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The decomposition of acidic and neutral cannabinoids in organic solvents

R. N. SMITH* AND C. G. VAUGHAN

Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London, SE1 7LP, U.K.

High-pressure liquid chromatography was used to study (a) the relative efficiencies of methanol, chloroform, light petroleum (B.P. 40–60°) and methanol–chloroform (9:1) for extracting neutral and acidic cannabinoids from cannabis resin; (b) the decomposition patterns of the resulting solutions under various storage conditions, and (c) the cannabinoid profile of a cross section through a block of cannabis resin. The results show that (a) methanol is the most effective extracting solvent of those tested; (b) acidic cannabinoids in solution decompose in darkness by varying amounts depending on the temperature, solvent, storage time and particular cannabinoid; (c) neutral cannabinoids in solution are relatively stable in darkness; (d) daylight causes appreciable decomposition of both acidic and neutral cannabinoids in solution, and (e) the cannabinoid profile of a resin is complex with lower levels of acidic material in the outer layers.

Previous studies of the decomposition of cannabinoids have been reviewed briefly by Fairbairn, Liebmann & Rowan (1976) whose own work showed that exposure to light was a more important factor than aerial oxidation or the effect of temperatures up to 20°. Most of the earlier work involved analysis of underivatized material by gas-liquid chromatography (g.l.c.) with the result that acidic cannabinoids were decarboxylated on injection to give the corresponding neutral compounds, and so it was not possible to distinguish the decomposition patterns of the acidic from those of the neutral cannabinoids.

A high-pressure liquid chromatographic (h.p.l.c.) method (Smith & Vaughan, 1976) has now been used to determine the decomposition patterns of both acidic and neutral cannabinoids.

MATERIALS AND METHODS

Cannabis resin

A single block of light brown, moderately friable cannabis resin thought to be of Middle-Eastern origin and approximately 2 years old was used for the extraction and decomposition experiments. It was finely ground and mixed to give a homogeneous material containing Δ^1-tetrahydrocannabinol (THC) 2·6%, Δ^1-tetrahydrocannabinolic acid (THCA) 3·5%, cannabidiol (CBD) 0·65%, cannabidiolic acid (CBDA) 1·3%, cannabinol (CBN) 0·76% and cannabinolic acid (CBNA) 0·36%. Also present (but not quantitated since pure standards were not available) were cannabichromene (CBCh), cannabichromenic acid (CBChA), cannabigerol (CBG) and cannabigerolic acid (CBGA).

Variation in cannabinoid content through a single block was determined using a Middle-Eastern resin of similar composition. 2 mm layers were cut from the 14 mm thick block and analysed separately.

Extraction and decomposition experiments

The solvents used were methanol (Analar grade), chloroform (spectroscopic grade), light petroleum (B.P. 40–60°; general purpose grade) and methanol–chloroform (9:1; Analar and spectroscopic grades respectively). Di-n-octyl phthalate (8 g litre^{-1}) was added to each solvent to provide an internal standard for both g.l.c. and h.p.l.c. There was no decomposition of the internal standard over the period of the experiments. 500 mg portions of the cannabis resin powder were extracted with 5 ml volumes of the solvents for 10 min in an ultrasonic bath. Solid debris was centrifuged down and 1 ml aliquots of the extracts were transferred to 1·7 ml clear-glass, screw-capped vials. Extracts in the four solvents were stored in darkness at −18°, 4° and *ca* 20° and in daylight at *ca* 20°. Aliquots were removed at intervals, quickly evaporated to dryness under a stream of nitrogen at room temperature, taken up in methanol–chloroform (9:1) and analysed in duplicate.

Analysis

The g.l.c. and h.p.l.c. methods of analysis have been described in detail elsewhere (Smith & Vaughan, 1976). H.p.l.c. was used for most analyses and g.l.c. was used as an occasional check. The coefficient of

* Correspondence.

variance of the h.p.l.c. method is about 2% for most of the cannabinoids. For CBN it is about 4–6%. Changes in CBCh and CBChA were calculated from peak height measurements relative to the internal standard. CBCh appears as a minor peak on h.p.l.c. so the results are accurate to only ±5–10%. The results for CBChA, which absorbs strongly at 254 nm, have a coefficient of variance of about 2%.

RESULTS AND DISCUSSION

Extraction of cannabinoids by organic solvents

Table 1 shows the percentage amounts of cannabinoids extracted from cannabis resin by the four solvents given relative to methanol to which was assigned an arbitrary extraction efficiency of 100% (ethanol (Analar) was found to be identical to methanol). Unlike Turner, Hadley & Davis (1973), we found alcohol-based solvents to be superior to chloroform for most of the cannabinoids. Exceptions were CBCh and CBChA for which chloroform was best. It may be that the ethanol used by Turner & others (1973) contained water. Chloroform has been widely used as a solvent for cannabis (cf. Parker, Borke & others, 1974), and the results in Table 1 confirm its suitability if the sample is extracted twice as recommended by Fairbairn & Liebmann (1973). A single extraction as used by Turner & others (1973) and Turner & Henry (1975) is likely to be less effective due to incomplete extraction of THCA, CBDA and CBNA.

We included methanol–chloroform (9:1) because it is suitable for the quantitative h.p.l.c. of cannabis (Smith & Vaughan, 1976) by virtue of its density and its efficiency as an extraction solvent. Light petroleum, while being more selective than methanol

Table 1. *Extraction of cannabinoids from cannabis resin by organic solvents.* Results are given as the percentage of cannabinoid extracted relative to the amount extracted by methanol = 100%*. The resin had the following composition by weight: THC 2·6%, THCA 3·5%, CBD 0·65%, CBDA 1·3%, CBN 0·76%, CBNA 0·36%. Absolute amounts of CBCh and CBChA were not determined since a pure standard was not available.

	THC	THCA	CBD	CBDA	CNB	CBNA	CBCh	CBChA
Chloroform	99	92	99	92	101	92	142	121
Light petroleum (B.P. 40–60°)	93	82	94	77	86	67	54	72
Methanol–chloroform (9:1)	100	99	99	99	100	100	110	100

* Ethanol was found to give identical results to methanol.

which extracts chlorophyll and other plant constituents as well as cannabinoids (Willinsky, 1973), is the least efficient (see Table 1) and we have found it to offer no advantage in h.p.l.c.

Decomposition in darkness at −18° (Table 2A). Slight increases in some of the neutral cannabinoids may be accounted for by partial decarboxylation of the corresponding acids, but over the 29 day period there were also some minor, unexplained increases and decreases. For the first few days of the experiment, however, the changes were minimal.

Decomposition in darkness at 4° (Table 2B). Particularly noticeable was the decrease in THCA in all solvents and the corresponding increase in THC. This was presumably due to decarboxylation of THCA. Similar but less marked changes occurred in CBD, CBN and the corresponding acids in some solvents. There were also some small, unexplained increases and decreases but, as in Table 2A, changes over the first few days were minimal.

Decomposition in darkness at ca *20°* (Table 2C). A decrease in THCA and an increase in THC occurred in all solvents and particularly in chloroform. The effect was greater than at −18° or 4°. Similarly there was a decrease in CBDA and an increase in CBD in all solvents, but the percentage changes were less than for THCA and THC. In chloroform and light petroleum, there was a decrease in CBNA and a corresponding increase in CBN but, in methanol and methanol–chloroform (9:1), a slow decrease in CBNA was accompanied first by a decrease then an increase in CBN. The reason is not known. CBCh and CBChA decreased with time in all solvents. Changes over the first few days were small.

Decomposition in daylight at ca *20°* (Table 2D). The decomposition patterns differed markedly from those in darkness. This was to be expected since daylight has a considerable effect on cannabinoid breakdown (Fairbairn & others, 1976). The most noticeable feature was a decrease in all cannabinoids, both acidic and neutral, with the exception of CBCh which increased in chloroform and light petroleum and remained relatively constant in methanol and methanol–chloroform (9:1). When the formula for quantitating CBN in the presence of CBGA (Smith & Vaughan, 1976) was applied to the chromatograms, negative values were obtained in some cases which indicated a build-up of one or more decomposition products with the same retention time as CBN on

Table 2. *Decomposition of cannabinoids in solution.* Results are given as percentage of cannabinoid remaining after the stated interval (Time 0 = 100%).

A. Storage in darkness at −18°

Day	THC	THCA	THC+ THCA	CBD	CBDA	CBD+ CBDA	CBN	CBNA	CBN+ CBNA	CBCh	CBChA
Methanol											
11	101	98	99	100	101	101	104	103	104	125	98
16	107	94	100	106	99	101	117	97	111	130	99
29	105	97	100	102	99	100	111	100	107	120	100
Chloroform											
11	102	106	105	98	108	104	99	109	102	122	104
16	101	101	101	97	101	100	107	103	106	110	98
29	106	98	102	103	98	100	117	97	111	104	96
Light petroleum (b.p. 40–60°)											
11	100	100	100	92	91	91	91	96	93	86	101
16	104	99	101	97	93	94	97	96	97	96	100
29	104	95	99	97	92	94	103	96	101	114	97
Methanol–chloroform (9 : 1)											
11	102	102	102	100	100	100	103	103	103	118	103
16	106	99	102	106	100	102	107	103	106	118	99
29	106	100	103	108	101	103	106	103	105	118	100

B. Storage in darkness at 4°

Day	THC	THCA	THC+ THCA	CBD	CBDA	CBD+ CBDA	CBN	CBNA	CBN+ CBNA	CBCh	CBChA
Methanol											
9	107	100	103	106	105	105	101	106	103	115	101
16	110	94	101	99	92	94	101	103	102	110	97
27	113	91	100	109	93	99	97	100	98	110	99
Chloroform											
9	118	90	102	103	96	98	109	94	105	104	93
16	132	81	103	109	94	100	108	88	102	107	91
27	148	68	103	116	88	97	104	79	96	93	82
Light petroleum (b.p. 40–60°)											
9	112	97	104	105	103	104	91	96	93	100	98
16	116	81	97	98	79	86	99	83	95	77	92
27	127	73	98	103	81	90	99	79	94	78	89
Methanol–chloroform (9 : 1)											
9	107	101	104	108	103	105	107	106	107	109	102
16	110	95	101	100	90	93	107	103	106	105	101
27	113	91	100	110	92	98	103	100	102	105	99

C. Storage in darkness at *ca* 20°

Day	THC	THCA	THC+ THCA	CBD	CBDA	CBD+ CBDA	CBN	CBNA	CBN+ CBNA	CBCh	CBChA
Methanol											
2	103	97	100	102	98	100	96	97	96	94	102
3	105	96	100	103	98	100	92	97	93	89	100
6	107	93	99	105	93	97	88	94	90	96	98
9	109	90	99	108	95	100	78	97	84	104	98
15	127	78	100	128	91	103	99	91	96	96	94
Chloroform											
2	113	86	98	106	92	97	109	88	102	89	99
3	119	81	98	106	91	96	104	85	98	100	96
6	138	69	100	119	89	96	107	74	96	91	87
9	150	57	99	125	82	96	109	65	94	89	78
15	184	35	102	159	73	102	123	41	96	83	63
Light petroleum (b.p. 40–60°)											
2	111	89	99	102	91	95	112	92	94	87	99
3	118	84	100	110	91	98	114	83	105	87	94
6	126	74	99	115	88	98	109	79	100	96	88
9	134	63	96	120	77	93	114	63	99	87	83
15	161	50	102	151	76	102	116	58	99	87	79
Methanol-chloroform (9 : 1)											
2	101	99	98	102	96	98	100	100	100	88	101
3	101	96	98	100	95	97	96	100	97	88	102
6	107	93	99	110	96	101	93	97	94	92	100
9	111	90	99	110	92	97	88	97	90	92	99
15	133	80	104	136	88	104	111	94	106	92	97

D. Storage in daylight at *ca* 20°

Day	THC	THCA	THC+ THCA	CBD	CBDA	CBD+ CBDA	CBN*	CBNA	CBN+ CBNA	CBCh	CBChA
Methanol											
2	82	95	78	97	89	91	—	92	—	105	90
8	80	62	70	98	84	89	—	86	—	100	68
13	77	51	63	89	76	80	—	83	—	100	61
24	70	34	49	92	64	74	—	72	—	85	41
37	44	16	28	75	48	57	—	53	—	115	28
Chloroform											
2	72	74	73	103	93	97	96	94	96	155	58
8	57	54	55	105	85	92	104	82	97	241	34
13	38	40	39	97	78	85	97	76	91	262	20
24	13	22	18	84	66	72	90	67	83	224	5
37	4	9	16	66	52	57	69	58	66	324	1
Light petroleum (b.p. 40–60°)											
2	81	82	82	103	89	94	—	83	—	409	85
8	61	69	65	97	80	86	—	75	—	400	69
13	46	58	53	90	69	77	—	67	—	255	65
24	42	43	43	92	57	70	—	58	—	236	54
37	27	33	30	89	50	64	—	50	—	209	44
Methanol–chloroform (9 : 1)											
2	82	73	77	102	84	90	—	86	—	91	78
8	73	57	64	94	79	84	—	86	—	96	60
13	64	44	52	80	69	73	—	75	—	96	51
24	46	24	33	81	52	61	—	64	—	96	33
37	30	13	20	64	39	47	—	47	—	109	19

* Interference from unidentified decomposition products in all solvents but chloroform precluded accurate measurement of CBN.

h.p.l.c. When the CBN in the 0 and 37-day extracts was quantitated at both 220 and 254 nm using appropriate calibration factors, discrepancies were revealed in all but the chloroform extracts which could not be attributed solely to changes in CBGA, thus confirming the presence of at least one additional component in the 37-day methanol, light petroleum and methanol–chloroform (9 : 1) extracts. A similar examination of THCA, CBDA and CBNA revealed no obvious interference by decomposition products. The CBN contents of the extracts stored in darkness at *ca* 20° were also re-examined but no discrepancies were found.

The use of measurements at two detector wavelengths to monitor the purity of separated cannabinoids is an advantage of h.p.l.c. over g.l.c., but it is only feasible for cannbinoids with reasonable ultraviolet absorbances at both wavelengths. THC and CBD cannot be quantitated at both 220 and 254 nm since they have a low absorbance at the latter wavelength, and so it was not possible to determine directly whether decomposition products interfered in their measurement. However, an indication that the values for THC in Table 2 are probably valid is given by comparison with the results of Fairbairn & others (1976) who found that pure THC in chloroform or light petroleum is relatively stable in darkness at 20° whereas it decomposes in light.

The question of CBD decomposition is more complex and controversial than that of THC. Turner & others (1973) and Turner & Henry (1975) claim that CBD is stable in chloroform for a number of days in daylight and artificial light at room temperature. Parker & others (1974) and Fairbairn & others (1976) however, report rapid decomposition of CBD in chloroform in darkness or daylight at room temperature. Turner & Henry (1975) suggested that impurities in certain grades of chloroform were responsible for the decomposition of CBD found by Parker & others (1974), but Fairbairn & others (1976) tried various grades of chloroform (including spectroscopically pure) and found rapid decomposition of CBD in daylight in all cases. We have studied the stability of pure CBD (1·0 mg ml^{-1}) in both chloroform (spectroscopic grade) and methanol (Analar) in darkness at *ca* 20° over five days. In methanol, there was no decomposition, but in chloroform only 18% of the original CBD remained after five days. No decomposition products were observed on h.p.l.c.

In view of the instability of CBD in chloroform, it was surprising to find that 66% of the original amount in a cannabis resin extract remained after 37 days in daylight (Table 2D). Possibly CBG, which has the same h.p.l.c. retention time as CBD, increased during the experiment due to decarboxylation of CBGA, but both CBG and CBGA are relatively minor components of cannabis resin and so their effect on the CBD peak would be small. Alternatively, unidentified decomposition products with the retention of CBD may have masked the decomposition of CBD, and another possibility is that pigments or other components of the extract partially stabilized the CBD against decomposition. In darkness (Table 2A, B & C), the CBD + CBDA content of all the extracts remained close to 100%. It is unlikely that a build-up of decomposition products would balance any loss of CBD + CBDA so precisely, and so it is reasonable to conclude that, in chloroform extracts of cannabis stored in darkness, the CBD is stabilized in some way. Our results agree qualitatively with those of Fairbairn & others (1976) who found that the CBD in ethanol or chloroform extracts of cannabis was stable in darkness but not in light.

Variation in composition through a block of cannabis resin

2 mm layers of a 14 mm thick block of cannabis resin were analysed. The results are given in Table 3. Variations between the layers were found which doubtless reflect the effects of manufacturing processes and storage conditions in addition to any heterogeneity of composition in the starting material. For instance, unequal exposure of the outer surfaces to heat or light during manufacture and storage could lead to variations in composition of the sort that were found. The measurement of such variation may have practical application in forensic work where it is sometimes necessary to determine whether different cannabis seizures could have originated from a single source. At present, the determination of gross composition by h.p.l.c. (Smith, 1975; Wheals & Smith, 1975) provides useful information but, where direct evidence is lacking or in long-term attempts to trace distribution chains, the additional parameters obtained by measuring variations through blocks of resin might well enhance the validity of the comparisons.

Fairbairn & others (1976) compared the surface and the inside of a block of resin. They found (a) more THC inside the block than in the surface layers; (b) a low THC content corresponded to a high CBN content and vice versa; (c) the total THC + CBN was higher inside the block than in the surface layers, and (d) the CBD content was the same

Table 3. *Variation in cannabinoid content through a block of cannabis resin.* Results are given as percentage of cannabinoid relative to the outer layer 1 = 100%.

	THC	THCA	THC + THCA	CBD	CBDA	CBD + CBDA	CBN	CBNA	CBN + CBNA	CBCh	CBChA	THC + THCA + CBN + CBNA
Outer layer*												
2	107	167	117	80	136	101	83	150	89	123	143	112
3	97	185	112	76	140	100	83	150	89	132	151	108
Centre layer*												
4	92	169	105	80	132	99	97	150	102	123	148	104
5	83	140	93	88	114	98	112	117	113	114	135	97
6	88	100	90	95	96	96	121	83	117	100	109	95
Outer layer*												
7	109	35	97	121	48	94	112	33	105	96	58	98

* 2 mm thick layers cut from 14 mm thick block or resin. Layer 1 had the following composition by weight: THC 2·3%, THCA 0·48%, CBD 0·84%, CBDA 0·50%, CBN 0·58%, CBNA 0·06%. Absolute amounts of CBCh and CBChA were not determined since a pure standard was not available.

throughout the block. By comparison, we found (a) more THCA in all the inner layers than in the surface layers, but in some of the inner layers there was less THC and THC + THCA than in the surface layers; (b) A low THC + THCA corresponded to a high CBN + CBNA and vice versa, but the variations in THC + THCA were far greater than those in CBN + CBNA when weights rather than percentages were considered; (c) the total THC + THCA + CBN + CBNA was higher in some inner layers compared with the surface layers but lower in others; and (d) the CBD + CBDA content was fairly constant throughout the block, but there were wide variations in the individual amounts of CBD and CBDA in the layers. In general, the acidic cannabinoids increased in concentration towards the centre of the block while the variations in the neutral cannabinoids were more complex.

CONCLUSIONS

The above experiments are a good illustration of the simplistic results given by g.l.c. compared with h.p.l.c. in cannabis analysis. G.l.c. is satisfactory in many instances, but it may not always be possible to ignore the complexities introduced by consideration of the acidic cannabinoids.

The extraction experiments provide useful data on the relative efficiences of the various solvents tested, and the results show that methanol (or ethanol) is a better solvent than chloroform or light petroleum.

An important feature of the decomposition experiments is the demonstration that solutions of acidic cannabinoids stored in darkness can decompose, presumably by decarboxylation, even at −18°. In general, the rate of decomposition increases with temperature, but there are wide variations depending on the cannabinoid and the solvent. The neutral cannabinoids are much more stable in darkness than the acidic cannabinoids but, like Fairbairn & others (1976) we found that exposure to daylight caused extensive decomposition. Such findings have obvious relevance in the preparation of dosage forms of natural or synthetic cannabinoids for pharmacological studies or clinical trials.

The determination of the cannabinoid profile through a block of cannabis resin shows that the variations are more complex than previously thought. Such variations may be useful in forensic applications of cannabis analysis.

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