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USE OF TRIMETHYLSILYL AND OTHER HOMOLOGOUS TRIALKYLSILYL DERIVATIVES FOR THE SEPARATION AND CHARACTERIZATION OF MONO- AND DIHYDROXYCANNABINOIDS BY COMBINED GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

A gas chromatographic separation of dihydroxy- from monohydroxycannabinoids by the use of homologous trialkylsilyl derivatives is discussed. Trimethylsilyl derivatives produced a group of peaks containing both sets of compounds, sometimes poorly resolved, whereas by increasing the alkyl chain length to *n*-butyl complete fractionation into two groups was achieved. The mass spectra of these derivatives resembled those of the trimethylsilyl derivatives with the addition of a set of ions resulting from estimation of the Si–alkyl chains as olefins.

INTRODUCTION

An effective method of resolving components of complex biological mixtures by gas–liquid chromatography is by the preparation of a set of derivatives such that a given group of compounds is selected and shifted relative to the other components of the mixture. This has been vividly illustrated by Devaux *et al.*[1], who have used benzyloxime-trimethylsilyl derivatives of steroids rather than methyloxime-trimethylsilyl derivatives in order to separate ketohydroxy- from hydroxy-steroids. Other, usually homologous, sets of derivatives useful for similar separation include the O-butyl- and O-pentyl-oximes[2,3], O-ethyl- and O-trimethylsilyl-oximes[4], cyclic alkyl boronates[5–7], homologous esters[8], and N-alkyl derivatives of barbiturates and xanthines[9,10].

Analyses incorporating this method can be carried out on a single, relatively low-resolution column rather than by employing a number of stationary phases. Moreover, recent work by Moffat *et al.*[11,12] has indicated that there is often little advantage to be gained by using multiple stationary phases, as elution patterns obtained are frequently similar. Such behaviour is exhibited by the cannabinoids[13–16]; polar phases such as OV-17, although producing longer retention times than phases such as SE-30, have little effect on the order of elution of the various cannabinoids.

In our studies of the composition of cannabis extracts, reasonably good sepa-

rations were obtained using trimethylsilyl (TMS) derivatives and a 3% SE-30 column. However, some poorly resolved peaks were present, but as these were frequently mixtures of mono- and dihydroxy compounds, it was possible to achieve separation by variation of the derivatives. Substitution of the TMS group by silyl moieties with higher alkyl substituents resulted in the production of longer retention times, multiplied in the case of the diols. By a suitable choice of the alkyl substituents, the diols in the cannabis extract could be shifted away from the other constituents into a relatively free area of the chromatogram.

A number of trialkylsilyl derivatives have been described as suitable for gas-phase analyses. These have been used mainly for the introduction of halogen atoms for electron-capture[17–22] and multiple-ion-monitoring[23–25] work (halomethyldimethylsilanes), improvement of stability (*tert.*-butyldimethylsilane)[26], and for reduction of molecular weight and retention index (dimethylsilanes)[27,28]. Chloromethyldimethylsilyl derivatives, proposed by Brooks and Middleditch[24] for the separation of steroid alcohols and diols, did not introduce a sufficiently large retention increment difference to separate all of the cannabinoids. Consequently for this study we investigated the following tri-*n*-alkylsilanes: triethyl-, tri-*n*-propyl-, tri-*n*-butyl-, and tri-*n*-hexylsilanes. These silyl derivatives were found to have good gas chromatographic properties and were stable, thus making them suitable for quantitative work.

EXPERIMENTAL

Cannabis extract

The cannabis sample used for these experiments was a commercial preparation of cannabis tincture (BPC 1949), an ethanolic extract of the flowering tops of *Cannabis sativa* L. grown in Pakistan. Its cannabinoid composition has been determined[29]. 0.1-ml samples of this tincture were added to a saturated sodium chloride solution (20 ml) and the cannabinoids and other lipid-like material were extracted thrice with 10 ml of ethyl acetate. Sugars and other polyhydroxy compounds remained in the aqueous layer. The combined ethyl acetate extracts were dried ($MgSO_4$) and the solvents were removed prior to derivatization.

Preparation of derivatives

Trimethylsilyl ethers. N,O-Bis(trimethylsilyl)trifluoroacetamide (40 μl), trimethylchlorosilane (20 μl) and acetonitrile (40 μl) were added to the sample of extracted cannabinoids. The mixture was allowed to stand at room temperature for 30 min. Aliquots (1 μl) were examined by gas chromatography.

Triethyl and other trialkylsilyl ethers. Pyridine (2 ml), diethylamine (0.5 ml) and the appropriate trialkylchlorosilane (1 ml) were mixed in a centrifuge tube. The mixture was cooled in ice-water and centrifuged for about 1 min to settle the white precipitate, and the clear supernatant was pipetted off. 0.1 ml of this reagent mixture was then added to the cannabis sample, prepared as above, and the mixture was left for 30 min at room temperature to complete the formation of the derivatives. Trialkylsilyl derivatives of a number of aliphatic alcohols and diols (Table I) and several standard cannabinoids (Table II) were prepared in a similar manner using 0.1 mg samples.

Gas chromatography

A Varian Model 2400 gas chromatograph fitted with dual flame ionization detectors was used. The columns were 6-ft. × 2-mm-I.D. glass coils packed with 3% SE-30 (Applied Science Labs., State College, Pa., U.S.A.) on 100–200 mesh Gas-Chrom Q. Nitrogen at 30 ml/min was used as the carrier gas and the column oven was programmed at 4°/min over the range 100–320°. The retention indices given in Tables I and II were determined by temperature programming using the above conditions.

Mass spectrometry

Mass spectra were recorded at 70 eV with a VG Micromass 12B mass spectrometer, interfaced via a glass jet separator to a Varian Model 2400 gas chromatograph fitted with an SE-30 column as described above. Spectra were recorded for each chromatographic peak using a 3-sec scan and an accelerating voltage of 2.5 kV.

RESULTS AND DISCUSSION

Formation of the derivatives was rapid and single, and well shaped chromatographic peaks were produced by all the compounds studied. The samples were stable and could be stored at 4° for several months without noticeable decomposition, although some darkening of the solution was observed.

The trialkylsilyl derivatives of a number of primary aliphatic alcohols and aliphatic diols were prepared in order to examine the effect on retention time produced by the various alkyl substituents. The results, expressed as retention indices on 3% SE-30, are given in Table I. Table II lists the retention index values of the trialkylsilyl derivatives of a number of standard cannabinoids. Similar results were obtained in each case. The largest change of retention increment was observed between the trimethyl and triethyl substituents (315 ± 5 in the case of the aliphatic alcohols and 280 ± 10 for the cannabinoids). A fall in the increment was observed between the triethyl and tri-*n*-propyl derivatives (180 ± 10 and 130 ± 10 for the alcohols and cannabinoids, respectively) and this was followed by a slight progressive rise as the chain length increased (Fig. 1). The tri-*n*-pentyl derivatives were not examined, but on the basis of published work[30] their retention indices would be

TABLE I

RETENTION INDICES OF THE R_3Si DERIVATIVES OF SOME ALIPHATIC ALCOHOLS AND DIOLS

Compound	*TMS*	*Et_3Si*	*Pr_3Si*	*Bu_3Si*	*Hex_3Si*
n-Decanol	1480	1790	1985	2205	2705
n-Undecanol	1575	1890	2080	2305	2795
n-Tetradecanol	1775	2090	2275	2500	2970
n-Octadecanol	2165	2485	2670	2890	3370
Propane-1,2-diol	—	1605	1965	2385	—
Propane-1,3-diol	—	1670	2045	2480	—
n-Pentane-1,5-diol	1260	1885	2250	2680	—
n-Heptane-1,7-diol	1455	2080	2440	2875	—

TABLE II

RETENTION INDICES OF THE R_3Si DERIVATIVES OF THE CANNABINOIDS

Compound	*TMS*	*Et_3Si*	*Pr_3Si*	*Bu_3Si*	*Hex_3Si*
Propylcannabidiol	2110	2635	2835	3180	—
Propyl-Δ^1-tetrahydrocannabinol	2170	2465	2590	2795	3250
Cannabidiol	2270	2780	2980	3325	—
Cannabicyclol	2280	2550	2680	2875	3330
$\Delta^{1(6)}$-tetrahydrocannabinol	2335	2615	2740	2935	3385
$\Delta^{1(7)}$-tetrahydrocannabinol	2335	2625	2750	2950	3405
Δ^1-tetrahydrocannabinol	2350	2635	2760	2965	3405
Cannabinol	2430	2725	2855	3055	3500
Cannabigerol	2440	2965	3175	3510	—
Heptyl-Δ^1-tetrahydrocannabinol	2545	2820	2940	3130	3570
7-Hydroxy-$\Delta^{1(6)}$-tetrahydrocannabinol	2650	3220	3440	—	—

expected to fall below the curve shown in Fig. 1. A similar trend has been observed in the retention index values of a series of tetraalkylsilanes[30]. The diols gave similar results with about twice the retention increment (Tables I and II).

Fig. 2 shows the gas chromatogram of the ethyl acetate extract of "cannabis tincture" as its TMS derivative. Identification of the components was made by both their retention times and mass spectra. The largest component (peak 7) was the monohydroxylated compound *trans*-Δ^1-tetrahydrocannabinol, with the diol, cannabidiol (peak 5) present in slightly smaller amounts. Other diols included the propyl analog of cannabidiol (peak 1) and cannabigerol (unresolved from cannabinol). Cannabicyclol, a monohydrated minor constituent, has a similar retention index to that of cannabidiol and its possible presence was thus obscured by the large peak produced by the latter compound. Consequently the gas chromatograms of the higher trialkyl-

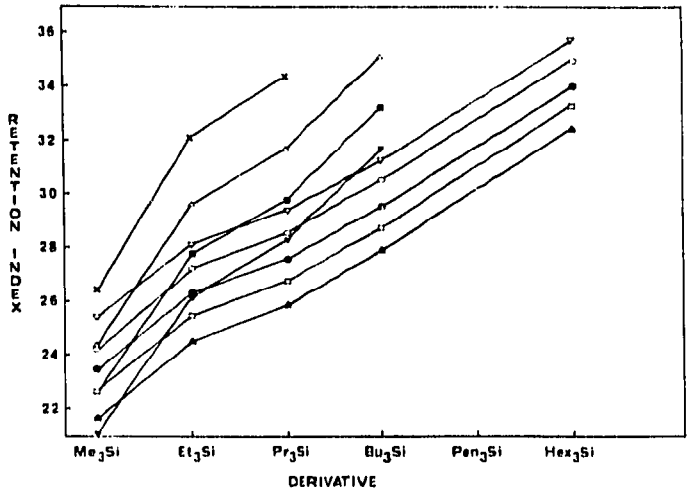

Fig. 1. Plot of retention index against derivative chain length for the various cannabinoid derivatives. ×, 7-Hydroxy-$\Delta^{1(6)}$-tetrahydrocannabinol; △, cannabigerol; ■, cannabidiol; ▼, propylcannabidiol; ▽, heptyl-Δ^1-tetrahydrocannabinol; ○, cannabinol; ●, Δ^1-tetrahydrocannabinol; □, cannabicyclol; ▲, propyl-Δ^1-tetrahydrocannabinol.

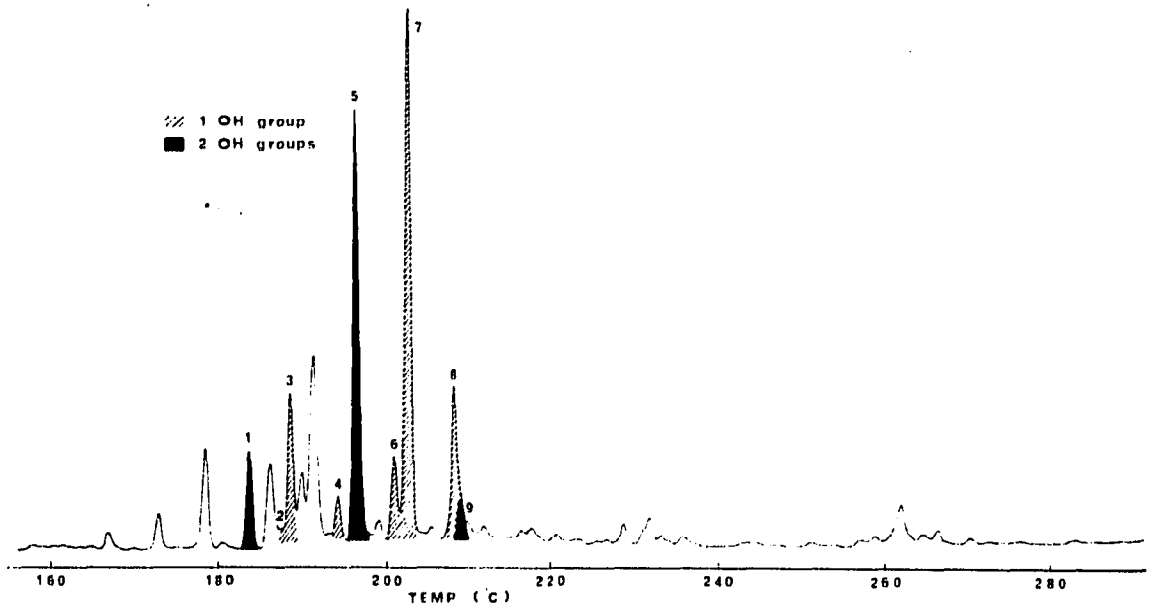

Fig. 2. Gas chromatogram of the TMS derivatives of an ethyl acetate extract of cannabis tincture obtained on a 6-ft. 3% SE-30 column, temperature programmed at 4°/min. The peaks were identified by mass spectrometry as: (1) propylcannabidiol; (2) propylcannabichromene; (3) propyl-Δ^1-tetrahydrocannabinol; (4) propylcannabinol; (5) cannabidiol; (6) cannabichromene; (7) *trans*-Δ^1-tetrahydrocannabinol; (8) cannabinol; (9) cannabigerol.

silyl homologues of this cannabis extract would be expected to show a shift of cannabidiol away from cannabicyclol and a separation of cannabigerol from cannabinol.

Figs. 3, 4 and 5 show the triethyl-, tri-*n*-propyl- and tri-*n*-butylsilyl derivatives of this cannabis extract, respectively. The progressive separation of cannabidiol, propylcannabidiol and cannabigerol from the monohydroxycannabinoids is clearly seen. Movement of the cannabidiol peak from the position occupied by cannabicyclol showed that the latter compound was absent. Complete separation was only achieved by the use of the tri-*n*-butyl derivatives, as propylcannabidiol co-chromatographed with Δ^1-tetrahydrocannabinol and cannabinol, respectively, during the analysis of the triethyl- and tri-*n*-propylsilyl derivatives.

Extension of the chain length further than C_4 was impractical for the separation of the cannabinoids because of interference by peaks produced by polyhydroxylated compounds from the lower end of the chromatogram. Also the temperatures required for elution were undesirably high. This latter point was the main disadvantage of this separation method and limited the application to compounds of moderately low molecular weight or possessing few hydroxy groups. However, when applicable, good separation of mono- and polyhydroxylated compounds could be achieved.

A relatively large molecular weight increment was introduced by the addition of the derivative (TMS = 72, triethylsilyl = 114, tri-*n*-propylsilyl = 156, tri-*n*-butylsilyl = 196 and tri-*n*-hexyl = 282). This set a limit to the molecular weight of the parent compound which could be examined by mass spectrometry, but could be an

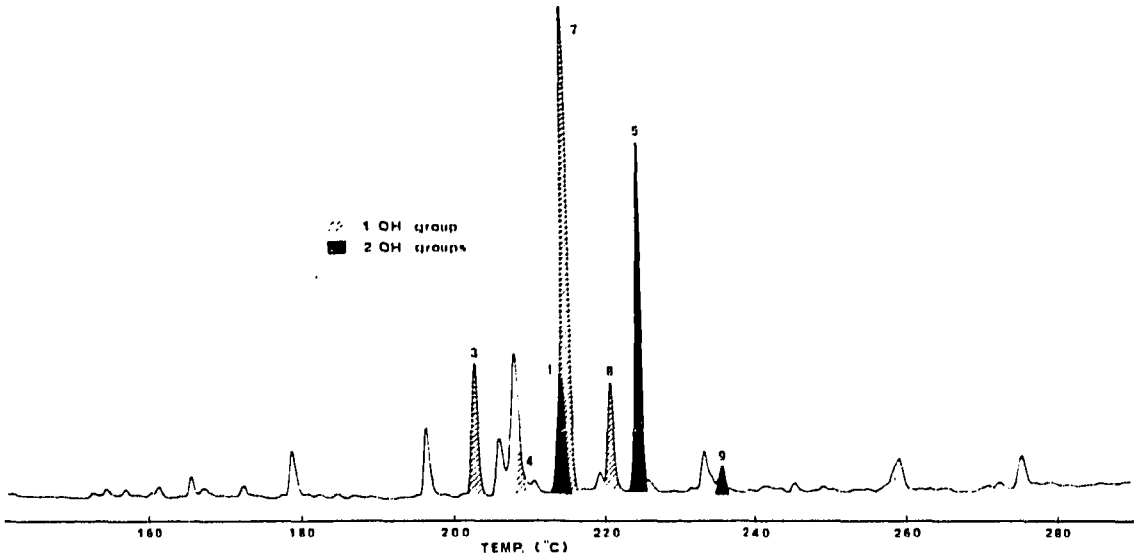

Fig. 3. Gas chromatogram of the triethylsilyl derivatives of an ethyl acetate extract of cannabis tincture. The conditions and peak identification are the same as for Fig. 2.

advantage for single- or multiple-ion work of low-molecular-weight compounds when an abundant ion clear of background ions is required. In general, the mass spectral characteristics of the higher alkylsilyl derivatives were similar to those of the TMS,[1] dimethylsilyl[31,32] and halomethyldimethylsilyl derivatives studied previously. Fragmentation was frequently initiated by charge localization on the silyloxy moiety for the alkyl alcohols; this resulted in a series of characteristic fragment ions containing

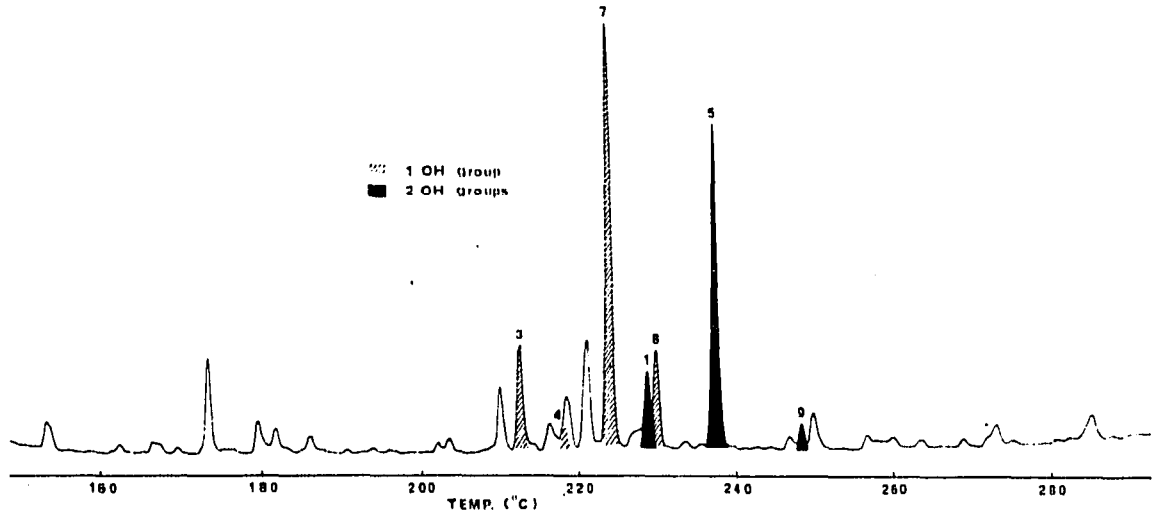

Fig. 4. Gas chromatogram of the tri-*n*-propylsilyl derivatives of an extract of cannabis tincture. The conditions and peak identification are the same as for Fig. 2.

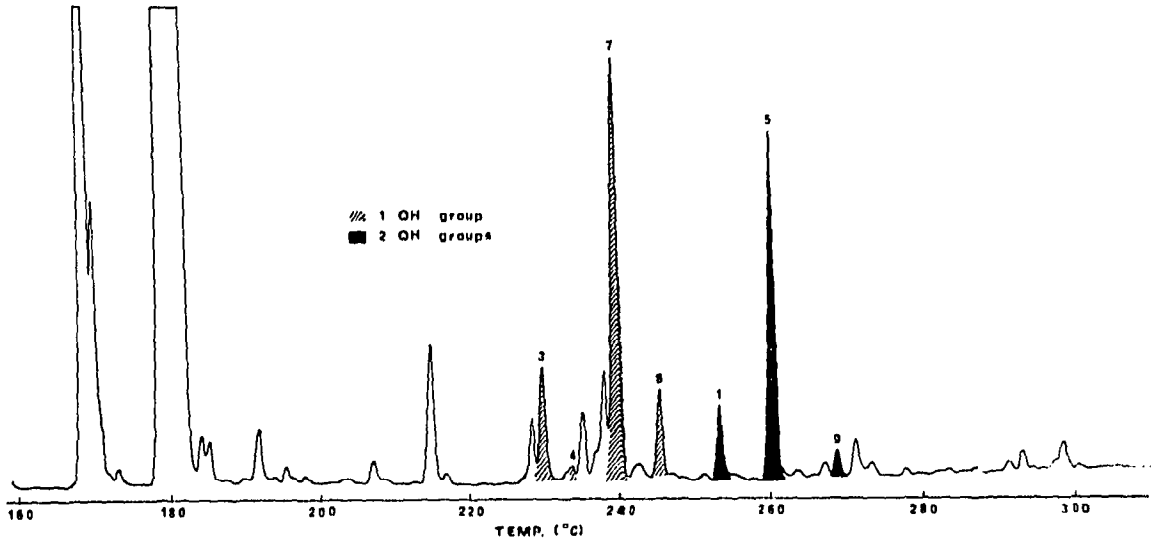

Fig. 5. Gas chromatogram of the tri-*n*-butylsilyl derivatives of an extract of cannabis tincture. The conditions and peak identification are the same as for Fig. 2.

silicon. Three earlier papers[34–36] have discussed an important additional set of fragment ions in the spectra of the triethylsilyl derivatives. These arose from elimination of ethylene from the triethylsilyl group. The higher alkylsilyl derivatives of aliphatic alcohols were also observed to fragment in this manner, with ions produced by the elimination of alkenes from several other fragment ions carrying a substantial proportion of the total ion current. Molecular ions were usually of low abundance or absent when the charge was localized on the derivatized function, a feature shared with the spectra of the TMS derivatives. The spectra of the higher alkylsilyl homologues of many of the cannabinoids were very similar to those of the TMS derivatives. Fragmentation was predominantly initiated by charge localization on the heterocyclic oxygen atom, the silyl moiety having little directing effect.

An interesting feature of the spectra of the derivatives of the aliphatic diols was the presence of a series of very abundant (up to 75% RI) doubly charged ions resulting from elimination of two alkyl groups, one from each silicon moiety (shown by the preparation of mixed triethyl- and tri-*n*-propylsilyl derivatives). These subsequently fragmented by the elimination of alkene groups in metastable transitions to further abundant doubly charged ions.

In addition to their use in separating groups of compounds, as exemplified by the application to the cannabinoids discussed above, the preparation of two different derivatives of an unknown compound reveals the number of hydroxyl groups present by virtue of the larger methylene unit shift exhibited by the polyhydroxy compounds. Fractionation of steroids with various degrees of hydroxylation by the preparation of chloromethyldimethylsilyl and TMS derivatives has previously been reported by Brooks and Middleditch[24]. Estimations of the degree of hydroxylation can thus be achieved without the necessity of preparing isotopically labelled compounds such as d_9-TMS derivatives and examining these by mass spectrometry.

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