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GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC STUDIES OF GINGER CONSTITUENTS

IDENTIFICATION OF GINGERDIONES AND NEW HEXAHYDROCURCUMIN ANALOGUES

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SUMMARY

The pungent principles of ginger (*Zingiber officinale* Roscoe), the gingerols and related compounds, were investigated by gas chromatography and gas chromatography–mass spectrometry using several chemical derivatives and deuterium labelling. Gingerdiones, postulated intermediates in the biosynthesis of the gingerols, were identified together with desmethylhexahydrocurcumin and the shogaol analogues of the hexahydrocurcumins.

INTRODUCTION

Previous studies on the composition of the pungent principles of ginger (*Zingiber officinale* Roscoe) have shown that the major constituents are the homologous gingerols (I) and the shogaols (II)[1–4]; the latter compounds are thought to be partly formed from the gingerols by decomposition[1,5,6]. Other related compounds present in smaller quantities include paradols (III), gingediols (IV), zingerone (V)[2,3,7], hexahydrocurcumin (VIa)[8] and the O-methyl ethers of many of these compounds[1,4]. Lower boiling extracts contain a range of terpenes and related compounds[9–12]. It has been proposed that the biosynthesis of the gingerols involves the condensation of dihydroferulic acid first with malonic acid and then with a short chain carboxylic acid such as hexanoic acid to give the intermediate gingerenedione (X, Fig. 1). This can either be reduced to the ketoalcohol (XI) and hydrogenated to gingerol (I), or hydrogenated to gingerdione (XII) and then reduced to gingerol[13–15]. Of these pathways, the first seems to be preferred as the reduction of gingerdione (XII) appears to be a relatively inefficient process[15]. However, neither gingerdione or the intermediate (XI) appears to have been identified in the ginger plant. In this paper, we describe the identification of three gingerdiones corresponding in chain lengths to the three major gingerols (Ia, $n = 4$, 6 and 8). Other new compounds identified were desmethylhexahydrocurcumin (VIb) and the shogaol equivalents (XIIIa, b) of both hexahydrocurcumin (VIa) and desmethylhexahydrocurcumin (VIb). These are probably produced

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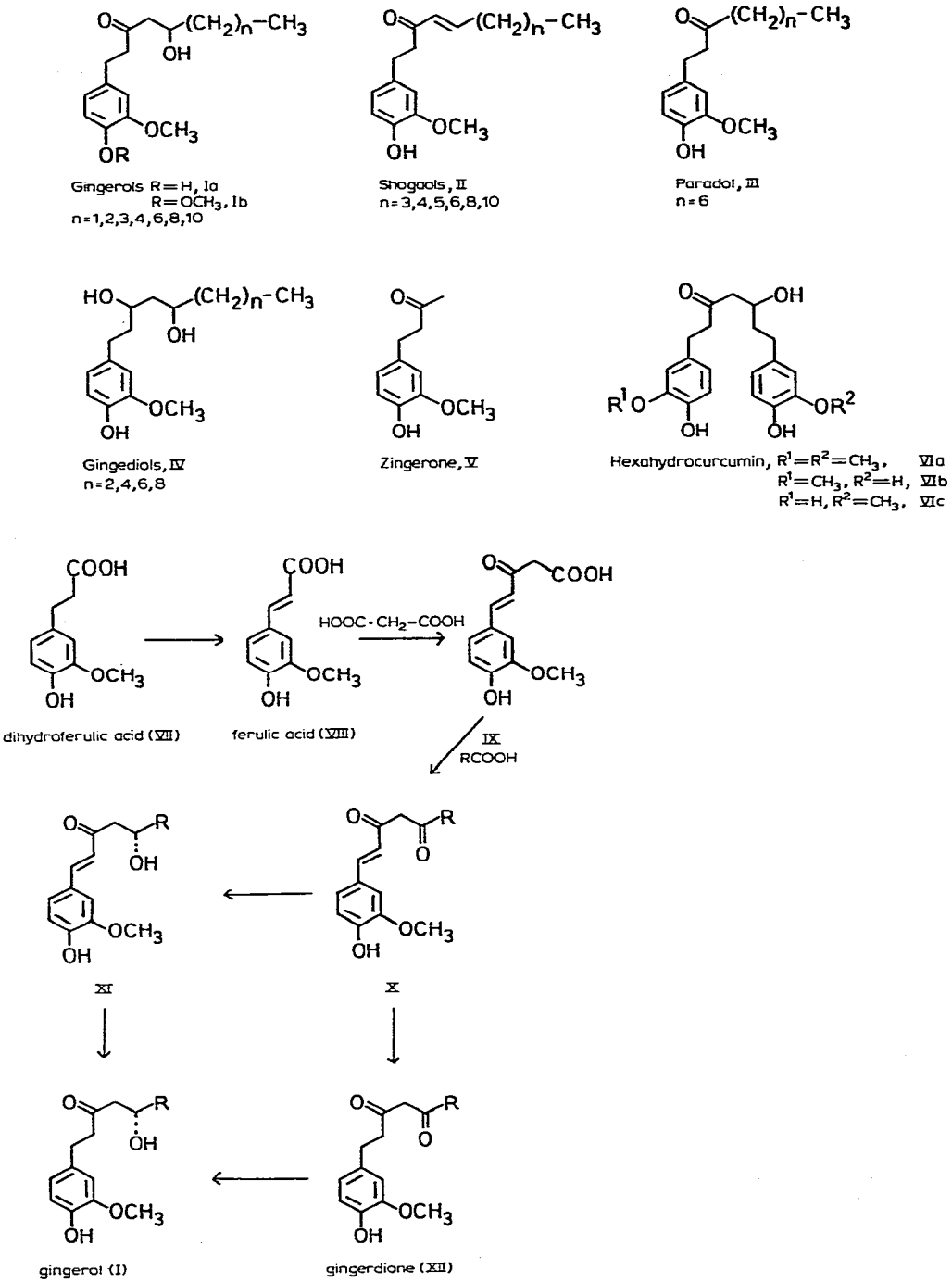
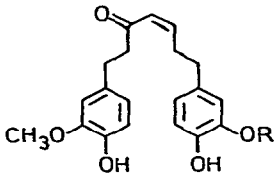
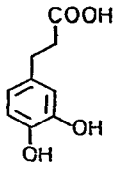


Fig. 1. Proposed scheme[14] for the biosynthesis of the gingerols and hexahydrocurcumins. Gingerols are produced when R is an aliphatic carboxylic acid such as hexanoic whereas the hexahydrocurcumins result from condensation with an aromatic acid such as dihydroferulic acid.

by the same biosynthetic pathway as the gingerols by condensation of the intermediate IX with either dihydroferulic or dihydrocaffeic acid (XIV).



R=CH$_3$, XIIIa
R=H, XIIIb



Dihydrocaffeic acid, XIV

EXPERIMENTAL

Preparation of samples

Root ginger from Jamaica was obtained locally. Gas chromatographic–mass spectrometric (GC–MS) studies on several samples showed similar profiles.

A 1.5-g amount of dry root was crushed and left to stand with ethyl acetate for 30 min. The solution was filtered and evaporated to dryness to yield 200 mg of oil. This was dissolved in 20 ml of ethyl acetate to give the stock solution. Aliquots of this were derivatized as described below.

Trimethylsilyl (TMS) derivatives. The sample (0.1 ml) was blown to dryness (nitrogen stream) and heated with a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 4 μl), trimethylchlorosilane (TMCS, 2 μl) and acetonitrile (4 μl) at 60°C for 10 min. Aliquots of the mixture were injected directly into the chromatograph.

[$^2H_9$]TMS derivatives[16]. The sample was heated with [$^2H_{18}$]bis(trimethylsilyl)-acetamide ([$^2H_{18}$]BSA, 5 μl) and acetonitrile (5 μl) for 10 min at 60°C.

Methyl ether–TMS derivatives. A 0.2-ml volume of the stock solution was blown to dryness and dissolved in dimethylformamide. Methyl iodide (10 μl) and potassium carbonate (*ca.* 50 mg) were added and the mixture was stood overnight at room temperature. The mixture was then diluted with water, the products were extracted with ethyl acetate, washed with water, dried ($MgSO_4$) and blown to dryness. The sample was then reacted with BSTFA as described above.

[$^2H_3$]Methyl ether–TMS derivatives. These were prepared as above using [$^2H_3$]-methyl iodide in place of the methyl iodide.

Methyloxime–TMS (MO-TMS) derivatives. The dry sample was dissolved in pyridine (20 μl) and heated for 1 h at 60°C with an excess of methoxyamine hydrochloride. The mixture was then blown to dryness and the TMS derivatives were prepared as described above.

Lithium aluminium hydride reduction. The sample was dissolved in ether (0.5 ml) and allowed to react with lithium aluminium deuteride (*ca.* 10 mg) for 1 h at room temperature. The products were extracted with ethyl acetate following destruction of the excess reagent by standard techniques. The solvent was removed from the dried ($MgSO_4$) solution with a nitrogen stream and the residue was trimethylsilylated as described above.

Deuterium exchange. The sample was dissolved in dioxane (0.5 ml), ^2H_2O (0.5 ml) and NaO^2H (trace) and allowed to stand at room temperature overnight. The

mixture was then diluted with ^2H_2O (2 ml) and acidified (H_2SO_4) and the products were extracted with ethyl acetate. The extract was washed with water, dried ($MgSO_4$) and converted into trimethylsilyl derivatives as described above.

Deuterium exchange, reduction. The deuterium exchange reaction was performed as above and the dried product was reduced with lithium aluminium deuteride as described under *Deuterium exchange.* The products were trimethylsilylated as described under *Trimethylsilyl (TMS) derivatives.*

Oxidation. The sample was dissolved in a mixture of dimethylsulphoxide (0.5 ml) and acetic anhydride[17] and left at room temperature overnight. Water was added and the products were extracted with ethyl acetate, washed with sodium bicarbonate solution, water and evaporated to dryness. The residue was hydrolysed in a mixture of methanol (1 ml) and dilute sodium hydroxide solution (0.1 ml, 0.1 *N*) for 1 h at 60°C, diluted with water and the products were extracted with ethyl acetate. This extract was washed with water, dried ($MgSO_4$) blown to dryness and converted into TMS derivatives as described above.

Gas–liquid chromatography

GC data were recorded with a Varian 2440 gas–liquid chromatograph fitted with dual flame-ionisation detectors and two 2 m × 2 mm glass columns packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.). Nitrogen at 30 ml/min was used as the carrier gas, the injector and detector temperatures were 300°C and the column oven was temperature programmed from 100 to 300°C at 4°C/min.

Gas chromatography–mass spectrometry

GC–MS data were recorded with a VG Micromass 12B mass spectrometer interfaced to a VG type 2040 data system and via a glass jet separator to a similar chromatographic system to that described above. Helium at 30 ml/min was used as

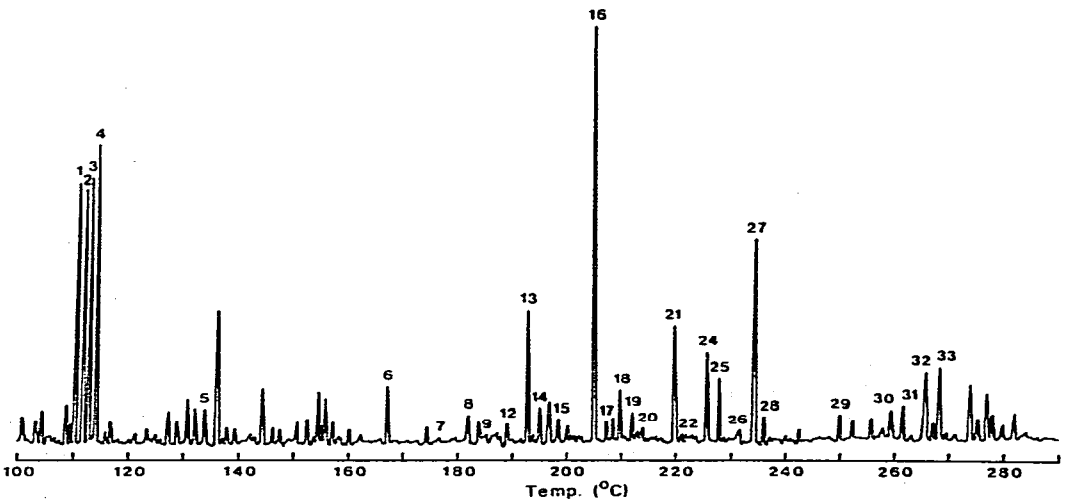


Fig. 2. Computer reprocessed[18] chromatogram of the ginger constituents separated on a 3% SE-30 packed column programmed from 100 to 300°C at 4°C/min. Peaks are identified in Tables I and II and in the text.

TABLE I

GC–MS DATA FOR THE TMS DERIVATIVES OF THE GINGEROLS AND RELATED COMPOUNDS

Compound type	*No.*	*n*	*Peak No.*	*MU (SE-30)*	M^+	*Base*	*Major ions*		
Gingerol	Ia	2	12	22.73	410* (31)	209	145** (50)	320*** (17)	222 (20)
Gingerol	Ia	4	16	24.57	438 (41)	209	173** (37)	348*** (33)	222 (25)
Gingerol	Ia	6	21	26.39	446 (44)	209	201** (31)	356*** (29)	222 (27)
Gingerol	Ia	8	27	28.35	494 (46)	209	229** (26)	404*** (34)	222 (25)
Gingerol	Ia	10	29	30.30	522 (24)	209	257** (13)	432*** (23)	222 (20)
Methylgingerol	Ib	4	15	–	380 (17)	151	173** (46)	290*** (21)	164 (33)
Methylgingerol	Ib	6	20	–	408 (14)	151	201** (40)	318*** (20)	164 (38)
Methylgingerol	Ib	8	26	–	436 (17)	151	229** (38)	346*** (28)	164 (45)
Shogaol	IIa	2	7	–	320 (27)	209	223 (15)		
Shogaol	IIa	4	13	23.24	348 (53)	209	223 (15)	277 (15)	
Shogaol	IIa	6	18	25.26	376 (43)	209	277 (15)	223 (13)	
Shogaol	IIa	8	24	27.28	404 (51)	209	277 (17)	223 (13)	
Methylshogaol	IIb	4	10	–	290 (83)	151	165 (72)	219 (71)	
Methylshogaol	IIb	8	22	–	346 (51)	151	219 (58)	165 (32)	
Paradol	III	4	11	–	350 (56)	209	320 (23)	223 (22)	
Gingediol	IV	4	17	24.91	512 (11)	209	173** (98)	332§ (92)	422*** (44)
Gingediol	IV	6	22	26.77	540 (11)	209	360§ (99)	201** (83)	450*** (43)
Gingediol	IV	8	28	28.69	568 (9)	209	388§ (99)	229** (71)	478*** (43)
Gingerdione	VII	4	14	23.62	364 (29)	209	179 (26)		
Gingerdione	VII	6	19	25.52	392 (30)	209	179 (24)		
Gingerdione	VII	8	25	27.55	420 (36)	209	179 (22)		

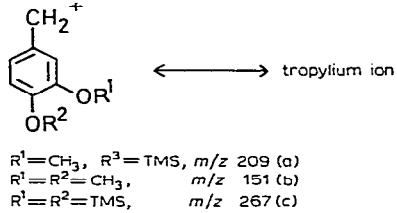
* *m/z* and (relative intensity).
** TMS-O^+=CH–$(CH_2)_n$–CH_3.
*** $[M - TMS\text{-}OH]^+$.
§ $[M - 2 \times TMS\text{-}OH]^+$.

the carrier gas. Operating conditions were: injector, separator and ion source temperatures, 300, 280 and 260°C, respectively; accelerating voltage, 2.5 kV; electron energy, 25 eV; trap current, 100 μA; scan, 3 sec/decade; exponential, down.

RESULTS AND DISCUSSION

Fig. 2 shows a computer reprocessed[18] total ion chromatogram of the ginger constituents separated as TMS derivatives on a 2-m SE-30 column. The compounds which were eluted in the low temperature region of the chromatogram were not studied in detail although a few identifications were made. Thus, peak 1 was identified as α-curcumene[19], peaks 2–4 were $C_{15}H_{24}$ sesquiterpenes, peak 5 was zingerone (V) and peaks 6, 8 and 9 were palmitic, oleic and stearic acids, respectively. Most of the other compounds in this region appeared to be hydroxylated derivatives of the sesquiterpenes (peaks 1–4).

Peaks 7, 10–29 were produced by the gingerols and related compounds (I–IV, VII), the compounds identified are listed in Table I together with their GC–MS characteristics. Identification was based on the GC–MS properties of the TMS, [$^2H_9$]TMS and MO–TMS derivatives. In addition the methylgingerols (Ib) and methylshogaols (IIb) were identified by comparing their GC–MS properties with synthetic samples prepared by methylation of the gingerols. The gingediols (IV) were identified similarly by reduction of the gingerols with lithium aluminium deuteride. The reduction yielded two diasteroisomers, epimeric about the reduced group, but only one of these was present in the ginger sample. Its stereochemistry was not determined. The compounds were located in the ginger fraction by plotting single ion chromatograms of characteristic ions; thus *m/z* 209 (a) was diagnostic of the compounds such as gingerol having one hydroxy and one methoxy substituent in the phenyl ring and *m/z* 151 (b) was characteristic of the methylgingerols and related compounds. Single ion chromatograms for the corresponding tropylium ions from other commonly occurring phenyl derivatives (*e.g.* methylenedioxy) failed to reveal any additional series of gingerols. Acetyl derivatives of the gingerols and gingediols recently found in samples of Japanese ginger[4] were not detected.



Further evidence that the shogaols are predominantly decomposition products of the gingerols was obtained from the experiments on labelling the α-hydrogens of the gingerols and shogaols with deuterium. GC–MS analysis of the products showed that most of the contribution to the shogaol peaks was by molecules which had incorporated 3 deuterium atoms. The shogaols should have incorporated 6 atoms as the result of double bond migration whereas the gingerols with 4 α-hydrogens incorporate 4 atoms. Thermal elimination of water from the gingerols, a 1:2 elimination, would remove one of these deuterium atoms (from C_4) to give a shogaol with

ABLE II

C–MS DATA FOR THE TMS DERIVATIVES OF THE HEXAHYDROCURCUMINS AND RELATE OMPOUNDS

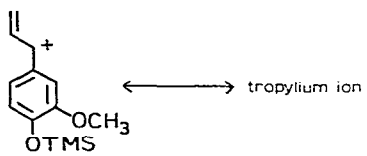
mpound	*No.*	*Peak No.*	*MU*	$M^{+\cdot}$	*Base*	*Major ions*		
exahydrocurcumin	VIa	32	32.55	590* (13)	209	500** (26)	179 (16)	235 (14)
esmethylhexahydrocurcumin	VIb	33	32.96	500 (10)	209	179 (17)		
	XIIIa	30	31.30	648 (21)	209	267 (47)	558** (37)	179 (31)
	XIIIb	31	31.86	558 (14)	209	267 (63)	179 (24)	

* *m/z* and (relative intensity).
** $[M - TMS\text{-}OH]^{+}$.

3 deuterium atoms. Thus, considerable dehydration took place after the completion of the exchange reaction, demonstrating the instability of the gingerols.

The compounds producing peaks 14, 19 and 25 were identified as gingerdiones (VII). They formed mono-TMS derivatives as shown by spectra of the $[^2H_9]$TMS derivatives, and the base peak in the spectrum of the TMS derivative at *m/z* 209 (a) showed that this hydroxy group was on the phenyl ring. Reaction with methoxyamine hydrochloride gave bis-oximes (2 peaks produced by *syn*- and *anti*-isomers) showing the presence of two ketone groups in the original molecules. Confirmation of the presence of these ketone groups in the 3 and 5 positions of the alkyl chain was obtained by oxidation of the gingerols with a mixture of acetic anhydride and dimethylsulphoxide[17]. The GC–MS characteristics of the products of oxidation (TMS derivatives) were identical to those of the diones in the ginger extracts. These compounds do not appear to have been reported before.

Peaks 30–33 were identified as hexahydrocurcumins and related compounds (Table II). Hexahydrocurcumin itself (VIa, peak 32) formed a tris–TMS derivative (molecular weight 590) and its mass spectrum (Fig. 3) showed an abundant loss of TMS-OH (*m/z* 500) and a base peak at *m/z* 209 (a). Ions at *m/z* 189, 193, 223, 249 and 251 were typical of the dihydroferulic acid residue and were also prominant in the spectra of the gingerols (Fig. 4). The ion at *m/z* 235 (d) which was absent from the spectra of the gingerols, came from the second dihydroferulic acid residue (C5–C7) by loss of TMS-OH (the site of the hydrogen loss was not determined) as shown by (a) the absence of exchangeable hydrogens and (b) the presence of the ion in the spectra of the TMS derivatives of the gingediols. Ion d is thus associated with the $Ar\text{–}CH_2\text{–}CH_2\text{–}CHO\text{-}TMS$- and not the $Ar\text{–}CH_2\text{–}CH_2\text{–}CO$-moiety.



d, *m/z* 235

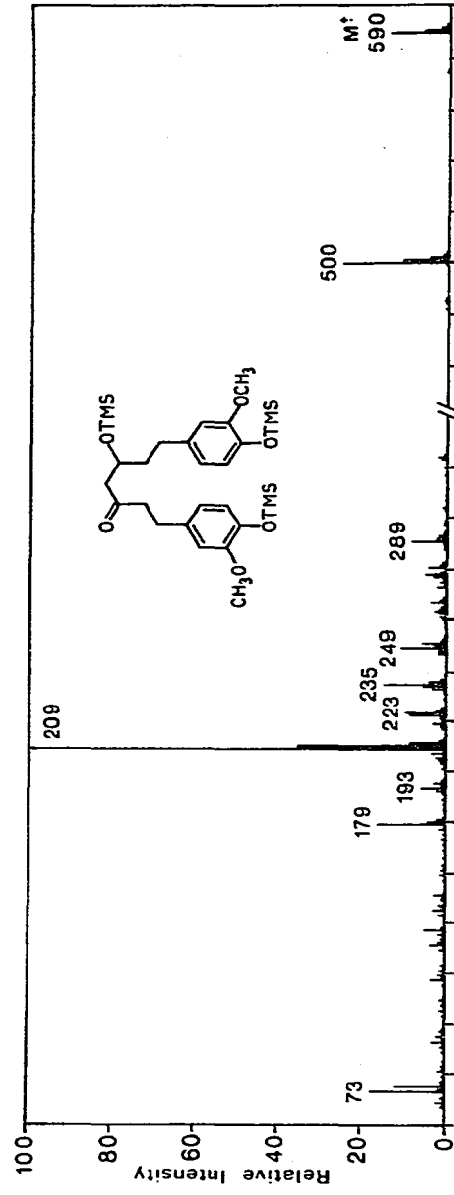


Fig. 3. 25 eV mass spectrum of the TMS derivative of hexahydrocurcumin.

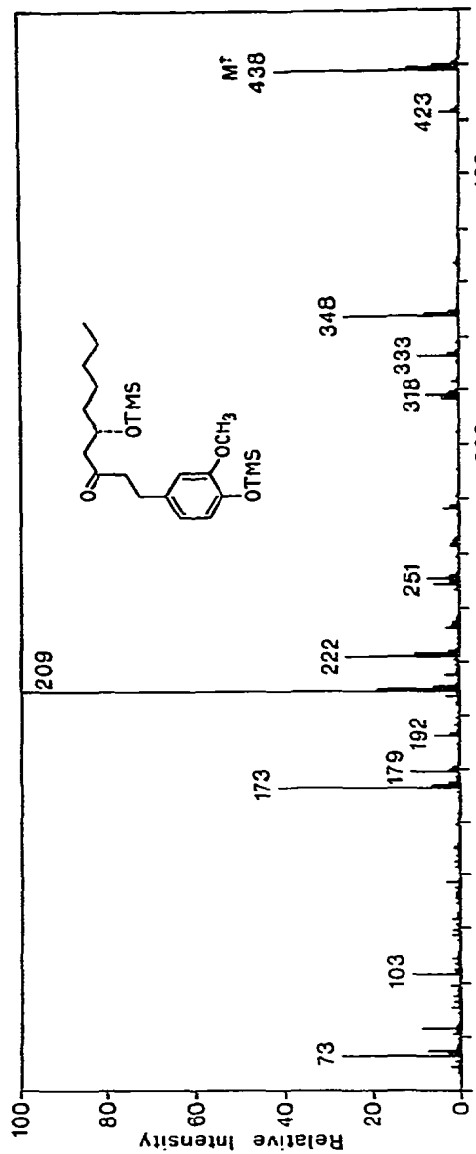


Fig. 4. 25 eV mass spectrum of the TMS derivative of [6]-gingerol (the name [6]-gingerol indicates the chain length of the acid condensed with intermediate IX).

The compound producing peak 33 formed a tetrakis-TMS derivative with a molecular weight of 648. Its mass spectrum was similar to that of the corresponding derivative of hexahydrocurcumin (VIa), but prominent ions at *m/z* 209 (a) and 267 (c) indicated that one of the methoxy groups had been replaced by a hydroxy group. Methylation with methyl iodide gave the same permethylhexahydrocurcumin from both hexahydrocurcumin and the compound producing peak 33. By the use of [$^2H_3$]-methyl iodide the two starting materials could be distinguished by their incorporation of two and three [$^2H_3$]methyl groups, respectively. Thus, peak 33 was produced by a desmethylhexahydrocurcumin. Two isomers (VIb and VIc) of this compound are possible but the mass spectrum of the TMS derivative showed that the compound was VIb. This was based on the shift of ion d (*m/z* 235) by 58 mass units. As this ion comes from the second aromatic residue (attached to C_7), then clearly this must contain the dihydroxy and not the hydroxy methoxy substituents.

A possible biosynthetic pathway for the production of these compounds can be proposed along the lines of the biosynthesis of the gingerols. Thus, condensation of dihydroferulic acid with the intermediate IX (Fig. 1) ultimately leads to hexahydrocurcumin (VIa) whereas condensation of IX with dihydrocaffeic acid (XIV) gives the desmethylhexahydrocurcumin (VIb).

Peaks 30 and 31 had GC–MS characteristics (Table II) fully consistent with their being produced by the hexahydrocurcumin equivalents to the shogaols (VIII). They formed bis-TMS derivatives and monomethyloximes. Results from the deuterium labelling experiments indicated that, like the shogaols, they were produced mainly by thermal dehydration of the hexahydrocurcumins.

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REFERENCES

1 D. W. Connell and M. D. Sutherland, *Aust. J. Chem.*, 22 (1969) 1033.
2 D. W. Connell and R. McLachlan, *J. Chromatogr.*, 67 (1972) 29.
3 Y. Masada, T. Inoue, K. Hashimoto, F. Fujioka and K. Shiraki, *J. Pharm. Soc. Japan.*, 93 (1973) 318.
4 Y. Masada, T. Inoue, K. Hashimoto, M. Fujioka and C. Uchino, *J. Pharm. Soc. Japan.*, 94 (1974) 735.
5 D. W. Connell, *Aust. Chem. Process Eng.*, 24 (1971) 27.
6 R. G. Raghuveer and V. S. Gouindarajan, *J. Food Qual.*, 2 (1979) 41.
7 H. Nomura, *J. Chem. Soc.*, (1917) 769.
8 T. Murata, M. Shinohara and M. Miyamoto, *Chem. Pharm. Bull.*, 20 (1972) 2291.
9 T. Kami, M. Nakayama and S. Hayashi, *Phytochemistry*, 11 (1972) 3377.
10 M. Kučera and H. Kučerová, *J. Chromatogr.*, 93 (1974) 421.
11 A. A. Bednarczyk, W. G. Galetto and A. Kramer, *J. Agr. Food Chem.*, 23 (1975) 499.
12 F. Sakamura and S. Hayashi, *J. Agr. Chem. Soc. Japan.*, 52 (1978) 207.
13 P. Denniff and D. A. Whiting, *Chem. Commun.*, (1976) 711.
14 I. Macleod and D. A. Whiting, *Chem. Commun.*, (1979) 1152.
15 P. Denniff, I. MacLeod and D. A. Whiting, *J. Chem. Soc., Perkin 1*, (1980) 2637.
16 J. A. McCloskey, R. N. Stillwell and A. M. Lawson, *Anal. Chem.*, 40 (1968) 233.
17 J. D. Albright and L. Goldman, *J. Amer. Chem. Soc.*, 87 (1965) 4214.
18 P. Powers, M. J. Wallington, J. A. V. Hopkinson and G. L. Kearns, *23rd Annual Conference on Mass Spectrometry and Allied Topics, Houston, TX, May 25–30, 1975*, Abstracts, p. 499.
19 E. Stenhagen, S. Abrahamsson and F. W. McLafferty, *Registry of Mass Spectral Data*, Vol. 2, Wiley, New York, 1974, p. 999.