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Note

Identification of synthetic cannabinoids by gas chromatography

K. BAILEY, D. LEGAULT and D. VERNER

Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa K1A OL2 (Canada)

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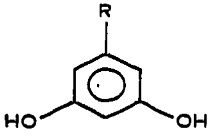
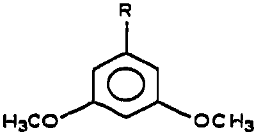
A recent communication by Vree *et al.*[1] indicated that gas chromatography (GC) offers a powerful technique for the preliminary identification of homologues of the cannabinoids which occur in Cannabis. Their work showed that the retention times of groups of cannabinoids formed a series in which the retention time increased by a factor of 0.42 per methylene unit extension in a straight side-chain. The ratio was stated to be independent of oven temperature, gas flow-rate, apparatus, and the stationary phases studied, and they used relative retention time factors to predict the GC retention times of uncharacterized cannabinoids.



Series a



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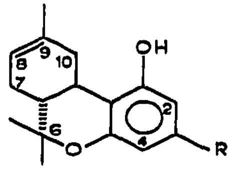

Series c

Series a, b, and c: I, R = H; II, R = CH_3; III, R = C_2H_5; IV, R = $n$$C_3H_7$; V, R = $n$$C_4H_9$; VI, R = $n$$C_5H_{11}$; VII, R = $n$$C_6H_{13}$; VIII, R = $n$$C_7H_{15}$; IX, R = HC·OH·$C_4H_9$; X, R = COC_4H_9; XI, R = CH_2·cyclohexyl; XII, R = CH_2·phenyl; XIII, R = HC·CH_3·phenyl.

We had prepared a series of homologues of Δ^8-tetrahydrocannabinol (VIc; Δ^8-THC) in which the side-chain varied continuously in methylene units from H to C_7H_{15} (Ic–VIIIc), and in which R was made more complex by branching (XIc–XIIIc). The products were obtained by the synthetic method of Petrzilka *et al.*[2], isolated by column chromatography, and identified by infrared and proton magnetic resonance (PMR) spectroscopy. The influence of the side-chain on the retention times at three temperatures for the straight-chain Δ^8-THC series (series c) is shown in Table I, in which results are also presented for the corresponding 1-alkyl-3,5-dihydroxybenzene (Ia–VIIIa) and 1-alkyl-3,5-dimethoxybenzene (Ib–VIIIb) series (these last compounds had been synthesized in these laboratories for the preparation of the Δ^8-THC analogues). A plot of log retention time *vs.* the number of carbon atoms in the side-chain of a homologous series would be a straight line if the factor by which retention times increase per methylene unit were constant, and parallel lines

TABLE I

RETENTION TIMES OF 1-ALKYL-3,5-DIHYDROXYBENZENES (SERIES a), 1-ALKYL-3,5-DIMETHOXYBENZENES (SERIES b), AND Δ^8-THC HOMOLOGUES (SERIES c)

The column was of glass, 6 ft. long × 3/16 in. I.D., containing 5% OV-7 on 80–100 mesh Chromosorb W as support; injection port temperature, 275°, FID temperature, 275°, nitrogen flow-rate 30 ml/min. Time is in minutes. The retention times for series b were obtained several months after those for series a and c, during which interval the column was repacked; all retention times were determined on multiple-component mixtures.

Structure	*Series*							
	a			*b*		*c*		
	175°	200°	220°	150°	180°	200°	220°	250°
I	2.05	1.3	1.1	1.6	1.0	12.6	7.3	3.7
II	2.8	1.6	1.3	2.2	1.3	17.2	9.6	4.6
III	4.1	2.0	1.6	3.0	1.6	23.2	12.5	5.8
IV	5.6	2.6	1.9	4.1	2.1	30.9	16.0	7.1
V	8.5	3.5	2.45	6.2	2.8	44.3	21.9	9.2
VI	12.7	4.7	3.2	9.4	4.0	64.0	29.5	12.0
VII	19.6	6.5	4.4	14.5	5.8		40.7	16.0
VIII	29.5	9.2	5.75	22.5	8.5		55.2	20.6

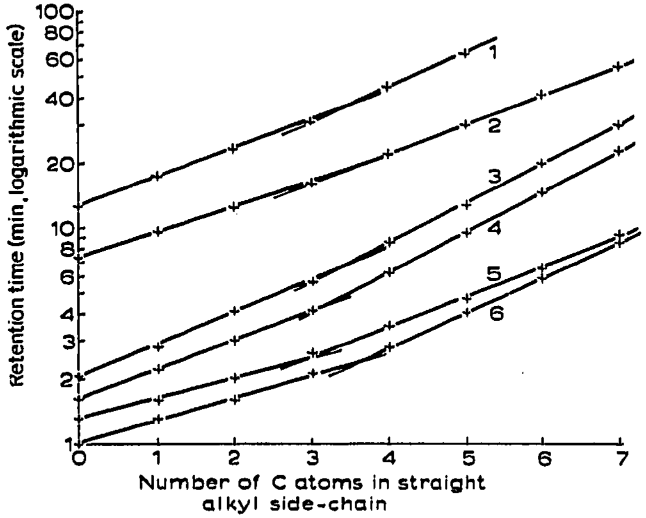

Fig. 1. Relationship between log retention time and number of carbon atoms in straight alkyl side-chain of 1-alkyl-3,5-dihydroxy- and dimethoxybenzenes and Δ^8-THC homologues. 1 = series c at 200°, 2 = series c at 220°, 3 = series a at 175°, 4 = series b at 150°, 5 = series a at 200°, 6 = series b at 180°. For column conditions, see Table I.

would result for measurements on a series at different column temperatures if the factor were temperature independent. The results in Table I are plotted in this way in Fig. 1. It is at once apparent that the curves are neither linear nor parallel to one another. The first three members of a series (H to C_2H_5) seem to obey a relationship different from that of the last four (C_4H_9 to C_7H_{15}). There appear to be two almost straight lines for a series at constant temperature, which intercept one another at or about the position for the propyl-substituted compound (an almost equally good fit to either line). The effect is emphasized by lower column temperatures. It therefore seems that there is some break in the regular change in properties which control GC behaviour[3] and that this occurs between the ethyl and propyl or propyl and butyl side-chains of these three series. This phenomenon, possibly conformational in origin, awaits further investigation.

Because of the discrepancies described above, a modification of the method of Vree *et al.*[1] was introduced which has proved to be of useful application for the identification of derivatives of Δ^8-THC including those with functionalized side-chains, in crude synthesis mixtures. In this method the retention times of the 1-substituted 3,5-dihydroxybenzenes (starting materials) are plotted graphically against the corresponding tetrahydrocannabinol (product) retention times measured at a convenient temperature. This effectively compares the influence of the basic C_{16} cannabinoid skeleton with that of the benzene ring on the retention times of the various compounds. The results (Figs. 2 and 3) show that smooth, almost straight-line plots result (possibly with a break at the propyl compound) even when the side-chain is branched or functionalized. It was not possible to obtain column conditions under which all pairs simultaneously had identical retention times. Determination of the

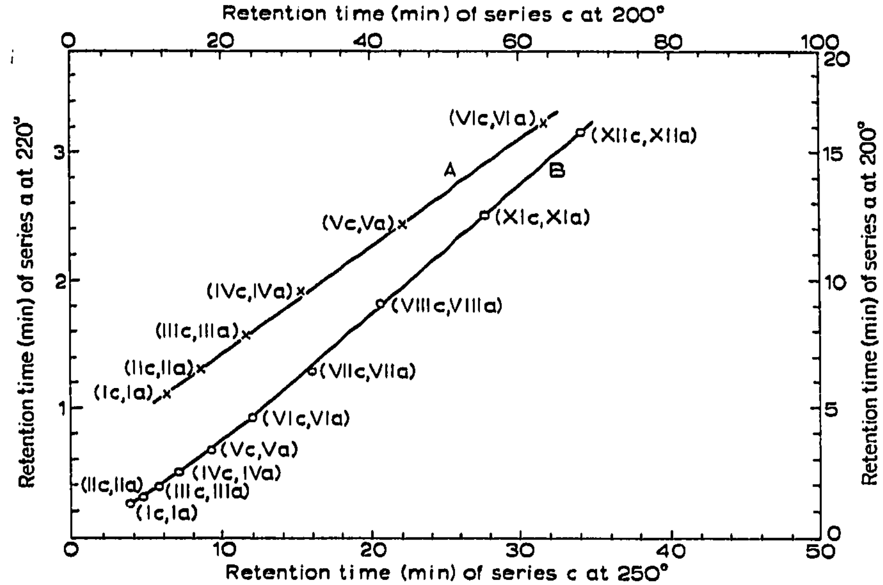

Fig. 2. Relationship between retention time of 1-substituted 3,5-dihydroxybenzene and that of corresponding Δ^8-THC homologue. For column conditions, see Table I. Line A: read upper abscissa and left-hand ordinate, Line B: read lower abscissa and right-hand ordinate.

retention time of a 1-substituted 3,5-dihydroxybenzene enables the retention time of the corresponding new Δ^8-THC analogue to be estimated from the curve and hence to be identified in the synthesis mixture. For example from Fig. 3, the estimated retention time for IXc from the other points, ignoring XIIIc *vs.* XIIIa, is 16.2 min and that found is 16.0 min.

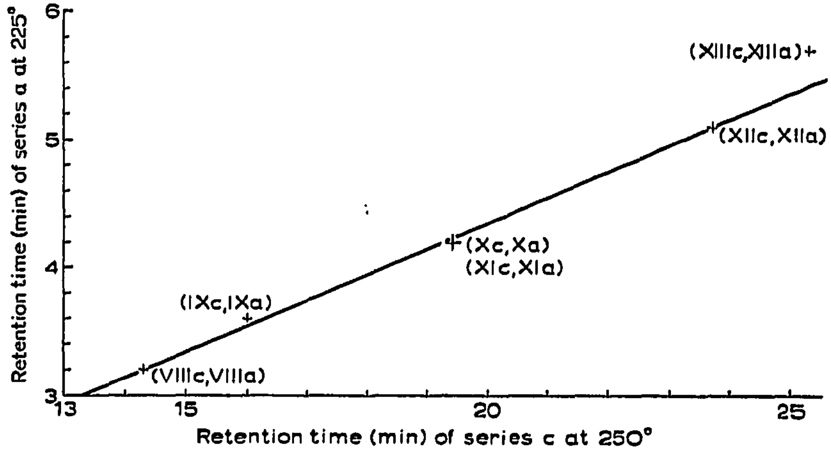

Fig. 3. Relationship between retention time of 1-substituted 3,5-dihydroxybenzene and that of corresponding Δ^8-THC homologue. These results were obtained later than those of Fig. 2. For column conditions, see Table I.

Compound IXc, a metabolite of VIc, was synthesized by a published method[4]. The PMR signals of the aromatic protons 2 and 4 indicated that both diastereoisomers were present (1′ R or S, apparently in equal amounts), but the GC peak of the mixture was always homogeneous under the conditions used. A similar situation was found by GC and PMR spectroscopy when the diastereomeric pair XIIIc was synthesised.

The 1′-bulkily substituted pair XIIIc *vs.* XIIIa does not closely obey the straight-line relationship IXc–XIIc *vs.* IXa–XIIa (Fig. 3): the retention time of XIIIc seems relatively short and/or that of XIIIa relatively long but by only about 5%. The employment of the ratio method developed by Vree *et al.*[1] for different cannabinoid skeletons may allow extension of the identification technique to cannabinoids in which the basic ring system also undergoes modification.

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