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NATURAL PUNGENT COMPOUNDS

IV. EXAMINATION OF THE GINGEROLS, SHOGAOLS, PARADOLS AND RELATED COMPOUNDS BY THIN-LAYER AND GAS CHROMATOGRAPHY

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SUMMARY

The gingerols, shogaols, paradols and related compounds have been examined by thin-layer (silica gel) and gas chromatography (SE-30 and OV-17 liquid phases) and reference retention times and R_F values reported. Thin-layer chromatography gives only partial separation of the various homologous series of compounds, whereas good resolution is obtained with gas chromatography. Compounds containing a β-hydroxyketone grouping, *e.g.*, the gingerols, decompose to aliphatic aldehydes and zingerone under the conditions of gas chromatography. The methods described are particularly applicable to the qualitative analysis of the oleoresins from ginger and grains of paradise.

INTRODUCTION

Recent studies on ginger (*Zingiber officinale*)[1,2] and grains of paradise (*Amomum melegueta*)[3] have resulted in the structural chracterization of the major pungent principles of these spices as O-methoxyphenols. Ginger was found to contain [6]-, [8]- and [10]-gingerols** (**1**, **2**, **3**, resp.), [6]-, [8]- and [10]-shogaols (**9**, **10**, **11**, resp.), zingerone (**12**) and [6]-paradol (**15**). In grains of paradise, [6]- and [8]-gingerols (**1** and **2**, resp.), [6]- and [8]-paradols (**15** and **16**, resp.) and [6]-shogaol (**9**) were identified.

The particular pungent compounds present and the quantitative ratio between them can vary considerably in different spice samples of the same type, depending principally on source material, method of preparation and also the period and method of storage. In general, the composition of the pungent compounds was found to have a marked effect on the quality of derived spice products[4].

However, the substances present difficulties in isolation since most are viscous oils and exist in closely related mixtures of homologues which cannot be readily

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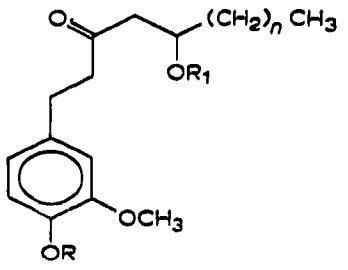
** The number bracketed before the name designates the number of carbons in the aldehyde which would be produced by a retroaldol reaction and thus distinguishes the gingerols and related compounds according to the length of the aliphatic side-chain.

separated by distillation or most preparative chromatographic techniques. In addition, since crystalline derivatives have not been prepared from a number of these compounds, characterization of purified materials cannot be readily accomplished.

Somewhat similar O-methoxyphenols have been analysed by gas (GC)[5], paper[6–8] and thin-layer chromatography (TLC)[5,9]; therefore chromatographic procedures have been investigated as an aid for the identification of the pungent O-methoxyphenols and as a means to provide rapid information on the composition of the pungent principles of spices.



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6; R = $CO{\cdot}CH_3$, R_1 = $CO{\cdot}CH_3$, n = 4
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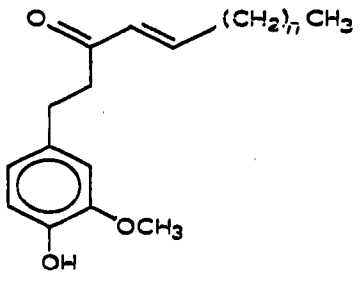

9; n = 4
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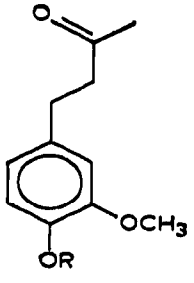
11; n = 8



12; R = H
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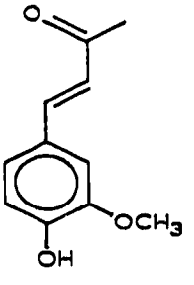

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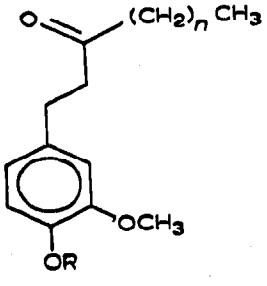

15; R = H, n = 6
16; R = H, n = 8
17; R = H, n = 10
18; R = $CO{\cdot}CH_3$, n = 6
19; R = $CO{\cdot}CH_3$, n = 8
20; R = $CO{\cdot}Ph$, n = 6

EXPERIMENTAL

Thin-layer chromatography

Glass plates (20 × 5 cm) were coated with a silica gel slurry (silica gel–water, 1:2) to a thickness of 0.6–0.7 mm and dried at 125° for 1 h 30 min. After cooling to room temperature (1 h) in a desiccator, samples (20–50 μg) in ether solution (4%) were spotted onto the plates and run with anhydrous diethyl ether–hexane solvent (4:1) in tanks at 20°. Developed plates were dried at 140° for 5 min, sprayed with concentrated sulphuric acid (5–10 sec), then heated at 140° for 10 min.

Densitometer curves from thin-layer chromatograms

TLC plates were prepared and developed as above, except that samples were placed on the slide as a band. Visualization was carried out with sulphuric acid spray followed by heating at 170° for 45 min. Plates were then allowed to cool, a layer of glycerol applied and stored in a vacuum desiccator for 30 min to remove entrapped air bubbles. This operation generally resulted in spreading the visualized bands with a consequent loss of resolution. The optical density of the chromatograms was measured by scanning with a manually operated densitometer having a slit width of 1 mm and a scanning width of 8 mm. Optical density measurements were made every 2 mm and the results plotted as in Fig. 1.

Gas chromatography

An Aerograph Model A-600-B instrument incorporating flame ionization detector and fitted with stainless-steel columns (1/8 in. diameter) was used. One column (6 ft. long) was packed with 1.6% SE-30 on 80–100 mesh acid-washed Chromosorb W and operated isothermally at 200°. The other (3 ft. long) containing 3% OV-17 on the same solid support was operated isothermally at 190°. Nitrogen was used as a carrier gas. A standard injector block temperature of 250° was maintained, although this was varied for experiments on the pyrolysis of [6]-gingerol.

In the pyrolysis experiments, the column containing SE-30 and maintained at 200° was used. Injections of between 0.05 and 0.10 μl were made of a carbon disulphide solution containing 10% (w/v) of [6]-gingerol. Similar results were obtained by the direct injection of pure [6]-gingerol, but the viscous nature of the gingerols made direct injection difficult.

Pyrolysis of [6]-gingerol (1)

[6]-Gingerol (0.3 g) was heated at 230°–250° in a sealed tube for 20 min. The tube was broken into diethyl ether, and the resultant solution was then extracted with dilute aqueous sodium hydroxide. This alkaline solution was neutralized with dilute hydrochloric acid, extracted with ether and the ether solution concentrated. The concentrate yielded a benzoate, m.p. 120°, not depressed on admixture with authentic O-benzoylzingerone. The ether solution of the pyrolysis product remaining after alkaline extraction was worked up and treated with Brady's reagent to yield orange needles (m.p. 103°) not depressed on admixture with an authentic *n*-hexanal derivative.

TABLE I

CHROMATOGRAPHIC DATA

Compound	*Structure*	*Thin-layer chromatography*			*Gas chromatogra retention times*[a]	
		Initial colour	*Final colour*	R_F *value*	*SE-30*	*OV-17*
[6]-Gingerol	1	brown	green-brown	0.26 ± 0.05	dec.	dec.
[8]-Gingerol	2	brown	green-brown	0.30 ± 0.04	dec.	dec.
[10]-Gingerol	3	brown	green-brown	0.30 ± 0.04	dec.	dec.
[6]-Gingeryl methyl ether	4	brown-green	brown-green	0.26 ± 0.04	dec.	dec.
[6]-Gingeryl methyl ether acetate	5	olive-green	olive-green	0.41 ± 0.03	1.0	not rec
[6]-Gingeryl diacetate	6	brown	green-brown	0.51 ± 0.04	1.4	1.6
[8]-Gingeryl diacetate	7	nor recorded	not recorded	not recorded	2.8	3.6
[10]-Gingeryl diacetate	8	not recorded	not recorded	not recorded	5.4	7.8
[6]-Shogaol	9	brown	green-brown	0.48 ± 0.03	1.0	1.0
[8]-Shogaol	10	brown	green-brown	0.51 ± 0.04	1.9	2.0
[10]-Shogaol	11	brown	green-brown	0.51 ± 0.04	3.7	3.9
Zingerone	12	olive-green	royal blue	0.29 ± 0.03	—[b]	—[b]
Zingerone acetate	13	purple	royal blue	0.31 ± 0.03	—[b]	—[b]
Dehydro zingerone	14	olive-green	dark purple	0.23 ± 0.03	—[b]	—[b]
[6]-Paradol	15	blue-green	turquoise	0.52 ± 0.03	0.9	0.7
[8]-Paradol	16	not recorded	not recorded	not recorded	1.7	1.6
[10]-Paradol	17	not recorded	not recorded	not recorded	3.5	3.3
[6]-Paradyl monoacetate	18	yellow-brown	blue-green	0.53 ± 0.03	1.1	1.4
[8]-Paradyl monoacetate	19	not recorded	not recorded	not recorded	2.2	not rec
[6]-Paradyl benzoate	20	green-brown	light blue	0.60 ± 0.03	> 7.3	not rec
Vanillin		light violet	light blue-grey	0.32 ± 0.03	—[b]	—[b]
Eugenol		crimson	crimson	0.55 ± 0.03	—[b]	—[b]

[a] Relative to [6]-shogaol = 1 (corrected for dead volume).
[b] Varies with the amount injected.

RESULTS AND DISCUSSION

Thin-layer chromatography

Investigation of a number of adsorbents and development solvents indicated that most satisfactory separations of the pungent phenols and related compounds were obtained with silica gel plates developed with diethyl ether–hexane solutions (see Table I). Under somewhat similar conditions alumina adsorbent gave partial separations of limited application. Solvents containing varying quantities of aqueous impurity can cause significant R_F variation since the presence of up to 1 % water in the ether–hexane solvent used with silica gel, was found to increase R_F values, *e.g.*, [6]-paradol to a mean R_F of 0.54, [6]-gingerol to 0.29 and [6]-shogaol to 0.51 (see Table I).

After development of the plates and visualization with the sulphuric acid spray, the initial colour of the bands was found to be modified on standing at room temperature (see Table I) and provided useful additional information for characterization. It is noteworthy that certain minor bands detected in some ginger oleoresins were found to be due to the presence of glucose, fructose and sucrose[10].

Clearing the chromatograms and scanning with a densitometer provided a profile (see Fig. 1) having two principal advantages. Firstly, a permanent record of the chromatograms was obtained and, secondly, the area under the curves due to

the various compounds gave some indication of quantitative composition. However, the use of this technique for qualitative work is limited since variable results were obtained when standard mixtures were analysed.

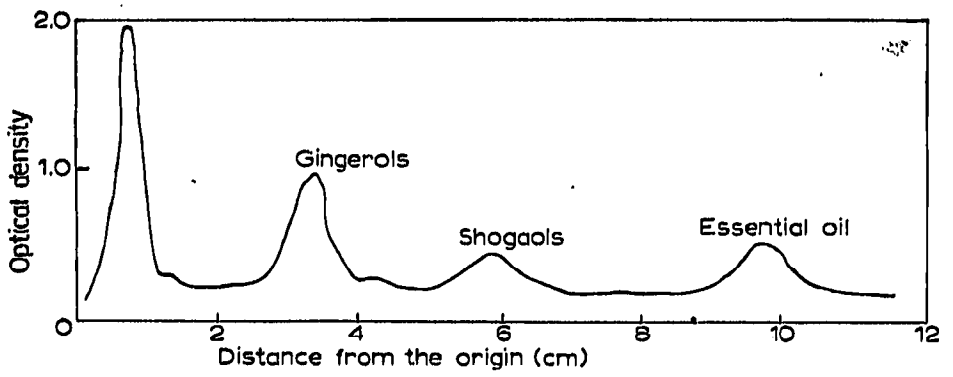

Fig. 1. Thin-layer chromatogram of a ginger oleoresin as evaluated by densitometer.

Gas chromatography

With comparatively low liquid phase loadings (1.6 and 3.0 %) and relatively high column temperatures (190° and 200°) some of the natural pungent compounds and derivatives were successfully subjected to GC examination (see Table I). Conventional semilog plots of retention time against carbon number were linear for those compounds forming a homologous series[11], *i.e.*, gingeryl diacetates, shogoals and paradols. These plots can be used to estimate the retention times of other homologous compounds in these series having either shorter or longer attached hydrocarbon chains. However, some limitations were apparent since, perhaps due to partial decomposition during analysis, the results obtained were semi-quantitative only.

With the lower-molecular-weight phenols — zingerone (**12**), dehydrozingerone (**14**), vanillin, and eugenol — retention time variations were noted according to the quantity of the compound injected onto the GC column. In the case of zingerone (**12**) retention times on 3 % SE-30 were found to decrease from 0.22 to 0.13 (relative to [6]-shogaol = 1) with increasing injection size.

When injector temperatures of 250° were maintained, the injection of a compound containing a β-hydroxyketone grouping (**1**, **2**, **3**, and **4**) resulted in the formation of two compounds as indicated by two peaks from the gas chromatograph. One peak at a shorter retention time was found to correspond with that due to the aliphatic aldehyde which would be formed from the compound by a retroaldol reaction, while the other corresponded with zingerone (**12**) or in the case of [6]-gingeryl methyl ether (**4**) with zingeryl methyl ether. The preparative-scale pyrolysis of [6]-gingerol (**1**) permitted the isolation and characterization of the decomposition products as zingerone (**12**) and *n*-hexanal. A minor GC peak was also obtained corresponding with [6]-shogaol (**9**), which is the dehydration product of [6]-gingerol.

An indication of the temperature necessary to initiate the fragmentation of compounds containing a β-hydroxyketone grouping was obtained by GC. The injector block of the gas chromatograph was maintained at various fixed temperatures while samples of [6]-gingerol were injected. There was no evidence, even at the lowest temperatures used, indicating that unreacted gingerol was passing through the GC column, however volatile decomposition products generated by the gingerol were

carried from the injector into the GC column and analysed in the usual way. I complete reaction occurred rapidly (*i.e.*, within approximately 10 sec) peaks wer obtained for zingerone and hexanal which corresponded closely with peaks obtaine from these compounds injected alone. However, if the fragmentation occurred at slower rate, the prolonged time of production of volatile products leads to a noticeabl broadening of the peaks due to zingerone and hexanal as compared to those due t the compounds injected along under the same conditions. These experiments indicate that injector block temperatures in excess of approximately 200° were necessary fo the rapid decomposition of [6]-gingerol, since at lowest temperatures considerabl broadening of the GC peaks occurred (see Fig. 2).

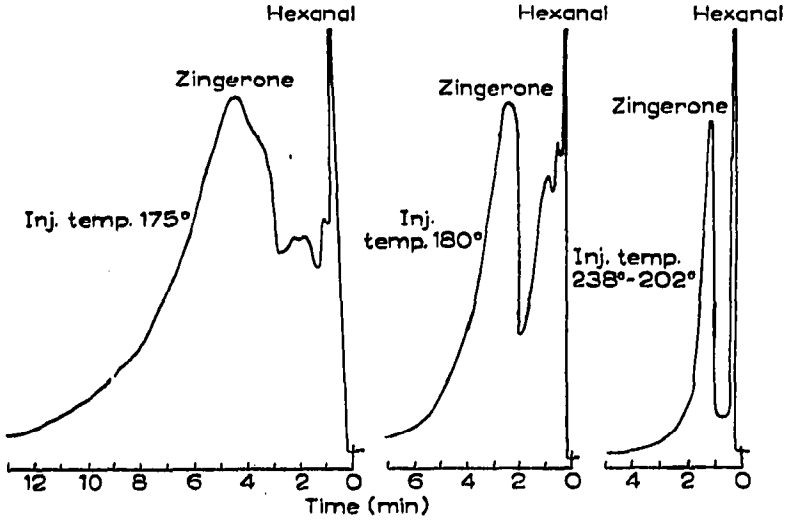

Fig. 2. Gas chromatograms obtained from [6]-gingerol by analysis with different injector bloc temperatures.

Although the gingerols cannot be analysed directly by GC, the homologou composition can be determined by measurement of the ratio between the aldehyde formed during GC procedures, similar to those outlined above. Injector temperature in excess of 200° are necessary, combined with GC conditions satisfactory for th analysis of low-molecular-weight aldehydes.

Chemical interconversions

Many of the pungent compounds are chemically related and previous work ha shown that they can be, to a certain extent, interconverted by suitable chemica treatment. The chromatographic characterization of these conversion products ca provide confirmatory evidence for identifications made on the basis of direct chroma tographic examination. For example, the gingerols produce zingerone and *n*-hexana pyrolytically, as previously described, and also by treatment with hot alkali[1]. I addition, under mild acid conditions, the gingerols (**1**, **2**, **3**) can be dehydrate to the shogaols (**9**, **10**, **11**), which can then be hydrogenated to form the paradol (**15**, **16**, **17**)[3].

CONCLUSIONS

The chromatographic procedures described provide a rapid method for the identification of the pungent O-methoxyphenols. However, for complete characterization, these chromatographic procedures should be substantiated by isolation and examination by chemical and spectroscopic techniques. The methods are particularly applicable to the analysis of the oleoresins from ginger and grains of paradise in which the major pungent principles have been well characterized.

The two methods are, to some extent, complementary, since TLC can yield information on all the oleoresin constituents while GC is restricted to the volatile components alone. Non-volatile components, *e.g.*, carbohydrates, are therefore not indicated by GC. The GC procedure gives a separation of the members of the various homologous series, or derived products, and therefore supplements the TLC procedure, which results in only partial separations of these compounds.

The gingerols, as diacetates, can be analysed by GC since acetylation of the hydroxyl group in the β-hydroxyketone grouping prevents pyrolytic fragmentation as occurs with the gingerols. Similar protection may occur on formation of the trimethyl silyl ethers[12,13]. Such a procedure may provide an alternative method of analysis with which the gingerols could be examined directly by GC.

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