Journal of Chromatography, 93 (1974) 421–428

CHROM. 7366

CONTRIBUTION TO THE KNOWLEDGE OF NIGERIAN MEDICINAL PLANTS

III. CHROMATOGRAPHIC STUDIES ON *ZINGIBER OFFICINALE* ROSCOE

M. KUČERA and H. KUČEROVÁ

Faculty of Pharmacy, University of Ife, Ife (Nigeria)

(First received July 16th, 1973; revised manuscript received January 21st, 1974)

SUMMARY

By using TLC and column chromatography, four groups possessing a hot taste were separated from ginger extracts. Those constituents isolated on a column were free from fluorescence. The specific detection of the isolated constituents was achieved with either 2,4-dinitrophenylhydrazine or Barton's reagent. Characteristic detection was achieved by spraying first with 2,4-dinitrophenylhydrazine and then with anisaldehyde. The hot principle was obtained in a rich fraction on a silica gel column with *n*-hexane–ethyl acetate (1 : 1).

The volatile oil (the yield from commercial Nigerian rhizomes was 1.25%) was also partitioned and the isolation of the phenolic hot principles on an ion-exchange resin was studied.

INTRODUCTION

The ginger used in pharmacy and the food industry consists of the peeled rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae), mainly of Jamaican origin. Ginger with a very pungent taste is also produced in Africa, *e.g.*, Nigeria.

The commercial Jamaican rhizomes contain up to 3% or more of volatile oil, the major components being the sesquiterpenes α- and β-zingiberene, the corresponding alcohols and smaller amounts of bisabolene, ar-curcumene, farnesene, citral, borneol, etc. Kami *et al.*[1] detected the following constituents in the low-boiling fraction: *n*-heptane, *n*-octane, *n*-nonane, acetaldehyde, propionaldehyde, *n*-butyraldehyde, isovaleraldehyde, acetone, *n*-propanol, *n*-nonanol, diethyl sulphide, ethyl isopropyl sulphide, allyl methyl sulphide, methyl and ethyl acetates, α-pinene, camphene, β-phellandrene and 1,8-cineol.

The hot pungent complex is a mixture of phenylpropane derivatives, the gingerols, shogaols, zingerone, methylzingerone and paradols. Resinous matter, 50–60% of starch, etc., are another group of ginger constituents.

It is mainly the hot pungent complex which irritates the heat-sensitive nerve endings of the mouth and gastric mucosa and contributes to digestion and it was of interest to separate this complex for further pharmacological studies.

This paper is concerned mainly with the chromatography of the hot constituents and with the general nature of the extract and the volatile oil of Nigerian ginger,

in order to obtain a method for further evaluation and comparative studies on Jamaican and Nigerian ginger. The hot complex, as a part of the non-volatile oleoresin, is present in the extract, and studies were therefore carried out separately on the extracts and the volatile oil.

Stahl[2] reviewed the TLC separation of ketones and reported the use of either benzene (hR_F 3), benzene–ethyl acetate–glacial acetic acid (90:5:5; hR_F 26), or chloroform (hR_F 30) for gingerone. Stahl[3] later recommended TLC involving TAS[2,3] oven sublimation in a double run, using two different solvents (first dichloromethane, followed by drying for 5 min, and then benzene). The hR_F value of zingerone is 5–10. He used silica gel GF_{254} with development at a relative humidity of air of 50% at 20°, using anisaldehyde for detection.

Connell and McLachlan[4] studied the pungent compounds and their homologues with 6-, 8- and 10-carbon side-chains and some of their derivatives, which are difficult to separate on thin-layer plates. In the solvent system anhydrous diethyl ether–*n*-hexane (4:1) the hR_F value of gingerols is 26–30, shogaols 48–51, zingerones 29 and paradiols about 62. They used 0.6–0.7 mm silica gel thin-layer plates activated for 1½ h at 125° and cooled for 1 h. The plates were dried at 140° for 5 min and the compounds were detected with sulphuric acid followed by heating for 10 min at 140°.

EXPERIMENTAL

Preparation of extracts

Dried unpeeled Nigerian ginger rhizomes were powdered and extracted in a Soxhlet apparatus for 16 h using methanol. The solvent was allowed to evaporate at room temperature to the required volume and this extract was used for further studies.

Extracts were also prepared using different solvents. The powdered ginger was extracted by shaking with light petroleum (b.p. 40–60°) (a), the mixture was decanted, the solvent was removed by evaporation and the residual ginger was extracted again in the same way with chloroform (b), acetone–water (95:5) (c), acetone–water (1:1) (d) and finally with water (e). In a further procedure a methanolic extract was shaken with light petroleum.

Preparation of thin-layer plates

Plates of dimensions 20 × 10 or 20 × 20 cm were coated with a 0.25 mm layer of silica gel (BDH, Poole, Great Britain) for thin-layer chromatography. For preparatory work plates with a 0.5 mm layer were used. The plates were activated for 1 h at 105° and kept in desiccator until required for use. Separations were carried out in solvent-saturated Camag tanks at room temperature (22–23°) with a development time of about 1 h. For two-dimensional separations, the plates were air-dried before being placed in the second solvent.

Separation and partition on silica gel

In order to obtain a fraction rich in the hot complex, the concentrated extract mixed with a small amount of silica gel was placed on the top of a silica gel column (20 cm × *ca.* 2 cm I.D.) and eluted with *n*-hexane–ethyl acetate (1:1). The hot complex was eluted in the first 150 ml and this semi-purified fraction was used for further studies in which four hot constituents were detected by TLC.

In an attempt to separate the hot complex, the extract was fractionated on the silica gel column with *n*-hexane (fractions 1–154) and additionally with 95:5 *n*-hexane–ethyl acetate (fractions 155–350). Fractions with similar chromatographic patterns were combined and the solvent was evaporated at room temperature and the residue used for further partitioning on thin-layer plates. The spots of interest were extracted from the layer with methanol and used for TLC studies.

TAS[2,3] *oven*

The cartridges were filled with 0.05 g and 0.1 g of the dried powdered rhizome. The temperatures studied were 200°, 250° and 300° and the times of application were 30, 60 and 90 sec.

Isolation of the volatile oil

The volatile oil was isolated from the rhizome by steam distillation, using the apparatus[8] for the quantitative determination of volatile oils; the collected oil was extracted with light petroleum, dried over anhydrous sodium sulphate and made up to the required volume. This was used for further experiments.

Solvent systems used for extracts and oil

(1) Light petroleum (b.p. 40°–60°).
(2) Light petroleum–ethyl acetate (2a = 98:2; 2b = 95:5).
(3) Light petroleum–ethanol (3a = 98:2; 3b = 94:6).
(4) Benzene.
(5) Benzene–ethyl acetate (5a = 98:2; 5b = 95:5; 5c = 90:10).
(6) Dichloromethane.
(7) Light petroleum–ethyl acetate–ethanol (95:2.5:2.5).
(8) Light petroleum–carbon tetrachloride (75:25).
(9) Chloroform.

For the extracts, solvents 1, 2a, 2b, 3a, 4, 5b, 5c and 6 were used, while for the oil solvents 1, 2b, 3a, 3b, 4, 5a, 5b, 5c, 7, 8, 9. The temperature in the laboratory was 27–28°.

Detection by general methods

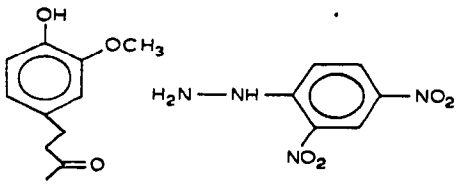
UV detector (*P. W. Allen, Liverpool, Great Britain*). The detector, utilizing above 3100 Å, was used before and after application of the reagents.

Anisaldehyde reagent. The reagent consisted of 0.5 ml of anisaldehyde, 50 ml of acetic acid and 1 ml of concentrated sulphuric acid. After spraying, the plates were heated at 100–110° for about 10 min until the appearance of coloured spots[2,3].

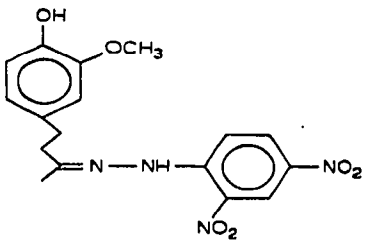
Detection by specific methods

Phenols. Barton's reagent[5] is prepared by mixing, just before use, equal volumes of a solution of 1 g of potassium hexacyanoferrate(III) in 100 ml of distilled water and a solution of 2 g of iron(III) chloride in 100 ml of distilled water.

Ketones and aldehydes[6]. A 1.5-g amount of 2,4-dinitrophenylhydrazine is dissolved in a cooled mixture of 10 ml of distilled water and 10 ml of concentrated sulphuric acid, and the solution is made up to 50 ml with distilled water, filtered and used fresh. This reagent is assumed to form a Schiff's base with the keto group, *e.g.*, zingerone dinitrophenylhydrazone.



Zingerone 2,4-Dinitrophenylhydrazine



Zingerone dinitrophenylhydrazone

Isolation of the hot principles

A 1-kg amount of finely powdered rhizome was extracted by shaking it with light petroleum to remove the volatile oil and other light petroleum-soluble constituents. The separated and dried powder was extracted by shaking it with methanol–water (1 : 1) until the hot taste disappeared. The organic solvent was evaporated on a rotary evaporator and the aqueous solution made alkaline with sodium hydroxide, shaken three times with diethyl ether to remove as much as possible of the accompanying constituents and acidified with hydrochloric acid to give a yellowish precipitate. The precipitate was separated, dissolved in diethyl ether, shaken with water, the ethereal portion was separated and the organic solvent evaporated. This alkali–acid procedure was repeated three times. The residue was an oily dark brown hot substance.

Isolation of the hot principles on an ion-exchange resin

Columns of dimensions 14 cm × 2 cm I.D. packed with the anionic resins Amberlite IRA-410 and De-acidite were converted into the OH^--form in the usual way with 4 *N* sodium hydroxide solution.

Powdered rhizome was macerated with water (1 : 10) on a boiling water-bath for 10 min, the water filtered off and the hot and only light yellow coloured extract was passed through the column. Also, the hot principles isolated via the alkali–acid method were passed through the column as a solution in ethanol–water (1 : 1). For washing the columns, 4 *N* hydrochloric acid and 4 *N* sodium hydroxide solution were used. The alkaline solution was acidified and shaken with diethyl ether to separate the hot principles, the ether being evaporated.

RESULTS AND DISCUSSION

As standards and reliable detection reagents were not available, it was necessary to detect the hot principles of the complex by taste. Using the solvent system 5b and occasionally also 6 (for double runs) it was possible to achieve a reasonable separation of the hot principles (Figs. 1 and 2).

On a silica gel column using *n*-hexane as eluent, fractions 1–154 were free from the hot constituents so that beginning with the fraction 155 the solvent system was changed to *n*-hexane–ethyl acetate (95:5). Fractions 165–204 contained the constituents with the highest hR_F values, fractions 205–244 those with the higher and middle hR_F values, fractions 245–264 those with the middle and the lower hR_F values and fractions 265–350 the hot constituents with the lowest hR_F values.

Using these fractions on thin-layer plates, four different groups of hot constituents were detected. The two top spots (1 and 2 in Fig. 1) were close together.

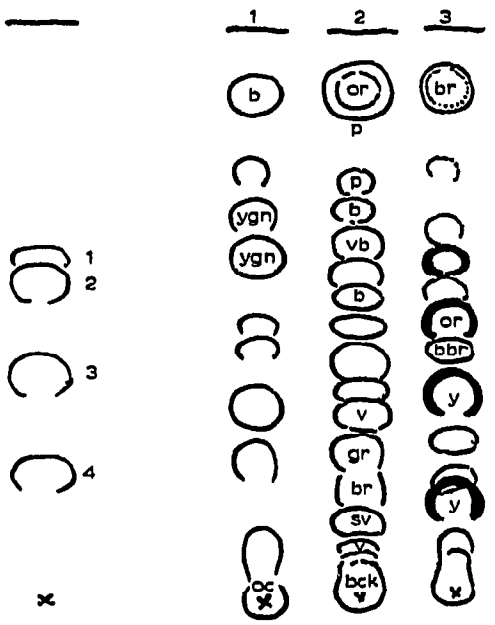


Fig. 1. Chromatography of hot fractions in a double run using solvent system 5b.

Fig. 2. Chromatography of acetone extract using solvent system 5b. Detection: 1 = UV light (unmarked spots yellow); 2 = anisaldehyde; 3 = 2,4-dinitrophenylhydrazine (thick lines represent hot principles). Colours: b = blue; bck = black; br = brown; gr = green; lb = light blue; oc = ochre; or = orange; p = pink; s = strong; v = violet; vb = violet-blue; y = yellow; ygn = yellow-green; yor = yellow-orange.

Using total extracts, the spots (the lowest one apparently being the major constituent of the hot complex) were detectable by their yellowish fluorescence (apparently accompanying compounds), but the hot principles from the column were free from fluorescence.

Using the anisaldehyde reagent, all four spots were identified by their violet colour, the intensity of which varied according to the concentration of the particular compound(s) present.

Selective detection was achieved by using 2,4-dinitrophenylhydrazine, reacting with ketonic or aldehydic groups yielding yellow condensation products. The side-chain of all the mentioned pungent principles is characterized by a keto-group. The second spot from the top seemed to be more orange in colour. Some impurities of ketonic or aldehydic character can interfere.

The phenolic character of zingerone and its derivatives offered the possibility of detecting these compounds, for example, by Barton's reagent, which produces a blue colour in the presence of these compounds. The semi-purified extract apparently contained some other reducing constituents, such as phenols and amines,

which interfered and gave diffused blue zones with the reagent. Using fractions from the column, this reaction was also specific.

Characteristic reactions of these compounds were found when two of the detection reagents were used successively. Using 2,4-dinitrophenylhydrazine as the first and anisaldehyde as the second spray reagent, the yellow colour of the spot changed to grey or olive green with different intensities, depending on the concentration of the compound on the plate.

Using Barton's reagent as the first and anisaldehyde as the second spray reagent, the blue-violet colour of the spot remained substantially unchanged.

It was of interest to compare the patterns of the extract and of the sublimate from the TAS oven, as the oven offered rapid separation of many constituents from the crude ginger and easy application on the plate. A temperature of 300° gave the sublimate richest in hot principles. The most convenient time was 30 sec, producing a pattern very similar to that of the methanolic extract and showing the least artefacts of pyrolysis. A load of 0.05 g of the powder gave better results than 0.1 g.

Unlike the extracts, the TAS oven produced, in UV light after two-dimensional thin-layer development, more than 35 spots if the specimen was heated for 90 sec, and only 10 spots more than the extract if heated for 30 sec. According to Connell and McLachlan[4], gingerol, containing a β-hydroxy group, was apparently decomposed to zingerone at high temperature.

2,4-Dinitrophenylhydrazine is not suitable for the detection of the hot principles on chromatograms resulting from the TAS oven procedure as there are too many yellow spots. The pyrolysis produces too many by-products that interfere with the reagent, which also reacts with some hydroxyphenylpropane derivatives. The spot marked SPV in Fig. 3 was only very weak, while that from the TAS oven was very strong and also the lower half of the plate contained many new spots. The top spot gave a very distinct colour reaction.

When the general anisaldehyde reagent was applied after the specific 2,4-dinitrophenylhydrazine reagent, there was a wide range of different colours. The spots of the hot constituents gave a dark olive green-grey colour after heating.

The successive extraction of the same specimen of ginger with (a) light petroleum, (b) chloroform, (c) and (d) acetone–water and finally (e) water was studied in order to achieve some fractionation, but it did not produce any new results. Extract (a) was rich in spots that apparently contained mainly the volatile oil fraction, extract (b) did not give these violet-blue spots, following the solvent front. In extract (c) the front spot was blue and extracts (d) and (e) gave only very few spots. The acetone extract (Fig. 2), prepared directly from the powder, showed spots similar to those from extract (c). When the methanolic extract was shaken with light petroleum, those spots characteristic for volatile oil were also removed.

In studies on the extractability and stability of the hot constituents, the lower spot was extracted from the gel layer first with light petroleum, then methanol and finally acetone. The hot constituent was in the methanolic fraction. When applied again on the thin layer, this extracted fraction also showed two spots with higher hR_F values, which supports the possible changes mentioned by Connell and McLachlan[4].

The gingerols possessing a saturated and hydroxylated side-chain are dehydrated under mild conditions to hydroxyl-free, unsaturated shogaols, which are possibly hydrogenated to saturated paradols[4].

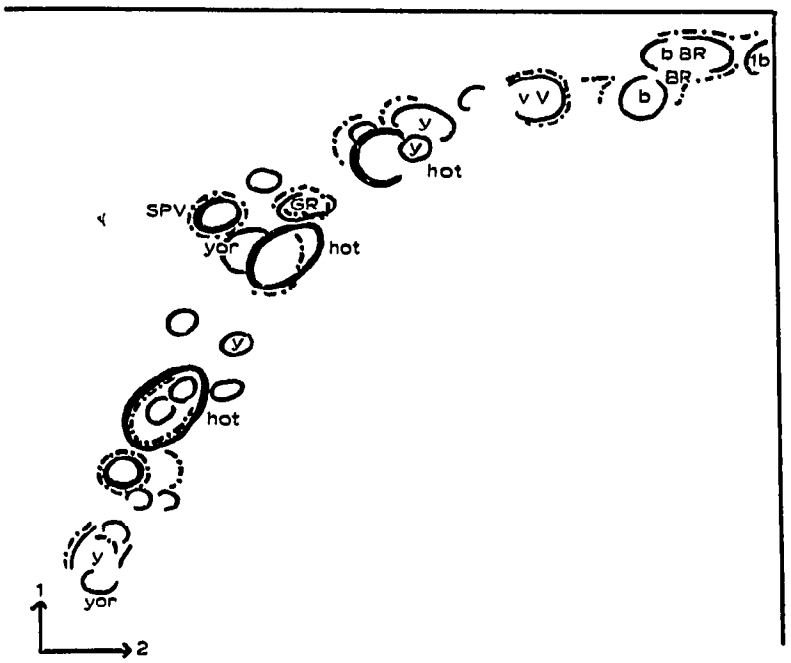


Fig. 3. Chromatography of extract and detection of keto-compounds. Detection: ———, UV light; ———, 2,4-dinitrophenylhydrazine (first spray); – – – –, anisaldehyde (second spray; colours in capitals). 1 = Run in dichloromethane; 2 = run in benzene–ethyl acetate (95:5). Colours as in Fig. 2.

The components of the hot principle, being phenolic in character, were separated by shaking with sodium hydroxide solution. On the thin-layer chromatograms more than four spots were detected, so that some more phenols were extracted. Using spray reagents for flavonoids, the result was negative[7].

The hot principles were removed from the solution on weakly anionic ion-exchange resin De-acidite. Better results were achieved by using the aqueous solution, which produced cleaner eluates than the fraction isolated by the alkali–acid method.

The column washed with 4 *N* sodium hydroxide solution produced an almost colourless eluate, while the eluate obtained using 4 *N* hydrochloric acid was yellowish. The alkaline eluate produced, after acidification and shaking with diethyl ether, a hot-tasting residue. The same procedure on Amberlite IRA-410 failed, in spite of the fact that the hot principles from the original solution were removed during passage through the column, and the eluate was completely tasteless. Elution from the column was not successful.

The Nigerian commercial unpeeled ginger rhizomes contained, on average, 1.25% of yellowish volatile oil. When this oil was studied, a regular distribution of spots was achieved using solvent system 5b and/or 6 (Fig. 4). The top spot, representing the major constituents, the sesquiterpenes, zingiberene, its corresponding alcohol, etc., were separated from the other constituents by solvent system 8.

Systems 3a and 7 gave a very good resolution, but the top spot, which was pink-violet with a brown centre in solvent systems 5b and 6, was strongly pink

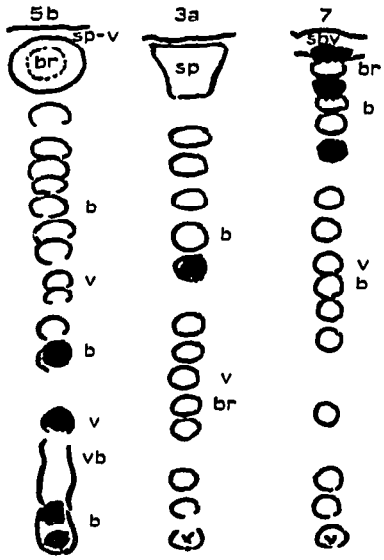


Fig. 4. Chromatography of volatile oil using solvent systems 3a, 5b and 7. Detection: unshaded spots, anisaldehyde; shaded spots, 2,4-dinitrophenylhydrazine. Unshaded spots were more or less pink. Colours as in Fig. 2.

when system 3a was used and strongly blue-violet when system 7 was used. Also, the spots that could be detected with 2,4-dinitrophenylhydrazine (*i.e.*, containing ketones and aldehydes) occurred as three distinct spots near the solvent front (system 7), while with solvent systems 5a and 6 they were near the start.

Solvent systems 3b, and less evidently 9, moved the constituents towards the second half of the layer, while systems 1 and 4 left the major part of the constituents in the lower half.

Double separation of the oil (solvent systems 5b and 6, both applied twice) yielded 25 spots detectable under UV light and by anisaldehyde.

In none of the systems used was the separation of the major top spot successful.

The TLC of the volatile oil sprayed with 2,4-dinitrophenylhydrazine showed, in addition to some very weak and brownish spots, one strong orange spot ($hR_F = 72$), which was apparently some aldehyde or ketone from the oil, but it did not correspond with the strongly violet-blue spot at the top (hR_F *ca.* 90–95) detectable with anisaldehyde.

REFERENCES

1 T. Kami, M. Nakayama and S. Hayashi, *Phytochemistry*, 11 (1972) 3377.
2 E. Stahl, *Thin-Layer Chromatography*, Springer, Berlin, Heidelberg, New York, 1969, p. 217.
3 E. Stahl, *Chromatographische und Mikroskopische Analyse von Drogen*, Fischer, Stuttgart, 1970, p. 147.
4 D. W. Connell and R. McLachlan, *J. Chromatogr.*, 67 (1972) 29.
5 G. M. Barton, R. S. Evans and J. A. F. Gardner, *Nature (London)*, 170 (1952) 249.
6 M. Luckner, *Prüfung von Drogen*, Fischer, Jena, 1966, p. 154.
7 M. Wichtl, *Die Pharmakognostisch-Chemische Analyse*, Akademie-Verlag, Frankfurt am Main, 1971, p. 150.
8 *British Pharmacopoeia*, 1958.