyzed directly or after being regenerated by TLC, GLC, and combined GLC–MS as described below. These analytical results are indicated in the appropriate columns of Table 1.

2.4-*Dinitrophenylhydrazones*

These derivatives prepared by treating the condensate (2 l.) were examined by TLC and GLC. TLC showed five carbonyl spots which could be assigned to acetaldehyde, propionaldehyde, *n*-butyraldehyde, isovaleraldehyde, and acetone, together with a slow uncharacterized spot, and the direct GLC of the 2,4-DNPHs exhibited five components attributable to those of the above five carbonyl compounds. Since the direct GLC of 2,4-DNPHs, however, is unable to separate a ketone from an aldehyde with the same carbon skeleton, the 2,4-DNPHs were decomposed by heating with α-ketoglutaric acid, and the regenerated substances gas-chromatographed to characterize six carbonyl compounds, that is acetaldehyde, propionaldehyde, *n*-butyraldehyde, isovaleraldehyde, acetone, and methyl isobutyl ketone.

The slow uncharacterized 2,4-DNPH component in TLC was separated into two spots by repeating TLC in toluene–EtOAc, and could then be assigned to glyoxal and methylglyoxal.

Acetaldehyde was the major carbonyl constituent; the 2,4-DNPH was isolated and identified by m.p. and I.R. spectrum.

3.5-*Dinitrobenzoates*

Both PC and direct GLC of 3,5-DNBs from the condensate revealed the presence of two components, one being *n*-nonanol. As to the other, PC indicated *n*-propanol, whereas GLC allowed assignment both to *n*-propanol and *sec*-butanol. *n*-Propanol fits the result of the GLC of the head space vapour. The tenth, single peak in the GLC of the head space vapour was attributed to *tert*-butanol by its R_t, but this component could not be detected in PC and GLC of the 3,5-DNBs. This failure may be due to the difficult formation of 3,5-DNB of *tert*-butanol.

Analysis of Sulfides

For the examination of sulfides, the mercuric chloride complex obtained by treating the condensate was decomposed with HI, and the gases formed were gas-chromatographed. Diethyl, ethyl isopropyl, and methyl allyl sulfides were thus identified, the last being the major component. All these components were also detected in GLC of the head space vapour as single or overlapping peaks.

Hydroxamic Acid

The esters, methyl and ethyl acetates and ethyl proprionate, were detected in GLC of the head space vapour, but as overlapping peaks. By the PC of hydroxamic acid prepared from the condensate, acetohydroxamic acid was detected, but propiohydroxamic acid was not. Accordingly, only the presence of methyl and ethyl acetates is confirmed. The failure in the detection of propiohydroxamic acid may be due to its low concentration.

Analysis of the Oily Residue

The oily residue condensed in the dry ice-cooled trap was analyzed by combined GC–MS, eight monoterpenes, α-pinene, camphene, β-pinene, sabiene, myrcene, limonene, β-phellandrene, and 1,8-cineole being identified. However, tricyclene and Δ^3-carene which were assigned in the GLC of the head space vapour, could not be identified in this procedure, because they were constituents in overlapping peaks or of very low concentration. Although the constituents of ginger oil have been examined by many workers,[1–6] the present investigation has newly identified many low-boiling constituents and four terpene hydrocarbons, namely β-pinene, sabinene, myrcene, and limonene. In comparison with the previous results on aerial parts of *Geranium* species[7] and of *Cryptotaenia japonica* Hassk,[8] the concentration of the low-boiling constituents is very low in the rhizome of *Zingiber officinale*.

EXPERIMENTAL

Materials. The present investigation was carried out on the large type of ginger rhizome which was harvested on a farm of Setocho of Fukuyama city in Hiroshima prefecture, September 1969. The ginger rhizome (14·5 kg), after being thinly sliced, was heated in 3 kg lots together with the equal wt of water. The distillate was passed into a series of water-, ice-, and dry ice-cooled traps for 75 min to collect 7·0 g of yellow oil and 11 l. of condensed water in the first trap, 39·5 g of condensed water along with trace of light yellow oil in the second, and 0·14 g of turbid water in the last. These collected substances were sealed into glass tubes at once and were kept at 3° until examined.

GLC of the head space vapour in the dry ice-cooled trap. An FID-equipped GC was operated at a column temp. of 50° and a 25 ml/min flow rate of carrier N_2 in a stainless-steel spiral tube (3 mm i.d. × 3 m long) packed with 10% Carbowax 1500 on Diasolid L. The gas chromatogram was taken by injecting 10 ml of the head space vapour at once from the dry ice-cooled trap in room temp. and the peak assignment was performed by comparison of R_t with authentic samples (Table 1). A GC taken in the same way from the head space vapour of the ice-cooled trap was inferior both as regards the number of peaks and their intensity.

TLC of 2,4-DNPH. 2 l. of the condensate in the water-cooled trap was treated with the 2,4-dinitrophenylhydrazine reagent[9] to give 0·17 g of a reddish yellow precipitate, which showed five spots with R_f 0·43, 0·51, 0·57, 0·64, and 0·72 on a silica gel TLC plate using *n*-hexane–Et_2O (1:1). These spots corresponded respectively to authentic 2,4-DNPHs of acetaldehyde (0·43), acetone (0·51), propionaldehyde (0·57), *n*-butyraldehyde (0·64), and isovaleraldehyde (0·72). For the slow, sixth spot see below.

Direct GLC of 2,4-DNPHs. By repeating the above TLC of 2,4-DNPHs the components other than the slowest one were collected. The yellow crystals thus obtained were dissolved in EtOAc and GLC on 10% SF-96 on Chromosorb W at 250° and 15·5 ml/min of N_2. Seven peaks were detected, and characterized as acetaldehyde (R_t 3·2 min), *n*-C_3 (3·8), *n*-C_4 (4·6), *iso*-C_5 (5·4), *iso*-C_6 (6·1), *iso*-C_7 (8·6), and *n*-C_8 (13·0) carbonyl compounds by comparison of the R_t with the authentic samples.

GLC of carbonyls regenerated from the 2-4-DNPHs. The 2,4-DNPHs from the condensate were heated with α-ketoglutaric acid to regenerate carbonyl compounds,[10] which were GLC on 10% Carbowax 1500 on Diasolid L at 45° and 25 ml/min of N_2. Eight peaks were detected: R_t in min 3·7, 5·4, 7·0, 9·6, 11·6, 23·4, 30·3, and 42·4. The first six corresponded to acetaldehyde (3·7), propionaldehyde (5·4), acetone (7·0), *n*-butyraldehyde (9·6), isovaleraldehyde (11·6), and methyl isobutyl ketone (23·4), the values in brackets being the R_t in min of authentic specimens.

Isolation of acetaldehyde 2,4-DNPH and detection of glyoxal and methyl glyoxal. By repeated TLC of the 2,4-DNPHs as above, only the major component (R_f 0·43) was successfully isolated in pure form. Its m.p. 168°, and IR spectrum were in agreement with those of acetaldehyde 2,4-DNPH. The slowest component similarly isolated was developed on silica gel TLC in toluene–EtOAc (9:1), when it separated into two spots, which agreed with authentic 2,4-DNPHs of glyoxal (R_f 0·46) and methylglyoxal (0·53) both in R_f and color.

Alcohols. 1·5 l. of the condensate in the water-cooled trap was treated with 3,5-dinitrobenzoyl chloride,[11] and 3,5-DNBs were obtained as a brownish viscous substance, which was GLC on 10% Silicone SE-30 on Diasolid L (3 mm i.d. × 3 m long) at 270° and 5 ml/min of N_2, after being injected as a solution in EtOAc. Two peaks were obtained: one corresponded to both 3,5-DNB of *n*-propanol and *sec*-butanol (R_t in min 7·0) and the other to that of *n*-nonanol (21·8). The 3,5-DNBs were also paper-chromatographed by using *N,N*-dimethylformamide–saturated decalin as solvent,[12] and two spots corresponding to those of *n*-propanol (R_f 0·48) and *n*-nonanol (0·87) were detected.

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Sulphur compounds. N_2 was bubbled into 3 l. of the condensate, the temperature being raised gradually up to 95°. The N_2 carrying the volatile components from the condensate was led into a series of three reaction tubes containing crystalline $Pb(OAc)_2$, and aqueous solutions of $Hg(CN)_2$ (4%) and $HgCl_2$ (3%) in this order; a gray precipitate accumulated slowly in the $HgCl_2$ tube, but the other tubes remained visually unchanged. The precipitate was separated by centrifugation, decomposed with HI,[13] and the regenerated gas was then gas-chromatographed on Carbowax 1500 (10%)/Diasolid L at 50° and 25 ml/min of N_2. Three peaks were obtained, corresponding in R_t to dimethyl (R_t 9·0) ethyl isopropyl (10·4), and methyl allyl (13·0) sulfides.

Esters. The condensate was heated in a stream of N_2 as in the sulfide analysis. The N_2 was led into a set of three tubes containing, respectively, 0·2 N H_2SO_4, a 0·2% solution of 2,4-dinitrophenylhydrazine in 2 N HCl, and a mixed solution of 5% $NH_2OH \cdot HCl$ in absolute EtOH and of EtONa (metallic Na 0·16 g/absolute EtOH 10 ml) in the ratio of 2:1.[14] The hydroxylamine solution of the third tube was thereafter acidified by adding drop by drop absolute EtOH which had been saturated with HCl gas. After the NaCl precipitated had been filtered off, the filtrate was paper chromatographed using *n*- BuOH as solvent, and a spot corresponding to acetohydroxamic acid (R_f 0·47) was detected.

Combined GC–MS on the oily residue. A Hitachi RMU-6E mass spectrometer was used in combination with a Hitachi K53 gas chromatograph, which was fitted with a U-shaped column (3 mm i.d. × 2 m long), packed with 10% Carbowax 1500 on Celite 545. Column temp. was programed from 30° to 130° at a rate of 3°/min, and ionization voltage, total emission and chamber temp. were respectively controlled 70 eV, 80 μA and 220°. The oily substance collected in the dry ice-cooled trap was examined by GC–MS and MS were taken for eleven components. Eight terpenes were characterized as α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, β-phellandrene, and 1,8-cineole by comparison of the MS patterns with authentic materials or with recorded data.[15]

Acknowledgements—The authors wish to express their gratitude to Mr. Shigeru Kôshimizu, Mr. Takeshi Yamato, and Mr. Tetsuya Yanai of Kawasaki Research Laboratories of T. Hasegawa & Co. Ltd for their kind measurement of the GC–MS.

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