Journal of Chromatography, 205 (1981) 444–450
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,390

Note

Use of Amberlite XAD-2 columns for the separation of cannabinoids from *Cannabis* extracts

H. HENDRIKS*, S. BATTERMAN, R. BOS, H. J. HUIZING and Th. M. MALINGRÉ
Laboratory for Pharmacognosy, Antonius Deusinglaan 2, 9713 AW Groningen (The Netherlands)
(First received May 8th, 1980; revised manuscript received October 3rd, 1980)

The adsorbent Amberlite XAD-2 (a polystyrene–divinylbenzene resin) is often used to isolate or concentrate organic compounds such as drugs and metabolites from aqueous solutions such as body fluids[1,2]. Recently the successful application of this adsorbent to the purification and separation of pyrrolizidine alkaloids has been reported[3]. A good separation between anthraglycosides and aglycones, present in anthracene derivative-containing drugs (*Rhamnus*, *Rheum* and *Cassia*), can also be obtained by this method[4]. In these cases aqueous extracts from the crude drug were used. However, in phytochemistry and pharmacognosy extracts are often prepared using more or less polar organic solvents. This might be a reason why Amberlite XAD-2 column chromatography has not often been applied in this field.

In *Cannabis sativa* L. both neutral cannabinoids and their corresponding acids may be present. Usually extracts from the drug are prepared with *n*-pentane, chloroform, diethyl ether, etc. Investigation of these extracts is often effected by means of different chromatographic techniques [liquid chromatography, thin-layer chromatography (TLC), gas chromatography (GC) and high-performance liquid chromatography][5–11]. Cannabinoid acids are completely decarboxylated during GC, leading to their neutral analogues[12,13], and we have found that this degradation also partly takes place when liquid chromatography with silica gel and aluminium oxide is used. This was a reason for extending the scavenging technique used by Grote and Spiteller[14] to linear-gradient Amberlite XAD-2 column chromatography for the separation of cannabinoid acids (as their salts) from neutral cannabinoids, present in extracts from *Cannabis*. For this separation, a water–ethanol gradient was used containing 1.0 *M* Tris, adjusted to pH 10.

In a second experiment, a water–ethanol gradient containing 0.5 *M* lithium chloride was used for the separation of neutral cannabinoids. In this way an alternative chromatographic method might be obtained because the GC retention times and TLC R_F values of cannabinoids may coincide.

EXPERIMENTAL

Preparation of Cannabis extracts

A 25-g amount of air-dried flowering tops of *Cannabis* (for botanical description see ref. 15) was extracted twice for 30 min with 100 ml of chloroform. The com-

0021-9673/81/0000–0000/$02.50

bined extracts were concentrated by means of a rotary evaporator below 40°C until the solvent had been removed. The residue was mixed with 100 ml of light petroleum (b.p. <40°C) and 4 g of silica gel (UCB 2301) to remove most of the chlorophyll. After filtration the solvent was removed again. The residue (extract I) was used for the separation of the cannabinoid acids from the neutral compounds.

A 15-g amount of a commercial *Cannabis* extract (Extractum Cannabis indicae spissum, ACF, Amsterdam, The Netherlands) was dissolved in 10 ml of a mixture of light petroleum and diethyl ether (9:1) and submitted to chromatography on a silica gel column (20 × 2.5 cm I.D.) using the same solvent mixture to remove the cannabinoid acids and chlorophyll. The eluate containing the neutral cannabinoids was concentrated by means of a rotary evaporator (extract II).

Amberlite XAD-2 column chromatography

The following eluents were used for the Amberlite XAD column chromatography: solution A1, 0.5 *M* Tris in 1.5 l of 30% ethanol, adjusted to pH 10 with concentrated hydrochloric acid; solution B1, 0.5 *M* Tris in 1.5 l of 70% ethanol, adjusted to pH 10 with concentrated hydrochloric acid; solution A2, 0.5 *M* lithium chloride in 1.5 l of 60% ethanol; solution B2, 0.5 *M* lithium chloride in 1.5 l of 90% ethanol.

Separation of the cannabinoid acids from the neutral cannabinoids. About 0.5 g of extract I was mixed with 2 ml of solution A1 and transferred on to the top of an Amberlite XAD-2 (50–100 μm) column (26 × 1.3 cm I.D.; Serva, Heidelberg, G.F.R.) that had previously been washed with methanol and solution A1. Gradient column chromatography was performed according to Fig. 1 using buffer solutions A1 and B1. Fractions of about 15 ml were collected at a flow-rate of 20 ml/h (45 min per fraction).

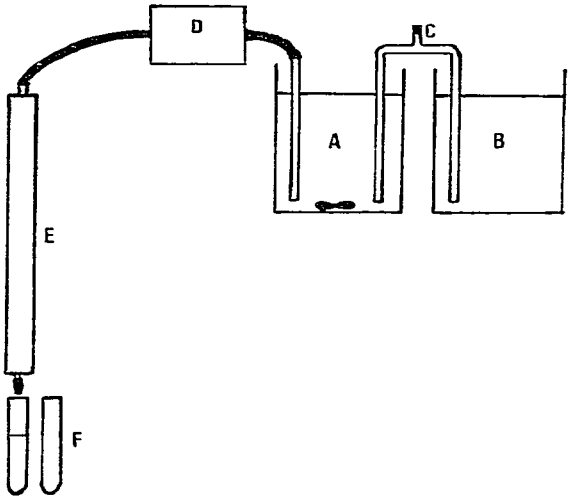

Fig. 1. Scheme for gradient liquid chromatography. A = solution A; B = solution B; C = bridge; D = multi-purpose peristaltic pump (HM 3208); E = Amberlite XAD-2 column; F = fraction collector.

Separation of the neutral cannabinoids. About 0.5 g of extract II was mixed with 2 ml of solution A2 and transferred on to the same Amberlite XAD-2 column, which had previously been washed with methanol and solution A2. Gradient column chromatography (Fig. 1) was performed using solutions A2 and B2. Fractions of about 15 ml were collected at a flow-rate of 20ml/h (45 min per fraction).

Investigation of the fractions by TLC, GC and GC–mass spectrometry (GC–MS)

Tris buffer fractions (3 ml) were diluted with 5 ml of water, acidified with concentrated hydrochloric acid and extracted with 5 ml of light petroleum (b.p. <40°C). The organic extracts of the fractions were concentrated to a small volume. The lithium chloride fractions were treated in the same way without acidification. The concentrated organic extracts of the fractions were examined by TLC, GC and GC–MS.

TLC of the extracts of the fractions. The sample was 1–10 μl of the extract. Pre-coated silica gel F_{254} plates (20 × 20 and 10 × 20 cm) (Merck, Darmstadt, G.F.R.) were used. The eluent was *n*-hexane–diethyl ether (70:30). Detection was effected with 0.5% Fast Blue Salt B in 96% ethanol (for cannabinoids) and anisaldehyde solution[16] (for terpenoids etc.). After TLC the fractions were combined and the combined fractions were examined again by TLC.

GC of the combined fractions on a Perkin-Elmer F30 gas chromatograph. The column (1.8 m × 2 mm I.D.) was packed with 3% OV-17 (Chrompack) on Chromosorb W HP (100–120 mesh) and the temperature was programmed from 200 to 270°C at 6°C/min, being maintained at the final temperature for 5 min. The flow-rate of the carrier gas (nitrogen) was 25 ml/min. The injection block and the flame-ionization detector temperatures were 250°C.

GC–MS. The column (1.8 m × 2 mm I.D.) was packed with 5% SE-52 (Chrompack) on Chromosorb W HP (100–120 mesh). The other GC conditions were as above. Mass spectra were taken with a Finnigan 3300/6110 computerized system by repetitive scanning (4 sec per cycle time) at 70 eV using a combined chemical ionization–electron impact source (electron impact mode).

RESULTS AND DISCUSSION

Fig. 2 shows that a good separation between the cannabinoid acids and the neutral analogues was obtained. This is easy to understand because the dissociated acids are more soluble than the neutral compounds in the Tris buffer solution. A good separation was also obtained between cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA), which may be explained by the differences in their hydrophobic adsorption[17]. In addition to the fractions containing CBGA and those containing neutral cannabinoids eluted from the column, fractions are present that probably contain some other cannabinoid acids and more polar neutral cannabinoids, as could be concluded from the results of TLC (low R_F values) and GC (long retention times).

The cannabinoid acids did not decompose when they were kept in the Tris buffer solution and stored at 4°C. In this way it was possible to obtain a pure solution of CBDA in Tris buffer using an Amberlite XAD-2 column with larger dimensions. From this solution CBDA and cannabidiol (CBD-C5) can be prepared as required.

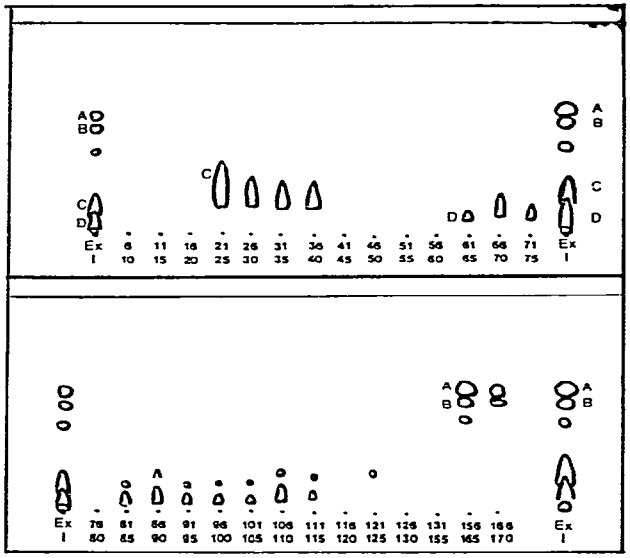

Fig. 2. TLC of the combined fractions (6–10, 11–15, etc.) obtained from the separation of cannabinoid acids and neutral cannabinoids on an Amberlite XAD-2 column. A = CBD-C5; B = CBG-C5; C = CBDA; D = CBGA; Ex I = original extract.

A clear separation of the neutral cannabinoids could not be achieved using Tris buffer and a water–ethanol gradient as described. To separate the neutral cannabinoids present in a cannabinoid acid-free *Cannabis* extract a water–ethanol gradient (60–90% ethanol) containing 0.5 *M* lithium chloride was used.

Figs. 3 and 4 show that, under these conditions, a clear separation can be obtained between cannabidivarol (CBD-C3) and CBD-C5 and between cannabivarol (CBN-C3) and cannabinol (CBN-C5). This separation might be explained by the effect of the side-chain, the pentyl chain being adsorbed more strongly than the propyl chain on Amberlite XAD-2 owing to the forces mentioned above. The fact that CBD-C3 and CBD-C5 elute earlier from the column than the corresponding CBN compounds can be ascribed to the presence of two phenolic hydroxyl groups and a methylene group in CBD.

The GC analysis of the fractions showed that the peak of CBD-C3 coincides with that of another component with the same molecular weight (286) (Fig. 4). This compound is present in fractions VI–VIII and is completely separated from CBD-C3 present in fractions I–IV. This was shown by means of GC–MS using an SE-52 column, a double peak being observed. Further, some differences in the mass spectra were observed (Fig. 5). Based on its chromatographic behaviour and its mass spectrum ($m/e = 218$ absent) the compound is probably the propyl analogue (CBC-C3) of cannabichromene (CBC-C5)[18].

An experiment with water–ethanol gradient containing 1.0 *M* (instead of 0.5 *M*) lithium chloride showed that the cannabinoids are retained longer on the column, owing to salting-out. A distinct effect on the separation could not be observed, but for such studies it would be desirable to have pure test compounds available.

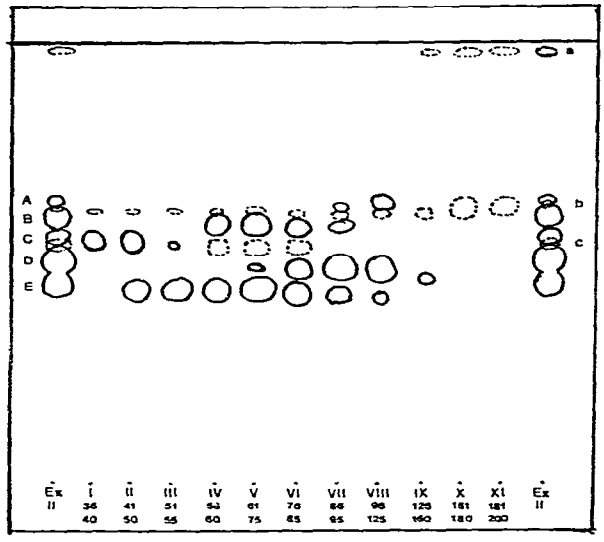

Fig. 3. TLC of the combined fractions I–XI (I = combination of fractions 36–40; II = combination of fractions 41–50, etc.) obtained from the separation of neutral cannabinoids on an Amberlite XAD-2 column. A = unknown; B = CBD-C5; C = CBD-C3; D = CBN-C5; E = CBN-C3. Ex II = original extract; a = hydrocarbone terpenes; b = diisooctyl phthalate; c = caryophyllene oxide.

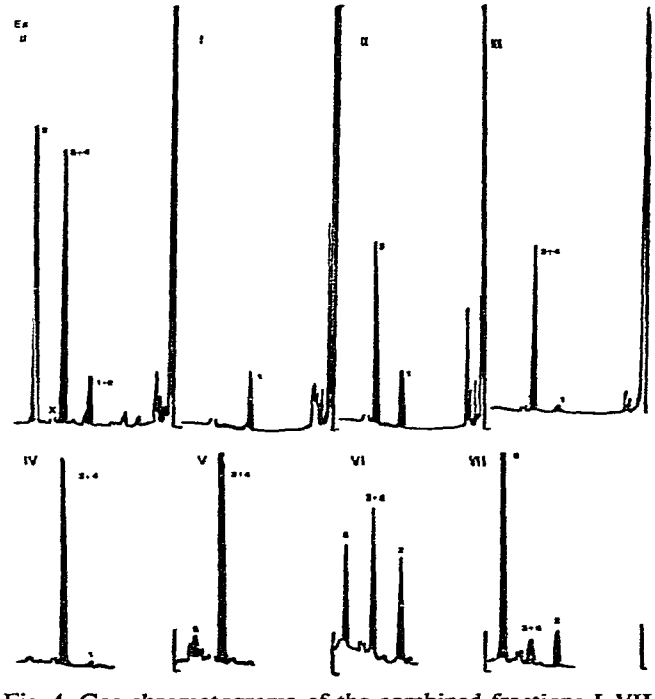

Fig. 4. Gas chromatograms of the combined fractions I–VII (I = combination of fractions 36–40, II = combination of fractions 41–50, etc.) and of the original extract (extract II) obtained from the separation of neutral cannabinoids on an Amberlite XAD-2 column. 1 = CBD-C3; 2 = CBC-C3; 3 = CBN-C3; 4 = CBD-C5; 5 = CBN-C6; X = diisooctyl phthalate.

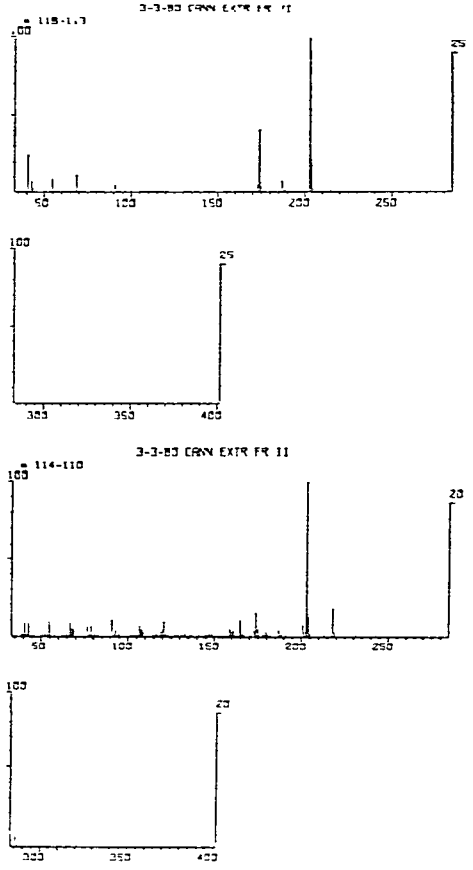

Fig. 5. Mass spectra of CBD-C3 in fraction II and of CBC-C3 (m/e = 218 absent) in fraction VI.

It should be borne in mind that other compounds (such as terpenoids) are also present in the original extracts. TLC using an anisaldehyde solution as a spray reagent showed that the essential oil component caryophyllene oxide[19] is present in fractions IV–VI (Fig. 3) whereas the less polar terpenes are present in fraction VIII and subsequent fractions. Further, the presence of phthalates, in this instance diisooctyl phthalate, which may be present as softeners in synthetic tube material, may interfere severely in GC using OV-17 columns because its peak coincides with Δ^1-tetrahydrocannabinol (THC-C5).

However, from the results obtained it can be concluded that Amberlite XAD-2 column chromatography offers possibilities for the separation and isolation of cannabinoids present in organic extracts from *Cannabis sativa* L.

ACKNOWLEDGEMENTS

The authors thank J. F. Visser for the cultivation of *Cannabis* in the experimental garden at Buitenpost, Dr. A. P. Bruins for the GC–MS work and J. J. Duitsch for the photography.

REFERENCES

1 F. Samuels Brusse, R. Furst and W. P. van Bennekom, *Pharm. Weekbl.*, 109 (1974) 921–928.
2 M. D. Grieser and D. J. Pietrzyk, *Anal. Chem.*, 45 (1973) 1348–1353.
3 H. J. Huizing and Th. M. Malingré, *J. Chromatogr.*, 176 (1979) 274–279.
4 R. Denee and H. J. Huizing, *Lloydia*, (1981) in press.
5 L. Hanus, L. Hruban, Z. D. Krejci, *Acta Univ. Olumuc. Fac. Med.*, 74 (1975) 167–172.
6 Z. Krejci and L. Hanus, *Acta Univ. Olumuc. Fac. Med.*, 76 (1976) 145–152.
7 M. Hoton-Dorge, *J. Pharm. Belge*, 29 (1974) 415–422.
8 R. Fowler, R. A. Gilhooley and P. B. Baker, *J. Chromatogr.*, 171 (1979) 509–511.
9 K. Fonseka, M. Widman and S. Agurell, *J. Chromatogr.*, 120 (1976) 343–348.
10 R. N. Smith, *J. Chromatogr.*, 115 (1975) 101–106.
11 S. L. Kanter, M. R. Musumeci and L. E. Hollister, *J. Chromatogr.*, 171 (1979) 504–508.
12 R. A. de Zeeuw, Th. M. Malingré and F. W. H. M. Merkus, *J. Pharm. Pharmacol.*, 24 (1972) 1–6.
13 M. Willinsky and L. di Simone, *Farmaco, Ed. Prat.*, 28 (1973) 441–448.
14 H. Grote and G. Spiteller, *J. Chromatogr.*, 154 (1978) 13–23.
15 Th. Malingré, H. Hendriks, S. Batterman, R. Bos and J. Visser, *Planta Med.*, 56 (1975) 56–61.
16 E. Stahl, *Chromatographische und Mikroskopische Analyse von Drogen*, Gustav Fischer Verlag, Stuttgart, 1979.
17 K. L. Cheng and H. Y. Guh, *Mikrochim. Acta*, No. 1 (1978) 55–68.
18 R. T. Coutts and G. R. Jones, *J. Forensic Sci.*, 24 (1979) 291–302.
19 H. Hendriks, Th. M. Malingré, S. Batterman and R. Bos, *Pharm. Weekbl.*, 113 (1978) 413–424.