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PUNGENT COMPOUNDS*

II. DETECTION AND IDENTIFICATION OF PARADOLS (ALKYL 4-HYDROXY-3-METHOXYPHENETHYL KETONES) BY COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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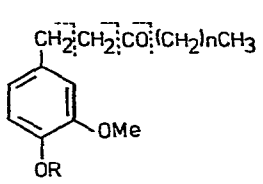
SUMMARY

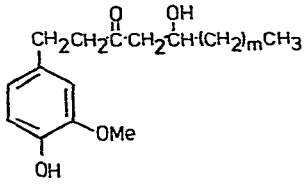
Combined gas chromatography–mass spectrometry is shown to be a suitable technique for the detection, separation and identification of paradols (alkyl 4-hydroxy-3-methoxyphenethyl ketones) as their trimethylsilyl derivatives. Mass spectrometric fragmentation pathways for the compounds are elucidated with the aid of accurate mass measurements and deuterium labelling.

INTRODUCTION

The paradols, alkyl 4-hydroxy-3-methoxyphenethyl ketones (I; R = H, n = 0–10), are pungent compounds, some of which have been isolated from, or detected in, *Zingiber officinale* Roscoe (commercial ginger)[2–5] and the seeds of another member of the Zingiberaceae, *Amomum melegueta* Roscoe[2–6], commonly known as Melegueta pepper, Grains of Paradise, or Guinea pepper[7].



(I)



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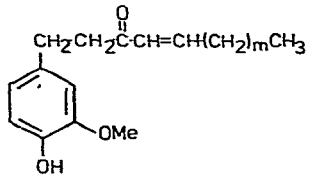

(III)

In the course of earlier studies aimed at correlating pungency with structure, one of us prepared eleven members of the paradol series (I; R = H, n = 0–10)[1]. This study showed that maximum pungency occurs in the range n = 5–7 and that the

* For Part I of this series, see ref. 1.

organoleptic qualities make these paradols very attractive alternatives to capsaicin (from *Capsicum* spp.) as pungent additives in certain foods and beverages.

Past experience[2–6,8–10] shows that paradols are not easily separable by thin-layer chromatography (TLC) or column chromatography, and gas–liquid chromatographic (GLC) separation of the unmodified compounds[1] is not satisfactory since higher members of the series have very long retention times even at 200°. Furthermore, thermal decomposition of the higher homologues probably occurs at this temperature, making the method unsuitable for analytical use. A considerable improvement in the volatility and stability of the related gingerols (II) and shogaols (III), which are also pungent constituents of ginger[2–5], was recently observed when the compounds were converted to trimethylsilyl derivatives before separation by GLC[11].

No parallel study of the trimethylsilyl derivatives of paradols has been made but these derivatives seemed to offer a basis for the rapid and convenient detection and identification of paradols, a process which we wished to develop before commencing a search for these compounds in certain natural materials. The trimethylsilyl derivatives were indeed completely separated from each other by GLC within a reasonable time and they gave characteristic mass spectra by means of which they could readily be identified. Combined gas chromatography and mass spectrometry (GC–MS) therefore provided the means for both separation and identification of the compounds.

EXPERIMENTAL

The paradols were synthesised by published methods[1]. A mixture of the paradols was silylated with "Trisil" and the mixture separated on a Pye 104 gas chromatograph directly coupled to an A.E.I. MS902S mass spectrometer via a silicone rubber membrane separator. The excess of silylating reagent in each injection was discarded via a "dump" valve between chromatograph and separator. Thereafter the arrival of peaks at the spectrometer source was indicated by a recorder connected to the total ion current monitor (Fig. 1) and a spectrum was scanned each time an ion current maximum was reached.

The chromatograph was equipped with an 18 ft. × 2 mm I.D. glass column packed with 3% SE-30 on Chromosorb W-HMDS. The column temperature was kept at 210° for 7 min, raised to 270° at 6°/min and then kept at this temperature. The separator was kept at 260° throughout. Helium was used as carrier gas at a flow-rate of 25 ml/min. The spectra were recorded with source temperature 220°, ionising voltage 70 eV, accelerating voltage 8 kV, and resolving power 1,000. Accurate mass measurements were made at a resolving power of 10,000 and the results obtained agreed with the values calculated for ion compositions shown in the reaction scheme to within 10 ppm.

The nonadeuterio derivative of 6-paradol was prepared by mixing 6-paradol (approx. 1 mg) with perdeuteriotrimethylchlorosilane (25 μl) and pyridine (0.2 ml) and warming the solution. The mass spectrum of the deuterio derivative was recorded by GC–MS as for the other paradols.

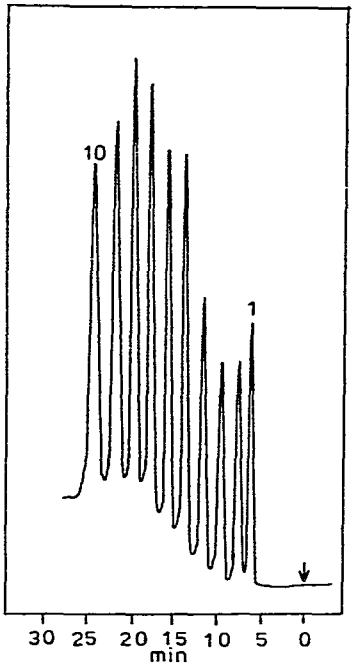

Fig. 1. Total ion current monitor trace during GC–MS of trimethylsilyl derivatives of mixed paradols (I; R = $SiMe_3$, n = 1–10).

RESULTS

Fig. 1 shows the chromatogram obtained from ten synthetic paradols (I; R = H, n = 1–10), which had been mixed together and treated with "Trisil", and Fig. 2 and Table I show examples of mass spectra recorded as the effluent peaks entered the mass spectrometer. Each spectrum shows a group of peaks at higher m/e ratios which are characteristic of a particular paradol and a group of peaks at lower m/e ratios which are common to all paradols.

Fragmentations

In an earlier study[1] of the mass spectral behaviour of the parent molecules, it was found that the paradols fragmented mainly in the predictable manner shown in I (R = H, n = 0–10). Additionally, when $n > 2$, the McLafferty rearrangement was observed as a minor fragmentation process.

With the trimethylsilylated derivatives, two main modes of fragmentation are discernable. One mode (Fig. 3) involves successive losses of two methyl groups from the substituted phenolic hydroxyl groups followed by cleavage of the ketonic side-chain at various points and the other mode (Fig. 4) involves similar side-chain cleavages without prior methyl loss. The fragmentation pathways are illustrated by a typical example, the trimethylsilyl derivative of 6-paradol (I; R = $SiMe_3$, n = 6).

The molecular ion (a), m/e 350, loses a methyl group in typical fashion for a trimethylsilyloxy substituent with a neighbouring group[13] to give the $M-15^+$ ion (b)

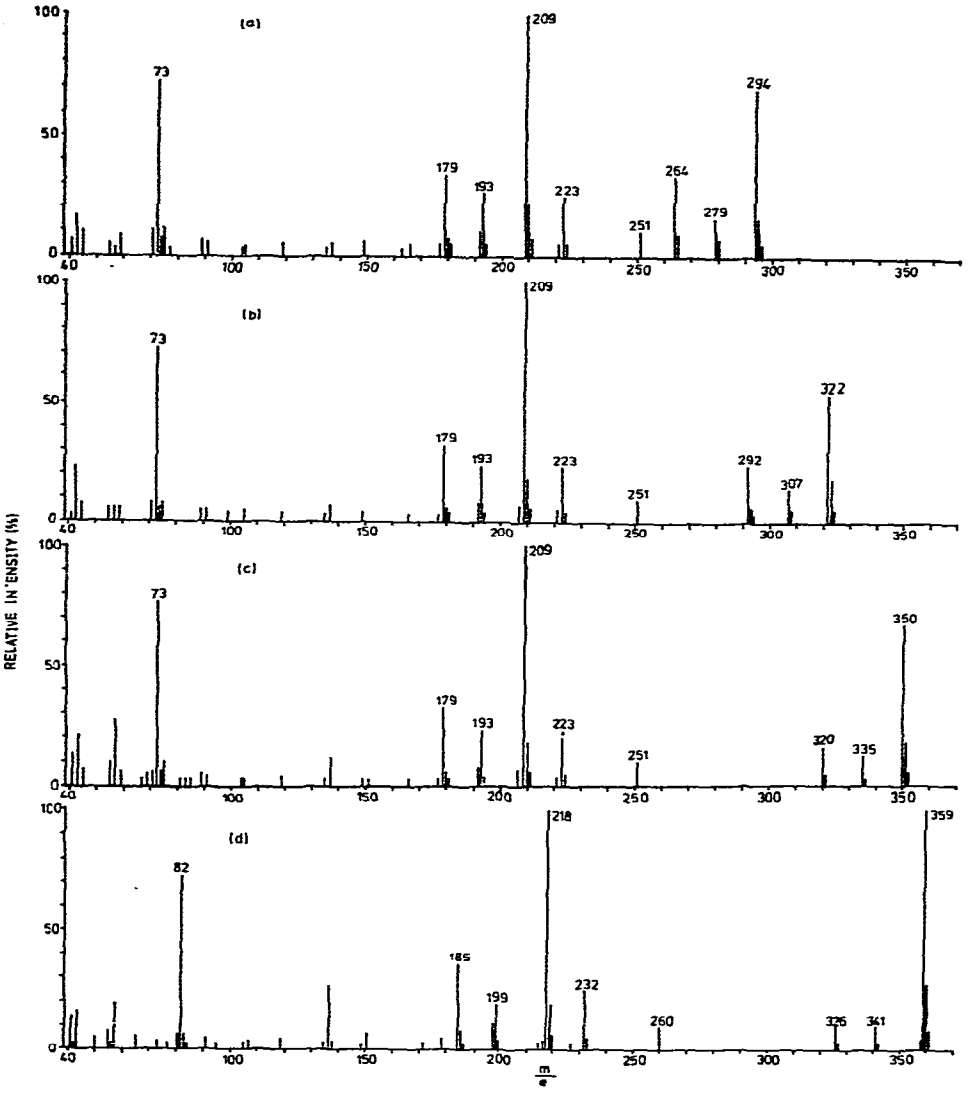

Fig. 2. Mass spectra of trimethylsilyl derivatives of paradols. (a) 2-paradol (I; R = $SiMe_3$, $n = 2$), (b) 4-Paradol (I; R = $SiMe_3$, $n = 4$), (c) 6-paradol (I; R = $SiMe_3$, $n = 6$), and (d) the nonadeuterio derivative (I; R = $Si(CD_3)_3$, $n = 6$).

at *m/e* 335 (Fig. 2c) and this loses the methoxymethyl group to give an ion, *m/e* 320, which probably has the bicyclic structure (c). The sequential nature of these methyl losses is shown by appropriate metastable peaks and the order in which they are lost is shown by the spectrum (Fig. 2d) of the $^2H_9$-trimethylsilyl derivative (I; R = $(CD_3)_3Si$, $n = 6$) in which the peaks at *m/e* 335 and *m/e* 320 are entirely shifted to *m/e* 341 and 326, respectively. Ion (c) undergoes cleavage of the side-chain at any one of the first three carbon atoms to give ion (d), *m/e* 221, (e), *m/e* 193, or (f), *m/e* 179. Direct formation of each of these ions from (c) is indicated by an appropriate metastable

TABLE I

RELATIVE INTENSITIES OF MAJOR PEAKS IN MASS SPECTRA OF PARADOL-TRIMETHYLSILYL DERIVATIVES

Paradol (n in formula I; R = SiMe₃)	*Relative intensity (%)*									
	M⁺	*M−15⁺*	*M−30⁺*	*m/e 251*	*m/e 223*	*m/e 221*	*m/e 209*	*m/e 193*	*m/e 179*	*m/e 73*
1	72	18	41	16	18	6	100	27	41	72
3	72	16	23	8	24	4	100	21	27	63
5	72	14	16	9	25	4	100	18	27	81
7	81	11	11	9	25	4	100	20	36	81
8	67	10	11	10	23	4	100	18	32	68
9	82	11	11	10	24	4	100	17	32	45
10	95	10	8	9	27	4	100	18	36	81

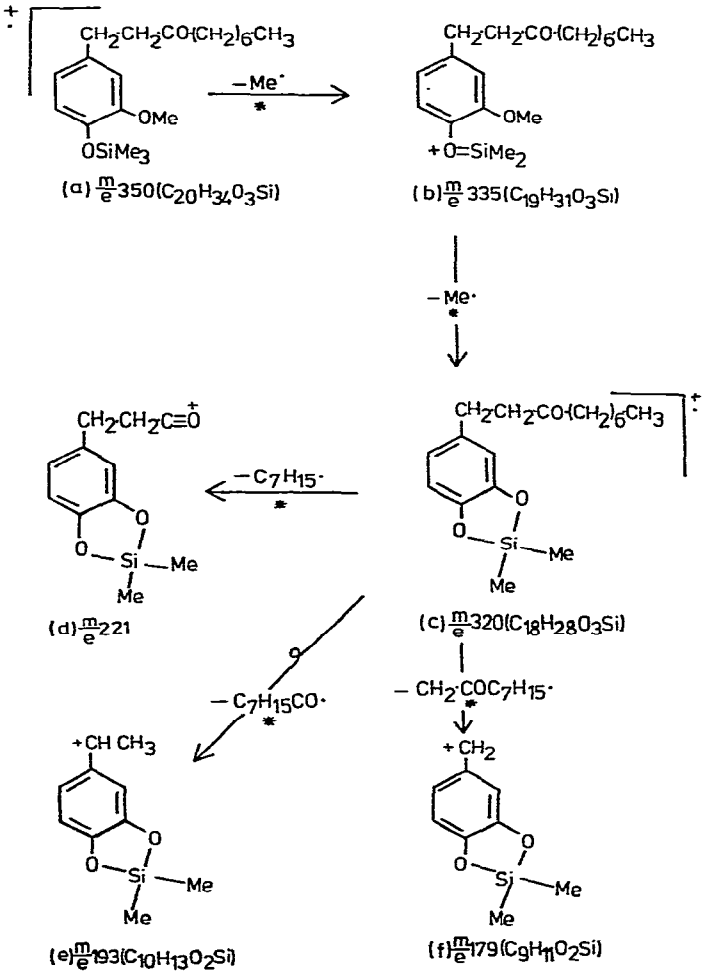

Fig. 3. Fragmentation involving successive losses of two methyl groups followed by cleavage of the ketonic side-chain.

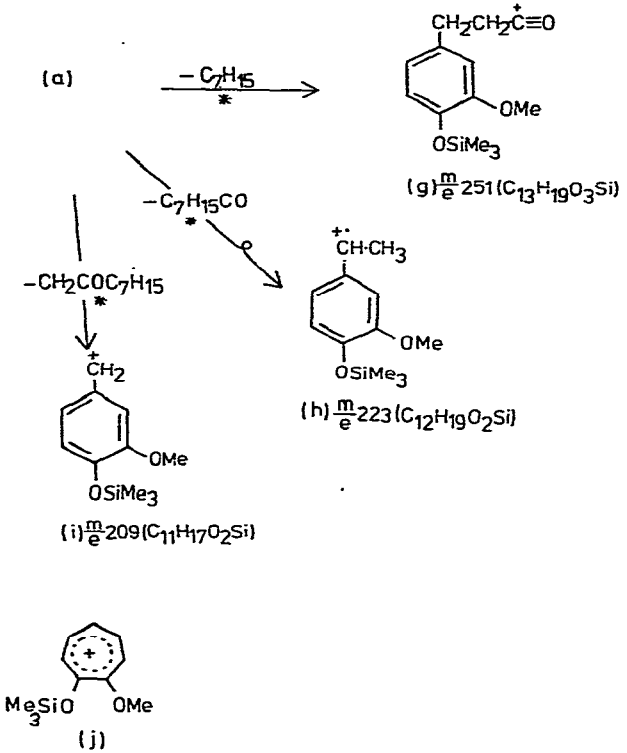

Fig. 4. Fragmentation involving cleavage of the ketonic side-chain without prior methyl loss.

peak. The peaks at *m/e* 221, 193, and 179 are all shifted upwards by six mass units in the spectrum of the nonadeuterio derivative (I; R = $(CD_3)_3Si$, $n = 6$), as required by the reaction scheme.

Alternatively, the side-chain of the intact molecular ion (a) may undergo side-chain cleavage at any one of the first three carbon atoms, in similar fashion to the M—30$^+$ ion (c), to give ion (g), *m/e* 251, (h), *m/e* 223, or (i), *m/e* 209 (base peak). In accordance with this scheme peaks at *m/e* 251, 223, and 209 are all moved upwards by nine mass units in the spectrum of the nonadeuterio derivative (I; R = $(CD_3)_3Si$, $n = 6$).

Compositions of the major fragment ions of *m/e* 335, 320, 251, 223, 209, 193, and 179 were confirmed by accurate mass measurements. Ions (f) and (i) are written as benzylic ions rather than the alternative tropylium ions (*e.g.*, j) because *p*-alkoxybenzyl ions formed from other compounds seem to retain their benzyl structures[14–17].

The lower limit to the amount of a paradol from which a recognisable mass spectrum could be obtained was about 0.1 μg on our instrument. This limit was imposed by bleed from the silicone rubber membrane molecular separator between the gas chromatograph and the spectrometer source which caused many strong background peaks to appear in the mass spectra at the high separator temperature required for the compounds of highest molecular weight. However, the fact that each trimethylsilylated paradol gives a series of strong peaks at *m/e* 251, 223, 209, 193, and

179 whose relative intensities are almost the same for all the compounds makes them very suitable for detection by mass fragmentography[18]. GC–MS, using monitoring of selected ions, should enable very small amounts of paradols to be detected and identified when mixed with larger amounts of other compounds and in the presence of strong background peaks from column or separator bleed.

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